# Solution Structures of Some ' Uridine Dialdehyde ' Derivatives

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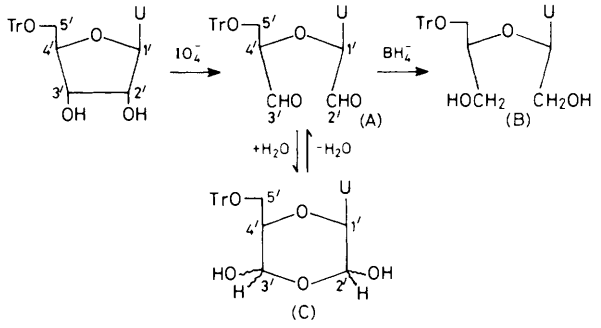
When uridine is oxidised with periodate, the product is ' uridine dialdehyde.' Contrary to a previous report we have shown by $^1$H n.m.r. spectroscopy that in solution, this compound is not polymeric but consists of a large number of isomers in dynamic equilibrium, although even at 400 MHz the spectrum is too complicated to analyse fully. However if the 5'-OH group is not present, such as in 5'-azido-5'-deoxyuridine dialdehyde or 5'-*O*-trityluridine dialdehyde, only three diastereoisomeric cyclic acetals are present. The $^1$H n.m.r. spectrum at 400 MHz of the latter compound has been completely analysed and the identity and amounts of each of the three diastereoisomers have been determined.

Oxidation of ribonucleosides with periodate gives α'-substituted derivatives of α-(hydroxymethyl)oxydiacetaldehyde (A), hereafter referred to as ribonucleoside dialdehydes.[1-3] Originally, these compounds were thought to be unstable, but recently they have been isolated and in some cases crystallised. However the precise structures of the molecules present in solution are still far from clear.

I.r. and n.m.r. spectroscopic analysis of adenosine dialdehyde, which is said to give broad peaks characteristic of polymeric aldehydes with asymmetric centres,[4] certainly confirms that the amount of free aldehyde present in the solution is very small,[5] although such a solution can be reduced quantitatively to the corresponding triol (B). The dialdehydes move on t.l.c. as single but elongated spots in several solvent systems and are more hydrophobic than the parent nucleosides. It is clear however from the most cursory inspection of a high-field n.m.r. spectrum of a nucleoside dialdehyde that, in solution, there are present several distinct species which give rise to sharp signals and there is no reason to suppose that any significant amount of polymeric species is present.

The evidence seems reasonably conclusive that in the solid state, nucleoside dialdehydes are polymeric[6] and various structures have been proposed for these polymeric forms. We have used the Bruker WH400 instrument to investigate the $^1$H n.m.r. (and briefly the $^{13}$C n.m.r.) spectrum of uridine dialdehyde and some of its derivatives in an attempt to clarify the nature of the individual species present in solution. However, at this resolution, the spectrum of uridine dialdehyde showed the presence of at least 12 different doublets from H-6 of uracil, each accounting for between 2 and 20% of the total species present (Figure 1). On raising the temperature from 22 to 70 °C, the proportions of the individual species present varied, demonstrating that one was looking at a complex equilibrium mixture. There was no, or very little, free aldehyde present but the mixture was far too complex to be analysed completely. Thus we turned to the study of some derivatives in the hope that a less complex mixture could be obtained.

The n.m.r. spectrum of the 5'-azido-5'-deoxy derivative of uridine dialdehyde was surprisingly simple, containing only three main signals (doublets) which could be assigned to H-6 (data not shown). Once again, if the temperature of the solution was increased, the proportions of the signals changed, showing that they were being produced by an equilibrium mixture of isomers (data not shown). Thus it appears that the removal of the 5'-OH group from the molecule prevents the formation of several species, which suggests that, in the free dialdehydes, at least some of the species present arise from




**Scheme 1.** Reaction of 5'-*O*-trityluridine dialdehyde with periodate and borohydride

reaction between the 5'-OH and the aldehyde groups. Once again, there was no evidence for the presence of any polymeric species.

At about this time, Lowe and Beechey[7] reported the $^1$H n.m.r. spectrum at 360 MHz of the dialdehyde of adenosine 5'-triphosphate. They also concluded that no free aldehyde or polymer was present and from a simple conformational analysis showed that the spectrum obtained was consistent with that expected for three of the four possible stereoisomeric forms of the cyclic hemiacetals formed in a equilibrium mixture of the dialdehyde hydrate (C). Some free dihydrate was also identified.

