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Synthesis and hybridization data of oligonucleotide analogs with triazole internucleotide linkages, potential antiviral and antitumor agents

Anna Varizhuk [a], Alexandr Chizhov [b], Vladimir Florentiev [a,*]

[a] Engelhardt Institute of Molecular Biology, Vavilov str., 32, 119991 Moscow, Russia
[b] N.D. Zelinsky Institute of Organic Chemistry, Leninsky prospect, 47, 119991 Moscow, Russia

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ABSTRACT

Triazolyl-functionalized oligonucleotide (ON) analogs have received much attention as potential antitumor and antiviral agents. The most promising of such analogs are those exhibiting high binding affinity toward native DNA/RNA, since they may prove to be efficient antisense or siRNA agents. To date, relatively few ON analogs with triazole internucleotide linkages have been described. In this paper, we report an improved synthesis of a modified dinucleoside phosphoramidite and hybridization data of ON analogs with four-bond triazole internucleotide linkages. We believe these data are essential for comprehensive analysis of the relation between the length of triazole internucleotide linkages and duplex stability.

1. Introduction

Click chemistry is a relatively new synthetic approach to joining organic molecules which mimics the approach used by nature. It takes advantage of reactions that are stereospecific and proceed in high yields under mild conditions in the presence of a diverse range of functional groups. Huisgen Cu^{I}-catalyzed [3 + 2] azide-alkyne cycloaddition (CuAAC), one of the most popular and efficient reactions within the concept of click chemistry [1], has recently gained significant importance in nucleic acids field, notably oligonucleotide labeling, immobilization, cross-linking and ligation [2,3]. The reaction leads to formation of 1,4-disubstituted 1,2,3-triazoles. A number of triazolyl-functionalized ON analogs have been synthesized using 1,3-dipolar cycloaddition, including base modified ON analogs, sugar modified analogs and the analogs with triazole rings in internucleotide linkages. Triazolyl-functionalized ON analogs have received much attention because of their potential antitumor and antiviral properties. In view of this therapeutic aspect, possibility of metal coordination, low toxicity of triazole rings and their remarkable stability may prove to be of use. Incorporation of triazole rings in internucleotide linkages is a way to generate promising ON analogs with enhanced resistance to chemical and enzymatic degradation. The most promising of such analogs are those exhibiting high binding affinity toward native DNA/RNA, since they may prove to be efficient antisense or siRNA agents [4,5].

To date, relatively few ON analogs with triazole internucleotide linkages have been described. One of the noteworthy triazoliyl-linked ONs is oligo-d*T* analog synthesized by Isobe et al. [6], which has been reported to form extremely stable duplex with its DNA complement (ΔTm per modification = 4.6 °C). It has been suggested that the six-bond backbone periodicity (from $C3'_i$ to $C3'_{i+1}$), characteristic of the abovementioned analog, is essential for duplex stability since longer triazole internucleotide linkages have been shown to destabilize duplexes [7,8]. The comparison between four- and five-bond (from C4′ to C3′) triazole linkages (Scheme 1) has not been made so far. Fully modified ON analogs with four-bond triazole linkages have been reported by Nuzzi et al. [9]. Their study has been continued by Chandrasekhar et al., who considered modification throughout the chain to be a limiting factor for further exploration and synthesized dinucleoside phosphoramidite **8** (Scheme 2) to overcome this [10]. Single and multiple incorporation of a modified dinucleoside in ON chains at requisite sites allows evaluating the influence of the number and the positions of modifications on duplex stability. Modified dinucleoside block **8** has been successfully utilized in solid-phase ON synthesis. However, no data on DNA binding affinity of thus obtained ON analogs has been reported. In this paper, we report on improved synthesis of modified dinucleoside phosphoramidite **8** and hybridization data of ON analogs with four-bond triazole internucleotide linkages. We believe these data are essential for comprehensive analysis of the relation between the length of triazole internucleotide linkages and duplex stability.

