Research Article

Rapid Communications in Mass Spectrometry

Received: 28 April 2014 Revised: 8 July 2014 Accepted: 11 July 2014 Published online in Wiley Online Library

Rapid Commun. Mass Spectrom. **2014**, *28*, 1964–1970
(wileyonlinelibrary.com) DOI: 10.1002/rcm.6984

Cleavage of phosphorus–carbon (P–C) bonds of α-amino phosphonates with intramolecular hydrogen migration in the gas phase using electrospray ionization tandem mass spectrometry

Yuzhen Gao[1], Jian Xu[1], Yaohui He[2], Guo Tang[1], Zhiwei Lin[1], Hongxia Liu[3], Xiang Gao[2]* and Yufen Zhao[1,2]

[1]Department of Chemistry and The Key Laboratory for Chemical Biology of Fujian Province, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, P. R. China
[2]School of Pharmaceutical Sciences, Xiamen University, Xiamen, 361102, P. R. China
[3]The Key Laboratory for Cancer Metabolomics of Shenzhen, Graduate School at Shenzhen, Tsinghua University, Shenzhen, 518055, P. R. China

RATIONALE: α-Amino phosphonates with intrinsic biological activities have been used in a wide variety of applications. Because of the widespread existence of natural organophosphorus compounds containing P–C bonds such as the α-amino phosphonates, it is important to investigate the gas-phase chemistry of P–C bonds in order to determine their basic properties, which might provide some insights into their biosynthesis and catalytic cleavage.
METHODS: Twenty α-amino phosphonates were successfully synthesized and their fragmentation behavior was systematically investigated using in-solution deuterium labeling in combination with high-resolution Fourier transform ion cyclotron resonance (FTICR) electrospray ionization tandem mass spectrometry.
RESULTS: The fragmentation pathways of twenty α-amino phosphonates with different chemical structures were systematically studied. In general, P–C bonds could be easily cleaved via a novel intramolecular hydrogen atom migration from the amino group to the phosphoryl group through a five-membered-ring intermediate in the gas phase. A possible mechanism of the rearrangement of α-amino phosphonates is proposed.
CONCLUSIONS: An interesting intramolecular hydrogen atom migration between the amino and phosphoryl groups was observed with cleavage of the P–C bond in the molecule through a five-membered-ring intermediate. This characteristic fragmentation pathway not only provides some insights into the basic chemistry of compounds with P–C bonds, but could also have some applications in the structural determination of the α-amino phosphonate analogues. Copyright © 2014 John Wiley & Sons, Ltd.

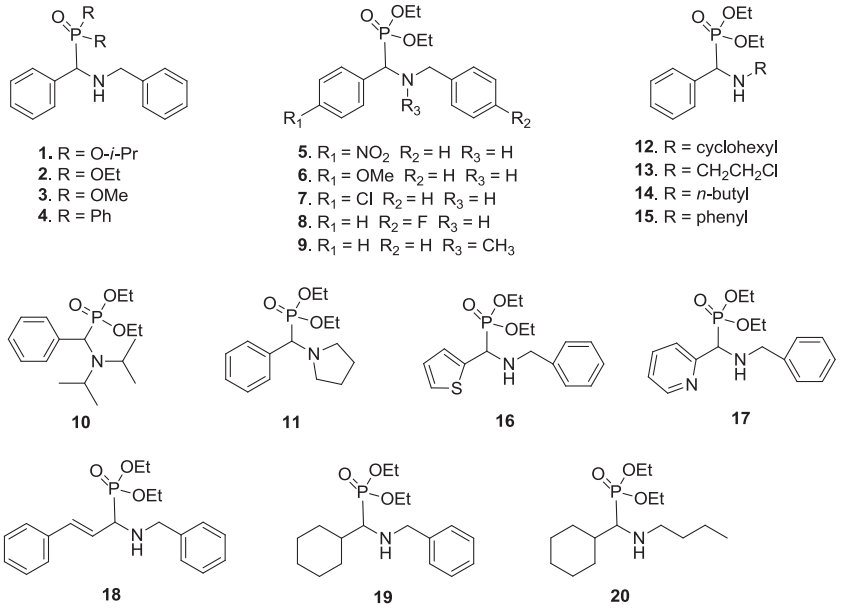
α-Amino phosphonates are bioactive natural products containing phosphorus–carbon (P–C) bonds. Since the first natural P–C compound, 2-aminoethylphosphonic acid, was isolated and identified from rumen Protozoa in 1959,[1] more such compounds have been synthesized.[2–8] Among these, α-amino phosphonates have been used in a wide variety of applications such as anticancer and antivirus drug development, and as antibiotics, herbicides, and fungicides,[9–13] and most of their biological activity could be attributed to the structural properties of their P–C bonds. Although P–C bonds are chemically stable, it has been reported that they can be cleaved by enzymes such as phosphonoacetate hydrolase (PhnA) and phosphonopyruvate hydrolase (PnPy).[14,15] Considerable efforts have been made to elucidate the mechanism of formation and cleavage of the P–C bonds, but the exact details remain unknown.[16,17]

