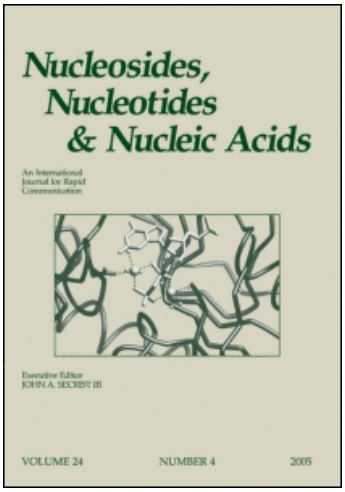

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Synthesis of Novel 3'-*C*-(Hydroxymethyl)thymidines and Oligodeoxynucleotide Analogues Containing Compressed 3'-*C*-Hydroxymethyl-Linked Phosphodiester Backbones

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SYNTHESIS OF NOVEL 3'-*C*-(HYDROXYMETHYL)THYMIDINES AND OLIGODEOXYNUCLEOTIDE ANALOGUES CONTAINING COMPRESSED 3'-*C*-HYDROXYMETHYL-LINKED PHOSPHODIESTER BACKBONES

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Abstract: Lombardo methylenation of the novel 2'-deoxy-3'-ketonucleosides **4** afforded 2',3'-dideoxy-3'-*C*-methylene nucleosides **5**, which were subjected to catalytic dihydroxylation reactions. In the case of 5'-deoxynucleoside **5a**, a 1:1 mixture of 3'-*C*-hydroxymethyl diastereoisomers **6a** and **7a** was obtained, whereas the 5'-*O*-silylated nucleoside **5b** afforded 3'-*C*-(hydroxymethyl)thymidine derivative **6b** as the only product. Sharpless asymmetric dihydroxylation of **5a** proceeded in low yield to give **6a** and **7a** as a 10:3 mixture. 5'-*O*-Silylated nucleoside **6b** was converted into the phosphoramidite synthon **9**, which was applied in automated syntheses of oligodeoxynucleotides containing novel compressed 3'-*C*-hydroxymethyl-linked phosphodiester backbones.

INTRODUCTION

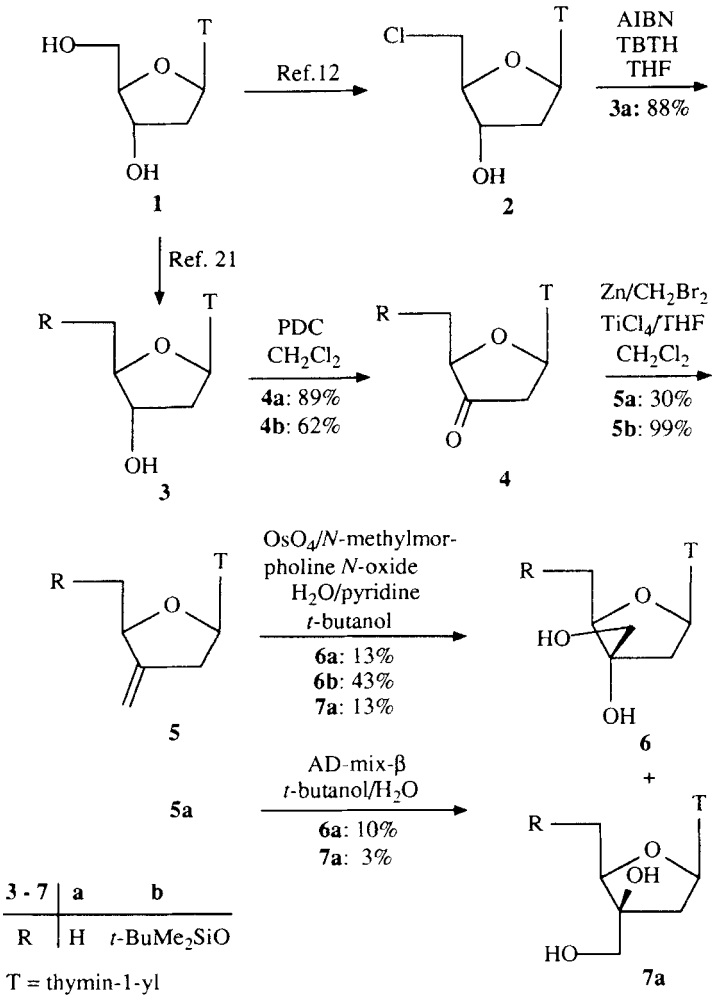
Since the discovery of nucleosides as promising antiviral and anticancer agents, considerable effort has been devoted towards developing novel analogues and improved synthetic methods. Recently, the inherent toxicity of anti-HIV active dideoxynucleosides has further emphasized the need for new derivatives. Although 3'-*C*-branched nucleoside derivatives, such as oxetanocin A,[1] 2',3'-dideoxy-3'-*C*-(hydroxymethyl)adenosine[2] and 2',3'-dideoxy-3'-(hydroxymethyl)cytidine,[3] have been reported to exhibit antiviral activity, 3'-*C*-(hydroxymethyl)thymidine[4] is the only 2'-deoxy-3'-*C*-hydroxymethyl pentofuranose

nucleoside synthesized so far. Therefore, as an attempt to develop a new class of potential biologically active nucleosides, we herein describe the synthesis of the novel 5'-deoxy- and 5'-*O*-silylated 3'-*C*-(hydroxymethyl)thymidines **6** and **7** in only three steps starting from the parent thymidines **3**.

