Mass Spectrometry of Modified Nucleic Acid Bases and Nucleosides. 2. Class II Mesoionic Nucleosides and Bases Derived From Thiazolo[3,2-*a*]pyrimidine-5,7-diones [1,2]

Ernst M. Schubert and Karl H. Schram*

Department of Pharmaceutical Sciences, College of Pharmacy, University of Arizona, Tucson, AZ 85721

Richard A. Glennon

Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University Richmond, VA 23298

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The mass spectral characteristics of novel Class II mesoionic heterocyclic bases and nucleosides based on the thiazolo[3,2-*a*]pyrimidine-5,7-dione system have been examined using low and high resolution mass spectrometry and metastable ion analysis. The mass spectra of these Class II mesoionic nucleosides differ significantly from the spectra of "normal" nucleosides by the absence of fragment ions associated with the base plus portions of the sugar. The difference in fragmentation is rationalized on the basis of exclusive localization of the radical-charge site in the aglycone, a result of the mesoionic structure of these molecules. The fast atom bombardment (FAB) mass spectra of a Class I mesoionic nucleoside, 7-methylguanosine, is compared to the FAB mass spectra of the Class II mesoionic nucleosides.

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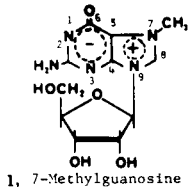
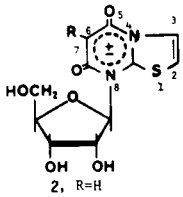
Introduction.

Mass spectrometry has been of significant value in the structure elucidation of modified nucleosides isolated from natural sources, particularly minor components of RNA [3], and the general fragmentation pattern shown by this biologically [4] and medicinally [5] important class of compounds is well documented [6,7].

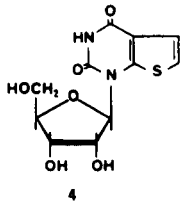
Although the mass spectra of "normal" nucleosides possessing a carbon-nitrogen glycosidic bond display, in general, similar fragmentation patterns, structural changes can radically alter the appearance of the mass spectrum. For example, *C*-nucleosides, *i.e.*, nucleosides with a carbon-carbon bond between the sugar and aglycone, yield an electron impact(EI) mass spectrum containing an intense $[B+30]^+$ ion[8-10] as a result of the increased stability of the glycosidic bond. Another example of a minor modification which results in a large change in mass spectral behavior is the addition of a methyl group to the N7 position of the purine ring. These mesoionic nucleosides, *e.g*, 7-methylguanosine (**1**), pose special problems for mass spectrometry because of their low volatility and, until recently, have been intractable to mass spectral analysis. Thus, little is known concerning the mass spectrometry of this class of mesoionic nucleosides.



1, 7-Methylguanosine



2, R=H
3, R=CH_3CH_2



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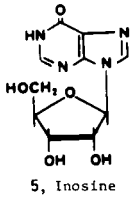
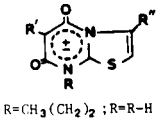
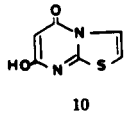


5, Inosine



6, R=$CH_3(CH_2)_2$; R=R-H
7, R=R=CH_3CH_2; R=H
8, R=R=CH_3CH_2; R=CH_3
9, 2,3 bond of 7 reduced



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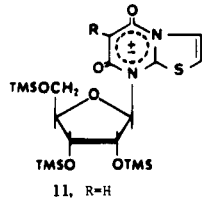
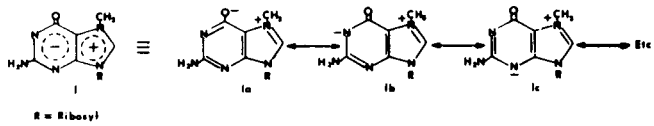


11, R=H
12, R=CH_3CH_2

Mesoionic nucleosides may be categorized as being Class I or Class II systems depending on the structure of the aglycone [11]. Class I mesoionic compounds are characterized by the presence of three pairs of pi-electrons delocalized in a five-membered ring while Class II mesoionic heterocycles possess three pairs of delocalized pi-electrons in a six-membered ring.

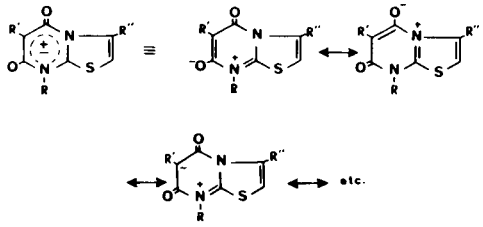
Neither Class I nor Class II mesoionic heterocycles can be adequately represented by a single covalent or dipolar structure [12], but are depicted by a generalized structure representing a combination of all possible resonance forms. For example, **1**, a Class I mesoionic nucleoside, is commonly drawn as generalized structure **1** [11] or as one of the representative resonance forms **1a-c** [13,14], as shown in Scheme 1 (additional forms are also possible). The dipolar structures **1a-c** with the positive and negative charges distributed over the two rings are responsible for the highly polar nature of this class of nucleoside [15]



Scheme 1. Important resonance forms of 7-methylguanosine. The charges are spread over two rings in Class I mesoionic structures.

which, in turn, renders these molecules non-volatile and, therefore, not amenable to mass spectral analysis. Earlier efforts to obtain the mass spectra of 7-methylpurine nucleosides resulted in pyrolysis of the sample with none of the commonly observed ions in the spectra of nucleosides being present [16]. Attempts to increase the volatility of **1** by preparation of the trimethylsilyl (TMS) derivative proceeds with incorporation of an oxygen atom, at the C8 position, thus altering the structure of the sample [17].

No representatives of the Class II mesoionic nucleosides have been available until the recently described synthesis of a series of 8-(α- and β-ribofuranosyl)thiazolo[3,2-*a*]-pyrimidine-5,7-diones [18,19]. This class of nucleoside, represented by **2** and **3**, differs from the Class I mesoionic systems in that the important resonance forms [18] have the charges localized in a single, six-membered ring, as shown in Scheme 2, rather than being distributed over both rings. Because of the unique structural differences of the Class II mesoionic nucleosides and bases relative to



Scheme 2. Important resonance forms of the Class II mesoionic systems contain the charges in the six membered ring only. The identities of R, R', R" are shown in the structures **2**, **3**, and **6-9**.

the Class I analogs and "normal" nucleosides, we have examined the mass spectral behavior of **2** and **3** and a commonly used [20] volatile derivative of **2** and **3**. The EI mass spectra of these Class II mesoionic nucleosides are compared to the mass spectrum of a closely related, non-mesoionic analog **4** [21] and to the mass spectra of nucleosides in general to determine if differences in fragmentation patterns attributable to the mesoionic heterocyclic system were apparent. In support of arguments on the mass spectral behavior of **2** and **3** is data obtained in the analysis of inosine (**5**). Since the mass spectra of the free nucleosides **2** and **3** are dominated by ions associated with fragmentation of the aglycone and because no detailed reports of the mass spectra of these mesoionic xanthine analogs have been described in the literature, the mass spectra of the mesoionic bases **6-9** were examined in detail. For comparison, the mass spectral characteristics of the non-mesoionic analog **10** are also included in this work. Finally, the fast atom bombardment (FAB) [22] spectra of **1**, **2** and **3** were compared in an effort to see if significant variations exist which may permit differentation of the Class I from the Class II mesoionic nucleosides using this new ionization technique.

Fragmentation schemes are proposed which account for the major ions observed in the mass spectra of the above compounds. Ion structures are based on high resolution and metastable ion data, but these structures should be considered tentative in the absence of stable isotope labeling studies.

EXPERIMENTAL

All samples examined were analytically pure based on elemental analysis or were of commercial origin. The preparation of compounds **2** and **3** has been described [18,19]. Compound **4** was a gift from Dr. L. B. Townsend of the University of Michigan and the base **6** was provided through the generosity of M. Hellberg. The mesoionic bases **7-9** and compound **10** were synthesized according to literature methods [23]. 7-Methylguanosine was of commercial origin and used without further purification.

Trimethylsilyl derivatives were prepared using *N,O*-bis(trimethylsilyl)-trifluoroacetamide containing 1% trimethylchlorosilane (Pierce Chemical Co., Rockford, IL) according to standard procedures [20].

Low and high resolution mass spectra were obtained via direct insertion probe using a Varian MAT 311A mass spectrometer operating at 70 eV with a source temperature of 250°. The sample probe, in all cases, was heated from ambient temperature to 400° in 200 seconds. High resolution mass measurements were performed under control of a Varian SS-200 data system at a scan rate of 25 seconds/decade with R = 7500. Metastable ion analyses were conducted using the linked scan mode with E/B a constant ratio at constant V which produced the daughter ion spectrum of the selected parent. FAB spectra were obtained using an Ion Tech B-11 NF saddle field gun with xenon as the bombarding gas at 8 keV. Samples ($\sim$50 μg) were dissolved in 100 μl of glycerol and 1-2 μl of the resulting solution were deposited on the stainless steel probe tip. The sample was introduced into the mass spectrometer with the source at ambient temperature. Data system subtraction was used to eliminate ions arising from the glycerol matrix. Gas chromatography was performed using a Varian 3700 gas chromatograph interfaced to the mass spectrometer with a single stage, all glass jet separator at 290° with an injection port temperature of 250°. The column, 6' OV-17(3%)(2 mm i.d.) on chromsorb W(100-200 mesh), was heated from and initial temperature of 180° to 300° at 4°/minute. Helium was used as the carrier gas at a flow rate of 30 ml/minute. Proper operation of the gcms system was checked by injection of a sample of adenosine-TMS_4 prior to injection of the TMS derivatives of **2** and **3**.

Results and Discussion.

Mass Spectra of the Free Nucleosides and Heterocyclic Bases.

