Positive and Negative Ion Electrospray Tandem Mass Spectrometry (ESI MS/MS) of Boc-Protected Peptides Containing Repeats of L-Ala-γ^4Caa/γ^4Caa-L-Ala: Differentiation of Some Positional Isomeric Peptides

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High-resolution electrospray ionization (ESI) quadrupole time-of-flight and ion trap tandem mass spectrometry has been used to distinguish the positional isomers of a new class of N-blocked hybrid peptides containing repeats of the amino acids, L-Ala-γ^4Caa $_{(l)}$/γ^4Caa$_{(l)}$-L-Ala [Caa$_{(l)}$ = Carbo (lyxose) amino acid, derived from D-mannose]. Both MS/MS and MS^3 of protonated isomeric peptides produce characteristic fragmentation involving the peptide backbone, Boc-group, and the side-chain. It is interesting to observe that the abundant y_n^+ ions are formed when the corresponding amide −NH does not participate in the helical structures in solution phase and relatively low abundance y_n^+ ions resulted when the amide −NH involves in the H-bonding. Thus, it was possible to identify the amide −NH hydrogens that participate in the helical structures through H-bonding in solution phase. Further, negative ion ESI MS/MS has also been found to be useful for differentiating these isomeric peptide acids. (J Am Soc Mass Spectrom 2007, 18, 651–662) © 2007 American Society for Mass Spectrometry

The design of non-natural oligomers that form diversified secondary structures is an active area of research [1–3]. While there are several reports on β-peptide class of foldamers, the γ-peptides, despite the possibility for a larger conformational space, have received less attention [4–8]. As a part of an ongoing program towards developing new oligomers [9, 10] of carbo-β and γ-amino acids with novel secondary structures [11, 12], one of us [13] has recently reported the synthesis of oligomers with dipeptide repeats of carbo-γ^4-amino acid [Caa$_{(l)}$ = Carbo (lyxose) amino acid derived from D-mannose ("S" configurations at the amine center)]/L-alanine (L-Ala) and demonstrated novel left-handed 10/12- and 12/10-helices. It may be noted that γ-amino butyric acid is a nonessential amino acid, found mainly in the human brain and eyes, and it is an inhibitory neurotransmitter in the mammalian central nervous system [14] that is referred to as the "brain's natural claiming agent". On the other hand, carbohydrates are the most abundant biomolecules in nature and are involved in many biological functions [15]. The combination of these two residues into a monomeric non-natural amino acid and the peptides derived thereof are expected to be of immense biological importance.

The mass spectrometry of natural amino acid peptides is well documented in the literature [16–18], and the tandem mass spectrometry of protonated [19–23] and deprotonated peptides [24–29] in electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) [30, 31] has been an established tool in determining amino acid sequence of peptides [19–23]. There are very few reports available in the literature on the mass spectral study of peptides derived from non-natural amino acids such as β-peptides [32–35]. We have reported a mass spectral study of a series of Boc-protected carbo-β^3-peptides, including differentiation of positional isomers of α,β-, and β,α hybrid peptides, and some of the diastereomeric dipeptides, by using electrospray tandem mass spectrometry (ESIMS/MS) [34, 35]. Following the interesting observations made in the foldamer chemistry of the new series of non-natural amino acid hybrid peptides comprising repeats of L-Ala-γ^4Caa$_{(l)}$ (α,γ-)/γ^4Caa$_{(l)}$-L-Ala (γ,α-) [13], we decided to investigate this series of hybrid

Published online January 16, 2007

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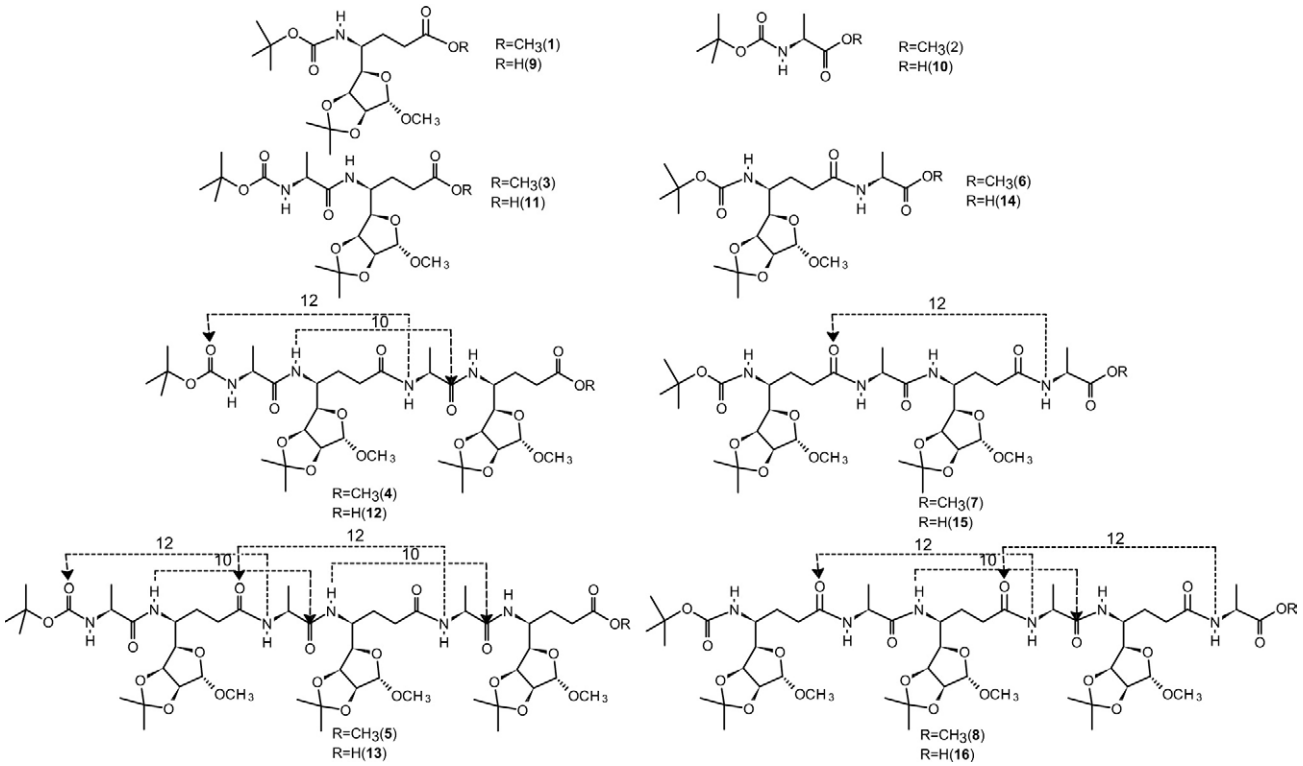
1044-0305/07/$32.00
doi:10.1016/j.jasms.2006.12.005

Received September 27, 2006
Revised November 19, 2006
Accepted December 1, 2006



Scheme 1. BocN-L-alanine-monomers (**2** and **10**), BocN-carbo-γ^4-monomers (**1** and **9**), BocN-α,γ-peptides (**3–5**; **11–13**), and BocN-γ,α-peptides (**6–8**; **14–16**).

peptides by high-resolution ESI tandem mass spectrometry and examine the possibility of correlating the mass spectrometric results with their helical structures.

Experimental

All experiments were performed using a Q-TOF mass spectrometer (QSTAR XL, Applied Biosystems/MDS Sciex, Foster City, CA), equipped with an electrospray ionization (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 (negative mode 4 kV); declustering potential, 50 V; focusing potential, 220 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. For the collision induced dissociation (CID) experiments, the precursor ion was selected using the quadrupole analyzer, and the product ions were analyzed by TOF analyzer. The collision energies were used at 20 to 40 eV unless otherwise stated. All the spectra reported here were recorded under identical experimental conditions for isomers, and are averages of 25 to 30 scans. Solvents used in the present study were purchased from Merck (Mumbai, India), and Sd. Fine Chemicals (Hyderabad, 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 10 to 20 μM each. All the samples were infused in to the ESI source at a flow rate of 15 to 20 μL/min by using an inbuilt syringe pump.