Antisense or antigene oligonucleotides are other classes of nucleic acid compounds with potential antiviral or anticancer activity. Since unmodified DNA or RNA segments are rapidly digested by nucleases *in vivo*, therapeutically useful oligonucleotide analogues have to be chemically modified, and a growing number of nucleic acid surrogates are being synthesized and evaluated for e.g. nuclease resistance and hybridization properties.[5-8] As part of our ongoing research program for synthesis and probing of *C*-branched hydroxymethyl oligodeoxynucleotide analogues as potential antisense agents,[4,9,10] we here report the use of the 5'-*O*-silylated 3'-*C*-(4,4'-dimethoxytrityl)oxymethyl phosphoramidite **9** for incorporation of 3'-*C*-hydroxymethyl-linked monomers containing a 5'-hydroxyl functionality in the middle and in the 3'-end of oligodeoxynucleotide 17-mers. The hybridization properties of the oligomers were examined by UV experiments.

RESULTS AND DISCUSSION

We tried several approaches for the 5'-deoxygenation of thymidine (**1**) including free-radical reduction of 5'-*O*-(phenoxythiocarbonyl)thymidine reported[11] to afford 5'-deoxythymidine (**3a**) in 71 % crude yield as a mixture with the corresponding 5',2-anhydro derivative. However, superior to this in our hands was deoxygenation of 5'-chloro-5'-deoxythymidine (**2**)[12] using tri-*n*-butyltin hydride (TBTH) and α,α'-azoisobutyronitrile (AIBN) to give **3a** in 88 % yield (Scheme 1). The conversion of **3a** to 3'-*C*-methylene derivative **5a** was performed as reported earlier for the corresponding 5'-dimethoxytrityloxy nucleoside.[4] Thus, oxidation of **3a** to the unstable 5'-deoxy-3'-ketothymidine (**4a**) was accomplished in 89 % yield by reaction with pyridinium dichromate (PDC) in the presence of 3A molecular sieve powder.[13] The known base lability of 2'-deoxy-3'-ketonucleosides[13,14] precludes Wittig reactions on these molecules, but Lombardo methylenation[15] afforded in 30 % yield the expected 3',5'-dideoxy-3'-*C*-methylenethymidine (**5a**) after column chromatographic purification. 5'-Deoxy-3'-*C*-(hydroxymethyl)-thymidine (**6a**) and the corresponding 3'-*C*-epimer **7a** were both obtained in 13 % yield



Scheme 1

by osmium tetroxide catalyzed dihydroxylation of **5a** in the presence of *N*-methylmorpholine *N*-oxide as co-oxidant[16,17] (Scheme 1). As a 1:1 mixture of diastereoisomers was obtained, the catalytic dihydroxylation was non-stereoselective contrary to the similar reaction involving the corresponding 5'-*O*-dimethoxytrityl-3'-*C*-methylene nucleoside as substrate.[4] As an attempt to control the stereochemistry of the introduction of the diol functionality, we applied the Sharpless asymmetric dihydroxylation reaction[18] on 5'-deoxy-3'-*C*-methylenethymidine (**5a**). The reaction was very slow at room temperature despite

the use of a large excess of the commercially available reagent. After 10 days' reaction, the expected epimer **6a** was isolated in only 10 % yield and the corresponding diastereoisomer **7a** in 3 % yield. Thus, although improving the stereoselectivity of the dihydroxylation, the usefulness of the Sharpless asymmetric dihydroxylation reaction on this substrate is probably hampered by steric problems resulting in low yields and slow conversion. In neither case, we observed nor isolated products originating from dihydroxylation in the nucleobase which has recently been reported for 3',5'-di-*O*-silylated thymidine.[19] Consequently, for both the catalytic and for the Sharpless dihydroxylation reactions, only unreacted starting material (nucleosides **5**) and 3'-*C*-hydroxymethyl products (**6** and **7a**) could be detected according to analytical TLC (10 % CH_3OH in CH_2Cl_2, heating with sulfuric acid). Earlier, we achieved analogous selectivity during dihydroxylation of the corresponding 5'-*O*-DMT-protected 3'-methylene nucleoside.[4] The different susceptibility of the thymine nucleobase towards dihydroxylation, observed by us and by Barvian and Greenberg,[19] can be explained by deviations in the reaction conditions used. Thus, for catalytic dihydroxylation, we applied a nucleoside:OsO_4 molar-ratio of 225 whereas Barvian and Greenberg applied a nucleoside:OsO_4 molar-ratio of only 27. For the Sharpless dihydroxylation of nucleoside **5a**, we used commercially available AD-mix-β; as Barvian and Greenberg were unsuccessful when trying to dihydroxylate the nucleobase with the commercial mixture, they needed to prepare an asymmetric dihydroxylation reagent containing additional osmium and ligand.[19] It is therefore obvious that the selective dihydroxylations obtained by us originate from the sluggishness by which the nucleobase reacts, compared to the 3'-methylene derivatized pentofuranose ring. Sterical hindrance around the nucleobase C_5-C_6 bond (especially in the preferred *anti*-conformation) and electronic factors (electron withdrawal by HNC_4=O) may contribute to this effect.

