Chemical and Enzymatic Synthesis of DNA Fragments Containing 5-(β-D-Glucopyranosyloxymethyl)-2′-deoxycytidine − a Modified Nucleoside in T4 Phage DNA

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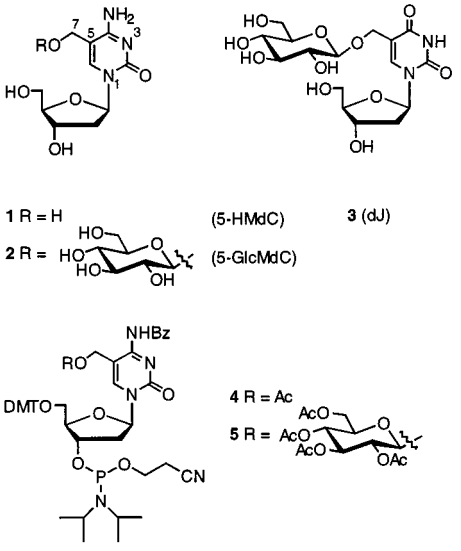
DNA fragments **23** and **25**, containing 5-hydroxymethyl-2′-deoxycytidine (5-HMdC) and 5-(β-D-glucopyranosyloxymethyl)-2′-deoxycytidine (5-GlcMdC) units, respectively, were assembled by means of a solid support-assisted synthesis using phosphoramidite building blocks **4** and **5**, respectively. In addition, 20-mer **23** was converted into fragment **26** by a glycosylation with uridine diphosphoglucose (UDPG), catalyzed by T4 β-glucosyltransferase (BGT).

Introduction

Bacteriophage T4 is extremely virulent for its host *E. coli*. When the infecting DNA has entered the host cell, T4 transcription is initiated at strong early promotors, leading very rapidly to a number of gene products involved in the transition from host to phage metabolism. For instance, specific endonucleases begin the degradation of cytosine-containing DNA,[1] while the viral genome is protected by the presence of 5-hydroxymethyl-2′-deoxycytidine (5-HMdC, **1**) instead of the common 2′-deoxycytidine.[2,3] In addition, both α- and β-glucosyltransferases instigate the post-replicative glucosylation of virtually all 5-HMdC residues. Thus, β-glucosyltransferase (BGT) catalyzes[4,5] the glucosyl transfer from uridine 5′-diphosphoglucose (UDPG) to the allylic hydroxyl group of 5-HMdC (**1**), resulting in the formation[6] of 5-(β-D-glucopyranosyloxymethyl)-2′-deoxycytidine (5-GlcMdC, **2**). It is also of interest to note that a similar glucosylation[7] takes place in the synthesis of 5-(β-D-glucopyranosyloxymethyl)-2′-deoxyuridine (dJ, **3**) residing in telomeric sites of the DNA of *Trypanosoma Brucei*, the causative agent of sleeping sickness.[8] It is generally accepted that glycosylated DNA plays a role in the regulation of gene expression, cell growth, and packaging of DNA.[1,9−12]

As part of an ongoing program to study the biosynthesis[4,5] and biological function[8,13] of glucosylated DNA, the synthesis of the phosphoramidite building blocks of 5-HMdC and 5-GlcMdC, **4** and **5**, is presented. The use of these compounds is demonstrated in the construction of oligodeoxynucleotides containing 5-HMdC (**1**) or 5-GlcMdC (**2**) at planned sites.



Results and Discussion

The solid support-assisted synthesis of DNA containing the modified nucleosides **1** or **2** requires the availability of suitably protected phosphoramidite building blocks. Recently, Sowers et al. reported the synthesis[14] of a 7-*O*-cyanoethyl-protected 5-HMdC derivative (**4**, R = CH_2CH_2CN), starting from the rather expensive 2′-deoxyuridine. The latter aspect and the prolonged reaction time (conc. aq. NH_4OH, 60 °C, 60 h) required for complete removal of the cyanoethyl group was an incentive for replacing the cyanoethyl group with an acetyl moiety and for using thymidine as the starting material. The synthesis of target compound **4** is presented in Scheme 1 and commences with radical bromination[15] of the known[13a] bis-silylated thymidine derivative **6**. Substitution of the resulting crude allylic bromide with cesium acetate in DMF afforded 5-HMdU derivative **7**, which was transformed into 5-HMdC **8** by the following

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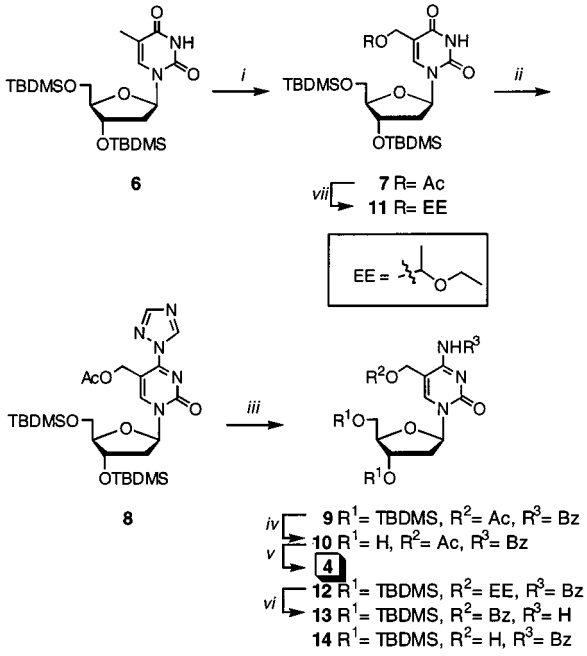
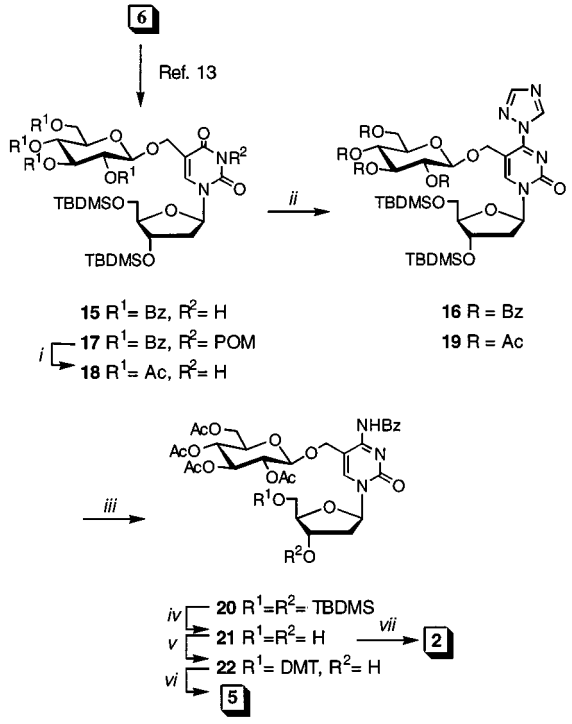


Scheme 1. Reagents and conditions: (*i*) a. Br_2, CCl_4, light, 2 h; b. CsOAc, DMF, 2 h, 51% (2 steps). (*ii*) $P(O)Cl_3$, 1,2,4-triazole, Et_3N, CH_3CN, 1 h. (*iii*) a. 25% NH_4OH/1,4-dioxane, 1:10, v/v, 30 min; b. BzCl, pyridine, 3 h, **9**: 65%, **12**: 70%, 3 steps. (*iv*) $Et_3N{\cdot}3HF$, pyridine, 16 h, 81%. (*v*) a. DMTCl, pyridine, 16 h, 76%; b. chloro(2-cyanoethoxy)(*N,N*-diisopropylamino)phosphane, D*i*PEA, CH_2Cl_2, 1 h, 81%. (*vi*) a. K_2CO_3, MeOH, 5 h, 87%; b. ethyl vinyl ether/CH_2Cl_2, 2:1, v/v, cat. *p*TsOH, 15 min, quant. (*vii*) MeOH, cat. *p*TsOH, 1.5 h, 50%.



Scheme 2. Reagents and conditions: (*i*) a. *t*BuOK, MeOH, 60 °C, 16 h; b. Ac_2O, pyridine, 2 h, 89%, 2 steps. (*ii*) $P(O)Cl_3$, 1,2,4-triazole, Et_3N, CH_3CN, **16**: 70 °C, 16 h, 29%; **19**: 16 h, 86%. (*iii*) a. 25% NH_4OH/1,4-dioxane, 1:10, v/v, 30 min; b. BzCl, pyridine, 16 h, 73%, 2 steps. (*iv*) $Et_3N{\cdot}3HF$, pyridine, 16 h, 76%. (*v*) DMTCl, pyridine, 16 h, 79%. (*vi*) chloro(2-cyanoethoxy)(*N,N*-diisopropylamino)-phosphane, D*i*PEA, CH_2Cl_2, 30 min, 85%. (*vii*) 25% NH_4OH/1,4-dioxane, 2:1, v/v, 50 °C, 16 h, 89%

three-step sequence. Treatment of **7** with phosphoryl chloride and 1,2,4-triazole[16] gave triazolide **8**, while subsequent mild ammonolysis of **8** gave, after benzoylation of the resulting amine, the fully protected cytidine derivative **9** in 65% overall yield. Desilylation of **9** with $Et_3N{\cdot}3HF$ in pyridine, followed by tritylation and subsequent phosphitylation[17] of the secondary hydroxyl group in **10** with the monofunctional reagent chloro(2-cyanoethoxy)(*N,N*-diisopropylamino)phosphane gave the requisite phosphoramidite **4**, in 50% yield from **9**.

