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Differentiation of some positional and diastereomeric isomers of Boc-carbo-β^3 dipeptides containing galactose, xylose and mannose sugars by electrospray ionization tandem mass spectrometry (ESI MS/MS)☆

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Abstract

Electrospray ionization (ESI) tandem mass spectrometry has been used to distinguish the positional and diastereomeric isomers of Boc-C-linked carbo-β^3 dipeptides (**1**–**38**) synthesized from glycine (Gly), β-h-glycine (β-hGly), β-h-alanine (β-hAla) and C-linked carbo-β^3-amino acid (Caa) that contain galactose, xylose and mannose sugars as side chains with "*R*" and "*S*" configurations at the amine center. The major fragmentation of $[M+H]^+$ of the dipeptides (**1**–**38**) yields mainly two ions: (i) $[M+H\text{-}C(CH_3)_3+H]^+$ (‘**a**’) and (ii) $[M+H\text{-Boc}+H]^+$ (‘**b**’) corresponding to losses of 2-methyl-prop-1-ene and -Boc moiety from $[M+H]^+$ ions, respectively. The diastereomeric dipeptide isomers with Caa (*R*) and (*S*) configurations at the N-terminus can easily be distinguished by the difference in the abundance of ion ‘**a**’ and ‘**b**’. The isomeric peptides with Caa (*R*) at the N-terminus gives prominent $[M+H\text{-}C(CH_3)_3+H]^+$ (‘**a**’) where as it is insignificant or totally absent for peptides which have Caa (*S*) at the N-terminus. This is presumably due to the different steric crowdings around the Boc-group in the different diastereomers. The positional isomers of dipeptides can also be differentiated by the difference in the abundance of ion ‘**a**’ and ‘**b**’ in the CID of $[M+H]^+$ ions. The CID of $[M+H\text{-Boc}+H]^+$ ions of the isomeric peptides also show y_1^+ ions at different *m*/*z* values. All these results suggest that the CID of $[M+H]^+$ ions is highly useful for distinguishing the Boc-NH-Caa-β^3 dipeptide isomers with Caa of "*S*" configuration from the isomers with Caa of "*R*" configuration and the positional isomers.

Keywords: Carbo-β dipeptides; Isomeric differentiation; Electrospray ionization; Tandem mass spectrometry; Diastereomeric–positional dipeptides

1. Introduction

Over the last few years there has been growing interest in oligomers of non-natural amino acids, which form a variety of secondary structures [1–5] derived from the β-amino acids. The presence of β-amino acids in place of α-amino acids in the naturally occurring pharmaceutically active peptides, known to increase the activity and the stability of the natural peptides [3,6,7]. 12/10-Mixed helix unique to the β peptides was discovered for the first time by Seebach et al. [8] in β^2/β^3 dipeptide repeats. In a later study, Sharma et al. [9] demonstrated the presence of robust right-handed mixed 10/12 as well as 12/10 helices in hetero-chiral dipeptide repeats from carbo-β^3-amino acid (Caa) with alternating chirality at Cβ carbon. They have also recently reported the synthesis of α/β hybrid peptides from *S*-epimer of Caa and L-alanine (L-Ala) and demonstrated the presence of novel 9/11 helices in these peptides [10].

Mass spectrometric characterization of peptides is well documented in the literature and the tandem mass spectrometry [11,12] of protonated peptides formed in fast atom bombardment (FAB) [13], electro spray ionization (ESI) mass spectrometry (MS) [14] and matrix assisted laser desorption ionization (MALDI) [15,16] is an established tool in determining

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amino acid sequence of peptides [17–23]. A number of studies have also been reported on the collision-induced dissociation (CID) of deprotonated small to larger peptides [24,25]. Applications of mass spectrometry to stereochemical problems is well known [26] and there are number of reports in the literature on the differentiation of stereoisomeric peptides by mass spectrometry. Tabet et al. reported the differentiation of *S*,*S* and *R*,*S* diastereomers of the acetylated dipeptide ester using MS/MS [27]. Tsunematsu et al. have demonstrated the differentiation of a pair of diastereomeric Boc-prolylproline ethyl esters by CID of sodium adduct ions in FAB [28,29] and ESI MS [30]. Cooks and co-workers have reported the differentiation and quantitation of isomeric dipeptides based on gas phase dissociation of Cu(II)-bound complexes by ESI MS [31]. The kinetic method [32,33] has also been applied to differentiate and quantify mixtures of isomeric tripeptides based on the competitive dissociations of divalent metal ion-bound clusters in an ion trap mass spectrometer [34,35]. Schug et al. have shown that arginine containing isomeric dipeptides can be distinguished by ESI-ion trap mass spectrometry [36]. de Person et al. have reported the use of an on-line LC–ESI MS/MS method for the identification and quantification of isomeric di- and tripeptides in champagne [37]. Lavanant and Hoppilliard [38] used Cu(I) and Cu(II) [39] to promote backbone fragmentations of argentine- and lysine-containing dipeptides. It was shown that dipeptides with the basic residue at the C-terminus were decarboxylated, while dipeptides with the arginine or lysine at the N-terminus fragmented by losing the entire C-terminal residue. Haselmann and co-workers have reported the use of electron capture dissociation (ECD) to distinguish a single D-amino acid in a protein [40]. Several groups have investigated elimination of small neutral molecules such as water, carbon monoxide and ammonia from protonated peptides by tandem mass spectrometry and theoretical calculations. Harrison and co-workers [41], Reid et al. [42] and Aviyente and co-workers [43] have shown that dipeptides with an underivatized COOH terminus and no serine or threonine residues primarily dehydrate at the C-terminus, yielding an N-protonated oxazolone. O'Hair et al. observed loss of NH_3 from the protonated methyl ester of CysGly, but not from the isomeric $[GlyCys\text{-}OCH_3 + H]^+$ ion which instead loses water [44]. Pingitore et al. have investigated by tandem mass spectrometry and density functional theory (DFT) calculations, the elimination of carbon monoxide and water from a series of protonated dipeptides [45]. They have shown that water loss takes place more efficiently when the more basic residue is at the C-terminus. Farrugia and O'Hair reported the differentiation of arginine-containing dipeptide isomers by tandem mass spectra and DFT calculations [46]. Recently, Pingitore and Wesdemiotis characterized mono- and dilithiated isomeric pairs of PheGly/GlyPhe dipeptide by tandem mass spectrometry [47]. We have demonstrated earlier the differentiation of a pair of positional and diastereomeric isomers of Boc-protected β,β and β,α dipeptides by using ESI MS/MS [48,49]. Here, we report on the differentiation of some positional and diastereomeric isomers of Boc-C-linked carbo-β^3 dipeptides (**1**–**38**) containing galactose, xylose and mannose sugars as side chains with "*R*" and "*S*" configuration at the amine center.

