# Substrates for Investigation of DNA Polymerase Function: Synthesis and Properties of 4′-*C*-Alkylated Oligonucleotides

**Ilka Detmer,[a] Daniel Summerer,[a] and Andreas Marx*[a]**

**Keywords:** DNA / DNA structures / Nucleotides / Oligonucleotides / Wittig reactions

In addition to their potential as diagnostic and therapeutic agents, modified oligonucleotides have also been shown to be highly valuable tools for examination of complex biological processes. Carefully designed nucleotide analogues have therefore found considerable application in investigations of DNA polymerase function and mechanism. To examine the contribution of primarily steric constraints on DNA polymerase selectivity, we have developed a new functional strategy based on the use of modified nucleotide analogues that differ primarily in their steric demand. Here we report the efficient synthesis of modified thymidine analogues bearing 4′-alkyl groups with varying steric demand, the effects of 4′-alkylation on sugar puckering, and the incorporation of these analogues into oligonucleotides by use of automated solid-phase DNA synthesis. We also studied the pairing properties of 4′-alkylated oligonucleotide duplexes in relation to those of their natural counterparts under a range of buffer conditions. In general, our studies indicate that 4′-alkylation of thymidines has little effect on nucleoside and oligonucleotide conformation. These results have relevance to the previously reported action of 4′-alkylated nucleotides and oligonucleotides as probes of DNA polymerase function and mechanism.



## Introduction

Apart from their potential as diagnostic and therapeutic agents, such as in the antisense and antigene strategies, modified oligonucleotides have also been shown to be highly valuable tools for investigation of complex biological processes.[1−4] This strategy has recently been finding considerable application in investigations of DNA polymerase function and mechanism.[5−7] Thus, nucleotide substrates were at first carefully modified to target specific substrate features such as hydrogen-bonding capability as precisely as possible. Subsequent in-depth functional investigation of various DNA polymerases gave rise to a plethora of new functional insights difficult or even impossible to access by other experimental means.

Recently, in order to investigate the contributions of primarily steric constraints on DNA polymerase selectivity, we have developed a new functional strategy.[8−12] This strategy is based on the use of modified nucleotide analogues differing primarily in their steric demand. This substrate feature in the nucleotide probes was achieved through substitution of the 4′-hydrogens of thymidines with alkyl groups of gradually increasing steric demand (Figure 1).

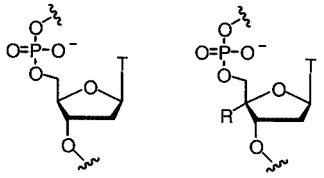



Figure 1. Thymidine (left) and 4′-alkylated thymidine (right) building blocks used as steric probes in enzymatic investigations. T: thymine, R: methyl, ethyl, isopropyl, isobutyl, respectively

Thanks to the employment of these probes in functional enzyme investigations, a number of new insights into DNA polymerase selectivity mechanisms have been gained. Furthermore, the 4′-alkylated probes turned out to enhance single mismatch discrimination through the polymerase chain reaction (PCR) significantly.[13] This feature might be exploitable for the development of highly accurate and robust analysis of genome variations such as point mutations and single nucleotide polymorphisms. Here we report a detailed description of the synthesis of 4′-alkylated thymidines, the impact of 4′-alkylation on sugar puckering, and the incorporation of these analogues into oligonucleotides. We also discuss the pairing properties of 4′-alkylated oligonucleotide duplexes in relation to those of their natural counterparts. The experiments reported here show that site-specifically 4′-alkylated oligonucleotides are capable of building stable duplexes under varying salt conditions and virtually independently of the position of the 4′-modifi-

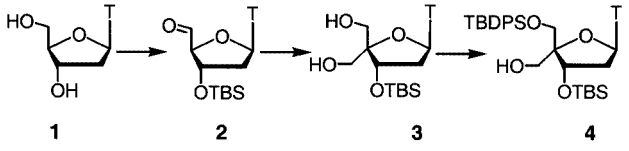
[a] Kekulé-Institut für Organische Chemie und Biochemie, Universität Bonn,
Gerhard-Domagk-Str. 1, 53121 Bonn, Germany
Fax: (internat.) + 49-(0)228-73-5388
E-mail: a.marx@uni-bonn.de

cation in the duplex. These findings have several implications for our recent studies concerning the usage of 4′-modified oligonucleotides as steric probes in studies of DNA polymerase fidelity mechanisms. Thus, the features of 4′-alkylated oligonucleotides described here indicate that the described action of 4′-alkylated probes on DNA polymerases can be assigned primarily to steric effects rather than to dramatically increased propensities of the DNA substrates to adopt aberrant conformations or to melting of the duplex promoted by a single 4′-modification.

## Results and Discussion

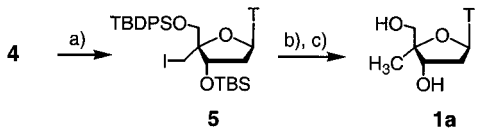
### Synthesis of 4′-Alkylated Thymidines

As reported, 4′-alkylated nucleotides have turned out to be highly valuable tools for investigation of the complex mechanisms involved in DNA polymerase selectivity. As a fundamental prerequisite for these studies, we have developed a synthetic route that allows access to the desired analogues in the amounts needed for the subsequent envisaged enzyme investigations. In the past, several strategies to synthesize 4′-modified nucleosides have been described.[14−30] In most of the known procedures the 4′-quaternary carbon center is constructed by means of a mixed aldol reaction between aldehyde **2** and formaldehyde to yield **3** (Scheme 1).



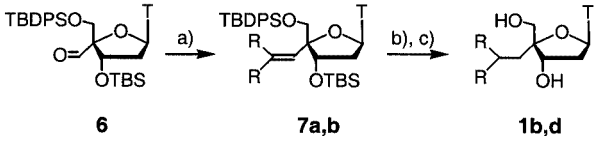

Scheme 1. General scheme for the synthesis of 4′-modified thymidines

After protection group manipulations, alcohol **4**[18] is obtained and serves as a basis for further functional group interconversions. Matsuda et al. have recently followed this route in the synthesis of several 4′-modified nucleosides starting with related compounds like the alcohol **4**.[15,16] Encouraged by these findings, we envisaged the synthesis of the desired thymidine probes bearing 4′-alkyl groups with varying steric demand. Our synthesis therefore started with alcohol **4**, which was converted into iodide **5** by treatment with $I_2$, $Ph_3P$, and imidazole at 50 °C. Hydrogenation with Pd/C in the presence of $Et_3N$ and subsequent cleavage of the silyl ethers with tetrabutylammonium fluoride (TBAF) gave 4′-*C*-methylthymidine (**1a**) in good yield (Scheme 2).



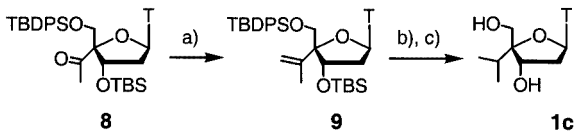

Scheme 2. Synthesis of 4′-methylthymidine **1a**; a) $Ph_3P$, $I_2$, imidazole, $C_6H_6$, 50 °C, 85%; b) Pd/C, $H_2$, EtOH, EtOAc, $NEt_3$; c) TBAF, THF, 81% (over two steps)

4′-Ethylated and 4′-isobutylated thymidines **1b** and **1d** were synthesized in high yields from easily available aldehyde **6**,[18] by sequential Wittig reaction, desilylation, and subsequent reduction of the aliphatic double bond (Scheme 3).




Scheme 3. Synthesis of 4′-ethylthymidine **1b** and 4′-isobutylthymidine **1d**; a) $CH_3PPh_3Br$, *n*BuLi, THF, −78 to 20 °C, 99% (R = H, **7a**), or $(CH_3)_2CHPPh_3I$, *n*BuLi, $Et_2O$, −78 to 20 °C, 83% (R = methyl, **7b**); b) TBAF, THF; c) Pd/C, $H_2$, $CH_3OH$, 88% (**1b**) or 89% (**1d**) over two steps, respectively

Finally, the synthesis of thymidine analogue **1c**, bearing a bulky isopropyl group α to the 4′-quaternary carbon center, was accomplished from the known ketone **8**[18] by means of a Wittig reaction and subsequent desilylation and hydrogenation of the aliphatic double bond (Scheme 4).




Scheme 4. Synthesis of 4′-isopropylthymidine **1c**; a) $CH_3PPh_3Br$, *t*BuOK, THF, 91%; b) TBAF, THF; c) Pd/C, $H_2$, $CH_3OH$, 99% (over two steps)

It is noteworthy that the choice of the base was crucial for this transformation. Thus, employment of reaction conditions similar to those applied in the synthesis of **1b** and **1d** with *n*BuLi resulted in little product formation. Bulky alkoxides have previously been reported to be the bases of choice in Wittig reactions involving sterically encumbered substrates.[31]

In order to gain insights into potential effects of the modifications on the sugar conformations we performed conformational analysis based on the coupling constants (Table 1) deduced from $^1H$ NMR spectroscopic data recorded in $D_2O$ by described methods.[32] Unmodified thymidine **1** was found to adopt about 70% southern conformation [(*S*)-type], while for **1a**−**d** approximately 50−60% (*S*)-type conformations were observed, indicating that 4′-alkylation had little impact on sugar puckering in solution.

### Synthesis of Site-Specifically 4′-Alkylated Oligonucleotides

Next, in order to incorporate 4′-alkylated thymidines **1a**−**d** into oligonucleotides, **1a**−**d** were converted into 2-(cyanoethyl)phosphoramidite building blocks by conversion into the respective 4,4′-dimethoxytrityl ethers **10a**−**d** and subsequent phosphitylation to form **11a**−**d** (Scheme 5).

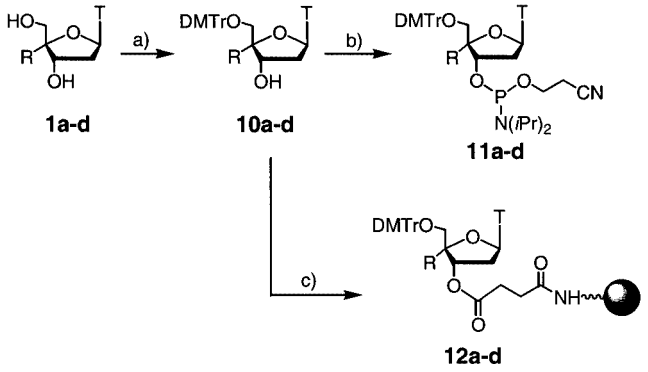
For the synthesis of oligonucleotides bearing the 4′-modified thymidines at the 3′-end of the strand we coupled

Table 1. Selected $^1$H NMR spectroscopic data (in $D_2O$, 500 MHz) for thymidine **1** and **1a**−**d**

| Coupling constants *J* [Hz] | 1′-H-2′-H | 1′-H-2′′-H | 2′-H-2′′-H | 2′-H-3′-H | 2′′-H-3′-H |
|---|---|---|---|---|---|
| **1** | 6.8 | 6.7 | n.a.[a] | 5.4 | 5.4 |
| **1a** | 7.1 | 5.8 | 14.1 | 6.3 | 6.7 |
| **1b** | 6.5 | 6.0 | 14.3 | 6.0 | 7.0 |
| **1c** | 7.0 | 6.8 | 14.1 | 6.9 | 4.4 |
| **1d** | 6.3 | 6.5 | n.a.[a] | 6.7 | 6.6 |

[a] n.a.: not accessible.




Scheme 5. Synthesis of 4′-alkylated building blocks suitable for automated solid-phase DNA synthesis; a) DMTrCl, pyridine, DMAP, 76−88%; b) ($i$Pr$_2$N)(NCCH$_2$CH$_2$O)PCl, $i$Pr$_2$NEt, CH$_2$Cl$_2$, 79−96%; c) succinylated long-chain alkylamine-modified controlled pore glass (LCAA-CPG), DMAP, EDC, NEt$_3$, pyridine; then 4-nitrophenol, then piperidine, then acetic anhydride, DMAP, pyridine

4,4′-dimethoxytrityl ethers **10a**−**d** to succinylated long-chain alkylamine-controlled pore glass (LCAA-CPG) support by standard procedures.[33] 4′-Modified oligonucleotides were synthesized by automated DNA synthesis from commercially available 2-(cyanoethyl)phosphoramidites and the modified building blocks. A standard method for 2-(cyanoethyl)phosphoramidites was used, with the exception that the coupling times of and from the modified nucleotides were extended to 10 min. The yields for modified oligonucleotides are similar to those obtained for unmodified oligonucleotides.

