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Oligodeoxynucleotides with extended zwitter-ionic internucleotide linkage

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Abstract—Two non-natural nucleoside analogues, *N*-(2-hydroxyethyl)-2′,5′-dideoxy-5′-aminothymidine (dT^{NH}) and *N*-(2-hydroxyethyl)-*N*-methyl-2′,5′-dideoxy-5′-aminothymidine (dT^{NMe}), have been prepared and used in the synthesis of oligodeoxythymidilates and mixed-sequence oligodeoxynucleotides, modified at internucleotide linkages. Both modified oligodeoxythymidilates and mixed-sequence oligodeoxynucleotides have been shown to form zwitter-ionic phosphate–amine pairs as evidenced by their decreased electrophoretic mobility in denaturing polyacrylamide gel.

1. Introduction

Synthetic oligonucleotide analogues represent significant interest in the development of new therapeutic agents and biosensor probes.[1,2] The enhancement of the specific affinity of complementary binding of modified oligonucleotides to target DNA or RNA represents the key task in the design of modified oligonucleotides. The substitution of charged phosphodiester internucleotide linkage by uncharged or positively charged fragments plays an important role in the mechanism of duplex stabilization. Substitution of phosphodiesters or entire sugar–phosphate backbone in PNA, guanidinium analogues, phosphoramidates and some other modifications has been shown to result in a considerable duplex stabilization effect.[3–5]

Here, we report the synthesis of two new thymidine derivatives with extended 5′-hydroxyl functionality and an amino group available for charge interaction with the adjacent phosphate in the oligodeoxynucleotide (Fig. 1).

2. Results and discussion

Modified nucleosides dT^{NH} and dT^{NMe} were designed to provide a possibility of charge interaction between the 5′-amino group of the nucleoside and the attached phosphodiester group without considerable conformational changes in the double helix. Model geometry optimization of the modified structure provided the suitable linker length between amino- and hydroxyl groups, which appeared to be two methylenes. The results of conformational modeling are shown in Fig. 2 and Table 1. The modified site did not disturb the original B-form, being arranged into the six-membered quasicycle in a chair conformation with electrostatic interaction between charged phosphate oxygen and protonated nitrogen. Some additional adjustment flexibility was assumed for the hydroxyalkyl chain due to possible inversions at nitrogen.

Calculated non-electrostatic components of potential energy for modified duplex differ insignificantly from those for natural duplex, indicating negligible strand distortions. On the other hand, considerable contribution of intramolecular electrostatic interactions in modified duplex results in lower

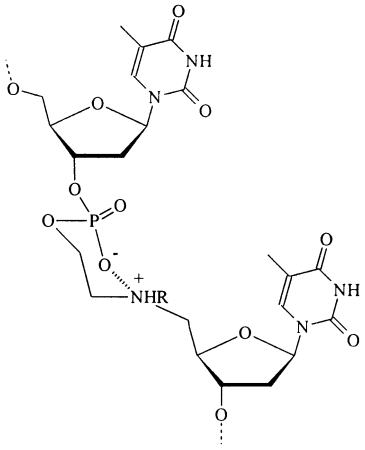

Figure 1. Phosphodiester linkage formed by dT^{NH} (R=H) or dT^{NMe} (R=Me) nucleoside analogues.

Keywords: nucleoside; oligodeoxynucleotide; modification.

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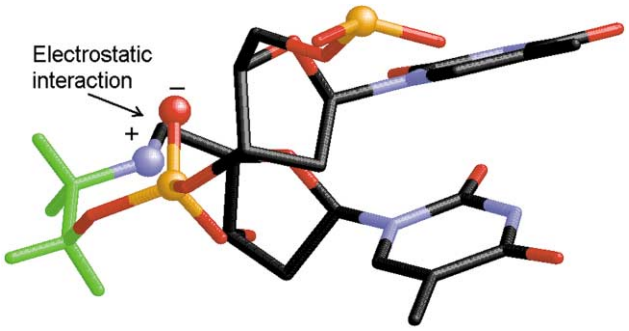


Figure 2. Fragment of duplex, containing modified internucleotide insert, generated by conformational modeling. Spheres mark electrostatically interacting atoms and phosphorus. Internucleotide insert collapses into quasicycle with chair conformation.

total potential energy. These data allow one to assume higher duplex stability for the modified oligomer.

