Amino-Functionalized Oligonucleotides with Peptide Internucleotide Linkages

Anna M. Varizhuk, Svetlana V. Kochetkova, Alexander U. Fedotov, Natalia A. Kolganova, Edward N. Timofeev and Vladimir L. Florentiev*

Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, ul. Vavilova 32, Moscow, 119991 Russia

Received December 14, 2010: Revised March 29, 2011: Accepted November 29, 2011

Abstract: Oligonucleotide analogs with L- and D-lysine residues incorporated in internucleotide linkages were synthesized and their affinity toward complementary DNA was studied. Stability of the duplexes formed by the modified oligonucleotides and their wild-type complements appeared to be close to that of the isosequential unmodified duplex, oligonucleotides carrying D-lysine residues forming generally more stable duplexes than L-lysine derivatives.

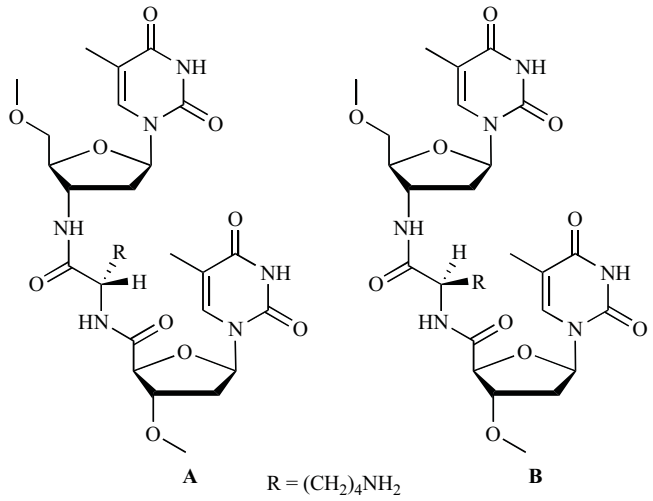
Keywords: Analogs, hybridization, oligonucleotides, peptide internucleotide linkages, synthesis, thermal stability.

INTRODUCTION

Modified oligonucleotides (ONs) have become an important instrument in molecular biology experiments. ON analogs are extensively used for manipulation of gene expression *via* antisense or RNA interference mechanisms [1-8]. However, the current ON analogs display limitations and there is a strong demand for new modified ONs. Much effort has been recently devoted to the problem of introduction of aminoalkyl side chains in ONs. This modification finds application in functionalization of nucleic acid aptamers and catalysts as well as nucleic acid labeling [9-11]. Furthermore, aminoalkyl-ONs display cationic side chains at appropriate pH and therefore can form with native DNA duplexes or triplexes with increased stability under certain conditions [12-15]. Most popular sites for appending amino- or aminoalkyl functionalities are 5-position of pirimidines [16, 17] and 2'-position of ribose [18, 19]. In this paper, we describe ON analogs in which aminoalkyl functionalities are introduced *via* peptide internucleotide linkages. Peptide fragments are known to protect ON analogs from nuclease digestion and facilitate their cellular uptake, thus helping to overcome the two main impediments in the use of ON-based therapeutics [20, 21]. In our previous work we have reported a general method for incorporation of hydrophobic amino acid residues in ON strands [22, 23]. Here we describe adaptation of this method for preparation of L- and D-lysine derivatives (Scheme **1**).

RESULTS AND DISCUSSION

Dinucleoside phosphoramidite blocks with trifluoroacetyl-protected ε-amino functionalities (Scheme **2**) were prepared and used in automated ON synthesis. Treatment with NH_3 at the end of the ON synthesis yielded ON analogs with free ε-amino groups.

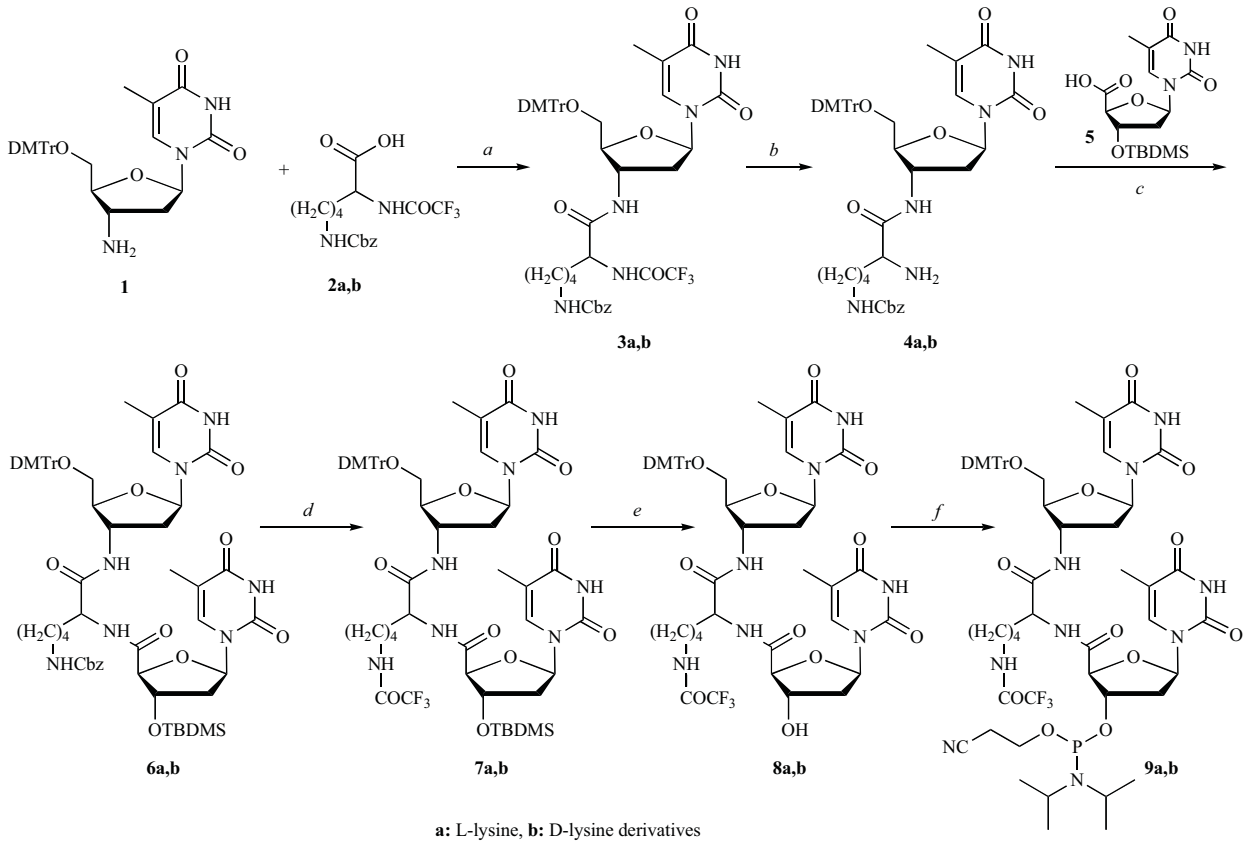


Scheme 1. Fragments of oligonucleotides with L-lysine (**A**) and D-lysine (**B**) residues in internucleotide linkages.

