The phototransformation of 5-*tert*-butyl-2′-deoxyuridine: an approach to the synthesis of new 1,2-dihydrocyclobuta[*d*]pyrimidin-2-ones[1]

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A new type of 2-oxo-1,2-dihydrocyclobuta[*d*]pyrimidin-1-yl-2′-deoxynucleoside 2a is obtained in 52% yield upon irradiation of aqueous solutions of 5-*tert*-butyl-2′-deoxyuridine 1a with short wavelength (254 nm) UV light. The identity and structure of the photoproduct is unequivocally established by ^{1}H and ^{13}C NMR (including a 2D INADEQUATE experiment), mass and UV spectroscopy. This new phototransformation represents an alternative route for the photolysis of 5-alkyl-substituted uracil derivatives where the side chain has more than one carbon atom. A mechanism for the phototransformation of compound 1a into 2a is proposed, and this requires the creation of a cyclobutanol-type intermediate (type-II photoprocess). A general pathway of UV-induced photolysis of 5-alkyl-substituted uracil derivatives (other than 5-methyl substituted) into 1,2-dihydrocyclobuta[*d*]pyrimidin-2-ones and photohydrated uracils is proposed.

Introduction

As part of a continuing study of 5-substituted uracils and their nucleosides, we have investigated the photoirradiation of the previously prepared[2] 5-*tert*-butyl-2′-deoxyuridine **1a**. 5-Substituted pyrimidine nucleosides are known to undergo three different types of phototransformations, depending on the character of the substituent. They are all related to the C(5)–C(6) double bond in a pyrimidine ring which is conjugated with the C(4)-carbonyl group.

Irradiation (254 nm) of dilute aqueous solutions of uracil derivatives yields predominantly 5,6-dihydro-6-hydroxyuracil derivatives[3,4] (Scheme 1; **6**→**8**, R^1 = H, alkyl, ribose, 2′dR). The phototransformation is reversible upon heating or by imposing alkaline conditions. Upon irradiation (254 nm) of thymine and its derivatives in aqueous solution or in an ice-matrix, a mixture of C(5)–C(6) *cis-syn* and *trans-syn* photodimers predominates rather than photohydration of the C(5)–C(6) double bond.[5a–c,6] Irradiation (254 nm) of dilute aqueous solutions of 5-alkyl substituted uracil derivatives with more than one carbon atom in the substituent (Scheme 1; **1**, R^1 = alkyl, ribose or 2′dR; 5-Et, Pr or Pr^i)[7–10] has so far been reported to result in the production of the corresponding 5,6-dihydro-6-hydroxyuracil derivative **8**; *cis*-2,4-diazabicyclo[4.2.0]-octane-3,5-diones **5** and uracils **6** were shown to be common intermediates.[9] Photolysis of 5-*tert*-butyl-2′-deoxyuridine **1a** (Scheme 2), which is presented in this paper, reveals a hitherto unsuspected phototransformation of 5-alkyluracil derivatives, and also opens an approach to the synthesis of new 1,2-dihydrocyclobuta[*d*]pyrimidin-2-ones at the nucleoside level.

Results and discussion

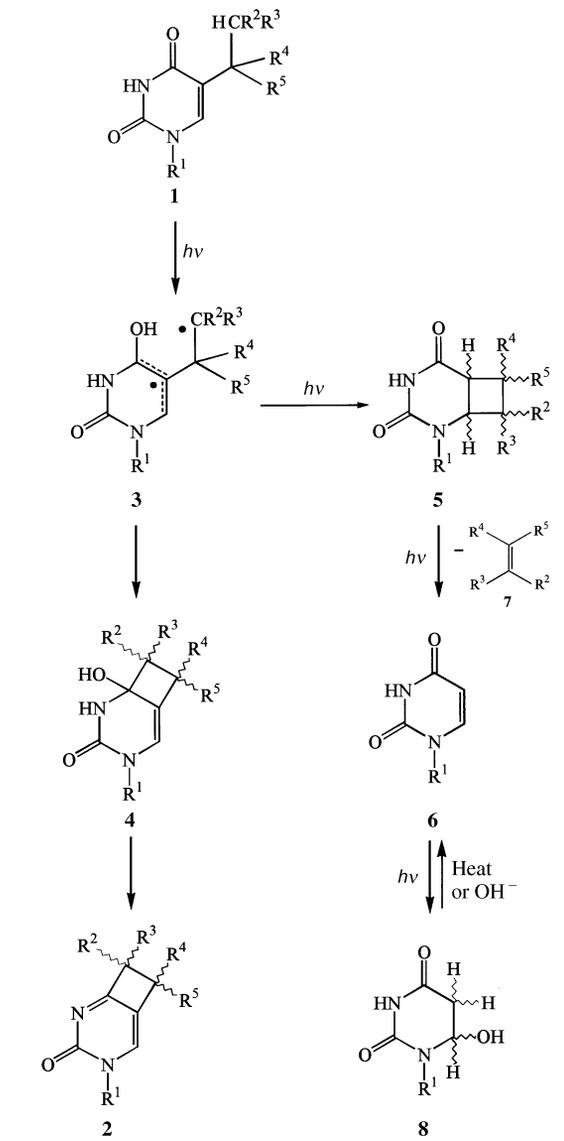
Phototransformation of 5-*tert*-butyl-2′-deoxyuridine 1a