The synthesis of the protected nucleoside derivative dT^{NMe} started from deoxythymidine and was easily accomplished in four steps without temporary protection of 3′ -hydroxyl group (Scheme 1). After substitution of the 5′-*p*-toluenesulfonate with *N*-methyl-ethanolamine, *N*-(2-hydroxyethyl)-*N*-methyl-2′,5′-dideoxy-5′-aminothymidine was separated from the excess of amine on Dowex-50 (in NH_4^+ form).

An alternative synthetic scheme, that uses a temporary 3′-hydroxyl protecting group, was used for *N*-(2-hydroxyethyl)-2′,5′-dideoxy-5′-aminothymidine. We attached a hydrophobic *t*BDMS tag to the 3′-hydroxyl of the thymidine 5′-tosylate (Scheme 2) in order to facilitate purification procedures for dT^{NH} derivatives.

Substitution of 5′-*p*-toluenesulfonates **1** and **5** was complicated by the formation of 2,5′-anhydro derivatives along with the target 5′-aminonucleosides dT^{NMe} and 3′-*t*BDMS-dT^{NH}, respectively. This effect appeared to be more expressed in the case of ethanolamine, resulting in a relatively low yield (26%) of compound **6**. A three-fold excess of either amine was sufficient for complete reaction and fast conversion of the tosylates, while larger excess did not influence the reaction efficiency and product distribution.

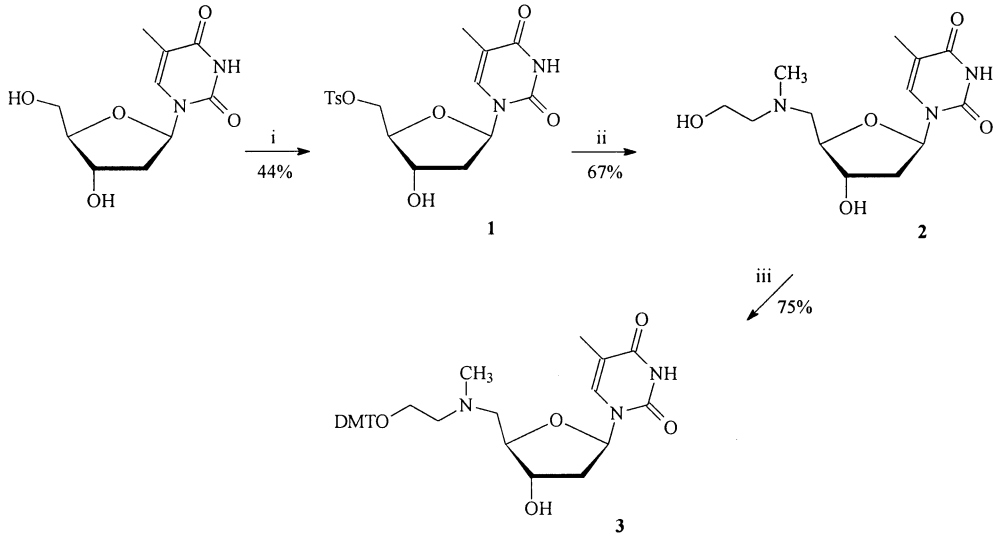
The aliphatic amino group of dT^{NH} analog was protected by conversion to the corresponding trifluoroacetamide, similarly to the commonly used aminomodifiers.[6]

Phosphoramidites of protected nucleoside analogues were prepared in situ following a known procedure[7] and used without isolation for the synthesis of modified oligodeoxynucleotides T1–T4 and M1–M2 (Table 2). We applied a standard synthetic protocol for all the modified nucleotides and did not observe a decrease in coupling efficiency as