* *Correspondence to:* X. Gao, School of Pharmaceutical Sciences, Xiamen University, Xiamen, 361102, P. R. China. E-mail: xgao@xmu.edu.cn

In our previous work, different classes of organophosphorus compounds with P–C bonds were synthesized and then investigated by electrospray ionization tandem mass spectrometry (ESI-MS/MS). It was found that the fragmentation pathways of P–C bonds were different from those of phosphates and phosphoramidates containing P–O and P–N bonds, respectively.[18–21] Several interesting rearrangement reactions have been observed, such as hydrogen-atom migration from the hydroxyl to the phosphoryl group for α-hydroxyl dialkylphosphonic acid esters,[22] neutral phosphoryl amine loss from phosphonate peptides,[23] and oxygen-atom migration to phosphoryl groups for [(4-substituted-benzoylamino)phenylmethyl]phosphonic acid diisopropyl esters.[24] Furthermore, it was found that P–C bonds could be easily cleaved in the gas phase under our experimental conditions. Because of the widespread existence of organophosphorus compounds such as α-amino phosphonates, it is valuable to investigate the gas-phase chemistry of their P–C bonds in order to obtain some insight into their biosynthesis and cleavage. In the present work, a series of α-amino phosphonates was synthesized and their fragmentation behavior was systematically investigated using

Rapid Communications in Mass Spectrometry

ESI-MS/MS in combination with stable isotope labeling. Simultaneously with the cleavage of P–C bonds, a novel intramolecular hydrogen-atom migration between the amino and phosphoryl groups through a five-membered ring was observed. The proposed mechanism of rearrangement of α-amino phosphonates was confirmed by deuterium labeling and high-resolution Fourier transform ion cyclotron resonance (FTICR) tandem mass spectrometry.



Scheme 1. Chemical structures of α-amino phosphonates **1–20**.

Table 1. ESI-MS^n data of $[M+Na]^+$ ions of α-amino phosphonates **1–20**

Compounds (MW)	Precursor ions (*m/z*)	Product ions (*m/z*, relative intensity percentage)				P–H bond formation
1 (361)	384	342 (9)	218 (97)	147 (9)	129 (11)	189 (100)
	342		218 (100)	147 (20)		
	189			147 (36)	129 (100)	
Exact mass[a]	384.1704	342.1235	218.0946			189.0657
HRMS[b]	384.1693	342.1224	218.0930			189.0646
2 (333)	356	218 (100)				161 (39)
d-**2**[c]	357.1444	218.0933				162.0394
3 (305)	328	218 (100)				133 (16)
4 (397)	420	218 (100)				224 (44)
5 (378)	401	263 (18)	115 (8)			161 (100)
6 (363)	386	248 (100)				161 (28)
7 (367)	390	252 (100)	115 (5)			161 (88)
8 (351)	374	236 (56)	115 (6)			161 (100)
9 (347)	370	210 (100)				
10 (327)	350	190 (100)	148 (5)			
11 (297)	320	160 (100)				
12 (325)	348	210 (20)	115 (2)			161 (100)
13 (305)	328	292 (70)	190 (100)	115 (7)		161 (76)
14 (299)	322	184 (9)				161 (100)
15 (319)	342	234 (4)	204 (14)	115 (11)		161 (100)
16 (339)	362	224 (100)				161 (12)
17 (334)	357	219 (100)				
18 (359)	382	244 (100)				161 (55)
19 (339)	362	224 (100)				161 (42)
20 (305)	328					161 (100)