The MS^n experiments were performed using a LCQ ion trap mass spectrometer (Thermo Finnigan, San Jose, CA), equipped with an ESI source. The data acquisition was under the control of Xcalibur software. The typical source conditions were: spray voltage, 5 kV; capillary voltage, 15 to 20 V; heated capillary temperature, 200 °C; tube lens offset voltage, 20 V; sheath gas (N_2) flow rate, 30 units; 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 parent 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 ac signal to resonantly excite them and so cause CID. The collision energies were used at 20 to 40%. The excitation time used was 30 ms. All the spectra reported here were recorded under identical experimental conditions for isomers, and averages of 25 to 30 scans. Stock (1 mM) solutions of peptides were prepared in HPLC-grade methanol, and diluted with methanol to achieve a final concentration of 10 μM each. All the samples were

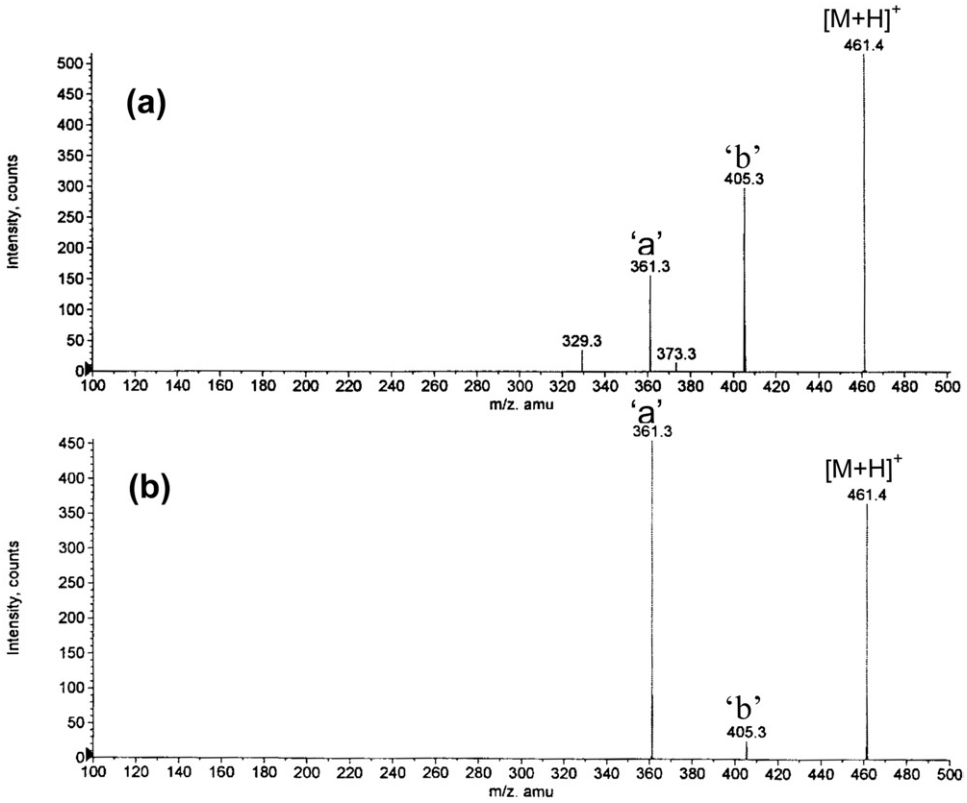


Figure 1. ESI MS/MS spectra of $[M + H]^+$ ions of compounds (**a**) **3**, (**b**) **6**, at a laboratory collision energy 10 eV.

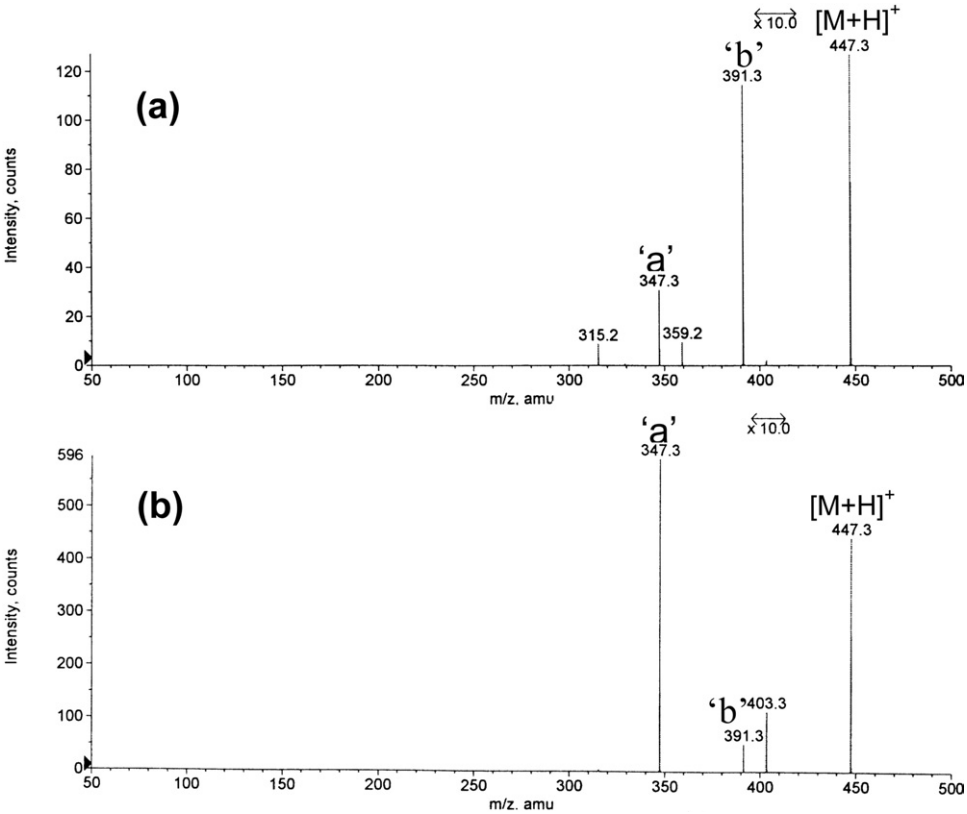


Figure 2. ESI MS/MS spectra of $[M + H]^+$ ions of compounds (**a**) **11**, (**b**) **14**, at a laboratory collision energy 10 eV.

