

Elimination of Neutral Lithium Atom from Lithiated Nucleobases upon Collision-induced Decomposition*

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Lithium cationization of cytosine, thymine, adenine and guanine leads to the formation of multiply lithiated nucleobases. Upon collision-induced decomposition, these lithium complexes eliminate neutral lithium atom, presumably from species in which lithium has replaced the exchangeable hydrogens. © 1998 John Wiley & Sons, Ltd.

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KEYWORDS: lithium cationization; fast atom bombardment; lithium exchange; nucleobases; lithium loss; collision-induced dissociation

INTRODUCTION

Mass spectrometry is a valuable tool in studying the gas-phase interactions of metal ions and various substrates.¹ Alkali metal ions play a very important role in nucleic acid metabolism. Although there are several reports on the complexation of alkali metal ions with nucleic acid constituents,^{2–9} mass spectral studies of alkali metal ions with nucleobases (NB) are very limited. Alkali metal ion cationized nucleosides decomposed to give $[\text{NB} + \text{Li}]^+$ ion on collision-induced decomposition (CID).^{7,8} Studies on metal chelation of dinucleotide analogues in the gas phase have been reported recently.⁹ The binding of alkali metal ions to nucleobases has been examined by two groups. Rodgers and Armentrout¹⁰ investigated the bonding of metal ions to nucleobases employing a guided ion beam mass spectrometer. The alkali metal ion affinities of nucleobases were determined by a kinetic method by Cerda and Wesdemiotis,¹¹ employing the dissociation of metal ion-bound heterodimers. The dissociation of metal ion complexes of nucleobases has not been studied so far. We have undertaken an investigation of the lithium cationization of the nucleobases cytosine (1), thymine (2), adenine (3) and guanine (4). The CID spectra of the resulting lithium ion complexes were also examined. Surprisingly, the CID spectra of the lithium ion complexes revealed the loss of neutral lithium atom.

EXPERIMENTAL

The nucleobases were commercially available samples. Fast atom bombardment (FAB) mass spectra, *B/E*

linked scan and mass-analysed ion kinetic energy (MIKE) spectra were recorded on a Jeol SX-102/DA-6000 double-focusing mass spectrometer with reverse geometry using a 6 kV xenon beam (10 mA). The matrix *m*-nitrobenzyl alcohol (NBA) was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). The FAB-desorbed ions were accelerated to 10 keV. The mass spectra were recorded by scanning the magnetic field over the mass range 0–2500 u with a scan slope of 10 s and a cycle time of 12 s at a resolution of 3000. The samples were dissolved in methanol. The CID spectra were recorded by *B/E* linked scanning or the MIKE technique using helium as the collision gas. The pressure of helium was adjusted so that the parent ion beam was suppressed by 50%. The MIKE spectra were recorded using a scan slope of 20 s and a cycle time of 25 s. Each spectrum reported here is an average of 4–5 scans.

RESULTS AND DISCUSSION

These nucleobases are reported to have high proton affinities^{12,13} and alkali metal ion affinities¹¹ and are therefore expected to give abundant $[\text{M} + \text{Li}]^+$ ions on lithium cationization. The abundances of the major ions in the lithium cationized FAB mass spectra of 1–4 recorded using LiCl-saturated NBA are given in Table 1. The lithium complexes observed correspond to $[\text{M} + \text{Li}]^+$, $[\text{M} + 2\text{Li} - \text{H}]^+$, $[\text{M} + 3\text{Li} - 2\text{H}]^+$, $[2\text{M} + \text{Li}]^+$, $[2\text{M} + 2\text{Li} - \text{H}]^+$ and $[2\text{M} + 3\text{Li} - 2\text{H}]^+$ ions. In the spectra recorded using ⁶LiCl these peaks showed the expected shifts confirming the presence of the assigned number of lithium atoms. In the pyrimidine bases the most intense peak corresponds to $[\text{M} + 2\text{Li} - \text{H}]^+$ ions, whereas in the purine bases it is due to the $[\text{M} + \text{Li}]^+$ ion. It is apparent that exchangeable protons are displaced by lithium ions.

