Kinetics and spectral properties of electron adducts of 2'-deoxyinosine: a comparison with other purine nucleosides

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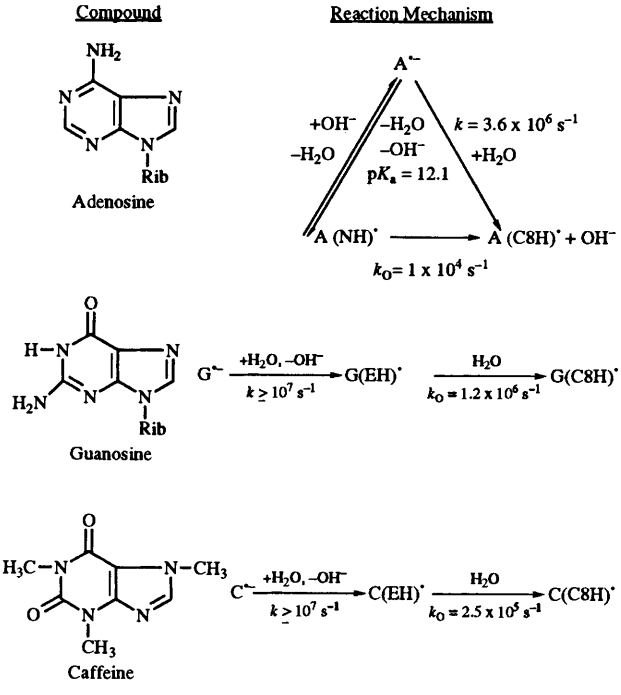
The heteroatom-protonated electron adducts of 2'-deoxyinosine formed following rapid protonation of the initially-produced radical anions in neutral solution exhibit a broad peak around 310 nm. In neutral solutions, these radicals transform spontaneously, in a slow process ($k \sim 2 \times 10^4$ s^{-1}), into *C*-protonated adducts. This reaction is catalysed by OH^- and the absorption changes at 350 nm *vs.* pH consist of two types of pK_a curve. The transformation rates in 2'-deoxyinosine are somewhat higher than those found for inosine and are in accord with the yields of $MV^{\bullet+}$. The spectrum at pH $\geqslant$ 13.5 closely resembles the H-adduct spectrum recorded at neutral pH. This spectrum with $\varepsilon_{315} = 5900$ and $\varepsilon_{350} = 4400$ dm^3 mol^{-1} cm^{-1} is assigned to the C-8 protonated adducts. This study suggests that the heteroatom-protonated and C-2 protonated electron adducts of 2'-deoxyinosine are probably less stable than the corresponding radicals of inosine.

Introduction

An understanding of the mechanism for the transformation reactions occurring in the electron adducts of pyrimidines[1-6] and purines[7-14] and their nucleosides is important in formulating the chemical basis[15] for radiation-induced DNA damage. Conversion of the initially-formed heteroatom-protonated electron adducts into final *C*-protonated species is a general phenomenon reported[10-14] for the nucleosides of all the purine systems. Such a transformation, however, was not observed with the nucleobases themselves (*e.g.* adenine, guanine and hypoxanthine). Furthermore, the rates of transformation from heteroatom-protonated species into *C*-protonated adducts of various nucleosides were found to be different. For example, the rate for the spontaneous *C*-protonation in neutral solutions of guanosine[11] was reported to be the fastest ($k = 1.2 \times 10^6$ s^{-1}) whereas it was only marginal in both adenosine[10] and inosine[13] ($k \sim 1 \times 10^4$ s^{-1}). In a recent study, we have shown[14] that fast protonation at carbon also takes place with the purine analogue, caffeine.

It is known[10,11] that both the kinetic (faster protonation at the heteroatoms than at carbon) and the thermodynamic (higher stability of the *C*-protonated adducts) factors control this transformation phenomenon. The reasons for the observed variation in the rates for the transformation processes are, however, not yet clearly understood, though the electron affinities and the ionisation potentials of the parent compounds seem to determine this variation. Thus, the nature and the position of the substituents obviously play a role in affecting this variation (Scheme 1).

In spite of detailed investigations reported on the transformation mechanism in adenosine,[10] guanosine[11] and inosine[13] (Ino) systems, not much attention has been paid to their deoxy derivatives. In this paper we show from a pulse radiolysis study of the reactions of e^-_{aq} with 2'-deoxyinosine (dIno) that differences in the sugar moiety affect, albeit to a minor extent, the rates of transformation and stability of the electron adducts.