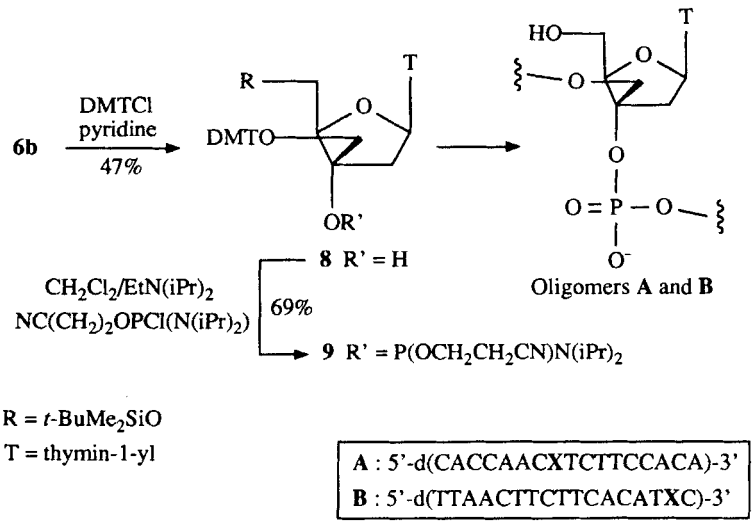
Epimers **6a** and **7a** were separated by use of preparative HPLC and their structures assigned on the basis of ^1H NOE experiments. Especially, the strong NOE's observed between 3'-*C*-CH_2O and H-2'β, combined with the absence of or only very weak effects between 3'-*C*-CH_2O and H-2'α or H-1', prove the positioning of the 3'-*C*-hydroxymethyl substituent at the β-face of the pentofuranose ring. The possible effect between 3'-*C*-CH_2O and H-6 was not observed, which may indicate that **6a** mainly exists in a $C_{2'}$-*endo* like conformation, as earlier reported for a β-D-*threo*-configurated 3'-deoxy-3'-*C*-hydroxy-

methyl nucleoside.[20] The opposite configuration at C-3' for nucleoside **7a** was confirmed by strong NOE effects between 3'-*C*-CH_2O and H-2'α and H-4'.

5'-*O*-(t-Butyldimethylsilyl)thymidine (**3b**)[21] was transformed into 5'-*O*-(t-butyldimethylsilyl)-3'-deoxy-3'-*C*-methylenethymidine (**5b**) by the same synthetic route as described for **5a** (Scheme 1). Thus, **3b** was oxidized in 62 % yield to 3'-ketonucleoside **4b** which was subsequently methylenated in quantitative yield to give **5b**. The catalytic dihydroxylation reaction on **5b** afforded 5'-*O*-(*t*-butyldimethylsilyl)-3'-*C*-(hydroxymethyl)-thymidine (**6b**) in 43 % yield as the only product after column chromatographic purification. The stereoselectivity of this reaction may be explained by the presence of the 5'-*O*-silyl protecting group forcing the osmium tetroxide to attack from the α-face of the pentofuranose ring.

The nucleosides **6a** and **7a** did not show any activity at 100 μM against Herpes Simplex Virus type 1 (HSV-1), strain McIntyre, when propagated in a continuous cell line from rabbit cornea (SIRC) which was maintained in Eagle's MEM containing 1 % fetal calf serum and test compounds. The same compounds were also devoid of activity at 100 μM against HIV-1 (strain HTLV-IIIB) in MT-4 cells, when MT-4 cells were incubated with virus, washed and added in a proportion of 1:10 to uninfected MT-4 cells which had been preincubated in test compound containing growth medium for 2 hours. The MT-4 cells were maintained with the culture medium likewise containing the test compound and expression of HIV in culture medium was quantitated by HIV antigen detection ELISA.

For the synthesis of oligodeoxynucleotides **A** and **B** containing one modified 3'-*C*-hydroxymethyl-linked monomer **X** in the 3'-end and in the middle, respectively, we used the phosphoramidite approach.[22] 3'-*C*-Hydroxymethyl nucleoside **6b** was reacted with 4,4'-dimethoxytrityl chloride affording the protected nucleoside **8** in 47 % yield after column chromatographic purification. The configuration of **8** (and hereby also **6b**) was verified by a ^1H NOE experiment showing e.g. strong effects between H-2'β and 3'-*C*-CH_2O. After reaction of **8** with 2-cyanoethyl *N,N*-diisopropylphosphoramido-chloridite in the presence of *N,N*-diisopropylamine,[23,24] the phosphoramidite building block **9** was isolated in 69 % yield after column chromatographic purification and precipitation from petroleum ether (Scheme 2). During automated DNA synthesis, the coupling efficiency for **9** was approximately 40 % as monitored by the release of the



