Conformationally Restricted Spin Labelled Nucleotides: a Model Study of the Synthesis and Properties of the 2',3'-*O*-Spiro Ketal of Uridine and 4-oxo-2,2,6,6-tetramethyl-1-piperidyloxy

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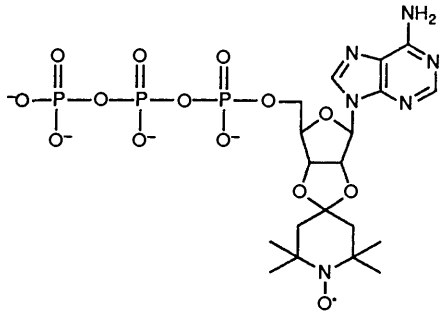
The synthesis of 2',3'-*O*-(1-oxy-2,2,6,6-tetramethyl-4-piperidylidene)uridine **13** is described in a model study directed at the corresponding spin labelled spiro ketal derivative of ATP (adenosine triphosphate) **1** and of other nucleotides. The synthesis proceeds *via* acid-catalysed addition of 5'-*O*-benzoyluridine to 1-acetoxy-4-methoxy-2,2,6,6-tetramethyl-1,2,5,6-tetrahydropyridine **9**. The spiro ketal **11** from this reaction exists as two slowly interconverting conformers at room temperature. Prolonged alkaline hydrolysis and concomitant aerial oxidation gives the required product **13**.

Spin labelled compounds have been used to address a variety of problems in biological research. For example, in organised systems such as muscle, electron paramagnetic resonance (EPR) has given information about relative orientations and movements of components,[1] while in structural studies using NMR of macromolecules such as proteins[2] and nucleic acids[3] the nuclear relaxation induced in nearby nuclei by a spin label has been used to obtain distance information. In any such study it is clear that local conformational movements and dynamics of the spin label relative to the macromolecule to which it is covalently or non-covalently attached may create uncertainties in the interpretation of spectroscopic data. Previous examples of nucleoside and nucleotide spin labelled derivatives in which relatively free rotation of the radical-bearing moiety is possible include compounds with nitroxyl groups attached by acylation of N^6 of adenosine[4] or of the 2'(3') hydroxy group of the ribose ring in ATP.[5] In connection with our interest in muscle physiology we wished to construct a spin labelled nucleotide in which the possibilities for rotational movement of the nitroxyl were severely constrained. We considered that such a derivative would be obtained if the vicinal hydroxy groups on the ribose ring of adenosine triphosphate were used to ketalise 4-oxo-2,2,6,6-tetramethyl-1-piperidyloxy (4-oxo-TEMPO), thereby affording the spiro derivative **1**. During the course of model studies to establish the synthesis of this compound, a number of unexpected results were encountered and these, together with spectral evidence for the spiroketal structure, are described here. Uridine was used as the model nucleoside throughout these experiments in order to avoid additional complications of acid-catalysed glycosidic cleavage of purine nucleosides. The synthesis and biochemical properties of the desired ATP derivative **1** will be reported elsewhere.

2',3'-Spiro ketals of ribonucleosides and alicyclic ketones are readily prepared by acid-catalysed condensation of the nucleoside and ketone or its derived dimethyl ketal, the latter being prepared either *in situ* or in a separate reaction according to the structure of the particular cycloalkanone.[6,7] Since nitroxyls are unstable under acidic conditions,[8] initial experiments were conducted using 4-oxo-2,2,6,6-tetramethylpiperidine **2** or its dimethyl ketal **3** but reactions of these compounds with uridine in dimethylformamide (DMF) under a variety of acidic conditions were unproductive. Protection of the amino group as its trifluoroacetyl derivative to give the ketone **4** and ketal **5** did not facilitate the desired condensation reaction, and in addition no conditions could be found for removal of the sterically hindred trifluoroacetyl group in the ketal **5** which would be compatible with maintaining the integrity of a subsequent nucleoside derivative. Because of this difficulty, we turned our attention to the *N*-acetoxy compound **7** in the expectation that eventual basic hydrolysis of the less sterically crowded acetyl group would be more readily achieved.



