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Structural Modification of Bioactive Compounds. II.[1] Syntheses of Aminophosphonoic Acids[2]

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To develop antagonists which show selectivity in blocking neurotransmitters, several aminophosphonoic acids, 2-amino-5-phosphonopentanoic acid (IVb), 2-amino-4-(2-phosphonomethylphenyl)butyric acid (VIII), 2-(2-amino-2-carboxy)ethylphenylphosphonic acid (XIc), and *N*-benzylproline-4-phosphonic acid (XIX), were synthesized. Compounds IVb, VIII, and XIc were prepared from the corresponding halides (V, Xa, and XIa, respectively) by treatment with sodium diethyl acetamidomalonate (VI). Compound XIX was synthesized *via* 1,3-dipolar cycloaddition of ethyl *N*-benzyl-*N*-phenylthiomethylglycinate (XV) to diethyl vinylphosphonate (XVI).

Keywords——aminophosphonoic acid; antagonist; diethyl acetamidomalonate; 1,3-cycloaddition; pyrazole

Many structural analogues of natural and synthetic compounds having significant biological activities have been synthesized in order to examine the structure–activity relationship and mechanisms of biological actions. In this series of studies, we intend to synthesize structurally modified bioactive compounds, especially vitamin, amino acid, steroid analogues. In the preceding paper,[1] we reported the syntheses of three kinds of vitamin D analogues. In this paper, the preparation of aminophosphonoic acid (I) is described.

Hemmi *et al.*[3] reported on the syntheses of some phosphonic acids (II) and their application to antibiotics. Kametani *et al.*[4] synthesized many phosphonopeptides (III) and examined their antibacterial activities. On the other hand, aminophosphonoic acids were studied as blockers of the central nervous system (CNS).[5] L-Glutamic and L-aspartic acids may be neurotransmitters in the vertebrate CNS. In order to obtain more definitive evidence for this possibility, and to assess the role of these compounds in neurotransmission at particular synapses, however, it will be necessary to develop antagonists which show selectivity in blocking one or other of these amino acids. D-α-Aminoadipic acid, diethyl L-glutamate, γ-D-glutamylglycine and *cis* 2,3-piperidine dicarboxylic acid were suggested to have antagonist activities. The most potent and selective antagonist available at present is considered to be aminophosphonic acid. The synthesis of 2-amino-4-phosphonobutyric acid (IVa) was reported,[6] and 2-amino-5-phosphonopentanoic acid (IVb) is also a known compound. However, experimental conditions for the preparation of IVb are not given in the literature. To find the optimum experimental conditions and to obtain physical data, IVa was at first synthesized by the known method (see Experimental). Based on the knowledge thus obtained, the preparation of IVb was attempted. Triethyl phosphite and 4 mol eq of propylene bromide were heated at 160 °C for 3 h to give only an unidentifiable resinous material. Next, 20 mol eq of the bromide was heated with triethyl phosphite, and the progress of the reaction was checked by gas chromatography (GC). After 2 h of heating, a large excess of propylene bromide was removed and diethyl 3-bromopropylphosphonate (V) was isolated by careful distillation under reduced pressure in a yield of 76.7%. Compound V was treated with sodium diethyl acetamidomalonate (VI) in toluene and diethyl carbonate to give diethyl 4-acetamino-4,4-bis(ethoxycarbonyl)propylphosphonate (VII), which exhibited signals at δ 2.00 (3H) and 6.80 (1H) ppm due to the acetamide group in the nuclear magnetic resonance (NMR) spectrum, in a yield of 25.4%. Compound VII was hydrolyzed under reflux in 1 N hydrochloric acid and purified through Dowex 50W-X8 resin to give IVb hydrochloride. Addition of epichlorohydrin to the salt gave IVb in a yield of 47.0% from VII. Compound IVb, mp 248 °C, exhibited a broad absorption band at 3600—2400 and a band at 1720 cm^{-1} due to the carboxyl group in the infrared (IR) spectrum, and two multiplet signals at δ 1.1—2.2 (1H) and 3.8—4.3 (6H) ppm due to the methine and methylene protons, respectively, in the NMR spectrum. The elemental analysis supported the formula of IVb.

A target compound, 2-amino-4-(2-phosphonomethylphenyl)butyric acid (VIII), was synthesized starting with (2-bromomethyl)phenethylbromide (IX), which was prepared according to the reported method.[7] This dibromide, IX, was treated with triethyl phosphite to give diethyl 2-(2-bromoethylphenyl)methylphosphonate (Xa), which exhibited a doublet signal ($J=22$ Hz) at δ 3.06 ppm due to the methylene protons of methylphosphonate coupling with phosphorus in the NMR spectrum. The yield was 63.0%. This bromophosphonate derivative was treated with VI to give the expected acetamidomalonylphosphonate derivative (Xb), which was purified through a silica gel column. The structure of Xb was determined from the NMR spectrum and mass spectrum (MS), and elemental analysis data. In the NMR spectrum, a doublet signal ($J=22$ Hz) at δ 3.10 ppm due to the methylene protons attached to the phosphorus atom was observed. The hydrolysis of Xb was performed under reflux with diluted hydrochloric acid. The product was purified by using ion-exchange resin and the neutrallized product was recrystallized from water to give VIII in a relatively high yield. Compound VIII showed a carbonyl band at 1728 cm^{-1} in the IR spectrum. The structure was confirmed by elemental analysis.

