Chemistry of Natural Compounds and Bioorganic Chemistry

Reagents for addressed modification of biopolymers 11.* The synthesis of N^{Im}-2,4-dinitrophenyl derivatives of histidine and urocanic and imidazolylacetic acids for anchoring imidazole residues to oligonucleotides

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Derivatives of histidine, imidazolylacetic and urocanic acids, containing an N^{Im}-2,4-dinitrophenyl moiety and a hydroxy group were synthesized and characterized. The applicability of these reagents for anchoring imidazole residues to oligonucleotides to give potential site-specific artificial ribonucleases was shown taking the synthesis of dinucleotide derivatives as an example.

Key words: 2,4-dinitrophenyl group, imidazole, histidine, imidazolylacetic acid, urocanic acid, artificial ribonucleases, oligonucleotide derivative.

The presence of histidine residues in the active sites of ribonucleases, together with the ability of the imidazole buffer to hydrolyze phosphodiester bonds in RNA,[2] have stimulated interest in the construction of artificial ribonucleases consisting of imidazole residues linked to polycations,[3,4] intercalators,[5,6] nucleotides,[7–11] and oligonucleotides.[12–15] The interest in these conjugates is due to the potential possibility of using them as therapeutic agents and reagents for the study of RNA structure and functions. Particular attention is attracted by oligonucleotide derivatives with anchored imidazole residues, which combine the hydrolytic activity of imidazole and the capacity for complementary linking to RNA.[16] Previously,[17,18] oligonucleotide derivatives containing imidazole fragments have been synthesized starting from 3′- or 5′-phosphorylated oligodeoxyribonucleotides without using protective groups for the imidazole fragments. A combination of solid-phase peptide and oligonucleotide syntheses has been reported[14]: in this synthesis, an oligonucleotide chain was assembled on the peptide after removal of the protective group from the N atom of the histidine imidazole ring. Nevertheless, it has been noted[7,9–11] that the imidazole residues in the monomer units still need to be protected for the oligonucleotide synthesis.

Several protective groups stable under conditions of oligonucleotide synthesis and cleavable at various steps of deprotection of the oligonucleotides synthesized can

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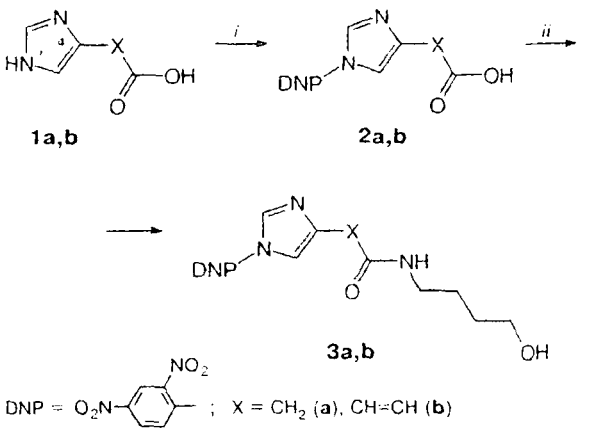
be used to protect the N(1) position of an imidazole ring. The possibility of using various protective groups based on triphenylmethane has been studied.[9] In the case of synthesis of nucleotide derivatives containing imidazole residues, the 2,4-dinitrophenyl group (DNP)[7,9] has also been used. This group is removed by concentrated ammonia in water–organic media or by treatment with benzenethiol.[9,14] Although the above-listed groups have been used in the synthesis of a number of mononucleotide derivatives, synthesis of oligonucleotides from these monomers has not been described.

In this work, we synthesized (Schemes 1 and 2) and characterized derivatives of histidine **6** and imidazolylacetic and urocanic acids (**3a** and **3b**) containing a DNP protective group at the N(1) atom of the imidazole ring and employed compounds **3b** and **6** in the synthesis of oligonucleotides by the phosphotriester method (Scheme 3).