Scheme 1

Experimental

High purity (> 98%) 2'-deoxyinosine obtained from Sigma was used in all the experiments. Solutions were prepared in water obtained from the Millipore-Milli-Q purification system. The solute concentration was usually maintained at 1×10^{-3} mol dm^{-3}. N_2-saturated solutions containing either propan-2-ol or 2-methylpropan-2-ol (*tert*-butyl alcohol) to scavenge the OH radical were pulse radiolysed using a linear accelerator delivering electron pulses of 7 MeV energy of 50 ns duration. A KSCN dosimeter was used to monitor the dose per pulse, with $G\varepsilon_{500} = 21\,520$ dm^3 mol^{-1} cm^{-1} and the dose per pulse normally kept at 15 Gy. The details of the pulse radiolysis set-up used are described elsewhere.[16]

Results and discussion

Evaluation of kinetic parameters

The e^-_{aq} monitored from its absorption at 720 nm decayed very quickly, as demonstrated by its reaction with dIno. The relevant

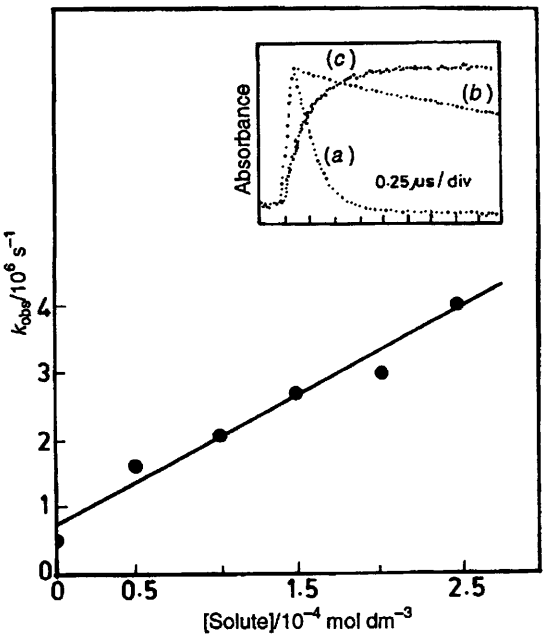


Fig. 1 Plot of k_{obs} *vs.* concentration of 2′-deoxyinosine. Inset: absorbance traces at 720 nm (*a*) with and (*b*) without solute and (*c*) build-up of absorbance at 310 nm.

Table 1 Second-order rate constants (k/10^9 dm^3 mol^{-1} s^{-1}) and the (ε/dm^3 mol^{-1} cm^{-1}) at different λ_{max}/nm values calculated in the reaction of e^-_{aq} with hypoxanthine and its nucleosides

Substrate	pH	k	λ	ε
Hypoxanthine[a]	6.5	9.6	300	4150
	11.0	1.3	310	3100
Inosine[a]	6.5	9.8	300	5400
	10.6	1.0	320	4600
	13.5	1.0	320	5800
			350	9900
2′-Deoxyinosine	6.0	10.0	310	5200
	13.5	1.2	350	5900

[a] Values for hypoxanthine and inosine are taken from ref. 13.

traces showing its decay with and without solute are depicted in the inset of Fig. 1. The bimolecular rate constant obtained from the plot of k_{obs} *vs.* concentration (Fig. 1) at neutral pH is 1×10^{10} dm^3 mol^{-1} s^{-1} and this value for dIno is similar to those reported [10,11,13] for the nucleosides of other purines. The intercept (5×10^5 s^{-1}) obtained in this plot is due to the reaction of e^-_{aq} with some impurities (*e.g.* acetone) present in water and/or by H^+ formed during the pulse.

A decrease in the rate in basic solutions was observed and the k value was found to be 1.2×10^9 dm^3 mol^{-1} s^{-1} at pH $\geqslant$ 11. Such a reduction in the rate constants with pH was also reported [13] with hypoxanthine and Ino which was attributed to the decrease in the electron affinity of the six-membered ring and the electrostatic repulsion resulting from deprotonation of the molecule. A comparison of the rate constants for the reaction of e^-_{aq} with hypoxanthine and its nucleosides at different pH values is given in Table 1.

