ISSN 1070-3632, Russian Journal of General Chemistry, 2015, Vol. 85, No. 1, pp. 71–74. © Pleiades Publishing, Ltd., 2015.
Original Russian Text © E.I. Lukashuk, E.R. Abdurakhmanova, K.M. Kondratyuk, A.V. Golovchenko, V.S. Brovarets, 2015, published in Zhurnal Obshchei Khimii, 2015, Vol. 85, No. 1, pp. 77–81.

Synthesis of Phosphorylated Dehydrotyrosine-Containing Tripeptides from 5-Amino-2-aminoalkyl-1,3-oxazole-4-phosphonic Acids Derivatives

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Received August 25, 2014

Abstract—Interaction of 4-phosphorylated derivatives of 5-amino-2-aminoalkyl-1,3-oxazole with 4-(4-acetoxybenzylidene)-2-phenyl-1,3-oxazol-5-one resulted in the formation of new 1,3-oxazole derivatives. The latter undergo 1,3-oxazole ring opening to give phosphorylated tripeptides with a dehydrotyrosine fragment.

Keywords: 1,3-oxazole, dehydroamino acids, tyrosine, phosphorylated peptidomimetics, phosphonoglycine

DOI: 10.1134/S1070363215010120

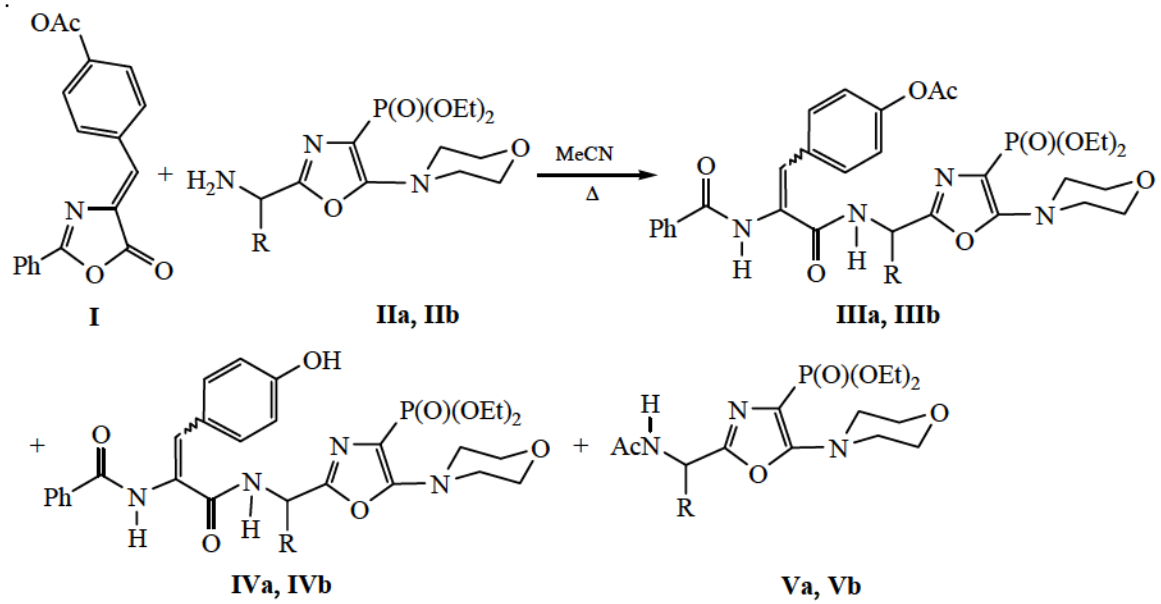
Peptides containing dehydroamino residues have a pronounced biological activity and play an important role in biochemical processes. Thus, a group of dehydroalanine-containing oligopeptides, which are effective inhibitors of protein phosphatases 1 and 2A, has been isolated from the blue-green algae [1–3]. Also dehydrovaline-containing heptapeptide antrimycin D_v [4] is known to exhibit antibiotic properties. Dehydrotyrosine residues are part of the blood pigments of molluscs, tunichromes An-1-3, Mm-1, Mm-2 [5, 6], as well as peptide alkaloids celenamides A and B isolated from *Cliona celata* sponge [7, 8]. It is known [9] that the introduction of phosphoryl groups into the polypeptide chain leads to the formation of new bioactive structures promising for further modification.

