Synthesis of Oligodeoxynucleotides Containing 5-(β-D-Glucopyranosyloxymethyl)-2′-deoxyuridine, a Modified Nucleoside in the DNA of *Trypanosoma Brucei*

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The synthesis of the recently discovered modified DNA base 5-(β-D-glucopyranosyloxymethyl)-2′-deoxyuridine (β-dJ, **1**) is described. TMSOTf mediated β-glucosylation of 5-hydroxymethyl-2′-deoxyuridine (5-HMdU) derivative **10** (obtained in 20% from 2′-deoxyuridine) with trichloroacetimidate **12** gave dimer **13** in 47% yield. On the other hand, condensation of **12** with N^3-POM-protected derivative **20**, readily available from thymidine in 48%, afforded the fully protected nucleoside **22** in 96% yield. The latter compound was converted into phosphoramidite **3** which was applied in the automated solid phase synthesis of several biological interesting β-dJ containing DNA fragments.

Introduction

Trypanosoma brucei, a unicellar parasitic eukaryote belonging to the order Kinetoplastida, is transmitted by tsetse flies and causes African sleeping sickness in mammals and Nagana disease in domestic cattle. The parasite is able to survive in the bloodstream by changing the variant surface glycoprotein (VSG) in its cell-surface coat, a process called antigenic variation.[1−4] The trypanosome genome contains numerous VSG genes which usually are expressed one at a time.[5] An active VSG gene is exclusively located in one of the telomeric expression sites, but the precise mechanism of its activation is still unexplained. The change of VSG coat can occur either by replacing the gene in the active site or by activating a new expression site, while silencing the previous one. Interestingly, antigenic variation in bloodstream form trypanosomes is accompanied by the occurrence of the modified nucleoside 5-(β-D-glucopyranosyloxymethyl)-2′-deoxyuridine (**1**, see Figure 1), called β-dJ.[6−8] This modified nucleoside is located in and around the inactivated VSG gene and is absent in (procyclic) insect-form trypanosomes which do not vary their surface coat. The correlation with antigenic variation strongly suggests that β-dJ is involved in the mechanism of gene expression of VSG.

With the objective to study in detail the biological function[9−12] and physical properties[13−15] of DNA fragments containing at predetermined positions the hypermodified nucleoside β-dJ (**1**), we here present an effective route to the synthesis of β-dJ phosphoramidite DNA building unit **3**.

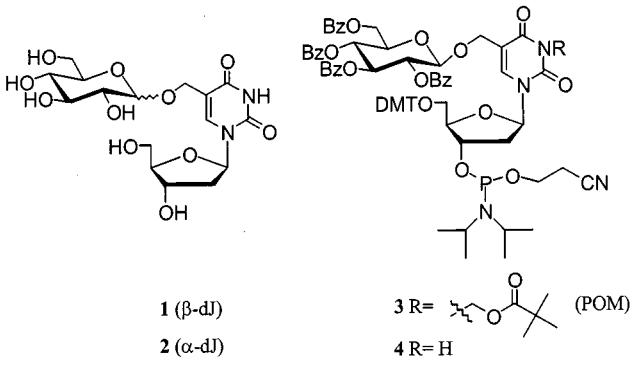

Figure 1

Results and Discussion

A previous report[16] from this laboratory revealed that 2′-deoxyuridine **5** could be transformed, as depicted in Scheme 1, by a four-step process into the key derivative **10** having a free allylic hydroxy function. Thus, hydroxymethylation[17] of commercially available **5**, followed by regioselective mono-acetylation[18] of **6** led to derivative **7**. Silylation of the latter compound with the bifunctional silylating reagent 1,3-dichlorotetraisopropyldisiloxane ($TiPDSCl_2$)[19] gave, after mild deesterification[20] of **8** with K_2CO_3/MeOH, acceptor **10** in an overall yield of 20% over the four steps.

Helferich condensation of acceptor **10** with fully benzoylated α-glucosyl bromide **11**[21] afforded the β-glucosylated derivative **13** in 50% yield.[22] A similar yield was obtained in the glycosylation of **10** using the corresponding α-trichloroacetimidate **12**[23] in the presence of the promoter trimethylsilyl triflate (TMSOTf). Transformation of **13** into the naturally occurring 5-(β-D-glucopyranosyloxymethyl)-2′-deoxyuridine (β-dJ, **1**) was effected by desilylation of **13** with $Et_3N \cdot 3\,HF$ in pyridine followed by ammonolysis of **14**. Purification by gel filtration gave compound **1** in 81% yield (based on **13**). The spectroscopic data of compound **1**

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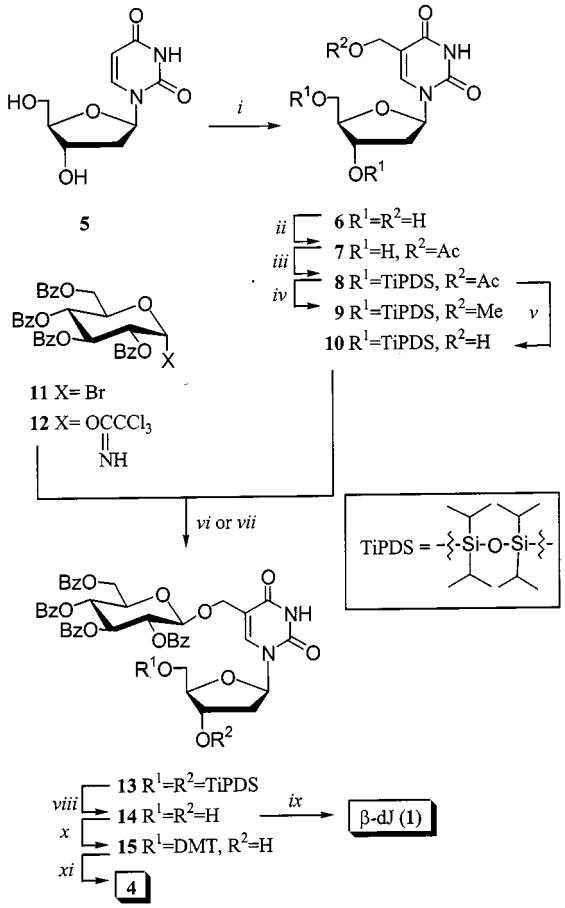
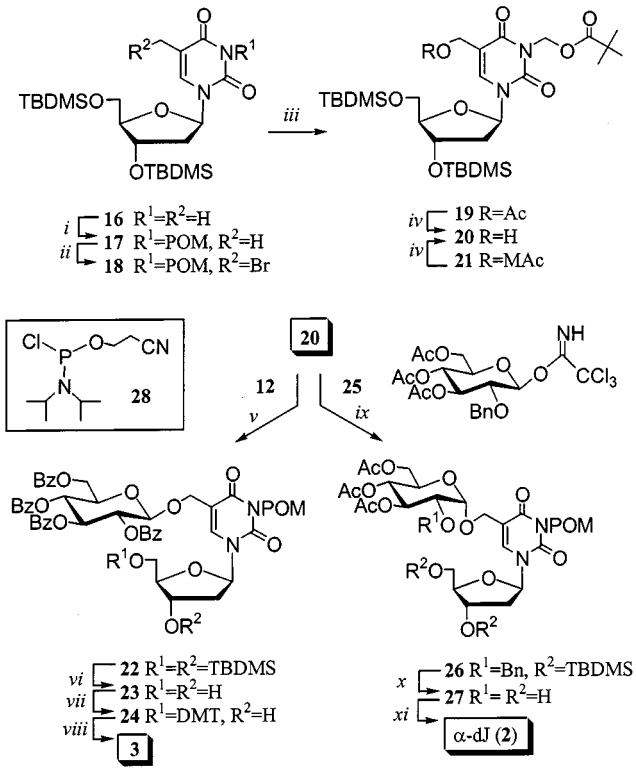


Scheme 1. Reagents and conditions: *i*. $(CH_2O)_n$, 0.5 N KOH, 40°C, 5 days, 40%. *ii*. CH_3COOH, cat. CF_3COOH, reflux, 84%. *iii*. TiPDSCl, pyridine, 90%. *iv*. NaOMe, MeOH, 96%. *v*. K_2CO_3, MeOH, 65%. *vi*. For **11**: $HgBr_2$, $Hg(CN)_2$, CH_3CN, 50%. *vii*. For **12**: cat. TMSOTf, $(CH_2Cl)_2$, 47%. *viii*. $Et_3N.3HF$, pyridine, 90%. *ix*. KO*t*Bu, MeOH, 91%. *x*. DMTrCl, pyridine, 71%. *xi*. **28**, DiPEA, CH_2Cl_2, 69%.

are in full accord with those reported[8] for isolated β-dJ (**1**). The structure of **1** was also confirmed independently by comparison of the spectroscopic data with those of the corresponding 1,2-*cis*-isomer α-dJ (**2**, vide infra).

