International Journal of Mass Spectrometry 282 (2009) 64–69

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Mass spectral study of hybrid peptides derived from (*R*)-aminoxy ester and β-amino acids: The influence of aminoxy peptide bond (CO–NH–O) on peptide fragmentation under electrospray ionization conditions

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ARTICLE INFO

Article history:
Received 12 January 2009
Received in revised form 7 February 2009
Accepted 9 February 2009
Available online 20 February 2009

Keywords:
Aminoxy peptide bond
Electrospray ionization
Tandem mass spectrometry
Hybrid peptide

ABSTRACT

A new class of Boc-protected aminoxy hybrid peptides containing repeats of β-hAla-(*R*)-Ama-, and β-Caa-(*R*)-Ama- (β-hAla = β^3-(*S*)-hAlanine, (*R*)-Ama = (*R*)-aminoxy ester, and β-Caa = (*R*)-C-linked carbo-β^3-amino acid) have been studied by electrospray ionization (ESI) ion-trap and quadrupole time-of-flight tandem mass spectrometry (Q-TOF MS/MS) of their protonated, cationized, and negative ions. MS^3 CID of protonated aminoxy peptides of β-hAla-(*R*)-Ama- yield intense β-amino acid characteristic retro-Mannich fragmentation. The b_n^+ and $[b_n$–methyl imine$]^+$ ($n = 3, 5$) ions formed by cleavage of aminoxy peptide bond (CO–NH–O) are more intense than b_n^+ ($n = 2, 4$) formed by that of peptide bond (CO–NH–C) cleavage. Another characteristic ion observed is due to loss of H_3NO from y_n^+ ions. The cationized (Li^+, and Na^+) peptides dissociate differently compared to protonated peptides. Intense cationized c_n and z_n ions are formed due to the cleavage of N–O bond. The deprotonated peptides also show abundant c_n^- and z_n^- ions ($n = 1, 3, 5$) and do not form any y_n^- ions. All these results clearly indicate the influence of aminoxy peptide bond on fragmentation of these hybrid peptides.

1. Introduction

Over the past one decade there has been growing interest in peptides derived from non-natural amino acids because of their importance in pharmaceutical and foldamer chemistry [1–3]. β-Peptides, obtained from β-amino acids have resulted in a variety of helices, sheets, and turns, received very significant attention [4]. Because β-peptides have excellent stability toward proteases, they are widely used as backbone-modified amino acids in drug design. To create skeletal diversity for attaining a variety of secondary structures leading to new classes of foldamers, modification of the backbone or the amino acid side chain are interesting protocols. Thus, the replacement of the C_α or C_β atom in β-amino acids by heteroatom such as an oxygen or nitrogen is an attractive extension to the β-peptides. Changes in their substitution pattern generate a variety of interesting structural features in these molecules, which have thus begun to prove immensely useful in bioactive peptide mimicry. α-Aminoxy acids [5] are analogs of β-amino acids in which the β-carbon atom is replaced by an oxygen atom. Because of repuslion between the lone pairs of electrons of the nitrogen and oxygen atoms, the backbone of α-aminoxy acid is more rigid than that of β-amino acid. Aminoxy amide bond is resistant to enzymatic degradation; therefore, α-aminoxy acids have been explored as potential peptide mimics in several studies. As a part of an ongoing program towards developing "new motif" oligomers of carbo-β^3-amino acids with novel secondary structures [4], one of us have recently reported the synthesis of oligomers with dipeptide repeats of β-hAla-(*R*)-Ama-, and β-Caa-(*R*)-Ama- which, revealed the existence of novel right-handed 12/10-mixed helices [6].

Mass spectral characterization of α-amino acid peptides is well documented in the literature [7–9] and the tandem mass spectrometry (MS/MS) of protonated peptides formed in fast atom bombardment (FAB), electrospray ionization (ESI) [10–15], and matrix assisted laser desorption ionization (MALDI) [16–18] mass spectrometry (MS) is an established tool in determining amino acid sequence of peptides. There are very few reports available in the literature on the mass spectral study of peptides derived from non-natural amino acids which are important from the view point of foldamer chemistry as described above [19–27]. We have reported earlier tandem mass spectrometry of hybrid carbopeptides [20–27] and differentiated positional and diastereomeric isomers of some of these peptides [20–26]. We have also shown that MS/MS of some of the α,γ-/γ,α-peptides can be used to identify the –NH hydrogen atoms that participate in the 'H' bonding leading to helical structures in the solution phase [25]. Recently we reported a study on

