

Electrospray tandem mass spectrometry of 3'-azido-2',3'-dideoxythymidine and of a novel series of 3'-azido-2',3',4'-trideoxy-4'-thio-5-halogenouridines and their respective α -anomers

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Abstract. Electrospray mass spectrometry has aided the structural characterization of 3'-azido-2',3'-dideoxythymidine (AZT) and the novel series of 3'-azido-2',3',4'-trideoxy-4'-thio-5-(bromo, chloro or fluoro)uridine nucleosides and their respective α -anomers. Low energy CAD MS/MS analysis of the protonated molecules $[M+H]^+$ confirmed the predicted fragmentation route for AZT and a series of related 4'-thio-5-halogenouridines. This MS/MS study also provided characteristic fingerprint patterns which permitted differentiation of anomers within the series of 3'-azido-2',3',4'-trideoxy-4'-thio-5-halogenouridine nucleosides.

1. Introduction

The human immunodeficiency virus (HIV) has been accepted as being the etiological agent of acquired immunodeficiency syndrome (AIDS) [1]. Nucleoside analogues such as 3'-azido-2',3'-dideoxythymidine (AZT) [2] and other 2',3'-dideoxynucleosides [3] are potent inhibitors of this retrovirus *in vitro* and *in vivo*. There is an ongoing search for novel nucleosides which are effective against the AIDS virus.

Systematic structure-reactivity relationship studies of AZT analogues have shown a pronounced enhancement of the anti-HIV activity when the 5-position of the pyrimidine ring is substituted by halogens such as Br or I [4]. It has also been shown that nucleoside analogues in which the O-4' has been replaced by either a methylene group or sulfur atom are more resistant to nucleoside phosphorylase cleavage [5,6]. Also, modifications of this type have conferred such analogues with either or both antiviral and anticancer activities.

2. Experimental

Compounds and sample preparation:

Synthesis of 3'-azido-2',3',4'-trideoxy-4'-thio-5-(bromo, chloro or fluoro)uridine nucleosides **2-7** were accomplished by a novel method [9]. The purified nucleoside derivatives were dissolved in methanol / water (1/1) containing 0.1 % formic acid . Solutions of 1 mg/mL of derivatives were prepared prior to mass spectral analysis.

Mass Spectrometry:

Conventional mass spectra were obtained by scanning the mass spectrometer from 50 to 1000 Da in 10 s. Assisted nebulized electrospray mass spectra were obtained using an API III triple quadrupole mass spectrometer (SCIEX, Thornhill, Ontario, Canada) equipped with an atmospheric pressure ionization (API) source operated in the ion spray mode (also known as assisted nebulized electrospray). For the sake of convenience, in this rationale, no distinction was made between the terms ion spray and electrospray which, nowadays, normally includes the assisted nebulizer notion. A Macintosh IIX computer was used for data acquisition and data processing. Samples were introduced to the mass spectrometer by infusing a solution at a flow rate of 5 μ L/min. The voltage of the ion spray needle was maintained at 5 kV, while the orifice voltage was typically 100V. A 0,6 L/min flow of high purity air was used as the nebulizing gas.

Tandem mass spectrometry experiments were also done using the API III instrument. Fragment ion spectra of mass-selected ions were induced by collisions with argon in the second (RF only) quadrupole. The resulting fragments were mass analyzed by the third quadrupole. The target thickness was typically 3×10^{14} atoms/cm² and collision energies of approximately 100 eV (laboratory frame) were used in all MS/MS experiments. Precursor ion scans were obtained by scanning the first quadrupole while selecting a given m/z value with the third quadrupole.

3. Results and discussion

The electrospray MS of 3'-azido-2',3'-dideoxythymidine **1** is shown in Figure 2a and summarized in Table 1. The spectrum showed abundant protonated molecules $[M+H]^+$ at m/z 268 as well as high intensities of dimer and trimer clusters corresponding to $[2M+H]^+$ and $[3M+H]^+$ at m/z 535 and 802 respectively. These peaks permitted the confirmation of the relative mass of AZT. Contrary to the FAB MS of nucleosides [20,21], thus far investigated, no characteristic fragment ions were detected which were derived from either the protonated molecules or clusters.

Table 1

Positive electrospray mass spectrum of 3'-azido-3'-deoxythymidine **1** (C₁₀H₁₃N₅O₄, M.Wt = 267.1)

Characteristic ion	m/z	%
$[3M + H]^+$	802	72.3
$[2M + H]^+$	535	100
$[M + H]^+$	268	84.8

