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LETTERS TO THE EDITOR

Synthesis of Pseudoalanylalanine

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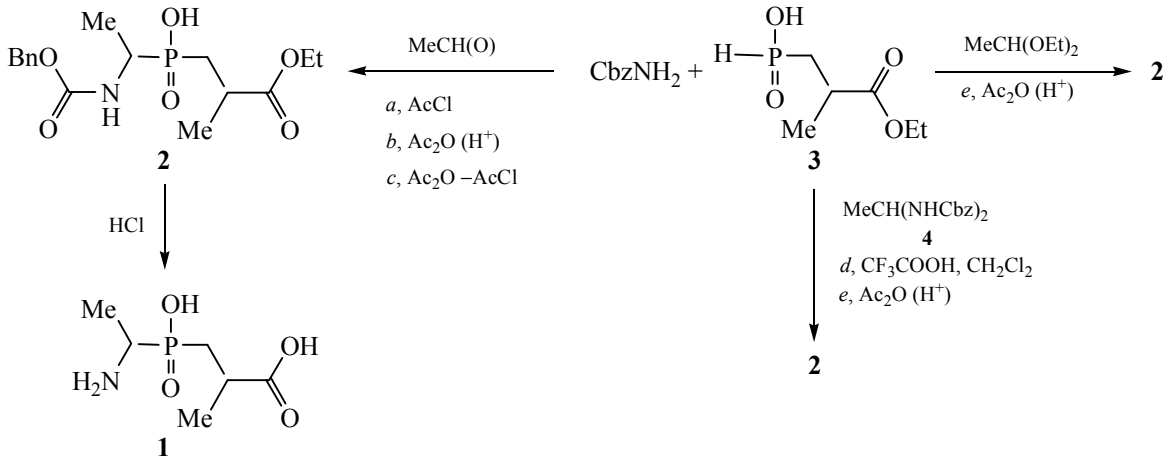
Synthetic methodology for the preparation of phosphinic peptide isosteres, based on the addition of P,N-protected α-aminophosphonous acid silyl esters to α-substituted acrylates according to Michael–Pudovik reaction, makes it possible to obtain a wide series of phosphinic pseudopeptides. However, it is not free from some drawbacks related to the necessity of introduction and subsequent removal of appropriate protecting groups to the phosphorus and nitrogen atoms of the α-aminophosphonous component [1–5]. We previously proposed and developed an alternative approach implying the reverse order of the construction of target molecules [6–11], namely initial 1:1 addition of bis(trimethylsilyl) phosphonite generated *in situ* to the corresponding α-substituted acrylates with formation of phosphonous acids containing an amino acid isostere fragment [7–9]. Amidoalkylation of the latter with alkyl carbamates and carbonyl compounds afforded phosphinic pseudopeptides (N-protected α-aminophosphinic acids) that are phosphinic analogs of the corresponding dipeptides in which the C(O)–NH peptide bond is replaced by a $P(O)CH_2$ methylenephosphoryl moiety [10, 11].

The present study continues further development of the above methodology through the synthesis of pseudoalanylalanine **1** which inhibits D-Ala-D-Ala ligase (bacterial enzyme involved in the cell wall biosynthesis) [12, 13]. Amino- and amidoalkylation of PH compounds with acetaldehyde as carbonyl component is usually complicated due to its high volatility. Therefore, the procedure proposed previously [10] for the amidoalkylation of functionally substituted phosphonous acids in acetic anhydride under acid catalysis at room temperature seems to be optimal as regards low-boiling aldehydes. In addition, an important problem is search for efficient but mild conditions for the amidoalkylation of phosphonous acids to minimize decomposition of carboxylic acid ester fragments. The proposed procedure ensured initial selective esterification of the hydroxyphosphoryl fragment of phosphinic acids like **2** and subsequent selective hydrolysis of the carboxylic ester group to obtain the corresponding pseudodipeptide block for peptide synthesis [2–5].

Herein we report amidoalkylation of phosphinic acid **3** to acid **2** which may be regarded as N-protected phosphinic pseudoalanylalanine ethyl ester, *N*-Cbz-Ala-ψ[$P(O)(OH)CH_2$]-Ala-OEt. The transformation **3**→**2** was accomplished in several ways with the use of benzyl carbamate and acetaldehyde in acetic anhydride, acetyl chloride, and a mixture of acetic anhydride with acetyl chloride, as well as with acetaldehyde diethyl acetal and *N*,*N*′-(ethane-1,1-diyl)bis(benzyl carbamate) (**4**) whose molecule is a combination of carbonyl and amide components (Scheme 1).