Scheme 2

dimethoxytrityl cation after each coupling step compared to approximately 99 % when using commercial unmodified phosphoramidites. The low coupling yield obtained with **9** is probably due to the tertiary nature of the phosphitylated hydroxyl group and steric hindrance from the dimethoxytrityl group. As an attempt to minimize this problem, the phosphoramidite **9** was dissolved in the activator solution (0.45 M tetrazole in acetonitrile) when applied on the DNA synthesizer to ensure optimum activation. The dimethoxytrityl protected oligodeoxynucleotides were removed from the solid support by treatment with concentrated ammonia at 20 °C for 72 h, which also removes the phosphate and nucleobase protecting groups. Subsequent purification by use of disposable reversed phase chromatography cartridges (which includes detritylation), desilylation and desalting followed standard protocols.[25] Oligodeoxynucleotides **A** and **B** displayed only one peak in analytical HPLC experiments, and the composition of oligomer **A** was confirmed by matrix assisted laser desorption mass spectrometry[9,26] (mass calculated: 5965.3 Da; mass found: 5066.2 Da).

Preliminary evaluation of the hybridization properties of oligodeoxynucleotides **A** and **B** by UV melting point (T_m) measurements indicates that incorporation of the modified monomer containing compressed 3'-*C*-hydroxymethyl to 3'-hydroxyl backbones

once in the middle (**A**) or in the 3'-end (**B**) of 17-mers significantly destabilizes the duplex formed with complementary DNA, compared to the unmodified controls (decrease observed in T_m = 14 and 4 °C, respectively). More promising hybridization results were obtained with the corresponding 3'-*C*-hydroxymethyl substituted oligodeoxynucleotides containing unmodified 5'-hydroxyl to 3'-hydroxyl backbones as these analogues exhibited unaltered melting points compared to the corresponding unmodified oligomers in duplexes with complementary DNA.[4]

In conclusion, syntheses of novel 2'-deoxy-3'-*C*-hydroxymethyl thymine nucleosides have been accomplished in only three steps, starting from the parent 2'-deoxynucleosides. Two novel oligodeoxynucleotide analogues containing compressed 3'-*C*-hydroxymethyl-linked phosphodiester backbones and an additional 5'-hydroxyl functionality have been synthesized using the phosphoramidite approach.

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 202.3 MHz for ^{31}P NMR on a Varian Unity 500 spectrometer. δ-Values 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. Positive FAB mass spectra were recorded on a Kratos MS 50 RF spectrometer using 8-9 kV xenon atoms. The silica gel (0.040-0.063 mm) used for column chromatography was purchased from Merck. HPLC was performed on a Waters Delta Prep 3000 HPLC system. Disposable purification cartridges (COP) was obtained from Cruachem Inc. Desalting was performed on NAP^{TM}-10 columns from Pharmacia Biotech AS. Melting points were determined on a Perkin-Elmer UV/VIS spectrometer fitted with a PTP-6 Peltiér temperature programming element.

5'-Deoxythymidine[11] (3a)

To a stirred solution of 5'-chloro-5'-deoxythymidine (**2**)[12] (2.00 g, 7.67 mmol) in anhydrous THF (100 mL) was added AIBN (0.52 g, 3.17 mmol) and TBTH (7.63 g, 26.22 mmol). The mixture was refluxed under nitrogen for 18 h and then evaporated to

dryness under reduced pressure. Cold petroleum ether (150 mL) was added, and the crude product was isolated by filtration, washed with cold petroleum ether (200 mL) and dried (Na_2SO_4). Recrystallization from water afforded **3a** as pure white crystals; yield: 0.61 g (35 %). The aqueous phase was evaporated to dryness under reduced pressure to give additional **3a** as a white solid that was used without further purification together with the recrystallized material in the next step; combined yield: 1.52 g (88 %).^{1}H NMR data were identical with earlier reported data.[11] R_f = 0.33 (CH_3OH/CH_2Cl_2, 1:9, v/v). ^{13}C NMR (DMSO-d_6): δ = 11.99 (CH_3), 18.68 (C-5'), 38.36 (C-2'), 74.28 (C-3'), 81.70, 83.29 (C-1', C-4'), 109.60 (C-5), 135.83 (C-6), 150.32 (C-2), 163.58 (C-4). MS (FAB): *m/z* = 227 ($M+1^+$, 100 %).

5'-Deoxy-3'-ketothymidine (4a)

