Synthesis of phosphamide conjugates of 5´-mononucleotides with carcinine and its analogs

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The reactions of histamine, the natural dipeptide carcinine (β-Ala-HA), and its analogs with 5´-monodeoxyribonucleotides (dNMP) in the presence of triphenylphosphine, 2,2´-dipyridyl disulfide, and *N*-methylimidazole were studied. The yield of phosphamide derivatives decreases from 72% to 17% as the length of the linker group between the imidazole ring and the terminal aliphatic amino group is increased. Hydrolytic stability of the resulting conjugates was examined. The stability of the bonds in the $—O—P(O)_2—NH—$ group linking the nucleotide and peptide portions of the conjugate depends on the nature of the heterocyclic base of the nucleotide and decreases in the series dTMP > dCMP > dAMP.

Key words: carcinine, histamine, mononucleotide, phosphamide, mononucleotide-peptide conjugate.

Oligonucleotide conjugates with peptides, peptide mimics, and a series of other ligands find wide application in different fields of molecular biology and are considered as promising therapeutic agents.[1,2] Recently, a radically new approach to the design of highly selective pharmaceuticals has been developed[3] based on complementary oligonucleotide complexes. In this case, one of the oligonucleotides is bound to the pharmaceutical molecule *via* a labile bond, whereas the second oligonucleotide bears the histamine residue, which serves as a catalyst of the cleavage of this labile bond. A release of the pharmaceutical molecule into the cell medium occurs only upon the complex formation on the corresponding RNA (DNA) template, for example, on the fragment of viral RNA.

Investigations aimed at searching for pharmaceuticals based on oligonucleotide derivatives called for the development of simple approaches to the construction of conjugates with different functional molecules. Two radically different strategies for synthesizing oligonucleotide conjugates with different ligands have gained wide acceptance. These involve either the introduction of ligands in one of the steps of the solid-phase oligonucleotide synthesis or postsynthetic modifications of oligonucleotides.[4] There are several versions of both the first and second approaches. Generally, the first strategy involves the automatic synthesis of oligonucleotides based on protected nucleosides as phosphoramidites and ligands. This procedure also shows promise in the successive synthesis of the oligonucleotide and peptide fragments of a conjugate on the same solid-phase support.[5] However, this strategy has limited utility for most potential consumers of these constructs because of the need for the synthesis of unique phosphoramidites in each particular case as well as due to the difficulties associated with the selection of protective groups and procedures for their removal in the successive synthesis of oligonucleotide conjugates. To the contrary, the synthesis of unmodified oligonucleotides was well developed by many biochemical companies due to which the postsynthetic modification of oligonucleotides shows considerable promise. The advantages of both strategies can be combined in the same procedure if one of the synthetic steps involves the introduction of functional groups into the oligonucleotide and these groups are selectively activated in the postsynthetic step. The oligonucleotide synthesis remains to be simple because a limited spectrum of simple phosphoramidites containing such groups can be used. The ligands are introduced at the postsynthetic step, which allows researchers in non-synthetic laboratories to readily synthesize series of conjugates based on a single oligonucleotide. Presently, oligodeoxyribonucleotides bearing the 5´- or 3´-terminal phosphate group are commercially available reagents. Taking into account this fact together with an available simple procedure for the activation of the terminal phosphate in the presence of other reactive centers of the deblocked oligonucleotide (nitrogen atoms of heterocyclic bases, exocyclic amino groups, internucleotide phosphates), the terminal phosphate can be considered as one of the most promising precursor groups. One commonly accepted procedure for the activation in oligonucleotides involves the