2-(2-Amino-2-carboxy)ethylphenylphosphonic acid (XIc) was obtained in a manner similar to that used in the preparation of Xb. The starting material, dimethyl 2-bromomethylphenylphosphonate (XIa), was prepared *via* two steps from 2-iodotoluene according to the literature,[11] and treated with VI followed by hydrolysis with 10% hydrochloric acid to give XIc in a satisfactory yield. In this case, we could not isolate the intermediate, dimethyl 2-[2-acetamido-2,2-bis(ethoxycarbonyl)]ethylphenylphosphonate (XIb), but obtained the partially hydrolyzed product.

We chose proline-4-phosphonic acid (XII) as the next target compound. *N*-Boc-4-iodo-L-proline methyl ester (XIIIa, Boc = *tert*-butoxycarbonyl), the starting material for XII, was prepared *via* three steps from 4-hydroxy-L-proline by the method described recently.[8] The structure of this starting material was confirmed by comparison of the melting point, NMR spectrum, and optical rotation with those reported. Several attempts to synthesize the corresponding ester of XII from XIIIa by treatment with triethyl phosphite were carried out under various conditions (heating at 160 °C without solvent, at reflux temperature in toluene, at reflux temperature in benzene in the presence of 4-dimethylaminopyridine). Unfortunately, we could not detect the target ester but only the dehydroiodated product, *N*-Boc-3,4- or -4,5-dehydro-L-proline methyl ester (XIV). Treatment of XIIIa with sodium dibutyl phosphite in toluene at 80—90 °C gave *N*-Boc-4-iodo-L-proline *n*-butyl ester (XIIIb), an ester-exchanged product.

Recently Achiwa *et al.*[9] reported an elegant synthesis of proline derivatives by means of the 1,3-dipolar cycloaddition reaction of ethyl *N*-phenylthiomethylglycinate to ethyl acrylate. We tried to apply this method to the synthesis of XII. Ethyl *N*-benzylglycinate (prepared from benzylamine and ethyl chloroacetate) was treated with thiophenol and formalin to give ethyl *N*-benzyl-*N*-phenylthiomethylglycinate (XV) in 46.0% yield, then XV was treated with diethyl vinylphosphonate (XVI) under the reported conditions. When XVI was added to a mixture of

