Heteroatom Chemistry
Volume 24, Number 4, 2013

A Novel Synthetic Approach to Phosphorylated Peptidomimetics

Olena I. Lukashuk, Kostyantyn M. Kondratyuk, Alexandr V. Golovchenko, Vladimir S. Brovarets, and Valery P. Kukhar

Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Sciences of Ukraine, Kiev-94 02660, Ukraine

Received 7 December 2012; revised 4 April 2013

ABSTRACT: *It has been shown that the derivatives of diethyl 5-amino-2-phthalimidoalkyl-1,3-oxazol-4-ylphosphonates can be employed in the synthesis of phosphorylated peptidomimetics containing the phosphonoglycine residue. The reaction of diethyl 5-alkylamino-2-aminoalkyl-1,3-oxazol-4-ylphosphonates with unsaturated azlactones was utilized to obtain phosphorylated peptidomimetics with dehydroamino acid moieties. The double bond in the latter was reduced with zinc in acetic acid to provide the corresponding saturated peptidomimetics containing a diethoxyphosphoryl group in the side chain.* Heteroatom Chem. 24:289–297, 2013; View this article online at wileyonlinelibrary.com. DOI 10.1002/hc.21093

INTRODUCTION

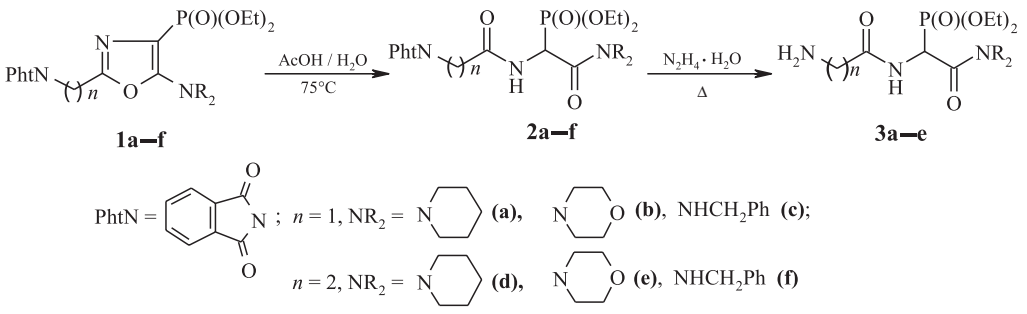
Phosphorylated peptides containing a phosphonoglycine moiety have received considerable attention of organic and bioorganic chemists [1,2]. This is due to the fact that compounds of peptide nature containing aminoalkylphosphonic acid residues have been found in biological systems [3]. On the other hand, phosphopeptides are known to efficiently inhibit enzymes [4].

Correspondence to: Vladimir S. Brovarets; e-mail: brovarets@bpci.kiev.ua.

Phosphopeptide mimetic molecules can be structurally divided into two types: one containing the peptide chain with a terminal phosphono group and the other with a nonterminal phosphorylated amino acid residue. The latter type has been much less studied, since phosphoamino acid residues are difficult to introduce at certain internal positions of polypeptides. Peptidomimetics of this kind were applied to the synthesis of natural peptide compounds such as desoxy-biphenomycin B [5], the vanadium- and iron-containing clam blood pigments (±)-tunichrome An-1 [6], and tunichromes Mm-1 and Mm-2 [7] as well as the alkaloids hexaacetylcelenamide A [8,9] and clionamide [10]. Moreover, some of these compounds are efficient glutathione transferase inhibitors [11,12], thus adding to the biological significance of phosphopeptide mimetics.

Peptidomimetics containing α,β-dehydroamino acid residues are equally important compounds from the fundamental and application point of view. They were employed in retrosynthetic routes to natural substances [13]. Members of this class, as for instance, the cyclopeptide phytotoxin tentoxin, the antitumor drugs cytoxin, and kahalalide F, and fungicide pseudomycin, are also quite notable for their biological activity. It is significant that the introduction of α,β-dehydroamino acid residues into synthetic counterparts of bioactive natural peptides makes them more resistant to cleaving enzymes [14].

Currently, several synthetic methods exist to obtain peptides with the phosphonoglycine residue. One of them involves the reaction



SCHEME 1

between the metallated derivatives of diethyl isocyanomethylphosphonates and methyl α-isocyanatocarboxylates at −70°C [15]. Another approach is based on the reaction of glyoxylic acid derivatives with *N*-benzylurethane, which leads to *N*-benzyloxycarbonyl-2-ethoxyglycine derivatives. These products, when treated with phosphorus trichloride and triethyl phosphite, afford the corresponding *N*-benzyloxycarbonyl-substituted phosphorylated glycines, which, on debenzylation with hydrogen on palladium, are applied to peptide synthesis [16] (see, for instance, the preparation of mucronine B [17] and chlamydocin [18]).

Here, we present a novel synthetic pathway to phosphorylated peptidomimetics containing the phosphonoglycine moiety; it includes, as a main step, acid-assisted oxazole ring cleavage.

RESULTS AND DISCUSSION

As shown previously, the derivatives of diethyl 5-amino-1,3-oxazol-4-ylphosphonates decompose hydrolytically in an acidic environment to yield phosphonoglycine amides [19, 20]. We have performed analogous conversions using easily available phthalimido derivatives of 5-alkylamino-2-aminoalkyl-1,3-oxazol-4-ylphosphonic acids **1** [21] (see Scheme 1).

On heating compounds **1a–f** in 70% aqueous acetic acid, the oxazole ring smoothly cleaves to form phthalimide-protected phosphopeptide mimetics **2a–f** in high yields (see Table 1). Compounds **2a–f** appear as white crystals soluble in methylene chloride and hexane. Their ^{1}H NMR spectra show a characteristic signal from the methine proton of the NH–CH–P moiety as a doublet of doublets at 5.11–5.59 ppm with $^2J_{HP}$ = 17.5–20.4 Hz and $^3J_{HH}$ = 7.3–9.5 Hz. The signals from the NH group of compounds **2a–e** appear at 7.01–7.83 ppm as a broad doublet with $^3J_{HH}$ = 7.3–9.6 Hz (see Table 2).

On treating phthalimido derivatives **2a–e** of hydrazine hydrate in ethanol, phosphopeptide mimetics **3a–e** result (see Table 1). Incubating the reaction mixture at 50–60°C for 3–4 h has been found to be the optimum condition for phthalimide deprotection. This convenient synthetic approach enables smooth formation of compounds **3a–e** and easy separation of the phthalazide produced in the reaction.

