ISSN 1070-3632, Russian Journal of General Chemistry, 2020, Vol. 90, No. 4, pp. 753–756. © Pleiades Publishing, Ltd., 2020.
Russian Text © The Author(s), 2020, published in Zhurnal Obshchei Khimii, 2020, Vol. 90, No. 4, pp. 645–649.

LETTERS TO THE EDITOR

# Synthesis of Phosphinic Analogue of Alanylleucine

**S. R. Golovash[a], O. S. Grigorkevich[b], G. S. Tsebrikova[c], M. E. Dmitriev[a], and V. V. Ragulin[a,*]**

[a] *Institute of Physiologically Active Substances of the Russian Academy of Sciences, Chernogolovka, 142432 Russia*
[b] *V.V. Zakusov Research Institute of Pharmacology of the Russian Academy of Sciences, Moscow, 125315 Russia*
[c] *A.N. Frumkin Institute of Physical Chemistry and Electrochemistry of the Russian Academy of Sciences, Moscow, 119071 Russia*
**e-mail: rvalery@dio.ru*

Received September 27, 2019; revised September 27, 2019; accepted October 5, 2019

**Abstract**—The amidoalkylation reactions of a phosphonous acid containing a structural isostere of leucine in acetyl chloride and(or) acetic anhydride were studied under conditions of acid catalysis. A two-component method for the synthesis of a phosphinic analogue of alanylleucine using ethylidenebis(benzylcarbamate) was proposed.

**Keywords**: pseudo-alanylleucine, matrix metalloproteinases (MMPs), zinc metalloproteinase inhibitors, amidoalkylation, phosphinic structural isosteres

**DOI:** 10.1134/S1070363220040313

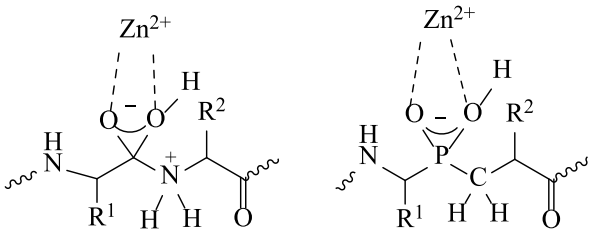
Phosphinic structural analogues of natural peptides are included in promising radiopharmaceuticals as ligand components [1]. They are also inhibitors of matrix metalloproteinases (MMPs), which are a family of zinc-containing endoproteases involved into various biological processes [2, 3]. Metalloproteinases of types 2 and 9 (MMP-2 and MMP-9), called gelatinases, play an important role in post-infarction myocardial remodeling [4]. Undesirable activity of gelatinases, which manifests itself in a negative effect on the tissues of the cardiovascular system, can be reduced or eliminated using inhibitors containing function that chelates zinc cation [5, 6]. Effective inhibitors of zinc-metalloproteinases are phosphinic pseudopeptides, structural isosteres of peptides in the molecule of which two amino acid components of the dipeptide are linked by a zinc chelating phosphinic fragment [2, 7]. In this case, methylenephosphorylic $CH_2P(O)(OH)$ fragment imitates a natural peptide bond with a tetra-coordinated carbon atom in the transition state of peptide hydrolysis (Scheme 1).

A widely used approach to the phosphinic modification of the peptide bond is the synthesis of the *N*,*P*-protected derivative **1**, the phosphorous isoster of the corresponding amino acid, developed by Ciba-Geigy company [8, 9] with its subsequent conversion to the silylic ether of the $P^{III}$-phosphonous analog of the amino acid and addition to corresponding α-substituted acrylates **2** (Scheme 2) [2, 7].