We therefore decided to analyse completely the $^1$H n.m.r. spectrum of a uridine dialdehyde derivative of a type such that some stereochemical control over the stereoisomer composition could be exerted. The derivative chosen was 5'-*O*-trityluridine dialdehyde.

The $^1$H n.m.r. spectrum obtained is shown in Figure 2, and the main peaks and their assignment are given in Table 1. The three diastereoisomer species present (I)—(III) are shown in Scheme 2.

To facilitate discussion, the $^1$H n.m.r. spectrum can be divided into four sections: δ 11.4—11.6 (N-3 protons), 6.7 – 7.9 (trityl protons and H-6), 4.8—5.8 (H-5, H-1', H-2', and H-3'), and 2.8—4.1 (H-4' and H-5').

$^1$H *N.m.r. Spectrum.*—(a) δ 11.4—11.6. In this range, there are three doublet resonances (1—3) which can be assigned to the N-3 protons of the uracil base, coupled to H-5 (resonances 13, 14, and 16) of the base [coupling constants for (I) and (II), $J_{NH,5}$ 2.3 Hz; for (III), $J_{NH,5}$ 2.0 Hz].

(b) δ 6.7—7.9. Resonances 4—12 and those of the trityl

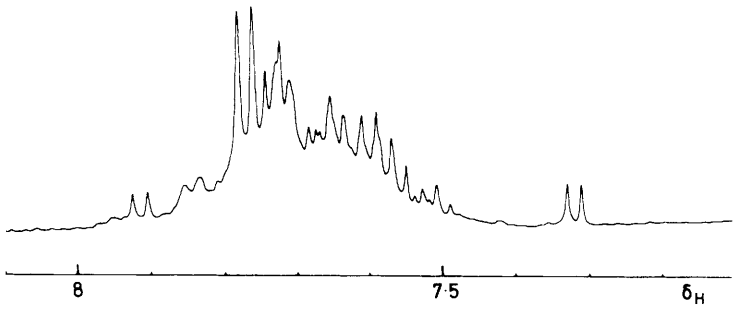



**Figure 1.** $^1$H N.m.r. spectrum from δ 8 to 7.5 (corresponding to uracil H-6) for uridine dialdehyde

**Table 1.** Chemical shifts and coupling constants of $^1$H n.m.r. spectrum of 5′-*O*-trityluridine in $(CD_3)_2SO$ †

| Resonance no. | Chemical shift (δ) | Integration | Multiplicity | *J*/Hz | Assignment: Proton | Assignment: Structure * |
|---|---|---|---|---|---|---|
| 1 | 11.495 | 13 | d | 2.3 | N–H | (I) |
| 2 | 11.478 | 7 | d | 2.3 | N–H | (II) |
| 3 | 11.456 | 12 | d | 2.0 | N–H | (III) |
| 4 | 7.824 | 59 | d | 8.2 | H-6 | (I) |
| 5 | 7.664 | } 61 | d | 8.0 | H-6 | (III) |
| 6 | 7.644 | | d | 8.0 | H-6 | (II) |
| 7 | } ~7.25 | | d | 6.3 | 2′-OH | (I) |
| 8 | | | | | | |
| | | | | | 2′-OH or 3′-OH | (II), (III) |
| 9 | 7.06 | 13 | d | 6.9 | 2′-OH | (III) |
| 10 | 7.02 | 9 | d | 7.2 | 3′-OH | (I) |
| 11 | 6.83 | } 19 | d | 6.3 | 2′-OH or 3′-OH | (II), (III) |
| 12 | 6.81 | | d | 8.0 | 3′-OH | (II) |
| 13 | 5.775 | } 85 | dd | 2.3, 8.0 | H-5 | (II) |
| 14 | 5.739 | | dd | 2.3, 8.2 | H-5 | (I) |
| 15 | 5.708 | 21 | d | 1.8 | H-1′ | (II) |
| 16 | 5.708 | 30 | dd | 8.0, 2.0 | H-5 | (III) |
| 17 | 5.383 | 42 | d | 7.55 | H-1′ | (III) |
| 18 | 5.328 | 60 | d | 7.55 | H-1′ | (I) |
| 19 | 5.206 | 32 | dd | 7.9, 8.0 | H-3′ | (II) |
| 20 | 5.144 | 39 | dd | 7.55, 6.9 | H-2′ | (III) |
| 21 | 5.063 | 57 } 244 | dd | 7.55, 6.5 | H-2′ | (I) |
| 22 | } 5.015 | 61 | m | 1.8, 6.3 | H-2′, H-3′ | (II), (III) |
| 23 | | | | | | |
| 24 | 4.905 | 55 | dd | 7.9, 7.2 | H-3′ | (I) |
| 25 | 4.077 | 35 | m | 1.8, 6.9, 6.4 | H-4′ | (III) |
| 26 | 3.803 | 25 } 114 | m | 7.9, 5.4, 2.0 | H-4′ | (II) |
| 27 | 3.624 | 54 | m | 7.9, 5.25, 2.6 | H-4′ | (I) |
| 28 | 3.13 + 3.09 | | m | 2.0, 5.4, 10.2 | 2H-5′ | (II) |
| 29 | 3.07 + 3.07 | 232 | m | 2.6, 5.25, — | 2H-5′ | (I) |
| 30 | 3.06 + 2.94 | | m | 4.9, 6.9, 10.0 | 2H-5′ | (III) |