Scheme 1

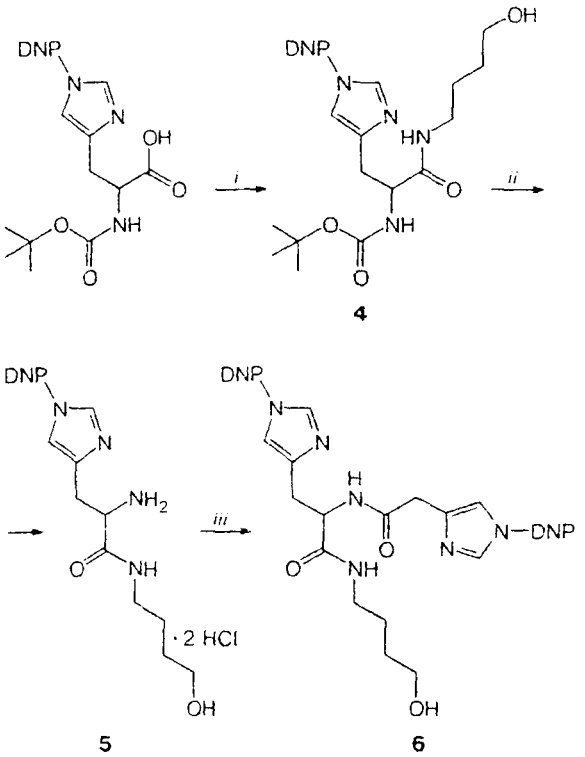


Reagents and conditions: *i*. 1-fluoro-2,4-dinitrobenzene (DNFB); *ii*. *N*-hydroxysuccinimide (NHS) (**a**), pentafluorophenol (PFP) (**b**), dicyclohexylcarbodiimide (DCC), $H_2N(CH_2)_4OH$.

The DNP protective group was introduced into the imidazole ring in compounds **1a,b** by treating the corresponding acids with 2,4-dinitrofluorobenzene (DNFB) in a MeOH—H_2O mixture in the presence of $NaHCO_3$, similarly to the procedure used for histidine.[19] Proteeted acids **2a,b** formed in 72 and 76% yields, respectively, were activated by dicyclohexylcarbodiimide (DCC) in the presence of *N*-hydroxysuccinimide (NHS) and pentafluorophenol (PFP), respectively. Activated esters were made to react with 4-aminobutan-1-ol without isolation (see Scheme 1).

The synthesis of **3a** was accompanied by the formation of a side product with R_f 0.75 (TLC, system **B**), whose ^1H NMR and mass spectra correspond to 4-amino-*N*-(2,4-dinitrophenyl)butan-1-ol (the data are not reported). The yield of this product was greater than 50% when 4-aminobutan-1-ol was taken in a threefold excess with respect to the activated ester of acid **2a** and the condensation was carried out for 12 h. When the excess of $H_2N(CH_2)_4OH$ was only 20% and the reaction time was only 30 min, compound **3a** was prepared in 72% yield (the course of the reaction was monitored by TLC). Acid **2b** was activated by PFP in the presence of DCC and the condensation was continued for 15 min with a 10% excess of $H_2N(CH_2)_4OH$ to give compound **3b** in 74% yield (see Scheme 1).

Scheme 2

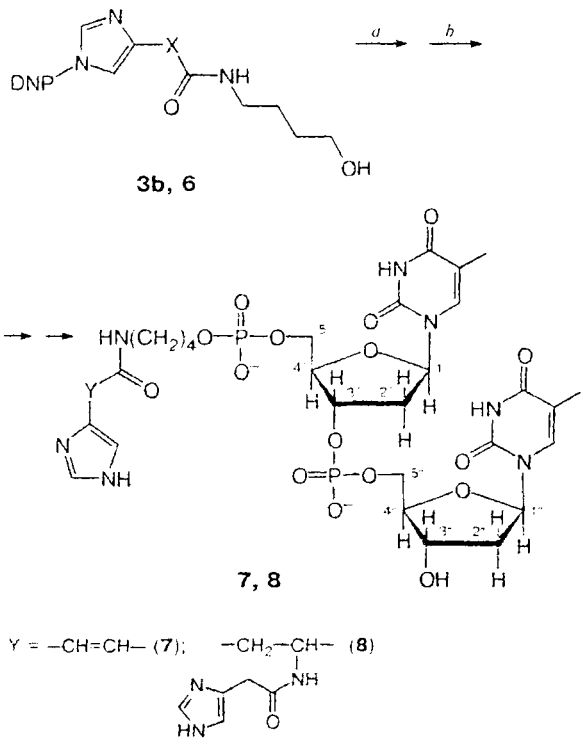


Reagents and conditions: *i*. DCC, PFP, $H_2N(CH_2)_4OH$ (**9**); *ii*. 2 *M* HCl, anhydr. MeOH; *iii*. **2a**, Et_3N, DCC, PFP.

We synthesized bisimidazolyl derivative **6** from DNP-N(1)-Im-protected histidine and imidazolylacetic acid (see Scheme 2). Amide **4** was prepared similarly to **3b**. After removal of the Boc-protective group on treatment with 2 *M* HCl in anhydrous MeOH, compound **5** was made to condense with pre-synthesized activated pentafluorophenyl ester of acid **2a** to give **6**.

