Russian Journal of General Chemistry, Vol. 74, No. 8, 2004, pp. 1297–1299. Translated from Zhurnal Obshchei Khimii, Vol. 74, No. 8, 2004, pp. 1400–1402.

LETTERS TO THE EDITOR

Enzymatic Synthesis of the Enantiomers of 2-Amino-2-methyl-4-phosphonobutyric Acid

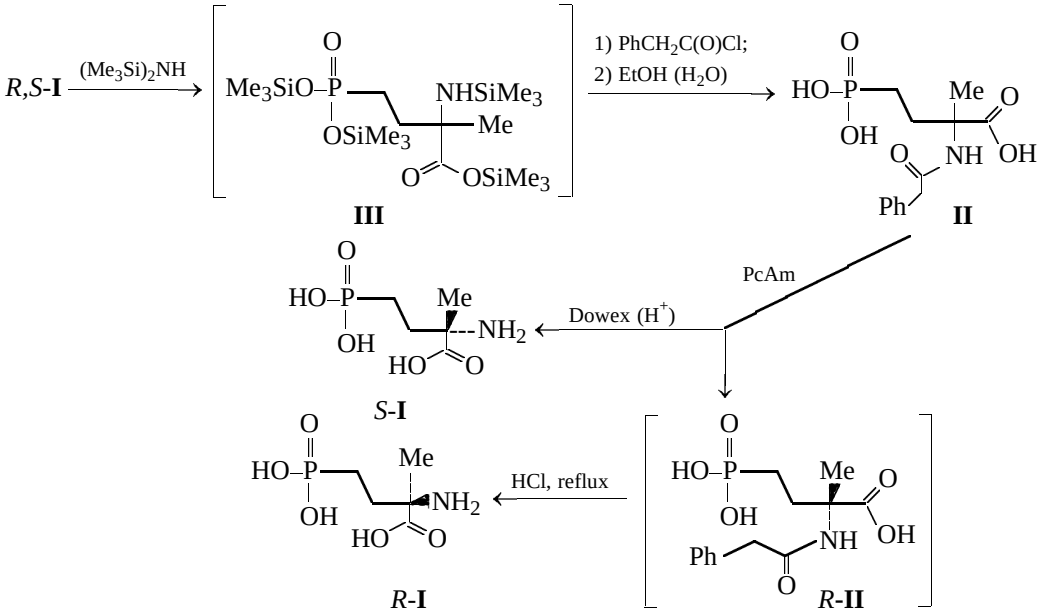
V. V. Ragulin

Institute of Physiologically Active Compounds, Russian Academy of Sciences, Chernogolovka, Russia

Received December 10, 2003

(*S*)-2-Amino-2-methyl-4-phosphonobutyric acid is known as an antagonist of metabotropic glutamate receptors III [1–3]. Stereoselectivity of these receptors is often unexpected [2, 3], and, therefore, both enantiomers are usually needed for complete biological investigation. To this end, the enzymatic method of resolution of racemic phosphonic aminocarboxylic acids [4–8] may prove more suitable than the asymmetric synthesis [7, 8]. Moreover, the stereoselective introduction of the β-phosphonoethyl radical [8] in the chiral *trans*-2-phenyloxazolidinone, aimed at preparing the *S* enantiomer of 2-amino-2-methyl-4-phosphonobutyric acid (**I**) may be accompanied by inversion of the chiral carbon center [7]. Ma *et al*. [8] have reported the following optical rotation data for *S*-**I**: $[\alpha]_{589}^{25}$ –12.3° (*c* 0.31, MeOH). However, the opposite value, $[\alpha]_{589}^{25}$ +12.4° (*c* 1.0, 6 N HCl), is also available in the literature [9].

In the present work we propose an enzymatic synthesis of the *R* and *S* enantiomers of compound **I** according to the following scheme. Penicillinamidase (PcAm) is an enzyme that exhibits an extremely high *S/R* stereoselectivity in hydrolysis of *N*-acyl derivatives of α-aminocarboxylic acids and peptides [4–6], which makes this synthetic procedure feasible for resolving the above-mentioned controversy.



In the enantioselective hydrolysis of *N*-phenylacetylated amino acid **II** according to our previously proposed procedure for resolution of racemic α-amino-ω-phosphonocarboxylic acids we made use of immobilized PcAm. Attempted acylation of amino acid **I** by the classical Schotten–Baumann procedure [10] failed.

1070-3632/04/7408-1297

Contrary to α-unsubstituted α-amino acids, amino acid **I** had to be preliminarily silylated [11, 12]. Silyl derivative **III** was then reacted *in situ* with phenylacetyl chloride to obtain target *N*-(phenylacetyl)amino acid **II** after alcoholysis and removal of the silyl group.

