**4´-Alkoxy Oligodeoxynucleotides: A Novel Class of RNA Mimics.**

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**1. Experimental**

*General.*

Unless stated otherwise, the solvents were evaporated at 40 °C and 13 kPa using rotary evaporator. The products were dried over phosphorus pentoxide at 40–50 °C and 13 Pa. The course of the reactions was followed by TLC on Merck Silica gel 60 F$_{254}$ aluminum sheets and the products were visualized by UV monitoring. Preparative column chromatography was performed on silica gel (40–60 µm, Fluka) whereby the amount of adsorbent used was 20–40 times the weight of the mixture separated. Elution was performed at the flow rate of 40 mL min$^{-1}$. In case of purification of dimethoxytrityl derivatives and phosphoramidites, the slurry of silica gel was prepared in the appropriate solvent containing triethylamine (2 ml per 100 ml of a silica gel bed).

*Materials and methods.*

The synthesis of oligonucleotides was performed in 0.5 micromolar scale on a GeneSyn synthesizer using solid support (CPG-dT) and standard phosphoramidite approach. Final oligonucleotides were purified by semipreparative HPLC on reverse phase column (Luna C18-5µ, 10x250 mm, Phenomenex) using a linear gradient of acetonitrile in 0.1M-TEAA pH 7.5. Thermal characteristics of oligonucleotide complexes were recorded on Cary 100 Bio spectrophotometer (THERMAL program). MALDI-TOF mass spectra were recorded on a Bruker Reflex4 spectrometer; N$_2$ laser 337 nm U.; 3-Hydroxypicolinic and picolinic acid (9:1) in acetonitrile and mQ-water (1:1, v/v) were used as matrices. Values of molecular mass of prepared oligonucleotides as determined by these measurements were all in accordance with the calculated ones, as follows:
Table S1 Molecular mass of oligomers obtained from MALDI TOF

<table>
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<th>Entry</th>
<th>T₁₅-strand</th>
<th>Molecular weight</th>
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<tr>
<td>4</td>
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<td>6</td>
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<td>4920.29</td>
<td>4919.65</td>
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Mass spectra (m/z) were recorded on ZAB-EQ (VG Analytical) instrument, using FAB+ and/or FAB- technique (Xe, 8 kV) with glycerol–thioglycerol (3:1) and 2-hydroxyethyl disulfide as matrices. MS HR ESI spectra (m/z) were recorded on LTQ Orbitrap XL (Thermo Fischer Scientific) instrument. Elemental analyses were carried out on a PE 2400 Series II analyzer.

NMR spectra were measured on Varian UNITY 500 spectrometer (¹H at 500 MHz; ¹³C at 125.7 and ³¹P at 202.3 MHz) and Bruker AVANCE 600 spectrometer (¹H at 600 MHz; ¹³C at 125.8 MHz) in DMSO, CDCl₃, D₂O or C₆D₆ at 27 °C. Homonuclear 2D-H,H-COSY spectra were used for the structural assignment of coupled protons and 2D-H,H-ROESY spectra for detection spatially closed protons (assignment of geminal H-2´ and H-2´´ and determination of configuration at C-4´ carbon atom). The 1D-¹³C-, attached proton test” (APT) spectra and heteronuclear 2D-H,C-HSQC and 2D-H,C-HMBC spectra were used for the structural assignment of carbon signals.

5'-Deoxy-5'-iodothymidine (4)¹
A mixture of thymidine (20 g, 82.6 mmol), triphenylphosphine (27 g, 103 mmol), iodine (26 g, 103 mmol), and pyridine (16 ml, 200 mmol) in dioxane (400 ml) was stirred for 7 h at room temperature, and then methanol (25 ml) was added. Solvents were removed in vacuo, the residue was dissolved in boiling ethanol (250 ml), and the solution was left to crystallize (4 °C, 12 h). First crop of the title compound (17.8 g) was recovered by filtration. Column chromatography of the evaporated mother liquors on silica gel in chloroform-ethanol (0-5 %) yielded a second crop (4.0 g) of the product (overall yield, 75 %); m. p. 170-173 °C corresponded to the literature data.

1-(2,5-Dideoxy-β-D-glycero-pent-4-enofuranosyl)thymine (5)¹
The iodo derivative 4 (21.8 g; 61.9 mmol) in 0.5M solution of sodium methoxide in methanol (400 ml) was heated at reflux for 8 h. The light brown solution was cooled to r. t. and neutralized by the addition of glacial acetic acid (9.5 ml). The solvent was evaporated, and the residue was crystallized from ethanol to give 11.2 g (81 %) of 5 as colorless needles; m. p. 207-209 °C corresponds to the literature data.
1-[3-O-(tert-Butyldimethylsilyl)-2,5-dideoxy-β-D-glycero-pent-4-enofuranosyl]thymine (6)

A solution of 5 (16.2 g, 72.2 mmol), tert-butyldimethylchlorosilane (14.5 g, 96 mmol), and imidazole (4.9 g, 72.2 mmol) in pyridine (360 ml) was stirred at room temperature for 8 h. The reaction was quenched with methanol (10 ml), and the solvents were evaporated. Traces of pyridine were removed by co-evaporation with toluene (2 x 400 ml). The residue was dissolved in chloroform (400 ml), and washed with 1M triethylammonium hydrogen carbonate (2 x 100 ml). The chloroform extract was dried with anhydrous MgSO₄, and concentrated in vacuo to afford the crude product (19.4 g). This product in toluene was filtered through a silica gel column (100 ml) in toluene to remove tert-butyldimethylsilyl methyl ether, and the product 6 was eluted with ethyl acetate to give 16.9 g (69 %) of 6 as a white foam. HR-ESI: for C₁₆H₂₆O₇N₂NaSi calculated: 361.15540; found: 361.15577; -0.3682 ppm

1H NMR spectrum (CDCl₃): 8.90 b, 1 H (NH); 6.99 q, 1 H, J(6,CH₃) = 1.2 (H-6); 6.49 t, 1 H, J(1',2') = 6.1, J(1',2'') = 6.1 (H-1'); 4.75 ddt, 1 H, J(3',2') = 6.3, J(3',2'') = 3.4, J(3',5'a) = 1.1, J(3',5'b) = 1.0 (H-3'); 4.51 dd, 1 H, J(5'a,5'b) = 2.2, J(5'a,3') = 1.1 (H-5'a); 4.21 dd, 1 H, J(5'b,5'a) = 2.2, J(5'b,3') = 1.0 (H-5'b); 2.39 ddd, 1 H, J(2',1') = 6.1, J(2',2') = 13.6, J(2',3') = 3.4 (H-2'); 2.19 dt, 1 H, J(2',1') = 6.1, J(2',2') = 13.6. J(2',3') = 6.3 (H-2'); 1.92 d, 3 H, J(CH₃,6) = 1.2 (5-CH₃); 0.89 s, 9 H (C(CH₃)₃); 0.11 s, 6 H (Si(CH₃)₂).

C NMR spectrum (CDCl₃): 164.03 (C-4); 162.62 (C-4'); 150.29 (C-2); 134.44 (C-6); 111.56 (C-5); 85.93 (C-1'); 84.82 (C-5'); 70.56 (C-3'); 40.51 (C-2'); 25.57 (3 x CH₃); -4.78 and -4.86 (Si(CH₃)₂).

3'-O-(tert-Butyldimethylsilyl)-4'-methoxythymidine (7a) and 1-[3-O-(tert-butyldimethylsilyl)-2-deoxy-4-methoxy-α-L-threo-pento-4-enofuranosyl]thymine (7b).