Saraswathi and Miller⁹ have shown that alkali metal ions which do not deprotonate amides in solution are able to displace amide protons under FAB. This is also

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Table 1. Ion abundances (m/z with relative abundances (%) in parentheses) in the lithium-cationized FAB mass spectra of 1–4

Ion	1	2	3	4
$[M + H]^+$	112 (11)	127 (71)	136 (59)	152 (38)
$[M + Li]^+$	118 (66)	133 (71)	142 (100)	158 (100)
$[M + 2Li - H]^+$	124 (100)	139 (100)	148 (43)	164 (46)
$[M + 3Li - 2H]^+$	130 (10)	145 (29)	—	—
$[2M + Li]^+$	229 (17)	259 (11)	277 (9)	309 (14)
$[2M + 2Li - H]^+$	235 (19)	265 (18)	283 (8)	315 (7)
$[2M + 3Li - 2H]^+$	241 (19)	271 (14)	289 (7)	—

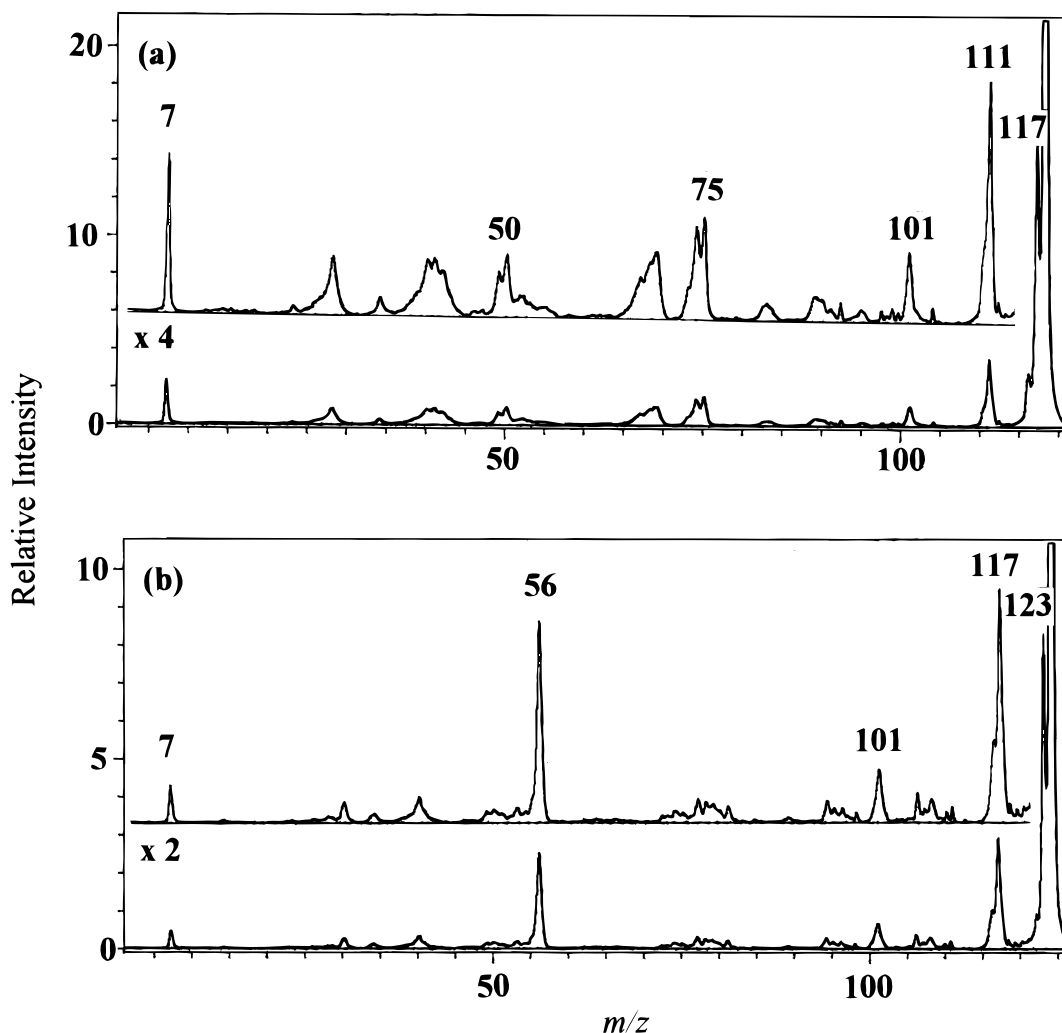
Table 2. Ion abundances (m/z with relative abundances (%) in parentheses) in the B/E CID mass spectra of the $[M + Li]^+$ and $[M + 2Li - H]^+$ ions of nucleobases 1–4