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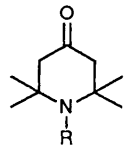
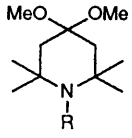
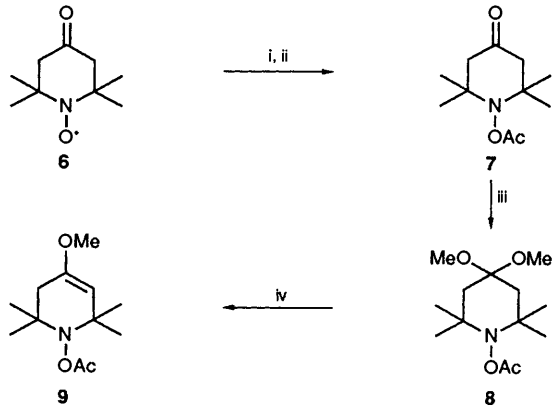


2; R = H
4; R = $COCF_3$



3; R = H
5; R = $COCF_3$

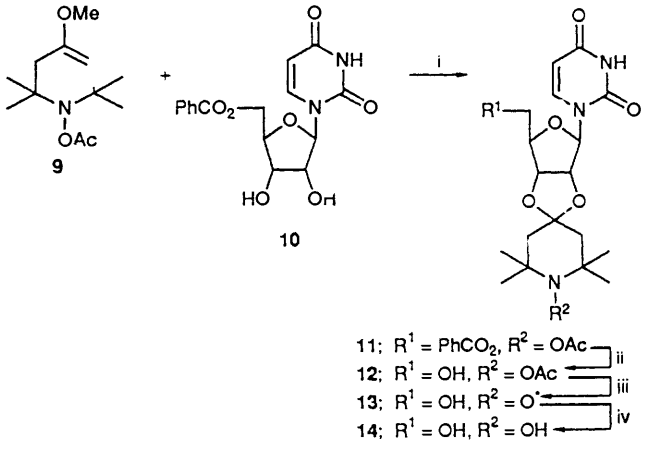
As shown in Scheme 1, 4-oxo-TEMPO **6** was reduced to the corresponding hydroxylamine with aqueous ascorbic acid[9] and acetylated *in situ* with acetic anhydride in the presence of



Scheme 1 *Reagents:* i, Na ascorbate; ii, Ac_2O–$NaHCO_3$; iii, $(MeO)_3$-CH–MeOH–TsOH; iv, TsOH–benzene

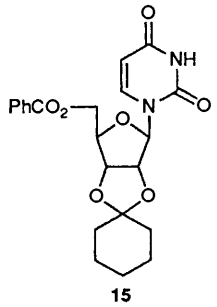
sodium hydrogen carbonate[10] to give the *N*-acetoxy ketone **7** in excellent yield. When the ketone **7** was treated with trimethyl orthoformate and toluenesulphonic acid, the product was a mixture of the dimethyl ketal **8** and the related enol ether **9**. Formation of the latter product had evidently occurred during concentration of the reaction mixture under reduced pressure at *ca.* 40 °C and could be minimised by prior neutralisation of the acid catalyst. The facile acid-catalysed loss of methanol from ketal **8** leading to the enol ether **9** was quite unexpected. For example the closely related 1-(2-fluorophenyl)-4-methoxy-1,2,5,6-tetrahydropyridine was prepared by treatment at 150 °C of its precursor dimethyl ketal with mesitylenesulphonic acid.[11] Evidently the relief of unfavourable 1,3-diaxial interactions between the axially disposed methyl and methoxy groups in the ketal **8** provides the driving force for elimination, and near quantitative conversion to the enol ether **9** was effected by brief treatment under reflux with toluenesulphonic acid in benzene.

Treatment of uridine with the ketal **8** and toluenesulphonic acid in DMF gave only a product tentatively identified as a mixed ketal in which one of the methoxy groups of **8** had exchanged with the 5′-hydroxy of the nucleoside. Under similar conditions the protected 5′-*O*-benzoyluridine **10** was recovered unchanged. However, when the latter compound was treated with the enol ether **9** in acidic tetrahydrofuran (THF) or dioxane[12] (Scheme 2), TLC analysis showed slow appearance



Scheme 2 *Reagents and conditions:* i, TsOH–THF; ii, KOH–aq. MeOH, 15 min; iii, KOH–aq. MeOH, 70 h; iv, $PhNHNH_2$

of two minor compounds less polar than the starting material and which were transformed slowly to a single still less polar compound, identified below as the required spiro ketal **11**. Under the same conditions, the reaction of 5′-*O*-benzoyluridine and 1-methoxycyclohexene showed an identical sequence of transformations to give 5′-*O*-benzoyl-2′,3′-*O*-cyclohexylideneuridine **15**, although *ca.* 10 times faster, since severe 1,3-diaxial interactions are absent in the 1,1-disubstituted cyclohexane ring. In each case the two transient species are presumed to be the isomeric mixed ketals formed by initial addition of the 2′- or



3′-hydroxy groups to the respective enol ethers. Conversion into the spiro ketals then requires acid-catalysed loss of the elements of methanol from the mixed ketals and intramolecular capture by the adjacent free hydroxy group of the ribose ring.