We have studied the photochemistry of 5-*tert*-butyl-2′-deoxyuridine **1a** in dilute aqueous solutions (2×10^{-4} mol l^{-1}; $h\nu$ = 254 nm, throughout all this study, unless specified). The course of photolysis was monitored by measurement of the UV spectra of the irradiated solution as presented in Fig. 1. At the end of the irradiation (2 h), the substrate **1a** (λ_{max} = 265 nm) had disappeared and a new λ_{max}, with a considerably lower absorbance, appeared at 314 nm. The isosbestic point at 282 nm indicated a fairly smooth transformation from compound **1a** into one major UV-absorbing photoproduct. TLC analysis of the irradiated solution revealed the presence of only one major UV-absorbing component. The phototransformation was repeated with a 5×10^{-2} mol l^{-1} solution of the starting material **1a** and was complete after irradiation for 48 h. A TLC-homogenous product was isolated (35%), which was identified by NMR, mass and UV spectroscopy as the previously unknown 1,2-dihydrocyclobuta[*d*]pyrimidin-2-one nucleoside **2a** {4-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-7,7-dimethyl-2,4-diazabicyclo[4.2.0]octa-1,5-dien-3-one}, shown in Scheme 2 and Fig. 2. The direct yield of compound **2a** in the irradiated solution was calculated to be 52% from its established molar extinction coefficient, as the photoproduct **2a** did not interfere with any other UV-absorbing component in the irradiated solution. As will be discussed later, the irradiated solution also contained small quantities of the photohydrate of 2′-deoxyuridine **8a** created apparently by the general mechanism of photodealkylation according to Scheme 1.

Structure of the photoproduct 2a

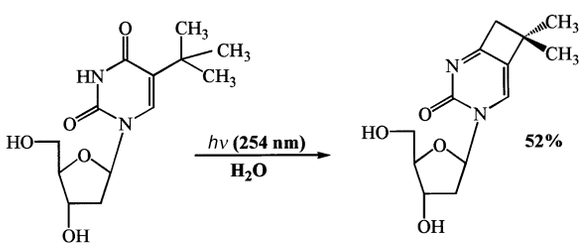
The structure of the photoproduct **2a** was established on the basis of the combined use of relevant analytical methods (UV spectra, MS), with a variety of NMR methods playing a key role.

NMR analysis. A one-dimensional ^{1}H NMR spectrum of the photoproduct in [$^2H_6$]DMSO was recorded at 400 MHz. The chemical shift values δ (ppm) and coupling constants J (Hz) are given in the Experimental section. The spectrum confirmed the presence of the 2′-deoxyribose moiety and all those protons and their coupling constants could be assigned as usual.[2,11,12] The anomeric proton, H-1′ (δ 6.08), was shifted slightly to lower frequency compared to the position of the anomeric proton H-1′ (δ 6.22) in the starting material **1a**[2] and was present as a clear pseudotriplet (dd, J 6.5) which is usually characteristic of the β-anomeric configuration.[12] Using the anomeric proton as the starting point, the chemical shift positions of all the other sugar ring protons were easily established from observable connectivity in the COSY 45 spectrum. The assignment of the two 2′-protons (H-2′, H-2″) was unequivocally made on the basis of NOE experiments (see below). The heterocyclic moiety of the photoproduct **2a** had the H-6 signal (δ 7.98) shifted significantly to higher frequency when compared with the same proton in compound **1a** (δ 7.64). The N(3)H signal was absent as



a R^1 = 2'dR; R^2 = R^3 = H; R^4 and R^5 = Me
b R^1 = 2'dR; R^2 = R^3 = R^4 = H; R^5 = Me

