

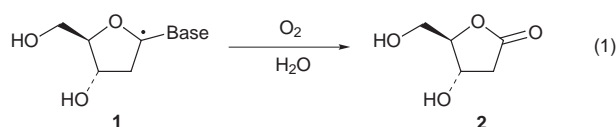
Fate of the C-1' peroxy radical in the 2'-deoxyuridine system

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The mechanism of 2-deoxyribonolactone formation from the reaction of photogenerated 2'-deoxyuridin-1'-yl radical with molecular oxygen in water has been investigated.

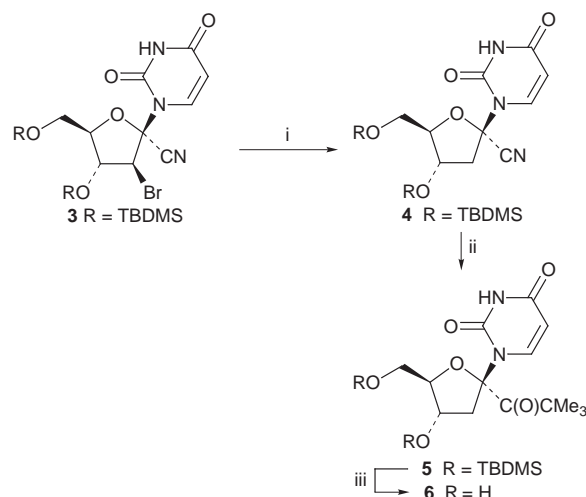
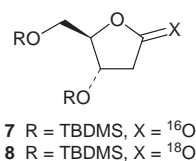
A number of agents are able to react with DNA to generate macromolecular radical species.¹ These processes are of considerable importance since they can lead to base modification or strand scissions. As research progresses in the area of the mechanism of attack of oxidative DNA cleavers, it becomes evident that hydrogen abstraction from the C-1' position is involved in many cases.^{2,3} Evidence for the existence of these radical species is mainly based on product studies,^{4,5} although spectroscopic and theoretical studies have been reported.^{6,7} Furthermore, based on the β -(acyloxy)alkyl radical rearrangement of a C-2' radical, we have suggested that C-1' radicals are stabilized substantially by the presence of the base and that the degree of stabilization is similar for purine and pyrimidine moieties.⁸ Under aerobic conditions, however, C-1' radicals **1** afford 2-deoxyribonolactone **2** [eqn. (1)] through a number of



currently disputed pathways.^{1,2,5} We report herein an *ex novo* synthesis of *tert*-butyl ketone **6**⁹ and the mechanism of the formation of **2** [eqn. (1)].

The recently reported synthesis of **6** has two major drawbacks:⁹ (i) a known psiconucleoside triol, prepared by a low yielding, laborious procedure, is used as the precursor,¹⁰ and (ii) a total lack of regioselectivity in the protection step of this triol is observed. For these reasons, we used as our precursor compound **3**, readily available in diastereomerically pure form from the corresponding protected 1',2'-didehydro-2'-deoxyuridine by a literature procedure.¹¹ When **3** was treated with (TMS)₃SiH¹² under normal free radical conditions, the crystalline cyanide **4** was obtained in excellent yield (94%) (Scheme 1).¹³ Short treatment of **4** with excess of Bu^tLi yielded, after silica gel purification, the protected *tert*-butyl ketone **5** as the major product (37% yield, 45% based on recovered starting material).[§] Finally, deprotection (NH₄F, MeOH, 60 °C, 24 h, 90%) produced the water soluble adduct **6**.