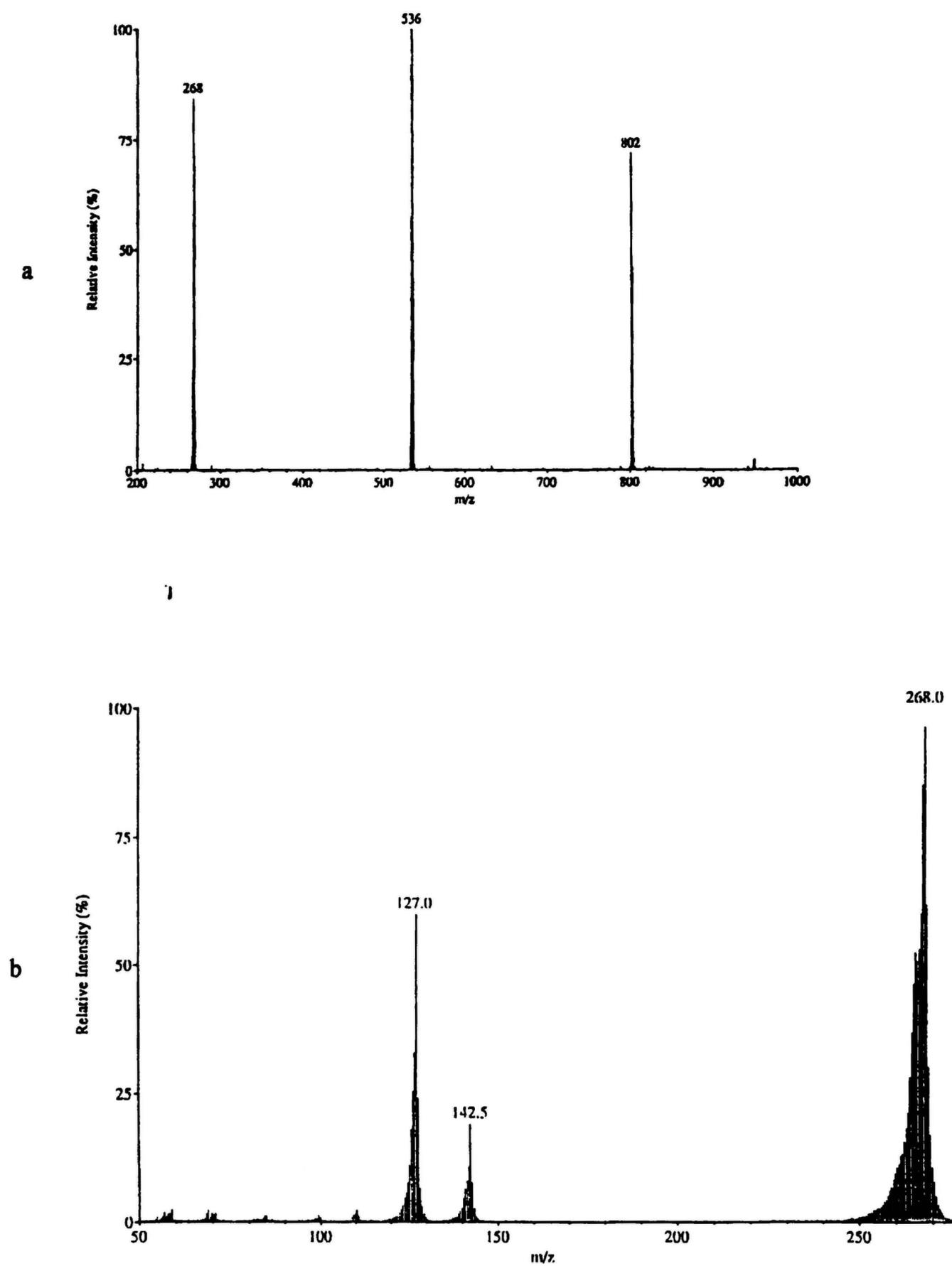


Figure 2. a) Positive electrospray mass spectrum of 3'-azido-3'-deoxythymidine 1. b) Low energy CAD MS/MS spectrum of the precursor ion $[M+H]^+$ at m/z 268 obtained from 3'-azido-3'-deoxythymidine 1.

Structural information, such as the sequence pattern of branched oligosaccharides [22-25] or differentiation of isomeric hexosyl units [26-33], can be obtained by tandem mass spectrometry [34,35]. The unique dissociation patterns of the precursor ions provide characteristic fragment-ions which facilitate differentiation of isomeric species. Application of high energy collision-induced dissociations (CID) has also proven to be of use in the structural characterization of native polysaccharide antigens [36], isomers of peracetylated saccharides [26-32], anomeric pairs and regioisomers of nucleosides [37-39], C-glycosides [40-43], series of diastereomeric 1,2-trans-2-deoxy-2-iodoglycosyl azides [44] and phosphoramidates [45]. Low energy collision induced dissociations (CID) have also been demonstrated by electrospray ionization for a series of distinct regioisomeric esters of sucrose [19]. Thus, low energy tandem mass spectrometric analysis was conducted on protonated AZT 1 molecules $[M+H]^+$ to explore the possible electrospray fragmentation and compare them with the various fragment-ions obtained by both conventional fast-atom bombardment (FAB) mass spectrometry and FAB MS/MS observed for other nucleosides [20]. A product ion spectrum, arising from fragmentation in the RF only middle quadrupole collision cell of the triple quadrupole instrument, was obtained.

The low energy CID MS/MS spectrum for the $[M+H]^+$ precursor ion at m/z 268 is shown in Figure 2b and summarized in Table 2. The fragmentation route for the $[M+H]^+$ parent-ion has been rationalized using the systematic nomenclature for nucleoside fragmentation in FAB-MS proposed by Crow *et al* [20]. Therefore, the main fragment-ion $[BH_2]^+$ at m/z 127 appears to be initiated by intramolecular transfer of a hydrogen atom from the sugar to the base, which is already protonated (Fig. 2b). The only other ion produced was the less abundant fragment-ion $[S]^+$ at m/z 142 obtained by cleavage of the glycosidic bond with charge retention on the sugar moiety (A-type cleavage) [20].

