Reinvestigation into the Synthesis of Oligonucleotides Containing 5-(β-D-Glucopyranosyloxymethyl)-2′-deoxyuridine

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A reinvestigation into the synthesis of oligonucleotides containing 5-(β-D-glucopyranosyloxymethyl)-2′-deoxyuridine revealed that existing procedures for the preparation of these DNA fragments suffered from decomposition at the final deprotection step. The decomposition product was identified as the corresponding 5-(aminomethyl)-2′deoxyuridyl derivative arising from amino substitution of the β-D-glucosyl moiety during ammonolysis. This was shown to be suppressed by the use of phosphoramidite **21** in place of **12** for solid-phase oligonucleotide synthesis, in conjunction with short ammonia treatment at room temperature.

Introduction

The kinetoplastid flagellate *Trypanosoma brucei*, transmitted by tsetse flies, causes African sleeping sickness in mammals and Nagana disease in domestic cattle. It manages to survive in the bloodstream of its host by virtue of its ability to change the variant surface glycoprotein (VSG) in its cell-surface coat so that it evades the host's immune systems, a process called antigenic variation.[1−4] It has been observed that the hypermodified nucleoside 5-(β-D-glucopyranosyloxymethyl)-2′-deoxyuridine (known as dJ) is present in inactive telomeric VSG gene expression sites of *T. brucei* but not in the active site.[5−7] This implies that dJ is involved in the suppression of VSG gene expression sites and consequently in antigenic variation.[8] However, the presence of dJ in other members of the Kinetoplastida family which do not undergo antigenic variation[9] suggests that dJ is involved in other biological processes.

In order to gain greater insight into the biological function of dJ at a molecular level, a synthetic program for the synthesis of short fragments of DNA containing dJ at predetermined sites was established. Use of these DNA fragments resulted in the discovery of a protein that specifically binds to dJ.[10] Moreover, it was also revealed that this binding is structure-specific and that recognition of the modified nucleoside only occurs when dJ is presented in double-stranded DNA.[11,12] During these studies, however, it was shown that some of the synthetic DNA fragments containing dJ were contaminated in varying amounts by an unspecified side product.[11] Here we present our investigations into the identification of this side product and the suppression of its formation.

Results and Discussion

Since the synthetic DNA fragments containing dJ were synthesised by a standard procedure, it is evident that the side product originates from modification of the J nucleobase. We have previously reported phosphoramidites **7**[13] and **12**[14] (Scheme 1), differing in the N^3 protection of the nucleobase, as suitable building blocks for the synthesis of DNA fragments containing dJ. The key step en route to **7** involved Helferich condensation of the 3′,5′-diprotected hydroxymethyldeoxyuridine (HMDU) **1** with the fully benzoylated α-glucosyl bromide **2** to give protected dJ **4** in a moderate yield (Scheme 1).[13] Endeavours to improve this procedure not only provided a cost-effective and convenient route towards a suitably protected HMDU derivative (**8**), but also an excellent efficiency with respect to the key glycosylation step (transformation **8** to **9**). In this regard, it was shown that N^3 protection of the nucleobase of HMDU with a pivaloyloxymethyl (POM) protecting group resulted in a twofold increase in yield (see Scheme 1, compare **1** to **4** with **8** to **9**, Step *ii*).[14] In addition, an improvement in the phosphoramidite formation (compare **6** to **7** with **11** to **12**, Step *v*) was also attributed to the use of the POM group, which has been reported by Reese et al.[15] to be completely cleaved with aqueous ammonia in 20 min at room tempera-

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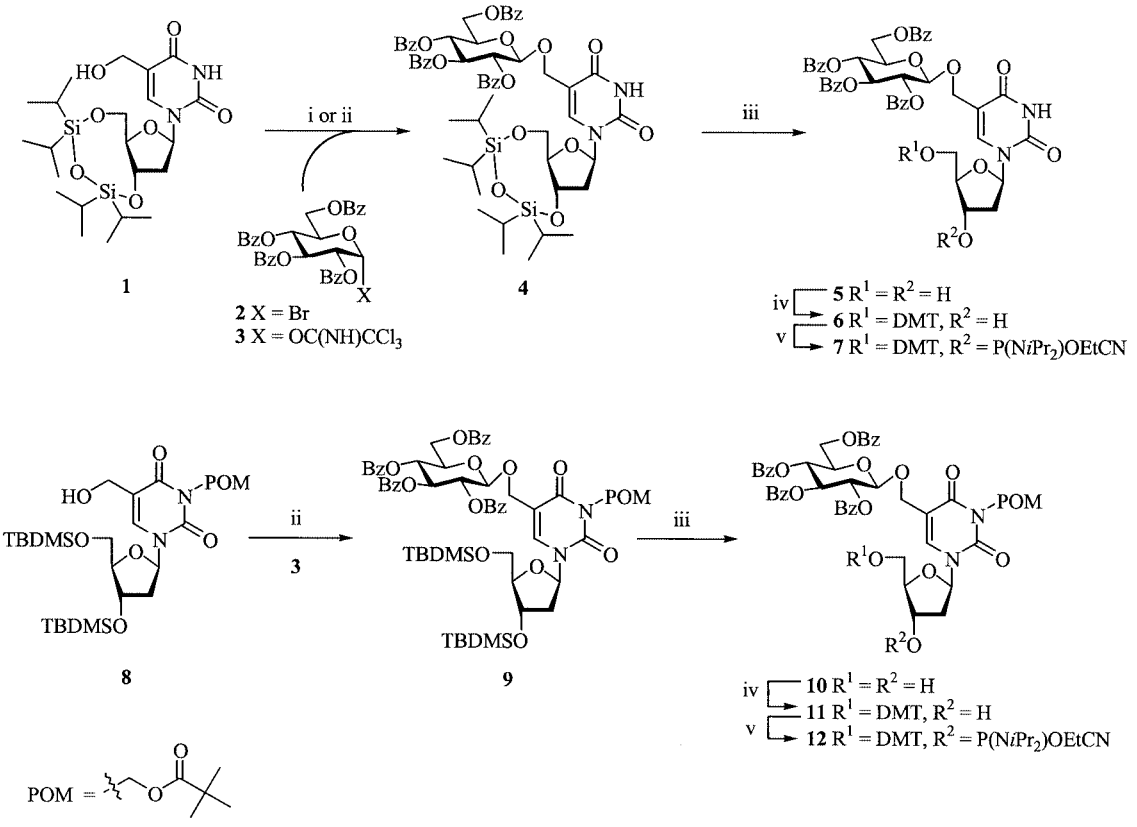
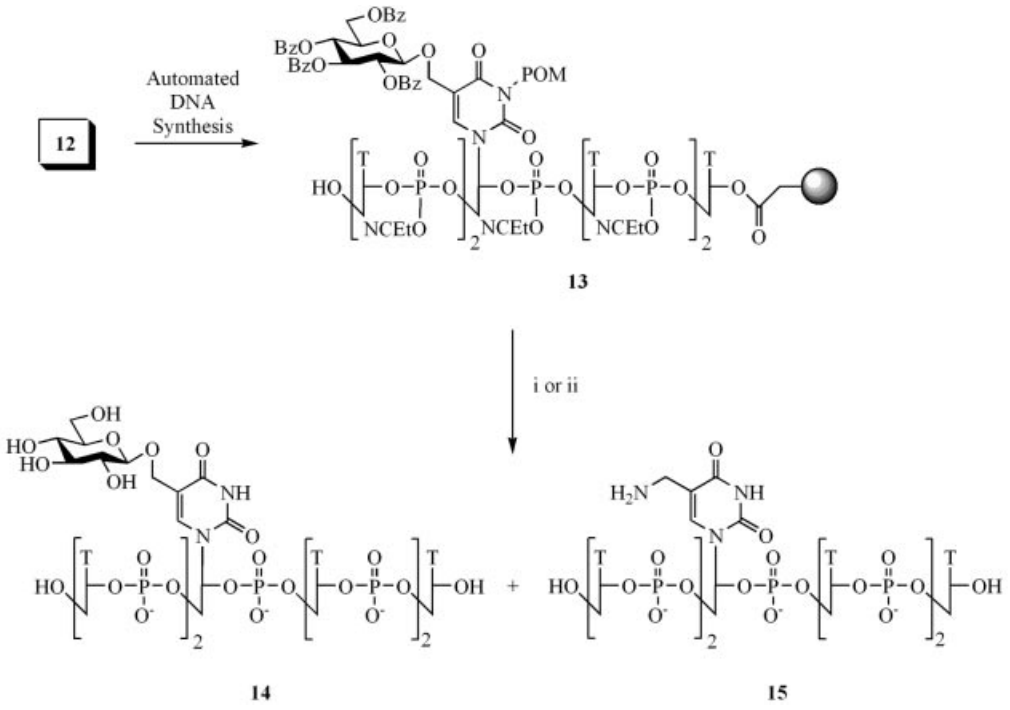


