Synthesis, structure and reactions of uridine 2′-*C*,3′-*O*-γ-butyrolactone: versatile intermediate for the synthesis of 2′-*C*-branched nucleosides

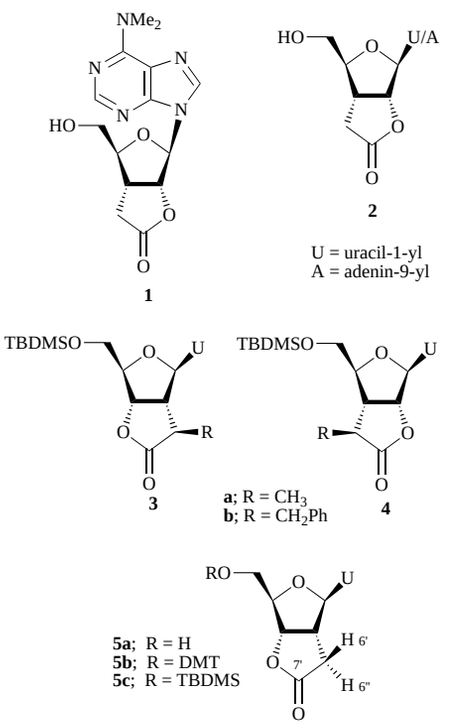
Anthony J. Lawrence,[a] John B. J. Pavey,[a] Mai-Yee Chan,[a] Robin A. Fairhurst,[b] Stephen P. Collingwood,[b] Julie Fisher,[c] Richard Cosstick *,†,[a] and Ian A. O'Neil *,[a]

[a] *Robert Robinson Laboratories, Department of Chemistry, University of Liverpool, PO Box 147, Liverpool, UK L69 3BX*
[b] *Novartis Pharmaceuticals UK Limited, Hulley Road, Macclesfield, UK SK10 2NX*
[c] *School of Chemistry, University of Leeds, Leeds, UK LS2 9JT*

Uridine 2′-*C*-3′-*O*-γ-butyrolactone 5a has been prepared by cyclisation of 2′-α-*C*-carboxymethyl-2′-deoxyuridine 8 in acetic acid–methanol. Detailed ^{1}H NMR studies on compound 5a clearly demonstrate *cis* fusion between the γ-lactone and the sugar moiety, and determination of conformationally averaged structural descriptors, *i.e.* the pseudorotation phase angle and the puckering amplitude, suggests that the dominant conformer of the furanose ring is *S*. The 2′-*C*,3′-*O*-lactone 5a and its 5′-protected derivatives (5b and 5c) readily undergo aminolyses to yield a range of amide derivatives. Reaction with 5′-amino-5′-deoxythymidine gave a 2′–5′-amide-linked analogue of a dinucleoside monophosphate. Under acidic conditions the amides slowly relactonised. The 5′-protected lactones were also shown to react with simple organometallic reagents.

Introduction

Naturally occurring *C*-branched nucleosides (oxetanocins[1]) and their synthetic analogues (2′,3′-dideoxy-3′-α-*C*-hydroxymethyl nucleosides[2,3]) have both been shown to exhibit potent antiviral activity. *C*-Branched nucleosides have also attracted considerable attention as precursors for backbone-modified antisense oligonucleotides. An interesting route into ribonucleoside-derived 2′-*C*- or 3′-*C*-branched nucleosides involves manipulation of a strained, nucleoside 2′,3′-fused γ-butyrolactone. This type of nucleoside γ-lactone (**1**) was first reported in 1973 as a precursor in the synthesis of a homologue of puromycin.[4] More recently the γ-lactones **2** have been used to prepare a 3′-*C*-amide-linked analogue of a dinucleoside monophosphate,[5] whilst Velázquez *et al.*[6] have proposed that 1″-alkyl nucleoside-γ-butyrolactones (**3** and **4**) could function as chiral synthons for the preparation of complex branched-chain nucleosides. In a recent communication from this laboratory the synthesis of uridine 2′-*C*,3′-*O*-γ-butyrolactone‡ **5a** was described.[7] This lactone can serve as an intermediate for the synthesis of oligonucleotide analogues that contain either 2′-alkyl substituents[8] or modified 2′ ⟶ 5′-linkages,[9,10] and is of interest since both these types of analogues are currently under investigation as potential antisense agents. We now report full details of the synthesis of lactone **5a**, its structure and reactions. In particular, we draw attention to the opening of the lactone with 5′-amino-5′-deoxythymidine to give a 2′ ⟶ 5′-amide-linked analogue of a dinucleoside monophosphate and provide the first clear demonstration that lactones of this type are useful intermediates for the synthesis of *C*-branched nucleosides.



Results and discussion

Synthesis of uridine 2′-*C*,3′-*O*-γ-butyrolactone 5a

The 3′,5′-*O*-tetraisopropyldisiloxanediyl (TIPS)-protected carboxymethyl nucleoside **7**, which served as a precursor for the lactone, was prepared in 94% yield from the readily available 2′-*C*-(formylethyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine[11] **6** by oxidation with $NaClO_2$ in the presence of KH_2PO_4 and 2-methylbut-2-ene in aq. *tert*-butyl alcohol.[12] Removal of the TIPS protecting group was accomplished using NEt_3·3HF, to give 2′-deoxy-2′-α-*C*-carboxymethyluridine **8** as the triethylammonium salt in 85% yield. The formation of 5-membered-ring lactones is extremely favourable and often spontaneous; therefore we were surprised to find that fully deprotected carboxymethyluridine **8** was relatively stable, and could easily be purified by column chromatography. However, TLC analysis of solutions in both aqueous and organic solvents revealed that this product underwent slow cyclisation to the

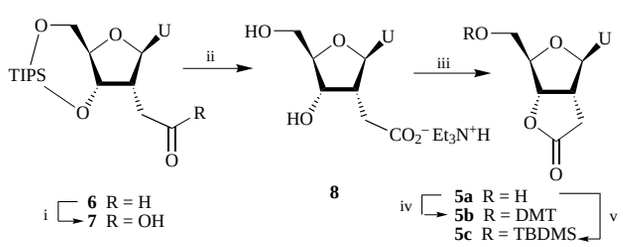
† E-Mail: rcosstic@liverpool.ac.uk
‡ *Systematic name:* 2,3-dihydro-(2′,3′-dideoxyuridino)[2′,3′-*b*]furan-5(4*H*)-one.

Table 1 Chemical shifts, coupling constants and dihedral angles for compound **5a**

	Chemical shift[a]		Coupling constant[a]		Dihedral angle		
H-1′	5.82	$J_{1',2'}$	6.6 (5.3)	$\varphi_{1',2'}$	151[b]	140[c]
H-2′	3.14	$J_{2',3'}$	7.3 (7.1)	$\varphi_{2',3'}$	−36[b]	−22[c]
H-3′	5.01	$J_{3',4'}$	2.7 (2.3)	$\varphi_{3',4'}$	−115[b]	−110[c]
H-4′	4.21	$J_{4',5'/5''}$	4.1	$\varphi_{4',5'/5''}$	121/52[b]	122/48[c]
H-5′/H-5″	3.63					
H-6′	2.89	$J_{2',6'}$	9.2	$\varphi_{2',6'}$	19[b]	160[c]
H-6″	2.61	$J_{2',6''}$	2.4 (3.0)	$\varphi_{2',6''}$	106[b]	107[c]
H-5	5.65	$J_{5,6}$	8.0			
H-6	7.81					

[a] Chemical shifts are in ppm, coupling constants are in Hz. The values given, excluding those for the base protons, are from the iterative spectral simulation using PANIC 86.[13] The final rms error for the 8 non-equivalent spins calculation was 0.29 Hz. The numbers in parentheses are those presented by Velázquez *et al.* for compound **3a**.[6] [b] Dihedral angle determined using the substituent electronegativity-modified Karplus equation. [c] Dihedral angle determined using the standard Karplus equation.