With the spiro ketal **11** in hand, it was possible to reassess some of the earlier failures. In particular it was found that both DMF and DMSO (dimethyl sulphoxide) had powerful inhibitory effects on the reaction between benzoyluridine and the enol ether **9**. Incremental additions of either solvent to the THF reaction medium caused progressive decreases in the extent of product formation and abolished it entirely at concentrations of 10–20%. We assume that proton solvation by the dipolar aprotic solvents reduces the effectiveness of the acidic catalyst. When the ketal **8** was treated with benzoyluridine in acidic THF, very slow conversion into the spiro ketal **11** was observed, although we did not specifically exclude the possibility that this reaction was proceeding *via* the intermediacy of the enol ether **9**. A range of catalysts for the spiro ketalisation was examined, including anhydrous hydrogen chloride, bis(4-nitrophenyl) phosphate[6] and boron trifluoride–diethyl ether: the first two offered no benefit over toluenesulphonic acid, while the Lewis acid was less effective.

Brief treatment of the spiro ketal **11** with alkali specifically hydrolysed the 5′-ester group which, if required, would allow subsequent phosphorylation at this site and hence conversion into nucleotides. Hydrolysis of the *N*-acetoxy group required prolonged treatment with alkali and in the presence of atmospheric oxygen was accompanied by concomitant oxidation of the hydroxylamine product to the nitroxyl **13**. Facile aerial oxidation of hydroxylamines, occurring in the absence of added transition metal ions,[13] has been reported previously.[14] The hydroxylamine **14** could be isolated almost pure after reduction of the nitroxyl **13** by ascorbate or phenylhydrazine, but gradually reverted to the nitroxyl on contact with air.

Confirmation of the spiro ketal structure of the series **11**–**14** was obtained by ^1H and ^{13}C NMR spectroscopy of compounds **11** and **12** but was complicated by the existence at room temperature in the latter two compounds of two equally populated conformers, which interconverted slowly on the NMR time scale (*vide infra*). The prime structural evidence for the spiro ketal formula came from the ^{13}C chemical shift of the ketal carbon (C-4″), which resonated at δ 112.6 and 112.0 in compounds **11** and **12** respectively. For comparison, the corresponding carbon atom in the dimethyl ketal **8** gave a signal at δ 98.1. This large downfield shift is characteristic of 1,3-dioxolanes[15] and similar values have been reported for a range of nucleoside 2′,3′-*O*-isopropylidene[16] and cyclohexylidene[17] ketals. The ^1H NMR spectra of the ribose protons in compounds **11** and **12** were also indicative of a 2′,3′-*O*-ketal system. Relevant features were the low values of $J_{1',2'}$ which ranged from being unresolvably small to 2.6 Hz and $J_{3',4'}$ which was 3.5–4.2 Hz. Gaudemer and co-workers[18] reported similar values for derivatives of 2′,3′-*O*-isopropylideneadenosine and interpreted them in terms of a rapidly equilibrating mixture of type N and S conformers.[19] Finally, both ^1H and ^{13}C NMR spectra of compound **11** and **12** showed evidence of differing environments for the two sides of the piperidine ring, as expected since the two sides have an *exo*/*endo* relationship to the ribose ring. For example, the ^1H NMR spectra show the geminal methyl groups almost completely resolved into eight resonances, *i.e.* four distinct methyl groups in each of two conformers (see Experimental section). In the ^{13}C NMR spectra, C-3″ and C-5″ differ by *ca.* 2.2 ppm, whereas C-2″ and C-6″ which are further removed from the ribose ring differ by only 0.1 ppm. Analogous anisochrony has been reported for the geminal methyl groups of 2′,3′-*O*-isopropylidene nucleosides,[16,18] and for the two sides of the cyclohexadienyl ring in the Meisenheimer spiro picryl complex of adenosine.[20]

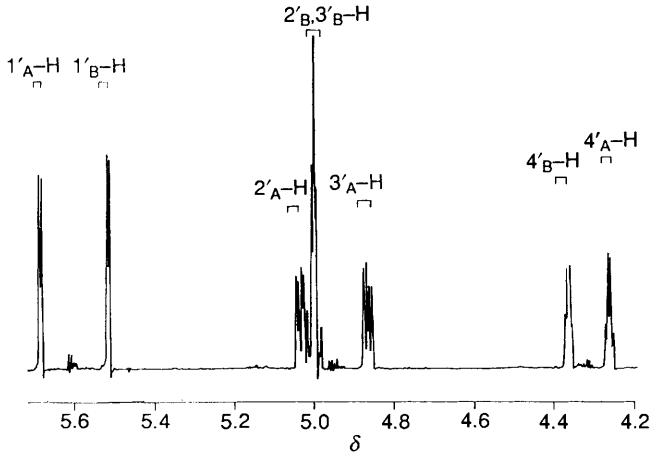


Fig. 1 Partial 500 MHz ^{1}H NMR spectrum of the *N*-acetoxy spiro ketal **12**, showing resonances of the ribose ring protons as doubled signals owing to the presence of two conformers (**A** and **B**)