A quartz tube containing ketone **6** (23 mg; 0.073 mmol) in H₂O (200 μ l) was photolyzed with a 500 W high pressure mercury lamp at room temperature. During the photolysis, continuous bubbling of air through the sample ensured the presence of O₂ in the reaction mixture. After 6 h of photolysis, ¹H NMR analysis (CD₃CN) of the lyophilized sample indicated a product ratio of 1 : 0.15 : 0.15 for **6** : **2** : uracil.¹⁴ The products were derivatized with Bu^tMe₂SiCl/imidazole/DMF and isolated by flash chromatography (hexane–ethyl acetate 9:1) to give **7**¹⁴



Scheme 1 Reagents and conditions: i, (TMS)₃SiH, AIBN, toluene, 80 °C, 2 h, 94%; ii, 3 equiv. Bu^tLi, THF, -78 °C, 5 min, 37%; iii, NH₄F, MeOH, 60 °C, 24 h, 90%

and **5** in 15 and 85% yield, respectively. GC–MS analysis of compound **7** showed an isotopic cluster of [M - 57]⁺ as reported in Fig. 1(a). A control experiment showed that photolysis of the sample under anaerobic conditions gave neither compound **2** nor uracil among the products.

The oxygen source of the carbonyl in the lactone **2** was determined by photolysis of **6** in the presence of ¹⁶O–¹⁸O and

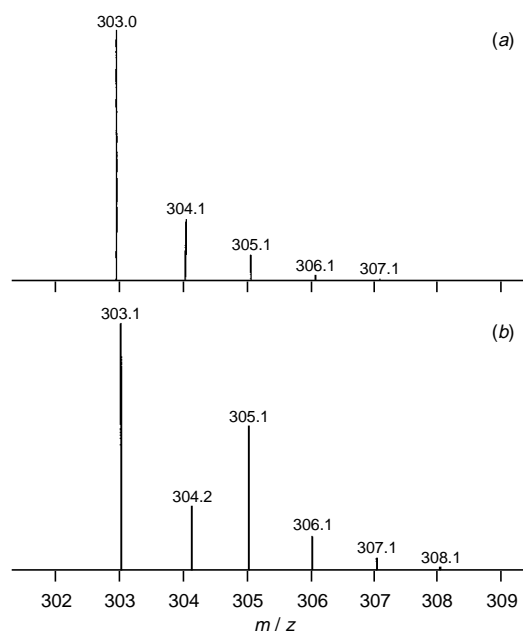
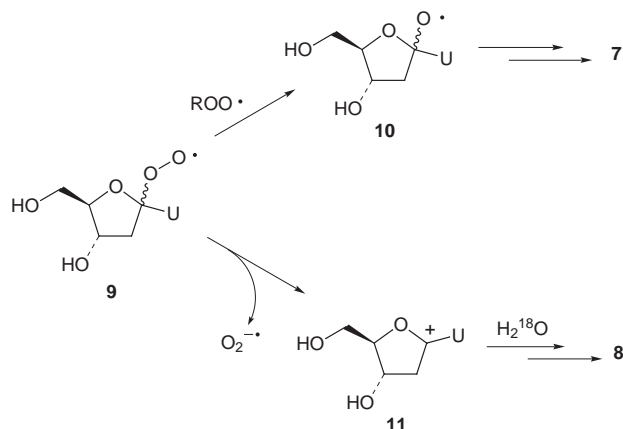


Fig. 1 Isotopic clusters of [M - 57]⁺ from the electron impact mass spectra of protected 2-deoxyribonolactone obtained by GC–MS analysis. (a) Sample isolated from the reaction in H₂¹⁶O (of natural isotopic distribution). (b) Sample isolated from the reaction in H₂¹⁸O (95 atom% ¹⁸O).



Scheme 2 Proposed mechanism for the formation of 2-deoxyribonolactone

H_2^{18}O . For this reason, the above experiment was performed in H_2^{18}O (95 atom% ^{18}O) as solvent. After work-up, the protected 2-deoxyribonolactone was analyzed by GC-MS. Inspection of the mass spectrum of the isotopic cluster of $[\text{M} - 57]^+$, shown in Fig. 1(b), indicates the presence of coeluting isotopomers **7** and **8**. Analysis of this isotopic cluster revealed that the product of interest contains 65 and 35% oxygen-16 and oxygen-18, respectively. A control experiment showed that the product lactone **2** does not exchange oxygen with the solvent under the conditions employed.