Loss/ion	1		2		3		4	
	118 ^a	124 ^a	133 ^a	139 ^a	142 ^a	148 ^a	158 ^a	164 ^a
Li	100	92	90	32	100	100	38	35
NH ₃	63	21	—	—	25	40	100	100
NH ₂ Li	7	47	—	—	6	—	10	8
HCN	35	3	50	10	90	25	18	25
(Li + HCN)	—	—	10	—	43	—	—	—
NHCO	72	17	54	—	—	—	37	49
$[NLiCOLi]^+$	—	100	—	100	—	—	—	14
$[NHCOLi]^+$	52	—	100	—	—	—	12	—
$[LiCNLi]^+$	—	20	—	10	—	70	—	15
$[HCNLi]^+$	7	7	18	5	15	11	7	4
$[HCNH]^+$	38	—	26	—	35	—	12	—
Li ⁺	18	10	33	8	24	23	8	6

^a Parent m/z .

evident from the B/E CID spectra of these nucleobase-metal complexes (Table 2). For example, apart from the expected losses of NH₃ and HCN there is a peak corresponding to $[HNCOLi]^+$ at m/z 50 in the CID spectrum of the $[M + Li]^+$ ion of 1, whereas this is shifted to m/z 56 corresponding to $[LiNCOLi]^+$ in the CID spectrum of the $[M + 2Li - H]^+$ ion, thus confirming that the amide hydrogen undergoes exchange with lithium. Similar displacement of active peptide protons by alkali metal cations has been reported to occur under electrospray ionization conditions.¹⁴ The observation of a peak at m/z 101 corresponding to losses of NH₃ and NH₂Li in the CID spectra of $[M + Li]^+$ and

$[M + 2Li - H]^+$, respectively, of 1 indicates that the proton on the amino group is also exchanged by Li⁺. The CID spectrum of the $[M + {}^6Li]^+$ ion at m/z 117 showed the elimination of ⁶Li (m/z 111), NH₃ (m/z 100), HCN (m/z 90) and NHCO (m/z 74). The $[NHCO{}^6Li]^+$

**Figure 1.** CID MIKE spectra of (a) $[M + Li]^+$ (m/z 118) and (b) $[M + 2Li - H]^+$ (m/z 124) of cytosine (1).

ion appeared at m/z 49. Similarly, upon CID the $[M + 2^6\text{Li} - \text{H}]^+$ ion at m/z 122 decomposed by elimination of ^6Li (m/z 116) and NH_2^6Li (m/z 100). The base peak in the spectrum corresponds to the ion $[\text{N}^6\text{LiCO}^6\text{Li}]^+$ at m/z 54. The lithium adducts of the purine bases adenine (3) and guanine (4) fragment mainly by elimination of NH_3 and HCN , as reported for the CID of adenine.¹⁵ Analogous to the formation of $[\text{H}_2\text{CN}]^+$ ion from $[\text{M} + \text{H}]^+$, $[\text{HCNLi}]^+$ (m/z 34) is formed during CID of the $[\text{M} + \text{Li}]^+$ ion of adenine, while it is shifted to m/z 40 corresponding to $[\text{LiCNLi}]^+$ in the spectrum of the $[\text{M} + 2\text{Li} - \text{H}]^+$ ion.

The most interesting observation is the elimination of a neutral lithium atom from these lithium complexes. This process results in the formation of M^+ from $[\text{M} + \text{Li}]^+$, $[\text{M} + \text{Li} - \text{H}]^+$ from $[\text{M} + 2\text{Li} - \text{H}]^+$, $[\text{M} + 2\text{Li} - 2\text{H}]^+$ from $[\text{M} + 3\text{Li} - 2\text{H}]^+$ ions. In order to avoid the ambiguity resulting from poor parent ion resolution under B/E scans,¹⁶ we recorded the CID MIKE spectra of these ions. These spectra also show the unexpected peaks corresponding to the elimination of neutral lithium atom. As representative examples, the CID MIKE spectra of $[\text{M} + \text{Li}]^+$ and $[\text{M} + 2\text{Li} - \text{H}]^+$ ions of 1 and 3 are given in Figs 1 and 2 respectively.