2. Experimental

Electrospray ionization mass spectra were recorded using a Quattro LC triple-quadrupole mass spectrometer (Micromass, Manchester, UK) interfaced to an electrospray ion source; data acquisition was under the control of Masslynx software (Version 3.2). Sample solutions were introduced into the source by an infusion pump (Harvard Apparatus) at a flow rate of 10 μL/min. The capillary voltage was maintained between 3.0 and 3.5 kV, and the cone voltage was kept at 20–40 V unless otherwise stated. Nitrogen was used as desolvation and nebulization gas. The source and desolvation temperatures were 100 °C. The mass spectra were recorded by scanning MS1, and the collision-induced dissociation spectra were obtained by selecting the precursor ion of interest with MS1 and scanning MS2. Argon was used as collision gas, and the pressure in the collision cell was maintained at 3.5×10^{-4} mbar. All the spectra reported here were recorded under identical experimental conditions, and are averages of 20–25 scans.

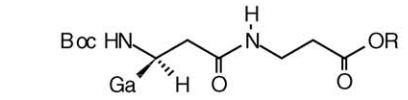
2.1. Materials

Solvents used in the present study were purchased from Aldrich (USA), Merck (Germany) and Sd. Fine Chemicals (India), and were used without further purification. Stock (1 mM) solutions of peptides were diluted with HPLC-grade methanol to achieve a final concentration of 100 μM of each.

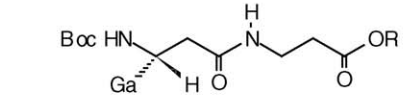
The syntheses of Boc-C-linked carbo-β^3 dipeptides studied in this work have been reported by some of us [9,10,50]. All the peptides were prepared from corresponding monomers by conventional method using 1-hydroxybenzotriazole hydrate (HOBt) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), in dichloromethane.

3. Results and discussion

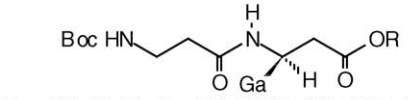
The BocN-carbo-β^3 dipeptides (esters, **1**–**19**; acids, **20**–**38**) studied in this work are shown in Scheme 1. These dipeptides were synthesized from β-h-glycine (β-hGly), glycine (Gly), β-h-alanine (β-hAla) and C-linked carbo-β^3-amino acid (Caa) containing galactose, xylose and mannose amino acids (Gaa, Xaa and Maa). The positive ion ESI mass spectra of all these peptides show abundant $[M + H]^+$, $[M + Na]^+$ and $[M + H\text{-Boc} + H]^+$ ions. A hydrogen migration from a methyl group of the *tert*-butyl group to the carbonyl oxygen (McLafferty-type rearrangement) in the BocN-moiety [48,49,51] followed by the loss of 2-methylprop-1-ene (56 Da) and subsequent loss of CO_2 leads to the formation of $[M + H\text{-Boc} + H]^+$ ions. Fragmentation of these peptides can be explained by using the nomenclature, which was originally proposed for α peptides by Roepstorff and Fohlman [17] and later modified by Biemann [18]. The ESI mass spectra of the dipeptide esters **1**–**12** show y_n^+ ions and **13**–**19** yield both y_n^+ and b_n^+ ions. Similarly, the ESI mass spectra of the dipeptide acids **20**–**31** give y_n^+ ions and **32**–**38** yield both y_n^+ and b_n^+ ions. Both the peptide esters and acids yield other peptide



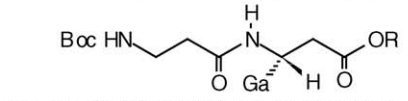
Boc-NH-Gaa(S)-ß-hGly-OR [R=CH_3(**1**);R=H(**20**)]



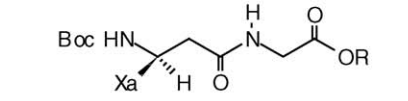
Boc-NH-Gaa(R)-ß-hGly-OR [R=CH_3(**2**);R=H(**21**)]



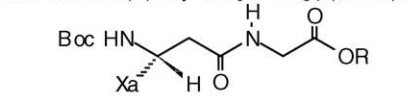
Boc-NH-ß-hGly-Gaa(S)-OR [R=CH_3(**3**);R=H(**22**)]



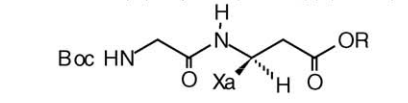
Boc-NH-ß-hGly-Gaa(R)-OR [R=CH_3(**4**);R=H(**23**)]



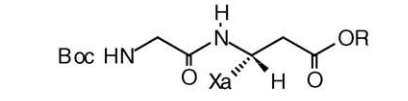
Boc-NH-Xaa(S)-Gly-OR [R=CH_3(**5**);R=H(**24**)]



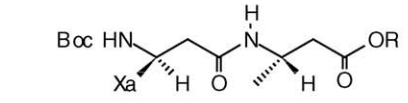
Boc-NH-Xaa(R)-Gly-OR [R=CH_3(**6**);R=H(**25**)]



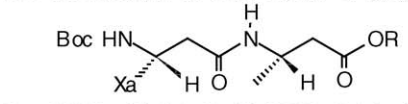
Boc-NH-Gly-Xaa(S)-OR [R=CH_3(**7**);R=H(**26**)]



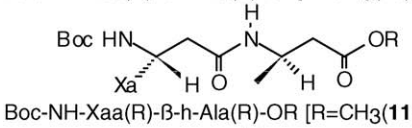
Boc-NH-Gly-Xaa(R)-OR [R=CH_3(**8**);R=H(**27**)]



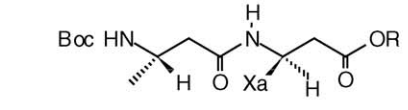
Boc-NH-Xaa(S)-ß-h-Ala(S)-OR [R=CH_3(**9**);R=H(**28**)]



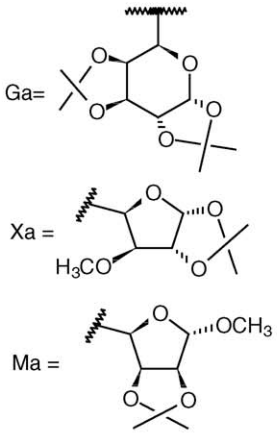
Boc-NH-Xaa(R)-ß-h-Ala(S)-OR[R=CH_3(**10**);R=H(**29**)]

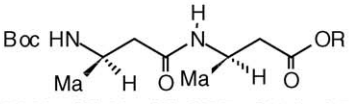


Boc-NH-Xaa(R)-ß-h-Ala(R)-OR [R=CH_3(**11**);R=H(**30**)]

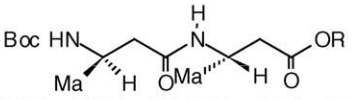


Boc-NH-ß-h-Ala(S)-Xaa(S)-OR [R=CH_3(**12**);R=H(**31**)]

