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Synthesis of Diastereomeric Mixtures of 5′-*C*-Hydroxymethylthymidine and Introduction of a Novel Class of *C*-Hydroxymethyl Functionalised Oligodeoxynucleotides

Jef Fensholdt and Jesper Wengel*

Department of Chemistry, Odense University, DK-5230 Odense M, Denmark

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Novel 5′-*C*-hydroxymethyl substituted derivatives of thymidine have been prepared by dihydroxylation of the 5′-*C*-methylene nucleoside **1**. Osmium tetraoxide catalysed dihydroxylation of **1** afforded a 3:2 epimeric mixture of diols **2**, whereas asymmetric dihydroxylation using AD-mix-α and AD-mix-β resulted in mixtures **3** and **4** of the two epimeric diols, both enriched with the same diastereomer. Nucleosides **2** were transformed into phosphoramidites **8** which were used for solid phase synthesis of oligodeoxynucleotides (ODNs) containing 5′-*C*-(hydroxymethyl) functionalised thymidine monomers. This novel class of *C*-hydroxymethyl modified ODN-analogues exhibited promising affinity towards both complementary DNA and RNA as well as resistance towards 3′-exonucleolytic degradation.

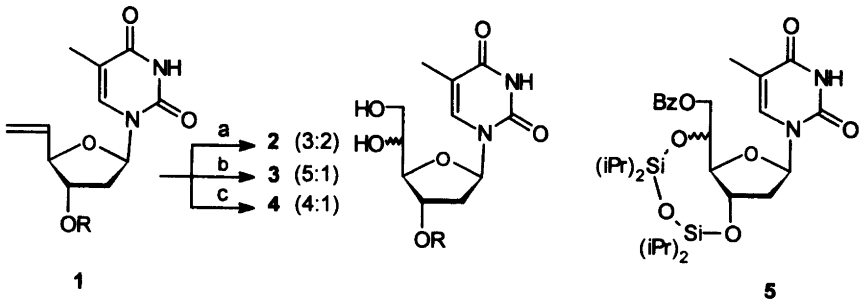
The sequence-specific blocking of the expression of genetic information by duplex- or triplex-forming oligonucleotides displays immense pharmaceutical potential, e.g., for antiviral and anticancer therapy.[1–5] Essential requirements for successful *in vivo* application of such antigene and antisense oligonucleotides are foremost high specificity and affinity for target nucleic acids and stability towards degradative nucleases. In this context, we have been interested in *C*-hydroxymethyl substituted nucleosides as monomeric substitutes in modified oligodeoxynucleotides (ODNs), and 3′-*C*- and 4′-*C*-hydroxymethyl functionalised ODNs have shown significant resistance towards 3′-exonucleolytic degradation combined with excellent hybridisation properties.[6–9] We envisaged that these *C*-hydroxymethyl groups, oriented towards the major[6,7] or minor[8,9] groove in duplexes, should provide versatile attachment sites for additional functionalities which may enhance membrane permeability or the affinity towards target nucleic acids. In this report, we describe our results on synthesis of novel 5′-*C*-(hydroxymethyl)-thymidines and 5′-*C*-functionalised-ODNs. Encouraging thermal stabilities for duplexes formed between this novel class of ODNs and both complementary DNA and RNA were obtained together with significant 3′-exonucleolytic resistance.

* To whom correspondence should be addressed.

Results and discussion

The synthesis of 1-(2-deoxy-β-D-*ribo*-hexofuranosyl)-thymine has been described in a communication[10] via coupling of 2,4-dimethoxythymine with a 1-bromo-3,5,6-tri-*O*-acyl-2-deoxy-D-*ribo*-hexofuranose derivative. However, as the starting material (methyl 2-deoxy-D-*ribo*-hexofuranoside) is not readily available and as the product nucleoside was obtained as a mixture of α- and β-anomers, this published procedure is far from ideal. Therefore, we decided to evaluate the possibility of introducing a 5′-*C*-hydroxymethyl group by dihydroxylation of 3′-*O*-(*tert*-butyldimethylsilyl)-5′-deoxy-5′-*C*-methylenethymidine (**1**) (Scheme 1). Following the published procedure for preparation of **1**,[11] Swern oxidation of 3′-*O*-silyl protected thymidine and subsequent reaction with methylenetriphenylphosphorane (generated under Li-salt free conditions) afforded the 5′-*C*-methylene compound **1** in 22% yield. However, we obtained a higher yield when the intermediate 5′-aldehyde used for the Wittig methylenation was prepared by Moffatt oxidation, purified as its 1,3-diphenylimidazolidine derivative, and regenerated before methylenation with *p*-toluenesulfonic acid.[12] Using this procedure, we obtained alkene **1** in 55% yield from 3′-*O*-silyl protected thymidine.

