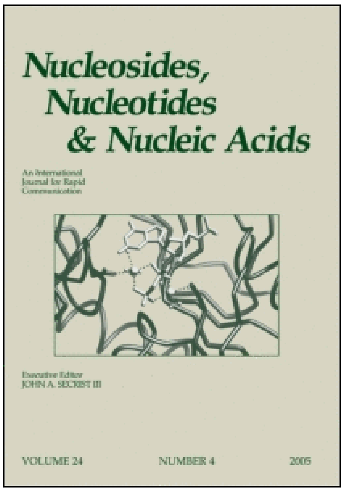

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TIME-OF-FLIGHT SECONDARY ION MASS SPECTROMETRY OF ISOMERIC O-ALKYLTHYMIDINES

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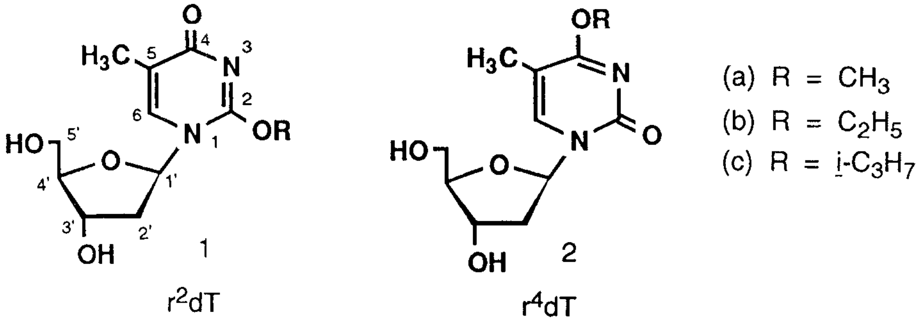
ABSTRACT: Positive ion mass spectra of the title compounds and their parent molecule, thymidine, show prominent $[M + H]^+$ and $[M + Na]^+$ ions, as well as base fragment ions resulting from cleavage of the N-glycosyl bond. In the case of alkylated nucleosides the base fragments can undergo further elimination of an alkene. In the negative ion spectra the prominent ions for all nucleosides are B^- and NCO^-. Molecular mass can be verified for thymidine by a prominent $[M - H]^-$ ion, which is not, or barely, detectable for the alkylated molecules. In addition, the NCO^- ion produces a prominent $[M + 41]^-$ adduct with a $(dT - H)^\bullet$ radical, but not with the alkylated species. Trends in the relative abundances of the ions are discussed.

Alkylating agents such as the N-nitroso-N-alkylamides have been implicated in carcinogeneis and mutagenesis. Such molecules react readily with the nucleophilic nitrogen and oxygen atoms of the DNA bases, and with the oxygen atoms of the phosphate internucleotide linkages[1,2]. Early interest focussed on the alkylation of the N7-nitrogen of the guanine base, the site most susceptible to attack by these agents, but evidence is accumulating that the formation of O6-alkylated guanine and the O2- and O4-alkylated thymines are the more significant events. It is known, for example, that the mutagenic and carcinogenic potential correlates with the tendency of the alkylating agent to attack oxygen[3] and with the size of the alkyl group[4]. The significance of O-alkyl modifications is evidenced by the existence of repair enzymes for these lesions[5]. Furthermore, the site of alkylation can greatly affect the lability of glycosyl bonds, as



1a, m^{2}dT
1b, e^{2}dT
1c, ip^{2}dT

2a, m^{4}dT
2b, e^{4}dT
2c, ip^{4}dT

FIGURE 1. O2- and O4-alkylthymidines (r^{2}dT and r^{4}dT)

observed with O-alkyl pyrimidine 2′-deoxynucleosides[4]. Kinetic and NMR studies have shown that alkylation of pyrimidines, in particular at O2, destabilizes the glycosyl bond[3,6,7].

To obtain information about the effects of alkylation on nucleic acid components we have initiated a study of O2- and O4-alkylated thymidines, r^{2}dT and r^{4}dT, **1** and **2**, (Fig. 1) by time-of-flight (TOF) secondary ion mass spectrometry (SIMS) on the Manitoba TOF instrument. We demonstrate that TOF spectra, especially when metastable ion studies are included, are useful for identifying these modified nucleosides, while revealing clear trends in the effects of the size of the alkyl group on fragmentation patterns.

In our initial study[8] of the positive SIMS of the m^{2}dT - m^{4}dT and e^{2}dT - e^{4}dT pairs, we described how the isomeric pairs could be differentiated by differences in first-order rate constants and half-lives for decomposition of their [M + Na]$^+$ ions, and we discussed some factors influencing the accuracy of these measurements. In the present work (for which preliminary results have been communicated[9]), both positive and negative ion spectra are described for the methylated and ethylated pairs as well as for their isopropylated analogues (ip^{2}dT and ip^{4}dT). Genetic relationships between several secondary ions have been established by using a correlation procedure, outlined below, analogous to the linked-scanning technique commonly used with sector instruments. This correlation technique, which we have described in more detail elsewhere[10], is useful for the identification of oligonucleotides containing these modified constituents.

EXPERIMENTAL

Sample preparation. The O2- and O4-alkylated nucleosides were prepared and purified chromatographically as described by Birnbaum *et al*[11]. For mass spectrometric analysis, they were dissolved, at a concentration of *ca* 1 mg/mL, in methanol containing less than 10% water. A few µL of each solution were then deposited on anodized aluminum foil that had been boiled in water to produce an AlO(OH) (boehmite) surface[12,13].

Mass spectrometry. The Manitoba TOF II secondary ion mass spectrometer[14] is shown schematically in Fig. 2. Sample targets were bombarded by a pulsed beam of primary Cs^+ ions (~ 2 ns pulses, repeated at a frequency of 2 kHz) at an ambient background pressure of ~ 10^{-7} Torr. The Cs^+ ions strike the target with an energy of 13 keV and 23 keV for the positive and negative ion studies, respectively. Of the secondary ions produced and ejected, those with a lifetime longer than *ca* 10^{-7} s can be accelerated through a grounded grid (not shown), by applying a voltage of +/- 5 kV to the target, and allowed to drift along the flight tube towards a "0° detector", in front of which is an ion mirror. During this stage of collision-free, field-free flight some parent ions may undergo unimolecular decomposition into ionic and neutral daughters having essentially the same velocity as the metastable parent ion. When the ion mirror is off, "direct" mass spectra are obtained in which the charged and neutral daughters of a given metastable parent ion are recorded at the same flight time, i.e. that of a non-decomposing parent ion. When an electric field is applied within the ion mirror, ions are reflected back along the flight tube toward a second "180° detector" located near the sample target. Neutral daughters are still recorded in the 0° detector, while the 180° detector records "reflected-ion" mass spectra in which stable parent ions are separated, in time, from daughter ions of metastable parent ions because the daughter ions spend less time in the mirror owing to their lower kinetic energy[10,15].

