4′-Epi-DNA: A DNA Mimic Containing 4′-hydroxymethyl-α-L-Xylo-Thymidine with Compact Backbone like RNA

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\textbf{ABSTRACT}

Synthesis of C4′-epi-DNA containing 3′→5″ linkages is reported for the first time. Crystal structure study of the monomer indicated that though the dihedral angle O3′-C3′-C4′-C5″ in this case would be like in RNA, the sugar conformation would remain like that in DNA. The study of the effect of this backbone configuration in DNA with respect to its binding to cDNA and RNA is reported in this note.

\textbf{GRAPHICAL ABSTRACT}

\textbf{Introduction}

Backbone-modified DNA oligomers as antisense oligonucleotides (ONs) are being explored for therapeutic applications since the last two decades.\textsuperscript{[1–3]} Numerous examples of modified ONs and their antisense action are reported in the literature.\textsuperscript{[4–7]} Different modifications of DNA, such as 2′′-O-alkyl,\textsuperscript{[7]} 2′′-O-aminoalkyl,\textsuperscript{[8,9]} LNA,\textsuperscript{[10,11]} as well as 2′′-fluoro,\textsuperscript{[12,13]} resulted in significant increase of the DNA:RNA duplex stability. Most of these modifications drive the sugar into the C3′-endo
conformation (Figure 1, N-type) typical for the A-type RNA/RNA duplex,\textsuperscript{[14]} which results in complete loss of susceptibility to RNase H activity. However, arabinonucleic acids and 2'-deoxy-2'-fluoro-arabinonucleic acids were shown\textsuperscript{[15–18]} to be substrates for RNase H-mediated cleavage of target RNA. In an NMR study, it was demonstrated that the 2'F-ANA adopted an eastern \( O^4\)-endo-sugar conformation, closer to B-type DNA when hybridized to RNA.\textsuperscript{[19]} The \( \alpha\)-L-LNA\textsuperscript{[20,21]} and CeNA\textsuperscript{[22]} were also reported to assume a DNA-like southern conformation. The crystal structure of RNase H complexed with a DNA:RNA duplex also later established that the enzyme specifically recognizes the A-form of the RNA strand and B-form of the DNA strand in the RNA:DNA duplex.\textsuperscript{[23]} It is apparent from the above discussion that an advantageous strategy would be to improve the affinity to the target RNA while maintaining the C2'-endo sugar pucker in the modified nucleosides (Figure 1, S-type).

ONs possessing a variously 4'-C-substituted 2'-deoxyribose moiety\textsuperscript{[24–27]} (Figure 2) have been reported. In these studies, the 5'-hydroxy→3'-hydroxy backbone substituted at C4'- (Figure 2, I) or the 5'-hydroxy→4'-C-hydroxymethyl backbone (Figure 2, II) were explored. In I, the geometry of the sugar remained S-type and the phosphodiester linkages remain \( \text{trans}(\text{pseudodiaxial})\), extended. Many of these resulted in enhanced stability of the resulting duplexes with DNA and RNA and also induced RNase H activity. The 4'-C-hydroxymethyl-\( \alpha\)-L-RNA nucleic acids were synthesized by Wengel's group (Figure 2, III).\textsuperscript{[28]} In this case, the C3'- and C4'-centers taking part in the phosphodiester linkages are inverted compared to natural linkages but still remain \( \text{trans}\)-oriented. The derived oligomers were found to form less stable duplexes with DNA and RNA, the latter also being good substrates for RNase H. The other isomer, 4'-C-hydroxymethyl-\( \beta\)-D-lyxofuranosyl...
nucleic acids (Figure 2, IV) where the C3' centre taking part in the phosphodiester linkages is inverted, has a cis disposition of the substituents involved in the backbone linkages.[28] Incorporation in a DNA strand led to RNA-selective binding, while incorporation in an RNA strand led to stable duplex formation with both DNA and RNA. In α-L-LNA, which is a locked analogue of 4'-C-hydroxymethyl-α-L-RNA[28], also, the stereochemistry at both C4'- and C3'-centers is inverted and remains trans. Similarly, 2'-deoxy-xylo-based nucleic acids[29] (Figure 2, V), in which the stereochemistry is inverted only at the C3'-center compared to DNA, with cis-orientation of the 5'- and 3'-backbone linkers, was investigated. Very recently, a xylo-nucleic acid analogue with a 2'-hydroxy group was also studied.[30] The backbone suggested for the present studies, containing modified 4'-hydroxymethyl-thymidine (or 4'-hydroxymethyl-α-L-xylo-thymidine), i.e., α-4'-C-hydroxymethyl→cis-3'-hydroxy backbone will be epimeric at the C4' position with respect to the DNA backbone (hence termed 4'-epi-DNA), has not yet been studied and therefore is of interest. We suppose that the cis orientation of C5'- and C3'-substitutions as phosphodiester linkers (pseudoequatorial, pseudoaxial, Figure 1) would lead to compact geometry as in the case of N-type sugar in RNA with pseudoequatorial orientation of phosphate groups. In this paper, we report the synthesis of this backbone as a DNA analogue containing 4'-substituted α-L-xylo-thymidine which is envisaged to have the sugar in a 2'-endo (S-type) pucker that is normally observed in DNA. The cis-geometry of the phosphodiester linker involving 4'-α-C5''H2OH and 3'-OH group may show internucleoside distance complementarity while binding to compact A-type RNA geometry (Figure 1).