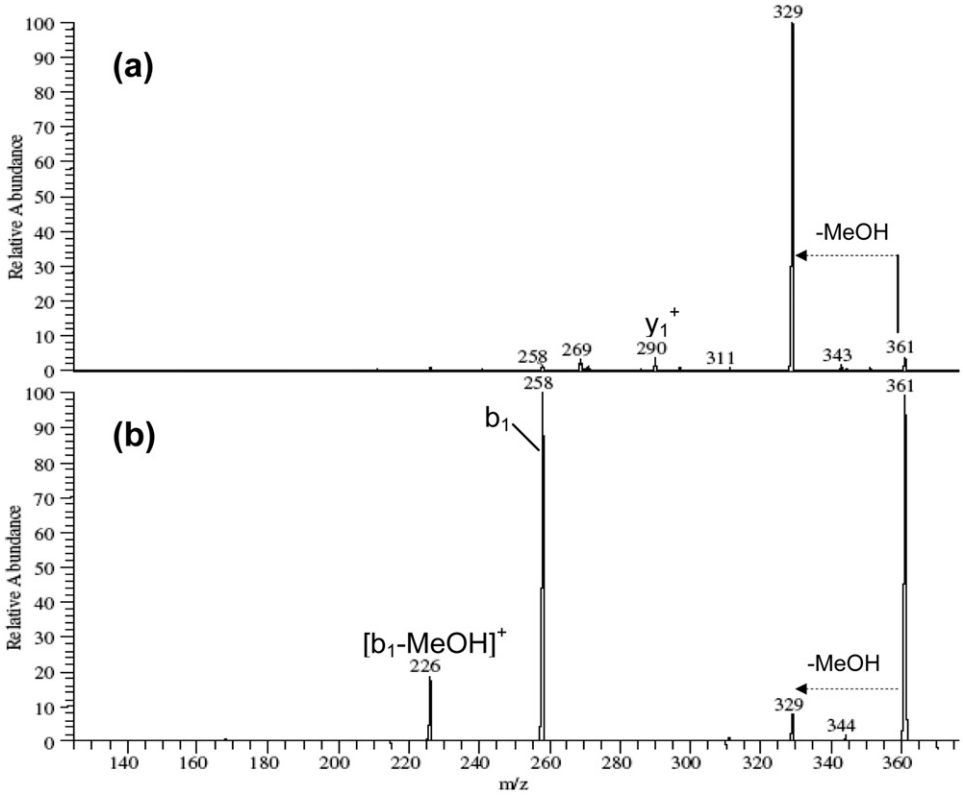


Figure 3. ESI MS^3 spectra of $[M + H - Boc + H]^+$ ions of compounds (**a**) **3**, (**b**) **6**.

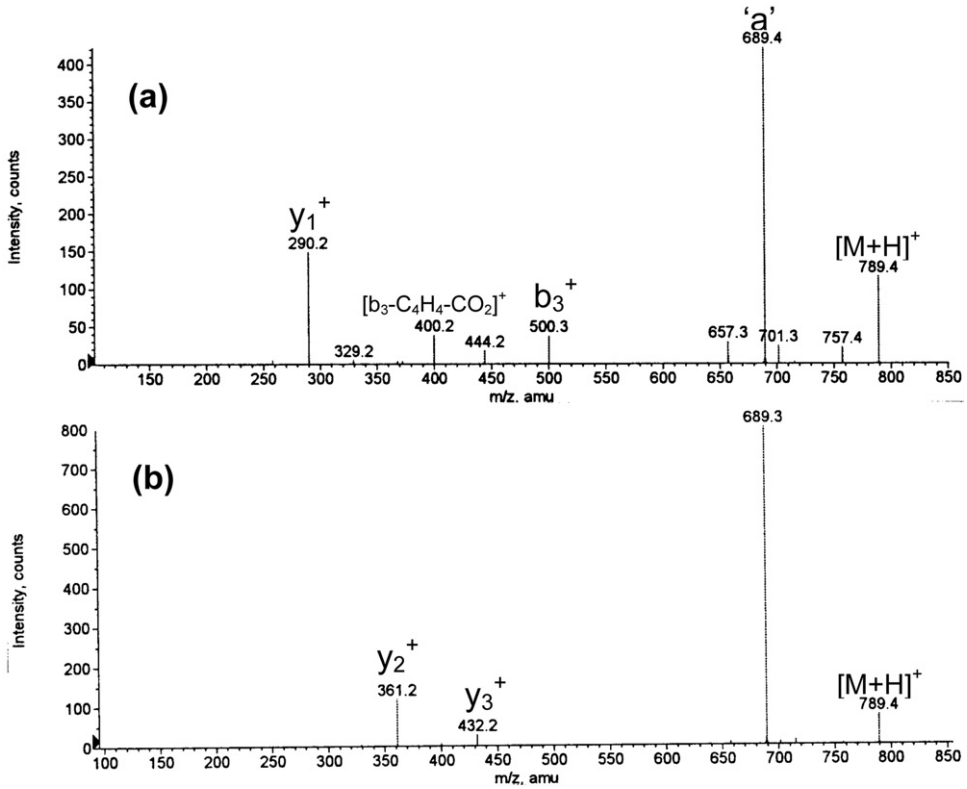


Figure 4. ESI MS/MS spectra of $[M + H]^+$ ions of compounds (**a**) **4**, (**b**) **7** at a laboratory collision energy 15 eV.

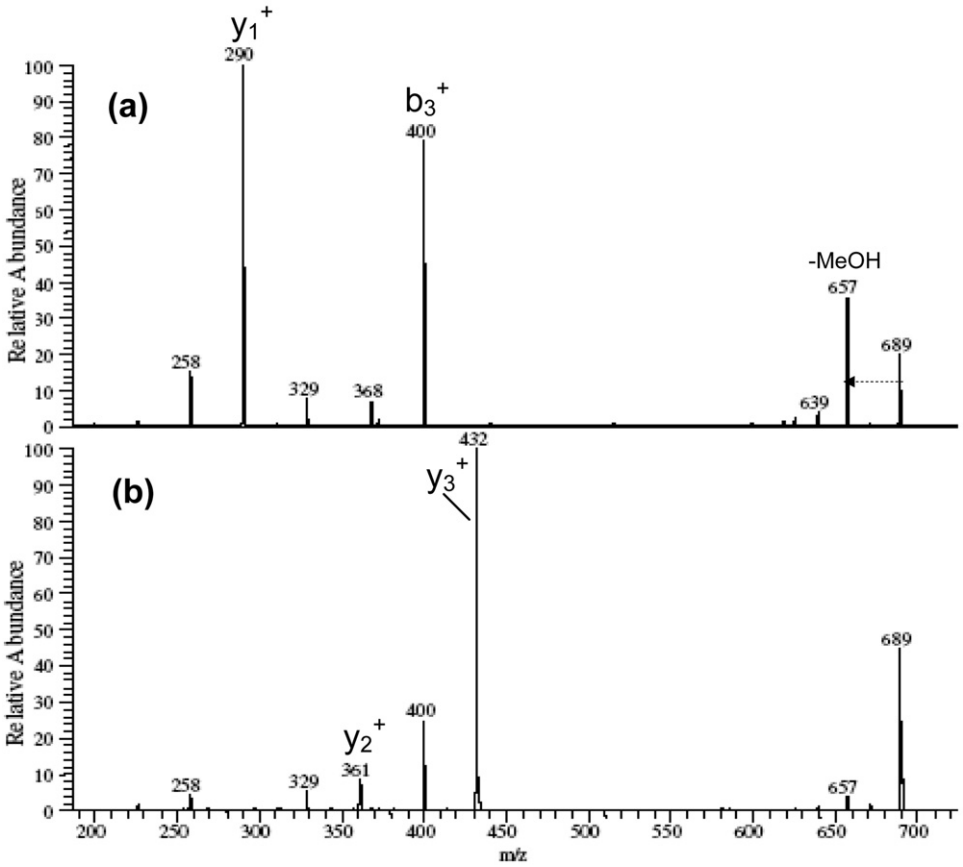


Figure 5. ESI MS^3 spectra of $[M + H - Boc + H]^+$ ions of compounds (**a**) **4**, (**b**) **7**.