Recently, we have developed a method for the synthesis of phosphorus-containing oligopeptides with residues of substituted dehydrophenylalanine by reacting 4-phosphorylated 5-amino-2-aminoalkyl-1,3-oxazoles with 4-(4-toluylidene)-2-phenyl-1,3-oxazol-5-one followed by acid cleavage of 1,3-oxazole ring [10]. In this work we investigated the possibility of applying the above 1,3-oxazoles for regioselective introduction of dehydrotyrosine residue into the molecule of phosphorylated tripeptide.

For this purpose, we studied a reaction of 4-(4-acetoxybenzylidene)-2-phenyl-1,3-oxazol-5-one **I** [11] with diethyl [2-(aminomethyl)-5-morpholino-1,3-oxazol-4-yl]phosphonate **IIa** as well as with diethyl [2-(1-aminoethyl)-5-morpholino-1,3-oxazol-4-yl]phosphonate **IIb** comprising optically active carbon atom. According to gas chromatography-mass spectrometry data the reaction afforded a mixture of compounds **III**, **IV**, and **V**. The formation of the latter two can be a result of acetylation of the amino group of 1,3-oxazoles **IIa** and **IIb** by the action of compounds **IIIa** and **IIIb** (Scheme 1).

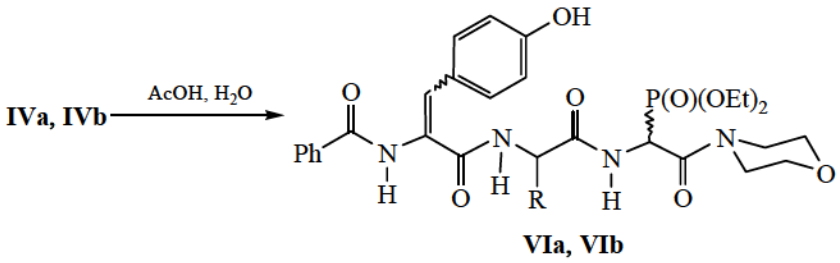
The reaction of **I** with 2-aminoalkyl-1,3-oxazoles **IIa** and **IIb** were performed in benzene, ethanol, acetonitrile, or dioxane in the temperature range of 20–110°C. It has been found that the optimum reaction conditions are heating the reactants in acetonitrile for 4–5 h at 50–60°C. The resulting mixture of compounds **III**, **IV**, and **V** (~6 : 2 : 1) without separating was subjected to short-term (~2 min) boiling in 25% aqueous ammonia to isolate dehydrotyrosine derivatives **IVa** and **IVb** in 68–71% yields. The specific rotation in **IVb** ($[\alpha]_D$ –22.4) indicates a stereoselective interaction of 2-(1-aminoethyl)-1,3-oxazole **IIb** with azlactone **I**.

Scheme 1.



R = H, Me.

Scheme 2.



R = H, Me.

Heating compounds **IVa** and **IVb** in a mixture of acetic acid–water (5 : 1) resulted in oxazole ring opening to form phosphorylated dehydrotyrosine-containing tripeptides **VIa** and **VIb** isolated in crystalline form in 90–93% yields (Scheme 2).

The structure of the compounds obtained was confirmed by IR, ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy. Thus, the ^{1}H NMR spectra of **VIa** and **VIb** contain the multiplets at 5.39–5.44 ppm, which correspond to NHCHP fragment. In addition, in the IR spectra of **VIa** and **VIb** there was no absorption of 5-aminooxazole ring in the range of 1611–1607 cm^{-1}. It should also be noted that tripeptide **VIb** was optically active ([α]$_D$ +5.2), and its ^{1}H and ^{31}P NMR spectra contained a double set of the signals of equal intensity that indicated the presence of a pair of diastereomers.