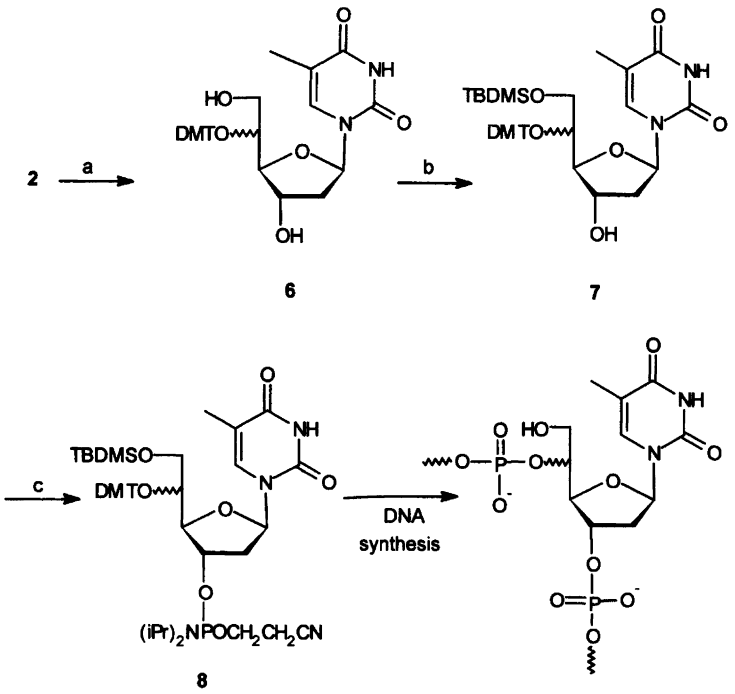
Dihydroxylation of **1** with a catalytic amount of osmium tetraoxide and *N*-methylmorpholine *N*-oxide (NMO) as co-oxidant[13] gave an inseparable mixture of



Scheme 1. (a) OsO_4, NMO, pyridine, H_2O, *t*-BuOH; (b) AD-mix-α, H_2O, *t*-BuOH; (c) AD-mix-β, H_2O, *t*-BuOH. R = TBDMS.

epimeric 5′,6′-diols **2** in 53% yield after column chromatographic purification (^{1}H NMR analysis indicated a ~3:2 ratio of epimers). Additional dihydroxylation in the thymine base, indicated by the presence of small amounts of polar by-products on analytical TLC, is one explanation for the rather moderate yield achieved.[14,15] Next, we investigated the possibility of synthesising each isomer stereoselectively by the Sharpless asymmetric dihydroxylation method[16–18] using two commercially available asymmetric dihydroxylation reagents AD-mix-α and AD-mix-β. Treatment of **1** with AD-mix-α in a 1:1 mixture of water and *tert*-butyl alcohol at 0 °C for 9 days gave the epimeric diol products **3** in 72% yield. From the ^{1}H NMR spectrum, the ratio of the two epimers was estimated as ~5:1. When a similar reaction was performed with AD-mix-β, all starting material was converted into product within 22 h according to analytical TLC, and a ~4:1 mixture **4** of the epimers was produced in 91% yield. Although increased diastereoselectivity (compared with the reaction using NMO as co-oxidant) was obtained, surprisingly, the same diastereomer was predominantly formed irrespective of the AD-mix used. As the major isomer was identical in all three dihydroxylation product mixtures (**2**–**4**, Scheme 1), and considering the difference in reaction time, apparently the approach of the AD-mix-α ligand towards its stereochemically preferred face of the double bond is sterically hindered. Thus, a comparatively slow reaction occurs (originating from a non-optimal interaction of the AD-mix-α ligand with the face that is normally preferred by the AD-mix-β ligand) resulting in a diastereoselectivity similar to that obtained with AD-mix-β. To determine the configuration of the predominant epimer at the new C-5′ stereocentre by ^{1}H NOE NMR experiments, an analytical sample of mixture **4** was converted into the conformationally locked derivatives **5** (Scheme 1) by the following reaction sequence: regioselective monobenzoylation at the primary 5′-*C*-hydroxymethyl functionality of **4** using 1.1 equiv. benzoyl chloride in pyridine at 0 °C; desilylation at the 3′-position with tetrabutylammonium fluoride in THF to give a monoprotected compound; reaction with 1.1 equiv. 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine for 3 days at r.t. to afford 3′,5′-*O*-bridged derivatives **5**. This gave a sufficient amount for NMR analysis. As these transformations were all efficient according to analytical TLC, the major epimer obtained at the end can be assumed to have the same configuration as the major epimer in the starting mixture **4**. The assignment of the signals in the ^{1}H NMR spectrum of **5** was verified by ^{1}H–^{1}H COSY NMR, and a ^{1}H NOE NMR experiment was performed. Saturation of the signal from H-5′ (4.30 ppm) gave enhancement (5%) of the signal at 3.75 ppm (H-4′) thus indicating an L-*lyxo* configuration of the major isomer. Because of overlapping signals in different solvents, no further proof of the predominant configuration could be obtained.