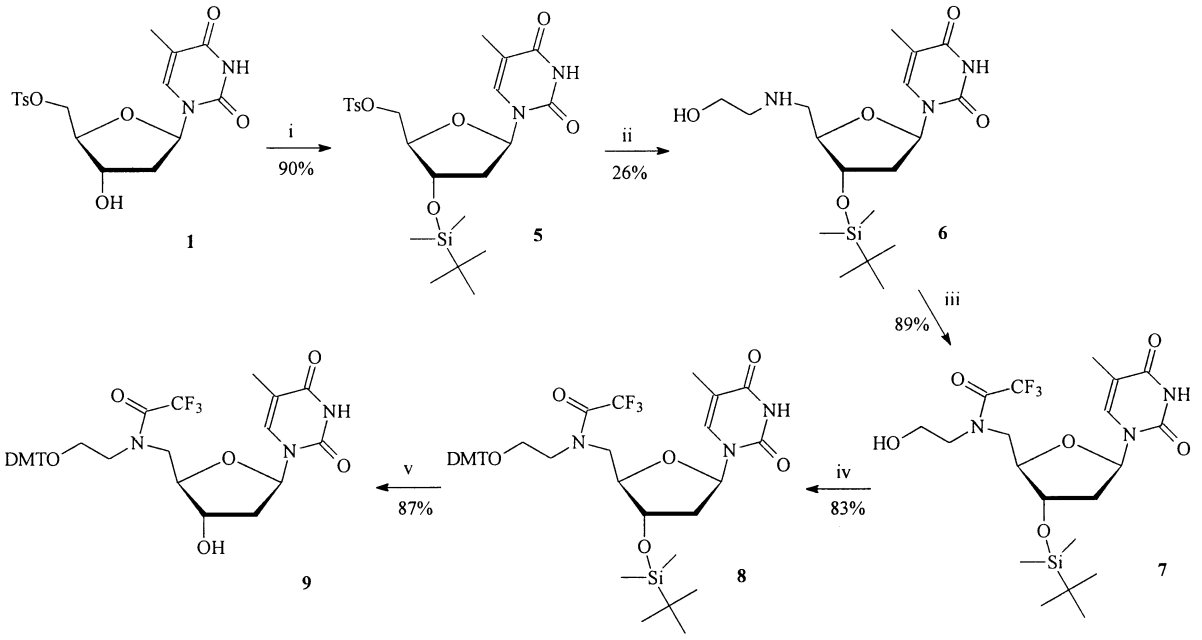
Table 1. Results of conformational modeling

Type of duplex	Energy components (kJ mol^{-1})					
	Total	Bond	Angle	Torsion	Van-der-Waals	Electro-static
Calculation without counter-ion						
Unmodified	−2379.9	6.3	306.9	1001.5	−780.5	−2956.4
Single modification[a]	−266.3	5.4	61.1	11.3	37.7	−381.9
Calculation with counter-ion						
Unmodified	−5878.1	45.6	360.1	1106.6	−729.8	−6661.1
Single modification[a]	−150.3	9.6	90.4	−16.3	72.9	−308.6

[a] For modified duplex differences in energy between modified and unmodified duplexes are given.



Scheme 1. *Reagents and conditions*: (i) *p*-TsCl in Py, 0°C; (ii) *N*-methyl ethanolamine in DMF, 100°C, 2 h; (iii) DMTCl in Py.



Scheme 2. *Reagents and conditions*: (i) *t*BDMSCl, Im in Py; (ii) ethanolamine in DMF, 100°C, 2 h; (iii) TFA anhydride, DMAP in acetonitrile, −10°C; (iv) DMTCl in Py; (v) 1 M TBAF in THF.

Table 2. Oligonucleotide sequences and mass data

ID	Sequence	M_{found} ($M_{calc.}$)
T0	5′-d(T)$_9$	–
T1	5′-d(TTTTT^{NH}TTTT)	2718 (2721)
T2	5′-d(TTTTT^{NMe}TTTT)	2734 (2735)
T3	5′-d(TT^{NH}TT^{NH}TT^{NH}TT^{NH}T)	2852 (2850)
T4	5′-d(TT^{NMe}TT^{NMe}TT^{NMe}TT^{NMe}T)	2910 (2906)
M0	5′-d(GATATCTTC)	–
M1	5′-d(GATAT^{NMe}CTTC)	2751 (2747)
M2	5′-d(GAT^{NMe}AT^{NMe}CT^{NMe}TC)	2867 (2861)