In summary, preparative synthesis was developed of new phosphorylated tripeptides containing dehydrotyrosine and phosphonoglycine residues from diethyl 2-(aminomethyl)- and 2-(1-aminoethyl)-5-morpholino-1,3-oxazol-4-ylphosphonic acids.

EXPERIMENTAL

NMR spectra were recorded on a Bruker AVANCE DRX-500 spectrometer [^{1}H (500 MHz), ^{31}P (202 MHz), ^{13}C (125 MHz)], internal reference TMS or external reference 85% phosphoric acid. IR spectra were obtained on a Vertex 70 spectrometer from KBr pellets. GC-MS spectra were registered using an Agilent 1100 Series HPLC system equipped with a diode array with a mass-selective detector Agilent LC\MSD SL [column Zorbax SB-C18 (1.8 microns, 4.6 × 15 mm, PN 821975-932); solvents: acetonitrile–water (95 : 5), 0.1% trifluoroacetic acid (A), 0.1% aqueous trifluoroacetic acid (B); eluent flow 3 mL min^{-1}; injection volume 1 μL; UV detection at 215, 254,

265 nm; chemical ionization at atmospheric pressure (APCI); scanning range *m/z* 80–1000]. Optical rotation was determined on a MCP 300 polarimeter (Anton Paar GmbH). Melting points were determined on a Fisher Johns instrument.

Elemental analysis was performed in the analytical laboratory of the Institute of Bioorganic Chemistry and Petrochemistry of National Academy of Sciences of Ukraine. The reaction progress was monitored by TLC on Silufol UV-254 plates, eluting with CH_2Cl_2–MeOH (95 : 5) and detecting with UV irradiation.

Diethyl [2-(1-aminomethyl)-5-morpholino-1,3-oxazol-4-yl]phosphonate **IIa** was obtained as described in [12].

Diethyl [2-(1-aminoethyl)-5-morpholino-1,3-oxazol-4-yl]phosphonate (IIb) was prepared as described in [22]. Yield 86%, pale yellow oil, $[\alpha]_D$ 3.6 (*c* 7.6 CH_2Cl_2). IR spectrum (ATR), ν, cm^{-1}: 2978 br (NH), 1606 s, 1570 s (C–N), 1265 s (P=O), 1018 br.sh (P–O–C), 959 s (P–O–C–C). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 4.04–3.95 m (4H, O$\underline{CH_2}CH_3$), 3.91–3.87 m (1H, CH), 3.69–3.67 m (4H, $2CH_2$), 3.48–3.46 m (4H, $2CH_2$), 1.31–1.29 d (3H, CH_3, $^3J_{HH}$ 6.6 Hz), 1.25–1.21 m (6H, OCH$_2\underline{CH_3}$). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 159.52 d (O$\underline{C}$=CP, $^2J_{PC}$ 37.4 Hz), 157.03 d (OC=N, $^3J_{PC}$ 20.4 Hz), 98.81 d (CP, J_{PC} 51.8 Hz), 63.65 (OCH_2, morpholine), 59.74 d (O$\underline{C}$H$_2$CH$_3$, $^3J_{PC}$ 5.8 Hz), 46.05 (NCH_2, morpholine), 42.62 (CH), 19.16 (CH_3), 13.94 d (OCH$_2\underline{C}$H$_3$, $^4J_{PC}$ 6.0 Hz). ^{31}P NMR spectrum (DMSO-d_6): δ_P 12.3 ppm. Mass spectrum: *m/z* 334.3 $[M + H]^+$. Found, %: C 46.75; H 7.36; N 12.55; P 9.39. $C_{13}H_{24}N_3O_5P$. Calculated, %: C 46.84; H 7.26; N 12.61; P 9.29.

Diethyl (2-{1-[2-benzoylamino-3-(4-hydroxyphenyl)acryloylamino]alkyl}-5-morpholino)-1,3-oxazol-4-yl phosphonates (IVa, IVb). To a solution of 0.015 mol of compound **II** in 50 mL of MeCN was added 3.0 g (0.01 mol) of 4-(4-acetylbenzylidene)-2-phenyl-4*H*-oxazol-5-one **I**. The mixture was refluxed for 4–5 h (TLC monitoring) and then evaporated to dryness at a reduced pressure. To the residue was added 30 mL of aqueous ammonia, the mixture was boiled for 2 min and cooled. The resulting crystalline precipitate was filtered off, dried in air and identified without further purification.

