Design of phosphonate analogs of short peptides by "click" chemistry*

*O. I. Artyushin,[a] E. V. Sharova,[a] A. N. Yarkevich,[b] G. K. Genkina,[a] N. V. Vinogradova,[a] and V. K. Brel[a]**

[a]*A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 28 ul. Vavilova, 119991 Moscow, Russian Federation. E-mail: brel@ipac.ac.ru*

[b]*Institute of Physiologically Active Compounds, Russian Academy of Sciences, 1 Severnyi proezd, 142432 Chernogolovka, Moscow Region, Russian Federation*

Methods were developed for the first time for the modification of the natural neuropeptides Ile—Gly—Leu and Leu—Gly—Leu simultaneously with the phosphonate moiety and the triazole ring or solely with the triazole ring by means of click chemistry. All of the peptidomimetics synthesized were isolated as a mixture of diastereomers and were characterized by spectroscopic methods.

Key words: peptidomimetics, peptides, "click" chemistry, phosphonates, nucleotides.

In recent years, the synthesis and applications of short peptides and their bioisosteres have attracted growing interest due primarily to utilization of these compounds as signaling molecules essential for regulatory processes in the body. In particular, it was found that the tripeptide Gly—His—Lys exerts a modulatory effect on the cell growth and differentiation[1] and the liver cell regeneration[2] and has anti-inflammatory[3] and neurotrophic[4] activities. A fairly broad range of short neuropeptides was shown to have highly specific and targeted action as effective neuroprotective agents, stimulators of cognitive functions, and modulators of memory processes. The "memory tripeptide" (Arg—Glu—Arg), which is of interest for the treatment of Alzheimer´s disease,[5] is a potential inhibitor of the urokinase/urokinase receptor system[6] and has antithrombotic activity.[7] Attempts to synthesize structural analogs of "memory tripeptides" were described in the literature.[8] These compounds may be of interest as potential drugs for the treatment of neurodegenerative disorders.[9] The modification of tripeptides by introducing heteroorganic moieties and the triazole heterocycle will extend the range of potentially active peptidomimetics. It should be noted that the formation of the triazole ring in the reaction of alkynes with azides has been known since the middle of the twentieth century.[10] Meanwhile, this reaction performed in the presence of copper catalysts provides the basis for the highly efficient "click" chemistry, which is widely used for the design of new materials and biologically active compounds,[11] in particular peptidomimetics.[12]

The methods and approaches, which we have chosen for the design of bioisosteric neuropeptides, rely on the concept of bioisosterism.[13] This methodology implies the chemical modification of neuropeptides in order to improve their physicochemical properties with the simultaneous retention or improvement of biological and pharmacological properties. The aim of this study is to design phosphorylated tripeptidomimetics — analogs of the tripeptide Ile—Gly—Leu — based on triazole as the structural unit of the peptide chain by means of "click" chemistry. Monoethyl azidomethylphosphonate was used as the starting phosphorylated azide. Earlier, a procedure has been reported[14] for the synthesis of this ester from diethyl 1-hydroxymethylphosphonate and hydrogen azide acid *via* the Mitsunobu protocol.[15] We developed an alternative approach based on the use of diethyl chloromethylphosphonate **1** as the starting compound, which was synthesized by a procedure described earlier[16] (Scheme 1).

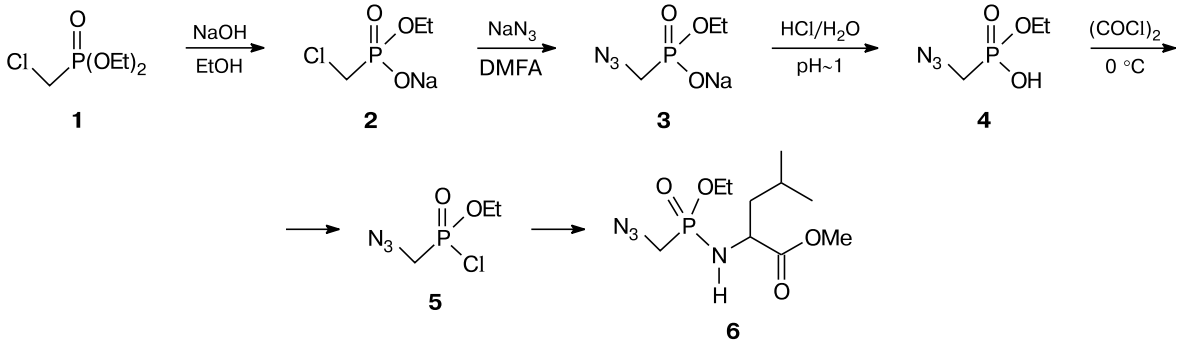
The characteristics of ester **4** were completely identical to the data published in the literature,[14] and this compound was then transformed into an acid chloride using oxalyl chloride as the chlorinating agent.[14] Azide unit **6** was synthesized using L-leucine hydrochloride in dichloromethane in the presence of triethylamine as the hydrogen chloride acceptor. Compound **6** was isolated by column chromatography, and its structure was established by NMR spectroscopy. Thus, the ^{1}H NMR spectra of compound **6** show characteristic proton signals of OCH_3 and OCH_2CH_3 groups at δ 3.72 and 3.73 (a mixture of diastereomers) and 3.95—4.20, respectively, and also proton signals of the PCH_2N_3 moiety that appear as a doublet with the chemical shifts of 3.50 and 3.51 ppm and the coupling constants $^2J_{H,H} = {}^2J_{H,P} = 12$ Hz. The ^{13}C NMR spectrum shows two doublets with the direct spin-spin coupling constant $^1J_{C,P} = 144.7$ Hz, which ultimately attests to the presence of the PCH_2N_3 group.

* Dedicated to Academician of the Russian Academy of Sciences N. S. Zefirov on the occasion of his 80th birthday.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 9, pp. 2172—2177, September, 2015.

1066-5285/15/6409-2172

Scheme 1



The second azide unit was synthesized according to Scheme 2. The key compound — azidoacetic acid chloride **9** — was prepared from bromoacetic acid by a procedure described earlier[17] (see Scheme 2).