Abbreviations: CuAAC, CuI-catalyzed [3 + 2] azide-alkyne cycloaddition; EDTA, ethylenediamine tetra-acetic acid; IBX, 2-iodoxybenzoic acid; ON, oligonucleotide; TEA, triethylamine; TEAA, triethylammonium acetate; TBAF, tetrabutylammonium fluoride; THF, tetrahydrofuran.

* Corresponding author. Fax: +7 499 135 1405.
E-mail address: flor@imb.ac.ru (V. Florentiev).

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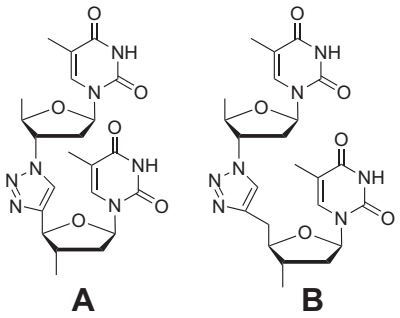
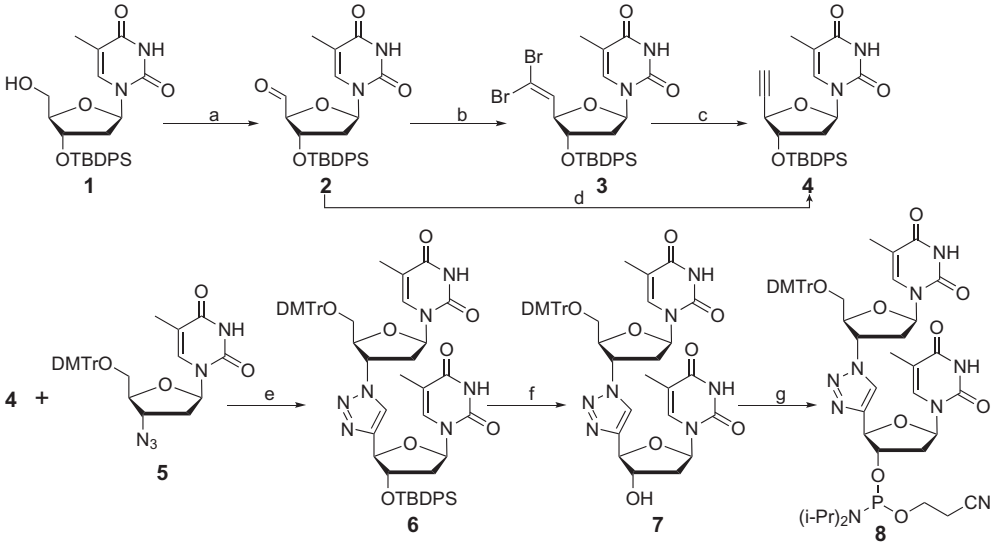


Scheme 1. Fragments of modified ONs with four-bond and five-bond triazole linkages.