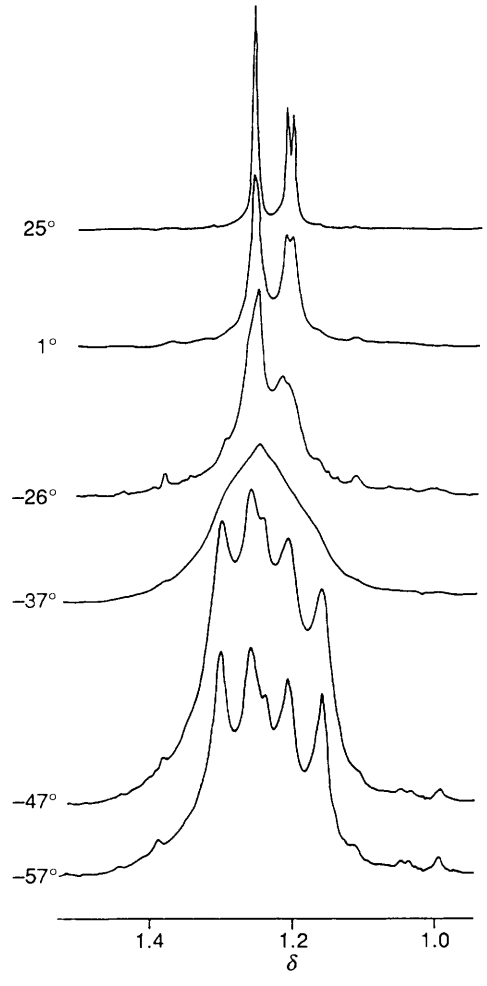


Fig. 2 Partial 500 MHz ^{1}H NMR spectra of the *N*-hydroxy spiro ketal **14** at various temperatures (°C), showing temperature dependence of the signals from the geminal methyl groups. Note that the vertical axis is not to scale in the different spectra.

The NMR spectra of the cyclohexylidene spiro ketal **15** were directly comparable in all the above features, *i.e.* ^{13}C chemical shift of the ketal carbon, magnitude of the proton–proton coupling constants in the ribose ring and anisochrony of the ^{13}C resonances for the two sides of the cyclohexane ring. However, as already mentioned, the ^{1}H and ^{13}C NMR spectra of the *N*-acetoxypiperidine spiro ketals **11** and **12** contained additional complexity since many of the resonances appeared as pairs of signals, which we ascribe to the presence of two slowly interconverting chair conformations (arbitrarily designated **A** and **B**) of the piperidine ring (*vide infra*). The phenomenon is illustrated in Fig. 1 which shows the ^{1}H NMR resonances of the ribose protons of compound **12**. Complete data are given in the Experimental section. Assignments to the **A** or **B** series were made by means of a series of single frequency decouplings, and the ^{13}C resonances (see Experimental section) were assigned with the aid of a heteronuclear shift correlation experiment. In contrast to the *N*-acetoxy compounds, the ^{1}H NMR spectrum of the *N*-hydroxy spiro ketal **14** showed no evidence for slow conformational exchange at room temperature. However, in a variable temperature study, shown in Fig. 2, it was possible to observe the temperature dependence of the conformational exchange and a coalescence temperature of approximately −30 °C for the resonances of the geminal methyl groups was found.

The conformational exchange behaviour described above is unlikely to arise from simple ring inversion of the piperidine ring, but is more likely to be due to slow inversion at the nitrogen. However we cannot at present exclude the possibility that the observed non-equivalence results from rotational isomers about the N–O bond. It is well established that electronegative substituents on a trigonal nitrogen atom cause an increase in the energy barrier to conformational change, which in hydroxylamines is generally ascribed to a barrier to nitrogen inversion rather than to rotation about the N–O bond.[21,22] We are aware of only one comparison of hydroxylamines and their *O*-acetates, where in the two examples studied the acetates had a slightly lower inversion barrier than the free hydroxylamines.[23] The tetramethylpiperidine derivatives in the present work appear to exhibit the contrary property of a significantly higher barrier for the *O*-acetates than for the free hydroxylamines. A more detailed study of model compounds is under way in order better to define the conformational processes in this series of compounds. Such information is likely to be relevant to the interpretation of results of EPR experiments with the spin labelled ATP derivative **1**.