We also adapted our method to access more complex structures **6** and **7** as shown in Scheme 2. It seemed reasonable to obtain them from unsaturated azlactones readily cleavage by *N*-nucleophiles to provide dehydroamino acids [22–26]. However, the interaction of azlactone **4** with 2-aminoalkyloxazoles **5** in glacial acetic acid does not proceed smoothly and products **6** could not be isolated from the reaction mixture. When this reaction was performed in boiling dioxane, peptidomimetics **6** and imidazolone derivatives **7** were formed in a ratio independent of the reaction time. In this case, compounds **7a,b** were isolated in a pure state (Table 1). When toluene was heated at 65°C, compounds **6** were solely formed and imidazolones **7** were not even detected by LS/MS.

Products **6a–e** are isolated as thick oils and crystallize within 1–2 weeks. The ^{1}H NMR signals from the methylene group of compounds **6a** and **6b** are observed as a doublet at 4.53 and 4.56 ppm, respectively, whereas compounds **6c–e** show the corresponding resonances as multiplets at 3.72–3.76 and 2.89–2.92 ppm.

Imidazolones **7a**,**b** are colorless crystalline solids. Their methylene protons give rise to a singlet ^{1}H resonance at 4.94–4.96 ppm. The IR spectra of compounds **7a** and **7b** show no absorption bands at 3100–3500 cm^{-1} characteristic of the NH group vibrations.

On heating oxazoles **6a–d** or **7a** in 70% aqueous acetic acid, the oxazole ring opens to give, in 79–94% yields, phosphorylated peptidomimetics **8a–d** or **9**, which need no further purification (see Table 1). The oxazole ring cleavage in the conversions **6a–d** → **8a–d** and **7a** → **9** is easy to monitor by ^{1}H NMR spectroscopy. Indeed, compounds **8a–d** and **9** exhibit a characteristic signal from the

TABLE 1 Physical and Analytical Data of Compounds **2, 3, 6–9, 11**

Compound (Product)	*Melting Point (°C)*	*Yield (%)*[a]	*Molecular Formula (Weight)*	*Elemental Analysis, Found (Calcd) (%)*			
				C	*H*	*N*	*P*
2a	191–192	95	$C_{21}H_{28}N_3O_7P$ (465.44)	54.30 (54.19)	6.17 (6.06)	9.19 (9.03)	6.53 (6.65)
2b	185–187	93	$C_{20}H_{26}N_3O_8P$ (467.41)	51.59 (51.39)	5.80 (5.61)	9.01 (8.99)	6.52 (6.63)
2c	197–198	88	$C_{23}H_{26}N_3O_7P$ (487.44)	56.52 (56.67)	5.55 (5.38)	8.68 (8.62)	6.34 (6.46)
2d	176–178	96	$C_{22}H_{30}N_3O_7P$ (479.47)	55.22 (55.11)	6.55 (6.31)	8.83 (8.76)	6.40 (6.43)
2e	195–196	90	$C_{21}H_{28}N_3O_8P$ (481.44)	52.43 (52.39)	5.98 (5.86)	8.77 (8.73)	6.32 (6.43)
2f	195–197	95	$C_{24}H_{28}N_3O_7P$ (501.47)	57.56 (57.48)	5.75 (5.63)	8.46 (8.38)	6.08 (6.18)
3a	oil	75	$C_{13}H_{26}N_3O_5P$ (335.34)	46.55 (46.56)	7.92 (7.81)	12.60 (12.53)	9.16 (9.24)
3b	oil	76	$C_{12}H_{24}N_3O_5P$ (337.31)	42.75 (42.73)	7.19 (7.17)	12.49 (12.46)	9.14 (9.18)
3c	oil	78	$C_{15}H_{24}N_3O_5P$ (357.34)	50.44 (50.42)	6.89 (6.77)	11.89 (11.76)	8.57 (8.67)
3d	oil	77	$C_{14}H_{28}N_3O_5P$ (349.36)	48.23 (48.13)	8.25 (8.08)	12.06 (12.03)	8.75 (8.87)
3e	oil	78	$C_{13}H_{26}N_3O_6P$ (351.34)	44.52 (44.44)	7.49 (7.46)	11.99 (11.96)	8.71 (8.82)
6a	69–71	86(93)	$C_{30}H_{37}N_4O_6P$ (580.61)	62.18 (62.06)	6.55 (6.42)	9.68 (9.65)	5.21 (5.33)
6b	79–80	83(95)	$C_{29}H_{35}N_4O_7P$ (582.59)	59.85 (59.79)	6.11 (6.06)	9.71 (9.62)	5.30 (5.32)
6c	83–85	92(95)	$C_{31}H_{39}N_4O_6P$ (594.64)	62.75 (62.61)	6.50 (6.61)	9.49 (9.42)	5.13 (5.21)
6d	75–77	85(92)	$C_{30}H_{37}N_4O_7P$ (596.611)	60.40 (60.39)	6.39 (6.25)	9.48 (9.39)	5.14 (5.19)
6e	65–66	79(90)	$C_{33}H_{37}N_4O_6P$ (616.64)	64.32 (64.28)	6.13 (6.05)	9.14 (9.09)	4.96 (5.02)
7a	183–185	10	$C_{30}H_{35}N_4O_5P$ (562.60)	64.09 (64.04)	6.35 (6.27)	10.07 (9.96)	5.47 (5.51)
7b	176–178	11	$C_{29}H_{33}N_4O_6P$ (564.57)	62.76 (61.69)	5.75 (5.89)	10.02 (9.92)	5.48 (5.49)
8a	86–87	88	$C_{30}H_{39}N_4O_7P$ (598.63)	60.25 (60.19)	6.63 (6.57)	9.39 (9.36)	5.11 (5.17)
8b	94–96	87	$C_{29}H_{37}N_4O_7P$ (600.60)	57.99 (57.99)	6.36 (6.21)	9.46 (9.33)	5.06 (5.16)
8c	93–95	88	$C_{31}H_{41}N_4O_7P$ (612.65)	60.86 (60.77)	6.89 (6.75)	9.19 (9.14)	5.03 (5.06)
8d	77–78	79	$C_{31}H_{41}N_4O_7P$ (612.65)	58.68 (58.62)	6.52 (6.40)	9.20 (9.12)	5.01 (5.04)
9	98–100	94	$C_{30}H_{37}N_4O_6P$ (580.61)	62.10 (62.06)	6.48 (6.42)	9.72 (9.65)	5.22 (5.33)
11a	80–82	64	$C_{30}H_{41}N_4O_7P$ (600.64)	60.02 (59.99)	6.98 (6.88)	9.39 (9.33)	5.11 (5.16)
11b	82–83	53	$C_{31}H_{43}N_4O_7P$ (614.67)	60.15 (60.07)	7.15 (7.05)	9.18 (9.11)	5.00 (5.04)

