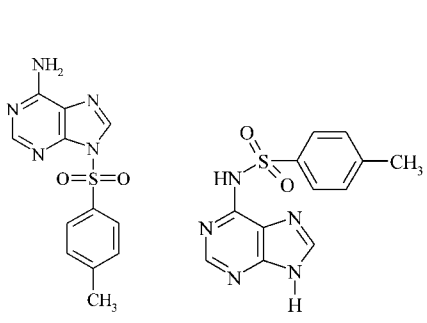
Mass spectrometric investigation of *N*-sulfonylated purine nucleic bases and nucleosides

Dunja Srzić, Marko Rožman, Irena Krizmanić and Biserka Žinić

The gas/phase behaviour of *N*-sulfonylated purine nucleic bases and nucleosides towards electron impact (EI) and matrix-assisted laser desorption/ionization (MALDI) occurring in a ion trap of a Fourier transform ion cyclotron resonance mass spectrometer is investigated. The influence of the storage time on the protonated molecule ($[M+H]^{+\cdot}$) abundance under EI conditions confirms that the formation of these ions proceeds through ion/molecule reactions. Using stored-waveform inverse Fourier transform (SWIFT) selective isolation of $M^{+\cdot}$ or H_3O^+, self-chemical ionization, $M^{+\cdot}/M$, and chemical ionization, $H_3O^{+\cdot}/M$, are detected. Investigation of specific EI expulsion of SO_2, S O $_2$H and/or SO_2H_2 from $M^{+\cdot}$ and/or $[M+H]^{+\cdot}$ shows that oxygen protonation in $-SO_2-$ pro-ceeds faster than nitrogen protonation. Expulsion of SO_2 from molecular ions is not observed in MALDI mass spectra of nucleosides.

N-Sulfonylated derivatives[1] of nucleic acid bases and nucleosides have been the subject of many studies due to their potential activity as enzyme inhibitors resulting in antitumor[2] and antiviral[3] activity. Recently, we have reported the synthesis of a series of *N*-sulfonylated pyrimidine[4–6] and purine[7] nucleic bases and nucleosides that showed promising *in vitro* antitumor activity.

In view of the biochemical importance of these compounds we report here the Fourier transform ion cyclotron resonance mass spectrometry (FTICRMS) investigation of some novel *N*-sulfonylated purine nucleic bases (**1**–**3**) and nucleosides (**4**–**7**). By using FTICRMS that allows ion storage, concomitant formation of molecular ions, $M^{+\cdot}$, and protonated molecules, $[M+H]^+$, has been observed under electron



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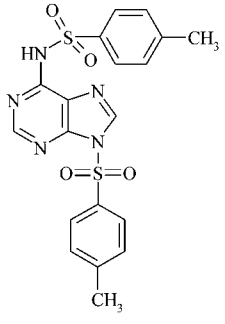

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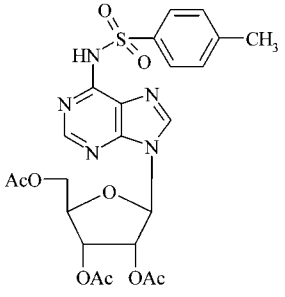

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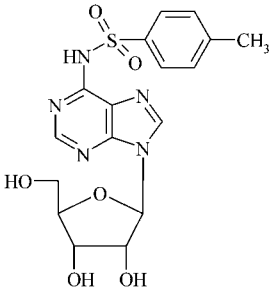

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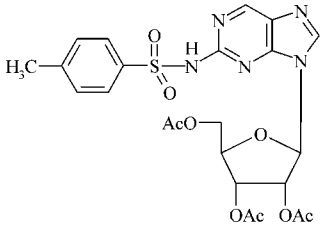

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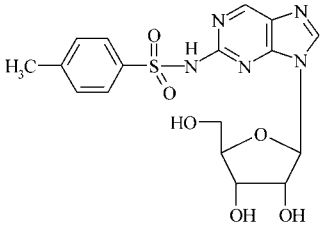

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impact (EI) conditions. The relative abundance of $[M+H]^+$ ions increases with longer delay time in the ion trap. This behaviour suggests that $[M+H]^+$ ions are produced by ion/molecule reactions between $M^{+\cdot}$ (self-chemical ionization) or

other ions (chemical ionization, CI) and neutral molecules M. Specific EI expulsion of SO_2, SO_2H and/or SO_2H_2 from $M^{+\cdot}$ and/or $[M+H]^+$ has shown that the structure of protonated molecules (site of protonation) depends on the storage time in the ion trap. In this report we use SWIFT excitation to isolate individual ions and to obtain separate EI product-ion spectra from each of the molecular ions produced in $M^{+\cdot}$/M and H_3O^+/M reactions. In contrast to EI spectra, under MALDI conditions, SO_2 elimination from the cationized molecules of nucleosides (**4**–**7**) is not observed.