lactone. A variety of conditions for the preparation of the fused-ring lactone were investigated. The most efficient conversion, in terms of speed of reaction and ease of purification, was achieved by warming the acid (salt) **8** at 60 °C in acetic acid–methanol (4:1) for 2 h, and under these conditions the lactone was obtained quantitatively after evaporation off of the solvents (see Scheme 1). The lactone was identified by a downfield



Scheme 1 *Reagents and conditions:* i, $NaClO_2$, KH_2PO_4, 2-methylbut-2-ene, aq. Bu^{t}OH; ii, NEt_3·3HF, THF; iii, AcOH–MeOH (4:1), 40 °C; iv, 4,4′-dimethoxytrityl chloride (DMTCl), pyridine, CH_2Cl_2; v, TBDMSOTf, pyridine

shift for both H-3′ (δ 4.35 for acid, 5.01 for lactone) and C-3′ (δ_C 74.62 for acid, 90.24 for lactone) in the ^{1}H and ^{13}C NMR spectra respectively.

NMR studies of uridine 2′-*C*,3′-*O*-γ-butyrolactone

The structure of the lactone **5a** was determined unambiguously using information acquired from a series of nuclear Overhauser enhancement (NOE) experiments together with a detailed analysis of the ^{1}H NMR spectrum (Fig. 1).

The resonance frequencies of all resolved peaks were systematically saturated to produce the following NOE enhancements. Irradiation of H-1′ enhanced the signal for H-2′ (4%), H-4′ (2%), H-6 of the base (3%) and H-6″ of the lactone (6%). Similarly, irradiation of H-2′ produced an enhancement of the signal for H-1′ (5%), H-3′ (6%) and H-6 (9%). Irradiation of H-3′ produced enhancements of the signals for H-4′ (2%) and H-2′ (6%). The chemical shifts for H-5′ and H-5″ were degenerate hence irradiation at this frequency could not provide 'quantitative' information but did support all other observations. Signal enhancements were detected at the chemical shift of H-6 (2%), H-3′ (4%) and H-4′ (7%). These data clearly demonstrate that H-2′ and H-3′ are on the upper face of the furanose ring and hence support the *cis* fusion between the γ-lactone and the sugar moiety. Unambiguous chemical-shift assignments could also be made for the lactone protons on the basis of NOEs (Table 1).

In order to determine the conformation of the sugar and lactone rings, the ^{1}H NMR spectrum was completely analysed *via* iterative spectral simulation using PANIC 86[13] (see Table 1); even at 500 MHz, second-order behaviour was observed. From this, accurate values for vicinal couplings could be determined for use in a Karplus-type approach to the estimation of dihedral angles. The method described by Haasnoot *et al.*[14] who

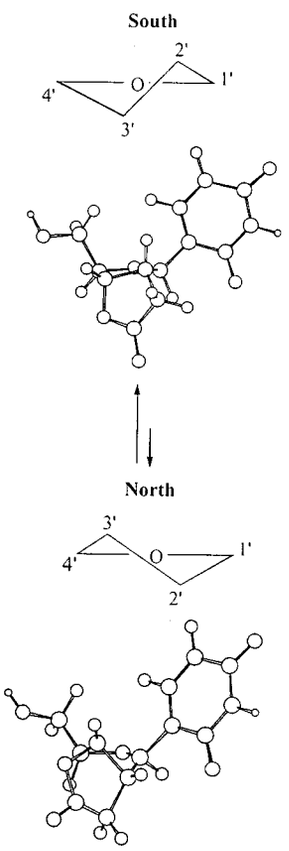


Fig. 1 Ball-and-stick representations of the conformational equilibrium of compound **5a**. The structures were generated in Chem3d based on dihedral angles and pseudorotational parameters calculated from experimental coupling constants. The structures were energy-minimised using the MM2 package within Chem3d. The south conformer has been shown to predominate in solution.

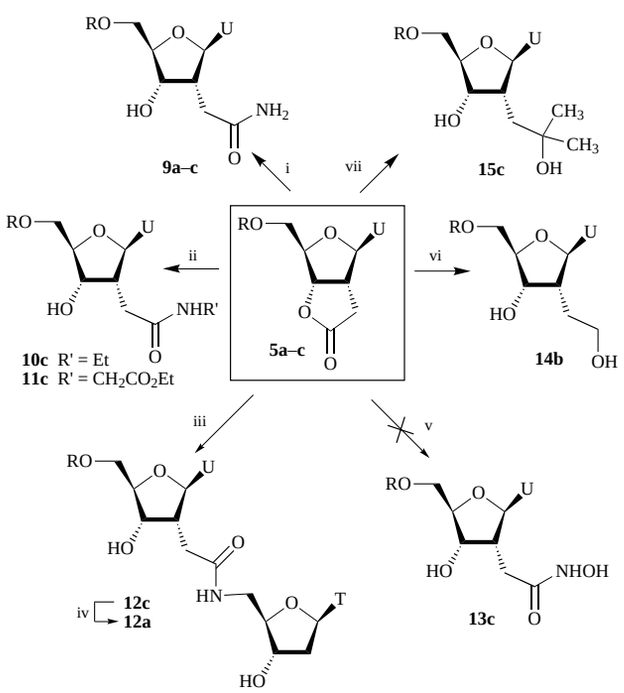
adopted a generalised Karplus equation modified to take account of substituent orientation and electronegativity, for substituents both α and β to the N–C–C–H–C–C bond, was followed. The dihedral angles thus calculated are also shown in Table 1. Knowledge of dihedral-angle constraints for the furanose ring enabled us to determine conformationally averaged structural descriptors, *i.e.* the pseudorotation phase angle, $\boldsymbol{P}$ ($\boldsymbol{P}_{av}$ = 185°) and the puckering amplitude, φ_m [$\varphi_{m(av)}$ = 34°).[14,15] It is known that, in general, in solution, furanose rings exist as a $C_{3'\text{-}endo}$ (*N*)/$C_{2'\text{-}endo}$ (*S*) equilibrium of two (or more) rapidly interconverting conformers, each with its own pseudorotational phase angle and puckering amplitude. Such conformational flexibility means that the coupling constants measured, and subsequently dihedral angles, are time-averaged and linearly related to these quantities for each conformer. No attempt was made to determine the proportion of *S* and *N* conformers

present but a ***P***-value of 185° suggests that the dominant conformer is *S*. Fig. 1 shows ball-and-stick representations of the conformational equilibrium of lactone **5a**.

It is interesting to compare the structural data obtained for compound **5a** with that obtained by Velázquez *et al*.[6] for two related compounds (Table 1). It is not advisable to compare magnitudes of NOEs but the trends are informative and the same pattern of connections is seen for lactone **5a** as was observed for 1-[5′-*O*-(*tert*-butyldimethylsilyl)-2′-*C*-[(*R*)-1-carboxyethyl]-2′-deoxy-β-D-ribofuranosyl]uracil 2′,3′-γ-lactone **3a**, and the carboxy(phenyl)ethyl equivalent **3b**. In all three cases the 3′ and 2′ hydrogens are on the same, upper, face. There is also broad agreement in the derived dihedral angle and pseudorotational constraints for these compounds; the differences that are noted are likely to be due to the effects of the different substituents on the lactone. Velázquez *et al*. carried out an analysis of the conformational equilibrium for the carboxy(phenyl)ethyl derivative and established that the dominant conformation was *S*. Here again our work is in agreement.