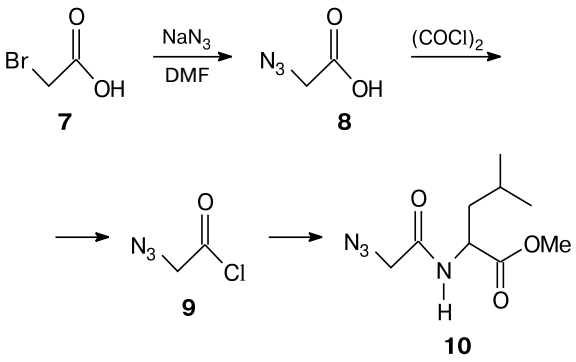
The condensation of acid chloride **9** with L-leucine methyl ester hydrochloride was performed by a known procedure.[18] Compound **10** was isolated by chromatography and characterized by ^{1}H and ^{13}C NMR spectra and elemental analysis. Apart from azide units, units containing acetylenic moieties are required for the assembly of the target tripeptides. For this purpose, we synthesized two propargyl esters — *N*-Boc-protected isoleucine[19] and *N*-Ac-protected leucine — by the alkylation of potassium salts of the appropriate amino acids with propargyl bromide[20] (Scheme 3).

The spectroscopic characteristics of acetylenic units **12** and **14** are in complete agreement with these structures. The most convincing evidence of these structures is the presence of the propargyl moiety, which appears in the ^{1}H NMR spectra as a signal of an acetylenic proton at δ 2.46 and proton signals of the OCH_2 group as a doublet of doublets at δ 4.64 and 4.65 with the coupling constants $^2J_{H,H}$ = 15.4 Hz and $^4J_{H,H}$ = 2.4 Hz. The ^{13}C NMR spectra show characteristic signals of carbon atoms of the triple bond at δ 75.04 and 79.50 and a signal of C atoms assigned to the OCH_2 group of different diastereomers at δ 52.

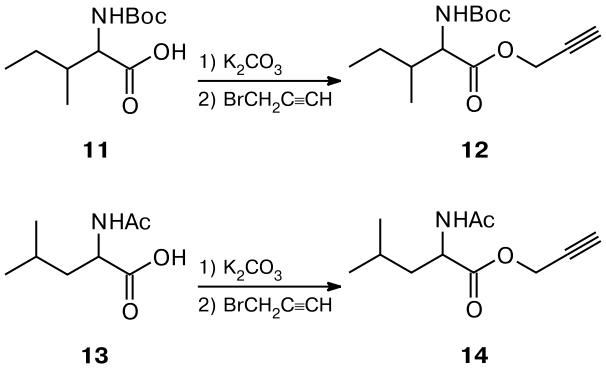
The final step of the synthesis of the phosphorylated peptidomimetic involves the condensation of azide unit **6** with propargyl esters of *N*-Boc-protected isoleucine **12** and *N*-Ac-protected leucine **14** (Schemes 4 and 5). It should be noted that the "click" chemistry is widely used for the design of peptidomimetics.[14] Compounds **15**, **17**, **19**, and **20** were synthesized by the "click" reaction of the acetylenic component with the azide one under the conditions described earlier.[21]

It is worthy of note that these reactions proceeded much more rapidly and were completed in 12 h at ~20 °C. Due to the presence of several asymmetric centers (primarily the asymmetric phosphorus atom) in the molecule, the resulting compounds exist as mixtures of diastereomers.