The above described synthetic route to **1** is not fully satisfactory in terms of time, cost, as well as yield. Starting from thymidine, instead of the rather expensive 2′-deoxyuridine, would be an attractive entry to a differentially protected 5-HMdU glucosyl acceptor. In addition, it was expected that protection of the lactam function in the uracil moiety would have a beneficial effect on the yield of the glycosylation.[24] In line with these considerations, known[25] 3′,5′-di-*O*-*tert*-butyldimethylsilyl thymidine **16** was treated, as depicted in Scheme 2, with chloromethyl pivalate (POMCl),[26][27] leading to the N^3-POM protected derivative **17**, which was subjected to radical bromination[28] to yield the allylic bromide **18**.[29] Substitution of the crude bromide using cesium acetate in DMF afforded the corresponding allylic acetate **19** in an overall yield of 53% based on **17**. Unfortunately, deacetylation of **19** with K_2CO_3/MeOH was accompanied by partial loss of the POM protecting group, resulting in the isolation of **20** in a yield of 60%. The latter unwanted side reaction could be suppressed completely by subjecting the similarly prepared methoxyacetate derivative **21** to the same basic conditions to give **20** in an overall yield of 52% instead of 32%.[30] It was also gratifying to establish that the TMSOTf-assisted glucosylation[23] of the N^3-POM protected acceptor **20** with trichloroacetimidate donor **12** led to the isolation of the β-glucosylated derivative **22** in a yield of 96%. In contrast, the yield of TMSOTf-assisted α-glucosylation of acceptor **20** (see Scheme 2) with the known[31] α-directing donor 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate (**25**) was less satisfactory. Thus, despite the observation that the reaction proceeded in a highly stereoselective manner, the fully protected α-glucosylated product **26** was obtained in a moderate yield of 66%. Deprotection of **26** to give α-dJ (**2**) was easily effected as follows. Hydrogenation of **26** with 10% Pd/C proceeded as expected[32][33] by complete removal of the benzyl and silyl protecting groups. Subsequent ammonolysis of **27** afforded α-dJ (**2**) in an overall yield of 65% (based on **26**).



Scheme 2. Reagents and conditions: *i*. POMCl, K_2CO_3, DMF, 98%. *ii*. Br_2, CCl_4, light. *iii*. CsOAc or CsOAcOMe, DMF, **19**: 53%, **21**: 56% (2 steps from **17**). *iv*. K_2CO_3, MeOH, 69% (from **19**), 93% (from **21**). *v*. **12**, cat. TMSOTf, $(CH_2Cl)_2$, 96%. *vi* $Et_3N.3HF$, pyridine, 90%. *vii*. DMTrCl, pyridine, 75%. *viii*. **28**, DiPEA, CH_2Cl_2, 90%. *ix*. **25**, cat. TMSOTf, Et_2O, 66%. *x*. H_2, 10% Pd-C, *i*PrOH, 74%. *xi*. 25% NH_4OH, 85%.

At this stage, the β-dJ derivative **22** was converted according to a well established procedure (see Scheme 2) into the fully protected DNA building unit **3**. Desilylation of **22** with $Et_3N \cdot 3$ HF in pyridine, and subsequent regioselective tritylation of **23** with dimethoxytrityl chloride (DMTrCl) gave, after phosphitylation of the 3′-OH in **24** with

Table 1. Chemical steps involved in each elongation cycle.

Step	Manipulation	Solvents and reagents	Time (min)
1	Detritylation	2% Trichloroacetic acid in $(CH_2Cl)_2$	2.5
2	Coupling	**3**[a] or amidite,[b] *1H*-tetrazole[c]	6 or 3
3	Oxidation	0.02 M I_2 in CH_3CN/sym-collidine/H_2O, 11:1:5 (v/v/v)	1
4	Capping	0.25 M DMAP[d] in Ac_2O/sym-collidine/ CH_3CN, 1:1:8 (v/v/v)	1.2

[a] 0.1 M amidite in CH_3CN/$(CH_2Cl)_2$ (5:4, v/v). – [b] 0.1 M commercially available (dC^{Bz}, dA^{Bz}, dG^{iBu}, T) amidite in CH_3CN. – [c] 0.5 M in CH_3CN. – [d] 4-(Dimethylamino)pyridine.

chloro(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphane[34] (**28**), the key phosphoramidite **3** in 60% overall yield. In this respect it is also of interest to note that the earlier reported[16] phosphitylation of derivative **15** with reagent **28** (see Scheme 1) led to phosphoramidite **4** in a yield of 69% (cf. phosphitylation of **24** gave **3** in 90% yield). The lower yield of **4** relative to **3** is in agreement with the observation that the unprotected lactam function of **15** gives rise to the formation[35] of an additional O^4-phosphitylated product.

Having the fully protected phosphoramidite **3** in hand, the assemblage of several β-dJ containing DNA fragments was undertaken. To this end, the appropriate d-nucleoside, immobilized via a 3′-*O*-succinyl bond to controlled pore glass (CPG), was elongated following the protocol summarized in Table 1 using an automated Pharmacia Gene Assembler Special. The molar amounts of the individual amidites and coupling reagent were in accordance with the ratios summarized in Table 2. Under these conditions, the coupling efficiency was higher than 95% as gauged spectrophotometrically by the released DMT cation. After completion of the elongation cycles the DNA fragments were deprotected and simultaneously cleaved from the resin by ammonolysis (50°C, 16 h). FPLC analysis of the crude products showed in each case one major product and purification was effected by gel filtration or reversed phase chromatography.

Table 2. Molar amounts of reagents with respect to the scale of the synthesis.

Scale (μmol)	amidite	**3**	*1H*-tetrazole[a]
10	4	5	10
1	10	15	15
0.2	25	38	40

[a] Relative to the amidite.

In this way, the eight β-dJ containing DNA fragments in Table 3 (entries 1–8) were prepared successfully and used as model compounds in physical[14] (e.g. entry 1) and biological[9] (e.g. entries 2–8) studies. The three other fragments in Table 3 (e.g. entries 9–11), which served as standards in the quantification of β-dJ in the genome of *Trypanosoma Brucei*, were readily accessible by a slight modification of the solid phase synthesis protocol.[13]

The results presented in this paper clearly show that the valuable 5-(β-D-glucopyranosyloxymethyl)-2′-deoxyuridine phosphoramidite derivative **3** can be attained in nine steps from thymidine in an overall yield of 29%. In addition, protection of the lactam function of the nucleobase enhanced the yield of both the glucosylation and phosphitylation (cf. the earlier reported[16] β-dJ derivative **4** was obtained from 2′-deoxyuridine in a yield of 4% via an eight-step process). The effectivity of the DNA building unit **3** is illustrated by the routine solid phase synthesis of a variety of DNA fragments containing at predetermined positions the hypermodified nucleoside β-dJ (**1**).

Table 3. Sequences of the synthetically prepared β-dJ containing DNA fragments.

Entry	Sequence	ref.
1	CG**J**ACG	[13,14]
2	$(GGG\mathbf{J}TA)_4$	[9,13]
3	$(GGGT\mathbf{J}A)_4$	[9,13]
4	$(GGG\mathbf{JJ}A)_4$	[9,13]
5	$(ACCC\mathbf{J}A)_4$	[9,13]
6	CAGAAGGCAG C**J**GCAACAAG	[13]
7	CTTGTTGCAG C**J**GCCTTCTG	[13]
8	CTTGT**J**GCAG CTGCCTTCTG	[13]
9	pT**J**A	[13]
10	p**J**Tp	[13]
11	pT**J**p	[13]

Experimental Section

General: ^1H-, ^{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. ^1H and ^{13}C chemical shifts are give in ppm (δ) relative to tetramethylsilane as internal standard and ^{31}P chemical shifts relative to 85% H_3PO_4 as external standard. – Electrospray mass spectra were recorded with a Finnigan MAT TSQ70 triple quadrupole mass spectrometer. – Optical rotations were determined with a Propol automatic polarimeter at room temperature. – Toluene, dichloromethane, and pyridine were distilled from P_2O_5 and stored over molecular sieves (4 Å). Diethyl ether was freshly distilled from $LiAlH_4$. 1,2-Dichloroethane, (Biosolve, HPLC grade), *N*,*N*-dimethylformamide (DMF, Baker, p.a.), acetonitrile (Rathburn, HPLC grade), and tetrachloromethane (Baker, p.a.) were stored over molecular sieves (4 Å). Methanol (Rathburn, HPLC grade) was stored over molecular sieves (3 Å). *N*,*N*,*N*-Diisopropylethylamine (DiPEA) was dried by refluxing with CaH_2 (5 g/L) 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.[34] Cesium methoxyacetate

was prepared by stirring cesium carbonate in an excess of methoxyacetic acid, followed by evaporation, crystallization (Et_2O) and drying. – 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 charring with 20% H_2SO_4 in EtOH. Prior to reactions that require anhydrous conditions, traces of water were removed by coevaporation with 1,2-dichloroethane, toluene, or pyridine.