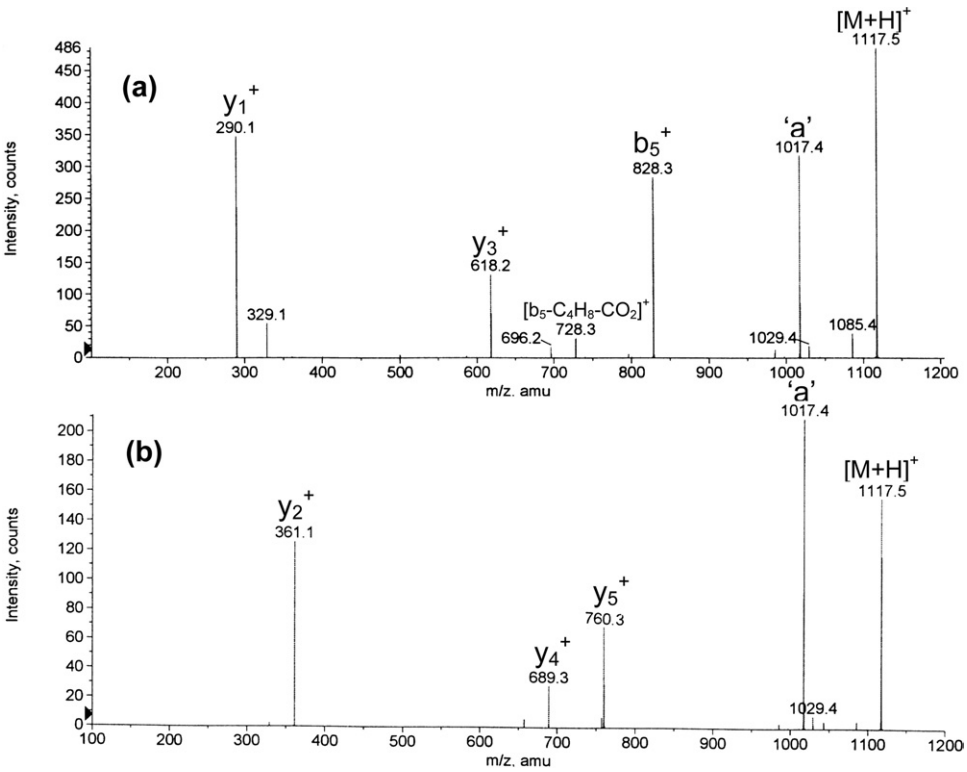


Figure 6. ESI MS/MS spectra of $[M + H]^+$ ions of compounds (**a**) **5**, (**b**) **8**, at a laboratory collision energy 25 eV.

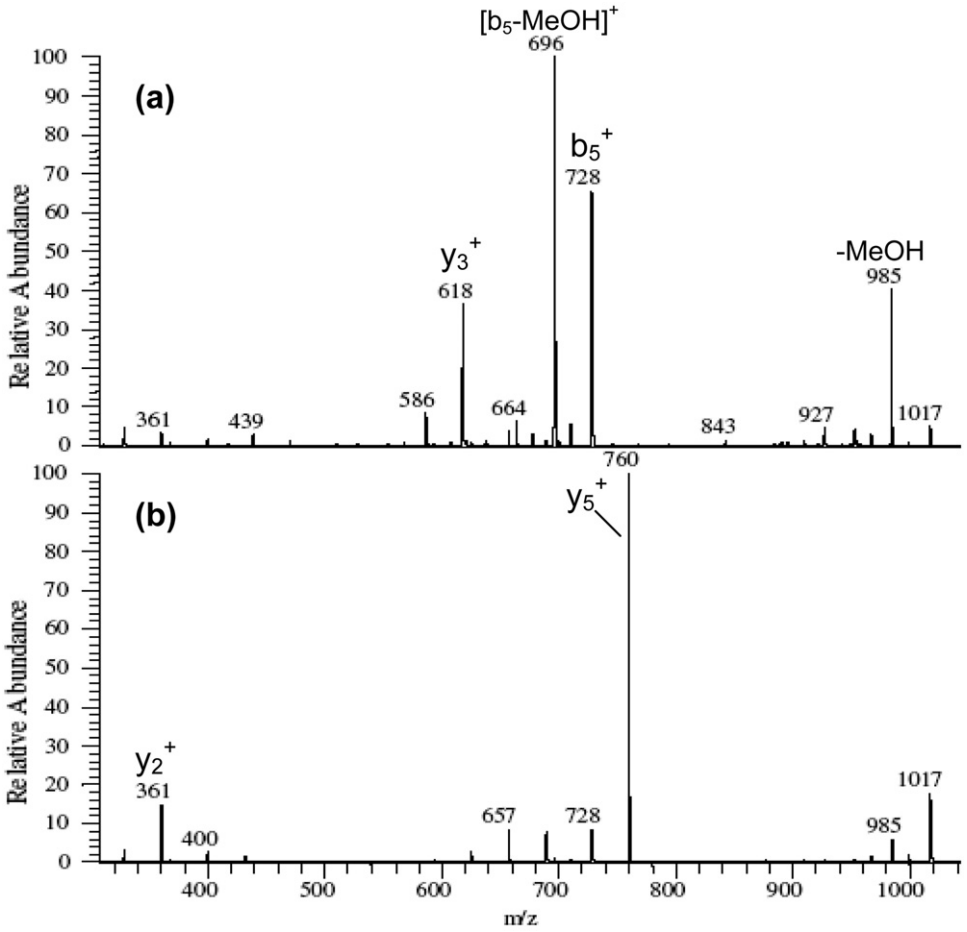


Figure 7. ESI MS^3 spectra of $[M + H - Boc + H]^+$ ions of compounds (**a**) **5**, (**b**) **8**.

infused in to the ESI source at a flow rate of 5 μl/min by using in built syringe pump.

Materials

All°the°peptides°[13]°were°synthesized°by°conventional solution phase procedure by using a fragment condensation strategy. Boc- and methyl ester groups were used for N- and C-terminal protection. For example, for the synthesis of a L-Ala-γ^4Caa$_{(l)}$ (Caa = carbo-γ^4-amino acid derived from D-mannose)-dipeptide, Boc N-protected α-amino acid (**10**) (1.0 mmol) dissolved in dry CH_2Cl_2 (6 mL) was treated with HOBt (1-hydroxybenzotriazole hydrate) (1.2 mmol) and EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) (1.2 mmol) at 0 °C under nitrogen atmosphere. After 15 min, the free amino γ^4Caa$_{(l)}$ ester (**1**) (1.0 mmol) was added to the above reaction mixture. It was allowed to reach room temperature and stirred for 6 h under nitrogen atmosphere. The reaction mixture was diluted with aq NH_4Cl, washed with 1N HCl water saturated $NaHCO_3$ and NaCl solutions. The organic layer was dried (Na_2SO_4) and evaporated to give the residue, which was purified by column chromatography (Silica gel) and the yields were quantitative.

Results and Discussion

The isomeric pairs of the Boc-protected di-, tetra-, and hexapeptides comprising repeats of L-Ala-γ^4Caa$_{(l)}$ and γ^4Caa$_{(l)}$-L-Ala° are° shown° in° the° Scheme° **1** [13].° The positive ion ESI mass spectra of all these peptides (**1**–**16**) show abundant $[M + H]^+$, $[M + Na]^+$, and $[M + H - Boc + H]^+$ ions. The formation of $[M + H - Boc + H]^+$ ions can be explained by the migration of "H" from the *tert*-butyl group to the carbonyl oxygen in the BocN-moiety°[34]°followed°by°the°loss°of°2-methylprop-1-ene (−56 Da) and subsequent loss of CO_2 from the $[M + H]^+$. The $[M + H - Boc + H]^+$ can also be formed by a stepwise mechanism involving the loss of CO_2 from $[M + H - C_4H_8]^+$ as evidenced by the MS/MS spectra of the latter ions.

