Synthesis of Pentapeptide Containing *DL*-1-Aminobenzylphosphonic Acid

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Six previously unreported peptides containing diethyl *DL*-1-aminobenzylphosphonate terminal unit were prepared. These are: Diethyl phthalyl-gly*-gly-*DL*-1-aminobenzylphosphonate, diethyl gly-gly-*DL*-aminobenzylphosphonate, diethyl phthalyl-gly-gly-gly-*DL*-1-aminobenzylphosphonate, diethyl phthalyl-gly-gly-gly-gly-*DL*-1-aminobenzylphosphonate, diethyl phthalyl-*DL*-1-phenylalanyl-*DL*-1-aminobenzylphosphonate, diethyl phthalyl-gly-*DL*-phenylalanyl-*DL*-1-aminobenzylphosphonate. All the compounds were obtained as white crystalline powder and characterized by means of elemental analysis, IR spectroscopy, NMR spectroscopy, and ninhydrin test.

Introduction

Aminophosphonic acid is analog of aminocarboxylic acid. Since 2-aminoethylphosphonic acid(2-AEP) was isolated from sheep rumen in 1959 by Horiguchi and his coworker,[1] some aminophosphonic acids were discovered from living organisms.[2-4]

Aminoethylphosphonic acid(2-AEP) was also discovered in mammalian tissues like human muscles, sheep liver and ox brain.[5-6] Its concentration in human tissues was higher in heart and skeletal muscle than in liver and brain. In particular, the acid was found in the highest concentration in the protein rich fraction.[7-8] Recently the antimetabolites, N-1409 which was isolated from the fermentation broth of *Streptomices plumbues* was identified as tripeptide containing 2-amino-5-phosphono-3-pentenoic acid which is one of aminophosphonic acid.[9]

Since then, the syntheses of peptides containing aminophosphonic acids have been extensively studied. The syntheses of some dipeptides and tripeptides were reported by Kim,[10-12] Martell,[13-14] Gilmore,[15] and Imoto[16-17] and their coworkers.

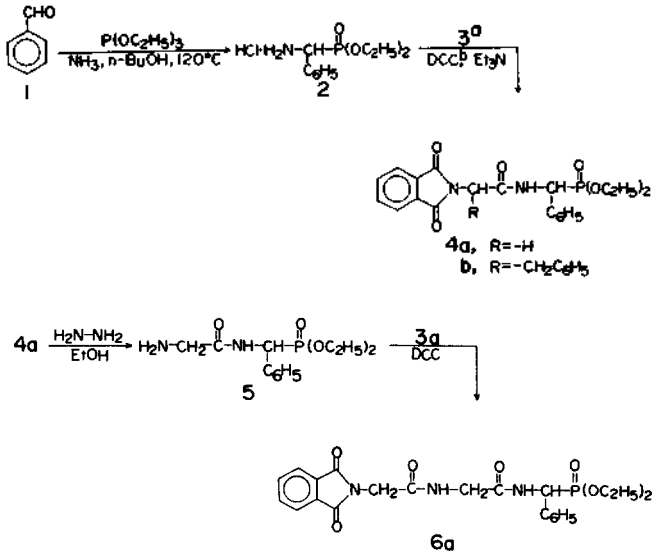
In this paper, we wish to report the synthesis of pentapeptide containing diethyl *DL*-aminobenzylphosphonate at the terminal.

Results and Disscussion

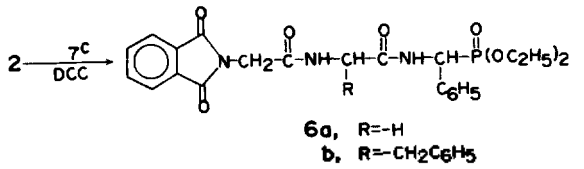
Diethyl *DL*-1-aminobenzylphosphonate hydrochloride (2) was early synthesized by Kosolapoff[18] under elevated pressure using diethyl phosphite, benzaldehyde and ammonia according to the Mannich-type reaction. The yield was 31 % in this case.

In this report the compound was prepared under atmospheric pressure using triethyl phosphite instead of diethyl phosphite by the above modified method. The over-all yield was 27 %.