[a]A value in brackets is yield by method B (see Experimental).

methine proton of the NH–CH–P moiety as a doublet of doublets at 5.43–5.54 ppm. The methylene proton resonances of the glycine residue in products **8a**,**b** are shifted upfield and appear as a multiplet at 4.15–4.16 ppm. The α- and β-methylene protons of the β-alanine moiety in compounds **8c**,**d** are nonequivalent and give rise to two groups of multiplets at 3.64–3.75 and 2.50–2.60 ppm, respectively. For imidazolone derivative **9**, the methylene proton resonances of the glycine residue are also shifted upfield to 4.47 ppm.

Several methods are available for the double bond reduction in α,β-dehydroamino acids [12, 27–29] but none concerning the case when their residues are incorporated into phosphopeptide mimetics. To obtain reduced analogues of compounds **8**, we attempted, unsuccessfully, their direct hydrogenation with zinc in an AcOH:HCl (10:3) mixture. In contrast, oxazoles **6a**,**c** were thus reduced directly to peptidomimetics **11a**,**b** in good yields (53%–64%), without isolation of intermediate products **10a**,**b (**Scheme 3 and Table 1).

Compounds **11a**,**b** containing two asymmetric carbon atoms are isolated as 1:1 diastereomeric mixtures. This is demonstrated well by their ^{31}P NMR spectra in which two separate equally intense singlets are observed at 18.3 and 18.4 ppm for compound **11a** and at 18.5 and 18.8 ppm for **11b**. In the corresponding ^1H NMR spectra, the methine proton of the NHCHP moiety manifests itself by a multiplet at 5.46 and 5.49 ppm for compounds **11a** and **11b**, respectively, thus also suggesting the oxazole ring cleavage and the second chiral center formation. The methine proton of the $NHCHCH_2$ moiety gives rise to a multiplet at 4.70 ppm for compound **11a** and to two multiplets at 5.01 and 4.87 ppm for **11b**.

To conclude, we have developed a novel synthetic route to peptidomimetics with a diethoxyphosphoryl group in the side chain. The method that is based on acid-assisted oxazole ring cleavage in 5-alkylamino-2-aminoalkyl-1,3-oxazol-4-ylphosphonic acid derivatives affords, in high yields and without laborious purification, new compounds of the peptide nature containing the phosphonoglycine moiety. A combined azlactone/oxazole strategy of the peptide synthesis has enabled the preparation of phosphorylated peptidomimetics with dehydroamino acid residues. Under the appropriate conditions chosen for C=C bond reduction, the latter products have been converted to their saturated analogues.