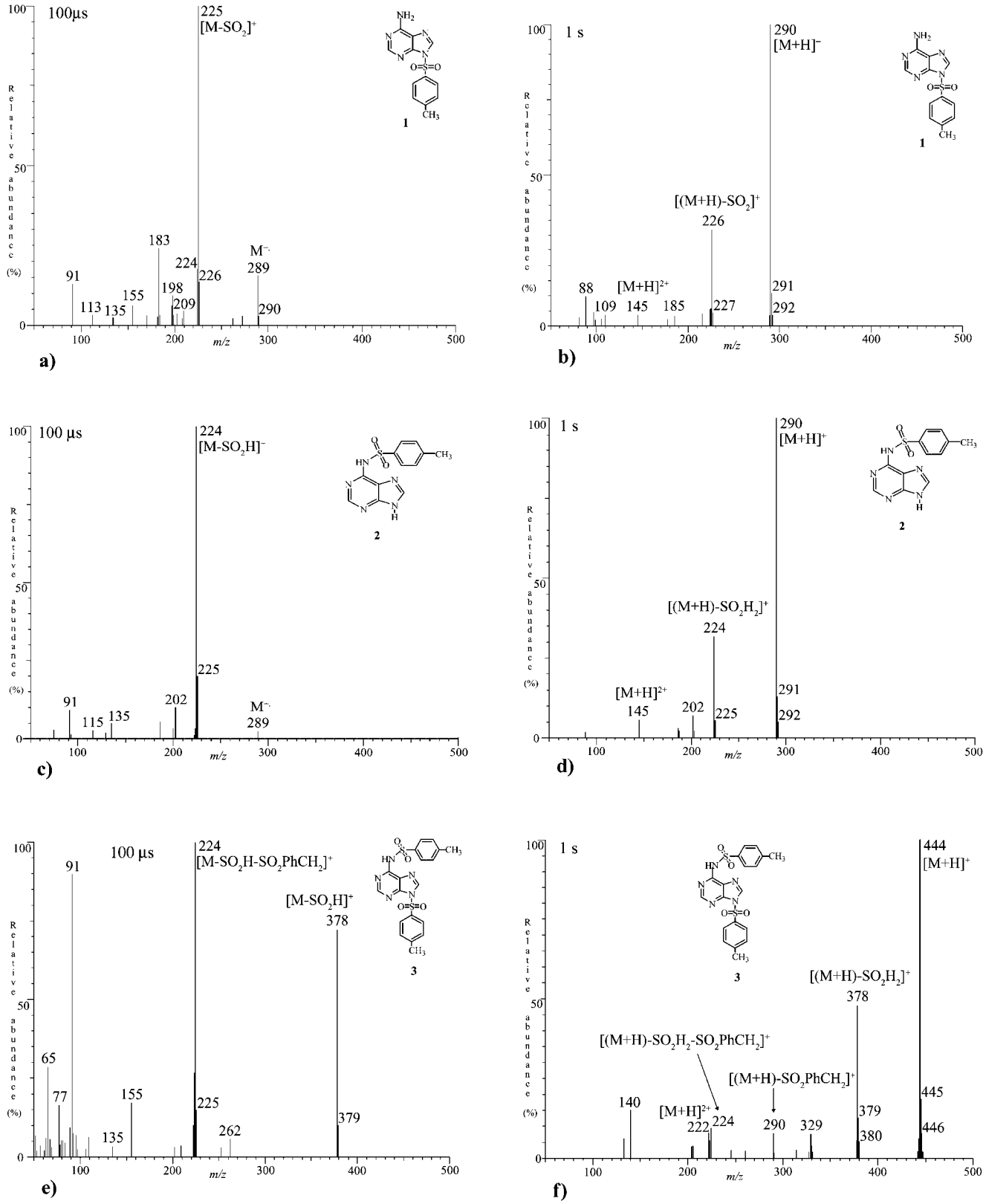

Figure 1. 70 eV EI-FTICR mass spectra of *N*-sulfonylated purine nucleic bases **1**–**3** recorded at 100 µs (left) and 1 s (right) time delay.