Recently, a communication describing the incorporation of a ~1:1 epimeric mixture of (5′*S*,5′*R*)-5′-*C*-methyl derivatives of thymidine and 2′-deoxyadenosine into ODNs has been published.[19] The hybridisation properties of these 5′-*C*-methyl-DNA analogues were as good as for unmodified controls. Stimulated by these results, we decided to transform the ~3:2 epimeric mixture of hexofuranosyl-modified nucleosides **2** into phosphoramidite synthons **8** suitable for solid phase synthesis of ODNs containing 5′-*C*-hydroxymethyl functionalised thymidine monomers (Scheme 2). Treatment of **2** with 0.97 equiv. benzoyl chloride in anhydrous pyridine at 0 °C afforded a 6′-*O*-benzoylated derivative that was subsequently reacted with 4,4′-dimethoxytrityl chloride (DMTCl) in the presence of $AgNO_3$ and pyridine in anhydrous THF to give a 6′-*O*-benzoyl-5′-*O*-dimethoxytrityl intermediate. Desilylation using tetra-*n*-butylammonium fluoride (TBAF) followed by debenzoylation with methanolic ammonia gave epimeric 5′-*O*-monoprotected nucleosides **6** in 63% yield (calculated from **2**). Resilylation[20] of **6** with 1.1 equiv. *tert*-butyldimethylsilyl chloride (TBDMSCl) and imidazole in anhydrous DMF afforded 6′-*C*-(*tert*-butyldimethylsilyloxy)methyl nucleosides **7** in 48% yield. The structure of **7** was confirmed by analytical scale acetylation of the free hydroxy group and subsequent ^{1}H NMR and ^{1}H–^{1}H COSY NMR analyses using a procedure described earlier.[9] Phosphitylation[21,22] of **7** in anhydrous dichloromethane by reaction with 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite and *N*,*N*-diisopropylethylamine afforded a mixture (~4:3 ratio of 5′-*C*-epimeric pairs according to ^{1}H NMR and ^{31}P NMR) of four diastereomeric phosphoramidite building blocks **8** in 86% yield after



Scheme 2. (a) 1, Benzoyl chloride, pyridine; 2, DMTCl, $AgNO_3$, pyridine, THF; 3, TBAF, THF; 4, NH_3, MeOH; (b) TBDMSCl, imidazole, DMF; (c) 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite, *N,N*-diisopropylamine, CH_2Cl_2.

column chromatographic purification and precipitation from petroleum ether.

Oligodeoxynucleotides **A–G** (Table 1) were synthesised by the solid phase phosphoramidite method[23] on an automated DNA synthesiser using **8** and commercial 2′-deoxynucleoside 2-cyanoethyl phosphoramidites. The coupling efficiency of the modified phosphoramidites **8** was 90–95%, as monitored by the release of the dimethoxytrityl cation during deprotection in each coupling cycle, compared with approximately 99% for unmodified amidites. The 5′-*O*-DMT-protected modified ODNs were released from the solid support with simultaneous removal of the acyl and 2-cyanoethyl protecting groups by treatment with 32% aqueous ammonia for 3 days at r.t. After desilylation with TBAF for 16 h and desalting, the oligomers were purified by use of disposable reversed-phase chromatography cartridges. As representative examples, the composition of ODNs **C** and **G** was verified by matrix-assisted laser desorption mass spectrometry as the observed relative mass of ODN **C** was 5096.9 Da and of **G** was 4314.7 Da, consistent with calculated masses of 5095.4 Da and 4315.9 Da, respectively.

The ability of 5′-*C*-hydroxymethyl modified ODNs **B**, **C**, **E** and **G** to form duplexes with complementary DNA and RNA was evaluated by determining melting temperatures (T_m) in medium salt buffer using conditions described earlier.[24] The results are outlined in Table 1. Incorporation of epimeric 5′-*C*-hydroxymethyl nucleoside derivative **X** once and twice in the middle, or once in the 3′-end, of 17-mers induces no significant decrease in the thermal stability of duplexes formed with complementary DNA, compared with unmodified controls **A**, **D** and **F**. Furthermore, a T_{14}-mer containing four modifications (ODN **G**) is capable of forming stable duplexes both with complementary DNA and RNA as only minor decreases in T_m-values were observed.