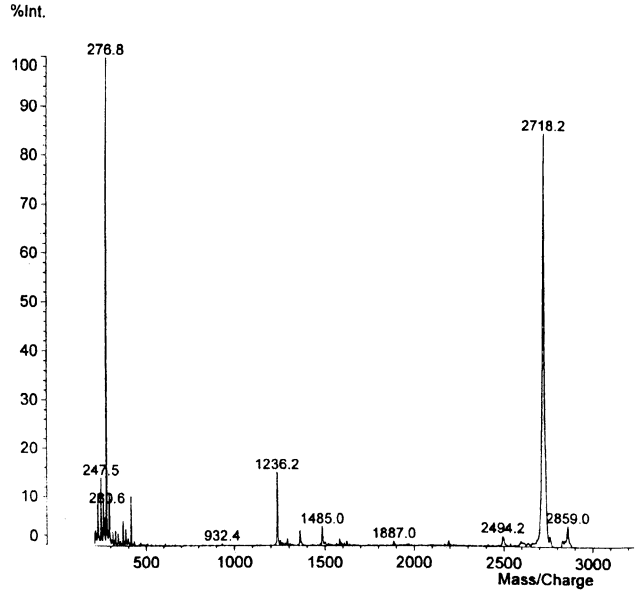


Figure 3. MALDI spectrum of the oligonucleotide T1.

estimated by trityl monitoring. Post synthetic procedures included usual ammonia deprotection (55°C, 6 h) and reverse phase HPLC. The structure of the oligonucleotides was examined by MALDI TOF mass spectrometry (Fig. 3 and Table 2), confirming the presence of modified nucleosides.

Protonation of the secondary amino-group in either dT^{NH}- or dT^{NMe}-modified oligonucleotides results in the zwitter-ionic structures that affect the chain charge. Electrophoretic mobility of the modified oligonucleotides in denaturing polyacrylamide gel reflects the effect of added positive charges (Fig. 4). The higher the content of modified nucleotides was, the lower was the mobility of zwitter-ionic

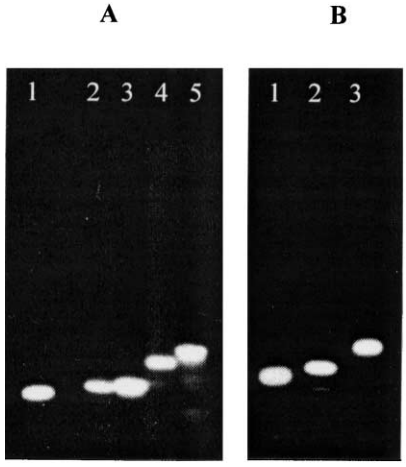


Figure 4. Denaturing polyacrylamide gel-electrophoresis of the unmodified oligomer T0 in lane 1 and oligonucleotides T1–T4 in lanes 2–5 (A). Electrophoresis of mixed-sequence oligonucleotides M0, M1 and M2 in lanes 1, 2 and 3, respectively (B).

oligomers in the electric field. Differences in the electrophoretic mobility between oligothymidilates T3 and T4 are most probably related to the differences in hydrodynamic volume of the molecules (Fig. 4(A)). This effect does not appear at the level of single replacement, while tetra-substituted oligomers differ enough to be separated in the gel given the same chain charge. Mixed-sequence oligodeoxynucleotides M1–M2 exhibit generally the same behavior as follows from Fig. 4(B).

Physico-chemical studies of the modified oligothymidilates, including thermal denaturation and circular dichroism experiments are currently being conducted.

We also note that despite the observed ability to induce intramolecular charge interaction, the thymidine derivative dT^{NH} provides an additional possibility for oligonucleotide functionalization. Secondary amino-group inserted into the phosphodiester backbone may be used for side-chain modifications of the oligonucleotide.

3. Conclusions

We have synthesized two new thymidine analogues that allow intramolecular charge interaction in the zwitterionic oligonucleotides. Corresponding phosphoramidites have been prepared and used in the synthesis of modified oligonucleotides. Denaturing gel-electrophoresis experiments provided evidence for the intramolecular zwitterionic interactions.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Brucker 400 MHz spectrometer. IR spectra were obtained from Specord M80 spectrophotometer. MALDI TOF analysis of modified oligonucleotides was performed on a Kratos Kompact MALDI IV mass spectrometer. TLC analysis was carried out on the Whatman AL SIL G/UV TLC plates.