5-Acetyloxymethyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyuridine (8): 1,3-Dichlorotetraisopropyldisiloxane (0.41 mL, 1.3 mmol) was added to a stirred solution of **7**[18] (0.35 g, 1.2 mmol) in pyridine (10 mL) under a nitrogen atmosphere. After 30 minutes the mixture was diluted with CH_2Cl_2 and washed with sat. aqueous $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residual oil was purified by column chromatography (light petroleum/diethyl ether, 9:1 to 1:1, v/v) to give **8**. Yield 0.58 g (1.1 mmol, 90%). – ^{1}H NMR ($CDCl_3$) δ = 7.75 (s, 1 H, 6-H), 6.03 (dd, 1 H, 1′-H, $J_{1',2a'}$ = 7.2 Hz, $J_{1',2b'}$ = 2.6 Hz), 4.82 (s, 2 H, 7-H), 4.51 (m, 1 H, 3′-H), 3.70–4.14 (m, 3 H, 4′-H, 5a-H), 2.40 (m, 2 H, 2′-H), 2.06 (s, 3 H, Ac), 1.05 (m, 28 H, TiPDS). – ^{13}C{^{1}H} NMR ($CDCl_3$) δ = 170.5 (C=O Ac), 162.7 (C-4), 149.9 (C-2), 139.9 (C-6), 108.7 (C-5), 84.9 (C-4′), 84.2 (C-1′), 68.1 (C-3′), 60.5 (C-7), 58.9 (C-5′), 39.7 (C-2′), 20.5 (CH_3 Ac), 16.6–17.1 (CH_3 TiPDS), 12.2–13.1 (CH TiPDS).

5-Methoxymethyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyuridine (9): Acetate **8** (1.60 g, 2.50 mmol) was dissolved in a 0.5 M solution of NaOMe in MeOH (20 mL). After 15 min the reaction mixture was neutralized with Dowex 50 W (H^+) and filtered. The filtrate was concentrated in vacuo and purified by column chromatography to give **9**. R_f = 0.32 (diethyl ether/light petroleum, 2:1). Yield 1.46 g (96%). – ^{1}H NMR ($CDCl_3$): δ = 7.59 (s, 1 H, 6-H), 6.09 (dd, 1 H, 1′-H, $J_{1',2a'}$ = 5.9 Hz, $J_{1',2b'}$ = 5.7 Hz), 4.35 (m, 1 H, 3′-H), 4.18 (AB, 2 H, 7-H, J = –13.0 Hz), 3.91 (dd, 1 H, 4′-H, $J_{3',4'}$ = 5.5 Hz, $J_{4',5'}$ = 2.7 Hz), 3.80 (ABX, 2 H, 5′-H, $J_{5a',5b'}$ = –14.4 Hz), 3.42 (s, 3 H, OCH_3), 2.40 (ddd, 1 H, 2a-H, $J_{2a',2b'}$ = –12.1 Hz, $J_{2a',3'}$ = 1.8 Hz), 2.05 (m, 1 H, 2b′-H), 1.05 (m, 28 H, TiPDS). – ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 163.2 (C-4), 150.0 (C-2), 137.9 (C-6), 111.4 (C-5), 86.8 (C-4′), 84.9 (C-1′), 68.3 (C-3′), 66.7 (C-7), 60.6 (C-5′), 58.5 (OCH_3), 39.7 (C-2′), 16.5–17.1 (CH_3 TiPDS), 12.1–13.0 (CH TiPDS). – ESI-MS: *m/z*: 515 $[M+H]^+$, 532 $[M+NH_4]^+$, 527 $[M+Na]^+$, 553 $[M+K]^+$.

5-Hydroxymethyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyuridine (10): Compound **8** (0.70 g, 1.3 mmol) was dissolved in a 0.05 M solution of K_2CO_3 in methanol (15 mL). When TLC analysis showed complete disappearance of the starting material, the mixture was carefully neutralized with Dowex 50 W (H^+), filtered, and concentrated in vacuo. After purification by silica gel column chromatography (EtOAc/light petroleum, 7:3 to 1:0, v/v) **10** was obtained as a white foam. Yield 426 mg (0.85 mmol, 65%). – ^{1}H NMR ($CDCl_3$) δ = 7.63 (s, 1 H, 6-H), 6.07 (dd, 1 H, 1′-H, $J_{1',2a'}$ = 7.2 Hz, $J_{1',2b'}$ = 2.4 Hz), 4.54 (m, 1 H, 3′-H), 4.38 (s, 2 H, 7-H), 3.60–4.14 (m, 3 H, 4′-H, 5′-H), 2.4 (m, 2 H, 2′-H), 1.05 (m, 28 H, TiPDS). – ^{13}C{^{1}H} NMR ($CDCl_3$) δ = 163.9 (C-4), 150.0 (C-2), 137.0 (C-6), 113.5 (C-5), 84.4 (C-4′), 81.9 (C-1′), 68.4 (C-3′), 60.9 (C-7), 57.7 (C-5′), 30.5 (C-2′), 16.6–17.1 (CH_3 TiPDS), 12.1–13.1 (CH TiPDS).

General Procedure for the Glycosylation of 5-HMdU Derivatives 10 and 20. – Method A: To a stirred mixture of $HgBr_2$ (0.40 g, 1.10 mmol), $Hg(CN)_2$ (0.28 g, 1.10 mmol), powdered molecular sieves (4 Å) and 1.00 mmol of acceptor in CH_3CN (6 mL) was slowly added bromide **11** (0.73 g, 1.10 mmol) in CH_3CN (4 mL), under an argon atmosphere. After stirring for 3 h, the reaction mixture was diluted with CH_2Cl_2, filtered through Hyflo, and washed with aqueous KF (1 M, 2 ×), sat. aqueous $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. **Method B:** A mixture of the acceptor (1.00 mmol) and donor **12** (0.83 g, 1.10 mmol) or **25** (0.60 g, 1.10 mmol) was dried by coevaporation with dichloroethane (2 × 5 mL). The mixture was dissolved in dichloroethane (10 mL) and stirred for 15 min with powdered molecular sieves (4 Å). TMSOTf (17 μL, 0.15 mmol) was added and the reaction mixture was stirred for 1.5 h under an atmosphere of argon. The mixture was quenched with Et_3N (0.10 mL) and filtered through Hyflo. The filtrate was washed with H_2O and brine, dried ($MgSO_4$), and concentrated.

5-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)oxymethyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyuridine (13): The title compound was prepared as described in the general procedure. The product was purified by column chromatography (diethyl ether/light petroleum, 1:1 to 9:1, v/v) to afford **13** as a white solid. Yield: **method A**: 1.10 g (1.02 mmol, 50%); **method B**: 0.86 g (47%). R_f = 0.64 (CH_2Cl_2/MeOH, 97:3, v/v). – ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.44–8.41 (m, 21 H, 6-H, CH-arom), 5.95–6.11 (m, 2 H, 1′-H, 4′′-H), 5.49 (m, 2 H, 2′′-H, 3′′-H), 5.13 (d, 1 H, 1-H, $J_{1'',2''}$ = 8.0 Hz), 4.41–4.68 (m, 5 H, 7-H, 3′′-H, 6′′-H), 4.20 (m, 1 H, 5′′-H), 4.00 (m, 2 H, 4′-H, 5a′-H), 3.78 (m, 1 H, 5b′-H), 2.38 (m, 2 H, 2′-H), 1.05 (m, 28 H, TiPDS). – ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 165.6, 165.1, 165.0, 164.9 (C=O, Bz), 161.3 (C-4), 149.6 (C-2), 144.5 (C-6), 133.3–128.3 (CH-arom, Bz), 129.1, 128.8 (Cq, Bz), 110.6 (C-5), 101.6 (C-1″), 85.2 (C-4′), 84.5 (C-1′), 72.8, 72.2, 71.9, 69.5, 68.9 (C-2″, C-3″, C-4″, C-5″, C-3′), 64.6, 62.8, 61.1 (C-6″, C-5′, C-7), 39.9 (C-2′), 17.4–16.8 (CH_3, TiPDS), 13.3, 13.0, 12.6, 12.4 (CH, TiPDS). $[\alpha]_D^{20}$ –0.4° (*c* = 2.0 $CHCl_3$). – ESI-MS: *m/z*: 1079 $[M + H]^+$. – *M. p.* 109°C. – $C_{56}H_{66}N_2O_{16}Si_2$ (1078): calcd. C 62.32, H 6.16, N 2.60; found C 62.01, H 6.19, N 2.69.