Compounds **3a, 3b**, key intermediates in the synthesis of target dinucleosides **9a, 9 b**, were prepared *via* condensation of 3'-amino-3'-deoxy-5'-*O*-dimethoxytritylthymidine **1** with L- and D-lysine derivatives **2a, 2b** in the presence of DCC and NHS (4 °C, 24 h). The usage of DCC and NHS enables avoidance of epimerization at α-carbon atoms of amino acid residues, observed when using other condensing agents (about 30% epimerization, determined by ^{1}H-NMR, occurred when BOP was used). Compounds **3a, 3b** were obtained in a yield of 83%. Optical purity of **3a, 3b** was no less than 98% (determined by ^{1}H-NMR spectroscopy). Trifluoroacetyl protection was removed by treatment with K_2CO_3 in a methanol/water mixture under reflux (4 h) to give compounds **4a, 4b**, bearing free α-amino groups, in a yield of 71-80%. The latter were condensed with 3'-*O*-(*t*-butyldimethylsilyl)thymidine 5'-carboxylc acid **5** (prepared following the published procedure [22]) in the presence of BOP and TEA in CH_2Cl_2 (20 °C, 1 h) to give dinucleoside analogs **6a, 6b** in a yield of 84-89%. Epimerization *via* the 5(4*H*)-oxazolone mechanism not being possible at this stage,

*Address correspondence to this author at the Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, ul. Vavilova 32, Moscow, 119991 Russia; Tel: +7 (499) 135-2182; Fax: +7(499)135-1405; E-mail: flor@imb.ac.ru

1875-6255/12 $58.00+.00



a: L-lysine, **b:** D-lysine derivatives

Scheme 2. Synthesis of the dinucleoside blocks containing L- and D-lysine residues in internucleoside linkages.

Reagents and conditions: (*a*) DCC, NHS, CH_2Cl_2, 4°C; (*b*) K_2CO_3, methanol/water, reflux; (*c*) BOP, TEA, CH_2Cl_2; (*d*) *i* - NH_4COOH, Pd/C, methanol, reflux; *ii* - CF_3COOEt, TEA, methanol; (*e*) TBAF, THF; (*f*) $NCCH_2CH_2OP(NPr^i_2)_2$, 1*H*-tetrazole, pyridine, CH_2Cl_2.

BOP was chosen as a condensing agent since it affords higher yields of **6a, 6b.** Benzyloxicarbonyl protection was substituted for trifluoroacetyl protection without isolation of intermediate dinucleosides with free ε-amino groups. Dinucleosides **6a, 6b** were deprotected by hydrogenation with ammonium formate in methanol in the presence of 10% Pd/C (reflux, 3h). Subsequent treatment with ethyl trifluoroacetate in the presence of TEA in methanol gave triflouroacetylated dinucleosides **7a, 7b** in a yield of 80-82%. The silyl protection was removed with 0.5 M TBAF in THF (20°C, 4 h). Resulting dinucleosides **8a, 8b** with free 3'-hydroxyl groups were treated with 2-cyanoethyl-*N,N,N′,N′*-tetraisopropylphosphoramidite in the presence of terazole and pyridine in dichloromethane (20°C, 2 h) to give amidites **9a, 9b** in a yield of 64-65%. Dinucleosides **6a,b** – **9a,b** were characterized by MALDI-TOF mass spectrometry and the structures of **6a,b - 8a,b** were confirmed by COSY NMR spectroscopy. COSY-spectra enabled unambiguous assignment of signals to protons of lysine, thymidine 5'-carboxylc acid and 3'-amino-3'-deoxythymidine moieties (Fig. **1**).

Dinucleoside amidite blocks **9a, 9b** were used in the synthesis of modified ONs on an automated synthesizer following standard phosphoramidite protocols. Coupling time was increased to 15 minutes for modified phosphoramidites. No decrease in coupling efficiency was observed (98-99% step-wise coupling yields). Along with the modified ONs, isosequential and complementary wild-type ONs were synthesized. Purification of the ONs was carried out by reverse-phase HPLC. All the ONs were characterized by MALDI-TOF mass spectrometry (Table **1**).

Thermal dissociation of the modified duplexes and their wild-type counterpart was measured. The resulting curves enabled evaluation of melting temperatures of the duplexes. The results are summarized in Table **2**.

As evident from Table **2**, ONs carrying D-lysine residues generally form more stable duplexes than L-lysine derivatives (ΔT_m = +0.4°C and -0.3°C per modification, on an average, respectively[1]). The effect of the modification on duplex stability depends on its position. Terminal modifications tend to increase duplex stability, while modifications in the middle of the strand have small destabilizing effect, more significant in the case of the L-lysine derivative.

CONCLUSION

In summary, we have described the facile synthesis of the novel ON analogs carrying L-and D-lysine residues in internucleotide linkages. The effect of the modifications on

[1] Average ΔT_m per modification was calculated as a sum of all corresponding ΔT_m values reported in Table 2 divided by the total number of modifications.