As mentioned above, increased resistance towards cellular nucleases, especially 3′-exonucleases, is essential

Table 1. Sequences synthesised, melting temperatures for DNA–DNA[a] and DNA–RNA[b] hybrids, and 3-exonucleolytic stabilities

Sequence	T_m[a]/°C	ΔT_m[a]/°C	T_m[b]/°C	ΔT_m[b]/°C	$t_{1/2}$/min
(**A**) 5′-CACCAACTTCTTCCACA-3′	59				<1
(**B**) 5′-CACCAAC**X**TCTTCCACA-3′	57	−2			<1
(**C**) 5′-CACCAAC**X**TCT**X**CCACA-3′	56	−1.5			<1
(**D**) 5′-TTAACTTCTTCACATTC-3′	50				<1
(**E**) 5′-TTAACTTCTTCACAT**X**C-3′	48	−2			>50
(**F**) 5′-TTTTTTTTTTTTTTT-3′	36		32		
(**G**) 5′-TT**X**TT**X**TT**X**TT**X**TT-3′	32	−1	26	−1.5	

A = 2′-deoxyadenosine; C = 2′-deoxycytidine; T = thymidine; **X** = epimeric mixture of 5′-*C*-hydroxymethyl modified thymidine monomer derived from amidite **8**; T_m = melting temperature; ΔT_m = change in T_m/modification; $t_{1/2}$ = hyperchromicity half-life.

for successful application of oligonucleotides *in vivo*. ODNs **B**, **C** and **E** were exposed to snake venom phosphodiesterase (3′-exonuclease) and the degradations were followed as described earlier.[24–26] The increase in absorbance measured at 260 nm, originating from reduced base-stacking as a result of 3′-exonucleolytic degradation,[25] was followed. From the hyperchromicity versus time plots (see Fig. 1), hyperchromicity half-lives ($t_{1/2}$) were estimated (Table 1). The oligomer containing a 5′-*C*-modified thymidine monomer in the 3′-end (**E**) is significantly more stable ($t_{1/2} > 50$ min) towards 3′-exonucleolytic digestion than the unmodified control **D** ($t_{1/2} < 1$ min). The mid-modified ODNs (**B** and **C**) are rapidly degraded ($t_{1/2} < 1$ min), but the overall hyperchromicity increase is less than for control **A** indicating inhibition of further degradation when the enzyme encounters a mid-modification.

In summary, a novel class of *C*-hydroxymethyl modified ODNs has been synthesised based on incorporation of a mixture of 5′-*C*-epimeric 5′-*C*-hydroxymethyl thymidines. Encouraging hybridisation properties both towards complementary DNA and RNA, and notable protection against 3′-exonucleolytic degradation, were observed. It was not possible to isolate or synthesise the 5′-*C*-epimeric nucleosides as pure diastereomers. As the possibility exists that the stereochemistry around C-5′ proves more favorable in one disastereomer than in the other with regard to thermal stability of duplexes, development of more effective stereoselective synthetic methods of 2′-deoxy-5′-*C*-hydroxymethyl nucleosides should be undertaken. In addition, based on the results reported here, future exploration of the possibility of tethering various functionalities to high-affinity ODNs via a 5′-*C*-hydroxymethyl group seems justified.

Experimental

NMR spectra were recorded at 250 MHz for ^{1}H NMR, 62.9 MHz for ^{13}C NMR and 202.3 MHz for ^{31}P NMR. Chemical shifts are in ppm relative to tetramethylsilane as an internal standard unless otherwise stated (^{1}H NMR and ^{13}C NMR) and relative to 85% H_3PO_4 as an external standard (^{31}P NMR). Microanalyses were performed at The H. C. Ørsted Institute, University of Copenhagen. The silica gel used for column chromatography (0.040–0.063 mm) was purchased from Merck. Oligodeoxynucleotides were synthesised on a Pharmacia Gene Assembler® Special DNA-synthesiser. Purification of 5′-*O*-DMT-ON oligodeoxynucleotides was accomplished using disposable Oligopurification Cartridges (COP, Cruachem) and desalting using NAP-10 columns (Pharmacia). Oligoribonucleotide rA_{14} was purchased from DNA Technology ApS, Aarhus, Denmark.