## Biophysical Properties of Duplexes Containing 4′-Alkylated Oligonucleotides

We next investigated the influence of 4′-alkyl groups in oligonucleotides on duplex stability. The presence of intensive hydration or metal-ion binding throughout the grooves of DNA double helices is well known from structural investigations.[34−39] Recent functional investigations employing chemical probes constructed on the principle of modified nucleobases with deleted hydrogen bonding sites have suggested that minor groove hydration has implications for DNA duplex stability.[40,41] However, alkyl groups covalently attached at the 4′-positions of 2′-deoxyribose residues should point towards the minor groove of double-stranded DNA and so should interfere with ligand binding in the groove through different mechanisms. We have recently investigated 4′-alkylated oligonucleotide duplexes in term of their abilities to form duplexes and have found that complementary duplexes are formed, with few overall helix deviations in relation to their unmodified counterparts.[42] Nevertheless, the introduction of several modified residues into oligonucleotide duplexes did decrease the duplex stability of the respective oligonucleotides. Here we report on the effect of varying salt concentrations on duplex stability.

By use of the procedure described above we first synthesized oligonucleotides **ON1a**−**d** and **ON2a**−**d**, bearing one or four unmodified or 4′-modified thymidine residues, respectively. We subsequently performed thermal denaturating studies and measured $T_m$ values (Table 2).

Table 2. Thermal denaturating experiments ($T_m$/°C values shown) of unmodified and 4′-alkylated oligonucleotides at varied buffer conditions

| Oligonucleotides | 0.1 M NaCl | 1 M NaCl | 4 M NaCl |
|---|---|---|---|
| 5′-d(ATA GCT$^R$ AAG ACC) | | | |
| 3′-d(TAT CGA TTC AGG) | | | |
| **ON1** (R = 4′-H) | 39.6 | 47.6 | 45.4 |
| **ON1a** (R = 4′-methyl) | 38.1 | 47.1 | 44.3 |
| **ON1b** (R = 4′-ethyl) | 38.7 | 47.4 | 44.4 |
| **ON1c** (R = 4′-isopropyl) | 37.2 | 46.6 | 43.8 |
| **ON1d** (R = 4′-isobutyl) | 38.1 | 48.3 | 45.3 |
| 5′-d(CGC GA A T$^R$T$^R$C GCG) | | | |
| 3′-d(GCG CT$^R$T$^R$A A G CGC) | | | |
| **ON2** (R = 4′-H) | 54.7 | 60.8 | 56.0 |
| **ON2a** (R = 4′-methyl) | 49.4 | 57.8 | 51.4 |
| **ON2b** (R = 4′-ethyl) | 47.2 | 53.7 | 49.7 |
| **ON2c** (R = 4′-isopropyl) | 45.1 | 53.8 | 48.4 |
| **ON2d** (R = 4′-isobutyl) | 46.4 | 56.2 | 52.7 |

Each measured profile obtained from thermal denaturation studies showed a single transition. We found that the presence of a single modification in the helix did not significantly alter the duplex stability from that of the unmodified oligonucleotides **ON1**. The $T_m$ value was most significantly decreased when 4′-isopropylated thymidine residues (**ON1c**) were present. However, four successive modified 4′-alkyl residues pointing into the minor groove, as present in **ON2a**−**d**, have more significant effects on duplex stability (Table 2). 4′-Ethyl- and 4′-isopropyl substituents appear to affect thermal stability most significantly in the sequence context investigated. Interestingly, at the highest salt con-

centration employed, 4′-isobutylated oligonucleotide **ON2d** showed the highest stability among the modified oligonucleotides, indicating that the presence of potential steric constraints in the minor groove might be compensated by other effects such as hydrophobic interactions. In general, the thermal denaturating studies are a further indication that oligonucleotides are able to form stable duplexes at high and low salt concentrations even with disruption of the hydration spine.

We have recently reported on the positional effects of site-specifically 4′-methylated primer template complexes on DNA polymerase function.[9,10,13] Thus, 4′-methylation is able to decrease DNA polymerase elongation efficiency by factors of up to several thousand times relative to the efficiencies of the unmodified primer template complexes. It was also found that the observed effects are strongly dependent on the position of the modification within the primer template stem of the DNA substrate.[9,10] Here we have investigated whether 4′-methyl modifications cause melting or aberrant conformations of the duplex depending on differential positioning of the modifications within the DNA duplex. For these investigations we synthesized oligonucleotides **ON3−6**, in the same sequence context as applied in our previous functional enzyme studies, and subsequently studied their apparent duplex properties. The data derived from thermal denaturation studies indicate little effect on duplex stability of the position of the 4′-modification relative to that of the unmodified duplex (Table 3).

Table 3. Thermal denaturating experiments ($T_m$/°C values shown) of oligonucleotides bearing unmodified or 4′-methylated thymidines at different positions

| Oligonucleotides[a] | 4′-H | 4′-methyl |
|---|---|---|
| **ON3** | | |
| 5′-d(TGA CAG ACA **T$^R$**) | | |
| 3′-d(ACT GTC TGT A CT GTC TGC) | 41.7 | 43.0 |
| **ON4** | | |
| 5′-d(TGA CAG ACA **T$^R$**G) | | |
| 3′-d(ACT GTC TGT A CT GTC TGC) | 49.1 | 47.7 |
| **ON5** | | |
| 5′-d(TGA CAG ACA **T$^R$**GA) | | |
| 3′-d(ACT GTC TGT A CT GTC TGC) | 50.9 | 51.9 |
| **ON6** | | |
| 5′-d(TGA CAG ACA **T$^R$**GA C) | | |
| 3′-d(ACT GTC TGT A CT GTC TGC) | 57.6 | 57.4 |

[a] Buffer contained 1 M NaCl, 20 mM $KH_2PO_4$ (pH = 7.0).

We next investigated whether 4′-methylation has a significant impact on overall DNA helix conformation and examined the circular dichroism (CD) characteristics of all native and 4′-methylated oligonucleotides (Figure 2).

The corresponding duplexes bearing 4′-methyl groups yield nearly superimposable CD spectra, indicating little if any dependence of overall helix conformation on the presence of 4′-methyl groups at differential positions. The results reported here have several implications for our recent studies concerning the usage of 4′-alkylated oligonucleotides as steric probes in studies of DNA polymerase fidelity

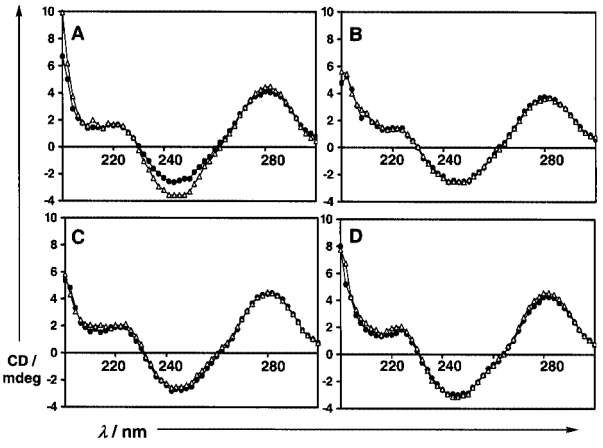



Figure 2. Circular dichroism spectra of native and 4′-methylated oligonucleotides; spectra of native oligonucleotides are displayed as closed circles, 4′-methylated as open triangles; A: **ON3**, B: **ON4**, C: **ON5**, D: **ON6**

mechanisms. The results indicate that the introduction of a single 4′-methyl modification in double-stranded DNA has little effect on intrinsic duplex properties such as overall helix conformation and stability, regardless of the position within the duplex. Thus, the effects of 4′-alkylated probes on DNA polymerase function[9,10,13] can probably be attributed primarily to steric effects rather than to dramatically increased propensities of the DNA substrates to adopt aberrant conformations or to promotion of melting of the duplex by a single 4′-modification.

## Conclusion

We describe an efficient synthesis of modified thymidine analogues bearing 4′-alkyl groups of varying steric demand. These analogues adopt sugar conformations similar to those of unmodified thymidine, as deduced from NMR spectroscopic data analysis. The 4′-alkylated thymidines were converted into building blocks suitable for automated solid-supported oligonucleotide synthesis. Several site-specifically 4′-modified oligonucleotide duplexes were synthesized in yields comparable to those obtained for unmodified strands. Subsequently, duplex properties such as stability and helix conformation were investigated in relation to those of the unmodified strands. It was found that duplexes comprising 4′-alkylated thymidine residues were able to form stable duplexes at various salt concentrations. Additionally, little effect of the positioning of 4′-methylated thymidines within DNA duplexes was found. Taken together, these studies indicate that 4′-alkylation of thymidines has little effect on nucleoside and oligonucleotide conformation.

## Experimental Section

**General:** All temperatures quoted are uncorrected. All reagents are commercially available and were used without further purification.

Solvents were purchased over molecular sieves (Fluka) and were used directly without further purification unless otherwise noted. All reactions were conducted with rigorous exclusion of air and moisture. IR spectra: Perkin−Elmer Lambda 2 FTIR spectrophotometer. NMR spectra: Bruker with the solvent peak as internal standard. FAB MS: Concept 1 H, matrix: 3-nitrobenzyl alcohol (NBA) and NBA + KCl. Microanalysis was performed at the Kekulé-Institut für Organische Chemie und Biochemie, Bonn University. Flash chromatography: Merck silica gel G60 (230−400 mesh). Thin layer chromatography: Merck precoated plates (silica gel 60 $F_{254}$). MALDI-ToF MS analysis of oligonucleotides was conducted by Metabion, Germany.
Abbreviations: TBDPS = *tert*-butyldiphenylsilyl, TBS = *tert*-butyldimethylsilyl.

**3′-*O*-*tert*-Butyldimethylsilyl-5′-*O*-*tert*-butyldiphenylsilyl-4′-(*C*-iodomethyl)thymidine (5):** $Ph_3P$ (50.4 mg, 0.19 mmol), imidazole (13.0 mg, 0.19 mmol), and **4** (100 mg, 0.16 mmol) were dissolved in dry benzene (2 mL), and iodine (45.7 mg, 0.18 mmol) was added in a stream of argon. The flask was sealed and heated to 50 °C, and stirring was continued for 18 h. After cooling to 20 °C, the mixture was poured into a solution of concentrated $NaHCO_3$/$Na_2S_2O_3$ and extracted three times with $CH_2Cl_2$. The combined extracts were dried over $MgSO_4$, concentrated, and purified by flash column chromatography (*tert*-butyl methyl ether/cyclohexane, 1:3 → 1:1) to yield **5** (100 mg, 85%) as a colorless foam. $R_f$ = 0.46 (ethyl acetate/cyclohexane, 1:2). $^1$H NMR (400 MHz, $CDCl_3$): δ = 0.07 (s, 3 H, SiMe), 0.08 (s, 3 H, SiMe), 0.90 (s, 9 H, $SiCMe_3$), 1.09 (s, 9 H, $SiCMe_3$), 1.64 (d, *J* = 1.3 Hz, 3 H, 5-Me), 2.14 (ddd, *J* = 13.4, 8.4, 5.8 Hz, 1 H, 2′′-H), 2.36 (ddd, *J* = 13.4, 5.7, 2.4 Hz, 1 H, 2′-H), 3.31 (d, *J* = 11.0 Hz, 1 H, 5′′-H), 3.48 (d, *J* = 10.9 Hz, 1 H, 5′-H), 3.83 (d, *J* = 1.5 Hz, 2 H, 4′-$CH_2$), 4.56 (dd, *J* = 5.7, 2.4 Hz, 1 H, 3′-H), 6.29 (dd, *J* = 8.5, 5.6 Hz, 1 H, 1′-H), 7.21 (q, *J* = 1.3 Hz, 1 H, 6-H), 7.33−7.49 (m, 6 H, $H_{arom}$), 7.61−7.69 (m, 4 H, $H_{arom}$), 8.26 (s, 1 H, NH) ppm. $^{13}$C NMR (101 MHz, $CDCl_3$): δ = −4.9 (Me-Si), −4.6 (Me-Si), 7.6 (4′-$CH_2$), 12.2 (5-Me), 18.0 (*C*$Me_3$), 19.4 (*C*$Me_3$), 25.7 (C*Me*$_3$), 27.0 (C*Me*$_3$), 41.6 (C-2′), 68.2 (C-5′), 72.6 (C-3′), 84.1 (C-1′), 86.9 (C-4′), 111.1 (C-5), 128.0, 128.0, 130.2, 130.2, 132.2, 132.7 ($C_{arom}$), 135.0 (C-6), 135.4, 135.7 ($C_{arom}$), 149.9 (C-2), 163.3 (C-4) ppm. IR (KBr): ṽ = 3049, 2928, 2855, 1692, 1471, 1113, 834, 701 cm$^{-1}$. MS (FAB): *m*/*z* (%) = 735 (81) [M + H$^+$].