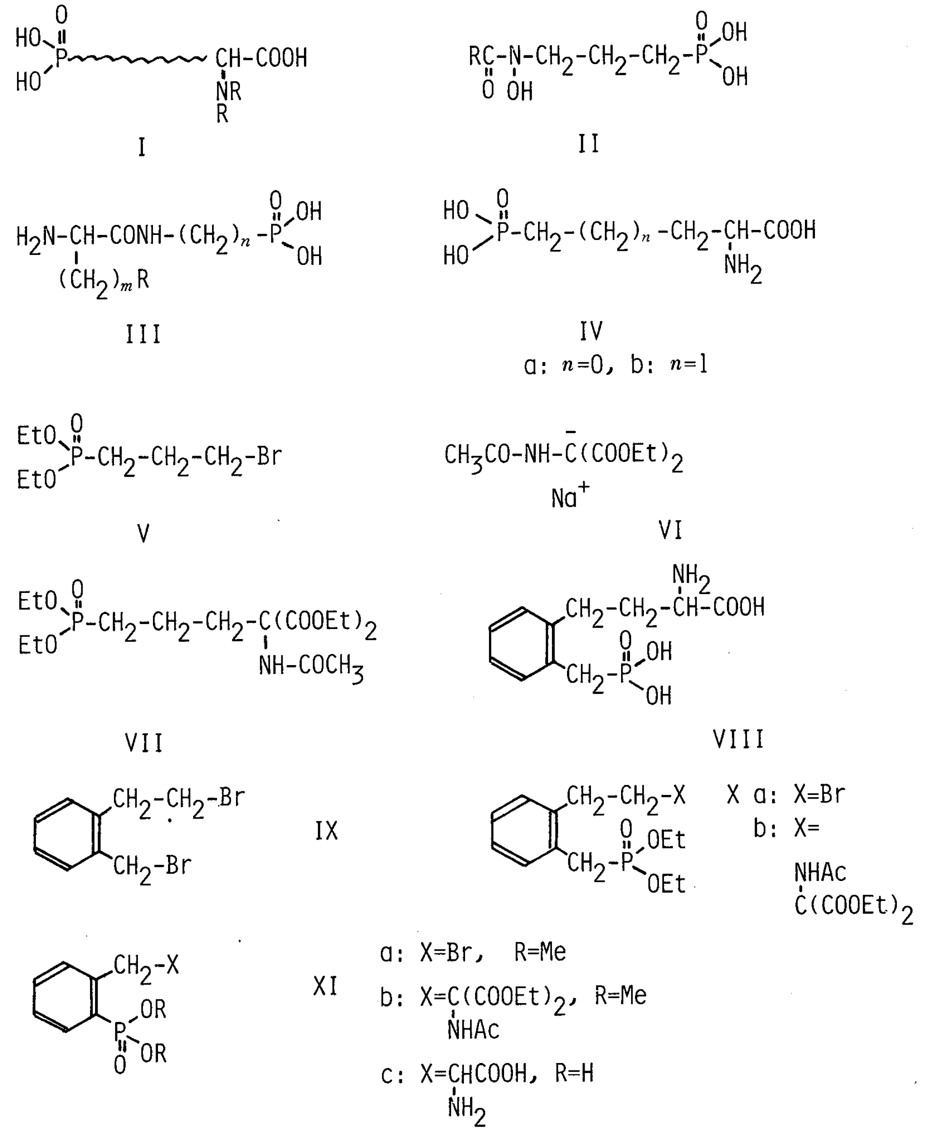

Chart 1

XV, sodium hydride (2 mol eq), and hexamethylphosphoramide (HMPA) in dimethoxyethane (DME), the cyclized product, diethyl 1-benzyl-2-ethoxycarbonylpyrrolidine-4-phosphonate (XVII), was obtained in 6.0% yield together with a large amount of diethyl 2-phenylthioethylphosphonate (XVIII). The formation of this Michael adduct, XVIII, was suppressed when XV was added to the mixture of XVI, sodium hydride, and HMPA in DME. In this case, the yield of XVII was improved to 25.5%. Compound XVII exhibited two peaks at retention times (t_R) of 4.1 and 4.8 min in gas chromatography (GC) in a ratio of *ca.* 3:5. Separation was achieved by medium pressure liquid chromatography using 30% ethyl acetate–benzene as an eluant. The structures of these products were determined from the NMR spectra and the elemental analyses. Although the relative configuration could not be determined from the NMR data, it was considered that the major product should be the *trans* isomer, based on molecular models. Attempts to debenzylate XVII using palladium–carbon under normal or medium pressure of hydrogen failed. Thus, the hydrolysis of XVII into *N*-benzylproline-4-phosphonic acid (XIX) was carried out by refluxing in 5% hydrochloric acid, with monitoring by high performance liquid chromatography (HPLC). After 14 h of refluxing, HPLC (reverse phase, methanol) revealed that the product was a mixture of half-

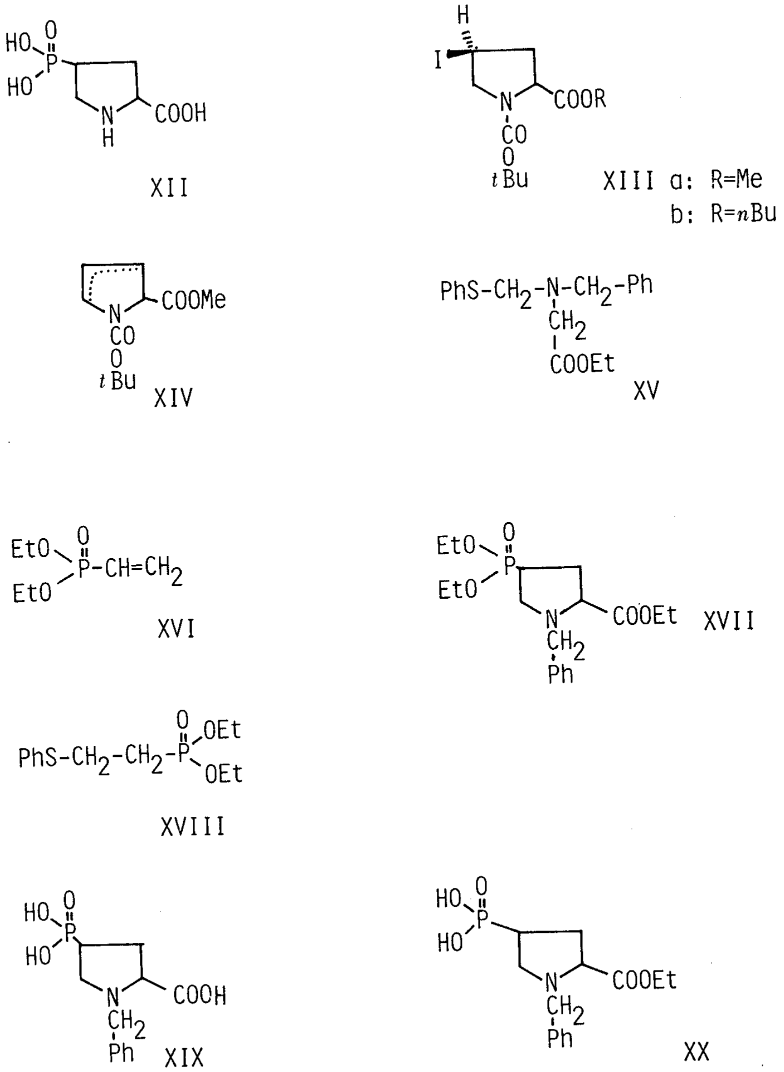

Chart 2

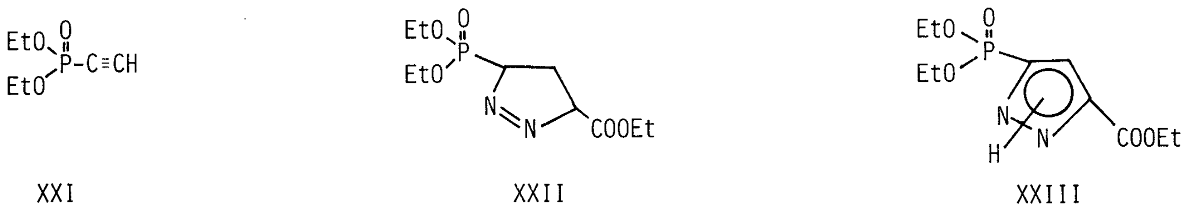

Chart 3

ester (XX) and XIX in a ratio of 13 : 7. In HPLC, XVII, XIX, and XX showed t_R 2.67, 3.00, and 2.10 min, respectively. To transform XX into XIX, treatment with concentrated hydrochloric acid was necessary. Compound XIX exhibited a double-doublet signal ($J=20$ and 8 Hz) at δ 4.34 ppm due to the C_2-proton in the NMR spectrum, measured in deuterium oxide. The elemental analysis revealed that XIX has one eq of crystalline water.