5-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)oxymethyl-2′-deoxyuridine (14): Compound **13** (1.0 g, 0.93 mmol) and $Et_3N \cdot 3$ HF (0.32 mL, 1.86 mmol) were stirred in 2.0 mL of pyridine. After 16 h the reaction mixture was concentrated, dissolved in EtOAc (25 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 (CH_2Cl_2/MeOH, 1:0 to 95:5, v/v) to afford **14** as a white foam. Yield 605 mg (0.72 mmol, 78%). – ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 8.24 (s, 1 H, 6-H), 8.03–7.30 (m, 20 H, CH-arom), 6.27 (t, 1 H, 1-H, $J_{1',2'}$ = 5.8 Hz), 6.01 (t, 1 H, 3′′-H, $J_{3'',4''} = J_{2'',3''}$ = 9.7 Hz), 5.75 (t, 1 H, 4′′-H, $J_{4'',5''}$ = 9.7 Hz), 5.52 (dd, 1 H, 2′′-H, $J_{1'',2''}$ = 8.0 Hz), 5.20 (d, 1 H, 1′′-H), 4.75–4.25 (m, 5 H, 6′′-H, 7-H, 3′-H), 3.98 (m, 4 H, 5′′-H, 4′-H, 5′-H), 2.44 (ddd, 1 H, 2a′-H, $J_{2a',2b'}$ = –7.6 Hz), 2.27 (ddd, 1 H, 2b′-H). – ^{13}C{^{1}H} NMR ($CDCl_3$) δ = 165.9, 165.6, 165.0 (C=O, Bz), 162.6 (C-4), 149.7 (C-2), 138.2 (C-6), 133.2–128.0 (CH-arom, Bz), 128.7, 128.6 (Cq, Bz), 110.0 (C-5), 101.1 (C-1″), 87.3 (C-4′), 84.9 (C-1′), 72.8, 72.4, 72.3, 70.7, 69.4 (C-2″, C-3″, C-4″, C-5″, C-3′), 65.0, 62.9, 60.8 (C-6″, C-5′, C-7), 39.9 (C-2′).

5-(β-D-Glucopyranosyloxymethyl)-2′-deoxyuridine (β-dJ, 1): KO*t*Bu (50 mg, 0.44 mmol) was added to solution of **14** (72 mg, 78 μmol) in methanol (3.0 mL). After stirring for 16 h the reaction mixture was neutralized with Dowex 50 W X4 (H^+). The mixture was filtered, concentrated in vacuo and applied to a Fractogel column (HW 40(s), 26:60) with triethylammonium carbonate buffer (0.15 M) as eluent. The appropriate fractions were concentrated in vacuo, co-evaporated with methanol/water (5 mL, 4:1, v/v, 3×) and lyophilized, affording **1** (30 mg, 71 μmol, 91%) as a white solid. – ^{1}H

NMR (D_2O, 600 MHz, H-H-COSY): δ = 8.02 (s, 1 H, 6-H), 6.28 (t, 1 H, 1′-H, $J_{1',2'}$ = 6.5 Hz), 4.64 (AB, 1 H, 7a-H, J_{AB} = −12.2 Hz), 4.44−4.52 (m, 3 H, 7b-H, 1′′-H, 3′-H), 4.04 (ddd, 1 H, 4′-H), 3.89 (dd, 1 H, 6′′a-H, $J_{6a,6b}$ = −12.2 Hz, $J_{6'',5''}$ = 2 Hz), 3.85 (dd, 1 H, 5′a-H, $J_{5'a,5'b}$ = −12.4 Hz, $J_{5'a,4'}$ = 3.4 Hz), 3.76 (dd, 1 H, 5′b-H, $J_{5'b,4'}$ = 4.7 Hz), 3.71 (dd, 1 H, 6′′b-H, $J_{6''b,5''}$ = 5.2 Hz), 3.34−3.51 (m, 3 H, 3′′-H, 4′′-H, 5′′-H), 3.27 (dd, 1 H, 2′′-H, $J_{2'',1''}$ = 7.9 Hz, $J_{2'',3''}$ = 9.1 Hz), 2.40 (m, 2 H, 2′-H). − $^{13}C\{^1H\}$ NMR (D_2O, 150 MHz) δ = 165.8 (C-4), 152.2 (C-2), 142.1 (C-6), 111.3 (C-5), 102.2 (C-1′′), 87.4 (C-4′), 86.2 (C-1′), 76.7 (C-5′′), 76.4 (C-3′′), 73.7 (C-2′′), 71.0 (C-3′), 70.3 (C-4′′), 64.8 (C-7), 61.8 (C-5′), 61.4 (C-6′′), 39.6 (C-2′). − ESI-MS: *m/z*: 443 $[M+Na]^+$. − $C_{16}H_{24}N_2O_{11}$ (420): calcd. C 45.72, H 5.75, N 6.66; found C 45.65, H 5.76, N 6.68.

5-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)oxymethyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine (15): To a stirred solution of **14** (0.67 g, 0.80 mmol) in pyridine (4 mL) was added DMTrCl (0.41 g, 1.2 mmol). After 3 h the reaction was quenched with methanol and after another 15 minutes the mixture was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and washed with brine (2×). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification of the crude product was accomplished by column chromatography (EtOAc/light petroleum/Et_3N, 50:50:1 to 90:10:1, v/v) to give **15**. Yield 0.65 g (0.57 mmol, 71%). R_f 0.38 (EtOAc/Et_3N, 99:1, v/v). − ^1H NMR ($CDCl_3$): δ = 8.00−7.80 (m, 8 H, CH-arom), 7.61 (s, 1 H, 6-H), 7.51−7.16 (m, 21 H, CH-arom Bz, DMTr), 6.82 (2 × d, 4 H, CH-arom, DMTr, *J* = 9.0 Hz), 6.10 (t, 1 H, 1′-H, $J_{1',2'}$ = 6.3 Hz), 5.91 (t, 1 H, 3′′-H, $J_{2'',3''} = J_{3'',4''}$ = 9.5 Hz), 5.69 (t, 1 H, 4′′-H, $J_{4'',5''}$ = 9.6 Hz), 5.45 (dd, 1 H, 2′′-H, $J_{1'',2''}$ = 8.0 Hz), 5.08 (d, 1 H, 1′′-H), 4.63 (dd, 1 H, 6a′′-H, $J_{5'',6a''}$ = 3.0 Hz, $J_{6a'',6b''}$ = −12.0 Hz), 4.48 (m, 3 H, 7-H, 3′-H), 4.24 (d, 1 H, 6b′′-H), 4.19 (dd, 1 H, 5′′-H), 3.97 (dd, 1 H, 4′-H, $J_{4',5'}$ = 4.4 Hz, $J_{3',4'}$ = 2.0 Hz), 3.76, 3.75 (2 × s, 6 H, OMe DMTr), 3.38 (ABX, 2 H, 5′-H, $J_{5a',5b'}$ = −10.2 Hz), 2.39 (m, 1 H, 2a′-H), 2.21 (m, 1 H, 2b′-H). − $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ = 164.9 (C-4), 166.0, 165.6, 165.0, 163.0 (C=O Bz), 158.5 (Cq DMTr), 149.9 (C-2), 144.5 (Cq DMTr), 139.5 (C-6), 135.4, 135.1 (Cq DMTr), 132.9−127.8 (CH arom), 129.1, 128.8, 128.7 (Cq, Bz), 113.1 (CH DMTr), 111.0 (C-5), 101.5 (C-1′′), 86.7 (Cq DMTr), 86.0 (C-4′), 85.3 (C-1′), 72.9, 72.0, 71.9, 69.5 (C-3′, C-2′′, C-3′′, C-4′′, C-5′′), 64.2 (C-7), 62.8(C-6′′), 59.8 (C-5′), 55.0 (OMe) 34.3 (C-2′).

5-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)oxymethyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine-3′-*O*-(2-cyanoethyl-*N*,*N*,-diisopropyl)phosphoramidite (4): To a stirred solution of **15** (0.44 g, 0.39 mmol) in CH_2Cl_2 (6 mL) were added DiPEA (0.26 mL, 1.5 mmol) and chlorophosphane **28** (0.11 mL, 0.48 mmol). After 1 h, the reaction mixture was diluted with CH_2Cl_2 and washed with aqueous $NaHCO_3$ (9%) and water. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude mixture 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 0.36 g (0.27 mmol, 69%). R_f 0.80, 0.68 (EtOAc/light petroleum/Et_3N, 80:19:1, v/v/v). − $^{31}P\{^1H\}$ NMR ($CDCl_3$) δ = 149.6, 149.3.