Table 2
Low energy CAD MS/MS spectrum of the precursor ion $[M+H]^+$ at m/z 268 obtained for AZT 1.

Characteristic ion	Type of ion	m/z	%
$[M + H]^+$	precursor ion	268	100
$[M + H - 126]^+$	3-azido-glycosyl or $[S]^+$	142	18.8
$[M + H - 142 + H]^+$	diprotonated base or $[BH_2]^+$	127	60.0

The electrospray mass spectra of the pair, 3'-azido-2',3',4'-trideoxy-4'-thio-5-bromouridine **2** and its α -anomer **3** are summarized in Table 3. It can be seen that ESMS for this anomeric pair are very similar. As expected, each of the anomers of 4'-thio-5-bromouridines **2** and **3** formed the protonated molecules $[M+H]^+$ at m/z 350 and 348 respectively in almost equal ratio and is in accordance with the isotopic abundance of bromine (ie. ^{79}Br and ^{81}Br). An interesting finding was the presence of clusters of $[2M+H]^+$ at m/z 699 and 695 corresponding to $[2(C_9H_{10}N_5O_3S^{81}\text{Br})+H]^+$ and $[2(C_9H_{10}N_5O_3S^{79}\text{Br})+H]^+$, respectively. Equally noteworthy was the presence of an intense mixed cluster of $[2M+H]^+$ formed by combination of the two bromine isotopes $[C_9H_{10}N_5O_3S^{81}\text{Br} + C_9H_{10}N_5O_3S^{79}\text{Br} + H]^+$ at m/z 697. The presence of these peaks permitted the determination of the relative

masses of this anomeric pair. It should be mentioned that some fragmentation of the protonated molecules and clusters occurred during the conventional ESMS and the same characteristic fragmentations, due to the loss of bromine atoms, were observed.

The protonated molecules $[\text{C}_9\text{H}_{10}\text{N}_5\text{O}_3\text{S}^{79}\text{Br}+\text{H}]^+$ at m/z 348 were selected for the recording of the low-energy collision induced (CID) mass analyzed ion kinetic energy (MIKE) spectra in order to probe further the electrospray fragmentation behaviour and also to see whether it would be possible to obtain discriminating differences, between the two related anomers **2** and **3**, by the metastable analysis. The low energy CID MS/MS of the precursor $[\text{M}+\text{H}]^+$ ions at m/z 348 are summarized in Table 4 and tentatively depicted in figure 3. Examination of Table 4 permitted us to deduce the fate of the parent ions and their dissociations into product ions. Interestingly, the product ions obtained, which are of diagnostic importance for the elucidation of this anomeric pair, were not formed in the conventional electrospray MS. As expected, the diagnostic product ions result from the sugar moiety $[\text{S}]^+$ product ion at m/z 158. We noted three ions originating from the product ion $[\text{S}]^+$ at m/z 115, 97 and 85, which were obtained by elimination of a molecule of hydrogen azide to form the $[\text{S}-\text{HN}_3]^+$ ion at m/z 115, by consecutive losses of molecules of hydrogen azide and water to form the $[\text{S}-\text{HN}_3-\text{H}_2\text{O}]^+$ ion at m/z 97 and by consecutive elimination of molecules of hydrogen azide and formaldehyde to form the $[\text{S}-\text{HN}_3-\text{HCHO}]^+$ ion at m/z 85. These last three product ions seem only to be present for all the members of the 4'-thio-5-halogenouridine nucleoside series and are absent in the case of AZT **1**.

Table 3

Positive electrospray mass spectra of 3'-azido-2',3',4'-trideoxy-4'-thio-5-bromouridine **2** and its corresponding α -anomer **3** ($\text{C}_9\text{H}_{10}\text{N}_5\text{O}_3\text{S}^{79}\text{Br}$, M.W.t = 347; $\text{C}_9\text{H}_{10}\text{N}_5\text{O}_3\text{S}^{81}\text{Br}$, M.Wt = 349).

Characteristic ion	Type of ion	m/z	β -anomer (%)	α -anomer (%)
$[2\text{M} + \text{H}]^+$	$[2(\text{C}_9\text{H}_{10}\text{N}_5\text{O}_3\text{S}^{81}\text{Br} + \text{H})]^+$	699	31.7	33.9
	$[\text{C}_9\text{H}_{10}\text{N}_5\text{O}_3\text{S}^{81}\text{Br} + \text{C}_9\text{H}_{10}\text{N}_5\text{O}_3\text{S}^{79}\text{Br} + \text{H}]^+$	697	50.7	56.2
	$[2(\text{C}_9\text{H}_{10}\text{N}_5\text{O}_3\text{S}^{79}\text{Br} + \text{H})]^+$	695	26.9	31.2
$[2\text{M} + \text{H} - \text{Br}]^+$	$[699 - 81]^+$, $[697 - 79]^+$	618	10.8	15.0
	$[697 - 81]^+$, $[695 - 79]^+$	616	9.1	10.1
$[\text{M} + \text{NH}_4]^+$	$[\text{C}_9\text{H}_{10}\text{N}_5\text{O}_3\text{S}^{81}\text{Br} + \text{NH}_4]^+$	367	12.5	7.1
	$[\text{C}_9\text{H}_{10}\text{N}_5\text{O}_3\text{S}^{79}\text{Br} + \text{NH}_4]^+$	365	7.5	6.3
$[\text{M} + \text{H}]^+$	$[\text{C}_9\text{H}_{10}\text{N}_5\text{O}_3\text{S}^{81}\text{Br} + \text{H}]^+$	350	100	100
	$[\text{C}_9\text{H}_{10}\text{N}_5\text{O}_3\text{S}^{79}\text{Br} + \text{H}]^+$	348	96.8	93.0
$[\text{M} + \text{H} - \text{Br}]^+$		269	28.2	27.0

Table 4

Low energy CAD MS/MS spectra of the precursor ions $[M+H]^+$ at m/z 348 of 3'-azido-2',3',4'-trideoxy-4'-thio-5-bromouridine **2** and its corresponding α -anomer **3**.