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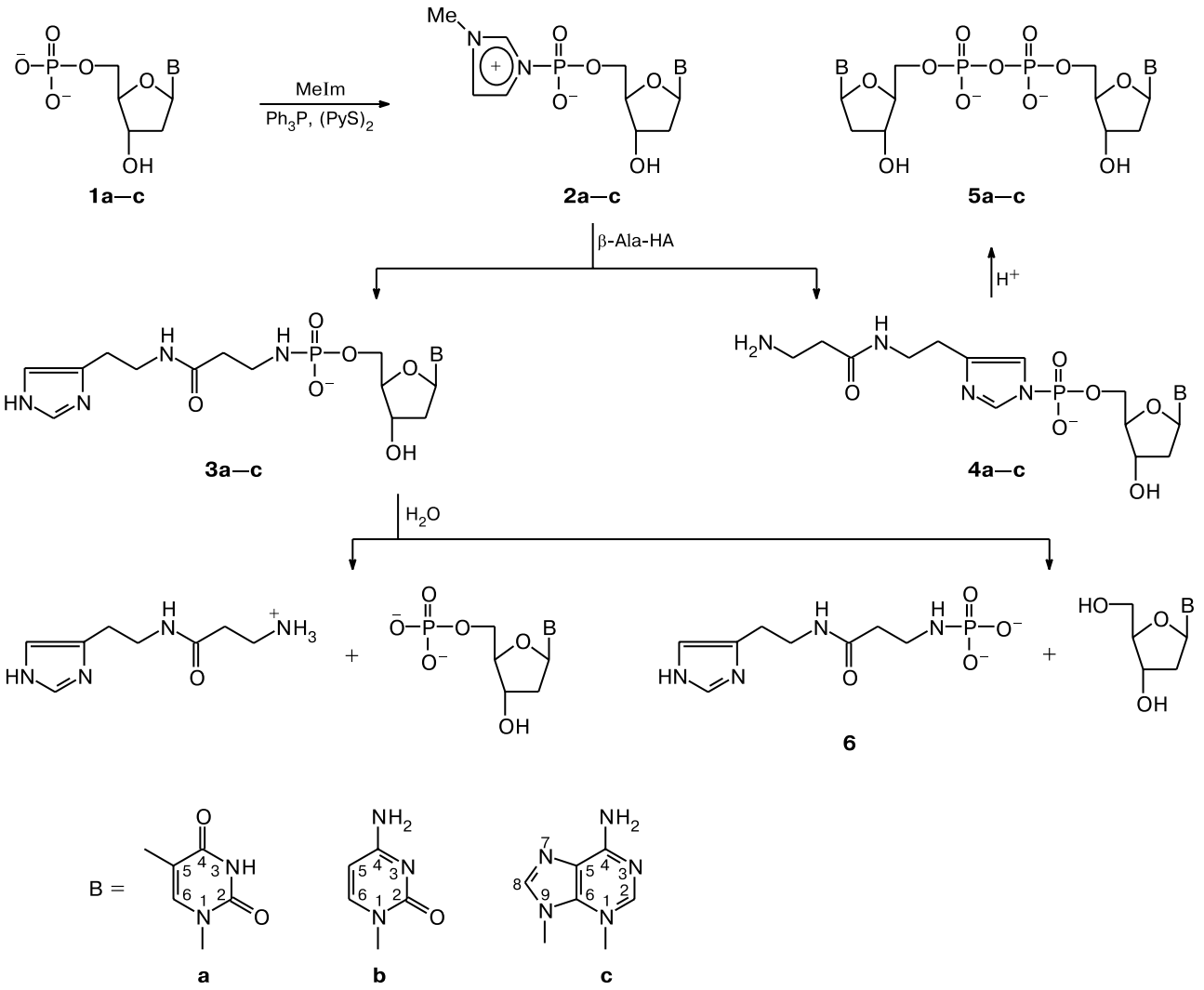
Mukayama method[6] modified by Godovikova and coworkers[7] with the use of the triphenylphosphine—2,2′-dipyridyl disulfide (Ph_3P—$(PyS)_2$) redox pair in the presence of *N*-methylimidazole (MeIm). The distinguishing feature of this modification is the formation of an intermediate (Scheme 1), which possesses high reactivity to aliphatic amino groups and does not virtually react with functional groups of the deblocked nucleotide. Previously, it has been demonstrated that this procedure made it possible to prepare oligonucleotide conjugates with short peptides containing the guanidine groups[8,9] as well as tetranucleotide conjugates with histamine[10] and with the natural antibiotic bleomycin.[11] However, the structures of the conjugates thus constructed were confirmed only by indirect methods.