Experimental

Analyses were carried out by Butterworth Laboratories, Teddington, Middlesex. NMR spectra were determined, unless otherwise stated, in $CDCl_3$ on JEOL FX90Q and Bruker WM 200, 400 and 500 spectrometers with tetramethylsilane as internal standard, *J* values are given in Hz. The EPR spectrum was run on a Bruker ER-200D spectrometer. High resolution mass spectra were obtained on a Kratos MS80RF instrument. Merck 9385 silica gel was used for flash chromatography. 5′-*O*-Benzoyluridine was purchased from Sigma Chemical Co. Ltd., Gillingham, Dorset. Light petroleum was the fraction boiling 40–60 °C.

1-*Acetoxy*-2,2,6,6-*tetramethyl*-4-*piperidone* **7**.—4-Oxo-2,2,6,6-tetramethyl-1-piperidyloxy (20 g, 188 mmol) was melted by gentle warming and treated with a solution of sodium ascorbate (37.6 g, 190 mmol) in water (320 cm^3). The solution was stirred vigorously at ambient temperature for 5 min, during which its colour changed rapidly from dark red to pale yellow, then diluted with saturated aqueous $NaHCO_3$ (800 cm^3) and cooled in ice. Acetic anhydride (64 cm^3, 679 mmol) was added over 2 min to the stirred mixture (pH 8). Portions of solid $NaHCO_3$ were added carefully to maintain the mixture at pH 8 until no further pH change occurred (*ca.* 1 h) and the mixture was extracted with $CHCl_3$ (3 × 200 cm^3). The combined $CHCl_3$ extract was washed with saturated aqueous $NaHCO_3$ (2 × 200 cm^3), dried (Na_2SO_4) and evaporated under reduced pressure,

to leave the *keto acetate* **7** as a pale solid (23.3 g, 94%). A sample recrystallised from light petroleum gave pale needles, m.p. 95–95.5 °C (Found: C, 62.0; H, 8.6; N, 6.6. $C_{11}H_{19}NO_3$ requires C, 61.9; H, 9.0; N, 6.6%); ν_{max}(Nujol)/cm^{-1} 1765, 1720 and 1195; δ_H(90 MHz) 2.78 and 2.25 (AXq, 4 H, J_{gem} 12.7, CH_2), 2.14 (s, 3 H, CH_3CO) and 1.21 (s, 12 H, CH_3).

1-*Acetoxy*-4,4-*dimethoxy*-2,2,6,6-*tetramethylpiperidine* **8**.—The crude keto acetate **4** (23.5 g, 110 mmol) and toluene-*p*-sulphonic acid monohydrate (2.1 g, 11 mmol) were dissolved in a mixture of methanol (250 cm^3) and trimethyl orthoformate (250 cm^3) and the solution was heated under reflux for 2 h and then cooled to room temperature. 3% Aqueous $NaHCO_3$ (750 cm^3) was added, the mixture was saturated with NaCl and extracted with ether (3 × 200 cm^3). The combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure. Distillation of the residual oil afforded the *ketal* **8** as a pale liquid (25.9 g, 91%), b.p. 84 °C (0.5 mmHg) which crystallised with considerable loss from light petroleum (−20 °C) as colourless prisms, m.p. 57–57.5 °C (Found: M$^+$, 259.1770. $C_{13}H_{25}NO_4$ requires *M*, 259.1784); ν_{max}(film)/cm^{-1} 1765, 1360, 1190, 1095 and 1045; δ_H(90 MHz) 3.16 (s, 6 H, OCH_3), 2.08 (s, 3 H, CH_3CO), 1.89 (ABq, 4 H, J_{gem} 12.6, CH_2), 1.28 (s, 3 H, CH_3) and 1.08 (s, 3 H, CH_3); δ_C(22.6 MHz) 170.6 (C=O), 98.1 (C-4), 59.5 (C-2,6), 47.6 (OCH_3), 47.3 (OCH_3), 43.4 (C-3,5), 32.6 (CH_3CO), 20.8 (CH_3) and 19.1 (CH_3).