3′,5′-Di-*O*-*tert*-butyldimethylsilyl-N^3-pivaloyloxymethyl-2′-deoxythymidine (17): To a solution of thymidine (7.27 g, 30.0 mmol) in DMF (150 mL) was added imidazole (8.90 g, 0.13 mol) and *tert*-butyldimethylsilyl chloride (9.96 g, 66.0 mmol). After stirring for 1 h at room temperature, water (5 mL) was added and the reaction mixture was extracted with diethyl ether. The organic phase was washed with water, dried ($MgSO_4$), and evaporated. Crude compound **16** (14.0 g, 29.7 mmol) was protected as described by Reese et al.[27] The crude oily product was purified by column chromatography (diethyl ether/light petroleum, 1:8 to 1:3, v/v) to give **17** as an oil. R_f 0.90 (diethyl ether/light petroleum, 3:1, v/v). Yield 17.2 g (98%, 2 steps). − ^1H NMR ($CDCl_3$): δ = 7.48 (s, 1 H, 6-H), 6.33 (t, 1 H, 1′-H, $J_{1',2'}$ = 6.0 Hz), 5.93 (AB, 2 H, CH_2 POM, *J* = −12.1 Hz), 4.38 (m, 1 H, 3′-H), 3.91 (m, 1 H, 4′-H), 3.79 (AB, 2 H, 5′-H, $J_{5a',5b'}$ = −18.0 Hz), 2.23 (ddd, 1 H, 2a′-H, $J_{2a',2b'}$ = −12.1 Hz, $J_{2a',3'}$ = 2.2 Hz), 1.99 (m, 1 H, 2b′-H), 1.19 (s, 3 H, CH_3 -dT), 1.14 (s, 9 H, CH_3 POM), 0.89, 0.85 (2 × s, 18 H, CH_3 *tBu* TBDMS), 0.07, 0.04 (2 × s, 12 H, CH_3 TBDMS). − $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 176.7 (C=O POM), 162.0 (C-4), 149.8 (C-2), 134.1 (C-6), 119.4 (C-5), 87.5 (C-4′), 85.1 (C-1′), 72.0 (C-3′), 64.6 (CH_2 POM), 62.6 (C-5′), 40.9 (C-2′), 38.3 (Cq POM), 26.6 (CH_3 POM), 25.5, 25.3 (CH_3 *tBu* TBDMS), 18.0, 17.6 (Cq TBDMS), 12.3 (CH_3 dT), −5.1, −5.3, −5.8, −5.9 (CH_3 TBDMS).

5-Bromo-3′,5′-di-*O*-*tert*-butyldimethylsilyl-N^3-pivaloyloxymethyl-2′-deoxythymidine (18): Compound **17** (9.0 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 on a flow of dry nitrogen gas during a 2 h period as described by Bärwolff et al.[28] The solution was cooled to room temperature and degassed for 30 min. The solvent was evaporated in vacuo (< 45 °C) and the resulting crude and oily product **18** was immediately used in the next reaction. R_f 0.61 (CH_2Cl_2/MeOH, 99:1, v/v). − ^1H NMR ($CDCl_3$): δ = 7.92 (s, 1 H, 6-H), 6.31 (t, 1 H, 1′-H, $J_{1',2'}$ = 6.5 Hz), 5.96 (AB, 2 H, CH_2 POM, *J* = −12.1 Hz), 4.47 (s, 2 H, 7-H), 4.40 (m, 2 H, 3′-H, 5a′-H), 3.91 (m, 2 H, 4′-H, 5b′-H), 2.34 (ddd, 1 H, 2a′-H, $J_{2a',2b'}$ = −12.2 Hz, $J_{2a',3'}$ = 2.1 Hz), 2.01 (m, 1 H, 2b′-H), 1.18 (s, 9 H, CH_3 POM), 0.95, 0.89 (2 × s, 18 H, CH_3 *tBu* TBDMS), 0.14, 0.08 (2 × s, 12 H, CH_3 TBDMS). − $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 177.1 (C=O POM), 160.2 (C-4), 149.6 (C-2), 138.0 (C-6), 110.7 (C-5), 88.1 (C-4′), 86.1 (C-1′), 72.3 (C-3′), 64.7 (CH_2 POM), 62.9 (C-5′), 41.7 (C-2′), 38.6 (Cq POM), 26.8 (CH_3 POM), 25.7 (CH_2Br), 25.8, 25.6 (CH_3 *tBu* TBDMS), 18.3, 17.8 (Cq TBDMS), −4.8, −5.0, −5.5 (CH_3 TBDMS).

5-Acetyloxymethyl-3′,5′-di-*O*-*tert*-butyldimethylsilyl-N^3-pivaloyloxymethyl-2′-deoxyuridine (19): Cesium acetate (1.10 g, 5.0 mmol) was added to a vigourously stirred solution of crude bromide **18** (2.0 mmol) in DMF (20 mL). After 30 min brine (15 mL) was added and the mixture was extracted with diethyl ether. The organic layer was washed with a sat. aqueous solution of $NaHCO_3$ and dried ($MgSO_4$). Purification was effected by column chromatography (diethyl ether/light petroleum, 1:5 to 1:2, v/v) to give **20** as an oil. Yield 0.71 g (53%, 2 steps from **18**). R_f = 0.57 (CH_2Cl_2/MeOH, 99:1, v/v). − ^1H NMR ($CDCl_3$): δ = 7.78 (s, 1 H, 6-H), 6.22 (dd, 1 H, 1′-H, $J_{1',2a'}$ = 5.8 Hz, $J_{1',2b'}$ = 5.9 Hz), 5.86 (AB, 2 H, CH_2 POM, *J* = −10.2 Hz), 4.75 (AB, 2 H, CH_2OAc, *J* = −14.8 Hz), 4.40 (m, 1 H, 3′-H), 3.90 (dd, 1 H, 4′-H, $J_{3',4'}$ = 5.3 Hz, $J_{4',5'}$ = 2.6 Hz), 3.75 (ABX, 2 H, 5′-H, $J_{5a',5b'}$ = −14.7 Hz), 2.34 (ddd, 1 H, 2a′-H, $J_{2a',2b'}$ = −12.6 Hz, $J_{2a',3'}$ = 1.9 Hz), 2.06 (m, 1 H, 2b′-H), 2.05 (s, 3 H, CH_3, Ac), 1.19 (s, 9 H, CH_3 POM), 0.91, 0.90 (2 × s, 18 H, CH_3, *tBu* TBDMS), 0.11, 0.08 (2 × s, 12 H, CH_3, TBDMS). − $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 177.0 (C=O POM), 170.4 (C=O, Ac), 161.0 (C-4), 149.7 (C-2), 139.6 (C-6), 108.2 (C-5), 88.0 (C-4′), 86.1 (C-1′), 72.2 (C-3′), 64.6 (CH_2 POM), 62.9 (C-5′), 59.4 (CH_2OAc), 41.3 (C-2′), 37.5 (Cq POM), 26.8 (CH_3, POM), 25.6, 25.5 (CH_3, *tBu* TBDMS), 18.2, 17.7 (Cq TBDMS), −4.9, −5.0, −5.7 (CH_3 TBDMS).

3′,5′-Di-*O*-*tert*-butyldimethylsilyl-5-methoxyacetyloxymethyl-N^3-pivaloyloxymethyl-2′-deoxyuridine (21): Crude **18** was treated with cesium methoxyacetate as described for the synthesis of **19**. Yield

2.0 g (56%, 2 steps from **18**). R_f = 0.43 (CH_2Cl_2/MeOH, 99:1, v/v). – ^{1}H NMR ($CDCl_3$): δ = 7.88 (s, 1 H, 6-H), 6.31 (dd, 1 H, 1′-H, $J_{1',2a'}$ = 5.8 Hz, $J_{1',2b'}$ = 5.9 Hz), 5.95 (AB, 2 H, CH_2 POM, J = −9.4 Hz), 4.92 (AB, 2 H, 7-H, J = −12.1 Hz), 4.40 (m, 1 H, 3′-H), 4.02 (s, 2 H, CH_2 MAc), 3.99 (dd, 1 H, 4′-H, $J_{3',4'}$ = 5.1 Hz, $J_{4',5'}$ = 2.6 Hz), 3.81 (ABX, 2 H, 5′-H, $J_{5a',5b'}$ = −11.6 Hz), 3.43 (s, 3 H, CH_3 MAc), 2.35 (ddd, 1 H, 2a′-H, $J_{2a',2b'}$ = −10.0 Hz, $J_{2a',3'}$ = 2.1 Hz), 2.05 (m, 1 H, 2b′-H), 1.19 (s, 9 H, CH_3 POM), 0.91, 0.90 (2 × s, 18 H, CH_3, *tBu* TBDMS), 0.11, 0.09 (2 × s, 12 H, CH_3, TBDMS). – ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 179.7 (C=O POM), 176.8 (C=O, MAc), 160.1 (C-4), 149.6 (C-2), 139.8 (C-6), 107.6 (C-5), 87.9 (C-4′), 86.0 (C-1′), 72.0 (C-3′), 69.3 (CH_2 MAc), 64.5 (CH_2 POM), 62.8 (C-5′), 59.7 (C-7), 41.3 (C-2′), 38.6 (Cq POM), 26.7 (CH_3, POM), 25.6, 25.4 (CH_3, *tBu* TBDMS), 18.1, 17.5 (Cq TBDMS), −5.0, −5.1, −5.7 (CH_3 TBDMS).