Diethyl (2-{[2-benzoylamino-3-(4-hydroxyphenyl)acryloylamino]methyl}-5-morpholino)-1,3-oxazol-4-yl phosphonate (IVa). Yield 71%, pale yellow crystals, mp 204–205°C. IR spectrum (KBr), ν, cm^{-1}: 3183 br (NH), 1648–1575 br (2C=O), 1611 s, 1566 s (C–N), 1278 s (P=O), 1017 br.sh (P–O–C), 976 s (P–O–C–C). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 9.87 s (1H, NH), 9.84 s (1H, OH), 8.60–8.58 m (1H, NH), 8.00 d (2H, Ar, $^3J_{HH}$ 7.4 Hz), 7.57 t (1H, Ar, $^3J_{HH}$ 7.4 Hz), 7.53–7.52 m (2H, Ar), 7.42 d (2H, Ar, $^3J_{HH}$ 8.8 Hz), 7.21 s (1H, CH), 6.71 d (2H, Ar, $^3J_{HH}$ 8.0 Hz), 4.30 d (2H, CH_2, $^3J_{HH}$ 5.3 Hz), 3.97–3.96 m (4H, O$\underline{CH_2}CH_3$), 3.66–3.65 m (4H, OCH_2, morpholine), 3.45–3.44 m (4H, NCH_2, morpholine), 1.22–1.20 m (6H, OCH$_2\underline{CH_3}$). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 165.9 (C=O), 165.6 (C=O), 160.9 d (O$\underline{C}$=CP, $^2J_{PC}$ 37.4 Hz), 158.3 (HC=$\underline{C}$), 151.7 d (OC=N, $^3J_{PC}$ 22.4 Hz), 133.8, 131.7, 131.4, 130.5, 128.4, 127.7, 126.6, 125.0 (C_{arom}), 115.4 ($\underline{CH}$=C), 100.9 d (CP, J_{PC} 251.8 Hz), 65.6 (OCH_2, morpholine), 61.8 d (O$\underline{C}$H$_2$CH$_3$, $^3J_{PC}$ 5.5 Hz), 48.1 (NCH_2, morpholine), 36.4 (CH_2), 16.2 d (OCH$_2\underline{C}$H$_3$, $^4J_{PC}$ 6.5 Hz). ^{31}P NMR spectrum (DMSO-d_6): δ_P 12.5 ppm. Mass spectrum: *m/z* 585.6 $[M + H]^+$. Found, %: C 57.44; H 5.87; N 10.06; P 5.12. $C_{28}H_{33}N_4O_8P$. Calculated, %: C 57.5; H 5.69; N 9.58; P 5.30.