At this stage, the synthesis of the 5-GlcMdC phosphoramidite building block **5** was undertaken, starting from the 5-HMdU derivative **7**. To this end, compound **7** was deacetylated with K_2CO_3/MeOH and treated[18] with ethyl vinyl ether and pyridinium *p*-toluenesulfonate (PPTS) catalyst to give the allylic ethoxyethyl (EE) ether derivative **11**, in 87% yield over the two steps. Conversion of **11**, as described above for the synthesis of **9**, afforded the orthogonally protected 5-HMdC derivative **12** in good yield. Unfortunately, acid-mediated removal[19] of the EE ether in **12** resulted in migration of the N^4-benzoyl function, resulting in O^7-benzoylated **13** instead of the expected N^4-benzoyl derivative **14**.

An alternative route to the 5-GlcMdC derivative **5** would entail transformation of the known[13a] glucosylated 5-HMdU building block **15** (Scheme 2). Surprisingly, subjection of the benzoylated gluconucleoside **15** to the same conditions as used for the synthesis of **8** did not produce the expected intermediate triazolide **16**. It did, however, prove possible to obtain **16**, in a poor yield (29%), by prolonged treatment of **15** with excess $POCl_3$ and 1,2,4-triazole at elevated temperature. Alternatively, treatment of the tetra-*O*-acetyl derivative **18**, easily accessible by conversion of N^3-pivaloyloxymethyl (POM)-protected **17**,[13a] could readily be converted under the originally applied mild conditions (cf. **7** → **9**) into the requisite triazolide **19**, in 86% yield. Ammonolysis of the triazolyl group in **19**, followed by N^4-benzoylation, afforded the fully protected 5-HMdC derivative **20**, the identity of which was corroborated by NMR spectroscopy. Desilylation of **20** with fluoride ion and subjection of diol **21** to the same sequence of reactions as described for the conversion of compound **10** into **4** afforded the requisite glucosyl phosphoramidite derivative **5**, in 67% yield over the last two steps. In addition, ammonolysis of partially acylated **21** gave, after gel filtration, the fully deprotected nucleoside 5-(β-D-glucopyranosyloxymethyl)-2′-deoxycytidine (**2**), the structure of which was unambiguously assigned by mass spectrometry, as well as by ^1H and ^{13}C NMR spectroscopy.

Solid-phase Synthesis

With the fully protected phosphoramidites **4** and **5** in hand, the assemblage of the DNA fragments **23** and **25** was undertaken (Figure 1). Firstly, attention was focused on the

synthesis of the DNA 20-mer 5′-GTT TAC TTC **X**TC GGT TAG TG-3′ (**23**, X= 5-HMdC), the length and sequence of which was based on a molecular modeling study using the X-ray structure of T4 BGT[4,5,20] to select suitable candidates for co-crystallization experiments. The appropriate deoxynucleoside, immobilized by means of a 3′-*O*-succinyl bond to controlled pore glass (CPG), was elongated according to previously reported[13a] methodology for the solid-phase synthesis of oligodeoxynucleotides containing dJ (**3**). After completion of the elongation cycles, the immobilized DNA fragment was deprotected and simultaneously cleaved from the resin by ammonolysis (50 °C, 16 h). FPLC analysis of crude **23** (Figure 1) showed the presence of two products (ratio ≈2:1). MALDI-TOF mass spectrometric analysis of the individual DNA fragments, obtained after purification with FPLC, revealed that the main product corresponded to the target fragment **23**, containing 5-HMdC (calcd. for $C_{197}H_{266}N_{65}O_{126}P_{19}$: 6149.5; found 6149.9±4.6 $[M - H]^-$). Mass spectrometric analysis of the minor product showed an increment of 99 mass units (M = 6249.0±4.6) relative to **23**, indicating the presence of a benzamide group (M + 103), as in **24**. The formation of this side product can be explained by migration of the N^4-benzoyl group to a putative N^7-aminomethyl intermediate, formed by nucleophilic displacement of the 7-*O*-acetate moiety[21] with ammonia. Indirect support for the proposed mechanism was obtained as follows. The immobilized fully protected DNA fragment containing 5-HMdC was treated with a 0.1 M solution of NaOH in 1,4-dioxane/H_2O (1:2, v/v) at room temp. for 16 h to give, after ammonolysis, the oligonucleotide **23** as the sole product as evidenced by FPLC analysis of the crude product. In this respect, it is of interest to note that displacement of the acetate moiety by an amine is not observed in ammonolysis of corresponding 7-*O*-acetyl protected 5-HMdU-containing ODNs.[22]

Phosphoramidite building block **5** was successfully used in the automated solid-phase synthesis of the DNA 19-mer 5′-CATTACTAC**X**GGAACTCAG-3′ (**25**, X= 5-GlcMdC), containing 5-(β-D-glucopyranosyloxymethyl)-2′-deoxycytidine, following the same coupling methodology as described for the synthesis of **23**. FPLC analysis of the crude 19-mer, obtained after deblocking of the immobilized DNA fragment and cleavage from the solid support by ammonolysis, clearly indicated the presence of a major product, which could readily be purified by anion exchange chromatography to give the homogeneous glucosylated DNA fragment **25**. The integrity of **25** was corroborated by mass spectrometric analysis (calcd. for $C_{191}H_{245}N_{71}O_{116}P_{18}$: 5949.0; found 5954.7±4.5 $[M + H]^+$).

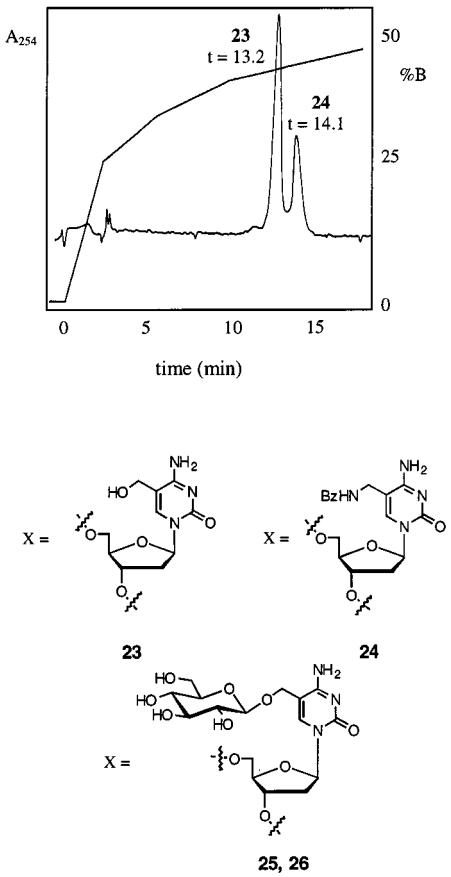


Figure 1. FPLC analysis of the crude DNA 20-mer **23**, containing 5-HMdC, after ammonolysis

BGT-Catalyzed Glucosylation of DNA Containing 5-HdMC Moieties

The above-described DNA 20-mer **23**, containing 5-HMdC, and the corresponding benzamide derivative **24**, were each hybridized with their complementary DNA strands. The feasibilities of the double-stranded (ds) ODNs as substrates for T4 β-glucosyltransferase (BGT) were next examined, by subjecting the ds-DNA fragments derived from **23** and **24** to an assay containing wild-type T4 BGT and radiolabeled uridine 5′-diphosphoglucose (^{14}C-UDPG, see Experimental Section). The reaction mixtures were analyzed by electrophoresis on a nondenaturing 20% polyacrylamide gel.

An autoradiograph of the electrophoresis gel is shown in Figure 2, A. The presence of a visible trace (highest spot) in lane 2 indicates that T4 phage DNA containing 5-HMdC (≈169 kbp) is glucosylated upon subjection to T4 BGT and ^{14}C-UDPG, proving that T4 BGT binds to DNA and is active. Interestingly, inspection of lane 3 clearly shows that synthetic ds-DNA fragment **23** has been converted into ^{14}C-glucosylated fragment **26**. On the other hand, the observation that ds-DNA **24** (lane 4) is not glucosylated strongly suggests the absence of an allylic hydroxyl group in **24**.