The mass spectra of the Class II mesoionic nucleosides **2** and **3** differ from those of the Class I analog **1** and from non-mesoionic nucleosides, *e.g.*, **4**, in a number of important aspects. First, in contrast to the Class I mesoionic nucleosides which decompose prior to vaporization and provide no molecular ions, the Class II compounds may be volatilized to provide readily identifiable molecular ions at

m/z 300 and 328 for **2** and **3**, respectively, as shown in Figure 1. This observed difference in volatility of the Class I *vs.* Class II mesoionic nucleosides may be a reflection of differences in charge localization of the two systems. The resonance form most commonly drawn [13,14] to represent **1** has the positive charge localized at N7 in the imidazole ring with the negative charge residing in the pyrimidine ring at the O^6 position. The resulting dipole may thus present two widely spaced polarized regions on the molecule permitting the formation of strong intermolecular ionic bonds. These strong intermolecular forces prevent vaporization of the sample, with the result that decomposition occurs prior to vaporization and no molecular ions are present in the mass spectra of the Class I mesoionic nucleo-

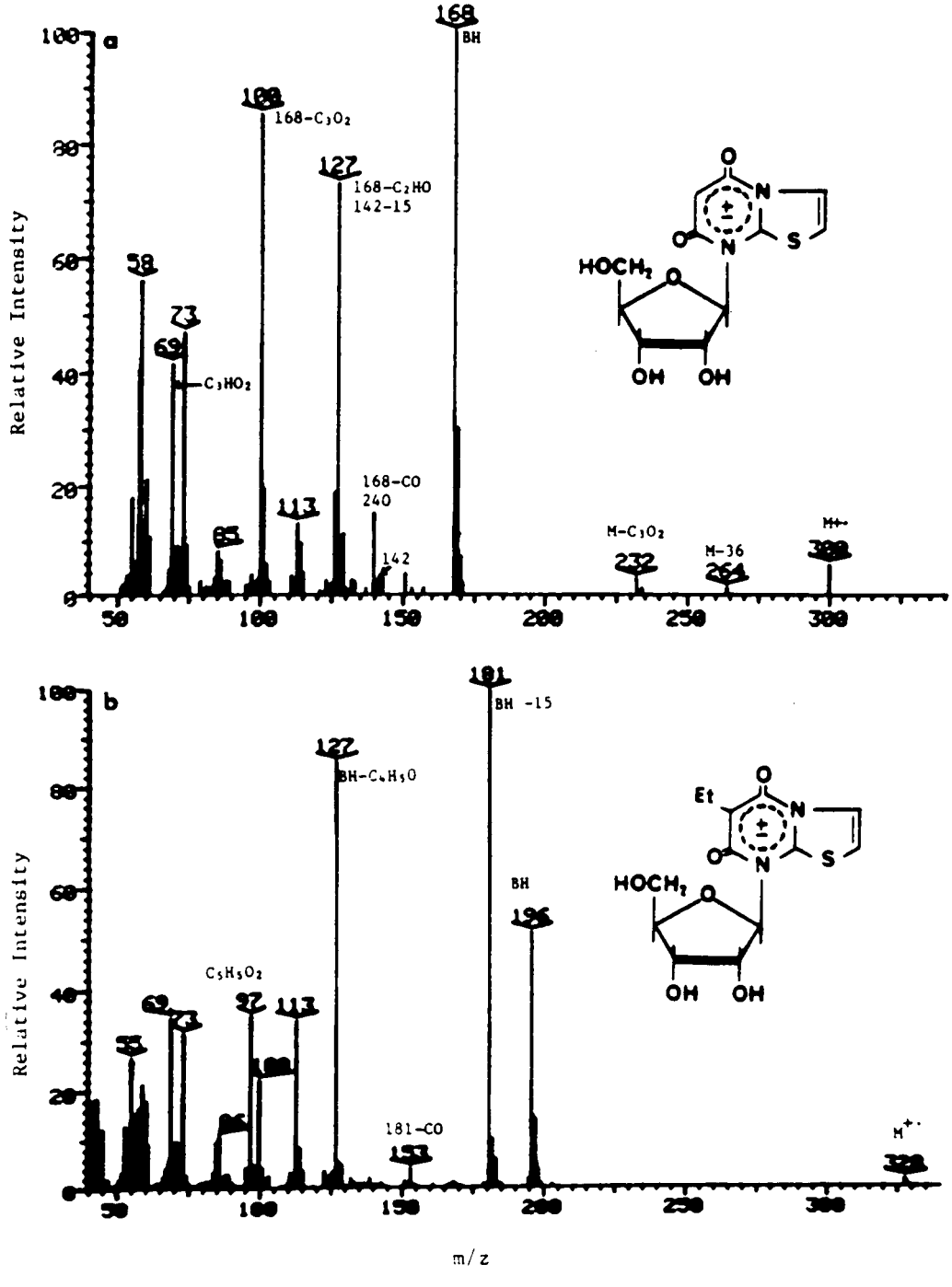


Figure 1. Mass spectra of **2** (top) and **3** obtained using electron impact ionization (70 eV).

sides. Contrariwise, the major resonance forms contributing to the structure of the Class II mesoionic nucleosides have both the positive and negative charges localized in the pyrimidine ring [18,19]. The concentration of charges in a single ring apparently allows internal compensation of the charges or reduces the area over which intermolecular interactions may occur to a degree sufficient to permit vaporization before decomposition. The increased volatility of the Class II mesoionic compounds therefore allows their mass spectral analysis, without derivitization, with molecular ions being observed in the EI spectra.

A second feature which may play a role in the production of molecular ions in the spectra of **2** and **3** is the presence of a sulfur atom in the aglycone. Previous work [24-26] has shown that inclusion of sulfur into a nucleoside may have a marked effect on the intensity of the molecular ion. The magnitude and direction of this effect is difficult to predict however, since results are not consistent with either the type of heterocyclic base or the position of sulfur substitution. Sulfur substitution in the ribose moiety may either increase or decrease the intensity of the molecular ion relative to that of adenosine (3.3%). Thus, 3′-thioadenosine desplays an $M^{+\cdot}$ of 13% relative intensity (RI) while the $M^{+\cdot}$ of 4′-thioadenosine (sulfur in the ribose ring) has a RI of only 0.3% [24]. Exchange of the exocyclic oxygen atoms in the C2 or C4 positions or uridine by sulfur results in either a decrease, *e.g.*, 2-thiouridine (0.5%) [25], or little effect, 4-thiouridine (9%) [25], on the molecular ion intensity relative to that of uridine (9%) [24].

A more pertinent example for determining the effect of the sulfur atom in **2** and **3** on the molecular ion intensity would be a heterocyclic system possessing the atom series N, O, S in the same position of a ring system. A number of examples of such systems are available which closely parallel the aglycone in **2** and **3**, *e.g.*, imidazole, oxazole and thiazole or the series benzimidazole, benzoxazole and benzthiazole. Unfortunately, the molecular ion in all of the above compounds is also the base peak in the spectra [26], thus precluding a valid comparison of the effect of the sulfur atom on the intensity of the molecular ion.

The mass spectra of **2** and **3** also differ significantly from the spectrum of the non-mesoionic analog **4**, and from nucleosides in general, by the absence of any fragments associated with cleavages across the carbohydrate ring [6]. Thus, the structurally informative $(M\text{-}30)^{+\cdot}$, $(B+30)^{+}$, $(B+44)^{+}$ and $(B+60)^{+}$ ions [27] usually observed in the mass spectra of nucleosides are completely absent in the spectra of **2** and **3**. The only peaks in the spectrum of **2** between the molecular and m/z 168 ions are of low intensity and appear at m/z 264, 234 and 232. The first of these ions represents the loss of two molecules of water from the $M^{+\cdot}$ ion and is probably pyrolytic in origin. The neighboring ion at m/z 234 has a composition (see high resolution data presented in Table 1) consistent with the further loss of CH_2O from the m/z 264 ion, most likely from the 5′-position. On the other hand, the m/z 232 peak, formed by expulsion of a molecule of carbon suboxide (C_3O_2) from $M^{+\cdot}$, appears to represent a true fragmentation since the ratio of the m/z 232/300 ions, 0.92, is approximately the same as the intensity ratio of the m/z 100/168 ions, 0.85. The latter ions represent the transition m/z 168 ($BH^{+\cdot}$) to m/z 100 by loss of C_3O_2 as suggested by high resolution and metastable ion data (see below). An alternative mechanism for the formation of m/z 232 could involve elision of a sulfur atom from the m/z 264 ion. The low intensity of the m/z 232 ion yields ambiguous high resolution data, but the operation of this second pathway is questionable since a similar process is not observed to occur from the $BH^{+\cdot}$ ion and is not present in the fragmentation of the heterocyclic bases. In either case, the fragmentation of the heterocyclic base prior to cleavage of the sugar bond is noteworthy because such processes are not normally observed and must be related to the unusual structure of the mesoionic nucleosides.

The spectrum of **3**, likewise, shows the complete absence of ions associated with the base with portions of the carbohydrate attached. Thus with the exception of the minor ions described above, the decomposition of the molecular ions of the mesoionic nucleosides **2** and **3** follow a uniquely different course relative to other "normal" nucleosides. That the presence of the mesoionic base play a major role in altering the fragmentation of this class of nucleoside is supported by a comparison with the mass spectrum of the isomeric, but non-mesoionic analog **4**.

