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Short communication

ESI-MS characteristics of *N*-methylpyrrole polyamide/IDB conjugates

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Abstract

The MS and MS^n fragmentation pathways of polyamide/IDB (IDB = bis(2-benzimidazolylmethyl)amine) conjugates, which are synthesized as potent artificial nucleases, are discussed. The main fragmentation pathways occur by the cleavage of the C—CO bonds between *N*-methylpyrrole and carbonyl groups. Electrospray ionization is proven to be a good method for the structural characterization and identification of this kind of compounds.
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Keywords: Polyamide; IDB; ESI/MS/MS; DNA

1. Introduction

Polyamides containing *N*-methylpyrrole (Py) moieties can reversibly bind to the minor groove of DNA by hydrogen bonds, van der Waals contacts and electrostatic interactions with a strong preference for adeninethymine (AT)-rich sequences containing at least four A/T base pairs [1–9]. The synthesis and biological activity of such compounds have been studied intensively over the last few years. Compounds containing benzimidazole such as the dye Hoechst 33258, are reported to have significant affinity to A/T stretches [10]. By the addition of one more benzimidazole unit to the structure of Hoechst 33258, the corresponding derivatives exhibit high AT-base pair selectivity [11,12]. Qiao et al. [13] have reported a new compound ($NO_2Py_4\gamma$-cyclen) as artificial nuclease, and described the mass spectral fragmentation mechanisms of this compound. Mass spectral fragmentation mechanisms of eight polyamides containing *N*-methylpyrrole and *N*-methylimidazole were reported by Li [14]. A novel series of polyamide/IDB conjugates as potent artificial nuclease have been first synthesized and evaluated in our laboratory. In this paper, the fragmentation of these compounds was investigated by electrospray ionization mass spectrometry (ESI-MS) combined with tandem mass spectrometry (ESI-MS/MS).

2. Experimental

Mass spectra were acquired in positive ion mode using a Bruker ESQUIRE-LCTM ion trap spectrometer equipped with a gas nebulizer probe, capable of analyzing ions up to *m*/*z* 6000. Nitrogen was used as drying gas at a flow rate of 8 L/min. The nebulizer pressure was 11.0 psi. The capillary was typically held at 4 kV and the source temperature was maintained at 300 °C. The instrument was operated at unit-mass resolution; calibration of *m*/*z* was performed using a standard ES-tuning-mix. Three scans were averaged for each spectrum. The samples dissolved in methanol were ionized by electrospray ionization and continuously infused into the ESI chamber by a Cole-Parmer 74900 syringe pump (Cole-Parmer Instrument Company).

3. Results and discussion

We synthesized a series of polyamide/IDB conjugates **1** and **2** (Scheme 1), which have been characterized by IR and ^1H NMR. The Zn(II) complexes of these compounds have been demonstrated to induce DNA cleavage and its activity is much

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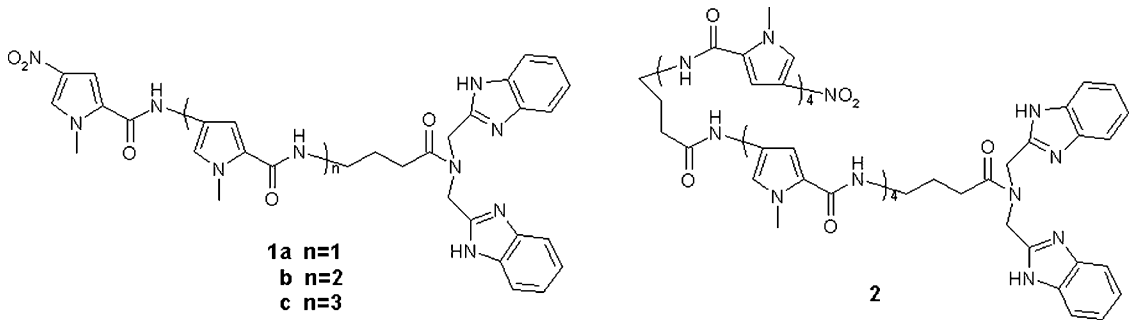


Scheme 1. The structures of polyamide/IDB conjugates.