5'-Deoxythymidine (**3a**) (0.85 g, 3.76 mmol) was added in one portion to a stirred suspension of 3A molecular sieve powder (2.80 g) and pyridinium dichromate (2.30 g, 6.11 mmol) in anhydrous CH_2Cl_2 (200 mL, freed from acids by filtration through basic alumina). The inside of the flask was washed with anhydrous CH_2Cl_2 (50 mL), and the mixture was stirred for 6 h at r.t. under nitrogen. The reaction mixture was filtered through 3A molecular sieve powder (deposited as a slurry with anhydrous CH_2Cl_2 on a glass filter), the plug was washed with anhydrous CH_2Cl_2 (100 mL) and the combined filtrates were evaporated under reduced pressure. The residue was suspended in EtOAc (700 mL), sonicated for 5 min and filtered through 3A molecular sieve powder (deposited as a slurry with EtOAc on a glass filter). The plug was washed with EtOAc (300 mL), and the solvent was evaporated under reduced pressure to give **4a** as a solid white material which was used without further purification in the next step; yield: 0.75 g (89 %). R_f = 0.41 (CH_3OH/ CH_2Cl_2, 1:9, v/v). ^{1}H NMR ($CDCl_3$): δ = 1.44 (d, 3H, *J* = 6.7 Hz, H-5'), 1.96 (s, 3H, CH_3), 2.65 (dd, 1H, *J* = 18.8, 6.6 Hz, H-2'β), 3.00 (dd, 1H, *J* = 18.7, 7.1 Hz, H-2'α), 4.13 (q, 1H, *J* = 6.7 Hz, H-4'), 6.19 (t, 1H, *J* = 6.8 Hz, H-1'), 7.16 (s, 1H, H-6), 8.46 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ = 12.43 (CH_3), 15.95 (C-5'), 40.48 (C-2'), 78.08 (C-4'), 82.92 (C-1'), 111.74 (C-5), 135.66 (C-6), 150.42 (C-2), 163.78 (C-4), 210.38 (C-3').

3',5'-Dideoxy-3'-*C*-methylenethymidine (5a)

To a stirred solution of nucleoside **4a** (700 mg, 3.12 mmol) in anhydrous CH_2Cl_2 (80 mL) under nitrogen at 0 °C, were added three portions (at 10 min intervals, 3 × 10 mL) of the slurry reagent prepared from: zinc (dust, 5.80 g, 88.7 mmol), CH_2Br_2 (2.02 mL, 28.8 mmol) and $TiCl_4$ (2.40 mL, 21.9 mmol) in anhydrous THF (50 mL). After additional stirring for 10 min, the ice-bath was removed and the reaction mixture stirred at r.t. for 4 h. The mixture was poured into a mixture of $CHCl_3$ (350 mL) and an ice-cold saturated aqueous solution of $NaHCO_3$ (300 mL), stirred for 10 min and filtered through silica gel (deposited as a slurry with $CHCl_3$ on a glass filter). The separated water phase and the plug were washed with $CHCl_3$ (5 × 50 mL), and the organic phases were combined and dried (Na_2SO_4). After evaporation of the solvent and column chromatographic purification (0.5-2 % CH_3OH in CH_2Cl_2, v/v), **5a** was obtained as an oil; yield: 208 mg (30 %). R_f = 0.49 (CH_3OH/CH_2Cl_2, 1:9, v/v). ^1H NMR ($CDCl_3$): δ = 1.45 (d, 3H, *J* = 6.2 Hz, H-5'), 1.92 (s, 3H, CH_3), 2.58 (dd, 1H, *J* = 16.5, 2.3 Hz, H-2'β), 3.13 (dd, 1H, *J* = 16.7, 6.1 Hz, H-2'α), 4.56 (q, 1H, *J* = 5.4 Hz, H-4'), 4.99 (d, 1H, *J* = 2.2 Hz, CH_2''-a), 5.09 (d, 1H, *J* = 2.1 Hz, CH_2''-b), 6.17-6.21 (m, 1H, H-1'), 7.17 (s, 1H, H-6), 9.65 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ = 12.50 (CH_3), 19.40 (C-5'), 38.50 (C-2'), 77.49 (C-4'), 82.75 (C-1'), 106.74 (CH_2''), 111.02 (C-5), 134.83 (C-6), 147.78 (C-3'), 150.56 (C-2), 163.92 (C-4). MS (EI): *m/z* = 222 (M^+, 3 %). HRMS (EI): *m/z*, $C_{11}H_{14}N_2O_3$ calcd. 222.1004; found 222.1007.

5'-Deoxy-3'-*C*-(hydroxymethyl)thymidine (6a) and 1-(2,5-Dideoxy-3-*C*-hydroxymethyl-β-D-*threo*-pentofuranosyl)thymine (7a)

Method 1: To a mixture of **5a** (200 mg, 0.90 mmol), H_2O (0.60 mL), pyridine (0.60 mL) and *t*-butanol (15 mL) was added *N*-methylmorpholine *N*-oxide (730 mg, 6.23 mmol) and OsO_4 (50 µL of a 2.5 % solution in *t*-butanol, 4.0 µmol). The reaction mixture was stirred at 76 °C for 6 h, cooled to r.t. and treated with 20 % aqueous sodium bisulfite (1.0 mL). The mixture was concentrated under reduced pressure, diluted with H_2O (5 mL) and a saturated aqueous solution of sodium chloride (5 mL) and extracted with EtOAc (3 × 20 mL). The combined organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. Purification by column chromatography (1-3 % CH_3OH in CH_2Cl_2, v/v)

afforded a mixture of isomers **6a** and **7a**, which was separated on reversed phase HPLC (20 % CH_3OH in H_2O, v/v) to give **6a** as a white foam; yield 30 mg (13 %), and **7a** as a white foam; yield 30 mg (13 %). HRMS (EI) on the mixture before HPLC: *m/z*, $C_{11}H_{16}O_5N_2$ calcd. 256.1059; found 256.1049.