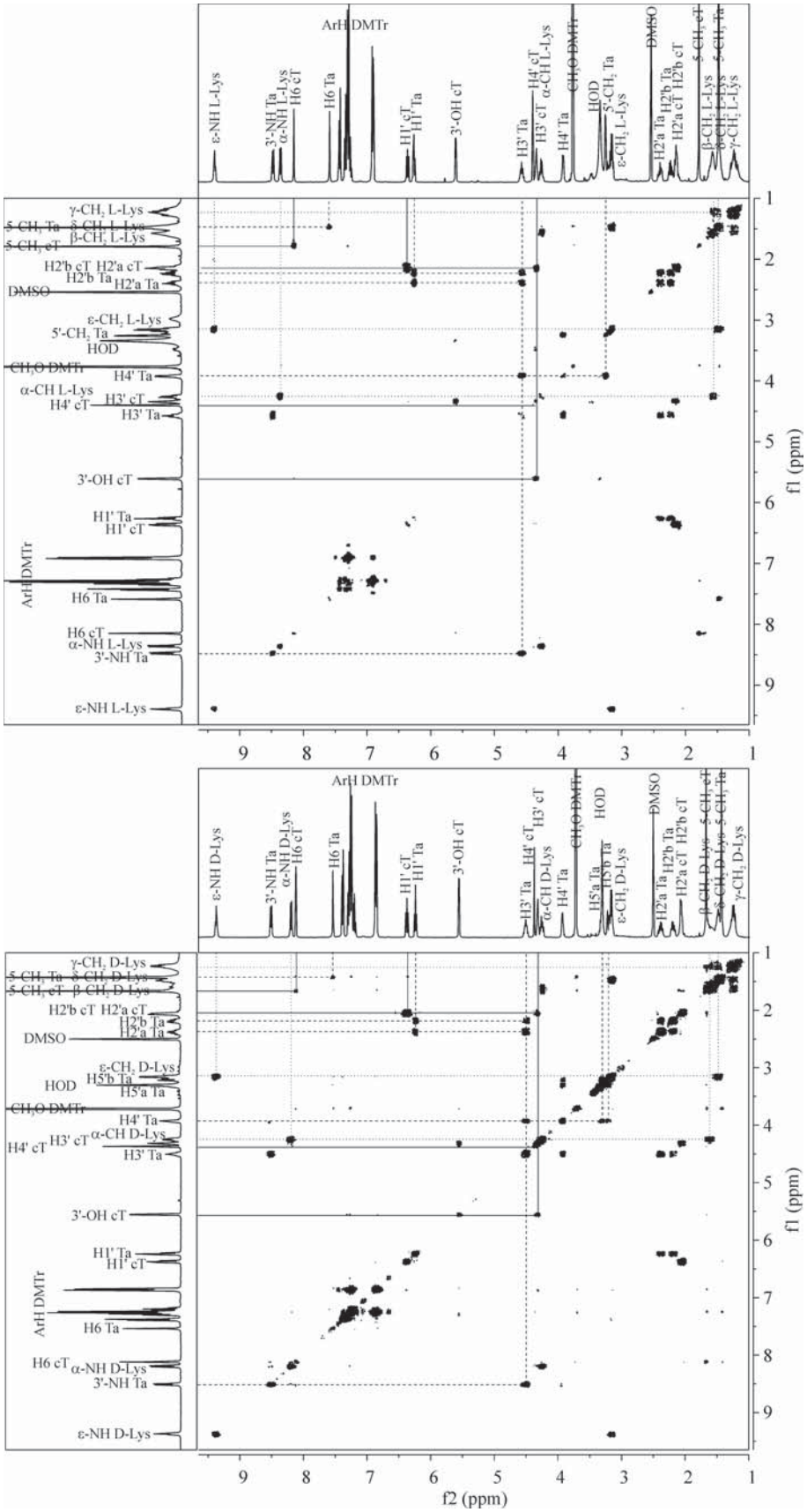


Fig. (1). COSY NMR spectrum of compounds **8a, 8b**. Abbreviations: cT - thymidine 5'-carboxylc acid moiety, Ta - 3'-amino-3'-deoxythymidine moiety.

Table 1. MALDI-TOF Mass Spectra of the Modified ONs

Sequence (5'→3')*	*m/z*, found (calculated for $[M - H]^-$)	
	X = L-Lys	X = D-Lys
TTAACTTCTTCACA**X**C	-	5131.6 (5130.51)
XAACTTCTTCACATTC	5131.2 (5130.51)	5135.1 (5130.51)
TTAACTTC**X**CACATTC	5129.9 (5130.51)	5132.3 (5130.51)
XAACTTCTTCACA**X**C	5192.0 (5191.70)	5191.0 (5191.70)
XAACTTC**X**CACA**X**C	5251.5 (5253.89)	5254.3 (5253.89)

Notes: *X – modified dinucleoside fragments containing L- or D- lysine residues in internucleotide linkages.

Table 2. Melting Temperatures of the Modified and Wild-Type Duplexes (Duplex Concentration $2.5 \cdot 10^{-6}$ M)

Sequence (5'→3')*	T_m, °C ± 0.5, (ΔT_m, °C**)	
	X = L-Lys	X = D-Lys
TTAACTTCTTCACATTC	50.3	
TTAACTTCTTCACA**X**C	-	51.0 (+0.7)
XAACTTCTTCACATTC	50.9 (+0.6)	50.6 (+0.3)
TTAACTTC**X**CACATTC	48.9 (-1.4)	50.2 (-0.1)
XAACTTCTTCACA**X**C	50.4 (+0.1)	51.7 (+1.4)
XAACTTC**X**CACA**X**C	49.2 (-1.1)	51.3 (+1.0)

Notes: *X – modified dinucleoside fragments containing L- or D- lysine residues in internucleotide linkages.
**T_m difference between modified and natural duplexes.

duplex stability has been assessed. Melting temperatures of the modified duplexes appeared to be close to that of the isosequential native duplex. Thus, the novel analogs meet the stability criterion set for modified ONs and could become a promising tool for the development of gene-targeted therapeutics. Considering that ε-amino groups of lysine residues in the novel analogs are accessible for conjugation reactions, such as fluorescent labeling, we believe, these analogs could also find application in diagnostics and in the study of biological functions of nucleic acids.

EXPERIMENTAL

All reagents were commercially available and used without further purification. 3'-Azydo-3'-deoxythymidine was provided by Association AZT (Russia). Dichloromethane was dried by distillation from phosphorus pentaoxide, pyridine was distilled from calcium hydride, and THF was distilled from lithium alumohydride prior to use. Silica gel flesh column chromatography was performed on silica gel Kieselgel 60 (0.040-0.063 mm, Merck). TLC was performed on silica gel Kieselgel 60 F_{254} precoated plates (Merck) with detection by UV using the following solvent systems (compositions expressed as *v*/*v*): ethanol - methylene chloride 1:65 (A), 1:32 (B), 1: 19 (C), 1:12 (D), 1:9 + 0.1% TEA (E). ^{1}H NMR spectra were recorded on a Bruker AMXIII-400 NMR spectrometer on solutions in deuterated dimethyl sulfoxide (DMSO-*d*6). Chemical shifts are given in parts per million (ppm). The coupling constants (*J*) are given in Hz. The signals were assigned using COSY experiments. Abbreviations used: cT (thymidine 5'-carboxylc acid moiety), Ta (3'-deoxy-3'-aminothymidine moiety). The ^{1}H NMR data were processed and presented using MestReNova version 6.2.0 (Mestrelab Research SL). MALDI-TOF mass spectra were acquired on a Kratos MS-30 spectrometer using linear flight path.

ONs were synthesized on an Aplied Biosystem DNA synthesizer 3400 using standard phosphoramidite protocols and purified using preparative scale reverse-phase HPLC on a 250 mm x 4.0 mm^2 Hypersil C18 column with detection at 260 nm. Chromatography of dimethoxytrytil-protected ONs was performed using 10-50% gradient of CH_3CN in 0.05 M TEAA. Melting curves of the duplexes were recorded on a Shimadzu UV 160-A spectrophotometer, equipped with a thermostatic system, in 20 mM sodium phosphate buffer, 100 mM NaCl, 01 mM EDTA, pH 7.0, concentration of each duplex being $2.5 \cdot 10^{-6}$M. Samples were denatured at 95°C for 5 min and slowly cooled to 20°C prior to measurements. A_{260} (duplex absorbance) was measured as a function of temperature. It was registered every 0.5°C from 20 to 70°C. Thermodynamic parameters of duplex formation were obtained by performing nonlinear regression analysis using DataFit version 9.0.059 (Oakdale Engineering, US). The calculation method taking into account temperature dependence of UV absorbance of duplexes and single strands was applied.

***N*-ε-Benzyloxycarbonyl-*N*-α-trifluoroacetyl-L-lysine (2a).** To a stirred suspension of *N*-ε-benzyloxycarbonyl-L-lysine [24] (2.8 g, 10 mmol) in 30 mL of abs. MeOH, ethyl trifluoroacetate (2.84 g, 2.4 mL, 20 mmol) and TEA (2.02 g, 2.8 mL, 20 mmol) were added. The mixture was stirred for 24 h. Solvent was evaporated, the residue was dissolved in 50 mL of ethyl acetate, washed with water, 10% $NaHSO_4$,

water, dried over Na_2SO_4 and evaporated. Compound **2a** was obtained as a colourless viscous oil. Yield 3.5 g (93%). ^{1}H NMR: 12.898 (1 H, s, COOH), 9.621 (1 H, d, *J* 7.7, α-NH), 7.397 – 7.271 (5 H, m, Ar-H Ph), 7.206 (1 H, t, *J* 5.5, ε-NH), 5.004 (2 H, s, CH_2 Bn), 4.209 (1 H, ddd, *J* 9.8, 7.7, 4.7, α-CH), 2.986 (2 H, q, *J* 6.4, ε-CH_2), 1.877 – 1.667 (2 H, m, β-CH_2), 1.463 – 1.363 (2 H, m, δ-CH_2), 1.341 – 1.249 (2 H, m, γ-CH_2).