2. Results and discussion

We started the synthesis of the target dinucleoside block with the preparation of a key intermediate, acetylenic derivative **4** (Scheme 2). Synthesis of **4** from easily accessible 3′-*O*-(*tert*-butyldiphenylsilyl)thymidine **1** [11] was realized in 2–3 steps. Alcohol **1** was oxidized with IBX in abs. ethyl acetate (4 h, 80 °C) [12]. Remarkably simple work-up procedure, including filtration and removal of the solvent, afforded aldehyde **2** in near quantitative yield. To prepare acetylenic derivative **4** from aldehyde **2**, we employed two methods. The first method included two steps. Like Chandrasekhar and et al., we converted aldehyde to terminal alkyne via intermediate dibromomethylene derivative. While Chandrasekhar et al. employed Corey–Fuchs [13] protocol to prepare the dibromomethylene derivative, we employed a Wittig-type condensation of aldehyde **2** with the ylide derived from dibromomethyltriphenylphosphonium bromide (prepared by Wolkoff's method [14]) in the presence of Zn in abs. dioxane under reflux (3 h), which gave compound **3** in 80% yield. (Condensation of aldehydes with the abovementioned ylide has been reported as the first stage of a one-pot procedure for synthesis of alkynes from aldehydes [15]. In that procedure, t-BuOK was used to generate ylide in the first stage and to convert dibromo derivatives to target alkynes in the second stage. However, the yield of compound **7** obtained by this general procedure was disappointingly low). Compound **3** was treated with BuLi in abs. THF (0.5 h, −70 °C) to give compound **4** in 83% yield. The second method we used for formyl-to-ethynil group transformation was Ohira–Bestmann reaction [16], and it enabled us to avoid the intermediate step. Aldehyde **2** was reacted with dimethyl-1-diazo-2-oxopropylphosphonate (Ohira–Bestmann reagent), generated in situ from tosyl azide (prepared following the published procedure [17]) and dimethyl-2-oxopropylphosphonate (prepared following the published procedure [18]), in the presence of K_2CO_3 and methanol in abs. acetonitrile (24 h, 20 °C) to give **4** in a yield of 87%. Compound **4** was subjected to cycloaddition with 3′-azido-3′-deoxy-5′-*O*-dimethoxytritylthymidine **5** in the presence of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate. Compounds **4** and **5** being poorly soluble in aqueous medium and such typical solvent systems for CuAAC as H_2O/*t*BuOH or H_2O/ethanol, we used a two-phase solvent system, CH_2Cl_2/H_2O [19]. Although CH_2Cl_2 is commonly used as a solvent for organic ligands in ligand-mediated CuAAC, in ligand-free CuAAC this solvent system is quite new. The use of this system has been reported to increase reaction rates. Indeed, the reaction of azide **5** and alkyne **4** in CH_2Cl_2/H_2O afforded triazole dinucleoside **6** in 75% yield after 2 h (20 °C). The silyl protection was removed with 0.5 M TBAF in THF (2 h, 20 °C). Treatment of thus obtained dinucleoside **7** with 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphoramidite in the presence of 1*H*-tetrazole and pyridine in dichloromethane (2 h, 20 °C) afforded target phosphoramidite **8** in 65% yield. Nucleoside **4** and dinucleosides **6**–**8** were characterized by ESI HRMS and the structures of **6** and **7** were confirmed by COSY NMR spectroscopy (see Fig. 1 for the spectrum of compound **7** and supplementary data for the spectrum of compound **6**). COSY NMR experiments enabled assignment of signals to protons of 5′-deoxy-5′-methylenethymidine and 3′-amino-3′-deoxythymidine moieties in ^{1}H NMR spectra of the dinucleosides, and CH correlation NMR experiments enabled assignment of ^{13}C NMR spectra of the dinucleosides (for ^{13}C and C–H correlation NMR spectra, see supplementary data).

Dinucleoside block **8** was utilized directly for solid-phase synthesis of modified ONs using standard phosphoramidite protocols. Coupling time was increased to 15 min for the modified phosphoramidite. No decrease in coupling efficiency was observed (98–99% step-wise coupling yields for both modified and unmodified amidites). Along with the modified ONs, a wild-type complement and isosequential ONs were synthesized. Purification of the ONs was carried out by reverse-phase HPLC. All the ONs were characterized by MALDI mass spectrometry (Table 1). Thermal dissociation of the modified duplexes and their wild-type counterpart



Scheme 2. Synthesis of the phosphoramidite dinucleosideblockwith a triazole internucleoside linkage. Reagents and conditions: (a) IBX, ethylacetate, 80 °C; (b) $PPh_3CHBr_2^+Br^-$, Zn, dioxane, reflux; (c) *n*-BuLi, THF, −70 °C;(d) Ohira–Bestmann reagent, K_2CO_3, MeOH, acetonitrile; (e) $CuSO_4$, ascorbate Na, CH_2Cl_2/H_20; (e) and (f) TBAF, THF; (g) $NCCH_2CH_2OP(NPr^i_2)_2$, 1*H*-tetrazole, pyridine, CH_2Cl_2.