Scheme 1. Reagents and conditions: (i) for **2**: $HgBr_2$, $Hg(CN)_2$, ACN, 3 h, 50%. (ii) for **3**: cat. TMSOTf, DCE, 1.5 h, **4**: 47%, **9**: 96%. (iii) $Et_3N{\cdot}3HF$, pyridine, 16 h, **5**: 90%, **10**: 91%. (iv) DMTCl, pyridine, 3 h, **6**: 71%, **11**: 75%. (v) $ClP(NiPr_2)OEtCN$, DIPEA, DCM, 1 h, **7**: 69%, **12**: 91%

ture. Consequently, the use of N^3 protection was considered a valuable addition to the synthesis of dJ building blocks and oligomers, as it was compatible with automated synthesis and the cleavage takes place during the aqueous ammonia treatment in the final stage of a standard DNA synthesis procedure.

In order to identify the side product in our dJ-containing DNA fragments, hexameric fragment **13** (Scheme 2) was selected as a model compound. Thus, fully protected dJ phosphoramidite **12** and commercially available dT phosphoramidite were used in a standard solid-phase DNA synthesis procedure and subjected to standard deprotection conditions (NH_3 aqu., 50 °C, 16 h). Subsequent HPLC/anion-exchange analysis of the crude mixture[16] revealed the presence of the expected product **14** and a significant amount of side product, in a ratio of 3:2 (Figure 1). MALDI-TOF measurements on the crude sample indicated a mass difference of 163 between **14** (calcd. for $C_{66}H_{89}N_{12}O_{46}P_5$: 1941.2; found 1943.4 $[M + H]^+$) and the side product, corresponding to aminomethyl derivative **15** (calcd. for $C_{60}H_{80}N_{13}O_{40}P_5$: 1778.2; found 1780.5 $[M + H]^+$). The formation of **15** can be readily explained in terms of competing substitution at the benzylic position of the J nucleobase during the ammonolysis step. This assumption was further substantiated by the ammonolysis of **13** at room temperature, which furnished a mixture of the two compounds in a ratio of 4:1 (Figure 2). To assess the lability of unprotected dJ incorporated in a DNA sequence, the mixture was separated by anion-exchange and pure **14** (Figure 3, A) was treated with saturated aqueous ammonia at different temperatures and for varying lengths of time (Figure 3). It was found that unprotected dJ, as present in the DNA sequence **14**, decomposes upon prolonged treatment with ammonia. Gratifyingly, it was established that after 16 hours at room temperature there is only 1% decomposition (Figure 3, B). It is also interesting to note that after 64 hours at room temperature the decomposition is of the order of 3% (Figure 3, C). However, subjection of **14** to the standard deprotection conditions used for DNA sequences (NH_3 aqu., 50 °C, 16 h) resulted in a significant amount (15%, Figure 3, D) of decomposition of **14** into the benzylic amine **15**.

In light of the above results, it is clear that the side product observed with the dJ-containing oligomers was the result of decomposition of dJ arising from the final deprotection step with ammonia. Furthermore, it can be surmised that two separate factors are responsible for this: the protecting groups on the J nucleobase and its own intrinsic instability towards ammonia even in its free state, particularly at high temperature. In order to assess the influence of the individual protecting groups (N^3, β-D-glucopyranosyl) in dJ on the decomposition of the J nucleobase, monomers **5**, **10**, **16**, **18**, and **19** were chosen for examination (Scheme 3).[17]



Scheme 2. Reagents and conditions: (i) NH_3 (aqu.), 50 °C, 16 h, **14/15** = 3:2 (see Figure 1); (ii) NH_3 (aqu.), room temp., 16 h, **14/15** = 4:1 (see Figure 2)

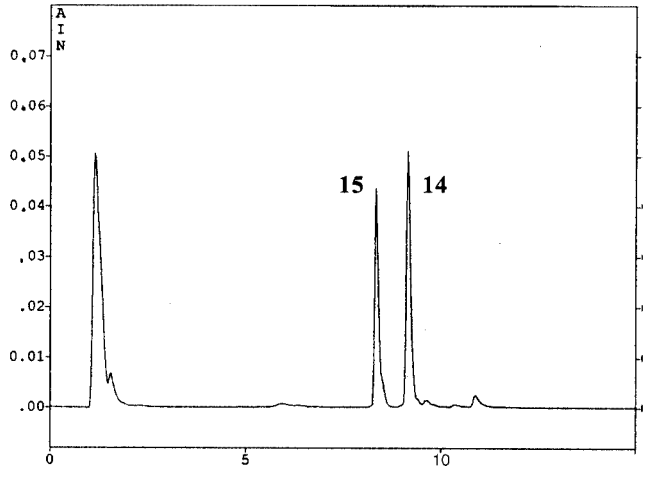


Figure 1. Crude HPLC chromatogram after treatment of **13** with aqu. ammonia at 50 °C for 16 h

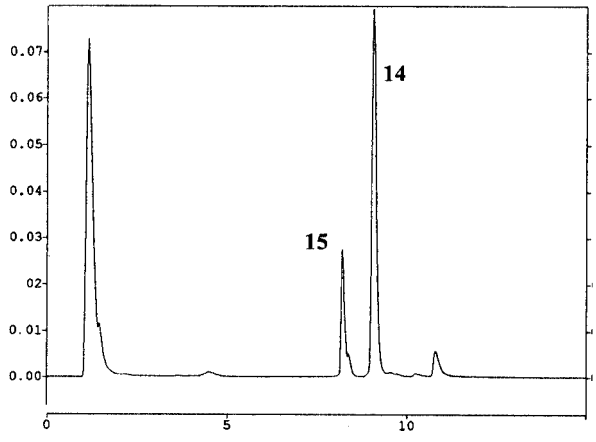


Figure 2. Crude HPLC chromatogram after treatment of **13** with aqu. ammonia at room temperature for 16 h

