Peptides Containing Aminophosphonic Acids. I. Reactivity of α-Aminobenzylphosphonic Acid

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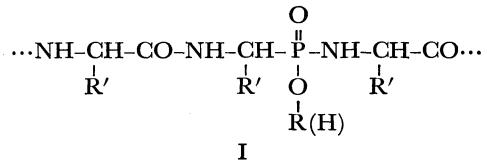
The chemical reactivity of α-aminobenzylphosphonic acid was investigated in order to survey the methods for incorporating aminophosphonic acids into a peptide chain. The investigation revealed that i) a dipeptide analog linked with a phosphonamide bond could be produced by the reaction of *C*-protected amino acid with the *N*-protected aminophosphonomonochloridate, and that ii) amino groups of the aminophosphonic acid possessed a reactivity similar to those of amino acids, and were capable of coupling with *N*-protected amino acids by means of dicyclohexylcarbodiimide.

Since the recent discovery of β-aminoethylphosphonic acid from sea animals, such as rumen protozoa and anthopleura elegantissima,[1,2] the aminophosphonic acids have attracted considerable biological interest not only because of their structural similarity to amino acids, but also because of their excellent metal-chelating properties, especially for calcium and ferric ions.[3]

This paper is concerned with the reactivity of aminophosphonic acids, and α-aminobenzylphosphonic acid was employed as a model compound to search the route for the preparation of phosphorus analogs of enzymes or peptides, which are chemically modified by replacing some amino acids with their phosphonic acid analogs or by inserting aminophosphonic acids into a peptide chain, as is shown below (I). α-Aminobenzylphosphonic acid can be obtained in a sizable quantity from a simple reaction of benzaldehyde, diethyl phosphite, and ammonia.[4]

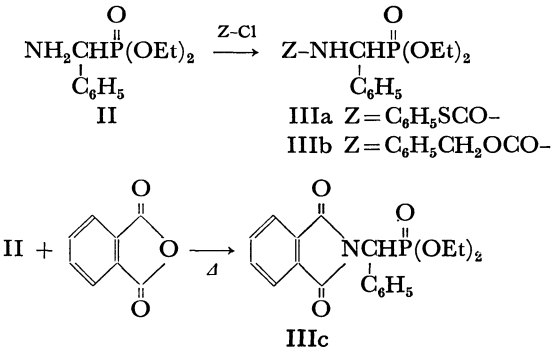


I

Results and Discussion

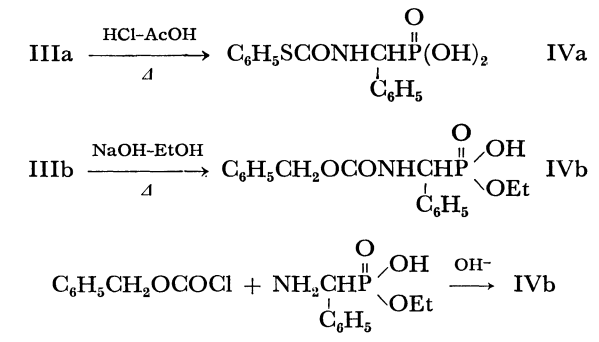
As in peptide synthesis, the reactivity of aminophosphonic acids for the following four steps must be studied in order to construct peptides with aminophosphonic acids; i) the protection of the amino and the phosphonic acid groups, ii) the activation of the phosphonic acid group, iii) coupling reactions to form an amide bond or a phosphonamide bond, and iv) the removal of the protecting groups.

For Step i, when diethyl α-aminobenzylphosphonate (II), in which the phosphonic acid function is already blocked by ethyl groups, was treated with the usual



masking reagents for the amino group[5] (*e.g.* thiophenoxycarbonyl chloride, benzyloxycarbonyl chloride, and phthalic anhydride), the corresponding *N*-protected derivatives (IIIa—c) were produced in 79%, 98%, and 51% yields respectively. These results and a coupling reaction with a free carboxyl group, which will be described later, indicate that amino groups of aminophosphonic acids have reactivity similar to those of amino acids.