Characteristic ion	Type of ion	m/z	β -anomer (%)	α -anomer (%)
$[M + H]^+$	Precursor ion	348	91.2	98.2
$[M + H - 158 + H]^+$	$[BH_2]^+$	191	100	100
$[M + H - 158 + H - 18]^+$	$[BH_2 - H_2O]^+$	173	10.8	9.8
$[M + H - 190]^+$	$[S]^+$	158	50.9	60.3
$[M + H - 190 - 43]^+$	$[S - HN_3]^+$	115	9.8	12.5
$[M + H - 190 - 43 - 18]^+$	$[S - HN_3 - H_2O]^+$	97	11.6	13.4
$[M + H - 190 - 43 - 30]^+$	$[S - HN_3 - HCHO]^+$	85	31.3	38.8

Table 5

Positive electrospray mass spectra of 3'-azido-2',3',4'-trideoxy-4'-thio-5-chlorouridine **4** and its corresponding α -anomer **5** ($C_9H_{10}N_5O_3S^{35}Br$, M.W.t = 303; $C_9H_{10}N_5O_3S^{37}Br$, M.Wt = 305).

Characteristic ion	Type of ion	m/z	β -anomer (%)	α -anomer (%)
	$[2(C_9H_{10}N_5O_3S^{35}Cl) + C_9H_{10}N_5O_3S^{37}Cl + H]^+$	912	-	10.7
$[3M + H]^+$	$[3(C_9H_{10}N_5O_3S^{35}Cl) + H]^+$	910	-	8.0
	$[C_9H_{10}N_5O_3S^{35}Cl + C_9H_{10}N_5O_3S^{37}Cl + H]^+$	609	-	47.3
$[2M + H]^+$	$[2(C_9H_{10}N_5O_3S^{35}Cl) + H]^+$	607	-	58.0
	$[C_9H_{10}N_5O_3S^{37}Cl + NH_4]^+$	323	18.7	-
$[M + NH_4]^+$	$[C_9H_{10}N_5O_3S^{35}Cl + NH_4]^+$	321	100	-
	$[C_9H_{10}N_5O_3S^{37}Cl + H]^+$	306	13.8	45.1
$[M + H]^+$	$[C_9H_{10}N_5O_3S^{35}Cl + H]^+$	304	34.4	100