The detectors were multichannel plate electron multipliers whose output was processed by a data system based on a LeCroy model 4208 time-to-digital converter (TDC) and a DEC LSI 11/23 computer[16]. After calibration, the time scale was converted to *m/z* values, and ion yields were displayed as ion counts in time bins a few ns wide.

RESULTS AND DISCUSSION.

Generation of Mass Spectra. Genetic relationships between secondary ions can be established by separating stable parent ions from neutral and charged daughters of decomposing parent ions, as illustrated (Fig. 2 (a-d)) for the positive ion spectra of ip^{4}dT. With the electrostatic mirror off [case A, Fig. 2(a)] neutral and charged daughters of a given fragmenting parent ion are detected by the 0° detector behind the mirror as a broadened peak centred at the same flight time as an isobaric non-fragmenting ion. For example, the "$[B + 2H]^+$ peak" contains both undissociated $[B + 2H]^+$ parent ions and the daughter species produced by parent ion decompositions in the first leg of the flight path. (B is the base moiety of the neutral molecule). When the appropriate voltage is applied to the mirror, the three components of the peak are separated. Figure 2(b) shows the neutral daughters (ND) formed in the field-free region between the acceleration grid and the mirror entrance. Because they are unaffected by the electric field in the mirror, the neutral daughters are detected in the 0° detector at flight times corresponding to those of their parent ions. For example, ND is a neutral daughter of the $[B + 2H]^+$ species, i.e. the neutral component of peak P in Fig. 2(a). The reflected ion spectrum (Fig. 2(c)) shows well-separated peaks for charged daughter (CD) and unfragmented parent ions. The difference in flight time between daughter and parent ions is a function of their masses.

It is also possible to record in the 180° detector the daughter ion that corresponds to arrival in the 0° detector of its complementary neutral fragment resulting from the same parent ion decomposition. These events are stored in dedicated sections of computer memory and, in this way, we obtain a spectrum of fragment ions "correlated" with the decomposition of a selected parent ion. In other words, such a spectrum is a "daughter ion spectrum" resembling those obtained with the more familiar types of tandem mass spectrometer. For example, Fig. 2(d) shows the spectrum of reflected ions that are correlated with the neutral fragments from peak ND in the 0° detector (Fig. 2(b)), i.e. this figure shows the daughter ion spectrum of ion P. In this example, the *m/z* values of P and CD are 169, designated as $[B + 2H]^+$, and 127, designated as $[B + 2H - C_3H_6]^+$. We have used this approach to establish genetic relationships between series of secondary ions.

In this paper, emphasis is placed on the interpretation of the direct spectra of the rdT but reference is made to their metastable decompositions whenever they provide clues to mechanisms of ion formation. Details of the metastable ion decomposition kinetics will be published elsewhere.

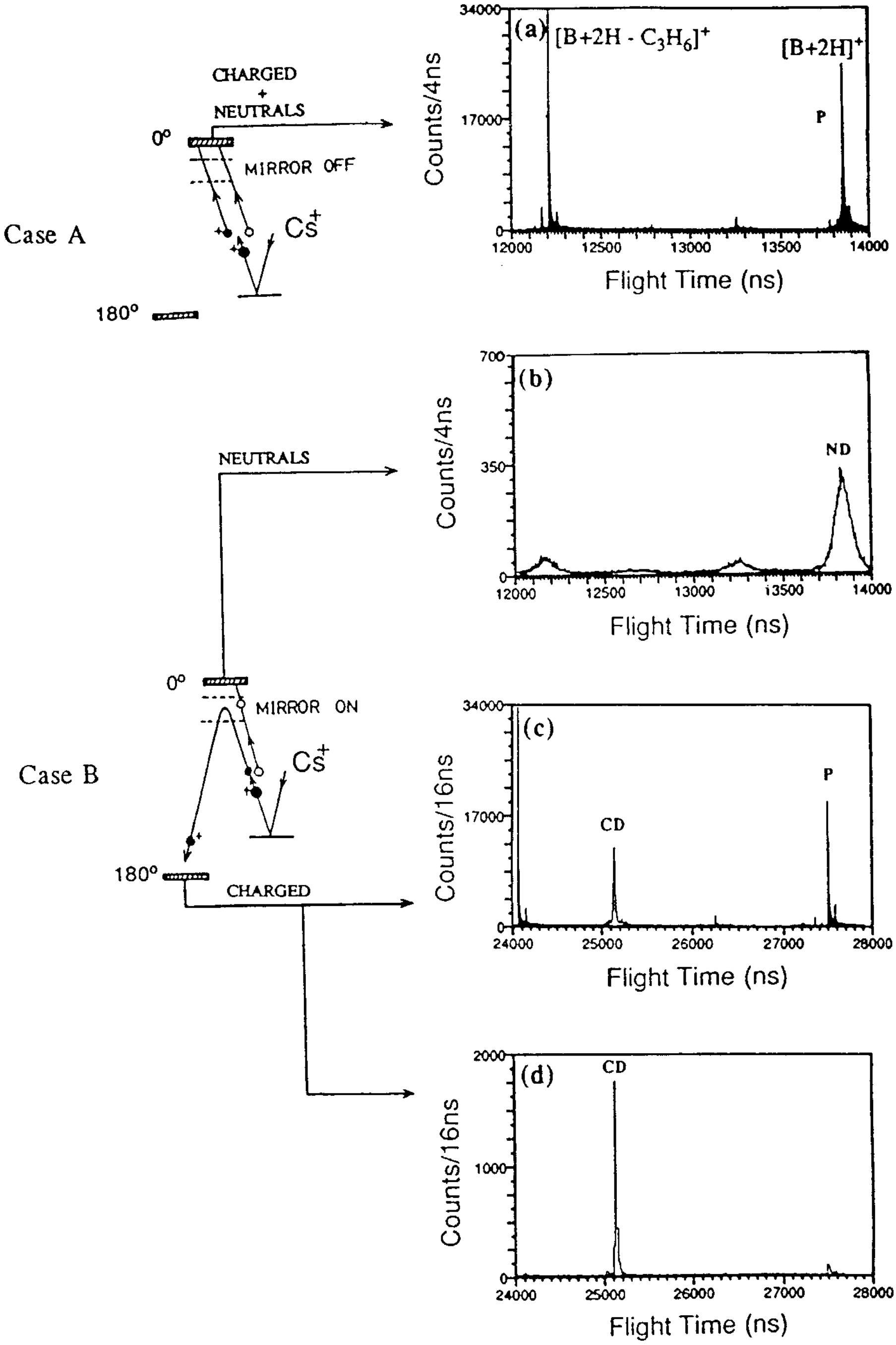


FIGURE 2. Analysis of ion decompositions in field-free flight. (a) Case A: Part of direct positive spectrum of ip^{4}dT recorded with the ion mirror "off". (b-d) Case B: Spectra recorded with mirror "on": (b) neutral daughters (ND) of $[B + 2H]^+$; (c) reflected parent (P) and charged daughter (CD) ions; (d) reflected CDs correlated with NDs.