To a stirred solution of 6 (1.015 g, 3 mmol) in anhydrous methanol (30 ml), 70% m-chloroperbenzoic acid (1.5 g, 6 mmol) was added. After 5 h at r. t. the mixture was diluted with chloroform (80 ml) and washed with 2M triethylammonium hydrogen carbonate (2x50 ml, 4 °C). The aqueous layer was washed with chloroform (80 ml) and washed with 2M triethyl ammonium hydrogen carbonate. The chloroform extract was dried with anhydrous MgSO₄, and concentrated in vacuo. The residue was purified by silica gel chromatography (stepwise gradient, chloroform-ethanol 0-5 %) yielded less polar epimer 7a (358 mg, 31 %), eluted at 4% ethanol in chloroform and the desired epimer 7b (520 mg, 45 %) at ~5% ethanol in chloroform; both as amorphous solids.

HR-ESI 7a: for C₁₆H₂₆O₇N₂NaSi calculated: 409.17653; found: 409.17649; -0.1143 ppm

7b: 1H NMR spectrum (CDCl₃): 8.41 bs, 1 H (NH); 7.26 q, 1 H, J(6,CH₃) = 1.2 (H-6); 6.63 dd, 1 H, J(1',2') = 8.3, J(1',2'') = 6.4 (H-1'); 4.33 dd, 1 H, J(3',2') = 4.6, J(3',2'') = 14.3 (H-3'); 3.88 bd, 1 H, J(5'a,5'b) = 12.1 (H-5'a); 3.84 d, 1 H, J(5'b,5'a) = 12.1 (H-5'b); 3.40 s, 3 H (4'-OCH₃); 2.24 ddd, 1 H, J(2',1') = 6.4, J(2',2') = 13.7, J(2',3') = 1.4 (H-2'); 2.33 ddd, 1 H, J(1',2') = 6.1, J(1',2'') = 6.1 (H-1').
J(2',1') = 8.3, J(2',2'') = 13.7, J(2',3') = 4.6 (H-2'); 1.95 d, 3 H, J(CH₃,6) = 1.3 (5-CH₃); 1.78 bs (5'-OH); 0.92 s, 9 H (C(CH₃)₃); 0.13 s, 3 H and 0.14 s, 3 H (Si(CH₃)₂).

¹³C NMR spectrum (CDCl₃): 163.21 (C-4); 150.46 (C-2); 135.54 (C-6); 111.72 (C-5); 110.73 (C-4'); 85.50 (C-1'); 76.31 (C-3'); 57.85 (C-5'); 49.47 (4'-OCH₃); 38.79 (C-2'); 25.64 (3 x CH₃ (t-Bu)); 17.91 (>C< (t-Bu)); 12.73 (5-CH₃); -4.73 and -5.20 (Si(CH₃)₂).

5'-O-(4,4'-Dimethoxytrityl)-4'-methoxythymidine (8a).

(a) Dimethoxytritylation of 7a. To a stirred solution of 7a (0.52 g, 1.3 mmol) in anhydrous pyridine (13 ml), 4,4'-dimethoxytrityl chloride was added in three portions (3 x 0.18 g, 1.6 mmol). The solution was left to stand overnight, then triethylamine (0.3 ml, 2.1 mmol) and methanol (0.2 ml) were added, and the suspension was concentrated in vacuo. The residue was taken up with chloroform (60 ml), washed with 1 M triethylammonium hydrogen carbonate (2 x 20 ml), and the aqueous layers were washed with chloroform (3 x 80 ml). The combined organic extracts were dried over anhydrous Na₂SO₄, and the solvent was evaporated to dryness. The obtained crude 3'-O-(tert-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)-4'-methoxythymidine was subjected to the desilylation reaction.

(b) Desilylation. The product from the previous step was dissolved in 0.5M tetra-n-butylammonium fluoride in THF (10 ml), and the solution was left to stand overnight at room temperature. Then the suspension of Dowex 50Wx8 (Et₃NH⁺ form, 20 ml) in aqueous 70% methanol (20 ml) was added, the mixture was stirred for 5 minutes, and the resin was filtered off. Sodium hydrogen carbonate (2 g) was added and the mixture was evaporated to dryness. The solid residue was extracted with chloroform (70 ml), and the chloroform extract was dried over anhydrous Na₂SO₄. Chromatography on a silica gel column using a stepwise gradient of ethanol in chloroform (0-4%) gave 0.72 g (96%, based on 7a) of 8a as a white foam.

HR-ESI: for C₃₂H₃₉O₈N₂Na calculated: 597.22074; found: 597.22060; -0.2258 ppm

¹H NMR spectrum (d₅-DMSO): 11.36 bs, 1 H (NH); 7.54 q, 1 H, J(6,CH₃) = 1.2 (H-6); 7.40 m, 2 H, 7.31 m, 2 H, and 7.25 m, 1 H (C₆H₅); 7.27 m, 2 H, 7.26 m, 2 H and 6.89 m, 4 H (2x C₆H₄); 6.13 dd, 1 H, J(1',2'a) = 3.6, J(1',2'b) = 7.7 (H-1'); 5.02 d, 1 H, J(3'-OH,3') = 8.1 (3'-OH); 4.70 bq, 1 H, J(3',2'a) = 8.6, J(3',2'b) = 9.1, J(3',OH) = 8.1 (H-3'); 3.74 s, 6 H (2x PhOCH₃); 3.16 s, 3 H (4'-OCH₃); 3.29 d, 1 H, and 3.08 d, 1 H, J(gem) = 9.8 (H-5'a + H-5'b); 2.34 ddd, 1 H, J(2'a,1') = 3.6, J(2'a,2'b) = 13.5, J(2'a,3') = 8.6 (H-2'a); 2.30 ddd, 1 H, J(2'a,1') = 7.7, J(2'a,2'b) = 13.5, J(2'a,3') = 9.1 (H-2'a); 1.50 d, 3 H, J(CH₃,6) = 1.2 (CH₃).

¹³C NMR spectrum (d₅-DMSO): 163.87 (C-4); 158.36, 158.35, 135.36, 135.34, 130.00 (2), 26.96 (2), 113.40 (2) and 113.39 (2) (2xC₆H₄); 150.50 (C-2); 144.82, 128.06(2), 127.89(2) and 126.98 (C₆H₅); 136.08 (C-6); 109.90 (C-5); 105.42 (C-4'); 82.38 (C-1'); 80.13 (>C< (DMTr)); 70.48 (C-3'); 61.85 (C-5'); 55.21(2xOCH₃ (DMTr)); 49.50 (4'-OCH₃); 36.86 (C-2'); 11.98 (CH₃).

1-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-4'-methoxy-a-L-threo-pentofuranosyl]thymine (8b)
The title compound was prepared according to the procedure described for 8a starting from 7b (0.4 g, 1.3 mmol). Yield of 8b 0.53 g (92%, white foam).

¹H NMR spectrum (d₅-DMSO): 11.30 bs, 1 H (NH); 7.26 q, 1 H, J(6,CH₃) = 1.2 (H-6); 7.33 m, 2 H, 7.30 m, 2 H and 6.88 m, 4 H (2xC₆H₄); 7.46 m, 2 H, 7.30 m, 2 H and 7.23 m, 1 H (C₆H₅); 6.44 dd, 1 H, J(1',2'a) = 8.5, J(1',2'b) = 6.5 (H-1'); 5.62 d, 1 H, J(OH,3') = 5.5 (3'-OH); 4.34 bt, 1 H, J(3',2'a) = 4.6, J(3',OH) = 5.5, J(3',2'b) < 1 (H-3'); 3.737 s, 3 H and 3.734 s, 3 H (2x PhOCH₃); 2.82 s, 3 H (4'-OCH₃); 3.42 d, 1 H, and 2.93 d, 1 H, J(gem) = 9.7 (H-5'a + H-5'b); 2.42 m, 1 H, J(2'a,1') = 8.5, J(2'a,2'b) = 13.6, J(2'a,3') = 4.6 (H-2'a); 2.17 m, 1 H, J(2'b,1') = 6.5, J(2'b,2'a) = 13.6, J(2'b,3') < 1 (H-2'b); 1.76 d, 3 H, J(CH₃,6) = 1.2 (5-CH₃).
$^{13}$C NMR spectrum ($d_6$-DMSO): 163.63 (C-4); 158.29(2), 135.68, 135.18, 130.12(2), 130.00(2), 113.21(2) and 113.12(2) (2xC$_6$H$_4$); 150.98 (C-2); 144.85, 127.98(2), 127.86(2) and 126.85 (C$_6$H$_5$); 135.82 (C-6); 110.44 (C-5); 110.37 (C-4'); 85.50 (>C< (DMTr)); 84.16 (C-1'); 74.18 (C-3'); 57.18 (C-5'); 55.19 (2xOCH$_3$ (DMTr)); 37.78 (C-2'); 12.45 (5-CH$_3$).