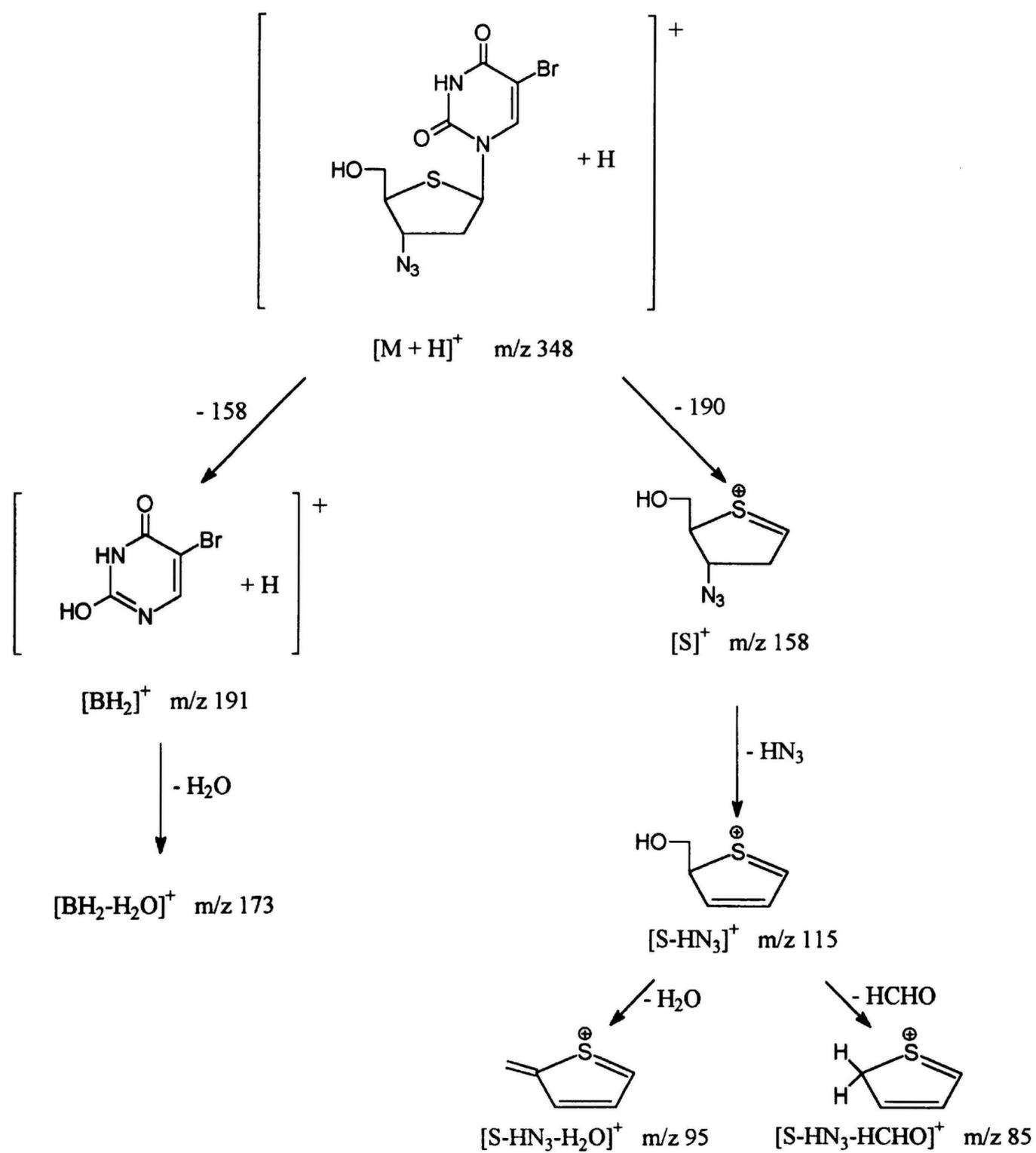


Figure 3. Proposed major fragmentation routes of the $[M+H]^+$ ion observed by CAD MS/MS of the 3'-azido-2',3',4'-trideoxy-4'-thio-5-bromouridine **2**.

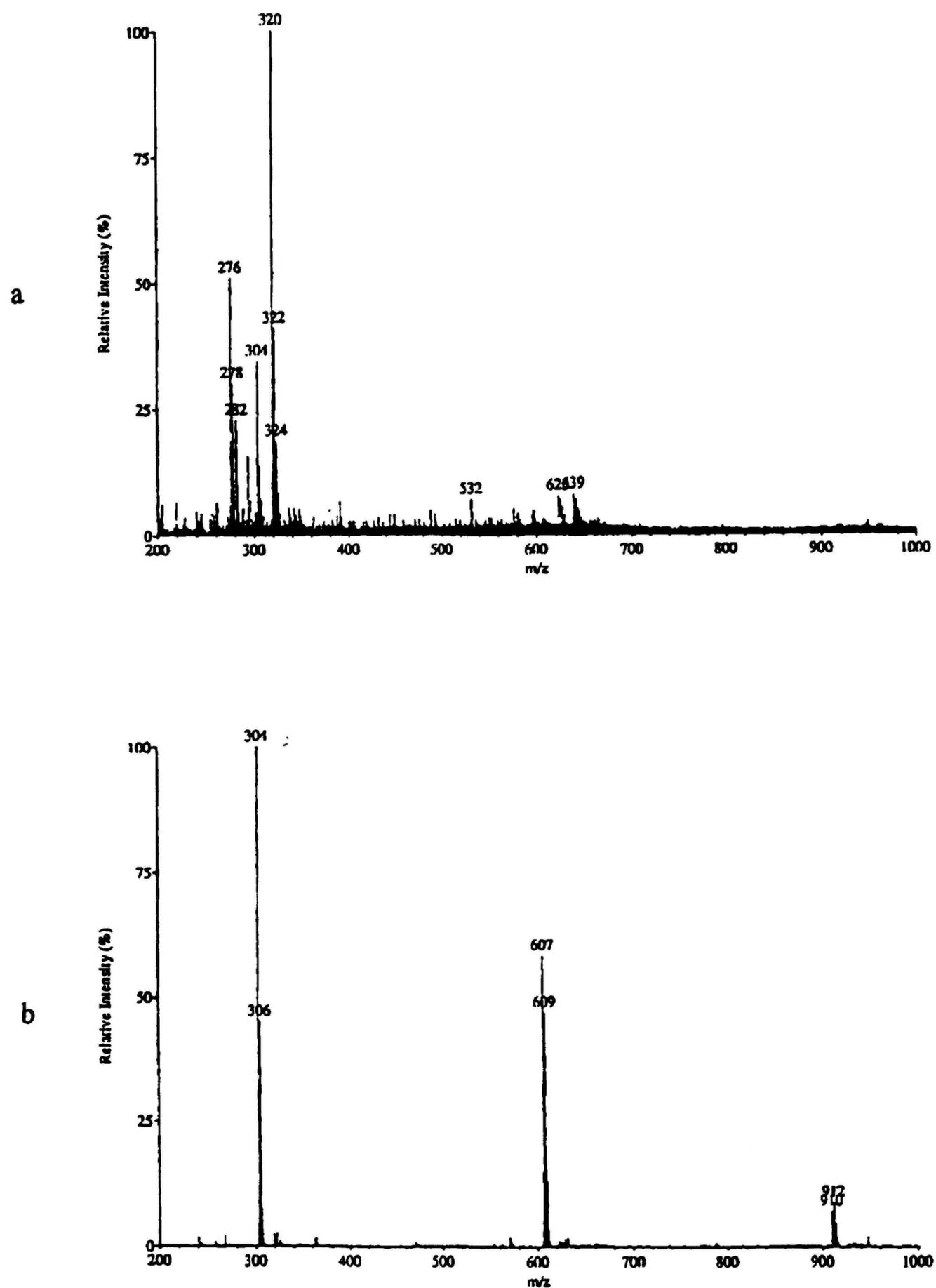


Figure 4. Positive electrospray mass spectra of (a) 3'-azido-2',3',4'-trideoxy-4'-thio-5-chlorouridine **4** and (b) its corresponding α -anomer **5**.