* See Scheme 2. † δ[$(CH_3)_2SO$] taken as 2.50.

protons are present here. Resonances 4—6 are doublets (*J* 8.0—8.2 Hz) and decoupling experiments show that they are coupled to resonances 13, 14, and 16. The total integrals of each of these sets are the same. Hence we assign resonances 4, 5, and 6 to H-6 of the uracil base and resonances 13, 14, and 16 to H-5 of the uracil base.

The trityl protons appear as a complex set of resonances between δ 7.2 and 7.4.

Resonances 7—12 are doublets (*J* 6.3—8.0 Hz) and decoupling experiments show that they are coupled to resonances 19—24. We assign 7—12 to hydroxy protons, as they are coupled to H-2′ and H-3′, and individually as in Table 1.

(c) δ 4.8—5.8. This region contains resonances 13—24 which correspond to H-5, H-1′, H-2′, and H-3′. There is a complex series of resonances (13—16) between δ 5.66 and 5.8 consisting of doubled doublets. Decoupling experiments show that resonances 13, 14, and 16 are coupled to the H-6 resonances (4, 5, and 6, *J* 8.0—8.2 Hz). Irradiation at the frequencies of these resonances (13—16) causes the resonance 22/23 to collapse. Thus resonance 15 is coupled to resonance 22/23 (*J* 1.8 Hz), which must be from a sugar moiety proton; therefore resonance 15 has been assigned to H-1′ for isomer (II), which has an axial-equatorial H-1′,H-2′ coupling. Resonances 13, 14, and 16 have been assigned to H-5. The further splitting