3′,5′-Di-*O*-*tert*-butyldimethylsilyl-5-hydroxymethyl-N^3-pivaloyloxymethyl-2′-deoxyuridine (20): Acetate **19** (1.59 g, 2.47 mmol) or methoxyacetate **21** (1.50 g, 2.22 mmol) was dissolved in a 0.05 M solution of anhydrous K_2CO_3 in MeOH (30 mL). The mixture was stirred for 1 h (**19**) or 20 min (**21**) and the solution was neutralized with Dowex 50 W (H^+), filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (diethyl ether/light petroleum, 1:3 to 1:1, v/v) which afforded **20** as a colorless oil. Yield from **19**: 1.02 g (69%); from **20**: 1.24 g (93%). R_f = 0.39 (diethyl ether/light petroleum, 2:1, v/v). – ^{1}H NMR ($CDCl_3$): δ = 7.69 (s, 1 H, 6-H), 6.30 (dd, 1 H, 1′-H, $J_{1',2a'}$ = 5.9 Hz, $J_{1',2b'}$ = 5.8 Hz), 5.92 (AB, 2 H, CH_2 POM, J = −10.0 Hz), 4.34 (m, 3 H, C-7, 3′-H), 3.94 (dd, 1 H, 4′-H, $J_{3',4'}$ = 5.1 Hz, $J_{4',5'}$ = 2.6 Hz), 3.70 (ABX, 2 H, 5′-H, $J_{5a',5b'}$ = −14.6 Hz), 2.28 (ddd, 1 H, 2a′-H, $J_{2a',2b'}$ = −13.1 Hz, $J_{2a',3'}$ = 2.5 Hz), 1.98 (m, 1 H, 2b′-H), 1.16 (s, 9 H, CH_3 POM), 0.89, 0.87 (2 × s, 18 H, CH_3, *tBu* TBDMS), 0.08, 0.05 (2 × s, 12 H, CH_3, TBDMS). – ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 176.8 (C=O POM), 161.6 (C-4), 149.6 (C-2), 135.8 (C-6), 112.7 (C-5), 87.6 (C-4′), 85.6 (C-1′), 71.9 (C-3′), 64.3 (CH_2 POM), 62.6 (C-5′), 58.2 (CH_2OH), 40.9 (C-2′), 38.3 (Cq, POM), 26.5 (CH_3, POM), 25.5, 25.3 (CH_3, *tBu* TBDMS), 17.5, 17.0 (Cq, TBDMS), −5.1, −5.3, −5.8 (CH_3, TBDMS).

5-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)oxymethyl-3′,5′-di-*O*-*tert*-butyldimethylsilyl-N^3-pivaloyl-oxymethyl-2′-deoxyuridine (22): The title compound was prepared as described in the general procedure. Purification by column chromatography (CH_2Cl_2 to CH_2Cl_2/MeOH, 99:1, v/v) gave **22** as a white solid. Yield **method A**: 1.77 g (60%); **method B**: 2.80 g (96%). R_f = 0.61 (CH_2Cl_2/MeOH, 199:1, v/v). – ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.03−7.81 (m, 8 H, CH-arom), 7.65 (s, 1 H, 6-H), 7.57−7.25 (m, 12 H, CH-arom), 6.11 (dd, 1 H, 1′-H, $J_{1',2a'}$ = 5.7 Hz $J_{1',2b'}$ = 5.8 Hz), 5.90 (t, 1 H, 3′′-H, $J_{2'',3''}$ = 9.7 Hz, $J_{3'',4''}$ = 9.7 Hz), 5.81 (AB, 2 H, CH_2 POM, J = −10.3 Hz), 5.69 (t, 1 H, 4′′-H, $J_{4'',5''}$ = 9.7 Hz), 5.49 (dd, 1 H, 2′′-H, $J_{1'',2''}$ = 8.0 Hz), 5.11 (d, 1 H, 1′′-H), 4.66 (dd, 1 H, 6a′′-H, $J_{5'',6a''}$ = 3.1 Hz $J_{6a'',6b''}$ = −12.3 Hz), 4.54 (AB, 2 H, 7-H), 4.45 (dd, 1 H, 6b′′-H, $J_{5'',6b''}$ = 4.7 Hz), 4.34 (dd, 1 H, 3′-H, $J_{2a',3'}$ = 2.2 Hz, $J_{3',4'}$ = 5.4 Hz), 4.18 (ddd, 1 H, 5′′-H), 3.93 (dd, 1 H, 4′-H), 3.74 (d, 2 H, 5′-H, $J_{4',5'}$ = 3.3 Hz), 2.28 (ddd, 1 H, 2a′-H, $J_{2a',2b'}$ = −7.8 Hz), 1.97 (ddd, 1 H, 2b′-H), 1.17 (s, 9 H, CH_3 *tBu* POM), 0.90, 0.85 (2 × s, 18 H, CH_3 *tBu*-Si), 0.08, 0.06 (2 × s, 12 H, CH_3Si). – ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 177.3 (C=O POM), 165.8, 165.5, 165.0, 164.9 (C=O, Bz), 161.3 (C-4), 149.6 (C-2), 138.0 (C-6), 133.3−128.1 (CH-arom, Bz), 129.0, 128.6 (Cq, Bz), 109.8 (C-5), 101.4 (C-1′′), 88.2 (C-4′), 86.5 (C-1′), 72.8, 72.6, 72.1, 71.7, 69.4 (C-2′′, C-3′′, C-4′′, C-5′′, C-3′), 64.9, 64.6, 63.0, 62.6 (C-6′′, CH_2 POM, C-5′, C-7), 41.3 (C-2′), 37.0 (Cq, POM), 26.8 (CH_3, POM), 25.7, 25.6 (CH_3, *tBu* TBDMS), 18.2, 17.8 (Cq, TBDMS). −4.8, −5.0, −5.4, −5.6 (CH_3,TBDMS). $[\alpha]_D^{20}$ = +5.2° (*c* = 2.0, $CHCl_3$). – ESI-MS: *m/z*: 1179 $[M + H]^+$, 1201 $[M + Na]^+$, 1217 $[M + K]^+$. – *M*. *p*. 71 °C. – $C_{62}H_{78}N_2O_{17}Si_2$ (1178): calcd. C 63.14, H 6.67, N 2.38; found C 62.88, H 6.73, N 2.42.

5-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)oxymethyl-2′-deoxy-N^3-pivaloyloxymethyluridine (23): Compound **22** (1.12 g, 0.95 mmol) was deprotected as described for the conversion of **13** into **14**. Purification by column chromatography (EtOAc/light petroleum, 1:1 to 1:0, v/v) afforded **23**. Yield: 0.82 g (91%). R_f = 0.68 (EtOAc). – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.00−7.81 (m, 11 H, CH-arom, 6-H), 7.57−7.26 (m, 10 H, CH-arom), 6.34 (t, 1 H, 1′-H, $J_{1',2'}$ = 6.6 Hz), 5.97 (t, 1 H, 3′′-H, $J_{2'',3''}$ = $J_{3'',4''}$ = 9.7 Hz), 5.83 (AB, 2 H, CH_2 POM, J = −9.8 Hz), 5.72 (t, 1 H, 4′′-H, $J_{4'',5''}$ = 9.7 Hz), 5.53 (dd, 1 H, 2′′-H, $J_{1'',2''}$ = 7.9 Hz), 5.02 (d, 1 H, 1′′-H), 4.78 (dd, 1 H, 6a′′-H, $J_{5'',6a''}$ = 1.1 Hz, $J_{6a'',6b''}$ = −13.1 Hz), 4.60 (m, 3 H, 7-H, 3′-H), 4.47 (dd, 1 H, 6b′′-H, $J_{5'',6b''}$ = 1.2 Hz), 4.22 (ddd, 1 H, 5′′-H), 4.03 (dd, 1 H, 4′-H, $J_{4',5'}$ = 2.1 Hz, $J_{3',4'}$ = 2.3 Hz), 3.95 (ABX, 2 H, 5′-H, $J_{5a',5b'}$ = −23.4 Hz), 2.31 (m, 2 H, 2′-H), 1.15 (s, 9 H, CH_3, *tBu* POM). – ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 177.4 (C=O POM), 166.0, 165.9, 165.5, 165.1 (C=O, Bz), 160.7 (C-4), 149.8 (C-2), 136.5 (C-6), 133.6−128.2 (CH-arom, Bz), 129.2, 128.5 (Cq, Bz), 109.9 (C-5), 101.0 (C-1′′), 87.1 (C-4′), 86.0 (C-1′), 72.4, 72.2, 72.1, 71.7, 69.4 (C-2′′, C-3′′, C-4′′, C-5′′, C-3′), 64.8, 64.5, 62.8, 62.0 (C-6′′, CH_2 POM, C-5′, C-7), 40.7 (C-2′), 38.8 (Cq, POM), 26.8 (CH_3, POM). – *M*. *p*. 90 °C.