CID of Isomeric Peptides

Dipeptides. The CID mass spectra of $[M + H]^+$ ions of dipeptide esters, BocN-L-Ala-γ^4Caa$_{(l)}$ (**3**) and γ^4Caa$_{(l)}$-L-Ala (**6**) mainly show the fragment ions, $[M + H - Boc + H]^+$ (**a**) (*m/z* 361) and $[M + H - C_4H_8]^+$ (**b**) (*m/z* 405). These isomeric peptides display distinct differences in their CID spectra. The ion **'a'** is highly abundant for **6**

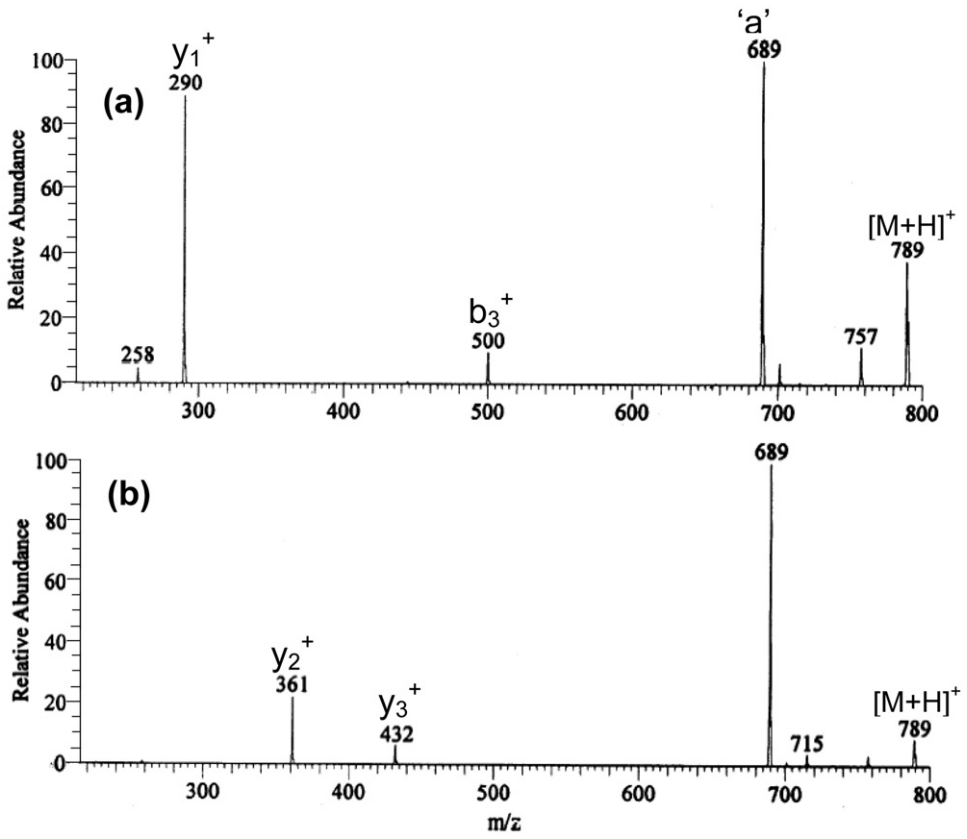


Figure 8. ESI MS/MS spectra of $[M + H]^+$ ions of compounds (**a**) **4**, (**b**) **7**.

compared with **3** and **'b'** is much less abundant for **6** than for **3**. Besides, the CID spectrum of protonated **3** shows additional fragment ions at *m/z* 373 $[M + H - C(CH_3)_3OH]^+$ and *m/z* 329 $[M + H - Boc + H - MeOH]^+$, which are absent for **6** (Figure 1). The lower abundance of **'b'** in **6** can be attributed to steric crowding caused by the sugar group at the N-terminus, which probably suppresses the "H" migration from the tert-butyl group to the carbonyl oxygen leading to the decreased abundance of $[M + H - C_4H_8]^+$. The greater abundance of **'a'** in **6** may perhaps be due to the increased product ion stability of $[M + H - Boc + H]^+$ following "H" bonding involving terminal $-NH_2$ and the Caa- at the N-terminus. It is also likely that after the loss of C_4H_8, the sugar group may act as a catalyst to transfer the proton from the carboxyl group to the −NH-, facilitating the loss of CO_2. This may not be possible in case of **3** due to the presence of L-Ala at the N-terminus. The absence of loss of *tert*-butanol (*m/z* 373) in **6** can be explained, due to likely suppression of "H" migration from −NH- to the "O" of the butyloxy group, presumably due to sugar moiety. Thus, these positional isomers can clearly be distinguished from one another by their characteristic fragmentation pattern. The CID mass spectra of $[M + H]^+$ ions of the dipeptide acids **11** and **14** also show distinct differences in the abundances of ions **'a'** and **'b'**. Similarly to the esters, the CID of protonated **11** shows additional fragment ions at *m/z* 359 $[M + H - C(CH_3)_3OH]^+$ and *m/z* 315 $[M + H - Boc + H - MeOH]^+$, which are absent for **14** (Figure 2). The CID of $[M + H]^+$ ions of **14** shows another ion at *m/z* 403 corresponding to $[M + H - CO_2]^+$ ion which is absent for **11**. It should be noted that these α,γ- and γ,α-peptide isomers behave differently from the α,β- and β,α-peptides (reference [34], [Figure 2b]). For example α,β- and β,α-peptides do not show $[M + H - C(CH_3)_3OH]^+$ and $[M + - CO_2]^+$, respectively. Another difference between α,β- and α,γ peptides (**3**–**5** and **11**–**13**) is the loss of methanol from $[M + H - Boc + H]^+$, which is significant in the latter due to the presence of lyxose side-chain with an anomeric −OMe group instead of xylose in the former [34].

Further, we wanted to examine the potential of differentiating these isomers by MS^3 product ion spectra using ion trap mass spectrometry. Figure 3 shows MS^3 of $[M + H - Boc + H]^+$ from **3** and **6**. It can be clearly seen that these CID spectra are different from one another. In keeping with the MS/MS spectra, the $[M + H - Boc + H]^+$ is much more abundant for **6** than **3**. In addition, the ion at *m/z* 329 (−MeOH) is highly abundant in **3** than **6**. This may perhaps be due to the proximity of −OMe on sugar group to the protonated −COOR at the C-terminus of **3**. Other low abundant ions in **3** are at *m/z* 343 ($-H_2O$), *m/z* 290 (y_1^+), and *m/z* 258 (y_1^+ − MeOH). In contrast, **6** shows abundant *m/z* 258 (b_1^+), *m/z* 226 (b_1 − MeOH)$^+$ and low abundant *m/z* 344