higher than that of IDB (without polyamide backbone). Take example for compound **1c**, the ESI-MS^n spectra was obtained (Fig. 1). The ESI-MS of IDB is acquired to confirm fragmentation pathways of the IDB group in these compounds. The main fragmentation are cleavages labeled as a, b, m and n (Scheme 2). The most striking feature of the MS/MS spectra of the $[M+H]^+$ ions is the fragment ions a_1–a_7 at *m*/*z* 389, 511, 633, 755, 963, 1084 and 1207, all due to cleavage of the C–CO bond between *N*-methylpyrrole and carbonyl group. Because of the introduction of IDB into the polyamide, the important features of the MS/MS spectra of compounds **1** and **2** are the fragment ions *m*/*z* 507, 629, 751, 1325 and 278, which are due to the cleavage

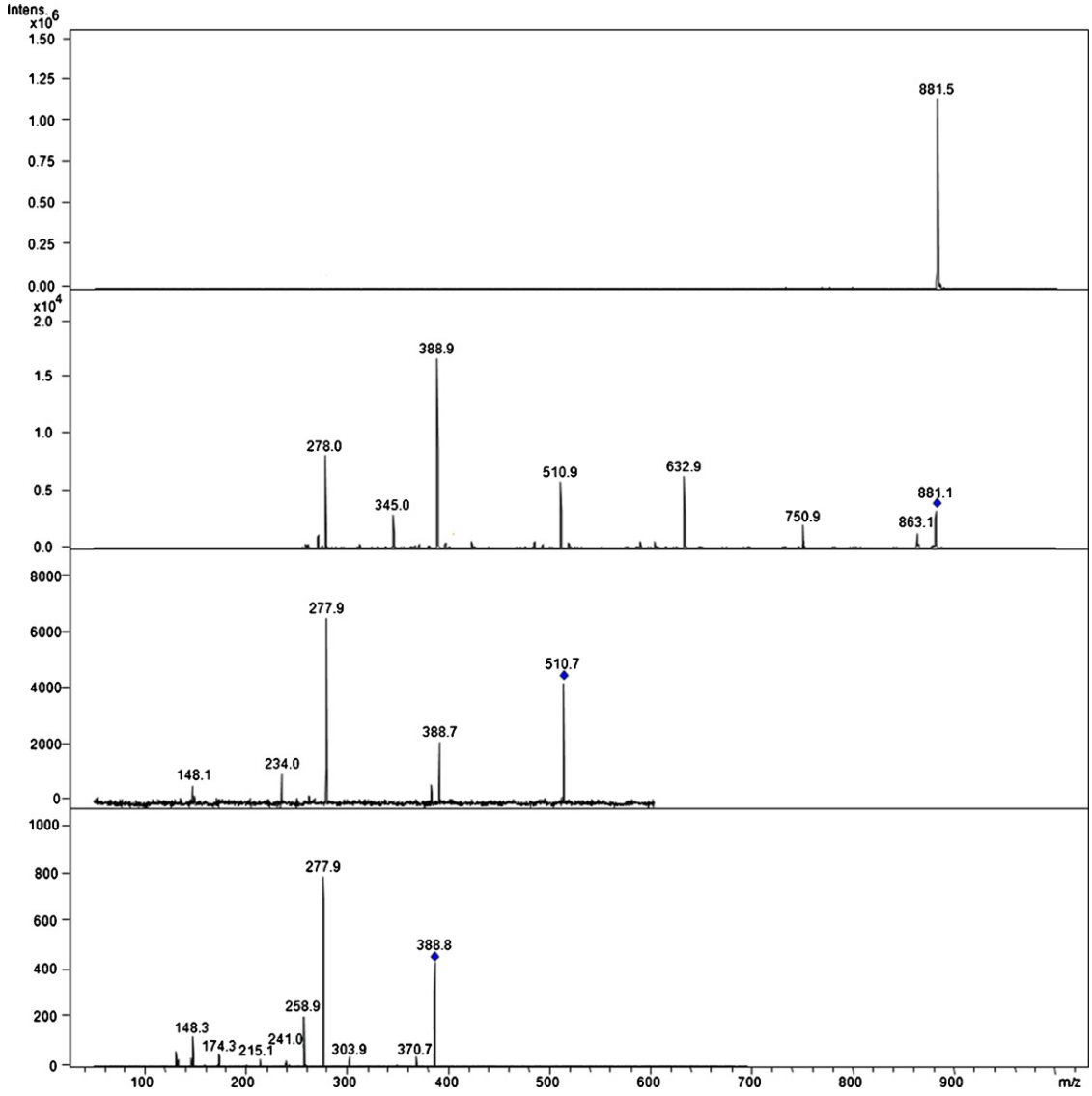
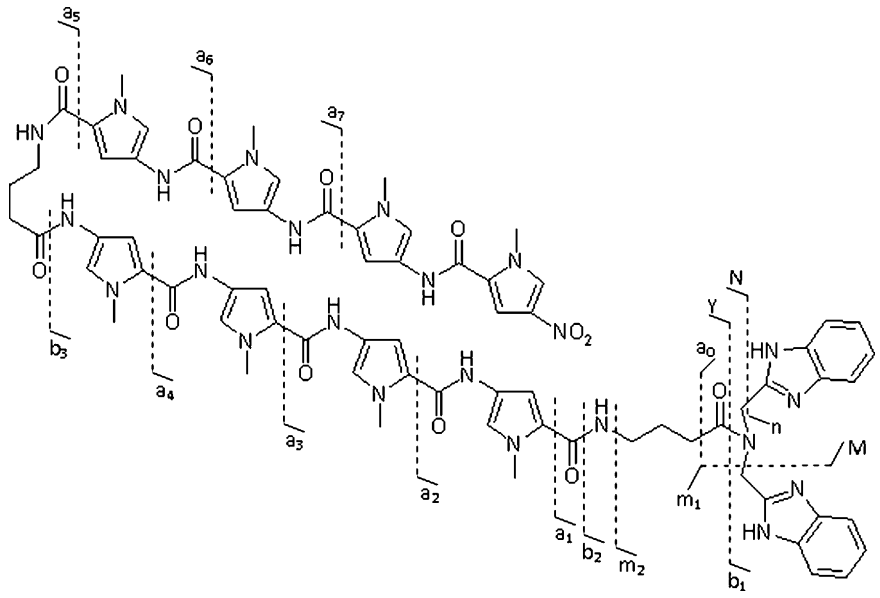