The present study was aimed at constructing conjugates of monodeoxyribonucleotides (dNMP) with histamine, the natural dipeptide carcinine (β-Ala-HA), and its analogs. One would expect that the observed main reaction pathways will take place also in the synthesis of peptide-oligonucleotide conjugates from phosphorylated oligodeoxyribonucleotides and unprotected peptides containing aliphatic amino groups and histidine residues.

The conjugates of dTMP (**1a**), dCMP (**1b**), and dAMP (**1c**) with carcinine were synthesized according to Scheme 1. Triethylammonium salts of 5′-phosphorylated mononucleotides were dissolved in a minimum volume of DMSO and then fivefold molar excesses of Ph_3P, $(PyS)_2$, and MeIm in DMF were added. Intermediate zwitterionic compounds **2a—c** were generated at room temperature during 10—15 min. The dipeptide dissolved in DMF (0.1—0.5 mol L^{-1}) was added to the reaction mixtures containing active derivatives **2a—c**. The reaction mixtures were stirred at room temperature for 15—20 min. The nucleotide material and, partially, carcinine were precipitated with a 6% $LiClO_4$ solution in acetone. After

Scheme 1



the removal of excesses of the activating reagents and their transformation products, the reaction mixtures were analyzed by reversed-phase and ion-exchange chromatography and NMR spectroscopy. Incubation of the reaction mixtures at room temperature over a longer period of time led to accumulation of impurities of the unknown nature whose ^{31}P NMR spectra have signals with a complex structure at δ from −10 to −16. The ratio of the major reaction products remained virtually unchanged.

^{31}P NMR spectroscopy is the most informative method, which enables one to estimate the quantitative compositions of the reaction mixtures. The assignment of the signals in the NMR spectra was made according to the data published in the literature.[12] After the removal of an excess of triphenylphosphine and its transformation products, the spectra of the reaction mixtures show signals assigned to the target phosphamides **3a—c** (δ_P 8.57—10.46), phosphoimidazolides **4a—c** (δ_P from −8.36 to −9.36), and symmetrical pyrophosphates **5a—c** (δ_P from −10.22 to −11.54). In addition to the above-mentioned signals, the spectra have signals at δ 7.7—7.9. The intensities of these signals were minimum for the thymidine derivative (2%) and maximum for the 2′-deoxycytosine derivative (13%) (Table 1). After the chromatographic separation of the reactions mixtures, individual products **3a—c** were characterized by ^{1}H spectroscopy and mass spectrometry. The ^{1}H NMR spectra of the resulting compounds correspond to those expected. However, in the spectra of compounds **3a** and **3b**, the signals for the proton in the second position of the imidazole rings are split (J = 12.5 Hz). This effect was reproducible and is, apparently, associated with the presence of different tautomeric forms, which were fixed for some reason. The correct explanation of the observed effect calls for the additional investigation.

The ^{31}P NMR spectra, which was measured within 15 min after the addition of a 0.1 *M* TFA solution to the reaction solution in an NMR tube (to pH 3), had no signals at δ from −8.36 to −9.36, and the main signals were those of phosphamides **3a—c** (δ_P 8.57—10.46) and pyrophosphates **5a—c** (δ_P from −10.22 to −11.54). Compounds **3a—c** and **5a—c** can be readily separated by HPLC. The ^{31}P NMR spectra of the corresponding fractions showed signals only of the individual compounds. However, the spectrum, which was measured after incubation of compound **3c** in an aqueous solution at neutral pH for 24 h, again had a signal at δ 7.89 (17%) along with a signal at δ 8.67 assigned to phosphamide **3c**. The former signal is most likely to be attributed to phosphorylation at the amino group of carcinine **6** formed through the intramolecular cleavage of the phosphodiester bond in compound **3c**. This assumption is evidenced by the fact that the mass spectrum of the sample obtained after evaporation of an aqueous solution had peaks corresponding to deoxyadenosine and phosphorylated carcinine. The data from ion-exchange chromatography are also indicative of the formation of the nucleoside. At the same time, compound **3a** appeared to be stable in the pH range of 4.6—8.2 for 30 h, whereas compound **3b** was stable at pH 6.8—8.2. However, a further decrease in pH led (according to the data from ion-exchange chromatography) to decomposition of compound **3b** to deoxycytidine and dCMP (pH 5.2, 30 h, 27% and 4%, respectively). Evidently, this difference in the properties of the resulting compounds is attributable to specific intramolecular interactions of the heterocyclic bases of adenine and cytidine with the phosphamide fragment.