The compounds synthesized were characterized by NMR spectroscopy. The signals in the ^1H NMR spectra of compounds **2a**, **3a**, and **4**—**6** were assigned in accordance with the available published data for histidine[9–11] and imidazolylacetic acid[9,10] derivatives and those for compounds **2b** and **3b** were assigned by comparing the spectra with the ^1H NMR spectrum of urocanic acid recorded beforehand. No ^{13}C NMR data for

Scheme 3



Reagents and conditions: *a.* $p^{*}Tp^{*}T$(Lev) (p^{*} is the *p*-chlorophenyl phosphoric acid residue, Lev is levulinoyl), TPSCl, MeIm; *b.* conc. NH_3, 48 h.

DNP-N(1)-histidine, DNP-N(1)-imidazolylacetic acid, or DNP-N(1)-urocanic acid can be found in the literature. The assignment of the chemical shifts in the ^{13}C NMR spectrum of compound **2a** was based on the comparison of the $^{13}C-^{1}H$ coupling constants measured in the ^{13}C NMR spectra recorded without proton decoupling with the published values for these constants[20] and on the magnitudes of residual splitting in the ^{13}C NMR spectra with off-resonance proton irradiation.[21] The signals of the DNP and Im groups in the ^{13}C NMR spectra of compounds **2b**, **3a,b**, and **4** were assigned by analogy with those for compound **2a**.

According to elemental analysis, even after prolonged drying *in vacuo* at 50 °C, compound **2a** exists as a crystal hydrate containing one water molecule per two molecules of **2a**. Although, according to chromatography, products **3a**, **3b**, and **4** were individual compounds, we were unable to obtain correct data of elemental analysis. Compounds **3a**, **3b**, and **4** were used without additional purification.

The mass spectrum (EI) of compound **3a** contains no $[M]^+$ peak but only $[M-OH]^+$. In the case of compound **3b**, whose molecular weight and melting point are greater than those of **3a**, the EI mass spectrum contains the $[M]^+$ peak with a relative intensity of 0.7%.

To find out whether the imidazole-containing conjugates prepared here can be used in the synthesis of oligonucleotide derivatives, amides **3b** and **6** were added to protected dinucleotide $p^{*}Tp^{*}T$(Lev) under conditions of phosphotriester oligonucleotide synthesis in solution. The condensation was carried out in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) and *N*-methylimidazole (MeIm), by analogy with a previous study.[22] Deprotection of the imidazole ring occurred simultaneously with the removal of the levulinoyl group during the standard treatment with concentrated aqueous ammonia. The corresponding derivatives **7** and **8** (see Scheme 3) were characterized by ^{1}H NMR spectroscopy and MALDI mass spectrometry.

Thus, compounds **3b** and **6** are suitable for anchoring imidazole residues to oligonucleotides by means of the phosphotriester synthesis of oligonucleotides. The possibility of using the resulting conjugates in the phosphoamidite synthesis of oligonucleotides and the properties of the conjugates are currently under investigation.

Experimental

The following reagents and equipment were used: 2,4-dinitro-1-fluorobenzene (DNFB) (Fluka); 4(5)-imidazolylacetic acid hydrochloride, 3-(imidazol-4-yl)propenoic (urocanic) acid, triisopropylbenzenesulfonyl chloride (TPSCl), and *N*-methylimidazole (MeIm) (Aldrich); N^{α}-Boc-N^{1}-Im(DNP)-L-histidine monohydrate (Fisher Biotech); dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (NHS) (Sigma); pentafluorophenol (PFP), and 4-aminobutan-1-ol ("pure" grade) distilled *in vacuo*. The protected dinucleotide $p^{*}Tp^{*}T$(Lev) was synthesized at the Institute of Bioorganic Chemistry, Siberian Branch of the Russian Academy of Sciences, by a procedure described previously.[23] Organic solvents were dried and purified by standard procedures.[24] Preparative HPLC was performed on a Waters 600 chromatograph with a Waters 486 UV detector using a 1×25 cm column with LiChroprep RP-18, 15–25 μm (Merck), gradient elution with solutions of MeCN (0 to 80%) in 0.03 *M* $LiClO_4$ over a period of 60 min, elution rate 6 mL min^{-1}. TLC was carried out on HPTLC-Alufolien Kieselgel 60 F_{254} plates (Merck) using the following solvent systems (v/v): $MeOH-CHCl_3-Et_3N$ 30 : 10 : 0.8 (**A**), 10 : 40 : 0.05 (**B**), CH_2Cl_2–MeOH 9 : 1 (**C**), and CH_2Cl_2–EtOH 4 : 1 (**D**). NMR spectra were recorded on Bruker WP-200-SY and Bruker AM-400 spectrometers; mass spectra were run on Finnigan-MAT-8200 (EI, 70 eV) and Vision 2000 (MALDI) mass spectrometers. Melting points were measured on a Kofler hot stage (VEB Analytik).

