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Incorporation of 3'-*C*-(Hydroxymethyl)thymidine into Novel Oligodeoxynucleotide Analogues

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Abstract: 3'-*C*-(Hydroxymethyl)thymidine (**1**) has been incorporated into novel oligodeoxynucleotide (ODN) analogues containing extended (**Y**, 5'-hydroxyl to 3'-hydroxymethyl) backbones using the phosphoramidite building block **4**. The hybridization properties and enzymatic stabilities were studied by UV experiments. ODNs containing the modified nucleotide **Y** exhibited minor decreases in the affinity towards complementary DNA, but Watson-Crick base pairing involving the modified nucleotide **Y** was supported by evaluation of ODNs containing the corresponding analogously modified N^3-methyl nucleotide **Z**. The results obtained for **Y** are compared with ODNs containing **1** with an unmodified backbone (**X**, 5'- to 3'-hydroxyl).

INTRODUCTION

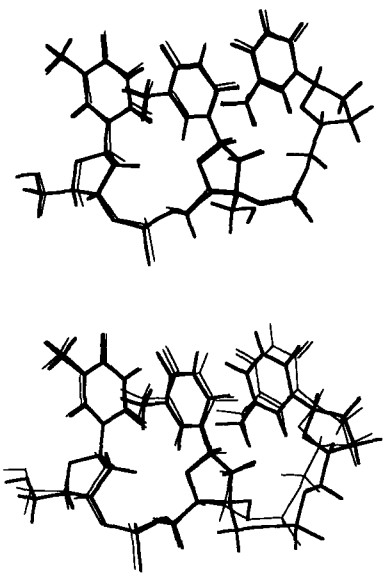
Synthesis of modified oligodeoxynucleotide (ODN) analogues has become an area of intense research[1] because of the potential to sequence specifically inhibit gene expression through high-affinity recognition of single or double stranded nucleic acids.[2-5] *In vivo* applications of ODNs depend on the introduction of chemical modifications in one or more nucleotide constituents in order to e.g. increase the hybridization properties, nuclease resistance and cellular uptake of the ODNs. We[6-8] and others[9-16] have developed ODN analogues containing carbohydrate modified nucleotides thus eliminating the problem of highly heterogeneous oligomers often encountered when synthesizing ODNs with modifications in the phosphate moiety (e.g. phosphorothioates,[17] phosphoramidates,[18,19] and methylphosphonates[20,21]) caused by introduction of uncontrolled chirality at phosphorus.

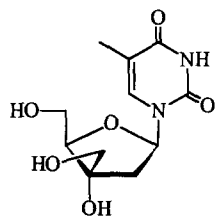
As an attempt to enhance the biological potential of carbohydrate modified ODNs, we have initiated a program aiming at design and synthesis of monomeric nucleotides containing extra functionalities which may prove useful as attachment sites for intercalating agents, lipophilic carriers, or additional ODN strands. As the first example of this class of ODNs, we decided to incorporate and evaluate 2'-deoxy-3'-*C*-hydroxymethyl

nucleosides, and Figure 1 shows the results from molecular modelling[22] studies on trinucleotides (T_3) containing 3'-*C*-(hydroxymethyl)thymidine (**1**)[7] in the middle. It is evident from superimposing the unmodified T_3 on the energy minimized modified trimers, that 5'-hydroxyl to 3'-hydroxyl linked 3'-*C*-(hydroxymethyl)thymidine (Figure 1, entry a, **X**) constitutes an interesting nucleotide substitute as a close fit between the geometries of two trimers is obtained. Although energy minimization of the trimer containing **1** incorporated through 5'-hydroxyl to 3'-*C*-hydroxymethyl (Figure 1, entry b, **Y**) results in a geometry less identical to that of the unmodified trimer, the possibility of efficient hydrogen bonding to complementary nucleic acids seems retained. This prompted us to incorporate 3'-*C*-(hydroxymethyl)thymidine (**1**) in two structurally distinct ways into ODNs. This paper describes the synthesis of the nucleoside phosphoramidite derivatives **4** and **6** and their incorporation into ODN analogues **E-H** and **I-L** containing extended backbones (monomers **Y** and **Z**). These novel ODNs are compared with regard to hybridisation properties and 3'-exonucleolytic stability to 3'-*C*-hydroxymethyl-ODNs containing unmodified backbones (monomers **X**).[7]

a) 5'-TXT-3'

b) 5'-TYT-3'