The removal of ethyl groups was then attempted from phosphonic acid groups of these compounds. Thus, IIIa, upon treatment with HCl-acetic acid, produced IVa, whereas upon alkaline hydrolysis IIIa only underwent decomposition.



On the other hand, when IIIb was treated with an ethanolic sodium hydroxide solution, the half ester (IVb) was generated; this half ester could also be synthesized through the reaction of ethyl hydrogen α-aminobenzylphosphonate with benzyloxycarbonyl chloride.

These *N*-protected aminobenzylphosphonic acid

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1) M. Horiguchi and M. Kandatsu, *Nature*, **184**, 901 (1959).

2) J. S. Kettredge, E. Roberts, and D. G. Simmon, *Biochemistry*, **1**, 624 (1963).

3) A. D. F. Toy and E. Uhing, Div. of Organic Chemistry, American Chemical Society Meeting, New York, September, 1963.

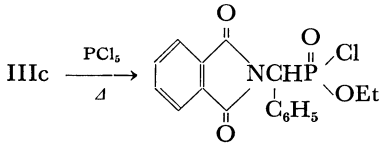
4) M. E. Chalmers and G. M. Kosolapoff, *J. Amer. Chem. Soc.*, **75**, 5278 (1953).

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

derivatives, however, did not condense to form any phosphonamide with ethyl glycinate by means of dicyclohexylcarbodiimide (DCC).

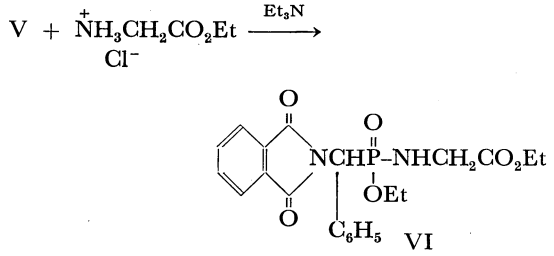
$$\text{IVa or IVb} + NH_2CH_2CO_2C_2H_5 \xrightarrow{DCC} \text{No reaction}$$

Therefore, another route was estimated–the activation of the phosphonic acid group of II (Step ii) through its conversion to its phosphonochloridate. This transformation was easily accomplished by the reaction of IIIc with a slight excess of phosphorus pentachloride, thus producing ethyl phthalimidobenzylphosphonochloridate (V). The process is quite similar to that used for chlorination of phthalylamino acids with the same reagent.[6]



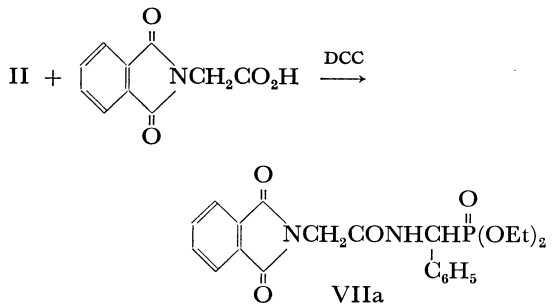
V

When the chloride (V) was subsequently allowed to react with ethyl glycinate, the dipeptide (VI) was formed in a 70% yield (Step iii), showing the extension of peptides from phosphinyl to amino groups.

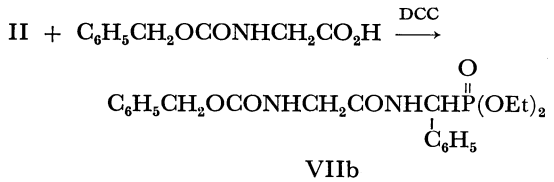


VI

The remaining step necessary for the introduction of aminophosphonic acids into a peptide chain is to place an amino acid to the left of the aminophosphonic acid. This process was examined by the reaction of II with *N*-protected amino acids, using DCC. The successful formation of the dipeptide analogs (VIIa—b) in 74—95% yields ensures that a peptide linkage can be obtained by procedures similar to those employed in the usual peptide synthesis.[5,7]



VIIa



VIIb

Using the experimental results above obtained, we have studied the synthesis of tripeptides with aminomethylphosphonic acid in the center. These results and the attempted removal of the protecting groups (Step iv) will be discussed in a subsequent paper.

