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Gas-phase fragmentation of di- and tripeptides via ion–molecule reactions with $ClPCl^+$

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Abstract

The reactivity of the phosphenium ion $ClPCl^+$ toward gaseous neutral di- and tripeptides was examined by Fourier transform ion cyclotron resonance mass spectrometry. $ClPCl^+$ was found to react with the peptides at collision rate. Extensive fragmentation of the peptides leads to only one (dipeptides) or two (tripeptides) dominant product ions. For each dipeptide, the major product ion has a molecular weight that is 34 u less than that of the dipeptide, i.e. the side chains are retained in the fragment ion. A substantial amount of effort was given to resolve the structure of this product ion, with a focus on the ion (*m/z* 98) generated from reaction of Gly–Gly (MW 132) with $ClPCl^+$. The molecular formula of the ion of *m/z* 98 was determined to be C_4H_6NO by exact mass measurements. The relative energies of a variety of isomeric ions with this elemental composition were examined by molecular orbital calculations in order to identify the most likely structures. Collision-activated dissociation experiments, mechanistic considerations, and comparison of calculated adiabatic recombination energies of isomeric ions to that determined experimentally for the unknown ion, led to the assignment of a six-membered, highly unsaturated ring structure to the product ion of *m/z* 98 of Gly–Gly. This assignment is supported by the finding that the experimentally determined acidity of the unknown ion is in excellent agreement with that calculated for the proposed six-membered structure. The most likely pathway for the formation of this product ion, as well as those obtained for the other dipeptides, involves an electrophilic attack of $ClPCl^+$ at the amide carbonyl oxygen of the dipeptide, followed by a hydride shift, proton transfer, and loss of H_2O and $Cl_2PO^{\cdot}$. The very high exothermicity of the initial addition (69 kcal mol^{-1}, AM1) allows the adduct to form high-energy intermediates (highest estimated to lie at 34 kcal mol^{-1} above the adduct) during the reaction that is exothermic by about 36 kcal mol^{-1} overall. An analogous mechanism readily rationalizes the formation of the two major product ions observed for tripeptides. These reactions involve the cleavage of each peptide bond, yielding product ions that contain the intact side chains of two adjacent amino acids. Similar observations were made for the $BrPBr^+$ reagent ion. The results presented here suggest that $ClPCl^+$ (and $BrPBr^+$) may provide useful sequence information for peptides. (Int J Mass Spectrom 195/196 (2000) 609–623) © 2000 Elsevier Science B.V.

Keywords: Phosphenium ion; Peptides; Ion-molecule reaction; FTICR

1. Introduction

Structural characterization of peptides and proteins by mass spectrometry is a challenging analytical problem. In the latter half of the 1980s, the development of matrix-assisted laser desorption ionization (MALDI) [1] and electrospray ionization (ESI) [2] led the way to evaporation and ionization of large involatile biomolecules in the mass spectrometer. When coupled with collision-activated dissociation (CAD), these methods make it possible to use mass spectrometry to sequence biopolymers [3,4]. For example,

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Dedicated to Bob Squires for his many seminal contributions to mass spectrometry and ion chemistry.

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peptide sequencing is carried out by identification of two series of "sequence ions" (N- and C-terminal fragment ions) formed from protonated peptides upon CAD [5]. In some instances, however, the interpretation of the spectra is hindered by the formation of various nonsequence fragment ions and by the absence of some key sequence ions (e.g. the b_1 ion is generally absent) [6–8]. Several groups are actively investigating the fragmentation mechanisms of protonated peptides in order to learn more about the factors that control peptide fragmentation [8,9,10a,10b,11].

Various alternatives to CAD have been explored in order to obtain complementary information on the structures of *protonated* peptides. These methods include photon [12] and surface-induced dissociation [13], blackbody infrared dissociation [14], ion mobility measurements [15], ion–molecule reactions [10c,16], and hydrogen/deuterium (H/D) exchange, as well as deprotonation reactions of singly [17] and multiply charged peptide ions [18]. However, examination of the reactions of *neutral* peptides with gas-phase reagent ions (chemical ionization) appears to be limited to proton transfer [19], electron transfer [20], and adduct formation [10c,21], in spite of several attractive characteristics associated with this approach. For example, the degree of fragmentation that results from chemical ionization reactions can be accurately controlled by the choice of the reagent ion [22]. Further, studies with substrates other than peptides have demonstrated the possibility of obtaining useful structural information by using chemical ionization reactions other than proton transfer [23].

We have examined fragmentation of di- and tripeptides induced by gas phase ion–molecule reactions with the dichlorophosphenium ion, $ClPCl^+$. This ion was found to efficiently fragment the peptides by analogous addition/elimination pathways that lead to one (dipeptides) or two (tripeptides) structurally diagnostic fragment ions.

2. Experimental

The chemicals were purchased from Aldrich (Milwaukee, WI) and Sigma (St. Louis, MO), and their identity and purity were verified by electron impact mass spectrometry (comparison to NIST MS data base revealed no visible impurities). The experiments were carried out in two different dual-cell Fourier transform ion cyclotron resonance mass spectrometers, an Extrel FTMS 2001 and a Finnigan FTMS 2001. A Sun IPX data station running ODYSSEY version 4.0 software controlled the Extrel instrument, whereas a Sun SPARC 20 data station running ODYSSEY version 4.0 software controlled the Finnigan mass spectrometer.

The Finnigan FTMS 2001 mass spectrometer contains two differentially pumped cells aligned within the magnetic field produced by a 3.0 T superconducting magnet. A nominal baseline pressure of $<1 \times 10^{-9}$ Torr was maintained in the differentially pumped cells by two Edwards diffusion pumps (800 L/s), each backed by an Alcatel 2012 mechanical pump. The two cells are separated by a conductance limit plate that contains a 2-mm hole in the center for transfer of ions. The Extrel FTMS 2001 mass spectrometer is very similar to the Finnigan instrument. It contains a differentially pumped dual cell that is aligned collinearly with a ~2.7 T magnetic field produced by a 3 T superconducting magnet. A nominal baseline pressure of $<1 \times 10^{-9}$ Torr is maintained by two Balzer turbomolecular pumps (330 L/s).

The conductance limit and the two end trapping plates were held at +2.0 V in both instruments unless otherwise specified. Liquid samples were introduced into the Extrel instrument by using two single batch inlet systems equipped with Andonian leak valves, whereas the peptide samples were introduced with a heated (100–200 °C), manually inserted solids probe. In the Finnigan instrument, liquid samples were introduced via a Varian leak valve or batch inlet systems equipped with Andonian leak valves, whereas the peptide samples were introduced with a commercial autoprobe heated up to 100–200 °C.