In keeping with the mechanism proposed previously for the amidoalkylation of PH compounds, the phosphorus–carbon bond is formed via Arbuzov-like reaction as a result of nucleophilic attack by the P(III)OAc derivative on the positively charged carbon atom of iminium ion (charged form of the Schiff base); both these species are generated *in situ* from the initial PH compound and *N*,*N*′-(alkane-1,1-diyl)bis(alkyl carbamate), respectively [10, 11]. Bis-carbamates were identified as stable intermediate products arising from the reaction of aldehyde with alkyl carbamate and were isolated from the reaction mixture [10]. Acetic

Scheme 1.



anhydride and acetyl chloride are excellent acylating agents capable of generating *in situ* nucleophilic P(III)OAc species; acetic acid and hydrogen chloride are formed, respectively, as by-products which catalyze the formation of bis-carbamates and subsequent generation of acyliminium cation as electrophilic component [10, 11].

Monitoring of the reaction progress by ^{31}P NMR spectroscopy revealed fairly fast amidoalkylation of phosphinic acid **3** in acetyl chloride or its mixture with acetic anhydride until complete disappearance of the signal belonging to acid **3**. However, partial dealkylation of the ester fragment of **2** was observed during the reaction in acetyl chloride and (to a lesser extent) in acetic anhydride–acetyl chloride mixture (3:1 by volume), which probably lowers the yield of **2** and its purity. Therefore, we tried procedures for the amidoalkylation of **3** in acetic anhydride under milder acid catalysis by *p*-toluenesulfonic acid. In this case, we succeeded in avoiding undesirable dealkylation of the carboxylic acid ester moiety, but the reaction was appreciably slower. Nevertheless, the yield and purity of target acid **2** remained fairly high.

Considerably worse results were obtained with the use of acetaldehyde diethyl acetal, which may be rationalized assuming formation of ethyl acetate via decomposition of acetaldehyde diethyl acetal into acetaldehyde and ethanol in AcCl–Ac_2O and impaired conditions for the generation *in situ* of the above reaction intermediates. The two-component version of amidoalkylation of phosphinic acid **3** with preliminarily prepared biscarbamate **4** in methylene chloride in the presence of an equimolar amount of trifluoroacetic anhydride afforded a lower yield of **2** than that obtained in the three-component version in acetic anhydride under catalysis by *p*-toluenesulfonic acid.

Thus, the optimal conditions for the synthesis of phosphinic acid **2** by amidoalkylation of acid **3** include the use of benzyl carbamate and acetaldehyde in acetic anhydride in the presence of *p*-toluenesulfonic acid. The two-component version involving bis-carbamate **4** provides better results, but it requires preliminary preparation of *N*,*N*′-(ethane-1,1-diyl)bis(benzyl carbamate) (**4**).

2-(Ethyloxycarbonyl)propylphosphonous acid (3) was synthesized by reaction of ethyl methacrylate with 3 equiv of bis(trimethylsilyl) hypophosphite generated *in situ* from ammonium hypophosphite according to modified procedure [6–8]. Yield 73%. ^{1}H NMR spectrum ($CDCl_3$ containing a drop of CD_3OD), δ, ppm: 1.19 d (3H, CH_3, $^3J_{HH}$ = 7.3 Hz), 1.24 t (3H, $^3J_{HH}$ = 7.3 Hz), 1.76 m and 2.14 m (1H each, PCH_2), 2.82 m (1H, CHCO), 4.10 q (2H, CH_2O, $^3J_{HH}$ = 7.3 Hz), 6.50 br.s (1H, POH), 7.12 d (1H, PH, $^1J_{PH}$ = 558.1 Hz). ^{13}C NMR spectrum ($CDCl_3$ containing a drop of CD_3OD), δ_C, ppm: 14.0 ($\underline{C}H_3CH_2$), 18.5 d ($\underline{C}H_3CH$, $^3J_{PC}$ = 10.7 Hz), 32.9 d (CH_2P, $^1J_{PC}$ = 94.7 Hz), 33.6 ($\underline{C}HCO$), 61.0 (CH_2O), 174.9 d (C=O, $^3J_{PC}$ = 8.8 Hz). ^{31}P NMR spectrum ($CDCl_3$ containing a drop of CD_3OD): δ_P 34.7 ppm. Found, %: C 39.67; 39.52; H 7.57; 7.46. $C_6H_{13}O_4P$. Calculated, %: C 40.01; H 7.27.