Experimental

The melting points (mp) are uncorrected. The infrared spectra were measured by means of a Jasco, Model IR-G spectrometer. The nuclear magnetic resonance spectra (NMR) were recorded with a Hitachi-Perkin-Elmer appratus, Model R-20.

Preparation of Diethyl α-Aminobenzylphosphonate (II). The hydrochloride of II was synthesized according to the procedure of Chalmers and Kosolapoff[4] using benzaldehyde (25.4 g, 0.24 mol), diethyl phosphite (33.0 g, 0.24 mol), and ammonia (excess); mp 158—159°C, 26.4 g, 40% (lit,[4] 159—160°C, 31%).

To the hydrochloride of II (10.0 g, 0.036 mol) suspended in ether (70 m*l*) was added 4N sodium hydroxide, keeping the temperature of the mixture below 3°C with an ice–water bath. Enough anhydrous potassium carbonate was then added to the mixture until the water layer disappeared. The ether layer gave a clear liquid of II (6.13 g, 70%) after the evaporation of the solvent. The decomposition of II took place during vacuum distillation. IR (neat), 3350 (w), 3275 (w), 3030 (w), 2980 (m), 1600 (w), 1490 (w), 1235 (s), 1030 (s), 960 (s), 690 (m) cm^{-1}; NMR ($CDCl_3$), τ 2.7 (complex m, 5, phenyl), 5.89 (d, 1, C<u>H</u>), 4.20 (complex m, 4, C<u>H</u>$_2$), 8.85 (qua, 6, C<u>H</u>$_3$). The ester was found pure from thin-layer and gas chromatographies.

Protection of Amino Group of II. *A) With Thiophenoxycarbonyl Chloride*: Thiophenoxycarbonyl chloride (3.50 g, 20.4 mmol) was added to a solution of II (9.50 g, 39.0 mmol) in THF (70 m*l*). After the mixture had been stirred at room temperature overnight, the resulting white crystals of the hydrochloride of II were filtered out, and the solvent was removed from the filtrate. The addition of petroleum ether to the residue produced diethyl *N*-thiophenoxycarbonyl-α-aminobenzylphosphonate (IVa) as white needle crystals; 6.0 g, 80%; mp 136—137°C (THF–petroleum ether); IR (KBr), 3350 (m), 3150 (m), 2950 (m), 1670 (s), 1540 (s), 1240 (s), 1020 (s), 970 (m), 700 (m) cm^{-1}; NMR ($CDCl_3$), τ 1.9 (broad d, N<u>H</u>), 2.5 (complex m, 5, phenyl), 4.67 (qua, 1, C<u>H</u>C$_6$H$_5$), 5.76 (qui, 2, C<u>H</u>$_2$CH$_3$), 6.13 (complex six, 2, C<u>H</u>$_2$CH$_3$), 8.64 and 8.91 (t, 3, CH$_2$C<u>H</u>$_3$).

Found: C, 56.95; H, 5.90; N, 3.84%. Calcd for $C_{18}H_{22}NO_4PS$: C, 56.98; H, 5.85; N, 3.69%.

B) With Benzyloxycarbonyl Chloride: To amine (II) (7.00 g, 28.8 mmol) dissolved in tetrahydrofuran (THF) (100 m*l*) was added benzyloxycarbonyl chloride (2.50 g, 14.7 mmol) at room temperature with stirring vigorously. Soon after the addition, the hydrochloride of II precipitated as white crystals. After stirring at room temperature overnight, the reaction mixture was filtered to remove the hydrochloride of II (3.80 g). The filtrate was then concentrated to give a yellow residue which was crystallized from ether to give needle crystals of IVb; 5.30 g, 98%; mp 108—109°C (from ether); IR (KBr); 3200 (m), 3050 (m), 1705 (s), 1550 (s), 1260 (s), 1020 (s), 970 (s), 690 (m) cm^{-1}; NMR ($CDCl_3$)

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

7) G. T. Young, "Peptides, Preceedings of the 5th European Symposium (1962)", Pergamon Press, Oxford, p. 1063.