The reactant ion $ClPCl^+$ was generated by electron ionization of Cl_3P (Br_3P was used for generation of $BrPBr^+$) in one of the cells. The ion signal was maximized by varying the electron beam time (30–50 ms), emission current (5–8 μA), and electron energy (20–70 eV). All ions were removed from the other

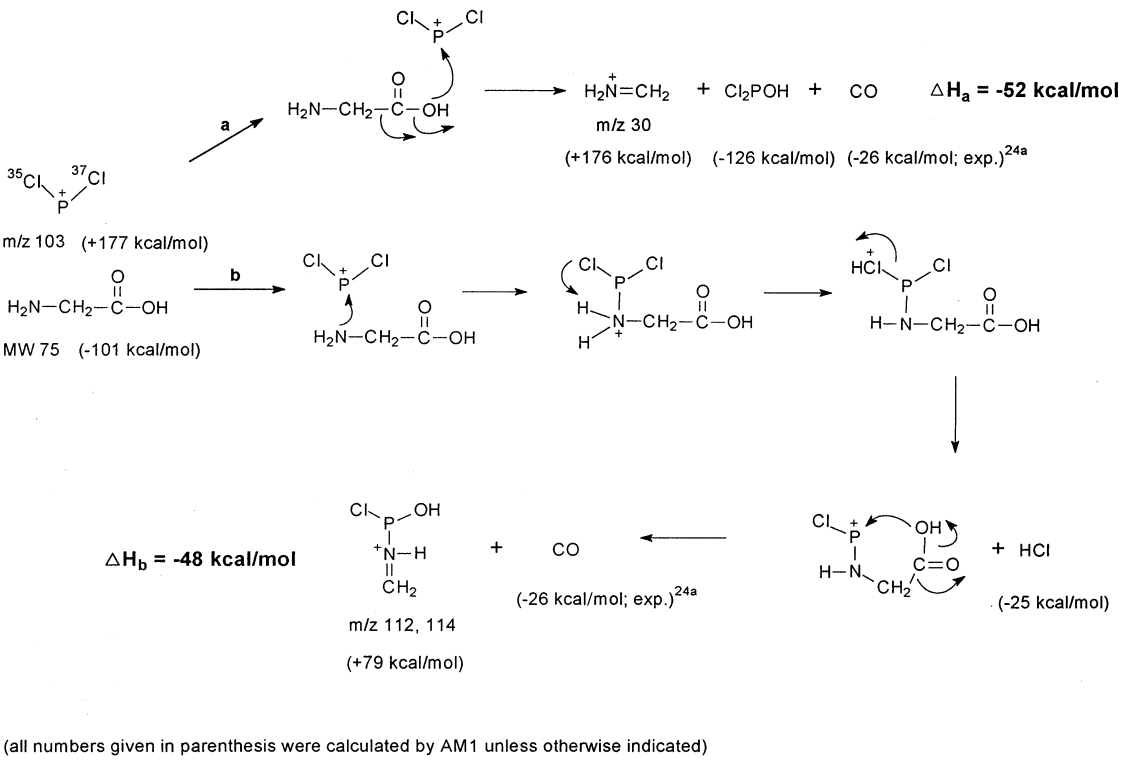
Table 1
Reaction efficiencies and major primary product ions (>6%) obtained for the reaction of $^{35}ClP^{37}Cl^+$ (*m/z* 103) with selected dipeptides and tripeptides

Neutral reagent (MW)	Primary products (*m/z*)	Branching ratios (%)	Reaction eff.[a]	Likely reaction
N,N-Dimethyl acetamide (87)	43	15	NA	$(CH_3)_2N^-$ abstraction
	86	15		H^- abstraction
	110,112	66		adduct − HCl, $H_2C{=}C{=}O$
Glycine (75)	30	49	NA	adduct − CO, Cl_2POH
	112,114	51		adduct − HCl, CO
Gly–Gly (132)	98	56	~1.0	adduct − H_2O, $Cl_2PO^{\cdot}$
	115	7		adduct − Cl_2POH
	151[b],153[b]	20		adduct − HCl, H_2O, CO
	179[b],181[b]	13		adduct − HCl, H_2O
Gly–Ala (146)	111	10	1.3	adduct − H_2O, Cl_2POH
	112	78		adduct − H_2O, $Cl_2PO^{\cdot}$
	165[b],167[b]	8		adduct − HCl, H_2O, CO
Gly–Val (174)	97	8	0.70	[c]
	129	11		adduct − CO, Cl_2POH
	140	48		adduct − H_2O, $Cl_2PO^{\cdot}$
	157	11		adduct − Cl_2POH
	193[b],195[b]	9		adduct − HCl, H_2O, CO
Pro–Ala (186)	152	98	~1.0	adduct − H_2O, $Cl_2PO^{\cdot}$
Ala–Ala (160)	126	89	0.8	adduct − H_2O, $Cl_2PO^{\cdot}$
Gly–Ala–Ala (217)	111	9	~1.0	adduct − H_2O, CO, $HN{=}CHCH_3$, Cl_2POH
	112	49		adduct − H_2O, CO, $HN{=}CHCH_3$, $Cl_2PO^{\cdot}$
	126	29		adduct − H_2O, CO, $HN{=}CH_2$, $Cl_2PO^{\cdot}$
Gly–Gly–Val (231)	72	33	0.9	adduct − 2CO, $H_2NCH_2C(O)N{=}CH_2$, Cl_2POH
	98	34		adduct − H_2O, CO, $HN{=}CH(CH_3)_2$, $Cl_2PO^{\cdot}$
	140	34		adduct − H_2O, CO, $HN{=}CH_2$, $Cl_2PO^{\cdot}$

[a] The reaction efficiency is given as k_{exp}/k_{coll}. The dipole moments used to calculate k_{coll} were estimated by molecular orbital calculations at AM1 level.
[b] Two products were observed, one containing ^{35}Cl and the other one ^{37}Cl.
[c] The identity of the product is unknown.

cell region prior to ion transfer by changing its end trapping plate voltage from +2.0 V to −2.0 V for five ms. The ions generated in the first cell were transferred into the other cell by temporarily grounding the conductance limit plate (~100 μs). The ions were cooled by collisions with argon pulsed into the cell through a pulsed valve system [24] (nominal peak pressure of about 10^{-6}–10^{-5} Torr). All unwanted ions were ejected by applying stored waveform inverse Fourier transform (SWIFT) [25] excitation pulses to the excitation plates. The isolated ions were allowed to react with a neutral peptide (nominal pressure in the cell was 3.0×10^{-8}–1.0×10^{-7} Torr) for a variable period of time. Each reaction spectrum was background corrected by using a previously described procedure [26]. The reactant and product ions were excited for detection by applying an excitation sweep of 124 $V_{p\text{-}p}$ amplitude, 2.7 MHz bandwidth, and 3.2 kHz μs^{-1} sweep rate. A minimum of 20 acquisitions were signal averaged to improve the signal-to-noise ratio. The spectra were recorded as 32k or 64k data points by using one zero-fill prior to Fourier transformation.

All reactions followed pseudo-first-order kinetics. The second-order rate constant ($k_{reaction}$) of each reaction was derived from a semilogarithmic plot of the relative abundance of the reactant ion versus time. The collision rate constants (k_{coll}) were calculated by using a parameterized trajectory theory [27]. The reaction efficiency is given as $k_{reaction}/k_{coll}$. The pres-



Scheme 1.

