An Improved Synthesis of *N*-(Phosphonoacetyl)-amino Acids

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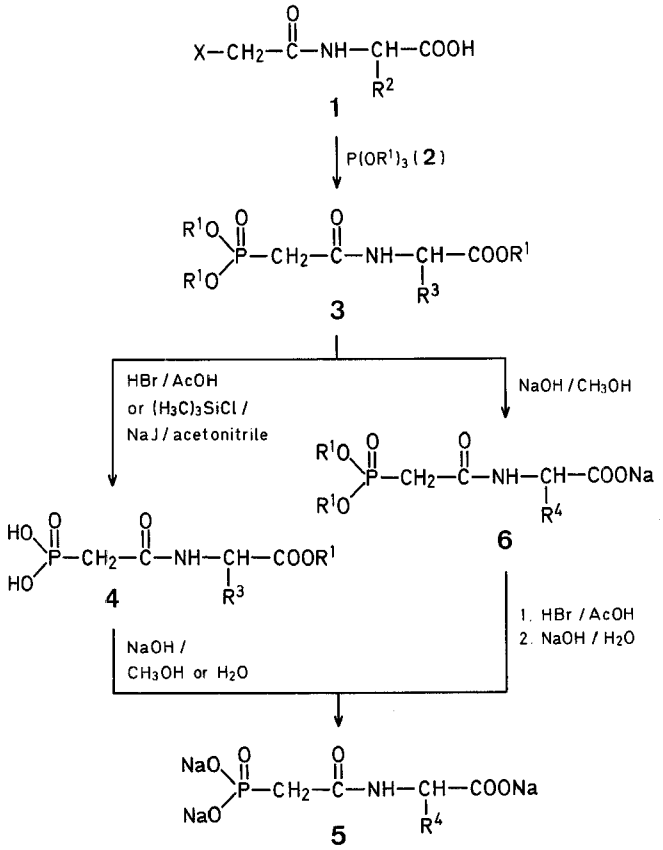
During experiments directed towards the development of structural analogues of carbamoyl phosphates which are potential inhibitors of aspartate transcarbamylase, the enzyme catalyzing the second step of the pyrimidine synthesis de novo, phosphonoacetic acid amides were prepared[1,2]. Among them, *N*-(phosphonoacetyl)-L-aspartic acid (PALA) which is a putative transition-state analogue of the above enzyme was synthesized[3]. PALA also showed strong anti-proliferative[4,5] and anti-tumor[6,7] activities. Recently, a transition-state analogue of ornithine transcarbamylase, *N*-(phosphonoacetyl)-L-ornithine, was prepared[8,9]. It is worth noting that phosphonoacetic acid has been found to be active against viruses[10].

As regards the availability of the *N*-(phosphonoacetyl)-amino acids required for studies of their biological activities, most of the literature syntheses of these compounds are rather complicated multistep procedures. Usually, amino acid esters are *N*-acylated with phosphonoacetic acid derivatives by standard methods[4,5,11,12,13].

We describe here a highly efficient and simple method for the preparation of *N*-(phosphonoacetyl)-amino acids. The reaction of *N*-(haloacetyl)-amino acids **(1)** with trialkyl phosphites **(2)** affords the alkyl esters of *N*-(dialkylphosphonoacetyl)-amino acids **(3)** in nearly quantitative yields (Table 1). Compounds **3** are used for the preparation of the sodium salts of the *N*-(phosphonoacetyl)-amino acids **(5)** by standard methods (Table 2). The salts **5** may be converted into the free *N*-(phosphonoacetyl)-amino acids in nearly quantitative yields by use of a strongly acidic ion-exchange resin.

A similar synthesis of *N*-(phosphonoacetyl)-amino acids, published recently[14,15], afforded *N*-(phosphonoacetyl)-L-aspartic acid salts in only 25–30% yields. We have found that the decrease in yield as compared to our method results from the thermal instability of the starting *N*-(chloroacetyl)-amino acid esters[14,15]. The overall yields of *N*-(phosphonoacetyl)-L-aspartic acid (PALA) and its analogs obtained by our method exceed 70%; the substrates are commercially available and the products are of high purity.