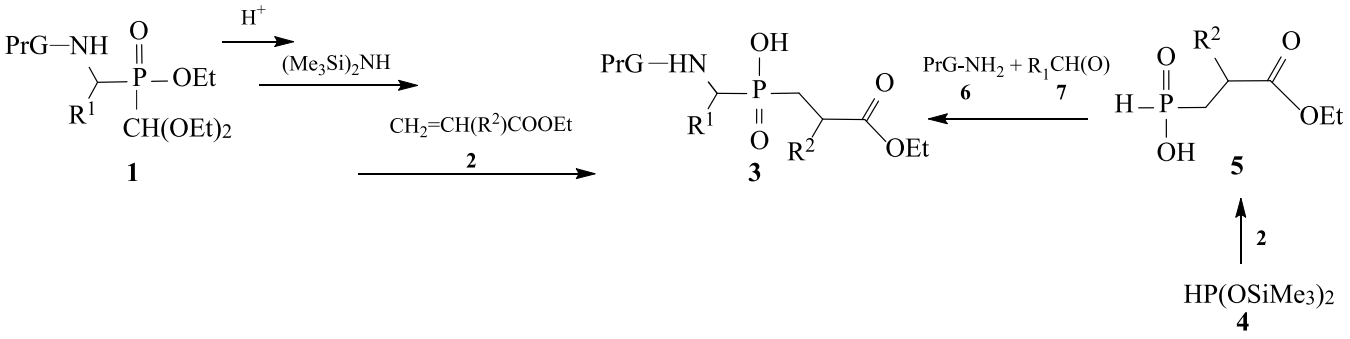
We have proposed a shorter synthesis with the reverse construction of the desired pseudopeptide **3** molecule. It consists in the initial addition of the in situ generated hypophosphite **4** to the corresponding α-$R^2$-substituted acrylates **2** with the formation of phosphonous acids **5** containing a structural isostere fragment of the corresponding amino acid, which is determined by the corresponding substituent ($R^2$) in the α-position of the acrylic system [10–13]. Amidoalkylation of phosphonous acids **5** using alkyl carbamates **6** and the corresponding carbonyl compounds **7** leads to target pseudopeptides **3** (Scheme 2) [11–13].

This work is a further development of the previously proposed methodology for the synthesis of phosphoisosteres of peptides from hypophosphites [10–13] and is devoted to the synthesis of pseudoalanyl-ψ[$P(O)(OH)CH_2$]-leucine **8**, which is a promising type

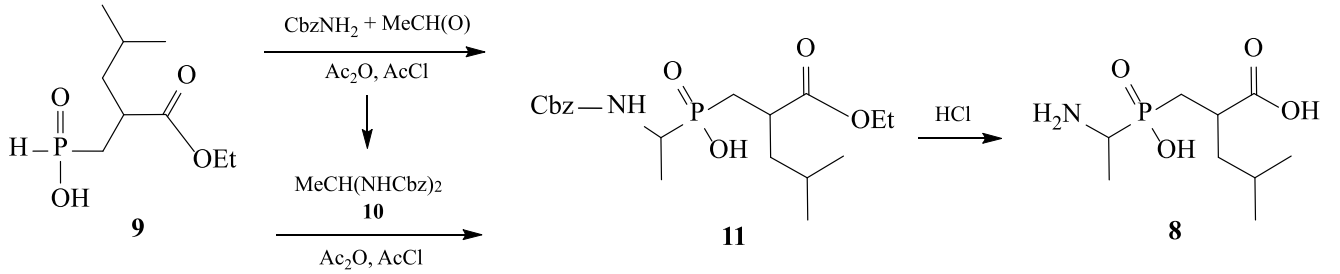
**Scheme 1.**



**Scheme 2.**




**Scheme 3.**




9 matrix-zinc metalloproteinase (MMP-9) inhibitor [14] (Scheme 3).

The process of amidoalkylation of phosphonous acid **9** containing structural leucine isostere was performed using benzyl carbamate and acetic aldehyde in acetyl chloride and (or) acetic anhydride as a three-component carbamate version of the Kabachnik–Fields reaction [11–13] for the synthesis of phosphinic acid **11**, which is ethyl ester of *N*-protected phosphinic pseudoalanylleucine *N*-Cbz-Ala-ψ[P(O)(OH)$CH_2$]-Leu-OEt. As an alternative method, a two-component synthesis was performed by reaction of the phosphonous component **9** with pre-synthesized *N*,*N*'-ethylidenebis(benzylcarbamate) **10** [12].

The reaction progress was monitored using $^{31}$P NMR spectroscopy by the ratio of the intensities of the signals of phosphinic acid **11** ($\delta_P$ ~55 ppm) and the starting phosphonous component **9** ($\delta_P$ ~32–33 ppm) in the spectrum of the reaction mixture. It was found that the process of amidoalkylation of phosphonous acid **9** proceeds rather quickly in acetyl chloride or in a mixture with acetic anhydride. However, in this case, side processes occur, associated with partial dealkylation of the ester fragment of acid **11** during the amidoalkylation of phosphonous acid **9**. Side reactions with the participation of acetaldehyde and the formation of phosphorus-free compounds are also observed. This leads to the fact that phosphonous component **9** is partially remains unchanged in the reaction mixture.