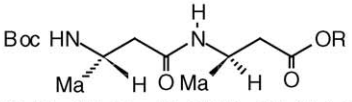




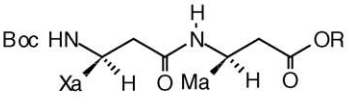
Boc-NH-Maa(S)-Maa(S)-OR[R=CH_3(**13**);R=H(**32**)]



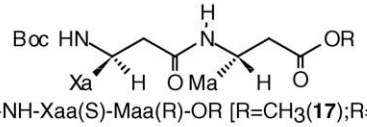
Boc-NH-Maa(S)-Maa(R)-OR[R=CH_3(**14**);R=H(**33**)]



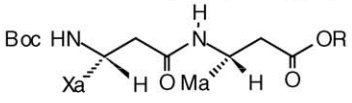
Boc-NH-Maa(R)-Maa(S)-OR[R=CH_3(**15**);R=H(**34**)]



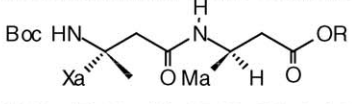
Boc-NH-Xaa(S)-Maa(S)-OR [R=CH_3(**16**);R=H(**35**)]



Boc-NH-Xaa(S)-Maa(R)-OR [R=CH_3(**17**);R=H(**36**)]



Boc-NH-Xaa(R)-Maa(R)-OR [R=CH_3(**18**);R=H(**37**)]



Boc-NH-Xaa(R)-Maa(S)-OR [R=CH_3(**19**);R=H(**38**)]

Scheme 1. Boc-NH-Caa-β^3 dipeptide (esters, **1**–**19**; acids, **20**–**38**), Caa (*R*), Caa (*S*) represent epimeric with respect to the amine center (Caa = Gaa, Xaa and Maa).

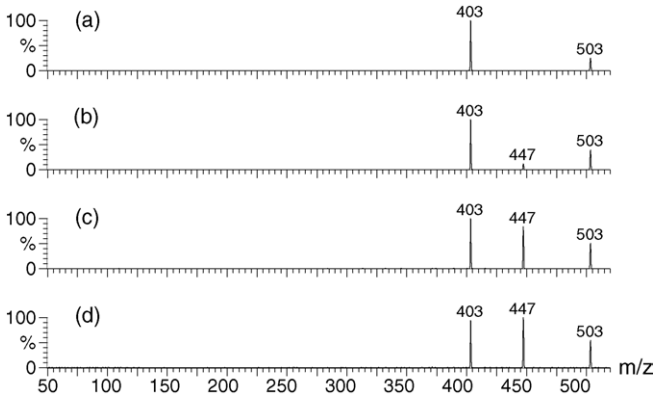


Fig. 1. ESI MS/MS spectra of $[M+H]^+$ ions of compounds: (a) **1**, (b) **2**, (c) **3** and (d) **4**, at 10 eV.

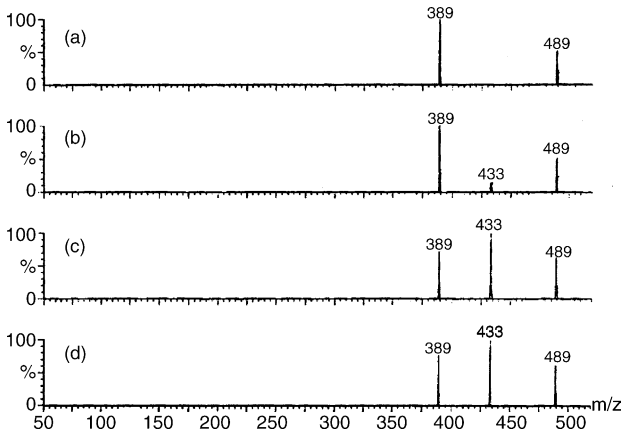


Fig. 2. ESI MS/MS spectra of $[M+H]^+$ ions of compounds: (a) **20**, (b) **21**, (c) **22** and (d) **23**, at 10 eV.

backbone fragment ions of low abundance and fragment ions characteristic of the C-linked sugar moiety.

3.1. Differentiation of diastereomeric and positional isomers of BocNH-Gaa-β-hGly/BocNH-β-hGly-Gaa-β³ dipeptides

In order to differentiate the diastereomeric dipeptide isomers **1** from **2**, the CID of these protonated dipeptides were compared. Similarly, the CID of $[M+H]^+$ ions of positional isomers **3** and **4** were compared with those of **1** and **2**, respectively.

The CID mass spectrum of $[M+H]^+$ ion of **2** with Gaa (*R*) (where configuration "*R*" refers to point of attachment) at the N-terminus, shows a moderately abundant fragment ion of *m/z* 447 corresponding to $[M+H\text{-}C(CH_3)_3+H]^+$ (**'a'**) and an intense ion at *m/z* 403, $[M+H\text{-Boc}+H]^+$ (**'b'**). Whereas for **1** with Gaa (*S*) at the N-terminus, **'b'** is more abundant and **'a'** is totally absent (Fig. 1; Table 1). The greater abundance of **'a'** for **2** may presumably due to the relatively less steric crowding around the Boc-group in '*R*' configuration. This probably facilitates the hydrogen migration leading to elimination of 2-methyl-prop-1-ene from the $[M+H]^+$ ion of **2** isomer to form **'a'**, whereas it appears to be suppressed in **1** due to higher steric crowding following "*S*" configuration at the amine center. This may also perhaps due to the increased stability of $[M+H\text{-Boc}+H]^+$ by hydrogen bonding between the $-NH_2$ and the Gaa (*S*) at the N-terminus. It is noteworthy that these protonated peptides dissociates similarly to those of peptides with xylose amino acid (Xaa) at the N-terminus [48,49].

The CID mass spectra of $[M+H]^+$ ion of **3** and **4** are different from those of their positional isomers **1** and **2**, respectively (Fig. 1; Table 1). Both **3** and **4** exhibit abundant **'a'** and **'b'** ions. Whereas **'a'** is absent for **1** and moderately abundant for **2**. It should be noted that **3** and **4**, which have Gaa (*S*) and Gaa (*R*) at the C-terminus, respectively, do not show any difference in their fragmentation. The observed differences in the CID mass spectra of $[M+H]^+$ ions of diastereomers (**1**, **2**) and two pairs of positional isomers (**1**, **3** and **2**, **4**) are retained at different relative collision energies (RCEs) (Table 1). These results clearly demonstrate that CID of $[M+H]^+$ ions is highly useful for distinguishing the diastereomeric peptides **1** from **2** and positional isomers **3** and **4** from **1** and **2**, respectively. Similar differences are observed between the CID mass spectra of $[M+H]^+$ of isomeric acids (**20**–**23**) (Fig. 2).

