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Peptides Containing Aminophosphonic Acids. II. The Synthesis of Tripeptide Analogs

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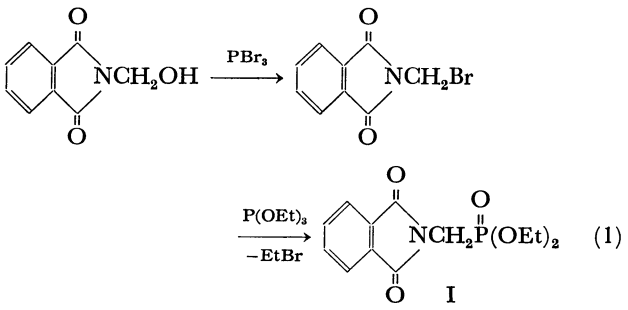
The synthesis of tripeptides containing aminophosphonic acid was attempted as follows. Diethyl phthalylimidomethyl phosphonate (I) was chlorinated with phosphorus pentachloride to the phosphonomonochloridate (II), which was then coupled with amino acid ethyl esters to give the corresponding dipeptides (IIIa—c). The phthalyl groups of III were removed with hydrazine, and the resulting free amines were subsequently combined with phthalyl-amino acids by means of dicyclohexylcarbodiimide to produce the tripeptide analogs (IVa—c) in good yields.

In the preceding paper[1] we described the reactivity of α-aminobenzylphosphonic acid in connection with exploring a synthetic route for incorporating aminophosphonic acids into a peptide chain; we found that i) the reaction of *C*-protected amino acids with the *N*-protected aminophosphonomonochloridate could produce the corresponding dipeptide analogs linked through the phosphonamide bond, and ii) the amino group of aminophosphonic acids possessed a reactivity similar to that of amino acids and was capable of coupling with carboxyl groups of amino acids by means of dicyclohexylcarbodiimide (DCC).

In this paper we wish to report the synthesis of tripeptide analogs containing aminomethylphosphonic acid in the center. Aminophosphonic acid may be considered as the simplest phosphonic acid analog of glycine, and it would give readily crystallizable products than those obtained from α-aminobenzylphosphonic acid[1] because of less steric hindrance and the lack of an asymmetric carbon. Furthermore, the synthesis of tripeptides may offer a suitable guideline for the preparation of phosphorus analogs of higher peptides.

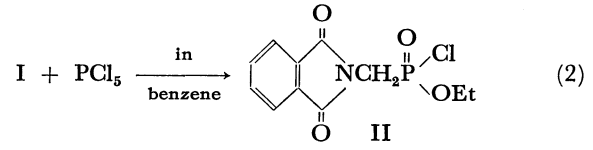
Results and Discussion

On the basis of the results of the previous work,[1] aminomethylphosphonic acid, the amino and phosphonic acid groups of which were blocked with phthalyl and ethyl groups respectively, was prepared by the following route. *N*-Hydroxylmethylphthalimide was brominated by phosphorus tribromide to produce *N*-bromomethylphthalimide in a 78% yield, the latter substance was then allowed to react with triethyl phosphite to generate diethyl phthalimidomethylphosphonate (I) in an 82% yield (Eq. (1)).



(1)

To activate the phosphonic-acid function for coupling with amino acids, compound I was treated with a slight excess of phosphorus pentachloride in benzene, giving crystalline ethyl phthalimidomethylphosphonochloridate (II) quantitatively (Eq. (2)). The chloride (II) showed a typical acid chloride character.[2]



(2)

Amino acid ethyl ester hydrochlorides were then coupled with II in the presence of triethylamine,

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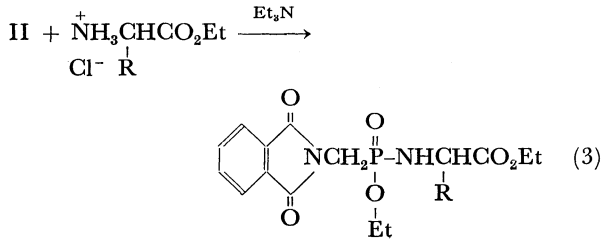
1) K. Yamauchi, M. Kinoshita, and M. Imoto, This Bulletin, **45**, 2528 (1972).