***N*-ε-Benzyloxycarbonyl-*N*-α-trifluoroacetyl-D-lysine (2b)** was prepared following the previous procedure from 2.8 g (10 mmol) of *N*-ε-benzyloxycarbonyl-D-lysine [24]. Yield 3.4 g (90.3%). ^{1}H NMR: 12.889 (1 H, s, COOH), 9.625 (1 H, d, *J* 7.7, α-NH), 7.401 – 7.268 (5 H, m, Ar-H Bn), 7.216 (1 H, t, *J* 5.7, ε-NH), 5.008 (2 H, s, CH_2 Bn), 4.213 (1 H, ddd, *J* 9.9, 7.7, 4.6, α-CH), 2.991 (2 H, q, *J* 6.6, ε-CH_2), 1.885 – 1.662 (2 H, m, β-CH_2), 1.466 – 1.365 (2 H, m, δ-CH_2), 1.344 – 1.251 (2 H, m, γ-CH_2).

(5*S*)-Benzyl 6-{(5'-*O*-(4,4'-dimethoxytrityl)-3'-deoxythymidine-3'-yl)amino}-6-oxo-5-(2,2,2-trifluoracetamido) hexylcarbamate (3a). To a stirred solution of **2a** (206 mg, 0.55 mmol) in dry CH_2Cl_2 (5 mL), nucleoside **1** (272 mg, 0.5 mmol) and NHS (63 mg, 0.55 mmol) were added. The mixture was cooled to 0 °C in an ice-water bath with stirring, and DCC (113 mg, 0.55 mmol) was added. The mixture was kept overnight at 4 °C, then filtered. The filtrate was diluted with water (30 mL) and extracted with ethyl acetate (3 x 30 mL). Combined organic layers were washed with saturated $NaHCO_3$ (30 mL) and water (30 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by silica gel flesh column chromatography in 3% of ethanol in CH_2Cl_2 + 0.1% TEA to give 3a as a hard foam (390 mg, 83.3% yield). R_f = 0.48 (Solvent system B). ^{1}H NMR: 11.303 (1 H, s, H3), 9.518 (1 H, d, *J* 7.8, α-NH L-Lys), 8.508 (1 H, d, *J* 7.8, 3'-NH Ta), 7.552 (1 H, d, ^4J 1.0, H6), 7.427 – 6.838 (18 H, m, Ar-H), 7.199 (1 H, t, *J* 5.2, ε-NH L-Lys), 6.229 (1 H, t, *J* 6.4, H1' Ta), 4.995 (2 H, s, CH_2 Bn), 4.594 – 4.487 (1 H, m, H3' Ta), 4.251 – 4.160 (1 H, m, α-CH L-Lys), 3.927 – 3.846 (1 H, m, H4' Ta), 3.728 (6 H, s, CH_3O DMTr), 3.223 (2 H, br. s, 5'-CH_2 Ta), 2.947 (2 H, q, *J* 5.9, ε-CH_2 L-Lys), 2.423 – 2.322 (1 H, m, H2'a Ta), 2.183 (1 H, dt, $J_{1',\,2'b}$ 6.6, $J_{3',\,2'b}$ 6.6, $^2J_{2'a,\,2'b}$ 13.2, H2'b Ta), 1.677 – 1.525 (2 H, m, β-CH_2 L-Lys), 1.449 (3 H, d, ^4J 1.0, 5-CH_3 Ta), 1.421 – 1.324 (2 H, m, δ-CH_2 L-Lys), 1.312 – 1.085 (2 H, m, γ-CH_2 L-Lys).

(5*R*)-Benzyl 6-{(5'-*O*-(4,4'-dimethoxytrityl)-3'-deoxythymidine-3'-yl)amino}-6-oxo-5-(2,2,2-trifluoracetamido) hexylcarbamate (3b) was prepared following the previous procedure from 1.087 g (2 mmol) of 3'-amino-3'-deoxy-5'-*O*-dimethoxytritylthymidine (**1**). Yield 1.503 g (83.2%). R_f 0.45 (Solvent system B). ^{1}H NMR: 11.302 (1 H, s, H3), 9.484 (1 H, d, *J* 7.8, α-NH D-Lys), 8.516 (1 H, d, *J* 7.4, 3'-NH Ta), 7.539 (1 H, d, ^4J 1.1, H6), 7.423 – 6.827 (18 H, m, Ar-H), 7.229 (1 H, t, *J* 5.4, ε-NH D-Lys), 6.225 (1 H, t, *J* 6.5, H1' Ta), 4.997 (2 H, s, CH_2 Bn), 4.555 – 4.463 (1 H, m, H3' Ta), 4.254 – 4.184 (1 H, m, α-CH D-Lys), 3.944 – 3.882 (1 H, m, H4' Ta), 3.725 (6 H, s, CH_3O DMTr), 3.307 – 3.252 (1 H, m, H5'a Ta[2]), 3.204 (1 H, dd, $J_{4',\,5'b}$ 2.4, $^2J_{5'a,\,5'b}$ 10.1, H5'b Ta), 3.023 (2 H, q, *J* 5.9, ε-CH_2 D-Lys), 2.429 – 2.316 (1 H, m, H2'a Ta), 2.175 (1 H, dt, $J_{1',\,2'b}$ 6.2, $J_{3',\,2'b}$ 6.2, $^2J_{2'a,\,2'b}$ 13.1, H2'b Ta), 1.767 – 1.635 (2 H, m, β-CH_2 D-Lys), 1.429 (3 H, d, ^4J 1.1, 5-CH_3 Ta), 1.429 – 1.358 (2 H, m, δ-CH_2 D-Lys), 1.324 – 1.196 (2 H, m, γ-CH_2 D-Lys).

(5*S*)-Benzyl 6-{(5'-*O*-(4,4'-dimethoxytrityl)-3'-deoxythymidine-3'-yl)amino}-6-oxo-5-aminohexylcarbamate (4a). Nucleoside **3a** (730 mg, 0.809 mmol) was dissolved in methanol (15 mL), water (1.5 mL) and K_2CO_3 (550 mg, 4 mmol) were added. The mixture was refluxed for 4 h, then cooled, diluted with water (50 mL) and extracted with chloroform (3×50 mL). Combined organic layers were washed with water (30 mL), dried over Na_2SO_4, filtered and evaporated. The residue was purified by CC in 7 - 10% of ethanol in CH_2Cl_2 + 0.1% TEA to give **4a** as a hard foam (468 mg, 71.7% yield). R_f = 0.37 (Solvent system E). ^{1}H NMR: 8.359 (1 H, d, *J* 7.4, 3'-NH Ta), 7.559 (1 H, d, ^4J 1.0, H6), 7.447 – 6.805 (18 H, m, Ar-H), 7.170 (1 H, t, *J* 5.5, ε-NH L-Lys), 6.226 (1 H, t, *J* 6.4, H1' Ta), 4.988 (2 H, s, CH_2 Bn), 4.586 – 4.458 (1 H, m, H3' Ta), 3.968 – 3.881 (1 H, m, H4' Ta), 3.726 (6 H, s, CH_3O DMTr), 3.234 (2 H, br. s, 5'-CH_2 Ta), 3.213 – 3.153 (1 H, m, α-CH L-Lys), 2.940 (2 H, q, *J* 5.9, ε-CH_2 L-Lys), 2.401 – 2.290 (1 H, m, H2'a Ta), 2.220 (1 H, dt, $J_{1',\,2'b}$ 6.6, $J_{3',\,2'b}$ 6.6, $^2J_{2'a,\,2'b}$ 13.2, H2'b Ta), 1.574 – 1.453 (2 H, m, β-CH_2 L-Lys), 1.461 (3 H, d, ^4J 1.0, 5-CH_3 Ta), 1.428 – 1.301 (2 H, m, δ-CH_2 L-Lys), 1.295 – 1.138 (2 H, m, γ-CH_2 L-Lys).