1-*Acetoxy*-4-*methoxy*-2,2,6,6-*tetramethyl*-1,2,5,6-*tetrahydropyridine* **9**.—A solution of toluene-*p*-sulphonic acid monohydrate (684 mg, 3.6 mmol) in benzene (400 cm^3) was heated under reflux in a flask fitted with a Dean–Stark trap until no further water separated (*ca.* 30 min). The water was run off and a solution of ketal **8** (14.4 g, 56 mmol) in benzene (25 cm^3) was added to the toluene-*p*-sulphonic acid solution. The mixture was heated under reflux for 30 min and the contents of the trap were run off twice during this period to remove entrained methanol. The mixture was then cooled to room temperature, washed with saturated aqueous $NaHCO_3$ (2 × 200 cm^3), dried (Na_2SO_4) and the solvent removed under reduced pressure. The residual oil was purified in batches by short-path distillation at 0.8 mmHg (Kugelrohr, oven temperature 150 °C) to give the *enol ether* **9** as a pale oil (11.5 g, 91%) which solidified to a waxy solid, m.p. 37–40 °C on storage at 4 °C (Found: M$^+$, 227.1519. $C_{12}H_{21}NO_3$ requires *M*, 227.1522); ν_{max}(film)/cm^{-1} 1765, 1677, 1375, 1360 and 1195; δ_H(90 MHz) 4.40 (d, 1 H, *J* 1, 3-H), 3.49 (s, 3 H, OCH_3), 2.53 (dd, 1 H, *J* 16.5 and 1, 1 × 5-H), 2.11 (s, 3 H, CH_3CO), 1.93 (d, 1 H, *J* 16.5, 1 × 5-H), 1.28 (s, 3 H, Me), 1.21 (s, 3 H, Me), 1.19 (s, 3 H, Me) and 1.16 (s, 3 H, Me); δ_C(22.6 MHz) 170.9 (C=O), 150.3 (C-4), 101.2 (C-3), 60.1 and 59.0 (C-2,6), 54.3 (OCH_3), 41.6 (C-5), 32.3 (CH_3CO), 29.4 (Me), 24.7 (Me), 22.2 (Me) and 19.1 (Me).

2′,3′-O-(1-*Acetoxy*-2,2,6,6-*tetramethyl*-4-*piperidylidene*)-5′-O-*benzoyluridine* **11**.—5′-*O*-Benzoyluridine (350 mg, 1 mmol) was added to a solution of toluene-*p*-sulphonic acid monohydrate (190 mg, 1 mmol) in dry THF (5.6 ml), followed by 1-acetoxy-2,2,6,6-tetramethyl-1,2,5,6-tetrahydropyridine (2.2 g, 9.7 mmol). The solution was left at room temperature for 7 d and then diluted with ethyl acetate (100 cm^3), washed with saturated $NaHCO_3$, dried (Na_2SO_4) and evaporated. The oily residue was purified by flash chromatography using ethyl acetate–light petroleum (3:2) as the eluting solvent to give the *title product* as a pale foam (311 mg, 57%) which was homogeneous by TLC ($CHCl_3$–MeOH 95:5) (Found: C, 59.2; H, 6.65; N, 7.3%; M$^+$ − CH_3, 528.1774. $C_{27}H_{33}N_3O_9$ requires C, 59.65; H, 6.1; N, 7.7%; M$^+$ − CH_3, 528.1878); ν_{max}($CHCl_3$)/cm^{-1} 3380, 1755, 1720, 1690, 1270 and 1090; δ_H(500 MHz) 8.02 and 8.01 (2 × d, 2 × 1 H, *J* 7.6, *ortho*-H), 7.58 (t, 1 H, *J* 7.4, *para*-H), 7.45 (t, 2 H, *meta*-H), 7.29 and 7.24 (2 × d, 2 × 0.5 H, $J_{5,6}$ 8.2, 6-H), 5.70 (br s, 0.5 H, W, 2.5 Hz, 1_A′-H), 5.66 (d, 1 H, 5-H), 5.60 (br s, 0.5 H, W 2.5 Hz, 1_B′-H), 5.09 (br d, 0.5 H, $J_{2',3'}$ 6, 2_A′-H), 5.02 (m, 2 × 0.5 H, 2_B′,3_B′-H), 4.88 (dd, 0.5 H, $J_{3',4'}$ 4.2, 3_A′-H), 4.55–4.60 (m, 2 H, 5′-H), 4.53 (m, 0.5 H, 4_B′-H), 4.44 (m, 0.5, 4_A′-H), 2.28 (d, 1 H, *J* 13, 1 H of 3″-H or 5″-H), 2.11 (s, 3 H, MeCO), 1.81–1.97 (m, 3 H of 3″-H and 5″-H), 1.35 (s, 1.5 H, 0.5 Me), 1.32 (s, 1.5 H, 0.5 Me), 1.30 (s, 1.5 H, 0.5 Me), 1.29 (s, 1.5 H, 0.5 Me), 1.13 (s, 1.5 H, 0.5 Me), 1.12 (s, 1.5 H, 0.5 Me), 1.07 (s, 3 H, 2 × 0.5 Me); δ_C(125.8 MHz) 170.8 (Me*C*O), 166.1 (Ph*C*O), 163.9 (C-4), 150.3 (C-2), 143.0 and 142.7 (C-$6_{A,B}$), 133.4 (C-4_{Ar}), 129.6 (C-2_{Ar}), 129.5 (C-1_{Ar}), 128.5 (C-3_{Ar}), 112.6 (C-4″), 102.7 (C-5), 95.4 (C-1_B′), 95.0 (C-1_A′), 86.0 (C-4_A′), 85.4 (C-4_B′), 85.2 (C-2_A′), 82.8 and 82.4 (C-2_B′,3_B′), 79.7 (C-3_A′), 64.6 (C-5′), 60.1 and 60.0 (C-2″,6″), 47.5, 47.2 and 45.3, 45.1 (C-$3''_{A,B}$,$5''_{A,B}$), 32.3 (CH_3CO) and 21.1, 20.6 and 19.0 (Me).