Scheme 1



Scheme 2

expected, and the remaining signals were assigned to two unresolved diastereotopic protons (δ 3.08, 2 H, s, H-8) and to the two resolved diastereotopic methyl groups (δ 1.39, 2 × 3 H, 2 s, Me-9 and 10) in the cyclobutane ring in compound **2a**. Qualitative information about the glycosidic bond C(1′)–N(1) conformation was obtained from NOE experiments, in which all the carbon-attached protons in compound **2a** were irradiated in turn. The strong NOE interaction between the anomeric proton (H-1′) and the multiplet at δ 2.27, together with the lack of such an interaction between H-1′ and the multiplet at δ 1.92, clearly assigned the multiplet at δ 2.27 to H-2″ and the multiplet at δ 1.92

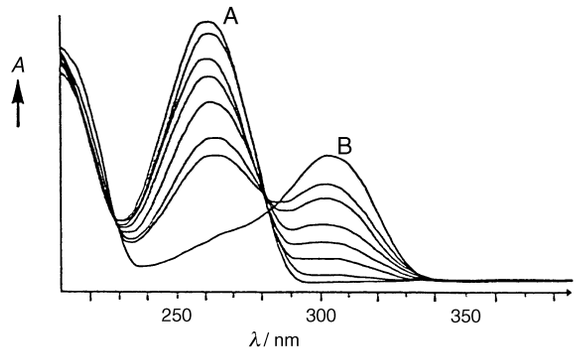


Fig. 1 UV Spectra of an irradiated solution of compound **1a**, from 0 (A) to 2 h (B)

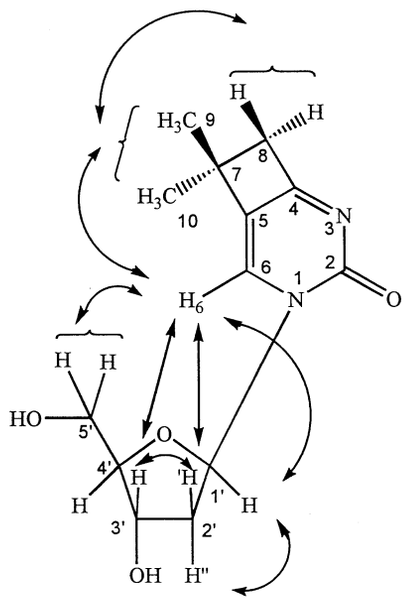


Fig. 2 The arrows indicate observed NOE's when NOE difference spectra were performed in [$^2H_6$]DMSO on compound **2a**

to H-2′. The observation of H-2″ at δ 2.27 is interesting in that it is resonating at a higher frequency than is usually observed in compounds of this type.[11] This assignment was further confirmed by the observed NOE interaction betwwen H-3′ and H-2′ and its absence between H-3′ and H-2″. As can be seen in Fig. 2, the overall NOE data are consistent with the H-6 proton being positioned over the sugar ring (*anti*-glycosidic conformation), which would account for the strong NOE interactions observed between the proton H-6 and the protons H-2′, H-3′, H-5′ and H-5″.[13] On irradiation of the diastereotopic methyl groups (at the positions 9 and 10), strong NOE interactions were observed with the H-6 and the two C(8)H. On irradiation of H-6, strong NOE interactions were now observed with the two diastereotopic methyl groups but no enhancement of the signal for the two C(8)H. This data is consistent with the structure containing the cyclobutapyrimidine moiety as presented for compound **2a** in Fig. 2.