[a]The theoretical mass by calculation.
[b]The mass measured by high-resolution ESI-FTICR-MS.
[c]Deuterium-labeled compound **2**.

EXPERIMENTAL

Synthesis and identification of α-amino phosphonates

Twenty α-amino phosphonates (compounds **1–20**, Scheme 1) were synthesized according to a novel method developed in our previous work.[25] All the compounds were characterized by ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), ^{31}P NMR (162 MHz) and ESI-MS. The general synthetic procedure was as follows. Amide (0.3 mmol) was treated in anhydrous tetrahydrofuran (3.0 mL) with Schwartz's reagent Cp_2ZrHCl (171 mg, 0.66 mmol) under nitrogen and stirred at room temperature. H-phosphonate (0.36 mmol) was added until the suspension cleared (about 5–15 min), and the mixture was stirred at 60 °C for about 12 h in a Schlenk tube. The reaction was quenched with saturated sodium bicarbonate (35.0 mL), and extracted with ethyl acetate (3 × 5.0 mL). The combined organic extracts were washed with brine (10 mL), dried over Na_2SO_4, and evaporated under reduced pressure to leave the crude product, which was subsequently purified by flash chromatography and eluted using petroleum ether/ethyl acetate (2:1, v/v) to give the product.

The NMR data for diisopropyl ((benzylamino)(phenyl) methyl)phosphonate (compound **1**) and diethyl ((benzylamino) (phenyl)methyl)phosphonate (compound **2**) were as follows.

Compound **1**: ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.46-7.45 (m, 2H), 7.39-7.29 (m, 8H), 4.74-4.66 (m, 1H), 4.55-4.48 (m, 1H), 4.00-3.95 (d, *J* = 20.4 Hz, 1H), 3.84-3.81 (d, *J* = 13.1 Hz, 1H), 3.58-3.54 (d, *J* = 13.3 Hz, 1H), 2.32 (s, 1H), 1.31-1.29 (m, 6H), 1.25-1.24 (d, *J* = 6.1 Hz, 3H), 1.00-0.99 (d, *J* = 6.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 139.6 (s), 136.1 (d, *J* = 4.3 Hz), 128.8 (d, *J* = 6.6 Hz), 128.3 (d, *J* = 6.6 Hz), 127.7 (d, *J* = 2.9 Hz), 127.2 (s), 71.3 (d, *J* = 7.4 Hz), 60.2 (d, *J* = 155.1 Hz), 51.4 (d, *J* = 17.5 Hz), 24.2 (t, *J* = 3.7 Hz), 23.9 (d, *J* = 5.3 Hz), 23.4 (d, *J* = 5.3 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ (ppm) 21.87.

Compound **2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.44-7.42 (m, 2H), 7.38-7.34 (m, 2H), 7.32-7.28 (m, 3H), 7.26-7.22 (m, 3H), 4.11-3.92 (m, 4H), 3.84-3.78 (m, 2H), 3.56-3.52 (d, *J* = 13.3 Hz, 1H), 2.31 (s, 1H), 1.27-1.25 (t, *J* = 7.0 Hz, 3H), 1.14-1.10 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 139.4 (s), 135.8 (d, *J* = 3.9 Hz), 128.8 (d, *J* = 6.5 Hz), 128.6 (d, *J* = 2.2 Hz), 128.4 (d, *J* = 2.2 Hz), 128.0 (d, *J* = 3.6 Hz), 127.2 (s), 63.0 (d, *J* = 6.8 Hz), 62.9 (d, *J* = 7.0 Hz), 59.7 (d, *J* = 153.4 Hz), 51.3 (d, *J* = 17.5 Hz), 16.5 (d, *J* = 5.8 Hz), 16.3 (d, *J* = 5.7 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ (ppm) 23.51.