5'-O-(4,4'-Dimethoxytrityl)-4'-methoxymethylidine-3'-O-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (9a)

Compound 8a (0.36 g, 0.62 mmol) was dried by co-distillation with anhydrous toluene (2 x 8 ml), dissolved in THF (3.2 ml), and N,N-diisopropylethylamine (0.43 ml, 2.47 mmol) followed by 2-cyanoethyl N,N-diisopropylphosphoramidochloridite (0.25 ml, 1.12 mmol) were added under argon. The solution was stirred at room temperature for 40 minutes (reaction complete, monitored by TLC), and then the mixture was partitioned between ethyl acetate (60 ml) and an ice-cold saturated sodium hydrogen carbonate solution (50 ml). The organic layer was washed with the same solution (3 x 50 ml), and the aqueous washings were re-extracted with ethyl acetate (30 ml). Combined ethyl acetate layers were shortly dried with anhydrous Na$_2$SO$_4$ and evaporated with addition of anhydrous toluene. The residue was then purified on a silica gel column (pre-treated with 2 % of triethylamine) using a toluene-ethyl acetate (0-8 %) gradient, to give, after lyophilisation from benzene, 0.42 g (86 %) of 9a as a pale yellow foam.

$^1$H NMR spectrum ($C_6D_6$): 9.37 b + 9.28 b, 1H, NH); ~7.74 m, 2H, Ar-H (DMTr); 7.67 q + 7.72 q, 1H, (H-1'); 5.34 m, 1H (H-3'); 3.96 d, 1H, $J$(5'a,5'b)=9.6 (H-5'a); 3.61 dh + 3.58 dh, 2H, $J$(CH,P)=10.2, $J$(CH,CH$_3$)=6.8 (>N-CH (N-iPr$_2$)); 3.54 m + 3.43 m and 3.54 m + 3.22 m, 2H (P-OCH$_2$); 3.54 d + 3.60 d, 1H, J(5'b,5'a)=9.6 (H-5'b); 3.43 s +3.41 s and 3.42 s + 3.40 s, 6H (2xOMe (DMTr)); 3.14 s + 3.17 s, 3H (4 '-OMe); 2.69 ddd + 2.75 ddd, 1H, J(2”,1')=8.0, J(2”,2”)=13.6, J(2”,3’)=8.5 (H-2’); 2.45 ddd + 2.63 ddd, 1H, J(2’,1’)=3.0, J(2’,2”)=13.6, J(2’,3”)=8.5 (H-2’); 1.89 m + 1.82 m, 2H (CH$_2$CN); 1.68 d + 1.74 d, 3H, $J$(CH$_3$,6)=1.2 (5-CH$_3$); 1.25 d + 1.16 d and 1.20 d + 1.16 d, 12H, J(CH$_3$)=6.8, 4xCH$_3$ (N-iPr$_2$)).

$^{31}$P NMR spectrum ($C_6D_6$): 151.65, 150.71.

1-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-4'-methyl-2-threo-pentofuranosyl]thymine

3'-O-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (9b)

The title compound was prepared according to the procedures described for 8a and 9. Starting from 7b (358 mg, 0.9 mmol), we obtained, in three steps, after freeze drying from benzene, 315 mg (46 %, 0.41 mmol) of the title phosphoramidite 9b. For C$_4$H$_{13}$N$_4$O$_9$P (774.85)
4'-(2-Methoxyethoxy)thymidine (10a) and 1-[4-methoxy-α-L-threo-pentofuranosyl]-5-methyluracil (10b).

Compound 5 (2.24 g, 10 mmol) was dissolved in anhydrous 2-methoxyethanol (100 ml) and then 70% m-chloroperbenzoic acid (5 g, 20 mmol) was added under stirring. After 4 h of stirring at r. t., the solution was concentrated, and the solid residue was treated with boiling diethyl ether (2 x 100 ml). Subsequent chromatography on a reversed phase column using a linear gradient of methanol in water (0-25 %) yielded 0.65 g (21 %) of the compound 10a, along with 0.32 g (10 %) of 10b which was not further exploited. For 10a, C_{13}H_{20}O_{5}N_{2}Na calculated: 339.11627; found: 339.11620; -0.22302 ppm.

1H NMR spectrum of 10a (d_{6}-DMSO): 11.29 bs, 1 H (NH); 7.66 q, 1 H, J(6,CH_{3})=1.2 (H-6); 6.12 dd, 1 H, J(1',2')=3.2, J(1',2'')=7.7 (H-1'); 5.23 b, 1 H and 4.98 b, 1 H (3'-OH and 5'-OH); 4.42 bt, 1 H, J(3',2')=8.2, J(3',2'')=9.1 (H-3'); 3.76 m, 1 H, 3.59 m, 1 H, 3.46 m, 1 H and 3.42 m, 1 H (O-CH_{2}-CH_{2}-O); 3.63 d, 1 H, J(5'a,5'b)=11.8 (H-5'a); 3.48 d, 1 H, J(5'b,5'a)=11.8 (H-5'b); 3.26 s, 3 H (OCH_{3}); 2.26 ddd, 1 H, J(2',1')=7.7, J(2'',2')=13.2, J(2',3')=9.1 (H-2'); 2.16 ddd, 1 H, J(2',1')=3.2, J(2',2'')=13.2, J(2',3'')=8.2 (H-2'); 1.75 d, 3 H, J(CH_{3})=1.2 (5-CH_{3}). 13C NMR spectrum of 10a (d_{6}-DMSO): 163.92 (C-4); 150.51 (C-2); 136.30 (C-6); 109.42 (C-5); 106.59 (C-4'); 82.27 (C-1'); 71.70 and 61.14 (O-CH_{2}-CH_{2}-O); 69.14 (C-3'); 59.84 (C-5'); 58.25 (OCH_{3}); 37.60 (C-2'); 12.47 (5-CH_{3}).

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2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-4'-(2-methoxyethoxy)thymidine-3'-O-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (12).

(a) Dimethoxytritylation of 10a. To a stirred solution of 10a (0.63 g, 2 mmol) in anhydrous pyridine (40 ml), 4,4’-dimethoxytrityl chloride was added in four portions (4 x 0.24 g, 2.8 mmol) during 24 h. The solution was left to stand at room temperature for additional 24 h, then triethylamine (1 ml, 7 mmol) was added, and the solvents were evaporated. The residue was purified on a silica gel column using a linear gradient of ethanol in chloroform (0-4 %) to give 1.16 g (94 %) of 11, which on checking by TLC was considered of satisfactory purity for the subsequent reaction.