We also examined the 1,3-dipolar cycloaddition reaction of ethyl diazoacetate to XVI or diethyl ethynylphosphonate (XXI). When a mixture of XVI and ethyl diazoacetate was refluxed in benzene, diethyl 5-ethoxycarbonyl-4,5-dihydro-3(3*H*)pyrazolylphosphonate (XXII) was obtained as an unstable yellow oil in 87.0% yield. On the other hand, a mixture of

XXI and ethyl diazoacetate was heated overnight in a sealed tube to give diethyl 5-ethoxycarbonyl-3(1*H*) or (2*H*)pyrazolylphosphonate (XXIII) in 78.4% yield as a relatively stable oil. Compound XXIII showed a doublet peak at δ 7.20 ppm due to the vinylic proton in the NMR spectrum. This coupling may be attributed to the phosphorus nucleus.[12)]

The biological assay of these synthetic aminophosphonoic acids, IVa, IVb, VIII, XIc, and XIX, is in progress.

Experimental

All melting points and boiling points are uncorrected. IR spectra were determined by using a JASCO IRA-1 diffraction grating spectrophotometer; absorption data are given in cm^{-1}. NMR spectra were recorded on JEOL PMX-60 and Varian XL-200 spectrometers with tetramethylsilane (TMS) as an internal standard. When D_2O was used as a solvent, TMS was used as an external standard. The chemical shifts and coupling constants (*J*) are given in δ and Hz, respectively. MS were measured with a JEOL JMS D-200 (700 eV, direct inlet system) spectrometer. GC was carried out using a Shimadzu GC-6AM instrument with a stainless steel column packed with 5% SE-30. The velocity of N_2 gas flow was 40 ml/min. HPLC was done on a Waters 244 instrument with a Radial Pak A (8 m/m × 10 cm) for reverse phase separation. The solvent (MeOH) flow rate was 1 ml/min. Specific rotation ($[\alpha]_D$) were obtained in $CHCl_3$ with a JASCO DIP-4 polarimeter. All solvents were removed by evaporation under reduced pressure after drying of the solution over anhyd. $MgSO_4$.

2-Amino-3-phosphonobutyric Acid (IVa)——A mixture of ethyl phosphite (5.6 g, 33 mmol) and ethylene dibromide (25 g, 134 mmol) was heated on an oil bath at 160 °C with stirring for 3 h, then diethyl 2-bromoethylphosphonate (5.4 g, 66.7%) was obtained by vacuum distillation. bp 105 °C (3 mmHg). NMR (CCl_4): 1.36 (6H, t, $J=7$), 1.9—2.7 (2H, m, PCH_2), 3.2—3.9 (2H, m, CH_2Br), 4.06 (4H, q, $J=7$). This bromophosphonate (5.4 g) was treated in diethyl carbonate with sodium ethyl acetaminomalonate (VI) [prepared from ethyl acetaminomalonate (4.5 g, 22 mmol) and Na (0.48 g, 21 mmol) in EtOH–toluene] as described in the literature[6)] to give crude phosphonomalonate, which was purified on an SiO_2 column using 30% AcOEt–benzene as an eluant. NMR (CCl_4): 0.7—2.8 (4H, m), 1.49 (12H, t, $J=6$), 1.99 (3H, s, >NAc), 4.07 (8H, q like $J=6$), 7.15 (1H, br s, >NH). The phosphonomalonate was refluxed overnight in 1 : 1 HCl. The mixture was concentrated, then fractionated on a Dowex 50W-X8 (H^+ form) column with 1 N HCl as an eluant. The salt was neutralized with epichlorohydrin to give IVa, which was recrystallized from aq. Me_2CO. mp 238 °C (lit.[6)] 226 °C). 1.5 g (35.0%). IR (Nujol): ν 3200—2300, $\nu_{C=O}$ 1705. NMR (D_2O): 0.8—2.0 (4H, m), 3.3—3.8 (1H, t like, $J=6.5$). MS, *m/e*: 96 [$(HO)_3P-CH_2$]. *Anal.* Calcd for $C_4H_{10}NO_5P+2/3H_2O$: C, 24.62; H, 5.85; N, 7.18. Found: C, 24.70; H, 5.88; N, 7.31.

Diethyl 3-Bromopropylphosphonate (V)——A mixture of ethyl phosphite (5 g, 30 mmol) and propylene bromide (125 g, 600 mmol) was heated on an oil bath at 160 °C, then carefully distilled using stirrer chip instead of a capillary. V: bp 100—110 °C (7 mmHg). 5.9 g (76.7%). GC (200 °C) t_R 2.0 min. *Cf.* propylene dibromide: t_R 0.9 min. Tetraethyl propylenediphosphonate: t_R 3.7 min. NMR (CCl_4): 1.30 (6H, t, $J=7.5$, $-CH_2-\underline{CH_3}$), 0.9—2.5 (4H, m, PCH_2CH_2), 3.2—3.7 (2H, m, CH_2Br), 3.7—4.3 (4H, m). MS, *m/e*: 259 (M^+), 261 (M^++2).