The synthesis of the monomer was accomplished from thymidine 1 (Scheme 1). Compound 4 was obtained following the reaction sequence as reported in literature.[31] We applied a simple oxidation protocol for the synthesis of the 5'-aldehyde using IBX[32] rather than Swern oxidation[33,34] (oxalyl chloride/DMSO/DIPEA) or Moffatt's oxidation (DMSO/DCC/TFA/pyridine) procedure.[35] The latter two oxidation procedures require polar solvents and tedious work-up conditions whereas IBX oxidation was simple and high yielding. Reaction of compound 4 with IBX
Scheme 1. Synthesis of C₄′-epi-DNA monomer.

gave corresponding aldehyde 5 which was followed by aldol condensation with formaldehyde in the presence of aqueous sodium hydroxide with concomitant reduction using sodium borohydride employing known reaction conditions \[36\] to give known compound 6. The α-4′-C-hydroxymethyl functionality (C₅′′-OH) was regioselectively protected by reaction with DMT-Cl to give nucleoside 7. Similar selectivity for the reactivity of C₅′′-OH was found earlier by Obika et al.\[37\] Protection of 5′-hydroxyl group as a silyl ether furnished compound 8. The DMT groups in compound 8 were then removed using 2% dichloroacetic acid in DCM to give 5′-O-( tert-butyldimethylsilyl)-4′-C-(hydroxymethyl)thymidine 9. The protection of the α-4′-C-hydroxymethyl group by reaction with DMT-Cl furnished compound 10 which was then converted to its phosphoramidite derivative 11. Compound 11 was found to be unstable for characterization and easily decomposed, probably due to the cis DMT-protected hydroxymethyl group and the reactive phosphoramidite. Its formation was confirmed by \(^{31}\)P NMR spectroscopy and was used in the synthesis of oligomers immediately.

The sugar conformation of compound 9 was calculated from NMR H1′ coupling constants (\(J_{\text{H1′-H2′}} = 8.84\) and 5.43 Hz) using Sum rule\[38\] and was found to have 76% S-type sugar pucker. We further confirmed the sugar conformation using crystal structure of compound 9 (Figure 3) which clearly indicated S-type sugar conformation and the envisaged proximity of the O3′ and O5′ centers to be engaged in phosphodiester linkages.

The unmodified oligomers\[39\] were synthesized using an automated Bioautomation MM4 DNA synthesizer and commercially available 5′-O-DMT-2′-deoxy-3′-phosphoramidite building blocks using the β-cyanoethylphosphoramidite method.\[40,41\] The chosen control 18mer oligonucleotide DNA1 was specific for the Splice correction of an aberrant β-globin intron (705 site). The modified monomer 11 was incorporated at pre-determined positions in the unmodified sequence DNA1.
using extended coupling time to yield the modified oligomers ON1 and ON2 (Table 1). The synthesized ONs were cleaved from the solid support with conc. NH₄OH and lyophilized. The 5'-O-silyl group was deprotected by treatment with 0.1 mL desilylation solution (1.5 mL NMP + 0.75 mL TEA + 1 mL 3HF: TEA) at 65°C for two hours. The reaction mixture was then lyophilized and desalted to