Mass spectrometric conditions

The ESI mass spectra were acquired in positive ion mode using a Bruker Esquire 3000plus ion trap mass spectrometer (Bruker Daltonic Inc., Billerica, MA, USA). Typically, $[M+H]^+$ and $[M+Na]^+$ ions were observed with high relative intensities for all twenty α-amino phosphonates studied. The α-amino phosphonates were dissolved in methanol at a concentration of about 5–10 ppm and infused continuously into the ESI chamber at a flow rate of 4 μL min^{-1} using a model 74900 syringe pump (Cole-Parmer Instrument Co., Vernon Hills, IL, USA). Nitrogen (N_2) was used as the drying gas at a flow rate of 4 L min^{-1} and the nebulizer gas with a

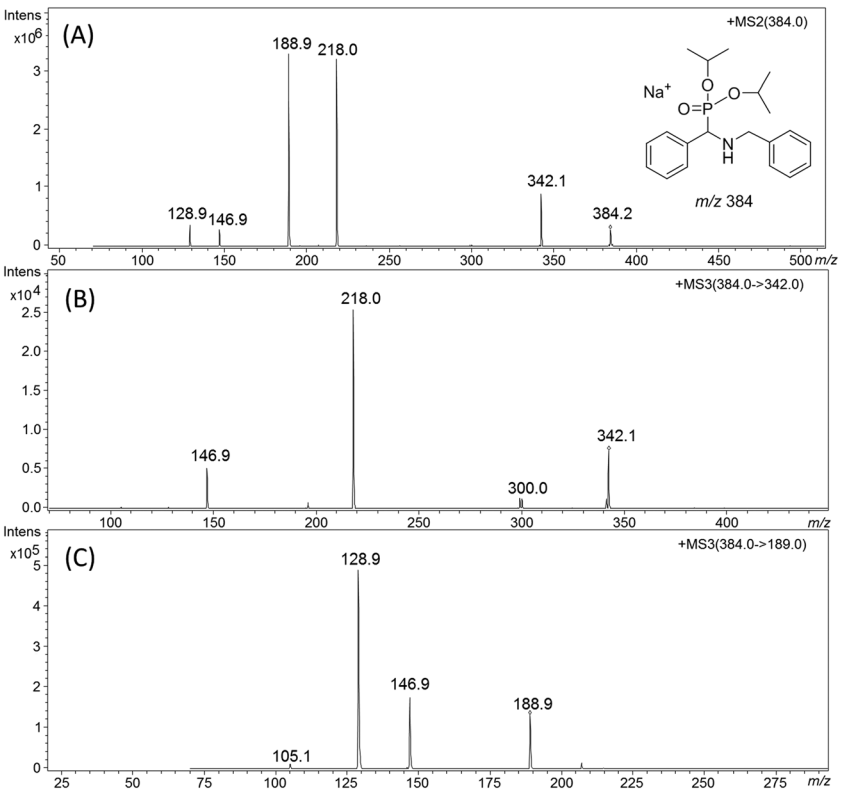
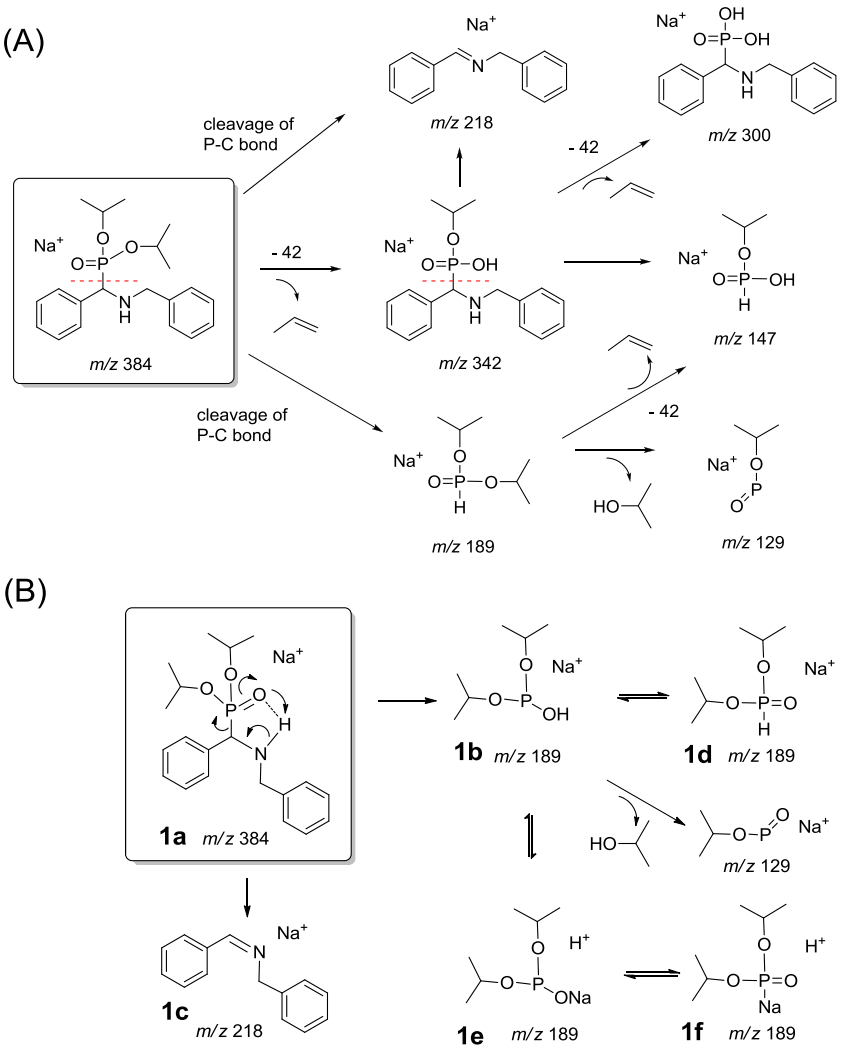