***N*,*N*′-(Ethane-1,2-diyl)bis(benzyl carbamate) (4)** was synthesized by reaction of acetaldehyde diethyl acetal with 2 equiv of benzyl carbamate in acetic anhydride according to the procedure described in [14]. Yield 77%, mp 206–207°C; published data [14]: mp 203–204°C. ^{1}H NMR spectrum (DMSO-d_6), δ,

ppm: 1.23 d (3H, CH_3, $^3J_{HH}$ = 6.6 Hz), 5.00 br.s (4H, CH_2O), 5.14 m (1H, CHN), 7.34 m (10H, Ph), 7.61 d (2H, NH, $^3J_{HH}$ = 5.0 Hz). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 21.2 (CH_3), 56.1 (CHN), 65.2 (CH_2O); 127.6, 127.7, 127.8, 128.4, 137.1 (C_{arom}); 154.6 (C=O).

{1-[(Benzyloxycarbonyl)amino]ethyl}(3-ethoxy-2-methyl-3-oxopropyl)phosphinic acid (2). *a*. A mixture of 3.6 g (20 mmol) of preliminarily dried phosphonous acid **3** and 3.0 g (20 mmol) of benzyl carbamate in 25 mL of acetyl chloride was cooled to 5°C, and 1.4 mL (25 mmol) of acetaldehyde was added. The mixture spontaneously warmed up and was stirred until it cooled down and then for several hours at room temperature. When the reaction was complete (according to the ^{31}P NMR data), the mixture was poured into 70 mL of an ice–water mixture and evaporated under reduced pressure. The residue was treated with 70 mL of a saturated solution of sodium hydrogen carbonate and extracted with diethyl ether (2×10 mL). The aqueous phase was carefully acidified to pH ~2 and extracted with chloroform (3×10 mL) or ethyl acetate. The organic extract was dried over magnesium sulfate and evaporated under reduced pressure. The residue crystallized either spontaneously or after treatment with diethyl ether or petroleum ether (40–70°C). Yield 4.4 g (62%), mp 112–115°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm : 1.22 t (3H, CH_3, $^3J_{HH}$ = 7.5 Hz), 1.24 d (3H, CH_3, $^3J_{HH}$ = 7.0 Hz), 1.34 d.d (3H, C<u>H</u>$_3$CH, $^3J_{HH}$ = 7.5, $^3J_{PH}$ = 14.9 Hz), 1.76 m and 2.24 m (1H each, PCH_2), 2.85 m [1H, CHC(O)], 4.02 m (1H, CHN), 4.11 q (2H, CH_2O, $^3J_{HH}$ = 7.5 Hz), 5.11 br.s (2H, OC<u>H</u>$_2$Ph), 5.38 d (1H, NH, $^3J_{HH}$ = 7.5 Hz), 7.33 m (5H, Ph), 9.72 br.s (1H, POH). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 13.9 d ($^3J_{PC}$ = 13.8 Hz), 18.6 d ($^2J_{PC}$ = 7.0 Hz), 18.8* d ($^2J_{PC}$ = 8.1 Hz), 29.3 d ($^1J_{PC}$ = 88.6 Hz), 29.4* d ($^1J_{PC}$ = 88.2 Hz), 33.4, 45.7 d ($^1J_{PC}$ = 105.8 Hz), 46.0* d ($^1J_{PC}$ = 105.8 Hz), 60.1, 65.6, 127.7 (2C), 127.8, 128.4 (2C), 137.1, 155.8 d ($^3J_{PC}$ = 3.7 Hz), 175.1 d ($^3J_{PC}$ = 9.5 Hz). ^{31}P NMR spectrum ($CDCl_3$), δ_P, ppm: 53.6,* 55.0. Hereinafter, signals of the minor diastereoisomer are marked with an asterisk. Found, %: C 53.68, 53.57; H 6.79, 6.87; P 8.53, 8.43. $C_{16}H_{24}NO_6P$. Calculated, %: C 53.78; H 6.77; P 8.67.

b. *p*-Toluenesulfonic acid, 0.03 g (0.2 mmol), was added with stirring at room temperature to a mixture of 1.8 g (10 mmol) of acid **3** and 1.5 g (10 mmol) of benzyl carbamate in 15 mL of acetic anhydride, 0.7 mL (12.5 mmol) of preliminarily cooled acetaldehyde was then added in portions, and the mixture was stirred at room temperature. The progress of the reaction was monitored by ^{31}P NMR. The product was isolated as described above in *a*. Yield 2.5 g (70%), mp 117–118°C.

c. Preliminarily cooled acetaldehyde, 0.7 mL (12.5 mmol), was added in portions to a mixture of 1.8 g (10 mmol) of anhydrous phosphonous acid **3** and 1.5 g (10 mmol) of benzyl carbamate in 12 mL of acetic anhydride under stirring at room temperature. Cold acetyl chloride, 4 mL, was then added, and the mixture was stirred at room temperature until the reaction was complete (^{31}P NMR). The product was isolated as described above in *a*. Yield 2.0 g (56%), mp 113–115°C.