TABLE 2 Spectroscopic Data of Compounds **2, 3, 6–9, 11**

Compound	*IR (KBr) (cm^{-1})*	*^{1}H NMR ($CDCl_3$/TMS) (ppm)*	*^{31}P NMR ($CDCl_3$/H_3PO_4) (ppm)*	*Mass-Spectrum: m/z, $[M]^+$ (ret. time, min)*
2a	3285,1727, 1677, 1638, 1254, 1026, 955	1.32 (m, 6H, $2OCH_2\underline{CH_3}$), 1.61–1.64 (m, 6H, $3CH_2$ piperidine), 3.54 (m, 1H, $^1/_2CH_2$ piperidine), 3.60 (m, 3H, CH_2 piperidine, $^1/_2CH_2$ piperidine), 4.16 (m, 4H, $2O\underline{CH_2}CH_3$), 4.48 (s, 2H, CH_2), 5.55 (dd, 1H, CH, $^2J_{HP} = 17.5$ Hz, $^3J_{HH} = 8.3$ Hz), 7.56 (d, 1H, NH, $^3J_{HH} = 8.3$ Hz), 7.75–7.89 (m, 4H, arom)	17.2	465 (0.86)
2b	3331,1725–1656, 253,1025,961	1.32 (m, 6H, $2OCH_2\underline{CH_3}$), 3.53 (m, 2H, CH_2 morpholine), 3,66 (m, 2H, CH_2 morpholine), 3.76 (m, 4H, CH_2 morpholine), 4.17 (m, 4H, $2O\underline{CH_2}CH_3$), 4.48 (s, 2H, CH_2), 5.52 (dd, 1H, NH, $^2J_{HP} = 18.3$ Hz, $^3J_{HH} = 8.6$ Hz), 7.75–7.88 (m, 4H, arom), 7.83 (d, 1H, NH, $^3J_{HH} = 8.6$ Hz)	17.0	467 (0.80)
2c	3256, 1717, 1634, 1266, 1017, 977	1.25 (m, 6H, $2OCH_2\underline{CH_3}$), 4.13 (m, 4H, $2O\underline{CH_2}CH_3$), 4.44–4.50 (m, 4H, CH_2 glycine, CH_2 benzylamine), 5.11 (dd, 1H, CH, $^2J_{HP} = 19.1$ Hz, $^3J_{HH} = 7.3$ Hz), 7.01 (d, 1H, NH, $^3J_{HH} = 7.3$ Hz), 7.16 (br s, 1H, NH), 7.23–7.86 (m, 9H, arom)	19.2	487 (0.79)
2d	3403, 1717, 1644, 1245, 1023	1.29 (m, 6H, $2OCH_2\underline{CH_3}$), 1.62–1.66 (m, 6H, $3CH_2$ piperidine), 2.73 (m, 2H, CH_2), 3.51 (m, 1H, $^1/_2CH_2$ piperidine), 3.57 (m, 2H, CH_2 piperidine), 3.72 (m, 1H, $^1/_2CH_2$ piperidine), 4.02 (m, 2H, CH_2), 4.11 (m, 4H, $2O\underline{CH_2}CH_3$), 5.59 (dd, 1H, NH, $^2J_{HP} = 18.3$ Hz, $^3J_{HH} = 8.6$ Hz), 7.31 (d, 1H, NH, $^3J_{HH} = 8.6$ Hz), 7.72–7.84 (m, 4H, arom)	17.6	479 (0.83)
2e	3401, 1720, 1651, 1245, 1026	1.28 (m, 6H, $2OCH_2\underline{CH_3}$), 2.73 (m, 2H, CH_2), 3.56 (m, 2H, NCH_2 morpholine), 3.66 (m, 1H, $^1/_2CH_2$, morpholine), 3.75 (m, 5H, $^1/_2CH_2$ morpholine, $2CH_2$ morpholine), 4.00 (m, 2H, CH_2), 4.12 (m, 4H, $2O\underline{CH_2}CH_3$), 5.57 (dd, 1H, CH, $^2J_{HP} = 19.4$ Hz, $^3J_{HH} = 9.6$ Hz), 7.63 (d, 1H, NH, $^3J_{HH} = 9.6$ Hz), 7.72–7.83 (m, 4H, arom)	17.6	481 (0.88)
2f	3288, 1721,1670, 1234, 102, 983	1.19–1.24 (m, 6H, $2OCH_2\underline{CH_3}$), 2.71 (m, 2H, CH_2), 3.98 (m, 4H, $2O\underline{CH_2}CH_3$), 4.13 (m, 2H, CH_2), 4.40 (dd, 1H, CH, $^2J_{HH} = 14.8$ Hz, $^3J_{HH} = 5.6$ Hz), 4.47 (dd, 1H, CH, $^2J_{HH} = 14.8$ Hz, $^3J_{HH} = 5.8$ Hz), 5.16 (dd, 1H, CH, $^3J_{HP} = 20.4$ Hz, $^3J_{HH} = 8.2$ Hz), 7.10 (d, 1H, NH, $^3J_{HH} = 8.2$ Hz), 7.26–7.28 (m, 5H, arom), 7.37 (m, 1H, NH), 7.68–7.78 (m, 4H, arom)	19.4	501 (0.86)
3a	3298, 1638, 233, 1049, 947	1.34 (m, 6H, $2OCH_2\underline{CH_3}$), 1.59–1.66 (m, 6H, $3CH_2$ piperidine), 1.97 (br s, 2H, NH_2), 3.42 (s, 2H, CH_2), 3.60 (m, 3H, CH_2, $^1/_2CH_2$ piperidine), 3.68 (m, 1H, $^1/_2CH_2$ piperidine), 4.18 (m, 4H, $2O\underline{CH_2}CH_3$), 5.56 (dd, 1H, CH, $^2J_{HP} = 17.1$ Hz, $^3J_{HH} = 7.3$ Hz), 8.13 (d, 1H, NH, $^3J_{HH} = 7.3$ Hz)	18.1	335 (0.70)
3b	3329, 1649, 1241, 1026, 965	1.33 (m, 6H, $2OCH_2\underline{CH_3}$), 3.04 (br s, 2H, NH_2), 3.44 (s, 2H, CH_2), 3.54–3.81 (m, 8H, $4CH_2$ morpholine), 4.18 (m, 4H, $2O\underline{CH_2}CH_3$), 5.53 (dd, 1H, CH, $^2J_{HP} = 17.2$ Hz, $^3J_{HH} = 6.2$ Hz), 8.13 (d, 1H, NH, $^3J_{HH} = 6.2$ Hz)	17.3	337 (0.49)

(Continued)