Transient absorption spectra of the electron adducts

Neutral solutions. The transient absorption spectrum recorded in N_2-saturated neutral solutions of 5×10^{-4} mol dm^{-3} dIno containing 0.2 mol dm^{-3} propan-2-ol after completion of the reaction of e^-_{aq} (1 μs) has a broad maximum centred around 310 nm (Fig. 2) whose rate of build-up corresponds to the decay of e^-_{aq} (inset of Fig. 1). The ε value at this wavelength was estimated to be 5200 dm^3 mol^{-1} cm^{-1} with $G(e^-_{aq}) = 2.8 \times 10^{-7}$ mol J^{-1}. This value is comparable to those found

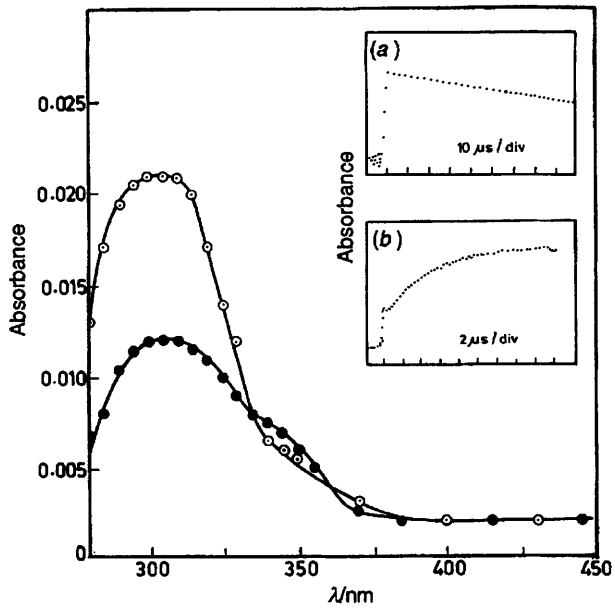


Fig. 2 Time-resolved absorption spectra obtained in N_2-saturated neutral solutions of 2′-deoxyinosine (5×10^{-4} mol dm^{-3}) containing 0.2 mol dm^{-3} propan-2-ol: (○) 1 and (●) 40 μs after the pulse. Inset: (*a*) decay of absorbance at 310 nm and (*b*) absorption build-up of $MV^{\bullet+}$ at 605 nm in N_2-saturated neutral solutions of 2′-deoxyinosine (1×10^{-3} mol dm^{-3}) containing 0.2 mol dm^{-3} *tert*-butyl alcohol and 5×10^{-5} mol dm^{-3} MV^{2+}. Dose per pulse = 15 Gy in all cases, except for (*a*) where it was 8 Gy.

for the heteroatom-protonated electron adducts of adenosine [10] (ε_{300} = 4800 dm^3 mol^{-1} cm^{-1}) and inosine [13] (ε_{300} = 5400 dm^3 mol^{-1} cm^{-1}).

The time-resolved absorption spectrum of the electron adducts of dIno in neutral solutions at 40 μs (Fig. 2) shows about 50% reduction in absorbance at 310 nm with a marginal increase of absorption at 350 nm. The rate of increase in absorption at 350 nm was found to be $\sim 2 \times 10^4$ s^{-1} which is twice that observed [13] in the case of Ino. The decay of the species absorbing at 310 nm is mainly a bimolecular process. For example, the first half-life for this decay is expected to be around 50 μs assuming a bimolecular rate constant of 2×10^9 dm^3 mol^{-1} s^{-1} and [radical] = 10^{-5} mol dm^{-3} at the doses used (15 Gy per pulse) in our experiments. Furthermore, the trace recorded under identical solution conditions but at a lower dose (8 Gy pulse^{-1}) shows only a marginal decay at 310 nm on a 100 μs scale (inset of Fig. 2).

Basic solutions. The absorption spectra recorded in basic solutions of dIno showed pH-dependent changes due to the transformation of *C*-centred radicals into *N*-centred adducts. The spectrum recorded in N_2-saturated solutions of 1×10^{-3} mol dm^{-3} dIno containing 0.2 mol dm^{-3} propan-2-ol at pH 11 shows two maxima at 315 and 350 nm. However, the rates of build-up (Fig. 3) at these two wavelengths are different. The rate of build-up at 315 nm ($k_{obs} = 1.3 \times 10^6$ s^{-1}) agrees well with the bimolecular rate constant evaluated for the reaction of e^-_{aq} with dIno at this pH ($k_{obs} \sim 1 \times 10^9$ dm^3 mol^{-1} s^{-1}) whereas the initial fast build-up at 350 nm was followed by a further growth of absorbance with $k_{obs} \sim 2 \times 10^5$ s^{-1}.