EXPERIMENTAL

All mass spectrometric investigations were performed using a FT/MS 2001 DD Fourier transform mass spectrometer (Finnigan, Madison, WI, USA) interfaced to a VSL-337 ND-S nitrogen laser (Laser Science Inc., Newton, MA, USA). Following a quench pulse applied to the trap plates to remove all ions from the cell, positive ions of **1**–**4** were formed by 70 eV EI ionization. MALDI of 4–7 was performed by single laser pulse using dihydrobenzoic acid matrix and at a wavelength of 337 nm. Subsequent to each ionization event, the mass spectra were recorded after various time delays of 100 μs to

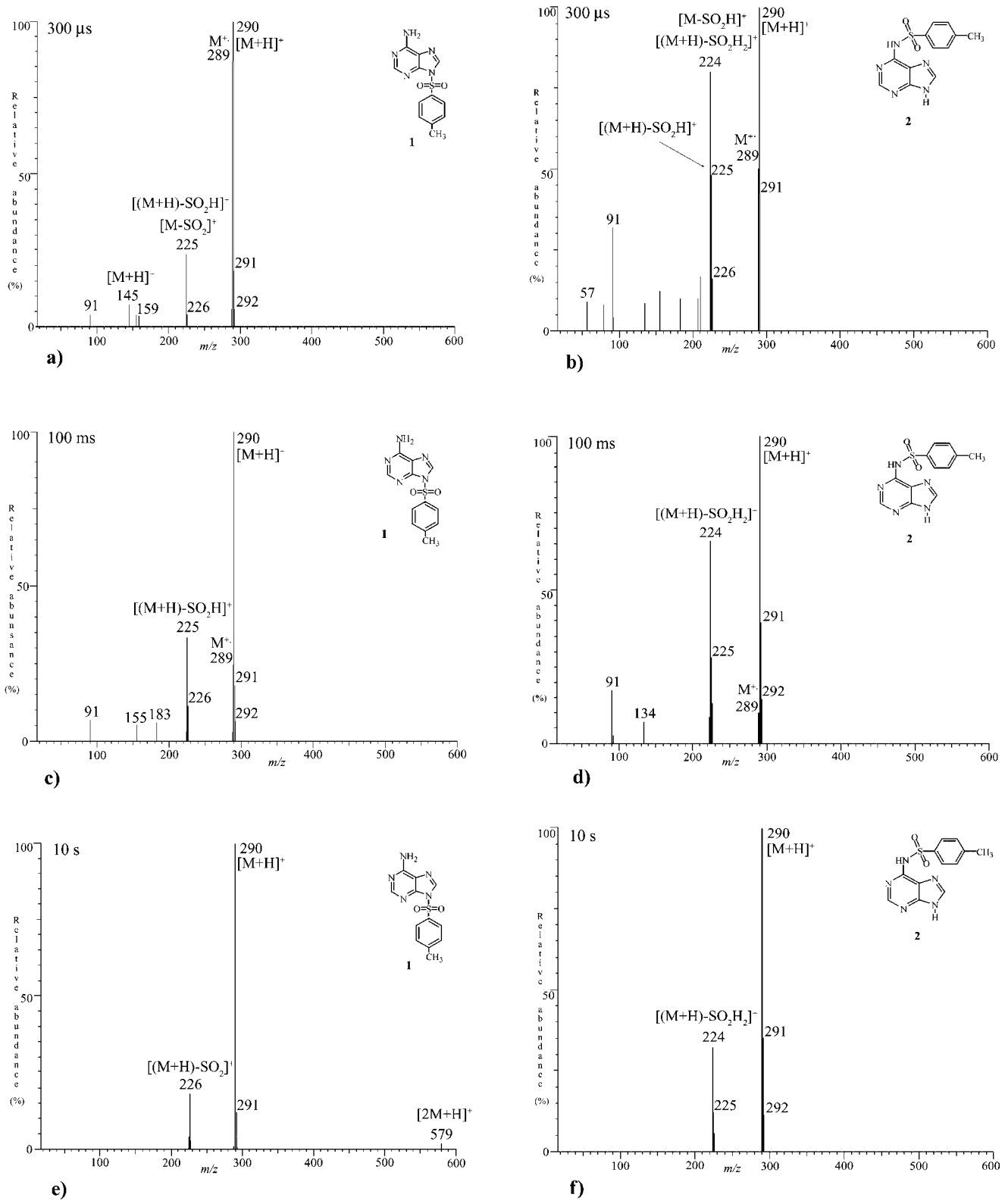

Figure 2. Self-CI-FTICR mass spectra of *N*-sulfonylated purine nucleic bases **1** and **2** recorded after SWIFT selective isolation of $M^{+\cdot}$ (delay times 300 μs (a and b), 100 ms (c and d) and 10 s (e and f)).

100 s allowing the disappearance of $M^{+\cdot}$, changes in fragmentations and formation of protonated and cationized species as result of ion/molecule reactions to be followed. To monitor particular ion/molecule reactions all ions except $M^{+\cdot}$ or H_3O^+ were removed from the cell shortly after the EI ionization by a SWIFT procedure.

The syntheses of 9-(*p*-toluenesulfonyl)adenine **(1)**, N^6-(*p*-toluenesulfonyl)adenine **(2)**, N^6,9-bis(*p*-toluenesulfonyl)adenine **(3)**, 9-(2,3,5-tri-*O*-acetyl-*β*-D-ribofuranosyl)-N^6-(*p*-toluenesulfonyl)adenine **(4)**, N^6-(*p*-toluenesulfonyl)adenosine **(5)**, 9-(2,3,5-tri–*O*-acetyl-*β*-D-ribofuranosyl)-N^2-(*p*-toluenesulfonyl)aminopurine **(6)**, and 9-(*β*-D-ribofuranosyl)-N^2-(*p*-toluenesulfonyl)aminopurine **(7)** have been reported previously.[7]

RESULTS AND DISCUSSION

EI mass spectra