The spectrum of 1-(β-D-ribofuranosyl)thieno[2,3-*d*]pyrimidine-2,4-dione (**4**) [21] follows decomposition routes more representative of nucleosides (See Figure 2a). Ions at m/z 210 and 227 represent, respectively, the $[B+43]^{+\cdot}$ and $[B+60]^{+}$ ions formed by fragmentation of the sugar ring. The appearance of a $[B+43]^{+\cdot}$ rather than a $[B+44]^{+}$ ion is interesting, but not unique to **4** since a similar ion, of unknown origin, is also present in the spectrum of 2′-deoxycytidine and, possibly, thymidine [6]. The intensity of the molecular ion of **4**, m/z 300 (2%), is approximately the same as observed for **2** and **3** indicating that the mesoionic aglycone has little effect on the intensity of the molecular ion. Note should also be made of the fact that the relative intensity of the molecular ion has little correlation with the intensity of the $[B+30]^{+}$, $[B+44]^{+}$ or $[B+60]^{+}$ ions. For example, relatively intense fragments corresponding to these ions are present in the spectrum of 5′,5′-di-C-methyladenosine ($M^{+\cdot}$, 0.3%) with relative intensities of 16, 20 and 0.4%, respectively, while 1-methyladenosine ($M^{+\cdot}$, 7.6%) provides the same ions with respective intensities of 4.6, 5.2 and 4.7% [24,25]. Therefore, nucleosides with only marginally detectable molecular ions may fragment to yield the structurally informative $[B+30]^{+}$, $[B+44]^{+}$ and

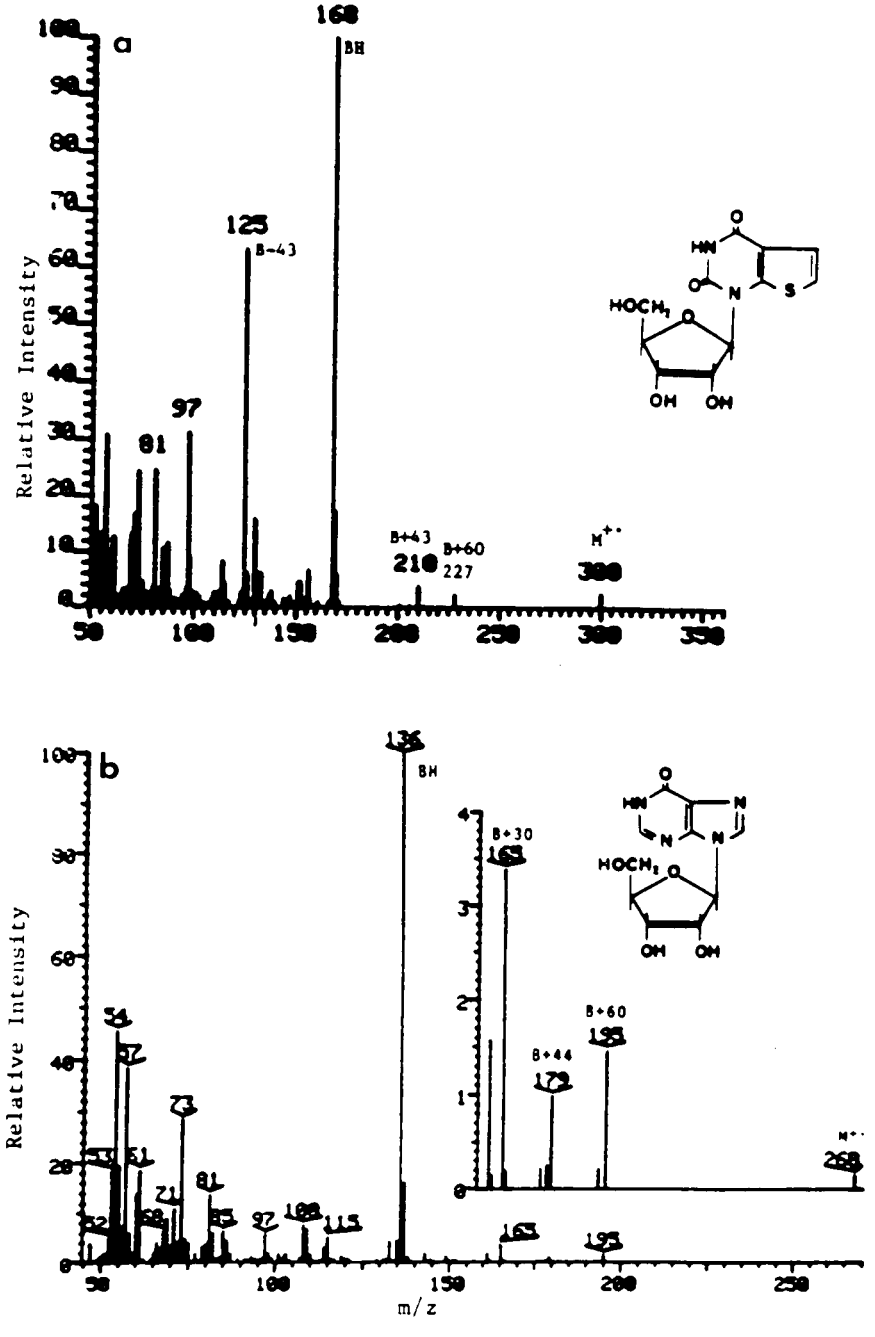


Figure 2. Electron impact (70 eV) mass spectra of a) **4** which is isomeric with **2** but is non-mesoionic and b) inosine showing the presence of the $(B+30)^+$, $(B+44)^+$, $(B+60)^+$ and $M^{+\cdot}$ ions. Both of these spectra were ob tained using conditions identical to those used to record the spectra of **2** and **3**.

$[B+60]^+$ ions and some other mechanism must be operating which accounts for the absence of these ions in the spectra of **2** and **3**.

The non-volatile nature of underivatized nucleosides presents the ever present possibility of pyrolysis of the sample to the free base, equivalent to the $BH^{+\cdot}$ ion, or other thermally derived product ions. One method of distinguishing decomposition from fragmentation is to plot the history of ions associated with the intact molecule and ions associated with thermal degradation [6]. Shown in Figure 3a are mass chromatograms of the m/z 268 ($M^{+\cdot}$), 136

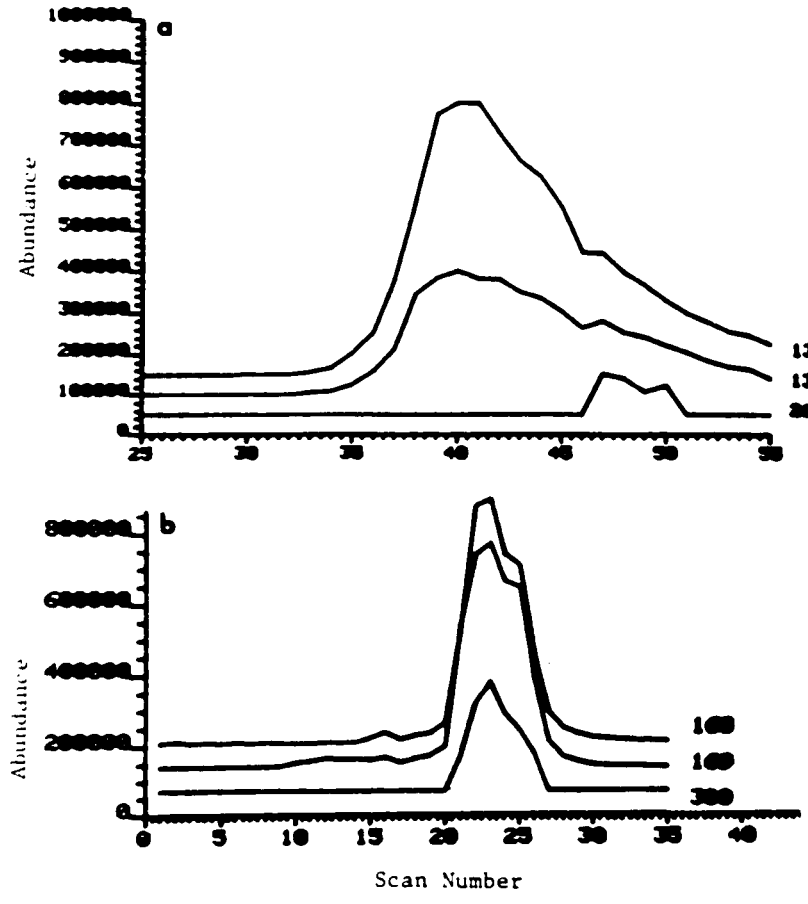


Figure 3. Mass chromatograms obtained by plotting ions at a) m/z 136 [(BH)$^+$], 137 [(B+2H)$^+$] and 268 (M$^+$) for Inosine (**5**) and b) m/z 168 [(BH)$^+$], 169 [(B+2H)$^+$] and 300 (M$^+$) in the spectrum of mesoionic nucleoside **3**.

(BH$^+$) and 137 (BH$_2^+$) ions of a sample of inosine (**5**), a nucleoside known to undergo extensive decomposition prior to ionization [6]. (Conditions used in acquisition of this data are as described in the experimental section and are unchanged in any of these experiments.) The rise in the ion currents of the m/z 136 and 137 ions during the earlier scans, *i.e.*, scan numbers 35-45, indicate decomposition is occurring prior to volatilization and ionization of the intact molecule. The ion profile of m/z 268 shows that spectra of the intact molecule are generated later in the run when higher probe temperatures are reached and beginning around scan number 46 and continuing to scan number 51. In contrast, Figure 3b shows the ion profiles of the m/z 300 (M$^+$·), 168 (BH$^+$·) and 169 (BH$_2^+$) ions during the vaporization of **2** using same experimetnal conditions as were used in the analysis of **5**. In this case, the ion profiles are seen to rise and fall in a coincident fashion, indicating that thermal decomposition plays a minimal role in the production of BH$^+$· in the spectrum of **2**.

As shown in Figure 3a, some molecular ions are formed with heating of the direct probe during the analysis of inosine and an average of scans 47-50 produces the spectrum shown in Figure 2b. Although the quality of this mass spectrum of underivatized inosine must be considered poor because of the decomposition mentioned above, the spectrum does provide a significant number of structurally informative ions. Of particular interest are the M$^+$· (m/z 268, 0.3%) and fragments containing the base plus portions of the sugar, *i.e.*, [B+30]$^+$ (m/z 165, 3.4%), [B+44]$^+$ (m/z 179, 1.0%) and [B+60]$^+$ (m/z 195, 1.5%). Thus, even in the case of inosine where pyrolytic effects are extreme, the characteristic nucleoside fragments are, to some degree, observed.

The inherent stability of the glycosidic bond is another factor to be considered as a potential source of free base. Under normal EI conditions, cleavage of the glycosidic bond is the most prominent route of fragmentation for nucleosides and this reaction leads directly to ions related to the heterocyclic base, BH$^+$· and BH$_2^+$, and/or ions derived from the sugar moiety [6]. In addition to permitting identification of the aglycone and carbohydrate portions of the molecule, the relative intensity of these ions provides information concerning the class of base or sugar present. More intense BH$^+$· ions are associated with a pur-

ine base while the BH_2^+ ion predominates in the spectra of pyrimidine nucleosides [6]. The intensity of the sugar ion (S^+, m/z 133) is also affected by the class of base with the purine analogs producing relatively weak S^+ ions because of charge stabilization by the electron rich purine ring system. The pyrimidines, in general, produce more intense S^+ ions. The spectra of **2**, **3**, and **4** are therefore of interest since they reflect a pronounced purine character, strong $BH^{+\cdot}$ and weak S^+ ions, even though the glycosidic bond is made with a pyrimidine ring. Differentiation of **2** or **3** from **4** is possible, however, based on differences in decomposition of the heterocyclic ring, most especially the loss of HNCO by a Retro-Diels Alder mechanism, common to uracil type compounds [6], to afford the m/z 125 ion in the spectrum of the latter compound.