Reactions of uridine 2′-*C*,3′-*O*-γ-butyrolactone 5a

Our interest in the reactions of the lactone **5a** was also stimulated by the work of Velázquez *et al*., which describes the synthesis of some 1″-alkyl nucleoside γ-butyrolactones (**3** and **4**) through a radical cyclisation. Their studies on the susceptibility of these lactones to ring-opening showed that they were resistant to aminolysis with dil. aq. ammonia, liquid ammonia or primary amines under standard conditions but could be opened by aluminium(III) chloride-promoted aminolysis with isobutylamine.[6] A similar lack of reactivity has also been described for the γ-butyrolactone nucleosides **2** and, in this case, formation of the amide with 5′-amino-5′-deoxy nucleosides was achieved only in the presence of 2-hydroxypyridine.[5] Both of these studies suggested that these nucleoside lactones react only under forcing conditions and therefore may not be generally useful synthetic intermediates. In contrast to these reports, lactone **5a** was opened within 10 min by treatment with 15% aq. ammonia at 0 °C to yield the amide **9a** quantitatively (Scheme 2).



a; R = H, **b**; R = DMT, **c**; R = TBDMS, T = thymin-1-yl, U = uracil-1-yl

Scheme 2 *Reagents and conditions:* i, **9a**: conc. aq. NH_3; **9b,c**: saturated NH_3–MeOH; ii, **10c**: 35% $EtNH_2$–EtOH; **11c**: $EtO_2CCH_2NH_2$·HCl, DMF–NEt_3 (5:1), 70 °C; iii, 5′-amino-5′-deoxythymidine, DMF, 80 °C; iv, NEt_3·3HF, THF; v, NH_2OH·HCl, NaOH, MeOH; vi, DIBAL-H, THF–$PhCH_3$, −78 °C ⟶ room temp.; vii, MeMgI, Et_2O–THF, 0 °C ⟶ reflux

In order to explore the reactions of the lactone more fully it was first converted into either the dimethoxytrityl (DMT) derivative **5b** or the *tert*-butyldimethylsilyl (TBDMS) derivative **5c** in yields of 92 and 70%, respectively. As expected, both compounds **5b** and **5c** could also be quantitatively converted into the corresponding primary amide, **9b** and **9c** respectively, with methanolic ammonia. The TBDMS-protected lactone **5c** was chosen for a more extensive investigation of ring-opening reactions of the lactone system. Treatment of compound **5c** with a solution of ethylamine in ethanol (33% w/v) for 10 min gave the ethylamide **10c** in 97% yield. The corresponding glycinyl derivative **11c** was prepared in 70% yield by heating the lactone with ethyl glycinate (5 mol equiv.) in dimethylformamide (DMF)–NEt_3 (5:1) at 70 °C for 16 h. The most interesting of the aminolyses involved opening of the lactone with 5′-amino-5′-deoxythymidine[16,17] to give a 2′ ⟶ 5′-amide-linked analogue of a dinucleoside monophosphate. Thus, reaction of the lactone with 5′-amino-5′-deoxythymidine (5 mol equiv.) in DMF at 80 °C for 2.5 h gave the pseudodimer **12c** in 70% yield. In contrast to previously reported and closely related reactions of the isomeric 2′-*O*,3′-*C*-lactone **2** with 5′-amino-5′-deoxyadenosine, formation of an amide was achieved in the absence of acylation promoters such as 2-hydroxypyridine. Attempts to open the lactone with NH_2OH·HCl in methanolic KOH were unsuccessful; no trace of the expected hydroxyamic acid **13c** was seen. Analysis of these reactions by TLC revealed that whilst the lactone was rapidly and quantitatively converted into a product with a lower R_f-value, after neutralisation (pH 5–6) with acetic acid, evaporation and chromatography, only the starting lactone was isolated. These observations are consistent with reversible lactone formation occurring possibly under mildly acidic conditions. We have previously noted that the amide **9a** undergoes slow lactonisation in solution, whilst Rosenthal and Baker[4] reported that a related dimethylamide was susceptible to thermal lactonisation at 60 °C. Since the relactonisation process is of considerable importance with regard to the stability of the amide-linked pseudodimers **12** and their suitability for use in antisense oligonucleotides, the lactonisation of both amides **9a** and **12a** (prepared in 50% yield by desilylation of compound **12c**) was investigated.

Solutions of the amide **9a** and the amide-linked pseudodimer **12a** were warmed in acetic acid–water (8:2) at 50 °C and the lactonisation process was followed by high-pressure liquid chromatography (HPLC). Under these conditions, the half-life for the lactonisation of compound **9a** was 115 min. In comparison, lactonisation of the pseudodimer **12a** was much slower (half-life 930 min), and when the compound was warmed to 50 °C in aq. citric acid buffer (pH 3.0) less than 2% lactonisation occurred over a period of 24 h. On the basis of these results the lactonisation of this linkage is not expected to be problematic with regard to its potential use as an antisense agent. Formation of duplexes with oligonucleotides containing this linkage is currently under investigation.

Reactions of the protected lactone with organometallic reagents were also investigated. Reduction of the DMT derivative **5b** to give the corresponding 2′-*C*-2-hydroxyethyl analogue **14b** was achieved in 70% yield by using an excess of diisobutylaluminium hydride (DIBAL) in tetrahydrofuran (THF) at −78 °C. This product was identical with that derived from aldehyde **6** by borohydride reduction, followed by subsequent desilylation and tritylation.[12] The tertiary alcohol **15c** was accessible in 50% yield by treatment of lactone **5c** with an excess of methylmagnesium iodide in refluxing THF. Both the ^1H and ^{13}C NMR spectra of compound **15c** showed characteristic singlets for the diastereotopic methyl groups.

Conclusions

Our studies show that the 2′-*C*,3′-*O*-lactone **5a** and its 5′-protected derivatives **5b** and **5c** readily undergo aminolyses to

yield a range of amide derivatives. The very low reactivity of the analogous 6′-alkyl nucleoside γ-butyrolactones **3**, under similar conditions, is almost certainly due to the alkyl substituent shielding the upper face of the lactone ring. The approach-vector analysis described by Baldwin[18] predicts that the alkyl substituent would substantially hinder the approach of the nucleophile. The poor reactivity of the 2′-*O*,3′-*C*-lactone **2** in comparison with the isomeric 2′-*C*,3′-*O*-lactone **5a** is more surprising, but may partly be the result of different furanoid conformations in the two compounds. The observation that the 5′-protected lactones also react with simple organometallic reagents demonstrates that these compounds are indeed versatile intermediates for the preparation of 2′-*C*-branched nucleoside analogues.

Experimental

FAB mass spectra were recorded on a VG Analytical 7070E mass spectrometer operating with a PDP 11/250 data system and an Ion Tech FAB ion gun working at 8 kV. High-resolution fast-atom bombardment (FAB) mass spectra were obtained on a VG ZAB/E spectrometer at the EPSRC Mass Spectrometry Service Centre (Swansea, UK). 3-Nitrobenzyl alcohol (3-NOBA) was used as a matrix unless stated otherwise. ^{1}H and ^{13}C NMR spectra were measured on either a Bruker AMX400, a Bruker AC200 or a Varian Gemini 300 spectrometer and chemical shifts are given in ppm downfield from an internal standard of tetramethylsilane (TMS). *J*-Values are in Hz. For structural studies on lactone **5a** ^{1}H NMR spectra were recorded on a GE Omega 500 and Bruker AM 400 operating at 500 MHz and 400 MHz, respectively, on samples prepared in dimethyl sulfoxide (DMSO) solution with TMS as internal reference. NOE experiments were performed on the Bruker AM 400 using a 90 degree pulse of 10.5 μs duration, recycle delay of 5 s and irradiation time of 6 s. Spectral simulations were performed using PANIC 86 available as part of the Bruker software suite. All other computations were carried out using either a PC486 or a Sun IPC Sparc station. The representation of the structure was performed using CHEM Office on a PC.