The spectrum recorded at pH 13 is similar to that obtained at pH 11, but shows an increase in the rate of growth as well as in the intensity of absorption at 350 nm (inset of Fig. 4). The spectrum was, however, modified when solutions containing a high concentration of NaOH (pH ~ 13.5) were pulse radiolysed (Fig. 4). In these solutions, the absorbance at 350 nm was found to be less than at 310 nm.

This spectrum is similar to those reported for C-8 protonated species in the case of guanosine [11] and caffeine, [14] but it is different from the spectra recorded for the electron adducts of

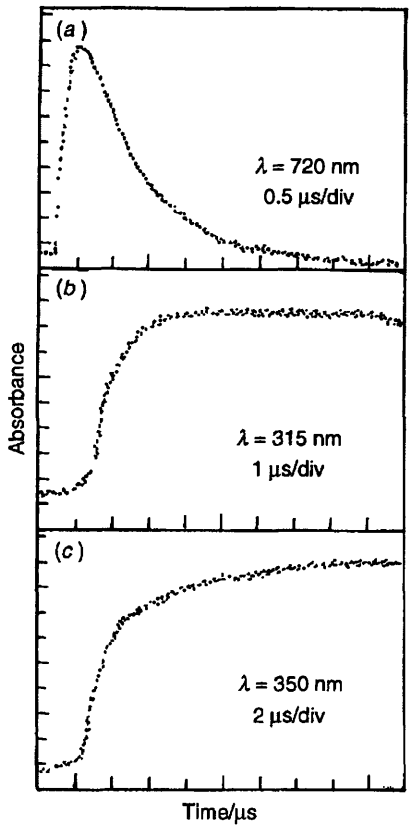


Fig. 3 Absorbance traces recorded at (*a*) 720; (*b*) 315 and (*c*) 350 nm on pulse radiolysis of N_2-saturated solutions of 2'-deoxyinosine (1×10^{-3} mol dm^{-3}) containing 0.2 mol dm^{-3} propan-2-ol at pH = 11. Dose per pulse = 15 Gy.

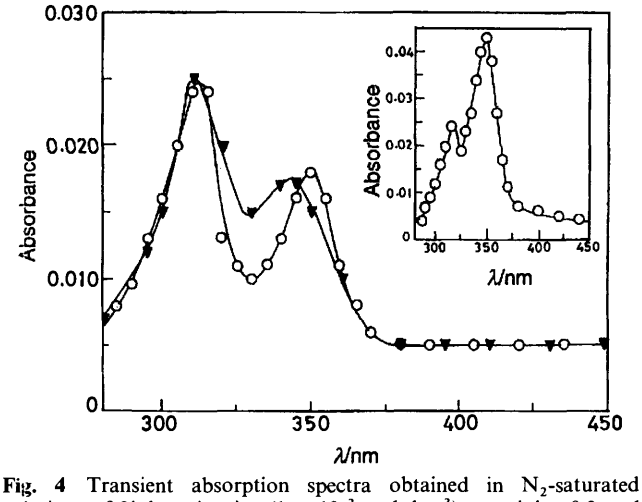


Fig. 4 Transient absorption spectra obtained in N_2-saturated solutions of 2'-deoxyinosine (1×10^{-3} mol dm^{-3}) containing 0.2 mol dm^{-3} *tert*-butyl alcohol at pH ~ 13.5 (○) at 2 μs and H-adduct spectrum recorded in neutral solutions (▼) at 8 μs after the pulse. Inset: transient absorption spectrum obtained at pH = 13 (○) at 2.5 μs after the pulse. All the spectra are normalised to $G = 2.8 \times 10^{-7}$ mol J^{-1} and dose per pulse = 15 Gy.

adenosine[10] and Ino[13] in basic solutions after the completion of transformation reaction where a reverse trend in the absorption intensities at the two wavelengths was observed.