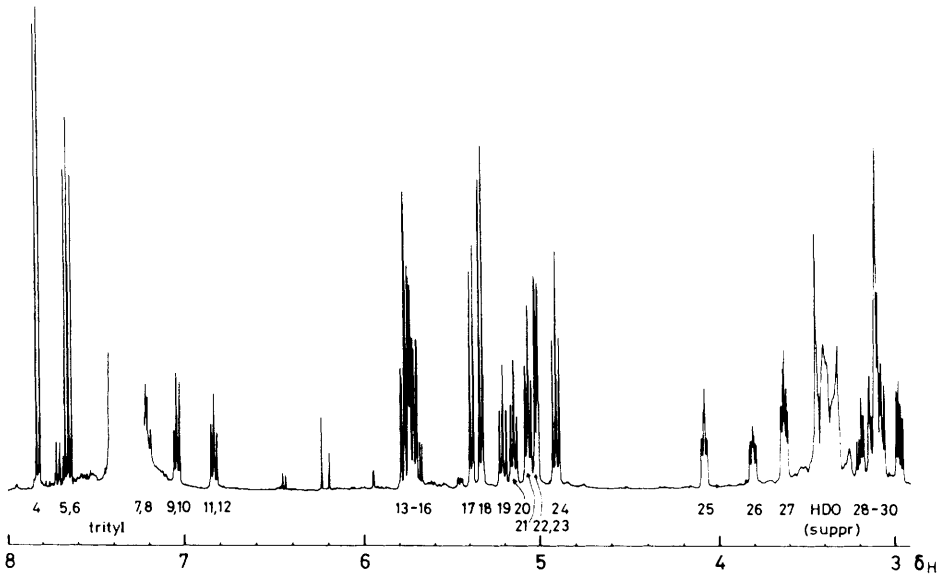
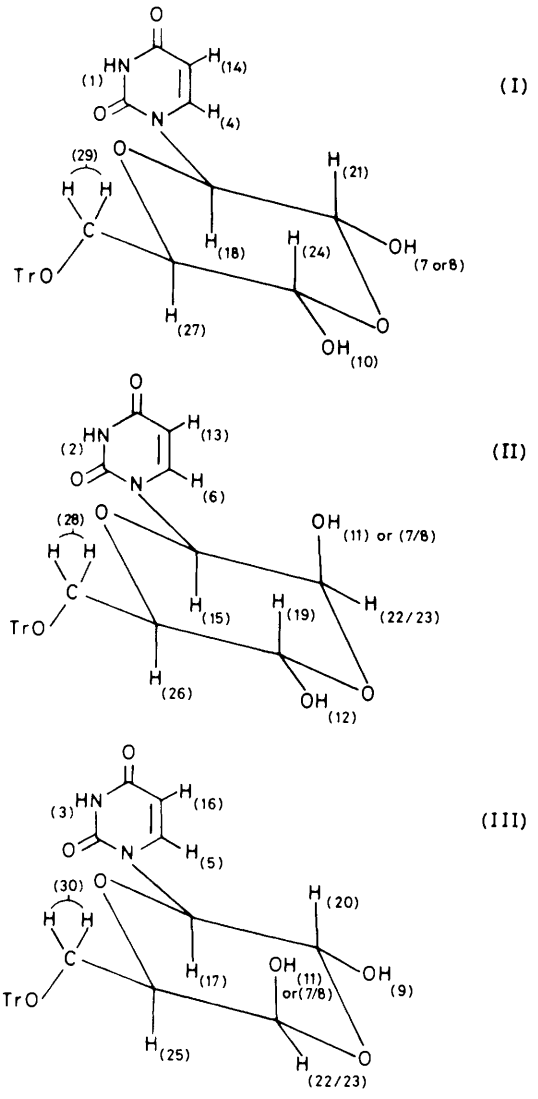



**Figure 2.** $^1$H N.m.r. spectrum of 5′-*O*-trityluridine dialdehyde




**Scheme 2.** Formulae of the three diastereoisomeric species present in a solution of 5′-*O*-trityluridine dialdehyde

(*J* 2.0, 2.3) is caused by coupling with the N–H of the uracil base.

Resonances 17—24 appear as a series of doublets and pseudo-triplets. Resonances 17 and 18 are doublets that are coupled to resonances 20 and 21 (*J* 7.55 Hz). The integral of resonances 13—18 is equivalent to that expected for two protons and thus resonances 15, 17, and 18 have been assigned to H-1′.

Resonances 19—21 and 24 are pseudo-triplets which are coupled to the hydroxy protons (resonances 7—12, *J* 6.3—8 Hz) and to resonances 17, 18, 26, and 27 (*J* 7.55—7.9 Hz). Resonances 20 and 21 have been assigned to H-2′ because they are coupled to resonances 17 and 18 (assigned to H-1′). The coupling between H-1′ and H-2′ is consistent with their being mutually axial.

Resonances 22 and 23 are not fully resolved and are coupled to resonances 15 (*J* 1.8 Hz) and 25 (*J* 1.8 Hz) and the hydroxy signals 7/8 and 11 (*J* 6.3 Hz). As one of these resonances is coupled to resonance 15 (H-1′) and a hydroxy signal, it must be due to H-2′ in an equatorial position. The other resonance (22/23) has been assigned to H-3′ in an equatorial position, *via* its small coupling.

Resonances 24 and 19 are pseudo-triplets coupled to hydroxy signals (10, *J* 7.2 Hz, and 12, *J* 8.0 Hz, respectively) and resonances 27 and 26 (*J* 7.9 Hz). These resonances must be due to the remaining H-3′ with a diaxial relationship to H-4′ in isomers (I) and (II). The total integral for resonances 19—24 is consistent with this assignment.

(d) δ 2.8—4.1. Resonances 25—27 are multiplets which have the form expected for an AMNX system and have a total integral equivalent to one proton. Resonances 25, 26, and 27 are coupled with resonances 22/23, 19, and 24, respectively (*J* 1.8, 7.9, and 7.9 Hz). The double doublets remaining after irradiation show that the protons are also coupled to two further protons (resonance 25, *J* 6.9 and 4.9 Hz; resonance 26, *J* 2.0 and 5.4 Hz; resonance 27, *J* 2.6 and 5.25 Hz).