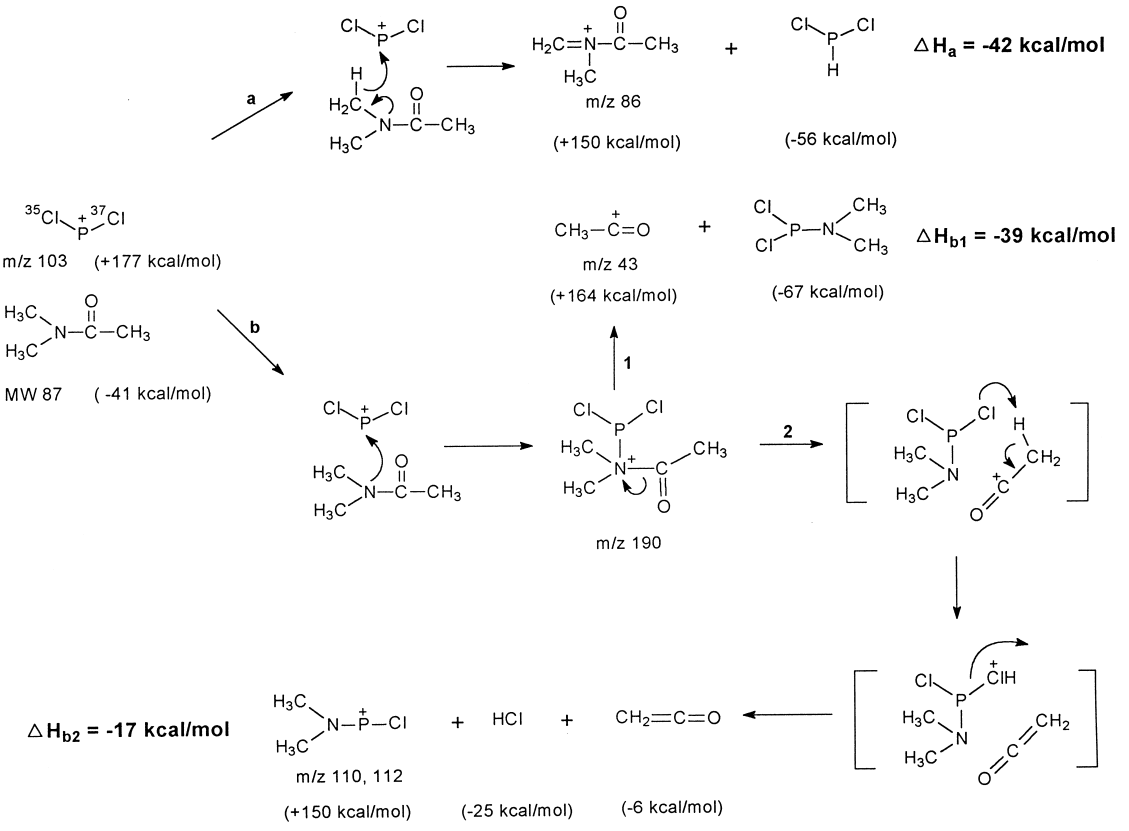
'sure readings of the ion gauges were corrected for their sensitivity toward the neutral peptides [28]. A correction factor for pressure differential between the cell and the ion gauge was obtained for each peptide by measuring rates of highly exothermic proton transfer reactions that can be expected to occur at collision rate. Primary products were identified on the basis of their fixed relative abundances (branching ratios) at short reaction times.

CAD experiments were performed with argon collision gas at nominal pressures of (5–10) $\times$ 10^{-8} Torr. The collision energy was varied from 0–25 eV (laboratory frame) [29].

Molecular orbital calculations were carried out using the semi-empirical method AM1 via Spartan 5.0.3, and density functional calculations via GAUSSIAN 94 Revision D [30]. The AM1 method was used to estimate the energies of the proposed reaction intermediates and the reaction exothermicities. The relative stabilities of various possible product ions were also examined at a higher level of theory. The geometries were fully optimized at the Becke3LYP/6-31G(d) level of theory. The energies were corrected for the zero-point vibrational energy obtained from the harmonic vibrational frequencies calculated at the same level of theory. The force constant matrices of the stationary points were confirmed to have the correct number of negative eigenvalues (zero for equilibrium structures). Isodesmic reactions [31] (involving acetone and acetamide) were used in the calculation of recombination energies and gas-phase acidities.

3. Results and discussion

The gaseous phosphenium ion $ClPCl^+$ has been previously characterized as a very strong electrophile. For example, the ion rapidly induces heterolytic C–O bond cleavages in organic ethers, yielding a variety of



Scheme 2.

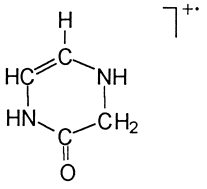
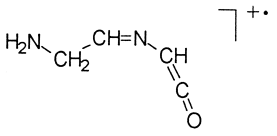
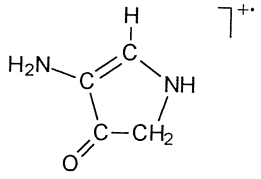
product ions in a Fourier transform ion cyclotron resonance mass spectrometer [32]. The high reactivity of this ion toward oxygen lone pairs inspired us to examine its reactions with nitrogen-containing compounds, specifically, amino acids, amides, and small peptides. Our ultimate desire is to find reagent ions that yield sequence information for peptides by fragmentation involving the peptide bonds.

The ion $ClPCl^+$ reacts readily with glycine to yield two primary product ions (Table 1). The ion $H_2N^+{=}CH_2$ (*m/z* 30) is likely formed by hydroxide abstraction followed by elimination of CO, whereas $ClP(OH){-}NH^+{=}CH_2$ (*m/z* 112, 114) is probably the result of addition of the amino group of glycine to $ClPCl^+$ followed by elimination of HCl and CO (Scheme 1). Therefore, the attack takes place at both the carboxylic group as well as the amino group of glycine. Similar observations have been reported earlier for reactions of glycine with a range of electrophiles [10d,10e]. In contrast, the $ClPCl^+$ ion appears to react with N,N-dimethyl acetamide by nearly exclusive attack at the amide oxygen (product ions of *m/z* 43, 110 and 112; Table 1). Feasible pathways for these reactions are shown in Scheme 2. This reaction is very facile, occurring at 75% efficiency (75/100 collisions lead to a reaction). Based on these findings, examination of reactions of $ClPCl^+$ with small di- and tripeptides seemed warranted.

In addition to $ClPCl^+$, reactions of $BrPBr^+$ were briefly examined to explore the generality of the findings.