Diethyl 4-Acetamino-4,4-bis(ethoxycarbonyl)propylphosphonate (VII)——V (4.9 g, 19 mmol) was treated with sodium ethyl acetaminomalonate (VI, 4.1 g, 17 mmol) under conditions similar to those used in the preparation of IVa (with monitoring by GC). Ethyl carbonate was distilled off, and the residue was fractionated by SiO_2 column chromatography. VII was eluted with AcOEt. 1.39 g. GC (250 °C) t_R: 3.4 min. *Cf.*, VI: t_R: 0.8 min. NMR ($CDCl_3$): 1.0—1.5 (12H, m, $CH_2-\underline{CH_3}$), 0.8—2.6 (6H, m), 2.00 (3H, s, AcN<), 3.6—4.5 (8H, $-\underline{CH_2}-CH_3$), 6.80 (1H, br s, >NH). MS, *m/e*: 396 (M^++1).

2-Amino-5-phosphonopentanoic Acid (IVb)——A mixture of VII (1.33 g, 3.4 mmol) and 6 N HCl (50 ml) was refluxed for 15 h, then concentrated and fractionated through a Dowex 50W-X8 column. IVb·HCl was eluted with 2% HCl solution. This salt was neutralized by the addition of epichlorohydrin to give crude IVb, which was recrystallized from water. IVb: mp 251—255 °C (dec.). 0.32 g (47.0%). IR (Nujol): ν 3600—2400, $\nu_{C=O}$ 1720. NMR (D_2O): 1.3—2.3 (6H, m, $-(CH_2)_3-$), 3.8—4.3 (1H, m, >CH–N<). MS, *m/e* (%): 96 [$CH_2=P(OH)_3$]. *Anal.* Calcd for $C_5H_{12}NO_5P+1/3H_2O$: C, 29.56; H, 6.29; N, 6.90. Found: C, 29.23; H, 6.31; N, 7.20.

Diethyl 2-(2-Bromoethyl)phenylmethylphosphonate (Xa)——2-Bromoethylphenethylbromide (IX) was prepared *via* two steps from phenethyl alcohol according to the literature.[7)] IX: NMR (CCl_4): 3.1—3.8 (4H, m, $-CH_2-CH_2-$), 4.55 (2H, s, $-CH_2-Br$), 7.30 (4H, s, aromatic H). A mixture of IX (10 g, 36 mmol) and triethyl phosphite (6 g, 36 mmol) was stirred for 15 h at room temperature, and then overnight at 70 °C. The mixture was fractionated through an SiO_2 column. The fraction eluted with 10% Et_2O in hexane was distilled to give crude Xa. 6.5 g. bp 140—150 °C (2 mmHg). NMR (CCl_4): 1.23 (6H, t, $J=7$, $-CH_2-\underline{CH_3}$), 3.06 (2H, d, $J=22$, $-CH_2-P$), 3.3—3.8 (4H, m, $-CH_2-CH_2-Br$), 3.8—4.3 (4H, m, $-OCH_2-$), 7.15 (4H, s, aromatic H). MS, *m/e*: 334 (M^+), 336 (M^++2).

Diethyl 2-{[3-Acetamido-3,3-bis(ethoxycarbonyl)]propyl}phenylmethylphosphonate (Xb)——A mixture of crude Xa (6.5 g) and VI [prepared from Na (0.42 g, 18.3 mmol)] was heated overnight in diethyl carbonate (*ca.* 50 ml) at

130 °C. GC-MS (250 °C): t_R 8.15 min, *m/e* 471 (M^+). Contaminants were removed by distillation to give crude XI (3.36 g, 43.6%) as the residue. NMR (CCl_4): 1.23 (12H, m), 2.03 (3H, s, Ac), 2.56 (4H, s, $-CH_2-CH_2-$), 3.10 (2H, d, $J=22$, CH_2P), 3.6—4.5 (8H, m), 7.13 (4H, s, aromatic H). *Anal.* Calcd for $C_{16}H_{30}NO_8P$: C, 56.04; H, 7.27; N, 2.97. Found: C, 56.15; H, 7.18; N, 3.18.

2-Amino-4-(2-phosphonomethylphenyl)butyric Acid (VIII)——Crude Xb (3.36 g) was hydrolyzed under reflux in 6 N HCl (*ca.* 100 ml) for 18 h. The mixture was concentrated and fractionated through a Dowex 50W-X8 column. The fraction eluted with 10% HCl was concentrated, and then epichlorohydrin and acetone were added to the residue to give a precipitate, which was recrystallized from water to give pure VIII. 0.82 g. VIII: IR (Nujol): ν 3300, ν 2800—2200, $\nu_{C=O}$ 1728, $\nu_{NH_3^+}$ 1615, 1558. NMR (D_2O with a few drops of trifluoroacetic acid (TFA)): 1.9—2.5 (2H, m), 2.5—3.1 (2H, m, CH_2Ph), 3.24 (2H, d, $J=22$, CH_2P), 3.9—4.3 (1H, m, >CHN<), 7.25 (4H, s, aromatic H). mp 269—271 °C. *Anal.* Calcd for $C_{11}H_{16}NO_5P$: C, 48.36; H, 5.90; N, 5.13. Found: C, 48.35; H, 5.96; N, 5.24.