In the first instance, fully protected nucleobase **10** was treated with aqueous ammonia at elevated temperature for 16 h. Analysis of the resulting mixture (LCMS) revealed (Table 1, entry 1) that the deprotected hypermodified nucleoside dJ **16** and 5-(aminomethyl)-2′-deoxyuridine **17**[18] were both present in a surprisingly high ratio in favour of the latter, indicating more than 60% decomposition. In contrast, subjection of compound **16** (isolated by RP-HPLC) to the same conditions (entry 2) resulted in just 15% decomposition, the same amount as for **14** (see Figure 3, D) under these conditions. In addition, very little decomposition occurred when **16** was subjected to ammonia treatment at room temperature for the same period of time (entry 3, Table 1), again, identical to that in the case of **14** (Figure 3, B), demonstrating that monomeric and incorporated dJ have comparable stabilities to decomposition. Subjection of partially protected dJ **5** (N^3 unprotected) to the same set of conditions (entries 4 and 5) revealed a close parallel, in terms of stability, to that of free dJ **16**. Furthermore, negligible decomposition was observed in the deprotection of **18** and **19**,[19] which, by virtue of their more labile acetyl and methoxyacetyl β-D-glucose hydroxy group protection, required just 2 h (entry 6) and 1 h (entry 7) treatments with aqueous ammonia at room temperature, respectively. The results depicted in Table 1 clearly demonstrate the influence of the N^3 POM protecting group on the decomposition of dJ, namely that its presence has a detrimen-

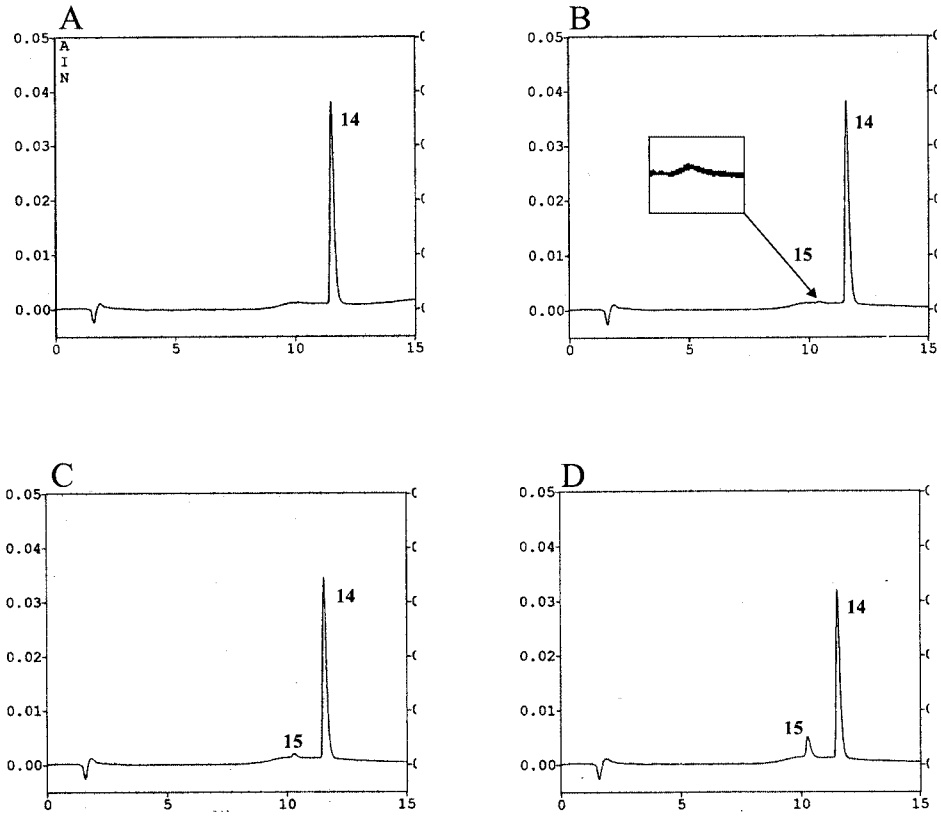
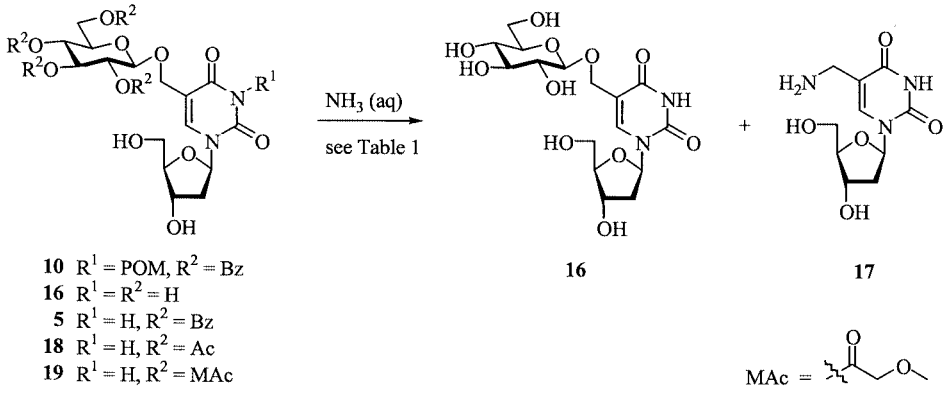


Figure 3. (A) Pure **14**, obtained by preparative RP-HPLC; (B) **14** after NH_3 (aqu.), room temp. 16 h; (C) **14** after NH_3 (aqu.), room temp. 64 h; (D) **14** after NH_3 (aqu.), 50 °C, 16 h



Scheme 3

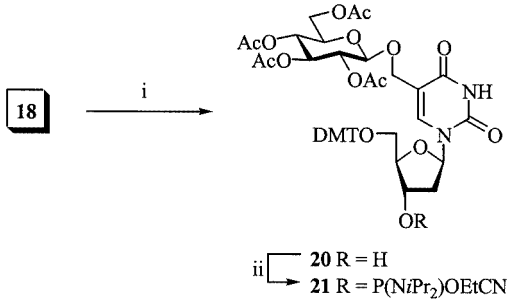
tal effect on the stability of dJ. In contrast, the β-D-glucose hydroxy group protection has a comparatively small influence, limited to the intrinsic lability of dJ in ammonia. In other words, although the benzoyl groups in **5** do not adversely affect the stability of dJ, the need to subject **5** to ammonia treatment overnight for complete deprotection causes more decomposition than the corresponding 2 h treatment for **18**, it having been established that both **14** and **16** are themselves sensitive to these conditions.

The lability of dJ in its free state as discussed above has a direct consequence with regard to the standard DNA phosphoramidite building blocks used for the synthesis of dJ-containing oligomers. Exocyclic amino groups for adenosine and cytidine are routinely protected with benzoyl groups, and the exocyclic amino of guanosine with an isobutyryl moiety. All these require overnight treatment with aqueous ammonia at elevated temperature (50 °C) for their complete removal, which would result in decomposition of

Table 1. Treatment of dJ monomers with differing N^3 and β-D-glucose hydroxy protection with NH_3 (aqu.)