The ^{13}C chemical shift values of compound **2a** are presented in the Experimental section. As expected, the values of the chemical shifts of the carbon atoms in the sugar moiety of compound **2a** are very close to those in the starting nucleoside **1a**.[2] On the other hand, in the heterocyclic base moiety, carbon atoms C-2, C-4 and C-5 are very much shifted to high frequency (by 6–17 ppm) when compared with the corresponding signals in compound **1a**. The chemical-shift values of C-8 (δ 49.9) and C-7 (δ 40.9) are within the range of values for similar carbon atoms in the cyclobutane moiety of the recently described cyclobutabenzene derivative.[14] The assignment of the signals was confirmed by way of an inverse-detected CH correlation

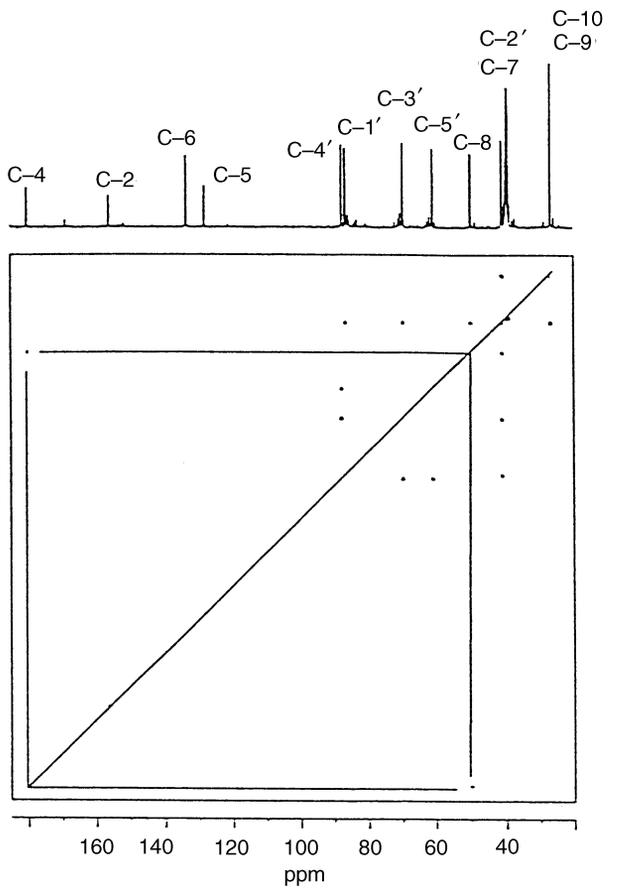


Fig. 3 The 2D-INADEQUATE spectrum of compound **2a** in [$^2H_6$]-DMSO clearly showing C-4/C-8 connectivity

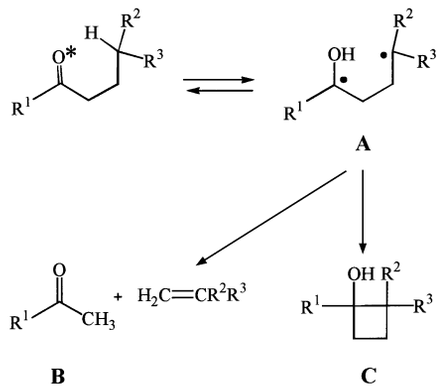
spectrum, performed with ^1H decoupling (HMQC experiment). The expected correlations from the proton-bearing carbon atoms were seen for all the carbon atoms of the sugar moiety, and also for C-6, C-8, C-9 and C-10. The remaining four signals of the quaternary carbon atoms in the heterocyclic base moiety were subsequently assigned on the basis of a further inverse CH correlation experiment, which was run without decoupling and which was optimized to show long-range proton–carbon correlations (HMBC experiment).

The final experimental proof of the structure of compound **2a**, which now conclusively established the C(4)–C(8) bond in the cyclobutane moiety of the molecule, was obtained from a 2D-INADEQUATE experiment which measures carbon–carbon connectivity directly. The cross-peaks for the C(4)–C(8) connectivity seen in the spectrum in Fig. 3 have been expanded and their doublet nature proved unequivocally. The clearly seen coupling $^1J_{C4,C8}$ 27 provided the final proof of the cyclobutane fragment in compound **2a**. The comparably bonded carbon atoms in cyclobutabenzene (aromatic C-1 and non-aromatic C-2) are coupled with the $^1J_{C1,C2}$ 35.4.[15] The following $^1J_{C,C}$ coupling constants between the carbons in compound **2a**, which are fully consistent with the already established structure, were also obtained: $^1J_{C7,C8}$ 26, $^1J_{C7,C9,10}$ 34, $^1J_{C1',C2'}$ 37, $^1J_{C2',C3'}$ 30, $^1J_{C3',C4'}$ 37, $^1J_{C4',C5'}$ 37.