Scheme 2

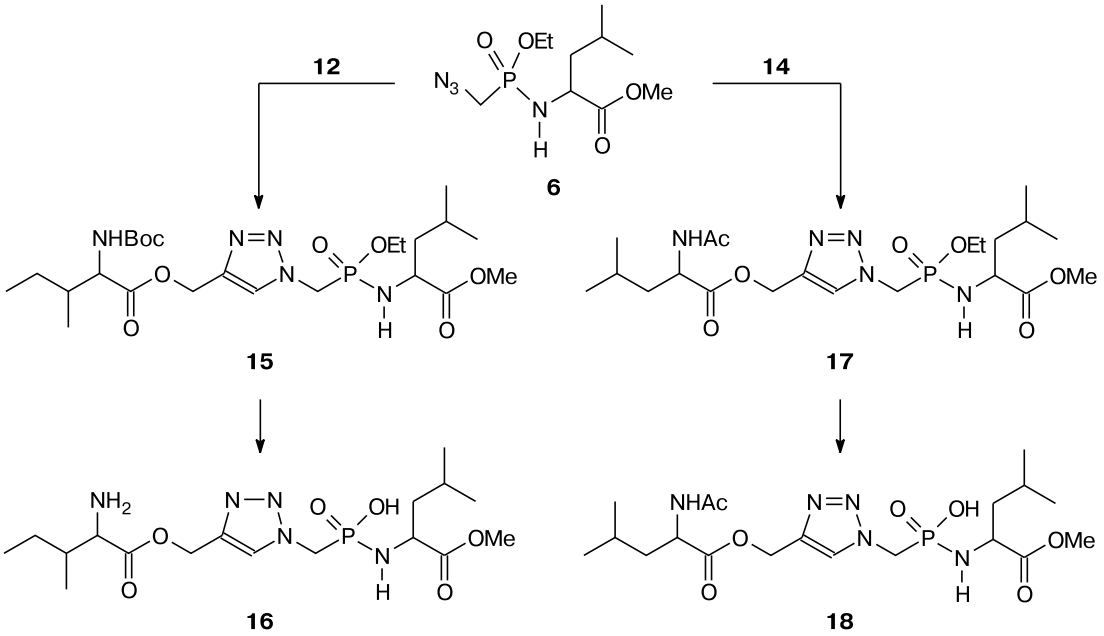


Scheme 3



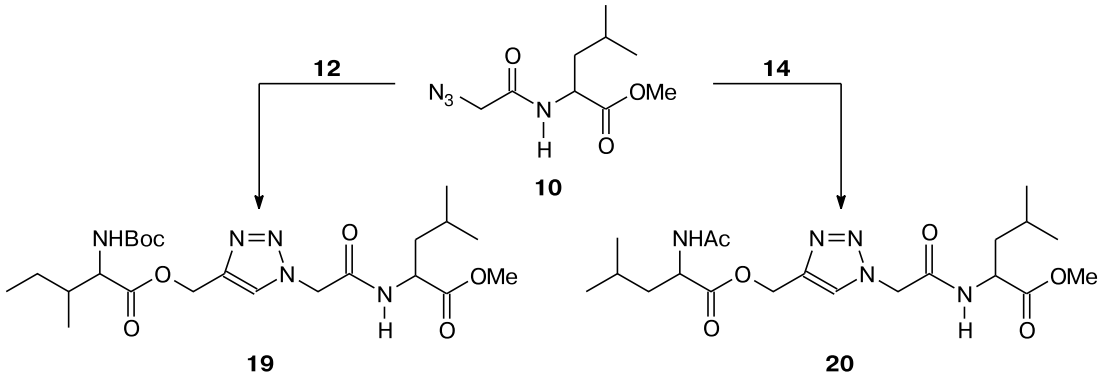
This interferes with the analysis of the spectroscopic characteristics of these compounds. However, the spectral pattern is substantially more simple in the absence of the asymmetric phosphorus atom (compounds **19** and **20**). The structures of compounds **15**, **17**, **19**, and **20** are confirmed by proton signals of the CH group in the ^{1}H NMR spectra and the signals for the carbon atoms of the double bond of the triazole ring in the ^{13}C NMR spectra at δ 7.8—8.0 and 124—146, respectively. In the spectra of compounds **15**, **17**, **19**, and **20**, the carbon signals of the triazole ring

Scheme 4



Reagents and conditions: $CuSO_4 \cdot 5H_2O$, Na ascorbate, Bu^tOH/H_2O.

Scheme 5



Reagents and conditions: $CuSO_4 \cdot 5H_2O$, Na ascorbate, Bu^tOH/H_2O.

are broadened, whereas all of the other signals have a usual shape. The absorption bands $\nu_{C\equiv C}$ at 2100—200 cm^{-1} characteristic of the starting acetylenic compounds **12** and **14** as well as intense absorption bands, which correspond to the azide group ν_{N3} at ~2100 cm^{-1} and are characteristic of the starting azides **6** and **10**, are absent in the IR spectra of compounds **15**, **17**, **19**, and **20**. Compounds **15** and **17** were transformed into free phosphonic acids **16** and **18** by the conventional treatment with Me_3SiBr. It should be noted that the Boc protecting group was unexpectedly removed from compound **16**. Apparently, this is due to the action of free HBr, which is always present as an impurity in Me_3SiBr. The spectra of compounds **16** and **18** are similar to those of **15** and **17**, except for the absence of the corresponding proton and carbon signals of the OC_2H_5 and Boc groups.

In summary, we developed for the first time methods for the modification of the natural neuropeptides Ile—Gly—Leu and Leu—Gly—Leu simultaneously with the phosphonate moiety and the triazole ring or solely with the latter by means of "click" chemistry.

Experimental

The NMR spectra were recorded on a Bruker AV-400 instrument in D_2O, $CDCl_3$, and CD_3OD using the signal of resid-

ual protons of the deuterated solvent as the internal standard (^{1}H, ^{13}C) and 85% H_3PO_4 as the external standard (^{31}P). The ^{13}C NMR spectra were measured using the JMODECHO mode; the signals of carbon atoms bearing odd and even numbers of protons have opposite polarities. The assignment of the signals in the NMR spectra was made based on the literature data.[20,21] The IR spectra were recorded on a Magna-IR 750 Fourier-transform infrared spectrometer (Nicolet), 2 cm^{-1} resolution, 128 scans (KBr pellets or a thin layer). The course of the reactions was monitored by TLC on Alumina TLC Plates w/UV254. The chromatographic purification was performed on Macherey-Nagel silica gel (MN Kieselgel 60, 70—230 mesh).

Propargyl esters **12** and **14** were prepared by a procedure described earlier.[20]

Sodium *O*-ethyl chloromethylphosphonate (2). A solution of NaOH (1.00 g, 0.025 mol) in water (4 mL) was added dropwise to a solution of diethyl chloromethylphosphonate (4.66 g, 0.025 mol) in anhydrous ethanol (20 mL) heated to boiling. The mixture was stirred at this temperature for 40 min. Then the solvent was removed *in vacuo*, the residue was dried over P_2O_5, and compound **2** was obtained in a yield of 4.50 g (100%). M.p. 182—183 °C (Pr^iOH). ^{31}P—{^{1}H} NMR (D_2O), δ: 16.5 (s). ^{1}H NMR (D_2O), δ: 1.24 (t, 3 H, $C\underline{H}_3CH_2OP$, $^3J_{H,H}$ = 7.1 Hz); 3.46 (d, 2 H, PCH_2Cl, $^2J_{H,P}$ = 12.0 Hz); 3.90—4.10 (m, 2 H, $CH_3C\underline{H}_2OP$).

Sodium *O*-ethyl azidomethylphosphonate (3). A suspension of compound **2** (6.32 g, 0.035 mol) and NaN_3 (2.73 g, 0.042 mol) in DMF (18 mL) was stirred for 5 h under slight reflux of the solvent. The precipitate of NaCl (1.96 g, 96%) was filtered off, the solvent was removed *in vacuo*, and the residue was washed with diethyl ether and then extracted with hot ethanol. The extract was concentrated, and the residue was dried over P_2O_5. The yield of ester **3** was 6.44 g (98%). ^{31}P—{^{1}H} NMR (D_2O), δ: 18.1 (s). ^{1}H NMR (D_2O), δ: 1.09 (t, 3 H, $C\underline{H}_3CH_2OP$, $^3J_{H,H}$ = = 7.1 Hz); 3.21 (d, 2 H, PCH_2N_3, $^2J_{H,P}$ = 11.0 Hz); 3.80—3.95 (m, 2 H, $CH_3C\underline{H}_2OP$).