Figure 1. ESI tandem mass spectra of compound **1** in positive ion mode: (A) ESI-MS/MS of $[M+Na]^+$ ion at *m/z* 384; (B) ESI-MS^3 of product ion at *m/z* 342; and (C) ESI-MS^3 of the rearrangement ion at *m/z* 189.

pressure of 8 psi. The electrospray capillary was typically held at 4 kV. The heated capillary temperature was 250 °C. The tandem mass spectra were obtained by collision-induced dissociation (CID) with helium after isolation of the appropriate $[M+H]^+$ and $[M+Na]^+$ precursor ions. Generally, the scan range was from *m/z* 50 to 800. The fragmentation amplitude values ranged from 0.3 to 0.8 eV and the fragmentation time was 40 ms. Five scans were averaged for each spectrum.

High-resolution positive ion ESI tandem mass spectra were acquired on a Bruker APEX-Ultra FTICR mass spectrometer (7.0 T). Nitrogen (N_2) was used as the drying gas and



Scheme 2. (A) Possible fragmentation pathways of $[M+Na]^+$ ion of compound **1**. (B) Proposed rearrangement mechanism for the intramolecular hydrogen migration.

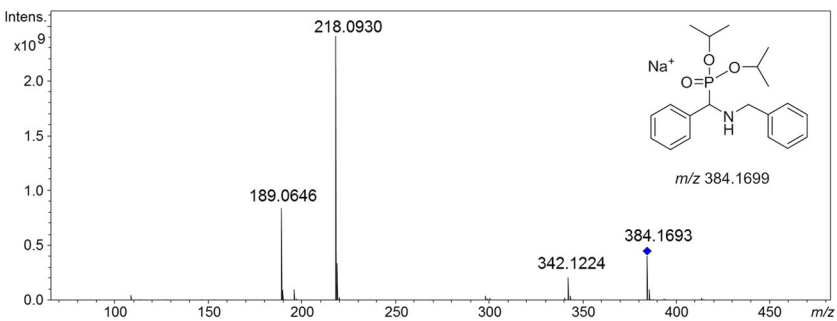


Figure 2. High-resolution ESI-FTICR tandem mass spectrum of $[M+Na]^+$ ion of compound **1** at *m/z* 384.1693.

nebulizer gas. The acquisition parameters were as follows: drying gas temperature, 180 °C; drying gas flow rate, 5.0 L min^{-1}; nebulizer gas flow rate, 1.0 L min^{-1}; ion accumulation time, 2.0 s. The capillary entrance voltage was 4.3 kV, the end plate voltage was 3.8 kV, and the collision energy was 13.5 eV. The precursor $[M+Na]^+$ ions were selected using an isolation width of 1.0 *m/z* unit. The scan range was from *m/z* 50 to 800. The sample dissolved in methanol was introduced into the source at a flow rate of 4 mL min^{-1}. Ten scans were averaged for each spectrum.

Deuterium-labeling experiment

Hydrogen/deuterium exchange was used to trace and determine the intramolecular hydrogen migration of α-amino phosphonates. Briefly, compound **1** (1 mg) was incubated with CD_3OD (1 mL) for 24 h at room temperature in order to exchange the labile protons completely. An aliquot of the reaction mixture (about 10 µL) was diluted with CD_3OD (1 mL) and analyzed by ESI-FTICR-MS/MS using the above MS conditions. Prior to analysis, CD_3OD (1 mL) was injected into the ESI source in order to remove active hydrogen atoms in the mass spectrometer system.

RESULTS AND DISCUSSION

ESI-MS and ESI-MS^n analysis of $[M+Na]^+$ ions of α-amino phosphonates

The ESI-MS^n data of the sodium adducts $[M+Na]^+$ of compounds **1–20** are summarized in Table 1. All α-amino phosphonates shared a general fragmentation pathway. As a representative example, the positive ion mode ESI mass spectra of compound **1** are shown in Fig. 1, and the corresponding fragmentation pathways are shown in Scheme 2(A). In ESI-MS/MS, the precursor $[M+Na]^+$ ion at *m/z* 384 fragmented to produce two abundant product ions at *m/z* 218 and 189 through cleavage of the P–C bond. Furthermore, by neutral loss of propene (42 Da), a minor product ion was observed at *m/z* 342, which in turn fragmented to form product ions at *m/z* 218 and 147 by cleavage of the P–C bond (Fig. 1(A)). A very low abundance product ion at *m/z* 300 was also formed by neutral loss of a second molecule of propene (Fig. 1(B)). In order to identify its structure, the product ion at *m/z* 189 was subsequently isolated and investigated by ESI-MS^3, as shown in Fig. 1(C). Two product ions at *m/z* 147 and 129 were generated by loss of either propene (42 Da) or isopropanol (60 Da), respectively. In the gas phase, the H-phosphonate ion at *m/z* 189 and the imine ion at *m/z* 218 were formed by P–C bond cleavage of compound **1** under collision conditions. Interestingly, P–C bonds could be easily formed through the Pudovik reaction used for the preparation of α-amino phosphonates by the reaction of imines with H-phosphonate under basic conditions in the solution phase.[26]

In order to determine the formulae of product ions, high-resolution ESI-FTICR-MS/MS was performed on the sodium adduct ion of compound **1** at *m/z* 384.1693 (theoretical mass 384.1704, relative error 2.8 ppm; Fig. 2 and Table 1). It was found that the measured masses of the ions at *m/z* 342, 218, and 189 (342.1224, 218.0930, and 189.0646) corresponded to the formulae $C_{17}H_{22}NNaO_3P$ (calculated 342.1235, relative