3.2. CID of [M + H-Boc + H]⁺

The CID mass spectra of $[M+H\text{-Boc}+H]^+$ ions of **1** and **2** show abundant y_1^+ at *m/z* 104, *m/z* 200 ($Ga–CH=NH_2^+$–acetone) and b_1^+ ions of insignificant abundance (Fig. 3a; Table 2). The spectra show moderately abundant ions characteristic of the sugar group at *m/z* 345 (loss of acetone), *m/z* 327 (loss of acetone plus H_2O), *m/z* 287 (loss of two acetone molecules), *m/z* 269 (loss of two acetone molecules plus H_2O), *m/z* 258 ($Ga–CH=NH_2^+$), *m/z* 251 (loss of two molecules of acetone plus $2H_2O$), *m/z* 242 ($Ga=CH^+$), *m/z* 200 ($Ga–CH=NH_2^+$–acetone), *m/z* 184 ($Ga=CH^+$–acetone), *m/z* 173 (loss of GaH), *m/z* 166

Table 1
CID of $[M+H]^+$ of **1**–**4** at different collision energies

Energy (eV)	Peptides							
	1		**2**		**3**		**4**	
	m/z 447[a]	*m/z* 403[a]	*m/z* 447[a]	*m/z* 403[a]	*m/z* 447[a]	*m/z* 403[a]	*m/z* 447[a]	*m/z* 403[a]
5	–	29	5.8	15.6	19.6	9.8	21.5	7.8
8	–	100	15.6	80	78	52.9	86.2	58.8
10	–	100	11.7	100	84	100	100	94.1
12	–	100	5	100	44	100	54.9	100

[a] Ions.

Table 2
CID of $[M + \text{H-Boc} + \text{H}]^+$ mass spectral data of compounds **1**–**12**; *m*/*z* with relative abundance (%) in parentheses

Ions	Peptides[a]											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
y_1^+	104 (100)	104 (82)	332 (48)	332 (15)	90 (66)	90 (82)	276 (11)	276 (9)	118 (100)	118 (100)	118 (100)	276 (8)
b_1^+	300 (–)	300 (2)	72 (6)	72 (4)	244 (–)	244 (–)	58 (1)	58 (–)	244 (–)	244 (–)	244 (–)	86 (2)
y_1^+–acetone	–	–	274 (100)	274 (18)	–	–	218 (14)	218 (14)	–	–	–	218 (16)
y_1^+–acetone–H_2O	–	–	256 (56)	256 (100)	–	–	200 (2)	200 (2)	–	–	–	200 (6)
y_1^+–acetone–CH_3OH	–	–	242 (26)	242 (6)	–	–	–	–	–	–	–	–
y_1^+–acetone–H_2O–CH_3OH	–	–	224 (16)	224 (7)	–	–	168 (30)	168 (40)	–	–	–	168 (10)
–H_2O	385 (–)	385 (1)	385 (6)	385 (5)	315 (–)	315 (1)	315 (6)	315 (19)	343 (3)	343 (2)	343 (2)	343 (4)
–CH_3OH	371 (–)	371 (–)	371 (2)	371 (10)	301 (2)	301 (–)	301 (2)	301 (4)	329 (1)	329 (2)	329 (1)	329 (–)
–Acetone	345 (18)	345 (24)	345 (20)	345 (8)	275 (24)	275 (24)	275 (4)	275 (20)	303 (23)	303 (13)	303 (10)	303 (1)
–Acetone–H_2O	327 (6)	327 (2)	327 (2)	327 (2)	257 (–)	257 (–)	257 (–)	257 (–)	285 (–)	285 (3)	285 (3)	285 (2)
–Acetone–H_2O–CH_3OH	295 (–)	295 (–)	295 (–)	295 (–)	225 (12)	225 (14)	225 (5)	225 (8)	253 (6)	253 (16)	253 (14)	253 (4)
–2 acetone	287 (4)	287 (3)	287 (3)	287 (–)	217 (–)	217 (2)	217 (–)	217 (–)	245 (–)	245 (–)	245 (–)	245 (–)
Su–CH=NH_2^+[b]	258 (13)	258 (5)	258 (5)	258 (10)	202 (10)	202 (2)	202 (12)	202 (16)	202 (6)	202 (14)	202 (46)	202 (1)
Su–CH^+	242 (16)	242 (15)	242 (15)	242 (6)	186 (30)	186 (56)	186 (32)	186 (28)	186 (20)	186 (50)	186 (46)	186 (10)
Su–CH=NH_2^+–acetone	200 (88)	200 (100)	200 (100)	200 (36)	144 (100)	144 (100)	144 (5)	144 (5)	144 (50)	144 (56)	144 (54)	144 (8)
Other ions	269 (6)	269 (16)	284 (85)	284 (32)	243 (3)	243 (5)	243 (100)	243 (100)	221 (12)	221 (14)	221 (16)	228 (100)
	251 (2)	251 (2)	313 (12)	313 (26)	112 (8)	112 (12)						260 (10)
	184 (12)	184 (16)	242 (10)	242 (5)								318 (12)
	173 (4)	173 (4)	226 (12)	226 (8)								
	166 (42)	166 (22)	216 (12)	216 (5)								
	142 (56)	142 (26)										

[a] For peptides **1**–**12**, CEs are same (25 eV).
[b] Su = Ga, Xa and Ma.

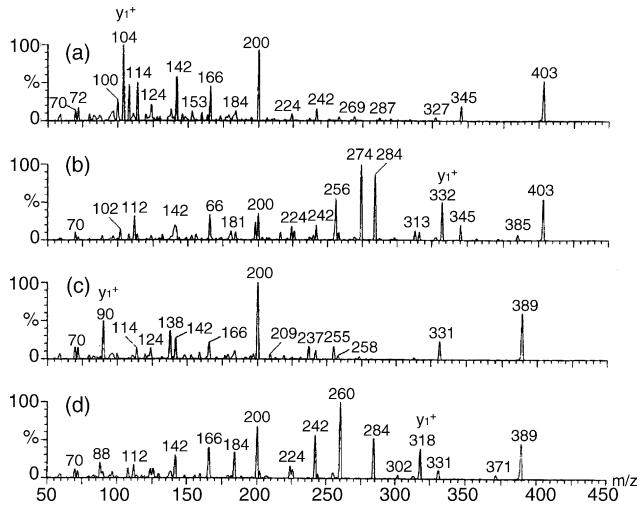


Fig. 3. ESI MS/MS spectra of $[M + \text{H-Boc} + \text{H}]^+$ ions of compounds: (a) **1**, (b) **3**, (c) **20** and (d) **22**, at 25 eV.

(Ga=CH^+–acetone–H_2O), *m/z* 153 (Ga–acetone–H_2O) and *m/z* 142 (Ga–CH=NH_2^+–2 acetone molecules). It is noteworthy that the presence of diacetonide feature is indicated by the loss of two acetone molecules from $[M + \text{H-Boc} + \text{H}]^+$ ions.