The synthesis of oligodeoxynucleotides was carried out on automatic DNA synthesizer ASM-102U (Biosset, Russia) using custom protocol. Reverse phase HPLC purification was performed using 25×4 mm^2 Hypersil C18 column in 0.05 M TEAA buffer. DMT-protected oligomers were purified in the gradient of acetonitrile from 10 to 50% over 30 min. Completely deprotected oligonucleotides were additionally purified in the gradient from 0 to 25% of acetonitrile (over 30 min) in the above buffer.

Denaturing gel-electrophoresis of the radioactively labeled (^{32}P) oligonucleotides was run in a 20% (19:1) polyacrylamide matrix and 100 mM tris-borate buffer, pH 7, and 7 M urea as the running buffer.

Conformational calculations were performed by molecular mechanic method with Hyper Chem 6.0 (AMBER second generation parameters[8]). Effect of water was approximated by distant dependence of dielectric constant. Atom charges were calculated by ab initio. (6-31G* basis set).

4.1.1. 5′-*O*-*p*-Toluenesulfonyl-2′-deoxythymidine (1). *p*-Toluenesulfonyl chloride (2.45 g, 12.9 mmol) was added to the cooled (0°C) solution of 2′-deoxythymidine (3.12 g, 12.9 mmol) in 50 mL of dry pyridine. The reaction mixture was stirred for 2 h at 0°C and left for slow warming up to room temperature and then concentrated in vacuo. Residue was recrystallized from ethanol, yielding 2.27 g (44%) of white solid, mp 174–176°C; (Found: C, 51.4; H, 5.08. $C_{17}H_{20}N_2O_7S$ requires C, 51.51; H, 5.09; N, 7.07%]; R_f (10% aq. NH_3/dioxan) 0.70; ν_{max} (KBr) 3190, 2990, 1720, 1670, 1440, 1320, 1270, 1210, 1140, 1020, 980 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 1.75 (s, 3H, thymine CH_3), 2.02–2.20 (m, 2H, 2′CH_2), 2.40 (s, 3H, CH_3–Ph), 3.85–3.90 (m, 1H, 3′CH), 4.12–4.22 (m, 2H, 5′CH_2), 4.23–4.30 (m, 1H, 4′CH), 5.38 (d, 1H, 3′OH, J=4.5 Hz), 6.15 (t, 1H, 1′CH, $J_{1',2'}$=7 Hz), 7.36 (s, 1H, 6CH), 7.47 and 7.79 (d, 2H, C_6H_4, J=8 Hz), 11.22 (s, 1H, NH); δ_C (100.6 MHz, DMSO-d_6) 165.1, 151.9, 151.8, 146.6, 145.5, 137.6, 137.3, 133.7, 131.6, 129.5, 129.0, 127.8, 127.0, 111.2, 110.8, 88.7, 85.5, 85.3, 84.7, 71.9, 71.6, 71.4, 62.8, 39.9, 22.5, 13.7, 13.5.

4.1.2. 5′-*N*-Methyl,*N*-(2-hydroxyethyl)-2′,5′-dideoxy-5′-aminothymidine (2). The solution of **1** (0.37 g, 0.94 mmol) and *N*-methyl-ethanolamine (0.21 g 2.82 mmol) in 5 mL DMF was heated at 100°C for 2 h. Then, DMF was removed in vacuo. Residue was dissolved in water (100 mL) and applied to a column with Dowex 50 in NH_4^+ form. Excess of amine was washed off with water. Aminonucleoside **2** was eluted with 1.5% aqueous ammonia. Corresponding fraction was evaporated to dryness to give 0.18 g (67%) as a white solid foam; [Found: C, 52.1; H, 7.1; N, 14.0. $C_{13}H_{21}N_3O_5$ requires C, 52.16; H, 7.07; N, 14.04%]; R_f (10% aq. NH_3/dioxan) 0.25; ν_{max} (KBr) 3290, 3010, 1710, 1680, 1440, 1270, 1210, 1150, 1020, 980 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 1.78 (s, 3H, thymine CH_3), 2.00–2.14 (m, 2H, 2′CH_2), 2.25 (s, 3H, NCH_3), 2.43–2.51 (m, 2H, $HOCH_2CH_2N$), 2.55–2.64 (m, 2H, 5′CH_2), 3.42–3.50 (m, 2H, $HOCH_2CH_2N$), 3.75–3.79 (m, 1H, 3′CH), 4.10–4.12 (m, 1H, 4′CH), 6.15 (t, 1H, 1′CH, $J_{1',2'}$=7 Hz), 7.40 (s, 1H, 6CH); δ_C (100.6 MHz, DMSO-d_6) 167.2, 153.4, 137.1, 110.9, 85.7, 85.2, 73.4, 61.5, 61.2, 60.4, 44.61, 39.9, 13.9.