The classical method of determining the relative stability of the glycosidic bond of nucleosides involves acid hydrolysis to determine the kinetics of the bond cleavage [28]. No kinetic studies have been reported in the case of the compounds of interest in this work. Chemical ionization mass spectrometry has also been used to determine the relative stabilities of isomeric pairs of nucleosides [29]. Although offering several advantages over the classical method, these CI studies have not been extended beyond the preliminary stage and have not been utilized in the present cse. Thus, no data is available concerning the stability of the glycosidic bond of **2** and **3**. However, elemental analysis of these samples, the presence of molecular ions in the EI spectra and the production of a strong MH^+ ion in the fast atom bombardment spectra of **2** and **3** argue against an inherent instability of the glycosidic bond in these samples.

In contrast to the above questionable possibility of an unstable glycosidic bond is the known facile cleavage of the base-sugar bond of nucleosides under EI conditions in the mass spectrometer. A unique feature of the Class II mesoionic nucleosides **2** and **3** is the presence of the localized charges in the pyrimidine ring. If the structure of **2** is drawn as one of the dipolar structures shown in Scheme 2, the ejection of an electron during electron impact ionization would be expected to occur from the position of highest electron density, *i.e.*, the site carrying the negative charge. Since the anionic site, which becomes the radical site following ionization, and the cationic site are both located in the aglycone, subsequent decompositions of the $M^{+\cdot}$ ion will reflect this exclusive localization of the radical-ion sites in the base moiety [30]. Fragmentation of the $M^{+\cdot}$ ion now proceeds by cleavage of the glycosidic bond with concommitent transfer of a hydrogen atom from the sugar to the base, as is the case with other nucleosides. That this reaction is extremely facile is indicated by the intensity of the $BH^{+\cdot}$ ion. Other pathways leading to cleavages across the ribose ring are thus suppressed by the rapid glycosidic bond rupture. In contrast, **4** and other normal nucleosides do not contain a localized negative charge from which an electron can be readily ejected. Mixed molecular ions are therefore produced in which the ion-radical sites are present in both the base and sugar portions of the molecular ions. Subsequent fragmentations are thus directed from both portions of the molecule depending on the extent of the localization of the ion-radical sites. Purines, being better able to stabilize the charge thus produce very few sugar related fragments while pyrimidines, with the ion-radical sites more evenly distributed between the aglycone and ribose rings, display more intense sugar related ions. The mesoionic nucleosides **2** and **3** may therefore be viewed as an extreme example of the purine system where the ion-radical sites are almost entirely contained in the aglycone. The unusual fragmentation of the mesoionic nucleosides **2** and **3** is thus a result of their unique structure which permits almost exclusive localization of the radical and charge sites in the aglycone resulting in an extremely facile cleavage of the glycosidic bond, following ionization and hydrogen transfer from a hydroxyl hydrogen of the sugar.

Decompositions of the $BH^{+\cdot}$ ions of **2** and **3** follow pathways also evident in the spectra of the mesoionic bases **6-9** and the non-mesoionic compound **10**. Similar spectral characteristics are present in samples **2**, **6** and **10** with compounds **3**, **7**, **8** and **9**, which contain an ethyl function in the C6 position, forming a second group. The EI mass spectra of **6**, **7** and **10** are shown in Figure 4.

Each of the three samples **2**, **6** and **10** display the m/z 168 ion as a strong peak in their spectra. As discussed above, concerted hydroxyl hydrogen transfer with glycosidic bond cleavage leads to the m/z 168 ion in the spectrum of **2**, while loss of a molecule of ethylene from the molecular ion (m/z 210) is required in the case of **6**. No losses are required to form this ion with sample **10** since this is the molecular ion. Following their formation, the m/z 168 ions decompose by four primary routes as established by high resolution mass measurements (Table 1) and/or metastable ion analysis (Table 2).

One major pathway of decomposition of the m/z 168 ion involves expulsion of a molecule of carbon suboxide to produce the m/z 100 ion. Although alternative mechanisms may be offered to explain this transition, rearrangement of the radical and charge sites with ring opening to structure m/z 168a with subsequent fragmentation by simple, known mechanisms readily accounts for the formation of not only m/z 100 but also the relatively intense m/z 69 ion (see Scheme 3). Thus, localization of the radical on O^7 initiates a cleavage of the N4-C5 bond leading to the opening of the pyrimidine ring. Charge site initiated, homolytic cleavage of the C7-N8 bond then produces the m/z 69 ion with loss of an aminothiazole radical. Alternatively, a McLafferty type rearrangement may take place with trans-

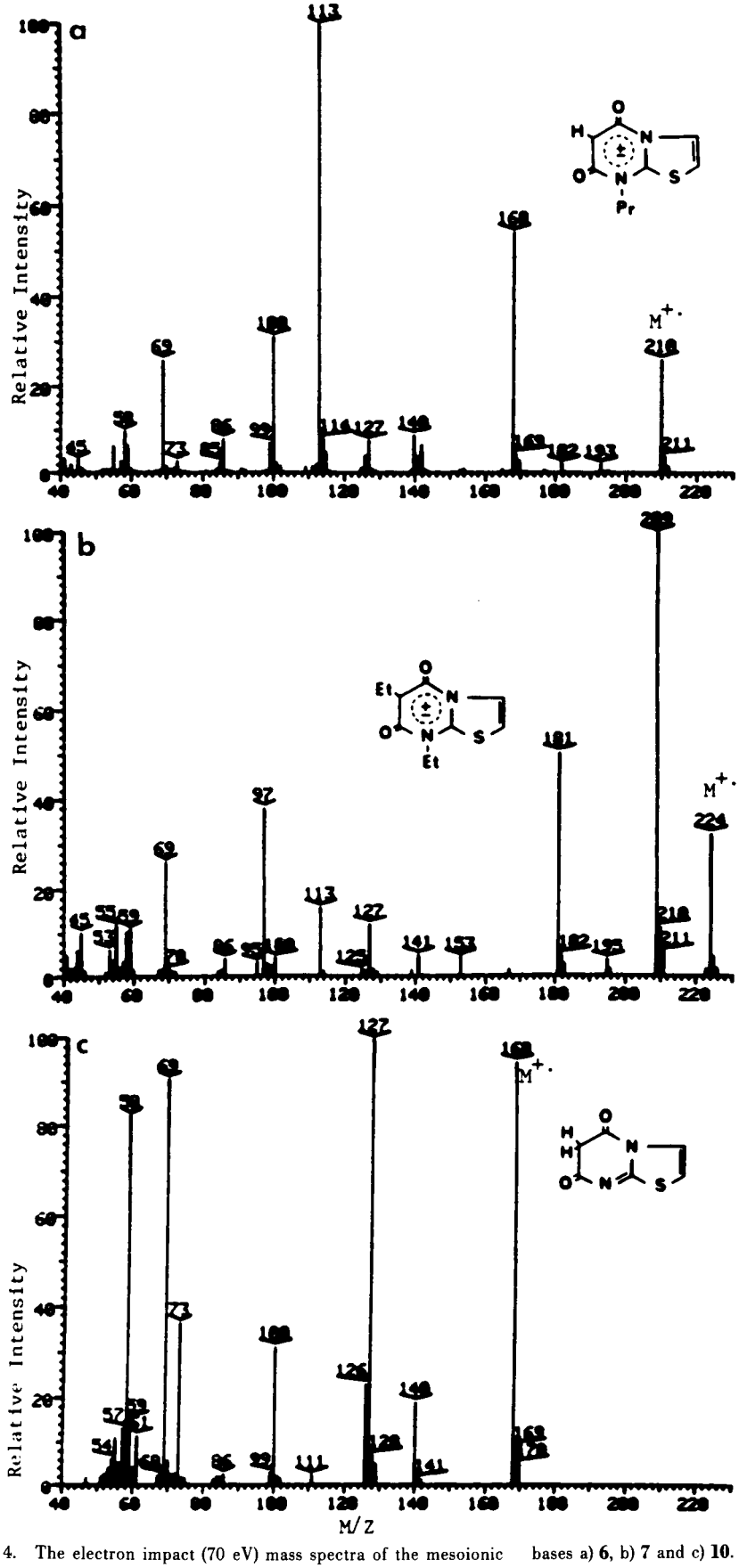


Figure 4. The electron impact (70 eV) mass spectra of the mesoionic bases a) **6**, b) **7** and c) **10**.

Table 1

High Resolution Mass Measurements of the Major Ions in the Spectra of Samples **2**,**3**,**6**,**7** and **10** [a]