Comparison between the low energy CID MS/MS analyses for this anomeric pair **2** and **3** (Table 4) showed small differences in the intensities of almost all product ions with the exception of the $[S]^+$ and $[S-HN_3-HCHO]^+$ product ions at m/z 158 and 85 respectively which showed pronounced differences in their relative intensities. These MS/MS characteristics allowed discrimination to be made between the two related anomers **2** and **3**.

In a similar fashion, 3'-azido-2',3',4'-trideoxy-4'-thio-5-chlorouridine **4** and its respective α -anomer **5** were also studied by electrospray. The characteristic ions obtained for this pair of anomers are presented in Table 5 and Figure 4. Comparison of these data shows very distinct spectral differences between the two anomers. Indeed, the only common ions for this anomeric pair are the protonated molecules $[M+H]^+$ at m/z 306 and 304 with different relative abundances (each anomer with the correct abundances for the isotopes of chlorine atoms). The exclusive presence of the diagnostic ammoniated adducts $[C_9H_{10}N_5O_3S^{37}Cl+NH_4]^+$ and $[C_9H_{10}N_5O_3S^{35}Cl+NH_4]^+$ can be seen, for the β -anomer only, at m/z 323 and 321 respectively. While the presence of these adducts, in the case of the β -anomer **4**, can be tentatively rationalized by the formation of a complex involving the ammonium ion held by H-bonding between 4'-S- and 5'-O- atoms of the sugar moiety and 2-carbonyl oxygen atom of the pyrimidine base, such a complex could not be formed in the case of the α -anomer **5**. However, for this anomer the exclusive formation of $[3M+H]^+$ cluster ions can be observed, resulting from the different permutation of the two isotopes of chlorine atoms to yield respectively: either the cluster ion $[2(C_9H_{10}N_5O_3S^{35}Cl)+C_9H_{10}N_5O_3S^{37}Cl+H]^+$ at m/z 912 containing two ^{35}Cl and one ^{37}Cl atoms or the related cluster ion $[3(C_9H_{10}N_5O_3S^{35}Cl)+H]^+$ at m/z 910 containing three ^{35}Cl atoms. Similarly, the formation of the $[2M+H]^+$ cluster ions at m/z 609 and 607 are formed exclusively for the α -anomer **5**.

In order to further establish the structural elucidation of this pair of anomeric nucleosides **4** and **5**, the protonated molecules $[C_9H_{10}N_5O_3S^{35}Cl+H]^+$ at m/z 304 were selected for recording by metastable MS/MS analysis. The low energy CAD MS/MS analysis of the precursor ions at m/z 304 are presented in Table 6 and Figure 5. Comparison between these two sets of data is in agreement with a fragmentation route typical of that which is expected for such kinds of 4'-thio-nucleosides. Thus, for both anomers, we observed the typical $[S]^+$ and $[BH_2]^+$ product ions, respectively, at m/z 158 and 147. As in the case of the anomeric pair **2** and **3**, identification can be made of the three fragment-ions at m/z 115, 97 and 85, originating from the sugar moiety product ions $[S]^+$, respectively obtained by either the loss of a molecule of hydrogen azide or "consecutive and perhaps concerted losses" of either both molecules of hydrogen azide and water or both molecules of hydrogen azide and formaldehyde. In this context, please note that "consecutive or concerted losses" of many of such product ions, in MS/MS experiments, simply means that they are both lost at the same time and within the same reaction region inside the tandem mass spectrometer. It is difficult to deduce the order of elimination reaction involving "consecutive or concerted losses" under the condition of MS/MS.

Examination of Table 6 permits the identification of pronounced and discriminating characteristic differences to be made between the relative abundances of the common product ions $[S]^+$, $[BH_2]^+$, $[S-HN_3]^+$ and $[S-HN_3-H_2O]^+$ respectively at m/z 158, 147, 115 and 97. Such distinct differences makes possible the clear differentiation of the pair of anomeric nucleosides **4** and **5**. The low energy CID MS/MS analysis of the chlorine-37 isotope of the ammoniated adduct $[M+NH_4]^+$ at m/z 321 of the 3'-azido-2',3',4'-trideoxy-4'-thio-5-chlorouridine β -anomer was also recorded and is presented in Table 7. In addition, Table 7 identifies the same type of product ions observed for the collision induced decomposition of the protonated molecules of $[M+H]^+$ precursor ions presented in Table 6.

Table 6

Low energy CAD MS/MS spectra of the precursor ions $[M+H]^+$ at m/z 304 of 3'-azido-2',3',4'-trideoxy-4'-thio-5-chlorouridine **4** and its corresponding α -anomer **5**.