Fig. 1. The MS and MS^n spectra of compound **1c**.



Scheme 2. Main fragmentation modes.

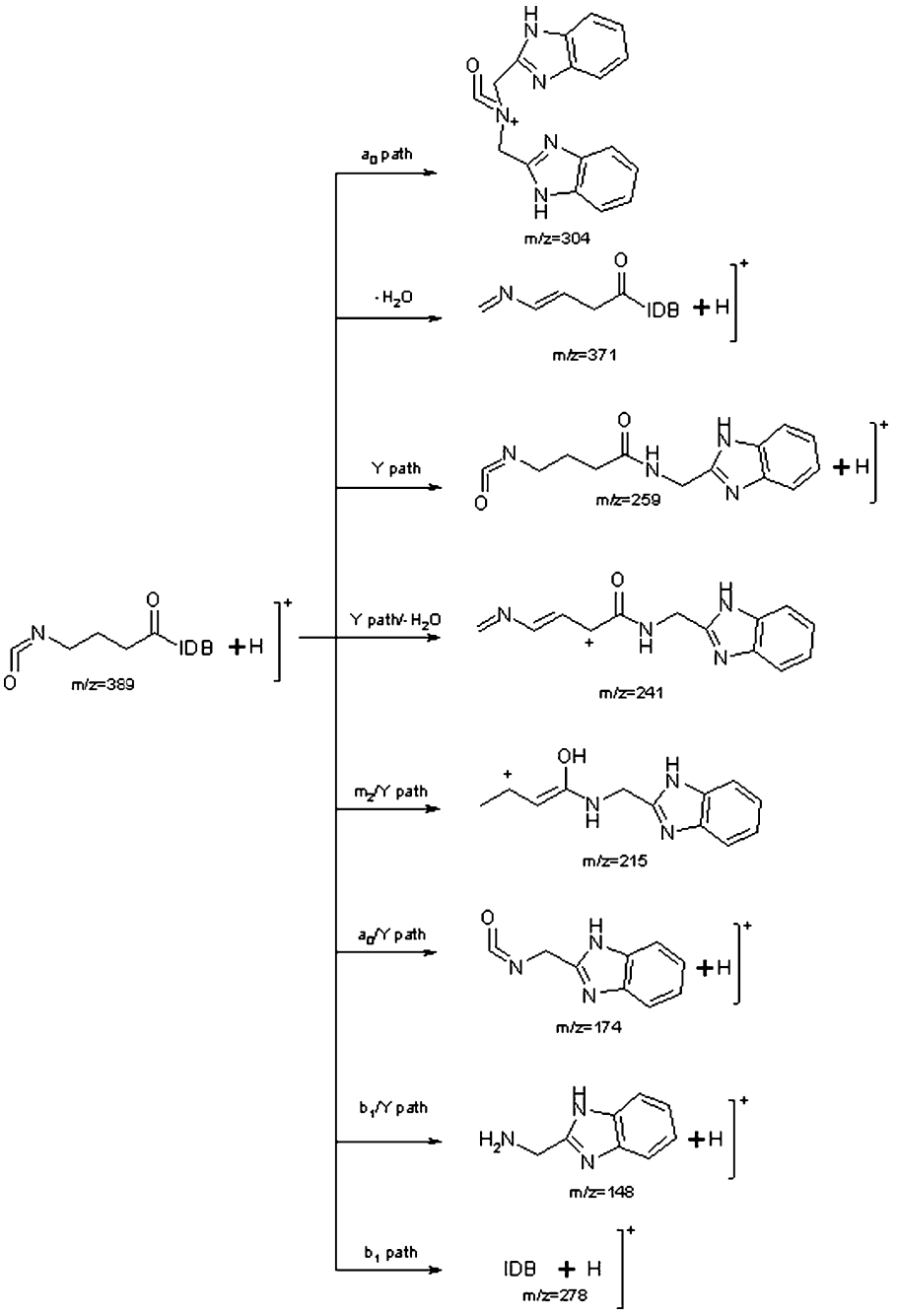
Table 1
MS^2 and MS^3 spectra data of the conjugates

Compounds	Precursor	Fragment ions (rel. int.)							
		$-H_2O$	M	a_7	a_6	a_5	a_4	a_3	a_2
2	1454(3)	1437(10)	1325(4)	1207(31)	1084(67)	963(14)	755(29)	633(100)	511(27)
	1206(3)						755(9)	633(9)	511(12)
	1084(4)						755(46)	633(100)	511(33)
1c	881(19)	863(8)	751(3)					633(38)	511(35)
	633(52)								511(30)
	511(70)								
	389(55)	370(5)	259(26)						
	278(62)								
1b	759(43)	741(6)	629(4)						511(21)
	511(42)								
	389(91)	370(3)	259(31)						
	278(77)								
1a	637(79)	619(5)	507(10)						
	389(83)	370(3)	259(31)						
	278(73)								
IDB	278(53)								
	148(89)								

Compounds	Fragment ions (rel. int.)										
	a_1	a_0	b_3	b_2	b_1	a_2/Y	b_1/M	m_2	a_1/M–H_2O	m_2/M	a_0/M
2		303(32)	851(8)								
	389(15)										
	389(57)			345(26)							
1c	389(100)			345(18)	278(49)						
	389(68)				278(100)	234(15)	148(20)				
	389(59)				278(100)	234(15)	148(20)				
		304(5)			278(100)		148(16)	131(8)	241(3)	215(3)	174(6)
							148(100)	131(23)			
1b	389(100)			345(20)	278(64)						
	389(17)				278(100)	234(17)	148(14)				
					278(100)		148(14)	131(11)	241(36)	215(3)	174(6)
							148(100)	131(24)			

Table 1 (*Continued*)

Compounds	Fragment ions (rel. int.)										
	a_1	a_0	b_3	b_2	b_1	a_2/Y	b_1/M	m_2	a_1/M–H_2O	m_2/M	a_0/M
1a	389(76)			345(7)	278(100) 278(100)		148(14) 148(100)	131(11) 131(24)	241(36)	215(4)	174(6)
IDB							148(100)	131(37) 131(100)			



Scheme 3. ESI-MS^2 fragmentation pathway of the ion at *m*/*z* 389.