R^1 = alkyl

R^2 = H, alkyl, or $-(CH_2)_n-COOH$

R^3 = H, alkyl, or $-(CH_2)_n-COOR^1$

R^4 = H, alkyl, or $-(CH_2)_n-COONa$

The mechanism of the reaction **1→3** is not quite clear yet. It may be assumed, however, than an Arbuzov reaction precedes the esterification step. Thus, we suppose that the intermediate quasi-phosphonium salt is stabilized rather by an intermolecular reaction with the carboxy group, resulting in esterification of the carboxy group than by nucleophilic attack of a halogen atom.

Alkyl 2-[*N*-(Dialkylphosphonoacetyl)-amino]-alkanoates (3); General Procedure:

The *N*-(haloacetyl)-amino acid **1** (0.05 mol) is suspended in the trialkyl phosphite **2** (0.2 mol), the suspension vigorously stirred, and gradually heated to 100 °C for chloroacetyl derivatives or to 85 °C for bromoacetyl derivatives. The exothermic reaction begins at this point and the temperature of the mixture rises to 120–130 °C. The resultant solution is refluxed for 1–3 h and the volatile components are removed in vacuo. The residual pale yellow oil is extracted with hexane (2 × 60 ml) and the hexane extract is evaporated to give product **3** of satisfactory purity. Further purification may be achieved by distillation[15] but this is not required for the use of products **3** in the following reaction.

Trisodium Salts of 2-[*N*-(Phosphonoacetyl)-amino]-alkanoic Acids (5):

Alkyl 2-[N-(Phosphonoacetyl)-amino]-alkanoates **(4):**

Method A: The respective compound **3** (0.015 mol) is dissolved in a 45% solution (20 ml) of hydrogen bromide in glacial acetic acid. The resultant solution is allowed to stand at room temperature overnight. The solvent and excess hydrogen bromide are then evaporated in vacuo and the oily residue is dissolved in ethanol (30 ml). The solvent is again evaporated to remove any traces of acetic acid. The crude product **4** thus obtained is used in the next step without further purification.

Method B: The respective compound **3** (0.01 mol) is dissolved in acetonitrile (100 ml) and sodium iodide (3.0 g, 0.02 mol) is added. The

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Table 1. Alkyl 2-[*N*-(Dialkylphosphono)-amino]-alkanoates (**3**)