4.1.3. 5′-*N*-Methyl,*N*-(2-(*O*-4,4′-dimethoxytrityl)ethyl),-2′,5′-dideoxy-5′-aminothymidine (3). 4,4′-Dimethoxytrityl chloride (0.27 g, 0.81 mmol) was added to a stirred solution of **2** (0.22 g, 0.77 mmol) in 2 mL of dry pyridine. In 8 h, the reaction was completed and reaction mixture was concentrated in vacuo. Residue was dissolved in chloroform. Solution was washed with water, dried over Na_2SO_4 and evaporated to dryness. Residue was purified by column chromatography on silicagel. Column was eluted consequently with 20% hexane in chloroform, chloroform and 2% ethyl alcohol in chloroform. Appropriate fractions were evaporated to give 0.33 g (75%) as a white dry foam; [Found: C, 68.0; H, 6.6; N, 6.9. $C_{34}H_{39}N_3O_7$ requires C, 67.87; H, 6.53; N, 6.98%]; R_f 0.4 (10% methanol/chloroform); ν_{max} (KBr) 3290, 3000, 1720, 1690, 1440, 1270, 1210, 1140, 1020, 980 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 1.69 (s, 3H, thymine CH_3), 2.22 (s, 3H, NCH_3), 2.03–2.12

(m, 2H, 2′CH_2), 2.50–2.65 (m, 4H, OCH_2CH_2N and 5′CH_2), 3.08 (t, 2H, OCH_2CH_2N, J=6 Hz), 3.73 (s, 6H, OCH_3), 3.78–3.82 (m, 1H, 3′CH), 4.06–4.10 (m, 1H, 4′CH), 5.16 (d, 1H, 3′OH, J=4.5 Hz), 6.12 (t, 1H, 1′CH, J=7 Hz), 7.18–7.39 (m, 13H, 4,4′-dimethoxytrityl aromatic), 7.45 (s, 1H, 6CH), 11.20 (s, 1H, NH); δ_C (100.6 MHz, DMSO-d_6) 163.7, 158.0, 150.4, 145.1, 136.0, 135.9, 129.6, 127.8, 127.7, 126.6, 113.1, 109.4, 85.5, 84.4, 83.9, 71.8, 61.6, 59.8, 57.2, 55.0, 43.1, 38.5, 12.1.

4.1.4. 5′-*O*-*p*-Toluenesulfonyl-3′-*O*-*t*-butyldimethylsilyl-2′-deoxythymidine (5). *t*-Butyldimethylsilyl chloride (0.86 g, 5.73 mmol) and imidazole (0.39 g, 5.73 mmol) were added to a stirred solution of **1** (2.27 g, 5.73 mmol) in 5 mL of dry pyridine. In 8 h, the reaction was completed and pyridine was evaporated. Residue was dissolved in chloroform; solution was washed with water, dried over Na_2SO_4 and evaporated to dryness in vacuo. Product, 2.61 g (90%), was obtained as a white solid foam and used without additional purification; [Found: C, 54.0; H, 6.7; N, 5.5. $C_{23}H_{34}N_2O_7SSi$ requires C, 54.10; H, 6.71; N, 5.49%]; R_f (10% methanol/chloroform) 0.8, ν_{max} (KBr) 3180, 3000, 1720, 1690, 1440, 1270, 1210, 1150, 980, 900 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 0–0.1 (m, 6H, $(CH_3)_2Si$), 0.84–0.92 (m, 9H, *t*Bu), 1.95 (s, 3H, thymine CH_3), 2.02–2.28 (m, 2H, 2′CH_2), 2.45 (s, 3H, CH_3–Ph), 3.95–3.97 (m, 1H, 3′CH), 4.11–4.24 (m, 2H, 5′CH_2), 4.32–4.34 (m, 1H, 4′CH), 6.28 (t, 1H, 1′CH, $J_{1'2'}$=6.5 Hz), 7.35 and 7.77 (d, 2H, C_6H_4, J=7 Hz), 7.35 (s, 1H, 6CH); δ_C (100.6 MHz, DMSO-d_6) 165.1, 151.7, 151.0, 146.7, 146.5, 137.6, 137.4, 133.5, 131.8, 131.6, 129.5, 129.4, 129.2, 129.0, 125.3, 111.2, 86.9, 85.7, 85.6, 84.7, 83.6, 74.3, 73.0, 71.6, 71.4, 70.9, 45.5, 39.7, 36.8, 27.3, 27.0, 22.5, 19.0, 13.5, −1.8, −3.4, −3.7.