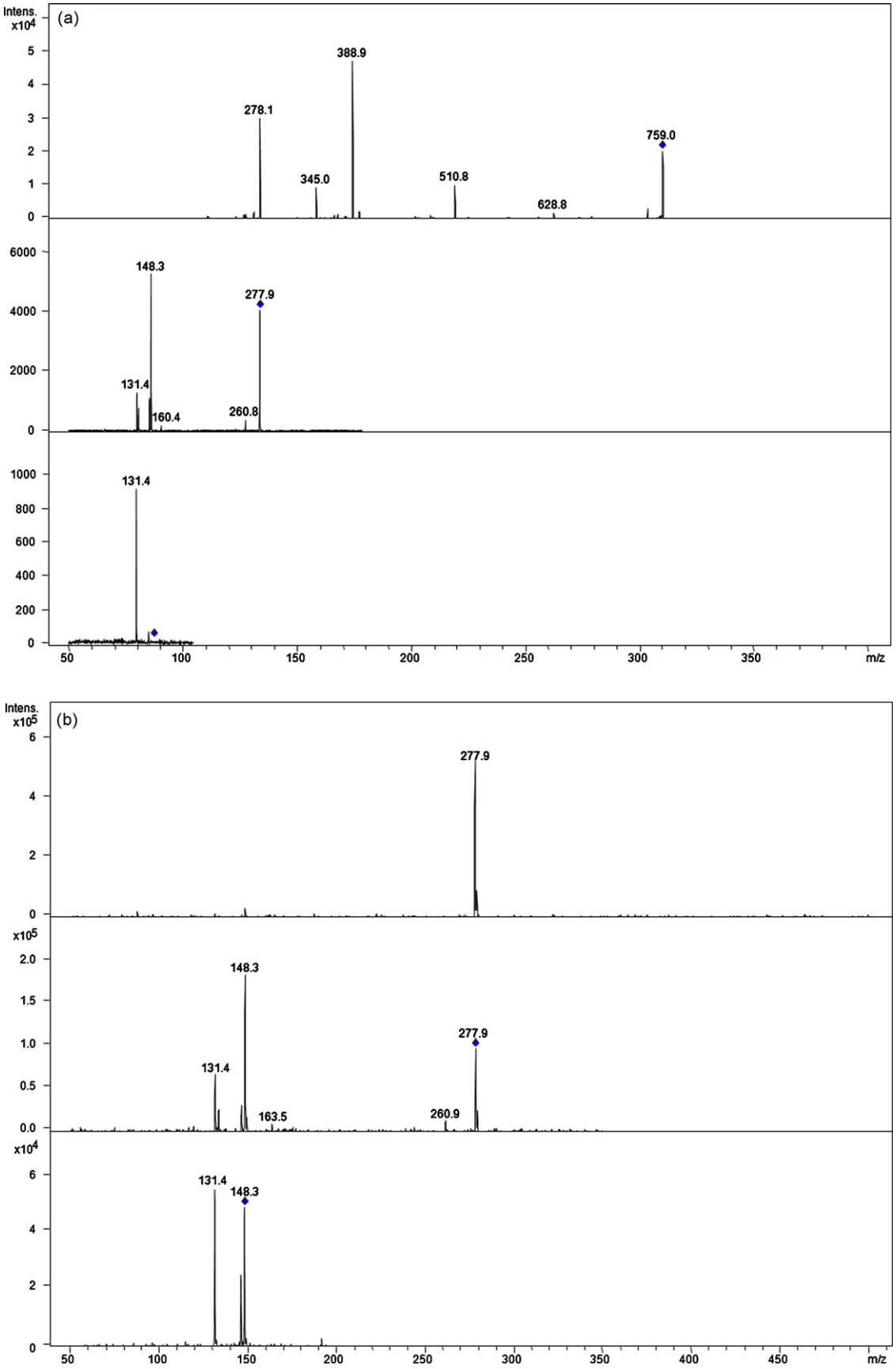


Fig. 2. The MS and MS^n spectra of ion *m*/*z* 278 (a, from precursor ion *m*/*z* 759; b, from IDB).

of IDB. The other characteristic fragmentation pathway is loss of water, which generates the ions *m/z* 619, 741, 863 and 1437. The MS/MS spectral data of the $[M+H]^+$ ions, and of the most significant fragmentation ions of these new conjugates and IDB, are summarized in Table 1.