***O*-Ethyl(azidomethyl)phosphonic acid (4).** A suspension of compound **3** (3.80 g, 0.02 mol) in CH_2Cl_2 (20 mL) was acidified with 20% HCl to pH 1, and then NaCl (1 g) was added. The organic layer was separated, the aqueous layer was twice extracted with CH_2Cl_2, the combined organic extracts were dried over Na_2SO_4, and the solvent was removed *in vacuo*. The yield of acid **4** was 2.59 g (78%), oil. ^{31}P—{^{1}H} NMR ($CDCl_3$), δ: 30.3 (s). ^{1}H NMR ($CDCl_3$), δ: 1.38 (t, 3 H, $C\underline{H}_3CH_2OP$, $^3J_{H,H}$ = 7.0 Hz); 3.51 (d, 2 H, PCH_2N_3, $^2J_{H,P}$ = 12.2 Hz); 3.90—4.20 (m, 2 H, $CH_3C\underline{H}_2OP$); 12.20 (br, 1 H, POH).

***O*-Ethyl(azidomethyl)phosphonic acid chloride (5).** A solution of oxalyl chloride (1.33 mL, 1.77 g, 0.014 mol) in CH_2Cl_2 (10 mL) was added with constant stirring at 0 °C to a solution of compound **4** (1.00 g, 0.006 mol) in CH_2Cl_2 (25 mL). The solution was stirred at this temperature for 1 h and then at 20 °C for 2 h. The solvent was removed *in vacuo*, and the residue was dried over P_2O_5. The yield of compound **5** was 2.57 g (100%). ^{31}P—{^{1}H} NMR ($CDCl_3$), δ: 33.20 (s). ^{1}H NMR ($CDCl_3$), δ: 1.46 (t, 3 H, $C\underline{H}_3CH_2OP$, $^3J_{H,H}$ = 7.1 Hz); 3.80—3.85 (m, 2 H, PCH_2N_3); 4.20—4.39 (m, 2 H, $CH_3C\underline{H}_2OP$).

***O*-Ethyl-1-azidomethylphosphonic acid *O*-methyl-L-leucinamide (6).** A solution of compound **5** (2.57 g, 0.014 mol) in CH_2Cl_2 (15 mL) was added on cooling (−2 °C) to a stirred mixture of leucine methyl ester hydrochloride (2.54 g, 0.014 mol) and Et_3N (5.83 mL, 4.25 g, 0.042 mol) in CH_2Cl_2 (40 mL) for 40 min. Then the mixture was stirred at this temperature for 1 h and allowed to stand at 20 °C for 12 h. The precipitate of $Et_3N \cdot HCl$ that formed was filtered off, and the organic layer was thrice washed with water and dried over Na_2SO_4. The solvent was removed *in vacuo*, and the residue was dried over P_2O_5. The yield of compound **6** was 3.40 g (83%), viscous oil. Found (%): C, 40.89; H, 7.18; N, 19.28; P, 10.90. $C_{10}H_{21}N_4O_4P$. Calculated (%): C, 41.09; H, 7.24; N, 19.17; P, 10.60. ^{1}H NMR ($CDCl_3$), δ: 0.92—0.96 (m, 6 H, CH_3, Pr^i); 1.34, 1.35 (both t, 3 H, $POCH_2C\underline{H}_3$, $^3J_{H,H}$ = 7.0 Hz); 1.45—1.65 (m, 2 H, $C\underline{H}_2CH(COOCH_3)$); 1.70—1.80 (m, 1 H, $CH_2C\underline{H}(CH_3)_2$); 3.16—3.39 (m, 1 H, $C\underline{H}NH$); 3.50, 3.51 (both dd, 2 H, PCH_2N_3, $^2J_{H,P} = {}^2J_{H,H}$ = 12.0 Hz); 3.72, 3.73 (both s, 3 H, OCH_3); 3.95—4.20 (m, 2 H, $POCH_2$). ^{13}C—{^{1}H} NMR ($CDCl_3$), δ: 16.20, 16.25 (both br.s, $POCH_2\underline{C}H_3$); 21.34, 21.50 (both s, CH_3, Pr^i); 22.61, 22.67 (both s, CH, Pr^i); 43.28, 43.70 (both d, $C\underline{H}_2CH$, $^3J_{C,P}$ = = 5.8 Hz); 47.16, 47.36 (both d, PCH_2N_3, $^1J_{C,P}$ = 144.7 Hz); 52.12 (s, OCH_3); 52.40 (d, CHNHP, $^2J_{C,P}$ = 10.0 Hz); 60.79, 60.98 (both d, $POCH_2$, $^2J_{C,P}$ = 7.0 Hz); 174.53 (s, C=O). ^{31}P—{^{1}H} NMR ($CDCl_3$), δ: 23.8 (s), 24.6 (s).

Azidoacetic acid *O*-methyl-L-leucinamide (10). Compound **10** was synthesized by a procedure described earlier[3] from leucine methyl ester hydrochloride (0.84 g, 4.62 mmol), chloroazidoacetic acid (0.60 g, 5.02 mmol), and triethylamine (0.95 g, 9.4 mmol) in CH_2Cl_2. The yield was 0.84 g (80%), viscous oil. Found (%): C, 47.27; H, 7.17; N, 24.65. $C_9H_{16}N_4O_3$. Calculated (%): C, 47.36; H, 7.07; N, 24.55. ^{1}H NMR ($CDCl_3$), δ: 0.83 (d, 6 H, CH_3, Pr^i, $^3J_{H,H}$ = 7.5 Hz); 1.49—1.56 (m, 3 H, $C\underline{H}_2CH$ + + CH, Pr^i); 3.63 (s, 3 H, OCH_3); 3.89 (s, 2 H, CH_2N_3); 4.52—4.56 (m, 1 H, $C\underline{H}NH$); 6.80 (d, 1 H, NH, $^3J_{H,H}$ = 7.7 Hz). ^{13}C—{^{1}H} NMR ($CDCl_3$), δ: 21.44, 22.41 (both s, CH_3, Pr^i); 24.48 (s, CH, Pr^i); 40.91 (s, $\underline{C}H_2CH$); 50.29 (s, OCH_3); 51.99 (s, CH_2N_3); 52.04 (s, CHNH); 166.44 (s, $\underline{C}(O)OCH_3$); 172.64 (s, C(O)NH).