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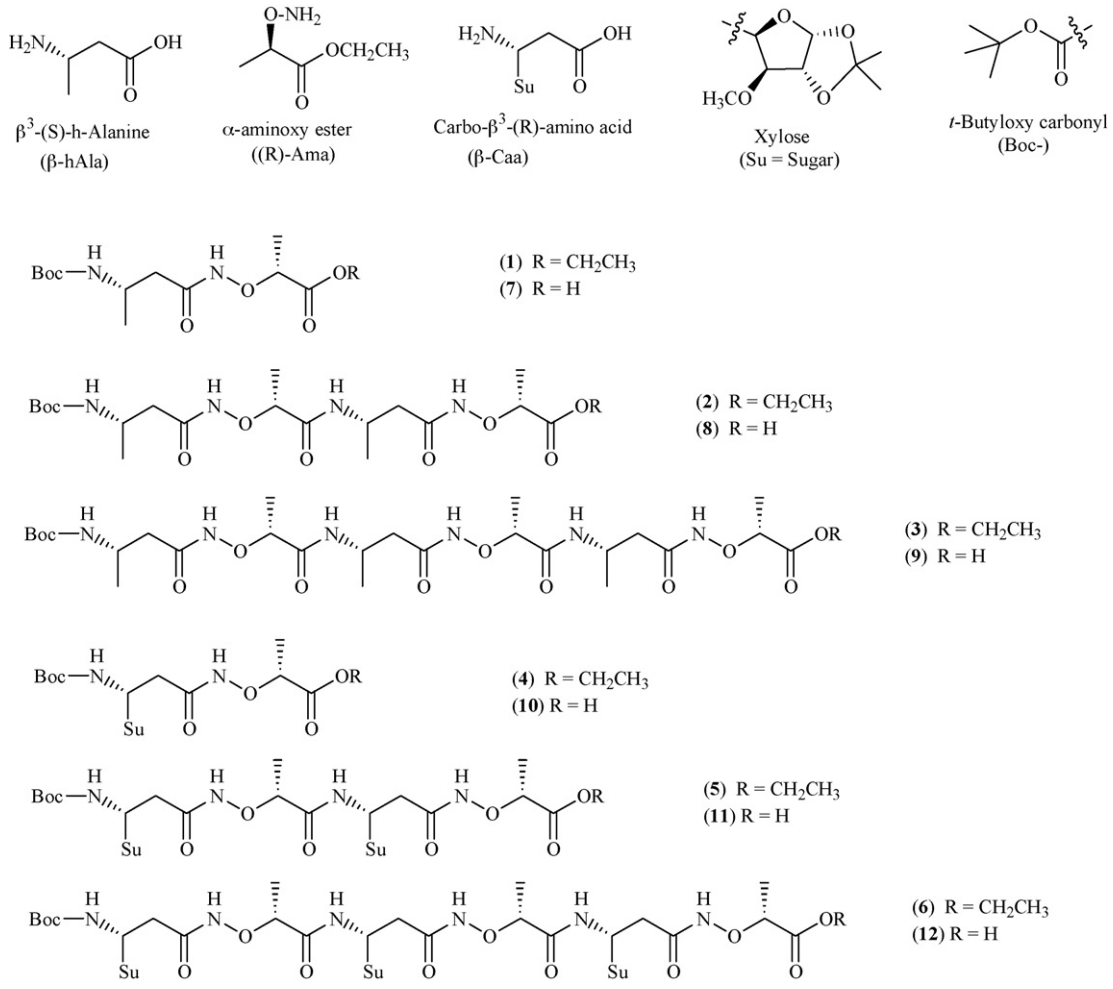
doi:10.1016/j.ijms.2009.02.006



Scheme 1. Structures of the studied peptides: β-hAla-(*R*)-Ama- (**1**–**3**, and **7**–**9**), β-Caa-(*R*)-Ama- (**4**–**6**, and **10**–**12**).

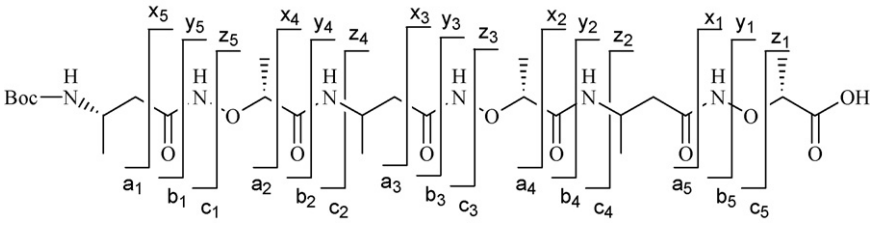
the effect of N-terminal β- and γ-carbo amino acids on fragmentation of γ-aminobutyric acid (GABA-) containing hybrid peptides [27]. In continuation of our studies on non-natural amino acid peptides, here we report a mass spectrometric study on the influence of aminoxy peptide bond on the fragmentation of aminoxy hybrid peptides consisting repeats of β-hAla-(*R*)-Ama- and β-Caa-(*R*)-Ama- using ESI MS/MS. As discussed above, these peptides have exhibited right-handed 12/10-mixed helices in solution phase (Scheme 1) [6].