R^1	R^3	Yield [%]	Molecular formula[a]	I.R. (film) ν [cm^{-1}]	^{1}H-N.M.R. ($CDCl_3$/HMDSO[b]) δ [ppm]
C_2H_5	H	85 85[c]	[15]	3300 (NH); 1750, 1678 (CO); 1545 (NH); 1245 (P=O); 1050, 1025 (POC)	1.20 (t, 3H, $^3J_{HH}$=7.5 Hz, C—O—CH_2—CH_3); 1.27 (t, 6H, $^3J_{HH}$=7.0 Hz, 2 H_3C—CH_2—O—P); 3.8–4.4 (m, 8H, 4 CH_2); 8.06 (br, 1H, $^3J_{HH}$=5.0 Hz, NH)
C_2H_5	CH_3	84	[15]	3295 (NH); 1745, 1675 (CO); 1545 (NH); 1235 (PO); 1040, 1015 (POC)	1.13 (t, 3H, $^3J_{HH}$=7.0 Hz, C—O—CH_2—CH_3); 1.18 (t, 6H, $^3J_{HH}$=7.0 Hz, 2 H_3C—CH_2—O—P); 1.23 (d, 3H, $^3J_{HH}$=7.0 Hz, NH—CH—CH_3); 2.84 (d, 2H, $^2J_{PH}$=21.5 Hz, P—CH_2); 3.7–4.4 (m, 7H, 3 O—CH_3, NH—CH); 8.17 (bd, 1H, $^3J_{HH}$=7.5 Hz, NH)
CH_3	*i*-C_3H_7	98	$C_{10}H_{20}NO_6P$ (281.3)	3200 (NH); 1725, 1665 (CO); 1530 (NH); 1240 (PO); 1015 (POC)	0.82 [d, 6H, $^3J_{HH}$=7.0 Hz, $CH(CH_3)_2$]; 1.24 [oct, 1H, $^3J_{HH}$=6.0 Hz, $CH(CH_3)_2$]; 2.99 (d, 2H, $^2J_{PH}$=21.5 Hz, P—CH_2); 3.44 (s, 3H, C—OCH_3); 3.70 (d, 6H, $^3J_{PH}$=10.5 Hz, 2 H_3C—O—P); 4.37 (dd, 1H, $^3J_{HH}$=6.0 Hz, $^3J_{HH}$=8.0 Hz, CH—CO); 7.87 (bd, 1H, $^3J_{HH}$=8.0 Hz, NH)
C_2H_5	*i*-C_3H_7	90	$C_{13}H_{26}NO_6P$ (323.4)	3300 (NH); 1730, 1675, (CO); 1530 (NH); 1235 (PO); 1030, 1005 (POC)	0.88 (d, 3H, $^3J_{HH}$=7.0 Hz, CH—CH_3); 0.91 (d, 3H, $^3J_{HH}$=7.0 Hz, CH—CH_3); 1.20 (t, 3H, $^3J_{HH}$=7.0 Hz, C—O—CH_2—CH_3); 1.27 (t, 6H, $^3J_{HH}$=7.5 Hz, 2 H_3C—CH_2—O—P); 1.7–2.5 [m, 1H, $CH(CH_3)_2$]; 2.93 (d, 2H, $^2J_{PH}$=21.0 Hz, P—CH_2); 3.6–4.6 (m, 7H, 3 O—CH_2, CH—NH); 7.48 (bd, 1H, $^3J_{HH}$=8.0 Hz, NH)
CH_3	—CH_2—C_6H_5	97	$C_{14}H_{20}NO_6P$ (329.3)	3160 (NH); 1710, 1645 (CO); 1520 (NH); 1230 (PO); 1025 (POC)	2.88 (d, 2H, $^2J_{PH}$=21.5 Hz, P—CH_2); 3.01 (bd, 2H, $^3J_{HH}$=7.0 Hz, CH—CH_2); 3.52 (s, 3H, C—O—CH_3); 3.57 (d, 6H, $^3J_{PH}$=10.5 Hz, 2 H_3C—O—P); 4.68 (tt, 1H, $^3J_{HH}$=7.0 Hz, $^3J_{HH}$=8.0 Hz, CH—NH); 7.13 (s, 5H_{arom}); 7.93 (bd, 1H, $^3J_{HH}$=8.0 Hz, NH)