| Sequence | HPLC t_R (min) | MALDI-TOF Mass Expected/Found | UV-T_m °C (ΔT_m °C) |
|----------|----------------|-------------------------------|---------------------|
| DNA1     | 8.66           | 5400/5401.9                   | 53.1                |
| ON1      | 7.95           | 5431/5431.3                   | 50.2 (−2.9)         |
| ON2      |                |                               | 46.8 (−6.3)         |
| DNA2     |                |                               | 56.5                |
| RNA      |                |                               | 50.4 (−6.1)         |
| T        |                |                               | 48.6 (−7.9)         |

Table 1. The synthetic oligomers, their characterization and T_m (°C) values of 18mer chimeric ONs: DNA/RNA duplexes.

Figure 3. X-ray Crystal structure of compound 9, the percentage of probability of the ellipsoids is 40%.

\[\text{DNA1: 5'}^\text{CCT TTT ACC TCA GTT ACA3'}, \text{ON1: 5'}^\text{CCT TTT ACC TCA GTT ACA3'} \text{ ON2: 5'}^\text{CCT TTT ACC TCA GTT ACA3'} \text{ DNA2: 5'}^\text{TGT AAC TGA GTG AAG AGG 3'}, \text{RNA: 5'}^\text{UGU UGC UGA GGG UGG AAG AGG 3'}, \text{T: represents modification.} \text{ T_m = melting temperature (measured in the buffer 10 mM sodium phosphate, 100 mM NaCl, pH = 7.2), of C4-epi-DNA-modified ONs:DNA/RNA complexes. The values reported here are the average of 3 independent experiments and are accurate to } \pm 1.0 \text{°C.} \text{ ΔT_m = T_m − T_m(control).} \]
get the crude ONs. The purity of the oligomers listed in Table 1 was checked by analytical RP-HPLC (C18 column, 0.1 N TEAA buffer-acetonitrile) which showed more than 75–80% purity. These oligomers were subsequently purified by reverse phase HPLC on a C18 column. The purity of the oligomers was again ascertained by analytical RP-HPLC and found to be >95%. The modified oligomers were characterized by MALDI-TOF mass spectrometric analysis (SI). The UV-melting profiles of the duplexes formed by oligonucleotides ON1 and ON2 with complementary DNA/RNA were studied (SI). The unmodified 18mer DNA1 sequence formed complexes with complementary DNA2 and RNA with higher melting temperature for DNA1:RNA complex over DNA1:DNA2 ($\Delta T_m = +3.4^\circ C$, Table 1). Introduction of a single monomeric unit towards the 3’-end in 18mer oligomer ON1, caused destabilization of 2.9$^\circ C$ and 6.1$^\circ C$ with complementary DNA2 and RNA, respectively. The effect of the second unit was less disturbing to the ON2:RNA duplex stability and much less destabilization was observed for the ON2:RNA duplex ($\Delta T_m = −1.8^\circ C$) compared to ON2:DNA2. Thus, intermittent change to 3’−5’ linkage in continuous 3’−5’ backbone at a single or more positions destabilized the duplexes with both DNA and RNA.

In summary, NMR and crystal structure studies confirmed the S-type sugar pucker for C4’-hydroxymethyl-thymidine derivative. Introduction of this 4’-substituted thymidine monomer into oligomers containing 3’ → 5’ linkages at selected positions, destabilized complexes with both DNA and RNA. We found that the experimental results did not corroborate with the proposed hypothesis that the compact geometry due to RNA-like O3’-C3’-C4’-C5” dihedral angle and cis axial-equatorial orientation of phosphodiester groups at the modified site would be effective for stabilizing the DNA:RNA duplexes. The duplexes formed with both DNA and RNA were largely destabilized similar to the instances of the other stereoisomers studied before.