Reaction of electron adducts with methyl viologen (MV^{2+})

On pulse radiolysis of N_2-saturated solutions of dIno (1×10^{-3} mol dm^{-3}) containing *tert*-butyl alcohol (0.2 mol dm^{-3}) and MV^{2+} (5×10^{-5} mol dm^{-3}) at pH = 6 (dose = 15 Gy pulse^{-1}), an increase in absorption at 605 nm due to electron transfer from heteroatom-protonated adducts was noticed (inset of Fig. 2). Further, the rate for this build-up was found to be dependent

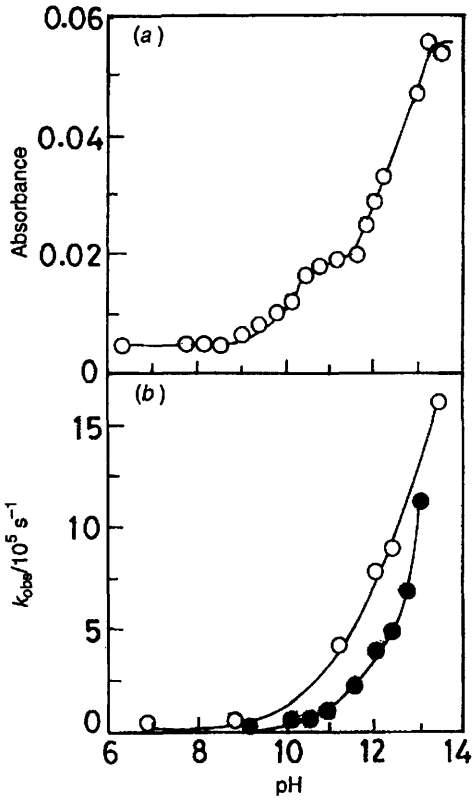


Fig. 5 Plots of (*a*) maximum absorbance values and (*b*) k_{obs} *vs.* pH at 350 nm (●) inosine and (○) 2'-deoxyinosine. Dose per pulse = 15 Gy. The values for inosine are taken from ref. 13.

on [oxidant] and the bimolecular rate constant evaluated from this dependence was 3.7×10^9 dm^3 mol^{-1} s^{-1}. This value is in between those reported for the oxidation of the electron adducts of adenosine[10] by MV^{2+} ($k = 2.3 \times 10^9$ dm^3 mol^{-1} s^{-1}) and Ino[13] by *p*-nitroacetophenone (*p*NAP) ($k = 4 \times 10^9$ dm^3 mol^{-1} s^{-1}).

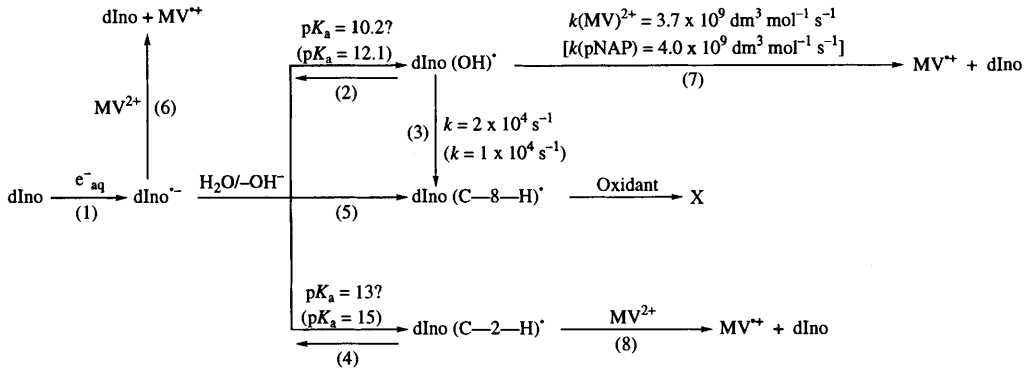
The yield of $MV^{\bullet+}$ determined at low doses (8 Gy pulse^{-1}) at high [MV^{+2}] ($0.5–1.0 \times 10^{-4}$ mol dm^{-3}) was found to be around ($2.8–3.3$) $\times 10^{-7}$ mol J^{-1} indicating that the electron transfer from the heteroatom-protonated adducts is quantitative. The loss of these radicals due to their bimolecular decay at high doses is reflected in the lowering of the yield of $MV^{\bullet+}$ at 15 Gy pulse^{-1} where a yield of 2.1×10^{-7} mol J^{-1} was obtained.