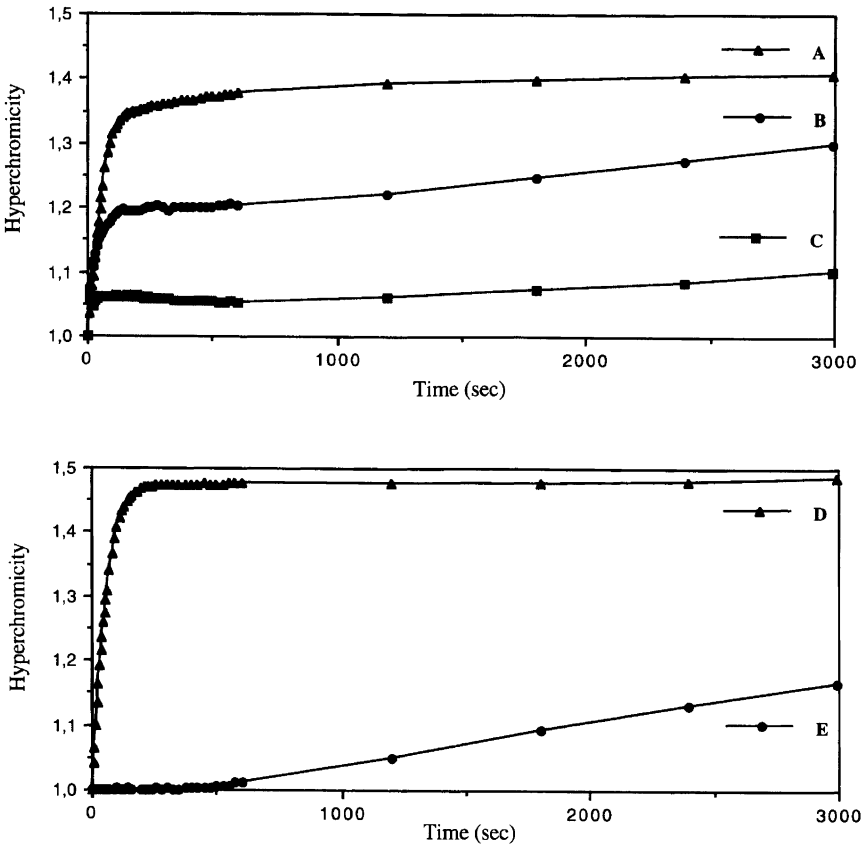


Fig. 1. Hyperchromicity versus time plots during 3′-exonucleolytic degradation.

3′-O-(tert-Butyldimethylsilyl)-5′-deoxy-5′-C-methylenethymidine (1).[11] To a solution of 3′-*O*-(*tert*-butyldimethylsilyl)-5′-deoxy-5′,5′-(*N*,*N*′-diphenylethylenediamino)thymidine[12] (4.2 g, 7.7 mmol) in anhydrous CH_2Cl_2 (260 ml) at 0 °C was added a solution of *p*-toluenesulfonic acid monohydrate (4.4 g, 23.1 mmol) in anhydrous acetone (130 ml). The resulting reaction mixture was stirred at r.t. under nitrogen for 60 min. The precipitate formed was filtered of and washed with CH_2Cl_2 (130 ml). The combined filtrate was washed with a saturated aqueous solution of $NaHCO_3$ (130 ml) and water (130 ml), dried (Na_2SO_4) and evaporated to dryness under reduced pressure to give crude 5′-aldehyde as a slightly yellow foam (2.7 g). Methyltriphenylphosphonium bromide (5.5 g, 15.4 mmol) was suspended in dry THF (25 ml) and cooled to 0 °C. Sodium bis(trimethylsilyl)amide (15.4 ml of an 1.0 M solution in THF, 15.4 mmol) was added dropwise under nitrogen and the bright yellow suspension was stirred for 30 min before the crude 5′-aldehyde (2.7 g) dissolved in dry THF (20 ml) was added at 0 °C. The reaction mixture was stirred at r.t. for 2 h and then diluted with ethyl acetate (70 ml). The solution was washed with water (2 × 30 ml) and brine (2 × 30 ml) and dried (Na_2SO_4). After evaporation, the residue was purified by column chromatography on silica gel (20% ethyl acetate in petroleum ether, v/v) to give the 5′-*C*-methylene nucleoside **1** as a white solid material. Yield 1.5 g (55%). ^{1}H NMR ($CDCl_3$): δ 0.08 (s, 6 H), 0.89 (s, 9 H), 1.94 (d, 3 H, J = 0.9 Hz), 2.08–2.16 (m, 1 H), 2.29–2.33 (m, 1 H), 4.17–4.28 (m, 2 H), 5.28–5.44 (m, 2 H), 5.83–5.97 (m, 1 H), 6.24 (t, 1 H, J = 6.4 Hz), 7.20 (d, 1 H, J = 1.1 Hz), 9.30 (s, 1 H). ^{13}C NMR ($CDCl_3$): δ −4.86, −4.80, 12.54, 17.86, 25.58, 40.46, 75.17, 85.10, 87.39, 110.88, 117.96, 135.01, 135.17, 150.15, 163.66. Anal $C_{17}H_{28}N_2O_4Si$: C, H, N.

