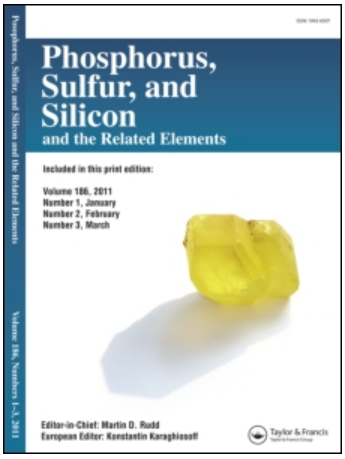

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SEPARATION OF RACEMIC PHOSPHONIC ANALOGUES OF GLUTAMIC ACID

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SEPARATION OF RACEMIC PHOSPHONIC ANALOGUES OF GLUTAMIC ACID

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α- and γ-phosphonic analogues of glutamic acid were separated to give all enantiomers. 2-Amino-4-phosphonobutyric acid enantiomers were separated as 2-*N*-benzoylamino-4-diethylphosphonobutyric acid derivatives by the action of papain. Resolution of 4-amino-4-phosphonobutyric acid was performed by crystallization of 4-*N*-carbobenzoxyamino-4-diethylphosphonobutyric acid salts of both optically active 1-phenylethylamines. Enantiomers of 4-amino-4-phosphonobutyric acid as *N*-carbobenzoxydiethylphospho derivatives, as *N*-carbobenzoxy-diethylphospho DCHA salts and as free amino acids are described.

Recently, a number of phosphorus analogues of amino acids have been studied in order to determine their biological activity.[1–4] These experiments required the resolved enantiomers of aminophosphonic acids. Several amino-phosphonic acids have been resolved[5–10] but there are no analogues of glutamic acid reported.

In this report, we present the results of our investigation on the resolution of two phosphonic analogues of glutamic acid.

We decided to resolve racemic 2-amino-4-phosphonobutyric acid (**1**) by the action of papain because we expected[11] its α-aminocarboxylic system to be a good enzymatic substrate. 2-*N*-Benzoylamino-4-phosphonobutyric acid (**2**) was obtained by the classical method from the free amino acid (**1**).

The triester (**3**) was obtained by the reaction with ethyl orthoformate and the corresponding diester (**4**) by basic hydrolysis with an equimolar amount of sodium hydroxide.

N-benzoyl protection was selected as the best substrate for papain. The anilide (**5**) was produced in the efficient enzymatic reaction as a thick oil. The acidic hydrolysis of compounds (**4a**) and (**5**) yielded optically active amino acids (**1a**) and (**1b**). The use of the papain method of resolution provided information about the absolute