In order to facilitate product ion identification, the ions $^{35}ClP^{37}Cl^+$ (*m/z* 103) and $^{79}BrP^{81}Br^+$ (*m/z* 191) were mass selected for the chemical ionization experiments, instead of the more abundant ions $^{35}ClP^{35}Cl^+$

Table 2
Calculated relative energies (at 0 K) and adiabatic recombination energies (RE) of the three most likely structures of the product ion of *m/z* 98

Ion structure ($A^{+\cdot}$)	Energy of $A^{+\cdot}$ in Hartree[a]	Relative energy in kcal mol^{-1}	Energy of A in Hartree[a]	Relative energy in kcal mol^{-1}	RE (eV)[a]	RE (eV)[b]
(a) 	−340.39460	0	−340.64582	0	6.84	7.34
(b) 	−340.31229	+51	−340.58373	+39	7.39	7.90
(c) 	−340.39104	+2.2	−340.63478	+6.9	6.63	7.14

[a] Becke 3LYP/6-31(*d*) + ZPVE.
[b] Recombination energies corrected by isodesmic reactions [31] with acetamide at the Becke 3LYP/6-31G(*d*) + ZPVE level (the calculated ionization energy of acetamide is 9.11 eV; the experimentally determined value is 9.62 eV; [33]).

(*m/z* 101) and $^{79}BrP^{79}Br^{+}$ (*m/z* 189). From now on, $ClPCl^{+}$ and $BrPBr^{+}$ are used to refer to the former isotopes (*m/z* 103, 191). The major product ions and reaction efficiencies are listed in Table 1.

3.1. Reactions of $ClPCl^{+}$ with dipeptides

The most abundant product ion generated from the dipeptides Gly–Gly, Gly–Ala, Ala–Gly, Gly–Val, Pro–Ala, and Ala–Ala has a mass-to-charge ratio that is 34 u less than the molecular weight of the dipeptide. The fact that the ion $(M - 34)^{+}$ was observed for all the dipeptides indicates that the side chains of the individual amino acids are retained within this product ion. This was a curious finding that warranted efforts toward the identification of the structure of the ion $(M - 34)^{+}$.

The ion of *m/z* 98 formed upon the reaction of Gly–Gly (MW 132) with $ClPCl^{+}$ was used as a representative case for the $(M - 34)^{+}$ type products. This ion was determined by exact mass measurements to have the formula $C_4H_6N_2O$. Hence, the product is a radical cation formed by a net loss of two hydrogen and two oxygen atoms from Gly–Gly. The same net element loss applies to the product ions generated from the other dipeptides.

The product ion, $C_4H_6N_2O^{+\cdot}$ (*m/z* 98), has various possible structures, including cyclic (five- and six-membered) and acyclic structures. Examination of its reactivity, as discussed later in this article, suggests that the ion population is isomerically pure (all the reactions follow pseudo-first-order kinetics). The relative stabilities of over a dozen possible structures with formula $C_4H_6N_2O$ were examined by AM1 calculations in order to identify the most likely candidates. Three structures with an N–C–C–N–C–C

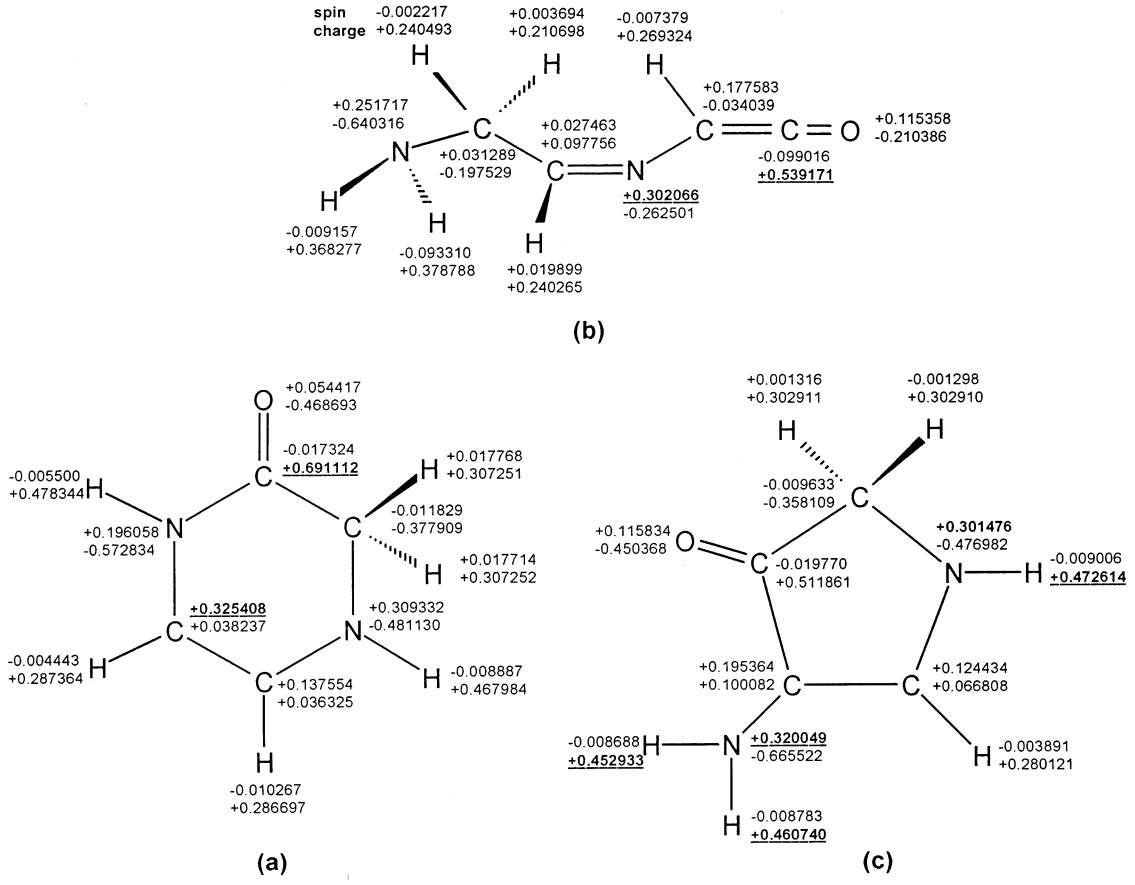


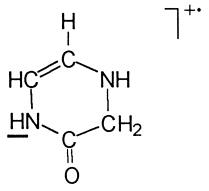
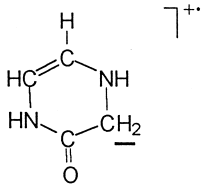
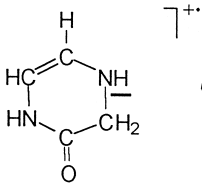
Fig. 1. The charge and spin distributions obtained from molecular orbital calculations (Becke 3LYP/6-31G(*d*) + ZPVE) for the three most likely structures of the reaction product.