The yield of $MV^{\bullet+}$ was lowered further in basic solutions. For instance, G ($MV^{\bullet+}$) is 0.5×10^{-7} mol J^{-1} at pH 11.1 indicating that 80% of the adducts are already converted into the non-reducing species. In solutions with pH ⩾ 13, no build-up due to $MV^{\bullet+}$ was seen. This is in contrast to the extent of oxidation observed[13] with the electron adducts of Ino where nearly 40% and 18% of *p*$NAP^{\bullet-}$ formation was seen at pH 11 and 13, respectively. The lower yield of $MV^{\bullet+}$ in dIno is due to the higher rate of conversion of reducing radicals into non-reducing species than in the case of Ino.

Mechanism

The initial radical anions ($dIno^{\bullet-}$) formed from the attack of e^-_{aq} can be assumed to be rapidly protonated by water [reaction (2) in Scheme 2] in neutral solutions as was suggested[10,11,13] for other purine nucleosides.

The change in maximum absorption at 350 nm with pH, recorded at 18 μs after the pulse at all values, follows a pK_a type of curve with superimposition of two pK_a values (Fig. 5). It is, however, difficult to measure accurately the pK_a values from the superimposed curve. Our absorption curve is very similar to that found[13] for the dependence of the $pNAP^{\bullet-}$ yield on the pH in the case of inosine. The first pK_a value in our study is centred around 10.2 with a plateau up to about pH 11.2. A



(The values in parentheses represent those reported[13] for inosine)

Scheme 2

further increase in absorption was observed with the maximum being obtained beyond pH 13. However, the inosine data could be fitted assuming the pK_a values to be 12.1 and 15. What is clear from our previous study[13] and the present studies is that the superimposition of two pK_a values is characteristic of hypoxanthine nucleosides in contrast to a single value observed[10] with the adenine nucleosides.

The dependence of k_{obs} on [OH^-] for the absorption growth at 350 nm in dIno (Fig. 5) is similar to that found from Ino where an intersection in the plot at pH 11.7 was reported.[13] Thus, more than one type of transformation reaction, as in the case of Ino, is involved [reaction (4)]. The k_{obs} values are somewhat higher (about 20%) than those reported[13] for inosine. These values were determined from traces recorded on the time-scale 20–5 μs in the pH range 11–13.5. Corrections for the bimolecular decay of the radicals were not applied as the first half-life in basic solutions above pH 11 is at least 200 μs at the dose rate employed, assuming that $2k$ is around 5×10^8 dm^3 mol^{-1} s^{-1} and that the concentration of the adduct radicals is about 9×10^{-5} mol dm^{-3}.

In order to obtain further information on the nature of the electron adducts of dIno, the reaction of the hydrogen atom was studied in N_2-saturated neutral solutions of dIno (1×10^{-3} mol dm^{-3}) containing *tert*-butyl alcohol (0.2 mol dm^{-3}) as a OH radical scavenger and using a 500 ns pulse with an absorbed dose of 30 Gy. The spectrum recorded after completion of the reaction ($k \sim 4 \times 10^8$ dm^3 mol^{-1} s^{-1}) in these solutions and normalised to the conditions employed in the e^-_{aq} reaction [dose = 15 Gy and $G(H) = 2.8 \times 10^{-7}$ mol J^{-1}] is shown in Fig. 4. This spectrum is identical to the electron adduct spectrum obtained at pH ~ 13.5. This spectrum can be attributed to the thermodynamically stable dIno (C-8-H) species which are formed by irreversible protonation [reaction (5)] and the extinction coefficients estimated for these and heteroatom protonated adducts are given in Table 1. The pK_a values and the reaction rates for transformation obtained in our studies with Ino[13] and dIno are depicted in Scheme 2.

Our data thus suggest that the transformation behaviour of the electron adducts of inosine and deoxyinosine is similar, though there are small differences in the pK_a values and the rates of transformation. It appears that the C-8 protonated adducts of deoxyinosine are relatively more stable than the corresponding adduct radicals of inosine leading to lower pK_a values for heteroprotonated and C-2 protonated adducts. These rates are known to be affected by substituents on the bases. However, such detailed studies dealing with the effects due to the changes in the sugar moiety on the transformation behaviour, as performed in the present investigation, are lacking with other nucleosides.

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