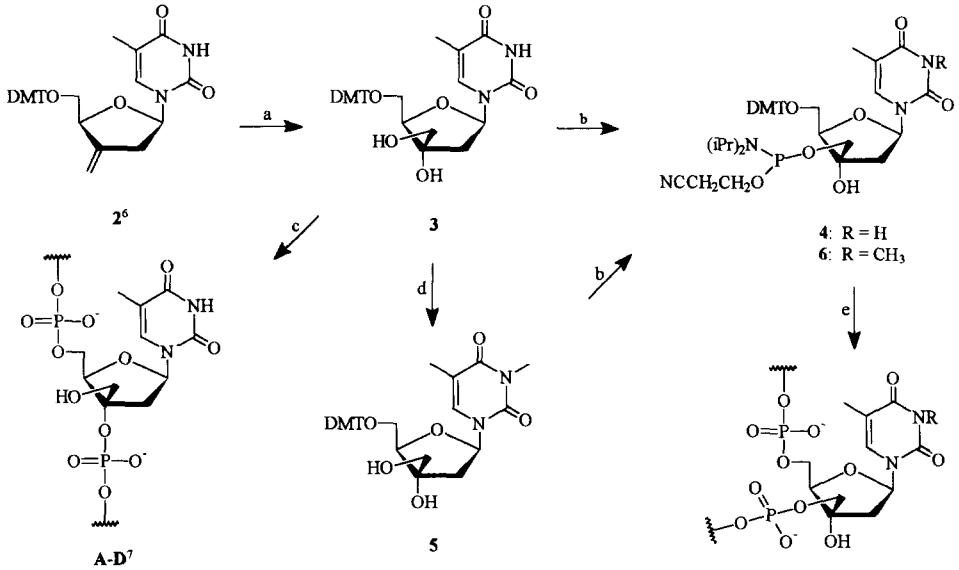


1[7]

Figure 1. Superimposition of energy minimized thymidine trinucleotides (faint line) on modified trimers containing 3'-*C*-(hydroxymethyl)thymidine (**1**) in the middle a) through 5'-hydroxyl to 3'-hydroxyl (bold) and b) through 5'-hydroxyl to 3'-*C*-hydroxymethyl (bold). **X** = nucleotide with 5'-hydroxyl to 3'-hydroxyl backbone, **Y** = nucleotide with 5'-hydroxyl to 3'-*C*-hydroxymethyl backbone

RESULTS AND DISCUSSION

5'-*O*-(4,4'-Dimethoxytrityl)-3'-*C*-(hydroxymethyl)thymidine (**3**) was obtained from 3'-*C*-methylene nucleoside **2**[6] in 70 % yield by stereoselective catalytic osmium tetroxide oxidation of **2** in basic aqueous *tert*-butanol using *N*-methylmorpholine *N*-oxide as co-oxidant. *N*-Methylmorpholine *N*-oxide is reported to be preferable compared to a variety of other known co-oxidants by avoiding overoxidation,[23-26] thus preventing the formation of keto or acid byproducts. No product originating from dihydroxylation in the thymine base was detected. The β-D-*erythro*-configuration of **3** was unambiguously established by a ^{1}H NOE difference experiment. Especially the key NOE contact between 3'-*C*-CH_2 and H-5' confirmed the positioning of the 3'-*C*-substituent at the β-face of the pentofuranose ring. Phosphitylation of **3** is possible at the 3'-*C*-hydroxyl as well as the 3'-*C*-hydroxymethyl group allowing the incorporation of this modified nucleoside in several ways into ODNs. We have earlier reported on the synthesis of ODNs containing 3'-*C*-(hydroxymethyl)thymidine (**1**) with an unmodified backbone using a 3'-*C*-hydroxymethyl protected 3'-*O*-phosphoramidite derivative.[7]



a) OsO_4, *N*-methylmorpholine *N*-oxide, pyridine, H_2O, *tert*-butanol; b) $NCCH_2CH_2OP(Cl)N(iPr)_2$, *N,N*-diisopropylethylamine, CH_2Cl_2; c) Ref. 7; d) CH_3I, BDDDP, CH_3CN; e) DNA-synthesizer