**4′-(*C*-Methyl)thymidine (1a):** Compound **5** (2.85 g, 3.87 mmol) and 10% Pd/C (1.15 g) were placed under argon in a flask, and ethyl acetate (8.40 mL), ethanol (8.40 mL), and $Et_3N$ (0.86 mL, 6.19 mmol) were added. The reaction mixture was subsequently floated with hydrogen (balloon). After 7 days stirring under hydrogen atmosphere at 20 °C, the mixture was diluted with $CH_2Cl_2$ and filtered through a short silica column. After removal of the solvent the residue was employed directly in the subsequent transformation, being dissolved in THF (26 mL) and a *n*$Bu_4$NF solution in THF (1 M, 8 mL, 8 mmol) being added at 25 °C. After stirring for 3 h the reaction mixture was poured on a silica column without further workup. Flash chromatography (ethyl acetate → ethyl acetate/methanol, 10:1) yielded **1a** (799 mg, 81%) as a colorless foam. $R_f$ = 0.15 (ethyl acetate). $^1$H NMR (400 MHz, $CD_3OD$): δ = 1.15 (s, 3 H, Me), 1.86 (q, *J* = 1.1 Hz, 3 H, 5-Me), 2.32 (dd, *J* = 6.2, 6.2 Hz, 1 H, 2′′-H), 2.32 (dd, *J* = 6.2, 6.2 Hz, 1 H, 2′-H), 3.55 (d, *J* = 11.8 Hz, 1 H, 5′′-H), 3.59 (d, *J* = 11.8 Hz, 1 H, 5′-H), 4.39 (dd, *J* = 5.9, 5.9 Hz, 1 H, 3′-H), 6.19 (dd, *J* = 6.3, 6.3 Hz, 1 H, 1′-H), 7.88 (q, *J* = 1.2 Hz, 1 H, 6-H) ppm. $^{13}$C NMR (101 MHz, $CD_3OD$): δ = 12.7 (5-Me), 18.2 (4′-Me), 41.4 (C-2′), 67.8 (C-5′), 72.6 (C-3′), 85.3 (C-1′), 89.3 (C-4′), 111.6 (C-5), 138.7 (C-6), 152.7 (C-2), 166.8 (C-4) ppm. IR (KBr): ṽ = 3418, 1700, 1475, 1276, 1062, 860, 775 cm$^{-1}$. MS (FAB): *m*/*z* (%) = 257 (19) [M + H$^+$]. $C_{11}H_{16}N_2O_5$ 1/3$H_2O$ (262.11): calcd: C 50.38, H 6.41; found C 50.78, H 6.61.

**3′-*O*-*tert*-Butyldimethylsilyl-5′-*O*-*tert*-butyldiphenylsilyl-4′-*C*-(vinyl)thymidine (7a):** $MePPh_3Br$ (1.88 g, 5.25 mmol) was suspended in dry THF (50 mL) under Ar and treated at 0 °C with *n*BuLi (1.75 mL of a 2.5 M solution in hexanes, 4.38 mmol). After the mixture had been stirred for 5 min the ice bath was removed and stirring was continued at 20 °C for 1 h. The yellow reaction mixture was then cooled to −78 °C, aldehyde **6** (1.09 g, 1.75 mmol) in dry THF (15 mL) was added, stirring at −78 °C was continued for 30 min, and the mixture was then allowed to warm to 0 °C. After stirring for a further 3 h, the reaction mixture was quenched with concentrated $NH_4Cl$ solution and extracted with diethyl ether. The combined extracts were dried over $MgSO_4$, concentrated, and purified by column chromatography on silica gel (*tert*-butyl methyl ether/cyclohexane, 3:2) to give **7a** (1.08 g, 99%) as a colorless foam. $R_f$ = 0.62 (ethyl acetate/cyclohexane, 1:1). $^1$H NMR (400 MHz, $CDCl_3$): δ = 0.06 (s, 6 H, Me-Si), 0.87 (s, 9 H, *t*Bu-Si), 1.09 (s, 9 H, *t*Bu-Si), 1.51 (d, *J* = 1.3 Hz, 3 H, 5-Me), 2.16 (ddd, *J* = 13.2, 7.2, 5.0 Hz, 1 H, 2′′-H), 2.28 (ddd, *J* = 13.3, 6.7, 6.7 Hz, 1 H, 2′-H), 3.70 (s, 2 H, 5′-H), 4.74 (dd, *J* = 6.9, 6.9 Hz, 1 H, 3′-H), 5.22 (dd, *J* = 10.9, 1.8 Hz, 1 H, 4′-CH−*CH*$_2$), 5.44 (dd, *J* = 17.4, 1.7 Hz, 1 H, 4′-CH−*CH*$_2$), 5.83 (dd, *J* = 17.3, 10.9 Hz, 1 H, 4′-*CH*−$CH_2$), 6.26 (dd, *J* = 7.1, 5.1 Hz, 1 H, 1′-H), 7.33−7.44 (m, 6 H, $H_{arom}$), 7.51 (q, *J* = 1.2 Hz, 1 H, 6-H), 7.62−7.69 (m, 4 H, $H_{arom}$), 8.72 (s, 1 H, NH) ppm. $^{13}$C NMR (101 MHz, $CDCl_3$): δ = −5.0 (Me-Si), −4.5 (Me-Si), 11.9 (5-Me), 17.9 (*C*$Me_3$), 19.5 (*C*$Me_3$), 25.7 (C*Me*$_3$), 27.1 (C*Me*$_3$), 40.6 (C-2′), 65.6 (C-5′), 71.0 (C-3′), 82.8 (C-1′), 89.3 (C-4′), 111.1 (C-5), 116.5 (4′-CH−*CH*$_2$), 127.9, 127.9, 130.0, 130.1, 132.6, 133.0 ($C_{arom}$), 134.2 (4′-*CH*−$CH_2$), 135.3 ($C_{arom}$), 135.4 (C-6), 135.5 ($C_{arom}$), 150.3 (C-2), 163.7 (C-4) ppm. IR (KBr): ṽ = 2930, 2857, 1699, 1469, 1428, 1279, 1116, 1068, 939, 837, 778, 740, 703, 609 cm$^{-1}$. MS (FAB): *m*/*z* (%) = 621 (28) [M + H$^+$]. $C_{34}H_{48}N_2O_5Si_2$ (620.9): calcd. C 65.77, H 7.79, N 4.51; found C 65.84, H 7.73; N 4.17.

**4′-*C*-(Ethyl)thymidine (1b):** Nucleoside **7a** (982 mg, 1.58 mmol) was dissolved in THF (10 mL), and a *n*$Bu_4$NF solution in THF (1 M, 3.50 mL, 3.50 mmol) was added at 20 °C. After stirring for 3 h the reaction mixture was poured on a silica column without further workup. Short column chromatography (ethyl acetate → ethyl acetate/methanol, 10:1) yielded the crude product as a gum that could be used directly in the subsequent reaction. The residue was dissolved in methanol (15 mL) under an Ar atmosphere, and 10% Pd/C (150 mg) was added. The reaction mixture was subsequently floated with hydrogen (balloon). After stirring under a hydrogen atmosphere for 2 h, the reaction mixture was poured on a silica column without further workup. Short-column flash chromatography (ethyl acetate → ethyl acetate/methanol, 10:1) yielded **1b** (375 mg, 88%) as a colorless foam. $R_f$ = 0.16 (ethyl acetate). $^1$H NMR (400 MHz, $CD_3OD$): δ = 0.95 (dd, *J* = 7.5, 7.5 Hz, 3 H, 4′-$CH_2$-*Me*), 1.58 (dq, *J* = 14.5, 7.4 Hz, 1 H, 4′-*CH*$_2$-Me), 1.71 (dq, *J* = 14.7, 7.4 Hz, 1 H, 4′-*CH*$_2$-Me), 1.87 (d, *J* = 1.1 Hz, 3 H, 5-Me), 2.29 (ddd, *J* = 13.6, 6.6, 5.0 Hz, 1 H, 2′′-H), 2.33 (ddd, *J* = 13.6, 6.5, 6.5 Hz, 1 H, 2′-H), 3.57 (d, *J* = 11.8 Hz, 1 H, 5′′-H), 3.67 (d, *J* = 11.8 Hz, 1 H, 5′-H), 4.45 (dd, *J* = 6.4, 5.0 Hz, 1 H, 3′-H), 6.19 (dd, *J* = 6.5, 6.5 Hz, 1 H, 1′-H), 7.87 (q, *J* = 1.2 Hz, 1 H, 6-H) ppm. $^{13}$C NMR (101 MHz, $CD_3OD$): δ = 8.8 (4′-$CH_2$-*Me*), 12.7 (5-Me), 25.3 (4′-*CH*$_2$-Me), 41.8 (C-2′), 65.3 (C-5′), 73.0 (C-3′), 85.5 (C-1′), 91.1 (C-4′), 111.6 (C-5), 138.7 (C-6), 152.7 (C-2), 166.8 (C-4) ppm. IR (KBr): ṽ = 3443, 2927, 1687, 1473, 1272,

1070, 776 $cm^{-1}$. MS (FAB): *m/z* (%) = 271 (18) [M + $H^+$]. $C_{12}H_{18}N_2O_5$ (270.3): calcd. C 53.33, H 6.71; found C 53.01, H 6.73.

**3′-*O*-*tert*-Butyldimethylsilyl-5′-*O*-*tert*-butyldiphenylsilyl-4′-(*C*-isopropenyl)thymidine (9):** $MePPh_3Br$ (2.00 g, 5.60 mmol) and *t*BuOK (550 mg, 4.90 mmol) were suspended in dry THF (20 mL) under Ar and stirred at 20 °C for 1 h. Compound **8** (890 mg, 1.40 mmol) in dry THF (5 mL) was then added to the reaction mixture. After stirring for 14 h at 20 °C, the reaction mixture was quenched with concentrated $NH_4Cl$ solution and extracted with $CH_2Cl_2$. The combined extracts were dried over $MgSO_4$, concentrated, and purified by column chromatography on silica gel (ethyl acetate/cyclohexane, 1:3) to give **9** (810 mg, 91%) as a colorless foam. $R_f$ = 0.31 (ethyl acetate/cyclohexane, 1:3). $^1H$ NMR (400 MHz, $CDCl_3$): δ = −0.02 (s, 3 H, Me-Si), 0.04 (s, 3 H, Me-Si), 0.85 (s, 9 H, *t*Bu−Si), 1.09 (s, 9 H, *t*Bu−Si), 1.60 (q, *J* = 1.6 Hz, 3 H, 5-Me), 1.66 (m, 3 H, 4′-CCH$_2$*Me*), 2.26 (ddd, *J* = 12.8, 6.1, 3.3 Hz, 1 H, 2′′-H), 2.30 (ddd, *J* = 12.8, 7.7, 5.0 Hz, 1 H, 2′-H), 3.81 (d, *J* = 11.2 Hz, 1 H, 5′′-H), 3.94 (d, *J* = 11.4 Hz, 1 H, 5′-H), 4.44 (dd, *J* = 4.8, 3.4 Hz, 1 H, 3′-H), 4.87 (m, 2 H, 4′-C*CH*$_2$Me), 6.34 (dd, *J* = 7.6, 6.1 Hz, 1 H, 1′-H), 7.35−7.47 (m, 6 H, $H_{arom}$), 7.61−7.66 (m, 4 H, $H_{arom}$), 7.67 (q, *J* = 1.5 Hz, 1 H, 6-H), 8.14 (s, 1 H, NH) ppm. $^{13}C$ NMR (101 MHz, $CDCl_3$): δ = −5.1 (Me-Si), −4.4 (Me-Si), 12.0 (5-Me), 17.9 (*C*Me$_3$), 19.4 (*C*Me$_3$), 20.9 (4′-CCH$_2$*Me*), 25.6 (C*Me*$_3$), 27.1 (C*Me*$_3$), 41.7 (C-2′), 67.6 (C-5′), 74.3 (C-3′), 84.5 (C-1′), 92.9 (C-4′), 110.8 (C-5), 112.5 (4′-C*CH*$_2$Me), 128.0, 128.0, 130.1, 130.3, 132.4, 132.8, 135.4, 135.6 ($C_{arom}$), 135.7 (C-6), 143.0 (4′-*C*CH$_2$Me), 150.1 (C-2), 163.5 (C-4) ppm. MS (FAB): *m/z* (%) = 635 (26) [M + $H^+$]. $C_{35}H_{50}N_2O_5Si_2$ (635.0): calcd. C 66.21, H 7.94, N 4.41; found C 66.24, H 7.94, N 4.21.