In this regard, we have studied the amidoalkylation of acid **9** in acetic anhydride under milder acid catalysis using *p*-toluenesulfonic acid. The side process of dealkylation of ester fragments in this case is absent, however, the amidoalkylation reaction proceeds noticeably slower. A high yield of target acid **11** was achieved using a two-component version of amidoalkylation using ethylidenebis(benzylcarbamate) **10**, which combines carbonyl and carbamate components in the molecule, since this avoids side processes involving acetaldehyde.

Ethylidenebis(benzylcarbamate) **10** was previously synthesized in accordance with our previously proposed procedure [12]. The synthesis of phosphonous acid **9** was carried out in accordance with the modified method proposed by us earlier [11]. In this case, it was also possible to isolate the product of double addition, phosphinic acid **9a**. Synthesis of ethyl ester of α-isobutylacrylic acid was carried out in accordance with a previously published procedure [15].

**2-(Ethoxycarbonyl)-4-methylamylphosphonic acid (9).** Yield 81%, oily substance. $^1$H NMR spectrum ($CCl_4$–$CD_3OD$), δ, ppm: 0.97–1.05 br. t (3H, $CH_3$), 1.35 d (6H, $CH_3$, $^3J_{HH}$ = 7.0 Hz), 1.48 m (1H, CH), 1.65 m (2H, C$\underline{H_2}$CH), 2.10 m (2H, $CH_2$P), 2.83 m [1H, CHC(O)], 4.17 m (2H, $CH_2O$), 7.05 d (1H, PH, $^1J_{PH}$ = 560.0 Hz), 12.68 br. s (1H, POOH). $^{31}$P NMR spectrum ($CCl_4$–$CD_3OD$): $\delta_P$ 31.1 ppm. Mass spectrum (ESI), *m*/*z*: 223.4

$[M + H]^+$ (caculated for $C_9H_{19}O_4P$: 223.2). Found P, %: 13.63, 13.43. $C_9H_{19}O_4P$. Calculated P, %: 13.94.

**Bis[2-(ethoxycarbonyl)-4-methylamyl]phosphinic acid (9a)** was obtained as a pale yellow oily substance. $^1$H NMR spectrum ($CCl_4$–$CD_3OD$), δ, ppm: 0.85–0.95 m (6H, $CH_3$), 1.25–1.35 m (12H, $CH_3$), 1.43 m (2H, CH), 1.60 m (4H, C<u>H</u>$_2$CH), 2.05 m (4H, $CH_2P$), 2.76 m [2H, 2CHC(O)], 4.12 m (4H, $CH_2O$). $^{31}$P NMR spectrum ($CCl_4$–$CD_3OD$): $\delta_P$ 47.1 ppm. Found, %: C 56.95, 57.05; H 9.45, 9.50; P 8.33, 8.21. $C_{18}H_{35}O_6P$. Calculated, % C: 57.13; H 9.32; P 8.18.

**2-(Ethyloxycarbonyl)-4-methylamyl-1-(*N*-benzyloxycarbonyl)aminoethylphosphinic acid (11).** *a*. To a mixture of 4.4 g (20 mmol) of pre-dried phosphonous acid **9** and 3.0 g (20 mmol) of benzyl carbamate in 25 mL of acetyl chloride, cooled to 5°C, was added in portions 1.4 mL (25 mmol) of acetaldehyde. The resulting mixture was stirred at room temperature for several hours, then poured into 70 mL of ice water and evaporated in vacuum at a bath temperature of not higher than 35°C. The residue was treated with 70 mL of saturated sodium bicarbonate solution. The aqueous phase was washed with diethyl ether (2 × 10 mL), carefully acidified with a solution of 1 N HCl to pH ~ 2 and then extracted with chloroform (3 × 10 mL) or ethyl acetate. The combined organic extract was dried with magnesium sulfate and evaporated in vacuum. The residue was chromatographed on silica gel [eluent—chloroform, chloroform–isopropanol (5–7%)]. The eluate was evaporated in vacuum, the residue crystallized spontaneously, and also after treatment with diethyl or petroleum ether (40/70). Phosphinic acid **11** was isolated after additional crystallization from petroleum ether. Yield 4.1 g (51%), mp. 74–77°C.