Scheme 1

Phosphitylation[27,28] of **3** afforded stereoselectively in 74 % yield the nucleoside phosphoramidite **4**, suitable for incorporation through the 5'-hydroxyl to 3'-*C*-hydroxymethyl. The stereoselective phosphitylation of the primary hydroxyl group of **3** was confirmed by the measured ^{31}P NMR shifts at 149.2 and 149.4 ppm which is approximately 10 ppm downfield to ^{31}P NMR shifts obtained for tertiary phosphoramidites.[7] Additionally, as the melting point (Table 1) measured e.g. for oligomer **E** differs from the one mesured for oligomer **A**, the position of phosphitylation of **3** is unambigously established as the primary hydroxyl group. As an attempt to verify the hybridization properties of 3'-*C*-(hydroxymethyl)thymidine incorporated using synthon **4**, the N^3-analogue **6** was synthesized. Introduction of a N^3-methyl group at the thymine base reduces the ability of the nucleobase to form hydrogen bonds with the complementary adenine.[29] Nucleoside **3** was reacted with methyl iodide in the presence of the organic base 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BDDDP) following a reported method[30] to give the N^3-methylated nucleoside **5** in 81 % yield after column chromatographic purification. The nucleoside phosphoramidite **6** was subsequently obtained in 98 % yield. (Scheme 1).

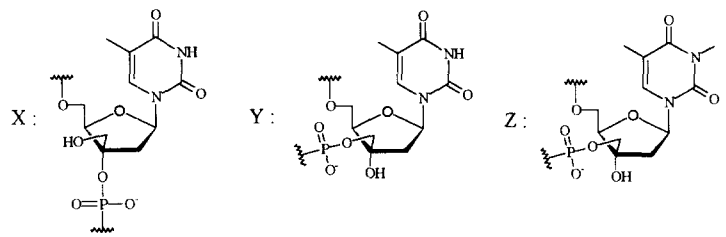
Synthesis of oligomers **E-N** (Table 1) was performed by use of standard phosphoramidite methodology on an automated DNA-synthesizer using the modified phosphoramidites **4** and **6** and commercial 2'-deoxynucleoside-β-cyanoethylphosphoramidites. The coupling efficiency of the modified phosphoramidites were approximately 70 % (12 min couplings) compared to approximately 99 % for standard phosphoramidites (2 min couplings). The presence of an unprotectected tertiary 3'-hydroxyl group during incorporation of amidites **4** and **6** does not impede the condensation on the solid support. We confirmed this by solution phase experiments with tetrazole activation of acetonitrile solutions of **4** and **6** resulting in no detectable self-condensation after 1 h. This regioselective phosphitylation and ODN-synthesis corresponds to a strategy applied earlier for synthesis of oligoarabinonucleotides.[31] The dimethoxytrityl protected ODNs were removed from the solid support by treatment with concentrated ammonia at 20 °C for 48 h, which also removed the phosphate and nucleobase protecting groups. Purification using disposable reverse phase chromatography cartridges (which includes detritylation), desilylation and desalting afforded the unprotected oligomers **E-N**. The purity of the modified ODNs was confirmed by reveres phase HPLC analysis.

The composition of the ODNs was verified by matrix assisted laser desorption mass spectrometry, which has proven useful as an analytical tool for mass analysis of ODNs.[6,32] The measured masses of oligomers **G** containing **Y** and **K** containing **Z** (5100.4 and 5115.4 Da, respectively) correspond with the calculated (5102.4 and 5115.7 Da, respectively), and we therefore conclude that the modified nucleotides have been incorporated in **G** and **K** as contemplated. Because of the homogeneous results from the syntheses of all the modified ODNs we hereby consider their composition verified.

