

Chimera of Dimethylene Sulfone-, Methyl Sulfide-, and Methyl Sulfoxide-Linked Ribonucleotides and DNA

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Introduction

Modified nucleic acids are important tools for studying structural and functional aspects of DNA, RNA, and protein–nucleic acid interactions. They can also help rationalize why Nature has selected RNA and DNA as the sole carriers of genetic information.¹ Analogs of nucleosides can also be powerful drugs,² and analogs of oligonucleotides have the potential for being used as sequence-specific inhibitors of gene expression.³

Our studies of modified nucleic acids have focused on backbone modified oligonucleotides where the phosphorus found in DNA and RNA has been replaced by sulfur and the bridging phosphodiester oxygens by methylene units.⁴ Crystal structures have shown that dimethylene sulfone-bridged ribonucleotides can form A-type, Watson–Crick-paired duplexes⁵ and, at elevated temperatures, extended single-stranded structures with only three torsion angles significantly different from A-type duplex RNA.⁶ Their preferred conformation appears thus to be similar to that of natural RNA. It was therefore of great interest to see how incorporation of this nonionic modification influences the properties of natural oligomers. We present here the synthesis of chimera of dimethylene sulfone-, methyl sulfoxide-, and methyl sulfide-linked RNA and natural DNA. The extent to which these modifications induce perturbations in double-stranded DNA and RNA was determined in hybridization experiments with unmodified oligonucleotide counterstrands.

Results and Discussion

Incorporation of a dimethylene sulfone-bridged ribonucleotide linkage into DNA was achieved using the phosphor-

amidite protocol. This required preparation of 2'-protected dimer **14** (Scheme 1). Synthesis of this dimeric building block made use of known olefin **1**,^{4a} which after ozonolysis and *in situ* reduction of the resulting aldehyde gave alcohol **2** in 85% yield. Cleavage of the acetal with iodine/methanol,⁷ regioselective silylation, and benzylation converted **2** to glycosyl donor **5**. Silylated uracil was reacted with **5** under Vorbrüggen conditions⁸ to afford 3'-homonucleoside **6** in 90% yield. Conventional ester hydrolysis gave **7**, and a Mitsunobu-type hydroxyl to thioester conversion at the 3' position yielded **8**, which was coupled with iodohomothymidine **9**⁹ using cesium carbonate in THF/DMF. Under these conditions, the liberation of the attacking thiolate occurs via migration of the protecting acetyl group to the 2'-hydroxyl group. As expected,^{4c} 30% of the thioether precursor of **11** was formed as the 2'-unprotected dimer due to intermolecular transesterification. After oxidation of the crude coupling product with buffered Oxone, alcohol **10** was isolated by chromatography, and acetylated with Ac₂O/pyridine, and the combined fractions of **11** were desilylated with HF/pyridine. The yield of isolated diol **12** was 50% over four steps. Dimethoxytritylation and phosphitylation with bis(diisopropylamino) (β -cyanoethoxy)phosphine¹⁰ afforded phosphoramidite **14** as the expected mixture of stereoisomers, which was characterized by FAB-MS and ¹H and ³¹P NMR spectroscopy.

Sulfone dimer **14** was incorporated into modified DNA decamers **17** and **21** (Scheme 2) using a standard phosphoramidite protocol (see Experimental Section). Interestingly, the all-pyrimidine oligomer **21** coeluted from RP₁₈ HPLC columns with shorter "failure sequences" and had to be purified on an ion-exchange resin. Structure confirmation for both oligomers was obtained from MALDI-TOF MS under established conditions.¹¹

Duplexes between the chimeras **17** and **21** and their complementary DNA strands **22** and **25** had lower melting points than the double helices of the unmodified controls (**16/22** and **20/25**) (Table 1). The lowering of the melting temperature (ΔT_m) was considerably more pronounced for the internally modified **17** than for **21**, which bears a terminal modification. The interpretation of this phenomenon is difficult in this case, as the oligomers have different sequences. Examples from the literature are known, however, where oligomers of a given sequence have shown a similar melting point depression upon positioning a terminal nonionic¹² or charged¹³ backbone modification in the middle of the sequence. Assuming that regularity in a duplex structure has energetic significance, a perturbation in backbone structure may be more destabilizing when in the middle of a strand than when at an end. It may be argued that the ribonucleoside units in chimera **17** and **21** cause A-type structural perturbations to otherwise B-type DNA duplexes, as

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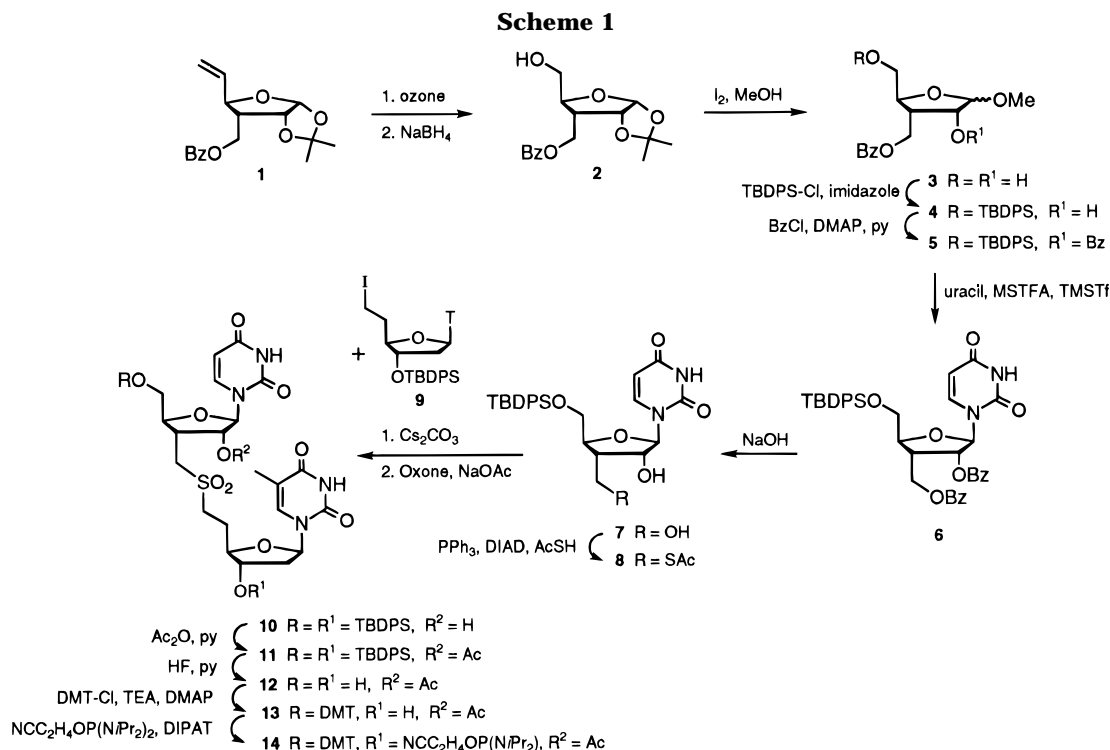
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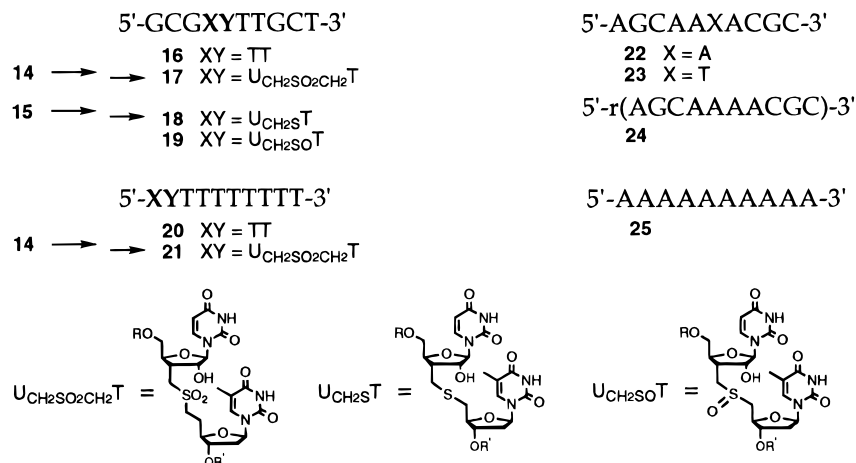
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Scheme 2



dimethylene sulfone-linked oligoribonucleotides have been observed to be conformationally similar to natural RNA,^{4c,5,6} which is found almost exclusively in an A or A' conformation.¹⁴ This effect could be expected to lead to improved binding of the ribo-modified oligomers to RNA counter strands, because DNA/RNA duplexes are known to adopt A-type conformations.¹⁴ The melting point of **17** with RNA-target **24** was only slightly elevated, however ($\Delta T_m -14^\circ\text{C}$ versus -16°C for **17/22**), a selectivity for RNA considerably less pronounced than that reported for an ethylenesulfide-linked RNA-DNA chimera.¹⁵ The lowering of the melting points of duplexes containing **17** and **21** may be caused by increased stability of self-structure in the modified oligonucleotides and not by decreased stability of the duplexes containing them.¹⁶ A high-temperature UV transition has been

observed for a non-self-complementary dimethylene sulfone-linked RNA octamer, corroborating this assumption.^{4c}