**4′-*C*-(Isopropyl)thymidine (1c):** Compound **9** (768 mg, 1.21 mmol) was dissolved in THF (10 mL) and a *n*Bu$_4$NF solution in THF (1 M, 2.70 mL, 2.70 mmol) was added at 20 °C. After stirring for 3 h, the reaction mixture was poured on a silica column without further workup. Short-column chromatography (ethyl acetate → ethyl acetate/methanol, 10:1) yielded the crude product as a gum that could be used directly in the subsequent reaction. The residue was dissolved in methanol (15 mL) under an Ar atmosphere, and 10% Pd/C (150 mg) was added. The reaction mixture was subsequently floated with hydrogen (balloon). After stirring under a hydrogen atmosphere for 2 h, the reaction mixture was poured on a silica column without further workup. Short-column chromatography (ethyl acetate/cyclohexane, 10:1 → ethyl acetate/methanol, 10:1) yielded **1c** (342 mg, 99%) as a colorless foam. $R_f$ = 0.23 (ethyl acetate). $^1H$ NMR (300 MHz, $CD_3OD$): δ = 1.00 (d, *J* = 7.0 Hz, 3 H, 4′-CH*Me*$_2$), 1.03 (d, *J* = 7.2 Hz, 3 H, 4′-CH*Me*$_2$), 1.83 (d, *J* = 1.2 Hz, 3 H, 5-Me), 2.17−2.29 (m, 1 H, 4′-C*H*Me$_2$), 2.23 (ddd, *J* = 13.2, 6.0, 2.7 Hz, 1 H, 2′′-H), 2.42 (ddd, *J* = 13.3, 8.2, 6.0 Hz, 1 H, 2′-H), 3.72 (s, 2 H, 5′-H), 4.54 (dd, *J* = 6.1, 2.7 Hz, 1 H, 3′-H), 6.19 (dd, *J* = 8.3, 5.9 Hz, 1 H, 1′-H), 7.90 (q, *J* = 1.2 Hz, 1 H, 6-H) ppm. $^{13}C$ NMR (75 MHz, $CD_3OD$): δ = 12.8 (5-Me), 18.7 (4′-CH*Me*$_2$), 19.3 (4′-CH*Me*$_2$), 32.3 (4′-*CH*Me$_2$), 43.1 (C-2′), 64.1 (C-5′), 74.4 (C-3′), 86.1 (C-1′), 93.2 (C-4′), 111.6 (C-5), 139.1 (C-6), 152.9 (C-2), 166.8 (C-4) ppm. IR (KBr): $\tilde{\nu}$ = 3444, 2964, 1686, 1474, 1275, 1206, 1079, 973, 869, 775 $cm^{-1}$. MS (FAB): *m/z* (%) = 285 (30) [M + $H^+$]. $C_{13}H_{20}N_2O_5 \cdot 0.5H_2O$ (293.3): calcd. C 53.23, H 7.22, N 9.55; found C 53.51, H 7.36, N 9.29.

**3′-*O*-*tert*-Butyldimethylsilyl-5′-*O*-*tert*-butyldiphenylsilyl-4′-*C*-(2-methylpropenyl)thymidine (7b):** Isopropylphosphonium iodide (1.78 g, 4.12 mmol) was suspended in dry diethyl ether (20 mL) under an Ar atmosphere, and the mixture was treated at 20 °C with *n*BuLi (1.34 mL of a 2.5 M solution in hexanes, 3.36 mmol). After stirring for 45 min the dark red reaction mixture was cooled to −78 °C, compound **6** (600 mg, 0.96 mmol) in dry diethyl ether (5 mL) was added, stirring was continued at −78 °C for 15 min, and the reaction mixture was subsequently allowed to warm to 20 °C. After stirring for a further 30 min, the reaction mixture was cooled to −78 °C and afterwards quenched with concentrated $NH_4Cl$ solution. After warming to 20 °C, the mixture was extracted with $CH_2Cl_2$. The combined extracts were dried over $MgSO_4$, concentrated, and purified by flash column chromatography (*tert*-butyl methyl ether/cyclohexane, 1:4 → 1:3) to yield **7b** (522 mg, 83%) as a colorless foam. $R_f$ = 0.14 (*tert*-butyl methyl ether/cyclohexane, 1:4). $^1H$ NMR (400 MHz, $CDCl_3$): δ = 0.07 (s, 3 H, Me-Si), 0.08 (s, 3 H, Me-Si), 0.88 (s, 9 H, *t*Bu-Si), 1.08 (s, 9 H, *t*Bu-Si), 1.53 (d, *J* = 1.3 Hz, 3 H, 5-Me), 1.67 (d, *J* = 1.4 Hz, 3 H, 4′-CHC*Me*$_2$), 1.72 (d, *J* = 1.3 Hz, 3 H, 4′-CHC*Me*$_2$), 2.10 (ddd, *J* = 13.2, 7.8, 4.5 Hz, 1 H, 2′′-H), 2.27 (ddd, *J* = 13.3, 7.6, 7.6 Hz, 1 H, 2′-H), 3.59 (d, *J* = 11.8 Hz, 1 H, 5′′-H), 3.85 (d, *J* = 11.6 Hz, 1 H, 5′-H), 4.67 (dd, *J* = 7.7, 7.7 Hz, 1 H, 3′-H), 5.30 (dq, *J* = 1.4, 1.4 Hz, 1 H, 4′-*CH*CMe$_2$), 6.09 (dd, *J* = 7.6, 4.4 Hz, 1 H, 1′-H), 7.32−7.45 (m, 6 H, $H_{arom}$), 7.39 (q, *J* = 1.3 Hz, 1 H, 6-H), 7.64−7.70 (m, 4 H, $H_{arom}$), 8.18 (s, 1 H, NH) ppm. $^{13}C$ NMR (101 MHz, $CDCl_3$): δ = −4.9 (Me-Si), −4.5 (Me-Si), 11.9 (5-Me), 18.0 (*C*Me$_3$), 18.6 (4′-CHC*Me*$_2$), 19.5 (*C*Me$_3$), 25.7 (C*Me*$_3$), 27.1 (C*Me*$_3$), 27.6 (4′-CHC*Me*$_2$), 40.1 (C-2′), 64.1 (C-5′), 70.1 (C-3′), 82.2 (C-1′), 88.0 (C-4′), 110.9 (C-5), 118.4 (4′-CH*C*Me$_2$), 127.8, 127.9, 129.9, 130.0, 132.9, 133.2, 135.4, 135.5 ($C_{arom}$), 135.7 (C-6), 139.6 (4′-*CH*CMe$_2$), 150.1 (C-2), 163.4 (C-4) ppm. IR (KBr): $\tilde{\nu}$ = 3050, 2929, 2857, 1701, 1470, 1428, 1279, 1113, 1062, 1008, 949, 838, 779, 741, 702, 611 $cm^{-1}$. MS (FAB): *m/z* (%) = 649 (18) [M + $H^+$]. $C_{36}H_{52}N_2O_5Si_2$ (649.0): calcd. C 66.63, H 8.08, N 4.32; found C 66.78, H 8.08, N 3.98.

**4′-*C*-(Isobutyl)thymidine (1d):** Compound **7b** (1.07 g, 1.65 mmol) was dissolved in THF (15 mL), and a *n*Bu$_4$NF solution in THF (1 M, 3.63 mL, 3.63 mmol) was added at 20 °C. After stirring for 4 h, the reaction mixture was poured on a silica column without further workup. Short-column chromatography (ethyl acetate → ethyl acetate/methanol, 10:1) yielded the crude product as a gum that could be used directly in the subsequent reaction. The gum was dissolved in methanol (15 mL) under an argon atmosphere, and 10% Pd/C (150 mg) was added. The reaction mixture was subsequently floated with hydrogen (balloon). After stirring under a hydrogen atmosphere for 48 h, the reaction mixture was poured on a silica column without further workup. Short column chromatography (ethyl acetate/cyclohexane, 10:1 → ethyl acetate/methanol, 10:1) yielded **1d** (438.7 mg, 89%) as a colorless foam. $R_f$ = 0.19 (ethyl acetate). $^1H$ NMR (300 MHz, $CD_3OD$): δ = 0.96 [d, *J* = 6.6 Hz, 3 H, 4′-CH$_2$−CH(*Me*)$_2$], 0.99 [d, *J* = 6.6 Hz, 3 H, 4′-CH$_2$−CH(*Me*)$_2$], 1.46 [dd, *J* = 14.8, 5.2 Hz, 1 H, 4′-*CH*$_2$−CH(Me)$_2$], 1.55 [dd, *J* = 14.9, 7.2 Hz, 1 H, 4′-*CH*$_2$−CH(Me)$_2$], 1.78 [dddq, *J* = 6.7, 6.8, 6.8, 5.0 Hz, 1 H, 4′-CH$_2$−*CH*(Me)$_2$], 1.83 (d, *J* = 1.3 Hz, 3 H, 5-Me), 2.30 (dd, *J* = 6.2, 6.2 Hz, 1 H, 2′′-H), 2.30 (dd, *J* = 6.2, 6.2 Hz, 1 H, 2′-H), 3.58 (d, *J* = 11.5 Hz, 1 H, 5′′-H), 3.75 (d, *J* = 11.7 Hz, 1 H, 5′-H), 4.47 (dd, *J* = 6.0, 6.0 Hz, 1 H, 3′-H), 6.18 (dd, *J* = 6.4, 6.4 Hz, 1 H, 1′-H), 7.90 (q, *J* = 1.2 Hz, 1 H, 6-H) ppm. $^{13}C$ NMR (75 MHz, $CD_3OD$): δ = 12.8 (5-Me), 24.9 [4′-CH$_2$−CH(Me)$_2$], 25.5 [4′-CH$_2$−CH(Me)$_2$], 25.8 [4′-CH$_2$−CH(Me)$_2$], 40.7 [4′-CH$_2$−*CH*(Me)$_2$], 41.3 (C-2′), 65.6 (C-5′), 73.1 (C-3′), 85.3 (C-1′), 90.9 (C-4′), 111.5 (C-5), 138.7 (C-6), 152.7 (C-2), 166.8 (C-4) ppm. IR (KBr): $\tilde{\nu}$ = 3423, 2955, 1 687, 1473, 1273, 1066, 963, 778 $cm^{-1}$. MS (FAB): *m/z* (%) = 299 (6) [M + $H^+$]. $C_{14}H_{22}N_2O_5$ (298.3): calcd. C 56.36, H 7.43, N 9.39; found C 56.08, H 7.61, N 9.13.