2) The chloride (II) showed the typical acid chloride character. Thus, when II was allowed to react with water, amines, and alcohols, the corresponding products, *e.g.*, ethyl hydrogenphosphonate, phosphonamides, and esters, were obtained in good yields.

thus producing dipeptide analogs (IIIa—c) in 70—88% yields (Eq. (3)).

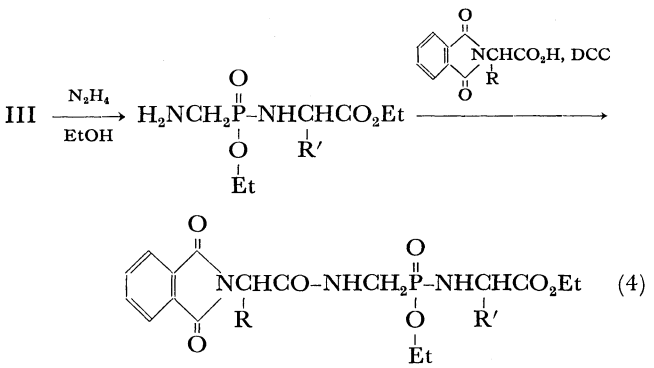


(3)

IIIa: R=H (PHT-gly-gly-OEt)
IIIb: R=*d*, *l*-CH_3 (PHT-gly-ala-OEt)
IIIc: R=*l*-$CH_2C_6H_5$ (PHT-gly-phe-OEt)

The attempted reactions of II with plain amino acids failed, though the procedure used was similar to that employed in the reactions of *N*-protected amino acid chlorides with amino acids, which has been reported to produce dipeptides.[3]

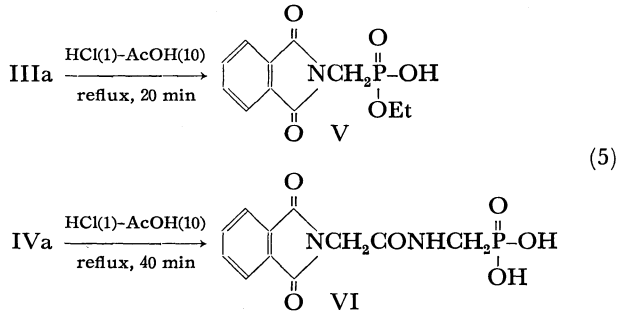
For the transformation of III into tripeptides which have aminomethylphosphonic acid in the center, the phthalyl group of III must be removed to give the corresponding free amines. This process was easily accomplished through the treatment of III with one equivalent of ethanolic hydrazine under mild conditions. The resulting amines were subsequently allowed to couple with phthalyl-amino acids, using DCC as the condensing agent, and produced tripeptide analogs (IVa—c) in 70—75% yields, (Eq. (4)). The procedure employed here was similar to those used in peptide synthesis.[4]



(4)

IVa: R=H, R′=H (PHT-gly-gly-gly-OEt)
IVb: R=H, R′=*l*-$CH_2C_6H_5$ (PHT-gly-gly-phe-OEt)
IVc: R=*l*-$CH_2C_6H_5$, R′=H (PHT-phe-gly-gly-OEt)

Concerning the removal of protecting groups, the phthalyl groups of the above compounds may be deblocked by hydrazine, as shown in the conversion of III to IV. The removal of the ethyl groups from carboxylic and phosphonic acid groups, however, was difficult, since P–N bond cleavage took place exclusively upon a mild acid treatment (Eq. (5)). Thus, when heated in a mixture of hydrochloric acid and acetic acid, IIIa produced the half ester (V) after 20 min, whereas IVa gave the phosphonic acid derivative (IV) within 40 min.