Table 1. Sequences synthesized, hybridization data and enzymatic stability

Sequence[a]		T_m(°C)[b]	ΔT_m(°C)[c]	$t_{1/2}$(sec)[d]
5'-(CACCAAC**X**TCTTCCACA)-3'	(**A**)[7]	60.0	0.0	50
5'-(CACCAAC**X**TCT**X**CCACA)-3'	(**B**)[7]	59.5	-0.5	100
5'-(TTAACTTCTTCACAT**X**C)-3'	(**C**)[7]	50.0	-2.0	200
5'-(TTAACTTCTTCACA**XX**C)-3'	(**D**)[7]	48.0	-2.0	400
5'-(CACCAAC**Y**TCTTCCACA)-3'	(**E**)	56.5	-3.5	30
5'-(CACCAAC**YYCY**TCCACA)-3'	(**F**)	45.0	-5.0	60
5'-(TTAACTTCTTCACAT**Y**C)-3'	(**G**)	49.5	-2.5	200
5'-(TTAACTTCTTCACA**YY**C)-3'	(**H**)	46.0	-3.0	300
5'-(CACCAAC**Z**TCTTCCACA)-3'	(**I**)	43.5	-16.5	-
5'-(CACCAAC**Z**TCT**Z**CCACA)-3'	(**J**)	33.5	-13.3	-
5'-(TTAACTTCTTCACAT**Z**C)-3'	(**K**)	46.0	-6.0	300
5'-(TTAACTTCTTCACA**ZZ**C)-3'	(**L**)	41.5	-5.3	>500
5'-(CACCAACTTCTTCCACA)-3'	(**M**)	60.0	-	40
5'-(TTAACTTCTTCACATTC)-3'	(**N**)	52.0	-	80

[a] A = 2'-deoxyadenosine, C = 2'-deoxycytidine, G = 2'-deoxyguanosine, T = thymidine, [b] T_m = melting temperature.
[c] ΔT_m= change in T_m per modification compared to unmodified ODNs. [d] $t_{1/2}$ = hyperchromicity half-life



Incorporation of the building block **4** results in a decrease in T_m of 3.5 and 15 °C when incorporated one and three times (**E**, **F**) in the middle of a 17-mer, respectively, whereas less pronounced destabilizations are observed for the 3'-end modified ODNs **G** and **H** (ΔT_m ~ -3 °C/**6** incorporated). The results obtained for **4** are similar to those obtained earlier for the corresponding 2',3'-dideoxy-3'-*C*-hydroxymethyl nucleotide modification.[6] Incorporation of 3'-*C*-(hydroxymethyl)thymidine with an unmodified backbone (5'-hydroxyl to 3'-hydroxyl) one or two times in the middle of a 17-mer (**A**,**B**) induces no destabilization of the duplexes formed with complementary DNA, whereas incorporation one or two times in the 3'-end (**C**,**D**) causes a minor destabilization (ΔT_m = -2 °C).[7] It is thus evident, that with regard to thermal stability the most promising way of incorporating 3'-*C*-(hydroxymethyl)thymidine into ODNs is through 5'-hydroxyl to 3'-hydroxyl (oligomers **A-D**) as shown clearly by the melting temperatures of oligomers **A** and **B**. As expected, a large destabilization is observed after

incorporation of the N^3-methylated nucleotide **Z** in the 3'-end or in the middle of 17-mers (**I-L**). Comparison of the results from incorporation of **4** and **6** indicates, that the nucleotide **Y** in oligomers **E-H** retain the ability to hybridize with complementary adenine bases.

As it has been reported that 3'-phosphodiesterase activity is the major cause of degradation of unmodified oligonucleotides *in vivo*,[33,34] the enzymatic stability of the modified ODNs was tested towards Snake Venom Phosphodiesterase (3'-exonuclease). The increase in absorbance (hyperchromity) at 260 nm was followed[6,8,13,35] during digestion and the half-life of the full-length oligomer estimated (Table 1). Incorporation of 3'-*C*-(hydroxymethyl)thymidine (**1**) through the 5'-hydroxyl and 3'-*C*-hydroxymethyl groups (**Y**) one or two times in the middle of a 17-mer (**E, F**) has no apparent effect on the enzymatic stability of the oligomers, while two 3'-end (**H**) substitutions result in a four-fold increase in half-life. Analogously, incorporation of **X** two times in the 3'-end (**D**) results in a three-fold increase in half-life.[7] Concerning effect on enzymatic stability, introduction of **Z** followed a pattern similar to **Y** indicating that the N^3-methyl group has no effect on the enzymatic stability.