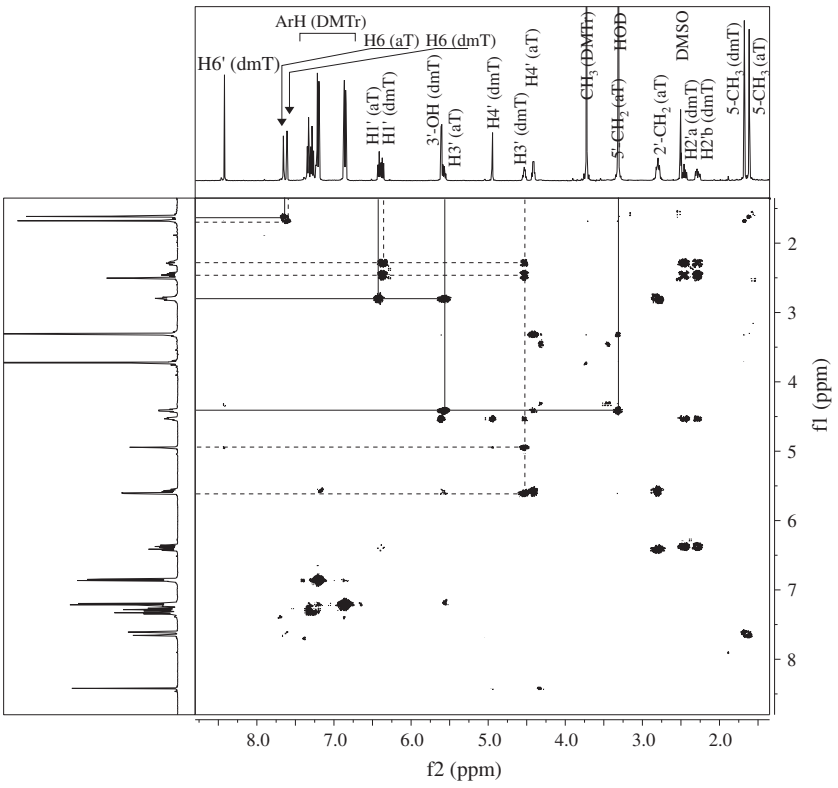


Fig. 1. COSY NMR spectrum of compound **6**. dmT = 5′-deoxy-5′-methylenethymidine moiety; aT = 3′-amino-3′-deoxythymidine moiety.

Table 1
MALDI-TOF mass spectra of ONs and melting temperatures of the corresponding duplexes (duplex concentration 2.5×10^{-6} M).

Sequence (5′ → 3′)*	*m*/*z*, found (calculated for $[M + H]^+$)	T_m, °C ± 0.5, (ΔT_m, °C**)
TTAACTTCACATTC		50.3
TTAACTTCACA**X**C	5030 (5028.37)-	47.3 (−3.0)
XAACTTCACATTC	5029 (5028.37)	49.6 (−0.7)
TTAAC**X**CACATTC	5030 (5028.37)	39.9 (−10.4)
XAAC**X**CACA**X**C	4944 (4942.45)	33.0 (−17.3)

Notes:
* X – modified dinucleoside fragment with a triazole internucleotide linkage.
** T_m difference between modified and natural duplexes.

was measured. The resulting curves enabled evaluation of the melting temperatures of the duplexes (Table 1).

As evident from Table 1, the modification causes destabilization, the extent of which depends on the position of the modified fragment. Modification in the middle of the strand dramatically decreases duplex stability, which is evident of serious distortion of duplex structure. 3′-Terminal modification also causes significant destabilization. Average ΔTm per modification is −5.3 °C (calculated as a sum of all ΔTm values divided by the total number of modifications). The overall effect of multiple modifications roughly equals the sum of the effects of single modifications.