Characteristic ion	Type of ion	m/z	β -anomer (%)	α -anomer (%)
$[M + H]^+$	Precursor ion	304	100	100
$[M + H - 146]^+$	$[S]^+$	158	57.1	60.1
$[M + H - 158 + H]^+$	$[BH_2]^+$	147	68.8	57.1
$[M + H - 146 - 43]^+$	$[S - NH_3]^+$	115	6.3	11.6
$[M + H - 146 - 43 - 18]^+$	$[S - HN_3 - H_2O]^+$	97	14.3	8.9
$[M + H - 146 - 43 - 30]^+$	$[S - HN_3 - HCHO]^+$	85	36.6	37.5

Table 7

Low energy CAD MS/MS spectrum of the ammoniated precursor ion $[M+NH_4]^+$ at m/z 321 of 3'-azido-2',3',4'-trideoxy-4'-thio-5-chlorouridine **4**.

Characteristic ion	Type of ion	m/z	β -anomer (%)
$[M + NH_4]^+$	Precursor ion	321	100
$[M + NH_4 - 146]^+$	$[S + NH_4]^+$	175	11.2
$[M + NH_4 - 175 + H]^+$	$[BH_2]^+$	147	6.3
$[M + NH_4 - 146 - 43]^+$	$[S + NH_4 - HN_3]^+$	132	15.1

Table 8

Positive electrospray mass spectra of 3'-azido-2',3',4'-trideoxy-4'-thio-5-fluorouridine **6** and its corresponding α -anomer **7** ($C_9H_{10}N_5O_3SF$, M.W.t = 287).

Characteristic ion	m/z	β -anomer 6 (%)	α -anomer 7 (%)
$[2M + NH_4]^+$	592	35.7	-
$[2M + H]^+$	575	32.1	59.3
$[M + NH_4]^+$	305	100	5.4
$[M + H]^+$	288	94.5	100

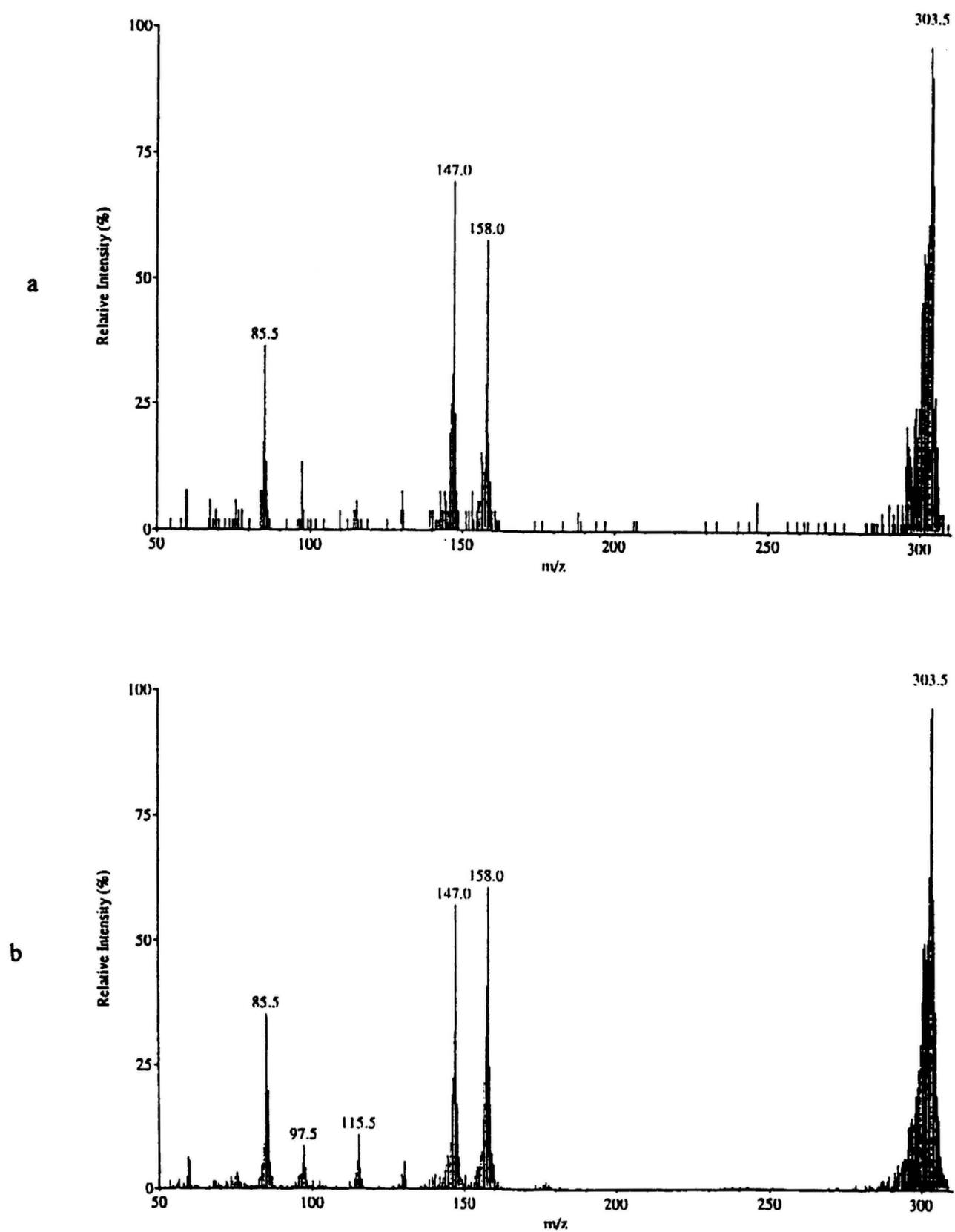


Figure 5. Low energy CAD MS/MS spectra of the precursor ions [M+H]⁺ at m/z 303.5 obtained from (a) 3'-azido-2',3',4'-trideoxy-4'-thio-5-chlorouridine **4** and (b) its corresponding α -anomer **5**.