***N-tert*-Butoxycarbonylisoleucine propargyl ester (12).**[19] Yield 60%, viscous oil. ^{1}H NMR ($CDCl_3$), δ: 0.89 (d, 3 H, $C\underline{H}_3CH$, $^3J_{H,H}$ = 7.0 Hz); 0.92 (t, 3 H, $C\underline{H}_3CH_2$, $^3J_{H,H}$ = 6.5 Hz); 1.18—1.26 (m, 1 H, $C\underline{H}HCH_3$); 1.43 (s, 9 H, CH_3—C—O); 2.49 (t, 1 H, ≡CH, $^4J_{H,H}$ = 2.5 Hz); 4.28 (br.s, 1 H, $C\underline{H}NH$); 4.65, 4.77 (both d, 2 H, OCH_2, $^2J_{H,H}$ = 15.6 Hz); 5.08 (br.s, 1 H, NH). ^{13}C—{^{1}H} NMR ($CDCl_3$), δ: 11.37 (s, $\underline{C}H_3CH_2$); 15.30 (s, $\underline{C}H_3CH$); 24.81 (s, $\underline{C}H_2CH$); 28.12 (s, CH_3, Bu^t); 37.92 (s, $\underline{C}HCH_3$); 52.15 (s, OCH_2); 57.65 (s, CHNH); 75.02 (s, ≡CH); 79.66 (s, —C≡); 155.33 (s, $\underline{C}(O)OCH_2$); 171.48 (s, C(O)OBu^t).

***N*-Acetylleucine propargyl ester (14)** was prepared from *N*-acetylleucine (0.013 mol) and propargyl bromide (0.013 mol). The yield was 65%, viscous oil. Found (%): C, 61.3; H, 9.0; N, 7.0. $C_{11}H_{17}NO_3 \cdot 0.17H_2O$. Calculated (%): C, 61.6; H, 9.4; N, 6.5. ^{1}H NMR ($CDCl_3$), δ: 0.87, 0.88 (both d, 3 H + 3 H, CH_3, Pr^i, $^3J_{H,H}$ = 6.3 Hz); 1.46—1.66 (m, 2 H + 1 H, CH, Pr^i + + $C\underline{H}_2CHNH$); 1.96 (s, 3 H, $CH_3C(O)$); 2.46 (t, 1 H, ≡CH, $^4J_{H,H}$ = 2.4 Hz); 4.55 (dt, 1 H, $C\underline{H}NH$, $^3J_{H,H}$ = 8.5 Hz, $^3J_{H,H}$ = = 4.7 Hz); 4.64, 4.65 (both dd, 2 H, OCH_2, $^2J_{H,H}$ = 15.4 Hz, $^4J_{H,H}$ = 2.4 Hz); 6.60 (br.s, 1 H, NH). ^{13}C—{^{1}H} NMR ($CDCl_3$), δ: 21.50 (s, CH_3, Pr^i); 22.49 (s, $\underline{C}H_3C(O)$); 24.48 (s, CH, Pr^i); 40.76 (s, $\underline{C}H_2CHN$); 50.39 (s, CHNH); 52.20 (s, OCH_2); 75.04 (s, HC≡); 79.50 (s, —C≡); 170.01 (s, C(O)O); 172.18 (s, C(O)N).

Compounds 15, 17, 19, and 20 (general procedure). The appropriate azide (1 mmol), $CuSO_4 \cdot 5H_2O$ (0.1 mmol, 10 mol.%), and sodium ascorbate (0.2 mmol, 20 mol.%) were successively added to a solution of compound **12** or **14** (1 mmol) in a 5 : 2 Bu^tOH—water mixture (4 mL). The reaction mixture was stirred

at 20 °C for 12 h, the course of the reaction being monitored by TLC. Then the reaction mixture was concentrated to dryness *in vacuo* and purified by chromatography on silica gel using the hexane—acetone gradient solvent system from 10 : 2 to 1 : 1 and then the 10 : 1 CH_2Cl_2—methanol mixture (to isolate the target products).

***P*-{[4-(*N*-*tert*-Butoxycarbonylisoleucine-*O*-methyl)-1*H*-1,2,3-triazol-1-ylmethyl]}-*N*-(*O*-methylleucine)-*O*-ethyl phosphonate (15).** Yield 63%, viscous oil. Found (%): C, 51.14; H, 8.38; N, 11.21. $C_{24}H_{44}N_5O_8P$. Calculated (%): C, 51.33; H, 7.90; N, 12.47. ^{1}H NMR ($CDCl_3$), δ: 0.86—0.91 (m, 12 H, CH_3, Pr^i + + C$\underline{H}_3$CH$_2$CH + C$\underline{H}_3$CHC$_2$H$_5$); 0.92, 0.96 (both d, 3 H, POCH$_2$C$\underline{H}_3$, $^3J_{H,H}$ = 6.6 Hz); 1.26—1.30 (m, 4 H, C$\underline{H}_2$CH); 1.42, 1.44 (both s, 9 H, CH_3CO); 1.51—1.85 (m, 2 H, C$\underline{H}$(CH$_3$)$_2$ + C$\underline{H}$C$_2$H$_5$ + CH$_3$C$\underline{H}_2$); 3.29 (br.d, 1 H, PNH, $^2J_{H,P}$ = 9.2 Hz); 3.74, 3.75 (both s, 3 H, OCH_3), 3.90—4.40 (m, 4 H, POCH$_2$ + + C(O)OCH$_2$); 4.71, 4.81 (both d, 2 H, PCH$_2$N, $^2J_{H,P}$ = 13.0 Hz); 5.04 (br.t, 1 H, C$\underline{H}$(NH)CH$_2$, $^3J_{HH}$ = 8.5 Hz); 5.31 (br.s, 1 H, C$\underline{H}$(NH)C(O)OBu^t); 7.85, 8.00 (both s, 1 H, =CH). ^{13}C—{^{1}H} NMR ($CDCl_3$), δ: 11.31 (s, C$\underline{H}_3$CH$_2$); 15.27, 15.30 (both s, C$\underline{H}_3$CHC$_2$H$_5$); 16.03 (d, POCH$_2$C$\underline{H}_3$, $^3J_{C,P}$ = 6.20 Hz); 21.11, 21.27 (both s, CH_3, Pr^i); 22.47, 22.61 (both s, C$\underline{H}$C$_2$H$_5$); 24.75, 24.78 (both s, C$\underline{H}_2$CH); 28.05 (s, CH_3, Bu^t); 37.60, 37.76 (both s, CH, Pr^i); 42.78, 43.24 (both d, PNHCH, $^2J_{C,P}$ = 5.8 Hz); 47.60, 47.83 (both d, PCH$_2$N, $^1J_{C,P}$ = 138.0 Hz); 52.34 (s, OCH_3); 57.77 (s, O—C, BOC); 57.81 (s, OCH_2); 61.31, 61.44 (both d, POCH$_2$, $^2J_{C,P}$ = 6.5 Hz); 124.5 (br.s, =C$\underline{H}$); 142.43 (br.s, =C); 155.29 (s, C=O, BOC); 171.90 (s, C$\underline{(O)}$OCH$_3$), 174,39 (s, C$\underline{(O)}$OCH$_2$). ^{31}P—{^{1}H} NMR ($CDCl_3$), δ: 18.94 (br.s), 19.40 (br.s). IR (thin layer), ν/cm^{-1}: 778, 968, 1036 (POC); 1155, 1229 (P=O); 1367, 1456, 1505, 1714, 1743 (C=O); 2875, 2933, 2962 (CH); 3276 (br, NH).