[1-(2,4-Dinitrophenyl)-1*H*-imidazol-4-yl]acetic acid (2a). Water (7 mL) and 5 *M* NaOH (625 μL) were added with ice cooling and stirring to a mixture of the hydrochloride of acid **1a** (505 mg, 3.11 mmol) and $NaHCO_3$ (700 mg, 8.34 mmol). A solution of DNFB (430 μL, 3.42 mmol) in 7 mL of MeOH was added dropwise to the resulting solution (pH 8.8) with stirring over a period of 30 min. The mixture was kept for 2 h at 20 °C and a solution of DNFB (150 μL, 1.19 mmol) in 3 mL of MeOH was added dropwise over a period of 15 min. Methanol (2 mL) was added to homogenize the reaction mixture, and the mixture was kept at 20 °C for 2 h. Methanol was evaporated *in vacuo*, and the aqueous residue was extracted with ether (5×10 mL) and acidified with 2 *M* HCl to

pH 3. The thick precipitate crystallized on trituration to give red-brown crystals. The yield of compound **2a** after recrystallization from AcOEt was 678 mg (72%), m.p. 83–84 °C, R_f 0.45 (A). Found (%): C, 43.64; H, 2.95; N, 18.24. $C_{11}H_8N_4O_6 \cdot 0.5H_2O$. Calculated (%): C, 43.86; H, 3.01; N, 18.60. ^{1}H NMR (CD_3OD), δ: 3.70 (s, 2 H, C$\underline{H}_2$COOH); 7.35 (s, 1 H, H(5)-Im); 7.94 (s, 1 H, H(2)-Im); 7.97 (d, 1 H, H(6)-DNP, $J_{6,5}$ = 8.9 Hz); 8.68 (dd, 1 H, H(5)-DNP, $J_{5,6}$ = 8.9 Hz, $J_{5,3}$ = 2.6 Hz); 8.98 (d, 1 H, H(3)-DNP, $J_{3,5}$ = 2.6 Hz). ^{13}C NMR (DMSO-d_6), δ: 34.00 (Im-$\underline{C}H_2$); 117.60 (C(5)-Im); 121.23 (C(3)-DNP); 128.65 (C(5)-DNP); 129.60 (C(6)-DNP); 134.70 (C(1)-DNP); 136.88 (C(2)-Im); 137.07 (C(4)-Im); 143.61 (C(2)-DNP); 146.30 (C(4)-DNP); 171.80 (C=O).

3-[1-(2,4-Dinitrophenyl)-1*H*-imidazol-4-yl]propenoic acid (2b). A mixture of acid **1b** (420 mg, 3.04 mmol) and $NaHCO_3$ (711 mg, 8.46 mmol) was dissolved in an ice-cooled mixture of 8 mL of H_2O and 3 mL of MeOH. A solution of DNFB (430 μL, 3.42 mmol) in 7 mL of MeOH was added dropwise with stirring to the resulting solution over a period of 30 min. Methanol was added until the mixture homogenized and the mixture was kept for 16 h at 20 °C. Methanol was evaporated *in vacuo*, and the aqueous residue was washed with ether (4× 5 mL) and acidified with 2 *M* HCl to pH 3. This gave an orange oily precipitate, which crystallized on stirring. The yield of compound **2b** after recrystallization from MeOH was 706 mg (76%), m.p. 190–191 °C, R_f 0.20 (B). Found (%): C, 47.43; H, 2.53; N, 18.23. $C_{12}H_8N_4O_6$. Calculated (%): C, 47.38; H, 2.65; N, 18.42. ^{1}H NMR (CD_3OD), δ: 6.65 (d, 1 H, C$\underline{H}$COOH, J_{trans} = 16.0 Hz); 7.61 (d, 1 H, Im-C$\underline{H}$, J_{trans} = 16.0 Hz); 7.76 (s, 1 H, H(5)-Im); 8.00 (d, 1 H, H(6)-DNP, $J_{6,5}$ = 8.9 Hz); 8.07 (s, 1 H, H(2)-Im); 8.71 (dd, 1 H, H(5)-DNP, $J_{5,6}$ = 8.9 Hz, $J_{5,3}$ = 2.6 Hz); 9.02 (d, 1 H, H(3)-DNP, $J_{3,5}$ = 2.6 Hz). ^{13}C NMR (DMSO-d_6), δ: 117.89 (=$\underline{C}$H–COOH); 121.90 (C(3)-DNP); 123.54 (C(5)-Im); 129.56 (C(5)-DNP); 130.47 (C(6)-DNP); 134.40 (C(1)-DNP); 135.70 (Im-$\underline{C}$H=); 138.65 (C(4)-Im); 139.24 (C(2)-Im); 143.56 (C(2)-DNP); 146.79 (C(4)-DNP); 167.71 (C=O).