The binding affinities of the (glucosylated) ds-DNA fragments for T4 BGT were examined by Coomassie blue/ethidium bromide staining of the gel (Figure 2, B). Comparison of the mobilities of T4 BGT (lane 1) in the presence of either **26** (lane 3) or **24** (lane 4) reveals that ds-DNA oligomer **24** exhibits binding affinity for the glucosyltransferase, as evidenced by the shifted T4 BGT in lane 4. Interestingly, the migration of T4 BGT is not affected by the 20-mer **26**

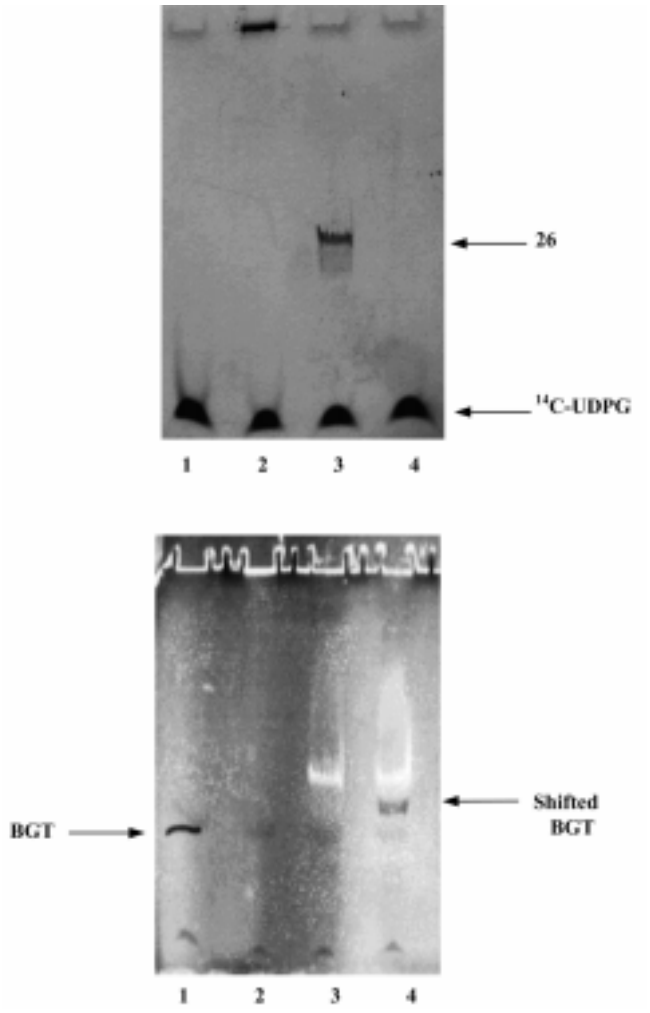


Figure 2. Polyacrylamide gel electrophoresis of ds-DNA 20-mers **23** (→ **26**) and **24** after incubation with T4 BGT and ^{14}C-UDPG. − (A) ^{14}C-Autoradiograph; (B) Coomassie/ethidium bromide staining − Lane 1: T4 BGT + ^{14}C-UDPG (control); Lane 2: T4* DNA, containing 5-HMdC (169 kbp); Lane 3: ds-DNA **23**, containing 5-HMdC (→ **26**); Lane 4: ds-DNA **24**, containing 7-benzamide

(lane 3), demonstrating that glucosylation of ds-DNA results in loss of binding affinity for T4 BGT.

Conclusions

This paper describes the synthesis of phosphoramidites **4** and **5** and their use in the solid-phase syntheses of the respective DNA fragments **23** and **25**. In addition, the first synthesis of the hypermodified nucleoside 5-(β-D-glucopyranosyloxymethyl)-2′-deoxycytidine (**2**) is reported. It was also established that the 5-HMdC-containing duplex DNA 20-mer **23** serves as a suitable substrate for in vitro T4 BGT-catalyzed glucosylation with UDPG (→ **26**), which may be useful for examining the activity of T4 BGT mutants to determine the amino acid residues involved in the glucosyltransfer reaction. Studies aimed at resolving a crystal structure of a ternary complex of BGT, UDPG, and ds-DNA fragment **23** are currently underway, while glucosylated ODNs similar to **25** and **26** may be useful tools for identifying novel proteins that specifically recognize glucosylated DNA.[12]

Experimental Section

General Procedures and Materials: Toluene, CH_2Cl_2, and pyridine were distilled from P_2O_5 and stored over 4-Å molecular sieves. Et_2O was freshly distilled from $LiAlH_4$. DCE (Biosolve, HPLC grade), DMF, and CCl_4 (Baker, p.a.) were stored over 4-Å molecular sieves. MeOH (Rathburn, HPLC grade) and CH_3CN (Rathburn, HPLC grade) were stored over 3-Å molecular sieves. D*i*PEA was dried by refluxing with CaH_2 (5 g·L^{-1}) for 16 h and then distilled. All chemicals (Acros, Belgium) were used as received. Chloro(2-cyanoethoxy)(*N,N*-diisopropylamino)phosphane was prepared as described.[17]− Column chromatography was performed with Baker silica gel (0.063−0.200 mm). TLC analysis was performed with "DC-Fertigfolien" (Schleicher & Schüll F1500, LS 254) with detection by UV absorption (254 nm) and by charring with 20% H_2SO_4 in EtOH. Prior to reactions requiring anhydrous conditions, traces of H_2O were removed by repeated coevaporation with DCE, toluene, or pyridine. − ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded with a Jeol JNM-FX-200 (200/50.1/80.7 MHz), a Bruker WM-300 (300/75.1/121.0 MHz) or a Bruker DMX-600 spectrometer (600/150/242.1 MHz). All spectra were recorded at 200/50.1/80.7 MHz, respectively, unless otherwise stated. ^{1}H and ^{13}C chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard and ^{31}P chemical shifts relative to 85% H_3PO_4 as external standard. − Elemental analyses were performed with a Perkin−Elmer Series II Analyzer 2400. − Mass spectra were recorded on a Finnigan MAT TSQ-70 or a PE-SIEX API 165 mass spectrometer equipped with an Electrospray Interface (ES). High resolution (ES) mass spectra were recorded with a Finnigan MAT 900 double focusing mass spectrometer equipped with an ES interface. − Optical rotations were determined with a Propol automatic polarimeter at room temp.

5-Acetyloxymethyl-3′,5′-di-*O*-*tert*-butyldimethylsilyl-2′-deoxyuridine (7): Compound **6** (7.05 g, 15.0 mmol) was irradiated until reflux in dry CCl_4 (275 mL) with a 250-Watt Philips heat lamp, and Br_2 (0.66 mL, 18.0 mmol) was passed through the solution in a flow of dry nitrogen gas over a 2 h period as described by Bärwolff et al.[15] The solution was cooled to room temp. and degassed for 30 min. The solvent was evaporated in vacuo (<45 °C) and the resulting crude, oily bromide, R_f = 0.43 (CH_2Cl_2/MeOH, 99:1, v/v), was immediately used in the next reaction. The bromide was dissolved in DMF (110 mL) and CsOAc (7.20 g, 37.5 mmol) was added to the vigorously stirred solution. After 1 h, brine (15 mL) was added and the mixture was extracted with Et_2O. The organic layer was washed with a sat. aq. solution of $NaHCO_3$ and dried ($MgSO_4$). Purification was effected by column chromatography (Et_2O/light petroleum, 1:4→1:1, v/v) to give **7** as an oil. Yield 4.08 g (51% for the 2 steps). R_f = 0.57 (CH_2Cl_2/MeOH, 99:1, v/v). − ^{1}H NMR ($CDCl_3$): δ = 9.52 (br. s, 1 H, NH), 7.72 (s, 1 H, H-6), 6.19 (t, 1 H, H-1′, $J_{1',2'}$ = 6.0 Hz), 4.72 (AB, 2 H, H-7, J = −12.6 Hz), 4.40 (m, 1 H, H-3′), 3.90 (dd, 1 H, H-4′, $J_{3',4'}$ = 5.3 Hz, $J_{4',5'}$ = 2.6 Hz), 3.86 (ABX, 2 H, H-5′, $J_{5a',5b'}$ = −14.7 Hz), 2.34 (ddd, 1 H, H-2a′, $J_{2a',2b'}$ = −12.6 Hz, $J_{2a',3'}$ = 1.9 Hz), 2.06 (m, 1 H, H-2b′), 1.94 (s, 3 H, CH_3 Ac), 0.83 (s, 18 H, CH_3 *t*Bu TBDMS), 0.11, 0.09 (2 × s, 12 H, CH_3 TBDMS). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 170.2 (C=O, Ac), 162.7 (C-4), 150.0 (C-2), 140.8 (C-6), 108.7 (C-5), 87.5 (C-4′), 85.0 (C-1′), 71.8 (C-3′), 62.6 (C-5′), 58.7 (C-7), 41.0

(C-2′), 25.5, 25.4 (CH_3 *t*Bu TBDMS), 20.4 (CH_3 Ac), 18.0, 17.6 (Cq *t*Bu TBDMS), −4.9, −5.0, −5.8 (CH_3 TBDMS). $C_{24}H_{44}N_2O_7Si_2$ (528.8): calcd. C 54.51, H 8.39, N 5.30; found C 54.68, H 8.52, N 5.19.