Entry	dJ monomer	Treatment with ammonia Time/h	temp/°C	Ratio of **16/17**
1	**10**	16	50	1:2
2	**16**	16	50	17:3
3	**16**	16	room temp.	99:1
4	**5**	16	50	3.65:1
5	**5**	16	room temp.	99:1
6	**18**	2	room temp.	>99:1
7	**19**	1	room temp.	>99:1

dJ. Replacement of these protecting groups with the considerably more labile 4-(*tert*-butyl)phenoxyacetyl (TAC) protecting group enables the deprotection time to be shortened to 2 h and the temperature to be lowered to ambient. It was therefore anticipated that suppression of 5-(aminomethyl)-2′-deoxyuridyl formation should be most effectively achieved by use of these building blocks for oligonucleotide synthesis, in conjunction with a dJ phosphoramidite building block with acetyl protection for the glucose moiety and − crucially − without POM N^3 protection.[20] To this end, compound **18** was treated with 4,4′-dimethoxytrityl chloride in pyridine, affording a 90% yield of **20** (Scheme 4), which in turn was converted into the corresponding phosphoramidite **21** in 96% yield. This DNA building block was then used to synthesise oligomers **14** and **22**−**24** (Table 2) through the use of standard DNA phosphoramidite building blocks with TAC exocyclic amino protection, without any decomposition product being detectable after deprotection with aqueous ammonia for two hours at ambient temperature.



Scheme 4. Reagents and conditions: (i) DMTCl, py, room temp., 16 h, 90%; (ii) ClP(N*i*Pr$_2$)OEtCN, DIPEA, DCM, room temp., 96%

Conclusion

The side product accompanying dJ-containing oligomers when the previously reported synthetic procedures from our laboratory[13,14] were used has been identified as the corresponding 5-(aminomethyl)-2′-deoxyuridyl derivative arising from amino substitution of the β-D-glucosyl moiety during the final deprotection step. The investigations disclosed in this paper have revealed that two key factors are responsible for this decomposition: the use of POM protection for N^3 and high-temperature aqueous ammonia treatment. With respect to the latter, it was shown that unprotected dJ, either as its monomer or incorporated into a DNA oligomeric sequence, is prone to significant decomposition under these conditions. Decomposition was shown to be suppressed by employment of a synthetic strategy of no N^3 POM protection but acetyl protection for the β-D-glucose hydroxy groups on the dJ phosphoramidite building block, in conjunction with TAC exocyclic amino protection for the standard DNA building blocks, followed by fast deprotection with ammonia at room temperature.

Experimental Section

General Remarks: ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AV-400 (400/100 MHz) spectrometer. ^{31}P NMR spectra were recorded with a Jeol JNM-FX-200 (80.7 MHz) spectrometer. ^{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. All ^{13}C and ^{31}P spectra given are proton decoupled. Electrospray mass spectra were recorded with a Perkin−Elmer/SCIEX API 165 mass instrument fitted with a custom-made electrospray interface (ESI). LCMS analysis was performed on a Jasco HPLC system (detection simultaneously at 214 and 254 nm) coupled to the aforementioned instrument, with an Alltima C_{18} column (Alltech, 4.0 mm D × 250 mm L, 5 μ particle size). Dichloromethane (DCM, Biosolve, HPLC-grade), 1,2-dichloroethane (Biosolve, HPLC-grade), pyridine (Baker, p.a.), 1,4-dioxane (Baker, p.a.) and toluene (Baker, p.a.) were stored over molecular sieves (4 Å). Methanol (MeOH, Biosolve, HPLC-grade) and acetonitrile (ACN, Biosolve, HPLC-grade) were stored over molecular sieves (3 Å). *N*,*N*,*N*-Diisopropylethylamine (DIPEA, Biosolve, p.a.) was dried by heating at reflux with CaH_2 (5 g/L) for 4 h and then distilled. All chemicals (Acros, Belgium) were used as received. Column chromatography was performed either on Fluka 60 (0.040−0.063 mm). TLC analysis was performed with DC-Fertigfolien (Schleicher & Schuell, F1500, LS254) or HPTLC aluminium sheets (Merck, silica gel 60, F254) with detection by UV absorption (254 nm) and charring with 20% H_2SO_4 in EtOH. Reac-

Table 2. ((Author: please give a Caption!))

Compound	Sequence	Mol. mass, calcd.	MALDI-TOF, $[M + H]^+$
14	TT**dJ**TTT	1941.2	1943.4
22	TG**dJ**TGT	1991.2	1992.8
23	TA**dJ**TCTTG**dJ**TGT	3810.4	3814.1
24	$(GGG\mathbf{dJ}TA)_4$	8286.0	8288.3

tions were run at room temperature unless stated otherwise. Prior to reactions requiring anhydrous conditions, traces of water were removed by coevaporation with 1,2-dichloroethane, 1,4-dioxane, toluene, or pyridine.

3′,5′-Bis(*O*-*tert*-butyldimethylsilyl)-2′-deoxy-5-(β-D-glucopyranosyl)oxymethyluridine (25): K_2CO_3 (166 mg, 1.2 mmol) was added to a solution of compound **9**[14] (1.18 g, 1.0 mmol) in MeOH (5 mL). After stirring for 4 h at room temperature, the reaction mixture was carefully neutralised with Dowex 50 W × 4 (H^+) and filtered, and the filtrate was concentrated in vacuo. The resulting oil was subjected to column chromatography (petroleum ether/EtOAc/MeOH, 3:1:0 to 0:1:1, v/v/v), affording **25** (474 mg, 0.73 mmol, 73%) and **26** (100 mg, 0.20 mmol, 20%) as colourless oils. Analytical data for **25**. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.76 (s, 1 H, H^6), 6.21 (dd, $J_{1′,2a′}$ = 7.8, $J_{1′,2b′}$ = 6.1 Hz, 1 H, $H^{1′}$), 4.57 (d, $J_{7a,7b}$ = −12.1 Hz, 1 H, H^{7a}), 4.46 (m, 1 H, $H^{3′}$), 4.42 (d, 1 H, H^{7b}), 3.38 (d, $J_{1′′,2′′}$ = 7.8 Hz, 1 H, $H^{1′′}$), 3.93 (m, 1 H, $H^{4′}$), 3.86 (dd, $J_{5′′,6a′′}$ = 2.0, $J_{6a′′,6b′′}$ = −11.9 Hz, 1 H, $H^{6a′′}$), 3.80 (d, $J_{4′,5′}$ = 4.4 Hz, 2 H, $H^{5′}$), 3.68 (dd, $J_{5′′,6b′′}$ = 5.1 Hz, 1 H, $H^{6b′′}$), 3.36 (m, 1 H, $H^{3′′}$), 3.32−3.29 (m, 2 H, $H^{4′′}$ and $H^{5′′}$), 3.22 (dd, $J_{2′′,3′′}$ = 8.9 Hz, 1 H, $H^{2′′}$), 2.27−2.16 (m, 1 H, $H^{2′}$), 0.92 and 0.92 (2 × s, 2 × 9 H, 2 × *t*Bu TBDMS), 0.12 (s, 12 H, 4 × Me TBDMS) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 165.2 (C^4), 151.9 (C^2), 141.0 (C^6), 112.1 (C^5), 103.9 ($C^{1′′}$), 89.2 ($C^{4′}$), 86.9 ($C^{1′}$), 77.8 ($C^{3′′}$), 77.8 ($C^{5′′}$), 74.8 ($C^{2′′}$), 73.9 ($C^{3′}$), 71.4 ($C^{4′′}$), 64.9 (C^7), 64.3 ($C^{5′}$), 62.6 ($C^{6′′}$), 41.2 ($C^{2′}$), 26.5 and 26.2 (CH_3 2 × *t*Bu TBDMS), 19.2 and 18.7 (2 × C_q TBDMS), −4.4, −4.5, −5.0, −5.1 (4 × Me TBDMS) ppm. MS (ESI): *m*/*z* = 649.4 $[M + H]^+$, 671.5 $[M + Na]^+$.