3. Conclusion

In summary, we have reported on improved synthesis of the dinucleoside phosphoramidite block, utilization of the modified block in ON synthesis and hybridization data of thus obtained ON analogs. The data reported provide the missing link in comparative analysis of various triazolyl modifications. ON analogs with four-bond (from C4′ to C3′) internucleotide linkages seem an attractive alternative to the analogs with five-bond linkages since preparation of the former requires less synthetic effort (no need in extra homologation step). However, short linkages distort the double helix. Our results add to the growing body of evidence that the six-bond (from $C3'_i$ to $C3'_{i+1}$) backbone periodicity is crucial for the double-strand formation.

4. Experimental

All reagents were commercially available unless otherwise mentioned and used without further purification. 3′-Deoxy-3′-azidothymidine was provided by Association AZT (Russia). All solvents were purchased from Khimmed (Russia). Dioxane was dried over sodium hydroxide and distilled, dichloromethane was distilled from phosphorus pentaoxide, pyridine was distilled from calcium hydride, and THF was distilled from lithium alumohydride prior to use. Flash column chromatography (CC) was performed on silica gel Kieselgel 60 (0.040–0.063 mm, Merck, Germany). 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), 1:49 (B), 1: 25 (C), 1:9 (D). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMXIII-400 NMR spectrometer (Germany). Chemical shifts are given in parts per million (ppm). The coupling constants (*J*) are given in Hz. Abbreviations used: dmT, 5′-deoxy-5′-methylenethymidine moiety; aT, 3′-amino-3′-deoxythymidine moiety. The signals were assigned to protons using COSY experiments. The NMR data were processed and presented using MestReNova version 6.1.1 (Mestrelab Research SL, Spain). MALDI TOF mass spectra were acquired on a Bruker Ultraflex II mass spectrometer (Germany) in a positive ion mode. ESI HR mass spectra were acquired on a Bruker maXis spectrometer (Germany) in a positive ion mode (capillary voltage – 4500 V, end plate offset – 500 V, interface capillary temperature 180 °C, nitrogen dry gas, 4.0 L/min, scanning range from *m*/*z* 50 to *m*/*z* 3000 Da, scanning frequency 1scan/s). Internal calibration was done using Electrospray Calibration Solution (Fluka).

ONs were synthesized on an Applied Biosystems 3400 DNA synthesizer (USA) using standard phosphoramidite protocols and purified using preparative scale reverse-phase HPLC on a 250 mm × 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. Detritylated oligonucleotides were further purified in 0–25% gradient of CH_3CN in TEAA buffer. Melting curves of the duplexes were recorded on a Shimadzu UV 160-A spectrophotometer (Japan), 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 2.5×10^{-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 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, USA). The calculation method taking into account temperature dependence of UV absorbance of duplexes and single strands was applied.

4.1. 1-(2-Deoxy-3-O-tert-butyldiphenylsilyl-β-D-erythro-pentadialdo-1,4-furanosyl)-5-methyluracil (2)

3′-*O*-(*tert*-Butyldiphenylsilyl)thymidine **1** (0.9 g, 1.87 mmol) was dissolved in abs. ethyl acetate (13 mL), and IBX (1.57 g, 5.62 mmol) was added. The resulting suspension was immersed in an oil bath set to 80 °C and stirred vigorously open to the atmosphere. After 3.5 h, the reaction mixture was cooled to room temperature and filtered. The filter cake was washed with 2 × 10 mL of ethyl acetate, and the combined filtrates were concentrated to provide 877 mg (97% yield, >95% pure by ^{1}H NMR) of the desired aldehyde **2** as a white foam. The aldehyde **2** was used without purification for the next step. R_f = 0.42 (Solvent system C). ^{1}H NMR (400 MHz, DMSO-d6): δ = 11.31 (1 H, s, H3), 9.35 (1 H, d, $J_{5',4'}$ 0.4, H5′), 7.69–7.38 (11 H, m, ArH and H6), 6.33 (1 H, t, *J* 7.1, H1′), 4.79–4.72 (1 H, m, H3′), 4.48 (1 H, d, $J_{3',4'}$ 1.6, H4′), 2.12 (2 H, m, H2′a and H2′b), 1.76 (3 H, d, ^4J 0.8, 5-CH_3), 1.06 (9 H, s, *t*-BuSi).