2. Experimental

Positive ion electrospray ionization mass spectra of aminoxy hybrid peptides (Scheme 1) were recorded using a LCQ ion-trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA), equipped with an ESI source. The data acquisition was under the control of Xcalibur software (Thermo Finnigan). The typical source conditions were: spray voltage, 5 kV; capillary voltage, 15–20 V; heated capillary temperature, 200 °C; tube lens offset voltage, 20 V; sheath gas (N_2) pressure, 20 psi; and helium was used as damping gas. For the ion-trap mass analyzer, the automatic gain control (AGC) settings were 2×10^7 counts for a full-scan mass spectrum and 2×10^7 counts for a full product ion mass spectrum with a maximum ion injection time of 200 ms. In the full-scan MS^2 and MS^3 modes, the precursor ion of interest was first isolated by applying an appropriate waveform across the end-cap electrodes of the ion-trap to resonantly eject all trapped ions, except those ions of the *m*/*z* ratio of interest. The isolated ions were then subjected to a supplementary alternating current (ac) signal to resonantly excite them and so cause collision induced dissociation (CID). The excitation time used was 30 ms. All the samples were infused into the ESI source at a flow rate of 5 μL/min using the instrument's syringe pump.

Negative ion ESI mass spectra of aminoxy carbopeptides (Scheme 1) were acquired using a quadrupole time-of-flight (Q-TOF) mass spectrometer (QSTAR XL, Applied Biosystems/MDS Sciex, Foster City, USA), equipped with an ESI source. The data acquisition was under the control of Analyst QS software (Foster City, CA). The typical source conditions were: capillary voltage, 5.00 kV; declustering potential, 60 V; focusing potential, 260 V; declustering potential 2, 10 V; resolution 8000 (full-width half-maximum). Ultra high pure nitrogen was used as the curtain gas and collision gas, whereas zero air was used as the nebulizer. The $[M - H]^-$ ions were selected as precursors by the quadrupole and allowed to collide with nitrogen gas in the collision cell. The product ions were then detected by a time-of-flight (TOF) analyzer. The samples were infused into the ESI source at a flow rate of 10 μL/min using an in-built syringe pump.

All the spectra were recorded an average of 25–30 scans. Stock (1 mM) peptide solutions were prepared in HPLC-grade methanol and were diluted with methanol to achieve a final concentration of 10 μM of each. Solvents used in the present study were purchased from Merck (Mumbai, India), and were used without further purification.



Scheme 2. Nomenclature for fragmentaion of aminoxy hybrid peptides.

The syntheses of the peptides studied in this work have recently been reported by one of us [6]. All the peptides were prepared from corresponding monomers β-hAla, (*R*)-Ama, and β-Caa, were derived from L-alanine, L-ethyl lactate, and D-glucose, respectively. The synthesis of oligomers involved coupling of monomers in the requisite sequence using standard coupling reagents 1-(3-dimethylaminopropyl)-3-ethylcarbodimide hydrochloride (EDCI), 1-hydroxy benzotriazole hydrate (HOBt) and diisopropylethylamine (DIPEA) in CH_2Cl_2. The compounds reported were purified by column chromatography over silica gel (60–120 mesh).

3. Results and discussion

Two new series of aminoxy hybrid peptides comprising repeats of β-hAla-(*R*)-Ama- and β-Caa-(*R*)-Ama- (β-hAla = β^3-hAlanine, (*R*)-Ama = α-aminoxy ester, β-Caa = (*R*)-C-linked carbo-β^3-amino acid derived from D-glucose, and β^3 refers to the presence of the side chain at C_β-position of β-amino acid) studied in this work are shown in Scheme 1. The positive ion ESI mass spectra of all these peptides (**1**–**12**) show abundant $[M+H]^+$, $[M+Na]^+$, $[M+H-i\text{-butene}-CO_2]^+$ and $[M+Na-i\text{-butene}-CO_2]^+$ ions. The formation of $[M+H-i\text{-butene}-CO_2]^+$ ions can be explained by a McLafferty-type rearrangement involving γ-H migration from the *tert*-butyl group to the carbonyl oxygen in the Boc-N- moiety followed by the loss of isobutene (−56 Da) and subsequent loss of CO_2 −44 Da) from the $[M+H]^+$ [19–27]. Fragmentation of these peptides can be explained by using the nomenclature (Scheme 2) which was originally proposed for α-peptides by Roepstorff, Fohlman and later modified by Biemann [7–9]. The negative ion ESI mass spectra of these peptide acids (**1**–**12**) show abundant $[M-H]^-$, $[M-H-(CH_3)_3COH]^-$, and $[2M-H]^-$ ions.