**Experimental section**

**General**: All the reagents were purchased from Sigma-Aldrich and used without purification. DMF, CH$_3$CN were dried over P$_2$O$_5$ and CaH$_2$, respectively, and stored by adding 4 Å molecular sieves. Pyridine, TEA were dried over KOH and stored by adding KOH. THF was passed over basic alumina and dried by distillation over sodium. Analytical TLCs were performed on Merck 5554 silica 60 aluminium sheets. Column chromatography was performed for purification of compounds on silica gel (60–120 mesh, Merck). For acid-sensitive (trityl-containing) compounds, the column was packed and equilibrated with 0.5% triethylamine. TLCs were performed using dichloromethane-methanol or petroleum ether–ethyl acetate solvent systems. Visualization was accomplished with UV light and/or by spraying with perchloric acid reagent and heating. $^1$H and $^{13}$C NMR spectra were obtained using Bruker ACF 200 (200 MHz) or 400 (400 MHz) spectrometers and all the chemical shifts (δ/ppm) are referred to internal TMS for $^1$H and chloroform-d for $^{13}$C NMR. $^1$H NMR data are reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; br, broad; br s, broad singlet; m, multiplet and/ or multiple
resonance), number of protons. Mass spectra were recorded on Thermo Finni-
gan Surveyor MS-Q spectrometer, while HRMS was recorded by using a Thermo
Scientific Q Exactive mass spectrometer. MALDI-TOF spectra were recorded on a Voyager-De-STR (Applied Biosystems)
MALDI-TOF instrument. UV experiments were performed on a Varian Cary 300
UV-VIS spectrophotometer fitted with a Peltier-controlled temperature program-
er and a water circulator.

5′-O-(tert-butyldimethylsilyl)-thymidine (2)
Thymidine 1 (10.0 g, 41.28 mmol) and imidazole (6.0 g, 90.75 mmol) were sus-
pended in 15 mL of dry DMF. TBS-Cl (6.5 g, 41.25 mmol) dissolved in 5 mL
of dry DMF was added dropwise at 0°C and the reaction was stirred at RT for
20 h. DMF was removed under vacuum and the residue was dissolved in ethyl
acetate, washed with water and then brine. The organic layer was dried over Na₂SO₄
and concentrated. Purification by column chromatography using 2% methanol in
dichloromethane gave the product 2 (12.1 g, Yield 80%).

1H NMR (200 MHz, CDCl₃) δ: 8.93 (s, 1H, exchanges with D₂O), 7.53 (s, 1H),
6.35–6.42 (dd, J = 8.21, 5.81 Hz, 1H), 4.48 (m, 1H), 4.05 (m, 1H), 3.89 (m, 2H),
2.65 (br s, 1H, exchanges with D₂O), 2.34–2.44 (m, 1H), 2.03–2.17 (m, 1H), 1.92 (s,
1H), 0.92 (s, 9H), 0.12 (s, 6H); 13C NMR (50 MHz, CDCl₃) δ: 164.1, 150.6, 135.5,
110.9, 87.3, 85.0, 72.7, 63.6, 41.0, 25.8, 18.3, 12.5, −5.4, −5.5; MS (EI)m/z 356.1767,
found 379.1659, found 379.1648.

5′-O-(tert-butyldimethylsilyl)-3′-O-(4,4′-dimethoxytrityl)thymidine (3)
A mixture of 2 (10.0 g, 28.0 mmol) and DMT-Cl (11.4 g, 33.7 mmol) in 25 mL dry
pyridine was heated at 50°C for 12 h. Pyridine was removed under vacuum and
the crude product was purified by column chromatography on neutralized silica gel
using 50% ethyl acetate in petroleum ether to give product 3 (17.1g, Yield 92%).

1H NMR (200 MHz, CDCl₃) δ: 8.32 (s, 1H, exchanges with D₂O), 7.32–7.47
(m, 10H), 6.82–6.86 (d, J = 8.85 Hz, 4H), 6.37–6.45 (dd, J = 9.34, 5.31 Hz, 1H),
4.28 (d, J = 5.30 Hz, 1H), 4.03 (s, 1H), 3.80 (s, 6H), 3.66 (m, 1H), 3.27 (m, 1H),
1.86 (s, 3H), 1.70 (m, 2H), 0.81 (s, 9H), −0.04 (s, 3H), −0.09 (s, 3H); 13C NMR
(50 MHz, CDCl₃) δ: 163.6, 158.6, 150.2, 145.0, 136.3, 136.2, 135.5, 130.2, 130.1,
128.2, 127.9, 127.0, 113.2, 110.8, 87.2, 86.6, 84.8, 74.9, 63.5, 55.2, 39.9, 25.7, 18.1,
12.3, −5.4, −5.7; MS (EI)m/z 658.3074, found 681.34 (M + Na⁺); HRMS (ESI):
calcd for C₁₆₆H₂₈₂₅N₂O₇SiNa: 681.2966, found 681.2956.