MS analysis. The FAB mass spectrum consisted of three intense peaks: 267 $[M + H]^+$ for the photoproduct **2a**, 533 $[M + H]^+$ for the dimer of the photoproduct **2a**, and the dominant peak 151 $[M - C_5H_9O_3]^+$ which corresponds to the protonated heterocyclic base moiety of compound **2a**. The dominance of this peak indicates the relative stability of the 1,2-dihydrocyclobuta[*d*]pyrimidin-2-one fragment at least under conditions of the FABMS analysis. The accurate mass analysis of the $[M + H]^+$ ion of compound **2a** fits with the expected formula $C_{13}H_{19}N_2O_4$ and the spectrum as a whole is fully consistent with the proposed structure **2a**.

Mechanism of phototransformation of compound 1a

A likely explanation for the phototransformation of compound **1a** into compound **2a** (Scheme 2) is proposed as follows. Photorearrangements *via* biradicals of simple carbonyl compounds have been extensively studied and are well understood.[16a–d] As is shown in Scheme 3, photoexcited carbonyl compounds con-



Scheme 3

taining γ-CH bonds undergo an intramolecular 1,5-hydrogen shift yielding a 1,4-biradical **A**, which can be stabilized *via* cleavage to give the olefin and a ketone **B** (Norrish type-II photoelimination) or alternatively, can undergo cyclization to a cyclobutanol **C**. Both processes usually occur simultaneously and are referred to as type-II photoprocesses.[16b] The ratio of all possible photoproducts (cyclobutanols *versus* olefins and ketones) depends mainly on the nature of the substrate. In the case of cyclic or aromatic ketones, the phototransformation has a synthetic value as one of the best routes to cyclobutanols. Mechanistic aspects of type-II photoprocesses have been studied in detail and triplet-derived photochemistry is dominant in the case of aryl ketones.[17] The reactivity of an aromatic carbonyl triplet-state towards γ-hydrogen abstraction followed by biradical cyclization is influenced by α, β and γ-substitution,[18] as well as by overall conformational flexibility.[19a,b]

5-*tert*-Butyl-2′-deoxyuridine **1a**, can be regarded as a carbonyl system with nine γ-hydrogen atoms ideally positioned for photoactivated abstraction by the carbonyl group in analogy to the mechanism in Scheme 3. As a result of such photoactivation, the cyclobutanol intermediate **4a** is created, which immediately eliminates a molecule of water producing the final photoproduct **2a** (Scheme 1). The driving force for this elimination is apparently the transformation of the strained sp^3-hybridized C(4) carbon atom in the intermediate **4a** into the sp^2 hybridised carbon atom in the highly conjugated product **2a**.

In order to ascertain whether the photoproduct **2a** was the sole product of the reaction or whether small quantities of the more expected products were formed, a series of phototransformation experiments were performed with aqueous solutions (2×10^{-4} mol l^{-1}) of 5-*tert*-butyl-2′-deoxyuridine **1a**, 5-isopropyl-2′-deoxyuridine **1b** and 2′-deoxyuridine **6a**. The phototransformation of compound **1a** again followed the pattern shown in Fig. 1. The phototransformation of compound **1b** into photoproduct **2b** followed the same pattern, as in the case of compound **1a**, but the reaction was significantly slower (for details see Experimental section); after irradiation for 7 h the photoproduct **2b** was formed in *ca.* 20% yield, compared to the 52% yield of the photoproduct **2a**. Under the same conditions, 2′-deoxyuridine **6a** was completely photohydrated into **8a** after irradiation for 1.5 h. The course of each phototransformation was monitored by measurement of the UV spectra of the irradiated solutions and the results are presented in Fig. 4.