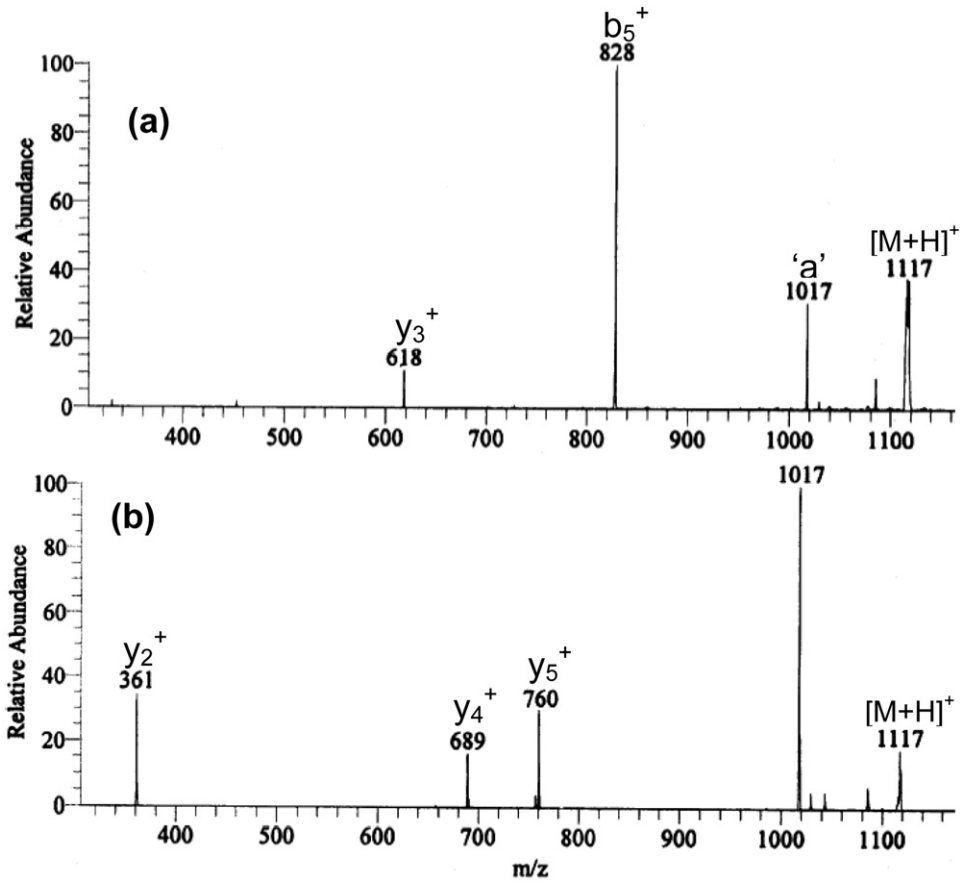


Figure 9. ESI MS/MS spectra of $[M + H]^+$ ions of compounds (**a**) **5**, (**b**) **8**.

($-NH_3$), and *m/z* 329 ($-$MeOH) (Figure 3). Similarly to the esters, the MS^3 spectra of isomeric peptide acids (**11** and **14**) exhibit distinct differences. Thus MS^3 spectra provide additional information for distinguishing these di-peptide isomers.

Tetrapeptides. The CID mass spectrum of $[M + H]^+$ of the tetrapeptide ester **4** shows abundant **'a'** and low abundance *m/z* 757 ($[M + H - MeOH]^+$), *m/z* 701 ($[M + H - MeOH\text{-}C_4H_8]^+$) and *m/z* 657 ($[M + H - Boc\text{-}MeOH]^+$). It should be noted that all these ions are insignificant for **7** except **'a'** (Figure 4). Other significant differences are, the CID of **4** gives *m/z* 500 (b_3^+), *m/z* 400 (b_3^+-Boc), and *m/z* 290 (y_1^+), which are absent for **7**, whereas the CID of isomer **7** yields *m/z* 432 (y_3^+) and *m/z* 361 (y_2^+). Similar differences are observed between the CID mass spectra of $[M + H]^+$ of isomeric tetrapeptide acids **12** and **15** (spectra not shown).

To probe further differentiation between these isomeric peptides, the MS^3 spectra were examined. The MS^3 spectra are given in Figure 5. It can be seen that the MS^3 of **4** is significantly different from that of **7**. The MS^3 of $[M + H - Boc + H]^+$ (*m/z* 689) of isomer **4** shows abundant ions at *m/z* 657 ($-$MeOH), *m/z* 400 (b_3^+), *m/z* 290 (y_1^+), and low abundant *m/z* 639 ($-H_2O$ plus $-$MeOH), *m/z* 368 ($b_3^+ -$ MeOH), *m/z* 329 (b_2^+) and *m/z* 258 ($y_1^+ -$ MeOH). Whereas the $[M + H - Boc + H]^+$ from **7** exhibits abundant *m/z* 432 (y_3^+) moderately abundant *m/z* 400 ($y_3^+ -$ MeOH), *m/z* 361 (y_2^+) and *m/z* 329 (b_2^+) and low abundant *m/z* 657 ($-$MeOH). It can be noted that *m/z* 657 ($-$MeOH) is much more abundant in **4** than **7**. Similarly to the esters, the MS^3 spectra of isomeric peptide acids (**12** and **15**) exhibit distinct differences (spectra not shown).

Hexapeptide isomers. The CID mass spectra of $[M + H]^+$ ions of isomeric hexapeptide esters **5** and **8** with repeats of L-Ala-γ^4Caa and γ^4Caa-L-Ala, respectively, shows abundant, **'a'**, $[M + H - Boc + H]^+$ (Figure 6). The CID spectrum of **5** is very much different from that of **8**. The CID spectrum of **5** shows low abundance $[M + H - MeOH]^+$ (*m/z* 1085), $[M + H - MeOH\text{-}C_4H_8]^+$ (*m/z* 1029), and $[M + H - Boc + H\text{-}MeOH]^+$ (*m/z* 985), which are low abundant in **8**. Other significant differences between the CID of **5** and **8** are, the former shows abundant fragment ions at *m/z* 828 (b_5^+), *m/z* 728 (b_5^+-Boc), *m/z* 618 (y_3^+), *m/z* 329 (b_2^+-Boc), and *m/z* 290 (y_1^+), and these ions are absent for **8**. Instead, the CID of **8** shows fragment ions *m/z* 760 (y_5^+), 689 (y_4^+), and *m/z* 361 (y_2^+) (Figure 6). Similar differences are observed between the CID mass spectra of $[M + H]^+$ of hexapeptide acids **13** and **16** and all the *m/z* values are shifted by 14 units less except that the N- terminal b_n^+ (n = 1–5) ions (spectra not shown). It should be noted that the pres-

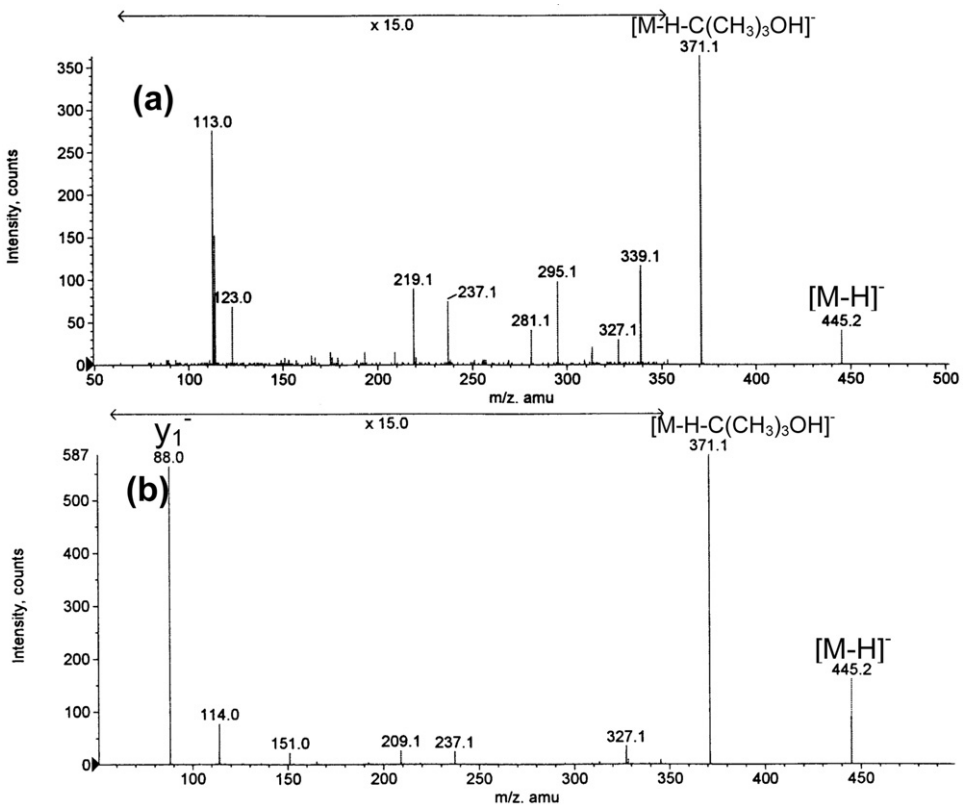


Figure 10. ESI MS/MS spectra of $[M - H]^-$ ions of compounds (**a**) **11**, (**b**) **14**, at a laboratory collision energy 20 eV.

ence of γ^4Caa and L-Ala at the N-terminus can also be easily identified by the characteristic product ions for **5** and **8** as discussed above.