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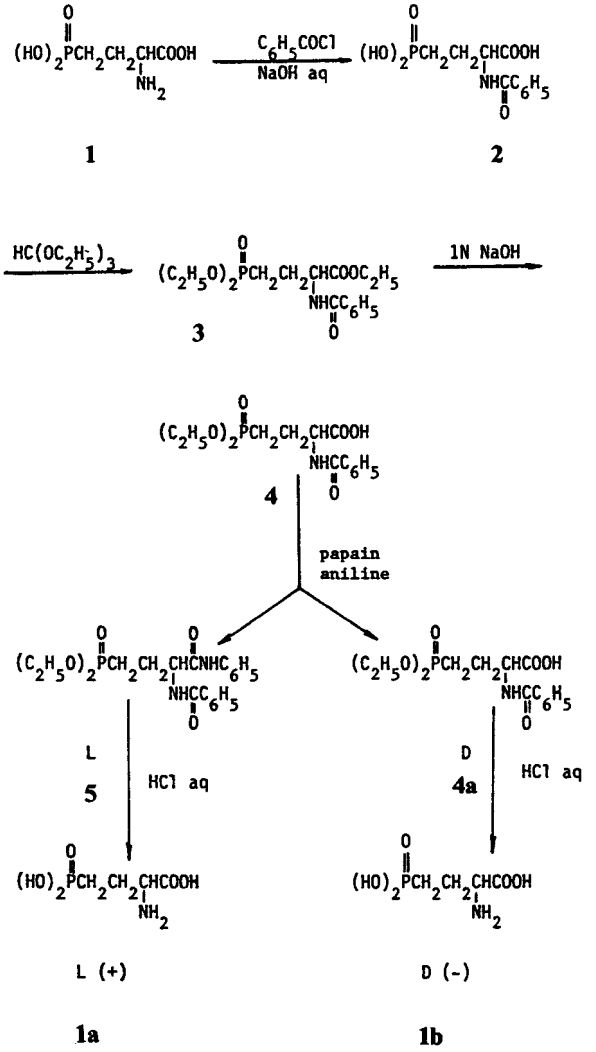
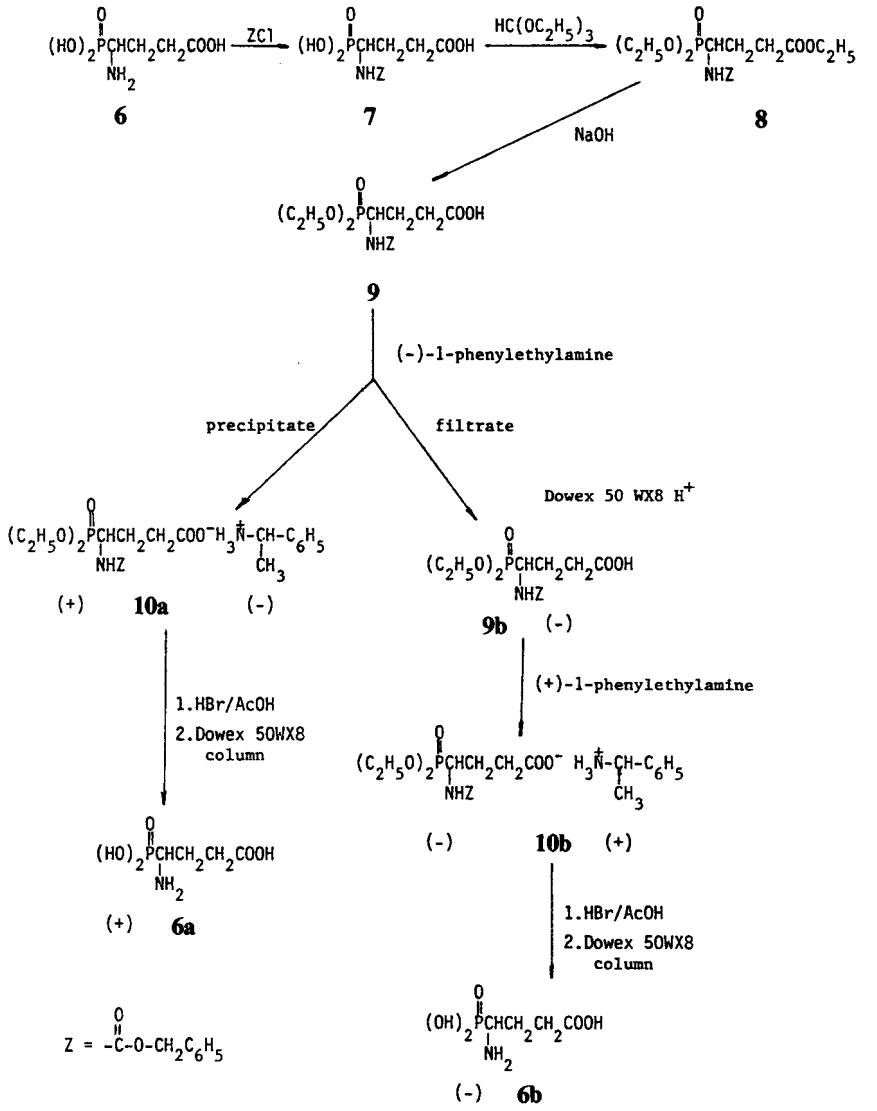


SCHEME 1 Separation of 2-amino-4-phosphonobutyric acid into enantiomers.

configuration of the enantiomers (**1a**) and (**1b**). Thus, dextrorotatory enantiomer (**1a**) obtained from the anilide (**5**) has L(S) configuration and levorotatory (**1b**) has D(R) configuration.