2	**3**	**6**	**7**	**10**
m/z 300, $C_{11}H_{12}N_2O_6S$ (-1.9)	m/z 328, $C_{13}H_{16}N_2O_6S$ (-0.4)	m/z 210, $C_6H_{10}N_2O_2S$ [b]	m/z 224, $C_{10}H_{12}N_2O_2S$ (1.7)	m/z 168, $C_6H_4N_2O_2S$ (-0.1)
m/z 264, $C_{11}H_8N_2O_4S$ (1.2)	m/z 196, $C_8H_8N_2O_2S$ (0.8)	m/z 182, $C_6H_{10}N_2OS$ (-0.5)	m/z 209, $C_9H_9N_2O_2S$ (1.2)	m/z 140, $C_5H_4N_2OS$ (-1.2)
m/z 234, $C_{10}H_6N_2O_3$ (-0.1)	m/z 181, $C_7H_5N_2O_2S$ (1.5)	m/z 168, $C_6H_4N_2O_2S$ (-0.1)	m/z 196, $C_8H_8N_2O_2S$ (0.8)	m/z 127, $C_4H_3N_2OS$ (2.3)
m/z 232, $C_8H_{12}N_2O_4S$ (-5.7)	m/z 153, $C_6H_5N_2OS$ (-1.6)	m/z 142, $C_6H_{10}N_2S$ (-0.6)	m/z 195, $C_8H_7N_2O_2S$ (-0.7)	m/z 100, $C_3H_4N_2S$ (-3.4)
$C_{11}H_8N_2O_4$ (-2.5)	m/z 127, $C_4H_3N_2OS$ (0.7)	m/z 140, $C_5H_4N_2OS$ (-0.8)	m/z 181, $C_7H_5N_2O_2S$ (1.5)	m/z 73, C_2H_3NS (1.1)
m/z 168, $C_6H_4N_2O_2S$ (-0.5)	m/z 100, $C_3H_4N_2S$ (0.4)	m/z 127, $C_4H_3N_2OS$ (0.6)	m/z 167, $C_7H_7N_2OS$ (1.6)	m/z 58, C_2H_2S (4.0)
m/z 140, $C_5H_4N_2OS$ (0.2)		$C_5H_7N_2S$ (0.5)	m/z 153, $C_6H_5N_2OS$ (-1.6)	
m/z 127, $C_4H_3N_2OS$ (0.8)		m/z 113, $C_4H_5N_2S$ (-0.5)	m/z 141, $C_5H_5N_2OS$ (2.5)	
m/z 100, $C_3H_4N_2S$ (0.4)		m/z 100, $C_3H_4N_2S$ (0.0)	m/z 127, $C_4H_3N_2OS$ (0.4)	
m/z 85, C_3H_3NS (1.4)		m/z 86, C_3H_4NS (-0.4)	$C_5H_7N_2S$ (0.7)	
m/z 73, C_2H_3NS (1.4)			m/z 113, $C_4H_5N_2S$ (0.5)	
			m/z 100, C_4H_6NS (-0.2)	
			$C_3H_4N_2S$ (0.4)	
			m/z 97, $C_5H_5O_2$ (-0.4)	
			m/z 86, C_3H_4NS (-0.8)	

[a] Nominal mass value is followed by elemental composition (error mmu). [b] High resolution data not obtained on this peak.

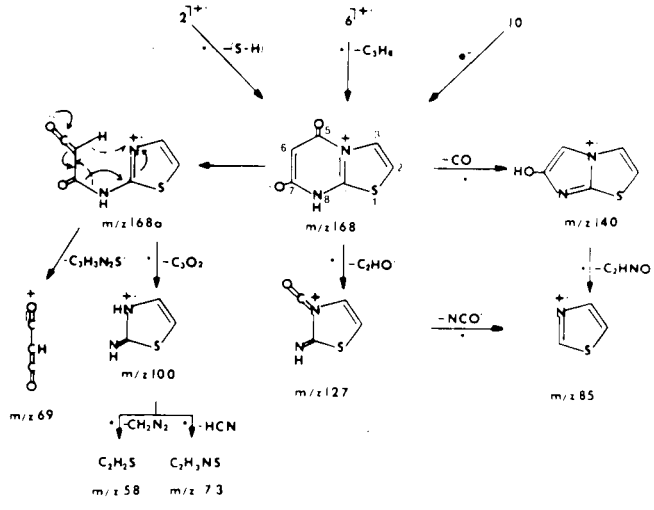
Table 2

Metastable Ion Data of Selected Mesoionic Bases and Nucleosides

Compound	Parent	Daughter(s)
3	328 (M^+)	197, 196
	196	181, 127, 97
	181	153, 127, 113
	127	100, 99, 86, 58
	97	69
6	210 (M^+)	193, 182, 168, 142
	182	154, 140
	168	140, 127, 100
	142	127, 113, 100, 99, 86
	140	113, 100, 85
	127	100, 86
	113	86
	100	73, 58
7	224 (M^+)	209, 196, 195, 127
	209	181, 167
	196	181, 127
	195	167, 127
	181	153, 127, 113
	167	139
	127	100, 86
	113	86
	97	69
10	168 (M^+)	140, 127, 100
	140	85
	127	100, 99, 86, 58
	100	73, 58

fer of the C6 hydrogen to the N4 nitrogen atom with concomitant cleavage of the C7-N8 bond to form the peak at m/z 100. The m/z 100 ion is identical to the molecular ion of 2-aminothiazole and further decompositions of this ion proceed by previously established routes [31,32] to yield strong ions at m/z 58 and 73.

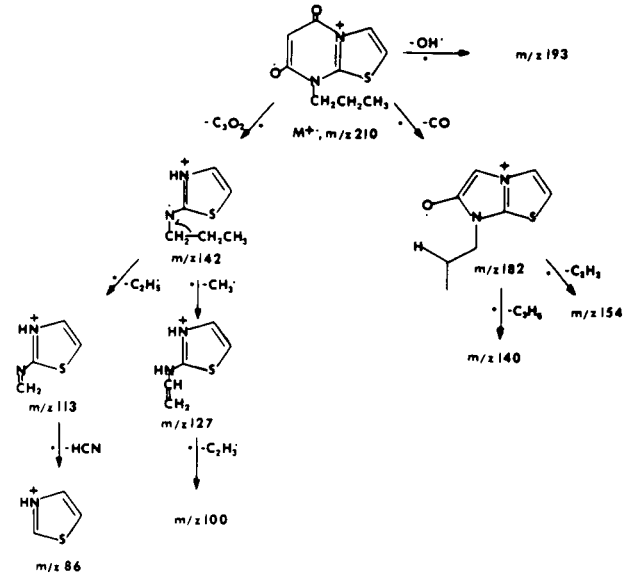
Two additional decompositions of the m/z 168 ion are established by metastable ion and high resolution data.



Scheme 3. Common pathways for the formation of major ions in the mass spectra of **2**, **6**, and **10**.

First loss of a ketene radical produces the m/z 127 ion which further expels an isocyanate radical to produce the m/z 85 ion. Although the elimination of two consecutive radicals violates the even-electron rule, numerous exceptions to this postulate have been noted [33], particularly when stable radicals are eliminated which result in the formation of highly stabilized systems. A second route for the production of the m/z 85 ion involves the initial expulsion of CO from the m/z 168 ion to yield the m/z 140 ion, which may exist in a bicyclic form. Isotopic labeling will be needed to establish which carbon and oxygen atoms are involved in this reaction. Breakdown of the imidazole ring in the m/z 140 ion by elision of C_2HNO then produces the m/z 85 ion.

The presence of the N8 propyl group in **6** offers alternate pathways for the decomposition of the molecular ion of this compound as shown in Scheme 4. Initial expulsion of carbon suboxide from the $M^{+\cdot}$ (m/z 210) forms an ion at

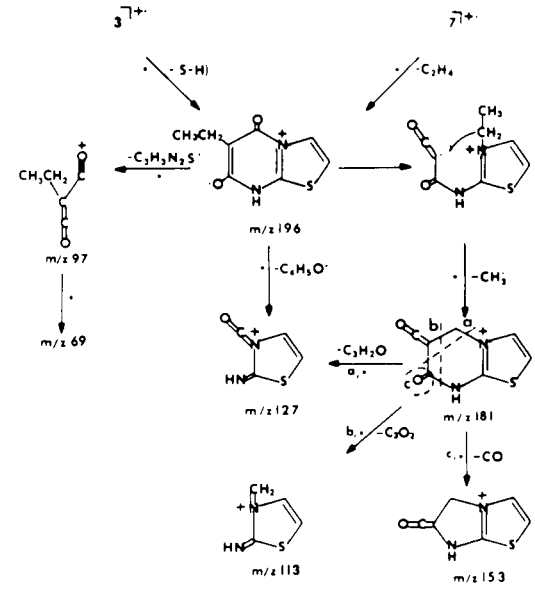


Scheme 4. Alternate decomposition pathways observed in the mass spectrum of **6**.

m/z 142 which retains the propyl group. Loss of an ethyl radical from m/z 142 then gives the m/z 113 ion, which is the most intense ion in the spectrum of **6**. Further elimination a molecule of HCN from m/z 113 gives the m/z 86 ion. Alternatively, the m/z 142 ion may expel a methyl radical to form a second m/z 127 ion with the composition $C_5H_7N_2$. This m/z 127 ion is indicative of alkyl substitution at N8 and is also observed in the spectrum of **7**. None of the samples which lack the alkyl substituent at N8 show the m/z 127 doublet. Expulsion of a C_2H_3 radical is indicated by a metastable ion and is a second pathway to the m/z 100 ion.

Minor pathways are also indicated by metastable ion data which involve the initial expulsion of CO from the $M^{+\cdot}$ ion of **6** to give the m/z 182 ion. The elimination of the C5 oxygen is used to illustrate this pathway, but loss of the C7 oxygen is also possible. The alkyl side chain may then be lost either by elimination of ethylene to provide the minor ion at m/z 154 or propylene may be expelled to give m/z 140 ion. The further decomposition of the m/z 154 ion was not examined because of the apparently minor nature of this pathway.

The major fragmentation pathways described above are also operating in the decomposition of **3** and **7-9** as shown in Scheme 5. Fragmentation of compounds **8** and **9** are the same as **7** with the expected mass shifts being observed and are not shown. Elimination of ethylene from the molecular ion of **7**, produces the m/z 196 ion which corresponds



Scheme 5. Common pathways of fragmentation observed in the mass spectra of **3** and **7**.