C_2H_5	—CH_2—C_6H_5	95	[15]	3290 (NH); 1740, 1675 (CO); 1545 (NH); 1230 (PO); 1055, 1015 (POC)	1.17 (t, 3H, $^3J_{HH}$=7.5 Hz, C—O—CH_2—CH_3); 1.29 (t, 6H, $^3J_{HH}$=7.5 Hz, 2 H_3C—CH_2—OP); 2.83 (d, 2H, $^2J_{PH}$=21.5 Hz, P—CH_2); 3.06 (d, 2H, $^3J_{HH}$=6.5 Hz, CH_2—C_6H_5); 4.03 (q, 2H, $^3J_{HH}$=7.5 Hz, C—O—CH_2—C_6H_5); 4.31 (qq, 4H, $^3J_{HH}$=7.5 Hz, $^3J_{PH}$=7.5 Hz, 2 C_6H_5—CH_2—O—P); 4.67 (b tt, 1H, $^3J_{HH}$=7.0 Hz, $^3J_{HH}$=6.5 Hz, CH—NH); 7.23 (s, 5H_{arom}); 7.76 (bd, 1H, $^3J_{HH}$=7.0 Hz, NH)
CH_3	—CH_2—$COOCH_3$	97	$C_{10}H_{18}NO_6P$ (311.3)	3300 (NH); 1700, 1635 (CO); 1505 (NH); 1210 (PO); 1015, 1000 (POC)	2.83 (bd, 2H, $^3J_{HH}$=6.0 Hz, NH—CH_2—CO); 3.00 (d, 2H, $^2J_{PH}$=19.5 Hz, P—CH_2); 3.63 (s, 3H, C—O—CH_3); 3.65 (d, 3H, $^3J_{PH}$=11.0 Hz, H_3C—O—P); 3.67 (s, 3H, C—O—CH_3); 3.72 (d, 3H, $^3J_{PH}$=11.0 Hz, H_3C—O—P); 4.78 (tt, 1H, $^3J_{HH}$=6.0 Hz, $^3J_{HH}$=8.0 Hz, NH—CH); 7.90 (bd, 1H, $^3J_{HH}$=8.0 Hz, NH)
C_2H_5	—CH_2—$COOC_2H_5$	92	[15]	3300 (NH); 1730, 1670 (CO); 1550 (NH); 1230 (PO); 1050, 1015 (POC)	1.18 (t, 3H, $^3J_{HH}$=7.5 Hz, C—O—CH_2—CH_3); 1.19 (t, 3H, $^3J_{HH}$=7.5 Hz, C—O—CH_2—CH_3); 1.24 (t, 6H, $^3J_{HH}$=7.5 Hz, 2 H_3C—CH_2—O—P); 2.73 (bd, 2H, $^3J_{HH}$=6.0 Hz, CH_2—CO); 2.88 (d, 2H, $^2J_{PH}$=21.5 Hz, P—CH_2); 3.7–4.4 (m, 8H, 4 CH_2); 4.63 (b tt, 1H, $^3J_{HH}$=6.0 Hz, $^3J_{HH}$=8.0 Hz, CH—NH); 8.07 (bd, 1H, $^3J_{HH}$=8.0 Hz, NH)
C_2H_5	—CH_2—CH_2—$COOC_2H_5$	84	[15]	3310 (NH); 1735, 1675 (CO); 1540 (NH); 1245 (PO); 1045, 1010 (POC)	1.19 (t, 3H, $^3J_{HH}$=7.5 Hz, C—O—CH_2—CH_3); 1.24 (t, 3H, $^3J_{HH}$=7.5 Hz, C—O—CH_2—CH_3); 1.29 (t, 6H, $^3J_{HH}$=7.0 Hz, 2 H_3C—CH_2—O—P); 1.7–2.5 (m, 4H, CH_2—CH_2—COO); 2.95 (d, 2H, $^2J_{PH}$=21.0 Hz, P—CH_2); 4.15 (q, 4H, $^3J_{HH}$=7.5 Hz, 2 C—O—CH_2—CH_3); 4.15 (qq, 4H, $^3J_{HH}$=7.0 Hz, $^3J_{PH}$=7.0 Hz, 2 H_2C—CH_2—O—P); 4.56 (b tt, 1H, $^3J_{HH}$=7.0 Hz, $^3J_{HH}$=7.5 Hz, CH—NH); 7.69 (bd, 1H, $^3J_{HH}$=7.5 Hz, NH)