3′-O-(4,4′-dimethoxytrityl) thymidine (4)
Compound 3 (15.0 g, 22.7 mmol) dissolved in 40 mL anhydrous THF and 1N TBAF
in THF (34.1 mL, 34.1 mmol) was added. The reaction was stirred at RT for 1 h.
THF was removed under reduced pressure and the residue was dissolved in DCM, washed with water and then brine. The organic layer was dried over Na₂SO₄ and concentrated. The product was purified by column chromatography using 1.5% of methanol in dichloromethane to yield product 4 as a white solid (9.8 g, Yield 79%).

**1H NMR** (200 MHz, CDCl₃) δ: 8.59 (s, 1H, exchanges with D₂O), 7.24–7.47 (m, 10H), 6.82 (d, J = 8.46 Hz, 4H), 6.09–6.17 (dd, J = 8.72, 5.81 Hz, 1H), 4.36 (m, 1H), 3.98 (s, 1H), 3.79 (s, 6H), 3.63–3.69 (m, 1H), 3.35 (m, 1H), 2.50 (br s, 1H, exchanges with D₂O), 1.90–1.95 (m, 1H), 1.85 (s, 3H), 1.69 (s, 1H); **13C NMR** (50 MHz, CDCl₃) δ: 163.7, 158.8, 150.3, 145.0, 137.1, 136.2, 136.1, 130.1, 128.2, 127.9, 127.0, 113.2, 110.8, 87.2, 87.1, 86.5, 74.2, 62.4, 55.2, 38.5, 12.3; **MS (EI)m/z** 544.2210, found 567.21 (M+ Na⁺); **HRMS (ESI)**: calcd for C₃₁H₃₂N₂O₁₇Na: 567.2102, found 567.2091.

**3’-O-(4, 4’-dimethoxytrityl)-5’-formyl Thymidine (5)**

Compound 4 (3.00 g, 5.51 mmol) was dissolved in 20 mL acetonitrile in a flask equipped with a reflux condenser. To it, IBX (4.63 g, 16.5 mmol) was added and the reaction mixture was heated at 80°C for 3 h. After completion of reaction, the reaction mixture was cooled to room temperature and filtered through a bed of celite. The filtrate was concentrated and subjected to filtration column purification on neutralized silica gel. The product eluted at 35–40% acetone in petroleum ether system to get compound 5 (2.65 g, yield 89%) as a white foam. The product obtained was immediately utilized for the next reaction.

**3’-O-(4,4’-dimethoxytrityl)-4’-hydroxymethylthymidine (6)**

To a stirred solution of 5 (2.62 g, 4.83 mmol) in dioxane, cooled in a waterbath, 35% HCHO (0.78 mL, 9.18 mmol) and 2N NaOH (4.6 mL, 9.18 mmol) were added. Stirring was continued for 5 min. The reaction mixture was then cooled in an ice bath and NaBH₄ (0.349 g, 9.18 mmol) was added in portions. Stirring was continued in an icebath for 1 h and then allowed to attain room temperature, with stirring continued for 3 h. To the reaction mixture, 1 mL water was added and the mixture was neutralized using Dowex H⁺ resin. The resin was filtered off and the filtrate evaporated to dryness. The residue was purified by column chromatography using 30% acetone in petroleum ether to give product 6 as a white solid (2 g, Yield 72%).