d. Trifluoroacetic anhydride, 2.1 g (10 mmol), was added with stirring to a cold solution of 1.8 g (10 mmol) of preliminarily dried phosphonous acid **3** and 3.3 g (10 mmol) of *N*,*N*′-(ethane-1,1-diyl)bis(benzyl carbamate) (**4**) in 15 mL in of anhydrous methylene chloride. The mixture was stirred at room temperature until the reaction was complete (^{31}P) and evaporated under reduced pressure, and the residue was treated with a mixture of 30 mL of a saturated solution of sodium hydrogen carbonate and 30 mL of diethyl ether. The resulting two-phase system was filtered from benzyl carbamate, the aqueous phase was carefully acidified to pH ~1–2 and extracted with chloroform and/or ethyl acetate (3×30 mL), and the extracts were combined with the organic phase, dried over magnesium sulfate, and evaporated under reduced pressure. The residue crystallized spontaneously or after treatment with diethyl ether. Yield 1.6 g (45%), mp 114–115°C.

e. *p*-Toluenesulfonic acid, 0.03 g (0.2 mmol), was added with stirring to a mixture of 1.8 g (10 mmol) of anhydrous phosphonous acid **3** and 3.3 g (10 mmol) of bis-carbamate **4** in 20 mL of acetic anhydride. The mixture was stirred at room temperature until the reaction was complete (^{31}P NMR) and evaporated under reduced pressure, and the residue was treated as described above in *d*. Yield 2.8 g (78%), mp 116–118°C.

f. A solution of 1.8 g (10 mmol) of phosphonous acid **3** and 1.5 g (10 mmol) of benzyl carbamate in a mixture of 15 mL of acetic anhydride and 5 mL of acetyl chloride was cooled to 5°C, and 1.6 mL (11 mmol) of acetaldehyde diethyl acetal was slowly added dropwise. The mixture was stirred at room temperature until the reaction was complete (^{31}P NMR) and was then treated as described above in *a*. Yield 1.1 g (31%), mp 108–111°C.

2-(Hydroxycarbonyl)propyl-1-aminoethylphosphinic acid (1, pseudoalanylalanine) was syn-

thesized by hydrolysis of acid **2** in 6 N HCl under reflux. The product was isolated by chromatography on a cation exchanger (using water and 0.3 N aqueous HCl as eluents), followed by crystallization from acetone. Yield 68%, mp 127–134°C (foaming with liberation of water), 218–221°C (decomp.). ^{1}H NMR spectrum (D_2O), δ, ppm: 1.20 d (3H, C<u>H</u>$_3$CHC, $^3J_{HH}$ = 7.3 Hz), 1.32 d.d (3H, C<u>H</u>$_3$CHP, $^3J_{PH}$ = 13.4, $^3J_{HH}$ = 7.3 Hz), 1.50–1.75 m and 1.92–2.14 m (1H each, PCH_2), 2.65–2.85 m [1H, CHC(O)], 3.12–3.28 m (1H, CHN). ^{13}C NMR spectrum (D_2O), δ_C, ppm: 11.9 d ($^3J_{PC}$ = 8.1 Hz), 18.0 d ($^2J_{PC}$ = 5.9 Hz), 18.2* d ($^2J_{PC}$ = 7.3 Hz), 29.7* d ($^1J_{PC}$ = 94.4 Hz), 29.8 d ($^1J_{PC}$ = 95.1 Hz), 33.3 d and 45.4* d ($^1J_{PC}$ = 93.6 Hz), 45.5 d ($^1J_{PC}$ = 95.1 Hz), 179.5 d ($^3J_{PC}$ 6.6 Hz), 180.5* d ($^3J_{PC}$ = 9.5 Hz). ^{31}P NMR spectrum: in D_2O: δ_P 35.2 ppm; in D_2O/DCl: δ_P 45.1 ppm Found, %: C 28.65, 28.56; H 7.02, 7.11; P 11.94, 12.13. $C_6H_{14}NO_4P \cdot HCl \cdot H_2O$. Calculated, %: C 28.87; H 6.86; P 12.41.

The ^{1}H, ^{31}P, and ^{13}C NMR spectra were recorded on a Bruker DPX-200 spectrometer with Fourier transform. Ion exchange chromatography was performed using KUICT (H^+) cation exchanger. The melting points were measured on a Boetius PHMK melting point apparatus.

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