TABLE 2 Continued

Compound	*IR (KBr) (cm^{-1})*	*^{1}H NMR ($CDCl_3$/TMS) (ppm)*	*^{31}P NMR ($CDCl_3$/H_3PO_4) (ppm)*	*Mass-Spectrum: m/z, $[M]^+$ (ret. time, min)*
3c	3309, 1675, 1247, 1025, 977	1.29 (m, 6H, $2OCH_2\underline{CH_3}$), 2.40 (br s, 2H, NH_2), 3.44 (s, 2H, CH_2), 4.08 (m, 2H, $O\underline{CH_2}CH_3$), 4.20 (m, 2H, $O\underline{CH_2}CH_3$), 4.44 (dd, 1H, CH, $^2J_{HH} = 15.3$ Hz, $^3J_{HH} = 5.2$ Hz), 4.54 (dd, 1H, CH, $^2J_{HH} = 15.3$ Hz, $^3J_{HH} = 6.4$ Hz), 5.18 (dd, 1H, $^2J_{HP} = 20.5$ Hz, $^3J_{HH} = 8.3$ Hz), 7.32 (m, 6H, arom; NH), 8.03 (d, 1H, NH, $^3J_{HH} = 8.3$ Hz)	17.1	357 (0.68)
3d	3301, 1663, 1242, 1028, 963	1.31 (m, 6H, $2OCH_2\underline{CH_3}$), 1.57–1.64 (m, 6H, $3CH_2$ piperidine), 2.61 (m, 2H, CH_2), 3.13 (m, 2H, CH_2), 3.56 (m, 4H, $2CH_2$ piperidine), 4.15 (m, 4H, $2O\underline{CH_2}CH_3$), 5.48 (dd, 1H, CH, $^2J_{HP} = 17.2$ Hz, $^3J_{HH} = 6.8$ Hz), 8.01 (d, 1H, NH, $^3J_{HH} = 6.8$ Hz), 8.17 (br s, 2H, NH_2)	18.5	349 (0.67)
3e	3291, 1652, 1231, 1026, 966	1.31 (m, 6H, $2OCH_2\underline{CH_3}$), 2.64 (m, 2H, CH_2), 3.14 (m, 2H, CH_2), 3.52–3.76 (m, 8H, $4CH_2$ morpholine), 4.16 (m, 4H, $2O\underline{CH_2}CH_3$), 5.48 (m, 1H, CH), 6.28 (br s, 2H, NH_2), 8.18 (m, 1H, NH)	18.2	351 (0.65)
6a	3250, 1652,1625,1578, 1278, 1024,966	1.30 (m, 6H, $2OCH_2\underline{CH_3}$), 1.63 (m, 6H, $3CH_2$ piperidine), 2.33 (s, 3H, CH_3), 3.48 (m, 4H, $2CH_2$ piperidine), 4.08 (m, 4H, $2O\underline{CH_2}CH_3$), 4.53 (d, 2H, CH_2, $^3J_{HH} = 6.2$ Hz), 7.13–7.90 (m, 9H, arom), 7.30 (s, 1H, CH), 7.38 (br s, 1H, NH), 8.20 (br s, 1H, NH)	14.2	–
6b	3258, 1662, 1578, 1151, 1271, 1024, 967	1.31 (m, 6H, $2OCH_2\underline{CH_3}$), 2.34 (s, 3H, CH_3), 3.58 (m, 4H, $2CH_2$ morpholine), 3.78 (m, 4H, $2CH_2$ morpholine), 4.10 (m, 4H, $2O\underline{CH_2}CH_3$), 4.56 (d, 2H, CH_2, $^3J_{HH} = 5.2$ Hz), 7.05 (br s, 1H, NH), 7.15–7.87 (m, 9H, arom), 7.24 (s, 1H, CH), 7.94 (br s, 1H, NH)	13.0	–
6c	3246, 1626, 1578, 1283, 1025, 966	1.27 (m, 6H, $2OCH_2\underline{CH_3}$), 1.63 (m, 6H, $3CH_2$ piperidine), 2.33 (s, 3H, CH_3), 2.89 (m, 2H, CH_2), 3.46 (m, 4H, $2CH_2$ piperidine), 3.75 (m, 2H, CH_2), 4.05 (m, 4H, $2O\underline{CH_2}CH_3$), 7.10–7.91 (m, 11H, arom, CH, NH), 8.20 (br s, 1H, NH)	14.1	–
6d	3255, 1604, 1579, 1275, 1025, 967	1.27 (m, 6H, $2OCH_2\underline{CH_3}$), 2.32 (s, 3H, CH_3), 2.90 (m, 2H, CH_2), 3.53 (m, 4H, $2CH_2$ morpholine), 3.72 (m, 2H, CH_2), 3.77 (m, 4H, $2CH_2$ morpholine), 7.02 (br s, 1H, NH), 7.12–7.88 (m, 10H, arom, CH), 8.16 (br s, 1H, NH)	13.4	–
6e	3249, 1637, 1281, 1024, 967	1.25 m, (6H, $2OCH_2\underline{CH_3}$), 2.34 (s, 3H, CH_3), 2.92 (m, 2H, CH_2), 3.76 (m, 2H, CH_2), 4.02 (m, 4H, $2O\underline{CH_2}CH_3$), 4.47 (d, 2H, CH_2, $^3J_{HH} = 5.7$ Hz), 6.32 (t, 1H, NH, $^3J_{HH} = 5.7$ Hz), 6.97–7.86 (m, 17H, arom, CH, 2NH), 7.56 (br s, 1H, NH)	14.5	–
7a	1720, 1643, 1255, 1036, 965	1.32 (m, 6H, $2OCH_2\underline{CH_3}$), 1.61 (m, 6H, $3CH_2$ piperidine), 2.42 (s, 3H, CH_3), 3.46 (m, 4H, $2CH_2$ piperidine), 4.07 (m, 4H, $2O\underline{CH_2}CH_3$), 4.94 (s, 2H, CH_2), 7.28–8.16 (m, 10H, arom, CH)	13.5	–
7b	1716, 1629, 1261, 1029, 966	1.33 (m, 6H, $2OCH_2\underline{CH_3}$), 2.42 (s, 3H, CH_3), 3.53 (m, 4H, $2CH_2$ morpholine), 3.76 (m, 4H, $2CH_2$ morpholine), 4.10 (m, 4H, $2O\underline{CH_2}CH_3$), 4.96 (s, 2H, CH_2), 7.28–8.15 (m, 10H, arom, CH)	12.3	–

(Continued)