**1H NMR** (200 MHz, CDCl₃) δ: 8.65 (s, 1H, exchanges with D₂O), 7.28–7.46 (m, 10H), 6.98 (s, 1H), 6.83–6.88 (m, 4H), 6.12–6.18 (m, 1H), 4.57 (m, 1H), 4.02 (m, 1H), 3.75–3.80 (m, 8H), 3.39 (m, 1H), 2.62 (br s, 1H, exchanges with D₂O), 2.02–2.15 (m, 1H), 1.81 (s, 3H), 1.41–1.55 (m, 1H); **13C NMR** (50 MHz, CDCl₃) δ: 163.6, 158.8, 150.5, 144.9, 136.6, 135.9, 135.8, 130.3, 128.2, 128.0, 127.2, 113.3, 111.1, 89.1, 87.3, 83.7, 72.6, 63.2, 62.1, 55.2, 38.8, 12.3; **MS (EI)m/z** 574.2315, found 597.17 (M+ Na⁺); **HRMS (ESI)**: calcd for C₃₂H₃₄N₂O₈Na: 597.2207, found 597.2196.
4′-C-[4,4′-dimethoxytrityl]oxomethyl–3′-O-[4,4′-dimethoxytrityl]thymidine (7)

To a solution of 6 (1.90 g, 3.31 mmol) in pyridine, DMT-Cl (1.34 g, 3.8 mmol) was added and reaction mixture was allowed to stir for 12 h. Pyridine was removed under vacuum and the crude product obtained was purified by column chromatography on neutralized silica gel using 45% ethyl acetate in petroleum ether to give product 7 (2.25 g, Yield 78%).

\[
\begin{align*}
\text{1H NMR} & \ (200 \text{ MHz, CDCl}_3) \ \delta: 8.60 (s, 1H, \text{exchanges with D}_2\text{O}), 7.51–7.54 (m, 2H), 7.39–7.45 (m, 4H), 7.09 (m, 1H), 6.84–6.89 (m, 4H), 6.71–6.77 (m, 4H), 6.26–6.32 (m, 1H), 4.33 (t, J = 8.09 Hz, 1H), 3.83 (m, 1H), 3.77 (s, 12H), 3.55 (d, J = 10.36 Hz, 1H), 3.30 (d, J = 10.36 Hz, 1H), 2.96–3.09 (m, 1H), 2.32–2.47 (m, 1H), 2.08 (br s, 1H, exchanges with D$_2$O), 1.84 (s, 3H), 1.49–1.62 (m, 1H); \\
\text{13C NMR} & \ (100 \text{ MHz, CDCl}_3) \ \delta: 163.6, 158.6, 158.4, 150.0, 145.1, 144.8, 136.5, 136.0, 135.9, 134.8, 135.7, 130.2, 130.1, 128.1, 127.9, 127.7, 127.0, 126.7, 113.2, 113.0, 110.7, 88.5, 87.0, 86.7, 84.1, 71.6, 64.8, 62.9, 55.1, 38.8, 12.4; \text{MS (EI)} m/z 876.3622, \text{found 875.50 (M}^+\text{)}. \\
\text{HRMS (ESI)}: \text{calcd for C}_{53}\text{H}_{52}\text{N}_2\text{O}_{10}\text{Na: 899.3514, found 899.3508.}
\end{align*}
\]

5′-O-(tert-butyldimethylsilyl)-4′-C-[4,4′-dimethoxytrityl]oxomethyl–3′-O-[4,4′-dimethoxytrityl]thymidine (8)

Compound 7 (2.000 g, 2.28 mmol), imidazole (0.446 g, 6.84 mmol) and TBS-Cl (0.515 g, 3.42 mmol) were suspended in 4 mL of dry DMF. The reaction mixture was stirred at RT for 3 h. DMF was removed under vacuum and the product was extracted in ethyl acetate. The organic layer was dried over Na$_2$SO$_4$, concentrated and the crude product was purified by column chromatography using 30% ethyl acetate in petroleum ether to give pure product 8 (1.71 g, Yield 76%).