In order to better understand the fragmentation mechanisms of these new conjugates, the ESI-MS^n spectra of compound **1c** was acquired (Fig. 1). The characteristic ions *m/z* 632, 511 and 389 are formed by a cleavage between *N*-methylpyrrole ring and carbonyl group (a pathway). The formation of the ion at *m/z* 751 and 863 occurs by the loss of a benzimidazolylmethyl group and a neutral fragment (H_2O) with rearrangement of a hydrogen atom, respectively. The ion *m/z* 345 formed corresponds to cleavage of the CO–NH bond between the first pyrrole ring and flexible linker and an elimination of water (b_2 pathway). The important feature of the MS/MS spectra of compounds **1** and **2** is the fragment ion *m/z* 278, which is due to the cleavage of the CO–NH bond between polyamide and IDB group. Because of the structure of these compounds **1** and **2** which contain polyamide and IDB group conjugated by flexible linker is similar, the ESI-MS^n spectra of the rest conjugates is similar to those of compound **1c** and the data of spectra is generalized in Table 1.

Interestingly, the decomposition of the ion *m/z* 389 yields ions *m/z* 371, 304, 278, 259, 241, 215, 174, 148 and 131 by various cleavages. The fragmentation pathways of the ion *m/z* 389, proposed in Scheme 3, can rationalize the spectra obtained. After the loss of a benzimidazolylmethyl group from the precursor ion *m/z* 389, the fragmentation pathways may involve the m_2 cleavage (*m/z* 215), a_0 cleavage (*m/z* 174), b_1 cleavage (*m/z* 148) and water loss (*m/z* 241) with rearrangement of a hydrogen atom. However, the whole IDB group can be saved, such as fragment ions *m/z* 304 and 371 observed just by a cleavage of the C–CO bond (a_0 pathway) and loss of water.

The MS/MS/MS spectra of IDB was recorded to better understand the fragmentation mechanism by comparing with the *m/z* 278 observed for a series of precursors ions. As shown in Fig. 2, the fragmentation pathway of the ion *m/z* 278 from the fragment ion and IDB are the same. The MS^3 spectrum of the ion *m/z* 278 and the MS^2 spectrum of IDB show four characteristic peaks at *m/z* 261, 160, 148 and 131 with the same fragmentation pathways. The ions *m/z* 131 and 148 are produced by cleavage of the C–N bond between two benzimidazolylmethyl groups. The peaks near the ion *m/z* 131 and 148 may be formed by the hydrogen atomic resonance on the imidazole. The characteristic ion *m/z* 261 is corresponding to the intramolecular elimination of a molecule of NH_3 from the precursor ion IDB group [13]. The ion *m/z* 160 is formed by expelling a benzimidazole group with rearrangement of a hydrogen atom.

Notably, the products of C–CO, CO–NH and NH–C cleavage were observed easily in many reports about polyamide and peptide analogues ESI-MS fragmentation pathways [13–15]. However, in this paper, it is difficult to obtain the products of CO–NH and NH–C cleavage under any conditions. Only the products of C–CO cleavage (a pathway) were observed in the ESI/MS^n spectra of all conjugates above. The dissimilarity cannot be solely explained by the energy difference of the molecular ion produced. It is possibility that introduction of IDB leads to the characteristic fragmentation pathways. Because of the rich-nitrogen structure of IDB specially the third amine, the additional proton available focuses on IDB field. Then the neutral terminals are needed in the cleavage site. So the cleavage fragments O=C=N– (a pathway) were liable to formed. This character is not observed in the ESI/MS^n spectra of polyamide derivations without IDB. This point offered a suitable tool for structure analysis of such compounds.

4. Conclusion

In this study, the ESI-MS data of the class of conjugates provided abundant structural information. The main fragmentation pathways involve the cleavage of the C–CO bonds between rings and carbonyl groups (a pathway), CO–NH amide bonds (b pathway). The important fragment ions are produced by loss of a benzimidazolylmethyl group due to the introduction of IDB into the polyamide. However, the cleavage products of CO–NH bonds between two rings are not observed in the ESI/MS^n spectra of all conjugates. The ESI-MS approach was proven to be a good method for the structural characteristic and identification of this new class of potent artificial nucleases.

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