***P*-{[4-(*N*-Acetylleucine-*O*-methyl)-1*H*-1,2,3-triazol-1-yl]-methyl}-*N*-(*O*-methylleucine)-*O*-ethyl phosphonate (17).** Yield 56%, viscous oil. Mixture of isomers. Found (%): C, 50.0; H, 7.8; N, 13.9; P, 6.1. $C_{21}H_{38}N_5O_7P$. Calculated (%): C, 50.1; H, 7.6; N, 13.9; P, 6.15. ^{1}H NMR ($CDCl_3$), δ: 0.80—0.95 (m, 12 H, CH_3, Pr^i); 1.19, 1.24 (both br.t, 3 H, P—OCH$_2$C$\underline{H}_3$, $^3J_{H,H}$ = = 8.5 Hz); 1.35—1.75 (m, 8 H, C$\underline{H}_2$CHNH + CH, Pr^i + PCH$_2$); 1.85, 1.89, 1.90 (all br.s, 3 H, CH$_3$C(O)N); 3.66, 3.68 (both s, 3 H, OCH_3); 3.85—4.15 (m, 4 H, POCH$_2$ + C$\underline{H}$NH); 4.65—4.80 (m, 2 H, C(O)OCH$_2$); 6.41—6.49 (m, 2 H, NH); 7.88, 7.95, 8.07 (all br.s, 1 H, =CH). ^{13}C—{^{1}H} NMR ($CDCl_3$), δ: 15.98 (d, POCH$_2$C$\underline{H}_3$, $^3J_{C,P}$ = 6.6 Hz); 20.90, 21.28, 21.48, 22.40, 22.52, 22.54, 22.67 (all s, CH_3, Pr^i + C$\underline{H}_3$C(O)N); 24.13, 24.27, 24.54 (all s, CH, Pr^i); 40.30, 40.46, 40.60, 40.63 (all s, C$\underline{H}_2$CHNH); 47.60, 47.87 (both d, P—CH$_2$N, $^1J_{C,P}$ = 137.8 Hz, $^1J_{C,P}$ = 142.5 Hz); 50.88, 50.96, 51.27 (all s, CHNH); 52.04, 52.12, 52.35, 52.51 (all s, OCH_3); 57.84 (br.s, OCH_2); 61.05, 61.16, 61.44 (all d, POCH$_2$, $^2J_{C,P}$ = 6.5 Hz); 125.50 (br.s, =CH Hz); 142.0 (br.s, =C—); 170.03, 170.07 (both s, C$\underline{(O)}$OCH$_2$); 172.45, 172.59 (both s, C$\underline{(O)}$OCH$_3$); 174.27, 174.35, 174.67 (all s, C$\underline{(O)}$CH$_3$). ^{31}P—{^{1}H} NMR ($CDCl_3$), δ: 19.1 (br.s), 19.4 (br.s), 19.5 (br.s). IR (KBr), ν/cm^{-1}: 964, 1037 (POC); 1154, 1229 (P=O); 1661, 1747 (C=O); 2872, 2959 (CH); 3270 (br, NH).

{[4-(*N*-*tert*-Butoxycarbonylisoleucine-*O*-methyl)-1*H*-1,2,3-triazol-1-yl]acetyl}-*N*-(*O*-methylleucine) (19). Yield 66%, viscous oil. Mixture of isomers. Found (%): C, 56.70; H, 8.26; N, 12.89. $C_{23}H_{39}N_5O_7$. Calculated (%): C, 55.52; H, 7.90; N, 14.07. ^{1}H NMR ($CDCl_3$), δ: 0.84—0.94 (m, 12 H, CH_3, Pr^i + + C$\underline{H}_3$CH$_2$); 1.43 (br.s, 9 H, CH_3, Bu^t); 1.10—1.40, 1.50—1.70 (both m, 6 H, CH$_3$C$\underline{H}_2$C$\underline{H}$ + C$\underline{H}_2$C$\underline{H}$CH$_3$); 3.72 (s, 3 H, OCH_3); 4.25—4.30, 4.59—4.64 (both m, 1 H + 1 H, OCH_2); 5.05—5.15 (m, 2 H, C$\underline{H}$NH); 5.31 (br.s, 2 H, NC$\underline{H}_2$C(O)); 6.65 (br.d, 2 H, NH, $^3J_{H,H}$ = 7.7 Hz); 7.84 (s, 1 H, =CH). ^{13}C—{^{1}H} NMR ($CDCl_3$), δ: 11.42 (s, C$\underline{H}_3$CH$_2$); 15.41 (s, C$\underline{H}_3$CHC$_2$H$_5$); 21.68, 22.55 (both s, CH_3, Pr^i); 24.70 (s, C$\underline{H}$C$_2$H$_5$); 24.83 (s, C$\underline{H}_2$CH); 28.17 (s, CH_3, Bu^t); 37.75 (s, C$\underline{H}$(CH$_3$)$_2$); 41.04 (s, NC$\underline{H}_2$C(O)); 50.98 (s, C$\underline{H}$(NH)C(O)OCH$_3$); 52.39 (s, OCH_3); 52.56 (s, OCH_2); 57.81 (s, C$\underline{H}$(NH)COCH$_2$); 57.85 (s, OC$\underline{(CH_3)_3}$); 125.39 (s, =CH); 142.92 (s, =C—); 155.44 (s, C$\underline{(O)}$OBoc); 164.55 (s, C$\underline{(O)}$OCH$_3$); 172.11 (s, C$\underline{(O)}$OCH$_2$); 172.62 (s, CH$_2$C$\underline{(O)}$NH). IR (thin layer), ν/cm^{-1}: 793, 1020, 1049, 1158, 1232, 1252, 1367, 1526 (br, C=O); 1699 (br, C=O); 1743 (C=O); 2876, 2963 (CH); 3318 (br, NH).