\[
\begin{align*}
\text{1H NMR} & \ (200 \text{ MHz, CDCl}_3) \ \delta: 8.27 (s, 1H, \text{exchanges with D}_2\text{O}), 7.50–7.53 (m, 2H), 7.34–7.43 (m, 4H), 7.22–7.31 (m, 3H), 7.07–7.18 (m, 9H), 7.09 (m, 1H), 6.80–6.86 (m, 4H), 6.67–6.72 (m, 4H), 6.38 (t, J = 6.84 Hz, 1H), 4.21–4.27 (dd, J = 7.70, 4.55 Hz, 1H), 3.92 (d, J = 10.7 Hz, 1H), 3.76 (s, 12H), 3.68 (d, merged with 3.76 peak, 1H), 3.24–2.31 (m, 2H), 1.87 (s, 3H), 1.63 (m, 2H), 0.77 (s, 9H), −0.07 (s, 3H), −0.16 (s, 3H); \text{13C NMR} (50 \text{ MHz, CDCl}_3) \ \delta: 163.5, 158.5, 158.4, 150.0, 145.3, 144.9, 136.0, 135.8, 130.3, 130.2, 130.1, 128.2, 128.1, 127.8, 127.7, 126.8, 126.7, 113.1, 113.0, 110.7, 88.7, 86.9, 86.5, 83.0, 77.3, 65.1, 64.5, 55.1, 39.2, 25.8, 18.2, 12.5, −5.4, −5.6; \text{MS (EI)} m/z 990.4487, \text{found 1013.54 (M}^+\text{Na}^+) ; \text{HRMS (ESI)}: \text{calcd for C}_{59}\text{H}_{66}\text{N}_2\text{O}_{10}\text{SiNa: 1013.4383, found 1013.4421.}
\end{align*}
\]

5′-O-(tert-butyldimethylsilyl)-4′-hydroxymethylthymidine (9)

Compound 8 (1.5 g, 1.51 mmol) was dissolved in minimum quantity of DCM and to it 2% dichloroacetic acid in DCM (total 18 mL + 0.3 mL of triisopropylsilane as scavenger) was added in portions. The reaction was monitored by TLC. After completion of the reaction, the mixture was diluted with DCM and the organic layer
was washed with saturated Na$_2$CO$_3$. The organic layer was dried over Na$_2$SO$_4$ and concentrated. The crude product was purified by column chromatography using 60% ethyl acetate in petroleum ether to give 9 as a white solid (0.438 g, Yield 75%).

$^1$H NMR (200 MHz, CDCl$_3$) $\delta$: 9.87 (s, 1H, exchanges with D$_2$O), 7.55 (s, 1H), 6.45–6.52 (dd, $J = 8.84, 5.43$ Hz, 1H), 4.50 (d, $J = 5.05$ Hz, 1H), 4.32 (br s, exchanges with D$_2$O, 1H), 3.70–3.88 (m, 5H), 2.39–2.48 (m, 1H), 2.16–2.30 (m, 1H), 1.90 (s, 3H), 0.92 (s, 9H), 0.12 (s, 6H); $^{13}$C NMR (50 MHz, CDCl$_3$) $\delta$: 163.9, 150.7, 135.4, 111.3, 88.8, 84.7, 74.0, 66.1, 63.2, 41.4, 25.8, 18.3, 12.4, $-5.4$, $-5.5$; MS (EI) $m/z$ 386.1873, found 409.16 (M$^+$ Na$^+$); HRMS (ESI): calcd for C$_{17}$H$_{30}$N$_2$O$_6$SiNa: 409.1771, found 409.1771.

5′-O-(tert-butyldimethylsilyl)-4′-C-[(4, 4′-dimethoxytrityl)oxomethyl]thymidine (10)

To a solution of 9 (0.40 g, 1.03 mmol) in pyridine, DMT-Cl (0.42 g, 1.24 mmol) was added and reaction mixture was allowed to stir for 12 h. Pyridine was removed under vacuum and the crude product was purified by column chromatography on neutralized silica gel using 40% ethyl acetate in petroleum ether to give product 10 (0.583 g, Yield 82%).

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 8.43 (s, 1H, exchanges with D$_2$O), 7.55 (s, 1H), 7.88 (d, $J = 7.33$ Hz, 2H), 7.22–7.33 (m, 8H), 6.84 (m, 4H), 6.43–6.46 (m, 1H), 4.50 (s, 1H), 3.90 (d, $J = 10.68$ Hz, 1H), 3.80 (s, 6H), 3.62 (d, $J = 10.68$ Hz, 1H), 3.35 (d, $J =$ 9.77 Hz, 1H), 3.24 (d, $J =$ 9.46 Hz, 1H), 2.88 (br s, exchanges with D$_2$O, 1H), 2.34–2.38 (m, 1H), 2.20–2.26 (m, 1H), 1.91 (s, 3H), 0.92 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$: 163.5, 158.7, 150.1, 143.8, 135.5, 134.8, 134.7, 129.8, 129.8, 128.1, 127.7, 127.1, 113.4, 110.8, 88.8, 87.2, 84.6, 73.9, 66.5, 63.2, 55.2, 40.8, 25.9, 18.4, 12.4, $-5.42$, $-5.45$; MS (EI) $m/z$ 688.3180, found 711.38 (M$^+$ Na$^+$); HRMS (ESI): calcd for C$_{38}$H$_{48}$N$_2$O$_8$SiNa: 711.3078, found 711.3098.