4.1.5. 5′-*N*-(2-Hydroxyethyl)-3′-*O*-*t*-butyldimethylsilyl-2′,5′-dideoxy-5′-aminothymidine (6). The solution of **5** (2.55 g, 5.00 mmol) and ethanolamine (0.92 g, 15.00 mmol) in 5 mL DMF was heated at 100°C for 2 h. Then, DMF was removed in vacuo. Residue was dissolved in diethyl ether; solution was washed twice with water, dried over Na_2SO_4 and evaporated to dryness. Compound **6** was purified by column chromatography on silicagel using, consequently 20% hexane in chloroform, chloroform and 5% methyl alcohol in chloroform. Appropriate fractions were evaporated to a colorless gum. Yield 0.52 g (26%); [Found: C, 53.9; H, 8.4; N, 10.4. $C_{18}H_{33}N_3O_5Si$ requires C, 54.11; H, 8.32; N, 10.52%]; R_f (10% methanol/chloroform) 0.25; ν_{max} (KBr) 3300, 3000, 1720, 1680, 1440, 1260, 1210, 1150, 1020, 980, 900 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 0.07 (s, 6H, $(CH_3)_2Si$), 0.86 (s, 9H, *t*Bu), 1.78 (s, 3H, thymine CH_3), 1.95–2.07 and 2.18–2.33 (m, 2H, 2′CH_2), 2.60 (t, 2H, CH_2CH_2N, J=6 Hz), 2.70–2.75 (m, 2H, 5′CH_2), 3.39–3.50 (m, 2H, $HOCH_2CH_2$), 3.71–3.79 (m, 1H, 3′CH), 4.33–4.36 (m, 1H, 4′CH), 6.11 (t, 1H, 1′CH, $J_{1'2'}$=7 Hz), 7.56 (s, 1H, 6CH); δ_C (100.6 MHz, DMSO-d_6) 205.5, 165.1, 151.9, 137.7, 111.1, 87.2, 85.2, 74.4, 61.6, 53.3, 52.2, 47.2, 27.1, 19.1, 13.6, 12.3, −3.3, −3.4.

4.1.6. 5′-*N*-Trifluoroacetyl-*N*-(2-hydroxyethyl)-3′-*O*-*t*-butyldimethylsilyl-2′,5′-dideoxy-5′-aminothymidine (7). Trifluoroacetic anhydride (0.09 mL, 0.63 mmol) in 2 mL of acetonitrile was added slowly to a stirred solution of **6** at −10°C (0.25 g, 0.63 mmol) and *N*,*N*-dimethylaminopyridine (0.08 g, 0.65 mmol) in 3 mL of dry acetonitrile. In 2 h, the reaction mixture was diluted by chloroform and washed with water. Organic layer was dried over Na_2SO_4 and evaporated to dryness, yielding 0.28 g (89%) of a colorless gum. Product was used without additional purification. [Found: C, 48.3; H, 6.7; N, 13.9. $C_{20}H_{32}F_3N_3O_6Si$ requires C, 48.47; H, 6.51; N, 14.04%]; R_f (10% methanol/chloroform) 0.5, ν_{max} (KBr) 3330, 2990, 1720, 1690, 1630, 1440, 1270, 1250, 1210, 1150, 1070, 970 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 0.08 (s, 6H, $(CH_3)_2Si$), 0.87 (s, 9H, *t*Bu), 1.80 (s, 3H, thymine CH_3), 2.06–2.13 and 2.33–2.41 (m, 2H, 2′CH_2), 3.30–3.60 (m, 6H, $HOCH_2CH_2$, CH_2CH_2N and 5′CH_2), 3.85–4.92 (m, 1H, 3′CH), 4.55–4.63 (m, 1H, 4′CH), 6.11 (t, 1H, 1′CH, $J_{1'2'}$=7 Hz), 7.48 (s, 1H, 6CH); δ_C (100.6 MHz, DMSO-d_6) 165.1, 151.9, 138.3, 137.8, 130.2, 111.3, 85.8, 84.6, 74.8, 73.8, 60.0, 58.8, 51.1, 50.1, 27.0, 19.1, 13.4, −3.3, −3.5.