TABLE 1. Direct positive and negative ion mass spectra of O-alkylated thymidines (%TIC, see text).

	ION ASSIGNMENTS	dT	m/z	m^2dT	m/z	m^4dT	e^2dT	m/z	e^4dT	ip^2dT	m/z	ip^4dT
(+)	$[M + Na]^+$	30.5	265	20.2	279	19.0	3.2	293	12.3	8.0	307	0.6
	$[M + H]^+$	24.5	243	7.4	257	15.1	20.1	271	13.1	7.1	285	18.6
	$[BHCH{=}CH_2]^+$	---	154	3.2	168	3.5	2.7	182	2.9	0.7	196	1.1
	$[BH + Na]^+$	---	149	11.1	163	2.5	1.6	177	2.3	2.9	191	---
	$[B + 2H]^+$	21.2	127	33.7	141	43.1	41.1	155	41.0	24.9	169	33.9
	$[B + 28 - (R - H)]^+$	1.6	153	9.4	153	0.4	2.6	153	1.0	2.5	153	2.2
	$[B + 2H - (R - H)]^+$	---	---	0.8	127	0.6	6.4	127	8.2	29.7	127	25.7
	S^+	14.5	117	9.8	117	10.3	14.4	117	10.7	16.3	117	11.8
	$[B - 15 - (R - H)]^+$	2.0	110	0.7	110	0.9	2.3	110	4.4	2.1	110	2.4
	$[S - H_2O]^+$	2.0	99	3.6	99	4.7	5.6	99	4.1	5.6	99	3.6
	ΣB	24.8		55.7		47.5	54.0		56.9	62.1		64.2
	ΣS	16.5		13.4		15.0	20.0		14.8	21.9		15.4
(-)	$[M - H]^-$	8.7	241	0.7	255	0.5	---	269	---	0.6	283	2.1
	$[M - R]^-$	---	---	1.7	241	2.5	3.3	241	2.1	0.6	241	3.7
	$[BCH{=}CHCHO - R]^-$	---	179	0.7	179	0.8	0.6	179	1.3	1.4	179	0.5
	B^-	25.9	125	50.5	139	27.2	31.0	153	17.1	17.9	167	12.7
	$[B - (R - H)]^-$	---	---	5.4	125	3.3	6.5	125	4.1	7.7	125	5.4
	$[B - (OR - H]^-$	---	109	2.3	109	1.4	1.7	109	0.9	1.8	109	0.9
	$[B - ROH]^-$?	---	107	1.3	107	3.1	0.7	107	3.4	1.2	107	1.0
	$[B - (R - H) - CO]^-$	---	97	2.0	97	4.4	4.8	97	7.5	5.2	97	5.1
	NCO^-	29.6	42	35.3	42	55.1	51.4	42	60.6	63.7	42	68.6
	other	31.3	U		W	1.6		X	1.0			
		4.4	V					Y	1.9			

Note: U = $[M + 41]^-$, V = $[M + 13]^-$, W = $[M + Cl]^-$, X = $[M - R - NCO]^-$, Y = $[M - R - 30]^-$

$\Sigma B = [BH + Na]^+ + [B + 2H]^+ + [B + 28 - (R - H)]^+ + [B + 2H - (R - H)]^+ + [B - 15 - (R - H)]^+$

$\Sigma S = S^+ + [S - H_2O]^+$

General spectral characteristics. Table 1 lists positive and negative secondary ion abundances for dT and the rdT. For positive ions, results for the range *m/z* 90 up to that of $[M + Na]^+$ are expressed in % of total ion current (%TIC) because this quantity seems here to best reflect differences between spectra. For the negative mode, the prominent NCO^- ion (*m/z* 42) was added to the list. In all instances, we find sensitivity to be lower in the negative ion mode than in the positive ion mode, as indicated by a lower rate of data accumulation under similar detection conditions. (A similar observation has been made for liquid secondary ion (LSI) mass spectra of nucleosides[17].)

The general features of the TOF spectra are illustrated in Fig. 3 by the positive and negative secondary ion mass spectra of ip^{4}dT. In both spectral modes many structurally diagnostic species are observed. The ions characteristic of the intact molecule (i.e. $[M + H]^+$, $[M + Na]^+$, and $[M - H]^-$) and those arising from glycosyl bond cleavage are also observed for nucleosides by other "soft" ionization methods: LSI (also known as fast atom bombardment (FAB))[17-19], laser desorption (LD)[20] and chemical ionization (CI)[21], although there are quantitative differences in the spectra. For alkylated nucleosides, ions resulting from alkene loss are also present. Table 2 contains a summary of the important trends in the data of Table 1, which we now discuss.

The positive mass ion spectra.

Ions characteristic of the intact molecule. The ions $[M + H]^+$ and $[M + Na]^+$ are both prominent (Table 1); in addition, some spectra show small signals for $[M - H + 2Na]^+$ ions. While the relative amounts of $[M + H]^+$ and $[M + Na]^+$ vary unpredictably from sample to sample (presumably due to variable amounts of adventitious Na^+), their combined %TICs show that alkylation at the O2 or O4 positions of dT increases the extent of fragmentation, i.e. their overall stability decreases according to the trend dT > mdT > edT > ipdT. Thus, %TIC($[M + Na]^+$ + $[M + H]^+$) varies from 55% for dT to 15-34% for the rdT. These values are slightly greater for r^{4}dT than for r^{2}dT.

Glycosyl bond cleavage. Using r^{4}dT as the example, Scheme 1 depicts four reactions involving glycosyl bond cleavage, three of them (a-c) leading to formation of a positive ion. It has been assumed that initial protonation or cationization is at N3, which is probably the most basic site of the nucleoside[6,21]. Relative ion yields and decomposition rates of metastable ions of these compounds indicate that reaction (a) occurs more readily for $[M + H]^+$ than for $[M + Na]^+$, a result explained[8] by chelation of Na^+ by three oxygens, i.e. O2, O4′ and O5′. However, the *decomposing* form of $[M + Na]^+$ may be