Analytical TLC was performed on silica gel-coated aluminium plates impregnated with a fluorescent indicator (254 nm). Nucleosides were visualised as a black spot by spraying with a solution of 5% (v/v) sulfuric acid and 3% (w/v) phenol in ethanol and charring at 120 °C. Flash column chromatogaphy was performed using silica gel 60 (230–400 mesh). HPLC was conducted using a Varian Star 9010 liquid chromatograph equipped with a Varian Star 9050 variable-wavelength UV detector recording at 260 nm for analytical purposes and 290 nm for preparative work. Unless stated otherwise analyses were performed on a Nucleosil C_{18} reversed-phase column, using a gradient of 0 ⟶ 25% MeCN in 50 mM aq. potassium phosphate (pH 6.5) over a period of 20 min, with a flow rate of 1 cm^3 min^{-1}. Data were recorded using a Varian 4400 recording integrator. Optical rotations ($[\alpha]_D$) were measured on an optical activity AA-100 polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}.

2′-Deoxy-2′-α-*C*-(carboxymethyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine 7

To a solution of 2′-deoxy-2′-*C*-(formylmethyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine **6** (10.32 g, 20.15 mmol) and 2-methylbut-2-ene (35 cm^3, 330 mmol) in Bu^{t}OH (300 cm^3) was added aq. $NaClO_2$ and KH_2PO_4 (27.45 g, 300 mmol and 30.18 g, 220 mmol, respectively, in 100 cm^3) and the mixture was stirred vigorously for 1 h. The organic solvents were removed *in vacuo* and the resultant residue was diluted with ethyl acetate (250 cm^3), washed successively with saturated aq. $NaHCO_3$ (2 × 200 cm^3), water (200 cm^3) and brine (200 cm^3), dried ($MgSO_4$), and evaporated under reduced pressure to yield the *carboxylic acid* as a foam (10.00 g, 94%), δ_H(200 MHz; $CDCl_3$) 1.01–1.08 (28 H, m, Pr^i), 2.49 (1 H, dd, *J* 12.1 and 19.8, H-6′), 2.78–2.84 (2 H, m, 2′- and 6′-H), 3.91 (1 H, m, H-5′), 4.05 (2 H, m, H-4′ and -5″), 4.53 (1 H, t, *J* 7.2, H-3′), 5.77 (1 H, d, *J* 8.8, H-5), 5.90 (1 H, d, *J* 2.5, H-1′), 7.66 (1 H, d, *J* 8.8, H-6) and 10.69 (1 H, br s, NH); δ_C(50.4 MHz; $CDCl_3$) 12.39–13.17 (4 × Me_2CH), 16.65–17.24 (8 × CH_3), 30.64 (C-6′), 45.30 (C-2′), 61.08 (C-5′), 69.21 (C-3′), 83.55 (C-4′), 88.30 (C-1′), 102.18 (C-5), 139.48 (C-6), 150.78 (C-2), 164.04 (C-4), 175.15 (C-7′) [Found: FAB HRMS, *m*/*z* (M + H)$^+$, 529.2393. $C_{23}H_{41}N_2O_8Si_2$ requires (M + H)$^+$, 529.2401].

Uridine 2′-deoxy-2′-α-*C*,3′-*O*-γ-butyrolactone 5a *via* 2′-deoxy-2′-α-*C*-(carboxymethyl)uridine triethylammonium salt 8

The TIPS-protected carboxymethyl nucleoside **7** (0.99 g, 1.87 mmol) was dissolved in dry THF (10 cm^3); to this was added NEt_3·3HF (0.62 cm^3, 20 mmol) and the solution was left for 16 h. The solution was concentrated *in vacuo*, diluted with methanol (20 cm^3) and evaporated onto silica gel; the product as its triethylammonium salt **8** was subsequently isolated by column chromatography (CH_2Cl_2 containing an increasing gradient of MeOH from 0–10%) as an oil (0.46 g, 64%), δ_H(400 MHz; CD_3OD) 1.30 (9 H, t, *J* 7.2, NCH_2CH_3), 2.31 (1 H, dd, $J_{6',2'}$ 5.8, $J_{6',6''}$ 15.5, H-6′), 2.62 (1 H, dd, $J_{6'',2'}$ 7.9, $J_{6'',6'}$ 15.5, H-6″), 2.70 (1 H, m, H-2′), 3.17 (6 H, q, *J* 7.2, NCH_2CH_3), 3.75 (2 H, d, $J_{5',4'}$ 3.5, 2 × H-5′), 4.00 (1 H, m, H-4′), 4.35 (1 H, m, H-3′), 5.73 (1 H, dd, *J* 2.2, $J_{5,6}$ 8.1, H-5), 6.04 (1 H, d, $J_{1',2'}$ 8.8, H-1′), 7.98 (1 H, d, H-6, $J_{6,5}$ 8.1); δ_C(50.4 MHz; CD_3OD) 9.34 (NCH_2CH_3), 33.10 (C-6′), 47.34 (C-2′), 47.70 (NCH_2CH_3), 63.85 (C-5′), 74.62 (C-3′), 89.00 (C-4′), 89.87 (C-1′), 103.27 (C-5), 143.07 (C-6), 152.81 (C-2), 166.24 (C-4), 178.59 (C-7′); *m*/*z* (FAB$^+$) 287 (M + H$^+$, 3.5%), 269 [M + H − H_2O^+ (lactone)]. HPLC retention time t_R 9.41 min. For conversion into lactone **5a**, the carboxymethyl nucleoside **8** (292 mg, 1.09 mmol) was dissolved in acetic acid–methanol (4:1; 29.2 cm^3) and the solution was heated at 60 °C for 3 h. The solution was evaporated *in vacuo* and the resultant residue was coevaporated with water (2 × 30 cm^3) and dried over P_2O_5 for 48 h. *Product* **5a** was isolated as a microcrystalline solid (273 mg, 96%), mp 221–222 °C; δ_C(100.6 MHz; [$^2H_6$]DMSO) 31.75 (C-6′) 44.76 (C-2′) 61.02 (C-5′), 84.08 (C-4′), 84.73 (C-1′), 90.24 (C-3′), 101.96 (C-5), 140.51 (C-6), 150.63 (C-2), 163.16 (C-4), 175.67 (C-7′); $[\alpha]_D^{22}$ +40 (*c* 0.01, MeOH); ν_{max}/cm^{-1} (KBr) 1780 (s, C=O [lactone]); *m*/*z* (FAB$^+$) 269 (M + H$^+$, 60.9%), 157 (M − uracil$^+$, 14.8) and 113 (uracil + 2H$^+$, 100) [Found: HRMS, *m*/*z* (M + H)$^+$, 269.0754. $C_{11}H_{13}N_2O_6$ requires (M + H)$^+$, 269.0774] (Found: C, 49.07; H, 4.55; N, 10.39. $C_{11}H_{12}N_2O_6$ requires C, 49.26; H, 4.51; N, 10.44%); HPLC t_R 15.8 min.