3′,5′-Bis(*O*-*tert*-butyldimethylsilyl)-2′-deoxy-5-(methoxymethyl)uridine (26): ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$, 1:1 v/v): δ = 7.71 (s, 1 H, H^6), 6.30 (dd, $J_{1′,2a′}$ = 8.0, $J_{1′,2b′}$ = 5.8 Hz, 1 H, $H^{1′}$), 4.46−4.43 (m, 1 H, $H^{3′}$), 4.17 (AB, 2 H, H^7), 3.98−3.96 (m, 1 H, $H^{4′}$), 3.82 (ABX, $J_{4′,5a′}$ = 3.1, $J_{4′,5b′}$ = 2.9, $J_{5a′,5b′}$ = −11.4 Hz, 2 H, $H^{5′}$), 3.40 (s, 3 H, OMe), 2.17 (ABXY, $J_{2a′,3′}$ = 6.0, $J_{2b′,3′}$ = 2.5, $J_{2a′,2b′}$ = −13.2 Hz, 2 H, $H^{2′}$), 0.94 and 0.91 (2 × s, 2 × 9 H, 2 × *t*Bu TBDMS), 0.13, 0.11, 0.11 (3 × s, 12 H, 4 × Me TBDMS) ppm. ^{13}C NMR (100 MHz, $CD_3OD/CDCl_3$, 1:1 v/v): δ = 164.1 (C^4), 151.2 (C^2), 139.0 (C^6), 112.0 (C^5), 88.5 ($C^{4′}$), 85.7 ($C^{1′}$), 72.9 ($C^{3′}$), 67.2 (C^7), 63.5 ($C^{5′}$), 58.6 (OMe), 41.7 ($C^{2′}$), 26.3 and 26.0 (CH_3, 2 × *t*Bu TBDMS), 18.8 and 18.4 (2 × C_q TBDMS), −4.5, −4.6, −5.2, −5.3 (4 × Me TBDMS) ppm. MS (ESI): *m*/*z* = 501.4 $[M + H]^+$, 523.6 $[M + Na]^+$, 1001.7 $[2 M + H]^+$, 1023.6 $[2 M + Na]^+$.

3′,5′-Bis(*O*-*tert*-butyldimethylsilyl)-2′-deoxy-5-(β-D-glucopyranosyl)oxymethyl-*N*^{3}-(pivaloyloxymethyl)uridine (27): K_2CO_3 (2 mg, 14 μmol) was added to a solution of compound **9**[14] (118 mg, 0.1 mmol) in MeOH (2 mL). After stirring for 16 h at room temperature the reaction mixture was carefully neutralised with Dowex 50 W × 4 (H^+) and filtered, and the filtrate was concentrated in vacuo. The crude material was subjected to column chromatography (petroleum ether/EtOAc/MeOH, 9:1:0 to 0:9:1, v/v/v) to give **27** (31 mg, 41 μmol, 41%) and **26** (7 mg, 11 μmol, 11%) as colourless oils, together with recovered starting material **9** (33 mg, 28 μmol, 28%). Analytical data for **27**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.81 (s, 1 H, H^6), 6.25 (dd, $J_{1′,2a′}$ = 5.8, $J_{1′,2b′}$ = 7.7 Hz, 1 H, $H^{1′}$), 5.93 (AB, 2 H, CH_2 POM), 4.56 (dd, $J_{7a,7b}$ = 11.6 Hz, 1 H, H^{7a}), 4.47−4.41 (m, 2 H, H^{7b} and $H^{1′′}$), 4.38 (m, 1 H, $H^{3′}$), 3.99 (m, 1 H, $H^{4′}$), 3.88−3.74 (m, 4 H, $H^{5′}$ and $H^{6′′}$), 3.57−3.55 (m, 2 H, $H^{3′′}$ and $H^{4′′}$), 3.41−3.37 (m, 2 H, $H^{2′′}$ and $H^{5′′}$), 2.34 (1/2ABXY, $J_{2a′,3′}$ = 2.3, $J_{2a′,2b′}$ = 13.2 Hz, 1 H, $H^{2a′}$), 2.08−2.02 (m, 1 H, $H^{2b′}$), 1.18 (s, 9 H, *t*Bu POM), 0.91 and 0.89 (2 × s, 2 × 9 H, 2 × *t*Bu TBDMS), 0.10 (s, 6 H, 2 × Me TBDMS), 0.08 and 0.08 (2 × s, 2 × 3 H, 2 × Me TBDMS) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.5 (C=O POM), 162.2 (C^4), 149.8 (C^2), 139.0 (C^6), 109.7 (C^5), 102.9 ($C^{1′′}$), 88.4 ($C^{4′}$), 86.7 ($C^{1′}$), 76.4, 75.9, 73.3, 72.5, 70.1 ($C^{3′}$/$C^{2′′}$/$C^{3′′}$/$C^{4′′}$/$C^{5′′}$), 65.1 (C^7), 64.9 (CH_2 POM), 63.2 ($C^{5′}$), 62.2 ($C^{6′′}$), 41.5 ($C^{2′}$), 38.8 (C_q POM), 27.0 (CH_3 *t*Bu POM), 26.0 and 25.7 (CH_3 2 × *t*Bu TBDMS), 18.4, 18.0 (2 × C_q TBDMS), −4.7, −4.8, −5.3, −5.4 (4 × Me TBDMS) ppm. MS (ESI): *m*/*z* = 785.6 $[M + Na]^+$.

3′,5′-Bis(*O*-*tert*-butyldimethylsilyl)-2′-deoxy-5-methoxymethyl-*N*^{3}-(pivaloyloxymethyl)uridine (28): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.69 (t, $J_{6,7}$ = 0.9 Hz, 1 H, H^6), 6.34 (dd, $J_{1′,2a′}$ = 5.7, $J_{1′,2b′}$ = 8.0 Hz, 1 H, $H^{1′}$), 5.95 (AB, 2 H, CH_2 POM), 4.41 (m, 1 H, $H^{3′}$), 4.20 (ABX, 2 H, H^7), 3.96 (m, 1 H, $H^{4′}$), 3.80 (ABX, $J_{4′,5a′}$ = 3.1, $J_{4′,5b′}$ = 2.9 Hz, 2 H, $H^{5′}$), 3.40 (s, 3 H, OMe), 2.16 (ABXY, $J_{2a′,3′}$ = 2.5, $J_{2b′,3′}$ = 5.9 Hz, 2 H, $H^{2′}$), 1.19 (s, 9 H, *t*Bu POM), 0.92 and 0.89 (2 × s, 2 × 9 H, 2 × *t*Bu TBDMS), 0.11 (s, 6 H, 2 × Me TBDMS), 0.08 and 0.08 (2 × s, 2 × 3 H, 2 × Me TBDMS) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.5 (C=O POM), 161.3 (C^4), 150.1 (C^2), 137.0 (C^6), 110.9 (C^5), 88.0 ($C^{4′}$), 85.9 ($C^{1′}$), 72.3 ($C^{3′}$), 67.2 (C^7) 64.8 (CH_2 POM), 63.0 ($C^{5′}$), 58.6 (OMe), 41.3 ($C^{2′}$), 38.8 (C_q POM), 27.0 (CH_3 POM), 25.9, 25.7 (CH_3 2 × *t*Bu TBDMS), 18.4, 18.0 (2 × C_q TBDMS), −4.7, −4.9, −5.5, −5.6 (4 × Me TBDMS) ppm. MS (ESI): *m*/*z* = 615.4 $[M + H]^+$, 637.6 $[M + Na]^+$, 1251.7 $[2 M + Na]^+$.