(5)

For the extension of the peptide chain, however, the presence of the carbonyl ester part does not provide any obstacles when the phthalyl groups is replaced with other *N*-protecting groups in the –CONH-bond-forming step (Eq. 4) and when an azide method is employed for the activation of carboxylic acid group. Work on these problems is now in progress and will be described later.

Experimental

The melting points (mp) are uncorrected. The infrared spectra (IR) were run on a Jasco Model IR-G spectrometer. The nuclear magnetic resonance spectra (NMR) were recorded with a Hitachi-Perkin-Elmer appratus, Model R-20. The optical rotations were obtained using a Yanagimoto Photomagnetic Polarimeter, Model OR-10.

Preparation of Diethyl Phthalimidomethylphosphonate (I). A) N-*Bromomethylphthalimide*: A mixture of *N*-hydroxymethylphthalimide (50.0 g, 0.282 mol) and phosphorus tribromide (77.0 g, 0.285 mol) was warmed until a dark-orange solution resulted. The cooled reaction mixture was poured into ice water to give a gray solid, which was then washed with water, dried, and crystallized from chloroform to produce *N*-bromophthalimide; 53.0 g, 78%; mp 149—150°C (lit,[5] 148°C).

B): *N*-Bromomethylphthalimide (39.5 g, 0.165 mol) and triethyl phosphite (27.4 g, 0.165 mol) were placed in a 200 m*l* round-bottom flask equipped with a distilling column, and then heated gently to initiate a reaction. After the exothermic reaction had subsided, the heating of the flask was continued for one hour, during which time the distillation of ethyl bromide stopped. Chloroform (100 m*l*) was added to the cooled reaction mixture, and the solution was washed with water (100 m*l*×3). The organic solution was dried over Drierlite and concentrated. The addition of petroleum ether to the residue gave white crystals (I); 40.0 g, 82%; mp 67°C (*n*-hexane); IR (KBr), 1770 (w), 1715 (s), 1240 (s), 1050 (s), 980 (m) cm^{-1}; NMR ($CDCl_3$) τ 1.85 (s, 4, phthal), 5.58 (qui, 4, $C\underline{H}_2$), 5.70 (d, 2, $C\underline{H}_2PO$), 8.60 (t, 6, $C\underline{H}_3$).

Found: C, 52.74; H, 5.44; N, 4.76%. Calcd for $C_{13}H_{16}NO_5P$: C, 52.52; H, 5.42; N, 4.71%.

Preparation of O-*Ethyl Phthalimidomethylphosphonochloridate (II)*. Compound I (6.27 g, 21.0 mmol), phosphorus pentachloride (4.67 g, 22.5 mmol), and dry benzene (20 m*l*) were refluxed for 15 hr. The solvent was then distilled out under reduced pressure to give a clear residue which, upon standing at

3) J. C. Sheehan and V. S. Frank, *J. Amer. Chem. Soc.*, **71**, 1856 (1949).

4) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2. John Wiley & Sons Inc., New York, N.Y. (1961).

5) G. W. Pucher and T. B. Johnson, *J. Amer. Chem. Soc.*, **44**, 817 (1922).

room temperature, solidified as white crystals. Recrystallization from dry tetrahydrofuran(THF)–ether gave needle crystals; 5.6 g, 93%; mp 92—94°C; IR ($CHCl_3$), 1780 (w), 1725 (s), 1600 (w), 1385 (s), 1300 (m), 1250 (m), 710 (m) cm^{-1}; NMR ($CDCl_3$), τ 1.8 (complex m, 4, phthalyl), 5.40 (d, 2, $C\underline{H}_2P$), 5.50 (complex m, 2, $POC\underline{H}_2$), 8.55 (qua, 3, $C\underline{H}_3$).