4.2. 1-(2,5,6-Trideoxy-6,6-dibromo-3-O-(tert-butyldiphenylsilyl)-β-D-erythro-hex-5-enofuranosyl)-5-methyluracil (3)

To a stirred solution of nucleoside **2** (0.86 g, 1.8 mmol) and dibromomethyltriphenylphosphonium bromide (1.85 g, 3.59 mmol) in abs. dioxane (4 mL), 260 mg (3.95 mmol) of Zn was added. The reaction mixture was stirred at reflux for 3 h, then cooled to r.t. and filtered. The filtrate was evaporated, dissolved in dichloromethane (50 mL) and washed with brine (30 mL). The organic layer was separated, dried over Na_2SO_4 and concentrated. Silica gel column chromatography (CC) in 1–3% of ethanol in CH_2Cl_2 afforded compound **3** as a colorless foam (912 mg, 80% yield). R_f = 0.55 (Solvent system A). ^{1}H NMR (400 MHz, DMSO-d6): δ = 11.27 (1 H, s, H3), 7.65–7.42 (10 H, m, ArH), 7.37 (1 H, d, ^4J 1.1, H6), 6.76 (1H, d, *J* 9.0, H5′), 6.21 (1 H, t, *J* 6.9, H1′), 4.50 (1H, dd, $J_{5',4'}$ 9.0, $J_{4',3'}$ 3.53, H4′), 4.23–4.37 (1 H, m, H3′), 2.18–2.13 (2 H, m, H2′a and H2′b), 1.75 (3 H, d, ^4J 1.1, 5-CH_3), 1.05 (9 H, s, *t*-BuSi).

4.3. 1-(2,5,6-Trideoxy-3-O-(tert-butyldiphenylsilyl)-β-D-erythro-hex-5-ynofuranosyl)-5-methyluracil (4)

4.3.1. From nucleoside 3

To a stirred solution of nucleoside **3** (1.07 g, 1.688 mmol) in abs. THF (17 mL), 6 mL of 1.6 M solution of butyllithium in abs. hexane was added dropwise at −70 °C. The reaction mixture was stirred for 30 min at −70 °C and allowed to heat to r.t. Saturated NH_4Cl (30 mL) was added. The mixture was extracted with dichloromethane (3 × 30 mL). Combined organic layers were washed with water (50 mL), dried over Na_2SO_4 and concentrated. The residue was purified by CC in 1% of ethanol in CH_2Cl_2 to give compound **4** as a colorless foam (665 mg, 83% yield). R_f = 0.48 (Solvent system A).

4.3.2. From nucleoside 2

To a suspension of K_2CO_3 (829 mg, 6 mmol) and tosyl azide (473 mg, 2.4 mmol) in abs. acetonitrile (20 mL), dimethyl-2-oxopropylphosphonate (399 mg, 2.4 mmol) in abs. acetonitrile (5 mL) was added. The reaction mixture was stirred for 2 h at r.t., then a solution of compound **2** (957 mg, 2 mmol) in abs. methanol (6 mL) was added, and the mixture was left overnight at r.t. The solvent was removed, and the residue was partitioned between water (30 mL) and dichloromethane (50 mL). The aqueous layer was extracted with dichloromethane (30 mL). Combined organic layers were washed with brine (30 mL), dried over Na_2SO_4 and concentrated. The residue was purified by CC in 1% of ethanol in CH_2Cl_2 to give compound **4** as a colorless foam (823 mg, 87% yield). R_f = 0.48 (Solvent system A). ^{1}H NMR (400 MHz, DMSO-d6): δ = 11.32 (1 H, s, H3), 7.67–7.42 (10 H, m, ArH6), 7.40 (1 H, d, ^4J 1.1, H6), 6.34 (1 H, t, *J* 7.1, H1′), 4.57 (1 H, t, $J_{4',3'}$ 2.2, $^4J_{4',6'}$ 2.2, H4′), 4.53–4.49 (1 H, m, H3′), 3.75 (1H, d, $^4J_{4',6'}$ 2.2, H6′), 2. 26–2.21 (2 H, m, H2′a and H2′b), 1.75 (3 H, d, ^4J 1.1, 5-CH_3), 1.04 (9 H, s, *t*-BuSi). MS: *m*/*z* Calcd for $C_{27}H_{30}N_2O_4Si$, $[M + Na]^+$ 497.1867. Found: 497.1871.