4.1.7. 5′-*N*-Trifluoroacetyl-*N*-(2-(*O*-4,4′-dimethoxytrityl)-ethyl)-3′-*O*-*t*-butyldimethylsilyl-2′,5′-dideoxy-5′-aminothymidine (8). The compound was synthesized as described above for **3** starting from 0.25 g of **7**. The product was purified by column chromatography on silicagel using, consequently, 50% hexane in chloroform, chloroform and 1% methyl alcohol in chloroform. Appropriate fractions were evaporated to a yellow gum. Yield 0.33 g (83%); [Found: C, 61.6; H, 6.4; N, 5.2. $C_{41}H_{50}F_3N_3O_8Si$ requires C, 61.72; H, 6.32; N, 5.27%]; R_f (10% methanol/chloroform) 0.8; ν_{max} (KBr) 3000, 1720, 1690, 1640, 1440, 1270, 1260, 1210, 1160, 1070, 980 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 0.03–0.09 (m, 6H, $(CH_3)_2Si$), 0.82–0.91 (m, 9H, *t*Bu), 1.75 (s, 3H, thymine CH_3), 2.00–2.15 and 2.30–2.42 (m, 2H, 2′CH_2), 3.30–3.35 (m, 2H, OCH_2CH_2N), 3.65–3.80 (m, 10H, OCH_3, CH_2CH_2N and 5′CH_2), 3.85–3.95 (m, 1H, 3′CH), 4.25–4.43 (m, 1H, 4′CH), 6.07–6.14 (m, 1H, 1′CH), 6.75–7.52 (m, 14H, 4,4′-dimethoxytrityl aromatic and 6CH); δ_C (100.6 MHz, DMSO-d_6) 161.8, 158.1, 148.9, 145.2, 136.9, 129.5, 127.7, 127.9, 126.6, 113.0, 109.8, 85.6, 83.8, 73.2, 72.1, 59.8, 58.4, 55.1, 50.0, 26.9, 19.0, 12.0, −3.3, −3.5.

4.1.8. 5′-*N*-Trifluoroacetyl-*N*-(2-(*O*-4,4′-dimethoxytrityl)-ethyl)-2′,5′-dideoxy-5′-aminothymidine (9). The compound was prepared by standard desilylation procedure[9] from **8** with a yield of 87% as a white solid foam; [Found: C, 61.6; H, 5.2; N, 6.1. $C_{35}H_{36}F_3N_3O_8$ requires C, 61.49; H, 5.31; N, 6.15%]; R_f (10% methanol/chloroform) 0.6; ν_{max} (KBr) 3330, 3000, 1710, 1640, 1440, 1260, 1210, 1150, 1060, 980 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 1.87 (s, 3H, thymine CH_3), 2.15–2.22 and 2.29–2.38 (m, 2H, 2′CH_2), 3.37 (t, 2H, OCH_2CH_2N, J=5.5 Hz), 3.50–3.95 (m, 11H, OCH_2CH_2N, 5′CH_2, 3′CH and OCH_3), 4.15–4.21 (m, 1H, 4′CH), 6.16 (t, 1H, 1′CH, J=6.0 Hz), 6.78–7.39 (m, 14H, 4,4′-dimethoxytrityl aromatic and 6CH); δ_C (100.6 MHz, DMSO-d_6) 163.7, 160.0, 150.6, 147.2, 138.3, 130.9, 129.1, 127.9, 114.1, 110.8, 86.4, 74.4, 73.2, 60.7, 59.5, 56.0, 50.9, 27.8, 12.1.

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