2-(2-Amino-2-carboxy)ethylphenylphosphonic Acid (XIc)——In a manner similar to that used in the preparation of VII from V, dimethyl 2-bromomethylphenylphosphonate (XIa, crude 13.45 g contaminated by *ca.* 10% 2-iodobenzylbromide (GC)) in diethyl carbonate (20 ml) was treated with VI (prepared from Na (1.1 g, 48 mmol) and diethyl acetamidomalonate (10.42 g, 48 mmol)). After being heated at 115 °C for 11 h, the mixture was diluted with water. The aq. soln. was extracted with Et_2O and $CHCl_3$. The oily residue (3.37 g) obtained after removal of the organic solvents was purified through an SiO_2 column. The fraction eluted with 20% AcOEt–hexane provided 2-[2-acetamido-2,2-bis(ethoxycarbonyl)]ethyl-1-iodobenzene (*ca.* 1 g). mp 89—90 °C (recrystallized from Et_2O–hexane). Beilstein test: positive. GC (200 °C), t_R: 5.5 min. NMR (CCl_4): 1.29 (6H, t, $J=7$, $-CH_2\underline{CH_3}$), 1.95 (3H, s, Ac), 3.27 (2H, s, $PhCH_2$), 4.20 (4H, q, $J=7$, OCH_2), 6.54 (1H, br s, >NH), 6.6—7.9 (4H, m, aromatic H). MS *m/e* (%): 434 ($M^+ + 1$, 8), 433 (M^+, 2), 306 ($M^+ - I$, base peak). *Anal.* Calcd for $C_{16}H_{20}INO_5$: C, 44.36; H, 4.65; N, 3.23. Found: C, 44.44; H, 4.73; N, *ca.* 2.8. The residue (0.15 g) from the fraction eluted with AcOEt consisted of dimethyl 2-[2-acetamido-2,2-bis(ethoxycarbonyl)]ethylphenylphosphonate (XIb), dimethyl and ethyl methyl esters. GC (200 °C), t_R: 8.4 and 9.3 min. NMR (CCl_4): 1.0—1.5 (q like, $J=6.5$, $-CH_2\underline{CH_3}$), 1.80 (3H, s, Ac), 3.63 (2H, s, CH_2Ph), 3.81 (s, OMe), 4.16 (q, $J=6.5$, OCH_2), 6.9—7.9 (4H, m, aromatic H). MS, *m/e* (%): 444 ($M^+ + 1$ of diethyl ester, 12), 430 ($M^+ + 1$ of ethyl methyl ester, 75), 416 ($M^+ + 1$ of dimethyl ester, 33), 228 (14), 214 (base peak), 200 (69). By concentration of the aqueous layer, a mixture of half esters was obtained. 6.7 g. IR (Nujol): ν 3600, 3400, 3200, $\nu_{C=O}$ 1760, 1735, 1670. NMR (D_2O): 1.15 (t, $J=7$, $-CH_2-\underline{CH_3}$), 1.95 (3H, s like, Ac), 3.36, 3.56, and 3.78 (each s, $PhCH_2-$ and OMe), 4.15 (q, $J=7$, OCH_2), 7.0—8.0 (4H, m, aromatic H). The crude half esters (6.7 g) were refluxed in 10% HCl (200 ml) overnight, then the mixture was evaporated to dryness. The residue was suspended in acetone and treated with epichlorohydrin to give a precipitate, which was collected on a filter. The MeOH-soluble part was recrystallized from aq. acetone to give XIc. mp 220—250 °C (dec.). 3.2 g. IR (KBr): ν 3600—2100, ν 1740—1660. NMR (D_2O): 3.3—3.8 (2H, m, $PhCH_2$), 4.2—4.5 (1H, m, >CHCO), 7.1—8.1 (4H, m, aromatic H). MS, *m/e*: 182 (base peak, $C_8H_7O_3P$). *Anal.* Calcd for $C_9H_{12}NO_5P$: C, 44.09; H, 4.93; N, 5.71. Found: C, 44.38; H, 5.12; N, 5.69.

***N*-Boc-3,4- or -4,5-dihydro-L-proline Methyl Ester (XIV)**——*N*-Boc-4-iodo-L-proline methyl ester (XIIIa) was prepared under conditions similar to those described in the literature.[8] mp 62 °C (lit. mp 63—64 °C). NMR (CCl_4): 1.40 (9H, s, *tert*-Bu), 2.1—2.6 and 2.7—3.1 (each 1H, m, C_3–H), 3.75 (3H, s, OMe), 3.6—4.5 (4H, m). IR (Nujol): $\nu_{C=O}$ 1720, 1695. A mixture of this XIIIa (0.44 g, 1.2 mmol) and triethyl phosphite (0.2 g, 1.2 mmol) was stirred overnight on an oil bath at 160 °C. The product mixture was fractionated by SiO_2 column chromatography to give XIV (25 mg) and crude XIV (170 mg). XIV: IR (film): $\nu_{C=O}$ 1720, 1760. NMR (CCl_4): 1.40 and 1.46 (each s, 2 : 1, 9H, *tert*-Bu), 3.72 (3H, s, OMe), 4.0—4.3 (2H, m, $>NCH_2-$), 4.7—5.1 (1H, m, >NCH<), 5.5—6.2 (2H, m, vinylic H).