Analysis of 70 eV mass spectra of *N*-sulfonylated purine nucleic bases (Fig. 1) and nucleosides (Figs. 4(a) and 4(b)) at different time delays indicates characteristic behaviour of these compounds under electron impact: (1) weak-abundant or nonexistent molecular ions at short storage time (Fig. 1, left, and Fig. 4(a), time delay 100 μs); (2) high-abundance protonated molecules at 300 μs and more (Fig. 1, right, and Fig. 4(b), time delay 1 s); and (3) expulsion of SO_2, SO_2H and SO_2H_2 from molecular species.

Protonated molecules

As a result of EI ionization, the odd-electron $M^{+\cdot}$ ions possess high ionization energy and fragment within a short storage time. Mass spectra of **1** and **2** display weak-abundance molecular ions at *m*/*z* 289 for a 100 μs time delay (Figs. 1(a) and 1(c)). In the mass spectra of **3** and **4** (Figs. 1(e) and 4(a)) corresponding molecular ions at *m*/*z* 443 and 547, respectively, are not observed for the same time delay.

By increasing the storage time protonated molecules become base peaks and lower abundance fragment ions are detected in the mass spectra of **1**–**4** (Fig. 1, right, and Fig. 4(b)). The influence of storage times on $[M+H]^+$ abundances and their stability suggests that their formation proceeds by an ion/molecule reaction. Present investigation gives evidence for self-chemical ionization and chemical ionization (CI) with H_3O^+ for their formation.

Self-chemical ionization

To investigate the possibility of self-chemical ionization, the $M^{+\cdot}$ ions at *m*/*z* 289 produced by EI and SWIFT isolated in the mass spectra of **1** and **2** were stored (from 100 μs to 10 s) in the ion trap to allow them to react with the neutral molecule,