6a: HPLC retention time = 23 min. ^1H NMR (CD_3OD): δ = 1.42 (d, 3H, *J* = 6.6 Hz, H-5'), 1.99 (s, 3H, CH_3), 2.19 (dd, 1H, *J* = 13.4, 8.5 Hz, H-2'β), 2.33 (dd, 1H, *J* = 13.6, 6.0 Hz, H-2'α), 3.72 (s, 2H, CH_2''), 4.16 (q, 1H, *J* = 6.7 Hz, H-4'), 6.32 (dd, 1H, *J* = 8.3, 6.1 Hz, H-1'), 7.63 (s, 1H, H-6). ^{13}C NMR (CD_3OD): δ = 12.74 (CH_3), 17.63 (C-5'), 41.78 (C-2'), 66.05 (CH_2''), 82.38 (C-3'), 85.28, 85.84 (C-1', C-4'), 111.92 (C-5), 137.85 (C-6), 152.76 (C-2), 166.81 (C-4).

7a: HPLC retention time = 33 min. ^1H NMR (CD_3OD): δ = 1.39 (d, 2H, *J* = 6.2 Hz, H-5'), 1.97 (s, 3H, CH_3), 2.05 (dd, 1H, *J* = 14.8, 2.6 Hz, H-2'β), 2.78 (dd, 1H, *J* = 14.7, 8.3 Hz, H-2'α), 3.60 (s, 2H, CH_2''), 4.10 (q, 1H, *J* = 6.3 Hz, H-4'), 6.13 (dd, 1H, *J* = 8.1, 2.5 Hz, H-1'), 7.98 (s, 1H, H-6), 8.63 (s, 1H, NH). ^{13}C NMR (CD_3OD): δ = 12.81 (CH_3), 13.92 (C-5'), 43.89 (C-2'), 65.71 (CH_2''), 80.22 (C-3'), 81.91 (C-1'), 85.37 (C-4'), 110.97 (C-5), 139.60 (C-6), 152.76 (C-2), 166.81 (C-4).

Method 2: A mixture of *t*-butanol (6.0 mL), H_2O (6.0 mL) and AD-mix-β (1.6 g) was stirred at r.t. until two clear phases were obtained, cooled to 0 °C and then added to **5a** (200 mg, 0.90 mmol). The heterogeneous slurry was stirred vigorously at 0 °C for 140 h, additional AD-mix-β (2.0 g) was added, and stirring at 0 °C was continued for additional 90 h. After addition of sodium sulfite (3.0 g) and stirring at 0 °C for 30 min, EtOAc (50 mL) was added. The phases were separated, and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phase was dried (Na_2SO_4), evaporated under reduced pressure and purified, as described above; yield **6a**: 23 mg (10 %); yield **7a**: 7 mg (3 %).

5'-*O*-(*t*-Butyldimethylsilyl)-3'-ketothymidine (4b)

5'-*O*-(*t*-Butyldimethylsilyl)thymidine (**3b**)[21] (5.70 g, 16.0 mmol) was added in one portion to a stirred suspension of 3A molecular sieve powder (9.0 g) and pyridinium dichromate (6.30 g, 16.7 mmol) in anhydrous CH_2Cl_2 (70 mL, freed from acids by filtration through basic alumina). The inside of the flask was washed with anhydrous CH_2Cl_2 (40 mL), and the mixture was stirred for 4 h at r.t. under argon. The reaction mixture was filtered

through 3A molecular sieve powder (deposited as a slurry with anhydrous CH_2Cl_2 on a glass filter), the plug was washed with anhydrous CH_2Cl_2 (150 mL), and the combined solvent was evaporated under reduced pressure. The residue was suspended in EtOAc (1250 mL), sonicated for 5 min and filtered through 3A molecular sieve powder (deposited as a slurry with EtOAc on a glass filter). The plug was washed with EtOAc (100 mL), and the solvent was evaporated under reduced pressure. The residue was suspended in petroleum ether (200 mL), stirred at r.t. for 12 h and filtered to afford **4b** as an off-white powder, which was used without further purification in the next step; yield: 3.50 g (62 %). ^{1}H NMR ($CDCl_3$): δ = 0.09 (s, 6H, $Si(CH_3)_2$), 0.90 (s, 9H, $C(CH_3)_3$), 1.95 (s, 3H, CH_3), 2.41 (dd, 1H, *J* = 18.2, 8.1 Hz, H-2'β), 2.98 (dd, 1H, *J* = 18.2, 5.7 Hz, H-2'α), 4.00 (s, 2H, H-5'a, H-5'b), 4.12 (s, 1H, H-4'), 6.47-6.50 (m, 1H, H-1'), 7.64 (s, 1H, H-6), 9.15 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ = -5.60 ($Si(CH_3)_2$), 12.39 (CH_3), 18.27 ($C(CH_3)_3$), 25.75 ($C(CH_3)_3$), 42.35 (C-2'), 62.70 (C-5'), 81.11, 82.81 (C-1', C-4'), 111.82 (C-5), 134.52 (C-6), 150.32 (C-2), 163.47 (C-4), 209.13 (C-3').