***N*-Boc-4-iodo-L-proline *n*-Butyl Ester (XIIIb)**——A solution of XIIIa (368 mg, 1 mmol) in toluene was added to a mixture of dibutyl phosphite (580 mg, 3 mmol) and Na (30 mg, 1 mmol). The mixture was heated overnight at 70—80 °C, then cooled and diluted with benzene. The organic layer was washed with brine and dried. The residue obtained after removal of the solvent was fractionated by SiO_2 column chromatography. XIIIb was obtained from the fraction eluted with 5% Et_2O–hexane, and then microdistilled. bp <130 °C (1 mmHg). NMR (CCl_4): 0.7—1.8 (7H, m, C_3H_7), 1.40 (9H, s, *tert*-Bu), 3.5—4.4 (5H, m, OCH_2, $>NCH_2-$, and >NCH<). *Anal.* Calcd for $C_{14}H_{24}NO_4P$: C, 42.33; H, 6.09; N, 3.53. Found: C, 42.20; H, 5.89; N, 3.34.

Ethyl *N*-Benzyl-*N*-phenylthiomethylglycinate (XV)——Formalin (0.9 g, 10 mmol) was added to a mixture of thiophenol (1.1 g, 10 mmol) and ethyl *N*-benzylglycinate[13] (1.93 g, 10 mmol). After being heated at 80 °C for 2 h, the mixture was extracted with Et_2O. The Et_2O layer was washed with 10% NaOH soln. and brine. The residue obtained after removal of the solvent was fractionated through an SiO_2 column. XV was obtained from the benzene fraction, then microdistilled. bp <120 °C (1 mmHg). GC (200 °C), t_R 3.4 min. IR (film): $\nu_{C=O}$ 1735. NMR (CCl_4): 1.20 (3H, t, $J=8$, $CH_2\underline{CH_3}$), 3.34 (2H, s, $>NCH_2CO$), 3.78 (2H, s, $>NCH_2Ph$), 4.40 (2H, q, $J=8$, OCH_2), 4.44 (2H, s, $SCH_2N<$), 7.0—7.5 (10H, aromatic H). *Anal.* Calcd for $C_{18}H_{21}NO_2S$: C, 68.54; H, 6.70; N, 4.44. Found: C, 68.20; H, 6.82; N, 4.53.

Diethyl 1-Benzyl-2-ethoxycarbonylpyrrolidine-4-phosphonate (XVII)——A solution of XV (3.15 g, 10 mmol) in DME was added with ice-cooling and stirring to a mixture of diethyl vinylphosphonate[10] (XVI) (1.64 g, 10 mmol), NaH (50% in oil, 0.96 g, 20 mmol), and HMPA (1.79 g, 10 mmol) in an appropriate amount of DME under an Ar atmosphere. After being stirred overnight at room temperature, the mixture was poured into ice–water. The oil derived from the NaH, XVII (*cis*:*trans*$=9:17$), and XVIII (*vide infra*) were obtained from the hexane extract. XVII (*cis*:*trans*$=3:5$) was obtained from the Et_2O extract. GC (250 °C), t_R: 4.1 (*cis*), 4.8 (*trans*) min. The former product mixture was fractionated through an SiO_2 column and the *cis* and *trans* mixture of XVII was obtained from the $CHCl_3$ eluate. The combined XVII (0.94 g, 25.5%) was further fractionated by medium pressure liquid chromatography with 30% AcOEt in benzene as an eluant. The *cis* and then the *trans* isomer were eluted successively (checked by GC and MS). *trans*: oily compound. GC (250 °C), t_R: 4.8 min. NMR ($CDCl_3$): 1.28 (9H, q like, $J=7$, $-CH_2-\underline{CH_3}$), 2.2—2.5 (2H, m, C_3–H), 2.5—2.8 (2H, m, C_4– and C_5–H), 3.20 (1H, q, $J=6$, C_5–H), 3.38 (1H, dd, $J=8$, 6, C_2–H), 3.56 and 3.97 (each 1H, d, $J=12$, $PhCH_2$), 4.0—4.2 (6H, m, OCH_2), 7.2—7.4 (5H, d like, $J=8$, aromatic H). *Anal.* Calcd for $C_{18}H_{28}NO_5P$: C, 58.54; H, 7.64; N, 3.79. Found: C, 58.24; H, 7.35; N, 3.70. MS, *m/e* (%): 369 (M^+, 2.4), 296 ($M^+-COOEt$, 60), 91 (base peak, $PhCH_2^+$). *cis*: oily compound. GC (250 °C), t_R: 4.1 min. NMR ($CDCl_3$): 1.2—1.4 (9H, m, $-CH_2-\underline{CH_3}$), 2.0—2.4 (2H, m, C_3–H), 2.4—2.7 (1H, m, C_4–H), 2.6—3.0 and 3.0—3.2 (each 1H, m, C_5–H), 3.70 (1H, dd, $J=16$, 8, C_2–H), 3.84 and 3.95 (each 1H, d, $J=12$, $PhCH_2$), 4.0—4.2 (6H, m, OCH_2), 7.2—7.4 (5H, d like, $J=7$, aromatic H). *Anal.* Calcd for $C_{18}H_{28}NO_5P$: C, 58.54; H, 7.64; N, 3.79. Found: C, 58.28; H, 7.42; N, 4.10. MS, *m/e* (%): 369 (M^+, 6.1), 296 ($M^+-COOEt$, 83), 91 ($PhCH_2$, base peak).