Due to the low solubility of the starting dGMP in a DMSO/DMF mixture, we failed to prepare the conjugate of carcinine with this nucleotide according to the above-described scheme.

The effect of the length of the linker binding the imidazole residue and the terminal aliphatic amino group was studied for interactions of histamine (HA), carcinine (β-Ala-HA), γ-aminobutyrylhistamine (γ-Abu-HA), and ε-aminocaproylhistamine (ε-Aca-HA) with 5′-dTMP. After separation of an excess of the activating reagents and their transformation products, the compositions of the reaction mixtures were analyzed by ^{31}P NMR spectroscopy and ion-exchange chromatography. The ratios of the reaction products are given in Table 2. The phosphamide derivatives were isolated from the reaction mixtures in pure form by HPLC and characterized by

Table 1. Compositions of the reaction mixtures (%) in the reactions of deoxynucleoside 5′-phosphates (dpA, dpC, or dpT) with carcinine (β-Ala-HA) according to the ^{31}P NMR spectroscopic data

Reaction product	dpA	dpC	dpT
Phosphamides **3a—c**	58	57	56
Imidazolides **4a—c**	18	14	15
Pyrophosphates **5a—c**	11	24	27
Phosphamides **6***	13	5	2

* Phosphamide **6** was not isolated in pure form and its structure was proposed based on the spectroscopic and chromatographic data obtained for the reaction mixtures.

Table 2. Compositions of the reaction mixtures (%) in the reactions of deoxythymidine-5′-phosphate (dpT) with histamine (HA) and its derivatives, *viz.*, β-Ala-HA (I), γ-Abu-HA (II), and ε-Aca-HA (III), according to the ^{31}P NMR spectroscopic data

Reaction product	HA	I	II	III
Phosphamide	72	56	41	17
Imidazolide	9	15	33	23
Pyrophosphate	19	27	25	59

^{31}P NMR spectroscopy and mass spectrometry. The above-considered data demonstrate that an increase in the length of the linker group between the imidazole ring and terminal amino group leads to a decrease in the yields of the phosphamide derivatives. In the case of ε-Aca-HA, symmetrical pyrophosphate was obtained as the major reaction product. This change in the ratio of the reaction products with increasing distance between the aliphatic amino group and imidazole ring is, apparently, associated with intramolecular interactions between the imidazole and phosphate groups of the conjugates.

To summarize, the main reaction pathways were exemplified by the synthesis of the conjugates of monodeoxyribonucleotides (dNMP) with histamine, the natural dipeptide carcinine (β-Ala-HA), and its analogs (γ-Abu-HA and ε-Aca-HA). These results are of importance for the construction of peptide-oligonucleotide conjugates from phosphorylated oligodeoxyribonucleotides and unprotected peptides containing the aliphatic amino group and histidine residues.

Experimental

In this study, the following reagents were used: deoxymononucleotides (dNMP) (Special Design Office of Biologically Active Compounds, Main Administration of the Microbiological Industry (Glavmikrobioprom), Novosibirsk, Russia); triphenylphosphine (Ph_3P), 2,2′-dipyridyl disulfide $(PyS)_2$, histamine, $LiClO_4$ (Fluka AG, Switzerland); *N*-methylimidazole (MeIm) (Sigma, USA); trifluoroacetic anhydride (TFAA), tributylamine (Bu_3N) (Aldrich, USA); 2-(*N*-morpholino)ethanesulfonic acid (MES); *N*-2-hydroxyethylpiperazine-*N*′-3-propanesulfonic acid (HEPPS) (FisherBiotech, USA); trifluoroacetic acid (TFA), succinic acid, and NaOH of high-purity grade. Carcinine (β-Ala-HA) and its analogs (γ-Abu-HA and ε-Aca-HA) were prepared according to a procedure described previously.[13] The organic solvents, *viz.*, *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), triethylamine (TEA), methanol (MeOH), chloroform, acetone, acetonitrile (MeCN), and diethyl ether, were purified and dried according to standard procedures.[14] In the reactions, bidistilled water was used.