τ 2.62 (complex m, 5, phenyl), 3.80 (broad, 1, NH), 4.80 (qua, 1, $C\underline{H}C_6H_5$), 4.85 (s, 2, $C\underline{H}_2C_6H_5$), 5.6—6.5 (complex m, 4, $C\underline{H}_2CH_3$), 8.75 and 8.94 (two t, 6, $CH_2C\underline{H}_3$).

Found: C, 60.44; H, 6.56; N, 3.93%. Calcd for $C_{19}H_{24}NO_5P$: C, 60.47; H, 6.41; N, 3.70%.

C) With Phthalic Anhydride: A mixture of II (6.51 g, 26.6 mmol) and phthalic anhydride (3.94 g, 26.6 mmol) was heated at 170—180°C for 2 hr. The resulting viscous liquid was dissolved in chloroform (7 m*l*) and chromatographed on alumina. Diethyl phthalimidobenzylphosphonate (IIIc) was eluted with ethyl acetate; 5.0 g, 51%; mp 101—103°C (ether); IR (KBr), 2990 (w), 1770 (w), 1710 (s), 1380 (s), 1255 (s), 1050 (s), 730 (m) cm^{-1}; NMR ($CDCl_3$), τ 2.20 (complex m, 5, phenyl), 2.65 (complex m, 4, phthalyl), 6.20 (d, 1, C$\underline{H}$), 5.75 (six, 4, two $C\underline{H}_2$), 8.75 (t, 6, $C\underline{H}_3$).

Found: C, 61.30; H, 5.41; N, 3.75%. Calcd for $C_{19}H_{20}NO_5P$: C, 61.12; H, 5.40; N, 3.75%.

Hydrolysis of IIIa. A mixture of IIIa (1.65 g, 4.20 mmol), concentrated hydrochloric acid (3 m*l*), and acetic acid (3.5 m*l*) was refluxed for 30 min. The reaction mixture was then diluted with water (8 m*l*) and the solution was stored in an ice-box. The resulting crystals (IVa) were separated, washed with water and dried; 150 g, 75%; mp 142—143°C ($CHCl_3$–acetone); IR (KBr), 3350 (w), 1650 (s), 1620 (m), 1520 (m), 1200 (s), 1050 (m), 950 (m), 750 (w) cm^{-1}; NMR (DMSO-d_6), τ 0.9 (broad, 1, POH), 2.60 (complex m, 10, two phenyl), 3.30 (broad, 1, NH), 4.90 (qua, 1, $C\underline{H}C_6H_5$).

Found: C, 50.93; H, 4.85; N, 4.25%. Calcd for $C_{14}H_{14}NO_4PS\cdot 0.5H_2O$: C, 50.61; H, 4.55; N, 4.21%.

Hydrolysis of IIIb. Compound IIIb (1.0 g, 2.65 mmol) was dissolved in a mixture of 0.4N sodium hydroxide (10 m*l*) and ethanol (10 m*l*), after which the solution was heated at 80°C for one hour. The solvent was then evaporated under reduced pressure, giving a white paste-like residue, which was dissolved in 20 m*l* of water, and pH of the solution was adjusted to about 3 by the addition of concentrated hydrochloric acid. The white solid (IVb) obtained was separated and recrystallized from petroleum ether–chloroform, 0.59 g, 64%, mp 182—183°C; IR (KBr), 3250 (w), 1710 (s), 1540 (s), 1250 (s), 1040 (m), 700 (m) cm^{-1}; NMR ($CDCl_3$), τ 2.5 (complex m, 10, two phenyl), 7.40 (broad s, 1, NH), 6.80 (qua, 1, C$\underline{H}$), 5.15 (s, 2, $C\underline{H}_2C_6H_5$), 5.95 (qua, 2, $C\underline{H}_2$), 8.75 (t, 3, $C\underline{H}_3$).