3′,5′-Bis(*O*-*tert*-butyldimethylsilyl)-2′-deoxy-5-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxymethyluridine (29): Acetic anhydride (0.4 mL, 429 mg, 4.2 mmol) was added to a solution of **25** (454 mg, 0.7 mmol) in pyridine (5 mL) and the reaction mixture was stirred overnight at room temperature. The reaction was quenched by the addition of MeOH (2 mL) and after a period of 15 min the mixture was concentrated in vacuo. The resulting residue was partitioned between EtOAc and water, and the organic layer was washed with brine, dried ($MgSO_4$), concentrated, and subjected to column chromatography (EtOAc/light petroleum) to afford the title compound as a colourless syrup (549 mg, 0.67 mmol, 96%). ^{1}H and ^{13}C NMR were in full accordance with that reported previously.[21] MS (ESI): *m*/*z* = 817.4 $[M + H]^+$, 839.6 $[M + Na]^+$.

3′,5′-Bis(*O*-*tert*-butyldimethylsilyl)-2′-deoxy-5-(2,3,4,6-tetra-*O*-methoxyacetyl-β-D-glucopyranosyl)oxymethyluridine (30): Methoxyacetic acid anhydride[22] (97 mg, 0.6 mmol) was added to a solution of **25** (65 mg, 0.1 mmol) in pyridine (1 mL), and the reaction mixture was stirred overnight at room temperature. The reaction was quenched by the addition of MeOH (0.2 mL) and after a period of 15 min the mixture was concentrated in vacuo. The resulting residue was partitioned between EtOAc and water, and the organic layer was washed with brine, dried ($MgSO_4$), concentrated, and subjected to column chromatography (EtOAc/light petroleum) to afford the title compound as a colourless syrup (81 mg, 87 μmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.13 (s, 1 H, NH), 7.70 (s, 1 H, H^6), 6.30 (dd, $J_{1′,2a′}$ = 5.7, $J_{1′,2b′}$ = 8.1 Hz, 1 H, $H^{1′}$), 5.28 (m, 1 H, $H^{3′′}$), 5.10 (m, 1 H, $H^{4′′}$), 5.00 (dd, $J_{1′′,2′′}$ = 8.1, $J_{2′′,3′′}$ = 9.5 Hz, 1 H, $H^{2′′}$), 4.75 (d, 1 H, $H^{1′′}$), 4.42 (AB, 2 H, H^7), 4.37 (m, 1 H, $H^{3′}$), 4.26 (ABX, $J_{5′′,6a′′}$ = 4.4, $J_{5′′,6b′′}$ = 2.2 Hz, 2 H, $H^{6′′}$), 4.09 (AB, 2 H, 1 × CH_2, MAc), 4.10−3.93 (m, 7 H, $H^{4′}$, 3 × CH_2 MAc), 3.83−3.72 (m, 3 H, $H^{5′}$, $H^{5′′}$), 3.45, 3.39, 3.36, 3.36 (4 × s, 4 × 3 H, 4 × CH_3 MAc), 2.30 (1/2ABXY, $J_{2a′,3′}$ = 2.3, $J_{2a′,2b′}$ = 13.2 Hz, $H^{2a′}$), 2.03−1.96 (m, 1 H, $H^{2b′}$), 0.89 and 0.88 (2 × s, 2 × 9 H, 2 × *t*Bu TBDMS), 0.08 (s, 6 H, 2 × Me TBDMS), 0.07 and 0.06 (2 × s, 2 × 3 H, 2 × Me TBDMS) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.9, 169.6, 169.2, 169.0 (4 × C=O MAc), 162.8 (C^4), 149.9 (C^2), 139.7 (C^6), 110.7 (C^5), 100.9 ($C^{1′′}$), 88.2 ($C^{4′}$), 85.7 ($C^{1′}$), 73.1 ($C^{3′′}$), 72.8 ($C^{3′}$), 71.7 ($C^{5′′}$), 71.3 ($C^{2′′}$),

69.2 (4 × CH_2 MAc), 68.4 ($C^{4''}$), 64.9 (C^7), 63.1 ($C^{5'}$), 62.0 ($C^{6''}$), 59.4, 59.3, 59.3, 59.2 (4 × CH_3 MAc), 41.5 ($C^{2'}$), 25.9, 25.7 (CH_3 2 × *t*Bu TBDMS), 18.3, 17.9 (2 × C_q TBDMS), −4.7, −4.9, −5.4, −5.5 (4 × Me TBDMS) ppm. MS (ESI): *m*/*z* = 959.4 $[M + Na]^+$.

2′-Deoxy-5-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxymethyluridine (18): $Et_3N{\cdot}3HF$ (0.22 mL, 1.30 mmol) was added to a stirring solution of **29** (533 mg, 0.65 mmol) in pyridine (3 mL). After 16 h at room temperature, the reaction mixture was concentrated, dissolved in EtOAc, and washed with brine (2 ×). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting residue was purified by column chromatography (EtOAc/MeOH, 1:0 to 9:1, v/v) to give **18** (347 mg, 0.59 mmol, 91%) as a syrup. ^1H NMR (400 MHz, $CDCl_3$): δ = 10.07 (br. s, 1 H, NH), 7.85 (s, 1 H, H^6), 6.30 (m, 1 H, $H^{1'}$), 5.26 (m, 1 H, $H^{3''}$), 5.11 (m, 1 H, $H^{4''}$), 5.03 (dd, $J_{1'',2''}$ = 8.0, $J_{2'',3''}$ = 9.6 Hz, 1 H, $H^{2''}$), 4.67 (d, 1 H, $H^{1''}$), 4.64 (d, $J_{7a,7b}$ = −12.9 Hz, 1 H, H^{7a}), 4.55−4.52 (m, 1 H, $H^{3'}$), 4.39 (d, 1 H, H^{7b}), 4.21 (ABX, $J_{5'',6a''}$ = 4.6, $J_{5'',6b''}$ = 1.4, $J_{6a'',6b''}$ = −12.3 Hz, 2 H, $H^{6''}$), 4.00 (m, 1 H, $H^{4'}$), 3.87 (ABX, $J_{4',5a'}$ = 2.7, $J_{4',5b'}$ = 2.6, $J_{5a',5b'}$ = −12.1 Hz, 2 H, $H^{5'}$), 3.80−3.75 (m, 1 H, $H^{5''}$), 3.41 (br. s, 2 H, 2 × OH), 2.40−2.35 (m, 1 H, $H^{2a'}$), 2.27−2.20 (m, 1 H, $H^{2b'}$), 2.09, 2.06, 2.03, 2.01 (4 × s, 12 H, 4 × CH_3 Ac) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.8, 170.7, 170.1, 169.6 (4 × C=O Ac), 162.6 (C^4), 150.4 (C^2), 137.9 (C^6), 110.8 (C^5), 100.6 ($C^{1''}$), 87.2 ($C^{4'}$), 85.3 ($C^{1'}$), 72.4, 71.8, 71.4, 70.9 ($C^{3'}$/$C^{2''}$/$C^{3''}$/$C^{5''}$), 68.4 ($C^{4''}$), 64.4 (C^7), 61.8 ($C^{5'}$/$C^{6''}$), 40.5 ($C^{2'}$), 20.7, 20.6, 20.5 (4 × CH_3 Ac) ppm. MS (ESI): *m*/*z* = 611.4 $[M + Na]^+$, 1199.5 $[2 M + Na]^+$.