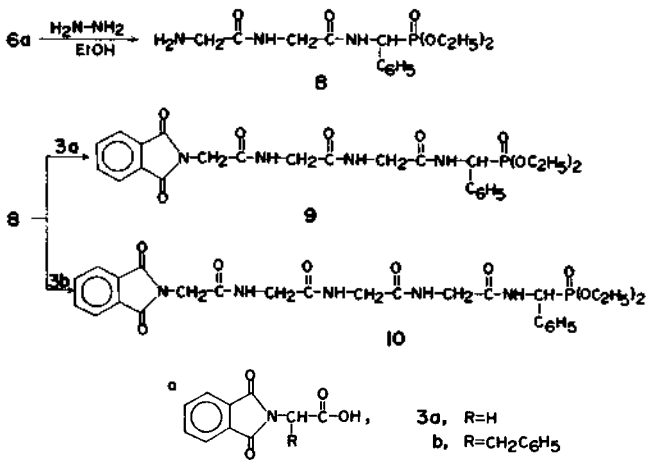
Scheme I:



Scheme II:

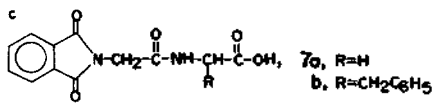


Scheme III:



* gly: glycyl

[b]dicyclohexylcarbodiimide



Diethyl phthalylglycyl-*DL*-1-aminobenzylphosphonate (4a) was prepared from diethyl *DL*-1-aminobenzylphosphonate hydrochloride(2) using dicyclohexylcarbodiimide(DCC) as coupling agent in 94.4% yield. Diethyl glycyl-*DL*-1-aminobenzylphosphonate(5) was generated by dephthalation using hydrazine hydrate (98%). Diethyl phthalylglycylglycyl-*DL*-1-aminobenzylphosphonate(6a) was produced from the crude state of compound **5**. When compound **6a** was prepared according to the routes indicated in scheme I, the overall yield was 38%. But if compound **6a** was prepared through the routes in Scheme II, we could obtain in higher yield(92%). Therefore, we come to the conclusion that the low yield was caused by dephthalation. Compounds **9** and **10** was obtained from compound **6a** according to the routes indicated in Scheme III. The overall yield was 17% and 15%, respectively.

The IR spectra of the unreported compounds **4,6,10** and **11** showed the absorption band of N-H at 3300 cm^{-1}, aromatic C-H at 3020 cm^{-1}, aliphatic C-H at 2960 and 2850 cm^{-1}, phthalyl C=O at 1775 and 1720 cm^{-1} and the characteristic band of peptide C=O at 1640 cm^{-1}. They also showed the absorption band of P=O at 1250 cm^{-1} and P-O-C at 1040 cm^{-1}.

The NMR spectra of them exhibited a multiplet for the phthalyl and phenyl groups at δ 7.85-7.25, a singlet for $-N-CH_2-$ at δ 4.5, a multiplet for $-P-CH_2-$ at δ 4.3 and a multiplet for methyl group at δ 1.5 and 1.2.

Experimental

1. Systheses of Starting Materials

Phthalylglycine (3a) was prepared by the procedure of Billmann and coworkers: m.p 191-192 °C (*lit.*[19] m.p 191-192 °C). Phthalyl-*DL*-1-phenylalanine (3b) was prepared by Sheehan and coworkers: m.p 174-175 °C (*lit.*[20] 176-178 °C). Phthalylglycylglycine (7a) and phthalylglycyl-*DL*-1-phenylalanine (7b) was obtained from phthalylglycine by the same method reported by Sheehan and coworkers: m.p 228-231 °C (*lit.*[21] 229-231 °C) and 197-199 °C (*lit*[21] 195-198 °C), respectively.