The CID mass spectra of $[M + \text{H-Boc} + \text{H}]^+$ ions of **3** and **4** exhibit different fragmentation compared to their positional isomers **1** and **2**, respectively (Fig. 3a and b; Table 2). The spectra show abundant y_1^+ at *m/z* 332, *m/z* 284 (Ga=CH–CH_2–CO^+), *m/z* 274 (y_1^+–acetone) and *m/z* 256 (y_1^+–acetone–H_2O). Fairly abundant peaks are observed at *m/z* 385 (loss of H_2O), *m/z* 345 (loss of acetone), *m/z* 313 (loss of acetone plus methanol), *m/z* 242 (Ga=CH^+), *m/z* 226 (Ga=CH–CH_2–CO^+–acetone), *m/z* 224 (y_1^+–acetone–H_2O–methanol), *m/z* 216 (y_1^+–2 acetone molecules), *m/z* 200 (Ga–CH=NH_2^+–acetone), *m/z* 198 (Ga–C≡NH^+–acetone), *m/z* 184 (Ga=CH^+–acetone) and *m/z* 166 (Ga=CH^+–acetone–H_2O), *m/z* 153 (Ga–acetone–H_2O), *m/z* 142 (Ga–CH=NH_2^+–2 acetone molecules). The CID mass spectra of $[M + \text{H-Boc} + \text{H}]^+$ ions of the dipeptide acids (**20**–**23**) are very much similar to those of dipeptide esters (**1**–**4**) except that *m/z* values of the C-terminal ions are decreased by 14 Da (Fig. 3c and d).

3.3. Differentiation of diastereomeric and positional isomers of BocNH-Xaa-Gly/β³ BocNH-Gly-Xaa-β³ dipeptides and BocNH-Xaa-β-hAla/BocNH-β-hAla-Xaa-β³ dipeptides

The CID mass spectra of $[M + \text{H}]^+$ ions of **6**, **10** and **11** with Xaa (*R*) at the N-terminus, show a moderately abundant '**a**' and an intense '**b**' ion. Whereas for **5** and **9** with Xaa (*S*) at the N-terminus, '**b**' is more abundant but '**a**' is totally absent (Figs. 4 and 5; Table 3).

The protonated dipeptides, **7** and **8** which are positional isomers of **5** and **6** shows '**a**' as the base peak and low abundance '**b**', whereas '**b**' is the base peak in both **5** and **6** and '**a**' is totally absent in the former and moderately abundant in the latter (Fig. 4). Similarly **12**, which is a positional isomer of **9** displays much abundant '**a**' whereas it is totally absent in the latter (Fig. 5). The peptides **10** and **11** which have Xaa and methyl groups at different positions with different configurations (at the point of attachment) compared to **12** can also be distinguished from the latter by the difference in the abundances of '**a**' and '**b**' (Fig. 5). However, **7** and **8** which contain Xaa with "*R*" and "*S*" configurations at C-terminus, do not show any difference in the fragmentation of their $[M + \text{H}]^+$ ions (Fig. 4c and d). It

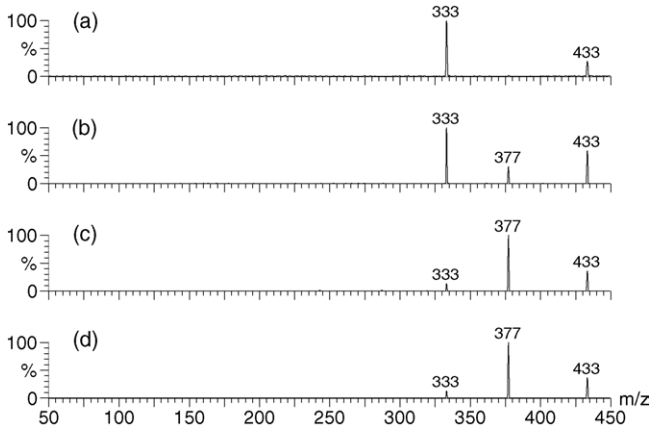


Fig. 4. ESI MS/MS spectra of $[M + \text{H}]^+$ ions of compounds: (a) **5**, (b) **6**, (c) **7** and (d) **8**, at 8 eV.

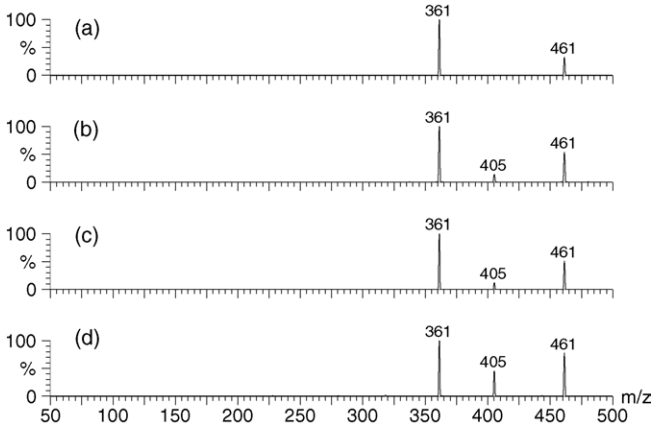


Fig. 5. ESI MS/MS spectra of $[M + \text{H}]^+$ ions of compounds: (a) **9**, (b) **10**, (c) **11** and (d) **12**, at 10 eV.

Table 3
CID of $[M + \text{H}]^+$ of **5**–**12** at different collision energies

Peptides[a]	5 eV		8 eV		10 eV		12 eV	
	'**a**'[b]	'**b**'[b]	'**a**'[b]	'**b**'[b]	'**a**'[b]	'**b**'[b]	'**a**'[b]	'**b**'[b]
5	–	58.8	–	100	–	100	–	100
6	17.6	23.5	41.1	100	13.7	100	3.9	100
7	51	3.9	100	13.7	100	45	88	100
8	60.7	3.9	100	11.7	100	29.4	74.5	100
9	–	19.6	–	100	–	100	–	100
10	3.9	9.8	13.7	56.8	13.7	100	1.9	100
11	3.9	9.8	11.7	54.9	11.7	100	1.9	100
12	9.8	7.8	29.4	41.1	43.1	100	11.7	100

[a] For peptides **5**–**8**, '**a**' (*m/z* 377) '**b**' (*m/z* 333); for peptides **9**–**12**, '**a**' (*m/z* 405) '**b**' (*m/z* 361).
[b] Ions.

should be noted that **7** and **8** which contain Gly at the N-terminus give an abundant peak corresponding to '**a**' (Fig. 4d). Whereas **12** with β-hAla at the N-terminus shows much less abundant '**a**' (Fig. 5d). This result suggests that the CID mass spectra of $[M+H]^+$ ions can also be used to distinguish between the N-terminus Gly and β-hAla. The observed differences in the CID mass spectra of $[M+H]^+$ ions of three pairs of diastereomers (**5**, **6**; **9**, **10**; **9**, **11**) and three pairs of positional isomers (**5** and **7**; **6** and **8**; **9** and **12**) are retained at different RCEs (Table 3). Similar difference is observed for the pairs of diastereomeric peptide acids **24**/**25**, **28**/**29** and **28**/**30** and the positional isomers of peptide acids **24**/**26**, **25**/**27** and **28**/**31**.