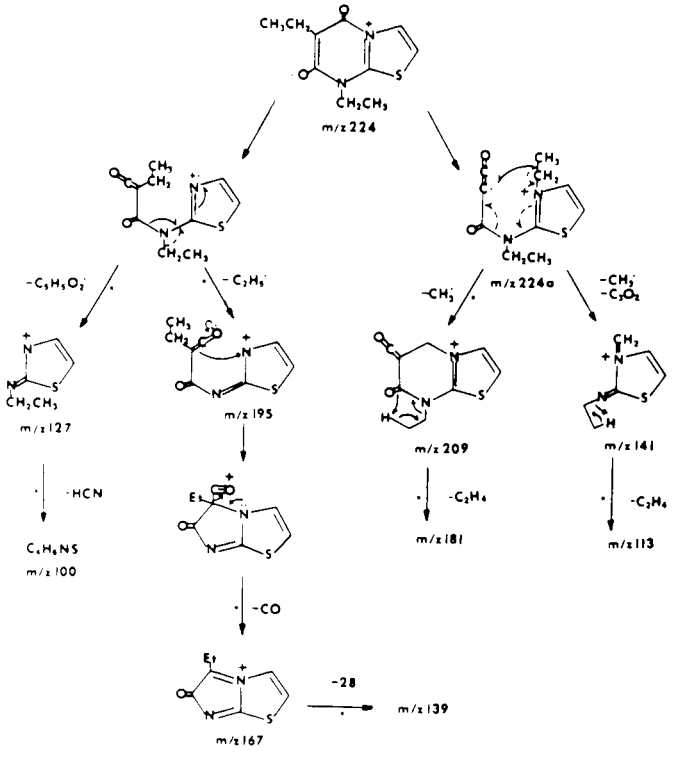
to the $BH^{+\cdot}$ ion present in the spectrum of **3**. The selective loss of the ethyl group located on N8 in **7** is strongly suggested by subsequent decompositions of the m/z 196 ion. For example, the direct formation of the m/z 97 ion from the m/z 196 ion is analogous to formation of the m/z 69 ion in the spectra of **2**, **6** and **10**, *i.e.*, fragmentation of the pyrimidine ring occurs with charge retention by the C5-C7 fragment which includes the ethyl group at C6. Likewise, the formation of an ion at m/z 100 from m/z 196 requires the ethyl group to be located at the C6 position (not shown in Scheme 5). Formation of the m/z 113 ion requires elimination of a molecule of carbon suboxide from the m/z 196 ion with loss of an additional methyl radical. A mechanism similar to that proposed for the formation of the m/z 100 ion in the spectra of **2**, **6** and **10** could account for this transition. Transfer of the ethyl group at C6 to the N4 position following opening of the pyrimidine ring would yield an intermediate capable of the concurrent and facile expulsion of both of the necessary elements. The m/z 113 ion so formed then further degrades to the m/z 86 ion by elimination of HCN.

The major route of decomposition of the m/z 196 ion is the loss of a methyl radical to form the m/z 181 ion, which is the base peak in the spectrum of **3** and is the second most intense ion in the spectrum of **7**. The appropriately shifted ion is also the second strongest ion in the spectra of **8** and **9**.

Formation of the m/z 181 ion is envisioned to proceed by transfer of an ethyl radical to the N4 position in a manner analogous to the transfer of a hydrogen in the C6 unsubstituted series. Ring closure of the diradical species

formed upon elimination of CH_3· then leads to the m/z 181 ion shown in Scheme 5. Although other structures may be easily rationalized for this ion, the structure shown in Scheme 5 may readily lose the required elements to form the m/z 113, 127 and 153 ions, shown by metastable ion analysis to be the daughter ions of the m/z 181 ion. The m/z 127 ion is also produced directly from the m/z 196 ion by elimination of a C_4H_5O radical, suggesting the presence of the ethyl group at C6.

The presence of the ethyl function at N8 adds to the complexity of the mass spectrum of **7** by affording alternate routes of decomposition illustrated in Scheme 6. Ring opening of the pyrimidine ring with loss of a $C_5H_5O_2$· fragment produces the second component of the m/z 127 doublet, with a composition of $C_5H_7N_2S$. This ion is a homolog



Scheme 6. Alternate routes for the mass spectral fragmentation of **7**.

of the m/z 113 ion in the spectrum of **6** and has the same elemental composition as the m/z 127 ion formed by elimination of a methyl radical from the m/z 142 ion in the spectrum of **6**. Elimination of a molecule of HCN from the m/z 127 ion of **7** produces the second component of the m/z 100 ion indicated by high resolution data. The m/z 127 ion, with the $C_5H_7N_2S$ composition, observed in the spectra of **6** and **7** is therefore indicative of alkyl substitution at the N8 position.

The ring opened intermediate may also expel an ethyl radical to provide the m/z 195 ion which subsequently eliminates CO to produce the m/z 167 ion, possibly through the bicyclic intermediate shown in Scheme 6. The only daughter ion of the m/z 167 ion is the minor m/z 139 ion of unknown composition.

Two final pathways for the decomposition of the $M^{+\cdot}$ of **7** are indicated by metastable ions. The base peak in the spectrum of **7** is formed by expulsion of a methyl radical following ring opening and transfer of the C6 ethyl group to the N4 position. The m/z 209 ion thus formed is analogous in structure to the m/z 181 ion but retains the N8 ethyl group; in fact elimination of ethylene to provide the m/z 181 ion by a second route is indicated by an appropriate metastable ion. Rather than undergoing ring closure, the intermediate m/z 224a may lose a methyl radical and a molecule of carbon suboxide in a concerted manner to provide the m/z 141 ion. Further loss of ethylene from m/z 141 then yields the m/z 113 ion which is isomeric with the m/z 113 ion present in the spectrum of **6**.

Mass Spectra of the Trimethylsilyl Derivatives of **2** and **3**.

The mass spectra of the trimethylsilyl (TMS) derivatives **11** and **12** (See Figure 5), like the free nucleosides **2** and **3**, show considerable variation from the patterns generally observed in the mass spectra of nucleoside-TMS derivatives [34]. Ions in the mass spectra of nucleoside-TMS derivatives have been divided into three groups: 1) those ions derived primarily from the $M^{+\cdot}$ ion; 2) ions associated with the base plus portions of the carbohydrate; and 3) ions derived from the sugar portion of the molecule. Of the 23 ions possible in the first two groups [34], only four are observed in the mass spectra of **11** and **12**.

Each of these samples incorporates three trimethylsilyl groups onto the sugar moiety as indicated by $M^{+\cdot}$ ions at m/z 516 and 544 for **11** and **12**, respectively, and the presence of a strong $[S\text{-}H]^{+\cdot}$ ion at m/z 348 in the spectra of both samples, indicating complete derivatization of the sugar. The absence of a TMS group in the heterocyclic base is surprising since the presence of the full negative charge, if located on one of the exocyclic oxygen atoms, was expected to react rapidly with the derivatizing reagent to incorporate a TMS group into the base.

The presence of an $[M\text{-}15]^+$ ion with an intensity less than the $M^{+\cdot}$ ion of TMS derivatized nucleosides is normally associated with the presence of a guanine type base [34]. The spectrum of **11** is, therefore, unique in that no $[M\text{-}15]^+$ is present, even when the spectrum is plotted to 0% relative intensity as shown in the insert Figure 5a. The virtual absence of an $[M\text{-}15]^+$ ion in the mass spectrum of a TMS derivative of a nucleoside has not been previously reported. In the case of **12**, the $[M\text{-}15]^+$ ion is observed but is very weak (0.29%) and the ratio of the $M^{+\cdot}/[M\text{-}15]^+$ ion is about seven, which is considerably greater than any of the guanosine-TMS derivatives reported thus far [34]. Factors other than the presence of an amino group at the C2 position of purine bases must therefore be responsible for the

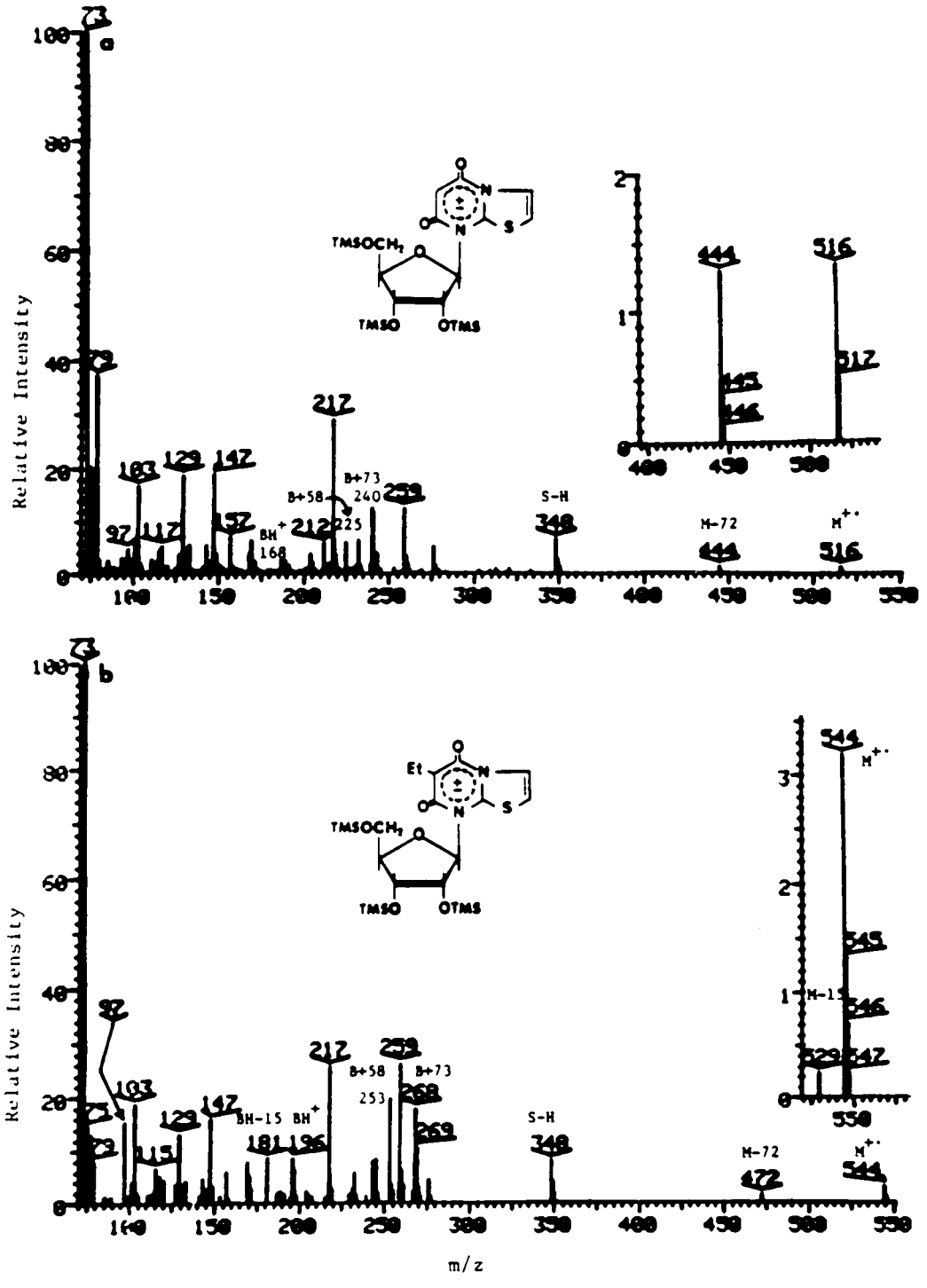


Figure 5. Mass spectra of the trimethylsilyl derivatized mesoionic nucleosides **11** (top) and **12**. Of particular interest is the $M^{+\cdot}$/M-15 ratio. The inserts show the $M^{+\cdot}$ ion region plotted to 0% relative intensity.

intensity of the $[M-15]^+$ ion relative to the $M^{+\cdot}$ ion [35]. In the present case, the rapid cleavage of the glycosidic bond may prevent any significant formation of even the $[M-15]^+$ ion.