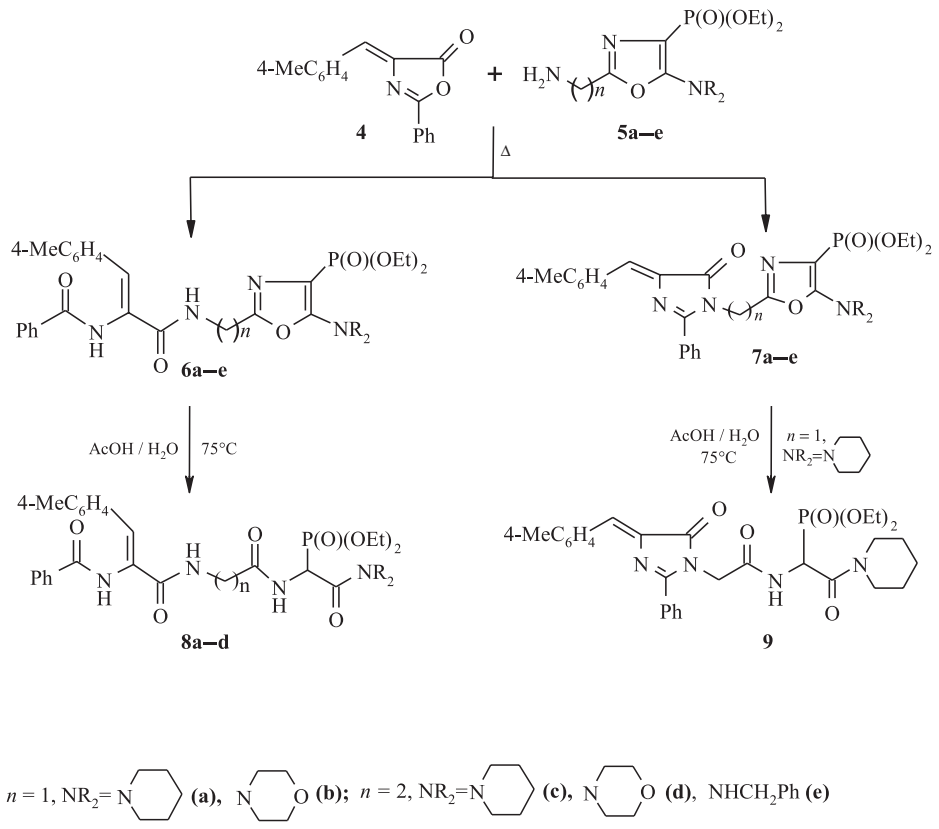
TABLE 2 Continued

Compound	*IR (KBr) (cm^{-1})*	*^{1}H NMR ($CDCl_3$/TMS) (ppm)*	*^{31}P NMR ($CDCl_3$/H_3PO_4) (ppm)*	*Mass-Spectrum: m/z, $[M]^+$ (ret. time, min)*
8a	3402, 1644, 1249, 1023, 976	1.29 (m, 6H, $2OCH_2\underline{CH_3}$), 1.55–1.63 (m, 6H, $3CH_2$ piperidine), 2.33 (s, 3H, CH_3), 3.54 (m, 4H, $2CH_2$ piperidine), 4.15 (m, 6H, $2O\underline{CH_2}CH_3$, CH_2), 5.54 (dd, 1H, CH, $^2J_{HP}$ = 17.6 Hz, $^3J_{HH}$ = 8.3 Hz), 7.13–7.94 (m, 11H, arom, 2NH), 8.21 (s, 1H, NH)	17.5	–
8b	3283, 1651, 1252, 1026, 975	1.31 (m, 6H, $2OCH_2\underline{CH_3}$), 2.34 (s, 3H, CH_3), 3.48–3.76 (m, 8H, $4CH_2$ morpholine), 4.16 (m, 6H, $2O\underline{CH_2}CH_3$, CH_2), 5.49 (dd, 1H, CH, $^2J_{HP}$ = 16.4 Hz, $^3J_{HH}$ = 7.5 Hz), 7.16–7.91 (m, 12H, arom, CH, 2NH), 8.01 (s, 1H, NH)	17.0	–
8c	3250, 1627,1260, 1025, 966	1.20 (m, 6H, $2OCH_2\underline{CH_3}$), 1.54–1.65 (m, 6H, $3CH_2$ piperidine), 2.32 (s, 3H, CH_3), 2.50 (m, 1H, CH), 2.60 (m, 1H, CH), 3.53 (m, 4H, $2CH_2$ piperidine), 3.64 (m, 1H, CH), 3.71 (m, 1H, CH), 4.08 (m, 4H, $2O\underline{CH_2}CH_3$), 5.45 (dd, 1H, CH, $^2J_{HP}$ = 17.6 Hz, $^3J_{HH}$ = 8.3 Hz), 7.11–7.95 (m, 12H, C_6H_5, C_6H_4, CH, 2NH), 8.55 (br s, 1H, NH)	18.3	–
8d	3286, 1649, 1250, 1025, 974	1.26 (m, 6H, $2OCH_2\underline{CH_3}$), 2.33 (s, 3H, CH_3), 2.58 (m, 2H, CH_2), 3.49–3.75 (m, 10H, $4CH_2$ morpholine, CH_2), 4.09 (m, 4H, $2O\underline{CH_2}CH_3$), 5.43 (dd, 1H, CH, $^2J_{HP}$ = 17.7 Hz, $^3J_{HH}$ = 8.0 Hz), 7.04–7.93 (m, 12H, arom, CH, 2NH), 8.23 (br s, 1H, NH)	17.0	–
9	3291, 1721, 1676, 1642, 1262, 1023, 968	1.29 (m, 6H, $2OCH_2\underline{CH_3}$), 1.66 (m, 6H, $3CH_2$ piperidine), 2.41 (s, 3H, CH_3), 3.56 (m, 4H, $2CH_2$ piperidine), 4.13 (m, 4H, $2O\underline{CH_2}CH_3$), 4.47 (s, 2H, CH_2), 5.50 (dd 1H, CH, $^2J_{HP}$ = 16.6 Hz, $^3J_{HH}$ = 8.0 Hz), 7.25–8.15 (m, 9H, arom), 7.39 (d, 1H, NH, $^3J_{HH}$ = 8.0 Hz)	17.2	–
11a	3332, 1643, 1235, 1019	1.21 (m, 6H, $2OCH_2\underline{CH_3}$), 1.44, 1.59 (m, 6H, $3CH_2$ piperidine), 2.22 (s, 3H, CH_3), 2.98, 3.09 (m, 2H, CH_2), 3.48 (m, 4H, $2CH_2$ piperidine), 3.85 (m, 2H, CH_2 glycine), 4.04 (m, 4H, $2O\underline{CH_2}CH_3$), 4.70 (m, 1H, CH), 5.46 (m, 1H, CHP), 7.05–7.82 (m, 9H, arom), 8.39 (m, 1H, NH), 8.44 (m, 1H, NH), 8.60 (m, 1H, NH).	18.3 18.4	–
11b[a]	3328, 1655, 1243, 1022	1.29 (m, 6H, $2OCH_2\underline{CH_3}$), 1.62 (m, 6H, $3CH_2$ piperidine), 2.29 (s, 3H, CH_3), 2.33, 2.52 (m, 2H, $NH\underline{CH_2}CH_2$), 3.10–3.31 (m, 4H, CH_2, 1H, $^1/_2NH\underline{CH_2}\underline{CH_2}CO$), 3.46–3.78 (m, 5H, $2CH_2$- piperidine, 1H, $^1/_2NHCH_2\underline{CH_2}CO$), 4.14 (m, 4H, $2O\underline{CH_2}CH_3$), 4.87, 5.01 (m, 1H, CH), 5.49 (m, 1H, CHP), 7.07- 7.72 (m, 12H, arom, 3NH).	18.5 18.8	–

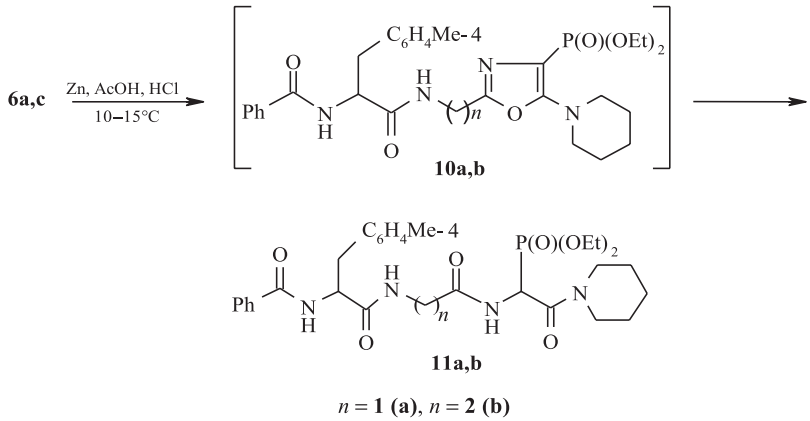
[a] ^{1}H and ^{31}P NMR spectra were recorded in DMSO-d_6.