5'-*O*-(*t*-Butyldimethylsilyl)-3'-deoxy-3'-*C*-methylenethymidine (5b)

To a stirred solution of nucleoside **4b** (3.49 g, 9.85 mmol) in anhydrous CH_2Cl_2 (60 mL) under argon at 0 °C, were added three portions (at 10 min intervals, 3 × 20 mL) of the slurry reagent prepared from: zinc (dust, 11.5 g, 176 mmol), CH_2Br_2 (4.04 mL, 57.6 mmol) and $TiCl_4$ (2.40 mL, 43.8 mmol) in anhydrous THF (100 mL). After additional stirring for 10 min, the ice-bath was removed, and the reaction mixture was stirred at r.t. for 2 h. The mixture was poured into a mixture of $CHCl_3$ (500 mL) and an ice-cold saturated aqueous solution of $NaHCO_3$ (750 mL), stirred for 10 min and filtered through silica gel (deposited as a slurry with $CHCl_3$ on a glass filter). The separated water phase and the plug were washed with $CHCl_3$ (5 × 200 mL), and the organic phases were combined and dried (Na_2SO_4). After evaporation of the solvent and column chromatographic purification (0-3 % CH_3OH in CH_2Cl_2, v/v), **5b** was obtained as a white solid; yield: 3.44 g (99 %). Anal. calcd. for $C_{17}H_{28}N_2O_4Si$: C, 57.92; H, 8.01; N, 7.95. Found: C, 57.90; H, 8.06; N, 7.57. ^{1}H NMR ($CDCl_3$): δ = 0.09 (s, 6H, $Si(CH_3)_2$), 0.91 (s, 9H, $C(CH_3)_3$), 1.93 (s, 3H, CH_3), 2.57 (dd, 1H, *J* = 15.7, 7.9 Hz, H-2'β), 3.01 (dd, 1H, *J* = 15.7, 6.0 Hz, H-2'α), 3.76 (dd, 1H, *J* = 11.1, 3.0 Hz, H-5'a), 3.98 (dd, 1H, *J* = 11.1, 2,7 Hz, H-5'b), 4.50 (br s, 1H, H-4'), 5.04 (d, 1H, *J* = 1.4 Hz, CH_2''-a), 5.17 (d,

1H, J = 1.4 Hz, CH_2''-b), 6.18 (dd, 1H, J = 7.9, 6.0 Hz, H-1'), 7.60 (s, 1H, H-6), 8.54 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ = -5.33 ($Si(CH_3)_2$), 12.51 (CH_3), 18.51 ($C(CH_3)_3$), 25.97 ($C(CH_3)_3$), 39.25 (C-2'), 66.09 (C-5'), 82.24, 83.87 (C-1', C-4'), 107.82 (CH_2''), 110.99 (C-5), 135.49 (C-6), 144.86 (C-3'), 150.34 (C-2), 163.64 (C-4).

5'-*O*-(*t*-Butyldimethylsilyl)-3'-*C*-(hydroxymethyl)thymidine (6b)

To a mixture of **5b** (964 mg, 2.73 mmol), H_2O (1.0 mL), pyridine (1.0 mL) and *t*-butanol (20 mL) was added *N*-methylmorpholine *N*-oxide (2.11 g, 18.01 mmol) and OsO_4 (100 µL of a 2.5 % solution in *t*-butanol, 8.0 µmol). The reaction mixture was stirred at 76 °C for 6 h, cooled to r.t. and treated with 20 % aqueous sodium bisulfite (2.5 mL). The mixture was concentrated under reduced pressure, diluted with a saturated aqueous solution of sodium chloride (5 mL) and extracted with EtOAc (3 × 20 mL). The combined organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. Purification by column chromatography (1-3 % CH_3OH in CH_2Cl_2, v/v) afforded **6b** as a white solid; yield: 459 mg (43 %). Anal. calcd. for $C_{17}H_{30}N_2O_6Si{\cdot}0.25H_2O$: C, 52.22; H, 7.86; N, 7.16. Found: C, 52.21; H, 7.90; N, 7.28. ^1H NMR ($CDCl_3$): δ = 0.24 (s, 6H, $Si(CH_3)_2$), 1.03 (s, 9H, $C(CH_3)_3$), 1.98 (s, 3H, CH_3), 2.09 (dd, 1H, J = 12.8, 9.2 Hz, H-2'β), 2.39 (dd, 1H, J = 12.8, 5.2 Hz, H-2'α), 3.93 (d, 1H, J = 5.0 Hz, H-5'a), 3.99 (m, 2H, CH_2''-a, CH_2''-b), 4.06 (d, 1H, J = 5.0 Hz, H-5'b), 4.08 (m, 1H, H-4'), 6.32 (dd, 1H, J = 9.2, 5.2 Hz, H-1'), 7.81 (s, 1H, H-6). ^{13}C NMR ($CDCl_3$): δ = -5.06, -4.97 ($Si(CH_3)_2$), 13.00 (CH_3), 19.44 ($C(CH_3)_3$), 26.79 ($C(CH_3)_3$), 43.19 (C-2'), 64.06, 65.90 (C-5', CH_2''), 82.76 (C-3'), 86.74, 89.28 (C-1', C-4'), 111.33 (C-5), 137.90 (C-6), 152.58 (C-2), 166.65 (C-4).