**General Procedure for 5′-*O*-Dimethoxytritylation of 4′-Modified Thymidines 1a−d:** The respective nucleoside **1a−d** was coevapo-

rated twice with pyridine and dissolved in pyridine (5 mL/mmol), and 4,4′-dimethoxytrityl chloride (1.5−2.0 equiv.) and a catalytic amount of 4-(dimethylamino)pyridine were then added at 20 °C. After TLC analysis indicated complete consumption of the starting material (3−5 h), the reaction was quenched by addition of access methanol and stirring was continued for 30 min. The reaction mixture was then poured into saturated $NaHCO_3$ solution and extracted with $CH_2Cl_2$. The combined extracts were dried over $MgSO_4$, concentrated, and purified by column chromatography on silica gel (ethyl acetate/cyclohexane, 2:1 → 3:1 containing 1% $Et_3N$) to yield the product as a colorless foam.

**5′-*O*-Dimethoxytrityl-4′-*C*-(methyl)thymidine (10a):** Compound **1a** (200 mg, 0.78 mmol) was converted into **10a** (383 mg, 88%). $R_f$ = 0.23 (ethyl acetate/cyclohexane, 2:1). $^1$H NMR (400 MHz, [D$_6$]DMSO): δ = 1.08 (s, 3 H, 4′-Me), 1.42 (d, *J* = 1.1 Hz, 3 H, 5-Me), 2.22−2.29 (m, 2 H, 2′-H), 3.02 (d, *J* = 9.9 Hz, 1 H, 5′-H), 3.07 (d, *J* = 9.9 Hz, 1 H, 5′′-H), 3.74 (s, 6 H, Me-O), 4.37 (dd, *J* = 11.6, 6.3 Hz, 1 H, 3′-H), 5.24 (d, *J* = 5.2 Hz, 1 H, OH), 6.10 (dd, *J* = 6.4, 6.4 Hz, 1 H, 1′-H), 6.85−6.91 (m, 4 H, $C_{arom}$), 7.23−7.42 (m, 9 H, $C_{arom}$), 7.45 (q, *J* = 1.3 Hz, 1 H, 6-H), 11.27 (s, 1 H, NH) ppm. $^{13}$C NMR (101 MHz, [D$_6$]DMSO): δ = 12.1 (5-Me), 18.5 (4′-Me), 55.5 (OMe), 60.2 (C-5′), 71.2 (C-3′), 82.8 (C-1′), 86.2 (C-4′), 86.6 [*C*Ph(C$_6$H$_4$OMe)$_2$], 109.7 (C-5), 113.6 ($C_{arom}$), 127.2, 128.2, 128.3, 130.2, 135.7, 135.9 ($C_{arom}$), 136.1 (C-6), 145.2 ($C_{arom}$), 150.8 (C-2), 158.6 ($C_{arom}$), 164.1 (C-4) ppm. IR (KBr): ṽ = 3554, 3037, 2835, 1694, 1606, 1509, 1471, 1371, 1299, 1250, 1176, 1117, 1081, 1034, 831, 754, 700 cm$^{-1}$. MS (FAB): *m*/*z* (%) = 558 (25) [M + H]$^+$. $C_{32}H_{34}N_2O_7$ (558.6): calcd. C 68.80, H 6.13, N 5.01; found C 68.49, H 6.10, N 4.86.

**5′-*O*-Dimethoxytrityl-4′-*C*-(ethyl)thymidine (10b):** Compound **1b** (205 mg, 0.76 mmol) was converted into **10b** (332 mg, 76%). $R_f$ = 0.26 (ethyl acetate/cyclohexane, 3:1). $^1$H NMR (400 MHz, [D$_6$]DMSO): δ = 0.76 (t, *J* = 7.5 Hz, 3 H, 4′-CH$_2$*Me*), 1.50 (d, *J* = 1.1 Hz, 3 H, 5-Me), 1.66 (m, 2 H, 4′-*CH*$_2$Me), 2.25 (ddd, *J* = 13.6, 6.6, 4.6 Hz, 1 H, 2′′-H), 2.35 (ddd, *J* = 13.6, 6.8, 6.8 Hz, 1 H, 2′-H), 3.06 (s, 2 H, 5′-H), 3.74 (s, 6 H, Me-O), 4.50 (ddd, *J* = 6.8, 4.9, 4.9 Hz, 1 H, 3′-H), 5.30 (d, *J* = 5.2 Hz, 1 H, OH), 6.16 (dd, *J* = 6.7, 6.7 Hz, 1 H, 1′-H), 6.85−6.92 (m, 4 H, $C_{arom}$), 7.22−7.41 (m, 9 H, $C_{arom}$), 7.44 (q, *J* = 1.3 Hz, 1 H, 6-H), 11.16 (s, 1 H, NH) ppm. $^{13}$C NMR (101 MHz, [D$_6$]DMSO): δ = 8.5 (4′-CH$_2$*Me*), 12.2 (5-Me), 24.7 (4′-*CH*$_2$Me), 55.5 (OMe), 66.1 (C-5′), 72.0 (C-3′), 83.2 (C-1′), 86.3 (C-4′), 88.4 [*C*Ph(C$_6$H$_4$OMe)$_2$], 109.8 (C-5), 113.6 ($C_{arom}$), 127.2, 128.2, 128.3, 130.2, 135.7, 135.9, ($C_{arom}$), 136.1 (C-6), 145.2 ($C_{arom}$), 150.8 (C-2), 158.6 ($C_{arom}$), 164.1 (C-4) ppm. IR (KBr): ṽ = 2927, 1686, 1607, 1509, 1464, 1251, 1176, 1034, 828, 700 cm$^{-1}$. MS (FAB): *m*/*z* (%) = 572 (13) [M + H]$^+$. $C_{33}H_{36}N_2O_7$ (572.7): calcd. C 69.21, H 6.34, N 4.89; found C 69.00, H 6.66, N 4.58.

**5′-*O*-Dimethoxytrityl-4′-*C*-(isopropyl)thymidine (10c):** Compound **1c** (200 mg, 0.70 mmol) was converted into **10c** (329 mg, 80%). $R_f$ = 0.40 (ethyl acetate/cyclohexane, 3:1). $^1$H NMR (400 MHz, [D$_6$]DMSO): δ = 0.78 [d, *J* = 7.0 Hz, 3 H, 4′-CH(*Me*)$_2$], 0.81 [d, *J* = 7.0 Hz, 3 H, 4′-CH(*Me*)$_2$], 1.39 (d, *J* = 1.1 Hz, 3 H, 5-Me), 2.10−2.24 [m, 2 H, 2′′-H, 4′-*CH*(Me)$_2$], 2.35 (ddd, *J* = 13.4, 8.0, 6.0 Hz, 1 H, 2′-H), 3.08 (d, *J* = 10.1 Hz, 1 H, 5′′-H), 3.22 (d, *J* = 10.2 Hz, 1 H, 5′-H), 3.72 (s, 6 H, Me-O), 4.22 (m, 1 H, 3′-H), 5.24 (d, *J* = 5.2 Hz, 1 H, OH), 6.15 (dd, *J* = 8.0, 6.0 Hz, 1 H, 1′-H), 6.86−6.92 (m, 4 H, $C_{arom}$), 7.21−7.34 (m, 9 H, $C_{arom}$), 7.47 (q, *J* = 1.2 Hz, 1 H, 6-H), 11.27 (s, 1 H, NH) ppm. $^{13}$C NMR (101 MHz, [D$_6$]DMSO): δ = 11.7 (5-Me), 17.9 [4′-CH(*Me*)$_2$], 18.5 [4′-CH(*Me*)$_2$], 30.8 [4′-*CH*(Me)$_2$], 41.2 (C-2′), 55.2 (OMe), 65.0 (C-5′), 72.4 (C-3′), 83.6 (C-1′), 86.8 (C-4′), 90.1 [*C*Ph(C$_6$H$_4$OMe)$_2$], 109.6 (C-5), 113.4 ($C_{arom}$), 127.9, 128.1, 128.3, 129.0, 135.3, 135.5 ($C_{arom}$), 135.8 (C-6), 144.7 ($C_{arom}$), 150.5 (C-2), 158.3 ($C_{arom}$), 163.8 (C-4) ppm. IR (KBr): ṽ = 3442, 2956, 2834, 1690, 1607, 1509, 1465, 1251, 1176, 1033, 828, 755, 729, 697 cm$^{-1}$. MS (FAB): *m*/*z* (%) = 586 (14) [M + H]$^+$. $C_{34}H_{38}N_2O_7$·0.75 $H_20$ (600.2): calcd. C 68.04, H 6.59, N 4.67; found C 68.02, H 6.84, N 4.48.

**5′-*O*-Dimethoxytrityl-4′-*C*-(isobutyl)thymidine (10d):** Compound **1d** (100 mg, 0.36 mmol) was converted into **10d** (165 mg, 75%). $R_f$ = 0.42 (ethyl acetate/cyclohexane, 3:1). $^1$H NMR (400 MHz, [D$_6$]DMSO): δ = 0.75 (d, *J* = 6.2 Hz, 3 H, 4′-CH$_2$−CH(*Me*)$_2$], 0.77 [d, *J* = 6.2 Hz, 3 H, 4′-CH$_2$−CH(*Me*)$_2$], 1.37 (d, *J* = 1.1 Hz, 3 H, 5-Me), 1.40−1.53 [m, 3 H, 4′-*CH*$_2$−*CH*(Me)$_2$], 2.19 (ddd, *J* = 13.5, 6.7, 5.1 Hz, 1 H, 2′′-H), 2.23−2.31 (m, 1 H, 2′-H), 3.03 (d, *J* = 9.5 Hz, 1 H, 5′′-H), 3.16 (d, *J* = 9.5 Hz, 1 H, 5′-H), 3.73 (s, 6 H, Me-O), 4.52 (dd, *J* = 6.8, 5.1 Hz, 1 H, 3′-H), 5.25 (s, 1 H, OH), 6.07 (dd, *J* = 6.6, 6.6 Hz, 1 H, 1′-H), 6.86−6.91 (m, 4 H, $C_{arom}$), 7.21−7.41 (m, 9 H, $C_{arom}$), 7.48 (q, *J* = 1.3 Hz, 1 H, 6-H), 11.25 (s, 1 H, NH) ppm. $^{13}$C NMR (101 MHz, [D$_6$]DMSO): δ = 11.8 (5-Me), 23.6 [4′-CH$_2$−CH(Me)$_2$], 24.0 [4′-CH$_2$−CH(Me)$_2$], 25.0 [4′-CH$_2$-CH(Me)$_2$], 55.2 (OMe), 65.9 (C-5′), 71.7 (C-3′), 82.7 (C-1′), 86.2 (C-4′), 87.9 [*C*Ph(C$_6$H$_4$OMe)$_2$], 109.5 (C-5), 113.4 ($C_{arom}$), 127.0, 127.9, 128.0, 129.9, 135.5, 135.5, ($C_{arom}$), 135.7 (C-6), 144.8 ($C_{arom}$), 150.5 (C-2), 158.3 ($C_{arom}$), 163.8 (C-4) ppm. IR (KBr): ṽ = 3480, 2951, 1698, 1607, 1509, 1471, 1298, 1251, 1176, 1131, 1033, 966, 901, 830, 755, 699 cm$^{-1}$. MS (FAB): *m*/*z* (%) = 600 (17) [M$^+$]. $C_{34}H_{38}N_2O_7$. 0.25 $H_2O$ (605.2): calcd. C 69.45, H 6.75, N 4.63; found C 69.55, H 6.75, N 4.30.

**General Procedure for the Synthesis of Phosphoramidites 11a−d:** The respective nucleoside **10a−d** was coevaporated twice with toluene and then dissolved in $CH_2Cl_2$ (10 mL/mmol), and *N*-ethyl diisopropylamine (5.0 equiv.) and 2-cyanoethyl-*N*,*N*-(diisopropylamino)chlorophosphite (2.0 equiv.) were then added at 20 °C. After TLC analysis indicated complete consumption of the starting material (3−5 h), the reaction mixture was quenched by addition of concentrated $NaHCO_3$ solution and extracted with $CH_2Cl_2$. The combined extracts were dried over $MgSO_4$, concentrated, and purified by column chromatography on silica gel (ethyl acetate/cyclohexane, 2:1 → 3:1 containing 1% $Et_3N$) to yield the product as a colorless foam.