3.4. CID of $[M + H\text{-Boc} + H]^+$

The CID mass spectra of $[M+H\text{-Boc}+H]^+$ ions of **5** and **6** display abundant y_1^+ at *m/z* 90, and b_1^+ ions of insignificant abundance (Table 2). The spectra show other important fragment ions at *m/z* 275 (loss of acetone), *m/z* 243 (loss of acetone plus methanol), *m/z* 225 (loss of acetone, methanol and H_2O), *m/z* 202 (Xa–CH=NH_2^+), *m/z* 186 (Xa=CH^+), *m/z* 144 (Xa–CH=NH_2^+–acetone) and *m/z* 112 (Xa–CH=NH_2^+–acetone, methanol). The $[M+H\text{-Boc}+H]^+$ ions of **7** and **8** which are positional isomers of **5** and **6** show different fragmentation in the way that y_1^+ appears at *m/z* 276 (Table 2). Other fragment ions are, *m/z* 315 (loss of H_2O), *m/z* 301 (loss of methanol), *m/z* 275 (loss of acetone), *m/z* 243 (loss of acetone plus methanol), *m/z* 225 (loss of acetone, methanol and H_2O), *m/z* 218 (y_1^+–acetone) and *m/z* 186 (Xa=CH^+).

The CID mass spectra of $[M+H\text{-Boc}+H]^+$ ions of **9**–**11** shows abundant y_1^+ (*m/z* 118) and *m/z* 144 (Xa–CH=NH_2^+–acetone) (Table 2). The spectra show other significant ions at *m/z* 343 (loss of H_2O), *m/z* 303 (loss of acetone), *m/z* 253 (loss of acetone, methanol plus H_2O), *m/z* 221 (loss of acetone, two methanol molecules and H_2O), *m/z* 202 (Xa–CH=NH_2^+) and *m/z* 186 (Xa=CH^+). The CID mass spectrum of $[M+H\text{-Boc}+H]^+$ ions of **12**, which is a positional isomer of **9**–**11** shows abundant y_1^+ at *m/z* 276, *m/z* 260 (loss of CH_3–CH=NH plus acetone) and *m/z* 228 (loss of CH_3–CH=NH plus acetone plus methanol) (Table 2). Other prominent fragment ions are at *m/z* 318 (loss of CH_3–CH=NH) and *m/z* 218 (y_1^+–acetone). The CID mass spectra of $[M+H\text{-Boc}+H]^+$ ions of the isomeric dipeptide acids (**28**–**31**) are very much similar to those of esters (**9**–**12**). These results indicate that the CID of $[M+H\text{-Boc}+H]^+$ ions of monosugar dipeptides (**1**–**12** and **20**–**31**) mainly gives y_1^+ and other characteristic fragment ions of carbohydrate moiety.

3.5. Differentiation of diastereomeric BocNH-Maa-Maa-β^3 and BocNH-Xaa-Maa-β^3 dipeptides

The CID mass spectra of $[M+H]^+$ ions of diastereomeric dipeptides with Maa (**13** and **14**) and Xaa (**16** and **17**) of "*S*" configuration at N-terminus, show intense '**b**' (*m/z* 519) and insignificant '**a**' (*m/z* 563) ions. Whereas dipeptides with Maa (**15**) and Xaa (**18** and **19**) of "*R*" configuration at the N-terminus, yield moderately abundant '**a**' in addition to an abundant '**b**' (Figs. 6 and 7; Table 4). The '**a**' ion is less abundant for these

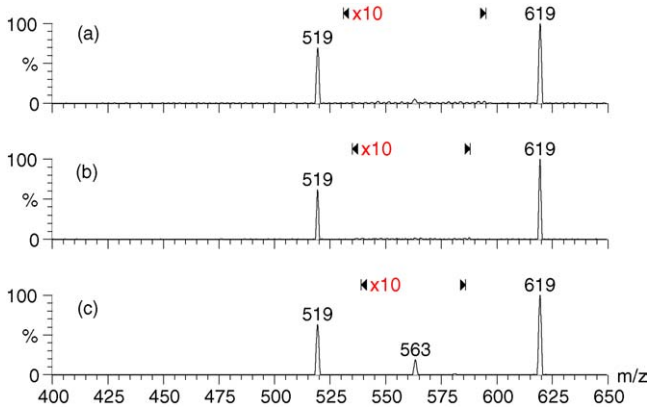


Fig. 6. ESI MS/MS spectra of $[M+H]^+$ ions of compounds: (a) **13**, (b) **14** and (c) **15**, at 10 eV.

disugar dipeptides compared to the monosugar dipeptides with "*R*" configuration at the N-terminus. For example, '**a**' is less prominent for **15**, **18** and **19** as compared to **2** which has Gaa (*R*) at the N-terminus. This may possibly due to the increased steric crowdings following the presence of two sugar groups. The observed differences in the CID mass spectra of the above isomers were reproducible at different RCEs (Table 4). The CID mass spectra of $[M+H]^+$ ions of the isomeric dipeptide acids (**32**–**38**), are very much similar to those of esters (**13**–**18**).

3.6. CID of $[M + H\text{-Boc} + H]^+$

The CID mass spectra of $[M+H\text{-Boc}+H]^+$ ions of **13**–**19** shows abundant fragment ions at *m/z* 286 (loss of Su=CH–NH_2 plus methanol) (Su = Ma or Xa), b_1^+ (*m/z* 244) and y_1^+ (*m/z* 276) ions. The abundance ratio of y_1^+, b_1^+ and *m/z* 286 ions are different from one another in **13**–**19** (Table 5). Compared to the monosugar sugar dipeptides, the disugar dipeptides shows abundant y_1^+ and b_1^+ ions.

Other important fragment ions that are common for **13**–**19** (Table 5) are at *m/z* 501 (loss of H_2O), *m/z* 487 (loss of methanol), *m/z* 469 (loss of methanol plus H_2O), *m/z* 455

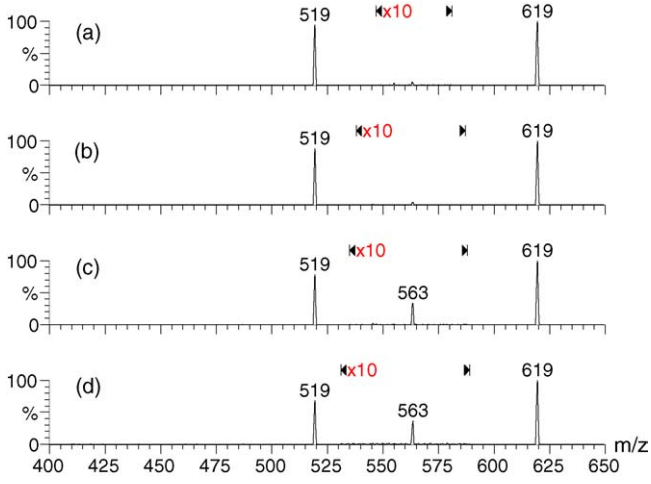


Fig. 7. ESI MS/MS spectra of $[M+H]^+$ ions of compounds: (a) **16**, (b) **17**, (c) **18** and (d) **19**, at 10 eV.