5'-*O*-(*t*-Butyldimethylsilyl)-3'-*C*-((4,4'-dimethoxytrityl)oxymethyl)thymidine (8)

To a stirred solution of nucleoside **6b** (413 mg, 1.06 mmol) in anhydrous pyridine (1 mL) was added 4,4'-dimethoxytrityl chloride (403 mg, 1.19 mmol). After 24 h at r.t., CH_3OH (0.2 mL) was added, and the solvents were evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (8 mL), washed with water (3 × 2 mL) and dried (Na_2SO_4). After evaporation of the solvent under reduced pressure, the crude product was purified by column chromatography (0-2 % CH_3OH, 0.5 % pyridine in CH_2Cl_2, v/v) to give **8** as a white solid; yield: 378 mg (47 %). Anal. calcd. for $C_{38}H_{48}N_2O_8Si{\cdot}0.5C_5H_5N{\cdot}$

$1.5H_2O$: C, 64.39; H, 7.14; N, 4.64. Found: C, 64.10; H, 7.07; N, 4.60. ^{1}H NMR ($CDCl_3$): δ = -0.08, -0.04 (2 × s, 6H, $Si(CH_3)_2$), 0.77 (s, 9H, $C(CH_3)_3$), 1.89 (s, 3H, CH_3), 1.94 (dd, 1H, *J* = 12.6, 9.1 Hz, H-2'β), 2.68 (dd, 1H, *J* = 12.6, 5.0 Hz, H-2'α), 3.26 (d, 1H, *J* = 9.0 Hz, CH_2''-a), 3.48 (m, 1H, H-5'a), 3.52 (d, 1H, *J* = 9.0 Hz, CH_2''-b), 3.77 (s, 6H, 2 × OCH_3), 3.80 (m, 1H, H-5'b), 4.03 (m, 1H, H-4'), 6.34 (dd, 1H, *J* = 9.0, 5.0 Hz, H-1'), 6.82 (m, 4H, aryl), 7.20-7.40 (m, 9H, aryl), 7.54 (s, 1H, H-6), 9.76 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ = -5.33 ($Si(CH_3)_2$), 12.53 (CH_3), 18.10 ($C(CH_3)_3$), 25.92 ($C(CH_3)_3$), 43.73 (C-2'), 55.19 (OCH_3), 62.13 (C-5'), 65.60 (CH_2''), 80.77 (C-3'), 85.23, 86.50, 86.94 (C-1', C-4', CAr_3), 110.25 (C-5), 113.31, 126,74, 127.02, 127.98, 128.09, 130.08, 144.47, 158.69 (aryl), 135.46 (C-6), 150.59 (C-2), 164.21 (C-4).

5'-*O*-(*t*-Butyldimethylsilyl)-3'-*O*-(2-cyanoethoxy(diisopropylaminophosphino))-3'-*C*-((4,4'-dimethoxytrityl)oxymethyl)thymidine (9)

Nucleoside **8** (136 mg, 0.18 mmol) was coevaporated with anhydrous CH_3CN (2 mL) and dissolved under argon in anhydrous CH_2Cl_2 (1 mL). *N,N*-Diisopropylethylamine (0.2 mL) was added followed by dropwise addition of 2-cyanoethyl *N,N*-diisopropylaminophosphoramidochloridite (0.2 mL, 0.90 mmol). After stirring at r.t. for 24 h, the reaction was quenched by addition of CH_3OH (0.5 mL), and the mixture was diluted with EtOAc (3.5 mL), washed successively with saturated aqueous solutions of $NaHCO_3$ (3 × 5 mL) and NaCl (3 × 5 mL), dried (Na_2SO_4) and evaporated under reduced pressure. Purification of the residue by column chromatography (EtOAc/CH_2Cl_2/Et_3N, 45:45:10, v/v/v) and precipitation in petroleum ether (50 mL) at -20 °C (after redissolution in anhydrous toluene (1 mL)) afforded **9** as a white foam; yield: 121 mg (69 %). ^{31}P NMR ($CDCl_3$): δ = 140.9, 142.5.

Synthesis of Oligodeoxynucleotides

The oligodeoxynucleotides **A** and **B** were synthesized on a Pharmacia Gene Assembler Special® DNA-synthesizer in 0.2 μmol scale (5 μmol amidite per cycle) by use of commercial β-cyanoethylphosphoramidites and compound **9**. The original protocol for the DNA-synthesizer was used, except that the coupling time for **9** was increased from 2 to 12 min and that **9** was dissolved directly in the activator solution (0.1 M solution of **9** in 0.45 M tetrazole in acetonitrile). The 5'-*O*-DMT-ON oligodeoxynucleotides were removed

from the solid support and deprotected with concentrated aqueous ammonia at room temperature for 72 h. Purification and detritylation on disposable purification cartridges, desilylation using 1 M tetra-*n*-butylammonium fluoride in THF and desalting afforded oligomers **A** and **B**. The purity of **A** and **B** was confirmed by analytical reversed 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 + 80 % 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**, 10 min 100-0 % **b** in **a**. Flow rate 1.0 mL/min. The purified ODNs eluted as one peak after approximately 23 min.

Melting experiments

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.

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