5-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)oxymethyl-5′-*O*-(4,4′-dimethoxytrityl)-N^3-pivaloyloxymethyl-2′-deoxyuridine (24): Compound **23** (0.91 g, 0.95 mmol) was converted into **24** as described for **15**. Purification was performed with column chromatography (EtOAc/light petroleum/Et_3N, 25:50:1 to 50:25:1, v/v/v) affording **24** as a foam. Yield 0.87 g, (73%). R_f = 0.30 (CH_2Cl_2/Et_3N, 99:1, v/v). – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.03−7.80 (m, 8 H, CH-arom), 7.62 (s, 1 H, 6-H), 7.56−7.14 (m, 21 H, CH-arom Bz, DMTr), 6.83 (2 × d, 4 H, CH-arom, DMTr, J = 9.0 Hz), 6.09 (t, 1 H, 1′-H, $J_{1',2'}$ = 6.5 Hz), 5.86 (t, 1 H, 3′′-H, $J_{2'',3''}$ = $J_{3'',4''}$ = 9.6 Hz), 5.76 (s, 2 H, CH_2 POM), 5.67 (t, 1 H, 4′′-H, $J_{4'',5''}$ = 9.6 Hz), 5.44 (dd, 1 H, 2′′-H, $J_{1'',2''}$ = 8.0 Hz), 5.04 (d, 1 H, 1′′-H), 4.61 (dd, 1 H, 6a′′-H, $J_{5'',6a''}$ = 3.1 Hz, $J_{6a'',6b''}$ = −12.1 Hz), 4.42 (m, 3 H, 7-H, 3′-H), 4.28 (d, 1 H, 6b′′-H), 4.18 (dd, 1 H, 5′′-H), 3.95 (dd, 1 H, 4′-H, $J_{4',5'}$ = 4.2 Hz, $J_{3',4'}$ = 2.0 Hz), 3.77, 3.76 (2 × s, 6 H, OMe DMTr), 3.34 (ABX, 2 H, 5′-H, $J_{5a',5b'}$ = −10.3 Hz), 2.36 (m, H, 2a′-H), 2.18 (m, 1 H, 2b′-H), 1.17 (s, 9 H, CH_3, *tBu* POM). – ^{13}C{^{1}H} NMR ($CDCl_3$) δ = 177.2 (C=O, POM), 165.8, 165.4, 164.9, 164.8 (C=O, Bz), 161.3 (C-4), 158.4 (Cq, DMTr), 149.5 (C-2), 144.3 (Cq-arom, DMTr), 138.5 (C-6), 135.1, 134.9 (Cq-arom, DMTr), 133.2−126.8 (CH-arom), 113.1 (CH-arom, DMTr), 109.9 (C-5), 101.3 (C-1′′), 86.6 (Cq, DMTr), 86.0, 85.9 (C-4′, C-1′), 72.8, 71.7, 69.4 (C-2′′, C-3′′, C-4′′, C-5′′, C-3′), 64.5, 63.2, 62.7, 60.1 (C-6′′, C-7, C-5′, CH_2 POM), 54.9 (OCH_3, DMTr), 40.8 (C-2′), 26.7 (CH_3, POM).

5-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)oxymethyl-5′-*O*-(4,4′-dimethoxytrityl)-N^3-pivaloyloxymethyl-2′-deoxyuridine-3′-*O*-(2-cyanoethyl-*N*,*N*,-diisopropyl)phosphoramidite (3): Compound **24** (0.26 g, 0.21 mmol) was phosphitylated as described for the synthesis of **4**. Yield 0.28 g (0.19 mmol, 91%). R_f = 0.89, 0.80 (CH_2Cl_2/Et_3N, 99:1, v/v). – ^{31}P{^{1}H} NMR ($CDCl_3$) δ 149.3, 148.9.

5-(2-*O*-Benzyl-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)oxymethyl-3′,5′-di-*O*-*tert*-butyldimethylsilyl-N^3-pivaloyloxymethyl-2′-deoxyuridine (26): Acceptor **20** was glycosylated with donor **25** according to method B of the general procedure. Purification by column chro-

matography (diethyl ether/light petroleum, 1:2 to 1:0, v/v) furnished **26** as a colorless oil. Yield 0.58 g (0.59 mmol, 66%). R_f = 0.61 (diethyl ether/light petroleum, 3:1, v/v). – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7, period77 (s, 1 H, 6-H), 7.27–7.35 (m, 5 H, CH, Bn), 6.27 (dd, 1 H, 1′-H, $J_{1',2a'}$ = 5.8 Hz $J_{1',2b'}$ = 7.7 Hz), 5.92 (AB, 2 H, CH_2, POM, J = –8.4 Hz), 5.39 (t, 1 H, 3′′-H, $J_{2'',3''}$ = 9.7 Hz, $J_{3'',4''}$ = 9.7 Hz), 5.19 (d, 1 H, 1′′-H, $J_{1'',2''}$ = 3.6 Hz), 4.98 (t, 1 H, 4′′-H, $J_{4'',5''}$ = 9.7 Hz), 4.60 (AB, 2 H, 7-H, J = –12.4 Hz), 4.43 (d, 1 H, 6a′′-H, $J_{6a'',6b''}$ = –11.7 Hz), 4.39 (dd, 1 H, 5′′-H), 4.30 (m, 1 H, 3′-H), 4.28 (d, 1 H, 6b′′-H), 4.10 (AB, 2 H, CH_2, Bn, J = –11.8 Hz), 3.97 (dd, 1 H, 4′-H, $J_{3',4'}$ = 3.7 Hz), 3.68 (ABX, 2 H, 5′-H, $J_{4',5'}$ = 3.0 Hz, $J_{5a',5b'}$ = –11.3 Hz), 3.57 (dd, 1 H, 2′′-H, $J_{2'',3''}$ = 10.0 Hz) 2.30 (ddd, 1 H, 2a′-H, $J_{2a',2b'}$ = –7.8 Hz), 2.07, 2.00, 1.97 (3 × s, 3 × 3 H, CH_3, Ac), 2.05 (m, 1 H, 2b′-H), 1.18 (s, 9 H, CH_3, POM), 0.93, 0.90 (2 × s, 18 H, CH_3, *t*Bu, TBDMS), 0.12, 0.11, 0.09, 0.08 (4 × s, 12 H, CH_3-Si). – ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 177.3 (C=O POM), 170.5, 169.9, 169.6 (C=O, Ac), 161.4 (C-4), 149.8 (C-2), 138.5 (C-6), 137.4 (Cq, Bn), 127.5–128.2 (CH, Bn), 109.6 (C-5), 96.9 (C-1′′), 88.0 (C-4′), 86.2 (C-1′), 72.1 (CH_2, Bn) 76.2, 72.2, 71.7, 68.5, 67.3 (C-2′′, C-3′′, C-4′′, C-5′′, C-3′), 64.6, 63.6, 62.9, 61.7 (CH_2, POM, C-6′′, C-5′, C-7), 41.3 (C-2′), 37.0 (Cq, POM), 26.8 (CH_3, POM), 25.8, 25.5 (CH_3, *tBu* TBDMS), 18.0, 17.9 (Cq, TBDMS), –4.7, –5.0, –5.3, –5.5 (CH_3,TBDMS). – ^{13}C NMR ($CDCl_3$): $J_{1'\text{-H, C-1'}}$ = 171.4 Hz. $[\alpha]_D^{20}$ = +49.6° (*c* = 1.0 $CHCl_3$). – ESI-MS: *m/z* $[M + H]^+$ 980, $[M + NH_4]^+$ 996, $[M + Na]^+$ 1001, $[M + K]^+$ 1018.