4.4. 1-(3′-Deoxy-5′-O-(4,4′-dimethoxytrityl)thymidine-3′-yl)-4-(3′-O-(tert-butyldiphenylsilyl)-4′-de(hydroxymethyl)thymidine-4′-yl)-1H-1,2,3-triazole (6)

To a stirred solution of compound **4** (300 mg, 0.63 mmol) and 3′-azido-3′-deoxy-5′-*O*-dimethoxytritylthymidine **5** (396 mg, 0.7 mmol) in dichloromethane (3 mL) and water (3 mL), $CuSO_4{\cdot}5H_2O$ (10 mg, 0.063 mmol) and sodium ascorbate (39 mg, 0.21 mmol) were added. The resulting solution was stirred for 2 h at r.t. The reaction mixture was diluted with dichloromethane (5 mL) and brine (5 mL). The organic layer was separated, dried over Na_2SO_4 and concentrated. The residue was purified by CC in 1% of ethanol in CH_2Cl_2 to give dinucleoside **6** as a hard foam (497 mg, 75% yield). R_f = 0.37 (Solvent system B). ^{1}H NMR (400 MHz, DMSO-d6): δ = 11.38 (1 H, s, H3), 11.30 (1 H, s, H3), 8.13 (1 H, s, H6′ dmT), 7.66 (1 H, d, ^4J 0.9, H6 aT), 7.54 (1 H, d, ^4J 1.0, H6 dmT), 7.62–6.81 (23 H, m, ArH DMTr and Ph_2Si), 6.46 (1 H, t, *J* 7.0, H1′ dmT), 6. 34 (1 H, t, *J* 6.0, H1′ aT), 5.55–5.47 (1 H, m, H3′ aT), 5.04 (1 H, d, $J_{4',3'}$ 2.6, H4′dmT), 4.64–4.60 (1 H, m, H3′ dmT), 4.36–4.30 (1 H, m, H4′ aT), 3.71 (6 H, s, CH_3O DMTr), 3.28 (2 H, d, $J_{4',5'\text{-}CH2}$ 3.9, 5′-CH_2 aT), 2.80 (1 H, ddd, $J_{1',2'a}$ 6.0, $J_{3',2'a}$ 8.6, $^2J_{2'a,2'b}$ 14.1, H2′a aT), 2.75–2.66 (1 H, m, H2′b aT), 2.42–2.31 (2 H, m, 2′-CH_2 dmT), 1.64 (3 H, d, ^4J 1.0, 5-CH_3 dmT), 1.62 (3 H, d, ^4J 0.9, 5-CH_3 aT), 1.02 (9 H, s, *t*-BuSi). MS: *m*/*z* Calcd for $C_{58}H_{61}N_7O_{10}Si$, $[M + Na]^+$ 1066.4141. Found 1066.4133.