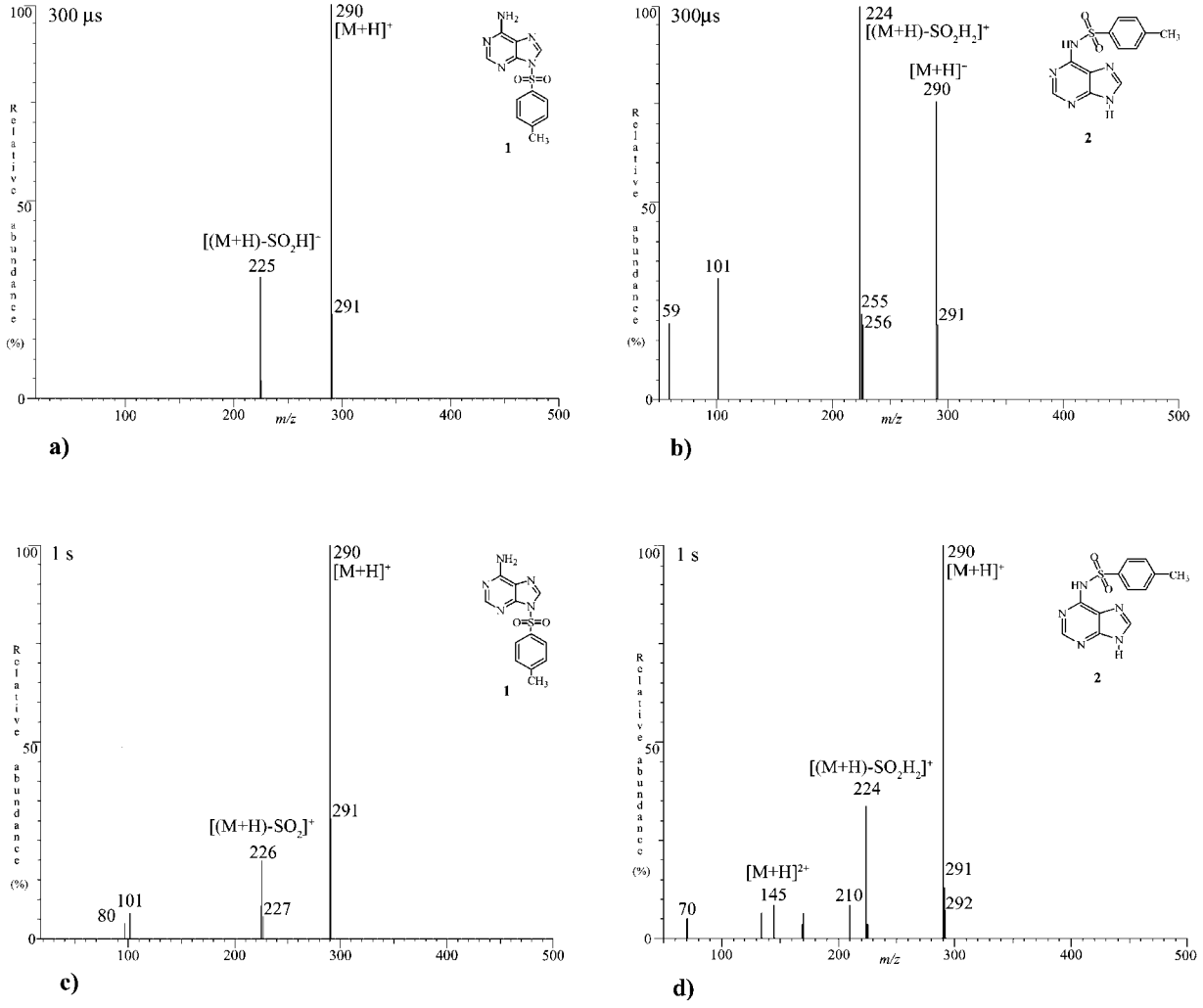

Figure 3. CI-FTICR mass spectra of *N*-sulfonylated purine nucleic bases **1** and **2** recorded after SWIFT selective isolation of H_3O^+ (delay times 300 μs (a and b) and 1 s (c and d)).