5-Acetyloxymethyl-*N*⁴-benzoyl-3′,5′-di-*O*-*tert*-butyldimethylsilyl-2′-deoxycytidine (9): Compound **7** (0.99 g, 1.88 mmol) and 1,2,4-triazole (2.60 g, 37.8 mmol) were dissolved in a mixture of CH_3CN (40 mL) and Et_3N (6.41 mL, 41.4 mmol). $P(O)Cl_3$ (0.45 mL, 4.70 mmol) was added under a nitrogen atmosphere and the reaction mixture was stirred for 1 h. The mixture was diluted with CH_2Cl_2 (25 mL) and then washed with sat. aq. $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$, concentrated, and purified by column chromatography (Et_2O/light petroleum, 1:1→1:0, v/v) to yield 0.93 g of the triazolide **8** (1.60 mmol, 85%). R_f = 0.38 (Et_2O). − ^1H NMR ($CDCl_3$): δ = 9.36 (s, 1 H, H triazole), 8.56 (s, 1 H, H-6), 8.17 (s, 1 H, H triazole), 6.31 (t, 1 H, H-1′, $J_{1',2'}$ = 5.8 Hz), 5.43 (AB, 2 H, H-7, J = −10.7 Hz), 4.47 (m, 1 H, H-3′), 4.18 (m, 1 H, H-4′), 3.91 (ABX, 2 H, H-5′, $J_{5a',5b'}$ = −11.0 Hz, $J_{4',5'}$ = 2.5 Hz), 2.73 (m, 1 H, H-2a′), 2.14 (m, 1 H, H-2b′), 2.07 (s, 3 H, CH_3 Ac), 0.86 (s, 18 H, CH_3 *t*Bu TBDMS), 0.12, 0.06 (2 × s, 12 H, CH_3 TBDMS). − $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 170.6 (C=O, Ac), 153.8 (C-4), 149.3 (C-6), 145.3 (C-2), 115.6 (CH triazole), 103.9 (C-5), 89.3 (C-4′), 88.7 (C-1′), 72.3 (C-3′), 61.6 (C-7), 63.1 (C-5′), 42.7 (C-2′), 26.3, 26.2 (CH_3 *t*Bu TBDMS), 21.1 (CH_3 Ac), 18.6, 18.3 (Cq TBDMS), −5.2 (CH_3 TBDMS). Triazolide **8** was dissolved in 1,4-dioxane (10 mL) and NH_4OH (25%, 1.0 mL) was added. The solution was stirred for 30 min, after which TLC analysis showed complete conversion into a lower-running product, R_f = 0.43 (EtOAc/MeOH, 92:8, v/v). The mixture was repeatedly coevaporated with pyridine (3 × 5 mL). The residue was dissolved in pyridine (8.0 mL) and treated with BzCl (0.30 mL, 2.64 mmol). The yellow solution was stirred overnight and quenched with NH_4OH (25%, 1 mL). After 5 min the solution was concentrated, dissolved in CH_2Cl_2, and washed with sat. aq. $NaHCO_3$ and brine. The crude product, obtained after drying ($MgSO_4$) and concentration of the organic layer, was purified by column chromatography (EtOAc/light petroleum, 1:4→1:1, v/v) to give cytidine **9**. Yield 76% (0.85 g, 1.34 mmol). R_f = 0.76 (EtOAc/light petroleum, 1:2, v/v). − ^1H NMR ($CDCl_3$): δ = 8.27 (dd, 2 H, H-arom Bz, J = 1.8 Hz and 8.4 Hz), 7.97 (s, 1 H, H-6), 7.52−7.41 (m, 3 H, H-arom Bz), 6.29 (dd, 1 H, H-1′, $J_{1',2a'}$ = 5.9 Hz, $J_{1',2'}$ = 7.3 Hz), 5.06 (AB, 2 H, H-7, J = −12.4 Hz), 4.40 (m, 1 H, H-3′), 4.02 (m, 1 H, H-4′), 3.82 (ABX, 2 H, H-5′, $J_{5a',5b'}$ = −11.7 Hz, $J_{4',5'}$ = 3.7 Hz), 2.40 (ddd, 1 H, H-2a′, $J_{2a',2b'}$ = −13.2 Hz, $J_{2a',3'}$ = 2.9 Hz), 2.08 (s, 3 H, CH_3 Ac), 2.04 (ddd, 1 H, H-2b′, $J_{2b',3'}$ = 1.5 Hz), 0.92, 0.90 (2 × s, 18 H, CH_3 *t*Bu TBDMS), 0.11, 0.08 (2 × s, 12 H, CH_3 TBDMS). − $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 179.4 (C=O, Bz), 170.7 (C=O, Ac), 158.4 (C-4), 147.2 (C-2), 140.9 (C-6), 136.6 (Cq Bz), 132.4, 129.8, 128.0 (CH-arom), 109.5 (C-5), 88.3 (C-4′), 86.1 (C-1′), 72.2 (C-3′), 62.9 (C-7), 59.4 (C-5′), 41.6 (C-2′), 25.8, 25.6 (CH_3 *t*Bu TBDMS), 20.8 (CH_3 Ac), 18.2, 17.8 (Cq TBDMS), −4.8, −5.6 (CH_3 TBDMS); ES-MS: 632 $[M + H]^+$. Calcd. for $C_{31}H_{49}N_3O_7Si_2$: C 58.92, H 7.82, N 6.65; found C 59.07, H 7.93, N 6.83.

5-Acetyloxymethyl-*N*⁴-benzoyl-2′-deoxycytidine (10): A solution of compound **9** (0.41 g, 0.65 mmol) in pyridine (3.0 mL) was treated with $Et_3N{\cdot}3HF$ (0.21 mL, 1.30 mmol). After stirring overnight, the mixture was concentrated, dissolved in CH_2Cl_2 (20 mL), and washed with brine (2 × 10 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was purified by column chromatography (EtOAc/CH_2Cl_2/MeOH, 20:5/1, v/v/v) to afford **10** as a white solid. Yield 0.21 g (81%, 0.53 mmol). R_f = 0.12 (EtOAc). − ^1H NMR (CD_3OD): δ = 8.40 (s, 1 H, H-6), 8.18 (d, 2 H, H-arom Bz, J 7.3 Hz), 7.57−7.39 (m, 3 H, H-arom Bz), 6.27 (t, 1 H, H-1′, $J_{1',2'}$ = 6.6 Hz), 5.05 (s, 2 H, H-7), 4.41 (m, 1 H, H-3′), 3.98 (m, 1 H, H-4′), 3.81 (ABX, 2 H, H-5′, $J_{5a',5b'}$ = −12.4 Hz, $J_{4',5'}$ = 2.9 Hz), 2.47−2.19 (m, 2 H, H-2′), 2.05 (s, 3 H, CH_3 Ac).

5-Acetyloxymethyl-*N*⁴-benzoyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)cytidine 3′-*O*-(2-Cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (4): DMTCl (0.26 g, 0.75 mmol) was added to a stirred solution of **10** (0.20 g, 0.50 mmol) in pyridine (4 mL). After 3 h, the reaction was quenched with MeOH (1.0 mL) and after 15 min the mixture was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (10 mL) and washed with sat. aq. $NaHCO_3$ (5 mL) and brine (2 × 5 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification was accomplished by column chromatography (EtOAc/light petroleum/Et_3N, 50:50:1→60:30:1, v/v/v) to give 5-acetyloxymethyl-*N*⁴-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine. Yield 0.13 g (0.38 mmol, 76%). R_f = 0.81 (EtOAc). − ^1H NMR ($CDCl_3$): δ = 8.26 (dd, 2 H, H-arom Bz, J = 1.5 Hz and 8.0 Hz), 8.05 (s, 1 H, H-6), 7.56−7.24 (m, 12 H, H-arom Bz, DMT), 6.84 (d, 4 H, H-arom DMT, J = 8.7 Hz), 6.38 (t, 1 H, H-1′, $J_{1',2'}$ = 5.9 Hz), 4.58 (m, 1 H, H-3′), 4.54 (AB, 2 H, H-7, J = −11.7 Hz), 4.11 (m, 1 H, H-4′), 3.79 (ABX, 2 H, H-5′, $J_{5a',5b'}$ = −10.2 Hz, $J_{4',5'}$ = 2.9 Hz), 2.53−2.19 (ddd, 1 H, H-2a′, $J_{2a',2b'}$ = −13.2 Hz, $J_{2a',3'}$ = 2.9 Hz), 2.38 (dd, H-2b′),1.86 (s, 3 H, CH_3 Ac). − $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 179.5 (C=O, Bz), 170.6 (C=O, Ac), 158.6 (Cq DMT), 158.3 (C-4), 147.7 (C-2), 144.0 (Cq DMT), 141.0 (C-6), 136.5 (Cq Bz), 135.2 (Cq DMT), 132.4−127.0 (CH-arom Bz, DMT), 113.1 (CH-arom DMT), 109.9 (C-5), 86.4 (C-4′), 85.6 (C-1′), 71.8 (C-3′), 63.2 (C-7), 59.0 (C-5′), 55.1 (OCH_3 DMT), 41.3 (C-2′), 20.5 (CH_3 Ac). D*i*PEA (0.10 mL, 0.58 mmol) was then added to the 5′-tritylated derivative (0.13 g, 0.38 mmol) in CH_2Cl_2 (4 mL), followed by chloro(2-cyanoethoxy)(*N*,*N*-diisopropylamino-)phosphane (95 μL, 0.39 mmol). After 1 h, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with sat. aq. $NaHCO_3$ and H_2O. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was purified by flash column chromatography (light petroleum/EtOAc/Et_3N, 40:59:1, v/v/v) to give **4** as a white foam. Yield 0.28 g (0.31 mmol, 81%). R_f = 0.81, 0.75 (EtOAc/light petroleum, 1:1, v/v). − $^{31}P\{^1H\}$ NMR ($CDCl_3$) δ = 149.6, 149.1.