[a] The microanalyses showed the following maximum deviations from the calculated values: N, ±0.32; P, ±0.31.
[b] Hexamethyldisiloxane.
[c] From *N*-(bromoacetyl)-glycine.

Table 2. Trisodium Salts of *N*-(Phosphonoacetyl)-amino Acids (**5**)

R^4	Yield [%]	Molecular formula[a]	I.R. (KBr) ν [cm^{-1}]	^{1}H-N.M.R. (D_2O/HMDSO) δ [ppm]
H	85	$C_4H_5NNa_3O_6P \cdot H_2O$ (281.1)	3380 (NH); 1630, 1605 (CO); 1535 (NH); 1165, 1285 ($PO_3^{\ominus\ominus}$)	2.86 (d, 2H, $^2J_{PH}$=20.0 Hz, P—C$\underline{H}_2$); 3.92 (s, 2H, C$\underline{H}_2$—CO)
CH_3	85	$C_5H_7NNa_3O_6P \cdot 7H_2O$ (403.2)	3700–2800; 1645, 1605 (CO); 1135, 1105 ($PO_3^{\ominus\ominus}$)	1.53 (d, 3H, $^3J_{HH}$=7.5 Hz, C$\underline{H}_3$); 2.75 (d, 2H, $^2J_{PH}$=19.0 Hz, P—C$\underline{H}_2$); 4.28 (q, 1H, $^3J_{HH}$=7.5 Hz, NH—C$\underline{H}$)
i-C_3H_7	87[b], 90[c]	$C_7H_{11}NNa_3O_6P \cdot H_2O$ (323.2)	3700–2800; 1635, 1590 (CO); 1549 (NH); 1130, 1100, 1090 ($PO_3^{\ominus\ominus}$)	1.15 (d, 6H, $^3J_{HH}$=6.5 Hz, 2 C$\underline{H}_3$); 1.9–2.6 [m, 1H, C$\underline{H}$(CH$_3$)$_2$]; 2.78 (d, 2H, $^2J_{PH}$=19.0 Hz, P—C$\underline{H}_2$); 4.24 (d, 1H, $^3J_{HH}$=5.5 Hz, C$\underline{H}$—CO)
—CH_2—C_6H_5	86[b], 74[c]	$C_{11}H_{11}NNa_3O_6P \cdot H_2O$ (371.2)	3700–2600; 1640, 1585 (CO); 1545 (NH); 1145, 1080 ($PO_3^{\ominus\ominus}$)	2.63 (d, 2H, $^2J_{PH}$=19.0 Hz, P—C$\underline{H}_2$); 3.00 (bd, 2H, $^3J_{HH}$=6.5 Hz, C$\underline{H}_2$—C$_6$H$_5$); 4.52 (t, 1H, $^3J_{HH}$=6.5 Hz, NH—C$\underline{H}$); 7.48 (s, 5H$_{arom}$)
—CH_2—COONa	84[b], 82[c]	$C_6H_6NNa_4O_8P \cdot 4H_2O$ (425.2)	3800–2700; 1600 br (CO); 1150, 1105, 1070 ($PO_3^{\ominus\ominus}$)	2.58 (d, 2H, $^2J_{PH}$=18.0 Hz, P—C$\underline{H}_2$); 2.63 (d, 2H, $^3J_{HH}$=6.0 Hz, C$\underline{H}_2$—CO); 4.38 (t, 1H, $^3J_{HH}$=6.0, CHCOO)

[a] The microanalyses showed the following maximum deviations from the calculated values: N, ±0.26; P, ±0.36.

[b] From the methyl ester.

[c] From the ethyl ester.

mixture is stirred and chlorotrimethylsilane (2.5 ml) is added. Stirring is continued for 15 min and the mixture then heated at 40–50 °C for an additional 15 min. The precipitated sodium chloride is filtered off and the volatile components are evaporated in vacuo. The residual crude product is suspended in water (30 ml) and the suspension allowed to stand at room temperature for 1 h. The water is evaporated in vacuo and the residual oil dissolved in ethanol. Ethanol is then removed in vacuo and the crude product **4** thus obtained is used in the next step without further purification.

Trisodium Salts of 2-[N-(Phosphonoacetyl)-amino]-alkanoic Acids (**5**): The acid **4** (0.01 mol) is suspended in a 2 molar solution (30 ml) of sodium hydroxide in methanol. The mixture is refluxed for 2 h, then allowed to cool to room temperature, and the product **5** isolated by suction. The crude salt **5** is purified by boiling with methanol/acetone (1/1). It is finally recrystallized by dissolving in a small amount of water and precipitation with acetone. The salts **5** are hygroscopic.

Sodium *N*-(Diethylphosphonoacetyl)-glycinate (6, $R^1=C_2H_5$, R^4=H): Ethyl *N*-(diethylphosphonoacetyl)-glycinate (**3**, $R^1=C_2H_5$, R^3=H; 3.0 g, 0.0114 mol) is dissolved in a 2 molar solution (6 ml, 0.012 mol) of sodium hydroxide in methanol. The solution is refluxed for 1.5 h and the precipitated product isolated by suction. The crude product is suspended in acetone, the suspension refluxed for 30 min, then allowed to cool, and the salt isolated by suction; yield: 2.2 g (74%).

$C_8H_{15}NNaO_6P$	calc.	N 5.09	P 11.26
(275.2)	found	4.86	11.03

^{1}H-N.M.R. (D_2O/HMDSO): δ=1.55 (t, 6H, $^3J_{HH}$=7.5 Hz, 2$\underline{H}_3$C—CH$_2$—O—P); 3.15 (d, 2H, $^2J_{PH}$=20 Hz, P—C$\underline{H}_2$); 4.27 (q, q, 4H, $^3J_{HH}$=7.5 Hz, $^3J_{PH}$=7.5 Hz, 2H$_3$C—C$\underline{H}_2$—O—P); 4.33 ppm (s, 2H, NH—C$\underline{H}_2$—CO).

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