Preparative HPLC was carried out on a Waters 600 chromatograph equipped with a Waters 486 UV detector and a 1×25-cm LiChroprep RP-18 column (15—25 μm; Merck, Germany); the gradient from 0 to 40% of MeCN in H_2O; 0.03 *M* $LiClO_4$, the rate of elution was 2 mL min^{-1}. Analytical ion-exchange chromatography was performed on a Milikhrom-4 chromatograph (Production Association "Nauchpribor", Orel, Russia) equipped with 2×62-mm columns with Polysil SA, 10 μm (Research and Production Association "Vektor", Novosibirsk, Russia); the gradient of a potassium phosphate buffer (KH_2PO_4/K_2HPO_4; pH 7.2) in 30% aqueous MeOH from 0 to 0.6 *M*; the rate of elution was 100 μL min^{-1}.

The NMR spectra were recorded on a Bruker AM-400 spectrometer (Germany) at 400 MHz for ^{1}H (Me_4Si as the internal standard) and at 162 MHz for ^{31}P (85% H_3PO_4 as the external standard). All spin-spin coupling constants are given in Hz. The chemical shifts are given in the δ scale. The mass spectra were obtained on a Vision 2000 spectrometer (MALDI).

Determination of hydrolytic stability of the conjugates. Hydrolytic stability of the resulting compounds was studied in the following buffer systems: succinic acid—NaOH (pH 4.6—5.8), MES—NaOH (pH 5.8—7.0), and HEPPS—NaOH (pH 7.0—8.2) at 20 °C over a period of time from 1 to 30 h. The reaction mixtures were analyzed by reversed-phase chromatography.

2′-Deoxythymidine-5′-*N*-(2-{*N*-[2-(1*H*-imidazol-4-yl)ethyl]carbamoyl}ethyl)phosphamide (3a). Compound **1a** (dTMP) (10 mg, 0.03 mmol, acid) was dissolved in H_2O (10 mL), then Et_3N (9 μL, 0.06 mmol) was added, and the solvent was evaporated *in vacuo*. The triethylammonium salt of the mononucleotide was dissolved in DMSO (300 μL), solutions of Ph_3P (39 mg, 0.15 mmol) and $(PyS)_2$ (42 mg, 0.15 mmol) in DMF (in 200 μL and 1 μL, respectively) were added, and then MeIm (52 μL, 0.6 mmol) was added. The reaction mixture was incubated for 15 min. A weighed sample of β-Ala-HA (29 mg, 0.15 mmol) was dissolved in DMF (100 μL), Et_3N (45 μL, 0.3 mmol) was added, and the reaction mixture was stirred for 10—15 min. A solution of β-Ala-HA was added to the activated monomer and the reaction mixture was kept at ~20 °C for 20 min. Then a 6% $LiClO_4$ solution in acetone (25 mL) was added to the reaction mixture. The precipitate that formed was separated by centrifugation (3—5 min, 9000 rpm), successively washed with acetone and Et_2O, dried in air, and dissolved in H_2O. Compound **3a** was isolated by preparative HPLC. The fraction containing the target product was concentrated to dryness and reprecipitated from H_2O with a 6% $LiClO_4$ solution in acetone. Compound **3a** was obtained as the lithium salt in 42% yield.

^{1}H NMR (D_2O), δ: 2.01 (m, 3 H, CH_3 of thymidine); 2.50 (m, 4 H, C(2′)H_2, C(O)$\underline{CH_2}CH_2$); 2.98 (t, 2 H, Im—$\underline{CH_2}$—CH_2, J = 7.0 Hz); 3.35 (m, 2 H, C(O)$CH_2\underline{CH_2}$—NH); 3.59 (t, 2 H, Im—CH_2—$\underline{CH_2}$, J = 7.0 Hz); 4.08 (m, 2 H, C(5′)H_2); 4.24 (m, 1 H, H(3′)); 4.56 (m, 1 H, H(4′)); 6.43 (t, 1 H, H(1′), J = 7.0 Hz); 7.20 (s, 1 H, H(5) of imidazole); 7.79 (s, 1 H, H(6)); 8.23 (d, 1 H, H(2) of imidazole, J = 12.5 Hz). ^{31}P NMR (D_2O), δ: 8.57 (s). MS of MALDI, *m/z*: 493.5 [M + H]. $C_{18}H_{27}LiN_6O_8P$. Calculated: [M + H] = 493.2.