Further, the MS^3 of $[M + H - Boc + H]^+$ of **5** is significantly different from that of **8** (Figure 7). The former shows abundant peaks at *m/z* 985 (−MeOH), *m/z* 728 (b_5^+), *m/z* 696 (b_5^+-MeOH), *m/z* 618 (y_3^+), *m/z* 586 (y_3^+-MeOH), and low abundant *m/z* 967 ($-H_2O$ plus -MeOH), *m/z* 953 (loss of 2 molecules of MeOH), *m/z* 946 (y_5^+), *m/z* 927 (loss of MeOH plus acetone), *m/z* 689 (y_4^+), *m/z* 657 (b_4^+), *m/z* 400 (b_3^+), *m/z* 361 (y_2^+), and *m/z* 329 (b_2^+) ions (Figure 7). It should be noted that due to low mass cut-off of the ion trap, the ion at *m/z* 290 is not seen in the spectrum. This fragmentation pattern is either insignificant or totally absent for isomer **8.** The CID of **8** shows different set of fragment ions at *m/z* 760 (y_5^+), *m/z* 728 $[y_5$-MeOH$]^+$, *m/z* 657 (b_4^+) *m/z* 689 (y_4^+), *m/z* 432 (y_3^+), *m/z* 361 (y_2^+), *m/z* 329 (b_2^+) ions and low abundant *m/z* 985 (−MeOH) (Figure 7). Thus these isomeric peptides can be distinguished by both MS^2 and MS^3 spectra. Similarly to the esters, the MS^3 of peptide acids **13** and **16** show significant differences (spectra not shown).

Analogy Between Solution Phase and Gas Phase Ion Chemistry of These Peptides

It is observed that the fragmentation pattern of the protonated tetra- and hexapeptides, as discussed above, provides an opportunity to identify the amide −NH hydrogens, which are reported to involve in H-bonding leading to various helical structures in solution phase. It is known that y_n^+ ions are formed by the cleavage of the peptide bond. It can be noticed that significant y_n^+ ions are formed when the corresponding amide −NH-does not participate in the helical structures through H-bonding, whereas the participation of amide −NH in the H-bonding results in the insignificant or relatively low abundant y_n^+ ions in the CID process. For example, the tetrapeptide **4** forms 12/10-helices [13] by H-bonding between the 3-NH and Boc-CO, and 2NH and 3CO, respectively (Scheme **1**). Accordingly, the cleavage of the peptide bond (3NH–CO) results in the formation of insignificant y_n^+ (n = 2 and 3) ions, whereas the y_1^+ (*m/z* 290) ion is more abundant in the CID mass spectra of $[M + H]^+$ of **4** (Figure 4). Other isomeric tetrapeptide **7** forms 12- helix by the H-bonding between the 4-NH and 1-CO. The cleavage of the peptide bond adjacent to 4-NH- should lead to y_1^+ ion, which is totally absent in the CID spectrum of protonated **7**, whereas abundant y_2^+ (*m/z* 361) and y_3^+ (*m/z* 432), corresponding to the cleavage of nonparticipating −NH–CO bonds, are observed (Figure 4). Similarly, the protonated tetrapeptide acid **12** yields y_1^+ ion at *m/z* 276 and **15** yields y_2^+ ion at *m/z* 347 and y_3^+ ion at *m/z* 418 ions. Other y_n^+ ions are insignificant or totally absent, indicating their participation in helical structures.

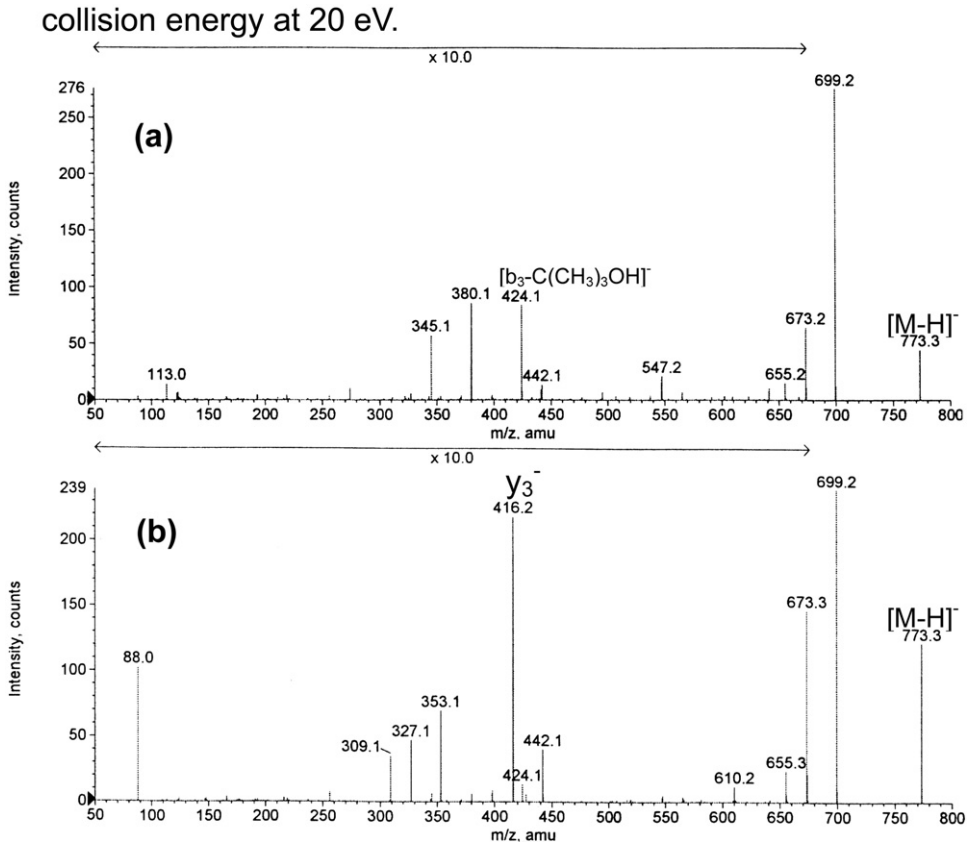


Figure 11. ESI MS/MS spectra of $[M - H]^-$ ions of compounds (**a**) **12**, (**b**) **15**, at a laboratory collision energy 40 eV.

Similarly, hexapeptide **5** displays abundant y_1^+ (*m/z* 290), and other y_n^+ ions are either insignificant or relatively low abundant. Note that all these amide −NH participate in H-bonding (Scheme **1** and Figure 6). The isomeric peptide **8** shows abundant y_2^+ and moderately abundant y_5^+ ions. Other y_n^+ ions are insignificant or relatively less abundant (Figure 5). The CID mass spectra of $[M + H]^+$ of the hexapeptide acids are very much similar to those of esters.

We obtained similar results using an ion trap mass spectrometer and different solvent conditions such as chloroform and acetonitrile. Figures 8 and 9 show the MS/MS of $[M + H]^+$ of tetra- and hexapeptides. It should be noted that the peak at *m/z* 290 (y_1^+) is not seen in the Figure 9a due to the low mass cut-off of the ion trap. Thus these results show the existence of some analogy between the solution phase and gas-phase ion chemistry of these peptides.