2′,3′-O-(1-*Acetoxy*-2,2,6,6-*tetramethyl*-4-*piperidylidene*)-*uridine* **12**.—A solution of the spiro ketal benzoate **11** (200 mg) in MeOH (65 cm^3) was treated with aqueous KOH (1.67 mol dm^{-3}; 0.15 cm^3) and the solution was kept at room temperature for 15 min, then acidified with glacial acetic acid (1.15 cm^3) and concentrated to a small volume under reduced pressure. The residue was dissolved in ethyl acetate, washed with $NaHCO_3$ and brine, dried and evaporated to leave the *spiro ketal* **12** as a pale foam (146 mg, 92%) which was homogeneous by TLC ($CHCl_3$–MeOH 9:1) (Found: M$^+$, 439.1954. $C_{20}H_{29}N_3O_8$ requires *M*, 439.1955); δ_H(500 MHz) 7.47 and 7.41 (2 × d, 2 × 0.5 H, *J* 8, $6_{A,B}$-H), 5.75 and 5.74 (2 × d, 2 × 0.5 H, $5_{A,B}$-H), 5.69 (d, 0.5 H, $J_{1',2'}$ 2.6, 1_A′-H), 5.51 (d, 0.5 H, $J_{1',2'}$ 2.5, 1_B′-H), 5.04 (dd, 0.5 H, $J_{2',3'}$ 6.3, 2_A′-H), 5.00 (m, 2 × 0.5 H, 2_B′,3_B′-H), 4.87 (dd, 0.5 H, $J_{3',4'}$ 3.5, 3_A′-H), 4.36 (m, 0.5 H, 4_B′-H), 4.26 (m, 0.5 H, 4_A′-H), 3.94–3.81 (m, 2 H, J_{gem} 12.1, 5′-H), 2.27 and 2.26 (2 × d, 2 × 0.5 H, J_{gem} 14, 2 × 0.5 of 3″- or -5″-H), 2.11 (s, 3 H, CH_3CO), 1.89–1.80 (m, 3 H, remainder of 3″, 5″-H), 1.34 (s, 1.5 H, 0.5 Me), 1.33 (s, 1.5 H, 0.5 Me), 1.29 (s, 1.5 H, 0.5 Me), 1.28 (s, 1.5 H, 0.5 Me), 1.12 (s, 3 H, Me) and 1.07 (s, 3 H, Me); δ_C(22.6 MHz), 171.0 (Me*C*O), 163.9 (C-4), 150.5 (C-2), 142.8 and 142.4 (C-$6_{A,B}$), 112.0 (C-4″), 102.4 (C-5), 95.3 (C-1_B′), 94.1 (C-1_A′), 87.6 (C-4_A′), 87.0 (C-4_B′), 85.3 (C-2_A′), 82.3 and 81.5 (C-2_B′,3_B′), 78.9 (C-3_A′), 62.6 and 62.3 (C-$5'_{A,B}$), 60.2 and 60.0 (C-2″,6″), 47.5 and 45.2 (C-3″,5″), 32.2 (CH_3CO) and 21.1, 20.8 and 19.0 (Me).