(b) Phosphitylation of 11. Compound 11 (619 mg, 1 mmol) was dried by co-distillation with anhydrous toluene (2 x 15 ml), then it was dissolved in THF (5 ml), and N,N-diisopropylethylamine (0.68 ml, 3.93 mmol) and 2-cyanoethyl amidochloridite (0.40 ml, 1.77 mmol) were subsequently added under argon. The solution was stirred at room temperature for 90 minutes, the mixture then partitioned between ethyl acetate (80 ml) and saturated ice-cold sodium hydrogen carbonate solution (80 ml), and the organic layer was washed with the same solution (3 x 80 ml). Ethyl acetate layer was dried over Na$_2$SO$_4$ and the solvent was evaporated with adding toluene. The residue was co-evaporated with anhydrous toluene and purified by flash chromatography on a silica gel column using a linear gradient of ethyl acetate in toluene (0-10 %) to give 566 mg (69 %, 0.41 mmol) of 12 after lyophilisation from benzene. HR-FAB: For C$_{43}$H$_{53}$N$_4$O$_{10}$PK calculated, 857.3772; found, 857.5016 (M+K$^+$).

$^1$H NMR spectrum (C$_6$D$_6$) – two diastereoisomers ~1:1; the most of signals are doubled: 9.66 bs, 1 H (NH); 7.71 q and 7.69 q, 1 H, J(6,CH$_3$) = 1.2 (H-6); 7.62-7.58 m, 4 H and 7.18 m, 1 H (C$_6$H$_3$); 7.75 m, 2 H, 7.34-7.31 m, 2 H and 6.92 m, 4 H (2x C$_6$H$_3$); 6.68 dd, J(1',2'') = 3.0, J(1',2'') = 8.1 and 6.64 dd, J(1',2'') = 2.7, J(1',2'') = 8.0 (H-1'); 5.36 dt, J(3',2') = 8.7, J(3',2'') = 9.5, J(3',3') = 10.9 and 5.32 q. J(3',2') = 8.6, J(3',2'') = 9.2, J(3',3') = 9.1 (H-3'); 3.987 d, J(5'a,5'b) = 9.9 and 3.984 d, J(5'a,5'b) = 9.9 (H-5'a); 3.85 m + 3.51 m and 3.77 m + 3.46 m, 2 H (4'-O-CH$_2$-); 3.64 d, J(5'b,5'a') = 9.9 and 3.57 d, J(5'b,5'a') = 9.9 (H-5'b); 3.62 m, 2 H (2x N-CH$_2$); 3.50 m, 3.46 m and 3.31 m, 2 H (P-O-CH$_2$-); 3.45 s, 3.44 s, 3.43 s and 3.42 s (2x OCH$_3$ (DMTr)); 3.35 m + 3.27 m and 3.27 m + 3.23 m, 2 H (-CH$_2$-OMe); 3.23 s and 3.19 s (OCH$_3$); 2.86 ddd, J(2',1') = 8.1, J(2',2'') = 13.6, J(2',3') = 9.2 and 2.81 ddd, J(2',1') = 8.0, J(2',2'') = 13.4, J(2',3') = 9.5 (H-2'); 2.64 ddd, J(2',1') = 3.0, J(2',2'') = 13.6, J(2',3') = 8.6 and 2.45 ddd, J(2',1') = 13.4, J(2',2'') = 13.4, J(2',3') = 8.7 (H-2'); 2.11 m + 2.04 m and 1.92 m, 2 H (-CH$_2$-CN); 1.747 d and 1.689 d, J(CH$_3$,6) = 1.2, 3 H (5-CH$_3$); 1.26 d, 1.21 d, 1.19 d and 1.18 d, J = 6.8 (2x CH$_2$(CH$_3$)$_2$).

$^13$C NMR spectrum (C$_6$D$_6$) – two diastereoisomers ~1:1; the most of signals are doubled: 163.36 and 163.30 (C-4); 159.02, 158.99, 158.94, 158.92, 135.47, 135.36, 135.21, 135.09, 128.44, 128.42, 127.86, 127.82, 113.24, 113.22, 113.19 and 113.15 (2x C$_6$H$_3$); 150.33 and 150.20 (C-2); 144.75, 144.60, 130.46, 130.41, 130.38, 130.34, 127.02 and 126.93 (C$_6$H$_3$); 135.14 and 134.73 (C-6); 117.33 and 117.32 (CN); 111.08 and 111.06 (C-5); 106.19 d, J(C,P) = 7.3 and 106.18 d, J(C,P) = 5.3 (C-4'); 87.09 and 87.05 (>C< (DMTr)); 83.45 and 83.25 (C-1'); 71.83 d, J(C,P) = 14.5 and 70.34 d, J(C,P) = 19.9 (C-3'); 71.63, 71.55, 61.62 and 61.52 (O-CH$_2$-CH$_2$-O); 62.04 and 61.68 (C-5'); 58.79 d, J(C,P) = 15.9 and 57.74 d, J(C,P) = 19.2 (P-0-CH$_2$-); 58.22 and 58.15 (OCH$_3$); 54.41, 54.40 and 54.34 (2 x OCH$_3$ (DMTr)); 43.13 d, J(C,P) = 12.8 and 43.03 d, J(C,P) = 12.7 (2x N-CH$_2$-); 37.41 d, J(C,P) = 2.7 and 36.94 d, J(C,P) = 2.5 (C-2'); 24.26 d, J(C,P) = 7.8, 24.24 d, J(C,P) = 6.8, 24.16 d, J(C,P) = 6.7 and 24.14 d, J(C,P) = 8.1 (2x CH(CH$_3$)$_2$); 19.61 d, J(C,P) = 6.5 and 19.44 d, J(C,P) = 5.1 (-CH$_2$-CN); 11.75 and 11.72 (5-CH$_3$).

$^{31}$P NMR spectrum (C$_6$D$_6$): 151.72, 150.55.
2. NMR data, and conformation analysis of compounds 3a and 3b

The configuration at carbon C(4’) and the preferred orientation of thymine in compounds 3a and 3b were determined from the observed NOE contacts in 2D-H,H-ROESY spectra in DMSO (see Figure S1). The absence of hydrogen at position 4’ reduces the number of proton vicinal couplings and limits a common NMR conformation analysis using PSEUROT program. Therefore, we used an approximate method described for the estimation of the population of “south”—type conformer (usually C(2’)-endo) based on the relation [1], as described for deoxyribo-nucleosides by Rinkel and Altona.3

\[
\text{% south C(2’)-endo conformer} = \left[31.5 \times J(1’,2’) - J(2’,2’) - J(2’,3’)\right] / 10.9 \times 100
\]

The observed J-values in 3a (Figure S1) then lead to a high preference of the “north”—type C(3’)-endo conformation (84 %). On the other hand, the observed J-values in 3b indicate a high population of the “south”—type C(2’)-endo conformer (83 %).

![Figure S1](image)

**Figure S1.** The selected nontrivial NOEs (shown with red arrows) and vicinal proton couplings in furanose ring observed in compounds 3a and 3b in DMSO.

**4’-Methoxythymidine (3a)**

\(^1\text{H NMR spectrum (600 MHz; } \text{d}_6\text{-DMSO)}: 11.30 \text{ bs, 1H (NH)}; 7.66 \text{ q, 1H, J(6,CH}_3) = 1.3 (H-6); 6.12 \text{ dd, 1H, J(1’,2’) = 3.5, J(1’, 2”) = 7.5 (H-1’); 5.21 dd, 1H, J(OH,5’a) = 5.5, J(OH,5’b) = 5.8 (5’-OH); 4.91 d, 1H, J(OH,3’) = 7.0 (3’-OH); 4.42 ddd, 1H, J(3’,OH) = 7.0, J(3’,2’) = 8.3, J(3’,2”) = 8.9 (H-3’); 3.65 dd, 1H, J(5’a,OH) = 5.5, J(5’a,5’b) = 11.7 (H-5’a); 3.47 dd, 1H, J(5’b,OH) = 5.8, J(5’b,5’a) = 11.7 (H-5’b); 3.28 s, 3H (OCH}_3); 2.24 ddd, 1H, J(2’,1’) = 7.5, J(2’,2’) = 13.4, J(2’,3’) = 8.9 (H-2’); 2.18 ddd, 1H, J(2’,1’) = 3.5, J(2’,2”) = 13.4, J(2’,3’) = 8.3 (H-2’); 1.76 d, 3H, J(CH_3,6) = 1.3 (5-CH_3). \(^{13}\text{C NMR spectrum (150.9 MHz; } \text{d}_6\text{-DMSO): 163.98 (C-4); 150.58 (C-2); 138.36 (C-6); 109.49 (C-5); 106.58 (C-4’); 82.30 (C-1’); 69.22 (C-3’); 59.40 (C-5’); 49.63 (4’-OCH}_3); 37.70 (C-2’); 12.52 (5-CH}_3).**