3.1. Positive ion CID of aminoxy hybrid peptides

To study the influence of aminoxy peptide bond (CO–NH–O) on the fragmentation of these hybrid peptides, the CID spectra of $[M+H]^+$ and $[M+H-i\text{-butene}-CO_2]^+$ ions were examined. The

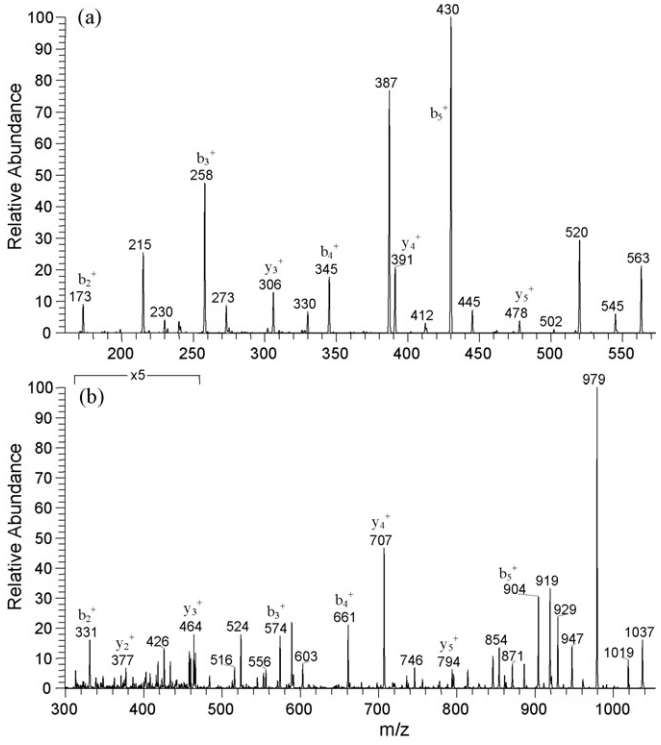
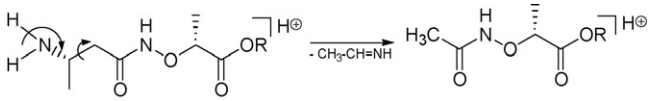


Fig. 1. MS^3 of $[M+H-i\text{-butene}-CO_2]^+$ ions of (a) **3** (*m*/*z* 563) at 31 eV, and (b) **6** (*m*/*z* 1037) at 30 eV.

MS/MS spectra of $[M+H]^+$ ions of di- (**1**), tetra- (**2**) and hexa- (**3**) peptides containing repeats of β-hAla-(*R*)-Ama- (Scheme 1), mainly show abundant $[M+H-i\text{-butene}]^+$ and $[M+H-i\text{-butene}-CO_2]^+$ ions. The MS^3 CID spectra of $[M+H-i\text{-butene}-CO_2]^+$ ions show abundant b_n^+, $[b_n-\text{methyl imine}]^+$ and $[M+H-i\text{-butene}-CO_2-\text{methyl imine}]^+$ ions (Table 1 and Fig. 1(a)). The loss of methyl imine corresponds to a retro-Mannich cleavage which seems to be a highly characteristic

Table 1
Partial CID of $[M+H-i\text{-butene}-CO_2]^+$ ion abundances (%), top values correspond to aminoxy hybrid peptide esters (**1**–**6**) and bottom values in parentheses correspond to aminoxy hybrid peptide acids (**7**–**12**).

Ion	**1** (**7**)	**2** (**8**)	**3** (**9**)	**4** (**10**)	**5** (**11**)	**6** (**12**)
b_1^+	2.5 (6.5)	–	–	17 (24)	–	–
b_2^+	–	5 (9)	11 (12)	–	11 (24)	3.5 (3)
b_3^+	–	100 (100)	53 (79)	–	66 (62)	17 (12.5)
b_4^+	–	–	19.5 (28)	–	–	21 (18)
b_5^+	–	–	100 (97)	–	–	30 (29)
y_1^+	3 (13)	–	–	–	–	–
y_2^+	–	3 (3)	–	–	28 (42)	3 (3)
y_3^+	–	6 (3)	14 (11)	–	30 (61.5)	3.5 (3)
y_4^+	–	–	22 (27)	–	–	47 (48)
y_5^+	–	–	3.5 (5)	–	–	6 (4)
b_3^+–methyl imine	–	52 (92)	29 (50)	–	–	–
b_5^+–methyl imine	–	–	84 (100)	–	–	–
$[M+H-i\text{-butene}-CO_2-\text{methyl imine}]^+$	100 (100)	25 (42)	28 (33)	–	–	–
$[M+H-i\text{-butene}-CO_2-\text{acetone}]^+$	–	–	–	8 (12)	55 (88)	100 (100)



Scheme 3. Proposed mechanism for retro-Mannich reaction in compounds **1**–**3** (R = CH_2CH_3) and **7**–**9** (R = H).