4-{[1-(2,4-Dinitrophenyl)-1*H*-imidazol-4-yl]acetyl}aminobutan-1-ol (3a). DCC (213 mg, 1.03 mmol) was added with stirring at 0 °C to a solution of compound **2a** (274 mg, 0.910 mmol) and NHS (119 mg, 1.03 mmol) in 5 mL of DMF, and the mixture was kept for 3 h at 20 °C. A solution of 4-aminobutan-1-ol (104 μL, 1.13 mmol) in 5 mL of DMF was added dropwise to the reaction mixture and the mixture was stirred for 30 min at 20 °C, cooled to 0 °C, filtered, and concentrated *in vacuo*. Ethyl acetate (50 mL) was added to the residue, the insoluble precipitate was filtered off, and the filtrate was washed with a saturated solution of Na_2CO_3 and water and dried with Na_2SO_4. The solvent was evaporated *in vacuo*, 15 mL of 1 *M* HCl was added to the residue, and the mixture was filtered. The pH of the aqueous phase was brought to 5 ($NaHCO_3$), the solution was washed with ether (3×5 mL), and the pH was brought to 7 ($NaHCO_3$). The solution was saturated with NaCl, filtered, and extracted with AcOEt (3× 15 mL). The organic layer was dried with Na_2SO_4 and the solvent was evaporated *in vacuo*. The yield of compound **3a** was 240 mg (72%), m.p. 54–56 °C, R_f 0.47 (B). ^{1}H NMR (CD_3OD), δ: 1.60 (m, 4 H, $CH_2(C\underline{H}_2)_2CH_2$); 3.27 (m, 2 H, NHC$\underline{H}_2$); 3.59 (m, 4 H, Im-C$\underline{H}_2$ and C$\underline{H}_2$OH); 7.32 (s, 1 H, H(5)-Im); 7.95 (s, 1 H, H(2)-Im); 7.98 (d, 1 H, H(6)-DNP, $J_{6,5}$ = 8.9 Hz); 8.69 (dd, 1 H, H(5)-DNP, $J_{5,6}$ = 8.9 Hz, $J_{5,3}$ = 2.6 Hz); 8.96 (d, 1 H, H(3)-DNP, $J_{3,5}$ = 2.6 Hz). ^{13}C NMR (CD_3OD), δ: 26.78 (NHCH$_2$C$\underline{H}_2$); 30.83 ($\underline{C}H_2CH_2OH$); 36.16 (Im-$\underline{C}H_2$); 40.41 (NH$\underline{C}H_2$); 62.46 ($\underline{C}H_2OH$); 119.57 (C(5)-Im); 122.37 (C(3)-DNP); 129.58 (C(5)-DNP); 131.13 (C(6)-DNP); 136.15 (C(1)-DNP); 138.57 (C(2)-Im); 138.65 (C(4)-Im); 145.64 (C(2)-DNP); 148.50 (C(4)-DNP); 172.28 (C=O). MS (EI), *m/z*: 346 $[M - OH]^+$, 275 $[M - NH(CH_2)_4OH]^+$.

4-{3-[1-(2,4-Dinitrophenyl)-1*H*-imidazol-4-yl]propenoyl}aminobutan-1-ol (3b). PFP (369 mg, 2.00 mmol) was added to a solution of compound **2b** (546 mg, 1.79 mmol) in 10 mL of DMF. The mixture was cooled to 0 °C and a solution of DCC (432 mg, 2.09 mmol) in 2 mL of DMF was added. The reaction mixture was stirred for 4 h at 20 °C and filtered. A solution of 4-aminobutan-1-ol (184 μL, 2.00 mmol) was added dropwise to the filtrate over a period of 15 min. The reaction mixture was kept for 15 min and DMF was evaporated *in vacuo*. AcOEt (70 mL) was added to the residue, the insoluble precipitate was filtered off, the product was extracted with brine (2×10 mL) and acidified with HCl to pH 2, the pH of the aqueous phase was brought to 6.5 ($NaHCO_3$), and the light yellow precipitate was filtered off, washed with water (3× 5 mL), and dried *in vacuo*. The yield of compound **3b** was 500 mg (74%), m.p. 121–122 °C, R_f 0.50 (B). ^{1}H NMR (CD_3OD), δ: 1.64 (m, 4 H, $CH_2(C\underline{H}_2)_2CH_2$); 3.32 (m, 2 H, NH$_2C\underline{H}_2$); 3.61 (m, 2 H, C$\underline{H}_2$OH); 6.68 (d, 1 H, C$\underline{H}$CONH, J_{trans} = 15.6 Hz); 7.45 (d, 1 H, Im-C$\underline{H}$, J_{trans} = 15.6 Hz); 7.67 (s, 1 H, H(5)-Im); 7.99 (d, 1 H, H(6)-DNP, $J_{6,5}$ = 8.9 Hz); 8.07 (s, 1 H, H(2)-Im); 8.70 (dd, 1 H, H(5)-DNP, $J_{5,6}$ = 8.9 Hz, $J_{5,3}$ = 2.4 Hz); 9.02 (d, 1 H, H(3)-DNP, $J_{3,5}$ = 2.4 Hz). ^{13}C NMR (DMSO-d_6), δ: 25.87 (NHCH$_2$C$\underline{H}_2$); 29.96 ($\underline{C}H_2CH_2OH$); 38.58 (NH$\underline{C}H_2$); 60.45 ($\underline{C}H_2OH$); 121.13 (=$\underline{C}$HCONH); 121.39 (C(3)-DNP, C(5)-Im); 128.85 (C(5)-DNP); 129.85 (Im-$\underline{C}$H=); 129.88 (C(6)-DNP); 134.43 (C(1)-DNP); 138.78 (C(2)-Im); 139.36 (C(4)-Im); 143.49 (C(2)-DNP); 146.57 (C(4)-DNP); 165.09 ($\underline{C}$ONH). MS (EI), *m/z*: 375 $[M]^+$, 358 $[M - OH]^+$, 287 $[M - NH(CH_2)_4OH]^+$.