The creation of a small amount of 2′-deoxyuridine **6a** during the photoirradiation of compound **1a** is the expected product from the alternative pathway for the photolysis of this compound, that is, photodealkylation according to Scheme 1

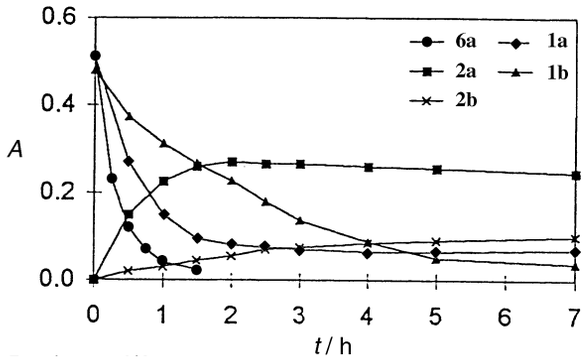


Fig. 4 Changes in amounts of compounds **1a**, **1b** and **6a** and photoproducts **2a** and **2b** during irradiation

(**1a**→**3a**→**5a**→**6a**). As is implied from Fig. 4, after irradiation for 7 h any 2′-deoxyuridine **6a** produced during irradiation would be photohydrated. To prove experimentally the presence of photohydrate **8a**, the irradiated solution of the nucleoside **1a** (7 h) was heated at 100 °C to regenerate 2′-deoxyuridine **6a** from the photohydrate **8a** since it is known that 2′-deoxyuridine can be regenerated by heating an aqueous solution of its photohydrate.[3] The appearance of a maximum at 262 nm in the UV spectra confirmed that indeed some uridine photohydrate had been present. This conclusion is supported by the result of another experiment, in which **6a** was completely regenerated from its irradiated solution under the same conditions.

The phototransformation of both 5-*tert*-butyl-2′-deoxyuridine **1a** and 5-isopropyl-2′-deoxyuridine **1b** into their corresponding photoproducts **2a** and **2b** accompanied by their photodealkylation to give 2′-deoxyuridine **6a** and its subsequent photohydration, links the phototransformation of 5-*tert*-2′-deoxyuridine **1a** and other 5-alkyl uracil derivatives **1**.[7–10] We can now propose a general pathway for the phototransformation of 5-alkyl-substituted uracil derivatives **1** (other than a 5-methyl substituent) to be as shown in Scheme 1. The substrate **1** is transformed by short wavelength UV light (254 nm) into a 1,3-biradical **3**, which can be stabilized by creating either of two cyclobutane intermediates **4** or **5**. In the case of the cyclobutane intermediate **4**, elimination of one molecule of water results in a stable 1,2-dihydrocyclobuta[*d*]pyrimidin-2-one derivative (**1**→**3**→**4**→**2**). In the case of the cyclobutane intermediate **5**, the general route of photodealkylation and photohydration is followed (**1**→**3**→**5**→**6**→**8**). Both phototransformations take place simultaneously with 5-alkyl substituted uracil nucleosides, although the proportion participating in each pathway depends upon the 5-alkyl substituent. Factors affecting the preference for either phototransformation route, as well as the scope of the reaction for synthetic purposes, are currently being investigated.

The photoproduct **2a** is an inhibitor of cytidine deaminase with an affinity ($K_i \sim 5 \times 10^{-6}$M)[20] comparable with that of zebularine (1,2-dihydropyrimidin-2-one ribonucleoside).[21]

Experimental

Photoirradiation was carried out in a photoreactor RQ 400 (Applied Photophysics, London, UK), with a quartz immersion well, gas-inlet and low-pressure UV-lamp model 3016 (16W, light output 3×10^{18} photon s^{-1}, >90% of irradiation at 254 nm). Dilute aqueous solutions of substrates (as specified) were irradiated in a working volume of 400 ml. Irradiated solutions were separated from the UV-lamp by a filter solution of 2 mol l^{-1} sodium acetate in water, which filters out all the irradiation <235 nm. The irradiated solution was stirred with a stream of argon. Samples for UV-monitoring were taken *via* a syringe and diluted with distilled water, as specified.