The ion corresponding to $[M-72]^{+\cdot}$ at m/z 444 in the spectrum of **11** and m/z 472 in **12** is also unique to the mesoionic nucleosides. Whether this ion is a result of incomplete derivatization or is an actual fragment ion is not

Table 3

Fragment Ions and Their Intensities in the Mass Spectra of Selected Thiazolo[3,2-*a*]pyrimidine-5,7-dione Bases and Nucleosides and Related Samples of Interest

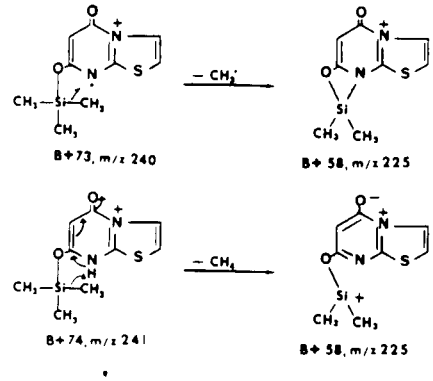
Compound	$M^{+\cdot}$	M-CH_3	M-CO	M-C_3O_2	M-CH_3-C_3O_2	M-$C_2R'O$	$BH^{+\cdot}$ [a]	BH-CH_3	BH-CO	BH-CH_3-CO	BH-$C_2R'O$ [b]	BH-C_3O_2	BH-CH_3-C_3O_2	C_3O_2R'	Other
2	300 (5) [c]	—	—	232 (3)	—	—	168 (100)	—	140 (15)	—	127 (73)	100 (86)	—	69 (42)	264 (1), M-$2H_2O$; 234 (1); 133 (2), $S^{+\cdot}$, 132 (3), S-H; 114 (9), S-H-H_2O, 86 (6), 85 (8); 73 (47); 58 (56)
3	328 (2)	—	—	—	—	—	196 (51)	181 (100)	—	153 (3)	127 (85)	—	113 (33)	97 (34)	100 (21), BH-$C_2R'O$-HCN, BH-$C_2R'O$-C_2H_3 [d]; 86 (10); 85 (7), 73 (30); 69 (33)
4	300 (2)	—	—	—	—	—	168 (100)	—	—	—	—	—	—	—	227 (2), B + 60; 210 (4), B + 43; 125 (63), BH-HNCO
5	268 (0.3)		—	—	—	—	136 (100)		—	—	—	—	—	—	195 (1.5), B + 60; 179 (1), B + 44; 165 (3), B + 30
6	210 (25)	—	182 (2)	142 (5)	—	141 (3)	168 (54)	—	140 (8)	—	127 [d] (7)	100 (30)	—	69 (24)	193 (2), M-OH; 113 (100), M-$C_3O_3C_2H_5$; 86 (7); 85 (2), 73 (2); 58 (8); 55 (5)
7	224 (31)	209 (100)	—	—	141 (4)	127 [d] (11)	196 (1)	181 (50)	168 (< 1)	153 (4)	127 [d] (11)	128 (< 1)	113 (14)	97 (37)	195 (3), M-C_2H_5; 167 (1), M-C_2H_5-CO; 86 (3); 69 (25); 55 (11)
8	238 (33)	223 (100)	—	—	155 (8)	141 (18)	210 (5)	195 (74)	182 (< 1)	167 (10)	141 (18)	142 (3)	127 (18)	97 (76)	209 (3), M-C_2H_5; 181 (1), M-C_2H_5-CO; 114 (8); 86 (6), 73 (13); 71 (21); 69 (48)
9	226 (27)	211 (100)	—	—	143 (7)	129 (7)	198 (< 1)	183 (41)	170 (< 1)	155 (3)	129 (7)	130 (< 1)	115 (26)	97 (8)	197 (5), M-C_2H_5; 95 (47); 88 (6), 86 (5); 69 (14)
10	[e]	—	—	—	—	—	168 (95)	—	140 (18)	—	127 (100)	100 (30)	—	69 (90)	73 (35); 58 (87)

[a] In the case of the bases this ion refers to loss of the alkyl group at N8 with transfer of a hydrogen to the heterocyclic ring. [b] This ion has same composition as B-CH_3-C_3H_2O. [c]Relative intensity given in brackets to nearest integer. [d] This ion is a doublet. [e] The $M^{+\cdot}$ and $BH^{+\cdot}$ ion series are the same.

at present known.

Ions of the base series are also different from those normally observed in the spectra of nucleoside-TMS derivatives. First, as was the case with the free nucleosides, is the complete absence of ions associated with the heterocyclic ring with portions of the sugar attached. Of the 15 ions reported [34] in the base series only the $[B+74]^+$, $[B+58]^+$ and $BH^{+\cdot}$ ions are present in the spectra of **11** and **12**. The $[B+74]^+$ ion, m/z 241 in the spectrum of **11** and m/z 269 in **12**, has been shown [34] to form from the molecular ion by transfer of a hydrogen and a trimethylsilyl group to the base and to be prevalent in the mass spectra of purine nucleosides. The $[B+58]^+$ ion is formed from the $[B+74]^+$ ion by loss of a molecule of methane. The presence of these two ions in the spectra of **11** and **12** thus confirms the purine type character of these nucleosides.

Of particular interest in the spectra of **11** and **12** is an ion corresponding to $[B+73]^{+\cdot}$ which has not been reported to be present in the spectra of other TMS derivatized nucleosides [34]. This ion occurs one mass unit lower than the $[B+74]^+$ ion and apparently forms from the molecular ion by transfer of a TMS group without concomitant transfer of a hydrogen. Transfer of a TMS radical to the oxygen at C6 concurrent with glycosidic bond cleavage yields an ion-radical at m/z 240 in the case of **11**, which is in contrast to the production of an even electron ion as normally occurs. As shown in Scheme 7, the $[B+73]^{+\cdot}$ ion may expel a methyl radical from the trimethylsilyl group to form the $[B+58]^+$ ion by a unique route. In fact, the $[B+73]^{+\cdot}$ ion



Scheme 7. Possible routes of formation of the B + 58 ion present in the mass spectra of mesoionic nucleosides **11** and **12** (only **11** shown).

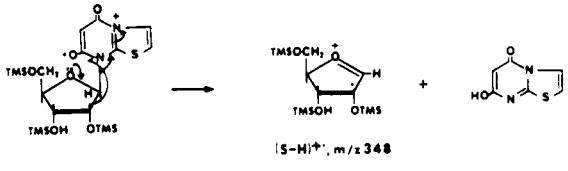
may be the major parent of the $[B+58]^+$ ion in the mesoionic nucleosides since elimination of CH_4 from the $[B+74]^+$ ion would produce the highly charged product ion shown at the bottom of Scheme 7.

The only other peaks in the mass spectra of **11** and **12** belonging to the molecular ion and base series are ions at m/z 168 and 196 which may represent the $BH^{+\cdot}$ ions observed in the underivatized nucleosides **2** and **3**. Ions observed in the spectrum of **11** correlated with decomposition of the $BH^{+\cdot}$ ion appear at m/z 181 and 97. No ions of significance in the fragmentation of the free nucleoside **2** are observed in the spectrum of **12**. Thus, in contrast to the spectra of the free nucleosides which are dominated by base related fragment ions, the spectra of the TMS deriva-

tized mesoionic nucleosides shows fragments derived from the sugar moiety.

The first ion of prominence below the $[M-72]^{+\cdot}$ ion in the spectra of both **11** and **12** is the $[S-H]^{+\cdot}$ ion at m/z 348. Formation of this ion directly from the $M^{+\cdot}$ and $[M-15]^{+}$ ions has been established earlier [34]. The two major factors involved in formation of the $[S-H]^{+\cdot}$ ion in the spectra of normal nucleosides are the transfer of the 2'H of the ribose ring and charge localization on the 4' oxygen of the ribose ring. The latter effect has been used to explain the greater intensity of the $[S-H]^{+\cdot}$ ion in the spectra of TMS derivatized pyrimidine nucleosides relative to the purine nucleosides since charge localization on the sugar is greater in the pyrimidines. The spectra of the TMS derivatized mesoionic nucleosides **11** and **12** thus display characteristics more akin to the pyrimidines nucleosides than the purine nucleosides, behavior which is opposite that observed in the free nucleosides **2** and **3**. The following considerations may explain this apparent inconsistency.

During formation of the $BH^{+\cdot}$ ion in the free nucleosides, transfer of a hydroxyl hydrogen occurs rapidly with concomitant facile cleavage of the glycosidic bond. Transfer of a TMS radical and the 2'C bound H is not as rapid as transfer of a hydroxyl hydrogen and competition for the charge and radical sites becomes possible. Normal cleavage of the glycosidic bond with retention of charge by the base, following transfer of the TMS radical, forms the $[B+73]^{+\cdot}$ ion, and to a lesser extent the $[B+74]^{+}$ ion. On the other hand, localization of the radical charge sites in the heterocyclic ring may also lead to formation of the $[S-H]^{+\cdot}$ ion as shown in Scheme 8. A shift of a lone pair of



Scheme 8. A suggested mechanism of formation of the $(S-H)^{+\cdot}$ ion in the spectra of **11** and **12** (only **11** shown).

electrons on the ribose oxygen toward the base results in cleavage of the glycosidic bond. Concurrent with glycosidic bond cleavage is the transfer of the 2' hydrogen to the base resulting in expulsion of the base as a neutral molecule with the charge and radical sites remaining on the sugar. The intensity of the $[B+73]^{+\cdot}$ ion confirms that transfer of a TMS radical is easier than transfer of a carbon bound H to the base, since the $BH^{+\cdot}$ ion is significantly smaller than the $[B+73]^{+\cdot}$ ion. The basic idea of charge and radical site localization almost exclusively in the base portion of the mesoionic nucleosides thus does not need to be altered to account for the differences noted in the mass spectra of the TMS derivatives relative to the free nucleosides.