4-Amino-4-phosphonobutyric acid (**6**) was resolved by the crystallization of the diastereoisomeric salts of 1-phenylethylamine. 4-*N*-Carbobenzoxyamino-4-phosphonobutyric acid (**7**), obtained from the amino acid (**6**), was esterified with ethyl orthoformate to triester (**8**). The basic hydrolysis of triester (**8**) gave the corresponding diester (**9**) which was resolved by the crystallization of its diastereoisomeric salts with 1-phenylethylamine (**10a, 10b**). The optical active forms of the corresponding derivative of amino acid (**6**) were identified as enantiomers of 4-*N*-carbobenzoxyamino-4-diethylphosphonobutyric acid (**9a, 9b**) and their DCHA salts. The



SCHEME 2 Separation of 4-amino-4-phosphonobutyric acid into enantiomers.

enantiomers of free 4-amino-4-phosphonobutyric acid (**6a, 6b**) were obtained by the acidolysis of the diastereoisomeric salts (**10a, 10b**).

EXPERIMENTAL

Melting points are uncorrected. ^{1}H NMR spectra were recorded on a Tesla BS 487 80 MHz spectrometer

2-Amino-4-phosphonobutyric acid (**1**) was obtained according to the literature.[12]

4-Amino-4-phosphonobutyric acid (**6**) was obtained by the general method described by Oleksyszyn and Tyka.[13] Yield. 36% M.p. 169–170°. ^{1}H NMR HMDSO (D_2O) 2.1–2.7 (m, 2, $C\underline{H}_2$, CH—P), 2.97

(t, J = 7 Hz, 2, C$\underline{H}_2$COOH), 3.4–3.9 (m, 1, CH—P). Anal. Calcd. for $C_4H_{10}NO_5P$: C, 26.23; H, 5.50; N, 7.65. Found: C, 26.46; H, 5.49; N, 7.73.

2-N-Benzoylamino-4-phosphonobutyric acid (**2**) was obtained according to the literature.[14] The recrystallization from water yielded white crystals, m.p. 201–203°, yield 80% ^{1}H NMR HMDSO (DMSO) 1.4–2.6 (m, 4, CH_2CH_2P), 4.2–4.8 (m, 1, C$\underline{H}$COOH), 7.4–8.2 (m, 5, C_6H_5), 9.00 (d, J = 7 Hz, 1, NH). Anal. Calcd. for $C_{11}H_{14}NO_6P$: C, 45.99; H, 4.91; N, 4.87. Found: C, 45.65; H, 4.98; N, 4.64.

4-N-Carbobenzoxyamino-4-phosphonobutyric acid (**7**) was obtained by the general method according to the literature.[15] The crude crystalline product was purified by the trituration with ethyl acetate. m.p. 157–8°. Yield 85% ^{1}H NMR HMDSO (DMSO + D_2O) 1.7–2.7 (m, 4, CH_2CH_2); 3.8–4.3 (m, 1, CH—P); 5.25 (s, 2, C$\underline{H}_2C_6H_5$); 5.6–6.5 (m, 1, NH) 7.55 (s, 5, C_6H_5). Anal. Calcd for $C_{12}H_{16}NO_7P$: C, 45.43; H, 5.08; N, 4.42. Found: C, 45.50; H, 5.13; N, 4.65.

Preparation of N-acylaminophosphonobutyric acid diethyl esters (**4**), (**9**). The triesters (**3**) and (**8**) were obtained with ethyl orthoformate according to reference.[15] The crude product (0.05 moles) was dissolved in 100 ml of ethanol and 50 ml aqueous 1N NaOH was added dropwise to this solution with vigorous agitation at 0°C. The stirring was continued for 1 h at room temperature. The ethyl alcohol was removed under reduced pressure below 25°C and the remaining aqueous solution was acidified with 6N HCl at 0°C and extracted three times with ethyl acetate. The acetate solution was dried with anhydrous Na_2SO_4 and then evaporated to dryness.