NMR spectra

NMR Spectra were recorded on a Bruker AMX 400 spectrometer in [$^2H_6$]DMSO at 31 °C. This instrument operates at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C. All 2D experiments were recorded with the sample non-spinning. ^{13}C NMR spectra were acquired with proton decoupling *via* the *J*-modulation (JMOD)[22] pulse sequence using a $^1J_{CH}$ coupling constant of 140 Hz. *J* Values were recorded in Hz throughout. The NOE experiments were performed by the steady-state method. NOE Difference spectra[23a,b] were obtained by irradiating the peak of interest for 5 s. The multiplet was digitized and each point was irradiated for 5 ms in a cycle that was repeated one thousand times. This procedure resulted in about 90% saturation of the multiplet with suitable adjustment of the attenuation. The COSY 45[24] spectrum we refer to was routine. The 2D CH correlation experiments were performed by inverse detection: one 'standard' experiment[25] and one which was optimized to show long-range coupling.[26] To establish the direct connectivities between C-8 and C-4, and hence to confirm the presence of the cyclobutane ring, a 2D-INADEQUATE[27] experiment was utilized. The sequence employed was developed by Turner *et al.*[28] using the autoprogram 'inadsy' in the Bruker UXNMR software. A final read pulse of 135° was employed as suggested by Mareci and Freeman[29] and the data were processed with a sure-bell window function shifted by $\pi/4$ in both domains prior to Fourier transformation.[30] A critical choice in this insensitive experiment is the choice of the carbon–carbon coupling constant so that maximum coherence is excited. A value of $^1J_{CC}$ 35 was chosen for the experiment with a relaxation delay between transients of 3 s. The experimental time was 84 h.

Mass spectra

Fast Atom Bombardment mass spectra (FABMS) were obtained from a VG Zabspec mass spectrometer. Samples were dissolved in a small volume of *m*-nitrobenzyl alcohol which had previously been coated onto a stainless steel probe and spectra were obtained in the positive-ion mode at a scan speed of 10 s per decade. For accurate mass measurement, narrow voltage scanning at a resolution of 5000 was employed and polyethylene glycol was used as reference.

UV spectra

UV Spectra were recorded on Perkin-Elmer 552 and Perkin-Elmer Lambda 2 UV–VIS Spectrometers, respectively, in the solvents as specified.

4-(2′-Deoxy-β-D-*erythro*-pentofuranosyl)-7,7-dimethyl-2,4-diazabicyclo[4.2.0]octa-1,5-dien-3-one 2a

A solution of 5-*tert*-butyl-2′-deoxyuridine **1a** (568 mg) in distilled water (400 ml; 5×10^{-2} mol l^{-1}) was irradiated for 48 h, after which time the $\lambda_{max} = 314$ nm had reached a maximum absorbance and the substrate **1a** ($\lambda_{max} = 265$ nm) had been completely consumed (UV spectra of the solutions are shown in Fig. 1). The irradiated solution was freeze-dried to give a white powder (489 mg, 86% recovery of starting material) which was subsequently purified on a silica column (4.5 cm × 25 cm) using ethyl acetate–methanol (4:1) as eluent. The homogeneous fractions (TLC on pre-coated Merck silica gel 60 F254 plates, solvent system ethyl acetate–methanol, 4:1; $R_f = 0.38$) were combined and evaporated *in vacuo*, leaving the pure photoproduct **2a** as a white powder (186 mg, 35%); λ_{max}(EtOH)/nm 313.7 (ε/dm^3 mol^{-1} cm^{-1} 13 080), λ_{max} 250 (ε 380); λ_{max}(0.1 mol l^{-1} HCl) 322 (ε 14 210), λ_{min} 260 (ε 1130); λ_{max}(0.1 mol l^{-1} NaOH) 347 (ε 79 500), λ_{min} 275 (ε 2050); δ_H([$^2H_6$]DMSO) 7.98 (s, 1 H, H-6), 6.08 (t, 1 H, H-1′, $J_{1',2'}$ 6.5, $J_{1',2''}$ 6.5). 5.20 (d, 1 H, OH-3′, $J_{OH,3'}$ 3.5), 5.00 (t, 1 H, OH-5′, $J_{OH,5'5''}$ 4.5), 4.19 (m, 1 H, H-3′, $J_{3',4'}$ 3.5, $J_{3',2'}$, 6.5, $J_{3',2''}$ 4.0, $J_{3',OH}$ 3.5), 3.83 (m, 1 H, H-4′, $J_{4',3'}$ 3.5, $J_{4',5'}$ 3.5, $J_{4',5''}$ 3.5), 3.64–3.55 (m, 2 H, H-5′,5″, $J_{5',4'}$ 3.5, $J_{5'',4'}$ 3.5, $J_{5'5'',OH}$ 4.5, $J_{5',5''}$ −11.5), 3.08 (s, 2 H, H-8), 2.27 (m, 1 H, H-2″, $J_{2'',1'}$ 6.5, $J_{2'',3'}$ 4.0, $J_{2'',2'}$ −13.5), 1.92 (m, 1 H, H-2′, $J_{2',1'}$ 6.5, $J_{2',3'}$