Hybridization experiments with **17** and DNA target strand **23**, which contains a T mismatch opposite the 3'-nucleoside of $\text{U}_{\text{CH}_2\text{SO}_2\text{CH}_2}\text{T}$, led to disappearance of an observable melting point (Table 1). When the experiment was run at 1 M NaCl, it could be shown that the sequence specificity of modified oligomer **17** was greater than that of the control oligomer **16**. The lowering of the melting point induced by the mismatch was greater than 24°C for sulfone-linked **17** versus 16°C for **16**. This suggests involvement of the nucleobases of the UT dimer in Watson-Crick base-pairing and virtually excludes bulging. The nonionic linkage in **17** does not reduce the dependence of the melting point on ionic strength significantly, since ΔT_m values remained almost unchanged when increasing the NaCl content from 0.1 to 1 M (Table 1, compare, e.g., rows 1, 2 and rows 5, 6). As modification

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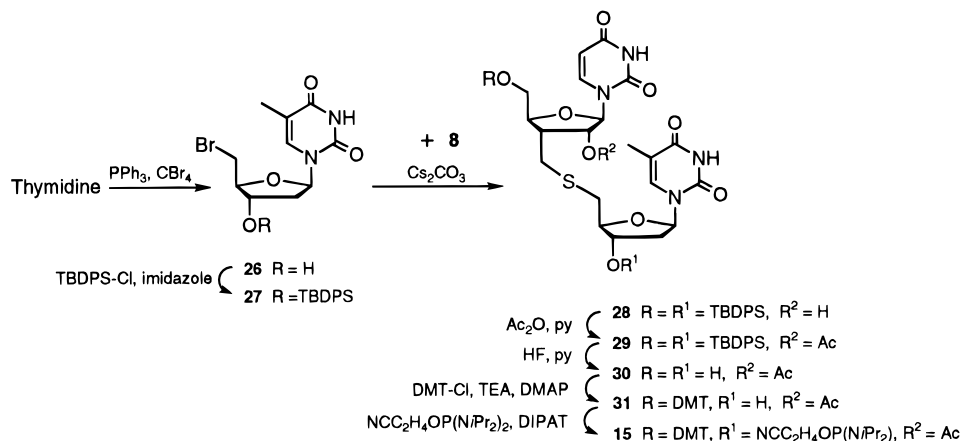
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Table 1. Melting Temperatures of Duplexes Containing Natural and Modified Oligonucleotides^a

oligomer	target	[NaCl] (M)	<i>T</i> _m (°C)	Δ <i>T</i> _m (°C)
5'-GCGTTTTGCT-3' (16)	3'-CGCAAAACGA-5' (22)	0.1	46	
5'-GCGU _{CH₂SO₂CH₂} TTTGCT-3' (17)	3'-CGCAAAACGA-5' (22)	0.1	30	-16
5'-GCGU _{CH₂S} TTTGCT-3' (18)	3'-CGCAAAACGA-5' (22)	0.1	30	-16
5'-GCGU _{CH₂SO} TTTGCT-3' (19)	3'-CGCAAAACGA-5' (22)	0.1	23	-23
5'-GCGTTTTGCT-3' (16)	3'-CGCAAAACGA-5' (22)	1	53	
5'-GCGU _{CH₂SO₂CH₂} TTTGCT-3' (17)	3'-CGCAAAACGA-5' (22)	1	39	-14
5'-GCGTTTTGCT-3' (16)	3'-CGCATAACGA-5' (23)	0.1	24	
5'-GCGU _{CH₂SO₂CH₂} TTTGCT-3' (17)	3'-CGCATAACGA-5' (23)	0.1	<15	<-9
5'-GCGU _{CH₂S} TTTGCT-3' (18)	3'-CGCATAACGA-5' (23)	0.1	<15	<-9
5'-GCGU _{CH₂SO} TTTGCT-3' (19)	3'-CGCATAACGA-5' (23)	0.1	<15	<-9
5'-GCGTTTTGCT-3' (16)	3'-CGCATAACGA-5' (23)	1	37	
5'-GCGU _{CH₂SO₂CH₂} TTTGCT-3' (17)	3'-CGCATAACGA-5' (23)	1	<15	<-22
5'-GCGTTTTGCT-3' (16)	3'- <i>r</i> (CGCAAAACGA)-5' (24)	0.1	45	
5'-GCGU _{CH₂SO₂CH₂} TTTGCT-3' (17)	3'- <i>r</i> (CGCAAAACGA)-5' (24)	0.1	31	-14
5'-GCGU _{CH₂S} TTTGCT-3' (18)	3'- <i>r</i> (CGCAAAACGA)-5' (24)	0.1	27	-18
5'-GCGU _{CH₂SO} TTTGCT-3' (19)	3'- <i>r</i> (CGCAAAACGA)-5' (24)	0.1	22	-23
5'-TTTTTTTTTT-3' (20)	3'-AAAAAAAAAA-5' (25)	0.1	22	
5'-U _{CH₂SO₂CH₂} TTTTTTTTTT-3' (21)	3'-AAAAAAAAAA-5' (25)	0.1	18	-4
5'-TTTTTTTTTT-3' (20)	3'-AAAAAAAAAA-5' (25)	1	35	
5'-U _{CH₂SO₂CH₂} TTTTTTTTTT-3 (21)	3'-AAAAAAAAAA-5' (25)	1	32	-3

^a Melting temperatures were determined as previously described.²⁴ Solutions were 1:1 mixtures of single strands (ca. 4 μM each), 10 mM NaH₂PO₄, adjusted to pH 7.0, and 0.1 mM EDTA. An estimate of experimental errors was obtained from experiments run in duplicate, which gave melting points identical within ±1.5 °C.

Scheme 3

of the DNA backbone with other nonionic linkages in most cases also lowers the melting temperature,¹⁷ abolition of the phosphate–phosphate repulsion appears to be a stability factor that is easily overcome by other, destabilizing effects.

The influence of backbone modifications on the thermal stability of oligonucleotide duplexes was subjected to further scrutiny. Sulfide-linked dinucleoside **15** was synthesized, which contains the ribonucleoside portion of **14** but lacks one CH₂ group in the ethylene linker of the 3'-nucleoside (Scheme 3). Synthesis of **15** started with bromination of thymidine using a modification of a known procedure.¹⁸ When bromide **26** was reacted with thioester **8**, the coupling yield never exceeded 40%. Silylation of the free 3'-position in **26** gave nucleoside **27**, which reacted smoothly with **8** to afford the desired thioether-linked dimer in almost 80% combined yield of 2'-acetylated **29** and 2'-deprotected **28**. The latter compound was converted to the former by acetylation with

Ac₂O/pyridine. Desilylation, dimethoxytritylation, and phosphitylation were performed as described for **11**, yielding **15**, which was used for the solid phase assembly of oligomer **18** (Scheme 2).