(*R*/*S*)-3′,5′-Di-*O*-*tert*-butyldimethylsilyl-2′-deoxy-5-(1-ethoxyethoxymethyl)uridine (11): Acetate **7** (0.91 g, 1.72 mmol) was dissolved in a 0.05 M solution of anhydrous K_2CO_3 in MeOH (15 mL). The mixture was stirred for 5 h and then neutralized with Dowex 50WX4-100 (H^+), filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (Et_2O/light petroleum, 1:2 to 2:1, v/v), affording the deacetylated product as a colorless oil. R_f = 0.52 (Et_2O). Yield 0.73 g (1.50 mmol, 87%). The alcohol was dissolved in a mixture of CH_2Cl_2/ethyl vinyl ether (20 mL, 2:1, v/v) and *p*TsOH (0.02 g, 0.11 mmol) was added. The mixture was stirred for 15 min, diluted with Et_2O (10 mL), and washed with sat. aq. $NaHCO_3$ and H_2O. The organic layer was dried ($MgSO_4$) and concentrated to give **11**, which was used in the next reaction without purification. Yield 0.84 g (1.50 mmol, quant.). R_f = 0.79 (Et_2O). − ^1H NMR ($CDCl_3$): δ = 9.21 (br. s, 1 H, NH), 7.59 (s, 1 H, H-6), 6.29 (t, 1 H, H-1′, $J_{1',2'}$ = 5.9 Hz), 4.78 (m, 1 H, CH EE), 4.42−4.09 (m, 3 H, H-3′, H-7), 3.92−3.44 (m, 5 H, H-5′, CH_2 EE, H-4′), 2.27 (m, 1 H, H-2a′), 2.01 (m, 1 H, H-2b′), 1.42−1.07 (m, 6 H, 2 × CH_3 EE), 0.86, 0.83 (2 × s, 18 H, CH_3 *t*Bu TBDMS), 0.10, 0.04 (2 × s, 12 H, CH_3 TBDMS). − $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 162.9 (C-4), 150.1 (C-2), 137.7 (C-6), 111.7 (C-5), 99.7 (CH EE), 87.7 (C-4′), 85.1 (C-1′),

72.1 (C-3′), 62.9, 60.9, 59.6 (CH_2 EE, C-5′, C-7), 40.9 (C-2′), 25.8, 25.6 (CH_3 *t*Bu TBDMS), 19.7, 18.2, 17.8, 15.1 (CH_3 EE, Cq *t*Bu TBDMS), −5.0, −5.6, (CH_3 TBDMS).

(*R*/*S*)-N^4-Benzoyl-3′,5′-di-*O*-*tert*-butyldimethylsilyl-2′-deoxy-5-(1-ethoxyethoxymethyl)cytidine (12): Uridine derivative **11** (0.84 g, 1.50 mmol) was converted as described for the preparation of **9** from **7**. Purification was established by column chromatography (light petroleum/EtOAc, 3:1, v/v). Yield 0.69 g (1.05 mmol, 70%). R_f = 0.67 (light petroleum/EtOAc, 3:1, v/v). − ^{1}H NMR (300 MHz, HH COSY, $CDCl_3$): δ = 8.28 (d, 2 H, H-arom Bz), 7.82 (s, 1 H, H-6), 7.37−7.56 (m, 3 H, H-arom Bz), 6.30 (t, 1 H, H-1′, $J_{1',2'}$ = 5.9 Hz), 4.91 (m, 1 H, CH EE), 4.55 (AB, 2 H, H-7, *J* = −12.5 Hz), 4.42 (m, 1 H, H-3′), 3.99 (m, 1 H, H-4′), 3.78 (d, 2 H, H-5′, $J_{4',5'}$ = 3.7 Hz), 3.63 (m, 1 H, CH_2 EE), 2.38 (ddd, 1 H, H-2a′, $J_{2a',2b'}$ = −13.2 Hz, $J_{2a',3'}$ = 2.5 Hz), 2.06 (m, 1 H, H-2b′), 1.39 (d, 3 H, CH_3 EE, *J* 5.4 Hz), 1.22 (t, 3 H, CH_3 EE, *J* 7.0 Hz), 0.90 (s, 18 H, CH_3 *t*Bu TBDMS), 0.11, 0.09 (2 × s, 12 H, CH_3 TBDMS). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 158.6 (C-4), 147.8 (C-2), 138.4 (C-6), 136.1 (Cq Bz), 132.4, 129.8, 128.1 (CH-arom), 112.2 (C-5), 100.0 (CH EE), 88.1 (C-4′), 86.1 (C-1′), 72.4 (C-3′), 63.1, 60.8, 59.4 (CH_2 EE, C-5′, C-7), 41.4 (C-2′), 25.6, 25.2 (CH_3 *t*Bu TBDMS), 19.7, 15.9 (CH_3 EE), 18.1, 17.8, (Cq *t*Bu TBDMS), −5.0, −5.4, −5.5, −5.6, (CH_3 TBDMS). − ES-MS: 662 [M + H^+], 684 [M + Na^+].

5-Benzoyloxymethyl-3′,5′-bis(*O*-*tert*-butyldimethylsilyl)-2′-deoxycytidine (13): A solution of compound **12** (0.45 g, 0.68 mmol) in MeOH (12 mL) containing *p*TsOH (15 mg, 0.08 mmol) was stirred for 1.5 h. The mixture was quenched with Et_3N (0.10 mL, 0.69 mmol), concentrated, and subjected to column chromatography (EtOAc) to give O^7-benzoyl derivative **13**. Yield 0.20 g (0.34 mmol, 50%). R_f = 0.14 (light petroleum/EtOAc, 1:3, v/v). − ^{1}H NMR ($CDCl_3$,): δ = 8.05−7.91 (m, 3 H, H-arom Bz, H-6), 7.54−7.30 (m, 3 H, H-arom Bz), 6.21 (t, 1 H, H-1′, $J_{1',2'}$ = 5.9 Hz), 5.04 (AB, 2 H, H-7, *J* = −12.3 Hz), 4.34 (m, 1 H, H-3′), 3.89 (m, 1 H, H-4′), 3.76 (ABX, 2 H, H-5′, $J_{4',5'}$ = 3.7 Hz, $J_{5a',5b'}$ = 3.1 Hz), 2.43 (ddd, 1 H, H-2a′, $J_{2a',2b'}$ = −11.4 Hz, $J_{2a',3'}$ = 2.2 Hz), 1.97 (m, 1 H, H-2b′), 0.89, 0.88 (2 × s, 18 H, CH_3 *t*Bu TBDMS), 0.09, 0.06, 0.04 (3 × s, 12 H, CH_3 TBDMS). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 159.7 (C-4), 157.9 (C=O Bz), 147.2 (C-2), 137.8 (C-6), 136.3 (Cq Bz), 132.6, 129.5, 128.2 (CH-arom), 113.3 (C-5), 88.2 (C-4′), 85.9 (C-1′), 72.1 (C-3′), 62.8, 59.9 (C-5′, C-7), 41.7 (C-2′), 25.8, 25.6 (CH_3 *t*Bu TBDMS), 18.3, 17.8, (Cq *t*Bu TBDMS), −4.8, −5.5 (CH_3 TBDMS). ES-MS: 590 [M + H^+], 612 [M + Na^+], 1180 [2M + H]$^+$, 1202 [2M + Na]$^+$.