EXPERIMENTAL

All starting materials were purchased from Acros, Merck, and Fluka. Solvents were purified according to the standard procedures. The progress of reactions was monitored by TLC (Silicagel ALUGRAM® SIL G/UV_{254} by MACHEREY-NAGEL, Germany). IR spectra were recorded on a Vertex 70 spectrophotometer in KBr tablets. The ^{1}H, ^{31}P, and ^{13}C NMR spectra were measured on a spectrometer Bruker Avance DRX-500 (Germany; 500, 202, and 125 MHz, respectively) in $CDCl_3$ solution. Chemical shifts are reported relative to internal $Si(CH_3)_4$ (^{1}H, ^{13}C) or external 85% H_3PO_4 (^{31}P).



SCHEME 2



SCHEME 3

LC/MS spectra were registered using a liquid chromatography/mass spectrometric system consisting of an Agilent 1100 series high-performance liquid chromatograph equipped with a diode matrix and an Agilent LC/MSD SL mass-selective detector. LC/MS analysis was performed on a 4.6 × 15 mm Zorbax SB-C18 column (Agilent Technologies, Santa Clara, CA), with 1.8 μm particle size (PN 821975-932); an acetonitrile:water (95:5) mixture with 0.1% trifluoroacetic acid was used as solvent A and 0.1% aqueous trifluoroacetic acid as solvent B; the eluent flow rate was 3 mL/min, and the injection volume was 1 μL; the elution was monitored simultaneously with three UV detectors, at 215, 254, and 285 nm; analytes were ionized by atmospheric pressure chemical ionization; the scan range was *m/z* 80–1000. Melting points were measured with a Fisher-Johns apparatus (Thermo Fisher Scientific, Dubuque, IA).

N-(Phthalimidoacetyl) diethoxyphosphorylglycine alkylamides **2a–c**

A solution of one of compounds **1a–c** (0.01 mol) in an AcOH:H_2O (5:1) mixture (50 mL) was heated at 75°C in a water bath for 7 h, followed by the evaporation of the reaction mixture to dryness under reduced pressure. To remove AcOH completely, the residue was treated with a 5% Na_2CO_3 solution (20 mL) and extracted with CH_2Cl_2 (3×50 mL). The extract was dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The resulting product was analyzed without further purification.

2a. ^{13}C NMR: δ = 16.3, 24.3, 25.5, 26.0, 40.3; 44.0, 48.5, 63.8, 123.6, 132.1, 134.1, 163.5, 165.5, 167.7.
2b. ^{13}C NMR: δ = 16.3, 40.2, 46.9, 47.2, 48.4, 64.1, 66.5, 123.6, 132.1, 134.2, 164.2, 165.9, 167.7.
2c. ^{13}C NMR: δ = 16.2, 40.3, 44.0, 50.1, 51.2, 64.4, 123.5, 127.4, 127.5, 128.6, 132.1, 134.1, 137.6, 164.5, 166.2, 167.6.

N-(β-Phthalimidopropionyl) diethoxyphosphorylglycine alkylamides **2d–f** were obtained from corresponding oxazoles **1d–f** similarly to compounds **2a–c**.

2e. ^{13}C NMR: δ = 16.3, 34.2, 34.3, 47.0, 47.1, 48.00, 66.5, 63.8, 123.3, 132.1, 134.0, 164.4, 168.0, 169.1.
2f. ^{13}C NMR: δ = 16.2, 34.2, 34.3, 43.8, 49.9, 51.0, 64.1, 123.2, 132.1, 134.1, 127.3, 127.5, 128.5, 137.7, 164.9, 168.0, 169.8.

N-Glycyldiethoxyphosphorylglycine alkylamides **3a–c**

To a solution of one of compounds **2a–c** (0.01 mol) in EtOH (50 mL), hydrazine hydrate (0.012 mol, 1.2 mL) was added and the mixture was heated at 50–55°C for 1–2 h under TLC control (Eluent system - CH_2Cl_2:CH_3OH as 95:5). The resulting white precipitate was filtered off, and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 mL), and the insoluble phthalazide was filtered off, followed by the evaporation of the filtrate to dryness under reduced pressure. The products appeared as thick oils.

N-(β-Alanyl)diethoxyphosphorylglycine alkylamides **3d,e** were obtained from corresponding compounds **2d,e** similarly to compounds **3a–c**.

Diethyl 5-alkylamino-2-{N-[N-benzoyl-(4-methylbenzylidene)glycyl]aminomethyl}-1,3-oxazol-4-yl-phos-phonates **6a**,**b** *and Diethyl 5-alkylamino-2-[4,5-dihydro-4-(4-methylbenzylidene)-5-oxo-2-phe-ny-limidazol-1-ylmethyl]-1,3-oxazol-4-ylphosphonates* **7a**,**b**

Method A. To a solution of one of compounds **5a**,**b** (0.015 mol) in anhydrous dioxane (50 mL), azlactone **4** (2.6 g, 0.01 mol) was added and the mixture was heated at reflux for 5–6 h under TLC control. The solvent was evaporated in vacuo, the residue was dissolved in toluene (20 mL), and the solution was diluted with 70–100C petroleum ether. The mixture was allowed to stand at 5–10°C for 2–3 h and decanted. The oily residue was concentrated under reduced pressure and treated with 50% aqueous EtOH to crystallize **7a** or **7b**. The toluene/petroleum ether solution was evaporated in vacuo to give pure final product **6a** or **6b.**

Method B. To a solution of one of compounds **5a**,**b** (0.015 mol) in anhydrous toluene (50 mL), azlactone **4** (2.6 g, 0.01 mol) was added and the mixture was heated at 60–65°C for 5–6 h under TLC control. The solvent was evaporated in vacuo to give a pure product **6a** or **6b.**

6a. ^{13}C NMR: δ = 16.3, 21.8, 23.9, 25.4, 37.8, 49.4, 62.22, 99.8, 100.2, 127.8, 130.9, 128.3, 128.5, 129.7, 132.0, 132.5, 133.2, 139.0, 142.4, 149.2, 150.3, 161.1, 161.9, 163.0, 167.8.

Diethyl 5-alkylamino-2-{N-[N-benzoyl-(4-methylbenzylidene)glycyl]aminoethyl}-1,3-oxazol-4-yl-phosphonates **6c–e** were obtained from corresponding compounds **5c–e** similarly to compounds **6a**,**b**.