2. Diethyl *DL*-1-Aminobenzylphosphonate Hydrochloride (2).

Into a mixture of benzaldehyde (16 g, 0.15 mol) and triethyl phosphite (16.6 g, 1.10 mol) in 30 m*l* of butyl alcohol under atmospheric pressure, dry ammonia gas was passed and refluxed for 12 hr. This yellowish reaction mixture was cooled until room temperature and was allowed to stand overnight. After removal of the solvent by rotary evaporator under the reduced pressure of water asprirator, the yellowish viscous residue was taken up in 100 m*l* of dry ethyl alcoho'-ether (v/v 1:1) mixture. The solution was cooled to 0-5 °C, and then dry hydrogen chloride gas was passed into the solution until the white crystal was not generated. The resultant precipitate obtained by filteration was recrystalized from dry ethyl alcohol-ether (v/v 1:1) to give compound 2 in the yield of 8.4 g(30%). The final white crystal had a melting point of 160°C(*lit.*[22] 159-160 °C).

3. Synthesis of Compound 4a

A solution of diethyl *DL*-1-aminobenzylphosphonate hydrochloride (1.84 g, 0.0030 mol) and dicyclohexylcarbodiimide (DCC, 0.69 g, 0.0033 mol) in 20 m*l* of anhydrous tetrahydrofuran was stirred, and to this reaction mixture was added 0.46 m*l*(0.003 mol) of triethyl amine. The mixture was stirred for 15 hr at room temperature, and the resulting white crystal of dicyclohexyl urea was filtered. The solvent was removed by evaporation under reduced pressure, and the residue was taken up in 25 m*l* of chloroform. The solution was washed three times with 25 m*l* of aqueous sodium bicarbonate and then water. The organic layer was dried over drierite for overnight and the drierite was filtered. The filterate was concentrated by rotary evaporator under reduced pressure. The resulting pale yellowish crystal of crude state compound **4a** was obtained in 94.4% (1.3 g) yield and the crystal was recrystallized from tetrahydrofuran. The final white crystal gave negative ninhydrin test and had a melting point of 185-187 °C.

IR(KBr) 3310 (N-H), 3050(aromatic C-H), 2930, 2830 (aliphatic C-H), 1770, 1710(phthalyl C=O), 1655(peptide C=O), 1530(N-H), 1230(P=O), 1025 cm^{-1}(P-O-C).

NMR($CDCl_3$) δ 7.9-7.2 (complex, *m*, phenyl and phthalyl), 5.7 (*qua*, $-CHC_6H_5$), 4.5 (*S*,$-CH_2-C-$), 4.35-3.5 (complex, *m*, $-P-O-CH_2-$), 1.45-1.18 (two, *t*,$-CH_3$).

Anal. Calcd for $C_{21}H_{23}N_2O_6P$: N, 6.5%; P,7.2% Found: N,6.7% P,7.3%.

In the same method as compound 4a, compound 4b was obtained in 63.4% yield from compound 3b: 178-180 °C.

IR(KBr) 3300(N-H), 3080(aromatic C-H), 2970, 2880 (aliphatic C-H), 1780, 1720(phthalyl C=O), 1640(peptide C=O), 1560(N-H), 1240(P=O), 1040 cm^{-1}(P-O-C).

Anal. Calcd for $C_{28}H_{29}N_2O_6P$: N,5.4%; P,6.0%.Found: N,5.7%; P,6.3%.

4. Synthesis of Compound 6a

Procedure A (Scheme I). To a solution diethyl phthalylglycyl-*DL*-1-aminobenzylphosphonate (4a) (1.43 g, 0.001 mol) in 8 m*l* ethyl alcohol was added 15 m*l* hydrazine hydrate (98%). The mixture was stirred for 10 hr at room temperature and refluxed for 2 hr. The obtained white crystal was filtered and the filterate was evaporated by rotary evaporator under reduced pressure. The resulting pale yellowish crystal of crude state compound 5 was obtained and the compound gave positive ninhydrin test. To a solution of compound 5 and phthalylglycine(0.22 g, 0.0011 mol) in 10 m*l* anhydrous tetrahydrofuran was added dicyclohexylcarbodiimide(0.23 g, 0.0011 mol), and the mixture was stirred for 22 hr at room temperature. To the reaction mixture was added 0.5 m*l* of aqueous acetic acid(40%) and the mixture was stirred

for 2 hr at the temperature. The resulting white crystal of dicyclohexyl urea was filtered, the filterate was concentrated by rotary evaporator under reduced pressure. The residue was taken up in 100 m*l* chloroform and the solution was washed three times with 10 m*l* of aqueous sodium bicarbonate and then water. The organic layer was dried over drierite for overnight and the drierite was filtered. The filterate was concentrated under reduced pressure. The resulting pale yellowish crystal of crude state compound **6a** was obtained in 41 %(0.2 g) yield and the crystal was recrystallized from tetrahydrofuran–ether. The final white crystal gave negative ninhydrin test and had a melting point of 206–208 °C.