Mass spectrometric analysis of **18** showed that during DNA synthesis approximately 60% of the thioether had been oxidized to the sulfoxide **19**. This was unexpected, as thioether-linked nucleoside dimers have been reported to be resistant to oxidation with I₂/pyridine/THF/water even after prolonged reaction times.^{15,19} Even more unexpected was the fact that thioether **18** could readily be separated from the mixture of sulfoxide diastereomers **19** by reversed-phase HPLC. This leads us to assume that a major conformational change occurs when the linking sulfur atom is oxidized. This was confirmed in the hybridization experiments (Table 1). Thioether-containing duplex **18/22** gave Δ*T*_m of -16 °C relative to the unmodified duplex, whereas the sulfoxide-containing duplex **19/22** gave a Δ*T*_m of -23 °C. The presence of two diastereomers in **19** did not lead to a biphasic melting curve. The modified oligomers **18** and **19** both displayed sequence specificity when hybridized to the mismatch-

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containing counterstrand **23**. While solvation effects have not been excluded, molecular modeling and force field calculations indicated that the T_m differences between the two duplexes may be due to different $\text{CH}_2\text{-S-CH}_2$ angles. The wider angle typically found in thioethers appears to distort the conformation of the UT dimer embedded in an A-type duplex less than the smaller angle in the sulfoxide. Attempts to oxidize the diastereomeric sulfoxides **19** to the corresponding sulfone led to decomposition of the oligomer, possibly via a mechanism proposed for ethylidene sulfone-linked analogs.²⁰

In conclusion, we have shown that ribo dimethylene sulfone, methyl sulfide, and methyl sulfoxide linkages can be incorporated into DNA oligomers. These linkages do not prevent the formation of DNA/DNA and DNA/RNA duplexes, and sequence-specific binding is observed. Therefore, incorporation of the nonionic dinucleosides presented here as well as longer sulfone-linked oligomers^{4c} may become a tool for studying nucleic acids and nucleic acid protein interactions.²¹ Their incorporation in synthetic RNA, in particular, merits exploration. In addition, we have shown that oxidation of a single thioether linker in a modified oligonucleotide can shift the melting point of its duplex with complementary DNA by 7 °C. Methyl sulfide-linked oligomers may thus have applications as chemotunable hybridization probes. Work in this area is in progress.²²

Experimental Section

General Procedures. Reactions involving air- or moisture-sensitive compounds were carried out in glassware dried either in an oven or under high vacuum (HV) and then placed under a positive pressure of argon. Molecular sieves (3 or 4 Å; grain size: 2–3 mm) were activated under high vacuum at 360 °C for 36 h. THF was distilled from sodium. DMF, pyridine and CH_3CN were puriss, absolute, over molecular sieves from Fluka, Buchs, Switzerland. Dry acetonitrile and 1,2-dichloroethane were from Rathburn Chemicals Ltd. All reagents were the best available quality from Fluka. Deoxyribonucleoside phosphoramidites, 5'-*O*-(dimethoxytrityl)thymidine LCAA-CPG, and DNA desalting cartridges were obtained from Glen Research Corporation. Diisopropylammonium tetrazolid²³ was kindly provided by B. Eschgfäller (University of Freiburg). Solvents for extraction and chromatography were distilled from anhydrous CaSO_4 (Sikkon, Fluka). Acetate buffer was made up from acetic acid (3 M), NaOAc (1 M), and deionized water. Analytical thin-layer chromatography was performed on E. Merck 60 F₂₅₄ precoated silica gel plates. Flash chromatography was performed under 0.2–0.3 bar pressure with Merck silica gel 60 (0.040–0.063 mm mesh). Eluent mixtures for chromatography are expressed as ratios of volumes. Reversed-phase HPLC purification of oligonucleotides was performed on a Supelcosil LC 18-S (RP 18, 250 × 10 mm) reversed-phase column with HPLC-grade CH_3CN (Merck) and 0.1 M triethylamine/acetate buffer (TEA/A buffer) pH 7.0. Anion-exchange chromatography was performed on a Nucleogel SAX 1000–8/46 (50 × 4.6 mm) anion-exchange column

(Macherey-Nagel) with 20 mM $\text{KHPO}_4/\text{CH}_3\text{CN}$ (4:1) and a gradient of KCl (0–1 M). Assignments (see the supporting information) are based on homonuclear decoupling, COSY, and DEPT experiments performed on selected compounds. Multiplets reported without spectral region cover ≤ 0.3 ppm. FAB spectra were obtained using a 3-nitrobenzyl alcohol (NOBA) matrix; MALDI-TOF spectra used a 2,4,6-trihydroxyacetophenone/diammonium hydrogen citrate matrix.¹¹ Experimental errors of the MALDI-TOF mass peaks are estimated to be $\pm 0.1\%$. Combustion analyses were performed by the Micro-analytical Laboratory ETH Zurich. HPLC-purified oligonucleotides **22–25** were obtained from Cruachem, Glasgow, Scotland, and were used without modification.

3-Deoxy-3-[(benzoyloxy)methyl]-1,2-isopropylidene- α -ribofuranose (2). Ozone/ O_2 gas was introduced into a stirred solution of alkene **1**^{4a} (66.3 mg, 0.21 mmol) in dry MeOH (5 mL) at –73 °C. A white precipitate formed during ozone treatment. After 30 min the solution turned blue, and the ozone introduction was terminated. N_2 was bubbled through the solution to remove the excess of ozone. A solution of NaBH_4 (21.7 mg, 0.57 mmol) in EtOH (2 mL) was added. The reaction mixture was allowed to warm to –15 °C and was stirred for another 60 min. Acetic acid (0.5 mL) was added dropwise to the solution, and the mixture was stirred for an additional 20 min at rt. The reaction mixture was diluted with ethyl acetate (20 mL) and was washed with aqueous saturated NaHCO_3 (20 mL) and brine (5 mL). The aqueous phase was reextracted twice with ethyl acetate. The combined organic phases were dried over MgSO_4 , filtered, and concentrated *in vacuo*, and the residue was chromatographed on silica (10 g) with petroleum ether/ethyl acetate (1:1). Alcohol **2** was obtained as a colorless oil (55.1 mg, 0.178 mmol). Yield: 85%. ¹H NMR (300 MHz, CDCl_3) δ : 1.33, 1.52 (2s); 2.3 (s, 1H); 2.57 (m, 1H); 3.68 (m, 1H); 3.95 (m, 1H); 4.11 (m, 1H); 4.41; 4.61 (dq, 2H); 4.79 (t, 1H); 5.86 (d, 1H); 7.42 (AA'BB'C-system, BB'-part, 2H); 7.55 (AA'BB'C-system, C-part, 1H); 8.02 (AA'BB'C-system, AA'-part, 2H). ¹³C NMR (75 MHz, CDCl_3) δ : 26.3, 26.7, 43.2, 61.4, 62.2, 80.7, 81.2, 105, 112.3, 128.4, 129.5, 129.8, 133.2, 166.4. MS *m/z* 307 ($\text{M} + \text{H}^+$, <1); 293 (29); 278 (0.32), 277 (1.9), 105 (100). Anal. Calcd for $\text{C}_{16}\text{H}_{20}\text{O}_6$: C, 62.33; H, 6.54. Found: C, 62.05; H, 6.26.

3-[(Benzoyloxy)methyl]-3-deoxy-1-*O*-methyl- α - and β -ribofuranose (3). To a solution of alcohol **2** (5.3 g, 17.2 mmol) in MeOH (170 mL) was added iodine (0.82 g, 3.2 mmol) under stirring at rt. The solution was refluxed for 4.5 h. The reaction mixture was allowed to cool to rt. Pyridine (3.3 mL) and $\text{Na}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ (1.6 g) were added, and the mixture was stirred for an additional 15 min before the solvents were removed by rotary evaporation. The residue was taken up in ethyl acetate (100 mL) and washed with brine (80 mL). The aqueous phase was reextracted twice with ethyl acetate. The combined organic phases were dried over MgSO_4 . After filtration and concentration *in vacuo*, the residue was chromatographed on silica (60 g) with ethyl acetate/petroleum ether (1:1–3:1). A mixture of the α - and β -anomers of **3** was obtained as a light yellow oil (3.02 g, 10.7 mmol). Yield: 62%. ¹H NMR (β -anomer, 300 MHz, CDCl_3) δ : 2.31 (q, 1H); 2.73 (m, 1H); 3.32 (d, 1H); 3.40 (s, 3H); 3.62, 3.86 (m, 2H); 4.18 (m, 2H); 4.24 (q, 1H); 4.81 (dd, 1H); 4.89 (s, 1H); 7.44 (t, 2H); 7.58 (m, 1H); 8.02 (d, 2H). ¹H NMR (α -anomer, 300 MHz, CDCl_3) δ : 2.11 (q, 1H); 2.60 (m, 1H); 2.78 (d, 1H); 3.49 (s, 3H); 3.62, 3.86 (m, 2H); 4.18 (m, 2H); 4.39 (m, 1H); 4.62 (dd, 1H); 5.00 (s, 1H); 7.44 (t, 2H); 7.58 (m, 1H); 8.02 (d, 2H). ¹³C NMR (β -anomer, 75 MHz, CDCl_3) δ : 40.9, 55.5, 61.3, 64.1, 75.4, 81.5, 109.2, 128.4, 129.6, 129.7, 133.4, 167.4. ¹H NMR (α -anomer, 75 MHz, CDCl_3) δ : 42.4, 55.2, 6.1, 63.9, 72.5, 81.2, 103.1, 128.3, 129.5, 129.9, 133.1, 166.5. MS *m/z*: 281 (M^+ , <1); 263; 251 (4.6); 233 (0.9); 176 (2.8); 105 (100). Anal. Calcd for $\text{C}_{14}\text{H}_{17}\text{O}_6$: C, 59.57; H, 6.43. Found: C, 59.17; H, 6.48.