**1-[2-Deoxy-4-methoxy-α-L-threo-pentofuranosyl]thymine (3b)**

\(^1\text{H NMR spectrum (600 MHz; } \text{d}_6\text{-DMSO): 11.35 bs, 1H (NH); 7.30 q, 1H, J(6,CH}_3) = 1.3 (H-6); 6.44 dd, 1H, J(1’,2’) = 8.7, J(1’,2”) = 6.5 (H-1’); 5.38 d, 1H, J(OH,3’) = 5.0 (3’-OH); 4.65 dd, 1H, J(OH,5’a) = 5.8, J(OH,5’b) = 6.0 (5’-OH); 4.08 bt, 1H, J(3’,OH) = 5.0, J(3’,2’) = 4.7, J(3’,2”) < 1 (H-3’); 3.62 dd, 1H, J(5’a,OH) = 5.8, J(5’a,5’b) = 12.1 (H-5’a); 3.57 dd, 1H, J(5’b,OH) = 6.0, J(5’b,5’a) = 12.1 (H-5’b); 3.24 s, 3H (OCH}_3); 2.33 ddd, 1H, J(2’,1’) = 8.7, J(2’,2”) = 13.7, J(2’,3’) = 4.7 (H-2’); 2.10 bdd, 1H, J(2’,1’) = 6.5, J(2’,2”) = 13.7, J(2’,3’) < 1 (H-2’); 1.80 d, 3H, J(CH_3,6) = 1.3 (5-CH}_3). \(^{13}\text{C NMR spectrum (150.9 MHz; } \text{d}_6\text{-DMSO):**

---

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We also performed the $^1$H NMR measurements of 3a and 3b in different solvents (CDCl$_3$, DMSO-$d_6$ and D$_2$O) and different temperatures (D$_2$O solutions at +7 °C, +27 °C, and +47 °C; see Tables S2 and S3). The change of solvent, as well as temperature, did not significantly change the conformer ratio, calculated using the relation [1].

Although the missing coupling constant $J(3',4')$ limits fitting of the experimental data by PSEUROT, we tried an alternative approach consisting in systematical stepwise changing of the north/south conformer ratio from 0:100 to 100:0 by 10 % per step and optimising all remaining parameters – phase angle $P$ and pucker amplitude $\phi_{\text{max}}$. Using this approach, we calculated the pseudorotation parameters of 3a and 3b in different solvents (Table S4). The sets of coupling constants obtained in D$_2$O at +7 °C, +27 °C and +47 °C enabled us to perform the PSEUROT conformation analysis and fit all the pseudorotation parameters, supposing that only the population of conformers changes with temperature (Table S5). The results show that the effect of solvent as well as temperature on the conformation is very small for both nucleosides 3a and 3b.

In order to support the results obtained from the $^1$H NMR conformation analysis using vicinal proton coupling constant, we decided to explore the conformation behaviour of compounds 3a and 3b by molecular modelling. We used the well-known concept of pseudorotation$^2$ for the sugar ring conformation of nucleosides. First, we performed “pucker-scan” that gave us information about optimal pucker amplitudes $\phi_{\text{max}}$ for the next step – the conformation analysis. For this purpose, we generated 13 conformers with phase angle $P = 180^\circ$ ($\text{I}_2\text{T}$ conformation) and $\phi_{\text{max}}$ stepwise changing from 0° to 60° by 5° per step. Geometries with restrained endocyclic torsion angles $\phi_0$ and $\phi_3$ were taken for geometry optimisation (B3LYP/6-31G** in vacuo). Optimal pucker amplitudes $\phi_{\text{max}} = 30^\circ$ for 3a and $\phi_{\text{max}} = 35^\circ$ for 3b were found as the energy minima on a curve of the calculated energy versus $\phi_{\text{max}}$ (see Figure S2).

In the next step, for each nucleoside 3a and 3b (with its optimised $\phi_{\text{max}}$) we generated a set of 20 conformers covering the whole pseudorotation pathway in 18 degree steps representing the envelope and twisted conformations with two restricted endocyclic dihedral angles $\phi_0$ and $\phi_3$. The geometry of the molecule was optimised for each conformer using DFT/B3LYP/6-31G** theory level. Predominant conformations can be found as the energy minima by plotting calculated energy against the phase angle $P$ (Figure S3). The energy minima were
then fully optimised in vacuo (DFT/B3LYP/6-31G**) and in water (DFT/B3LYP/6-31G* with PCM model for solvation) and Gibbs free energy was calculated for such two predominant conformers. Equilibrium constants were then calculated providing population of “north” and “south” conformers for both 3a and 3b nucleosides. The results of conformation analysis are shown in Table S6. The populations obtained by this procedure were in very good agreement with the results obtained from the NMR data.
Table S2. Proton coupling constants of nucleosides 3a and 3b in CDCl$_3$, DMSO and D$_2$O at +27 °C and calculated ratio of “south” and “north” conformer using relation [1]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solvent</th>
<th>$J(1',2')$</th>
<th>$J(1',2'')$</th>
<th>$J(2',2'')$</th>
<th>$J(2',3')$</th>
<th>$J(2'',3')$</th>
<th>South / North (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>CDCl$_3$</td>
<td>5.28</td>
<td>6.54</td>
<td>13.86</td>
<td>7.95</td>
<td>7.74</td>
<td>31 : 69</td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td>3.54</td>
<td>7.56</td>
<td>13.40</td>
<td>8.30</td>
<td>8.90</td>
<td>15 : 85</td>
</tr>
<tr>
<td></td>
<td>D$_2$O</td>
<td>4.56</td>
<td>6.80</td>
<td>14.02</td>
<td>8.70</td>
<td>8.90</td>
<td>16 : 84</td>
</tr>
<tr>
<td>3b</td>
<td>CDCl$_3$</td>
<td>8.13</td>
<td>6.80</td>
<td>14.18</td>
<td>4.68</td>
<td>1.60</td>
<td>82 : 18</td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td>8.75</td>
<td>6.56</td>
<td>13.75</td>
<td>4.70</td>
<td>0.80</td>
<td>95 : 5</td>
</tr>
<tr>
<td></td>
<td>D$_2$O</td>
<td>8.34</td>
<td>6.84</td>
<td>14.70</td>
<td>5.04</td>
<td>0.87</td>
<td>83 : 17</td>
</tr>
</tbody>
</table>

Table S3. Proton coupling constants of nucleosides 3a and 3b in D$_2$O at +7 °C, +27 °C and +47 °C and calculated ratio of “south” and “north” conformer using relation [1]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Temp.</th>
<th>$J(1',2')$</th>
<th>$J(1',2'')$</th>
<th>$J(2',2'')$</th>
<th>$J(2',3')$</th>
<th>$J(2'',3')$</th>
<th>South / North (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>7 °C</td>
<td>4.32</td>
<td>7.09</td>
<td>14.05</td>
<td>8.82</td>
<td>9.07</td>
<td>12 : 88</td>
</tr>
<tr>
<td></td>
<td>27 °C</td>
<td>4.56</td>
<td>6.80</td>
<td>14.02</td>
<td>8.70</td>
<td>8.90</td>
<td>16 : 84</td>
</tr>
<tr>
<td></td>
<td>47 °C</td>
<td>4.86</td>
<td>6.65</td>
<td>14.05</td>
<td>8.76</td>
<td>8.83</td>
<td>18 : 82</td>
</tr>
<tr>
<td>3b</td>
<td>7 °C</td>
<td>8.40</td>
<td>6.84</td>
<td>14.70</td>
<td>5.04</td>
<td>0.80</td>
<td>84 : 16</td>
</tr>
<tr>
<td></td>
<td>27 °C</td>
<td>8.34</td>
<td>6.84</td>
<td>14.70</td>
<td>5.04</td>
<td>0.87</td>
<td>83 : 17</td>
</tr>
<tr>
<td></td>
<td>47 °C</td>
<td>8.28</td>
<td>6.87</td>
<td>14.73</td>
<td>5.16</td>
<td>0.90</td>
<td>83 : 17</td>
</tr>
</tbody>
</table>