{[4-(*N*-Acetylleucine-*O*-methyl)-1*H*-1,2,3-triazol-1-yl]acetyl}-*N*-(*O*-methylleucine) (20). Yield 81%, viscous oil. Mixture of isomers. Found (%): C, 54.35; H, 7.24; N, 15.26. $C_{20}H_{33}N_5O_6 \cdot 0.1CH_2Cl_2$. Calculated (%): C, 53.90; H, 7.60; N, 15.60. ^{1}H NMR ($CDCl_3$), δ: 0.93—0.99 (m, 12 H, CH_3, Pr^i); 1.52—1.69 (m, 6 H, C$\underline{H}_2$C$\underline{H}$(CH$_3$)$_2$); 2.02 (s, C(O)CH$_3$); 3.75 (s, OCH_3); 4.55—4.69 (m, 2 H, CHNH); 5.11, 5.15 (both d, 2 H, C$\underline{H}_2$C(O), $^2J_{H,H}$ = = 16.7 Hz); 5.30, 5.35 (both d, 2 H, OC$\underline{H}_2$, $^1J_{H,H}$ = 12.8 Hz); 6.00, 6.75 (both br.s, 1 H + 1 H, NH); 8.05 (s, 1 H, =CH). ^{13}C—{^{1}H} NMR ($CDCl_3$), δ: 21.62, 21.65 (both s, CH_3, Pr^i); 22.58, 22.66, 22.81 (all s, CH, Pr^i); 24.69 (s, C$\underline{H}_3$C(O)); 40.87 (s, C$\underline{H}_2$CHNH); 50.85, 51.02 (both s, CHNH); 52.30 (s, OCH_3); 52.39 (s, OCH_2); 58.07 (s, C$\underline{H}_2$C(O)); 125.69 (br.s, =CH); 142.86 (br.s, =C—); 164.91 (s, C$\underline{(O)}$OCH$_3$); 170.20 (s, C$\underline{(O)}$OCH$_2$); 172.76 (s, NHC$\underline{(O)}$CH$_3$). IR (KBr), ν/cm^{-1}: 963, 1053, 1154, 1231, 1372, 1439, 1545, 16581, 1748 (C=O); 2872, 2960 (CH); 3291 (br, NH).

Compounds 16 and 18 (general procedure). Trimethylbromosilane (1.5 mmol) was added to a solution of compound **15** or **17** (1 mmol) in anhydrous CH_2Cl_2 (5 mL). The mixture was stirred at 20 °C for 8 h and allowed to stand at this temperature for 48 h. The solvent was removed *in vacuo*, CH_3OH (2 mL) was added to the residue, and the mixture was kept for 2 h. Then all volatile products were removed *in vacuo*, and the residue was dried with a vacuum oil pump over P_2O_5.

***P*-{[4-(*O*-Methylisoleucine)-1*H*-1,2,3-triazol-1-yl]methyl}-*N*-(*O*-methylleucine) phosphonic acid (16).** Yield 95%, viscous oil. Mixture of isomers. Found (%): C, 47.11; H, 7.12. $C_{17}H_{32}N_5O_6P$. Calculated (%): C, 47.11; H, 7.44. ^{1}H NMR (CD_3OD), δ: 0.94—1.03 (m, 12 H, CH_3, Pr^i + C$\underline{H}_3$CH$_2$CN + C$\underline{H}_3$CHC$_2$H$_5$); 1.31—1.38, 1.49-1.54 (both m, 2 H, CH$_3$C$\underline{H}_2$CH); 1.72—1.85 (m, 3 H, C$\underline{H}_2$C$\underline{H}$(CH$_3$)$_2$); 2.00—2.10 (m, 1 H, C$\underline{H}$—C$_2$H$_5$); 3.66 (d, 2 H, PCH$_2$N, $^2J_{H,P}$ = 10.8 Hz); 3.86 (s, 3 H, OCH_3); 4.05—4.09 (m, 2 H, C(O)OCH$_2$); 4.82—4.87 (m, 1 H, C$\underline{H}$(NH)CH$_2$); 5.37—5.45 (m, 1 H, CH(NH$_2$)); 8.19, 8.22 (s, 1 H, =CH). ^{13}C—{^{1}H} NMR (CD_3OD), δ: 10.39 (s, C$\underline{H}_3$CH$_2$); 13.29, 13.64 (both s, C$\underline{H}_3$CHC$_2$H$_5$); 20.83, 20.96 (both s, CH_3, Pr^i); 23.95 (s, C$\underline{H}$C$_2$H$_5$); 25.07 (s, C$\underline{H}_2$CH); 36.12, 36.17 (both s, CH, Pr^i); 45.58 (d, PCH$_2$N, $^1J_{P,C}$ = 145.8 Hz); 52.08 (s, OCH_3); 58.01 (s, OCH_2); 125.66, 125.69, 125.76 (all s, =CH); 141.24, 141.27, 141.32 (all s, =C—); 168.00 (s, C$\underline{(O)}$OCH$_3$); 169.77 (s, C$\underline{(O)}$OCH$_2$). ^{31}P—{^{1}H} NMR (CD_3OD), δ: 12.4 (s), 13.3 (s).