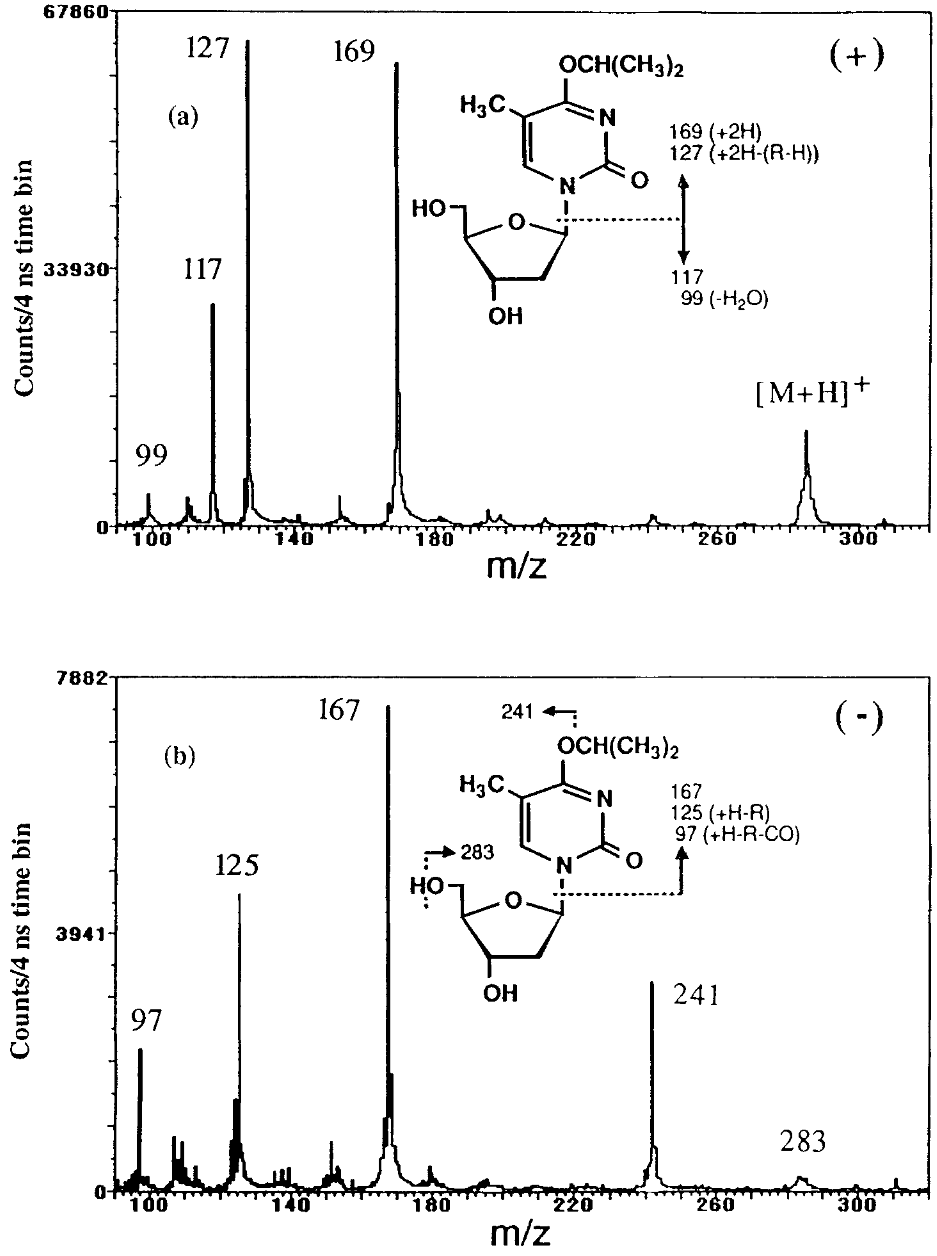


FIGURE 3. Direct spectra of ip^{4}dT: (a) positive ions, (b) negative ions.

TABLE 2. Direct mass spectra of O-alkylthymidines: Highlights

Ions characteristic of the intact molecule:

+ve - $[M + Na]^+$ and $[M + H]^+$ usually present.
-ve - $[M + Cl]^-$ and $[M + Cl]^-$ sometimes present, but in very low amounts.

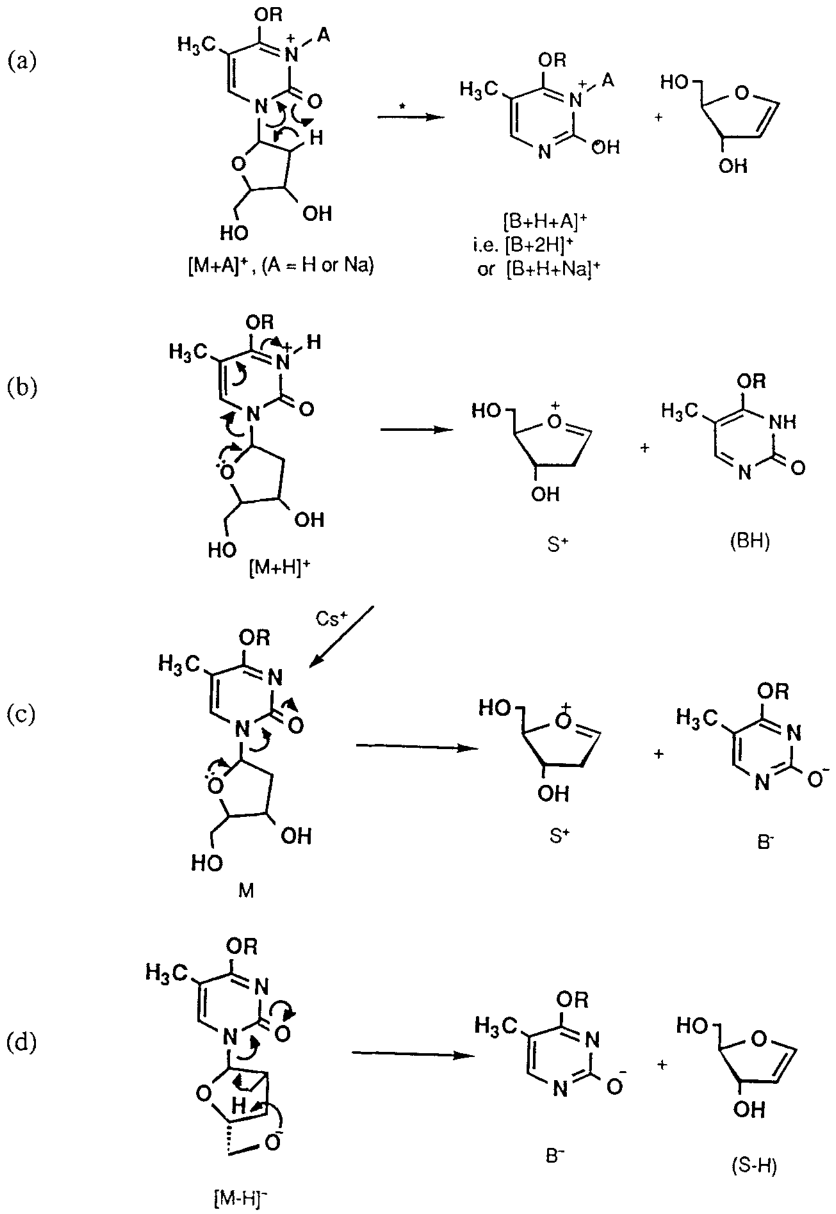
Fragmentation patterns:

+ve - glycosyl bond cleavage precedes alkene loss.
-ve - glycosyl bond cleavage usually precedes, but sometimes follows alkene loss.