(5*R*)-Benzyl 6-{(5'-*O*-(4,4'-dimethoxytrityl)-3'-deoxythymidine-3'-yl)amino}-6-oxo-5-aminohexylcarbamate (4b) was prepared following the previous procedure from 1.143 g (1.56 mmol) of nucleoside **3b**. Yield 1.07 g (83.0%). R_f 0.31 (Solvent system E). ^{1}H NMR: 8.219 (1 H, d, *J* 7.2, 3'-NH Ta), 7.613 (1 H, d, ^4J 1.2, H6), 7.506 – 6.853 (18 H, m, Ar-H), 7.227 (1 H, t, *J* 5.6, ε-NH D-Lys), 6.284 (1 H, t, *J* 6.4, H1' Ta), 5.057 (2 H, s, CH_2 Bn), 4.636 – 4.502 (1 H, m, H3' Ta), 4.026 – 3.964 (1 H, m, H4' Ta), 3.785 (6 H, s, CH_3O DMTr), 3.322 (1 H, dd, $J_{4',\,5'a}$ 4.7, $^2J_{5'a,\,5'b}$ 10.5, H5'a Ta), 3.258 (1 H, dd, $J_{4',\,5'b}$ 2.4, $^2J_{5'a,\,5'b}$ 10.5, H5'b Ta), 3.127 (1 H, t, $J_{CH,\,CH2}$ 6.12, α-CH D-Lys), 3.023 (2 H, q, *J* 5.9, ε-CH_2 D-Lys), 2.441 – 2.341 (1 H, m, H2'a Ta), 2.272 (1 H, dt, $J_{1',\,2'b}$ 6.7, $J_{3',\,2'b}$ 6.7, $^2J_{2'a,\,2'b}$ 13.3, H2'b Ta), 1.615 – 1.520 (2 H, m, β-CH_2 D-Lys), 1.522 (3 H, d, ^4J 1.0, 5-CH_3 Ta), 1.477 – 1.377 (2 H, m, δ-CH_2 D-Lys), 1.380 – 1.242 (2 H, m, γ-CH_2 D-Lys).

***N*-[(2*S*)-6-Benzyloxycarboxamido-1-{(5'-*O*-(4,4'-dimethoxytrityl)-3'-deoxythymidine-3'-yl)amino}-1-oxohexan-2-yl]-3'-*O*-(*tert*-butyldimethylsilyl)thymidine-5'-carboxamide (6a).** Nucleoside **4a** (1 g, 1.2 mmol) was suspended in dry CH_2Cl_2 (12 mL), 5'-carboxynucleoside **5** (489 mg, 1.3 mmol), TEA (392 μL, 2.8 mmol) and BOP (619 mg, 1.4 mmol) were added. The mixture was stirred for 1 h at 20 °C, then diluted with water (50 mL) and extracted with ethyl acetate (3×40 mL). Combined organic layers were washed with water (30 mL) and saturated $NaHCO_3$ (30 mL), dried over Na_2SO_4, filtered and evaporated. The residue was purified by silica gel flesh column chromatography in 4% of ethanol in CH_2Cl_2 + 0.1% TEA to give 6a as a hard foam (1.218 g, 87.6% yield). R_f = 0.35 (Solvent system C). ^{1}H NMR: 11.295 (2 H, s, H3 Ta, H3 cT), 8.840 (1 H, d, *J* 7.8, 3'-NH Ta), 8.225 (1 H, d, *J* 8.1, α-NH L-Lys), 7.948 (1 H, d,

[2]H5'a Ta signal is hidden under HOD signal. Identification by COSY-spectrum.

^4J 0.9, H6 cT), 7.549 (1 H, d, ^4J 1.0, H6 Ta), 7.439 – 6.812 (18 H, m, Ar-H), 7.160 (1 H, t, *J* 5.4, ε-NH L-Lys), 6.296 – 6.175 (2 H, m, H1' cT, H1' Ta), 4.976 (2 H, s, CH_2 Bn), 4.595 – 4.483 (1 H, m, H3' Ta), 4.473 (1 H, dt, $J_{3',\,2'a}$ 5.1, $J_{3',\,2'b}$, $J_{3',\,4'}$ 2.1, H3' cT), 4.302 (1 H, d, $J_{3',\,4'}$ 2.0, H4' cT), 4.263 – 4.175 (1 H, m, α-CH L-Lys), 3.917 – 3.842 (1 H, m, H4' Ta), 3.715 (6 H, s, CH_3O DMTr), 3.209 (2 H, br. s, 5'-CH_2 Ta), 2.931 (2 H, q, *J* 5.9, ε-CH_2 L-Lys), 2.451 – 2.357 (2 H, m, H2'a Ta, H2'a cT), 2.178 (1 H, dt, $J_{1',\,2'b}$ 6.7, $J_{3',\,2'b}$ 6.7, $^2J_{2'a,\,2'b}$ 13.8, H2'b Ta), 2.062 (1 H, ddd, $J_{1',\,2'b}$ 5.9, $J_{3',\,2'b}$ 2.1, $^2J_{2'a,\,2'b}$ 13.5, H2'b cT), 1.754 (3 H, d, ^4J 0.9, 5-CH_3 cT), 1.590 – 1.465 (2 H, m, β-CH_2 L-Lys), 1.431 (3 H, d, ^4J 1.0, 5-CH_3 Ta), 1.407 – 1.311 (2 H, m, δ-CH_2 L-Lys), 1.278 – 1.114 (2 H, m, γ-CH_2 L-Lys), 0.873 (9 H, s, *t*-BuSi), 0.092 (3 H, s, CH_3Si), 0.081 (3 H, s, CH_3Si). MS: *m/z* 1158.2. Calc. 1157.37 $[M-H]^-$ ($C_{61}H_{74}N_6O_{14}Si$).