Found: C, 46.21; H, 4.03; N, 5.10; Cl, 12.76%. Calcd for $C_{11}H_{11}ClNO_4P$: C, 45.93; H, 3.85; N, 4.87; Cl, 12.33%.

Since the chloride (II) was produced quantitatively in an almost pure state, it was used for the successive reactions directly, without recrystallization.

Preparation of Phosphorus Analogs of Dipeptide (III). *PHT-gly-gly-OEt (IIIa)*: To a chloroform (70 m*l*) solution of ethyl glycinate hydrochloride (4.65 g, 0.033 mol) was added triethylamine (15 m*l*) with cooling by an ice-water bath. Chloride (II) (9.65 g, 0.034 mol), which was dissolved in THF (70 m*l*), was then added drop by drop over 15 min with stirring vigorously. After the completion of the addition, the reaction mixture was stirred for 10 hr. The resulting triethylamine hydrochloride was filtered out; the filtrate was concentrated to about 20 m*l* and diluted with chloroform to 100 m*l*, after which the solution was washed once with 10% sodium bicarbonate and then with water. The evaporation of the solvent and the addition of ether to the residue yielded white crystals. Recrystallization from ether–THF gave 10.5 g (88%) of IIIa; mp 132—133°C; IR (KBr), 3200 (w), 1750 (w), 1710 (s), 1220 (s) cm^{-1}; NMR ($CDCl_3$); τ 1.8 (complex m, 4, phthalyl), 5.6 (complex m, 4, $POC\underline{H}_2$, $CO_2C\underline{H}_2$), 5.77 (s, 2, $NC\underline{H}_2CO$), 4.02 (d, 2, $NC\underline{H}_2P$), 8.75 (two t, 6, $POC\underline{H}CH_3$, $COCH_2C\underline{H}_3$).

Found: C, 51.09; H, 5.20; N, 7.68%. Calcd for $C_{15}H_{19}N_2O_6P$: C, 50.84; H, 5.40; N, 7.90%.

PHT-gly-ala-OEt (IIIb): The procedure similar to that used for the preparation of IIIa was employed, using II (5.80 g, 0.020 mol) and *d,l*-alanine ethyl ester hydrochloride (3.10 g, 0.020 mol) to produce IIIb as clear crystals; 5.1 g, 70%; mp 126—127°C) petroleum ether–THF); IR (KBr), 3150 (w), 1740 (w), 1710 (s), 1400 (s), 1200 (s), 1150 (s), 1040 (s), 720 (s) cm^{-1}. NMR ($CDCl_3$) τ 2.15 (complex m, 4, phthalyl), 5.6—6.2 (complex m, 7, $NC\underline{H}_2POC\underline{H}_2$, $C\underline{H}CH_3$, $COC\underline{H}_2$), 6.4 (broad, 1, $PN\underline{H}$), 8.55 (d, 3, $CHC\underline{H}_3$), 8.70 and 8.73 (t, 3, $COCH_2C\underline{H}_3$, or $POCH_2C\underline{H}_3$).

Found: C, 52.24; H, 5.66; N, 7.57%. Calcd for $C_{16}H_{21}N_2O_6P$: C, 52.19; H, 5.75; N, 7.61%.

PHT-gly-l-phe-OEt (IIIc): The chloride (7.00 g, 0.0243 mol) was allowed to react with *l*-phenylalanine ethyl ester hydrochloride (5.60 g, 0.025 mol) in a manner similar to that used in the synthesis of IIIa to form IIIc as clear crystals; 9.2 g, 83%; mp 94—95°C (ether–THF); IR (KBr), 3350 (w), 1760 (w), 1710 (s), 1390 (s), 1220 (s), 1110 (s), 960 (s) cm^{-1}; NMR ($CDCl_3$), τ 2.20 (complex m, 4, phthalyl), 5.6—6.5 (complex m, 7, $NC\underline{H}_2P$, $POC\underline{H}_2$, $C\underline{H}CO_2C\underline{H}_2$), 6.90 (d, 2, $C\underline{H}_2C_6H_5$), 8.80 (two t, 6, $POCH_2C\underline{H}_3$, $COCH_2C\underline{H}_3$); $[\alpha]_D^{21}$ +8.97° ($CHCl_3$, *c* 4.2).