Effect of alkyl group (presence and position):

+ve - %TIC of ($[M + Na]^+ + [M + H]^+$) ions decreases according to: dT > mdT > edT > ipdT; it is greater for O4-alkyl isomers.
- %TIC of ($[B + 2H]^+ + [BH + Na]^+$) increases according to dT < ipdT < edT ~ mdT.
- %TIC of the dealkenated base ion (m/z 127) increases as the size of R increases, but is independent of its position.
- %TIC of sugar ion increases as the size of R increases for O2-alkyl isomers; this effect is not observed for O4-alkyl isomers.

-ve - %TIC of B^- decreases according to the trend: mdT > edT > ipdT; it is greater for O2-alkyl isomers than for O4-alkyl isomers, although this difference decreases as the size of R increases.
- %TIC of $[BH - R]^-$ is not influenced by the size of R, although its relative abundance is slightly greater for O2-alkyl isomers.
- S^- is not detected.
- %TIC of NCO^- increases as R gets bigger; it is always slightly greater for O4-alkyl isomers, but this difference decreases as the size of R increases.



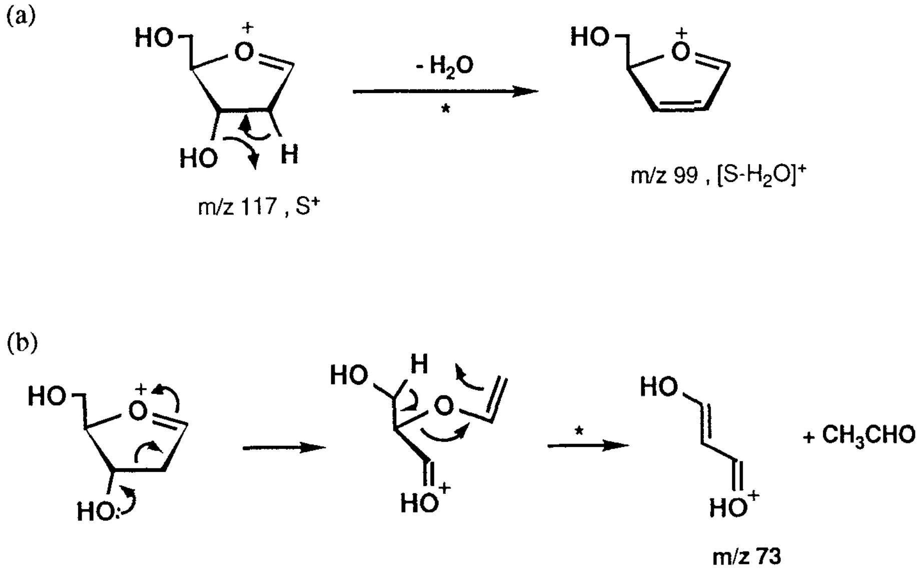
SCHEME 1

that represented in reaction (a). The formation of $[B + 2H]^+$ and $[BH + Na]^+$ by reaction (a) involves a well-documented hydrogen transfer from C2′ to O2[17,19,21].

While formation of $[B + 2H]^+$ and $[BH + Na]^+$ ions from the respective $[M + H]^+$ and $[M + Na]^+$ ions, reaction (a), is supported by detection of appropriate metastable ion decompositions, similar support for formation of S^+ ions from these precursors is lacking. Thus, S^+ ions must be formed at, or very near, the surface in order to receive full acceleration, and within *ca* 10^{-8} s of cesium ion impact. Reactions (b) and (c) of Scheme 1 are proposed formation modes. Reaction (b) occurs only for a high energy population of $[M + H]^+$ ions; lower internal energy populations can decompose by alternative, slower, low-energy pathways, such as the rearrangement shown in reaction (a). Formation of S^+ by a direct cleavage mechanism is consistent with these observations, and seems more probable than a rapid, high-energy rearrangement. The other proposed route, reaction (c), invokes ion pair production, i.e. formation of S^+ and B^- at, or near, the sample surface, following energization by primary Cs^+ ion impact. Such reactions are expected to be very rapid.

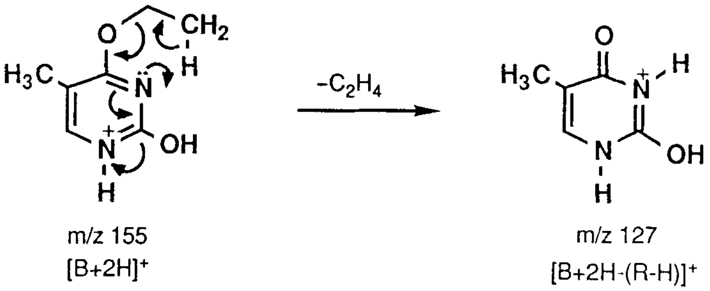
For discussion of fragment ions derived from glycosyl bond cleavage, it is useful to define (a) ΣB as the sum of the %TICs of $[BH + Na]^+$, $[B + 2H]^+$, $[B + 28 - (R - H)]^+$, $[B + 2H - (R - H)]^+$, and $[B - 15 - (R - H)]^+$ and (b) ΣS as the sum of the %TICs of S^+ and $[S - H_2O]^+$. These numbers are included in Table 1. For all molecules, $\Sigma B > \Sigma S$, indicating that scission of the glycosyl bond favors positive charge retention by the base moiety rather than by the sugar fragment. Furthermore, charge retention by the base is strikingly enhanced by O2 or O4 alkylation. Charge retention by the base was shown by Crow *et al*[17] to be a general feature of the LSI-collisional activation (CA) spectra of thirty nucleosides. In fact, for *purine* bases, which are particularly able to accommodate a positive charge, S^+ ions could not even be detected. In so far as isomer differences are concerned, we note that when the alkyl group is located on O4, its nature (m, e, or ip) does not seem to have much influence on the %TIC of S^+. On the other hand, for the O2 isomers, the %TIC increases slightly according to the trend mdT < edT < ipdT (Tables 1 and 2).

Both products of glycosyl bond cleavage undergo further decomposition. The S^+ ion decomposes in at least two ways, as shown in Scheme 2, both of which are supported by observation of metastable ion decompositions. Reaction (a) depicts formation of the ion of *m/z* 99 by the loss of H_2O, and reaction (b) formation of the ion of m/z 73 by loss of C_2H_4O . The $[B + 2H]^+$ ion derived from the rdT undergoes alkene loss (see below). This fragmentation significantly lowers its abundance and prevents further analysis of the effect of alkyl group size on N-glycosyl bond scission.



SCHEME 2

Alkene loss. Fragment ions resulting from dealkylation or dealkenation of $[M + H]^+$ or $[M + Na]^+$ are not detected. However, alkene loss from the $[B + 2H]^+$ or $[BH + Na]^+$ ions of e^4dT or ip^4dT probably proceeds through a cyclic six-membered rearrangement (illustrated for e^4dT in Scheme 3) involving transfer of hydrogen from the alkyl group to N3. (In the analogous rearrangement for the O2-alkylated derivatives, hydrogen can be transferred to whichever of N1 or N3 is unprotonated). By this mechanism, for which metastable ion decompositions give support, an alkene is expelled from the base. Propene loss from ipdT is significantly greater than ethene loss from edT in both of the O2 and O4 series. In the case of ip^2dT the resulting fragment at *m/z* 127 gives the most intense peak of the spectrum. A rationale for this trend was presented in an earlier publication[8]. However, there is no consistent trend in the effects of the *position* of the alkyl group. Note that this rearrangement is not applicable to the mdT because an alkene cannot be formed, and a mechanism involving a five-membered cyclic transition state would lead to the unlikely loss of $:CH_2$. It is probable that the $[B + 2H - (R - H)]^+$ species, observed with small %TIC in the mdT spectra, results by an alternative route. In addition, a metastable ion decomposition was not observed for such a loss.