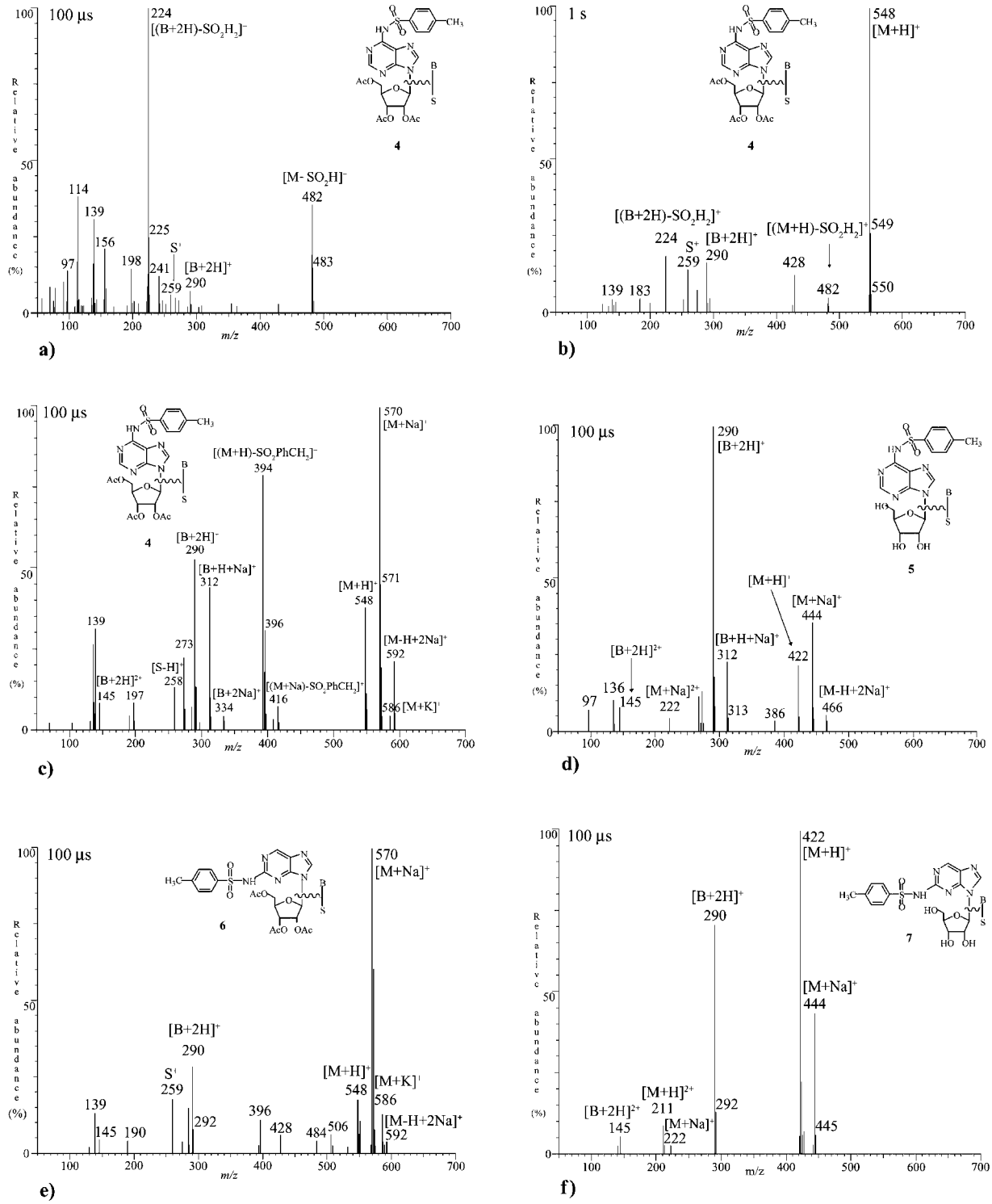

Figure 4. FTICR mass spectra of nucleosides **4**–**7**; (a and b) 70 eV EI-FTICR mass spectra of **4** recorded at 100 µs and 1 s time delay, respectively; (c–f) MALDI-FTICR mass spectra of **4**–**7** recorded at 100 µs time delay.

M. The $M^+/[M+H]^+$ ratio strongly depends on storage time (Fig. 2) especially for **1**. At higher storage times (>1 s), the signal corresponding to $[2M+H]^+$ is observed in the mass spectra of **1**.

H_3O^+ chemical ionization

Following SWIFT isolation of H_3O^+, formation of $[M+H]^+$ is highly favoured at short storage times as a result of the chemical ionization process of M with H_3O^+(Fig. 3). This behaviour suggests that H_3O^+ plays a major role in forming the $[M+H]^+$ species.