Recently, Vaisar *et al.*¹⁴ reported the elimination of neutral Na, K and Rb atoms during CID from dipeptide metal complexes generated by electrospray ionization. Elimination of a neutral lithium from lithium ion complexes is uncommon. Previous examples include loss of LiH , Li_2O , LiOAc , LiBr and LiNO_2 .¹⁷ The only reported loss of lithium atom as such is from lithium carboxylate radical anions resulting in carboxylate anions.¹⁸ The $[\text{M} + \text{Li}]^+$ ion could, in principle, consist of two species, $[\text{M} + \text{Li}^+]$ formed by Li^+ attachment or $[\text{M} - \text{H} + \text{Li} + \text{H}^+]$ produced by protonation of the lithium-exchanged molecule. The latter could then lose a lithium atom in place of a hydrogen atom from the former.

The ratio $[\text{M} + \text{Li} - \text{Li}]^+ / [\text{Li}]^+$ is always greater in the CID B/E linked scan spectra than in the corresponding CID MIKE spectra. For example, this ratio is 5.5 in the CID B/E linked scan spectrum of the $[\text{M} + \text{Li}]^+$ ion of 1, whereas it is 1.5 in its CID MIKE spectrum. Similarly, the ratio $[\text{M} + 2\text{Li} - \text{H} - \text{Li}]^+ / [\text{Li}]^+$ is 9.2 in the CID B/E spectrum of its $[\text{M} + 2\text{Li} - \text{H}]^+$ ion, whereas it is 5.7 in the corresponding CID MIKE spectrum. This could be due to a discrimination against the Li^+ ion in the B/E linked scan, to the differences in the population of ions being examined in the first field-free region (B/E scan) and the