The mechanism we envisage for the formation of the ^{18}O -labelled 2-deoxyribonolactone is outlined in Scheme 2. Reaction of C-1' radical **1** with O_2 gives the peroxy radical **9**. Laser flash photolysis studies showed that rate constant for oxygen trapping of the C-1' radical is about $1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$.⁷ The peroxy radical **9** decays either *via* a bimolecular reaction with another peroxy radical to generate the alkoxyl radical **10** or *via* a unimolecular path (heterolytic cleavage) to generate the carbocation **11** and superoxide radical anion.^{15a} The heterolytic cleavage of peroxides or alternatively the reaction of electron-rich carbon-centered radicals to give superoxide and carbocations is not without precedent.¹⁵ The cationic intermediate **11** was trapped by H_2^{18}O , thus demonstrating the partition between the two channels.

An important consequence of the mechanism in Scheme 2 is that the C-1' peroxy radical generated on DNA, in the absence of good hydrogen donors, should mainly undergo heterolytic cleavage since the probability that two macromolecular peroxy radicals meet is low. This finding accentuates the different chemical reactivity exhibited by the C-1' radical species when compared with the one observed in the more studied C-5' and C-4' positions,¹ a reactivity which is mainly due to the presence of the two α -heteroatoms present in the anomeric position. Further work on the reactions of compounds **3** and **4** with a variety of electrophiles and on the kinetics of radical reactions associated with the C-1' position is in progress.

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Notes and References

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§ Selected data for **5**: white solid, mp (pentane) 179–181 °C. δ_{H} (200 MHz; C_6D_6) –0.06, –0.04 (3 H each, s, SiMe), –0.01 (6 H, s, $2 \times \text{SiMe}$), 0.86, 0.91 (9 H each, s, SiBu^t), 1.27 (9 H, s, Bu^t), 2.26 (1 H, dd, J 13.8, 7.4, 2' α -H), 3.46 (1 H, dd, J 12.3, 2.4, 5' α -H), 3.62 (2 H, m, 4', 5' β -H), 3.75 (1 H, dd, J 13.8, 8.8, 2' β -H), 4.41 (1 H, ddd, J 8.8, 7.4, 3'-H), 5.73 (1 H, d, J 8.2, 5-H), 8.15 (1 H, d, J 8.2, 6-H), 9.29 (1 H, bs, NH); δ_{C} (50 MHz; C_6D_6) –5.63, –5.57, –5.02, –4.42 (each CH_3), 18.0, 18.4 (each C), 25.78, 25.79, 29.0 (each $3 \times \text{CH}_3$), 42.5 (CH_2), 43.5 (C), 60.2 (CH_2), 68.5, 87.0 (each CH), 97.5 (C), 102.0, 139.0 (each CH), 150.5, 163.2, 201.2 (each C).