Found: C, 57.24; H, 5.81; N, 4.47%. Calcd for $C_{17}H_{20}NO_5P\cdot 0.5H_2O$: C, 56.98; H, 5.90; N, 3.91%.

Reaction of Ethyl Hydrogen α-Aminobenzylphosphonate with Benzyloxycarbonyl Chloride. To the ester (1.58 g, 0.075 mol) suspended in water (30 m*l*) was mixed with benzyloxycarbonyl chloride (1.5 g, 0.088 mol) at room temperature. 4N Sodium hydroxide (5 m*l*) was then added to the mixture and a solution was stirred for 10 min. After the unreacted chloride had been removed by extraction with ether, the pH of the aqueous solution was adjusted to 5 by the addition of concentrated hydrochloric acid, the solution was then concentrated as much as possible under reduced pressure. The extraction of the residue with chloroform and the evaporation of the solvent from the organic layer gave a clear substance which upon mixing with petroleum ether afforded IVb as white crystals; 0.8 g, 31%; mp 183—184°C (acetone). The IR and NMR spectra were identical with those of an authentic sample produced from the hydrolysis of IIIb.

Attempted Coupling of IVa or IVb with Ethyl Glycinate. A mixture of IVa (0.64 g, 1.9 mmol) or IVb (0.40 g, 0.11 mmol), an equivalent amount of ethyl glycinate, and DCC (2 equivalents) in DMF (10 m*l*) was stored at room temperature for a week. No precipitate of dicyclohexylurea appeared, showing that the coupling reaction did not take place in this system.

O-Ethyl Phthalimidobenzylphosphonochloridate (V). A mixture of IIIc (2.70 g, 7.25 mmol), phosphorus pentachloride (1.52 g, 7.30 mmol) and benzene (5 m*l*) was heated for 6.5 hr. The light-boiling substances were then removed under reduced pressure. The residue[8] was mixed with petroleum ether–ether (1:1) and cooled in a methanol–dry ice bath to give a white solid (V); 2.1 g, 80%; mp up to 85°C; IR (0.1 mm $CHCl_3$ liquid cell), 3030 (w), 1770 (m), 1725 (s), 1470 (m), 1370 (s), 1275 (s), 1030 (s), 970 (s), 545 (m) cm^{-1}; NMR ($CDCl_3$) τ 2.2 (complex m, 5, phenyl), 2.6 (complex m, 4, phthalyl), 4.05 (qua, 1, CH), 5.70 (m, 2, CH_2), 8.70 (qua, 3, CH_3). The elemental analysis of V was replaced with that of *O*-ethyl hydrogen phthalimidobenzylphosphonate, which had been obtained from the reaction of V with an excess of water in an 80% yield; mp 181—183°C; IR (KBr), 2980 (w), 1760 (w), 1710 (s), 1375 (s), 1200 (s), 1020 (s), 720 (m) cm^{-1}: NMR ($CDCl_3$), τ 1.8—3.0 (complex m, 9, phthalyl and phenyl), 4.20 (d, 1, C$\underline{H}$), 5.87 (qua, 2, $C\underline{H}_2$), 8.80 (qua, 3, $C\underline{H}_3$).

Found: C, 59.42; H, 4.68; N, 3.81%. Calcd for $C_{17}H_{16}NO_5P$: C, 59.13; H, 4.67; N, 4.05%.