fragmentation for the presence of β[3]-hAlanine at the N-terminus (Scheme 3) [19,20,22,26,28]. For example: the hexapeptide shows b_n^+ (n = 2–5) ions at m/z 173, m/z 258, m/z 345 and m/z 430, respectively, and the [b_n–methyl imine]$^+$ (n = 3, 5) ions at m/z 215 and m/z 387. It is interesting to note that b_3^+ and b_5^+ ions are more abundant than b_2^+ and b_4^+ ions. The difference may be due to that the b_3^+ and b_5^+ ions are formed by the cleavage of aminoxy peptide (CO–NH–O) bond, whereas b_2^+ and b_4^+ ions are formed by the cleavage of peptide (CO–NH–C) bond. This indicates that the presence of oxygen in the peptide backbone induces a facile cleavage producing abundant b_n^+ (n = 3, 5) ions. The elimination of methyl imine which is abundant from b_3^+ (m/z 215) and b_5^+ (m/z 387), is insignificant from b_2^+ and b_4^+ ions. Besides, moderately abundance y_n^+ (n = 3–5) ions appear at m/z 306, m/z 391, and m/z 478 in the spectrum (Table 1). The y_n^+ ions with H_3NO at the N-terminus exhibit characteristic loss of H_3NO to form [y_n–H_3NO]$^+$ ion. These fragmentations are absent when the N-terminus contains a normal peptide (CO–NH–C) bond. For example: the MS[3] spectrum of the hexapeptide shows m/z 445 [y_5–H_3NO]$^+$, and m/z 273 [y_3–H_3NO]$^+$ ions, whereas this fragmentation is not seen in case of y_4^+ ions.

In case of other series of di- (**4**), tetra- (**5**) and hexa- (**6**) peptides containing the repeats of β-Caa-(*R*)-Ama-, the MS[3] spectrum of [M + H–*i*-butene–CO_2]$^+$ ions of **6** display both the N- and C-terminal fragment ions, i.e., b_n^+ (n = 2–5) ions at m/z 331, m/z 574, m/z 661, and m/z 904, and y_n^+ (n = 2–5) ions at m/z 377, m/z 464, m/z 707 and m/z 794 (Table 1 and Fig. 1(b)). Besides, side chain fragment ions are formed at m/z 1019 (loss of H_2O), m/z 979 (loss of acetone), m/z 947 (loss of acetone + MeOH), m/z 929 (loss of H_2O from m/z 947), m/z 846 (b_5^+–acetone), m/z 814 (loss of MeOH from m/z 846), m/z 736 (y_5^+–acetone), m/z 603 (b_4^+–acetone), m/z 556 (b_3^+–H_2O), m/z 524 (loss of MeOH from m/z 556), m/z 516 (b_3^+–acetone), and m/z 484 (loss of MeOH from m/z 516), respectively. It can be noted that the fragment ions formed by loss of methyl imine and H_3NO, which are significant for **2** and **3** are absent in these peptides. The difference in the fragmentation may be attributed to the presence of β-Caa at the N-terminus which probably catalyzes the transfer of mobile proton [14] giving rise to extensive dissociation of the side chain and suppression of influence of N–O bond.

As the peptides **1**–**3** contain β-hAla and (*R*)-Ama alternatively from the N-terminus, the peptide sequencing b_n^+ and the resulting y_n^+ ions occur at a mass difference of 85 and 87 Da, respectively. Similarly **4**–**6** contains β-Caa and (*R*)-Ama alternatively and the peptide sequencing b_n^+ and the resulting y_n^+ ions occur at a mass difference of 243 and 87 Da, respectively. For example: in compound **6** (Fig. 1(b)) the difference between y_4^+ (m/z 707), y_3^+ (m/z 464), and b_2^+ (m/z 331), b_3^+ (m/z 574) ions is 243 Da. Similarly, the difference between y_3^+ (m/z 464), y_2^+ (m/z 377), and b_3^+ (m/z 574), b_4^+ (m/z 661) ions is 87 Da.

To investigate the interaction of cations (lithium (Li), and sodium (Na)) with aminoxy hybrid peptides, we studied the MS/MS of cationized peptides and compared with those of protonated peptides. The fragmentation of cationized peptides is found to be distinctly different from that of protonated spectra. The MS/MS spectrum of [M + Na]$^+$ ions of **1**, **2** and **3** shows abundance [M + Na–*i*-butene–CO_2]$^+$, and an insignificant [M + Na–*i*-butene]$^+$. The spectra display low abundance y_n^+ and b_n^+ ions. The b_3^+ and b_5^+ ions which were abundant for protonated spectra are totally absent in the CID of cationized peptides. Instead these cationized spectra show [b_4 + Na–H–*i*-butene–CO_2]$^+$ (m/z 367) (Fig. 2(a)). Thus the MS/MS of cationized peptides provide complementary sequencing information. Interestingly the spectra also exhibit significant cationized c- and z-ions corresponding to N–O bond cleavage which occur at m/z 123 [c_1 + Na–H–*i*-butene–CO_2]$^+$, m/z 295 [c_3 + Na–H–*i*-butene–CO_2]$^+$, m/z 395 [c_3 + Na–H]$^+$, m/z 467 [c_5 + Na–H–*i*-butene–CO_2]$^+$ and m/z 567 [c_5 + Na–H]$^+$. The complementary z-ions appear at m/z 485 [z_5 + Na + H]$^+$, m/z 313 [z_3 + Na + H]$^+$, m/z 141 [z_1 + Na + H]$^+$ and m/z 413 [y_4 + Na + H]$^+$, m/z 339 [d_3 + Na–H]$^+$. Formation of intense cationized c- and z-ions can be attributed to facile cleavage of N–O bond in these peptides as compared to N–C_α bond in natural peptides. The formation of c- and z-ions is interesting in view of the earlier studies of Siu and co-workers [29] who reported that copper amine complexes of oligopeptides containing tyrosine or tryptophan residues produce abundant c- and z-ions.