SO_2, SO_2H and SO_2H_2 expulsion

The base peaks in the mass spectra of **1** and **2** at 100 µs delay correspond to the expulsion of SO_2 and SO_2H from the molecular ions, respectively (Figs. 1(a) and 1(c)). This behaviour suggests an intramolecular nitrogen-to-oxygen hydrogen transfer to occur within the sulfonamide group, $-SO_2-NH-$, of the molecular ion $M^{+\cdot}$ of **2** before the SO_2H expulsion.

The loss of SO_2 and SO_2H_2 from the $[M+H]^+$ ions stored for 1 s or longer before recording indicates nitrogen protonation of the amino group of **1** and of the sulfonamide group of **2**,

Table 1. High-resolution mass determination of fragment ions formed by loss of SO_2, S O_2H and SO_2H_2 from molecular ions of **1**–**3**

Compound	*m/z*	Proposed fragmentation	Measured value	Calculated value	Elemental composition	Time delay
1	225	$[M-SO_2]^+$	225.093369	225.100896	$C_{12}H_{11}N_5$	100 μs
1	226	$[(M+H)-SO_2]^+$	226.105592	226.108721	$C_{12}H_{12}N_5$	1 s
2	224	$[M-SO_2H]^+$	224.082800	224.093071	$C_{12}H_{10}N_5$	100 μs
2	224	$[(M+H)-SO_2H_2]^+$	224.082800	224.093071	$C_{12}H_{10}N_5$	1 s
3	224	$[M-SO_2H-SO_2PhCH_2]^+$	224.095610	224.093071	$C_{12}H_{10}N_5$	100 μs
3	224	$[M-SO_2H_2-SO_2PhCH_2]^+$	224.088632	224.093071	$C_{12}H_{10}N_5$	1 s

respectively (Figs. 1(b) and 1 (d)). Intramolecular transfer of two hydrogen atoms from nitrogen to oxygen in $-SO_2-NH_2-$ is part of the $[(M+H)-SO_2H_2]^+$ process for **2**.

The most interesting expulsion of SO_2H from $[M+H]^+$ ions of **1** for storage periods higher than 100 μs and below 1 s indicates a protonation of oxygen taking place in the $-SO_2-$ group (Figs. 2(a), 2(c) and 3(a)). Therefore, the rate of the sulfo group oxygen protonation must be higher than amino group protonation because for storage times longer than 1 s again only expulsion of SO_2 from $[M+H]^+$ is observed in mass spectra of **1** (Figs. 1(b), 2(e) and 3(c)).

Expulsion of SO_2H (time delay 100 μs) and SO_2H_2 (time delay 1 s) from the sulfonamide group of $M^{+\cdot}$ and $[M+H]^+$, respectively, is observed in the mass spectra of **3** (Figs. 1(e) and 1 (f)) and **4** (Figs. 4(a) and 4(b)). The other SO_2 group in **3** is not expelled analogously but as part in the secondary fragmentation process

$$[(M+H)-SO_2H_2-SO_2PhCH_2]^+$$

The composition of $[M-SO_2]^+$, $[M-SO_2H]^+$ and $[M-SO_2H_2]^+$ ions is checked by high-resolution mass measurements for compounds **1** and **2** (Table 1).

MALDI mass spectra of nucleosides

In contrast to EI mass spectra recorded after a 100 μs time delay, nucleosides **4**–**7** give abundant cationized molecules (base peaks for **4**, **6** and **7**) with MALDI at the same time delay (Figs 4(c)–4(f)). The high intensity of the $[M+Na]^+$ signal may, like the highly abundant $[M+H]^+$, be ascribed to the energetically favourable even-electron system. In this respect, the attachment of a positive ion to a neutral molecule and the detection of an intense signal from the complex provide an interesting parallel between MALDI-MS and CI-MS because the process of cationization may be the result of laser-induced ion/molecule reaction in the surface layers.

The main cleavage in nucleosides **5**–**7** occurs at the bond between the sugar (S) and the base (B) residues with transfer of two hydrogen atoms to the base. However, in the MALDI spectrum of **4**, this process is accompanied by strong fragmentation resulting in formation of $[(M+H)-SO_2PhCH_2]^+$ (Fig. 4(c)).

In the MALDI mass spectra of nucleosides no expulsion of SO_2 from the molecular ions is seen.

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