**Thymidine Derivative (11a):** Compound **10a** (350 mg, 0.63 mmol) was converted into **11a** (383 mg, 81%).

**Diastereomer a:** $R_f$ = 0.47 (ethyl acetate/cyclohexane, 2:1). $^1$H NMR (400 MHz, [D$_6$]acetone): δ = 1.19 (d, *J* = 4.6 Hz, 6 H, N−CH*Me*$_2$), 1.20 (s, 3 H, 4′-Me), 1.21 (d, *J* = 4.6 Hz, 6 H, N−CH*Me*$_2$), 1.46 (d, *J* = 1.3 Hz, 3 H, 5-Me), 2.51 (dd, *J* = 6.5, 6.5 Hz, 1 H, 2′′-H), 2.51 (dd, *J* = 6.5, 6.5 Hz, 1 H, 2′-H), 2.62 (dd, *J* = 6.1, 6.0 Hz, 2 H, *CH*$_2$CN), 3.25 (d, *J* = 10.0 Hz, 1 H, 5′′-H), 3.30 (d, *J* = 10.0 Hz, 1 H, 5′-H), 3.60−3.77 [m, 4 H, N(*CH*Me$_2$)$_2$, POCH$_2$], 3.79 (s, 6 H, Me-O), 4.86 (ddd, *J* = 10.7, 6.6, 6.6 Hz, 1 H, 3′-H), 6.24 (dd, *J* = 6.3, 6.3 Hz, 1 H, 1′-H), 6.85−6.91 (m, 4 H, $C_{arom}$), 7.23−7.42 (m, 9 H, $C_{arom}$), 7.59 (q, *J* = 1.2 Hz, 1 H, 6-H) ppm. $^{13}$C NMR (101 MHz, [D$_6$]acetone): δ = 11.2 (5-Me), 18.2 (4′-Me), 19.8 (*C*H$_2$CN), 23.9 [N(CH*Me*$_2$)$_2$], 38.6 (C-2′), 42.9 [N(*C*HMe$_2$)$_2$], 54.5 (O*Me*), 58.6 (*C*H$_2$OP), 67.1 (C-5′), 72.8 (C-3′), 82.8 (C-1′), 86.1 (C-4′), 86.3 [*C*Ph(C$_6$H$_4$OMe)$_2$], 109.8 (C-5), 113.0 ($C_{arom}$), 117.8 (CN), 126.8, 127.7, 128.2, 130.1, ($C_{arom}$), 135.4 (C-6), 144.9 ($C_{arom}$), 150.2 (C-2), 158.8 ($C_{arom}$), 163.2 (C-4) ppm. $^{31}$P NMR (162 MHz, [D$_6$]acetone): δ = 151.3 ppm.

**Diastereomer b:** $R_f$ = 0.34 (ethyl acetate/cyclohexane, 2:1). $^1$H NMR (400 MHz, [D$_6$]acetone): δ = 1.11 (d, *J* = 6.8 Hz, 6 H, NCH*Me*$_2$), 1.18 (d, *J* = 6.8 Hz, 6 H, NCH*Me*$_2$), 1.20 (s, 3 H, 4′-Me), 1.47 (d, *J* = 1.3 Hz, 3 H, 5-Me), 2.48 (ddd, *J* = 13.8, 7.5,

6.1 Hz, 1 H, 2′′-H), 2.57 (ddd, $J$ = 14.0, 6.9, 5.7 Hz, 1 H, 2′-H), 2.73−2.82 (m, 2 H, $CH_2$CN), 3.22 (d, $J$ = 10.0 Hz, 1 H, 5′′-H), 3.28 (d, $J$ = 10.0 Hz, 1 H, 5′-H), 3.58−3.96 [m, 4 H, N($CH$Me$_2$)$_2$, PO$CH_2$], 3.78 (s, 6 H, Me-O), 4.81 (ddd, $J$ = 9.8, 7.2, 5.7 Hz, 1 H, 3′-H), 6.26 (dd, $J$ = 6.8, 5.9 Hz, 1 H, 1′-H), 6.86−6.91 (m, 4 H, $C_{arom}$), 7.20−7.52 (m, 9 H, $C_{arom}$), 7.56 (q, $J$ = 1.2 Hz, 1 H, 6-H) ppm. $^{13}$C NMR (101 MHz, [D$_6$]acetone): δ = 12.2 (5-Me), 19.2 (4′-Me), 20.8 ($C$H$_2$CN), 24.8 [N(CH$Me_2$)$_2$], 39.8 (C-2′), 44.0 [N($C$HMe$_2$)$_2$], 55.5 (O$Me$), 59.2 ($C$H$_2$OP), 68.2 (C-5′), 74.8 (C-3′), 83.7 (C-1′), 86.8 (C-4′), 87.4 [$C$Ph(C$_6$H$_4$OMe)$_2$], 110.9 (C-5), 114.0 ($C_{arom}$), 119.0 (CN), 127.7, 128.7, 129.1, 131.1 ($C_{arom}$), 136.5 (C-6), 145.9 ($C_{arom}$), 151.2 (C-2), 158.7 ($C_{arom}$), 164.2 (C-4) ppm. $^{31}$P NMR (162 MHz, [D$_6$]acetone): δ = 151.6 ppm. IR (mixture of diastereomers, KBr): $\tilde{\nu}$ = 2967, 1689, 1608, 1509, 1464, 1365, 1252, 1178, 1033, 978, 878, 830, 727 cm$^{-1}$. MS (mixture of diastereomers, FAB): $m/z$ (%) = 759 (10) [M + H]$^+$. $C_{41}H_{51}N_4O_8P$ (mixture of diastereomers, 758.8): calcd. C 64.89, H 6.77, N 7.38; found C 64.49, H 6.62, N 7.14.

**Thymidine Derivative 11b:** Compound **10b** (250 mg, 0.44 mmol) was converted into **11b** (282 mg, 83%).

**Diastereomer a:** $R_f$ = 0.51 (ethyl acetate/cyclohexane, 2:1). $^1$H NMR (400 MHz, [D$_6$]acetone): δ = 0.83 (dd, $J$ = 7.5, 7.5 Hz, 3 H, 4′-CH$_2$$Me$), 1.20 (d, $J$ = 3.7 Hz, 6 H, NCH$Me_2$], 1.21 (d, $J$ = 3.5 Hz, 6 H, NCH$Me_2$), 1.45 (d, $J$ = 1.3 Hz, 3 H, 5-Me), 1.64 (dq, $J$ = 14.7, 7.4 Hz, 1 H, 4′-$CH_2$Me), 1.79 (dq, $J$ = 14.6, 7.5 Hz, 1 H, 4′-$CH_2$Me), 2.51 (dd, $J$ = 6.4, 6.4 Hz, 1 H, 2′′-H), 2.51 (dd, $J$ = 6.4, 6.4 Hz, 1 H, 2′-H), 2.64 (dd, $J$ = 6.0, 6.0 Hz, 2 H, $CH_2$CN), 3.23 (d, $J$ = 10.0 Hz, 1 H, 5′′-H), 3.36 (d, $J$ = 10.0 Hz, 1 H, 5′-H), 3.65 (q, $J$ = 3.4 Hz, 1 H, N$CH$Me$_2$), 3.68 (q, $J$ = 3.4 Hz, 1 H, N$CH$Me$_2$), 3.72−3.83 (m, 2 H, PO$CH_2$), 3.79 (s, 6 H, Me-O), 4.96 (ddd, $J$ = 10.8, 6.4, 6.4 Hz, 1 H, 3′-H), 6.24 (dd, $J$ = 6.4, 6.4 Hz, 1 H, 1′-H), 6.88−6.93 (m, 4 H, $C_{arom}$), 7.20−7.51 (m, 9 H, $C_{arom}$), 7.57 (q, $J$ = 1.2 Hz, 1 H, 6-H) ppm. $^{13}$C NMR (101 MHz, [D$_6$]acetone): δ = 8.3 (4′-$C$H$_2$Me), 12.1 (5-Me), 20.8 ($C$H$_2$CN), 24.9 [N(CH$Me_2$)$_2$], 25.8 (4′-$C$H$_2$Me), 39.9 (C-2′), 43.9 [N($C$HMe$_2$)$_2$], 55.6 (O$Me$), 59.7 ($C$H$_2$OP), 66.1 (C-5′), 74.5 (C-3′), 84.0 (C-1′), 87.5 (C-4′), 89.0 [$C$Ph(C$_6$H$_4$OMe)$_2$], 110.8 (C-5), 114.0 ($C_{arom}$), 118.8 (CN), 127.8, 128.7, 129.2, 131.2 ($C_{arom}$), 136.4 (C-6), 145.9 ($C_{arom}$), 151.2 (C-2), 159.8 ($C_{arom}$), 164.2 (C-4) ppm. $^{31}$P NMR (162 MHz, [D$_6$]acetone): δ = 151.3 ppm.

**Diastereomer b:** $R_f$ = 0.41 (ethyl acetate/cyclohexane, 2:1). $^1$H NMR (400 MHz, [D$_6$]acetone): δ = 0.83 (dd, $J$ = 7.5, 7.5 Hz, 3 H, 4′-CH$_2$$Me$), 1.14 (d, $J$ = 6.8 Hz, 6 H, NCH$Me_2$), 1.19 (d, $J$ = 6.8 Hz, 6 H, NCH$Me_2$), 1.46 (d, $J$ = 1.1 Hz, 3 H, 5-Me), 1.62 (dq, $J$ = 14.6, 7.3 Hz, 1 H, 4′-$CH_2$Me), 1.77 (dq, $J$ = 14.6, 7.3 Hz, 1 H, 4′-$CH_2$Me), 2.49 (ddd, $J$ = 13.8, 7.0, 6.9 Hz, 1 H, 2′′-H), 2.56 (ddd, $J$ = 14.0, 6.8, 5.0 Hz, 1 H, 2′-H), 3.21 (d, $J$ = 10.0 Hz, 1 H, 5′′-H), 3.34 (d, $J$ = 10.0 Hz, 1 H, 5′-H), 3.63 [q, $J$ = 6.8 Hz, 1 H, N($CH$Me$_2$)$_2$], 3.67 [q, $J$ = 6.8 Hz, 1 H, N($CH$Me$_2$)$_2$], 3.78 (s, 6 H, Me-O), 3.77−3.97 [m, 3 H, N($CH$Me$_2$)$_2$, PO$CH_2$], 4.89 (ddd, $J$ = 9.5, 7.1, 5.1 Hz, 1 H, 3′-H), 6.26 (dd, $J$ = 6.7, 6.7 Hz, 1 H, 1′-H), 6.87−6.91 (m, 4 H, $C_{arom}$), 7.19−7.50 (m, 9 H, $C_{arom}$), 7.55 (q, $J$ = 1.3 Hz, 1 H, 6-H) ppm. $^{13}$C NMR (101 MHz, [D$_6$]acetone): δ = 8.4 (4′-$C$H$_2$Me), 12.1 (5-Me), 20.8 ($C$H$_2$CN), 24.9 [N(CH$Me_2$)$_2$], 25.9 (4′-$C$H$_2$Me), 40.1 (C-2′), 44.0 [N($C$HMe$_2$)$_2$], 55.5 (O$Me$), 59.2 ($C$H$_2$OP), 66.2 (C-5′), 75.6 (C-3′), 83.9 (C-1′), 87.5 (C-4′), 88.8 [$C$Ph(C$_6$H$_4$OMe)$_2$], 110.9 (C-5), 114.0 ($C_{arom}$), 119.0 (CN), 127.7, 128.7, 129.2, 131.1 ($C_{arom}$), 136.5 (C-6), 145.9 ($C_{arom}$), 151.2 (C-2), 159.8 ($C_{arom}$), 164.2 (C-4) ppm. $^{31}$P NMR (162 MHz, [D$_6$]acetone): δ = 151.6 ppm. IR (mixture of diastereomers, KBr): $\tilde{\nu}$ = 2967, 1689, 1608, 1509, 1464, 1364, 1252, 1179, 1127, 1037, 978, 891, 828, 726, 702 cm$^{-1}$. MS (mixture of diastereomers, FAB): $m/z$ (%) = 773 (8) [M + H]$^+$. $C_{42}H_{53}N_4O_8P$·0.5H$_2$O (mixture of diastereomers, 781.4): calcd. C 64.52, H 6.96, N 7.17; found C 64.81, H 7.26, N 7.07.