Table 4
CID of $[M+H]^+$ of **13**–**19** at different collision energies

Peptides	5 eV		10 eV		15 eV		20 eV	
	m/z 563[a,b]	*m/z* 519[b]	*m/z* 563[a,b]	*m/z* 519[b]	*m/z* 563[a,b]	*m/z* 519[b]	*m/z* 563[a,b]	*m/z* 519[b]
13	–	12	3	76	4	100	–	100
14	–	7.8	3	70.5	3	100	–	100
15	10	7.8	39.2	78.4	10	100	4	100
16	–	9.8	5.8	100	–	100	–	100
17	–	9.8	3.9	100	–	100	–	100
18	19	10	54.9	76.4	17.6	100	4	100
19	18	10	58.8	68.6	19.6	100	4	100

[a] *m/z* 563 was 16 times magnified.
[b] Ions.

Table 5
CID of $[M+H\text{-Boc}+H]^+$ mass spectral data of compounds **13-19**; *m/z* with relative abundance (%) in parentheses

Ions	Peptides[a]						
	13	**14**	**15**	**16**	**17**	**18**	**19**
y_1^+	276 (60)	276 (34)	276 (100)	276 (60)	276 (66)	276 (60)	276 (89)
b_1^+	244 (40)	244 (46)	244 (64)	244 (40)	244 (38)	244 (50)	244 (42)
–Ma=CH–NH_2, –CH_3OH	286 (100)	286 (100)	286 (80)	286 (100)	286 (100)	286 (100)	286 (66)
–H_2O	501 (–)	501 (–)	501 (–)	501 (2)	501 (4)	501 (2)	501 (2)
–CH_3OH	487 (6)	487 (6)	487 (24)	487 (1)	487 (3)	487 (6)	487 (1)
–2CH_3OH	455 (3)	455 (5)	455 (15)	455 (5)	455 (6)	455 (3)	455 (2)
–Acetone, –CH_3OH	429 (14)	429 (6)	429 (14)	429 (3)	429 (2)	429 (14)	429 (2)
–2CH_3OH			455 (16)				
–2SuH[b]	345 (–)	345 (2)	345 (–)	345 (2)	345 (–)	345 (2)	345 (–)
Su–CH=NH_2^+	202 (10)	202 (12)	202 (30)	202 (20)	202 (12)	202 (20)	202 (16)
Su–CH^+	186 (32)	186 (34)	186 (50)	186 (70)	186 (50)	186 (93)	186 (100)
Su–CH=NH_2^+–CH_3OH	170 (30)	170 (36)	170 (40)	170 (25)	170 (14)	170 (18)	170 (14)
Su–CH=NH_2^+–acetone	144 (26)	144 (24)	144 (24)	144 (90)	144 (46)	144 (46)	144 (40)
Su–CH^+–CH_3OH	154 (34)	154 (50)	154 (44)	154 (34)	154 (24)	154 (32)	154 (26)
–CH_3OH, –H_2O	469 (2)	469 (5)	469 (4)	469 (8)	469 (5)	469 (5)	469 (4)
b_1^+–CH_3OH	212 (24)	212 (50)	212 (12)	212 (12)	212 (3)	212 (5)	212 (12)
b_1^+–H_2O	226 (18)	226 (22)	226 (5)	226 (15)	226 (2)	226 (8)	226 (10)

[a] For peptides **13**–**19**, CEs are same (25 eV).
[b] Su = Ga, Xa and Ma.

(loss of two methanol molecules), *m/z* 429 (loss of methanol plus acetone), *m/z* 226 (b_1^+–H_2O), *m/z* 212 (b_1^+–methanol), *m/z* 202 (Su–CH=NH_2^+), *m/z* 186 (Su=CH^+), *m/z* 170 (Su–CH=NH_2^+–methanol), *m/z* 154 (Su=CH^+–methanol), *m/z* 144 (Su–CH=NH_2^+–acetone). The CID mass spectra of $[M+H\text{-Boc}+H]^+$ ions of the dipeptide acids (**32**–**38**), are very much similar to those of esters except that *m/z* values of the y_1^+ ions are decreased by 14 Da (**13**–**19**).

4. Conclusions

All the mono- and disugar diastereomeric Boc-NH-Caa-β^3 dipeptides (Caa = Gaa, Xaa and Maa) studied in this work mainly show two ions: (i) $[M+H\text{-C}(CH_3)_3+H]^+$ (‘**a**’) and (ii) $[M+H\text{-Boc}+H]^+$ (‘**b**’) corresponding to losses of 2-methyl-prop-1-ene and -Boc moiety from $[M+H]^+$ ions. The diastereomeric isomers of the dipeptides with Caa (*R*) and Caa (*S*) configurations at the N-terminus have clearly been distinguished by the difference in the abundance of ions ‘**a**’ and ‘**b**’. The positional isomers, BocNH-Gaa-β-hGly/BocNH-β-hGly-Gaa-β^3 dipeptides, BocNH-Xaa-Gly/BocNH-Gly-Xaa-β^3 dipeptides and BocNH-Xaa-β-hAla/BocNH-β-hAla-Xaa-β^3 dipeptides could also be differentiated based on the difference in the abundance of ‘**a**’ and ‘**b**’, and *m/z* values of y_1^+ ions. While b_1^+ ions are insignificant or totally absent in monosugar sugar dipeptides, the disugar dipeptides show abundant y_1^+ and b_1^+ ions.

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References

[1] D. Seebach, J.L. Matthews, Chem. Commun. (1997) 2015.
[2] W.F. DeGrado, J.P. Schneider, Y. Hamuro, J. Peptide Res. 54 (1999) 206.
[3] R.P. Cheng, S.H. Gellman, W.F. DeGrado, Chem. Rev. 101 (2001) 3219.