As resonances 19, 22/23, and 24 have been assigned to H-3′, then resonances 25, 26, and 27 can be assigned to H-4′, further split by the two anisochronous 5′-protons.

Resonances 28—30 are due to the two 5′-protons coupling to H-4′ (coupling constants already given) and to each other (resonance 28, *J* 10.2 Hz; resonance 29, *J* not obtainable; resonance 30, *J* 10 Hz).

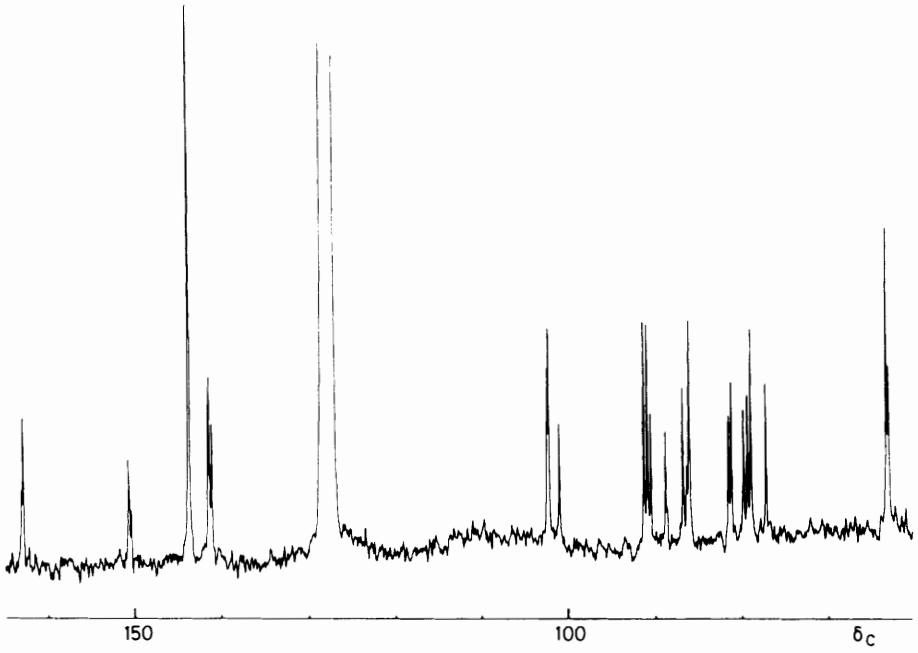



**Figure 3.** $^{13}C$ N.m.r. spectrum of 5′-*O*-trityluridine dialdehyde

The orientation of the 5′-methylene group and the trityl group can be estimated from the value of the 4′,5′-coupling constants. In the structures where the 3′-OH group is equatorial [(I) and (II)], the 5′-oxygen atom is below the plane of the ring. When the 3′-OH group is axial [in (III)] the 5′-oxygen atom is in the plane of the ring, pointing away from the OH group.

*Nuclear Overhauser Effect Difference Spectra.*—The abundances of the three isomers [(I)—(III)] are very similar; therefore n.O.e. difference spectra were required to confirm the relationship between protons.

Irradiation at the frequency of resonance 24 (H-3′) gave an n.O.e. at resonance 21 (H-2′) which thus allowed the protons of structure (I) to be assigned.

Irradiation at the frequency of resonance 26 (H-4′) gave an n.O.e. at resonance 15 (H-1′), which permitted unequivocal assignment of the protons in structure (II).

Irradiation at the frequency of resonance 17 (H-1′) gave an n.O.e. at resonance 25 (H-4′), which confirmed the proton assignment in structure (III).

$^{13}C$ *N.m.r.*—There are no resonances between 180 and 210 p.p.m., the region in which free carbonyl carbons would resonate. All resonances (Figure 3) can be grouped in threes, as would be predicted for an equilibrium mixture of the three isomers (I)—(III).

*Variable-temperature Spectra.*—Spectra taken at different temperatures show that the three isomers are in dynamic equilibrium in solution, which must prohibit the isolation of individual isomers. The percentage of each isomer present at two different temperatures is given in Table 2.