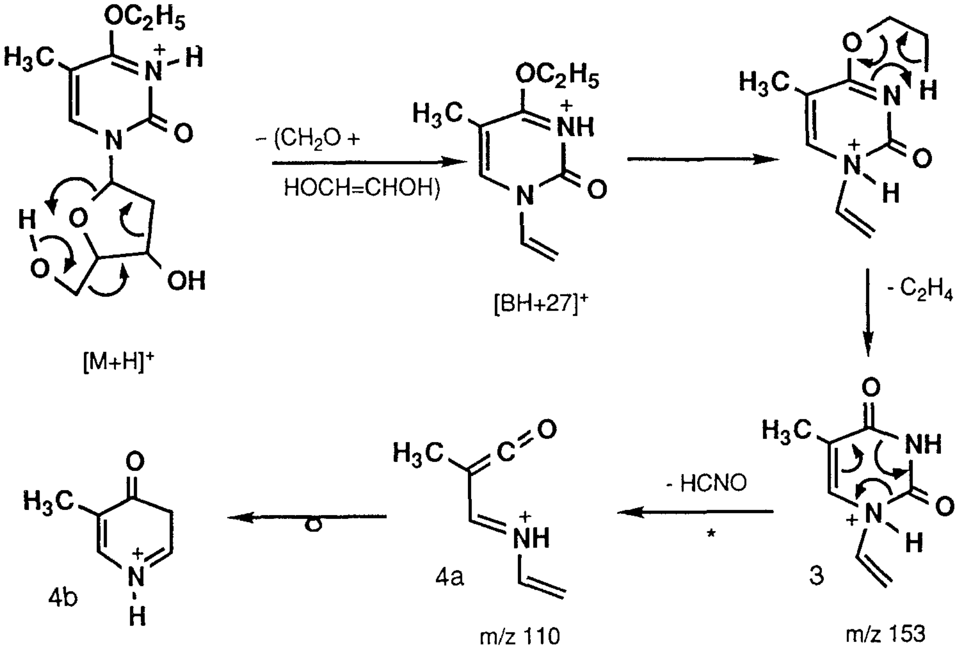
SCHEME 3

Alternative decomposition pathways for [M + H]$^+$. Table 1 gives data for three genetically related minor ions of the rdT, namely [B + 28]$^+$ (listed as [BHCH=CH$_2$]$^+$) and two unalkylated fragments at *m/z* 153 and *m/z* 110. Formation of [B + 28]$^+$ is widely known in electron ionization (EI)[22], CI[21] and LSI[17,19] mass spectra of unmodified nucleosides. Here, with the rdT, it is formed in much smaller yields, because of the increased importance of glycosyl bond cleavage and introduction of the alkene loss pathway. Scheme 4 depicts subsequent alkene loss from the [B + 28]$^+$ ion of e^{4}dT through a six-membered cyclic rearrangement[8] to produce [B + 28 - (R - H)]$^+$ (*m/z* 153), **3**, which could then form [B - 15 - (R - H)]$^+$ (*m/z* 110), **4a** or **4b**, by a retro-Diels-Alder (RDA) elimination of HNCO[22].

The negative ion spectra.

The [M - H]$^-$ ion of thymidine is prominent (%TIC ~ 9%) whereas [M - H]$^-$ ions of rdT can barely be detected (%TIC < 1%). For the alkylated derivatives, B$^-$ and NCO$^-$ ions are the most abundant ions in the spectra. In addition, the largest peak in the spectrum of thymidine corresponds to [M + 41]$^-$, which we assign to an NCO$^-$ adduct of [M - H]$^\bullet$ (see below). This adduct is not detected in the spectrum of any rdT.

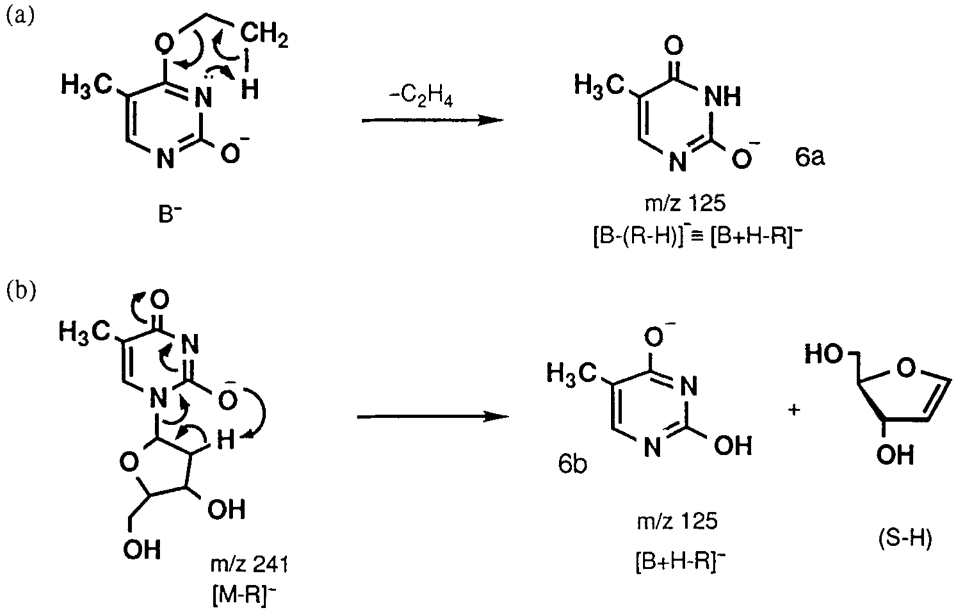
Glycosyl bond cleavage. One of the products of glycosyl bond cleavage, S$^-$, is not observed in any spectrum. The %TIC of the other product ion, B$^-$, is always greater for r^{2}dT than for the corresponding r^{4}dT isomer (Table 1) but, as the size of the alkyl group increases, its importance diminishes, with a concomitant increase in %TIC of NCO$^-$. Because, in positive ion spectra, the %TIC for S$^+$ is usually greater for the r^{2}dT than for the corresponding r^{4}dT isomer, we suggest a mechanism involving a common source for the B$^-$ and S$^+$ ions (reaction (c) of Scheme 1). We note that, in the ion pair production,