5′-*O*-Dimethoxytrityluridine 2′-deoxy-2′-α-*C*,3′-*O*-γ-butyrolactone§ 5b

The lactone **5a** (1.0 g, 3.73 mmol) was dried by coevaporation with dry pyridine (2 × 10 cm^3), then was dissolved in the same solvent (10 cm^3). 4,4′-Dimethoxytrityl chloride (DMTCl) (2.21 g, 6.52 mmol) was dissolved in a dry mixture of pyridine and dichloromethane (1.8 cm^3 and 6.8 cm^3, respectively) and added to the nucleoside solution dropwise over a period of 30 min. After stirring of the mixture for 1 h, methanol (0.1 cm^3) was added and after a further 15 min saturated aq. $NaHCO_3$ (0.5 cm^3) was also added; the mixture was then concentrated *in vacuo* to give a thick oil. The residue was coevaporated with toluene (2 × 20 cm^3), diluted with CH_2Cl_2 (20 cm^3) and washed successively with saturated aq. $NaHCO_3$ (2 × 20 cm^3) and brine (25 cm^3), the aqueous layers were combined and back-extracted with CH_2Cl_2 (5 cm^3), and the organics were dried ($MgSO_4$) and evaporated. The product was isolated by column chromatography (CH_2Cl_2 containing an increasing gradient of MeOH

§ *Systematic name:* 2,3-dihydro-[2′,3′-dideoxy-5′-*O*-(4,4′-dimethoxytrityl)uridino][2′,3′-*b*]furan-5(4*H*)-one.

from 0 to 3%) to yield the *required DMT derivative* **5b** as a foam (1.95 g, 92%), δ_H(400 MHz; $CDCl_3$) 2.92 (1 H, dd, $J_{6',2'}$ 8.4, $J_{6',6''}$ 18.5, H-6′), 2.99 (1 H, dd, $J_{6'',2'}$ 2.3, $J_{6'',6'}$ 18.5, H-6″), 3.16 (1 H, m, H-2′), 3.48 (1 H, dd, $J_{5',4'}$ 3.0, $J_{5',5''}$ 10.8, H-5′), 3.55 (1 H, dd, $J_{5'',4'}$ 2.8, $J_{5'',5'}$ 10.8, H-5″), 3.80 (6 H, s, OCH_3), 4.49 (1 H, m, H-4′), 5.12 (1 H, dd, *J* 1.4 and 7.0, H-3′), 5.45 (1 H, d, $J_{5,6}$ 8.1, H-5), 5.97 (1 H, d, $J_{1',2'}$ 6.3, H-1′), 6.85 (4 H, d, *J* 8.9, ArH *o* to OCH_3), 7.23–7.35 (9 H, m, ArH) and 7.70 (1 H, d, $J_{6,5}$ 8.1, H-6); δ_C(100.6 MHz; CD_3OD) 32.66 (C-6′), 47.42 (C-2′), 55.90 (OCH_3), 63.89 (C-5′), 84.85 (C-4′), 85.21 (C-1′), 87.89 ($OCAr_3$), 92.03 (C-3′), 103.30 (C-5), 114.00 (ArC), 127.94 (ArC), 128.55 (ArC), 128.71 (ArC), 130.60 (ArC), 135.40 (ArC), 135.47 (ArC), 140.02 (C-6), 144.54 (ArC), 151.12 (C-2), 159.44 (ArC), 163.82 (C-4) and 175.06 (C-7′); *m*/*z* (FAB^+) 571 ($M + H^+$, 2.6%), 570 (M^+, 3.3) and 303 (DMT^+, 100) [Found: FAB HRMS, *m*/*z* ($M + H^+$), 571.2055. $C_{32}H_{31}N_2O_8$ requires (M + H), 571.2080].

5′-*O*-(*tert*-Butyldimethylsilyl)uridine 2′-deoxy-2′-α-*C*,3′-*O*-γ-butyrolactone ¶ 5c

Lactone **5a** (1.10 g, 4.10 mmol) was dried by coevaporation with dry pyridine (2 × 10 cm^3) then was dissolved in the same solvent (20 cm^3). *tert*-Butyldimethylsilyl trifluoromethanesulfonate (TBDMSOTf) (2.8 cm^3, 12.30 mmol) was added to the nucleoside solution and the mixture was stirred for 1 h. The reaction mixture was quenched by the addition of water (1 cm^3), left for 5 min, diluted with CH_2Cl_2 (40 cm^3), washed successively with saturated aq. $NaHCO_3$ (2 × 20 cm^3) and brine (20 cm^3), dried ($MgSO_4$), then was concentrated *in vacuo* to afford a thick oil. The residue was coevaporated with toluene (3 × 20 cm^3) and the *pure product* **5c** was obtained by column chromatography [elution initially with hexane–CH_2Cl_2 (50:50) followed by CH_2Cl_2 containing an increasing gradient of MeOH from 0 to 1%] as a foam (1.10 g, 70%), δ_H(400 MHz; $CDCl_3$) 0.10 (6 H, s, $SiCH_3$), 0.89 (9 H, s, Bu^t), 3.02 (1 H, dd, $J_{6',2'}$ 8.9, $J_{6',6''}$ 18.6, H-6′), 2.97–3.04 (2 H, m, H-2′ and -6″), 3.88 (1 H, dd, $J_{5',4'}$ 2.2, $J_{5',5''}$ 11.5, H-5′), 3.98 (1 H, dd, $J_{5'',4'}$ 2.1, $J_{5'',5'}$ 11.5, H-5″), 4.50 (1 H, m, H-4′), 5.02 (1 H, dd, *J* 1.0 and 6.8, H-3′), 5.72 (1 H, d, $J_{5,6}$ 8.2, H-5), 5.95 (1 H, d, $J_{1',2'}$ 6.0, H-1′), 7.81 (1 H, d, $J_{6,5}$ 8.2, H-6) and 10.35 (1 H, br s, NH); δ_C(100.6 MHz; $CDCl_3$) −5.79 ($SiCH_3$), 17.97 [Si*C*(CH_3)$_3$], 25.58 [C(*C*H_3)$_3$], 31.93 (C-6′), 47.18 (C-2′), 63.38 (C-5′), 84.33 (C-4′), 85.65 (C-1′), 91.86 (C-3′), 102.12 (C-5), 139.27 (C-6), 150.51 (C-2), 163.58 (C-4) and 174.54 (C-7′); *m*/*z* (FAB^+) 383 ($M + H^+$, 0.6%), 271 (M − uracil$^+$, 2.6), 115 ($TBDMS^+$, 8.0) and 73 (Me_3Si^+, 100); (FAB^-) 535 (M + 3-NOBA$^-$, 13.5%), 381 ($M - H^-$, 100) and 111 (uracil$^-$, 57.5) [Found: FAB HRMS, *m*/*z* ($M + H^+$), 383.1635. $C_{17}H_{27}N_2O_6Si$ requires ($M + H^+$), 383.1638].

2′-α-*C*-Carbamoylmethyl-2′-deoxyuridine 9a

Lactone **5a** (35 mg, 0.13 mmol) was dissolved in cooled (0 °C) 15% (w/v) aq. ammonia (3.5 cm^3). After 10 min the water and excess of ammonia were removed *in vacuo* and the residue was coevaporated with water (10 cm^3) to yield the *required amide* **9a** as an oil (37 mg, 100%), δ_H(400 MHz; [$^2H_6$]DMSO) 2.10 (1 H, dd, $J_{6',2'}$ 6.1, $J_{6',6''}$ 15.7, H-6′), 2.43 (1 H, dd, $J_{6'',2'}$ 7.7, $J_{6'',6'}$ 15.7, H-6″), 2.58 (1 H, m, H-2′), 3.55 (2 H, d, $J_{5',4'}$ 3.8, 2 × H-5′), 3.85 (1 H, dd, $J_{4',3'}$ 3.1, $J_{4',5'}$ 3.8, H-4′), 4.15 (1 H, m, H-3′), 5.11 (1 H, br s, OH), 5.35 (1 H, br s, OH), 5.67 (1 H, d, $J_{5,6}$ 8.2, H-5), 5.91 (1 H, d, $J_{1',2'}$ 9.4, H-1′), 6.82 (1 H, s, NH amide), 7.34 (1 H, s, NH amide) and 7.84 (1 H, d, $J_{6,5}$ 8.2, H-6); δ_C(100.6 MHz; CD_3OD) 33.68 (C-6′), 46.87 (C-2′), 63.79 (C-5′), 74.56 (C-3′), 89.28 (C-4′), 89.68 (C-1′), 103.46 (C-5), 142.96 (C-6), 152.95 (C-2), 166.36 (C-4) and 176.80 (C-7′) [Found: FAB HRMS, *m*/*z* $(M + H)^+$, 286.1031. $C_{11}H_{16}N_3O_6$ requires $(M + H)^+$, 286.1039]; HPLC t_R 9.48 min.