2′,3′-O-(1-*Oxy*-2,2,6,6-*tetramethyl*-4-*piperidylidene*)*uridine* **13**.—The *N*-acetoxy spiro ketal **12** (0.25 mmol) was treated with aqueous methanolic KOH exactly as for the benzoate **11**, but the solution was left exposed to air at room temperature for 50–72 h; it was then acidified with glacial acetic acid (0.76 cm^3) and concentrated to approx. 5 cm^3 under reduced pressure. The residual solution was saturated with NaCl and extracted with ethyl acetate. The extracts were washed with $NaHCO_3$-saturated aqueous KCl and brine, dried and evaporated to leave the nitroxyl **13** as a pale orange gum which was obtained in 99% yield as determined by quantitative UV analysis at λ 261 nm, and was essentially homogeneous by TLC ($CHCl_3$–MeOH, 9:1) (Found: M$^+$, 396.1746. $C_{18}H_{26}N_3O_7$ requires *M*, 396.1771). The EPR spectrum recorded at 9.81 GHz (100 × 10^{-3} mol dm^{-3} imidazole–HCl, pH 7.0) showed a triplet centred at 0.347 058 T, with hyperfine splitting of 1.68 mT. The compound was further characterised by reduction to the hydroxylamine as described below.

2′,3′-O-(1-*Hydroxy*-2,2,6,6-*tetramethyl*-4-*piperidylidene*)-*uridine* **14**.—A portion (19.6 mg) of the nitroxyl **13** was dissolved in methanol (0.26 cm^3) and treated with 20 × 10^{-3} mol dm^{-3} phenylhydrazine in chloroform (2.32 cm^3). The solvent was evaporated and the residue purified by flash chromatography in CH_2Cl_2–MeOH (9:1). The recovered *N*-hydroxy spiro ketal **14**

(16 mg), R_f 0.33 (*cf.* nitroxyl **13** has R_f 0.65) in CH_2Cl_2–MeOH (9:1) was used directly for NMR spectroscopy; δ_H(500 MHz; $CDCl_3$–CD_3OD 9:1) 7.61 (d, 1 H, $J_{5,6}$ 8, 6-H), 5.75 (d, 1 H, $J_{1',2'}$ 2.6, 1'-H), 5.72 (d, 1 H, 5-H), 4.88 (m, 2 H, 2',3'-H), 4.30 (m, 1 H, 4'-H), 3.86 (dd, 1 H, J_{gem} 12, $J_{4',5'^a}$ 2.6, 5'^{a}-H), 3.77 (dd, 1 H, $J_{4',5'}$ 3.4, 5'^{b}-H), 1.99 (ABq, 2 H, J_{gem} 13.7, CH_2), 1.77 (ABq, 2 H, J_{gem} 14, CH_2), 1.25 (s, 6 H, Me), 1.21 (s, 3 H, Me) and 1.20 (s, 3 H, Me).

5'-O-Benzoyl-2',3'-O-cyclohexylideneuridine **15**.—1-Methoxycyclohexene[24] (1.05 g, 9.4 mmol) was added to a solution of 5'-*O*-benzoyluridine (0.20 g, 0.57 mmol) and toluene-*p*-sulphonic acid (0.12 g, 0.63 mmol) in dry dioxane (3.4 cm^3) and the mixture was kept at room temperature overnight, then diluted with ethyl acetate, washed with saturated $NaHCO_3$, dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography using ethyl acetate in light petroleum (3:2) as the eluting solvent to give the cyclohexylidine ketal **15** as a pale foam (240 mg, 98%); δ_H(200 MHz) 9.80 (s, 1 H, NH), 8.02 (m, 2 H, *ortho*-ArH), 7.62–7.38 (m, 3 H, ArH), 7.28 (d, 1 H, *J* 8, 6-H), 5.72 (d, 1 H, $J_{1',2'}$ 1.7, 1'-H), 5.63 (d, 1 H, 5-H), 5.04 (dd, 1 H, $J_{2',3'}$ 6.3, 2'-H), 4.94 (dd, 1 H, $J_{3',4'}$ 3.65, 3'-H), 4.67–4.45 (m, 3 H, 4',5'-H) and 1.85–1.35 (m, 10 H, cyclohexyl-H); δ_C(22.6 MHz) 166.1 (Ph*C*O), 163.6 (C-4), 150.1 (C-2), 142.2 (C-6), 133.4 ($C\text{-}4_{Ar}$), 129.7 ($C\text{-}2_{Ar}$), 129.5 ($C\text{-}1_{Ar}$), 128.5 ($C\text{-}3_{Ar}$), 115.3 (C-1''), 102.6 (C-5), 94.9 (C-1'), 85.6 (C-4'), 84.2 (C-2'), 80.8 (C-3'), 64.6 (C-5'), 37.1, 34.8, 25.0, 24.0 and 23.6 (C-2''–6'').

Brief alkaline treatment as described for the hydrolysis of the benzoyl ketal **11** gave 2',3'-*O*-cyclohexylideneuridine as prisms, m.p. 164–156 °C (from toluene–acetone) (lit.,[7] 160–163 °C).

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