4-[N^{α}-*tert*-Butyloxycarbonyl-$N^{im(1)}$-2,4-dinitrophenyl-L-histidyl]aminobutan-1-ol (4). DCC (1.11 g, 5.38 mmol) was added at 0 °C to a solution of N^{α}-Boc-N^{1}-Im(DNP)-L-His · H_2O (2.10 g, 4.77 mmol) and PFP (994 mg, 5.40 mmol) in 40 mL of CH_2Cl_2. The mixture was stirred for 3 h at 20 °C and filtered. The precipitate was washed with 5 mL of CH_2Cl_2. A solution of 4-aminobutan-1-ol (480 μL, 5.22 mmol) in 3 mL of CH_2Cl_2 was added dropwise to the filtrate. The mixture was stirred for 40 min at 20 °C, washed with brine (3×20 mL) acidified by citric acid to pH 3.5 and with water (2×20 mL), and dried with Na_2SO_4. Then CH_2Cl_2 was evaporated *in vacuo* and the oily precipitate was washed with ether (3×20 mL). Ethyl acetate (25 mL) was added to the residue, the mixture was stirred for 10 min and filtered, the precipitate was washed with 5 mL of AcOEt, and the combined filtrate was concentrated *in vacuo*. The yield of compound **4** was 1.84 g (3.74 mmol, 78%). ^{1}H NMR (CD_3OD), δ: 1.45 (s, 9 H, Boc); 1.55 (m, 4 H, $CH_2(C\underline{H}_2)_2CH_2$); 3.01 (m, 2 H, Im-C$\underline{H}_2$); 3.21 (m, 2 H, NHC$\underline{H}_2$); 3.56 (m, 2 H, C$\underline{H}_2$OH); 4.33 (m, 1 H, C$\underline{H}$-α); 7.20 (s, 1 H, H(5)-Im); 7.94 (d, 1 H, H(6)-DNP, $J_{6,5}$ = 8.9 Hz); 7.96 (s, 1 H, H(2)-Im); 8.69 (dd, 1 H, H(5)-DNP, $J_{5,6}$ = 8.9 Hz, $J_{5,3}$ = 2.5 Hz); 8.98 (d, 1 H, H(3)-DNP, $J_{3,5}$ = 2.5 Hz). ^{13}C NMR (CD_3OD), δ: 26.86 (NHCH$_2$C$\underline{H}_2$); 28.67 ($\underline{C}H_3$(Boc)); 30.82 ($\underline{C}H_2CH_2OH$); 31.68 (Im-$\underline{C}H_2$); 40.26 (NH$\underline{C}H_2$); 56.07 ($\underline{C}$H-α); 62.52 ($\underline{C}H_2OH$); 80.56 ($(CH_3)_3\underline{C}$); 119.22 (C(5)-Im); 122.35 (C(3)-DNP); 129.48 (C(5)-DNP); 131.01 (C(6)-DNP); 136.14 (C(1)-DNP); 138.54 (C(2)-Im); 140.58 (C(4)-Im); 145.83 (C(2)-DNP); 148.66 (C(4)-DNP); 157.47 ($\underline{C}$=O(Boc)); 174.00 ($\underline{C}$=O(His)).