- DNA and RNA Cleavers and Chemotherapy of Cancer and Viral Diseases*, ed. B. Meunier, Kluwer, Dordrecht, 1996; for a review, see G. Pratviel, J. Bernadou and B. Meunier, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 746.
- For a recent review, see C. Chatgililoglu and T. Gimisis, in *Free Radicals in Biology and Environment*, ed. F. Minisci, Kluwer, Dordrecht, 1997, pp. 281–292.
- For some recent work, see X. Zeng, Z. Xi, L. S. Kappen, W. Tan and I. H. Goldberg, *Biochemistry*, 1995, **34**, 12435; Y.-J. Xu, Z. Xi, Y.-S. Zhen and I. H. Goldberg, *Biochemistry*, 1995, **34**, 12451; M. Pitić, J. Bernadou and B. Meunier, *J. Am. Chem. Soc.*, 1995, **117**, 2935; H. Sugiyama, K. Fujimoto and I. Saito, *J. Am. Chem. Soc.*, 1995, **117**, 2945; H. Sugiyama, K. Fujimoto, I. Saito, E. Kawashima, T. Sekine and Y. Ishido, *Tetrahedron Lett.*, 1996, **37**, 1805; G. P. Cook and M. M. Greenberg, *J. Am. Chem. Soc.*, 1996, **118**, 10025; T. Melvin, S. W. Botchway, A. W. Parker and P. O'Neil, *J. Am. Chem. Soc.*, 1996, **118**, 10031; M. M. Meijler, O. Zelenko and A. S. Sigman, *J. Am. Chem. Soc.*, 1997, **119**, 1135; H. Sugiyama, K. Fujimoto and I. Saito, *Tetrahedron Lett.*, 1997, **38**, 8057; O. Zelenko, J. Gallagher and A. S. Sigman, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 2776.
- T. Gimisis and C. Chatgililoglu, *J. Org. Chem.*, 1996, **61**, 1908; T. Gimisis, C. Castellari and C. Chatgililoglu, *Chem. Commun.*, 1997, 2089.
- I. H. Goldberg, *Acc. Chem. Res.*, 1991, **24**, 191; B. K. Goodman and M. M. Greenberg, *J. Org. Chem.*, 1996, **61**, 2.
- E. G. Hole, W. H. Nelson, E. Sagstuen and D. M. Close, *Radiat. Res.*, 1992, **129**, 119; D. M. Close, W. H. Nelson, E. Sagstuen and E. O. Hole, *Radiat. Res.*, 1994, **137**, 300; K. Miaskiewicz and R. Osman, *J. Am. Chem. Soc.*, 1994, **116**, 232; A.-O. Colson and M. D. Sevilla, *J. Phys. Chem.*, 1995, **99**, 3867.
- C. Chatgililoglu, T. Gimisis, M. Guerra, C. Ferreri, C. J. Emanuel, J. H. Horner, M. Newcomb, M. Lucarini and G. F. Pedulli, *Tetrahedron Lett.*, in the press.
- T. Gimisis, G. Ialongo, M. Zamboni and C. Chatgililoglu, *Tetrahedron Lett.*, 1995, **36**, 6781; T. Gimisis, G. Ialongo and C. Chatgililoglu, *Tetrahedron*, 1998, **54**, 573.
- M. M. Greenberg, D. J. Yoo and B. K. Goodman, *Nucleosides Nucleotides*, 1997, **16**, 33.
- A. Holy, *Nucleic Acids Res.*, 1974, **1**, 289.
- K. Haraguchi, Y. Itoh, H. Tanaka, K. Yamaguchi and T. Miyasaka, *Tetrahedron Lett.*, 1993, **34**, 6913; Y. Itoh, K. Haraguchi, H. Tanaka, E. Gen and T. Miyasaka, *J. Org. Chem.*, 1995, **60**, 656.
- C. Chatgililoglu, *Acc. Chem. Res.*, 1992, **25**, 118; C. Chatgililoglu, *Chem Rev.*, 1995, **95**, 1229.
- The reduction using Bu_3SnH was reported to give 77% yield, see Y. Yoshimura, F. Kano, S. Miyazaki, N. Ashida, S. Sakata, K. Haraguchi, Y. Itoh, H. Tanaka and T. Miyasaka, *Nucleosides Nucleotides*, 1996, **15**, 305.
- For compounds **2** and **7**, see F. J. Lopez-Herrera, M. Valpuesta-Fernandez and S. Garcia-Claros, *Tetrahedron*, 1990, **46**, 7165; A. P. Kozikowski and A. K. Ghosh, *J. Org. Chem.*, 1984, **49**, 2762.
- (a) C. von Sonntag and H.-P. Schuchmann, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1229; (b) K. U. Ingold, T. Paul, M. J. Young and L. Doiron, *J. Am. Chem. Soc.*, 1997, **119**, 12364.

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