**Thymidine Derivative (11c):** Compound **10c** (300 mg, 0.55 mmol) was converted into **11c** (340 mg, 79%).

**Diastereomer a:** $R_f$ = 0.57 (ethyl acetate/cyclohexane, 2:1). $^1$H NMR (300 MHz, [D$_6$]acetone): δ = 0.89 (d, $J$ = 6.8 Hz, 3 H, 4′-CH$Me_2$), 0.96 (d, $J$ = 7.0 Hz, 3 H, 4′-CH$Me_2$), 1.23 (d, $J$ = 6.8 Hz, 6 H, NCH$Me_2$), 1.23 (d, $J$ = 6.8 Hz, 6 H, NCH$Me_2$), 1.43 (d, $J$ = 1.3 Hz, 3 H, 5-Me), 2.28 (dq, $J$ = 6.9, 6.9 Hz, 1 H, 4′-$CH$Me$_2$), 2.55 (ddd, $J$ = 13.6, 7.4, 6.1 Hz, 1 H, 2′′-H), 2.62 (ddd, $J$ = 13.6, 6.5, 3.8 Hz, 1 H, 2′-H), 2.70 (dd, $J$ = 5.9, 5.9 Hz, 2 H, $CH_2$CN), 3.34 (d, $J$ = 10.2 Hz, 1 H, 5′′-H), 3.41 (d, $J$ = 10.2 Hz, 1 H, 5′-H), 3.66 (q, $J$ = 6.8 Hz, 1 H, N$CH$Me$_2$), 3.69 (q, $J$ = 6.8 Hz, 1 H, N$CH$Me$_2$), 3.75−3.91 (m, 2 H, PO$CH_2$), 3.79 (s, 6 H, Me-O), 4.93 (ddd, $J$ = 10.2, 6.1, 4.1 Hz, 1 H, 3′-H), 6.29 (dd, $J$ = 7.6, 6.3 Hz, 1 H, 1′-H), 6.88−6.95 (m, 4 H, $C_{arom}$), 7.25−7.53 (m, 9 H, $C_{arom}$), 7.59 (q, $J$ = 1.3 Hz, 1 H, 6-H) ppm. $^{13}$C NMR (101 MHz, [D$_6$]acetone): δ = 12.1 (5-Me), 18.5 (4′-CHMe$_2$), 18.7 (4′-CHMe$_2$), 20.8 ($C$H$_2$CN), 24.9 [N(CH$Me_2$)$_2$], 40.9 (C-2′), 44.0 [N($C$HMe$_2$)$_2$], 55.6 (O$Me$), 59.6 ($C$H$_2$OP), 65.7 (C-5′), 76.2 (C-3′), 84.8 (C-1′), 88.2 (C-4′), 91.4 [$C$Ph(C$_6$H$_4$OMe)$_2$], 110.8 (C-5), 114.0 ($C_{arom}$), 118.8 (CN), 127.8, 128.7, 129.2, 131.2 ($C_{arom}$), 136.5 (C-6), 145.7 ($C_{arom}$), 151.2 (C-2), 159.8 ($C_{arom}$), 164.2 (C-4) ppm. $^{31}$P NMR (162 MHz, [D$_6$]acetone): δ = 151.0 ppm.

**Diastereomer b:** $R_f$ = 0.43 (ethyl acetate/cyclohexane, 2:1). $^1$H NMR (300 MHz, [D$_6$]acetone): δ = 0.85 (d, $J$ = 7.0 Hz, 3 H, 4′-CH$Me_2$), 0.94 (d, $J$ = 7.0 Hz, 3 H, 4′-CH$Me_2$), 1.17 (d, $J$ = 6.8 Hz, 6 H, NCH$Me_2$), 1.21 (d, $J$ = 6.8 Hz, 6 H, NCH$Me_2$), 1.43 (d, $J$ = 1.3 Hz, 3 H, 5-Me), 2.23 (dq, $J$ = 7.0, 7.0 Hz, 1 H, 4′-$CH$Me$_2$), 2.52−2.67 (m, 2 H, 2′-H), 2.81 (dd, $J$ = 6.3, 6.3 Hz, 2 H, $CH_2$CN), 3.33−3.41 (m, 2 H, 5′-H), 3.65 (q, $J$ = 6.8 Hz, 1 H, N$CH$Me$_2$), 3.68 (q, $J$ = 6.9 Hz, 1 H, N$CH$Me$_2$), 3.78 (s, 6 H, Me-O), 3.79−3.99 (m, 2 H, PO$CH_2$), 4.84 (ddd, $J$ = 8.2, 6.0, 3.9 Hz, 1 H, 3′-H), 6.31 (dd, $J$ = 7.6, 6.3 Hz, 1 H, 1′-H), 6.88−6.94 (m, 4 H, $C_{arom}$), 7.26−7.53 (m, 9 H, $C_{arom}$), 7.58 (q, $J$ = 1.3 Hz, 1 H, 6-H) ppm. $^{13}$C NMR (101 MHz, [D$_6$]acetone): δ = 12.0 (5-Me), 18.4 (4′-CHMe$_2$), 18.8 (4′-CHMe$_2$), 20.9 ($C$H$_2$CN), 24.9 [N(CH$Me_2$)$_2$], 41.2 (C-2′), 44.1 [N($C$HMe$_2$)$_2$], 55.6 (O$Me$), 59.2 ($C$H$_2$OP), 65.6 (C-5′), 76.9 (C-3′), 84.6 (C-1′), 88.3 (C-4′), 90.9 [$C$Ph(C$_6$H$_4$OMe)$_2$], 110.9 (C-5), 114.0 ($C_{arom}$), 119.0 (CN), 127.8, 128.7, 129.2, 131.2, ($C_{arom}$), 136.6 (C-6), 145.7 ($C_{arom}$), 151.2 (C-2), 159.8 ($C_{arom}$), 164.2 (C-4) ppm. $^{31}$P NMR (162 MHz, [D$_6$]acetone): δ = 152.7 ppm. IR (mixture of diastereomers, KBr): $\tilde{\nu}$ = 2966, 1690, 1608, 1509, 1464, 1365, 1252, 1179, 1037, 979, 893, 829, 727 cm$^{-1}$. MS (mixture of diastereomers, FAB): $m/z$ (%) = 787 (6) [M + H]$^+$. $C_{43}H_{55}N_4O_8P$·0.5H$_2$O (mixture of diastereomers, 786.38): calcd. C 64.89, H 7.09, N 7.04; found C 64.87, H 7.17, N 6.96.

**Thymidine Derivative 11d:** Compound **10d** (300 mg, 0.55 mmol) was converted into **11d** (537 mg, 96%).

**Diastereomer a:** $R_f$ = 0.54 (ethyl acetate/cyclohexane, 2:1). $^1$H NMR (300 MHz, [D$_6$]acetone): δ = 0.82 (d, $J$ = 6.2 Hz, 3 H, 4′-CH$_2$CH$Me_2$), 0.88 (d, $J$ = 6.2 Hz, 3 H, 4′-CH$_2$CH$Me_2$), 1.21 (d, $J$ = 6.8 Hz, 6 H, NCH$Me_2$), 1.22 (d, $J$ = 6.8 Hz, 6 H, NCH$Me_2$), 1.41 (d, $J$ = 1.1 Hz, 3 H, 5-Me), 1.52−1.67 (m, 3 H, 4′-$CH_2$CHMe$_2$, 4′-CH$_2$$CH$Me$_2$), 2.50 (dd, $J$ = 6.6, 6.6 Hz, 1 H, 2′′-H), 2.50 (dd, $J$ = 6.6, 6.6 Hz, 1 H, 2′-H), 2.64 (dd, $J$ = 6.0, 6.0 Hz, 2 H, $CH_2$CN), 3.21 (d, $J$ = 10.0 Hz, 1 H, 5′′-H), 3.50 (d, $J$ = 10.0 Hz, 1 H, 5′-H), 3.67 (q, $J$ = 6.8 Hz, 1 H, N$CH$Me$_2$), 3.68 (q, $J$ = 6.9 Hz, 1 H, N$CH$Me$_2$), 3.72−3.81 (m, 2 H, PO$CH_2$), 3.79 (s, 6 H, Me-O), 5.05 (ddd, $J$ = 10.6, 6.8, 6.8 Hz, 1 H, 3′-H), 6.23 (dd, $J$ = 6.3, 6.3 Hz, 1 H, 1′-H), 6.88−6.94 (m, 4 H, $C_{arom}$), 7.25−7.55 (m, 9 H, $C_{arom}$), 7.62 (q, $J$ = 1.2 Hz, 1 H, 6-H) ppm. $^{13}$C NMR

(101 MHz, [D$_6$]acetone): δ = 12.1 (5-Me), 20.8 (*C*H$_2$CN), 24.2 (4′-CH$_2$CHMe$_2$), 24.9 [N(CH*Me*$_2$)$_2$], 25.4 (4′-CH$_2$CHMe$_2$), 27.5 (4′-CH$_2$CHMe$_2$), 40.9 (C-2′), 43.9 [N(*C*HMe$_2$)$_2$], 55.6 (O*Me*), 59.7 (*C*H$_2$OP), 65.9 (C-5′), 74.4 (C-3′), 83.7 (C-1′), 87.6 (C-4′), 88.8 [*C*Ph(C$_6$H$_4$OMe)$_2$], 110.8 (C-5), 114.0 (C$_{arom}$), 118.8 (CN), 127.8, 128.7, 129.2, 131.2 (C$_{arom}$), 136.4 (C-6), 145.8 (C$_{arom}$), 151.2 (C-2), 159.8 (C$_{arom}$), 164.2 (C-4) ppm. $^{31}$P NMR (162 MHz, [D$_6$]acetone): δ = 151.3 ppm.

**Diastereomer b:** $R_f$ = 0.43 (ethyl acetate/cyclohexane, 2:1). $^1$H NMR (300 MHz, [D$_6$]acetone): δ = 0.82 (d, *J* = 6.2 Hz, 3 H, 4′-CH$_2$CH*Me*$_2$), 0.88 (d, *J* = 6.2 Hz, 3 H, 4′-CH$_2$CH*Me*$_2$), 1.15 (d, *J* = 6.8 Hz, 6 H, NCH*Me*$_2$), 1.20 (d, *J* = 6.8 Hz, 6 H, NCH*Me*$_2$), 1.42 (d, *J* = 1.3 Hz, 3 H, 5-Me), 1.50−1.68 (m, 3 H, 4′-*CH*$_2$CHMe$_2$, 4′-CH$_2$*CH*Me$_2$), 2.46 (ddd, *J* = 13.7, 6.8, 6.8 Hz, 1 H, 2′′-H), 2.54 (ddd, *J* = 14.0, 6.9, 5.6 Hz, 1 H, 2′-H), 2.78 (dd, *J* = 6.0, 6.0 Hz, 2 H, *CH*$_2$CN), 3.19 (d, *J* = 10.0 Hz, 1 H, 5′′-H), 3.47 (d, *J* = 10.0 Hz, 1 H, 5′-H), 3.63 (q, *J* = 6.8 Hz, 1 H, N*CH*Me$_2$), 3.66 (q, *J* = 6.8 Hz, 1 H, N*CH*Me$_2$), 3.78 (s, 6 H, MeO), 3.80−3.97 (m, 2 H, PO*CH*$_2$), 4.97 (ddd, *J* = 9.6, 7.2, 5.6 Hz, 1 H, 3′-H), 6.25 (dd, *J* = 6.5, 6.5 Hz, 1 H, 1′-H), 6.88−6.94 (m, 4 H, C$_{arom}$), 7.21−7.54 (m, 9 H, C$_{arom}$), 7.57 (q, *J* = 1.3 Hz, 1 H, 6-H), 9.89 (s, 1 H, NH) ppm. $^{13}$C NMR (101 MHz, [D$_6$]acetone): δ = 12.1 (5-Me), 20.8 (*C*H$_2$CN), 24.2 (4′-CH$_2$CHMe$_2$), 24.9 [N(CH*Me*$_2$)$_2$], 25.5 (4′-CH$_2$CHMe$_2$), 27.5 (4′-CH$_2$CHMe$_2$), 41.1 (C-2′), 44.0 [N(*C*HMe$_2$)$_2$], 55.5 (O*Me*), 59.2 (*C*H$_2$OP), 66.1 (C-5′), 75.6 (C-3′), 83.7 (C-1′), 87.6 (C-4′), 88.5 [*C*Ph(C$_6$H$_4$OMe)$_2$], 110.9 (C-5), 114.0 (C$_{arom}$), 119.0 (CN), 127.8, 128.7, 129.2, 131.2 (C$_{arom}$), 136.5 (C-6), 145.8 (C$_{arom}$), 159.8 (C$_{arom}$), 164.2 (C-4) ppm. $^{31}$P NMR (162 MHz, [D$_6$]acetone): δ = 151.6 ppm. IR (mixture of diastereomers, KBr): $\tilde{\nu}$ = 2964, 1690, 1608, 1509, 1464, 1364, 1252, 1179, 1129, 1033, 979, 899, 831, 702 cm$^{-1}$. MS (mixture of diastereomers, FAB): *m*/*z* (%) = 801 (21) [M + H]$^+$. C$_{44}$H$_{57}$N$_4$O$_8$P·0.5H$_2$O (mixture of diastereomers, 809.9): calcd. C 65.25, H 7.22, N 6.92; found C 65.16, H 7.21, N 6.92.