2-N-benzoylamino-4-diethylphosphonobutyric acid (**4**) was recrystallized from $CHCl_3$: m.p. 104–106°. Yield 15.6 g (91%). ^{1}H NMR HMDSO (DMSO) 1.5 (t, J = 6 Hz, P—OCH$_2$C$\underline{H}_3$), 1.7–2.6 (m, 4, CH_2CH_2), 3.8–4.5 (m, 4, C$\underline{H}_2$CH$_3$), 4.5–5.0 (m, 1H, CH—P), 7.3–8.3 (m, 5, C_6H_5) 8.87 (d, J = 7 Hz, 1, NH). Anal. Calcd. for $C_{15}H_{22}NO_6P$: C, 52.50; H, 6.46; N, 4.08. Found: C, 52.25; H, 6.52; H, 3.80.

4-N-Carbobenzoxyamino4-diethylphosphonobutyric acid (**9**). The bright yellow oil was obtained in 89% yield i.e. 16.8 g. ^{1}H NMR HMDSO ($CDCl_3$) 1.0–1.3 (m, 6, CH$_2$C$\underline{H}_3$); 1.6–2.5 (m, 4, CH_2CH_2); 3.7–4.3 (m, 5, C$\underline{H}$—P, C$\underline{H}_2$CH$_3$); 5.00 (s, 2, C$\underline{H}_2C_6H_5$); 6.00 (d, J = 10 Hz, 1, NH); 7.22 (s, 5, C_6H_5), 10.00 (s, 1, COOH). The DCHA salt of (**9**) was obtained for analysis. Recrystallization was from ethanol–ethyl ether, m.p. 126–127°. Anal. Calcd. for $C_{28}H_{47}N_2O_7P$: C, 60.62; H, 8.54; N, 5.05. Found: C, 60.34; H, 8.62; N, 4.99.

Optically active 2-amino-4-phosphonobutyric acid (**1a**) (**1b**). Separation of 8.58 g (0.025 mol) of (**4**) by the action of papain with aniline was performed according to the literature.[11] The anilide (**5**) was hydrolyzed by refluxing with 6N HCl for 18 h. The hydrolysate was cooled; the precipitate of benzoic acid was discarded. The filtrate was evaporated to dryness.

Product was purified by column chromatography (Dowex 50WX8) with water as eluent: Crude acid was recrystallized from 70% ethanol and gave 1.6 g (35% yield) of (+)-2-amino-4-phosphonobutyric acid (**1a**). m.p. 205–207°C. $[\alpha]_D^0$ = +11.2 (c = 2, H_2O). Anal. Calcd. for $C_4H_{10}NO_5P$: C, 26.23; H, 5.50; N, 7.65. Found: C, 26.15; H, 5.24; N, 7.38. The solution obtained after the decantation from anilide /5/ was refluxed and the denaturated protein was filtered.

The filtrate was evaporated to dryness and the residue was hydrolyzed with 6N HCl for 18 h. (−)-2-Amino-4-phosphonobutyric acid (**1b**) was isolated as described above. After two recrystallizations from 60% ethanol, 1.1 g (24% yield) of (−)-2-amino-4-phosphonobutyric acid (**1b**) was obtained. m.p. 205–207°C. $[\alpha]_D^0$ = −11.0° (c = 2, H_2O). Anal. Calcd. for $C_4H_{10}NO_5P$: C, 26.23; H, 5.50; N, 7.65. Found: C, 26.15; H, 5.25; N, 7.38.