***P*-{[4-(*N*-Acetylleucine-*O*-methyl)-1*H*-1,2,3-triazol-1-yl]-methyl}-*N*-(*O*-methylleucine) phosphonic acid (18).** Yield 100%, powder. Mixture of isomers. M.p. 80—90 °C. Found (%): C, 36.59; H, 6.04; N, 10.42; P, 4.86. $C_{19}H_{34}N_5O_7P \cdot 1.8$ HBr. Calculated (%): C, 36.70; H, 5.80; N, 11.00; P, 4.98. ^{1}H NMR (D_2O),

δ: 0.65—0.85 (m, 12 H, CH_3, Pr^i); 1.40—1.75 (m, 8 H, $C\underline{H}_2CHNH$ + CH, Pr^i + PCH_2); 1.87 (br.s, 3 H, $CH_3C(O)N$); 3.69 (br.s, 3 H, OCH_3); 4.02, 4.24 (both br.s, 2 H, $C\underline{H}NH$); 4.60—4.65 (m, 2 H, OCH_2); 8.00 (br.s, 1 H, =CH). $^{13}C—\{^1H\}$ NMR (D_2O), δ: 20.62, 20.97, 21.43, 21.87 (all s, CH_3, Pr^i + $\underline{C}H_3C(O)N$); 23.86, 24.23 (both s, CH, Pr^i); 38.72, 38.98 (both s, $\underline{C}H_2CHNH$); 48.00 (d, $P—CH_2N$, $^1J_{C,P}$ = 142.5 Hz); 51.39, 51.50 (both s, CHNH); 53.47 (s, OCH_3); 57.69 (s, OCH_2); 126.45 (br.s, =CH); 143.00 (br.s, =C—); 171.23 (s, $\underline{C}(O)OCH_2$); 173.97 (s, $\underline{C}(O)OCH_3$); 174.25 (s, $\underline{C}(O)CH_3$). $^{31}P—\{^1H\}$ NMR (D_2O), δ: 10.09 (br.s), 11.6 (br.s), 13.3 (br.s).

This study was financially supported by the Russian Foundation for Basic Research (Project No. 15-03-00663).

References

1. L. Pickart, J. Biomater, *Sci. Polym. Ed.,* 2008, **19**, 969.
2. M. Yu. Smakhtin, A. I. Konoplya, L. A. Sever´yanova, I. I. Bobyntsev, A. A. Kurtseva, V. Yu. Cherdakov, *Kursk. Nauch.-Prakt. Vestn. "Chelovek i Ego Zdorov´e"* [*Kursk Sci. Practic Bull "Man and Its Health"*], 2006, **3**, 11 (in Russian).
3. M. R. Ahmed, S. H. Basha, D. Gopinath, R. Muthusamy, R. Jayakumar, *J. Peripher. Nerv. Syst.*, 2005, **10**, 17.
4. V. Kh. Khavinson, A. Yu. Solov´ev, S. I. Tarnovskaya, N. S. Lin´kova, *Biol Bull Rev.*, 2013, **3**, 451 [*Usp. Sovrem. Biol.*], 2013, **133**, 310.
5. R. Mileusnic, C. L. Lancashire, A. N. B. Johnston, S. P. R. Rose, *Eur. J. Neurosci.,* 2000, **12**, 4487.
6. K. Bifulco, I. Longanesi-Cattani, L. Gargiulo, O. Maglio, M. Cataldi, M. De Rosa, M. P. Stoppelli, V. Pavone, M. V. Carriero, *FEBS Lett.,* 2009, **582**, 1141.
7. R. Mileusnic, S. P. R. Rose, Eur. Pat. EP 1381627, 2004.
8. E. D. Matveeva, E. A. Podrugina, M. V. Prisyazhnyi, S. O. Bachurin, N. S. Zefirov, *Russ. Chem. Bull.* (*Int. Ed.*), 2010, **59**, 200 [*Izv. Akad. Nauk, Ser. Khim.*, 2010, 196].
9. R. Mileusnic, C. L. Lancashire, S. P. R. Rose, *Ann. N. Y. Acad. Sci.*, 2005, **1048**, 149.
10. R. Huisgen, G. Szeimies, L. Meldal, *J. Org. Chem.*, 1967, **100**, 2494.
11. V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. Sharpless, *Angew. Chem., Int. Ed.,* 2002, **41**, 2596.
12. Yu. L. Angell, K. Burgess, *Chem. Soc. Rev.*, 2007, **36**, 1674.
13. O. N. Zefirova, N. S. Zefirov, *Vestn. Mosk. Univ.*, *Ser. Khim.*, 2002, **43**, No. 4, 251 [*Vestn. Mosk. Univ.*, *Ser. Khim.* (*Engl. Transl.*), 2002, **43**].
14. D. Sikora, T. Nonas, T. Gayda, *Tetrahedron*, 2001, **57**, 1619.
15. D. A. McLeod, R. I. Brinkworth, J. A. Ashley, K. D. Janda, P. Wirsching, *Med. Chem. Lett.,* 1991, **1**, 653.
16. T. Gajda, *Synthesis*, 1990, **8**, 717.
17. S. Haridas, R. P. Verma, S. Sadanandan, B. G. Kacheshwar, *Tetrahedron,* 2011, **67**, 1873.
18. R. Srinivasan, L. P. Tan, H. Wu, P.-Y. Yang, K. A. Kalesh, S. Q. Yao, *Org. Biomol. Chem.*, 2009, **7**, 1821.
19. R. Ramapanicker, R. Gupta, R. Megha, S. Chandrasekaran, *Int. J. Peptides*, 2011, doi: 10.1155/2011/854952.
20. S. P. Bew, G. D. Hiatt-Gipson, *J. Org. Chem.*, 2010, **75**, 3897.
21. E. V. Sharova, G. K. Genkina, E. V. Matveeva, I. B. Goryunova, E. I. Goryunov, O. I. Artyushin, V. K. Brel, *Russ. Chem. Bull.* (*Int. Ed.*), 2014, **63**, 2546 [*Izv. Akad. Nauk*, *Ser. Khim.*, 2014, 2546].

Received April 30, 2015;
in revised form September 3, 2015