Dihydroxylation of alkene **1** *using catalytic OsO_4 and NMO to give the epimeric mixture 1-[3-O-(tert-butyldimethylsilyl)-2-deoxy-β-*D*-ribo-hexofuranosyl]thymine and 1-[3-O-(tert-butyldimethylsilyl)-2-deoxy-α-*L*-lyxo-hexofuranosyl]thymine (***2***).* To a solution of compound **1** (730 mg, 2.1 mmol) in *t*-BuOH (30 ml), H_2O (1.0 ml) and pyridine (1.2 ml), was added *N*-methylmorpholine *N*-oxide (NMO, 1.5 g, 12.8 mmol) and OsO_4 (0.5 ml of a 2.5% solution in *t*-BuOH, 0.04 mmol). The resulting mixture was stirred under reflux for 6 h, whereafter a 20% aqueous solution of $Na_2S_2O_5$ (4.0 ml) was added. The mixture was evaporated under reduced pressure, and brine (20 ml) and water (20 ml) were added. This mixture was extracted with ethyl acetate (2 × 150 ml) and the combined organic extract was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The crude product was column chromatographed on silica gel (1–3% CH_3OH in CH_2Cl_2, v/v) to give the epimeric diol mixture **2** as a white foam (~3:2 ratio of epimers as estimated from the integral of the individual H-6 signals in the ^{1}H NMR spectrum). Yield 426 mg (53%). ^{1}H NMR (CD_3OD; spectrum referenced to methanol at δ 3.45; chemical shifts for protons belonging to the predominant epimer is marked with an asterisk for separate signals identified by ^{1}H–^{1}H COSY NMR): δ 0.27 [s, $Si(CH_3)_2$], 1.06 [s, $C(CH_3)_3$], 2.01 (d, J = 1.1 Hz, CH_3), 2.02* (d, J = 1.1 Hz, CH_3), 2.23–2.43 (m, H-2′α, H-2′β), 3.69–3.84 (m, H-6′a, H-6′b), 3.88–3.95 (m, H-5′), 4.03–4.06* (m, H-4′), 4.10–4.12 (m, H-4′), 4.69–4.76 (m, H-3′), 4.77–4.86* (m, H-3′), 6.41 (m, H-1′), 7.87* (d, J = 1.2 Hz, H-6), 8.11 (d, J = 1.2 Hz, H-6). ^{13}C NMR (CD_3OD): δ −4.67, −4.65, −4.57, −4.51, 12.43, 12.45, 18.72, 18.80, 26.24, 41.39, 41.72, 64.44, 64.60, 72.38, 73.40, 73.59, 74.61, 86.30, 86.33, 88.32, 89.57, 111.54, 111.77, 138.07, 138.47, 152.45, 166.30, 166.37. Anal $C_{17}H_{30}N_2O_6Si$: C, H, N.

Asymmetric dihydroxylation of alkene **1** *using AD-mix-α to give the epimeric mixture* **3**. AD-mix-α (2.8 g) dissolved in H_2O (10 ml) and *t*-BuOH (10 ml) was stirred at r.t. until two clear phases were observed (~10 min). The mixture was cooled to 0 °C and the 5′-*C*-methylene nucleoside **1** (350 mg, 0.99 mmol) was added. The reaction mixture was stirred vigorously for 9 days at 5 °C. Na_2SO_3 (3.0 g) was added, and the temperature was raised to r.t. and stirring was continued for 1 h. H_2O (10 ml) and ethyl acetate (100 ml) were added, and after separation of the phases the aqueous solution was extracted with ethyl acetate (3 × 100 ml). The combined organic phase was dried (Na_2SO_4) and the solvent evaporated under reduced pressure. The residue was column chromatographed on silica gel (1–3% CH_3OH in CH_2Cl_2, v/v), affording the epimeric diol mixture **3** as a white foam (~5:1 ratio of epimers, as estimated from the integral of the individual H-6 signals in the ^{1}H NMR spectrum). Yield 274 mg (72%). ^{1}H NMR and ^{13}C NMR data were identical with data given for mixture **2**.

Asymmetric dihydroxylation of alkene **1** *using AD-mix-β to give the epimeric mixture* **4**. The same procedure and amounts as described for the preparation of **3** were used. According to analytical TLC, the reaction with AD-mix-β was complete after 22 h. Column chromatographic purification on silica gel (1–3% CH_3OH in CH_2Cl_2, v/v) gave the epimeric diol mixture **4** as a white foam (~4:1 ratio of epimers, as estimated from the integral of the individual H-6 signals in the ^{1}H NMR spectrum). Yield 349 mg (91%). ^{1}H NMR and ^{13}C NMR data were identical with data given for mixture **2**.