Resolution of 4-N-carbobenzoxyamino-4-diethylphosphonobutyric acid (**9**) 3.73 g (0.01 mol) of (**9**) and 1.3 ml (0.01 mol) of (−)-1-phenylethylamine was dissolved in 5 ml of methanol and then 30 ml of ethyl ether was added to this solution. The reaction mixture was left for 24 h at room temperature. The precipitate was filtered and recrystallized from a mixture of 5 ml CH_3OH and 25 ml ethyl ether 1.73 g (35% yield) of the corresponding salt (**10a**) was obtained. m.p. 142–142.5°. $[\alpha]_D^0$ = +9.8° (c = 2.44, CH_3OH. Anal. Calcd. for $C_{24}H_{35}N_2O_7P$: C, 58.28; H, 7.13; N, 5.66. Found: C, 57.98; H, 7.22; N, 5.51. The salt (**10a**) was dissolved in methanol and Dowex 50WX8 (H^+) was added. The resin was filtered and the optically active (+)-4-N-carbobenzosy-amino-4-diethylphosphonobutyric acid (**9a**) was obtained as colorless oil by removing the methanol. $[\alpha]_D^0$ = +20.6° (c = 2.5, methanol). The DCHA salt of (**9a**) was obtained. m.p. 123–123.5°. $[\alpha]_D^0$ = +6.0° (c = 2, methanol). Anal. Calcd. for $C_{28}H_{47}N_2O_7P$: C, 60.62; H, 8.54; N, 5.05. Found: C, 60.50; H, 8.70; N, 4.91. In order to remove phenylethylamine from the collected filtrates obtained after the filtration of the salt (**10a**), Dowex 50WX8 (H^+) resin was added. Then the resin was filtered and the resulting filtrate was mixed with 0.85 ml (0.0065 mol) (+)-1-phenylethylamine. 1,88 g (38% yield) of salt (**10b**) m.p. 142–142.5° was obtained by the procedure described above. $[\alpha]_D^0$ = −9.6°

(c = 2.5, methanol). Anal. Calcd. for $C_{24}H_{35}N_2O_7P$: C, 58.28; H, 7.13; N, 5.66. Found: C, 58.04; H, 7.28; N. 5.38. (−)-4-*N*-carbobenzoxy-4-diethylphosphonobutyric acid (**9b**) as colorless oil was obtained from the salt (**10b**) by the method described above. $[\alpha]_D^0 = -20.2°$ (c = 2, methanol). The DCHA salt of (**9b**) was obtained. M. p. 123–123.5°. $[\alpha]_D^0 = 5.9°$ (c = 2, methanol). Anal. Calcd. for $C_{28}H_{47}N_2O_7P$: C, 60.62; H, 8.54; N, 5.05. Found: C, 60.45; H, 8.62; N, 5.00.

Optically active 4-amino-4-phosphonobutyric acid. 0.988 g (0.002 mol) of the salt (**10a**) or (**10b**) was dissolved in 10 ml of 45% HBr/CH_3COOH. The mixture was left for 72 h at room temperature. Then the solution was evaporated to dryness. The residue was dissolved in water and was passed through a Dowex 50WX8 (H^+) column. Recrystallization from water–ethyl alcohol gave the optically active 4-amino-4-phosphonobutyric acid.

A. 0.28 g (78% yield) of (+)-4-amino-4-phosphonobutyric acid (**6a**) was obtained from the salt (**10a**) M.p. 163–165°. $[\alpha]_D^0 = +17.2$ (c = 5, 1*N* NaOH). Anal. Calcd. for $C_4H_{10}NO_5P$: C, 26.23; H, 5.50; N, 7.65. Found: C, 26.04; H, 5.71; N, 7.40.

B. 0.3 g (82% yield) of (−)-4-amino-4-phosphonobutyric acid (**6b**) was obtained from the salt (**10b**) M.p. 164–166°C. $[\alpha]_D^0 = -17.8$ (c = 5, 1*N* NaOH). Anal. Calcd. for $C_4H_{10}NO_5P$: C, 26.23; H, 5.50; N, 7.65. Found: C, 26.01; H, 5.72; N, 7.39.

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