2′-Deoxycytidine-5′-*N*-(2-{*N*-[2-(1*H*-imidazol-4-yl)ethyl]carbamoyl}ethyl)phosphamide (3b). Compound **3b** was prepared from dCMP (10 mg, 0.03 mmol) and β-Ala-HA (29 mg, 0.15 mmol) analogously to compound **3a**. The yield as the lithium salt was 58%. ^{1}H NMR (D_2O), δ: 2.44 (m, 4 H, C(2′)H_2, C(O)$\underline{CH_2}CH_2$); 2.90 (t, 2 H, Im—$\underline{CH_2}$—CH_2, J = 7.0 Hz); 3.11 (m, 2 H, C(O)$CH_2\underline{CH_2}$—NH); 3.53 (t, 2 H, Im—CH_2—$\underline{CH_2}$ J = 7.0 Hz); 4.05 (m, 2 H, C(5′)H_2); 4.24 (s, 1 H, H(3′)); 4.50 (m, 1 H, H(4′)); 6.14 (d, 1 H, H(5), J = 8.0 Hz); 6.41 (t, 1 H, H(1′), J = 7.0 Hz); 7.10 (s, 1 H, H(5) of imidazole); 7.97 (d, 1 H, H(6), J = 8.0 Hz); 8.04 (s, 1H, H(2) of imidazole). ^{31}P NMR (D_2O), δ: 10.46 (s). MS of MALDI, *m/z*: 478.5 [M + H]. $C_{17}H_{26}LiN_7O_7P$. Calculated: [M + H] = 478.2.

2′-Deoxyadenosine-5′-*N*-(2-{*N*-[2-(1*H*-imidazol-4-yl)ethyl]carbamoyl}ethyl)phosphamide (3c). Compound **3c** was prepared from dAMP (10 mg, 0.03 mmol) and β-Ala-HA (29 mg, 0.15 mmol) analogously to compound **3a**. The yield as the lithium salt was 59%. ^{1}H NMR (D_2O), δ: 2.44 (m, 4 H, C(2′)H_2, C(O)$\underline{CH_2}CH_2$); 2.91 (t, 2 H, Im—$\underline{CH_2}$—CH_2, J = 7.0 Hz); 3.09

(m, 2 H, C(O)$CH_2\underline{CH_2}$NH); 3.54 (t, 2 H, Im—CH_2—$\underline{CH_2}$, J = 7.0 Hz); 4.03 (m, C(5´)H_2); 4.21 (s, 1 H, H(3´)); 4.70 (m, 1 H, H(4´)); 6.41 (t, 1 H, H(1´), J = 7.0 Hz); 7.10 (s, 1 H, H(5) of imidazole); 7.78 (s, 1 H, H(2)); 8.03 (s, 1 H, H(2) of imidazole); 8.08 (s, 1 H, H(8)). ^{31}P NMR (D_2O), δ: 8.67 (s). MS of MALDI, m/z: 493.5 [M + H]. $C_{18}H_{26}LiN_9O_6P$. Calculated: [M] = 495.2.

2´-Deoxythymidine-5´-*N*-[2-(1*H*-imidazol-4-yl)ethyl]phosphamide. The compound was prepared from dTMP (10 mg, 0.03 mmol) and histamine (17 mg, 0.15 mmol) analogously to compound **3a**. The yield as the lithium salt was 72%. ^{1}H NMR (D_2O), δ: 1.70 (s, 3 H, CH_3 of thymidine); 2.25 (m, 2 H, C(2´)H_2); 2.85 (t, 2 H, Im—$\underline{CH_2}$—CH_2, J = 7.0 Hz); 3.16 (t, 2 H, Im—CH_2—$\underline{CH_2}$, J = 7.0 Hz); 3.84 (m, C(5´)H_2); 4.04 (m, 1 H, H(3´)); 4.46 (m, 1 H, H(4´)); 6.24 (t, 1 H, H(1´), J = 7.0 Hz); 6.92 (s, 1 H, H(5) of imidazole); 7.54 (d, 1 H, H(2) of imidazole, J = 12.5 Hz); 7.82 (s, 1 H, H(6)). ^{31}P NMR (D_2O), δ: 9.03 (s).