*b*. Amidoalkylation of phosphonous acid **9** in a mixture of acetyl chloride and acetic anhydride allowed to isolate the target product **11** with a yield of 58%, mp 77–78°C.

*c*. The synthesis in the medium of acetic anhydride with the addition of *p*-toluenesulfonic acid (3 mol %) made it possible to obtain acid **11** with a yield of 71%, mp 78–80°C.

The two-component synthesis was carried out using pre-synthesized *N*,*N*'-ethylidenebis(benzylcarbamate) **10** [12] also using *p*-toluenesulfonic acid (3 mol %). To a stirred mixture of 2.2 g (10 mmol) of anhydrous phosphonous acid **9** and 3.3 g (10 mmol) of *N*,*N*'-ethylidenebis(benzylcarbamate) **10** in 20 mL of acetic anhydride was added 0.05 g (0.3 mmol) of *p*-toluenesulfonic acid. The reaction mixture was stirred at room temperature, monitoring the reaction progress by $^{31}$P NMR. Upon completion of the reaction, the mixture was filtered, the filtrate was treated similarly to procedure *a*. Yield 3.3 g (83%), mp 80–81°C, $R_f$ ~ 0.20–0.35 ($CHCl_3$ : EtOH = 5 : 1) (two spots due to the presence of diastereomers). $^1$H NMR spectrum ($CDCl_3$), δ, ppm: 0.84 d (3H, $CH_3$, $^3J_{HH}$ = 6.4 Hz), 0.88 d (3H, $CH_3$, $^3J_{HH}$ = 7.5 Hz), 1.21 t (3H, $CH_3$, $^3J_{HH}$ = 7.0 Hz), 1.34 d. d (3H, C<u>H</u>$_3$CH, $^3J_{HH}$ = 6.5, $^3J_{PH}$ = 14.5 Hz), 1.45–1.80 m (4H, $PCH_2$ + CHC<u>H</u>$_2$CH), 2.00–2.27 m (1H, C<u>H</u>CH$_3$), 2.70–2.92 m [1H, CHC(O)], 3.90–4.05 m (1H, PCHN), 4.11 q (2H, $CH_2O$, $^3J_{HH}$ = 7.0 Hz), 5.10 br. s (2H, OC<u>H</u>$_2$Ph), 5.38 br. d (1H, NH, $^3J_{HH}$ = 8.6 Hz), 6.50 br. s (1H, POOH), 7.33 s (5H, Ph). $^{13}$C NMR spectrum ($CDCl_3$), $\delta_C$, ppm: 13.84* (hereafter, an asterisk indicates the signal of a minor diastereomer), 14.05, 20.88*, 21.84, 22.32*, 22.67, 25.06*, 25.73, 28.67 d ($^1J_{PC}$ = 95.1 Hz), 28.96* d ($^1J_{PC}$ = 91.6 Hz), 37.12, 43.17* d ($^3J_{PC}$ = 8.8 Hz), 43.37 d ($^3J_{PC}$ = 11.1 Hz), 45.10* d ($^1J_{PC}$ = 103.5 Hz), 45.85 d ($^1J_{PC}$ = 104.3 Hz), 60.71, 67.04, 127.97, 128.09, 128.44, 136.18, 155.86 d ($^3J_{PC}$ = 5.0 Hz), 175.1. $^{31}$P NMR spectrum ($CDCl_3$), $\delta_P$, ppm: 54.7*, 55.0. Mass spectrum (ESI), *m*/*z*: 400.5 $[M + H]^+$ (caculated for $C_{19}H_{30}NO_6P$: 400.4). Found, %: C 56.68, 56.92; H 7.78, 7.87. $C_{19}H_{30}NO_6P$. Calculated, %: C 57.13; H 7.57.