***N*-[(2*R*)-6-Benzyloxycarboxamido-1-{(5'-*O*-(4,4'-dimethoxytrityl)-3'-deoxythymidine-3'-yl)amino}-1-oxohexan-2-yl]-3'-*O*-(*tert*-butyldimethylsilyl)thymidine-5'-carboxamide (6b)** was prepared following the previous procedure from 284 mg (0.35 mmol) of nucleoside **4b**. Yield 322 mg (84.4%). R_f 0.30 (Solvent system C). ^{1}H NMR: 11.354 (2 H, s, H3 Ta, H3 cT), 8.570 (1 H, d, *J* 7.4, 3'-NH Ta), 8.412 (1 H, d, *J* 8.1, α-NH D-Lys), 8.093 (1 H, d, ^4J 1.1, H6 cT), 7.590 (1 H, d, ^4J 1.1, H6 Ta), 7.473 – 6.880 (18 H, m, Ar-H), 7.244 (1 H, t, *J* 5.9, ε-NH D-Lys), 6.392 (1 H, dd, $J_{1',\,2'a}$ 9.1, $J_{1',\,2'b}$ 5.5, H1' cT), 6.297 (1 H, t, *J* 6.6, H1' Ta), 5.047 (2 H, s, CH_2 Bn), 4.618 – 4.543 (1 H, m, H3' Ta), 4.547 – 4.489 (1 H, m, H3' cT), 4.427 (1 H, d, $J_{3',\,4'}$ 1.0, H4' cT), 4.361 – 4.281 (1 H, m, α-CH D-Lys), 4.013 – 3.954 (1 H, m, H4' Ta), 3.779 (6 H, s, CH_3O DMTr), 3.412 – 3.366 (1 H, m, H5'a Ta[3]), 3.263 (1 H, dd, $J_{4',\,5'b}$ 2.2, $^2J_{5'a,\,5'b}$ 10.4, H5'b Ta), 3.032 (2 H, q, *J* 6.1, ε-CH_2 D-Lys), 2.492 – 2.389 (1 H, m, H2'a Ta), 2.298 – 2.195 (2 H, m, H2'b Ta, H2'a cT), (1 H, ddd, $J_{1',\,2'b}$ 5.5, $J_{3',\,2'b}$ 1.8, $^2J_{2'a,\,2'b}$ 13.5, H2'b cT), 1.710 (3 H, d, ^4J 1.1, 5-CH_3 cT), 1.702 – 1.588 (2 H, m, β-CH_2 D-Lys), 1.466 (3 H, d, ^4J 1.1, 5-CH_3 Ta), 1.500 – 1.456 (2 H, m, δ-CH_2 D-Lys), 1.380 – 1.240 (2 H, m, γ-CH_2 D-Lys), 0.936 (9 H, s, *t*-BuSi), 0.158 (3 H, s, CH_3Si), 0.145 (3 H, s, CH_3Si). MS: *m/z* 1157.8. Calc. 1157.37 $[M-H]^-$ ($C_{61}H_{74}N_6O_{14}Si$).

***N*-[(2*S*)-1-{(5'-*O*-(4,4'-Dimethoxytrityl)-3'-deoxythymidine-3'-yl)amino}-1-oxo-6-trifluoracetamidohexan-2-yl]-3'-*O*-(*tert*-butyldimethylsilyl)thymidine-5'-carboxamide (7a).** To a stirred solution of dinucleoside **6a** (659 mg, 0.56 mmol) in dry methanol (10 mL), ammonium formate (150 mg, 2.3 mmol) and 10 % Pd/C (66 mg) were added. The mixture was refluxed for 3 h, then cooled to r.t. and filtered through Celite. Ethyl trifluoroacetate (203 mL, 1.70 mmol) and TEA (236 mL, 1.70 mmol) were added to a filtrate and the mixture was stirred for 2 h at 20 °C. Methanol was evaporated, the residue was dissolved in ethyl acetate (50 mL), washed with water (30 mL) and saturated $NaHCO_3$ (30 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by silica gel flesh column chromatography in 3% of ethanol in CH_2Cl_2 + 0.1% TEA to give **7a** as a hard foam (520 mg, 82% yield). R_f = 0.53 (Solvent system A). ^{1}H NMR: 11.302 (2 H, s, H3 Ta, H3 cT), 9.336 (1 H, t, *J* 5.6, ε-NH L-Lys), 8.425 (1 H, d, *J* 7.8, 3'-NH Ta), 8.224 (1 H, d, *J* 8.1, α-NH L-Lys), 7.955 (1 H, s, H6 cT), 7.556 (1 H, s, H6 Ta), 7.462 – 6.800 (13 H, m, Ar-H), 6.302 – 6.167 (2 H, m, H1' cT, H1' Ta), 4.562 – 4.482 (1 H, m, H3' Ta), 4.473 (1 H, dt, $J_{3',\,2'a}$ 5.2, $J_{3',\,2'b}$, $J_{3',\,4'}$ 2.2, H3' cT), 4.299 (1 H, d, $J_{3',\,4'}$ 1.6, H4' cT), 4.289 – 4.184 (1 H, m, α-CH L-Lys), 3.919 – 3.859 (1 H, m, H4' Ta), 3.729 (6 H, s, CH_3O DMTr), 3.215 (2 H, br. s, 5'-CH_2 Ta), 2.931 (2 H, q, *J* 5.9, ε-CH_2 L-Lys), 2.402 – 2.256 (2 H, m, H2'a Ta, H2'a cT), 2.183 (1 H, dt, $J_{1',\,2'b}$ 6.4, $J_{3',\,2'b}$ 6.4, $^2J_{2'a,\,2'b}$ 13.1, H2'b Ta), 2.076 (1 H, ddd, $J_{1',\,2'b}$ 5.8, $J_{3',\,2'b}$ 2.3, $^2J_{2'a,\,2'b}$ 13.4, H2'b cT), 1.760 (3 H, s, 5-CH_3 cT), 1.609 – 1.498 (2 H, m, β-CH_2 L-Lys), 1.492 – 1.407 (2 H, m, δ-CH_2 L-Lys), 1.455 (3 H, s, 5-CH_3 Ta), 1.278 – 1.126 (2 H, m, γ-CH_2 L-Lys), 0.860 (9 H, s, *t*-BuSi), 0.095 (3 H, s, CH_3Si), 0.084 (3 H, s, CH_3Si). MS: *m/z* 1120.0. Calc. 1119.25 $[M-H]^-$ ($C_{55}H_{67}F_3N_7O_{13}Si$).

***N*-[(2*R*)-1-{(5'-*O*-(4,4'-Dimethoxytrityl)-3'-deoxythymidine-3'-yl)amino}-1-oxo-6-trifluoracetamidohexan-2-yl]-3'-*O*-(*tert*-butyldimethylsilyl)thymidine-5'-carboxamide (7b)** was prepared following the previous procedure from 498 mg (0.43 mmol) of dinucleoside **6b**. Yield 385 mg (80%). R_f 0.51 (Solvent system A). ^{1}H NMR: 11.300 (2 H, s, H3 Ta, H3 cT), 9.350 (1 H, t, *J* 5.6, ε-NH D-Lys), 8.527 (1 H, d, *J* 7.3, 3'-NH Ta), 8.356 (1 H, d, *J* 8.1, α-NH D-Lys), 8.027 (1 H, d, ^4J 1.1, H6 cT), 7.541 (1 H, d, ^4J 1.1, H6 Ta), 7.420 – 6.777 (13 H, m, Ar-H), 6.338 (1 H, dd, $J_{1',\,2'a}$ 9.1, $J_{1',\,2'b}$ 6.0, H1' cT), 6.245 (1 H, t, *J* 6.6, H1' Ta), 4.568 – 4.471 (1 H, m, H3' Ta), 4.487 – 4.439 (1 H, m, H3' cT), 4.371 (1 H, d, $J_{3',\,4'}$ 1.1, H4' cT), 4.338 – 4.227 (1 H, m, α-CH D-Lys), 3.992 – 3.900 (1 H, m, H4' Ta), 3.772 (6 H, s, CH_3O DMTr), 3.321 (1 H, dd, $J_{4',\,5'a}$ 4.4, $^2J_{5'a,\,5'b}$ 10.9, H5'a Ta), 3.205 (1 H, dd, $J_{4',\,5'b}$ 2.4, $^2J_{5'a,\,5'b}$ 10.9, H5'b Ta), 3.159 (2 H, q, *J* 6.0, ε-CH_2 D-Lys), 2.437 – 2.336 (1 H, m, H2'a Ta), 2.244 – 2.125 (2 H, m, H2'b Ta, H2'a cT), 2.043 (1 H, ddd, $J_{1',\,2'b}$ 5.6, $J_{3',\,2'b}$ 1.8, $^2J_{2'a,\,2'b}$ 13.5, H2'b cT), 1.654 (3 H, d, ^4J 1.1, 5-CH_3 cT), 1.655 – 1.553 (2 H, m, β-CH_2 D-Lys), 1.481 (3 H, d, ^4J 1.1, 5-CH_3 Ta), 1.437 – 1.406 (2 H, m, δ-CH_2 D-Lys), 1.319 – 1.194 (2 H, m, γ-CH_2 D-Lys), 0.883 (9 H, s, *t*-BuSi), 0.110 (3 H, s, CH_3Si), 0.095 (3 H, s, CH_3Si). MS: *m/z* 1120.2. Calc. 1119.25 $[M-H]^-$ ($C_{55}H_{67}F_3N_7O_{13}Si$).