Negative Ion CID of Isomeric Peptide Acids

The peptide acids are known to form negative ions readily, hence, we examined the potential of differentiating α,γ- from γ,α-peptide acids under negative ion ESI conditions. The negative ion ESI of the peptide acids (**11**–**13** and **14**–**16**) give $[M - H]^-$ ion as the base peak. Figure 10 shows the MS/MS of $[M - H]^-$ ions of the isomeric dipeptide acids **11** and **14**. It can be seen that the spectra are different from one another. Both the dipeptide acids give $[M - H - C(CH_3)_3OH]^-$ (*m/z* 371) as the base peak. The CID of the former (**11**) display significant ions formed from *m/z* 371 by side-chain fragmentation involving loss of MeOH (*m/z* 339), CO_2 (*m/z* 327), acetone (*m/z* 313), $CH_3OH + CO_2$, (*m/z* 295) CH_3OH + acetone (*m/z* 281), $CH_3OH + CO_2$ + acetone (*m/z* 237), and $CH_3OH + CO_2$ + acetone + H_2O (*m/z* 219) and *m/z* 113 $[O{=}C{=}N\text{-}CH(CH_3)\text{-}CO\text{-}NH]^-$. These fragment ions are insignificant or totally absent for **14**. In contrast CID of **14** shows abundant *m/z* 88 (y_1^-) and low abundant *m/z* 327 $[M - H - C(CH_3)_3OH\text{-}CO_2]^-$, *m/z* 237 $[M - H - C(CH_3)_3OH\text{-}CH_3OH\text{-}CO_2\text{-acetone}]^-$, and *m/z* 209 $[M - H - C(CH_3)_3OH\text{-}CH_3OH\text{-}CO_2\text{-acetone-}CO]^-$. Compared with the β,α-peptides (reference [34], Figure 4b), which show abundant $[M - H - C(CH_3)_3OH\text{-}HNCO]^-$, γ,α-peptides (**14**–**16**) do not show this fragment ion. The absence of loss of *tert*-butanol followed by the −HNCO in γ,α-peptides (**14**–**16**) may perhaps be due to the nonparticipation of $-CH_2-$ readily in this process.

The CID mass spectra of $[M - H]^-$ ions of isomeric tetrapeptide acids **12** and **15** are given in Figure 11. Both the tetrapeptide acids give abundant $[M - H - C(CH_3)_3OH]^-$ and other fragment ions of low abun-

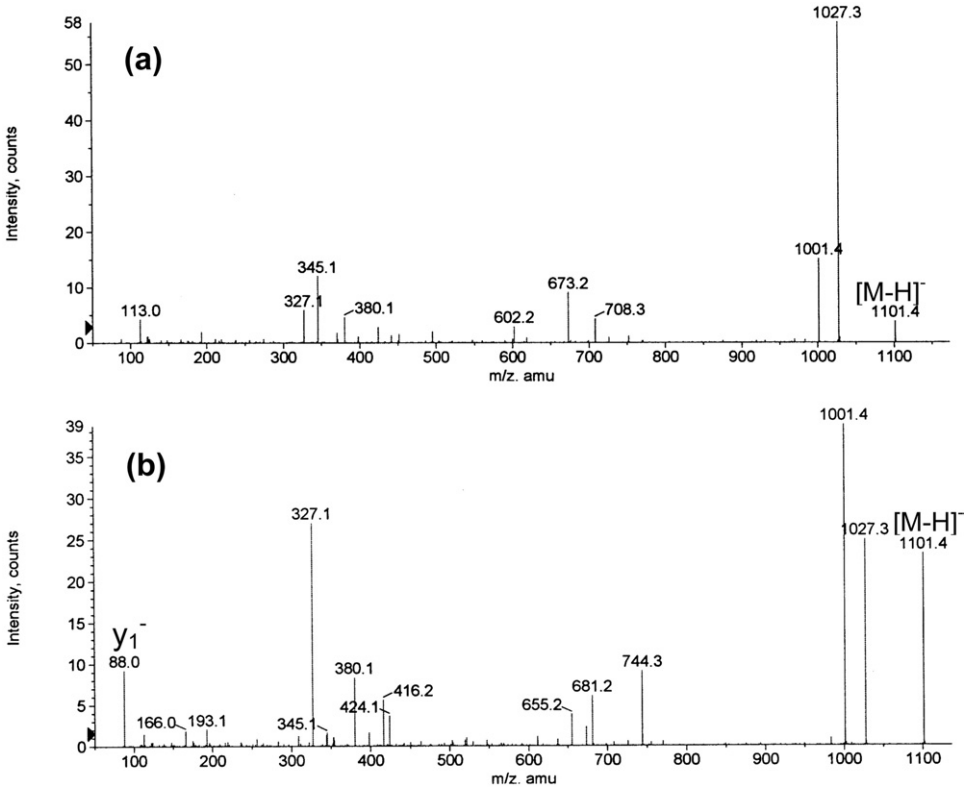


Figure 12. ESI MS/MS spectra of $[M - H]^-$ ions of compounds (**a**) **13**, (**b**) **16**, at a laboratory collision energy 70 eV.

dance. The CID of $[M - H]^-$ ions **12** shows characteristic ions at *m/z* 673 (−Boc), *m/z* 655 (−Boc + H_2O), *m/z* 641 (−Boc + MeOH), *m/z* 424 [b_3-$C(CH_3)_3OH]^-$, *m/z* 380 [b_3-Boc-$H_2O]^-$, *m/z* 345 (y_2^-), whereas these ions are insignificant or totally absent for **15**. The CID of **15** shows a different set of fragment ions at *m/z* 416 (y_3^-), *m/z* 353 [b_2-$C(CH_3)_3OH]^-$, *m/z* 327 [b_2- Boc]$^-$, *m/z* 309 [b_2-Boc-$H_2O]^-$, *m/z* 88 (y_1^-)°(Figure°11).

The CID mass spectra of $[M - H]^-$ ions of hexapeptide°acids°**13** and°**16** are°given°in°Figure°12.°Both°the hexapeptide acids give abundant $[M - H - C(CH_3)_3OH]^-$ and $[M - H - Boc]^-$ ions with the former being more abundant in **13** and the latter in **16**. The CID mass spectrum of $[M - H]^-$ of **13** shows other fragment ions at *m/z* 708 [b_5-Boc − $H_2O]^-$, *m/z* 673 (y_4^-), *m/z* 602 (y_3^-), *m/z* 380 (b_3-Boc-H_2O), *m/z* 345 (y_2^-), and *m/z* 327 (b_2^-Boc), which are insignificant or totally absent in **16**. The CID mass spectrum of $[M - H]^-$ ions of **16** shows other fragment ions at *m/z* 744 (y_5^-), *m/z* 681 [b_4-$C(CH_3)_3OH]^-$, *m/z* 655 (b_4^--Boc), *m/z* 416 (y_3^-), *m/z* 327 (b_2^--Boc), and *m/z* 88 (y_1^-)°(Figure°12).°It°can°be°concluded that ESI tandem mass spectrometry of both the protonated and deprotonated peptides is highly useful for differentiating these isomeric di-, tetra- and hexapeptides.

Conclusions

Six pairs of isomeric peptides containing repeats of L-Ala-γ^4Caa/γ^4Caa-L-Ala have been differentiated by both positive and negative ion ESI tandem mass spectrometry. The major difference between these isomeric peptides is in the abundances of the product ions formed by the dissociation of the Boc-group and the side-chain fragmentation. Also these isomeric peptides give rise to characteristic $y_n^{\pm}$ and $b_n^{\pm}$sequencing ions. The fragmentation pattern is also useful in identifying the amide −NH hydrogens, which participate in the H-bonding leading to helical structures in solution phase. Thus, these results point to some analogy between the solution-phase and gas-phase ion chemistry of these peptides.

Acknowledgments

The authors thank Dr. J. S. Yadav, Director, IICT, Hyderabad, for their facilities and Dr. M. Vairamani for cooperation. PNR, MRK, and VBJ thank CSIR, New Delhi, for the award of Senior Research Fellowships.

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