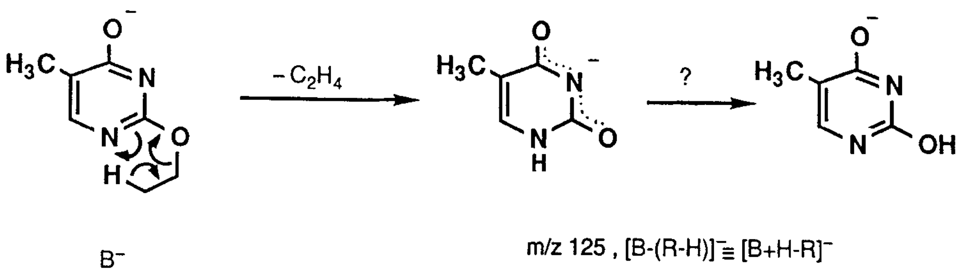
SCHEME 4.

the formation of B$^-$ would be facilitated by its conjugated π-electron system. On the other hand, B$^-$ ions can also be obtained from [M - H]$^-$ ions, as proposed in reaction (d) (Scheme 1), but this concerted mechanism could not be confirmed by metastable ion studies because the %TIC of [M - H]$^-$ was too low for daughter ions to be detected. However, it represents a well-documented fragmentation pathway for other nucleosides[17,19].

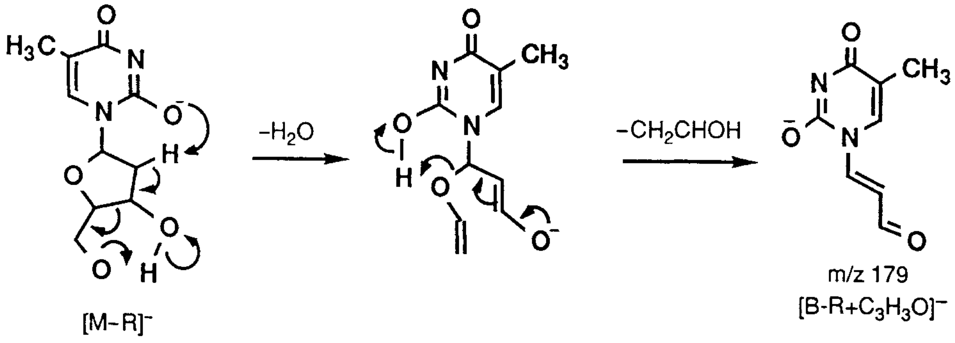
Alkene loss. In the negative ion mode, alkene loss from [M - H]$^-$ (or possibly dealkylation of M$^{-\bullet}$) produces [M - R]$^-$ ions (*m/z* 241). Alkene loss from B$^-$ produces ions at *m/z* 125 corresponding to [B + H - R]$^-$. Mechanistic rationalizations (Scheme 5) leading to the latter ion involve either simultaneous H transfer and alkene loss from B$^-$ to give **6a** (depicted in reaction (a) for e^{4}dT) or hydrogen transfer *after* the loss of R to give **6b** (reaction (b)). Easily rationalized by reaction (b) are *(i)* the presence of both [M - R]$^-$ and [B + H - R]$^-$, and *(ii)* the observation that the %TIC values of [B + H - R]$^-$ for the mdT are almost the same as for the other rdT. On the other hand, reaction (a) better



SCHEME 5



SCHEME 6



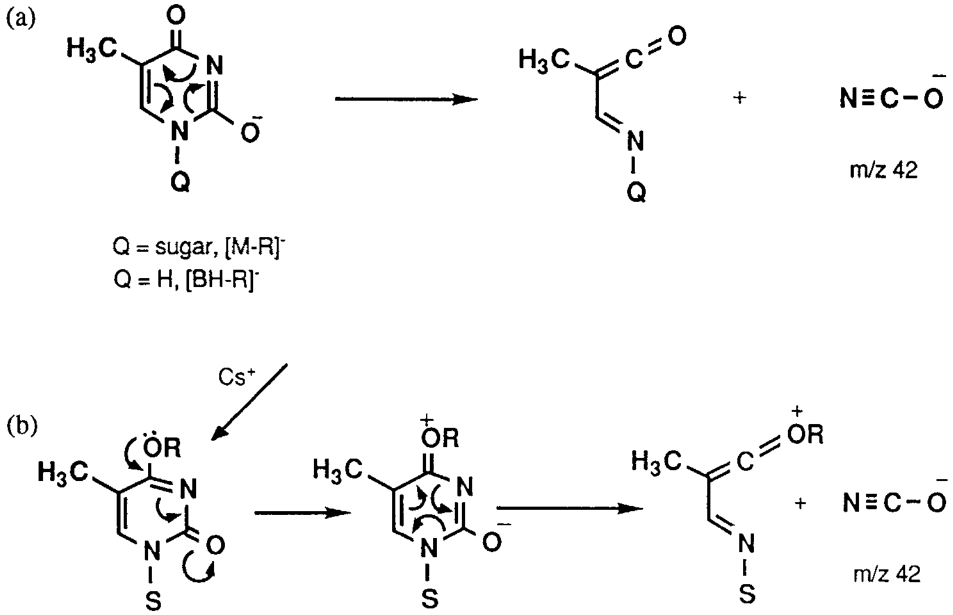
SCHEME 7

explains why the %TIC values of $[B + H - R]^-$ are greater for r^2dT than for r^4dT, because both N1 and N3 can serve as hydrogen acceptors during alkene loss from the O2 isomers. Transfer to N1 for e^2dT is shown in Scheme 6. An alternative mechanism (Scheme 7) for decomposition of $[M - R]^-$ provides a rationale for formation of the ion of *m/z* 179 (assigned as $[B - R + C_3H_3O]^-$), found in small yields in all rdT negative ion spectra.

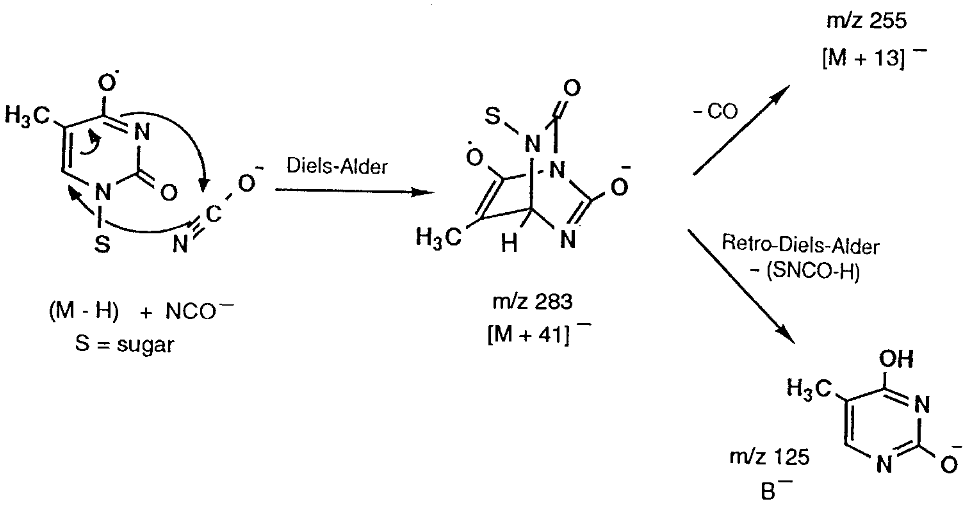
Formation of NCO⁻ ions. A striking feature of all spectra is the prominent *m/z* 42 peak, assigned to NCO^-, which is the base peak except in the spectra of dT and m^2dT. Crow *et al*[17] observed NCO^- and $[M - H - HNCO]^-$ in the collisional activation (CA) negative ion spectrum of uridine, and McCloskey[22] observed $[M - HNCO]^+$ in the EI positive spectra of thymine and uracil. Both studies presented strong evidence that the NCO fragment originated from N3-C2-O2 of the pyrimidine base, and RDA reactions were proposed for the losses of HNCO. However, in the absence of isotopic labelling we cannot discount alternative origins (N1-C2-O2, N3-C4-O4) for the NCO^- ion.