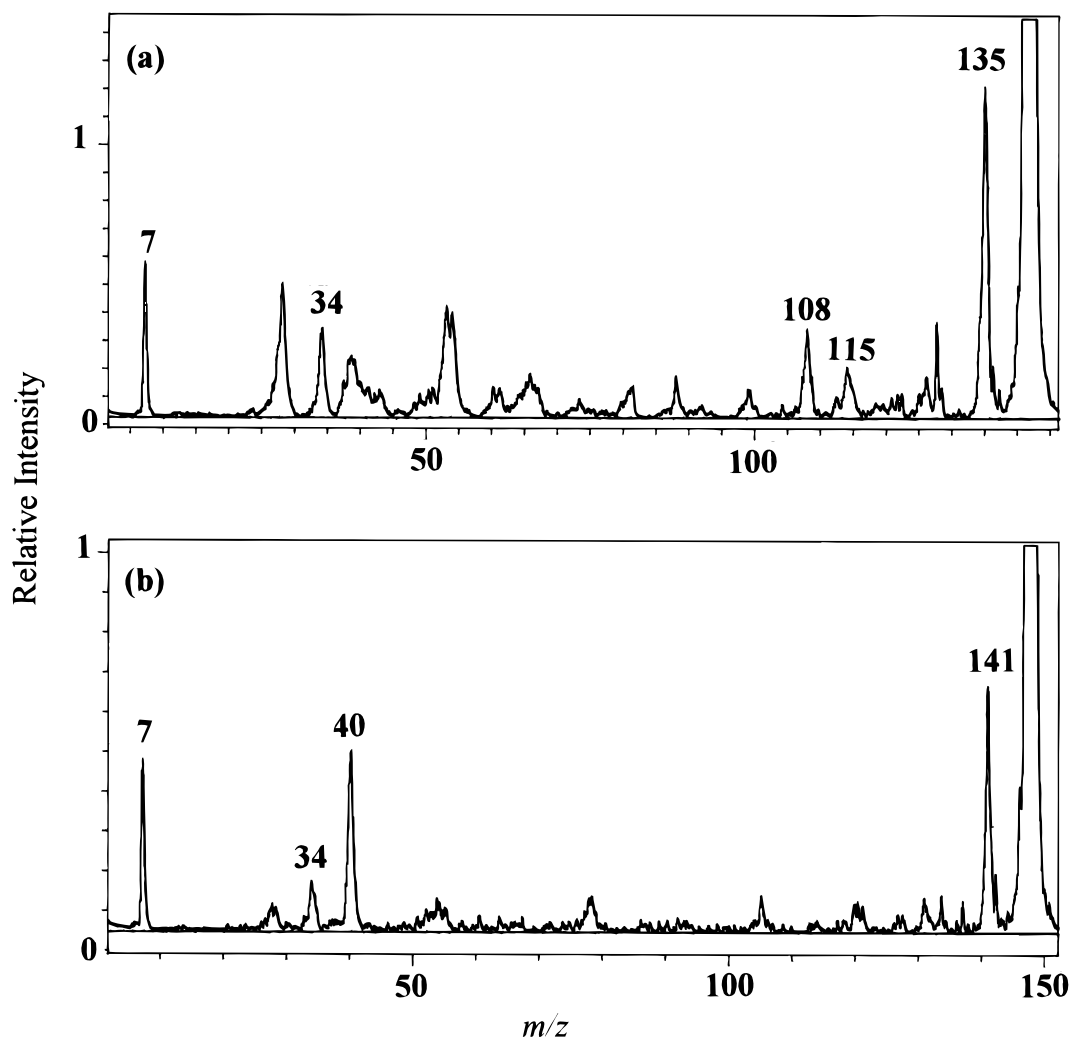


Figure 2. CID MIKE spectra of (a) $[\text{M} + \text{Li}]^+$ (m/z 142) and (b) $[\text{M} + 2\text{Li} - \text{H}]^+$ (m/z 148) of adenine (3).

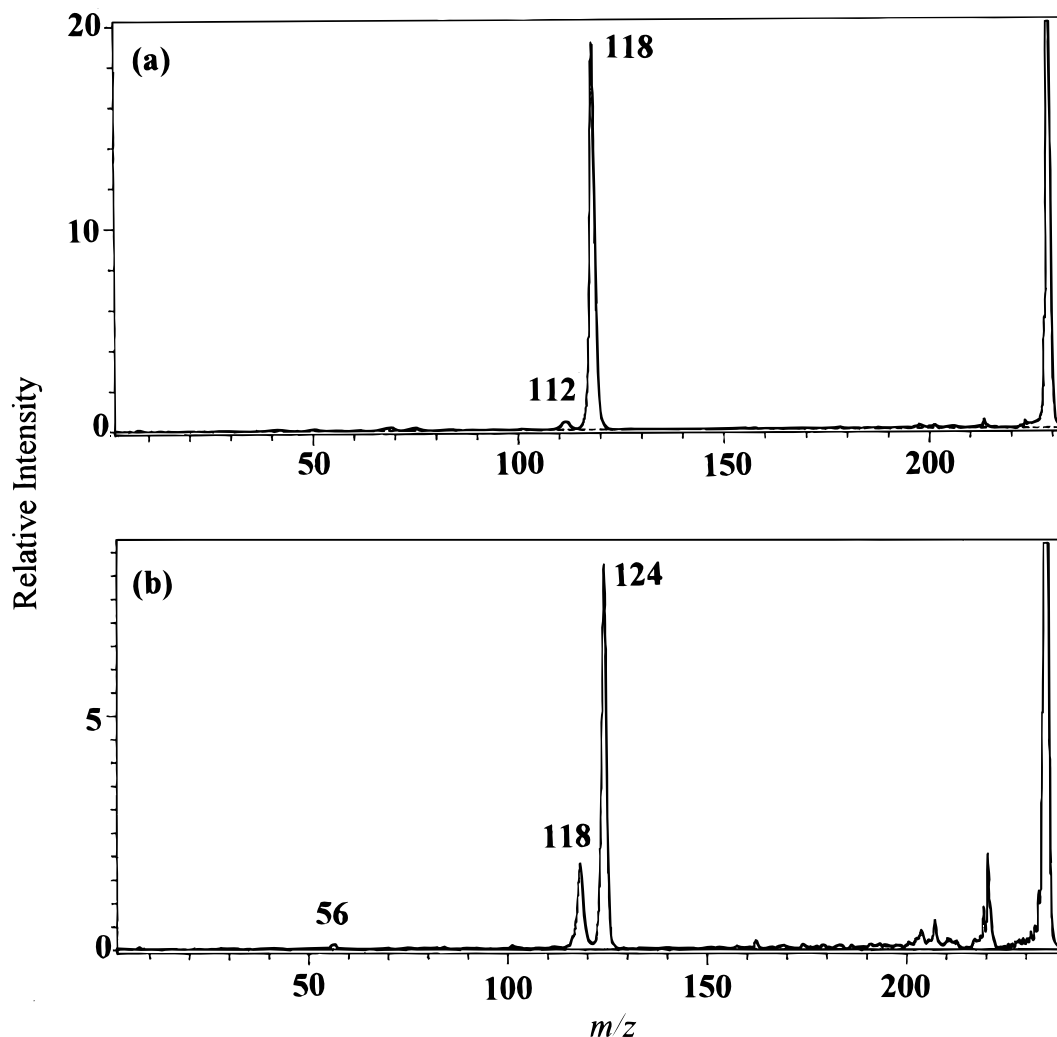
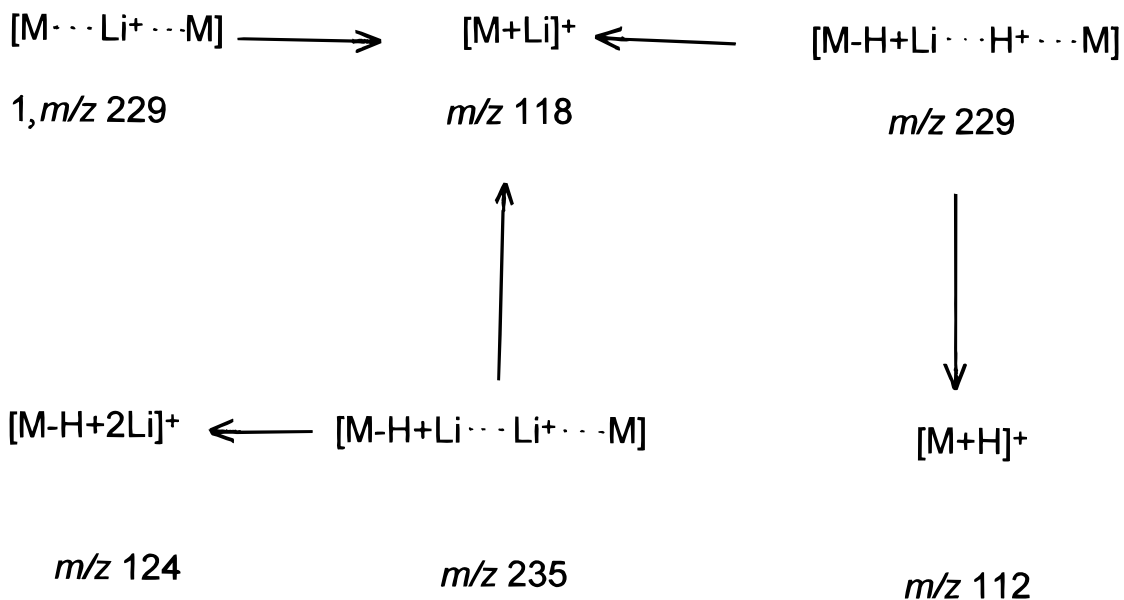