CONCLUSION

In conclusion, a generally applicable stereoselective dihydroxylation of 3'-*C*-methylene nucleoside **2** has been accomplished. Incorporation of 3'-*C*-(hydroxymethyl)thymidine (**1**) into ODNs (**E-H**) through an extended backbone (**Y**, 5'-hydroxyl to 3'-*C*-hydroxymethyl) has been achieved using the primary phosphoramidite **4** containing an unprotected 3'-hydroxyl group. Although minor decreases in thermal stability are observed upon incorporation of **Y**, results from the corresponding N^3-methylated ODNs (**I-L**) support the existence of Watson-Crick basepairing between modified monomer **Y** and complementary adenine bases in duplexes between **E-H** and complementary ODNs. Comparison between ODNs containing 3'-C-(hydroxymethyl)thymidine (**1**) with an unmodified backbone (**X**, 5'-hydroxyl to 3'-hydroxyl)[7] and ODNs with an extended backbone (**Y**) reveals, that concerning duplex stability the former seems to be the more promising. However, the modification (**Y**) reported here contains an unprotected 3'-hydroxyl group which by virtue of its low reactivity during phosphitylation and standard automated DNA-synthesis may prove useful as an post-synthetic attachment site for reactive agents to be oriented into the minor groove of duplex DNA.

EXPERIMENTAL

NMR spectra were recorded at 250 MHz for ^{1}H NMR and 62.9 MHz for ^{13}C NMR on a Bruker AC-250 spectrometer, and at 500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR and 202.3 MHz for ^{31}P NMR on a Varian Unity 500 spectrometer. Chemical shifts are in ppm relative to tetramethylsilane as internal standard (^{1}H NMR and ^{13}C NMR) and relative to 85% H_3PO_4 as external standard (^{31}P NMR). EI Mass spectra were recorded on a Varian Mat 311A Spectrometer. The silica gel (0.040-0.063 mm) used for column chromatography was purchased from Merck. ODNs were synthesized on a Pharmacia Gene Assemble Special® DNA-Synthesizer.

Purification of 5'-*O*-DMT-ON ODNs was accomplished using disposable Oligopurification Cartridges (COP, Cruachem) and desalting using NAP-10^{R} columns (Pharmacia). Snake Venom Phosphodiesterase (*Crotalus Adamanteus*) was obtained from Pharmacia. Matrix assisted laser desorption mass spectra were obtained on a prototype laser desorption mass spectrometer from Applied Biosystem Sweden AB, Uppsala, Sweden. Analytical reverse phase HPLC (C-18 column) was performed on a Waters Delta Prep 3000 Preparative Chromatography System. Melting profiles were obtained on a Perkin-Elmer UV/VIS spectrometer fitted with a PTP-6 Peltiér temperature programming element.

5'-*O*-(4,4'-Dimethoxytrityl)-3'-*C*-(hydroxymethyl)thymidine (3)

To a mixture of nucleoside **2**[6] (1.2 g; 2.22 mmol) in *tert*-butanol (20 mL) was added *N*-methylmorpholine *N*-oxide (1.8 g; 15.38 mmol), pyridine (1 mL), H_2O (1.3 mL) and osmium tetroxide (100 µl of a 2.5 % solution in *tert*-butanol, 8.0 µmol). The reaction was stirred under argon at 76 °C for 6 h, cooled to 25 °C and treated with 20 % aqueous sodium bisulfite (2.5 mL). The mixture was concentrated to dryness under reduced pressure, diluted with saturated aqueous NaCl (5 mL) and extracted with EtOAc (3 X 20 mL). The combined organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. Purification using silica gel column chromatography (0-2.5 % CH_3OH, 0.5 % triethylamine in CH_2Cl_2, v/v/v) afforded **3** as a white solid. Yield 892 mg (70 %). ^{13}C NMR ($CDCl_3$) δ: 11.47 (CH_3), 41.73 (C-2'), 55.16 (2 × OCH_3), 62.30 (C-5'), 64.89 (CH_2OH), 81.25 (C-3'), 84.05 (C-1'), 86.16 (C-4'), 87.49 (CAr_3), 111.37 (C-5), 113.27, 127.26, 127.99, 128.19, 130.12, 134.74, 143.72, 158.77 (Ar), 135.91 (C-6), 150.81 (C-2), 164.12 (C-4). ^1H NMR ($CDCl_3$) δ: 1.33 (s, 3H, CH_3), 2.11 (dd, 1H, J = 12.9, 9.3 Hz, H-2'β), 2.41 (dd, 1H, J = 12.9, 5.2 Hz, H-2'α), 3.25 (dd, 1H, J = 10.8, 2.4 Hz, H-5'a), 3.56 (dd, 1H, J = 10.8, 4.6 Hz, H-5'b), 3.57 (d, 1H, J = 11.3 Hz, CH_2a), 3.67 (d, 1H, J = 11.3 Hz, CH_2b), 3.76 (s, 6H, 2 × OCH_3), 4.13 (dd, 1H, J = 4.6, 2.4 Hz, H-4'), 6.48 (dd, 1H, J = 9.2, 5.2 Hz, H-1'), 6.83-6.88 (m, 4H, Ar), 7.21-7.39 (m, 9H, Ar), 7.70 (s, 1H, H-6), 9.80 (s, 1H, NH). HRMS: calcd. 574.2315; found 574.2299.