3-[(Benzoyloxy)methyl]-5-(*tert*-butyldiphenylsilyl)-3-deoxy-1-*O*-methyl- α and β -ribofuranose (4). To a solution of diol **3** (2.97 g, 10.5 mmol) and imidazole (1.72 g, 25.2 mmol) in anhydrous THF (60 mL) was added *tert*-butyldiphenylsilylchloride (TBDPSCI) (3.33 g, 11.6 mmol) under stirring at 0 °C. A white precipitate formed during the addition. The reaction mixture was allowed to warm to rt and was stirred for another 60 min. Dry MeOH (4 mL) was added and the solvents were removed by rotary evaporation. The residue was taken up in CH_2Cl_2 (140 mL), washed quickly with 1 N HCl, saturated aqueous NaHCO_3 , and brine (100 mL each), and dried over

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MgSO₄. Filtration and concentration *in vacuo* yielded crude **4** (5.9 g) in quantitative yield containing approximately 10% silyl methyl ether. The reaction product was submitted directly to benzoylation. An analytical sample was prepared by chromatography on silica with petroleum ether/ethyl acetate (2:1). ¹H NMR (β -anomer, 300 MHz, CDCl₃) δ : 1.11 (s, 9H); 2.68 (m, 1H); 3.28 (s, 3H); 3.82 (m, 2H); 4.01–4.08 (m, 2H); 4.29 (dd, 1H, J = 11.5, 4.5 Hz); 4.81 (dd, 1H, J = 11.5, 10.5 Hz); 4.83 (s, 1H); 7.32–7.49 (m, 8H); 7.58 (m, 1H); 7.71 (m, 4H); 8.02 (d, 2H). ¹³C NMR (β -anomer, 75 MHz, CDCl₃) δ : 19.2, 26.8, 45.4, 54.5, 61.3, 66.9, 75.3, 80.1, 108.7, 127.7, 128.3, 128.5, 129.8, 133.2, 133.3, 135.6, 129.6, 133.4, 167.5. MS m/z : 519 (M⁺, <1); 489 (5.6); 463 (6.9); 431 (9.5); 105 (100); 77 (30). Anal. Calcd for C₃₀H₃₆SiO₆: C, 69.20; H, 6.97. Found: C, 69.14; H, 6.95.

2-Benzoyl-3-[(benzoyloxy)methyl]-5-(tert-butylidiphenylsilyl)-3'-deoxy-1-O-methyl- α - and β -ribofuranose (5). Crude **4** (5.6 g, containing 10.2 mmol of pure compound) was dissolved in pyridine (105 mL). (Dimethylamino)pyridine (DMAP) (5 mg, 41 μ mol) was added, and the mixture was cooled to 0 °C. Benzoyl chloride (2.37 g, 20.4 mmol) was slowly added, and the reaction mixture was allowed to warm to rt. After 2.5 h, saturated aqueous NaHCO₃ (40 mL) was added at 0 °C, and the mixture was stirred for an additional 10 min. The solvent was partly evaporated before the mixture was diluted with ethyl acetate (150 mL). The mixture was washed with water, 1 N HCl, saturated aqueous NaHCO₃, and brine (100 mL each) and dried over MgSO₄. After filtration and concentration *in vacuo*, the residue was chromatographed on silica (320 g) with petroleum ether/ethyl acetate (7:1–2:1). Dibenzate **5** was obtained as a colorless oil (5.17 g, 8.27 mmol). Yield: 78% over silylation and benzoylation. ¹H NMR (β -anomer, 300 MHz, CDCl₃) δ : 1.08 (s, 9H); 3.04 (m, 1H); 3.35 (s, 3H); 3.88 (m, 2H); 4.34 (quint, 1H); 4.55 (m, 2H); 5.01 (s, 1H); 5.55 (d, 1H, J = 4.2 Hz); 7.32–7.47 (m, 10H); 7.55 (m, 2H); 7.71 (m, 4H); 8.02 (m, 4H). ¹³C NMR (β -anomer, 75 MHz, CDCl₃) δ : 19.2, 26.8, 42.0, 54.8, 61.2, 66.6, 77.8, 82.0, 106.8, 127.7, 128.3, 128.37, 128.43, 129.4, 129.6, 129.7, 133.0, 133.2, 133.3, 135.6, 129.4, 133.4, 166.2, 165.6. MS m/z : 593 (M⁺ – OCH₃, 28); 567 (20); 547 (2); 105 (100); 77 (30). Anal. Calcd for C₃₇H₄₀SiO₇: C, 71.13; H, 6.45. Found: C, 71.20; H, 6.52.

1-[2'-Benzoyl-3'-(benzoyloxy)methyl]-5'-(tert-butylidiphenylsilyl)-3'-deoxy- β -ribofuranosyl]uracil (6). Uracil (538 mg, 4.8 mmol) was dried under high vacuum at 60 °C and then suspended in dry CH₃CN (17.5 mL). *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) (3.5 mL, 19.2 mmol) was added under stirring, resulting in a clear solution after 10 min. Glycosyl donor **5** (1.5 g, 2.4 mmol), which had been coevaporated with toluene and dried under high vacuum at 50 °C, was dissolved in dry CH₃CN (17.5 mL) and added to the silylated base. The resulting solution was cooled to 0 °C, and trimethylsilyl triflate (TMSTf) (0.65 mL, 3.6 mmol) was added. The solution was warmed to 40 °C over 30 min and stirred at this temperature. After 3 h and after an additional 4 h, TMSTf (0.4 mL/2.2 mmol, and 0.3 mL/1.7 mmol) was added. After 5 h, the reaction was cooled to 0 °C and the solution was poured into a mixture of saturated NaHCO₃ solution (160 mL), ice (120 g), and CH₂Cl₂ (250 mL). The organic layer was separated, and the aqueous layer was reextracted twice with CH₂Cl₂. The combined organic phases were washed with brine, which was again reextracted twice with CH₂Cl₂. The organic layer was dried over MgSO₄, filtered, and concentrated *in vacuo* and the residue chromatographed on silica (35 g) with petroleum ether/ethyl acetate (2:1) and a stepwise MeOH gradient (1–6%). Removal of residual *N*-methyltrifluoroacetamide under high vacuum yielded homouridine **6** (1.6 g, 2.2 mmol) as a colorless glass: yield 90%. ¹H NMR (300 MHz, CDCl₃) δ : 1.12 (s, 9H); 3.23 (quint, 1H); 3.86 (dd, 1H, J = 12.1, 2.5 Hz); 4.22 (dd, 1H, J = 11.9, 2.2 Hz); 4.34 (m, 1H); 4.42 (m, 2H); 5.42 (dd, 1H, J = 7.1, 2.3 Hz); 5.82 (dd, 1H, J = 6.8, 3.2 Hz); 6.22 (d, 1H, J = 3.2); 7.32–7.47 (m, 10H); 7.55 (m, 2H); 7.68 (m, 4H); 7.85 (d, 1H); 7.99 (m, 4H); 8.77 (s, 1H). ¹³C NMR (50 MHz, CDCl₃) δ : 19.8, 27.0, 40.0, 60.6, 63.7, 77.2, 82.7, 89.0, 102.6, 127.2, 127.9, 128.0, 128.2, 128.4, 128.5, 128.7, 129.3, 129.6, 129.9, 130.1, 130.2, 132.0, 132.7, 133.2, 133.7, 135.4, 135.5, 135.6, 135.7, 139.8, 149.9, 162.8, 165.6, 166.2. FABMS m/z : 647 (M⁺ – *t*-Bu, 10); 627 (4); 593 (22); 105 (100); 77 (29). UV (CH₂Cl₂): λ_{\max} 260 (ϵ 11 300).