2´-Deoxythymidine-5´-*N*-(3-{*N*-[2-(1*H*-imidazol-4-yl)ethyl]carbamoyl}propyl)phosphamide. The compound was prepared from dTMP (10 mg, 0.03 mmol) and γ-Abu-HA (30 mg, 0.15 mmol) analogously to compound **3a**. The yield as the lithium salt was 43%. ^{31}P NMR (D_2O), δ: 10.58 (s). MS of MALDI, m/z: 508.5 [M + 2H]. $C_{19}H_{29}LiN_6O_8P$. Calculated: [M + 2H] = 508.2.

2´-Deoxythymidine-5´-*N*-(5-{*N*-[2-(1*H*-imidazol-4-yl)ethyl]carbamoyl}pentyl)phosphamide. The compound was prepared from dTMP (10 mg, 0.03 mmol) and ω-Aca-HA (35 mg, 0.15 mmol) analogously to compound **3a**. The yield as the lithium salt was 15%. ^{31}P NMR (D_2O), δ: 11.03 (s). MS of MALDI, m/z: 536.3 [M + 2 H]. $C_{21}H_{34}LiN_6O_8P$. Calculated: [M + H] = 536.2.

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References

1. C.-H. Tung, *Bioconjugate Chem.*, 2000, **11**, 605.
2. G. N. Grimm, A. S. Boutorine, and C. Helene, *Nucleosides Nucleotides*, 2000, **19**, 1943.
3. Z. Ma and J.-S. Taylor, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 11159.
4. A. S. Butorin, G. N. Grimm, and K. Elen, *Mol. Biol.*, 2000, **34**, 946 [*Russ. J. Mol. Biol.*, 2000, **34** (Engl. Transl.)].
5. J. Robles, E. Pedroso, and A. Grandas, *Nucleic Acids Res.*, 1995, **23**, 4151.
6. T. Mukayama, *Phosphorus and Sulfur*, 1976, **1**, 371.
7. T. S. Godovikova, V. F. Zarytova, T. V. Mal´tseva, and L. M. Khalimskaya, *Bioorg. Khim.*, 1989, **15**, 1246 [*J. Bioorg. Chem. USSR*, 1989, **15** (Engl. Transl.)].
8. D. V. Pyshnyi, M. N. Repkova, S. G. Lokhov, E. M. Ivanova, A. G. Ven´yaminova, and V. F. Zarytova, *Bioorg. Khim.*, 1997, **23**, 497 [*Russ. J. Bioorg. Chem.*, 1997, **23** (Engl. Transl.)].
9. D. Pyshnyi, M. Repkova, S. Lokhov, E. Ivanova, A. Venyaminova, and V. Zarytova, *Nucleosides Nucleotides*, 1997, **16**, 1571.
10. D. G. Knorre, E. V. Bichenkova, V. V. Koval´, P. V. Alekseev, V. D. Knorre, E. Nordkhof, and T. S. Godovikova, *Bioorg. Khim.*, 1998, **24**, 663 [*Russ. J. Bioorg. Chem.*, 1998, **24** (Engl. Transl.)].
11. V. F. Zarytova, D. S. Sergeyev, and T. S. Godovikova, *Bioconjugate Chem.*, 1993, **4**, 189.
12. A. V. Lebedev and A. I. Rezvukhin, *Bioorg. Khim.*, 1983, **9**, 149 [*J. Bioorg. Chem. USSR*, 1983, **9** (Engl. Transl.)].
13. E. A. Rozhkova, S. A. Ogrel´, D. N. Grigor´ev, V. E. Nebol´sin, G. A. Zheltukhina, and R. P. Evstigneeva, *Bioorg. Khim.*, 1996, **22**, 838 [*Russ. J. Bioorg. Chem.*, 1996, **22** (Engl. Transl.)].
14. A. J. Gordon and R. A. Ford, *The Chemist´s Companion. A Handbook of Practical Data, Techniques, and References*, J. Wiley and Sons, New York, 1972.

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