The enzymatic hydrolysis of compound **II** was much slower compared with α-unsubstituted amino acids [5]. Hydrolyzed form *S*-**I** and *N*-phenylacetylated form *R*-**II** were resolved by ion-exchange chromatography. Acid hydrolysis of *R*-**II** gave the target *R* enantiomer of **I** [4, 5].

Synthesis of 2-methyl-2-(phenylacetyl)amino]-4-phosphonobutyric acid (*R,S*-II). A mixture of 6.7 g of (*R,S)*-**I** and 21.5 ml of hexamethyldisilazane was stirred for 0.5 h at room temperature and then gradually brought to the boiling point, stirred with boiling for 2 h, cooled under argon, and evaporated in a vacuum. The residue was dissolved in 10 ml of absolute toluene, and a solution of 5.2 ml of phenylacetyl chloride in 5 ml of toluene was slowly added dropwise. The resulting mixture was boiled for 3 h, cooled, and 10 ml of aqueous ethanol was slowly added dropwise with vigorous stirring. The solvent was removed in a vacuum, and the residue was passed through a column (3 × 30 cm) of Dowex 50WX8-200 (H^+) using water as eluent. A fraction with a negative ninhydrin test was collected and evaporated in a vacuum. Crystallization of the residue from ethereal ethanol gave 8.9 g (82.4%) of product **II**, mp 181–183°C. ^{1}H NMR spectrum (D_2O, pH ~2), δ, ppm: 1.33 s (2H, Me), 1.57 m (2H, CH_2), 1.92 m (1H, CH_2), 2.12 m (1H, CH_2), 3.47 s (2H, CH_2Ph), 7.22 m (5H, Ph). ^{1}H NMR spectrum (C_3OD), δ, ppm: 1.47 s (3H, Me), 1.62 m (2H, CH_2), 2.18 m (2H, CH_2), 3.52 br.s (2H, CH_2Ph), 7.27 m (5H. Ph). ^{31}P NMR spectrum, δ_P, ppm: (D_2O, pH 1–2) 30.6, (CD_3OD) 30.2. Found, %: C 49.27, 49.37; H 5.97, 5.83; N 4.46, 4.50. $C_{13}H_{18}NO_6P$. Calculated, %: C 49.53; H 5.75, N 4.44.

Enzymatic synthesis of the enantiomers of 2-methyl-2-amino-4-phosphonobutyric acid (I). *R,S*-**II**, 13.5 g, was dissolved in 70 ml of water at pH 7.5 which was maintained with 1 N KOH. Preliminary prepared immobilized PcAm, 5–7 g, was added to the solution, and the mixture was stirred at 25°C. Reaction progress was controlled by HPLC by the formation of phenylacetic acid [5]. After the reaction was complete, the enzyme was filtered off, and the filtrate was washed with ether (2 × 20 ml). The aqueous layer was concentrated in a vacuum to 30 ml and treated with 1 N HCl to pH 5, was washed with ether (2 × 20 ml), and passed through a column (3 × 40 cm) of Dowex 50WX8-200 (H^+) using water as eluent.

Strongly acidic fractions with a negative ninhydrin test were evaporated in a vacuum. The oily residue (about 7 g), *R*-**II** [the ^{1}H and ^{31}P NMR spectra are identical to those of *R,S*-**II**], was dissolved in 30 ml of 8 N HCl, and the resulting solution was boiled for 10 h and evaporated in a vacuum. The residue was partitioned between water (50 ml) and ether (30 ml). The aqueous layer was additionally washed with ether (2 × 30 ml) and evaporated to dryness. The residue was treated with excess propylene oxide in aqueous ethanol. Additional crystallization from aqueous ethanol gave 2.4 g of *R*-**I**, yield 57% per *R,S*-**II**, mp 203–205°C (decomp.). R_f 0.2 (pyridine–acetic acid–water–2-methylpropan-1-ol 1 : 3 : 5 : 15). ^{1}H NMR spectrum (D_2O + DCl), δ, ppm: 1.42 s (3H, Me), 1.58 m (2H, CH_2), 1.97 m (2H, CH_2). ^{13}C NMR spectrum (D_2O + DCl), δ_C, ppm: 21.7 s (Me), 21.9 d (C^4, J_{PC} 136.1 Hz), 30.8 d (C^3, J_{PC} 3.3 Hz), 60.3 d (C^2, J_{PC} 18.7 Hz), 173.7 (C^1). ^{31}P NMR spectrum (D_2O + DCl), δ_P, ppm: 25.0. Found, %: C 30.13, 30.17; H 6.21, 6.19; N 7.07, 7.03. $C_5H_{12}NO_5P$. Calculated, %: C 30.47; H 6.14, N 7.11. $[\alpha]^{25}_{546}$ –8.5° (*c* 1.2, H_2O); $[\alpha]^{25}_{546}$ –13.4° (*c* 1.1, 6 N HCl); $[\alpha]^{25}_{589}$ –12.1 (*c* 1.1, 6 N HCl).