4-[$N^{im(1)}$-2,4-Dinitrophenyl-L-histidyl]aminobutan-1-ol dihydrochloride (5). Compound **4** (1.40 g, 2.84 mmol) was suspended in 5 mL of anhydrous MeOH and 5 mL of a 4 *M* solution of HCl in anhydrous MeOH was added. The mixture was stirred for 1.5 h at 20 °C. The solvent was evaporated *in vacuo* and the residue was concentrated three times with

10 mL of anhydrous MeOH and dried *in vacuo*. The yield of compound **5** was 1.32 g (2.83 mmol, 99.6%), m.p. 110–114 °C. Found (%): C, 41.81; H, 5.20; N, 17.74; Cl, 13.93. $C_{16}H_{20}N_6O_6 \cdot 2HCl$. Calculated (%): C, 41.30; H, 4.77; N, 18.06; Cl, 15.24. ^{1}H NMR (CD_3OD), δ: 1.60 (m, 4 H, $CH_3(C\underline{H}_2)_2CH_2$); 3.28–3.36 (m, 2 H, $NHC\underline{H}_2$); 3.46–3.50 (m, 2 H, Im-$C\underline{H}_2$); 3.55–3.62 (m, 2 H, $C\underline{H}_2OH$); 4.35 (m, 1 H, $C\underline{H}$-α); 7.96 (d, 1 H, H(5)-Im, $J_{5,2(Im)}$ = 1.5 Hz); 8.30 (d, 1 H, H(6)-DNP, $J_{6,5(DNP)}$ = 8.6 Hz); 8.84 (dd, 1 H, H(5)-DNP, $J_{5,6(DNP)}$ = 8.6 Hz, $J_{5,3(DNP)}$ = 2.5 Hz); 9.18 (d, 1 H, H(3)-DNP, $J_{3,5(DNP)}$ = 2.5 Hz); 9.48 (d, 1 H, H(2)-Im, $J_{2,5(Im)}$ = 1.5 Hz).

4-{N^{α}-[$N^{Im(1)}$-(2,4-Dinitrophenyl)-1*H*-imidazol-4-yl]acetyl-$N^{Im(1)}$-(2,4-dinitrophenyl)-L-histidyl}aminobutan-1-ol (6). DCC (256 mg, 1.24 mmol) was added to a solution of compound **2a** (325 mg, 1.08 mmol) and PFP (208 mg, 1.13 mmol) in 5 mL of DMF cooled to 0 °C and the mixture was stirred for 3 h at 20 °C. The cooled reaction mixture was filtered and the filtrate was added dropwise to a solution of compound **5** (511 mg, 1.10 mmol) and Et_3N (306 μL, 2.20 mmol) in 4 mL of DMF, which had been filtered from the $Et_3N \cdot HCl$ precipitate. The resulting reaction mixture was stirred for 25 min at 20 °C and the solvent was evaporated *in vacuo*. The residue was covered with 30 mL of 1 *M* HCl and extracted with ether (3×15 mL). The pH of the aqueous phase was brought to 5.6 ($NaHCO_3$) and the product was extracted with AcOEt (5×25 mL). The combined organic solutions were dried with Na_2SO_4 and filtered and the solvent was evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2 and purified by chromatography on Silasorb 600 silica gel (5 μm, Lachema, Czech Republic) using gradient elution with 0 to 15% solutions of EtOH in CH_2Cl_2. The fraction containing a product with R_f 0.52 (**D**) was concentrated *in vacuo* and dried *in vacuo*. The yield of compound **6** was 541 mg (0.812 mmol, 75%), m.p. 104–106 °C. Found (%): C, 48.03; H, 3.88; N, 20.25. $C_{27}H_{26}N_{10}O_{11}$. Calculated (%): C, 48.65; H, 3.93; N, 21.01. ^{1}H NMR (CD_3OD), δ: 1.56 (m, 4 H, $CH_2(C\underline{H}_2)CH_2$); 3.09 (m, 2 H, Im_{His}-$C\underline{H}_2$-CH-α); 3.24 (m, 2 H, $NHC\underline{H}_2CH_2$); 3.56 (m, 2 H, $C\underline{H}_2OH$); 3.66 (s, 2 H, Im-$C\underline{H}_2CONH$); 4.72 (m, 1 H, $C\underline{H}$-α); 7.20 (s, 1 H, H(5)-Im_{His}); 7.32 (s, 1 H, H(5)-Im); 7.94 (m, 4 H, 2 H(2)-Im, 2 H(6)-DNP); 8.65 (m, 2 H, 2 H(5)-DNP); 8.93 (m, 2 H, 2 H(3)-DNP).