*Spectra in* $(CD_3)_2SO$–$D_2O$.—To a solution of the dialdehyde in $(CD_3)_2SO$ was added a drop of $D_2O$ in an attempt to produce the dihydrated isomer. There was no evidence for the formation of such a species; the only changes in the spectra could be attributed to exchange of N–H and O–H protons. The ratios of the three isomers (I)—(III) were affected by addition of water such that the isomer ratio at 298 K with $D_2O$ added is very similar to the isomer ratio at 320 K in the absence of $D_2O$.

**Table 2.** Isomer composition of 5′-*O*-trityluridine at 298 and 320 °C

| | Isomer (%) | | |
|---|---|---|---|
| *t*/°C | (I) | (II) | (III) |
| 298 | 44 | 26 | 30 |
| 320 | 40 | 33 | 26 |

*Conclusion.*—An unambiguous assignment has been made for all significant resonances in the $^1H$ n.m.r. spectrum of 5′-*O*-trityluridine dialdehyde. This shows that, in solution, the dialdehyde consists of a dynamic equilibrium mixture of three of the four possible forms of the cyclic hemiacetal of the dialdehyde monohydrate. The absent isomer is thermodynamically very unfavourable; it would possess two axial hydroxy groups on the six-membered ring. The $^1H$ n.m.r. spectrum of 5′-azido-5′-deoxyuridine dialdehyde is also consistent with three similar species being present; it is not until there is a free 5′-OH group in the molecule, such as in uridine dialdehyde itself, that many more isomers, once again in a dynamic equilibrium, are present. The spectrum of uridine dialdehyde is too complicated to be analysed fully but it seems reasonable to suppose that many of these additional resonances are due to forms in which the 5′-OH group is involved in cyclic hemiacetal formation. There is no need to invoke the presence of polymeric species in solution; indeed the evidence suggests that such species are not present.

## Experimental

N.m.r. spectra were obtained at 400.13 MHz ($^1H$) and at 100.62 MHz ($^{13}C$) using a Bruker WH400 spectrometer, under standard conditions. The HOD peak was suppressed by pre-irradiation. The sample for $^{13}C$ n.m.r. was maintained at

305 K. $^1$H Nuclear Overhauser effect difference spectra typically required 256 transients with the irradiation position alternating at every eighth.

Uridine dialdehyde was prepared as previously described.[5]

5′-O-*Trityluridine Dialdehyde*.—To 5′-*O*-trityluridine (5.0 g) in acetone (350 ml) was added sodium periodate (2.27 g) in water (150 ml). The solution was left for 24 h at 20 °C in the dark, then evaporated to dryness under reduced pressure to give a white solid which was extracted twice with water. The residual solid was dissolved in acetone. The suspension was filtered and the filtrate evaporated to dryness. The product was dried *in vacuo* at 70 °C for 24 h ($P_2O_5$) to give a white *solid* (4.6 g, 89%) (Found: C, 65.6; H, 5.2; N, 5.5. $C_{28}H_{24}N_2O_6 \cdot 1.6H_2O$ requires C, 65.5; H, 5.3; N, 5.45%).

5′-O-*Trityluracil Triol*.—To 5′-*O*-trityluridine dialdehyde (1.0 g) in ethanol–water (100 ml; 7 : 3) was added sodium borohydride (0.97 g), and the solution was kept at 20 °C for 16 h in the dark. The pH was then adjusted to 7 with HCl, and the solution evaporated to dryness under reduced pressure. The residual solid was purified by fractionation on a silica column eluted with chloroform to remove impurities and then with chloroform–ethanol (9 : 1) to give the required *compound* (0.83 g, 82%) (Found: C, 69.15; H, 5.4; N, 5.4. $C_{28}H_{28}N_2O_6$ requires C, 68.85; H, 5.75; N, 5.75%).

5′-*Azido-5′-deoxyuridine Dialdehyde*.—To 5′-azido-5′-deoxyuridine (2.3 g) in water (200 ml) was added sodium periodate (1.88 g) and the solution was kept for 24 h at 20 °C in the dark. It was then evaporated to dryness under reduced pressure to yield a pale yellow solid. This was extracted with acetone and the organic solution was evaporated to dryness to give the required *compound* (2.55 g, 100%) (Found: C, 38.6; H, 3.6; N, 24.5. $C_9H_9N_5O_5 \cdot 0.85H_2O$ requires C, 38.3; H, 3.8; N, 24.8%).

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