***N*-[(2*S*)-1-{(5'-*O*-(4,4'-Dimethoxytrityl)-3'-deoxythymidine-3'-yl)amino}-1-oxo-6-trifluoracetamidohexan-2-yl]thymidine-5'-carboxamide (8a).** To a stirred solution of dinucleoside **7a** (570 mg, 0.5 mmol) in dry THF (1 mL), 1M solution of tetrabutylammonium fluoride in dry THF (1 mL) was added. The mixture was stirred for 4 h at 20 °C, diluted with saturated $NaHCO_3$ (50 mL) and extracted with $CHCl_3$ (3×40 mL). Combined organic layers were washed with water, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flesh column chromatography in 7-9% of ethanol in CH_2Cl_2 + 0.1% TEA to give **8a** as a hard foam (492 mg, 95.8% yield). R_f = 0.57 (Solvent system D). ^{1}H NMR: 11.329 (1 H, s, H3 cT), 11.315 (1 H, s, H3 Ta), 9.393 (1 H, t, *J* 5.5, ε-NH L-Lys), 8.4775 (1 H, d, *J* 7.9, 3'-NH Ta), 8.359 (1 H, d, *J* 8.0, α-NH L-Lys), 8.150 (1 H, s, H6 cT), 7.588 (1 H, s, H6 Ta), 7.466 – 6.879 (13 H, m, Ar-H), 6.363 (1 H, dd, $J_{1',\,2'a}$ 8.5, $J_{1',\,2'b}$ 6.0, H1' cT), 6.266 (1 H, t, *J* 6.3, H1' Ta), 5.610 (1 H, d, *J* 4.2, 3'-OH cT), 4.621 – 4.522 (1 H, m, H3' Ta), 4.400 (1 H, s, H4' cT), 4.371 – 4.312 (1 H, m, H3' cT), 4.303 – 4.218 (1 H, m, α-CH L-Lys), 3.962 – 3.887 (1 H, m, H4' Ta), 3.762 (6 H, s,

[3] H5'a Ta signal is hidden under HOD signal. Identification by COSY-spectrum.

CH_3O DMTr), 3.258 (2 H, br. s, 5'-CH_2 Ta), 3.159 (2 H, q, *J* 5.9, ε-CH_2 L-Lys), 2.447 – 2.354 (1 H, m, H2'a Ta), 2.234 (1 H, dt, $J_{1',\ 2'b}$ 6.3, $J_{3',\ 2'b}$ 6.3, $^2J_{2'a,\ 2'b}$ 12.7, H2'b Ta), 2.179 – 2.098 (2 H, m, H2'a cT, H2'b cT), 1.792 (3 H, s, 5-CH_3 cT), 1.669 – 1.517 (2 H, m, β-CH_2 L-Lys), 1.548 – 1.454 (2 H, m, δ-CH_2 L-Lys), 1.481 (3 H, s, 5-CH_3 Ta), 1.414 – 1.216 (2 H, m, γ-CH_2 L-Lys). MS: *m/z* 1005.6. Calc. 1004.99 $[M - H]^-$ ($C_{49}H_{53}F_3N_7O_{13}$).

***N*-[(2*R*)-1-{(5'-*O*-(4,4'-Dimethoxytrityl)-3'-deoxythymidine-3'-yl)amino}-1-oxo-6-trifluoracetamidohexan-2-yl]thymidine-5'-carboxamide (8b)** was prepared following the previous procedure from 420 mg (0.37 mmol) of dinucleoside **7b**. Yield 370 mg (98.1%). R_f 0.56 (Solvent system D). ^{1}H NMR: 11.301 (2 H, s, H3 cT), 11.273 (2 H, s, H3 Ta), 9.371 (1 H, t, *J* 5.5, ε-NH D-Lys), 8.509 (1 H, d, *J* 7.4, 3'-NH Ta), 8.195 (1 H, d, *J* 8.0, α-NH D-Lys), 8.119 (1 H, d, ^4J 1.1, H6 cT), 7.537 (1 H, d, ^4J 1.0, H6 Ta), 7.447 – 6.799 (13 H, m, Ar-H), 6.374 (1 H, t, *J* 7.3, H1' cT), 6.239 (1 H, t, *J* 6.6, H1' Ta), 5.556 (1 H, d, *J* 4.1, 3'-OH cT), 4.579 – 4.458 (1 H, m, H3' Ta), 4.370 (1 H, s, H4' cT), 4.335 – 4.289 (1 H, m, H3' cT), 4.271 – 4.185 (1 H, m, α-CH D-Lys), 3.969 – 3.886 (1 H, m, H4' Ta), 3.718 (6 H, s, CH_3O DMTr), 3.351 – 3.274 (1 H, m, H5'a Ta), 3.199 (1 H, dd, $J_{4',\ 5'b}$ 2.4, $^2J_{5'a,\ 5'b}$ 10.8, H5'b Ta), 3.172 (2 H, q, *J* 5.9, ε-CH_2 D-Lys), 2.451 – 2.292 (1 H, m, H2'a Ta), 2.246 – 2.114 (1 H, m, H2'b Ta), 2.106 – 1.993 (2 H, m, H2'a cT, H2'b cT), 1.668 (3 H, d, ^4J 1.1, 5-CH_3 cT), 1.703 – 1.553 (2 H, m, β-CH_2 D-Lys), 1.544 – 1.412 (2 H, m, δ-CH_2 D-Lys), 1.429 (3 H, d, ^4J 1.0, 5-CH_3 Ta), 1.326 – 1.122 (2 H, m, γ-CH_2 D-Lys). MS: *m/z* 1005.8. Calc. 1004.99 $[M - H]^-$ ($C_{49}H_{53}F_3N_7O_{13}$).

***N*-[(2*S*)-1-{(5'-*O*-(4,4'-Dimethoxytrityl)-3'-deoxythymidine-3'-yl)amino}-1-oxo-6-trifluoracetamidohexan-2-yl]-3'-deoxy-3'-[(2-cyanoethoxy)(diisopropylamino)phosphinooxy]thymidine-5'-carboxamide (9a).** Dinucleoside **8a** (492 mg, 0.49 mmol) and tetrazole (39 mg, 0.55 mmol) were evaporated with dry pyridine (2×5 mL), dissolved in dry CH_2Cl_2 (5 mL), 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphoramidite (127.5 μL, 0.55 mmol) was added, and the mixture was stirred for 2 h at 20 °C. TEA (0.1 mL) was added, the mixture was diluted with water (20 mL) and extracted with ethyl acetate (3×20 mL). Combined organic layers were washed with $NaHCO_3$ (30 mL) and water (30 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flesh column chromatography in 3-5% of ethanol in CH_2Cl_2 + 0.1% TEA to give **9a** as a hard foam (386 mg, 65.4% yield). R_f = 0.48 (Solvent system C). ^{31}P NMR: 152.47, 151.361. MS: *m/z* 1205.7. Calc. 1205.21 $[M - H]^-$ ($C_{58}H_{70}F_3N_9O_{14}P$).