Finally, the electrospray mass spectra of the anomeric pair of 3'-azido-2',3',4'-trideoxy-4'-thio-5-fluorouridine **6** and **7** were also recorded. The characteristic ions, obtained for this pair of anomers, are presented in Table 8 which shows very distinct spectra. It can be noted that for both anomers the protonated molecules and the $[2M+H]^+$ cluster ions, at m/z 288 and 575 respectively, have different relative abundances. As in the case of 4'-thio-5-chlorouridine **4** (β -anomer), the β -anomer **6** also shows the presence of two diagnostic ammoniated adducts, namely the $[M+NH_4]^+$ and $[2M+NH_4]^+$ ions at m/z 305 and 592 respectively. The formation of these ions can be rationalized by complexation with an ammonium ion.

The low energy CID MS/MS spectra for the $[M+H]^+$ ions of the anomers **6** and **7** are summarized in Table 9. Examination of this table showed pronounced differences in the intensities of particular fragment-ions, which permitted discrimination between these two anomers. Thus, relative intensities of the product ions $[S]^+$ and $[S-HN_3-HCHO]^+$ respectively at m/z 158 and 85, proved to be of diagnostic value in permitting discrimination between the β -anomer **6** and the corresponding α -anomer **7**.

Table 9

Low energy CAD MS/MS spectra of the precursor ions $[M+H]^+$ at m/z 288 of 3'-azido-2',3',4'-trideoxy-4'-thio-5-fluorouridine **6** and its corresponding α -anomer **7**.

Characteristic ion	Type of ion	m/z	β -anomer 6 (%)	α -anomer 7 (%)
$[M + H]^+$	Precursor ion	288	100	100
$[M + H - 130]^+$	$[S]^+$	158	33.9	51.8
$[M + H - 158 + H]^+$	$[BH_2]^+$	131	17.8	19.6
$[M + H - 130 - 43]^+$	$[S - HN_3]^+$	115	6.3	9.8
$[M + H - 130 - 43 - 18]^+$	$[S - HN_3 - H_2O]^+$	97	7.1	7.1
$[M + H - 130 - 43 - 30]^+$	$[S - HN_3 - HCHO]^+$	85	20.5	27.7

4. Conclusion

Mass spectral analyses of AZT **1** and a novel series of 3'-azido-2',3',4'-trideoxy-4'-thio-5-(bromo, chloro and fluoro)uridines **2-7** have been effected using electrospray ionization. Abundant signals, corresponding to the protonated molecules and molecular clusters, were observed in all cases using this ionization technique. In addition, the conventional electrospray MS permitted differentiation between the anomeric pairs: (**4/5**) and (**6 / 7**). The β -anomers in both cases formed an ammoniated adduct $[M+NH_4]^+$.

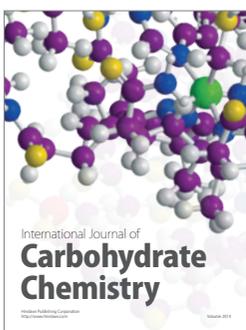
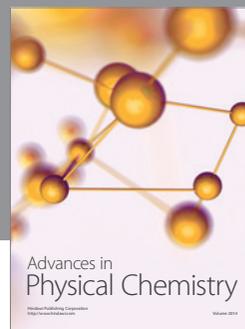
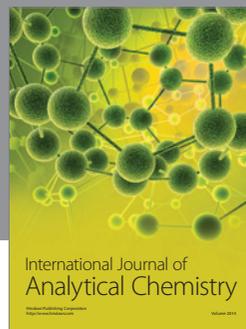
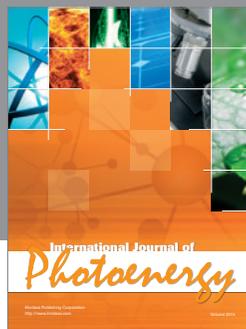
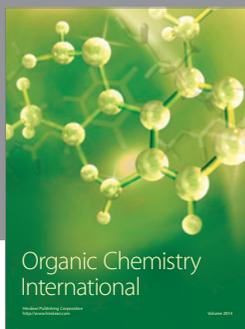
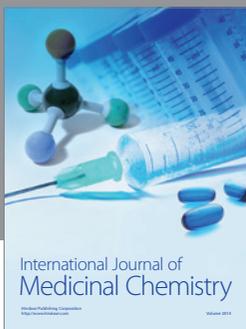
MS/MS spectra of this novel series of 4'-thio-5-halogenouridines, obtained using low energy collision activation, permitted differentiation between the various anomeric pairs. The difference in the relative abundance of same product ions obtained in the low energy CID MS/MS analyses reflects the inequalities of the chemical free energy, and

stereochemical differences between the anomeric configurations at C-1' of the 3'-azido-2',3',4'-trideoxy-4'-thio-glycososyl moiety, which contribute to the differences in the tertiary structures of the protonated molecular ions generated.

5. References

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