1-[5'-(tert-Butylidiphenylsilyl)-3'-deoxy-3'-(hydroxymethyl)- β -ribofuranosyl]uracil (7). Homouridine **6** (288.8

mg, 0.41 mmol) was dissolved in a mixture of MeOH (5.7 mL) and THF (5 mL) and treated with aqueous 2 N NaOH (2 mL, 4 mmol). The reaction mixture was stirred at rt for 1 h and diluted with acetate buffer (1.2 mL). The mixture was concentrated to one-third of its original volume by rotary evaporation, diluted with CH₂Cl₂ (40 mL), and washed with brine (30 mL) and aqueous 50 mM Na₂CO₃ (20 mL). The organic layer was separated, and the aqueous phase was reextracted four times with CH₂Cl₂. The combined organic layers were dried *in vacuo*, and the residue was chromatographed on silica (30 g) with CH₂Cl₂/MeOH (92:8) to yield **7** (143 mg, 0.29 mmol) as a colorless foam. Yield: 70%. ¹H NMR (300 MHz, CDCl₃) δ : 1.10 (s, 9H); 2.49 (m, 1H); 3.29 (s, 1H); 3.63–3.89 (m, 3H); 4.22 (d, 1H, J = 15 Hz); 4.41 (d, 1H, J = 9 Hz); 4.53 (d, 1H, J = 5 Hz); 5.39 (d, 1H, J = 8 Hz); 5.51 (s, 1H); 5.80 (s, 1H); 7.36–7.51 (m, 6H); 7.77 (m, 4H); 8.19 (d, 1H); 10.71 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ : 19.3, 27.1, 41.7, 58.1, 62.5, 78.4, 82.1, 92.4, 102.0, 128.0, 128.1, 130.1, 130.3, 132.2, 132.7, 135.4, 135.6, 140.2, 151.3, 164.0. FABMS m/z : 519 (M + Na⁺, 24); 497 (23); 439 (22); 111 (15); 77 (48); 57 (57). UV (CH₂Cl₂): λ_{\max} 265 (ϵ 7200).

1-[3'-(Acetylthio)methyl]-5'-(tert-butylidiphenylsilyl)-3'-deoxy- β -ribofuranosyl]uracil (8). Homouridine diol **7** (300 mg, 604 μ mol) was coevaporated from toluene, dried under high vacuum, and dissolved in dry CH₃CN (3 mL) and dry THF (2 mL). PPh₃ (328 mg, 1.2 mmol) was dissolved in dry CH₃CN (2 mL), cooled to 0 °C, and treated with diisopropyl azodicarboxylate (DIAD) (220 μ L, 1.15 mmol). After 15 min, the solution of the diol and thioacetic acid (96 mg, 90 μ L, 1.22 mmol) were added at the same time, and the reaction was allowed to warm to rt. After 2 h, the solvents were removed by rotary evaporation. The residue was chromatographed on silica (50 g) with petroleum ether/THF (3:2 and 9:5) to yield thioester **8** as a colorless foam (220 mg, 396 μ mol). Yield: 65%. ¹H NMR (300 MHz, CDCl₃) δ : 1.16 (s, 9H); 2.49 (m, 1H); 2.97 (m, 2H); 3.81 (dd, 1H, J = 2, 12.5 Hz); 4.13–4.26 (m, 2H); 4.32 (d, 1H, J = 4.5 Hz); 5.16 (s, 1H); 5.32 (d, 1H, J = 8.1 Hz); 5.81 (s, 1H); 7.38–7.50 (m, 6H); 7.72 (m, 4H); 8.19 (d, 1H, J = 8.1 Hz); 10.45 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ : 19.3, 23.7, 27.0, 30.5, 40.5, 62.0, 77.1, 84.7, 92.4, 102.0, 128.0, 128.1, 130.1, 130.2, 132.2, 132.8, 135.4, 135.7, 140.0, 150.8, 164.0, 195.6. FABMS m/z : 577 (M + Na⁺, 10); 555 (25); 497 (16); 111 (8); 77 (22); 57 (27). UV (CH₂Cl₂): λ_{\max} 264 (ϵ 10 200).

Compound 11. Homothymidine iodide **9**⁹ (169 mg, 279 μ mol), homouridine thioester **8** (170 mg, 307 μ mol), and CsCO₃ (293 mg, 0.9 mmol) were dried under high vacuum at 50 °C and then pulverized in the flask with a stirring bar. THF (5 mL) was added, and the fine white slurry was stirred for 3 h at rt. DMF (2 mL) was added, and the mixture was stirred for an additional 2.5 h. Acetate buffer (5 mL) and CH₂Cl₂ were added, and the mixture was washed with brine. The aqueous phase was reextracted three times with CH₂Cl₂. The combined organic phases were concentrated *in vacuo*. The crude product, containing 2'-OH thioether and 2'-OAc thioether, was submitted directly to oxidation. The crude mixture of thioethers (approximately 270 μ mol) was dissolved in MeOH (40 mL) and THF (6 mL), and a freshly prepared solution of Oxone (2KHSO₅·K₂SO₄·KHSO₄) (737 mg, 1.12 mmol) and NaOAc (428 mg, 5 mmol) in water (9 mL) was added under stirring. The resulting white slurry was stirred for 2 h at rt. Then, approximately 2/3 of the organic solvent was evaporated, and aqueous saturated Na₂S₂O₃ and CH₂Cl₂ were added. The organic phases were separated, and the aqueous phase was extracted four times with CH₂Cl₂. The combined organic phases were concentrated *in vacuo*, and the residue was chromatographed on silica (38 g) with CH₂Cl₂/ethyl acetate/water (80:20:0.25) and a stepwise MeOH gradient of 3–8%. Fractions containing 2'-OAc dimer **11** (108 mg, 102 μ mol) were concentrated *in vacuo*. Fractions containing the 2'-OH dimer **10** (84 mg, 75 μ mol) were concentrated *in vacuo* and submitted to acetylation. Combined yield over coupling and oxidation: 63%. The sample of 2'-OH dimer **10** (130 mg, 127 μ mol) was coevaporated from pyridine, dried under high vacuum at rt, and dissolved in dry pyridine (2 mL). Acetic anhydride (500 μ L) was added, and the reaction mixture was stirred at rt overnight. Then MeOH (4 mL) was added, and the solvents were removed by rotary evaporation. The residue was taken up in CH₂Cl₂ and was washed twice with aqueous saturated NaHSO₄. The aqueous phases were reextracted with CH₂Cl₂, and the combined organic phases were concentrated *in vacuo* yielding

quantitatively crude 2'-OAc dimer **11**. This product was submitted directly to desilylation. ¹H-NMR (300 MHz, CDCl₃) δ: 1.08 (s, 9H); 1.09 (s, 9H); 1.79 (m, 2H); 1.87 (d, 3H, *J* = 1.2 Hz); 2.12 (s, 3H); 2.21 (m, 2H); 2.74–2.94 (m, 3H); 3.18–3.32 (m, 2H); 3.82–3.93 (m, 1H); 4.00–4.10 (m, 3H); 4.12–4.18 (m, 1H); 5.43 (d, 1H, *J* = 8.2 Hz); 5.54 (m, 1H); 5.75 (t, 1H, *J* = 0.6 Hz); 6.08 (t, 1H, *J* = 7 Hz); 6.90 (d, 1H, *J* = 1.1 Hz); 7.33–7.49 (m, 12H); 7.51 (d, 1H, *J* = 8.2 Hz); 7.61–7.67 (m, 8H); 8.92 (s, 1H); 9.04 (s, 1H). ¹³C-NMR (75 MHz, CDCl₃) δ: 12.4, 19.1, 19.3, 20.7, 25.2, 26.88, 26.9, 35.4, 38.5, 49.0, 50.2, 63.4, 75.9, 76.5, 83.7, 84.6, 87.7, 91.0, 103.0, 111.4, 128.0, 128.1, 130.1, 130.2, 130.3, 130.35, 132.2, 132.7, 132.8, 132.9, 135.4, 135.7, 135.9, 136.8, 140.5, 150.0, 150.2, 163.6, 163.7, 169.4. FABMS *m/z*: 1085 (M + Na⁺, 20%); 1063 (M⁺, 6); 1005 (12); 937 (13); 623 (18); 603 (46); 569 (23); 511 (21); 491 (21); 461 (11); 401 (17); UV (CH₂Cl₂): λ_{max} 261 (ε 17 400).