Table 3
Bracketing the adiabatic recombination energy (RE) and gas-phase acidity (ΔH_{acid}) of the product ion of *m/z* 98 by reactions with various neutral reagents

Neutral reagent (MW)	Ionization energy (eV)[a]	Proton affinity (kcal mol^{-1})[a]	Product (*m/z*)	Reaction
di-*tert*-Butyl nitroxide (144)	6.77	[b]	144	Electron transfer
N,N-Dimethyl aniline (121)	7.12	224.9	121	Electron transfer
			122	Proton transfer
p-Toluidine (107)	7.24	214.3		No reaction
N-Methyl aniline (107)	7.32	219.1		No reaction
Triethyl amine (101)	7.53	234.7	102	Proton transfer
Aniline (92)	7.72	210.9		No reaction
Trimethyl phosphite (124)	8.4	222.2		No reaction
Acetophenone (120)	9.28	205.8		No reaction

[a] The ionization energy and proton affinity values are from [34].
[b] The proton affinity of di-*tert*-butyl nitroxide is not available.

Table 4
Gas-phase acidities (ΔH_{acid}; kcal mol^{-1}) obtained by molecular orbital calculations for the six-membered ring isomer (deprotonation sites are underlined)

Deprotonation site	ΔH_{acid}[a,b]
(**x**) 	237
(**y**) 	226
(**z**) 	230

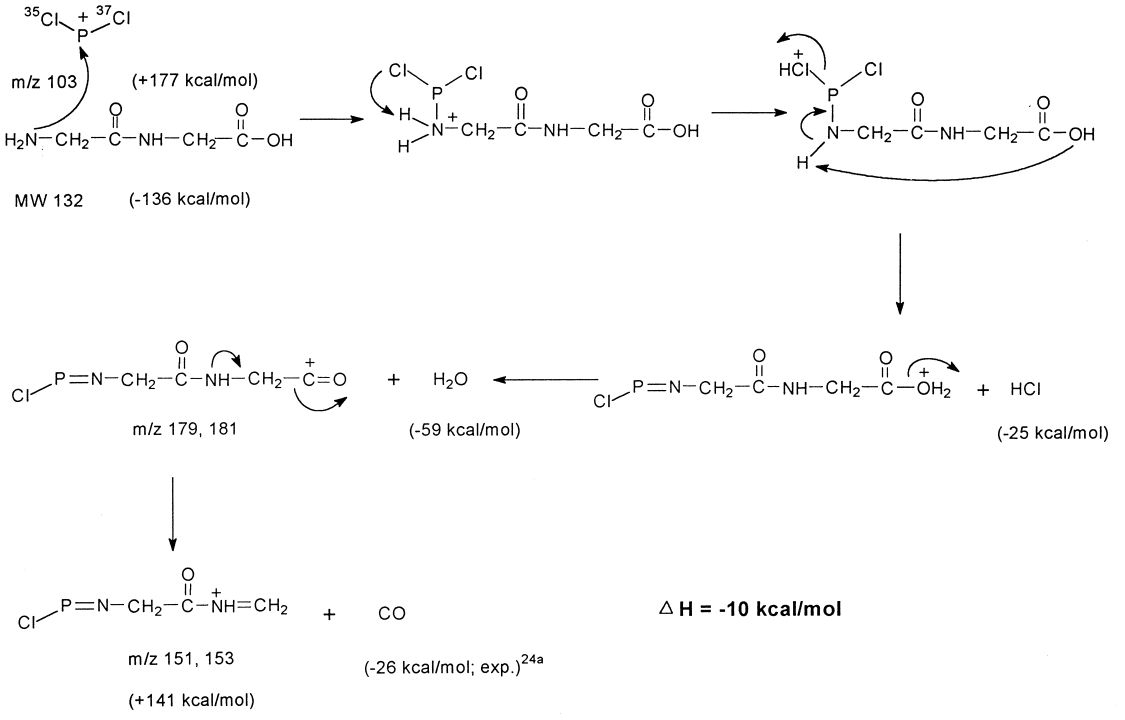
[a] Becke 3LYP/6-31(*d*) + ZPVE.
[b] ΔH_{acid} is corrected by isodesmic reaction approach using [acetamide − H$^{\cdot}$] (**x, z**) or [acetone − H$^{\cdot}$] (**y**).

skeleton (as in Gly–Gly) were selected for further evaluation: the most stable acyclic isomer [(**b**), Table 2)] and the two most stable cyclic isomers, one with a five-membered (**c**) and one with a six-membered ring (**a**). Molecular orbital calculations at a higher level of theory (Becke3LYP/6-31G(*d*) + ZPVE) were used to obtain a more accurate estimate for the relative energies (Table 2) and charge and spin distributions of these three structures (Fig. 1). The carbonyl carbon carries most of the positive charge in each isomeric ion. The spin is mostly found on the nitrogen atoms, except for structure (**a**) wherein a significant odd spin density resides on one of the carbon atoms (Fig. 1). The six-membered cyclic structure is estimated to be more stable than either the five-membered ring ($\Delta\Delta$H = 2.2 kcal mol^{-1}) or the linear structure ($\Delta\Delta$H = 51 kcal mol^{-1}; Table 2). Based on thermodynamic factors alone, this would be expected to be the structure of the product ion of *m/z* 98.

CAD was used to investigate the structure of the product ion $(M - 34)^{+\cdot}$ formed in the reaction of Gly–Gly, Gly–Ala, Ala–Gly, and Ala–Ala with $ClPCl^+$. Each precursor ion was found to yield two major fragment ions, a structurally uninformative ion from loss of an H atom, and another one from loss of HN=CHR (R = H for Gly–Gly, R = H and/or CH_3 for Gly–Ala, Ala–Gly, and Ala–Ala). The ions formed from Gly–Ala and Ala–Gly yield similar dissociation product distributions. The proposed five- (**c**) and six-membered ring isomers (**a**) contain -NH–CHR- as part of their structures, and the linear isomer contains H_2N–CHR. All three ions can reasonably be expected to lose HN=CHR upon fragmentation. Hence, although these CAD results support the proposed three structures, they do not allow distinction to be made among them.

In order to further characterize the ion of *m/z* 98, its adiabatic recombination energy (RE) was determined by allowing it to react with a series of neutral molecules with known ionization energies, and observing the formation of electron-transfer products [34]. These bracketing experiments yield a recombination energy of 7.1–7.2 eV for the ion (Table 3). Molecular orbital calculations yield an estimated RE of 7.34 eV, 7.90 eV, and 7.14 eV for the isomeric structures (**a**), (**b**), and (**c**), respectively (an isodesmic reaction with acetamide was employed in the calculations). The experimental RE is in agreement with either one of the cyclic structures (**a**) and (**c**), but not with the acyclic structure (**b**). Hence, this structure was not considered further.

Mechanistic rationale can be used to rule out the five-membered ring structure (**c**). Formation of this ion from Gly–Gly would require the N-terminal residue of the peptide to lose the two hydrogens of the CH_2 group either as part of the neutral product or via proton or hydride shifts to other atoms in the ion. For the other dipeptides, formation of the analogous ion would involve either (1) an unprecedented shift of an alkyl side chain from a carbon atom to another, or, alternatively, (2) elimination of the side chain, which is not supported by the experimental results (Table 1).