IR(KBr) 3300(N-H), 3050(aromatic C-H), 2930, 2858(aliphatic C--H), 1780, 1730(phthalyl C=O), 1640(peptide C=O), 1550(N-H), 1240(P=O), 1040 cm^{-1}(P-O-C).

NMR($CDCl_3$) δ 7.85-7.10(complex, *m*, phenyl and phthalyl), 4.45(*S*, $-CH_2C-$), 4.45(*d*, $-NH-CH_2-$), 4.2-3.6 (complex, *m*, $-P-O-CH_2-$), 1.8-1.35(two, *m*, $-CH_3$).

Anal. Calcd for $C_{23}H_{26}O_7N_3P$: N,8.6 %; P,6.4 %. Found: N,8.5 %; P,6.7 %.

Procedure B (Scheme II). To a solution diethyl *DL*-1-aminobenzylphosphonate hydrochloride (0.839 g, 0.0033 mol) and phthalylglycylglycine(7a) (0.87 g, 0.0033 mol), and dicyclohexylcarbodiimide(0.69 g, 0.0033 mol) in 30 m*l* anhydrous tetrahydrofuran was added 0.46 m*l*(0.0033 mol) of triethylamine and the mixture was stirred for 15 hr at room temperature. To the reaction mixture was added 1.5 m*l* of aqueous acetic acid(40 %) and the mixture was stirred for 2 hr at the temperature. The pale yellowish crystal of crude state compound **6a** was obtained in 92 %(1.3 g) yield by the same procedure A. The crystal was recrystallized from tetrahydrofuran–ether.

5. Synthesis of Compound 6b

Compound **6b** was obtained in 44 %(0.76 g) yield from phthalylglycyl-*DL*-1-phenylalanine(7b) (1.16 g, 0.0033 mol) by the same procedure B. The final white crystal gave negative ninhydrin test and had a melting point of 157–158 °C.

IR(KBr) 3280(N-H), 3080(aromatic C-H) 2980, 2850(aliphatic C-H), 1775, 1720(phthalyl C=O), 1650(peptide C=O), 1530(N-H), 1230(P=O) 1040 cm^{-1}(P-O-C).

Anal. Calcd for $C_{30}H_{32}N_3O_7P$: N,7.3 %, P,5.4 %. Found: N,7.6 %, P,5.8 %

6. Synthesis of Compound 9

Crude state compound **8** was obtained by the same procedure A and the compound gave positive ninhydrin test. The compound was dissolved in 10 m*l* of tetrahydrofuran and to the solution was added phthalylglycine(3a) (0.23 g, 0.0011 mol) and dicyclohexylcarbodiimide (0.25 g, 0.0012 mol). The reaction mixture was treated by the same procedure A, and then the pale yellowish crystal of crude state compoud **9** was obtained in 17 %(0.093 %) yield. The crude product was recrystallized from tetrahydrofuran–ether. The final white crystal gave negative ninhydrin test and had a melting point of 160–163 °C.

IR(KBr) 3350(N-H), 2950, 2870(C-H), 1780, 1730(phthalyl C=O), 1640(peptide C=O), 1560(N-H), 1240 (P=O), 1040 cm^{-1} (P-O-C).

NMR($CDCl_3$) δ 7.85-7.15(complex, *m*, phenyl and phthalyl), 4.5(*S*, $-N-CH_2C-$), 4.35-4.1(*m*, $-CH_3$), 3.75-3.4(*m*, $-NH-CH_2-P-$), 1.85-1.45(*m*, $-CH_3$)

Anal. Calcd for $C_{25}H_{29}N_4O_8P$: N,10.3 %, P,5.7 %. Found: N,10.1 %, P,5.4 %

7. Synthesis of Compound 10

Crude state compound **10** was obtained in 15 %(0.098 g) yield from phthalylglycylglycine (7a) (0.29 g, 0.0011 mol) instead of phthalylglycine by the same procedure as the preparation of compound **9**. The crude product was recrystallized from tetrahydrofuranether. The final white crystal gave negative ninhydrin test and had a melting point of 125–127 °C.