3'-*C*-(*O*-(2-Cyanoethoxy(diisopropylaminophosphino))hydroxymethyl)-5'-*O*-(4,4'-dimethoxytrityl)thymidine(4)

Nucleoside **3** (150 mg; 0.26 mmol) was dried by coevaporation with dry CH_3CN (3 X 2 mL) and dissolved under argon in anhydrous CH_2Cl_2 (1.5 mL). *N,N*-Diisopropylethylamine (0.23 mL) was added followed by dropwise addition of 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.10 mL; 0,57 mmol). After 1 h, CH_3OH (0.06 mL) was added and the reaction mixture was diluted with EtOAc (10 mL) containing triethylamine (0.1 mL), washed with saturated aqueous solutions of $NaHCO_3$ (3 X 5 mL) and NaCl (3 X 5 mL), dried (Na_2SO_4) and evaporated under reduced pressure. Purification using silica gel column chromatography (EtOAc/CH_2Cl_2/Et_3N, 45:45:10, v/v/v) followed by precipitation in petroleum ether (250 mL) at -20 °C (after redissolution in anhydrous toluene (1.5 mL)) afforded **6** as a white solid. Yield 150 mg (74 %). ^{31}P NMR ($CDCl_3$) δ: 149.2, 149.4.

5'-*O*-(4,4'-Dimethoxytrityl)-3'-*C*-hydroxymethyl-N^3-methylthymidine (5)

To a solution of nucleoside **3** (199 mg; 0.35 mmol) in anhydrous CH_3CN (1.2 mL) under argon at 0 °C was added BDDDP (100 µL; 345 nmol) followed immediately by CH_3I (20 µL; 0.32 mmol), and the reaction mixture was stirred for 1 h at 0 °C. After stirring at r.t. for 24 h, additional BDDDP (20µL, 69 nmol) and CH_3I (4 µL, 0.06 mmol) was added and stirring was continued for 5 h. CH_3OH (1 mL) was added and the mixture concentrated to dryness. The insoluble hydroiodide salt of BDDDP was precipitated in EtOAc (3 mL) and filtered off. Evaporation of the organic phase and precipitation of the salt was repeated three times. Purification using silica gel column chromatography (0-3 % CH_3OH, 0.5 % pyridine in CH_2Cl_2, v/v/v) afforded **7** as a white solid. Yield 203 mg (81 %). ^{13}C NMR ($CDCl_3$) δ: 12.42 (CH_3), 27.78 (N^3CH_3), 41.47 (C-2'), 55.12 (2 × OCH_3), 62.39 (C-5'), 64.86 (CH_2OH), 81.04 (C-3'), 84.73 (C-1'), 86.00 (C-4'), 87.61 (CAr_3), 110.19 (C-5), 113.27, 127.21, 127.98, 129.73, 129.93, 134.69, 143.61, 158.76 (Ar), 133.41 (C-6), 151.04 (C-2), 163.55 (C-4). ^1H NMR ($CDCl_3$) δ: 1.49 (s, 3H, CH_3), 2.02 (dd, 1H, J = 13.1, 9.1 Hz, H-2'β), 2.37 (dd, 1H, J = 13.1, 5.3 Hz, H-2'α), 3.32 (s, 3H, N^3CH_3), 3.27 (dd, 1H, J = 10.7, 2.5 Hz, H-5'a), 3.50 (dd, 1H, J = 10.7, 5.1 Hz, H-5'b), 3.61-3.66 (m, 2H, CH_2a, CH_2b), 3.78 (s, 6H, 2 × OCH_3), 4.12 (dd, 1H, J = 5.1, 2.5 Hz, H-4'), 6.46 (dd, 1H, J = 9.1, 5.3 Hz, H-1'), 6.84-6.90 (m, 4H, Ar), 7.36-7.42 (m, 9H, Ar), 7.57 (s, 1H, H-6).