Found: C, 58.15; H, 5.55; N, 6.16%. Calcd for $C_{22}H_{25}N_2O_6P\cdot 0.5H_2O$: C, 58.27; H, 5.78; N, 6.17%.

Preparation of Phosphorus Analogs of Tripeptide. *PHT-gly-gly-gly-OEt (IVa)*: Compound IIIa (1.50 g, 4.22 mol) was dissolved in ethanol (15 m*l*) and the solution was mixed with 1N ethanolic hydrazine (4.5 m*l*). After the reaction mixture had been kept at room temperature overnight, the resulting solid was filtered out and the filtrate was concentrated by a rotary evaporator at the water-bath temperature below 45°C. Since a white solid appeared upon the addition of THF to the residue, the solution was again filtered. The evaporation of the solvent from the filtrate gave gly-gly-OEt as a liquid. The amine thus obtained and phthalyl-glycine (0.83 g, 4.05 mmol) were dissolved in dry THF (10 m*l*), with which dicyclohexylcarbodiimide (1.0 g, 4.85 mmol) had been mixed. The solution was then kept at room temperature overnight. Water (1 m*l*) and acetic acid (0.5 m*l*) were subsequently added to the reaction mixture, after which it was allowed to stand at room temperature for 2 hr. The resulting dicyclohexylurea was removed, and the remaining solution was concentrated as much as possible. The residue was diluted with chloroform (20 m*l*) and neutrallized with 10% sodium bicarbonate. After the organic solution had then been dried over Drierite, the solvent was removed to give a light-yellow liquid. Upon the addition of ether, IVa appeared as white crystals; 1.1 g, 70%; mp 186—187°C (THF); IR (KBr), 3300 (w), 3200 (w), 1770 (w), 1710 (s), 1660 (m), 1560 (m), 1420 (m), 1200 (s), 1040 (m), 960 (m) cm^{-1}; NMR ($CDCl_3$), τ 2.3 (m, 4, phthalyl), 5.50 (s, 2, $C\underline{H}_2$-phthalyl), 5.90 (qua, 4, $COC\underline{H}_2$, $POC\underline{H}_2$), 6.0—6.6 (complex m, 5, $NHC\underline{H}_2PN\underline{H}C\underline{H}_2$), 8.70 (t, 3, $COCH_2C\underline{H}_3$), 8.76 (qua, 3, $POCH_2C\underline{H}_3$).

Found: C, 49.60; H, 5.28; N, 10.07%. Calcd for $C_{17}H_{22}N_3O_7P$: C, 49.63; H, 5.39; N, 10.21%.

PHT-gly-gly-phe-OEt (IVb): The phthalyl group was removed from IIIc (1.58 g, 3.50 mmol) with 1N ethanolic hydrazine (4.0 m*l*) in a manner similar to the procedure described in the preparation of IVa. The amine (about 1.59 g) was then coupled with phthalyl-glycine (0.70 g, 3.4 mmol) by DCC (1.0 g). The reaction mixture was processed as described above to produce IVb; 1.45 g, 75%; mp 164—166°C (THF); IR (KBr), 3250 (w), 3200 (w), 1715 (s), 1660 (m), 1550 (w), 1410 (m), 1190 (s), 1030 (s), 700 (m) cm^{-1}; NMR ($CDCl_3$), τ 2.1 (complex m, 5, phthalyl, CONH), 2.6 (complex m, 5, phenyl), 5.5—7.2 (complex m, 12, $C\underline{H}_2$ phthalyl, $C\underline{H}_2P(OC\underline{H}_2-)N\underline{H}C\underline{H}C\underline{H}_2C_6H_5$, $COC\underline{H}_2$), 8.5—9.2 (complex m, 6, $POCH_2C\underline{H}_3$, $COCH_2C\underline{H}_3$); $[\alpha]_D^{19}$ −27.3° ($CHCl_3$, *c* 6.1).