Diethyl (2-{1-[2-benzoylamino-3-(4-hydroxyphenyl)acryloylamino]ethyl}-5-morpholino)-1,3-oxazol-4-yl phosphonate (IVb). Yield 68%, colorless crystals, mp 138–140°C, $[\alpha]_D$ –22.4 (*c* 0.9 CH_2Cl_2). IR spectrum (KBr), ν, cm^{-1}: 3240 br (N–H), 1648–1580 br (2C=O), 1607 s (C–N), 1278 s (P=O), 1023 br.sh (P–O–C), 971 s (P–O–C–C). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 9.79 br.s (2H, NH, OH), 8.50 d (1H, NH, $^3J_{HH}$ 7.0 Hz), 8.00 d (2H, Ar, $^3J_{HH}$ 6.0 Hz), 7.58–7.56 m (1H, Ar), 7.51–7.50 m (2H, Ar), 7.41 d (2H, Ar, $^3J_{HH}$ 6.8 Hz), 7.14 s (1H, CH), 6.71 d (2H, Ar, $^3J_{HH}$ 7.0 Hz), 5.06–5.05 m (1H, CH), 3.97–3.96 m (4H, O$\underline{CH_2}CH_3$), 3.65–3.64 m (4H, $2OCH_2$, morpholine), 3.44 m (4H, $2NCH_2$, morpholine), 1.42 d (3H, CH_3, $^3J_{HH}$ 6.6 Hz), 1.21–1.20 m (6H, 2OCH$_2\underline{CH_3}$). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 165.8 (C=O), 165.1 (C=O), 160.1 d (O$\underline{C}$=CP, $^2J_{PC}$ 38.4 Hz), 158.2 (HC=$\underline{C}$), 154.7 d (OC=N, $^3J_{PC}$ 22.4 Hz), 133.8, 131.7, 131.4, 129.9, 128.4, 127.9, 127.0, 125.1 (C_{arom}), 115.5 ($\underline{CH}$=C), 100.8 d (CP, J_{PC} 246.3 Hz), 65.6 (OCH_2, morpholine), 61.8 d (O$\underline{C}$H$_2$CH$_3$, $^3J_{PC}$ 5.5 Hz), 48.0 (NCH_2, morpholine), 42.7 (CH), 18.2 (CH_3), 16.2 d (OCH$_2\underline{C}$H$_3$, $^4J_{PC}$ 6.5 Hz). ^{31}P NMR spectrum (DMSO-d_6): δ_P 12.8 ppm. Mass spectrum: *m/z* 599.6 $[M + H]^+$. Found, %: C 58.35; H 5.78; N 9.50; P 5.35. $C_{29}H_{35}N_4O_8P$. Calculated, %: C 58.19; H 5.89; N 9.36; P 5.17.

Diethyl (1-{2-[2-benzoylamino-3-(4-hydroxyphenyl)acryloylamino]alkylamino}-2-morpholino-2-oxoethyl)phosphonates (VIa, VIb). A solution of 0.01 mol of compound **IVa** or **IVb** in 50 mL of a mixture acetic acid–water (5 : 1) was heated for 7 h at 75°C on a water bath. The reaction mixture was evaporated to dryness at a reduced pressure. Then the residue was treated with 5% solution of $NaHCO_3$ (20 mL) and extracted with dichloromethane (3 × 50 mL). The extract was dried over $MgSO_4$, the solvent was removed at a reduced pressure. Compounds **VIa** and **VIb** were identified without further purification.

Diethyl (1-{2-[2-benzoylamino-3-(4-hydroxyphenyl)acryloylamino]acetylamino}-2-morpholino-2-oxoethyl)phosphonate (VIa). Yield 90%, yellow crystals, mp 129–130°C. IR spectrum (KBr), ν, cm^{-1}: 3273 br (N–H), 1649–1615 br (4C=O), 1277 s (P=O), 1026 br.sh (P–O–C), 978 s (P–O–C–C). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 9.91 br.s (2H, NH, OH), 8.35 d (1H, NH, $^3J_{HH}$ 8.5 Hz), 8.20–8.19 m (1H, NH), 8.03 d (2H, Ar, $^3J_{HH}$ 8.0 Hz), 7.58 t (1H, Ar, $^3J_{HH}$ 7.0 Hz), 7.52–7.51 m (2H, Ar), 7.42 d (2H, Ar, $^3J_{HH}$ 8.5 Hz), 7.22 s (1H, CH), 6.72 d (2H, Ar, $^3J_{HH}$ 8.8 Hz), 5.44 d. d (1H, CHP, $^3J_{HH}$ 8.5, $^2J_{HP}$ 19.3 Hz), 4.08–4.04 m (4H, OCH_2CH_3), 3.84–3.82 m (2H, CH_2), 3.59–3.39 m (8H, morpholine), 1.20–1.18 m (6H, OCH_2CH_3). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 168.8 d (C=O, $^2J_{PC}$ 3.5 Hz), 166.0 (C=O), 165.6 (C=O), 164.1 d (C=O, $^3J_{PC}$ 1.5 Hz), 158.4 (CH=C), 133.8, 131.8, 131.4, 130.3, 128.4, 128.0, 126.6, 132.1 (C_{arom}), 115.5 (CH=C), 66.0 (OCH_2, morpholine), 63.3 d (OCH_2CH_3, $^3J_{PC}$ 7.0 Hz), 62.9 d (OCH_2CH_3, $^3J_{PC}$ 6.5 Hz), 55.3 d (CP, J_{PC} 154.6 Hz), 48.2 (NCH_2, morpholine), 40.5 (CH_2), 16.4 d (OCH_2CH_3, $^4J_{PC}$ 5.5 Hz), 16.3 d (OCH_2CH_3, $^4J_{PC}$ 4.5 Hz). ^{31}P NMR spectrum (DMSO-d_6): δ_P 18.0 ppm. Mass spectrum: m/z 603.6 $[M + H]^+$. Found, %: C 55.94; H 5.98; N 9.44; P 5.25. $C_{28}H_{35}N_4O_9P$. Calculated, %: C 55.81; H 5.85; N 9.30; P 5.14.