Preparation of VI. Compound (IIIc) (2.22 g, 5.95 mmol) was allowed to react with phosphorus pentachloride (1.23 g, 5.90 mmol) in benzene (10 m*l*) to give the chloride as an oily substance, it was dissolved in THF (10 m*l*) and added to a chloroform (30 m*l*) solution of ethyl glycinate hydrochloride (0.9 g, 6.5 mmol) containing triethylamine (5.0 g, 50 mmol) at room temperature. The solution was then stirred overnight and washed with water. The chloroform layer was dried over Drierite and concentrated to about 5 m*l*. Column chromatography on silicic acid was then carried out on the solution. Elution with ether afforded VI (1.8 g, 70%) as a clear oil. The distillation of the product failed because of decomposition, its IR and NMR spectra were however, consistent with the assigned structure; IR (neat), 3350 (w), 3050 (w), 2980 (m), 1765 (w), 1710 (s), 1620 (w), 1495 (w), 1375 (s), 1250 (s), 1030 (s), 750 (m), 710 (s) cm^{-1}; NMR ($CDCl_3$) τ 2.2 (complex m, 5, phenyl), 2.65 (complex m, 4, phthalyl), 4.20 (qua, 1, CH), 5.5—6.3 (complex m, 7, $POC\underline{H}_2$, $N\underline{H}C\underline{H}_2CO_2C\underline{H}_2$), 8.78 and 8.85 (two t, 6, $C\underline{H}_3$).

Preparation of VIIa. A mixture of phthalyl-glycine (0.95 g, 0.467 mmol), II (1.13 g, 0.467 mmol), DCC (1.50 g, 0.73 mmol), and THF (15 m*l*) was stirred overnight at room temperature. Acetic acid (1 m*l*) and water (2 m*l*) were then added to the reaction mixture, and stirring was continued for 2 hr. The resulting dicyclohexylurea was filtered out, and the solvent was removed from the filtrate by means of a rotary evaporator at a water-bath temperature below 50°C. Chloroform (30 m*l*) was added to the residue, and the solution was washed with 10% sodium bicarbonate and water. The organic solution was concentrated to about 2.5 m*l*, giving a viscous oil which was subsequently crystallized from benzene to generate clear crystals; 1.90 g, 95%; mp 195—196°C; IR 3250 (m), 3030 (w), 2980 (m), 1730 (s), 1555 (m), 1420 (m), 1230 (s), 1030 (s), 720 (m) cm^{-1}; NMR ($CDCl_3$) τ 0.80 (d, 1, NH), 1.9—2.7 (complex m, 9, phenyl and phthalyl), 4.33 (qua, 1, $C\underline{H}C_6H_5$), 5.50 (s, 2, $C\underline{H}_2CO$), 5.60—6.60 (complex m, 4, $C\underline{H}_2CH_3$), 8.55 and 8.92 (two, t, 6, $C\underline{H}_3$).

Found: C, 58.82; H, 5.40; N, 6.30%. Calcd for $C_{21}H_{23}$-

8) The successive reactions were carried out using the oily residue directly, since which was almost pure according to the NMR spectrum but showed considerable difficulty in crystallization.

N_2O_6P: C, 58.60; H, 5.38; N, 6.51%.

Preparation of VIIb. A procedure similar to that used for the preparation of VIIa was carried out for a mixture of *N*-benzyloxycarbonylglycine (1.50 g, 7.18 mmol), II (1.75 g, 7.18 mmol), DCC (3.19 mmol), and THF (15 m*l*) to form VIIb as white crystals; 2.30 g, 74%; mp 88—89°C (ether); IR (KBr), 3250 (m), 3050 (w), 1720 (s), 1680 (s), 1550 (s), 1220 (s), 1160 (m), 1020 (s), 700 (m) cm^{-1}. NMR ($CDCl_3$) τ 1.45 (broad d, 1, N$\underline{H}$), 2.7 (complex m, 10, two, phenyl), 4.20 (broad t, 1, CONH$\underline{C}H_2$), 4.42 (qua, 1, PC$\underline{H}C_6H_5$), 4.92 (s, 2, C$\underline{H}_2C_6H_5$), 5.85 (qui, 2, C$\underline{H}_2CH_3$), 6.10 (qui, 2, C$\underline{H}_2CH_3$), 8.72 and 8.96 (two t, 6, C$\underline{H}_3$).

Found: C, 58.28; H, 6.48; N, 6.45%. Calcd for $C_{21}H_{27}N_2O_6P$: C, 58.19; H, 6.78; N, 6.46%.