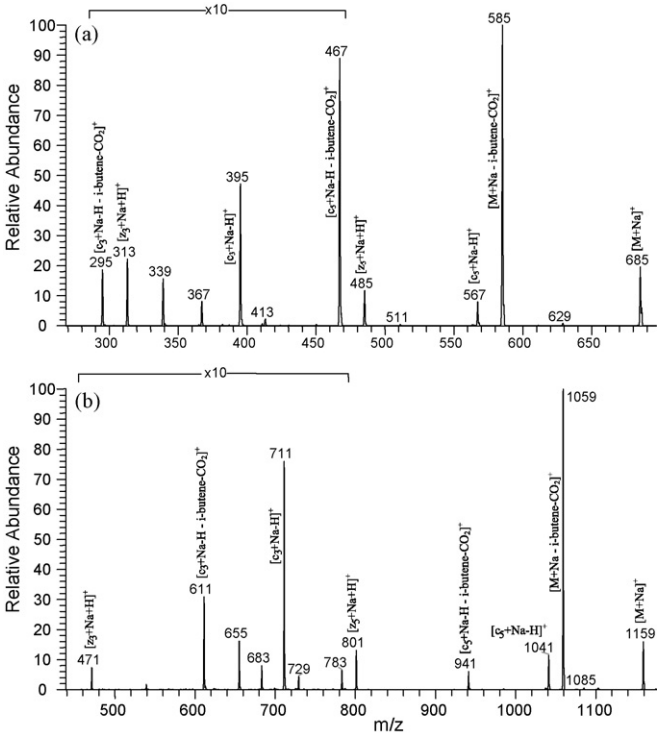


Fig. 2. MS[2] of [M + Na]$^+$ ions of (a) **3** (m/z 685) at 31 eV, and (b) **6** (m/z 1159) at 30 eV.

The MS/MS of [M + Na]$^+$ ions of **4**, **5** and **6** show abundance [M + Na–*i*-butene–CO_2]$^+$, and insignificant [M + Na–*i*-butene]$^+$. These peptides also display m/z 281 [c_1 + Na–H–*i*-butene–CO_2]$^+$, m/z 611 [c_3 + Na–H–*i*-butene–CO_2]$^+$, m/z 711 [c_3 + Na–H]$^+$, m/z 941 [c_5 + Na–H–*i*-butene–CO_2]$^+$, m/z 1041 [c_5 + Na–H]$^+$, m/z 141 [z_1 + Na + H]$^+$, m/z 471 [z_3 + Na + H]$^+$, m/z 801 [z_5 + Na + H]$^+$, m/z 683 [b_4 + Na–H–*i*-butene–CO_2]$^+$, m/z 783 [b_4 + Na–H]$^+$ and low abundance ions observed at m/z 729 [y_4 + Na + H]$^+$, m/z 655 [d_3 + Na–H]$^+$ (Fig. 2(b)). A similar fragmentation pattern was observed in the CID mass spectra of lithium cationized peptides (Fig. 3). The aminoxy hybrid peptide acids (**7**–**12**) display similar fragmentation to that of esters, except that all the m/z values of the C-terminal ions are shifted to lower masses by 28 m/z units, including the retro-Mannich product ion (Table 1).

3.2. Negative ion CID of aminoxy acid hybrid peptides

In contrast to the positive ion mass spectrometry, MS[2] of [M – H]$^-$ ions of di-, tetra- and hexa-peptide acids (**7**–**12**) mainly