Diethyl (1-{2-[2-benzoylamino-3-(4-hydroxyphenyl)acryloylamino]propionylamino}-2-morpholino-2-oxoethyl)phosphonate (VIb). Yield 92% (diastereomer mixture), colorless crystals, mp 125–126°C, $[\alpha]_D$ 5.2 (c 0.6 CH_2Cl_2). IR spectrum (KBr), ν, cm^{-1}: 3278 br (N–H), 1649–1616 br (4C=O), 1246 s (P=O), 1027 br.sh (P–O–C), 977 s (P–O–C–C). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 9.94 br.s (2H, NH, OH), 8.54 d (1H, NH, $^3J_{HH}$ 8.5 Hz), 8.34 d (1H, NH, $^3J_{HH}$ 8.5 Hz), 8.01–7.59 m (2H, Ar), 7.93 d (1H, NH, $^3J_{HH}$ 7.2 Hz), 7.58–7.56 m (1H, Ar), 7.51–7.50 m (2H, Ar), 7.42–7.40 m (2H, Ar), 7.19 s (1H, CH), 7.15 s (1H, CH), 6.73–6.72 m (2H, Ar), 5.40–5.37 m (1H, CHP), 4.59–4.56 m (1H, CH), 4.51–4.49 m (1H, CH), 4.02–4.04 m (4H, OCH_2CH_3), 3.59–3.39 m (8H, morpholine), 1.16–1.19 m (9H, CH_3, $2OCH_2CH_3$). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 172.1 d (C=O, $^2J_{PC}$ 4.0 Hz), 172.0 d (C=O, $^2J_{PC}$ 4.0 Hz), 165.9, 165.1, 164.9 (C=O), 164.0 m (C=O), 158.4 (CH=C), 133.8, 133.7, 131.9, 131.8, 131.7, 131.4, 130.2, 129.8, 128.5, 127.9, 127.8, 127.0, 126.8, 126.6, 125.0, 124.9 (C_{arom}), 115.5, 115.5 (CH=C), 66.0, 65.9 (OCH_2, morpholine), 63.2 d (OCH_2CH_3, $^3J_{PC}$ 6.5 Hz), 62.8 d (OCH_2CH_3, $^3J_{PC}$ 6.5 Hz), 62.7 d (OCH_2CH_3, $^3J_{PC}$ 6.0 Hz), 62.6 d (OCH_2CH_3, $^3J_{PC}$ 6.0 Hz), 48.8, 48.5 (NCH_2, morpholine), 47.6 d (CP, J_{PC} 148.6 Hz), 47.4 d (CP, J_{PC} 148.6 Hz), 42.4, 40.5 (CH), 18.5, 18.3 (CH_3), 16.4 d (OCH_2CH_3, $^4J_{PC}$ 4.5 Hz), 16.2 d (OCH_2CH_3, $^4J_{PC}$ 5.0 Hz). ^{31}P NMR spectrum (DMSO-d_6), δ_P, ppm: 18.01, 17.98. Mass spectrum: m/z 617.6 $[M + H]^+$. Found, %: C 56.55; H 5.99; N 9.30; P 5.15. $C_{29}H_{37}N_4O_9P$. Calculated, %: C 56.49; H 6.05; N 9.09; P 5.02.

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