4.5. 1-(3′-Deoxy-5′-O-(4,4′-dimethoxytrityl)thymidine-3′-yl)-4-(4′-de(hydroxymethyl)thymidine-4′-yl)-1H-1,2,3-triazole (7)

To a stirred solution of dinucleoside **6** (842 mg, 0.81 mmol) in dry THF (1.7 mL), 1 M solution of tetrabutylammonium fluoride in dry THF (1.7 mL) was added. The mixture was stirred for 2 h at r.t., 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 CC in 7% of ethanol in CH_2Cl_2 + 0.1% TEA to give dinucleoside **7** as a hard foam (640 mg, 98% yield). R_f = 0.57 (solvent system D). ^{1}H NMR (400 MHz, DMSO-d6): δ = ^{1}H NMR (DMSO): 11.36 (1 H, s, H3), 11.28 (1 H, s, H3), 8.41 (1 H, s, H6′

dmT), 7.65 (1 H, d, ^4J 1.1, H6 aT), 7.60 (1 H, d, ^4J 1.2, H6 dmT), 7.39–6.82 (13 H, m, ArH DMTr), 6.40 (1 H, t, J 6.50, H1′ aT), 6.36 (1 H, dd, $J_{1',2'a}$ 7.6, $J_{1',2'b}$ 6.2, H1′ dmT), 5.59 (1 H, d, $J_{3'\text{-OH}',3'}$ 4.3, 3′-OH), 5.59–5.53 (1 H, m, H3′ aT), 4.94 (1 H, d, $J_{4',3'}$ 2.9, H4′dmT), 4.55–4.49 (1 H, m, H3′ dmT), 4.43–4.38 (1 H, dt, $J_{3',4'}$ 6.6, $J_{4',5'\text{-CH2}}$ 4.0, H4′ aT), 3.72 (6 H, s, CH_3O DMTr), 3.31 (2 H, d, $J_{4',5'\text{-CH2}}$ 4.0, 5′-CH_2 aT), 2.83–2.75 (2 H, m, J 8.54, 2′-CH_2 aT), 2.44 (1 H, ddd, $J_{1',2'a}$ 7.6, $J_{3',2'a}$ 6.1, $^2J_{2'a,2'b}$ 13.8, H2′a dmT), 2.27 (1 H, ddd, $J_{1',2'b}$ 6.1, $J_{3',2'a}$ 3.3, $^2J_{2'a,2'b}$ 13.8, H2′b dmT), 1.67 (3 H, d, ^4J 1.2, 5-CH_3 dmT), 1.61 (3 H, d, ^4J 1.1, 5-CH_3 aT). MS: m/z Calcd for $C_{42}H_{43}N_7O_{10}$, $[M + Na]^+$ 828.2964. Found 828.2963.

4.6. 1-(3′-Deoxy-5′-O-(4,4′-dimethoxytrityl)thymidine-3′-yl)-4-(3′-deoxy-3′-((2-cyanoethoxy)(diisopropylamino)phosphinooxy)-4′-de(hydroxymethyl)thymidine-4′-yl)-1H-1,2,3-triazole (8)

Dinucleoside **7** (580 mg, 0.72 mmol) was dissolved in abs. dichloromethane, pyridine (2 × 5 mL) and tetrazole (39 mg, 0.55 mmol) were added, and the mixture was stirred over molecular sieves for 30 min at r.t. 2-Cyanoethyl-*N,N,N′,N′*-tetraisopropylphosphoramidite (127.5 μL, 0.55 mmol) was added. The mixture was stirred for 30 min at r.t., then poured into 50 mL of cold saturated $NaHCO_3$ (50 mL) and extracted with CH_2Cl_2 (3 × 40 mL). Combined organic layers were washed with water, dried over Na_2SO_4, filtered and concentrated. The residue was purified by CC in 2–3% of ethanol in CH_2Cl_2 + 0.1% TEA to give amidite **8** as a hard foam (470 mg, 65% yield). R_f = 0.49 (Solvent system C). ^{31}P NMR (133 MHz, DMSO-d6): δ = 150.991, 150.575. MS: m/z Calcd for $C_{51}H_{60}N_9O_{11}P$, $[M + Na]^+$ 1028.4042. Found 1028.4033.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bioorg.2011.03.002.

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