3'-*C*-(*O*-(2-Cyanoethoxy(diisopropylaminophosphino))hydroxymethyl)-5'-*O*-(4,4'-dimethoxytrityl)-N^3-methylthymidine (6)

Nucleoside 7 (106 mg; 0.18 mmol) was dried by coevaporation with dry CH_3CN (3 X 2 mL) and dissolved under argon in anhydrous CH_2Cl_2 (1 mL). *N,N*-Diisopropylethylamine (0.16 mL) was added followed by dropwise addition of 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.20 mL; 0.114 mmol). After 5 h, CH_3OH (0.5 mL) was added and the reaction mixture diluted with EtOAc (3.5 mL) containing triethylamine (0.1 mL), washed with saturated aqueous solutions of $NaHCO_3$ (3 X 5 mL) and NaCl (3 X 5 mL), dried (Na_2SO_4) and evaporated under reduced pressure. Purification using silica gel column chromatography (EtOAc/CH_2Cl_2/Et_3N, 45:45:10, v/v/v) followed by precipitation in petroleum ether (250 mL) at -20 °C (after redissolution in anhydrous toluene (0.5 mL)) afforded **8** as a white solid. Yield 140 mg (98 %). ^{31}P NMR ($CDCl_3$) δ: 148.8, 149.2.

Synthesis of modified oligodeoxynucleotides

The synthesis of ODNs (**E-N**) was carried out in 0.2 µmol scale (5 µmol amidite per cycle, Pharmacia Primer Support™) using **4**, **6** and commercial β-cyanoethylphosphoramidites. The regular protocol of the DNA-synthezier for β-cyanoethylphosphoramidites was followed with an increase in coupling time from 2 to 12 min for the modified phosphoramidites. The ODNs were removed from the support and deblocked by treatment with concentrated ammonia at room temperature for 48 h. Purification and detritylation was achieved on Cruachem oligonucleotide purification cartridges using the standard procedure. The purity of the modified ODNs was confirmed by analytical reverse phase HPLC. The solvent systems consisting of 0.1 M NH_4HCO_3 + 5 %

CH_3CN, pH = 9.0 (A) and 0.1 M NH_4HCO_3 + 81 % CH_3CN, pH = 9.0 (B) were used in the following order: 5 min 100 % A, 40 min linear gradient of 0-100 % B in A, 5 min 100 % B, 1 min 100-0 % B in A, 9 min 100 % A. Flow rate 1.0 ml/min The purified ODNs eluted as one peak after approximately 16 min.

Melting experiments

The melting experiments were carried out in medium salt buffer, 1 mM EDTA, 10 mM Na_2HPO_4, 140 mM NaCl, pH 7.2. The increase in absorbance at 260 nm as a function of time was recorded while the temperature was raised from 10 to 80 °C with a rate of 1 °C per min. The melting temperatures were determined as the maximum of the first derivative plots of the 260 nm transitions.

Enzymatic stability

A solution of the ODNs (0.2 - 0.3 OD) in 2 mL of the following buffer (0.1 M Tris.HCl; pH 8.6; 0.1 M NaCl; 14 mM $MgCl_2$) was digested with 1.2 U Snake Venom Phosphodiesterase (SV PDE) (34 µl of a solution of the enzyme in the following buffer: 5 mM Tris.HCl; pH 7.5; 50 % glycerol (v/v)) at 25 °C. The increase in absorbance at 260 nm was followed for a period of 20 min. The absorption versus time curve of the digestion was plotted, and the half-life ($t_{1/2}$) of the full-length oligomer estimated.

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