*1-[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-β-*D*-ribo-hexofuranosyl]thymine and 1-[2-deoxy-5-O-(4,4′-dimethoxytrityl)-α-*L*-lyxo-hexofuranosyl]thymine (***6***).* The epimeric nucleosides **2** (411 mg, 1.06 mmol) were dissolved in anhydrous pyridine (4 ml) under argon and cooled to 0 °C. Benzoyl chloride (0.12 ml, 1.03 mmol) was added and the mixture was stirred at 0 °C for 15 min. After addition of BuOH (0.2 ml), the mixture was diluted with CH_2Cl_2 (30 ml) and washed with a saturated aqueous solution of $NaHCO_3$ (3 × 10 ml) and brine

(3 × 10 ml). The organic phase was dried (Na_2SO_4) and evaporated to dryness, and the residue was chromatographed on silica gel (0–1% CH_3OH in CH_2Cl_2, v/v) yielding 6-*O*-monobenzoylated epimeric products (380 mg) containing trace amounts of pyridine according to NMR analysis. To a solution of these 6-*O*-benzoyl nucleosides (360 mg) in anhydrous THF (20 ml) were added pyridine (0.6 ml, 7.4 mmol), $AgNO_3$ (250 mg, 1.47 mmol) and 4,4′-dimethoxytrityl chloride (500 mg, 1.48 mmol), and the resulting mixture was stirred at r.t. under argon. After 19 h, the mixture was filtered into a 5% aqueous solution of $NaHCO_3$ (15 ml) and the product was extracted with CH_2Cl_2. After drying (Na_2SO_4) and evaporation of the solvent under reduced pressure, the residue was dissolved in anhydrous THF (7.0 ml) under nitrogen and tetrabutylammonium fluoride was added (0.80 ml of a 1.1 M solution in THF, 8.8 mmol). The mixture was stirred for 16 h at r.t. and the solvent was evaporated. The remaining material was redissolved under nitrogen in saturated methanolic ammonia (50 ml) and stirred at r.t. for 24 h. After evaporation of the solvents and column chromatographic purification of the crude products on silica gel (0–3% CH_3OH in CH_2Cl_2, v/v), the epimeric mixture **6** was isolated as a white foam [~5:4 ratio of epimers as estimated from the integral of the individual (tentatively assigned) H-3′ signals at 4.55 and 4.82 ppm in the ^1H NMR spectrum]. Yield 364 mg (~63% calculated from **2**). ^1H NMR ($CDCl_3$): δ 1.52 (d, J=0.7 Hz), 1.83 (d, J=0.6 Hz), 2.10–2.42 (m), 3.29–3.52 (m), 3.66–3.72 (m), 3.76 (s), 3.77 (s), 3.82–3.86 (m), 3.96 (t, J=4.8 Hz), 4.55 (dd, J=5.2 and 11.5 Hz), 4.82 (dd, J=5.3 and 10.7 Hz), 6.23 (dd, J=6.5 and 11.7 Hz), 6.79–6.83 (m), 7.04 (d, J=1.1 Hz), 7.17–7.71 (m). ^{13}C NMR ($CDCl_3$): δ 11.92, 12.43, 39.18, 39.99, 55.18, 61.87, 62.12, 69.33, 70.86, 72.83, 73.38, 83.26, 84.49, 86.89, 87.03, 87.37, 87.79, 111.15, 111.24, 113.20, 113.26, 123.73, 127.10, 127.84, 127.92, 128.18, 130.16, 130.23, 130.34, 130.37, 135.48, 135.79, 136.03, 136.12, 136.32, 136.39, 145.58, 145.99, 150.33, 150.38, 158.81, 163.64, 163.75. Mixture **6** was used without further purification for the preparation of **7**.

