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Bromination of Pyrimidines using Bromide and Monoperoxysulfate: A Competition Study between Cytidine, Uridine and Thymidine

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Abstract: Bromination of pyrimidine nucleotides in aqueous conditions can be conveniently carried out using a mixture of potassium bromide and potassium monoperoxysulfate (Oxone). Deoxycytidine can also be selectively brominated in reaction mixtures containing uridine and thymidine. NMR studies show the course of the reaction and formation of 5-bromopyrimidine products.
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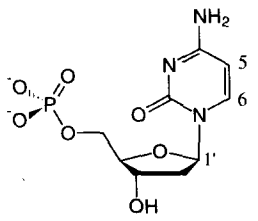
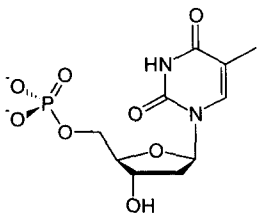
Halogenation of pyrimidines has been a much-studied reaction since initially being reported in 1907.[1] This stems in part from the interesting chemotherapeutic, biochemical and biophysical properties that halogenated nucleotides have been shown to exhibit,[2] such as inhibitory effects upon the growth of bacteria and tumors.

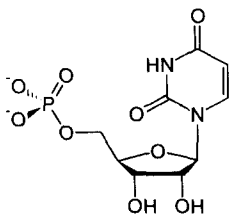
We have recently reported[3] the use of bromide and monoperoxysulfate as a cytosine-specific chemical probe of DNA. Reaction of micromolar concentrations of potassium bromide/potassium monoperoxysulfate with single-stranded DNA leads to bromination at cytosine residues, forming piperidine-labile sites that can be observed by polyacrylamide gel electrophoresis. Selectivity can also be observed for bulged (*i.e.* non-base-paired) cytosine residues in double-stranded DNA. No reactivity is observed at thymine residues in single or double-stranded DNA, although bulged (non-base-paired) thymine residues are more reactive than base-paired cytosine residues in the same strand.[4] These results prompted us to investigate the relative reactivity of $KBr/KHSO_5$ with the three pyrimidine nucleotides, 2'-deoxycytidine-5'-monophosphate (dCMP), 2'-deoxythymidine-5'-monophosphate (dTMP) and uridine-5'-monophosphate (rUMP).



dCMP



dTMP



rUMP

Dieter and co-workers[5] have also reported recently the use of mixtures of monoperoxysulfate and bromide or chloride to generate Br_2 or Cl_2 *in situ* under aqueous conditions for the halogenation of alkenes and α,β-enones. Interestingly, no modification of DNA was observed with *chloride* and monoperoxysulfate in the DNA reactions

described above, even at millimolar concentrations of chloride ion, which is routinely used in reactions to maintain ionic strength.

Reaction of KBr with $KHSO_5$ gave rise to a bright yellow-orange solution that is decolorized instantly upon addition of any of the three pyrimidine nucleotides (dCMP, dTMP or rUMP). Similarly, aqueous solutions of Br_2 are also decolorized, and Br_2 is believed to be the active reagent formed from Br^-/HSO_5^-. The reaction with nucleotides was monitored by ^1H NMR which gave identical results for the $KBr/KHSO_5$ mixture or for aqueous Br_2 solutions. For example, dCMP exhibits 2 doublets at 6.0 and 7.8 ppm (H5 and H6 respectively) that disappear upon addition of excess $KBr/KHSO_5$. Several new peaks are observed in the region 4.6 to 5.5 ppm corresponding to addition products across the 5,6-double bond. The mechanism for the action of aq. Br_2 on pyrimidines (Fig. 1) has long been established[6] as generation of a bromonium ion, followed by attack of water at the 6 position to produce a bromohydrin. This species can then eliminate water (the rate of which is pH dependent) to generate a 5-bromopyrimidine, which may be further brominated in the presence of excess Br_2.

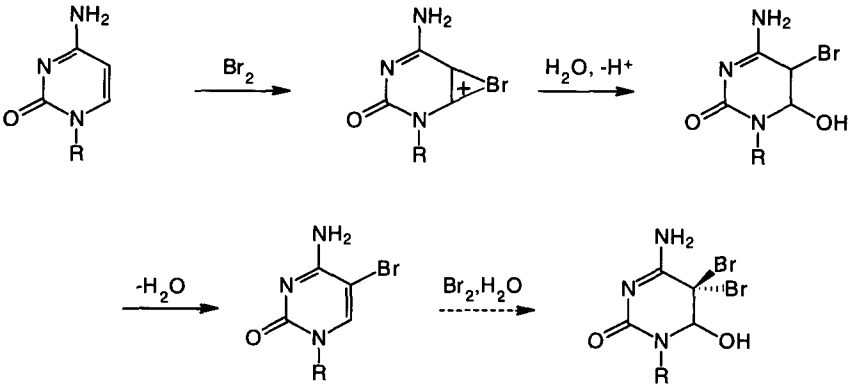


Fig. 1. Mechanism of pyrimidine bromination (R = phosphoribose moiety).