¶ *Systematic name:* 2,3-dihydro-[5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxyuridino][2′,3′-*b*]furan-5(4*H*)-one.

2′-α-*C*-Carbamoylmethyl-2′-deoxy-5′-*O*-(dimethoxytrityl)-uridine 9b

Lactone **5b** (1.13 g, 1.98 mmol) was dissolved in saturated methanolic ammonia (113 cm^3) and left for 8 h. The solution was evaporated *in vacuo* and the residue was coevaporated with methanol (2 × 50 cm^3) to yield the *pure amide* **9b** as a foam (1.16 g, 100%), δ_H(400 MHz; $CDCl_3$) 2.56 (1 H, m, H-6′), 2.69 (2 H, m, H-2′ and -6″), 3.37 (2 H, m, 2 × H-5′), 3.74 (6 H, s, OCH_3), 4.15 (1 H, m, H-4′), 4.46 (1 H, m, H-3′), 5.40 (1 H, d, $J_{5,6}$ 8.1, H-5), 6.07 (1 H, d, $J_{1',2'}$ 7.6, H-1′), 6.58 (1 H, br s, NH amide), 6.81 (4 H, d, *J* 8.8, ArH *o* to OCH_3), 6.93 (1 H, br s, NH amide), 7.17–7.39 (9 H, m, ArH), 7.65 (1 H, d, $J_{6,5}$ 8.1, H-6) and 8.34 (1 H, br s, NH uracil); δ_C(100.6 MHz; CD_3OD) 31.83 (C-6′), 47.40 (C-2′), 55.89 (OCH_3), 64.47 (C-5′), 74.06 (C-3′), 86.59 (C-4′), 87.49 ($OCAr_3$), 88.82 (C-1′), 103.57 (C-5), 112.67 (ArC), 113.95 (ArC), 127.71 (ArC), 128.65 (ArC), 128.82 (ArC), 130.76 (ArC), 135.96 (ArC), 136.14 (ArC), 141.02 (C-6), 144.98 (ArC), 152.36 (C-2), 159.26 (ArC), 164.45 (C-4) and 175.74 (C-7′) [Found: FAB HRMS, *m*/*z* $(M + H)^+$, 588.2330. $C_{32}H_{34}N_3O_8$ requires $(M + H)^+$, 588.2346].

5′-*O*-(*tert*-Butyldimethylsilyl)-2′-α-*C*-carbamoyl-2′-deoxy-uridine 9c

Lactone **5c** (10 mg, 0.03 mmol) was dissolved in saturated methanolic ammonia (1 cm^3) and left for 8 h. The solution was evaporated *in vacuo* and the residue was coevaporated with methanol to yield the *pure amide* **9c** as a foam (10 mg, 100%), δ_H(400 MHz; $CDCl_3$) 0.10 (6 H, s, $SiCH_3$), 0.91 (9 H, s, *t*-Bu), 2.57–2.69 (3 H, m, H-2′, and 2 × H-6′), 3.81 (1 H, dd, $J_{5',4'}$ 2.4, $J_{5',5''}$ 11.4, H-5′), 3.89 (1 H, dd, $J_{5'',4'}$ 2.3, $J_{5'',5'}$ 11.4, H-5″), 4.13 (1 H, m, H-4′), 4.39 (1 H, m, H-3′), 5.68 (1 H, d, $J_{5,6}$ 8.2, H-5), 6.07 (1 H, d, $J_{1',2'}$ 7.6, H-1′), 6.89 (1 H, br s, NH amide), 7.27 (1 H, br s, NH amide) and 7.89 (1 H, d, $J_{6,5}$ 8.2, H-6); δ_C(75.5 MHz; $CDCl_3$) −5.68 ($SiCH_3$), 18.23 [Si*C*(CH_3)$_3$], 25.80 [C(*C*H_3)$_3$], 31.11 (C-6′), 47.59 (C-2′), 63.76 (C-5′), 73.47 (C-3′), 87.32 (C-4′), 88.59 (C-1′), 102.92 (C-5), 140.46 (C-6), 151.72 (C-2), 163.68 (C-4) and 175.05 (C-7′); *m*/*z* (FAB^-) 797 (2M − H^-, 7.8%), 552 (M + 3-NOBA$^-$, 23.1), 398 ($M - H^-$, 52.3) and 111 (uracil$^-$, 100) [Found: FAB HRMS, *m*/*z* ($M + H^+$), 400.1913. $C_{17}H_{30}N_3O_6Si$ requires ($M + H^+$), 400.1904].

5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-2′-α-*C*-(*N*-ethyl-carbamoylmethyl)uridine 10c

Lactone **5c** (104 mg, 0.27 mmol) was dissolved in a 33% (w/v) solution of ethylamine in ethanol (10 cm^3) and left for 10 min. The solution was evaporated *in vacuo* and the residue was coevaporated twice with chloroform (2 × 25 cm^3). The residue was taken up into CH_2Cl_2 (25 cm^3) and the solution was washed with water (2 × 25 cm^3) and brine (25 cm^3), dried ($MgSO_4$), and the solvent was removed *in vacuo* to yield the *amide* **10c** as a pale brown foam (111 mg, 97%), δ_H(400 MHz; $CDCl_3$) 0.10 (6 H, s, $SiCH_3$), 0.91 (9 H, s, *t*-Bu), 1.14 [3 H, t, $J_{(CH_3,CH_2)}$ 7.3 CH_3], 2.38 (1 H, m, H-2′), 2.59 (1 H, dd, $J_{6',2'}$ 3.9, $J_{6',6''}$ 14.6, H-6′), 2.68 (1 H, dd, $J_{6'',2'}$ 10.4, $J_{6'',6'}$ 14.6, H-6″) 3.28 [2 H, dq, $J_{(CH_2,NH)}$ 5.6, $J_{(CH_2,CH_3)}$ 7.3, CH_2], 3.85 (1 H, dd, $J_{5',4'}$ 2.1, $J_{5',5''}$ 11.4, H-5′), 3.93 (1 H, dd, $J_{5'',4'}$ 2.1, $J_{5'',5'}$ 11.4, H-5″), 4.18 (1 H, m, H-4′), 4.42 (1 H, dd, *J* 2.4 and 6.6, H-3′), 5.67 (1 H, d, $J_{5,6}$ 8.1, H-5), 6.01 (1 H, d, $J_{1',2'}$ 7.8, H-1′), 6.92 [1 H, t, $J_{(NH,CH_2)}$ 5.6, NH amide] and 7.97 (1 H, d, $J_{6,5}$ 8.1, H-6); δ_C(75.5 MHz; $CDCl_3$) −5.77 ($SiCH_3$), 14.26 (NCH_2CH_3), 18.15 [Si*C*(CH_3)$_3$], 25.74 [C(*C*H_3)$_3$], 31.84 (C-6′), 34.14 (NCH_2CH_3), 48.75 (C-2′), 63.76 (C-5′), 73.04 (C-3′), 87.22 (C-4′), 88.80 (C-1′), 102.61 (C-5), 140.62 (C-6), 151.69 (C-2), 163.68 (C-4) and 172.29 (C-7′); *m*/*z* (FAB^+) 428 ($M + H^+$, 19.8%), 316 (M − uracil$^+$, 7.3), 115 ($TBDMS^+$, 25.3) and 73 (Me_3Si^+, 100) [Found: FAB HRMS, *m*/*z* $(M + H)^+$, 428.2219. $C_{19}H_{34}N_3O_6Si$ requires (M + H), 428.2217; Found: C, 53.66; H, 7.84; N, 9.75. $C_{19}H_{33}N_3O_6Si$ requires C, 53.37; H, 7.78; N, 9.83%].