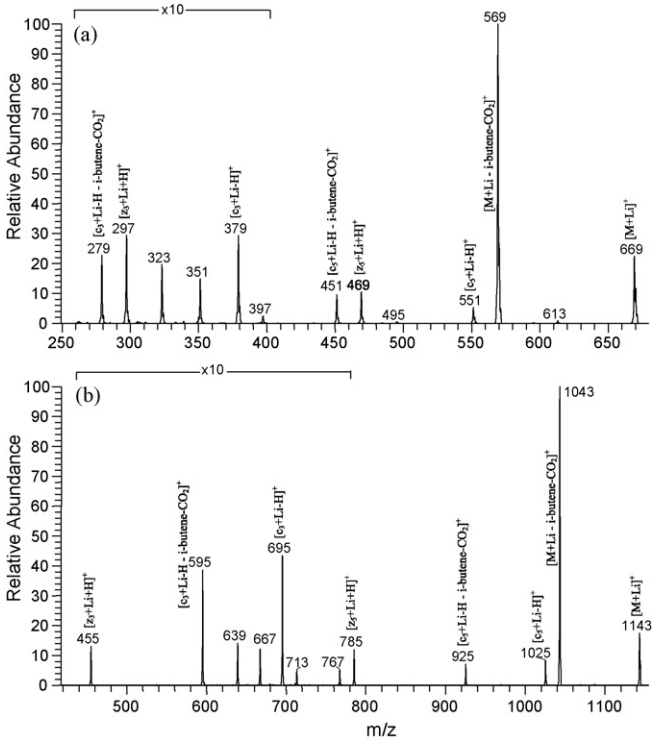


Fig. 3. MS^2 of $[M+Li]^+$ ions of (a) **3** (*m/z* 669), and (b) **6** (*m/z* 1143) at 30 eV.

show $[M-H-(CH_3)_3COH]^-$, c_{n-1}^-, $[c_n-(CH_3)_3COH]^-$ ($n=1$–5), and z_n^- ($n=1$, 3, 5) ions (Table 2). The y_n^- ions are totally absent. For example: hexapeptide (**9**) shows significant ions at *m/z* 545 (c_5^-), *m/z* 471 $[c_5-(CH_3)_3COH]^-$, *m/z* 428 $[c_5-(CH_3)_3COH-HNCO]^-$, *m/z* 343 $[c_4-(CH_3)_3COH-HNCO]^-$, *m/z* 299 $[c_3-(CH_3)_3COH]^-$, *m/z* 256 $[c_3-(CH_3)_3COH-HNCO]^-$, *m/z* 214 $[c_2-(CH_3)_3COH]^-$, *m/z* 171 $[c_2-(CH_3)_3COH-HNCO]^-$, and *m/z* 127 $[c_1-(CH_3)_3COH]^-$, the z_n^- ions at *m/z* 433 (z_5^-), *m/z* 261 (z_3^-), and *m/z* 88 (z_1^-), and the b_n^- ions ($n=2$, 4) at *m/z* 273, and *m/z* 445, and $[b_n-i\text{-butene-}CO_2]^-$ ($n=2$, 4) ions at *m/z* 173, and *m/z* 345 (Fig. 4(a)). Similarly, **12** also display *m/z* 1019 (c_5^-), *m/z* 945 $[c_5-(CH_3)_3COH]^-$, *m/z* 902 $[c_5-(CH_3)_3COH-HNCO]^-$, *m/z* 659 $[c_4-(CH_3)_3COH-HNCO]^-$, *m/z* 615 $[c_3-(CH_3)_3COH]^-$, *m/z* 589 $[c_3-i\text{-butene-}CO_2]^-$, *m/z* 572 $[c_3-(CH_3)_3COH-HNCO]^-$, *m/z* 372 $[c_2-(CH_3)_3COH]^-$, *m/z* 329 $[c_2-(CH_3)_3COH-HNCO]^-$, *m/z* 749 (z_5^-), *m/z* 419 (z_3^-), and *m/z* 88 (z_1^-), and the $[b_n-i\text{-butene-}CO_2]^-$ ($n=2$, 4) at *m/z* 331 and *m/z* 661 (Fig. 4(b)). The formation of c_n^- ions can be explained by the mechanism shown in Scheme 4. It may be noted that the carbanion adjacent to the N–O appears to induce the cleavage of N–O bond leading to c_n^- and z_n^- ($n=1$, 3, and 5) ions, respectively.

Table 2
Partial CID of $[M-H]^-$ ion abundances (%) of compounds **7**–**12**.

Ion	7	8	9	10	11	12
c_5^-			16			10
$[c_5-(CH_3)_3COH]^-$			27			30
c_3^-		5				
$[c_3-(CH_3)_3COH]^-$		100	6		100	18
$[c_2-(CH_3)_3COH]^-$			18			3
$[c_1-(CH_3)_3COH]^-$	100	3	7	100	4	
z_5^-			24			27
z_3^-		3	9.5		3	46
z_1^-		11	43	6	2	12
b_4^-			5			
$[b_4-(CH_3)_3COH]^-$			100			20
b_2^-		2	23			
$[b_2-(CH_3)_3COH]^-$		22	51		9	38
$[M-H-(CH_3)_3COH]^-$	16	3	5	68	11	5
$[M-H-(CH_3)_3COH-HNCO]^-$	3	2		6	3	