[2′-Deoxythymidylyl(5′→3′)-2′-deoxythymidin-5′-yl] {4-[3-(1*H*-imidazol-4-yl)propenoyl]aminobutyl} phosphate (7). Compound **7** was prepared by condensation of a protected derivative of dinucleotide *p*ᵀTpᵀT(Lev) with compound **3b** in the presence of TPS and MeIm, by analogy with a previous publication.[22] *p*ᵀTpᵀT(Lev) (50 mg, 50 μmol) was dissolved in 1 mL of anhydrous Py. Then TPS (100 mg, 0.33 mmol), MeIm (55 μL, 0.69 mmol), and compound **3b** (22.6 mg, 60.2 μmol) were added. The course of the reaction was monitored by TLC (R_f of the protected product was 0.44 (**C**)). After 1 h, several drops of water were added to the reaction mixture and the solution was concentrated. The residue was dissolved in 20 mL of CH_2Cl_2 and washed with 2×20 mL of 1 *M* KH_2PO_4, pH 7.0. The organic layer was concentrated, 10 mL of concentrated aqueous NH_3 was added to the residue, and the mixture was allowed to stand for 2 days at 20 °C with stirring at intervals. The reaction mixture was concentrated, the product was isolated by preparative HPLC, and the fractions containing the target compound were concentrated. The residue was dissolved in water and the product was precipitated by a 10-fold volume of a 2% solution of $LiClO_4$ in acetone. The precipitate was washed with acetone, dried *in vacuo*, and dissolved in water. The yield of the Li salt of compound **7** was 375 OE_{260} (45%, 21.5 μmol). ^{1}H NMR (D_2O), δ: 1.70 (m, 4 H, $CH_2(C\underline{H}_2)_2CH_2$); 2.00 (s, 6 H, 2 CH_3); 2.32–2.60 (both m, 4 H, 2 H(2′), 2 H(2″))*; 3.43 (m, 2 H, $NHC\underline{H}_2$); 3.99–4.09 (m, 2 H, 2 H(4′)); 4.19–4.26 (m, 4 H, 2 H(5′), 2 H(5″)); 4.28 (m, 2 H, $C\underline{H}_2OP$); 4.42–4.51 (m, 1 H, H(3′)); 4.97–5.08 (m, 1 H, H(3″)); 6.34–6.43 (m, 2 H, H(1′), H(1″)); 6.57 (d, 1 H, =C$\underline{H}$CONH, J_{trans} = 15.6 Hz); 7.47 (d, 1 H, Im-C$\underline{H}$=, J_{trans} = 15.6 Hz); 7.50 (s, 1 H, H(5)-Im); 7.80 (m, 2 H, H(6)-Thy); 7.90 (s, 1 H, H(2)-Im). MALDI MS, *m/z*: 818.3 [M + H$^+$]. Calculated $[C_{30}H_{41}N_7O_{16}P_2 + H]^+$: 818.22.

[2′-Deoxythymidylyl(5′→3′)-2′-deoxythymidin-5′-yl] {4-[N^{α}-(1*H*-imidazol-4-ylacetyl)-L-histidyl]aminobutyl} phosphate (8). Compound **8** was prepared from *p*ᵀTpᵀT(Lev) (50 mg, 50 μmol) and compound **6** (40 mg, 60 μmol), similarly to compound **7**. The R_f of the protected product was 0.38 (**C**). The yield of the Li salt of compound **8** was 195 OE_{260} (22%, 11 μmol). ^{1}H NMR (D_2O), δ: 1.56 (m, 4 H, $CH_2(C\underline{H}_2)_2CH_2$); 2.00 (s, 6 H, 2 CH_3); 2.38–2.72 (both m, 4 H, 2 H(2′), 2 H(2″)); 3.12 (m, 2 H, Im_{His}-$C\underline{H}_2$); 3.25 (m, 2 H, $NHC\underline{H}_2$); 3.71 (m, 2 H, Im-$C\underline{H}_2$); 3.89–4.00 (m, 2 H, 2 H(4′)); 4.16 (m, 4 H, 2 H(5′), 2 H(5″)); 4.22 (m, 2 H, $C\underline{H}_2OP$); 4.40–4.50 (m, 1 H, H(3′)); 4.97–5.08 (m, 1 H, H(3″)); 6.38–6.48 (m, 2 H, H(1′), H(1″)); 7.01 (s, 1 H, H(5)-Im_{His}); 7.12 (s, 1 H, H(5)-Im); 7.77 (m, 2 H, 2 H(6)-Thy); 7.89 (m, 2 H, 2 H(2)-Im). MALDI MS, *m/z*: 943.4 [M + H$^+$]. Calculated $[C_{35}H_{48}N_{10}O_{17}P_2 + H]^+$: 943.28.

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* The character (′) refers to the ribose fragment of thymidine having 3′-, 5′-phosphate residues and the character (″) implies the second ribose fragment.

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