Compound 12. 2'-OAc dimer **11** (181 mg, 170 μmol) was dissolved in dry pyridine (0.5 mL) in a 10 mL polypropylene tube under Ar. An HF/pyridine solution (0.7 mL, 3.29 mmol, 4.7 M in pyridine) was added, and the resulting solution was stirred for 3 h at rt before methoxytrimethylsilane (4 mL, 28 mmol) was added. A white precipitate formed. Stirring continued for 30 min before the mixture was concentrated *in vacuo*. The residue was chromatographed on silica (22 g) with CH₂Cl₂/EtOH/MeOH/water (86:7:7:0.25 and 83:9:8:0.25) to yield diol **12** as a white glass (79.5 mg, 135 μmol). Yield: 79%. ¹H-NMR (400 MHz, DMSO) δ: 1.87 (d, 3H, *J* = 1.1 Hz); 1.98 (m, 1H); 2.04–2.25 (m, 2H); 2.08 (s, 3H); 2.24 (m, 1H); 3.04 (m, 1H); 3.28 (m, 1H); 3.42 (m, 2H); 3.61 (m, 1H); 3.73 (m, 2H); 3.97 (m, 1H); 4.13 (m, 1H); 4.33 (s, 1H); 5.15 (s, 1H); 5.36 (dd, 1H, *J* = 2.6, 6.8 Hz); 5.66 (d, 1H, *J* = 8.1 Hz); 5.75 (d, 1H, *J* = 2.7 Hz); 6.15 (t, 1H, *J* = 7 Hz); 7.42 (d, 1H, *J* = 1 Hz); 7.84 (d, 1H, *J* = 8 Hz); 11.31 (s, 2H). ¹³C-NMR (100 MHz, DMSO) δ: 11.9, 20.4, 24.8, 34.5, 38.0, 47.9, 49.9, 60.3, 72.7, 75.6, 83.2, 83.4, 83.7, 89.7, 101.9, 109.9, 136.1, 141.4, 150.0, 150.4, 163.0, 163.6, 169.1. FABMS *m/z*: 609 (M + Na⁺, 7); 587 (3.5); 391 (3); 369 (3); 329 (3); 307 (11); 289 (7); 176 (21); 154 (64). UV (CH₂Cl₂/MeOH 1:1) λ_{max} 262 (ε 16 000).

Compound 13. Dimer **12** (75.8 mg, 129 μmol) was coevaporated with dry pyridine, dried under high vacuum at rt, and dissolved in dry pyridine (2 mL). TEA (45 mL, 320 μmol), DMAP (ca. 5 mg), and molecular sieves (6 beads, 4 Å) were added, and the solution was stirred for 15 min at rt before dimethoxytriphenylchloride (DMT-Cl) (90 mg, 258 μmol) was added. The yellow solution was stirred for 2.5 h at rt. Then, MeOH (2 mL) was added, and the solution was stirred for an additional 30 min before the mixture was concentrated *in vacuo*. The residue was chromatographed on silica (24 g) with CH₂Cl₂/iPrOH/MeOH/TEA (94:3:3:0.5 and 92:4:4:0.5) to yield 5'-DMT dimer **13** as a colorless solid (112.5 mg, 126 μmol). Yield: 97%. ¹H-NMR (400 MHz, CDCl₃) δ: 1.79 (d, 3H, *J* = 0.9 Hz); 1.88–2.05 (m, 1H); 2.04 (s, 3H); 2.15–2.26 (m, 3H); 2.91 (m, 1H); 3.08–3.36 (m, 5H); 3.49 (m, 1H); 3.80 (m, 1H); 3.97 (m, 1H); 3.67 (2Ys, 6H); 4.13 (m, 1H); 5.42 (d, 1H, *J* = 8.2 Hz); 5.52 (d, 1H, *J* = 1.5 Hz); 5.62 (d, 1H, *J* = 6.7 Hz); 6.02 (t, 1H, *J* = 6.7 Hz); 6.74 (d, 4H); 6.99 (s, 1H); 7.11 (m, 1H); 7.20 (m, 6H); 7.31 (d, 2H); 7.49 (d, 1H, *J* = 8.2 Hz); 9.63 (s, 1H); 9.80 (s, 1H). ¹³C-NMR (100 MHz, CDCl₃) δ: 12.4, 20.7, 25.1, 36.4, 39.0, 48.9, 49.5, 55.3, 62.5, 73.9, 76.4, 82.3, 83.9, 85.9, 92.6, 102.8, 111.5, 113.4, 127.2, 128.05, 128.12, 130.12, 130.15, 135.21, 135.24, 136.3, 141.7, 144.2, 150.5, 150.6, 158.7, 163.5, 164.0, 169.8. FABMS *m/z*: 990 (M + H⁺, 4.7); 888 (1.4); 492 (11); 391 (33); 303 (100). UV (CH₂Cl₂): λ_{max} 259 (ε 16 900); 238 (ε 18 450).

Compound 14. Dimer **13** (251 mg, 28 μmol) and diisopropylammonium tetrazolidate (DIPAT) (3 mg) were dried under high vacuum at 40 °C and then dissolved in dry CH₃CN (150 μL). Bis(diisopropylamino)-(β-cyanoethoxy)phosphine (15 μL, 47 μmol) was added at rt, and the solution was stirred for 3 h. Aqueous saturated NaHCO₃ (5 mL) and CH₂Cl₂ (5 mL) were added, and the phases were separated. The organic phase was washed with brine. The aqueous phase was reextracted twice with CH₂Cl₂. The combined organic phases were dried over MgSO₄, filtered, and concentrated *in vacuo* to a volume of 1 mL. The product was precipitated twice with hexane (5 mL) from the concentrated solution to yield phosphoramidite **14** (two diastereomers) as a colorless solid (18.5 mg, 17 μmol). Yield: 60%. ¹H-NMR (selected signals of diastereomeric mixture, 300 MHz, CDCl₃) δ: 1.12–1.34 (m, 2H); 1.91 (m, 3H, *J* = 0.9 Hz); 2.14 (2Ys, 3H); 2.38, 2.63 (dt, 4H); 3.58 (dt, 4H); 3.69 (2Ys, 6H); 5.45 (d, 1H);

5.62 (m, 1H); 5.76, 5.80 (dd, 1H); 6.03 (dt, 1H); 6.82 (d, 4H); 7.04 (m, 1H); 7.22 (m, 1H); 7.30 (m, 6H); 7.41 (dd, 2H); 7.66 (dd, 1H). ³¹P-NMR (121 MHz, CDCl₃) δ: 148.8 (s); 149.5 (s). FABMS *m/z*: 1661 (0.49); 1190 (M + (iPr)₂N⁺, 0.13); 1013 (M + Na⁺, 0.25); 1090 (M + H⁺, 0.3); 303 (100).