6c. ^{13}C NMR: δ = 16.3, 21.4, 24.0, 25.4, 27.7, 36.7, 49.5, 61.2, 98.6, 100.7, 127.7, 128.5, 128.5, 128.7, 129.2, 129.4, 131.0, 132.1, 133.2, 152.3, 152.4, 161.6, 161.9, 162.0, 165.9, 166.3.
6d. ^{13}C NMR: δ = 16.3, 21.3, 27.6, 36.8, 48.4, 62.2, 66.2, 66.3, 62.33, 101.1, 101.2, 127.8, 128.4, 128.5, 128.9, 129.3, 130.9, 131.9, 132.9, 139.0, 153.0, 153.2, 162.4; 166.4, 166.7.

N-{N-[N-Benzoyl-(4-methylbenzylidene)glycyl] glycyl} diethoxyphosphorylglycine alkylamides **8a**,**b** were obtained from corresponding compounds **6a**,**b** similarly to compounds **2a–c**.

8a. ^{13}C NMR: δ = 16.4, 21.4, 43.0, 46.8, 47.3, 48.6, 64.1, 66.5, 127.7, 127.9, 128.8, 129.0, 128.9, 129.3, 129.6, 130.8, 132.4, 132.9, 139.4, 164.1, 164.2, 165.9, 168.4.

N-{N-[N-Benzoyl-(4-methylbenzylidene)glycyl]-β-alanyl}diethoxyphosphorylglycine alkylamides **8c**,**d** were obtained from corresponding compounds **6c**,**d** similarly to compounds **8a**,**b**.

N-[4,5-Dihydro-4-(4-methylbenzylidene)-5-oxo-2-phenylimidazol-1-ylacetyl]diethoxyphosphorylglycine piperidide **9** was obtained from compound **7a** similarly to compounds **2a–c**.

N-{N-[N-Benzoyl-(4-methylbenzyl) glycyl]glycyl} diethoxyphosphorylglycine piperidide **11a**

To a stirred and cooled (5–10°C) solution of compound **6a** (0.58 g, 0.001 mol) in an AcOH:HCl (10:3) mixture (13 mL), powdered Zn (2g) was added in 25-mg portions during 5–10 min, followed by stirring the reaction mixture at 5–10°C for another 10 min under TLC control. The resulting precipitate was filtered off and washed with anhydrous AcOH. After evaporation of the solvent in vacuo, the product was purified by recrystallization from CH_3CN.

N-{N-[N-Benzoyl-(4-methylbenzyl)glycyl]-β-alanyl}diethoxyphosphorylglycine piperidide **11b** was obtained from compound **6c** similarly to compound **11a**.

REFERENCES

[1] Kukhar, V.; Hudson, H. Aminophosphonic and Aminophosphinic acids; Wiley: New York, 1999.
[2] Hugles, A. Amino Acids, Peptides and Proteins in Organic Chemistry; Wiley-VCH: Weinheim, Germany, 2009.
[3] Kafarski, P.; Lejezak, B. Curr Med Chem 2001, 1, 301–312.
[4] Lejezak, B.; Kafarski, P. Top Heterocyclic Chem 2009, 20, 31–64.
[5] Berwe, M.; Winfried, J.; Krüger, J.; Cancho-Crande, Y.; Lampe, T.; Michels, M.; Paulsen, H.; Raddatz, S.; Weigand, S. Org Proc Res Dev 2011, 15, 1348–1357.
[6] Horenstein, B.; Nakanishi, K. J Am Chem Soc 1989, 111, 6242–6247.
[7] Kim, D.; Li, Y.; Horenstein, B.; Nakanishi, K. Tetrahedron Lett 1990, 31, 7119–7122.
[8] Schmidt, U.; Wild, I. Angew Chem 1984, 96, 996–997.
[9] Schmidt, U.; Wild, I. Liebigs Ann Chem 1985, 9, 1882–1894.
[10] Schmidt, U.; Lieberknecht, A.; Griesser, H.; Bökens, H. Liebigs Ann Chem 1985, 4, 785–793.
[11] Kunze, T.; Heps, S. Biochem Pharm 2000, 59, 973–981.
[12] Kazmaier, V.; Amino Acids, Peptides and Proteins in Organic Chemistry; Hunghes A., Ed.; Wiley-VCH: Weinheim, Germany, 2009, 3–34.
[13] Saavedna, C.; Boto, A.; Hernandes, R. Org Lett, 2012, 14, 3788–3791.
[14] Kunze, T. Arch Pharm 1996, 329, 503–509.
[15] Köhler, B.; Shöllkopf, U. Liebigs Ann Chem 1987, 3, 267–269.
[16] Schmidt, U.; Lieberknecht, A.; Wild, J. Synth Commun 1984, 1, 53–60.
[17] Schmidt, U.; Schanbacher, U. Angew Chem 1983, 95, 150–151.
[18] Schmidt, U.; Beuttler, T.; Lieberknechtt, A.; Criesser, H. Tetrahedron Lett 1983, 24, 3573–3576.
[19] Röhr, G.; Schnell, M.; Köckritz, A. Synthesis 1992, 10, 1031–1034.
[20] Kondratyuk, K. M.; Lukashuk, E. I.; Golovchenko, A. V.; Rusanov, E. V.; Brovarets, V. S. Zh Obshch Khim 2012, 82, 556–565.
[21] Kondratyuk, K. M.; Lukashuk, E. I.; Golovchenko, A. V.; Brovarets, V. S. Tetrahedron (in press).
[22] Greenstein, I. P.; Winitz, M. Chemistry of the Amino Acids; Wiley: New York, 1961, 2, p. 823.
[23] Gross, E.; Meienhoter, I. The Peptides; Academic Press: New York, 1979, 1, p. 2.
[24] Phelps, D. I.; Godreau, P. V.; Nicholas, E. S. J Chem Soc, Perkin Trans 2, 1981, 1, 140–142.
[25] Tripathy, P. K.; Mukerjee, A. K. Synthesis 1985, 3, 285–288.
[26] Abdallah, M. A.; Zayed, M. E.; Shawali, A. S. Indian J Chem, Sect B: Org Chem Med Chem 2001, 40, 3, 187–190.
[27] Bergel, F.; Stock, I. A. J Chem Soc 1957, 4563–4567.
[28] Lisichkina, I. N.; Vinogradova, A. I.; Bachurina, I. B.; Kurkovska, L. N.; Belikov, V. M. Izv Akad Nauk SSSR, Ser Khim 1994, 5, 884–886.
[29] Davies, J. S.; Eaton, M. C.; Ibrahim, M. N. J Heterocyclic Chem 1980, 17, 1813–1814.