Fractions with a positive ninhydrin test were collected and evaporated in a vacuum. The residue was passed through a column (3 × 20 cm) of Dowex 50WX8-200 (H^+), and fractions with a positive ninhydrin test were evaporated. The residue was crystallized from 2 N HCl–acetone, 9 : 1, to obtain 3.1 g of *S*-**I** HCl, yield 62.0% per *R,S*-**II**, mp 198–199°C (decomp.). R_f 0.3 (pyridine–acetic acid–water–2-methylpropan-1-ol 1 : 3 : 5 : 15). The ^{1}H, ^{13}C, and ^{31}P NMR spectra are identical to those of *R*-**I**. Found, %: C 25.50, 2553; H 5.77, 5.70; N 6.07, 6.03. $C_5H_{12}NO_5P \cdot HCl$. Calculated, %: C 25.71; H 5.61; N 6.00. $[\alpha]^{25}_{589}$ +7.9° (*c* 1.0, H_2O); $[\alpha]^{25}_{546}$ +9.1° (*c* 1.0, H_2O); $[\alpha]^{25}_{589}$ +12.2°C (*c* 0.1, MeOH); $[\alpha]^{25}_{589}$ +12.3° (c^1 1.0, 6 N HCl).

The optical rotations of (*S*)-**I** in various solvents agree with those reported in [9]. The present work offers the first example of the application of PcAm for resultion of enantiomers of α-substituted α-amino acids.

The ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker DPX-200 Fourier spectrometer. The optical rotations were measured on a Perkin Elmer

[1] On an account of the free amino acid.

241 and Polamat A polarimeters. The enzymatic stage was carried using immobilized PcAm with a specific activity of 10^3 U/g dry biocatalyst [4–6]. Analytical HPLC was carried out on a Gilson chromatograph with a UV-Vis-118 detector at 254 nm. Quantitative analysis was carried out on a Diasorb C16/T column preliminary calibrated by phenylacetic acid.

REFERENCES

1. Jane, D.E., Pittaway, K., Sunter, D.C., Thomas, N.K., and Tse, H.W., *Phosphorus, Sulfur, Silicon Relat. Elem.*, 1996, vols. 109–110, nos. 1–4, pp. 313–316.
2. Bushell, T.J., Jane, D.E., Tse, H.W., Watkins, J.C., Davies, C.H., Garthwaite, J., and Collingridge, G.L., *Neuropharmacology*, 1995, vol. 34, no. 2, pp. 239–241.
3. Sekiyama, N., Hayashi, Y., Nakanishi, S., Jane, D.E., Tse, H.W., Birse, E.F., and Watkins, J.C., *Br. J. Pharmacol.*, 1996, vol. 117, no. 7, pp. 1493–1503.
4. Solodenko, V.A., Kasheva, N.A., Kukhar, V.P., Kozlova, E.V., Mironenko, D.A., and Svedas, V.-K., *Tetrahedron*, 1991, vol. 47, no. 24, pp. 3989–3998.
5. Rozhko, L.F., Klochkov, S.G., Ragulin, V.V., and Tsvetkov, E.N., *Zh. Obshch. Khim.*, 1999, vol. 69, no. 7, pp. 1134–1137.
6. Margolin, A.L., Svedas, V.-K., and Berezin, I.V., *Biochim. Biophys. Acta.*, 1980, vol. 616, no. 2, pp. 283–289.
7. Fadel, A. and Salaun, J., *Tetrahedron Lett.*, 1987, vol. 28, no. 20, pp. 2243–2246.
8. Ma, D., Ma, Z., Jiang, J., Yang, Z., and Zheng, C., *Tetrahedron: Asymmetry*, 1997, vol. 8, no. 6, pp. 889–893.
9. Warkins, J.C. and Jane, D.E., GB Patent 9515940, *Chem. Abstr.*, 1995, N 957967.
10. Schotten, C., *Ber.*, 1884, vol. 17, pp. 2544–2547; Baumann, E., *Ber.*, 1886, vol. 19, pp. 3218–3221.
11. Mucha, A., Kafarski, P., Plenat, F., and Cristau, H.-J., *Phosphorus, Sulfur, Silicon Relat. Elem.*, 1995, vol. 105, nos. 1–4, pp. 187–191.
12. Kankorat, T., Neda, I., Jones, P.G., and Schmutzler, R., *Phosphorus, Sulfur, Silicon Relat. Elem.*, 1996, vol. 112, nos. 1–4, pp. 247–251.
13. Saratovskikh, I.V., Kalashnikov, V.V., and Ragulin, V.V., *Zh. Obshch. Khim.*, 1999, vol. 69, no. 7, pp. 1218–1220.