*1-[6-*O*-(*tert*-Butyldimethylsilyl)-2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-ribo-hexofuranosyl]thymine and 1-[6-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-α-*L*-lyxo-hexofuranosyl]thymine (**7**).* A mixture of nucleosides **6** (329 mg, 0.57 mmol) and imidazole (117 mg, 1.72 mmol) dissolved in anhydrous DMF (2.0 ml) was added to *tert*-butyldimethylsilyl chloride (95 mg, 0.63 mmol) under argon. The reaction mixture was stirred at r.t. for 30 min, diluted with ethyl acetate (25 ml) and washed successively with H_2O and brine, and the organic phase was dried (Na_2SO_4). After evaporation of the solvents, the crude product was purified by column chromatography on silica gel (0–1% CH_3OH in CH_2Cl_2, v/v) to give an epimeric mixture of 6-*O*-monosilylated nucleosides **7** [~4:3 ratio of epimers as estimated from the integral of the individual (tentatively assigned) H-3′ signals at 4.37–4.48 and 4.60–4.69 ppm in the ^1H NMR spectrum] as a white foam after co-evaporation with anhydrous acetonitrile (2 × 5 ml). Yield 194 mg (48%). ^1H NMR ($CDCl_3$): δ −0.10 (s), −0.05 (s), −0.01 (s), 0.82 (s), 0.89 (s), 1.57 (d, J= 0.4 Hz), 1.81 (d, J=0.6 Hz), 2.04–2.35 (m), 2.51 (br s), 2.82 (br s), 3.34 (dd, J=2.3 and 10.4 Hz), 3.48–3.62 (m), 3.77 (s), 3.78 (s), 3.79 (s), 3.90 (t, J=4.2 Hz), 4.37–4.48 (m), 4.60–4.69 (m), 6.21–6.27 (m), 6.79–6.84 (m), 6.90 (d, J=1.0 Hz), 7.20–7.54 (m), 8.68 (br s). ^{13}C NMR ($CDCl_3$): δ −5.62, −5.40, 12.01, 12.43, 18.29, 25.81, 25.91, 39.60, 40.35, 55.21, 62.50, 62.81, 70.44, 71.00, 73.86, 73.97, 83.12, 83.93, 86.27, 86.45, 87.34, 110.86, 111.08, 113.12, 113.25, 123.73, 127.04, 127.13, 127.74, 127.90, 128.05, 128.39, 130.27, 130.37, 130.47, 130.52, 135.24, 135.47, 135.96, 136.14, 136.33, 136.38, 136.72, 145.63, 146.13, 149.77, 150.16, 158.79, 163.45. Anal. $C_{38}H_{48}N_2O_8Si \cdot 0.4CH_3CN$: C, H, N. MS (EI): m/z=688 (M^+, 0.2%).

*1-[6-*O*-(*tert*-Butyldimethylsilyl)-3-*O*-[2-cyanoethoxy(diisopropylamino)phosphino]-2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-ribo-hexofuranosyl]thymine and 1-[6-*O*-(*tert*-butyldimethylsilyl)-3-*O*-[2-cyanoethoxy(diisopropylamino)phosphino]-2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-α-*L*-lyxo-hexofuranosyl)thymine (**8**).* The epimeric mixture **7** (160 mg, 0.23 mmol) was dissolved under argon in anhydrous CH_2Cl_2 (0.7 ml) and *N,N*-diisopropylethylamine (0.2 ml, 1.14 mmol). 2-Cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.1 ml, 0.45 mmol) was added and the reaction mixture was stirred at r.t. for 1 h. After addition of CH_3OH (0.04 ml) and ethyl acetate (5 ml), the mixture was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 3 ml) and brine (2 × 3 ml), and the separated organic phase was dried (Na_2SO_4). After evaporation under reduced pressure, the residual clear oil was purified on a short silica gel column (1% Et_3N and 50–60% CH_2Cl_2 in petroleum ether, v/v/v). The resulting oil was dissolved in anhydrous toluene (1.0 ml) and the amidites produced were precipitated from petroleum ether (50 ml) at −70 °C to give **8** as a white solid material after filtration under nitrogen (~4:3 ratio of 5′-*C*-epimeric pairs). Yield 175 mg (86%). ^{31}P NMR ($CDCl_3$): δ 148.59, 149.35, 150.15, 150.26. ^{13}C NMR ($CDCl_3$): δ = −5.50, 12.02, 12.14, 18.02, 19.92, 20.19, 20.30, 20.38, 24.45, 24.50, 24.53, 24.65, 25.74, 42.85, 43.10, 43.14, 43.20, 43.30, 43.35, 43.40, 55.17, 55.23, 57.94, 58.08, 58.26, 61.25, 61.49, 73.67, 74.07, 83.74, 84.20, 87.04, 111.05, 113.06, 113.16, 117.5, 126.91, 127.15, 127.70, 127.77, 128.04, 128.23, 128.30, 130.27, 130.36, 130.45, 135.15, 135.89, 136.23, 136.42, 136.54, 136.62, 145.64, 146.01, 150.28, 150.33, 158.68, 158.81, 158.85, 163.58.

Oligodeoxynucleotide synthesis. Synthesis of oligodeoxynucleotides was carried out on a 0.2 μmol scale (5 mol amidite per cycle, Pharmacia Primer Supports) using **8** and commercial 2-cyanoethyl phosphoramidites. The regular protocol of the DNA synthesiser was followed

(the coupling time for the modified amidites **8** was increased from 2 min to 12 min). Cleavage from the solid support, deblocking, desilylation (except for unmodified ODNs), desalting and purification were performed as described earlier.[6]

Evaluation of melting temperatures (T_m) and hyperchromicity half-lives ($t_{1/2}$). Melting experiments,[24] and determination of hyperchromicity half-lives ($t_{1/2}$) during 3′-exonucleolytic degradation,[24–26] were carried out as previously described.[24–26]

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