IR(KBr) 3300(N-H), 2955, 2860(C-H), 1780, 1730 (phthalyl C=O) 1630(peptide C=O), 1570(N-H), 1240 (P=O), 1030 cm^{-1}(P-O-C).

Anal. Calcd for $C_{27}H_{32}N_5O_9P$; N, 11.6 %, P,5.1 %. Found: N, 11.2 %, P,4.8 %

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The Molecular Complexes (XI). The Complexes of Toluidines and N-Methyltoluidines with Iodine in Carbon Tetrachloride*

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The interactions of iodine with toluidines (*o*-, *m*-, and *p*-) and N-methyltoluidines (*o*-, *m*-, and *p*-) in CCl_4 solution have been investigated through spectrophotometric measurements. The results indicate that toluidines and N-methyltoluidines form the one-to-one charge-transfer complexes with I_2 in solution. By comparing the values of the formation constants of the complexes, it is concluded that the relative stabilities of the I_2-amine complexes decrease in the following orders: *p*-toluidine>*m*-toluidine>aniline>*o*-toluidine, N-methyl-*p*-toluidine>N-methyl-*m*-toluidine>N-methylaniline >N-methyl-*o*-toluidine, N-methyltoluidines>toluidines. These results can be explained by the electron-releasing character and the steric effect of methyl group in the amine molecules.

Introduction

The formation of molecular complexes of aromatic amines with iodine in solution was studied by several investigators through spectrophotometric measurements.[1-4] In a previous paper of this series,[4] we reported that I_2 formed one-to-one complexes with aromatic amines such as aniline and *o*-toluidine in CCl_4.

$$RNH_2 + I_2 \rightleftharpoons RNH_2 \cdot I_2$$

The observed values of formation constants of the complexes were as follows:

$$K_f(C_6H_5NH_2 \cdot I_2) = 12.8\,l/\text{mole}$$
$$K_f(o\text{-}CH_3C_6H_4NH_2 \cdot I_2) = 9.31\,l/\text{mole}$$

These complexes were considered to be formed by electron donor-acceptor interaction or charge-transfer interaction at the nitrogen atoms of the amines. If it was assumed that the electron-releasing property of methyl group was the only factor to affect relative stabilities of the complexes, iodine should be expected to form more stable complex with *o*-toluidine than with aniline. This was contrary to the experimental observations, however. It was concluded that the steric effect of the *ortho*-substituent also played important role for the formation of the charge-transfer complexes. Although the electron-releasing character of the methyl group might increase the electron density of nitrogen atoms of the amines to enhance the basicity of the amines toward the complex formation, the steric hindrance of *o*-methyl group might be considered to inhibit possible interaction between the nitrogen atoms of the amines and the iodine molecule.

It appeared of interest to extend the studies to the corresponding systems of toluidines and N-methyltoluidines in order to compare the steric effect with the electron-releasing power of the methyl substituent towards the charge-transfer complex formation. Thus, the present study was undertaken on the complexes of *o*-, *m*- and *p*-toluidines and *o*-, *m*- and *p*-N-methyltoluidines with iodine in carbon tetrachloride.

Experimental

Material. N-Methylaninline, *o*-, *m*- and *p*-toluidines and *o*-, *m*- and *p*-N-methyltoluidines (all Reagent grades, Eastman Organic Chemical Co.) were treated with aqueous solution of potassium hydroxide, dried with calcium chloride and fractionated under reduced pressure. The middle fractions were taken and used for the experimental studies.

Experimental Procedures. Experimental procedures for the preparation of various stock solutions and the UV spectrophotometric measurements were described in previous papers.[5-7] In each system examined, UV absorption spectra were measured in the region of 300-600 nm, and the wave lengths of the miximum absorption were observed. The absorbancy of each system at its absorption miximum was measured

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