Ethyl 2-Phenylthioethylphosphonate (XVIII)——A mixture of XV (5.22 g, 16.6 mmol), NaH (50% in oil, 1.59 g, 33.1 mmol), HMPA (2.88 ml, 16.6 mmol), and XVI (2.71 g, 16.6 mmol) in DME was refluxed for 1 h, then the reaction mixture was poured into ice-water. The extracted benzene layer was washed with 10% HCl soln. and then brine. Pure XVIII was obtained by bulb-to-bulb distillation. 1.5 g (44%). NMR (CCl_4): 1.29 (6H, t, $J=7$, $-CH_2\underline{CH_3}$), 1.6—2.3 (2H, m, SCH_2), 2.9—3.4 (2H, m, PCH_2), 3.8—4.2 (4H, m, OCH_2), 7.0—7.4 (5H, m, aromatic H). *Anal.* Calcd for $C_{12}H_{19}O_3PS+0.2H_2O$: C, 51.67; H, 7.01. Found: C, 51.71; H, 6.86.

1-Benzyl-2-carboxypyrrolidine-4-phosphonic Acid (XIX)——The *cis* and *trans* mixture (0.9 g) of XVII was dissolved in 5% HCl and the solution was refluxed overnight then concentrated. The residue was dissolved in conc. HCl and the solution was refluxed overnight. HPLC, t_R: 2.10 min (*cf.*, XVII: 2.67 min). The resulting soln. was concentrated and fractionated through a Dowex column. XIX–HCl was eluted with 3% HCl. mp 165—170 °C. *Anal.* Calcd for $C_{12}H_{16}NO_5P+HCl+0.2H_2O$: C, 44.31; H, 5.39; N, 4.31. Found: C, 44.20; H, 5.35; N, 4.46. This salt was neutralized with epichlorohydrin and recrystallized from water. 0.1 g. mp 158—164 °C. NMR (D_2O): 2.2—2.5 (2H, m, C_3–H), 2.7—3.0 (1H, m, C_4–H), 3.3—3.6 and 3.6—4.0 (each 1H, m, C_5–H), 4.34 (1H, dd, $J=20$, 8, C_2–H), 4.4—4.8 (2H, m, $PhCH_2$), 7.51 (5H, br s, aromatic H). *Anal.* Calcd for $C_{12}H_{16}NO_5P+H_2O$: C, 47.53; H, 5.98; N, 4.62. Found: C, 47.42; H, 5.81; N, 4.63.

Diethyl 5-Ethoxycarbonyl-4,5-dihydro-3(3*H*)-pyrazolylphosphonate (XXII)——A mixture of XVI (2 g, 12.2 mmol) and ethyl diazoacetate (1.4 g, 12.2 mmol) in benzene was stirred and refluxed for 3 h under an Ar atmosphere. The concentrated mixture was fractionated through an SiO_2 column. XXII was eluted with benzene. 2.95 g (87.0%). Yellow oil. GC (170 °C): t_R 3.4 min. NMR (CCl_4): 1.31 (9H, t, $J=7$, $-CH_2\underline{CH_3}$), 1.9—2.7 (2H, m, $>CH_2$), 3.2—3.9 (2H, m, $2\times CH<$), 3.9—4.5 (6H, m, OCH_2). MS, *m/e* (%): 278 (M^+, 7.5), 205 ($M^+-COOEt$, 48), 177 (*m/e* 205 $-CH_2=CH_2$, 30), 149 (*m/e* 177 $-CH_2=CH_2$, base peak).

Diethyl Ethynylphosphonate (XXI)[14)]——Diethyl phosphonochloridate (42.6 g, 247 mmol) was added to a THF soln. of ethynyl magnesium bromide (prepared from Mg (6 g, 247 mmol), EtBr (30 g, 275 mmol), and acetylene). After being refluxed for 1 h, the mixture was poured into ice-water. The Et_2O extract was washed with brine and evaporated to give crude XXI. bp 76.5—78 °C (5 mmHg). 3.02 g. GC (100 °C), t_R: 2.1 min (single peak, *cf.* diethyl phosphorochloridate, 1.4 min). IR (film): $\nu_{C\equiv C}$ 2080, $\nu_{C\equiv CH}$ 3180. NMR (CCl_4): 1.32 and 1.36 (each 3H, t, $J=8$, $CH_2\underline{CH_3}$), 3.63 (1H, d, $J=13$, $C\equiv CH$), 3.7—4.4 (4H, m, OCH_2). *Anal.* Calcd for $C_6H_{11}O_3P+1.3H_2O$: C, 38.84; H, 7.38. Found: C, 38.84; H, 7.33.

Diethyl 5-Ethoxycarbonyl-3(1*H*) or (2*H*)pyrazolylphosphonate (XXIII)——A mixture of ethyl diazoacetate (1.0 g, 8.7 mmol) and XXI (2.82 g, 17.4 mmol) was heated overnight at 100 °C in a sealed tube. Meterials having low bp were removed by distillation. bp <100 °C (5 mmHg), 0.9 g. The residue showed a single peak on GC (180 °C), t_R 10.6 min. The yield was 1.89 g (78.4%). XXIII: bp 150 °C (0.08 mmHg). NMR ($CDCl_3$): 1.35 (9H, t, $J=7$, CH_3), 3.9—4.6 (6H, m, OCH_2), 7.20 (1H, d, $J=2$, vinylic H), 14.04 (1H, br s, $>NH$). *Anal.* Calcd for $C_{10}H_{17}N_2O_5P$: C, 43.48; H, 6.20; N, 10.14. Found: C, 43.19; H, 6.21; N, 9.64. *Cf.*, Calcd for $C_{10}H_{17}N_2O_5P+0.2H_2O$: C, 42.92; H, 6.27; N, 10.01. MS, *m/e* (%): 277 (M^++1, 43), 204 (*m/e* 277-COOEt, base peak).

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