3′,5′-Bis(*O*-*tert*-butyldimethylsilyl)-2′-deoxy-5-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyloxymethyl)-4-(N^1-1,2,4-triazolyl)uridine (16): Compound **15**[13a] (3.19 g, 3.0 mmol) was treated, essentially as described for the preparation of **9**, at 70 °C for 16 h with 8.3 equiv. of $P(O)Cl_3$ and 38 equiv. of 1,2,4-triazole. Purification of the triazolide was effected by column chromatography (light petroleum/EtOAc, 2:1, v/v) to give **16**. Yield 2.91 g (0.87 mmol, 29%). R_f = 0.35 (Et_2O). − ^{1}H NMR ($CDCl_3$) δ = 9.13, 8.45 (2 × s, 2 H, H-triazole), 8.03−7.78 (m, 9 H, H-arom Bz, H-6), 7.54−7.54 (m, 12 H, H-arom Bz), 6.11 (dd, 1 H, H-1′, $J_{1',2a'}$ = 6.6 Hz), 5.94 (t, 1 H, H-3′′, $J_{2'',3''}$= $J_{3'',4''}$ = 9.5 Hz), 5.68 (t, 1 H, H-4′′, $J_{4'',5''}$ = 9.6 Hz), 5.51 (dd, 1 H, H-2′′, $J_{1'',2''}$ = 7.9 Hz, $J_{2'',3''}$ = 9.7 Hz), 5.09 (AB, 2 H, H-7, *J* = −12.2 Hz), 5.02 (d, 1 H, H-1′′), 4.64 (dd, 1 H, H-6a′′, $J_{5'',6a''}$ = 4.7 Hz, $J_{6a'',6b''}$ = −11.9 Hz), 4.51 (dd, 1 H, H-6b′′, $J_{5'',6b''}$ = 2.6 Hz), 4.38 (m, 1 H, H-3′), 4.23 (ddd, 1 H, H-5′′), 4.12 (m, 1 H, H-4′), 3.88 (ABX, 2 H, H-5′, $J_{5a',5b'}$ = −11.1 Hz, $J_{4',5a'}$= $J_{4',5b'}$ = 3.2 Hz), 2.72 (ddd, 1 H, H-2a′, $J_{2a',2b'}$ = −13.2 Hz, $J_{2a',3'}$ = 4.0 Hz), 2.09 (ddd, 1 H, H-2b′, $J_{2b',3'}$ = 6.7 Hz), 0.92, 0.85 (2 × s, 18 H, CH_3 *t*Bu-Si), 0.07, 0.06 (2 × s, 12 H, CH_3Si). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 166.0, 165.7, 165.2, 164.9 (4 × C=O Bz), 156.1 (C-4), 148.8 (C-2), 147.2 (CH triazole), 133.6 (C-6), 133.3−128.1 (CH-arom, Bz), 129.1, 128.6, 128.4 (Cq Bz), 104.5 (C-5), 101.0 (C-1′′), 89.5 (C-4′), 89.1 (C-1′), 72.8, 72.6, 72.3, 71.7, 69.6 (C-2′′, C-3′′, C-4′′, C-5′′, C-3′), 64.9, 66.2, 63.0, 62.9 (C-6′′, C-5′, C-7), 42.4 (C-2′), 25.7 (CH_3 *t*Bu TBDMS), 18.2 (Cq TBDMS), −4.9, −5.0, (CH_3 TBDMS).

3′,5′-Bis(*O*-*tert*-butyldimethylsilyl)-2′-deoxy-5-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxymethyl)uridine (18): A solution of **17**[13a] (1.60 g, 1.36 mmol) was stirred at 60 °C in a 0.1 M solution of *t*BuOK in MeOH (15 mL). After 16 h the reaction mixture was neutralized with Dowex 50WX4-100 (H^+), concentrated, and redissolved in a mixture of pyridine and Ac_2O (6 mL, 1:1, v/v). TLC analysis indicated the formation of a single product, and after 1 h the solution was repeatedly coevaporated with toluene (3 × 10 mL). The crude oily product was purified by column chromatography (light petroleum/EtOAc, 4:1→1:1, v/v) to give tetraacetate **18**. Yield 0.99 g (1.21 mmol, 89%). R_f = 0.70 (light petroleum/EtOAc, 1:3, v/v). − ^{1}H NMR (H-H COSY, 300 MHz, $CDCl_3$) δ = 8.10 (s, 1 H, NH), 7.67 (s, 1 H, H-6), 6.24 (dd, 1 H, H-1′, $J_{1',2a'}$ = 5.7 Hz, $J_{1',2b'}$ = 8.0 Hz), 5.21 (t, 1 H, H-3′′, $J_{2'',3''}$= $J_{3'',4''}$ = 9.5 Hz), 5.07 (t, 1 H, H-4′′, $J_{4'',5''}$ = 9.6 Hz), 4.97 (dd, 1 H, H-2′′, $J_{1'',2''}$ = 8.0 Hz, $J_{2'',3''}$ = 9.5 Hz), 4.68 (d, 1 H, H-1′′), 4.45 (AB, 2 H, H-7, *J* = −12.4 Hz), 4.38 (m, 1 H, H-3′), 4.25 (dd, 1 H, H-6a′′, $J_{5'',6a''}$ = 4.5 Hz, $J_{6a'',6b''}$ = −12.5 Hz), 4.13 (dd, 1 H, H-6b′′, $J_{5'',6b''}$ = 2.4 Hz), 3.97 (m, 1 H, H-4′), 3.79 (ABX, 2 H, H-5′, $J_{4',5'}$ = −3.2 Hz, $J_{5a',5b'}$ = −11.3 Hz), 3.70 (ddd, 1 H, H-5′′), 2.31 (ddd, 1 H, H-2a′, $J_{2a',2b'}$ = −13.2 Hz, $J_{2a',3'}$ 2.2 Hz), 2.01 (m, 1 H, H-2b′), 2.08, 2.02, 1.99 (3 × s, 12 H, 4 × CH_3 Ac), 0.90, 0.89 (2 × s, 18 H, CH_3 *t*Bu-Si), 0.10, 0.08 (2 × s, 12 H, CH_3Si). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 170.2, 169.8, 169.0, 168.5 (4 × C=O Ac), 163.1 (C-4), 149.9 (C-2), 138.8 (C-6), 110.3 (C-5), 100.5 (C-1′′), 91.3 (C-4′), 87.8 (C-1′), 72.3, 72.2, 71.4, 70.8, 69.4 (C-2′′, C-3′′, C-4′′, C-5′′, C-3′), 66.3, 62.7, 61.4 (C-6′′, C-5′, C-7), 40.9 (C-2′), 25.5, 25.4 (CH_3 *t*Bu TBDMS), 20.1 (CH_3 Ac), 18.0, 17.6 (Cq *t*Bu TBDMS), −5.1, −5.2, −5.9 (CH_3 TBDMS). ES-MS: 840 [M + Na^+]. $[\alpha]_D^{20}$ = −4.6° (*c* = 1.0 $CHCl_3$). Calcd. for $C_{36}H_{60}N_2O_{15}Si_2$: C 52.92, H 7.40, N 3.43; found C 53.01, H 7.45, N 3.51.

3′,5′-Bis(*O*-*tert*-butyldimethylsilyl)-2′-deoxy-5-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxymethyl)-4-(N^1−1,2,4-triazolyl)uridine (19): Tetraacetate **18** (0.49 g, 0.60 mmol) was treated as described for the preparation of **9** from **7**. Crude triazolide **20** was purified by column chromatography (light petroleum/EtOAc, 1:1→0:1, v/v). Yield 0.45 g (0.52 mmol, 86%). R_f = 0.30 (light petroleum/EtOAc, 1:3, v/v). − ^{1}H NMR ($CDCl_3$) δ = 9.29, 8.43 (2 × s, 2 H, CH triazole), 8.12 (s, 1 H, H-6), 6.19 (t, 1 H, H-1′, $J_{1',2'}$ = 6.6 Hz), 5.22 (t, 1 H, H-3′′, $J_{2'',3''}$= $J_{3'',4''}$ = 9.5 Hz), 5.17−4.92 (m, 4 H, H-4′′, H-2′′, H-7), 4.72 (d, 1 H, H-1′′, $J_{1'',2''}$ = 7.3 Hz), 4.36 (m, 1 H, H-3′), 4.28 (dd, 1 H, H-6a′′, $J_{5'',6a''}$ = 5.1 Hz, $J_{6a'',6b''}$ = −12.4 Hz), 4.13 (m, 1 H, H-4′), 4.09 (dd, 1 H, H-6b′′, $J_{5'',6b''}$ = 2.2 Hz), 3.84 (ABX, 2 H, H-5′, $J_{4',5'}$ = −3.7 Hz, $J_{5a',5b'}$ = −11.0 Hz), 3.74 (ddd, 1 H, H-5′′), 2.73 (ddd, 1 H, H-2a′, $J_{2a',2b'}$ = −13.0 Hz, $J_{2a',3'}$ = 5.9 Hz), 2.14 (m, 1 H, H-2b′), 2.07, 2.05, 2.02, 1.98 (4 × s, 12 H, 4 × CH_3 Ac), 0.89, 0.84 (2 × s, 18 H, CH_3 *t*Bu-Si), 0.08, 0.07 (2 × s, 12 H, CH_3Si). − ^{13}C NMR ($CDCl_3$): δ = 170.5, 170.1, 169.4, 169.2 (4 × C=O Ac), 156.4 (C-4), 153.6 (CH triazole), 147.2 (C-2), 144.8 (CH triazole), 138.9 (C-6), 104.6 (C-5), 100.6 (C-1′′), 89.6 (C-4′), 89.1 (C-1′), 72.8, 72.6, 71.8, 71.1, 68.2, 66.3, 62.8, 61.8 (C-2′′, C-3′′, C-4′′, C-5′′, C-3′, C-6′′, C-5′, C-7), 41.9 (C-2′), 25.8, 25.7 (CH_3 *t*Bu TBDMS), 20.5 (CH_3 Ac), 18.2 (Cq *t*Bu TBDMS), −5.0, −4.7 (CH_3 TBDMS). ES-MS: 869

[M + H$^+$], 891 [M + Na$^+$]. Calcd. for $C_{38}H_{61}N_5O_{14}Si_2$: C 52.58, H 7.08, N 8.07; found C 52.78, H 7.23, N 7.87.