5′-O-(tert-butyldimethylsilyl)-3′-O-[(2-cyanoethoxy)diisopropylaminophosphino]−4′-C-[(4, 4′-dimethoxytrityl)oxomethyl]thymidine (11)

Compound 10 (0.200 g, 0.29 mmol) was dissolved in dry dichloromethane (5 mL) followed by the addition of diisopropylethylamine (0.126 mL, 0.726 mmol). Then 2-cyanoethyl-$N$, $N′$-diisopropyl-chlorophosphine (77 $\mu$L, 0.34 mmol) was added to the solution at 0°C and the reaction mixture was stirred at room temperature for 3 h. The contents were then diluted with DCM and washed with 5% NaHCO$_3$ solution. The product was purified by column chromatography using 1% triethylamine in 30% petroleum ether in DCM to give compound 11 (0.153 g, yield 60%).

$^{31}$P NMR (400 MHz, CDCl$_3$) $\delta$: 149.3, 148.5.

**Solid phase synthesis of DNA oligonucleotides by phosphoramidite method**

Control 18mer oligonucleotide DNA1 chosen was specific for the Splice correction of an aberrant $\beta$-globin intron (705 site). The unmodified oligomers were
synthesized using an automated Bioautomation MM4 DNA synthesizer and commercially available 5’-O-DMT-2’-deoxy-3’-phosphoramidite building blocks using β-cyanoethylphosphoramidite method. The modified monomer unit 11 was incorporated at pre-determined positions using an extended coupling time of 6 min to yield the modified oligomers efficiently. ON1 and ON2 were synthesized with one and two modifications, respectively.

**Purification and MALDI-TOF characterization of modified oligomers**

The synthesized oligonucleotides were cleaved from the solid support with conc. NH₄OH, lyophilized. The silyl group was deprotected by adding 0.1 mL desilylation solution (1.5 mL NMP + 0.75 mL TEA + 1 mL 3HF:TEA) and heated at 65°C for two hours. The reaction mixture was then cooled and neutralized with 0.1 mL NH₄HCO₃ (0.5 M), lyophilized and desalted to get the crude oligonucleotides. The purity of the oligomers listed in Table 1 was checked by analytical RP-HPLC (C18 column, 0.1 N TEAA buffer-acetonitrile) which showed more than 75–80% purity. These oligomers were subsequently purified by reverse phase HPLC on C18 column. The purity of the oligomers was again ascertained by analytical RP-HPLC and found to be >95%. Their integrity was confirmed by MALDI-TOF mass spectrometric analysis. THAP (2,4,6-trihydroxy acetophenone) matrix with diammonium citrate as additive was used for MALDI-TOF (matrix assisted laser desorption ionization time-of-flight) mass analysis of oligomers. HPLC retention time and observed values of mass in MALDI-TOF spectrometry are listed in Table 1.

**Biophysical studies of modified oligomers**

UV-\(T_m\) studies were carried out to investigate the binding of the modified oligomers to complementary DNA and RNA. The \(T_m\) experiments of duplexes were carried out in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl and 0.1 mM EDTA. Chimeric DNA oligonucleotides were individually hybridized with the complementary DNA and RNA strands, to obtain duplexes.

The binding affinity of 18mer chimeric ONs (ON1 and ON2) with complementary DNA2 and RNA was investigated by measuring the melting temperatures (\(T_m\)) of the duplexes.

**Supporting Information.** X-ray data collection and refinement parameters, \(^1\)H, \(^13\)C NMR spectra of compounds 2–10, HRMS of compounds 2–10, \(^31\)P NMR spectrum of compound 11, HPLC profiles and MALDI-TOF spectra of ON1 and ON2, UV melting graphs.

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