2′-Deoxy-5-(2,3,4,6-tetra-*O*-methoxyacetyl-β-D-glucopyranosyl)oxymethyluridine (19): Compound **30** (62 mg, 66 μmol) was deprotected as described for the conversion of **29** into **18**. Purification by column chromatography (EtOAc/MeOH, 1:0 to 1:1, v/v) afforded **19** (36 mg, 51 μmol, 77%). ^1H NMR (400 MHz, CD_3OD): δ = 8.07 (s, 1 H, H^6), 6.24 (m, 1 H, $H^{1'}$), 5.38 (m, 1 H, $H^{3''}$), 5.14 (dd, $J_{3'',4''}$ = 9.3, $J_{4'',5''}$ = 10.1 Hz, 1 H, $H^{4''}$), 4.97 (dd, $J_{1'',2''}$ = 8.0, $J_{2'',3''}$ = 9.6 Hz, 1 H, $H^{2''}$), 4.83 (d, 1 H, $H^{1''}$), 4.49 (1/2AB, 1 H, H^{7a}), 4.46−4.36 (m, 3 H, H^{7b}, $H^{3'}$, $H^{6a''}$), 4.27 (1/2ABX, $J_{5'',6b''}$ = 2.2 Hz, 1 H, $H^{6b''}$), 4.12−3.91 (m, 10 H, 4 × CH_2 MAc, $H^{4'}$, $H^{5''}$), 3.78 (ABX, $J_{4',5a'}$ = 3.1, $J_{4',5b'}$ = 3.6 Hz, 2 H, $H^{5'}$), 3.41, 3.37, 3.35, 3.34 (4 × s, 4 × 3 H, 4 × CH_3 MAc), 2.33−2.20 (m, 2 H, $H^{2'}$) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 171.8, 171.4, 171.1, 171.0 (4 × C=O MAc), 165.0 (C^4), 152.1 (C^2), 141.9 (C^6), 111.4 (C^5), 101.0 ($C^{1''}$), 89.0 ($C^{4'}$), 86.7 ($C^{1'}$), 74.5 ($C^{3''}$), 72.9 ($C^{2''}$), 72.6 ($C^{5''}$), 72.0 ($C^{3'}$), 70.2 (4 × CH_2 MAc), 69.8 ($C^{4''}$), 65.7 (C^7), 62.8 ($C^{6''}$), 62.7 ($C^{5'}$), 59.6 (4 × CH_3 MAc), 41.5 ($C^{2'}$) ppm. MS (ESI): *m*/*z* = 731.4 $[M + Na]^+$, 1439.5 $[2 M + Na]^+$.

2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-5-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxymethyluridine (20): DMTrCl (0.31 g, 0.9 mmol) was added to a stirred solution of **18** (353 mg, 0.6 mmol) in pyridine (3 mL). After 3 h the reaction was quenched with MeOH (1 mL) and after 15 min the mixture was concentrated in vacuo. The resulting residue was partitioned between EtOAc and water, and the organic layer was washed with brine (2 ×), dried ($MgSO_4$), and concentrated. The crude product was purified by column chromatography (EtOAc/light petroleum/Et_3N, 50:50:1 to 100:0:1, v/v/v) to give **20** (480 mg, 0.54 mmol, 90%). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.77 (s, 1 H, H^6), 7.37−6.82 (m, 14 H, H_{arom} DMT), 6.29 (m, 1 H, $H^{1'}$), 5.21 (m, 1 H, $H^{3''}$), 5.08 (m, 1 H, $H^{4''}$), 4.92 (dd, $J_{1'',2''}$ = 8.1, $J_{2'',3''}$ = 9.6 Hz, 1 H, $H^{2''}$), 4.63 (d, 1 H, $H^{1''}$), 4.54 (m, 1 H, $H^{3'}$), 4.21 (dd, $J_{5'',6a''}$ = 4.2, $J_{6a'',6b''}$ = −12.4 Hz, 1 H, $H^{6a''}$), 4.17 (d, $J_{7a,7b}$ = −12.2 Hz, 1 H, H^{7a}), 4.08−4.06 (m, 2 H, $H^{4'}$ and $H^{6b''}$), 3.88 (d, 1 H, H^{7b}), 3.78 (s, 6 H, OMe DMT), 3.68−3.64 (m, 1 H, $H^{5''}$) 3.37 (ABX, $J_{4',5a'}$ = 3.3, $J_{4',5b'}$ = 3.5, $J_{5a',5b'}$ = −10.5 Hz, 2 H, $H^{5'}$), 2.51−2.45 (m, 1 H, $H^{2a'}$), 2.34−2.25 (m, 1 H, $H^{2b'}$), 2.01, 2.01, 1.99, 1.92 (4 × s, 4 × 3 H, 4 × CH_3 Ac) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.6, 170.1, 169.4, 169.4 (4 × C=O Ac), 162.9 (C^4), 158.6 (2 × C_q DMT), 150.2 (C^2), 144.5 (C_q DMT), 139.5 (C^6), 135.4, 135.2 (2 × C_q DMT), 130.0, 128.1, 127.9, 126.9, 113.3 (CH_{arom} DMT), 110.9 (C^5), 100.7 ($C^{1''}$), 86.8 (C_q DMT), 86.1 ($C^{4'}$), 85.4 ($C^{1'}$), 72.8 ($C^{3''}$), 72.2 ($C^{3'}$), 71.6 ($C^{5''}$), 71.2 ($C^{2''}$), 68.3 ($C^{4''}$), 64.1 (C^7), 63.5 ($C^{5'}$), 61.7 ($C^{6''}$), 55.1 (OMe DMT), 40.8 ($C^{2'}$), 20.5 (4 × CH_3 Ac) ppm. MS (ESI): *m*/*z* = 913.6 $[M + Na]^+$.