Table S4. Conformation analysis using PSEUROT and experimental $^3J(H,H)$ from Table S2.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solvent</th>
<th>$\phi_{\text{max}}$ (N) [deg]</th>
<th>X (N) [%]</th>
<th>$\phi_{\text{max}}$ (S) [deg]</th>
<th>X (S) [%]</th>
<th>rms [Hz]</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>CDCl$_3$</td>
<td>61</td>
<td>100</td>
<td>192</td>
<td>38</td>
<td>0.927</td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td>48</td>
<td>100</td>
<td>192</td>
<td>38</td>
<td>0.678</td>
</tr>
<tr>
<td></td>
<td>D$_2$O</td>
<td>55</td>
<td>100</td>
<td>192</td>
<td>38</td>
<td>1.061</td>
</tr>
<tr>
<td>3b</td>
<td>CDCl$_3$</td>
<td>-10</td>
<td>8</td>
<td>196</td>
<td>38</td>
<td>0.246</td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td>-10</td>
<td>0</td>
<td>196</td>
<td>38</td>
<td>0.253</td>
</tr>
<tr>
<td></td>
<td>D$_2$O</td>
<td>-10</td>
<td>0</td>
<td>196</td>
<td>38</td>
<td>0.290</td>
</tr>
</tbody>
</table>

Table S5. Conformation analysis using PSEUROT and three sets of $^3J(H,H)$ at +7 °C, +27 °C and +47 °C in D$_2$O from Table S3.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Temp.</th>
<th>$\phi_{\text{max}}$ (N) [deg]</th>
<th>X (N) [%]</th>
<th>$\phi_{\text{max}}$ (S) [deg]</th>
<th>X (S) [%]</th>
<th>rms [Hz]</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>7 °C</td>
<td>91</td>
<td>91</td>
<td>40</td>
<td>91</td>
<td>1.513</td>
</tr>
<tr>
<td></td>
<td>27 °C</td>
<td>32</td>
<td>90</td>
<td>186</td>
<td>90</td>
<td>1.581</td>
</tr>
<tr>
<td></td>
<td>47 °C</td>
<td>90</td>
<td>90</td>
<td>186</td>
<td>90</td>
<td>1.711</td>
</tr>
<tr>
<td>3b</td>
<td>7 °C</td>
<td>-27</td>
<td>1</td>
<td>99</td>
<td>99</td>
<td>0.176</td>
</tr>
<tr>
<td></td>
<td>27 °C</td>
<td>-27</td>
<td>2</td>
<td>199</td>
<td>2</td>
<td>0.174</td>
</tr>
<tr>
<td></td>
<td>47 °C</td>
<td>-27</td>
<td>2</td>
<td>199</td>
<td>2</td>
<td>0.152</td>
</tr>
</tbody>
</table>
Table S6  Results of conformation analysis of furanose ring in compound 3a and 3b using molecular modeling in water and in vacuo (values in brackets).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conformer</th>
<th>$P$ [deg]</th>
<th>$\phi_{\text{max}}$ [deg]</th>
<th>rel $\Delta G$ $^a$ [kcal/mol]</th>
<th>$X_{\text{calc}}$ $^b$ [%]</th>
<th>$X_{\text{NMR}}$ $^c$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>north</td>
<td>53 (31)</td>
<td>40 (36)</td>
<td>0 (0)</td>
<td>98 (97)</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>south</td>
<td>192 (186)</td>
<td>34 (33)</td>
<td>2.31 (2.10)</td>
<td>2 (3)</td>
<td>16</td>
</tr>
<tr>
<td>3b</td>
<td>north</td>
<td>345 (353)</td>
<td>30 (34)</td>
<td>0.99 (0.31)</td>
<td>16 (37)</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>south</td>
<td>196 (193)</td>
<td>38 (33)</td>
<td>0 (0)</td>
<td>84 (63)</td>
<td>83</td>
</tr>
</tbody>
</table>

$^a$ rel $\Delta G$ – difference between Gibbs free energies of optimised conformers (for more stable conformer $G = 0$)

$^b$ $X_{\text{calc}}$ – percentage of conformer calculated from rel $\Delta G$ (at 25 °C)

$^c$ $X_{\text{NMR}}$ – percentage of conformer in D$_2$O at 27 °C calculated from $^3J$(H,H) using relation [1]
3. Copies of 1H and 13C NMR spectra

9

OTEDMS

CDCl₃

H NMR in CDCl₃

OTEDMS

CDCl₃

13C NMR in CDCl₃
$^1$H NMR in C$_6$D$_6$

$^{13}$C NMR C$_6$D$_6$

Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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$1^H$ NMR in DMSO

$13^C$ NMR in DMSO
4. Hybridisation study - measurement of $T_m$ values (Table 1)

Thermal experiments were performed at 260 and 295 nm on a CARY 100 Bio UV Spectrophotometer (Varian Inc.) equipped with a Peltier temperature controller and thermal analysis software. The samples were prepared by mixing of equal molar amounts of modified T$_{15}$ and natural dA$_{15}$ (or rA$_{15}$) strands to give a 4 $\mu$M final concentration in 50 mM TRIS-HCl pH 7.2, 1 mM EDTA either with 100 mM Na$^+$ or 10 mM Mg$^{2+}$ ions. A heating-cooling cycle in the range 18–70 °C with a gradient of 0.2 °C/min was applied. $T_m$ values were determined from the maxima of the first derivative plots of absorbance versus temperature ($T_m \pm 0.5$ °C). In all cases, we observed single transition profiles. Characterization of the type of the complexes (see Table 1) was performed by a native PAGE at 15 °C. (for details and patterns, see SM bellow).
$[dT_{15}]^*[dA_{15}]$ at 260 nm

$A_{260}$

$10$ mM Mg$^{2+}$

$100$ mM Na$^+$

Temperature (°C)

$Tm = 48$ oC

$Tm = 38$ oC

$[dT_{15}]^*[dA_{15}]$ at 295 nm

$A_{295}$

Temperature (°C)
[(T₂-Ome)₁₄T]*[dA₁₅] at 260 nm

- 10 mM Mg²⁺
- 100 mM Na⁺

Temperature (°C)

[(T₂-Ome)₁₄T]*[dA₁₅] at 295 nm

Temperature (°C)

Tm = 54 oC

Tm = 35 oC
[(T-T₂-Ome)₇][rA₁₅] at 260 nm

\[ \text{A}_{260} \]

Temperature (°C)

10 mM Mg²⁺

100 mM Na⁺

\[ \text{dA}_{260}/\text{dt} \]

Tm = 33 °C

Tm = 38 °C

[(T-T₂-Ome)₇][rA₁₅] at 295 nm

\[ \text{A}_{295} \]

Temperature (°C)
[(4'-MeOE6O-T-4'-MeOT7)T]'[dA15] at 260 nm

Temperature (°C)

[(4'-MeOE6O-T-4'-MeOT7)T]'[dA15] at 295 nm

Temperature (°C)
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Tm = 28 °C

Tm = 50 °C
[(4'-MeOT-T-2'-OMe)₇T]⁺[rA₁₅] at 260 nm

Temperature (°C)