5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-2′-α-*C*-[*N*-(ethoxycarbonylmethyl)carbamoylmethyl]uridine 11c

To a solution of the lactone **5c** (47 mg, 0.12 mmol) in dry DMF–NEt_3 (5:1; 0.6 cm^3) was added glycine ethyl ester (84 mg, 0.60 mmol) and the vigorously stirred mixture was heated at 70 °C. After 16 h the reaction mixture was allowed to cool to room temperature, diluted with CH_2Cl_2 (30 cm^3) and extracted successively with water (4 × 30 cm^3) followed by brine (30 cm^3); the organic phase was dried ($MgSO_4$), and then concentrated *in vacuo* to give a thick oil. The required *nucleoside–amino acid conjugate* **11c** was obtained following column chromatography (elution with CH_2Cl_2 containing an increasing gradient of MeOH from 0 to 2%) as an oil (42 mg, 70%), δ_H(400 MHz; $CDCl_3$) 0.11 (6 H, s, $SiCH_3$), 0.91 (9 H, s, *t*-Bu), 1.26 (3 H, t, *J* 7.2, CH_2CH_3), 2.50 (1 H, m, H-2′), 2.57 (1 H, dd, $J_{6',2'}$ 4.4, $J_{6',6''}$ 14.4, H-6′), 2.71 (1 H, dd, $J_{6'',2'}$ 10.8, $J_{6'',6'}$ 14.4, H-6″), 3.81–3.94 (3 H, m, CH and H_2-5′), 4.11–4.24 (4 H, m, CH, H-4′ and CH_2CH_3), 4.46 (1 H, m, H-3′), 5.70 (1 H, d, $J_{5,6}$ 8.2, H-5), 6.08 (1 H, d, $J_{1',2'}$ 8.3, H-1′), 7.44 (1 H, t, *J* 5.5, NH amide) and 7.93 (1 H, d, $J_{6,5}$ 8.2, H-6); δ_C(75.5 MHz; $CDCl_3$) −5.72 ($SiCH_3$), 13.93 (CH_2CH_3), 18.20 [$SiC(CH_3)_3$], 25.76 [$C(CH_3)_3$], 31.53 (C-6′), 41.38 ($NCH_2C{=}O$), 48.22 (C-2′), 61.65 (CH_2CH_3), 63.77 (C-5′), 73.08 (C-3′), 87.18 (C-4′), 88.44 (C-1′), 102.83 (C-5), 140.38 (C-6), 151.27 (C-2), 163.44 (C-4), 170.50 (C=O) and 172.58 (C-7′) [Found: HRMS, *m/z* $(M + H)^+$, 486.2270. $C_{21}H_{36}N_3O_8Si$ requires $(M + H)^+$, 486.2270].

5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-2′-α-*C*-[*N*-(5′-deoxythymidin-5′-yl)carbamoylmethyl]uridine 12c

5′-Amino-5′-deoxythymidine[16,17] (318 mg, 1.31 mmol) was suspended in a solution of lactone **5c** (100 mg, 0.26 mmol) in DMF (1.5 cm^3) and the mixture was heated to 80 °C. After 2.5 h the mixture was diluted with CH_2Cl_2 and evaporated onto silica. The required *compound* **12c** was isolated following column chromatography (elution with CH_2Cl_2 containing an increasing gradient of MeOH from 0 to 6%) as a powder (114 mg, 70%), δ_H(400 MHz; CD_3OD) 0.14 (6 H, s, $SiCH_3$), 0.95 (9 H, s, *t*-Bu), 1.91 (3 H, s, CH_3), 2.25 (2 H, m, H_2-2′T), 2.41 (1 H, dd, $J_{6',2'}$ 7.3, $J_{6',6''}$ 15.3, H-6′U), 2.63 (1 H, dd, $J_{6'',2'}$ 7.0, $J_{6'',6'}$ 15.3, H-6″U), 2.75 (1 H, m, H-2′U), 3.37 (1 H, dd, $J_{5',4'}$ 4.7, $J_{5',5''}$ 14.1, H-5′T), 3.42 (1 H, dd, $J_{5'',4'}$ 6.7, $J_{5'',5'}$ 14.1, H-5″T), 3.87 (3 H, m, H-4′T and H_2-5′U), 4.03 (1 H, m, H-4′U), 4.22 (1 H, m, H-3′T), 4.29 (1 H, m, H-3′U), 5.65 (1 H, d, $J_{5,6}$ 8.1, H-5U), 6.17 (1 H, t, $J_{1',2'}$ 6.8, H-1′T), 6.09 (1 H, d, $J_{1',2'}$ 8.9, H-1′U), 7.54 (1 H, d, *J* 1.2, H-6T) and 7.93 (1 H, d, $J_{6,5}$ 8.1, H-6U); δ_C(101.6 MHz; CD_3OD) −5.37 ($SiCH_3$), 12.53 (CH_3 T), 19.28 [$SiC(CH_3)_3$], 26.50 [$C(CH_3)_3$], 32.22 (C-6′U), 40.01 (C-2′T), 40.98 (C-5′T), 47.28 (C-2′U), 65.26 (C-5′U), 72.84 (C-3′), 74.77 (C-3′), 86.70 (C-4′), 86.84 (C-4′), 88.91 (C-1′), 89.56 (C-1′), 103.15 (C-5U), 111.80 (C-5T), 138.37 (C-6), 142.29 (C-6), 152.35 (C-2), 152.54 (C-2), 165.96 (C-4), 166.47 (C-4) and 173.81 (C-7′U) [Found: HRMS, *m/z* $(M + H)^+$, 624.2689. $C_{27}H_{42}N_5O_{10}Si$ requires $(M + H)^+$, 624.2701].