2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-5-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxymethyluridine 3′-*O*-(2-Cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (21): DIPEA (0.35 mL, 2 mmol) was added under an atmosphere of argon, to a stirred solution of **20** (445 mg, 0.45 mmol) in DCM (10 mL), followed by 2-cyanoethyl diisopropylchlorophosphoramidite[23] (0.13 mL, 0.57 mmol). After 1 h, the reaction mixture was diluted with DCM and washed with aqueous $NaHCO_3$ (9%) and water. The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo, and the resulting residue was purified by flash column chromatography (EtOAc/light petroleum/Et_3N, 50:150:1 to 150:50:1, v/v/v), affording **21** (475 mg, 0.43 mmol, 96%) as a white foam. ^{31}P NMR (80.7 MHz, DCM-acetone capillary): δ = 149.1 and 149.0.

Solid-Phase Synthesis of Oligonucleotides: The polymer-supported synthesis of dJ-containing DNA fragments was performed on a fully automated synthesiser (Pharmacia Gene Assembler Special). Phosphoramidite **12** was employed for the construction of **13** by the same procedure as reported previously.[14] The improved synthesis of dJ-containing DNA fragments through the use of **21** and commercially available (Proligo) 4-(*tert*-butyl)phenoxyacetyl-protected 2′-deoxynucleoside 3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidites is summarised in Table 3. Controlled pore glass (CPG-AP) loaded with the appropriate nucleoside (Proligo) was used as solid support. Capping solutions Tac_2O [4-(*tert*-butyl)-phenoxyacetic acid anhydride] 50 g/L in THF and NMI/py/THF (1:1:8, v/v/v) were obtained from Proligo.

Ammonolysis of (Immobilised) Oligonucleotides: The cleavage of freshly prepared oligonucleotides from the solid phase and concomitant deprotection was effected by placing the immobilised DNA fragment in concentrated aqueous ammonia in a sealed vial. For DNA fragments synthesised with phosphoramidite **21** this was performed at room temperature for 2 h, furnishing pure **14** and **22**−**24** after purification (see Analysis and Purification of Oligonucleotides). For test sequence **13** ammonolysis was performed at 50 °C (Figure 1) and at room temperature for 16 h (Figure 2). The resulting oligomers **14** and **15** were separated (see Analysis and Purification of Oligonucleotides) and **14** was subjected to the same treatment as **13** but at varying temperatures and times (see Figure 3).

Analysis and Purification of Oligonucleotides: Analysis of (crude) oligonucleotides was performed with a DNAPac™ PA-100 (4 mmD × 250 mmL) anion-exchange column (Dionex) connected to a Jasco HPLC system (detection at 254 nm). Elution at pH 12: buffer A (10 mM NaOH + 20 mM NaCl), buffer B (10 mM NaOH + 1.2 M NaCl) − gradient 20−50% buffer B in 15 min. Elution at pH 7: buffer A (20 mM NaOAc + 20 mM NaCl), buffer B (20 mM NaOAc + 1 M NaCl) − gradient 10−30% buffer B in 15 min. Purification of oligonucleotides was achieved as for the analysis but with a DNAPac™ PA-100 (9 mmD × 250 mmL) anion-exchange column (Dionex) connected to a BioCAD "Vision" automated HPLC system (PerSeptive Biosystems, Inc), with elution at pH 12. Desalting was performed with Sephadex G25 (Pharmacia) and the

Table 3. Chemical steps involved in each elongation cycle modified for phosphoramidite **21**

Step	Manipulation	Solvents and reagents	Time (min)
1	Detritylation	2% Trichloroacetic acid in DCM	2.5
2	Coupling	**21**[a] or amidite,[b] 4,5-dicyanoimdazole[c]	6[d]or 3
3	Oxidation	0.02 M I_2 in ACN/sym-collidine/H_2O. 11:1:5 (v/v/v)	1
4	Capping	0.06 M Tac_2O in pyridine/NMI[e] /THF (1:1:18, v/v/v)	0.5

[a] 0.1 M amidite in ACN. [b] 0.1 M commercially available (dC^{TAC}, dA^{TAC}, dG^{TAC}, T) amidite in ACN. [c] 0.25 M in ACN. (DCI) [d] Only for **21**. [e] *N*-Methylimidazole.

oligonucleotides were obtained in pure form after lyophilisation. The integrity of the oligonucleotides was confirmed by MALDI-TOF mass spectrometry (positive mode) with a "Voyager System 6069" (Applied Biosystems).

Ammonolysis of dJ Monomers: Compounds **5**,[14] **10**,[14] **16**,[14] **18**, and **19** were treated with concentrated aqueous ammonia and sealed in vials for varying times and at different temperatures (see Table 1). Analysis of the crude mixtures was performed by LCMS with H_2O/ACN/0.5% TFA (aqu.) as eluent: Gradient 90:0:10 to 75:15:10, v/v/v in 15 min.

5-(β-D-Glucopyranosyloxymethyl)-2′-deoxyuridine (dJ, 16): LCMS: R_t 10.30, m/z = 421.2 $[M + H]^+$.

5-(Aminomethyl)-2′-deoxyuridine (17): LCMS: R_t 7.38, m/z = 258.2 $[M + H]^+$.

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[16] It should be noted that a higher-resolution ion-exchange column than previously was used (Dionex-Pac).[14] In addition, the buffer was changed from pH 12 to pH 7 in order better to visualise and quantify the side product **15**, which at pH 12 is a shoulder on the right hand side of the main peak (**14**).
[17] No protection of the 2-deoxyribose moiety hydroxy groups was used, as these are not responsible for decomposition and leaving them unmasked aids solubility in the aqueous medium.
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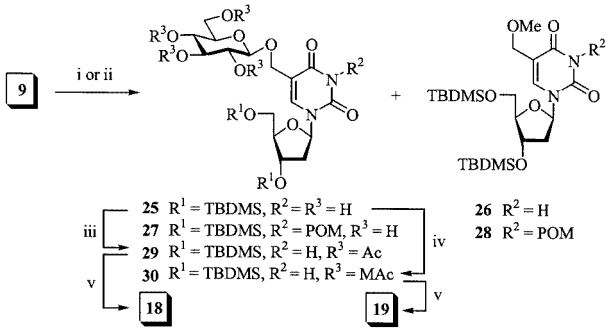
[19] Compounds **18** and **19** were prepared as depicted in Scheme 5. In contrast to earlier findings,[21] concomitant substitution of the glucosyl moiety was also observed, resulting in a mixture of **25** and the corresponding 5-(methoxymethyl)-2′-deoxyuridine derivative **26**. The amount of the latter was reduced by the replacement of KO*t*Bu with K_2CO_3 and a reduction in temperature. It is of interest to note that removal of the POM protecting group is not catalytic, as evidenced by the isolation and characterisation of both **27** and **28** when catalytic amounts of base are used. Nevertheless, the addition of 1.2 equiv. K_2CO_3 resulted in the reaction going to completion within 4 h with **20** and **21** being isolated in 73% and 20% yields, respectively.



Scheme 5. Reagents and conditions: (i) *t*BuOK 0.1 M in MeOH, 60 °C, 16 h, **25/26** = 5:3. (ii) K_2CO_3 (1.2 equiv.), MeOH, room temp., 4 h. **25**: 73%, **26**: 20% (from **9**). (iii) Ac_2O, py, room temp., 16 h. (iv) $(MeOCH_2CO)_2O$, py, room temp., 16 h. (v) $Et_3N{\cdot}3HF$, py, room temp., 16 h

[20] Use of the more labile methoxyacetyl hydroxy group protection would provide no further advantage, as TAC protection requires 2 h for complete removal.
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