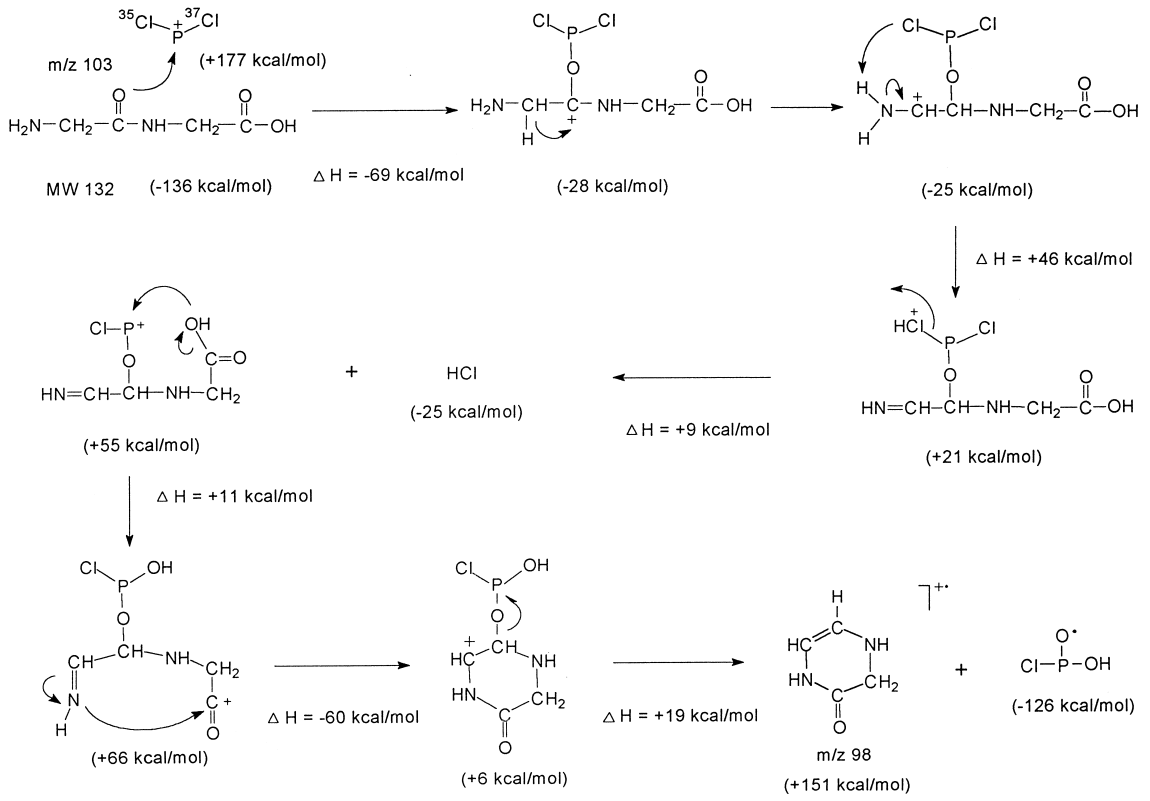


Scheme 3.

The gas-phase acidity (ΔH_{acid}) of the unknown ion of *m/z* 98 was bracketed between 222 kcal mol^{-1} and 225 kcal mol^{-1} by examining proton transfer reactions of the ion with a series of neutral molecules with known proton affinities (Table 3). This value is in an excellent agreement with that predicted by molecular orbital calculations for the most acidic site, CH_2, of the ion (**a**) (226 kcal mol^{-1}; Table 4). Furthermore, the experimental observation of exchange of exactly two hydrogen atoms with deuteriums in the ion upon reactions with ND_3 is readily rationalized based on structure (**a**).

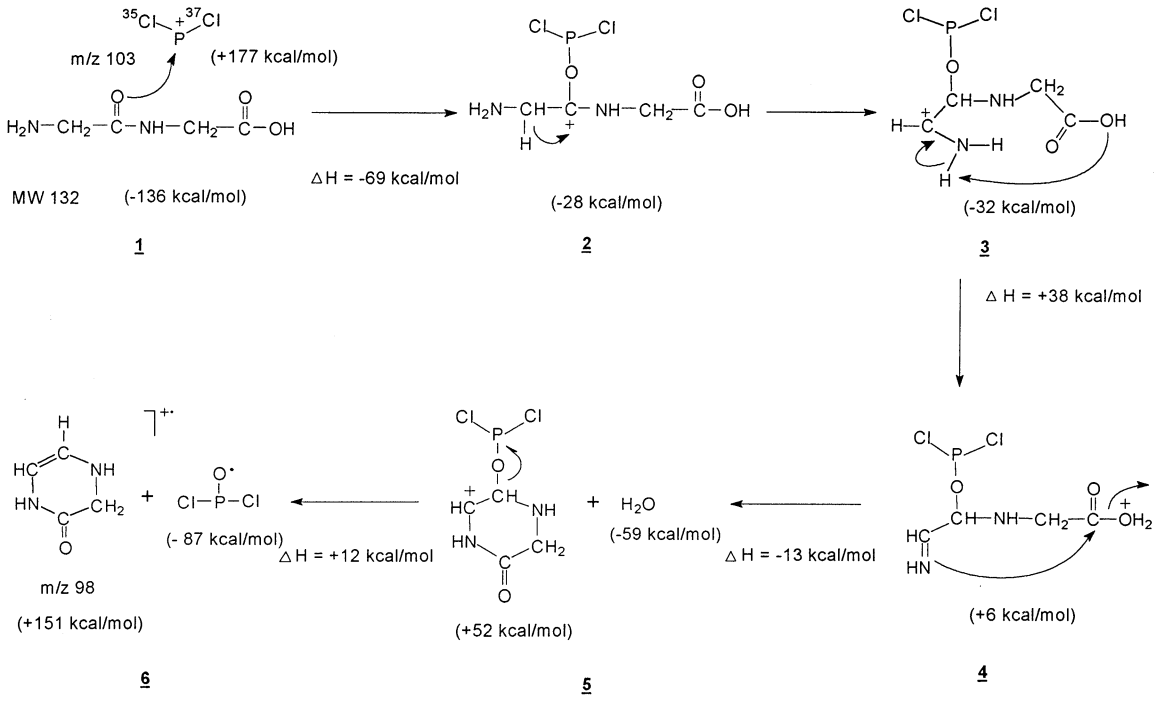
Based on all the above evidence, it is reasonable to conclude that the most likely structure for the ion of *m/z* 98 is the six-membered structure (**a**) (Table 2). Formation of this ion upon reaction of Gly–Gly with $ClPCl^+$ likely occurs either by loss of HCl and $ClP(O)OH^{\cdot}$ or loss of H_2O and $Cl_2PO^{\cdot}$.