Figure 3. CID MIKE spectra of (a) $[2M + Li]^+$ (m/z 229) and (b) $[2M + 2Li - H]^+$ (m/z 235) of cytosine (1).

second field-free region (MIKE scan) or to a combination of both.

It was also of interest to study the decomposition of the $[2M + Li]^+$ and $[2M + 2Li - H]^+$ ions. The

spectra in Fig. 3 serve as examples to illustrate the decompositions of the dimeric species. Upon CID, the $[2M + Li]^+$ ion at m/z 229 decomposes to give mainly $[M + Li]^+$ (100%) and traces of $[M + H]^+$ (3%), while



Scheme 1

the $[2M + 2Li - H]^+$ ion at m/z 235 produces $[M + 2Li - H]^+$ at m/z 124 and $[M + Li]^+$ at m/z 118 in the ratio 4:1. This observation is in accordance with the expectation that the metal ion affinity of metal-exchanged nucleobases will be greater than that of the original nucleobase. The Li^+ -bound dimer of cytosine upon CID gives rise to the $[M + Li]^+$ ion at m/z 118, whereas the proton-bound dimer of the lithium-exchanged cytosine with another molecule of cytosine should lead to both $[M + Li]^+$ at m/z 118 and $[M + H]^+$ at m/z 112. Similarly, decomposition of the Li^+ -bound dimer of lithium-exchanged cytosine with another molecule of cytosine can result in the formation of $[M + Li]^+$ at m/z 118 and $[M - H + 2Li]^+$ at m/z

124 (Scheme 1). The fractions of $[M - H + Li \cdots H^+ \cdots M]$ and $[M - H + Li \cdots Li^+ \cdots M]$ are relatively small.

In conclusion, the exchangeable hydrogens in these nucleobases undergo lithium exchange under FAB. The lithium complexes decompose by elimination of neutral lithium atom, giving rise to radical cations.

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