We carried out a series of ^1H NMR titrations in D_2O with 1:1 mixtures of the nucleotides (C:T, C:U and U:T) reacting with $KBr/KHSO_5$. Reactions were either carried out in phosphate buffer (pH 6.9, 200 mM) or in the absence of buffer. Initial concentrations of nucleotides were 50 mM in a total volume of 2 mL. KBr and $KHSO_5$ were then added in aliquots of 0.2 molar equiv. in 15 μL of D_2O. Monoperoxysulfate is a 2-electron oxidant per mole of HSO_5^-, thus the monoperoxysulfate concentration was half that of the KBr concentration. Unbuffered reactions generally gave a final pH of 3-4 after addition of 1 molar equiv. of monoperoxysulfate.

^1H NMR spectra were recorded after the addition of each aliquot of $KBr/KHSO_5$. 1:1 mixtures of dCMP: dTMP or dCMP:rUMP both exhibited a pronounced decrease in intensity in the signals for the cytosine H5 and H6 protons before reaction was observed with the thymidine or uridine nucleotides. This selectivity was maintained in the presence or absence of buffer. Fig. 2 illustrates the buffered reaction, with the 2'-deoxycytidine

H5 and H6 protons disappearing at 6.0 and 7.8 ppm, while the thymidine H6 proton at 7.6 ppm and the thymidine methyl group protons (not shown) are unchanged. Further addition of $KBr/KHSO_5$ causes the H6 proton of thymidine to diminish and the methyl group to split into 2 signals. The H1' proton of the deoxyribose of both C and T is coincident at 6.2 ppm initially, although as the $KBr/KHSO_5$ is added these peaks can be observed to separate as the 5,6 bond of the 2'-deoxycytidine reacts. A very slight downfield shift of all peaks is also observed due to lowering of pH even though the reaction is buffered.

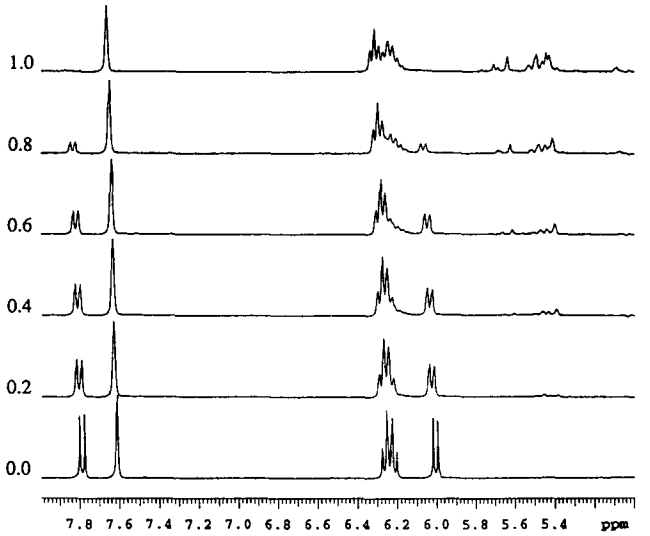


Fig. 2. ^1H NMR titration showing reaction of 1:1 mixture of dCMP:dTMP with 0, 0.2, 0.4, 0.6 and 1.0 equiv. $KBr/KHSO_5$ added. See text for reaction conditions.

1:1 mixtures of rUMP:dTMP showed equal reactivity of these nucleotides in the presence or absence of buffer, with the H5 and H6 protons of uridine and the H6 proton of thymidine decreasing in intensity in proportion to each other.

We are thus confident that the selectivity for C residues that we observe in our DNA reactions can be attributed to a difference in the rate of reactivity of cytosine bases with $KBr/KHSO_5$ compared to thymine bases due to electronic factors affecting the electron density of the 5,6 double bond, since thymidine (and uridine) nucleotides have been shown to react if no cytidine is present. The difference in rates must be at least one order of magnitude to account for the selectivity observed in the ^1H NMR competition reactions.

The significance of this work is two-fold. First, the observation of high cytosine reactivity compared to uracil suggests that the $KBr/KHSO_5$ reagent can be applied as a probe of exposed cytosine residues in folded RNA structures as it has been for DNA.[3] Secondly, the clean conversion of dCMP to 5-BrdCMP might be used to post-synthetically modify only exposed cytosines in folded DNA or RNA structures since 5-halopyrimidines can serve as a starting material for photo-cross-linking to proteins or chemical cross-linking to other nucleobases.[7-10]

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