The gas chromatographic properties of nucleosides TMS derivatives has been examined extensively [3,20] and the gcms analysis of nucleosides is a relatively common process. However, attempts to obtain gcms data on the TMS derivatives **11** and **12** were unsuccessful. Although mass spectra could be easily obtained from the direct insertion probe, the presence of base localized charges apparently allows sufficient interaction with the stationary phase of the gc column to prevent these samples from passing through the column. Even if a TMS function had been added to the aglycone, the residual positive charge would most likely have prevented gas chromatography of the sample. (To insure the proper operation of the gas chromatographic system a sample of the TMS derivative of adenosine was analyzed prior to and following the attempted analysis of the derivatized mesoionic nucleosides.)

Fast Atom Bombardment Spectra of Free Mesoionic Nucleosides.

Fast atom bombardment(FAB) mass spectrometry has been used to examine the spectra of the eight major nucleosides found in RNA and DNA and some related analogs and the spectra obtained using this new ionization method have been compared to the EI and CI mass spectra [36]. In each case, the FAB mass spectrum was dominated by the MH^{+} and BH_2^{+} ions. However, each spectrum also contained peaks corresponding to the $[B+30]^{+}$ and $[B+44]^{+}$ ions, representing the base with portions of the carbohydrate attached. These ions, although reduced in intensity relative to the EI spectra, were unmistakably sample related. Similar observations have been made during the analysis of a number of nucleoside analogs using FAB ms/ms [37], which has the advantages of eliminating solvent and other related artifact peaks from the spectrum and increasing the intensity of the fragment ions when used in conjunction with collisional activation. The formation of the $[B+30]^{+}$ and $[B+44]^{+}$ ions in the mass spectra of "normal" nucleosides using FAB ionization is therefore established.

The positive ion FAB mass spectra of **1**, **2** and **3** are shown in Figure 6. The spectrum of **1** is included for comparison although this spectrum has been published and described previously [38]. The FAB spectrum of **1** obtained in this laboratory does however differ significantly from the literature spectrum in the presence of $[B+14]^{+}$, $[B+30]^{+}$ and $[B+44]^{+}$ ions at m/z 180, 194 and 208, respectively. The difference in the spectra may possibly lie in the choice of the relative intensity used as a minimum in plotting the spectra. The ions shown in Figure 6 are to the 3% relative intensity level while the literature spectrum of **1** appears to include only peaks with an intensity greater than approximately 8%. Thus, the FAB mass spectrum of the Class I mesoionic nuceloside, like other "normal"

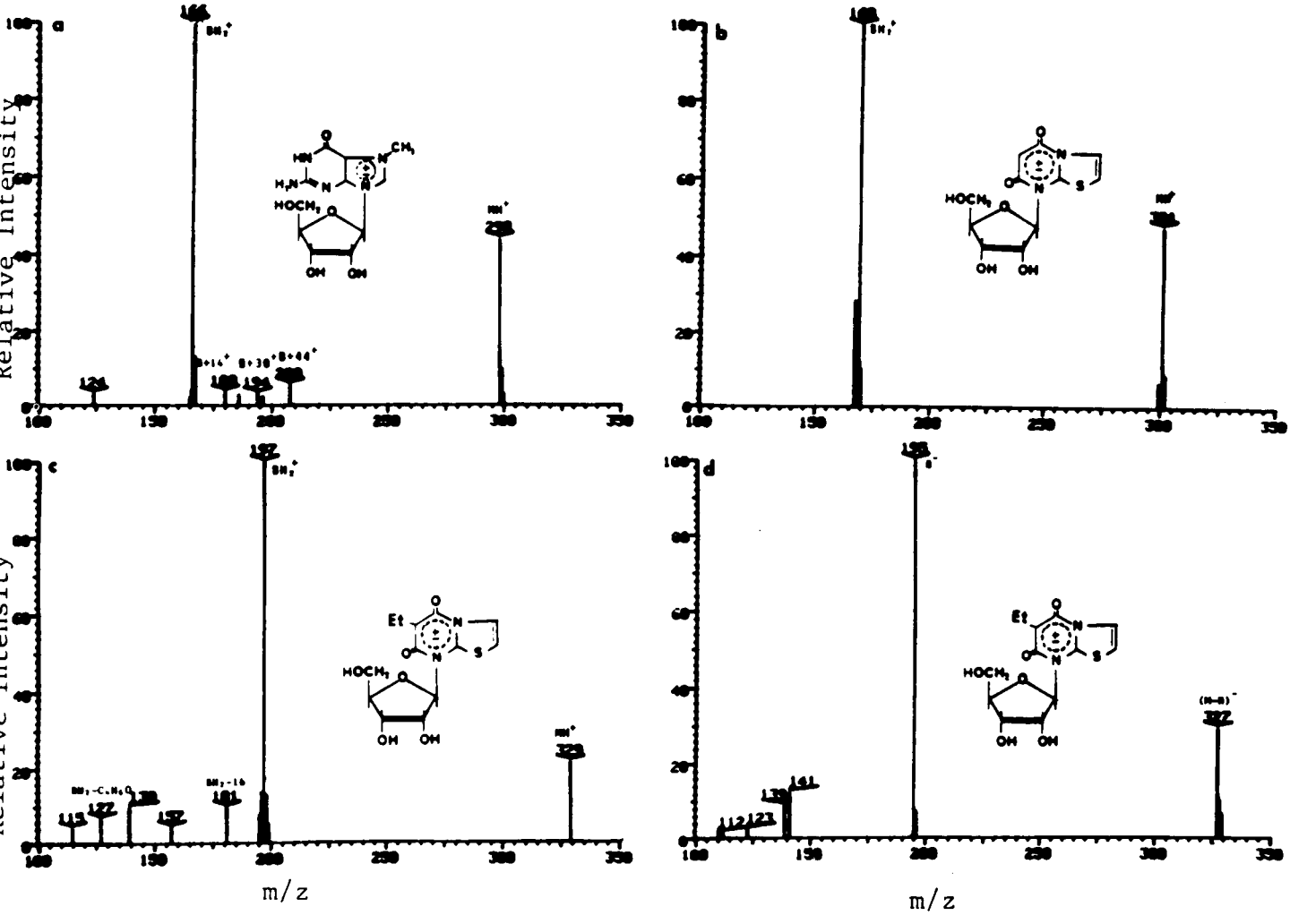


Figure 6. Fast atom bombardment (FAB) spectra of a) 7-methylguanos ine (**1**) showing the $(B+30)^+$ and $(B+44)^+$ ions, b) mesoionic nucleosides **2**, c) **3** and d) the negative ion FAB mass spectrum of **3**. Panels a-c were obtained using positive ion detection. The absence of the $(B+30)^+$ and $(B+44)^+$ ions in the FAB spectra of **2** and **3** is noteworthy.

nucleosides, is seen to contain peaks associated with the base plus portions of the sugar. In contrast, the FAB mass spectra of **2** and **3** show the absence of any peaks associated with the base with portions of the carbohydrate attached. Even when the raw data was examined with ions being plotted to 0% relative intensity, no peaks were observed at the appropriate m/z values. Other ions in the spectrum of **3** which are related to decomposition of the BH_2^+ ion are the m/z 181 and 127 ions. The remaining ions in the spectrum of **3** are not rationally associated with the sample and are thought to be artifacts or related to the solvent matrix. The same comment is made concerning the m/z 124 peak in the spectrum of **1**. Only the MH^+ and BH_2^+ ions are present in the spectrum of **2**, except as noted below.

Of interest, but not shown in Figure 6, are some other ions in the FAB spectra of **1**, **2** and **3** which may be used for confirmation of molecular weight. These ions correspond to the addition of a sodium ion to M to give the $[M+Na]^+$ ion, the formation of an $[M+H+glycerol]^+$ ion and an ion produced by dimerization to yield the $[M_2H]^+$ ion. The presence of these ions has been noted previously [36].

The absence of the $[B+30]^+$ and $[B+44]^+$ ions in the FAB mass spectrum of the Class II mesoionic nucleoside **3** was confirmed by performing a linked-scan for daughter ions analysis on the MH^+ ion. This technique is used to determine the daughter ions of a selected parent ion by examining the metastable ions formed during the decomposition of the selected parent ion. Focusing of the mass spec-

trometer on the MH^+ ion at m/z 329 and scanning the B(magnetic) and E(electric sector) fields in a constant E/B ratio and constant V(accelerating voltage) will thus produce a spectrum indicating all daughter ions formed from the MH^+ ion. The results of this study showed only one ion is formed from the MH^+ of **3** and this ion is the BH_2^+ ion. No indication of the formation of any other ions was observed. The same experiment was performed by obtaining the daughter ion spectrum of the MH^+ ion of **1**. The formation of the BH_2^+, $[B+30]^+$ and $[B+44]^+$ was indicated with a relative intensity of the peaks being 100%, 12% and 26% respectively. Thus, analysis of the free nucleosides **2** and **3** by FABMS substantiates the EI data in indicating the absence of any ions formed by decomposition of the sugar ring prior to cleavage of the glycosidic bond.

Conclusions.

The mass spectral behavior of the Class II mesoionic nucleosides **2** and **3**, either as the free nucleosides or as their TMS derivatives, is seen to differ significantly from other nucleoside classes examined to date. These differences are expressed independent of the ionization mode, EI or FAB, used to obtain the mass spectrum and are a result of the extremely facile cleavage of the glycosidic bond under the conditions used for ionization. The facile cleavage of the glycosidic bond is, in turn, a consequence of the unique structure of the Class II mesoionic nucelosides which, because of the localization of the positive and negative charges in the pyrimidine ring, afford sites for the almost exclusive ionization (EI) or protonation (FAB) of the base. The mass spectra of these compounds are therefore dominated by fragments associated with the decomposition of the heterocyclic ring. The differences in the mass spectra of the Class II mesoionic nucleosides relative to other nucleoside classes once again illustrated that subtle changes in structure may have a significant effect on the mass spectral behavior of nucleosides.

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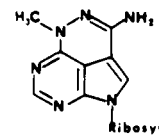

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