What site in the peptide is involved in the initial attack? Proton affinity considerations may be misleading here because the possibility of internal hydrogen bonding at certain sites increases their proton affinity but similar interactions are not possible for $ClPCl^+$. Indeed, although the amino nitrogen of glycine is more basic than either the carboxyl carbonyl oxygen or the hydroxyl group [35], $ClPCl^+$ reacts equally fast at the amino group and the hydroxyl group (Scheme 1). The ion reacts with the di- and tripeptides in a much more selective manner. Preferred attack is at the amide oxygen, although the computationally predicted basicity order of the various functionalities in, for example, Gly–Gly, is amino nitrogen $\geq$ amide oxygen $\gg$ amide nitrogen $\gg$ carboxyl carbonyl oxygen [35]. Attack at the amino nitrogen of Gly–Gly likely leads to two minor product ions due to loss of HCl and H_2O, and loss of HCl, H_2O, and CO (Scheme 3), in a largely analogous manner as elimination of HCl and H_2O occurs from glycine (Scheme 1). Analogous products were observed for several of the dipeptides studied.



Scheme 4.

The formation of the ion of *m/z* 98 from Gly–Gly likely involves attack at a carbonyl oxygen because two oxygen atoms are lost from Gly–Gly upon reaction with $ClPCl^+$. Such losses can be explained by addition of $ClPCl^+$ at the amide carbonyl oxygen. Among various possible reaction pathways, two were selected for more detailed examination (Schemes 4 and 5). AM1 calculations were carried out to evaluate the thermodynamic feasibility of these pathways. Both are estimated to be exothermic (Scheme 4: -41 kcal mol^{-1}; Scheme 5: -36 kcal mol^{-1}). The initial addition is highly exothermic (-69 kcal mol^{-1}) and likely barrierless (a simple electrophilic attack on a coordinatively unsaturated P atom; the ion has a singlet electronic ground state). After addition, a barrierless 1,2-hydride shift [36] occurs from a CH_2 group to the adjacent carbonyl carbon. The pathways differ from each other only in the final steps that lead to elimination of HCl and ClP(O)OH (Scheme 4), or H_2O and Cl_2PO (Scheme 5). The former reaction pathway is assessed to be the less likely one because this pathway involves more intermediates, some of which are of very high energy. In fact, formation of one of the intermediates (heat of formation 66 kcal mol^{-1}; Scheme 4) raises the energy level of the system up to the level of the separated reactants, and hence is not energetically feasible. Further, this pathway cannot be used to rationalize the reaction products of the tripeptides, whereas the other pathway can. An estimated (AM1) potential energy diagram for the reaction sequence shown in Scheme 5 is illustrated in Fig. 2. This diagram indicates that all proposed intermediate steps are readily accessible to the internally excited addition product.



Scheme 5.

Finally, another phosphenium ion, $BrPBr^+$, was found to react with the dipeptides to yield analogous products as obtained for $ClPCl^+$. This finding provides some additional support for the proposed reaction pathways and the identities of the product ions.

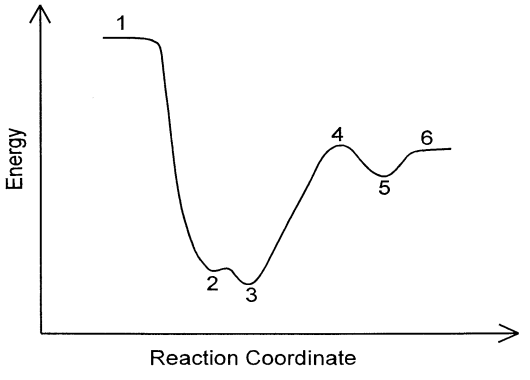


Fig. 2. The energies of the reactants, reaction intermediates, and final products were estimated by semiempirical calculations at AM1 level for the reaction of $ClPCl^+$ with Gly–Gly (Scheme 5).

3.2. Reactions of $ClPCl^+$ with tripeptides

$ClPCl^+$ yields two major product ions upon reaction with tripeptides. These products are analogous to those obtained for the corresponding dipeptides (Table 1). For example, the two most abundant products formed upon reaction of $ClPCl^+$ with the tripeptide Gly–Ala–Ala are the ions of *m/z* 112 and 126. The dipeptide Gly–Ala yields a major ion of *m/z* 112, while Ala–Ala yields an ion of *m/z* 126. The major product ions (*m/z* 98, 140) obtained for Gly–Gly–Val match those observed for Gly–Gly (*m/z* 98) and Gly–Val (*m/z* 140; Fig. 3). These observations demonstrate that upon reaction with $ClPCl^+$, a tripeptide (A_1–A_2–A_3) fragments via cleavage of each of the peptide bonds to lose either the C-terminus or the N-terminus amino acid residue (to form A_1–A_2 and A_2–A_3, respectively).

The results obtained for the dipeptides suggest that addition of $ClPCl^+$ to an amide carbonyl oxygen is followed by elimination of H_2O and $Cl_2PO^{\cdot}$ from the