**2-(Hydroxycarbonyl)-4-methylamyl-1-aminoethylphosphinic acid (pseudo-alanylleucine) (8).** Phosphinic acid **11** (1.1 g, 2.8 mmol) was boiled in 15 mL of 6 N HCl for 17 h. The cooled solution was treated with chloroform (2 × 5 mL). The aqueous phase was evaporated in vacuum and the residue was chromatographed on cation exchange resin (eluent—water, then 0.5 N HCl). The ninhydrin-positive eluate was evaporated in vacuum. The residue (~ 0.6 g) was dissolved in 5.5 mL of aqueous alcohol (~ 1 : 10), treated with 0.5 mL (7 mmol) of propylene oxide, and 0.4 g (62%) of aminophosphinic acid **8** was isolated. Amino acid **8** is a crystalline powder, sparingly soluble in water; therefore, the spectral data are given below in an acidic and alkaline medium; in the latter case, the presence of diastereomers manifests to a greater extent. Yield 62%, mp 197–199°C (decomp.). $^1$H NMR spectrum ($D_2O$–DCl, pH = 1), δ, ppm: 0.48 d (3H, C<u>H</u>$_3$CHC, $^3J_{HH}$ = 5.9 Hz), 0.52 d (3H, C<u>H</u>$_3$CHC, $^3J_{HH}$ = 6.5 Hz), 1.08 d. d (3H, C<u>H</u>$_3$CHP, $^3J_{PH}$ = 14.7, $^3J_{HH}$ = 7.1 Hz), 1.14–1.32 m [3H, <u>CH$_2$CH</u>(CH$_3$)$_2$], 1.56–1.74 m (1H, $PCH_2$), 1.78–1.98 m (1H, $PCH_2$), 2.35–2.57 m [1H, CHC(O)], 3.10–3.30 m (1H, CHN). $^1$H NMR spectrum

($D_2O$–NaOD, pH ~9), δ, ppm: 0.78 d (3H, C<u>H</u>$_3$CHC, $^3J_{HH}$ = 5.9 Hz), 0.82 d (3H, C<u>H</u>$_3$CHC, $^3J_{HH}$ = 5.3 Hz), 1.12 d. d (3H, C<u>H</u>$_3$CHP, $^3J_{PH}$ = 14.7, $^3J_{HH}$ = 7.1 Hz), 1.14* d. d (3H, C<u>H</u>$_3$CHP, $^3J_{PH}$ = 13.5, $^3J_{HH}$ = 7.1 Hz), 1.25–1.60 m [4H, $PCH_2$ + <u>$CH_2$</u><u>CH</u>$(CH_3)_2$], 1.71–1.99 m (1H, $PCH_2$), 2.42–2.60 m [1H, CHC(O)], 2.68–2.87 m (1H, CHN). $^{13}$C NMR spectrum ($D_2O$–DCl, pH = 1), $\delta_C$, ppm: 12.24*, 12.30, 21.29, 21.86*, 21.93, 25.3, 28.50* d ($^1J_{PC}$ = 92.7 Hz), 28.64 d ($^1J_{PC}$ = 93.1 Hz), 37.20 d ($^2J_{PC}$ = 4.8 Hz), 42.61 d ($^3J_{PC}$ = 11.6 Hz), 45.28* d ($^1J_{PC}$ = 97.0 Hz), 45.35 d ($^1J_{PC}$ = 96.6 Hz), 179.44 d ($^3J_{PC}$ = 5.2 Hz). $^{13}$C NMR spectrum ($D_2O$–NaOD, pH ~9), $\delta_C$, ppm: 13.18*, 14.24, 21.41, 21.60*, 22.62*, 22.86, 25.94*, 26.10, 31.16* d ($^1J_{PC}$ = 90.9 Hz), 31.56 d ($^1J_{PC}$ = 90.9 Hz), 41.42 d ($^2J_{PC}$ = 2.7 Hz), 43.74 d ($^3J_{PC}$ = 7.7 Hz), 43.91 d ($^3J_{PC}$ = 9.6 Hz), 45.66* d ($^1J_{PC}$ = 93.2 Hz), 46.16 d ($^1J_{PC}$ = 92.8 Hz), 184.92 d ($^3J_{PC}$ = 8.4 Hz), 185.02* d ($^3J_{PC}$ = 6.5 Hz). $^{31}$P NMR spectrum ($D_2O$–DCl, pH = 1): $\delta_P$ 44.3 ppm. $^{31}$P NMR spectrum ($D_2O$–NaOD, pH ~9), $\delta_P$, ppm: 43.1, 43.5*. Found,%: C 45.35, 45.26; H 9.02, 9.17; N 6.04, 6.13. $C_9H_{20}NO_4P$. Calculated, %: C 45.57; H 8.50; N 5.90.