5'-Bromo-5'-deoxythymidine (26). (Procedure modified after ref 18). A mixture of thymidine (441.5 mg, 1.823 mmol) and triphenylphosphine (956 mg, 3.65 mmol) was coevaporated from pyridine (20 mL) and dried, *in vacuo*, at 55 °C for 2 h. The solid was subsequently dissolved in pyridine (35 mL), and a solution of tetrabromomethane (1.088 g; 3.28 mmol) in pyridine (5 mL) was added. The yellow solution was stirred for 1 h (TLC: ethyl acetate/CH₂Cl₂/methanol, 60:40:10, *R_f* thymidine 0.28, *R_f* product 0.53). The mixture was then evaporated to dryness, *in vacuo*. The resulting yellow-brown oil was chromatographed on silica gel (50 g), with CH₂Cl₂/ethyl acetate (2:3) and a stepwise MeOH gradient (4–6%). The first product fractions contained some triphenyl phosphinoyl that was removed by washing the solid obtained after evaporation with CH₂Cl₂ (3 × 5 mL). The combined product fractions gave **26** (368 mg; 1.21 mmol) as a colorless foam. Yield: 66%. Mp: 157–158 °C (lit.¹⁶ mp 157–158 °C). ¹H-NMR (200 MHz, DMSO-*d*₆) δ: 1.79 (s, 3H); 2.09, 2.28 (2 × m, 2H); 3.65 (dd, 1H, *J* = 6.3, 10.2 Hz); 3.75 (dd, 1H, *J* = 5.7, 10.7 Hz); 3.93 (m, 1H); 4.23 (m, 1H); 5.49 (br d, 1H); 6.23 (t, 1H, *J* = 6.4 Hz); 7.51 (s, 1H); 11.33 (br s, 1H). ¹³C-NMR (50 MHz, DMSO-*d*₆) δ: 11.78, 33.38, 37.59, 71.57, 83.49, 84.73, 109.47, 135.62, 150.06, 163.25. UV (MeOH): λ_{max} (nm) 266 (ε 18 000); 226 (ε 5 000).

5'-Bromo-5'-deoxy-3'-(tert-butylidiphenylsilyl)thymidine (27). Bromide **26** (528.3 mg, 1.73 mmol) was dissolved in warm MeOH and coevaporated from toluene. Imidazole (353.6 mg, 5.19 mmol) was added, and the mixture was dried, *in vacuo*, at 50 °C for 1 h. Subsequently, the solid was dissolved in tetrahydrofuran (10.5 mL), and *tert*-butylidiphenylsilyl chloride (665 μL, 2.60 mmol) was slowly added, causing precipitation of imidazolium chloride. The suspension was heated to 40 °C for 14 h (TLC: CH₂Cl₂/ethyl acetate/methanol, 75:25:5, *R_f* **26** 0.22, *R_f* product 0.79). MeOH (4 mL) was added, and the solution was stirred for 10 min and then evaporated to dryness, *in vacuo*. The resulting solid was taken up in CH₂Cl₂ (20 mL) and water (1 mL), and the organic phase was extracted with ice-cold 0.1 M hydrochloric acid (2 × 10 mL) and saturated aqueous sodium bicarbonate (10 mL). The aqueous phases were back-extracted with CH₂Cl₂, and the combined organic phases were evaporated to dryness. The resulting foam was purified by column chromatography on silica (95 g) with CH₂Cl₂ and a stepwise MeOH gradient (1.5–7%). Silyl ether **27** was isolated as a white foam (882 mg, 1.62 mmol, 94% yield). ¹H-NMR (200 MHz, CDCl₃) δ: 1.10 (s, 9H); 1.89 (s, 3H); 1.99 (m, 1H); 2.37 (ddd, 1H, *J* = 2.4, 5.9, 13.1 Hz); 2.84 (dd, 1H, *J* = 3.4, 11.3 Hz); 3.34 (dd, 1H, *J* = 3.5, 11.3 Hz); 4.09 (m, 1H); 4.36 (m, 1H); 6.41 (dd, 1H, *J* = 5.8, 8.2 Hz); 7.33 (s, 1H); 7.43 (m, 6H); 7.66 (m, 4H); 8.84 (br s, 1H). ¹³C-NMR (75 MHz, CDCl₃) δ: 12.80, 19.19, 27.10, 33.60, 40.86, 74.86, 84.72, 85.25, 111.67, 128.37, 128.41, 130.58, 130.68, 133.14, 133.38, 135.80, 136.14, 150.76, 164.19; FAB-MS *m/z*: 545, 543 (M + H⁺). UV (CH₂Cl₂) λ_{max} 265 (ε 10 700); 228 (ε 7 100).

Compound 29. Cesium carbonate (29.4 mg, 0.0901 mmol, flame dried, *in vacuo*, for 2 min), bromide **27** (11.3 mg, 0.0207 mmol), and thioester **8** (10.0 mg, 0.0180 mmol) were mixed and dried, *in vacuo*, at 40 °C for 1 h. Subsequently, tetrahydrofuran (0.7 mL) was added, and the suspension was stirred at rt. The reaction was followed by TLC (methanol/hexane/CH₂Cl₂, 5:20:75, *R_f* bromide 0.51, *R_f* thioester 0.22, *R_f* 2'-OAc-product 0.18). After 10 h acetate buffer (45 μL; 3 M acetic acid, 1 M sodium acetate) was added, and the reaction mixture was evaporated to dryness, *in vacuo*. The resulting solid was taken up in half-saturated brine (3 mL) and CH₂Cl₂ (3 mL). The aqueous phase was extracted with CH₂Cl₂ (4 × 3 mL). The combined organic phases were washed with brine (10 mL) and evaporated to dryness, *in vacuo*. The residue was purified by column chromatography on silica (3 g) with methanol/ethanol/water/CH₂Cl₂ (1:1:0.2:98, stepwise gradient to 3:3:0.2: 94). Compound **29** (9.6 mg, 0.0094 mmol; 52%) was isolated as a white foam. Fractions containing compound **28**, which eluted after **29**, were combined, dried *in vacuo*, and acetylated. To this end, a 0.07 M solution of **28** in pyridine was treated with 2 equiv of acetic anhydride. The solution was stirred overnight, quenched with MeOH, and

evaporated to dryness, *in vacuo*. The compound was redissolved in CH_2Cl_2 and extracted twice with saturated sodium bicarbonate, and evaporated to dryness, *in vacuo*, affording a second crop of **29** (90% pure according to TLC), which was combined with the first crop obtained from the reaction. $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 1.08 (s, 18H); 1.82 (s, 3H); 1.93 (m, 1H); 2.09 (s, 3H); 2.32 (m, 3H), 2.48 (m, 2H); 2.69 (quintet, 1H, $J = 7$ Hz); 3.73 (dd, 1H, $J = 2.2, 11.8$ Hz); 4.02 (m, 2H); 4.09 (dd, 1H, $J = 2, 11.8$ Hz); 4.26 (m, 1H); 5.35 (d, 1H, $J = 8.1$ Hz); 5.42 (dd, 1H, $J = 2.8, 6.3$ Hz); 5.91 (d, 1H, $J = 2.9$ Hz); 6.28 (t, 1H, $J = 6.8$ Hz); 7.11 (s, 1H); 7.41 (m, 12H); 7.63 (m, 8H); 7.77 (d, 1H, $J = 8.1$ Hz); 8.99 (br s, 1H); 9.08 (br s, 1H). $^{13}\text{C-NMR}$ (50 MHz, CDCl_3) δ : 12.76, 19.24, 20.87, 27.01, 29.65, 35.11, 40.29, 44.3, 63.86, 74.76, approximately 77 (under CDCl_3), 84.42, 85.0, 85.95, 88.92, 102.92, 111.54, 128.36, 130.56, 132.51–133.43, 135.73–136.0, 136.08, 140.1, 150.57, 150.70, 163.65, 164.18, 169.64; FAB-MS m/z 1017 ($\text{M} + \text{H}^+$); 959; 905; 891; UV (CH_2Cl_2 , qualitative) λ_{max} (nm) 264 (100); 227 (84).

Compound 30. Protected dimer **29** (186.4 mg; 0.183 mmol) was dissolved in CH_2Cl_2 , transferred to a 15 mL plastic tube, and evaporated to dryness. The residue was dissolved in pyridine (1.3 mL), and a 4.7 M solution of hydrogen fluoride in pyridine (2.9 mL) was added. The solution was stirred overnight at rt. (TLC: ethyl acetate/ CH_2Cl_2 /methanol, 60:40:10, R_f starting material 0.78, R_f product 0.14). Methoxytrimethylsilane (4.8 mL, 34.77 mmol) was added to quench excess HF, and the solution was stirred for 30 min and evaporated to dryness, *in vacuo*. The crude product was used directly for the following tritylation. An analytical sample of **30** was purified by column chromatography on silica with CH_2Cl_2 /ethanol/water (85:15:0.5). $^1\text{H-NMR}$ (300 MHz, CD_3OD) δ : 1.88 (s, 3H); 2.10 (s, 3H); 2.26 (m, 2H); 2.71, 2.81 (2 \times m, 3H); 2.89 (dd, 2H, $J = 4.4, 6.2$ Hz); 3.75 (dd, 1H, $J = 3.4, 12.5$ Hz); 3.92–4.05 (m, 3H); 4.32 (m, 1H); 5.47 (dd, 1H, $J = 2, 5.7$ Hz); 5.68 (d, 1H, $J = 8.1$ Hz); 5.78 (d, 1H, $J = 2.2$ Hz); 6.23 (t, 1H, $J = 6.9$ Hz); 7.52 (s, 1H); 7.98 (d, 1H, $J = 8.1$ Hz). FAB-MS m/z : 541 ($\text{M} + \text{H}^+$).