Found: C, 58.23; H, 5.50; N, 8.43%. Calcd for $C_{24}H_{28}N_3O_7P$: C, 57.48; H, 5.62; N, 8.37%.

PHT-l-phe-gly-gly-OEt (IVc): After IIIa (2.0 g, 5.65 mmol) had been treated with 1N hydrazine (6 m*l*), the resulting amine (1.22 g) was coupled with phthalyl-*l*-phenylalanine (1.67 g, 5.65 mmol) by DCC (1.5 g) in THF (20 m*l*). The mixture was handled in a way similar to that used with IVa to generate a light-yellow oil; this oil was then column-chromatographed on silicic acid to give IVc as a clear oil; 2.0 g, 70%; IR ($CHCl_3$); 3200 (m), 1770 (w), 1740 (m), 1710 (s), 1670 (m), 1520 (m), 1375 (s), 1190 (s), 1130 (s), 960 (m), 710 (s) cm^{-1}; NMR ($CDCl_3$) τ 2.0 (broad m, 1, $CON\underline{H}$), 2.3 (complex m, 4, phthalyl), 2.78 (s, 5, phenyl), 4.80 (qua, 1, $PON\underline{H}$), 5.6—6.7 (complex m, 11, $C\underline{H}C\underline{H}_2$-$C_6H_5$, $C\underline{H}_2POC\underline{H}_2$, $C\underline{H}COC\underline{H}_2$), 8.6—9.0 (two t, 6, $COCH_2$-$C\underline{H}_3$, $POCH_2C\underline{H}_3$); $[\alpha]_D^{19}$ −75.47° ($CHCl_3$, *c* 14.5).

Found: C, 57.10; H, 5.91; N, 8.12%. Calcd for $C_{24}H_{28}N_3O_7P$: C, 57.48; H, 5.62; N, 8.37%.

Acid Hydrolysis of IIIa. Compound IIIa (0.67 g, 18.9 mmol) was dissolved in 7 m*l* of a mixture of concentrated hydrochloric acid and acetic acid (1: 10), and then the solution was refluxed for 20 min. Water was added to the cooled reaction mixture, and the resulting solution was concentrated under a reduced pressure to give a white solid, this solid subsequently crystallized from THF to produce ethyl hydrogen phthalimidomethylphosphonate; 0.3 g, 59%; mp 205°C; IR (KBr), 3000—2500 (broad w), 1775 (w), 1725 (s), 1400 (m), 1205 (m), 1035 (s), 720 (s) cm^{-1}.

Found: C, 48.92; H, 4.54; N, 5.01%. Calcd for $C_{11}H_{12}NO_5P$: C, 49.07; H, 4.49; N, 5.20%.

Acid Hydrolysis of IVa. A similar procedure was employed for IVa (0.055 g, 0.13 mmol) using 5 m*l* of the

acid solution and taking a refluxing time of 40 min to produce phthalyl-glycylaminomethylphosphonic acid; 0.03 g, 75%; mp 192—194°C, IR (KBr), 3250 (w), 1770 (w), 1725 (s), 1660 (m), 1420 (m), 1100—1200 (m), 1000 (m), 960 (m), 720 (m) cm^{-1}; NMR (D_2O), 2.30 (s, 4, phthalyl), 5.70 (s, 2, $NC\underline{H}_2CO$), 6.55 (d, 2, $C\underline{H}_2P$).

Found: C, 44.62; H, 3.98; N, 9.05%. Calcd for $C_{11}H_{11}N_2O_6P$: C, 44.30; H, 3.71; N, 9.40%.