$^1$H, $^{31}$P, and $^{13}$C NMR spectra were recorded on a Bruker DPX-200 Fourier spectrometer. For ion exchange chromatography, Purolite C100E ($H^+$) cation exchanger was used. Melting points were determined in a block in an open capillary. Chromatographic analysis was performed on an Agilent 1100 Series LC/MSD system equipped with DAD, ELSD and a single quadrupole mass-selective electrospray ionization detector.

## FUNDING

This work was performed as part of the governmental task of the Institute of Physiologically Active Substances of the Russian Academy of Sciences for 2019 (topic no. 00902019-008) with partial financial support from the Presidium of the Russian Academy of Sciences no. 14P, the Russian Foundation for Basic Research (grants no. 18-03-00959, 18-03-01123) and the Russian Science Foundation (grant no. 19-13-00294, chromatographic, spectral and elemental analysis).

## CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

## REFERENCES

1. Notni, J., Simecek, J., Hermann, P., and Wester, H.-J., *Chem. Eur. J.*, 2011, vol. 17, p. 14718. https://doi.org/10.1002/chem.201103503
2. Mucha, A., *Molecules*, 2012, vol. 17, p. 13530. https://doi.org/10.3390/molecules171113530
3. *Zinc Metalloproteases in Health and Disease*, Hooper, N.M., Ed., London: Taylor and Francis, 1996, p. 153.
4. Hori, M. and Nishida, K., *Cardiovasc. Res*., 2009, vol. 81, no. 3, p. 457. https://doi.org/10.1093/cvr/cvn335
5. Whittaker, M. and Ayscough, A., *Celltransmissions (Sigma Aldrich Inc.)*, 2001, vol. 17, no. 1, p. 3.
6. Pirad, B. and Matter, H., *J. Med. Chem*., 2006, vol. 49, no. 1, p. 51. https://doi.org/10.1021/jm050363f
7. Georgiadis, D. and Dive, V., *Top. Curr. Chem*., 2015, vol. 360, p. 1. https://doi.org/10.1007/128_2014_571
8. Baylis, E.K., Campbell, C.D., and Dingwall, J.G., *J. Chem. Soc. Perkin Trans. 1*, 1984, p. 2845. https://doi.org/10.1039/P19840002845
9. McCleery, P.P. and Tuck, B., *J. Chem. Soc. Perkin Trans. 1*, 1989, p. 1319. https://doi.org/10.1039/P19890001319
10. Rozhko, L.F. and Ragulin, V.V., *Amino Acids*, 2005, vol. 29, no. 2, p. 139. https://doi.org/10.1007/s00726-005-0194-9
11. Dmitriev, M.E. and Ragulin, V.V., *Tetrahedron Lett*., 2010, vol. 51, no. 19, p. 2613. https://doi.org/10.1016/j.tetlet.2010.03.020
12. Dmitriev, M.E., Rossinets, E.A., and Ragulin, V.V., *Russ. J. Gen. Chem*., 2011, vol. 81, no. 6, p. 1092. https://doi.org/10.1134/S1070363211060041
13. Dmitriev, M.E. and Ragulin, V.V., *Tetrahedron Lett*., 2012, vol. 53, no. 13, p. 1634. https://doi.org/10.1016/j.tetlet.2012.01.094
14. Eckhard, U., Huesgen, P.F., Schilling, O., Bellac, C.L., Butler, G.S., Cox, J.H., Dufour, A., Goebeler, V., Kappelhoff, R., Keller, U., Klein, T., Lange, P.F., Marino, G., Morrison, C.J., Prudova, A., Rodriguez, D., Starr, A.E., Wang, Y., and Overall, C.M., *Matrix Biol*., 2016, vol. 49, p. 37. https://doi.org/10.1016/j.matbio.2015.09.003
15. Stetter, H. and Kuhlmann, H., *Synthesis*, 1979, no. 1, p. 29. https://doi.org/10.1055/s-1979-28537