[(4'-MeOT-T-2'-OMe)₇T]⁺[rA₁₅] at 295 nm

Temperature (°C)
5. Normalized thermal difference spectra (TDS)

\[ \text{dA}_{15} - \text{Na} \]

\[ \text{rA}_{15} - \text{Na} \]
6. Determination of the type of complex by PAGE

Modified oligonucleotides (mT₁₅) were mixed with (³²P) 5'-end labelled dA₁₅ or rA₁₅ in 10 mM Tris, pH 7.4 and 150 mM NaCl (or 10 mM MgCl₂) at 1:1 molar ratios to obtain 100 nM final concentrations. Individual annealings were performed by heating the mixtures to 80 °C and slow cooling to room temperature. The samples were mixed with 10 mM Tris, pH 7.4 and 20% glycerol in 1:1 ratio and loaded onto 20% native polyacrylamide gels. The electrophoresis was run in 1xTBE (or 1x TB with 10 mM MgCl₂) at 12.5 V/cm for 10 hours at 15 °C. The gels were dried and visualized by autoradiography (Figure S4).
Figure S4. The native electrophoretic analysis of the mT\textsubscript{15} multimeric states in complex mixtures with dA\textsubscript{15} (Panels A and C) or rA\textsubscript{15} (Panels B and D; the abbreviation mT\textsubscript{15} stands for the “modified” oligothymidylates.

Panel A: Individual annealings (mT\textsubscript{15}-dA\textsubscript{15}) proceeded in the absence of MgCl\textsubscript{2}. Lane 1, dA\textsubscript{15} alone; lane 2, [T(4'-MeO\textsubscript{T})\textsubscript{7}]\textsubscript{T} hybridized with dA\textsubscript{15}; lane 3, [T(T\textsubscript{2}-OMe)\textsubscript{7}]\textsubscript{T} with dA\textsubscript{15}; lane 4, (4'-MeOE\textsubscript{T})\textsubscript{14}T with dA\textsubscript{15}; lane 5, T(T-pc\textsubscript{T})\textsubscript{7} with dA\textsubscript{15}; lane 6, (4'-MeO\textsubscript{T})\textsubscript{14} T with dA\textsubscript{15}; lane
7, (4′-Me)14T with dA15; lane 8, T15 with dA15; lane 9, [d(xyloT)]14T with dA15; lane 10, dA15 alone; lane 11, (T2′-OME)14T with dA15; lane 12, T15 with dA15.

Panel B: Individual annealings (mT15-rA15) proceeded in the absence of MgCl2. Lane 1, rA15 alone; lane 2, [T(4′-MeO)T]14T hybridized with rA15; lane 3, [T(T2′-OME)]14T with rA15; lane 4, (r-MeO-EtO)T14T with rA15; lane 5, T(T-p, T)7 with rA15; lane 6, (r-MeO)T14T with rA15; lane 7, (r-Me)T14T with rA15; lane 8, T15 with rA15; lane 9, [d(xyloT)]14T with rA15; lane 10, rA15 alone; lane 11, (T2′-OME)14T with rA15; lane 12, T15 with rA15.

Panel C: Individual annealings (mT15-dA15) proceeded in the presence of 10 mM MgCl2. Lane 1, dA15 alone; lane 2, (T(4′-MeO)T)7 hybridized with dA15; lane 3, [T(T2′-OME)]14T with dA15; lane 4, (r-MeO-EtO)T14T with dA15; lane 5, T(T-p, T)7 with dA15; lane 6, (r-MeO)T14T with dA15; lane 7, (r-Me)T14T with dA15; lane 8, T15 with dA15; lane 9, [d(xyloT)]14T with dA15; lane 10, dA15 alone; lane 11, T15 with dA15; lane 12, (T2′-OME)14T with dA15.

Panel D: Individual annealings (mT15-rA15) proceeded in the presence of 10 mM MgCl2. Lane 1, rA15 alone; lane 2, [T(4′-MeO)T]7 hybridized with rA15; lane 3, [T(T2′-OME)]14T with rA15; lane 4, (r-MeO-EtO)T14T with rA15; lane 5, T(T-p, T)7 with rA15; lane 5, (r-MeO)T14T with rA15; lane 7, (r-Me)T14T with rA15; lane 8, T15 with rA15; lane 9, [d(xyloT)]14T with rA15; lane 10, rA15 alone; lane 11, T15 with rA15; lane 12, (T2′-OME)14T with rA15.

Modified oligonucleotides [d(xyloT)]14T and T(T-p, T)7 (ref.6) in mixtures with dA15 or rA15 were used as reference entities to be run in native gels as markers indicating approximate migration positions of oligonucleotide duplexes and triplexes. The oligomer T(T-p, T)7 formed a mixture of duplexes and triplexes with dA15, and only duplexes with rA15 (all in the presence of Mg2+), while [d(xyloT)]14T formed triplexes with dA15 or rA15 in both the absence and presence of Mg2+. The existence of the above mentioned indicative forms occurring under similar in vitro conditions was independently determined earlier using surface plasmon resonance experiments (data not shown).

7. Molecular dynamics simulation (MDS) – methodology and additional figures

Molecular dynamics simulations (MDS) lasting for 5ns with model oligonucleotide helical structures carrying chemical modifications on either C4’ or C2’ atoms ([T(4′-MeO)T]8T)WC•rA10*[T(4′-MeO)T]8TWC • rA10* [T(4′-MeO)EtO]8T)WC•rA10* [T(4′-MeO)EtO]8T)WC • rA10* [T(T2′-OME)8T)Hestm - see Figures S5-S10) were produced at 310 K.

Additional groups of atoms (either -Oα-CH3 or -Oα-CH2-CH2-CH3) were anchored to the C2’ and C4’ atoms and manipulated manually (using VMD7) into reasonable starting configurations.

Simulated systems were surrounded by TIP3P water molecules,8 which extended to a distance of 10Å in each direction from solute atoms. This gives a periodic box size of 65Å x 60Å x 52Å consisting of approximately 15,000 atoms.

We dealt with MD simulations of nucleic acids carrying chemical modifications on either C4’ or C2’ atoms. Therefore, necessary *.pre files describing topology of the modified residues were derived from those describing topology of natural nucleic acids in the AMBER force field.

Further, additional force constants were incorporated into the AMBER force field partially on the basis of analogy with force constants presented in literature.9,10 Total energies of simple model systems were computed for either 2’-endo or 3’-endo conformers. It was found as
inevitable to introduce the CT-OS-CT-OS force constant to mimic the anomeric effect (i. e. preference for the gauche conformer consider the C1'-O4'-C4'-O₄A torsion angle) and to impel the C4' modified sugars to prefer the 3'-endo conformation within MD runs.

Large concentrations of ions, which are, however, obvious in the context of melting temperatures of the antisense oligonucleotides, were used in our experiments (10 mM Mg²⁺ or 100 mM Na⁺). Identity of ions (Na⁺/Mg²⁺) was found to be influential, considering the stability of helical structures carrying various chemical modifications. We checked a hypothesis that the binding cavities for ions (consisting of O₃', O₄A and O₄B atoms) are localised in the close proximity of the C4'-O₄A-CH₃, C4'-O₄A-CH₂-CH₂-O₄B-CH₃ moieties. Binding of water molecules in similar binding sites was observed in the case of C2’-modified oligonucleotides. The O₃', O₄A and O₄B atoms compete in binding of ions with another potential ligands – oxygen atoms of water molecules (OW), as well as with the non-bridge oxygen atoms of the phosphate groups (O1P) situated in close proximity. Therefore, partial charges on all oxygen atoms must be carefully established to secure reliable relative binding frequencies within a MD run. Classical force fields were developed during the last three decades by a gradual accumulation of force constants from different sources (their detail consistency is not a matter, of course, up to now).