6.5, $J_{2',2''}$ −13.5 Hz) and 1.39 (2 s, 6 H, Me-9,10); δ_C([$^2H_6$]-DMSO) 180.2 (C-4), 156.2 (C-2), 133.6 (C-6), 128.1 (C-5), 87.7 (C-4′), 86.6 (C-1′), 69.8 (C-3′), 60.8 (C-5′), 49.9 (C-8), 41.0 (C-2′), 40.9 (C-7) and 26.7 (C-9,10); *m/z* 533 (dimer of **2a** $[M + H]^+$), 267 (monomer of **2a** $[M + H]^+$) and 151 $[M - C_5H_9O_3]^+$ (Found: *m/z* 267.1345. Calc. for $C_{13}H_{18}N_2O_4$: 267.1339). This material gave an elemental analysis for the monohydrate of **2a** (Found: C, 54.3; H, 6.5; N, 10.4. Calc. for $C_{13}H_{18}N_2O_4 \cdot H_2O$: C, 54.9; H, 7.1; N, 9.9%).

Phototransformations of 5-Bu^t-2′-dU 1a, 5-Pr^i-2′dU 1b and 2′-dU 6a: a comparative study

Aqueous solutions of substrates **1a**, **1b** or **6a** (400 ml of 2×10^{-4} mol l^{-1} each), were irradiated under standard conditions (see above). At timed intervals an aliquot (1 ml) of the irradiated solution was taken and made up to 5 ml with distilled water in a volumetric flask and the UV spectrum measured. The absorbance at 265 and 314 nm in the case of substrate **1a**, at 267 and 307 nm in the case of substrate **1b** and at 262 nm in the case of 2′-dU **6a**, were plotted against time (Fig. 4). The UV spectra of the resulting solutions of substrates **1a** and **1b** at the end of irradiation (7 h) had the following parameters. In the case of substrate **1a** the ratio of absorbance at λ_{max} 314 (**2a**) to λ_{max} 265 (**1a**) was $A_2/A_1 = 3.3$ and the isosbestic point was at 282 nm. In the case of substrate **1b** the ratio of absorbance at λ_{max} 307 (**2b**) to $\lambda_{max} = 267$ (**1b**) was $A_4/A_3 = 1.7$ and the isosbestic point was at 292 nm. The relative yield of the photoproducts **2a** and **2b** was established from the ratio of absorbance at λ_{max} 314 (**2a**) to absorbance at λ_{max} 307 (**2b**). This ratio was $A_2/A_4 = 2.7$, which corresponds to a 20% yield of the photoproduct **2b** assuming a molar extinction coefficient of ε 13 080. In the case of 2′-dU **6a** after irradiation for 1.5 h the absorbance at λ_{max} 262 dropped to zero due to complete photohydration.

Regeneration of 2′-dU 6a in the irradiated solutions of 5-Bu^t-2′-dU 1a and 2′-dU 6a

Aqueous solutions of **1a** and **6a** (400 ml of 2×10^{-4} mol l^{-1} each) were irradiated for 7 h. Then 1 ml of irradiated solution was transferred (*via* pipette) into a 5 ml volumetric flask which was covered with aluminium foil and heated to 100 °C on a boiling water-bath. After heating was finished the volumetric flask was cooled to room temperature, made up to 5 ml with distilled water and the UV spectrum of the resulting solution measured. The complete regeneration of 2′-deoxyuridine **6a** was observed after 30 min of heating. The UV spectra of the irradiated solution of the compound **1a**, which was heated for 30 min, 4 h and 8 h respectively, confirmed an immediate regeneration of 2′-deoxyuridine **6a** as was indicated by the new λ_{max} 262, accompanied by the slow decomposition of the photoproduct **2a** (decrease of the λ_{max} 314 was clearly seen).

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