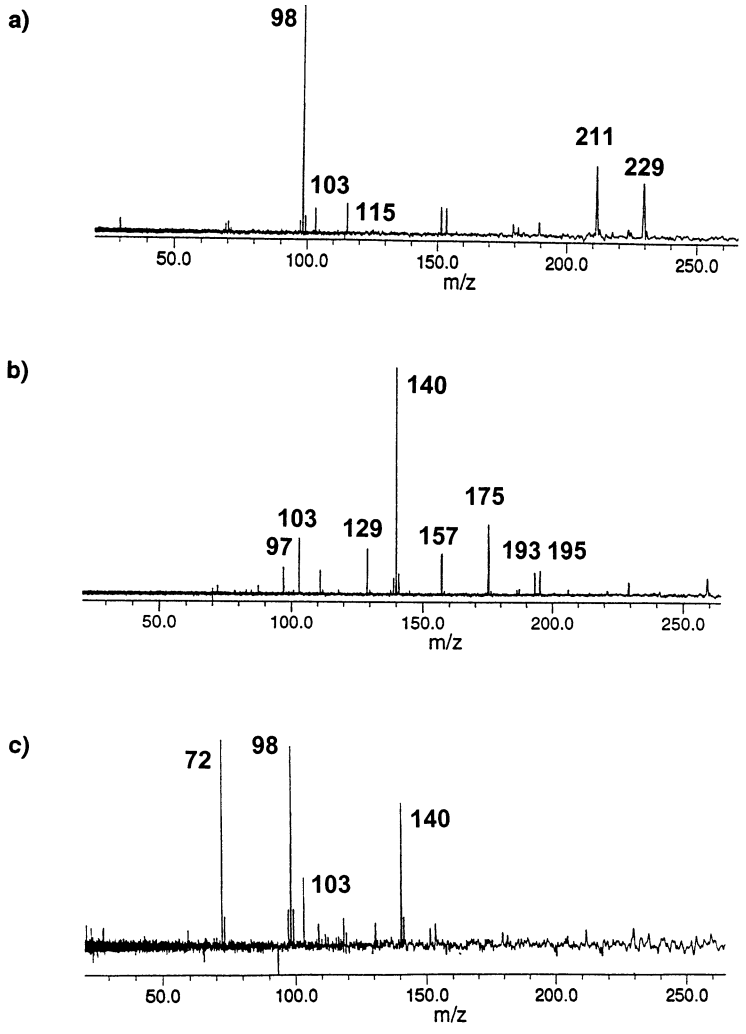
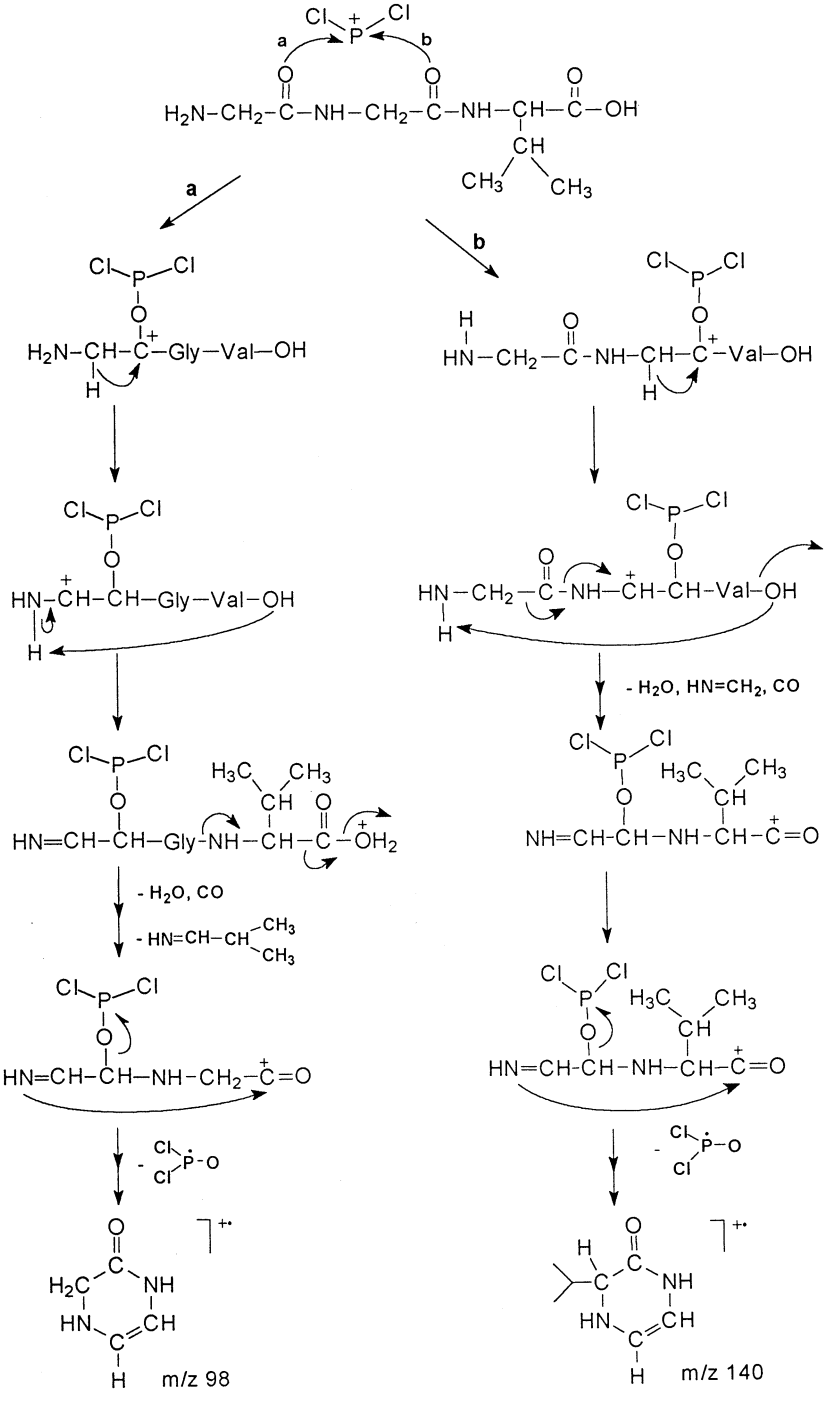


Fig. 3. (**a**) Reaction of $^{35}ClP^{37}Cl^+$ (*m/z* 103) with Gly–Gly (MW 132) yields the major product ion of *m/z* 98 (see Table 1 for the identities of the primary product ions). (**b**) Reaction of $^{35}ClP^{37}Cl^+$ with Gly–Val (MW 174) yields the ion of *m/z* 140 as the most abundant product. (**c**) Reaction of $^{35}ClP^{37}Cl^+$ with Gly–Gly–Val yields the ions of *m/z* 98, 140, and 72 as the most abundant products [the ion of *m/z* 72 may be H_2N^+=CH–CH$(CH_3)_2$].

C terminus. A tripeptide has two amide carbonyl groups, each of which could be attacked by $ClPCl^+$. These attacks appear to lead to fragmentation in an analogous manner for dipeptides (Scheme 6), resulting in elimination of H_2O, CO, NH=CHR (R = side chain) and $Cl_2PO^{\cdot}$. The side chains of two adjacent amino acids in the tripeptide appear as part of each product ion. As a result, the fragmentation products obtained for the tripeptides provide sequence information. The above conclusions are supported by the finding that $BrPBr^+$ yields the same products as $ClPCl^+$ upon reactions with the tripeptides.

4. Conclusions

The phosphenium ion $ClPCl^+$ induces extensive fragmentation of gaseous di- and tripeptides, yielding only one (dipeptides) or two major products (tripeptides) that each contain the intact side chains of two


a
b
H2N–CH2–C–NH–CH2–C–NH–CH–C–OH
CH
CH3 CH3
Gly–Val–OH
Val–OH
- H2O, HN=CH2, CO
- H2O, CO
- HN=CH–CH
m/z 98
m/z 140

Scheme 6.

adjacent amino acids. For the tripeptides, formation of these product ions involves cleavage of peptide bonds. Experimental and computational evidence supports a highly delocalized, six-membered ring structure for the product ion formed from Gly–Gly. An analogous product ion, with the side chain intact and hence of a different *m/z* value, is likely formed from the other peptides studied (Gly–Ala and Ala–Gly yield the same major product ion). A feasible pathway leading to this type of a product ion for dipeptides involves initial addition of the amide carbonyl oxygen to $ClPCl^+$, followed by loss of H_2O and $Cl_2PO^{\cdot}$. An analogous mechanism, involving attack at either one of the amide carbonyl oxygens, leads to fragmentation of the tripeptides. These findings suggest that $ClPCl^+$ and $BrPBr^+$ have the potential to provide useful sequence information for peptides. We are currently exploring the applicability of this approach to larger peptides. The key issue here is evaporation of the neutral peptide. This can be carried out, for example, by the substrate-assisted laser desorption method introduced by Amster and co-workers [19,37,38].

Acknowledgement

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