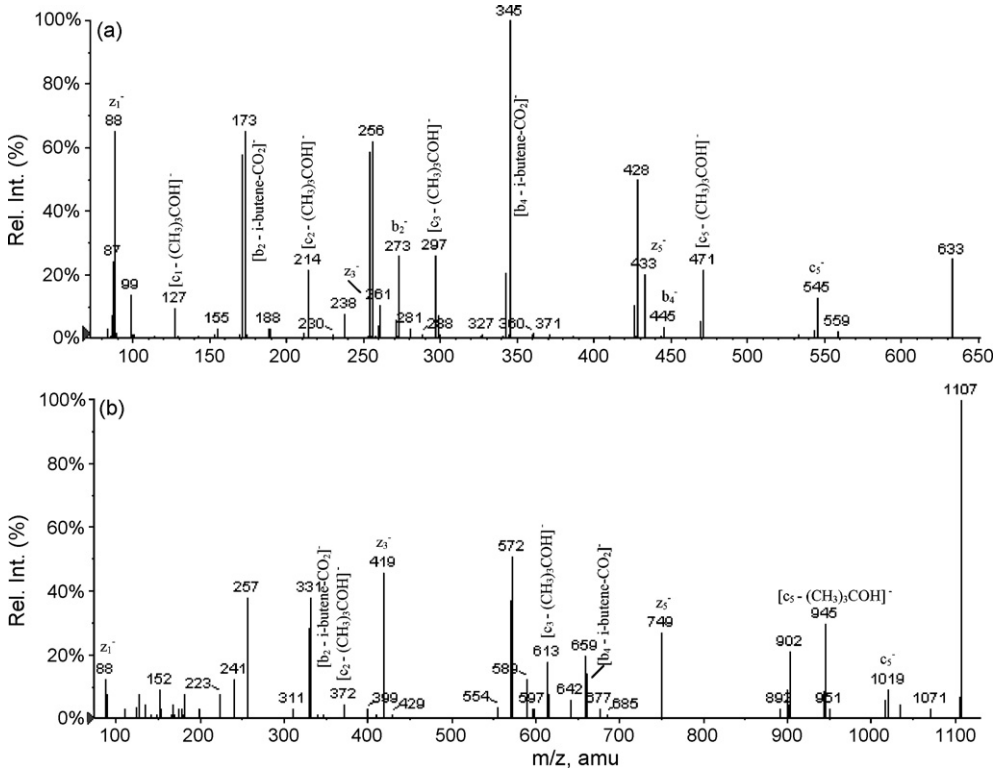
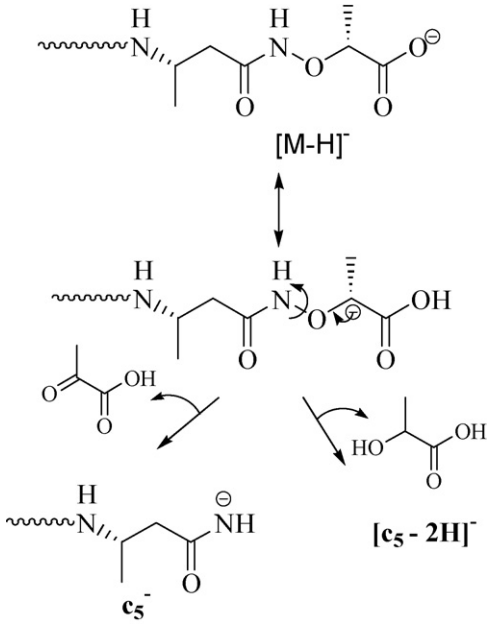


Fig. 4. CID MS/MS of $[M-H]^-$ ions of (a) **9** (*m/z* 633) at 40 eV, and (b) **12** (*m/z* 1107) at 52 eV.



Scheme 4. Proposed mechanism for formation of c_5^- and $[c_5 - 2H]^-$ ions in **9** (*m*/*z* 545 and *m*/*z* 543) and **12** (*m*/*z* 1019 and *m*/*z* 1017).

4. Conclusions

In conclusion the influence of N–O bond on the fragmentation behavior of these hybrid peptides can be clearly seen from the distinct fragmentaion of these peptides under positive and negative ion conditions. In case of peptides containing β-hAla-(*R*)-Ama- retro-Mannich fragmentation is prominent. The b_n^+ ions formed by CO–NH–O bond cleavage are more intense than CO–NH–C bond cleavage. Another characteristic ion is y_n^+–H_3NO. The cationized peptides dissociate differently compared to protonated peptides. Prominent cationized c_n and z_n ions are formed due to the cleavage of N–O bond. It is also noteworthy that abundant c_n^- and z_n^- ions are observed in negative ion spectra and y_n^- ions are totally absent. Thus it can be clearly seen that the aminoxy hybrid peptides exhibit characteristic fragmentation and the ESI MS/MS study of protonated, cationized, and negative ion peptides provide complementary structural information.

Acknowledgements

The authors thank Dr. J.S. Yadav, Director, IICT, Hyderabad, for facilities. V.R., and V.M. thank CSIR, New Delhi for the award of Senior Research Fellowships, and M.R. thankful to DST, New Delhi for the award of Junior Research Fellowship.

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