We took into account the partial charges determined in the previous studies of oligonucleotides bearing –OCH₃ and –OCH₂CH₂OCH₃ groups on C2' atoms. Molecular mechanical (MM) partial charges (developed using the AMBER/RESP methodology - fitting of partial point charges to reproduce surrounding ab initio electrostatic potential) on O₄A and O₄B (-0.35), O₃' (-.5232), O1P (-0.7761) and OW (-0.834) atoms are rather manifold. The so called Mulliken charges determined by ab initio calculations show lower dispersion (reaching from -0.59 to -0.85). Mulliken charges (comparing to AMBER/RESP charges) determined for O₄A, O₄B, O₃' and O1P atoms show somewhat lower values. In contrast, the MM partial charge on the OW oxygen atom from the TIP3P water molecule is very close to its ab initio Mulliken value.

Therefore, we found necessary to unify a little bit partial charges of atoms considered here to compete in binding of ions. Total energies of several model systems were optimized using ab initio calculations: Mg²⁺.6H₂O, Mg²⁺.5H₂O, H₂O, Mg²⁺.5H₂O.CH₃OCH₃ a CH₃OCH₃. Ab initio energy of 36 kcal/mol was found to stabilize H₂O as well as CH₃OCH₃ binding toward Mg²⁺.5H₂O. In contrast, molecular mechanical calculations using original partial charges (-0.35 for O₄A/O₄B atoms⁹,¹⁰) produced energy gap of 27 kcal/mol for CH₃OCH₃ and 42 kcal/mol for the TIP3P water molecule. Molecular mechanical calculations using refined partial charges (this means -0.5 for O₄A/O₄B atoms) pushed the stabilization energy toward the 42 kcal/mol too. Interestingly, the resulting gap between the old and new values of O₄A, O₄B charges is the same as the difference between AMBER/CHARMM partial charges for atom O₄' of ribose moiety (-0.3691/-0.5). It should be noted, that CHARMM partial charges were derived to reproduce ab initio interaction energies between model compounds and water.

New *.inpcrd (initial coordinates) and *.prmtop (molecular topology, force field etc.) files for the whole simulated system were created by use of the TLEAP module (AMBER software package¹¹). Fully solvated trajectories were computed with the aid of the NAMD software package. Conventional computational procedures were used: periodic boundary conditions, cut off distances of 10Å for the nonbonded interactions and the particle-mesh-Ewald method¹³ for the summation of the coulombic interactions (PME grid size was chosen 64 in each direction), MD time step 0.002 ps. Initially, for 5 ps, the system was heated up to 310 K using
a Langevin temperature equilibration scheme while restraining the position of the solute. The MD was then continued for 5 ns at constant T and constant P (using Langevin Piston) with all restraints removed. Figures were produced with the aid of the VMD software package.  

**Figures S5-S14:**

Triple helical structures used as models in molecular dynamics simulations:

\[
[T(T_2'\text{OMe})_8T]^{W\cdot C}\cdot r_{A_{10}}^* [T(T_2'\text{OMe})_8T]^{\text{Hgstn}} \quad \text{(Figure S5, S6)}
\]

\[
([T(4'\text{MeO})_8T]^{W\cdot C}\cdot r_{A_{10}}^* [T(4'\text{MeO})_8T])^{\text{Hgstn}} \quad \text{(Figure S7, S8)}
\]

\[
[T(4'\text{MeOEtO})_8T]^{W\cdot C}\cdot r_{A_{10}}^* [T(4'\text{MeOEtO})_8T]^{\text{Hgstn}} \quad \text{(Figure S9, S10)}
\]

Binding of Mg\textsuperscript{2+} ions toward the \([T(4'\text{MeOEtO})_8T]^{W\cdot C}\cdot r_{A_{10}}^* [T(4'\text{MeOEtO})_8T]^{\text{Hgstn}}\) triple helical structure (Figure S11, S12). Two modes of contacts between Mg\textsuperscript{2+} ions and one and/or two oxygen atoms of C4'-OCH\textsubscript{2}CH\textsubscript{2}OCH\textsubscript{3} group in \([T(4'\text{MeOEtO})_8T]^{W\cdot C}\cdot r_{A_{10}}^* [T(4'\text{MeOEtO})_8T]^{\text{Hgstn}}\) (Figure S13). Na\textsuperscript{+} binding to the C4'-OCH\textsubscript{3} moiety in \([T(4'\text{MeO})_8T]^{W\cdot C}\cdot r_{A_{10}}^* [T(4'\text{MeO})_8T]^{\text{Hgstn}}\) triple helical structure (Figure S14).
Figure S5: \([T(T_2\text{-OMe})_8T]^W\cdot rA\text{10}^9[T(T_2\text{-OMe})_8T]^{\text{Hg}10}\) – hydrogen atoms omitted - stereo view
Figure S6: $[T(T_{2-OMe})_8T]^W_{\text{W}} \cdot rA_{10}^* [T(T_{2-OMe})_8T]^{H_{\text{Hg}}}$ – all atoms depicted – stereo view
Figure S7: $[T(\text{MeO}T)_8T]^{w-c} \cdot rA_{10}^g[T(\text{MeO}T)_8T]^{H_{8\text{stn}}}$ – hydrogen atoms omitted - stereo view
Figure S8: $[T(_4'MeOT)_8T]^W\cdot rA_{10}\cdot[T(_4'MeOT)_8T]^H_{gstn}$ – all atoms depicted – stereo view
Figure S9: $[T(\gamma'-\text{MeOEtO} T)_{8}T]^W \cdot rA_{10}^\ast [T(\gamma'-\text{MeOEtO} T)_{8}T]^H_{\text{pmn}}$ – hydrogen atoms omitted - stereo view
**Figure S10:** \([T(\tau'\text{MeOE}OT)_8T]^{W-C \bullet rA_{10} \ast}[T(\tau'\text{MeOE}OT)_8T]^{H_{s_{tn}}} \) – all atoms depicted – stereo view
Figure S11: $[T(4’\text{-MeOE}_{8}T)_{8}T]^{W}rA_{10}*[T(4’\text{-MeOE}_{8}T)_{8}T]^{\text{Hgstn}}$ vs. Mg$^{2+}$ ions– hydrogen atoms omitted - stereo view
Figure S12: \[\{T(4'-\text{MeOEtO}T)\}_8 T\}^{W-C} \cdot rA_{10}^* \{T(4'-\text{MeOEtO}T)\}_8 T\}^{H_{\text{est}}} \text{ vs. Mg}^{2+} \text{ ions – all atoms depicted – stereo view}\]
Figure S13: (4’-MeOEiT)26 and (4’-MeOEiT)27 and rA4-rA7 parts of [T-(4’-MeOEiT)8T]_{WC}\cdot A_{10}^{\cdot8}[T(4’-MeOEiT)_{8}\cdot T]_{Hgstn} (where A_{10} consists of residues 1-10, Watson-Crick strand – residues 11-20, Hoogsteen strand – residues 21-30) bridged by Mg^{2+} ions – (top) stereo view, (bottom) oxygen atoms serving as ligands for Mg^{2+} are indicated by red lines.
Figure S14: The \((4\text{-}\text{MeO}T)_{26}\) and \((4\text{-}\text{MeO}T)_{27}\) part of \([T(4\text{-}\text{MeO}T)_{8}T]^\text{W}-\text{rA}_{10}^\text{r}[T(4\text{-}\text{MeO}T)_{8}T]^\text{Hgstn}\) (where rA\text{10} consists of residues 1-10, Watson-Crick strand – residues 11-20, Hoogsteen strand – residues 21-30) + Na\text{+}.4H\text{2}O - (top) stereo view, (bottom) oxygen atoms serving as ligands for Na\text{+} are indicated by blue lines.

8. REFERENCES