***N*^{4}-Benzoyl-3′,5′-bis(*O*-*tert*-butyldimethylsilyl)-2′-deoxy-5-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxymethyl)cytidine (20):** Triazolide **19** (0.33 g, 0.38 mmol) was dissolved in a mixture of 1,4-dioxane and 25% NH_4OH (10 mL, 10:1, v/v). The solution was stirred for 1 h and concentrated with pyridine (3 × 5 mL). The intermediate cytidine (R_f = 0.05, EtOAc) was dissolved in pyridine (4.0 mL) and BzCl (0.11 mL, 0.95 mmol) was added. The yellow solution was stirred overnight and quenched with MeOH (0.5 mL). After 5 min the mixture was concentrated, dissolved in CH_2Cl_2 (20 mL), and washed with sat. aq. $NaHCO_3$ (2 × 10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated. Purification by column chromatography (EtOAc/light petroleum, 1:3→1:1, v/v) gave **20** in 73% yield (0.25 g, 0.28 mmol). R_f = 0.85 (EtOAc). − ^{1}H NMR ($CDCl_3$) δ = 8.20 (m, 2 H, CH-arom Bz), 7.88 (s, 1 H, H-6), 7.50 (3 H, CH-arom Bz), 6.24 (dd, 1 H, H-1′, $J_{1',2a'}$ = 5.8 Hz, $J_{1',2b'}$ = 7.3 Hz), 5.19 (t, 1 H, H-3′′, $J_{2'',3''}$= $J_{3'',4''}$ = 9.5 Hz), 5.08 (t, 1 H, H-4′′, $J_{4'',5''}$ = 9.5 Hz), 5.02 (dd, H-2”, $J_{1",2"}$ = 7.3 Hz), 4.79 (d, 1 H, H-1"), 4.71 (AB, 2 H, H-7, J = −12.2 Hz), 4.41 (m, 1 H, H-3′), 4.26 (dd, 1 H, H-6a′′, $J_{5'',6a''}$ = 4.2 Hz, $J_{6a'',6b''}$ = −12.3 Hz), 4.16 (dd, 1 H, H-6b′′, $J_{5'',6b''}$ = 2.7 Hz), 4.04 (m, 1 H, H-4′), 3.82 (m, 2 H, H-5′), 3.69 (ddd, 1 H, H-5′′), 2.39 (ddd, 1 H, H-2a′, $J_{2a',2b'}$ = −13.1 Hz, $J_{2a',3'}$ = 2.4 Hz), 2.08 (m, 1 H, H-2b′), 2.08, 2.03, 2.01, 1.92 (4 × s, 12 H, 4 × CH_3 Ac), 0.89, 0.87 (2 × s, 18 H, CH_3 *t*Bu-Si), 0.09, 0.07 (2 × s, 12 H, CH_3Si). − ^{13}C NMR ($CDCl_3$): δ = 170.0, 169.6, 168.8, 168.7 (4 × C=O Ac), 158.2 (C-4), 146.9 (C-2), 139.6 (C-6), 136.2, 133.7 (Cq Bz), 133.0, 132.0, 129.5, 129.1, 127.8, 127.6 (CH-arom Bz), 110.2 (C-5), 100.2 (C-1′′), 88.1 (C-4′), 86.1 (C-1′), 72.2, 71.2, 70.6, 67.6, 64.0, 62.5, 61.2 (C-2′′, C-3′′, C-4′′, C-5′′, C-3′, C-6′′, C-5′, C-7), 41.1 (C-2′), 25.2, 25.1 (CH_3 *t*Bu TBDMS), 20.0 (CH_3 Ac), 17.7, 17.4 (Cq *t*Bu TBDMS), −5.3, −6.0 (CH_3 TBDMS). ES-MS: 921 [M + H$^+$], 943 [M + Na$^+$]. − $C_{36}H_{60}N_2O_{15}Si_2$: calcd. C 56.13, H 7.12, N 4.57; found C 56.08, H 7.09, N 4.69.

***N*^{4}-Benzoyl-2′-deoxy-5-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxymethyl)cytidine (21):** Compound **20** (0.28 g, 0.28 mmol) was desilylated and purified as described under the preparation of **10**. Yield 76% (0.14 g, 0.21 mmol). R_f = 0.15 (EtOAc). − ^{1}H NMR (300 MHz, H-H COSY, $CDCl_3$) δ = 8.23 (dd, 2 H, CH-arom Bz, J = 1.1 and 8.1 Hz), 8.08 (s, 1 H, H-6), 7.58−7.41 (3 H, CH-arom Bz), 6.36 (t, 1 H, H-1′, $J_{1',2'}$ = 6.6 Hz), 5.28 (t, 1 H, H-3′′, $J_{2'',3''}$ = $J_{3'',4''}$ = 9.5 Hz), 5.12 (t, 1 H, H-4′′, $J_{4'',5''}$ = 9.5 Hz), 5.07 (dd, H-2”, $J_{1",2"}$ = 7.9 Hz), 4.75 (AB, 2 H, H-7, J = −13.2 Hz), 4.71 (d, 1 H, H-1"), 4.62 (m, 1 H, H-3′), 4.34 (dd, 1 H, H-6a′′, $J_{5'',6a''}$ = 4.8 Hz, $J_{6a'',6b''}$ = −12.4 Hz), 4.16 (dd, 1 H, H-6b′′, $J_{5'',6b''}$ = 2.3 Hz), 4.05 (m, 1 H, H-4′), 4.02 (dd, 1 H, H-5a′, $J_{5a',5b'}$ = −11.1 Hz, $J_{4',5a'}$ = 2.3 Hz), 3.88 (dd, 1 H, H-5b′, $J_{4',5b'}$ = 2.5 Hz), 3.81 (ddd, 1 H, H-5′′), 2.42 (ddd, 1 H, H-2a′, $J_{2a',2b'}$ = −13.6 Hz, $J_{2a',3'}$ = 3.9 Hz), 2.29 (ddd, 1 H, H-2b′, $J_{2b',3'}$ = 6.7 Hz), 2.08, 2.05, 2.04, 2.03 (4 × s, 12 H, 4 × CH_3 Ac). − ^{13}C NMR ($CDCl_3$): δ = 170.7, 170.6, 170.0, 169.4 (4 × C=O Ac), 157.6 (C-4), 147.7 (C-2), 137.9 (C-6), 136.5 (Cq Bz), 132.5, 129.6, 128.0 (CH-arom Bz), 110.9 (C-5), 100.5 (C-1′′), 87.1 (C-4′), 85.5 (C-1′), 72.2, 71.8, 71.4, 70.8, 68.2 (C-2′′, C-3′′, C-4′′, C-5′′, C-3′), 64.8, 61.6 (C-6′′, C-5′, C-7), 41.1 (C-2′), 20.7, 20.5 (CH_3 Ac). ES-MS: 692 [M + H$^+$], 714 [M + Na$^+$]. − $[\alpha]_D^{20}$ = −9.2 (c = 0.5 $CHCl_3$).