Compound 31. Dimer **30** (39 mg crude product; approximately 0.05 mmol) was dissolved in pyridine (1 mL) and evaporated to dryness, *in vacuo*. A catalytic amount of 4-(*N,N*-dimethylamino)pyridine (DMAP) (ca. 0.5 mg) was added, and the mixture was dried, *in vacuo*, at 40 °C for 1 h. Pyridine (1.3 mL) and molecular sieves (4 Å) were added, and the solution was stirred for 15 min followed by addition of triethylamine (17.4 μL , 0.125 mmol) and 4,4'-dimethoxytriphenylmethyl chloride (33.9 mg, 0.1 mmol). After full conversion of the educt (2.5 h, TLC (MeOH/triethylamine/ CH_2Cl_2 , 10:0.5:89.5, R_f educt 0.25, R_f product 0.55), MeOH (2 mL) was added. The solution was evaporated to dryness, and the residue was chromatographed on silica (10 g) with MeOH/2-propanol/triethylamine/ CH_2Cl_2 (4:4:0.5:91.5). Dimer **31** was obtained as a slightly yellow foam (39.2 mg; 0.0465 mmol; approximately 93%). $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 1.89 (s, 3H); 2.14 (s, 3H); 2.20 (m, 1H); 2.41, 2.62, 3.01 (3 \times m, 4H); 2.78 (d, 2H, $J = 6.1$ Hz); 3.36 (dd, 1H, $J = 3.2, 10.9$ Hz); 3.66 (dd, 1H, $J = 1.9, 10.9$ Hz); 3.79 (s, 6H); 4.02 (m, 2H); 4.38 (m, 1H); 5.46 (d, 1H, $J = 8.1$ Hz); 5.64 (dd, 1H, $J = 1.6, 5.8$ Hz); 5.75 (d, 1H, $J = 1.7$ Hz); 6.16 (t, 1H, $J = 6.8$ Hz); 6.85 (dd, 4H, $J = 1.0, 9.0$ Hz); 7.29, 7.40 (2 \times m, 10H); 7.82 (d, 1H, $J = 8.2$ Hz). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ : 12.58, 20.84, 28.32, 34.98, 39.63, 41.32, 55.29, 62.35, 72.81, 77.63, 83.14, 84.88, 85.21, 86.95, 90.89, 102.49, 111.19, 113.31, 127.17, 128.03, 128.14, 130.16, 135.18, 144.26, 158.69, 135.82, 141.08, 150.32, 150.45, 163.54, 163.93, 169.87. FAB-MS m/z : 843 ($\text{M} + \text{H}^+$). UV (CH_2Cl_2): λ_{max} 261 (ϵ 25 100); 239 (ϵ 25 600).

Compound 15. A mixture of dimer **31** (39.1 mg, 0.0464 mmol) and diisopropylammonium tetrazolide²³ (DIPAT) (4 mg, 0.0232 mmol) was dried, *in vacuo*, at 40 °C for 1 h (a small amount of DIPAT was probably lost due to sublimation). Most of the solid was dissolved in acetonitrile (200 μL), and bis-(diisopropylamino)(β -cyanoethoxy)phosphine (20 μL , 0.063 mmol) was added. The mixture was stirred at rt for 5 h (TLC: acetone/hexane/triethylamine, 45:45:10, R_f starting material 0.08, R_f product 0.34) and then transferred to a separatory funnel with CH_2Cl_2 (5 mL) and saturated sodium bicarbonate (5 mL). The

aqueous phase was further extracted with CH_2Cl_2 (2 \times 5 mL), and the combined organic phases were washed with brine (10 mL) and dried over Na_2SO_4 for 30 min. After filtration, the solution was concentrated to approximately 1 mL and cooled to 0 °C, and the product was precipitated with hexane (5 mL). After 20 min at –20 °C, the suspension was centrifuged, the solvent was decanted off, and the residue was dried, *in vacuo*, overnight. Phosphoramidite **15** was obtained as a colorless foam (43.2 mg, 0.0414 mmol). Yield: 90%. FAB-MS indicated that some residual bis(diisopropylamino)(β -cyanoethoxy)phosphine was present. FAB-MS m/z : 303 (bis(diisopropylamino)(β -cyanoethoxy)phosphine); 1043 ($\text{M} + \text{H}^+$).

Modified DNA Oligomers. Oligonucleotides were prepared on a 1.0 μmol scale by solid-phase synthesis on a DNA synthesizer using the protocol recommended by the manufacturer (Pharmacia, Gene Assembler Plus, 1992). Phosphoramidites **14** and **15** were dried for 12 h under vacuum over P_2O_5 and dissolved in CH_3CN (0.1 M solutions), and molecular sieves (3Å, ca. 10% v/v of beads) were added to the solutions. Coupling times were 1 min for natural nucleosides, 2 min for **14** and 10 min for **15**. Oligomers were synthesized "trityl-on". At the end of the synthesis, oligomers were deprotected and released from the support with 25% aqueous ammonium hydroxide at 55 °C overnight. The crude deprotection solutions were decanted from the CPG beads, evaporated to dryness, *in vacuo*, rehydrated, and purified by reversed-phase HPLC. Product-containing fractions were combined, lyophilized, redissolved in water (100 μL), and detritylated with acetic acid (400 μL). After 20 min, deprotection was terminated by lyophilization, followed by redissolving in water (200–600 μL) and washing with diethyl ether (3 \times 500 μL) to remove the DMT alcohol. Following this procedure, compound **17** was obtained >95% pure by RP-HPLC (4–18% CH_3CN in 30 min, peak at 21 min) and MS analysis. UV (H_2O): λ_{max} 261 nm. MALDI-TOFMS: calcd average mass 3023.1, found m/z 3020.2 ($(\text{M} - \text{H}^+)^-$, 1508.5 ($\text{M} - 2\text{H}^+)^{2-}$). Oligopyrimidine **21** was further purified by anion-exchange chromatography and desalted. RP-HPLC, 0–25% CH_3CN in 30 min, peak at 23 min. UV (H_2O): λ_{max} 260 nm. MALDI-TOFMS: calcd average mass 2977.6, found m/z 2977.0 ($(\text{M} - \text{H}^+)^-$, 1487.2 ($\text{M} - 2\text{H}^+)^{2-}$). The mixture of **18** and **19** obtained following the above-given procedure was further purified on a Hamilton 79425, 150 \times 4.1 mm, 10 μm column, at 50 °C with a gradient of 100% A for 5 min, 100% A to 100% B in 40 min (A, 5% CH_3CN in 0.1 M NH_4HCO_3 , pH 9.0; B, 80% CH_3CN in 0.1 M NH_4HCO_3 , pH 9.0); retention times, **19**, 3.8 min; **18**, 5.1 min. Compound **18**. UV (H_2O): λ_{max} 261 nm. MALDI-TOFMS: calcd average mass 2978.1, found 2978.4 ($(\text{M} - \text{H}^+)^-$, 1490.2 ($\text{M} - 2\text{H}^+)^{2-}$). Compound **19**. UV (H_2O): λ_{max} 260 nm. MALDI-TOF MS: calcd average mass 2994.1, found 2994.5 ($(\text{M} - \text{H}^+)^-$, 1497.4 ($\text{M} - 2\text{H}^+)^{2-}$).

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Supporting Information Available: Assigned NMR and mass spectral data, selected IR spectral data, copies of NMR spectra of **6–8**, **11**, **13**, **26**, **27**, and **30**, and MALDI-TOF spectra of **17–19** and **21** (18 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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