Table 1 shows that the %TIC of NCO^- increases in both the r^2dT and r^4dT series according to the trend dT < mdT < edT < ipdT. Furthermore, the %TIC is always greater for an O4-alkyl compound relative to its O2-alkyl isomer but the difference between them decreases as the size of the alkyl group increases. A simple rationale for these observations can be given if the origin of the NCO^- ion is the N3-C2-O2 fragment in both the r^2dT and r^4dT series, as shown in reaction (a) of Scheme 8 for its formation from dealkenated r^2dT and r^4dT precursor ions. However, for the r^4dT, a dealkenated precursor ion is not essential for NCO^- formation. Thus, Scheme 8(b) depicts NCO^- formation by ion pair production in the substrate shortly after energization by the primary Cs^+ ions. On the other hand, for the r^2dT, conversion of the N3-C2-O2 fragment into NCO^- would require prior elimination of the O2 alkyl group and hence would be expected to proceed less readily. But, as the size of the alkyl group increases, its elimination becomes easier and thus the isomer difference should diminish.

Formation of adduct ions. In the negative ion spectrum of thymidine (Fig. 2), two unusual peaks are observed at *m/z* 255, $[M + 13]^-$, and at *m/z* 283, $[M + 41]^-$, the latter being, as noted above, the most intense peak of the spectrum (Table 1). These ions are not detected in the rdT spectra, nor have they been reported in published LSI[19] or EI[23] mass spectra of thymidine. The formation of these ions might be explained by a reaction at the surface of the sample where, in addition to formation of ions, energetic particle bombardment also produces and desorbs larger quantities of energized neutral molecules and radicals. Possibly, in the present matrix-less samples, the readily formed NCO^- ions combine with $[M - H]^\bullet$ radicals by a Diels-Alder reaction to give $[M - H + NCO]^{-\bullet}$, i.e. $[M + 41]^{-\bullet}$, ions (Scheme 9). These radical ions could, by losing CO, yield the observed



SCHEME 8



SCHEME 9

[M + 13]$^-$ ions, and also act as another source of B$^-$ ions. In view of these observations, it is noteworthy that a [B + 42]$^-$ ion species reported in the LSI mass spectrum[19] of this compound was not observed here. (However, its probable assignment as [B + C_2H_2O]$^-$ is different from that expected from our proposed [M + 41]$^-$ precursor.) Decompositions of metastable [M + 41]$^-$ ions were not detected in our reflected ion spectra and any reactions, if they do occur at all, must be very rapid (< 10^{-8} s). Isotopic labelling could shed some light on this matter. We note that, in our VG 7070E-HF mass spectrometer, we could not detect an [M + 41]$^-$ ion in the LSI mass spectrum of dT samples in a glycerol matrix in which NaOCN had been dissolved. It is possible that, in this latter experiment, the polar matrix inhibits the putative reaction, either by solvent effects or by physical separation of the supposed reactants.

Relative rates of alkene loss and glycosyl bond cleavage. In the positive ion spectra of the rdT the absence of the dealkenation products of [M + H]$^+$ and [M + Na]$^+$ and the prominence of the glycosyl bond cleavage ionic products [B + 2H]$^+$, [BH + Na]$^+$ and S$^+$, indicate that glycosyl bond cleavage proceeds more rapidly than alkene loss; subsequent dealkenation of [B + 2H]$^+$ yields [(B + 2H) - (R - H)]$^+$ (i.e. [B + 3H - R]$^+$). On the other hand, all rdT negative ion spectra do show a small percentage of the dealkenation product [M - R]$^-$, a percentage not influenced by the size or the position of the alkyl group. Thus, alkene loss is competitive with glycosyl bond cleavage, meaning that the [B - R]$^-$ ion can be formed from a common precursor by, at least, two competing pathways. While the glycosyl bond cleavage product ion B$^-$ is present, sugar-containing ions, as noted above, were not observed.

Summary and comparison of TOF/SIMS with LSI/CA in sector-field instruments. In our TOF-SIMS experiments, matrix-free samples were bombarded by 13 keV or 23 keV Cs$^+$ ions. In the *positive* ion mass spectra, molecular masses can be verified by the presence of prominent [M + H]$^+$ and [M + Na]$^+$ ions. Alkylation leads to a significant increase in fragmentation of these ions as the size of the alkyl group increases. The fragmentation due to cleavage of the glycosyl bond is more facile for r^{4}dT and, in particular, for r^{2}dT than for dT. In addition, the extent of alkene loss increases as the size of the alkyl group increases but is independent of its position. Glycosyl bond cleavage occurs more rapidly than alkene loss. In the *negative* ion spectra, molecular mass can be verified for dT by a prominent [M - H]$^-$ ion, which is not, or barely, detectable for the rdT. (Thus, in the analysis of an oligomer containing these modified units, an ion diagnostic of the molecular mass might not be detected.) The prominent ions are B^- (of greater abundance for r^2dT than for r^4dT) and NCO^-. In addition, NCO^- appears to form an adduct with $(dT - H)^\bullet$ to give a prominent $[M + 41]^-$ ion. For the rdT, alkene loss and glycosyl bond cleavage occur at competitive rates.

In some respects, the capabilities of sector-field mass spectrometers and our reflecting TOF instrument are similar, e.g. in their ability to detect ion decompositions on the µs time scale. In our TOF instrument we can study only unimolecular ion decompositions, whereas collisionally-activated decompositions can be routinely studied with a sector-field instrument. Nevertheless, the greatest differences between the spectra recorded by the two techniques are likely to be more dependent upon the sample (e.g. matrix or matrix-free) than upon the type of mass spectrometer employed.

These results have demonstrated the utility of TOF-SIMS for providing molecular mass and structural information for modified nucleosides. By using both positive and negative ion modes, confirmatory and complementary information can be obtained. We have described other applications of the TOF-SIMS technique to nucleotides and oligonucleotides elsewhere.[24-28]

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