2′-Deoxy-5-(β-D-glucopyranosyloxymethyl)cytidine (2): Cytidine derivative **21** (54 mg, 78 μmol) was stirred for 16 h at 50 °C in a solution of 25% NH_4OH/1,4-dioxane (10 mL, 2:1, v/v). The mixture was concentrated in vacuo and loaded onto a Fractogel column [HW40(s), 26:60], with triethylammonium bicarbonate buffer (0.15 M) as eluent. The appropriate fractions were concentrated in vacuo, repeatedly coevaporated with MeOH/H_2O (3 × 5 mL, 4:1, v/v), and lyophilized, affording glucosylcytidine **1** (29 mg, 69 μmol, 89%) as a white fluffy solid. − ^{1}H NMR (D_2O, 600 MHz, H-H-COSY) δ = 7.95 (s, 1 H, H-6), 6.21 (t, 1 H, H1′, $J_{1',2'}$ = 6.5 Hz), 4.70 (AB, 1 H, H-7a, J = −12.6 Hz), 4.48 (d, 1 H, H-1”, $J_{1",2"}$ = 8.0 Hz), 4.40, (m, 1 H, H-3′), 4.02 (m, 1 H, H-4′), 3.88 (dd, 1 H, H-6a”, $J_{6a",6b"}$ = −12.3 Hz, $J_{6a",5''}$ = 2.1 Hz), 3.82 (dd, 1 H, H-5a′, $J_{5'a,5'b}$ = −12.5 Hz, $J_{5'a,4'}$ = 3.5 Hz), 3.73 (dd, 1 H, H-5b′, $J_{5'b,4'}$ = 5.0 Hz), 3.70 (dd, 1 H, H-6b”, $J_{6''b,5''}$ = 5.8 Hz), 3.45 (t, 1 H, H-3′′, $J_{3",4"}$ = $J_{2",3"}$ = 9.2 Hz), 3.42 (m, 1 H, H-5′′), 3.36 (t, 1 H, H-4”, $J_{2",3"}$ = 9.5 Hz), 3.27 (dd, 1 H, H-2′′, $J_{2'',3''}$ = 9.1 Hz), 2.41 (ddd, 1 H, H-2a′, $J_{2a',2b'}$ = −14.1 Hz, $J_{2a',3'}$ = 4.3 Hz), 2.28 (ddd, 1 H, H-2b′, $J_{2b',3'}$ = 6.7 Hz. − ^{13}C{^{1}H} NMR (D_2O) δ = 165.9 (C-4), 158.1 (C-2), 142.9 (C-6), 104.2 (C-5), 101.6 (C-1′′), 87.5 (C-4′), 87.0 (C-1′), 76.9, 76.5, 73.7, 71.1, 70.3 (C-5′′, C-3′′, C-2′′, C-3′, C-4′′), 66.0 (C-7), 61.9, 61.5 (C-5′, C-6′′), 40.3 (C-2′). − ES-MS: *m*/*z*: 420 [M + H]$^+$, 442 [M + Na]$^+$, 458 [M + K]$^+$, 861 [2M + Na]$^+$. − HRMS (ES) calcd. $C_{16}H_{26}N_3O_{10}$ [M + H]$^+$ 420.1609; found 420.1614 (± 0.0023).

***N*^{4}-Benzoyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-5-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxymethylcytidine 3′-*O*-(2-Cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (5):** Tritylation of **21** (0.11 g, 0.17 mmol) was performed as described under the synthesis of **5**. Purification by column chromatography (EtOAc/light petroleum, 1:2→2:1, v/v) furnished the monotritylated product in a yield of 79% (0.12 g, 0.13 mmol). R_f = 0.68 (EtOAc). − ^{1}H NMR (300 MHz, H-H COSY, $CDCl_3$) δ = 8.26 (dd, 2 H, CH-arom Bz, J 1.2 and 8.1 Hz), 7.92 (s, 1 H, H-6), 7.56−7.22 (16 H, CH-arom Bz, DMT), 6.85 (d, 2 H, H-arom, DMT), 6.27 (t, 1 H, H-1′, $J_{1',2'}$ = 6.7 Hz), 5.17 (t, 1 H, H-3′′, $J_{2'',3''}$ =$J_{3'',4''}$ = 9.3 Hz), 5.08 (t, 1 H, H-4′′, $J_{4'',5''}$ = 9.3 Hz), 4.94 (dd, H-2”, $J_{1",2"}$ = 8.0 Hz), 4.72 (d, 1 H, H-1"), 4.54 (m, 1 H, H-3′), 4.38 (AB, 2 H, H-7, J = −12.0 Hz), 4.21−4.12 (m, 3 H, H-6′′, H-4′), 3.79 (s, 6 H, OMe DMT), 3.62 (ddd, 1 H, H-5′′, $J_{5",6a"}$ = 4.4 Hz, $J_{5",6b"}$ = 2.5 Hz), 3.43 (m, 2 H, H-5′), 2.58 (ddd, 1 H, H-2a′, $J_{2a',2b'}$ = −13.2 Hz, $J_{2a',3'}$ = 3.8 Hz), 2.35 (ddd, 1 H, H-2b′, $J_{2b',3'}$ = 6.3 Hz), 2.04, 2.02, 2.00, 1.99 (4 × s, 12 H, 4 × CH_3 Ac). − ^{13}C NMR ($CDCl_3$): δ = 170.6, 170.1, 169.4 (4 × C=O Ac), 158.9 (C-4), 158.6 (Cq DMT), 147.6 (C-2), 140.2 (C-6), 136.7 (Cq Bz), 135.4 (Cq DMT), 132.7−127.0 (CH-arom Bz, DMT), 113.3 (CH DMT), 111.0 (C-5), 100.6 (C-1′′), 86.2 (C-4′, C-1′), 72.7, 72.2, 71.7, 71.1, 68.2 (C-2′′, C-3′′, C-4′′, C-5′′, C-3′), 64.1, 63.4, 61.7 (C-6′′, C-5′, C-7), 41.1 (C-2′), 20.5 (CH_3 Ac). − ES-MS: 995 [M + H$^+$], 1017 [M + Na$^+$]. − $[\alpha]_D^{20}$ = +24.8 (c = 0.5 $CHCl_3$). − Phosphitylation of the 3′-alcohol group (0.12 g, 0.13 mmol) was performed as described under the synthesis of **5**. The requisite amidite **4** was purified by flash column chromatography (light petroleum/EtOAc/Et_3N, 80:40:1, v/v/v). Yield 0.12 g (0.11 mmol, 85%). R_f = 0.88, 0.90 (light petroleum/EtOAc, 4:1, v/v).^{31}P{^{1}H} NMR: δ = 149.5, 149.1.

Solid-Phase Syntheses of ODNs 23 and 25, Containing 5-HMdC and 5-GlcMdC: The polymer-supported syntheses of the DNA fragments **23**: 5′-GTT TAC TTC **X**TC GGT TAG TG-3′ (**X** = 5-HMdC) and **25**: 5′-CAT TAC TAC **X**GG AAC TCA G-3′ (**X** = 5-GlcMdC) were performed on a fully automated synthesizer (Pharmacia Gene Assembler Special) at 1 μmol scale, using 15 equiv.[13a] of phosphoramidite **4** or **5**, respectively, and commercially available (PerSeptive Biosystems, USA) 2′-deoxynucleoside 3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidites (10 equiv.). Controlled pore glass, loaded with the appropriate nucleoside, was used as the solid support. Clean deprotection of the acetyl-protected ODN containing 5-HMdC (→ **23**) was achieved by treating the solid sup-

port with a 0.1 M solution of NaOH in 1,4-dioxane/H_2O (1:2, v/v) for 16 h at room temp. Complete deprotection and cleavage from the solid support was effected by (additional) treatment with concentrated ammonia in a sealed vial and heating at 50 °C for 16 h. Purification was performed by Fast Protein Liquid Chromatography (FPLC) with a Pharmacia (Uppsala, Sweden) LCC-500 liquid chromatograph. Analytical anion exchange FPLC was performed on a Mono Q HR 5:5 column (Pharmacia), elution at pH 12.0: gradient of buffer A (0.01 N NaOH) buffer B (0.01 N NaOH + 1.2 N NaCl). Desalting was performed with Sephadex G25 (Pharmacia) and the oligonucleotides were obtained in pure form after lyophilization. The integrity of the oligodeoxynucleotides was confirmed by MALDI-TOF mass spectrometry (negative mode for **23** and **24,** positive mode for **25**) at Eurogentec S.A. (Belgium).

T4 BGT-Catalyzed Glucosylation of the DNA 20-mer 23, Containing 5-HMdC: The following methodology was applied for preparation of the assays for the gel shift experiments (Figure 2). A mixture was prepared, containing 12 μL of Tris-HCl, pH 7.9 (from a 1.0 M solution), 6μL of $MgCl_2$ (from a 1.0 M solution), 1 μL of β-mercaptoethanol, 2 μL of UDPG (unlabeled), 20 μL of BGT (20 mM, 4 mg/mL), 10 μL of UDPG (U-^{14}C; Amersham-Biotech #B104). The mixture was divided into four aliquots, each of 12 μL, which were added to: **1**) 18 μL of H_2O; **2**) 18 μL of T4* DNA (unglucosylated mutant, containing 5-HMdC); stock sol. OD 6.4; **3**) 6 μL of duplex oligo **23** + 12 μL of H_2O; **4**) 6 μL of duplex oligo **24** + 12 μL of H_2O (Figure 2). Prior to the preparation of the above mixtures, the ODNs had been annealed for 5 min at 90 °C and allowed to cool to room temp.. The above aliquots were then allowed to react for 20 min at 30 °C, their volumes were reduced by running for 30 min in a Speed Vac® drying apparatus, 3 μL of dye mix was added to each aliquot, and the samples were loaded onto a nondenaturing 20% PAGE gel. After running for 1 h under standard conditions, the gel was stained with coomassie and ethidium bromide, dried, and autoradiographed overnight. The results are presented in Figure 2 and clearly show that the 20-mer **23**, containing 5-HMdC, is glucosylated (→ **26**) in the assay. Note that the glucosylated T4* DNA (Figure 2, A, lane 2) does not migrate into the gel (169,000 base pairs, ds DNA).

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