2′-Deoxy-2′-α-*C*-[*N*-(5′-deoxythymidin-5′-yl)carbamoylmethyl]uridine 12a

The TBDMS-protected dimer **12c** (55 mg, 0.088 mmol) was dissolved in dry THF (0.5 cm^3) and NEt_3·3HF (0.144 cm^3) was added. After 6 h at room temperature the solution was evaporated onto silica gel and the *required compound* **12a** was isolated following column chromatography (CH_2Cl_2 containing an increasing gradient of MeOH from 0 to 9%) as a foam (25 mg, 55%), δ_H(400 MHz; CD_3OD) 1.87 (3 H, s, CH_3), 2.21 (2 H, m, H_2-2′T), 2.36 (1 H, dd, $J_{6',2'}$ 7.6, $J_{6',6''}$ 15.3, H-6′U), 2.59 (1 H, dd, $J_{6'',2'}$ 7.0, $J_{6'',6'}$ 15.3, H-6″U), 2.73 (1 H, m, H-2′U), 3.32 (1 H, dd, $J_{5',4'}$ 5.1, $J_{5',5''}$ 14.0, H-5′T), 3.39 (1 H, dd, $J_{5'',4'}$ 6.4, $J_{5'',5'}$ 14.0, H-5″T), 3.70 (2 H, m, H_2-5′U), 3.82 (1 H, m, H-4′T), 3.94 (1 H, m, H-4′U), 4.18 (1 H, m, H-3′T), 4.26 (1 H, m, H-3′U), 5.65 (1 H, d, $J_{5,6}$ 7.8, H-5U), 6.03 (1 H, d, $J_{1',2'}$ 8.9, H-1′U), 6.12 (1 H, t, $J_{1',2'}$ 7.0, H-1′T), 7.48 (1 H, s, H-6T) and 7.91 (1 H, d, $J_{6,5}$ 7.8, H-6U); δ_C(101.6 MHz; CD_3OD) 13.32 (CH_3 T), 33.31 (C-6′U), 40.99 (C-2′T), 43.38 (C-5′T), 47.62 (C-2′U), 64.44 (C-5′U), 73.92 (C3′), 75.38 (C-3′), 87.41 (C-4′), 88.01 (C-4′), 89.86 (C-1′), 90.59 (C-1′), 104.25 (C-5U), 112.78 (C-5T), 139.30 (C-6), 143.46 (C-6), 153.30 (C-2), 153.61 (C-2), 166.91 (C-4), 167.36 (C-4) and 174.97 (C-7′U); *m/z* (FAB^+) 510 ($M + H^+$, 4.83%) and 398 ($M -$ uracil$^+$, 3.3) [Found: HRMS, *m/z* $(M + H)^+$, 510.1829. $C_{21}H_{28}N_5O_{10}$ requires (M + H), 510.1836]; HPLC t_R 17.44 min.

2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2′-α-*C*-(2-hydroxyethyl)uridine 14b

To a stirred suspension of lactone **5b** (207 mg, 0.36 mmol) in dry THF (7 cm^3) at −78 °C was added a 1 M solution of lithium diisobutylaluminium hydride in toluene (1.10 cm^3) and the reaction mixture was allowed to warm to room temperature. After 30 min the mixture was cooled to 0 °C and saturated aq. potassium sodium (+)-tartrate (5 cm^3) was added dropwise. The mixture was extracted with CH_2Cl_2 (3 × 10 cm^3) and the organic layers were then washed successively with saturated aq. $NaHCO_3$ (2 × 10 cm^3) and brine (10 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. The required compound **14b** was isolated following column chromatography (CH_2Cl_2 containing an increasing gradient of MeOH from 0 to 3%) as a yellow foam (146 mg, 70%), δ_H(200 MHz; $CDCl_3$) 1.67 (1 H, m, H-6′), 2.03 (1 H, m, H-6″), 2.39 (1 H, m, H-2′), 3.43 (2 H, m, H_2-7′), 3.76–3.91 (5 H, m, OCH_3 and H_2-5′), 4.16 (1 H, m, H-4′), 4.50 (1 H, m, H-3′), 5.37 (1 H, d, $J_{5,6}$ 8.3, H-5), 6.06 (1 H, d, $J_{1',2'}$ 7.7, H-1′), 6.78–6.85 (4 H, m, ArH *o* to OCH_3), 7.25–7.39 (9 H, m, ArH) and 7.76 (1 H, d, $J_{6,5}$ 8.3, H-6); δ_C(75.5 MHz; $CDCl_3$) 26.48 (C-6′), 48.96 (C-2′), 55.22 (OCH_3), 61.10 (C-7′), 63.77 (C-5′),72.81 (C-3′), 85.24 (C-4′), 86.98 ($OCAr_3$), 88.41 (C-1′), 102.53 (C-5), 113.15 (ArC), 127.74 (ArC), 127.97 (ArC), 128.13 (ArC), 130.07 (ArC), 135.25 (ArC), 135.41 (ArC), 139.46 (ArC), 140.42 (C-6), 144.33 (ArC), 150.86 (C-2), 158.65 (ArC) and 163.31 (C-4); *m/z* (FAB^+) 575 ($M + H^+$, 0.4%), 574 (M^+, 0.6) and 303 (DMT^+, 100) [Found: FAB HRMS, *m/z* $(M + H)^+$, 575.2378. $C_{32}H_{35}N_2O_8$ requires (M + H), 575.2393. Found: FAB HRMS, *m/z* M^+, 574.2317. $C_{32}H_{34}N_2O_8$ requires M, 574.2315].

5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-2′-α-*C*-(2-hydroxy-2-methylpropyl)uridine 15c

Magnesium turnings (80 mg, 3.33 mmol) were suspended in dry diethyl ether (1 cm^3) and the solvent and apparatus were thoroughly flushed with N_2. Methyl iodide (0.23 cm^3, 1.36 mmol) was dissolved in dry diethyl ether (2 cm^3) and this solution was added dropwise to the suspension of magnesium; the mixture was left for a further 30 min, then cooled to 0 °C. Lactone **5c** (100 mg, 0.26 mmol), as a solution in dry THF (7 cm^3), was added dropwise to the Grignard reagent and the mixture was then heated at reflux for 1 h. The reaction mixture was poured onto ice–water (20 cm^3) and the aqueous mixture was extracted with CH_2Cl_2 (2 × 20 cm^3). The combined organic fractions were subsequently washed successively with water (20 cm^3), 10% aq. $Na_2S_2O_3$ (20 cm^3), saturated aq. $NaHCO_3$ (20 cm^3) and brine (20 cm^3), dried ($MgSO_4$), and evaporated *in vacuo* to give an oil. The desired product **15c** was obtained following purification by column chromatography (CH_2Cl_2 containing an increasing gradient of MeOH from 0 to 2%) as a foam (54 mg, 50%), δ_H(200 MHz; $CDCl_3$) 0.10 (6 H, s, $SiCH_3$), 0.91 (9 H, s, *t*-Bu), 1.26 (3 H, s, CH_3), 1.34 (3 H, s, CH_3), 1.43 (1 H, dd, $J_{6',2'}$ 3.9, $J_{6',6''}$ 14.7, H-6′), 2.05 (1 H, dd, $J_{6'',2'}$ 12.3, $J_{6'',6'}$ 14.7, H-6″), 2.28 (1 H, m, H-2′), 3.82 (1 H, dd, $J_{5',4'}$ 1.9, $J_{5',5''}$ 11.3, H-5′), 3.93 (1 H, dd, $J_{5'',4'}$ 2.1, $J_{5'',5'}$ 11.3, H-5″), 4.17 (1 H, m, H-4′), 4.45 (1 H, m, H-3′), 5.71 (1 H, d, $J_{5,6}$ 8.2, H-5), 6.08 (1 H, d, $J_{1',2'}$ 8.7, H-1′), 7.99 (1 H, d, $J_{6,5}$ 8.2, H-6) and 8.98 (1 H, br s, NH); δ_C(75.5 MHz; $CDCl_3$) −5.70 ($SiCH_3$), 18.16 [$SiC(CH_3)_3$], 25.76 [$C(CH_3)_3$], 27.61 (CH_3), 32.41 (CH_3), 36.05 (C-6′), 47.67 (C-2′), 64.51 (C-5′), 71.30 (C-7′), 73.49 (C-3′), 86.41 (C-4′), 88.90

(C-1′), 102.73 (C-5), 140.63 (C-6), 150.87 (C-2) and 163.45 (C-4) [Found: HRMS, *m*/*z* $(M - OH)^+$, 397.2180. $C_{19}H_{33}N_2O_5Si$ requires (M − OH), 397.2159].

Lactonisation studies

Solutions of amides **9a** and **12a** (0.2 mg cm^{-3}) were prepared in acetic acid–water (4:1) or 40 mM citrate buffer (pH 3.0). Reaction mixtures were incubated at temperatures described in the Results and discussion section and were analysed at appropriate time intervals by HPLC. Components of the reaction mixtures had the following retention times: **5a**, 15.8 min; **9a**, 9.48 min; **12a**, 17.4 min and 5′-amino-5′-deoxythymidine, 10.05 min.

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