[4] D. Seebach, M. Overhand, F.N.M. Kuhnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, Helv. Chim. Acta 79 (1996) 913.
[5] D.H. Appella, L.A. Christianson, I.L. Karle, D.R. Powell, S.H. Gellman, J. Am. Chem. Soc. 118 (1996) 13071.
[6] T. Yamazaki, Y.F. Zhu, A. Probstl, R.K. Chadha, M. Goodman, J. Org. Chem. 56 (1991) 6644.
[7] Z. Huang, A. Probstl, J.R. Spencer, T. Yamazaki, M. Goodman, Int. J. Peptide Protein Res. 42 (1993) 352.
[8] D. Seebach, S. Abele, K. Gademann, G. Guichard, T. Hintermann, B. Juan, J.L. Matthews, J.V. Schreiber, L. Oberer, U. Hommel, H. Widmer, Helv. Chim. Acta 81 (1998) 932.
[9] G.V.M. Sharma, K.R. Reddy, P.R. Krishna, A.R. Shankar, K. Narsimulu, S.K. Kumar, P.J. Prakash, B. Jagannath, A.C. Kunwar, J. Am. Chem. Soc. 125 (2003) 13670.
[10] G.V.M. Sharma, P. Nagender, P.J. Prakash, P.R. Krishna, K.V.S.R. Krishna, B. Jagannath, A.C. Kunwar, Angew. Chem. Int. Ed. 44 (2005) 5878.
[11] F.W. McLafferty (Ed.), Tandem Mass Spectrometry, Wiley, New York, 1983.
[12] K.L. Busch, G.L. Glish, S.A. McLuckey, Techniques and Applications of Tandem Mass Spectrometry, VCH Publishers, New York, 1988.
[13] M. Barber, R.S. Bordoli, R.D. Sedgwick, A.N. Tyler, Nature 293 (1981) 270.
[14] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, Science 246 (1989) 64.
[15] T. Yoshida, K. Tanaka, Y. Ido, Y. Akita, Y. Yoshida, Shitsutyo Bunseki 36 (1988) 59.
[16] M. Karas, F. Hillenkamp, Anal. Chem. 60 (1988) 2299.
[17] P. Roepstorff, J. Fohlman, Biomed. Mass Spectrom. 11 (1984) 601.
[18] K. Biemann, Biomed. Environ. Mass Spectrom. 16 (1988) 99.
[19] K. Biemann, Primary studies of peptides and proteins, in: T. Matsuo, R.M. Caprioli, M.L. Gross, T. Seyama (Eds.), Biological Mass Spectrometry: Present and Future, Wiley, New York, 1993, p. 275.
[20] P. Roepstorff, W.J. Richter, J. Mass Spectrom. Ion Process. 118/119 (1992) 789.
[21] I.A. Papayannopoulos, Mass Spectrom. Rev. 14 (1995) 49.
[22] M. Kinter, N.E. Sherman, Protein Sequencing and Identification Using Tandem Mass Spectrometry, Wiley/Interscience, New York, 2000.
[23] B. Paizs, S. Suhai, Mass Spectrom. Rev. 24 (2005) 508, and references cited therein.
[24] J.H. Bowie, C.S. Brinkworth, S. Dua, Mass Spectrom. Rev. 21 (2002) 87.
[25] R.J. Waugh, J.H. Bowie, Rapid Commun. Mass Spectrom. 8 (1994) 169.
[26] J.S. Splitter, F. Turecek (Eds.), Applications of Mass Spectrometry to Organic Stereochemistry, VCH Publishers, New York, 1994, p. 83.
[27] J.C. Tabet, H.B. Kagan, J.C. Poulin, D. Meyer, D. Fraisse, Spectrosc. Int. J. 4 (1985) 81.
[28] H. Tsunematsu, R. Isobe, H. Hanazono, M. Inagaki, R. Higuchi, M. Yamamoto, J. Mass. Spectrom. Soc. Jpn. 49 (2001) 224.
[29] H. Tsunematsu, R. Isobe, H. Hanazono, Y. Soeda, M. Inagaki, N. Ito, R. Higuchi, M. Yamamoto, Chem. Pharm. Bull. 47 (1999) 1040.
[30] H. Tsunematsu, H. Ikeda, H. Hanazono, M. Inagaki, R. Isobe, R. Higuchi, Y. Goto, M. Yamamoto, J. Mass. Spectrom. 38 (2003) 188.
[31] W.A. Tao, L. Wu, R.G. Cooks, J. Am. Soc. Mass Spectrom. 12 (2001) 490.
[32] R.G. Cooks, P.S.H. Wong, Acc. Chem. Res. 31 (1998) 379.
[33] R.G. Cooks, J.T. Koskinen, P.D. Thomas, J. Mass Spectrom. 34 (1999) 85.
[34] L. Wu, K. Lemr, T. Aggerholm, R.G. Cooks, J. Am. Soc. Mass Spectrom. 14 (2003) 152.
[35] L. Wu, E.C. Meurer, B. Young, P. Yang, M.N. Eberlin, R.G. Cooks, Int. J. Mass Spectrom. 231 (2004) 103.
[36] K.A. Schug, W. Lindner, K. Lemr, J. Am. Soc. Mass Spectrom. 15 (2004) 840.
[37] M. de Person, A. Sevestre, P. Chaimbault, L. Perrot, F. Duchiron, C. Elfakir, Anal. Chim. Acta 520 (2004) 149.
[38] H. Lavanant, Y. Hoppilliard, Eur. J. Mass Spectrom. 5 (1999) 41.
[39] C.A. Schalley, D. Schroeder, H. Schwarz, Organometallics 14 (1995) 316.
[40] C.M. Adams, F. Kjeldsen, R.A. Zubarev, B.A. Budnik, K.F. Haselmann, J. Am. Soc. Mass Spectrom. 15 (2004) 1087.
[41] T. Yalcin, C. Khouw, I.G. Csizmadia, M.R. Peterson, A.G. Harrison, J. Am. Soc. Mass Spectrom. 6 (1995) 1165.
[42] G.E. Reid, R.J. Simpson, R.A.J. O'Hair, J. Am. Soc. Mass Spectrom. 9 (1998) 945.
[43] B. Balta, V. Aviyente, C. Lifshitz, J. Am. Soc. Mass Spectrom. 14 (2003) 1192.
[44] R.A.J. O'Hair, M.L. Styles, G.E. Reid, J. Am. Soc. Mass Spectrom. 9 (1998) 1275.
[45] F. Pingitore, M.J. Polce, P. Wang, C. Wesdemiotis, B. Paizs, J. Am. Soc. Mass Spectrom. 15 (2004) 1025.
[46] J.M. Farrugia, R.A.J. O'Hair, Int. J. Mass Spectrom. 222 (2003) 229.
[47] F. Pingitore, C. Wesdemiotis, Anal. Chem. 77 (2005) 1796.
[48] R. Srikanth, P.N. Reddy, R. Narsimha, R. Srinivas, G.V.M. Sharma, K.R. Reddy, P.R. Krishna, J. Mass Spectrom. 39 (2004) 1068.
[49] P.N. Reddy, R. Srikanth, N.S. Swamy, R. Srinivas, G.V.M. Sharma, P. Nagendar, P.R. Krishna, J. Mass Spectrom. 40 (2005) 1429.
[50] G.V.M. Sharma, P. Nagendar, K.R. Reddy, P.R. Krishna, K. Narsimulu, A.C. Kunwar, Tetrahedron Lett. 45 (2004) 8807.
[51] C. Wolf, C.N. Villalobos, P.G. Cummings, S. Kennedy-Gabb, M.A. Olsen, G. Trescher, J. Am. Soc. Mass Spectrom. 16 (2005) 553.