**Synthesis of 4′-Modified Oligonucleotides:** The synthesis of oligonucleotides was carried out on a 0.2 μmol scale on an Applied Biosytems Model 392 DNA synthesizer, with commercially available 2-(cyanoethyl)phosphoramidites. A standard method for 2-(cyanoethyl)phosphoramidites was used, with the exception that the coupling times of and from the modified nucleotides were extended to 10 min. Yields for modified oligonucleotides are similar to those obtained for unmodified oligonucleotides. After synthesis (*trityl-on*) the oligonucleotides were cleaved from the support by treatment with conc. NH$_4$OH at 55 °C for 12 h. After removal of NH$_4$OH the residue was purified by RP HPLC (Knauer) with 5−80% MeCN in 0.1 M triethylammonium acetate (TEAA) buffer. Fractions containing product were freeze-dried, dissolved in 80% acetic acid (20 μL/OD), and incubated for 20 min at 20 °C. Sodium acetate solution (3 M, 5 μL/OD) and cooled (0 °C) ethanol (60 μL/OD) were added to the solution, which was stored after intensive mixing at −80 °C for 15 min. After centrifugation and decantation, the DNA pellet was washed with ethanol and the remaining solvent was evaporated. The oligonucleotides were quantified by absorption measurements at 260 nm. Total yields of purified oligonucleotides were in the 17−50% range. The integrity of all modified oligonucleotides was confirmed by MALDI-ToF MS

**General Procedure for Coupling between 10a−d and Succinylated LCAA-CPG:** Compounds **10a−d** were coupled to succinylated LCAA-CPG by published procedures.[33] Briefly, succinylated LCAA-CPG, the respective nucleosides **10a−d**, DMAP (each 0.1 mmol/1.0 g CPG), and EDC (1.0 mmol/1.0 g CPG), were combined, pyridine (10 mL/1.0 g CPG) and NEt$_3$ (80 μL/1.0 g CPG) were added, and the reaction mixture was stirred under Ar overnight. Afterwards, 4-nitrophenol (0.5 mmol/1.0 g CPG) was added and shaking was continued for an additional 24 h. Piperidine (5 mL/1.0 g CPG) was then added, and stirring was continued for 5 min. Afterwards, the beads were filtered off and intensively washed, first with pyridine, and then with methanol and CH$_2$Cl$_2$. After drying, the beads were suspended in pyridine (10 mL/1.0 g CPG) and acetic anhydride (14 mmol/1.0 g CPG), and a catalytic amount of DMAP was added. After having been shaken for 5 h, the beads were filtered off and intensively washed as described above. After drying, the loading was determined by trityl analysis of a small portion of the collected beads by known methods.[33] The integrity of all modified oligonucleotides was confirmed by MALDI-ToF MS.

**DNA Thermal Denaturation Studies:** Melting curves were recorded on a Lambda 2 (Perkin−Elmer) instrument fitted with a PTP-6 temperature-control device. Data were obtained from three individual cooling/heating cycles. Melting temperatures ($T_m$/ °C values) were obtained from the maximum of the first derivative of the melting curve ($A_{260}$ vs. temperature). Measurements were conducted in buffer made up of NaCl (concentrations are indicated in the respective table), 20 mM KH$_2$PO$_4$ (pH, 7.0), and containing 600 nM duplex DNA. The mixtures were heated to 95 °C for 5 min and allowed to cool slowly to room temperature prior to the melting curve measurements. A measurement of the buffer was conducted separately and subtracted from the spectra resulting from the sample.

**CD Spectra:** CD spectra were recorded on a Jasco 720 in buffer made up of 1 M NaCl and 20 mM KH$_2$PO$_4$ (pH 7.0), which contained 15 μM duplex DNA. The mixtures were heated to 95 °C for 5 min and allowed to cool slowly to room temperature prior to measurements. A spectrum of the buffer was measured separately and subtracted from the spectra resulting from the samples. An average of 12 spectra were recorded in each experiment.

## Acknowledgments

Financial support by the VolkswagenStiftung, the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie, and the Dr. Otto Röhm Gedächtnisstiftung is gratefully acknowledged. We thank Dr. M. Zelgert for critical reading of the manuscript and Professor Dr. M. Famulok for his support.

[1] A. DeMesmaeker, R. Häner, P. Martin, H. Moser, *Acc. Chem. Res.* **1995**, *28*, 366−374.
[2] Y. S. Sanghvi, in *DNA and Aspects of Molecular Biology* (Ed.: E. T. Kool), Pergamon, Elsevier Science, Oxford, **2002**, chapter 8.
[3] S. Verma, F. Eckstein, *Annu. Rev. Biochem.* **1998**, *67*, 99−134.
[4] L. W. McLaughlin, M. Wilson, in *DNA and Aspects of Molecular Biology* (Ed.: E. T. Kool), Pergamon, Elsevier Science, Oxford, **2002**, chapter 7.
[5] E. T. Kool, J. C. Morales, K. M. Guckian, *Angew. Chem. Int. Ed.* **2000**, *39*, 990−1009.
[6] E. T. Kool, *Annu. Rev. Biochem.* **2002**, *71*, 191−219.
[7] E. T. Kool, *Cupp. Opin. Chem. Biol.* **2000**, *4*, 602−608.
[8] D. Summerer, A. Marx, *Angew. Chem. Int. Ed.* **2001**, *40*, 3693−3695.
[9] M. Strerath, D. Summerer, A. Marx, *ChemBioChem* **2002**, *3*, 578−580.
[10] D. Summerer, A. Marx, *J. Am. Chem. Soc.* **2002**, *124*, 910−911.
[11] M. Strerath, J. Cramer, T. Restle, A. Marx, *J. Am. Chem. Soc.* **2002**, *124*, 11230−11231.

[12] J. Cramer, M. Strerath, A. Marx, T. Restle, *J. Biol. Chem.* **2002**, *277*, 43593−43598.
[13] M. Strerath, A. Marx, *Angew. Chem. Int. Ed.* **2002**, *41*, 4766−4769.
[14] C. O-Yang, W. Kurz, E. M. Eugui, M. J. McRoberts, J. P. H. Verheyden, L. J. Kurz, K. A. M. Walker, *Tetrahedron Lett.* **1992**, *33*, 41−44.
[15] I. Sugimoto, S. Shuto, S. Mori, S. Shigeta, A. Matsuda, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 385−388.
[16] M. Nomura, S. Shuto, M. Tanaka, T. Sasaki, S. Mori, S. Shigeta, A. Matsuda, *J. Med. Chem.* **1999**, *42*, 2901−2908.
[17] C. O-Yang, H. Y. Hu, E. B. Fraser-Smith, K. A. M. Walker, *Tetrahedron Lett.* **1992**, *33*, 37−40.
[18] A. Marx, P. Erdmann, M. Senn, S. Körner, T. Jungo, M. Petretta, P. Imwinkelried, A. Dussy, K. J. Kulicke, L. Macko, M. Zehnder, B. Giese, *Helv. Chim. Acta* **1996**, *79*, 1980−1994.
[19] H. Ohrui, S. Kohgo, K. Kitano, S. Sakata, E. Kodama, K. Yoshimura, M. Matsuoka, S. Shigeta, H. Mitsuya, *J. Med. Chem.* **2000**, *43*, 4516−4525.
[20] M. Kanazaki, Y. Ueno, S. Shuto, A. Matsuda, *J. Am. Chem. Soc.* **2000**, *122*, 2422−2432.
[21] M. Montembault, N. Bourgougnon, J. Lebreton, *Tetrahedron Lett.* **2002**, *43*, 8091−8094.
[22] J. Wengel, *Acc. Chem. Res.* **1999**, *32*, 301−310.
[23] C. J. Leumann, *Bioorg. Med. Chem. Lett.* **2002**, *10*, 841−854.
[24] R. D. Youssefyeh, J. P. H. Verheyden, J. G. Moffatt, *J. Org. Chem.* **1979**, *44*, 1301−1306.
[25] G. H. Jones, M. Taniguchi, D. Tegg, J. G. Moffatt, *J. Org. Chem.* **1979**, *44*, 1309−1317.
[26] T. Imanishi, S. Obika, *Chem. Commun.* **2002**, 1653−1659.
[27] T. Kendrick, B. Giese, *Chem. Commun.* **2002**, 2016−2017.
[28] M. Spormann, B. Giese, *Synthesis* **2001**, 2156−2164.
[29] D. Crich, X. L, *J. Org. Chem.* **1999**, *64*, 4016−4024.
[30] M. E. Jung, A. Toyota, *J. Org. Chem.* **2001**, *66*, 2624−2635.
[31] L. Fitjer, U. Quabeck, *Synth. Comm.* **1985**, *15*, 855−864.
[32] L. J. Rinkel, C. Altona, *J. Biomol. Struct. Dyn.* **1987**, *4*, 621−648.
[33] R. T. Pon, in *Current Protocols in Nucleic Acids Chemistry* (Eds.: S. L. Beaucage, D. E. Bergstrom, G. D. Glick, R. A. Jones) John Wiley & Sons Inc., New York, **2000**, chapter 3.2.1−3.2.23.
[34] H. M. Berman, B. Schneider, in *Oxford Handbook of Nucleic Acid Structure* (Ed.: S. Neidle,) Oxford University Press, New York, **1999**, chapter 9.
[35] R. Wing, H. Drew, T. Takano, C. Broka, S. Takana, K. Itakura, R. E. Dickerson, *Nature* **1980**, *287*, 755−758.
[36] M. Soler-López, L. Malinina, J. Lui, T. Huynh-Dinh, J. A. Subirana, *J. Biol. Chem.* **1999**, *274*, 23683−23686.
[37] V. Tereshko, G. Minasov, M. Egli, *J. Am. Chem. Soc.* **1999**, *121*, 470−471.
[38] S. Neidle, *Nat. Struc. Biol.* **1998**, *5*, 754−756.
[39] W. Saenger, *Principles of Nucleic Acid Structure* (Ed.: C. R. Cantor), Springer, Berlin, **1983**.
[40] T. Lan, L. W. McLaughlin, *J. Am. Chem. Soc.* **2000**, *122*, 6512−6512.
[41] T. Lan, L. W. McLaughlin, *Biochemistry* **2001**, *40*, 968−976.
[42] I. Detmer, D. Summerer, A. Marx, *Chem. Commun.* **2002**, 2314−2315.

Received November 15, 2002