5-(3,4,6-Tri-*O*-acetyl-α-D-glucopyranosyloxymethyl)-N^3-pivaloyloxymethyl-2′-deoxyuridine (27): A suspension of compound **26** (0.25 g, 0.26 mmol) and palladium on carbon (50 mg, 10 wt%) in *i*PrOH (3 mL) was stirred under an atmosphere of H_2. After 3 h the reaction mixture was filtered over Hyflo and concentrated in vacuo to give, after purification by column chromatography (EtOAc/MeOH, 15:1, v/v), compound **27** (0.13 g, 74%). – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.28 (s, 1 H, 6-H), 6.39 (t, 1 H, 1′-H, $J_{1',2'}$ = 6.3 Hz), 5.98 (s, 2 H, CH_2, POM), 5.22 (t, 1 H, 3′′-H, $J_{2'',3''}$ = $J_{3'',4''}$ = 9.6 Hz), 5.04 (t, 1 H, 4′′-H, $J_{4'',5''}$ = 9.6 Hz), 5.02 (d, 1 H, 1′′-H, $J_{1'',2''}$ = 3.5 Hz), 4.62 (m, 3 H, 7-H, 3′-H), 4.39 (d, 1 H, 6a′′-H, $J_{6a'',6b''}$ = –13.1 Hz), 4.24 (m, 3 H, 6b′′-H, 5′-H), 4.03 (dd, 1 H, 4′-H), 3.78 (m, 3 H, OH, 5′′-H), 3.52 (dd, 1 H, 2′′-H, $J_{2'',3''}$ = 10.0 Hz) 3.30 (br. s, 1 H, OH), 2.32 (m, 2 H, 2′-H), 2.18, 2.15, 2.09 (3 × s, 9 H, CH_3, Ac), 1.24 (s, 9 H, CH_3, POM. – ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 177.1 (C=O POM), 171.9, 170.8, 169.4 (C=O, Ac), 160.9 (C-4), 149.9 (C-2), 136.4 (C-6), 109.6 (C-5), 98.2 (C-1′′), 87.0 (C-1′), 86.0 (C-4′), 73.6, 70.7, 67.9, 67.7, 67.3 (C-2′′, C-3′′, C-4′′, C-5′′, C-3′), 64.6, 63.0, 61.8, 61.6 (CH_2, POM, C-6′′, C-5′, C-7), 41.9, 41.1 (C-2′, Cq, POM), 26.9 (CH_3, POM).

5-(α-D-Glucopyranosyloxymethyl)-2′-deoxyuridine (α-dJ, 2): Compound **28** (0.13 g, 0.19 mmol) was dissolved in 25% NH_4OH (2 mL). After stirring for 5 h, the mixture was concentrated. Purification as described for **1** afforded α-dJ **2**. Yield: 71 mg (85%). – ^{1}H NMR (600 MHz, D_2O): δ = 7.92 (s, 1 H, 6-H), 6.27 (t, 1 H, 1′-H, $J_{1',2'}$ = 6.6 Hz), 4.98 (d, 1 H, 1′′-H, $J_{1'',2''}$ = 3.8 Hz), 4.44 (dd, 1 H, 3′-H, $J_{3',4'}$ = 4.4 Hz), 4.43 (AB, 2 H, 7-H, J = –12.3 Hz), 4.01 (dd, 1 H, 4′-H, $J_{4',5a'}$ = 3.4 Hz), 3.82 (dd, 1 H, 5a′-H, $J_{5a',5b'}$ = 9.0 Hz), 3.75 (m, 4 H, 5b′-H, 5′′-H, 6′′-H), 3.66 (t, 1 H, 3′′-H, $J_{3'',4''}$ = 9.5 Hz), 3.53 (dd, 1 H, 2′′-H, $J_{2'',3''}$ = 6.1 Hz), 3.38 (t, 1 H, 4′′-H, $J_{4'',5''}$ = 9.5 Hz), 2.36 (m, 2 H, 2′-H). – ^{13}C{^{1}H} NMR (150 MHz, D_2O): δ = 165.5 (C-4), 153.8 (C-2), 141.3 (C-6), 111.4 (C-5), 98.4 (C-1′′), 87.1 (C-4′), 86.1 (C-1′), 73.6, 72.5, 71.8, 70.9, 70.1 (C-5′′, C-3′′, C-2′′, C-3′, C-4′′), 63.3 (C-7), 61.7, 61.0 (C-5′, C-6′′), 39.4 (C-2′). – ESI-MS: *m/z*: 443 $[M + Na]^+$. – $C_{16}H_{24}N_2O_{11}$: calcd. C 45.72, H 5.75, N 6.66; found C 45.38, H 5.70, N 6.48.

Synthesis of Oligonucleotides

The polymer supported synthesis of β-dJ containing DNA fragments (entries 1–8, see Table 3) was performed on a fully automated synthesizer (Pharmacia Gene Assembler Special) using phosphoramidite **3** and commercially available (PerSeptive Biosystems, USA) 2′-deoxynucleoside 3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)-phosphoramidites according to conditions listed in Table 1 and 2 (see text). Controlled pore glass (CPG-AP), loaded with the appropriate nucleoside, was used as solid support. Cleavage from the solid phase and complete deprotection was effected by placing the solid support in 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 HPLC 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 smaller nucleotides (entries 9–11, Table 3) were prepared with 3′-phosphate CPG (Glen Research/Eurogentec, Seraing, Belgium) and an additional phosphorylation step[35] to introduce the 5′-phosphate. The fragments were purified by reversed phase chromatography (Lichrospher C18, Merck, Darmstadt, Germany).

d(CGJACG): The oligonucleotide was prepared on a 10 μmol scale (see Table 2) and purified as described above. The structure was ascertained by mass spectrometry, X-ray crystallography[14] and H-H COSY NMR spectroscopy: ^{1}H NMR (600 MHz, 333 K, D_2O): δ = 8.40 (s, 1 H, 2-H, *A*), 8.22 (s, 1 H, 8-H, *A*), 8.06 (br. s, 2 H, 2 × 8-H, G^2, G^6), 7.85 (s, 1 H, 6-H, ***J***), 7.80 (d, 1 H, 6-H, C^1, $J_{5,6}$ = 7.5 Hz), 7.71 (d, 1 H, 6-H, C^5, $J_{5,6}$ = 7.5 Hz), 6.42 (t, 1 H, 1′-H, *A*, $J_{1',2'}$ = 7.3 Hz), 6.28 (t, 1 H, 1′-H, G^6, $J_{1',2'}$ = 6.7 Hz), 6.26 (t, 1 H, 1′-H, ***J***, $J_{1',2'}$ = 6.9 Hz), 6.19 (m, 2 H, 2 × 1′-H, G^2, C^1), 6.13 (dd, 1 H, 1′-H C^5, $J_{1',2a'}$ = 8.0 Hz, $J_{1',2b'}$ = 6.3 Hz), 6.07 (d, 1 H, 5-H, *C*, *J* = 7.5 Hz), 6.03 (d, 5-H, *C*, *J* = 7.5 Hz), 5.06 (m, 2 H, 2 × 3′-H, *A*, G^2), 4.87 (m, 2 H, 2 × 3′-H, ***J***, C^5), 4.76 (ddd, 1 H, 3′-H, G^6), 4.70 (m, 1 H, 3′-H, C^1), 4.58 (d, 1 H, α-1′′-H, glucose, $J_{1'',2''}$ = 8.0 Hz), 4.57 (AB, 2 H, 7-H, ***J***), 4.47 (m, 1 H, 4′-H, G^2), 4.44 (m, 1 H, 4′-H, *A*), 4.33 (m, 1 H, 4′-H, ***J***), 4.29 (m, 1 H, 4′-H, C^5), 4.26 (m, 1 H, 4′-H, G^6), 4.18–4.08 (m, 11 H, 5 × 5′-H, 4′-H C^1), 3.95 (dd, 1 H, 6a′′-H, $J_{6a'',6b''}$ = –12.3 Hz, $J_{5'',6a''}$ = 2.0 Hz), 3.80–3.76 (m, 2 H, 6b′′-H, 5a′-H C^1), 3.72 (dd, 1 H, 5b′-H, C^1, $J_{5',6a'}$ = –12.5 Hz, $J_{4',5'}$ = 5.0 Hz), 3.59 (t, 1 H, 3′′-H, $J_{2'',3''}$ = $J_{3'',4''}$ = 8.1 Hz), 3.51 (m, 1 H, 5′′-H), 3.47 (t, 1 H, 4′′-H, $J_{4'',5''}$ = 8.0 Hz), 3.37 (t, 1 H, 2′′-H), 2.83 (m, 3 H, 3 × 2a′-H, *A*, G^2, G^6), 2.75 (dd, 1 H, 2b′-H, G^2, $J_{2a',2b'}$ = –14.0 Hz, $J_{1,2a'}$ = 6.0 Hz), 2.56 (m, 2 H, 2b′-H, G^6, 2a′-H, ***J***), 2.50 (m, 1 H, 2a′-H, C^1), 2.45 (dd, 1 H, 2a′-H, C^5, $J_{2a',2b'}$ = –14.1 Hz, $J_{1,2a'}$ = 5.9 Hz), 2.19 (m, 1 H, 2b′-H, ***J***), 2.13 (m, 1 H, 2b′-H, C^5), 2.00 (m, 1 H, 2b′-H, C^1).

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