***N*-[(2*R*)-1-{(5'-*O*-(4,4'-Dimethoxytrityl)-3'-deoxythymidine-3'-yl)amino}-1-oxo-6-trifluoracetamidohexan-2-yl]-3'-deoxy-3'-[(2-cyanoethoxy)(diisopropylamino)phosphinooxy]thymidine-5'-carboxamide (9b)** was prepared following the previous procedure from 328 mg (0.33 mmol) of dinucleoside **8b**. Yield 252 mg (64.1%). R_f 0.41 (Solvent system C). ^{31}P NMR: 150.55, 150.42. MS: *m/z* 1206.5. Calc. 1205.21 $[M - H]^-$ ($C_{58}H_{70}F_3N_9O_{14}P$).

ACKNOWLEDGEMENT

The work was supported by a grant from the Russian Foundation for Basic Research No 11-04-0031-a.

ABBREVIATIONS

BOP = (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate

DCC = *N,N'*-dicyclohexylcarbodiimide

EDTA = ethylenediaminetetraacetic acid

NHS = N-hydroxysuccinimide

ON = oligonucleotide

TEA = triethylamine

TEAA = triethylammonium acetate

TBAM = tetrabutylammonium fluoride

THF = tetrahydrofuran

DISCLOSURES

Part of information included in this chapter has been previously published in Nucleosides, Nucleotides and Nucleic Acids Volume 30, Issue 1, 2011.

REFERENCES

[1] Leonetti, C.; Zupi, G. Targeting Different Signaling Pathways with Antisense Oligonucleotides Combination for Cancer Therapy. *Curr. Pharm. Des*., **2007**, *13*, 463-470.

[2] Dean, N.M.; Bennett, C.F. Antisense oligonucleotide-based therapeutics for cancer. *Oncogene*, **2003**, *22*, 9087-9096.

[3] Dias, N.; Stein, C.A. Antisense Oligonucleotides: Basic Concepts and Mechanisms, *Mol. Cancer Ther.*, **2002**, *1*, 347-355.

[4] Aboul-Fadl T. Antisense Oligonucleotides: The State of the Art. *Curr. Med. Chem*., **2005**, 12, 763-771.

[5] Crooke, S.T. Progress in antisense technology. *Annu. Rev. Med*., **2004**, *55*, 61-95.

[6] Sazani, P.; Ko R. Therapeutic potential of antisense oligonucleotides as modulators of alternative splicing. *J. Clin. Invest.*, **2003**, *112*, 481-486.

[7] Tamm, I.; Wagner, M. Antisense Therapy in Clinical Oncology. Preclinical and Clinical Experiences. *Mol. Biotechnol.*, **2006**, *33*, 221-238.

[8] Agrawal, S.; Iyer, R.P. Modified oligonucleotides as therapeutic and diagnostic agents. *Curr. Opin. Biotechnol.*, **1995**, *6*, 12-19.

[9] Agrawal, S.; Zamecnik P.C. Site specific functionalization of oligonucleotides for attaching two different reporter groups. *Nucleic Acids Res.*, **1990**, *18*, 5419-23.

[10] Davies, M.J.; Shah, A.; Bruce, I.J. Synthesis of fluorescently labelled oligonucleotides and nucleic acids. *Chem. Soc. Rev*., **2000**, *29*, 97-107.

[11] Brazier, J.A.; Shibata, T.; Townsley, J.; Taylor, B. F.; Frary, E.; Williams, N.H.; Williams, D.M. Amino-functionalized DNA: the properties of C5-amino-alkyl substituted 2'-deoxyuridines and their application in DNA triplex formation. *Nucleic Acids Res*., **2005**, *33 (4)*, 1362–1371.

[12] Hashimoto, H.; Nelson, M.G.; Switzer, C. Formation of chimeric duplexes between zwitterionic and natural DNA. *J. Org. Chem.*, **1993**, *58*, 4194–4195.

[13] Bijapur, J.; Keppler, M.D.; Bergqvist, S.; Brown, T.; Fox, K.R. 5-(1-Propargylamino)-20-deoxyuridine (u-p): a novel thymidine analogue for generating DNA triplexes with increased stability. *Nucleic Acids Res.*, **1999**, *27*, 1802–1809.

[14] Sollogoub, M.; Darby, R.A.J.; Cuenoud, B.; Brown, T.; Fox, K.R. Stable DNA triple helix formation using oligonucleotides containing 20-aminoethoxy,5-propargylamino-U. *Biochemistry*, **2002**, *41*, 7224–7231.

[15] Heystek, L.E.; Zhou, H.Q.; Dande, P.; Gold, B. Control over the localization of positive charge in DNA: the effect on duplex DNA and RNA stability. *J. Am. Chem. Soc.*, **1998**, *120*, 12165–12166.

[16] Ruth, J. In: *Oligonucleotides and Analogues. Oligonucleotides with reporter groups attached to the base*; Eckstein, F. (Ed.); 1st ed.; Oxford University Press: New York, **1991**, pp. 255–282.

[17] Ito, T.; Ueno, Y.; Komatsu, Y.; Matsuda A. Synthesis, thermal stability and resistance to enzymatic hydrolysis of the oligonucleotides containing 5-(*N*-aminohexyl)carbamoyl-2'-*O*-methyluridines. *Nucleic Acids Res*., **2003**, *31 (10)*, 2514-2523.

[18] Sigurdson, S.T.; Eckstein F. Site specific labelling of sugar residues in oligoribonucleotides: reactions of aliphatic isocyanates with 2'-amino groups. *Nucleic Acids Res*., **1996**, *24(16)*, 3129-33.

[19] Printz, M.; Richert, C. Optimizing the Stacking Moiety and Linker of 2'-Acylamido Caps of DNA Duplexes with 3'-Terminal Adenine Residues. *J. Comb. Chem*., **2007**, *9 (2)*, 306-320.

[20] Venkatesan, N.; Kim, B.H. Peptide Conjugates of Oligonucleotides: Synthesis and Applications, *Chem. Rev*., **2006**, *106*, 3712-3761.

[21] Juliano, R.; Alam R.; Dixit V.; Kang H. Mechanisms and Strategies for Effective Delivery of Antisense and siRNA Oligonucleotides, *Nucleic Acids Res.*, **2008**, *36 (12)*, 4158–4171.

[22] Varizhuk, A.M.; Kochetkova, S.V.; Kolganova, N.A.; Timofeev, E.N.; Florentiev, V.L. Oligonucleotides containing a peptide internucleotide linkage. A Novel Class of Modified Oligonucletides, *Russian J. Bioorg. Chem.*, **2010**, *36 (4)*, 528-531.

[23] Varizhuk A.M.; Kochetkova S.V.; Kolganova N.A.; Timofeev E.N.; Florentiev V.L. Oligonucleotides Analogs with Peptide Internucleotide Linkages. *Nucleosides, Nucleotides Nucleic Acids*, accepted for publication.

[24] Lederer, R.; Stewart, F.H.C. The Use of Sequestering Agents in the Preparation of ε-Acyl-L-lysine and ε-Acyl-L-ornithine Derivatives. *Aust. J. Chem.,* **1965**, *18*, 933-935.