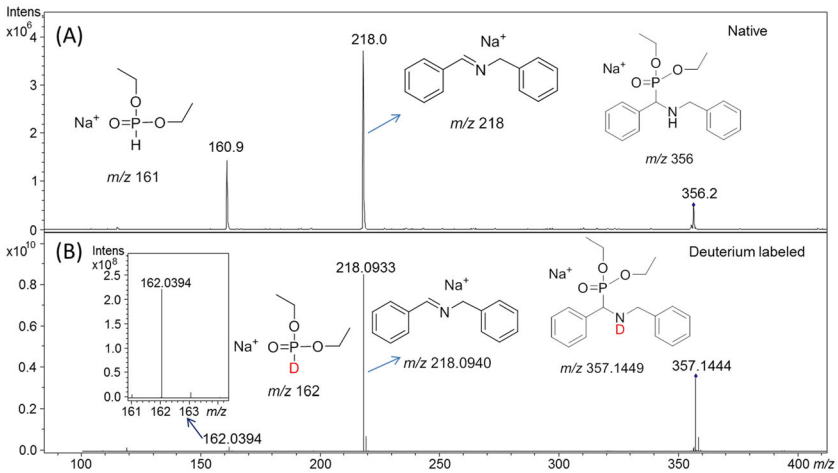


Figure 3. ESI tandem mass spectra of compound **2** in positive ion mode: (A) ESI-MS/MS of $[M+Na]^+$ ion at *m/z* 356 and (B) ESI-FT-ICR-MS/MS of deuterium-labeled $[d\text{-}M+Na]^+$ ion at *m/z* 357.1449.

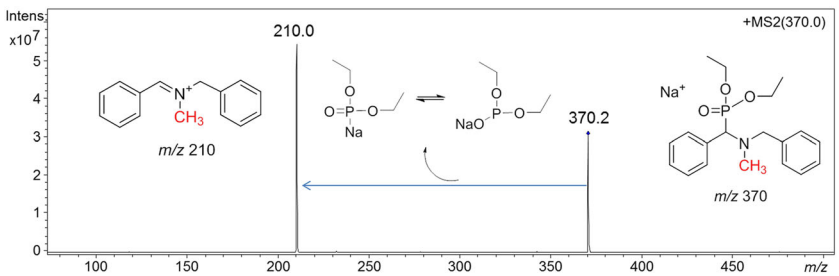


Figure 4. ESI tandem mass spectrum of $[M+Na]^+$ ion of compound **9** at *m/z* 370.

Rapid Communications in Mass Spectrometry

error 3.2 ppm), $C_{14}H_{13}NNa$ (calculated 218.0946, relative error 7.3 ppm), and $C_6H_{15}NaO_3P$ (calculated 189.0657, relative error 5.8 ppm), respectively, in agreement with the proposed structures shown in Scheme 2(A).

Mechanisms of P–C bond cleavage and intramolecular hydrogen migration

Based on these experimental results, a general intramolecular rearrangement mechanism is proposed. As shown in Scheme 2(B), the active hydrogen of the amino group might be coordinated with the phosphoryl oxygen atom to form an intermediate ion **1a** with a five-membered ring. Subsequently, the P–C bond would be cleaved in parallel with hydrogen-atom transfer to form the trivalent phosphite ion **1b** and imine ion **1c** in a coordinated process. Moreover, **1b** could exist as three exchangeable isomers, **1d**, **1e**, and **1f**, which might favor its stability, resulting in its high relative abundance of up to 100%. Based on this proposed mechanism of rearrangement, the formation of the bi-coordinated phosphate ion at *m/z* 129 could also be explained by neutral loss of propanol from **1b**. The five-membered phosphonate ring proposed is characteristic of the above intramolecular hydrogen migration, which is close to that in the McLafferty rearrangement with a six-membered ring in the transition state.[27]

Table 2. ESI-MS^n data of $[M+H]^+$ ions of α-amino phosphonates 1–20

Compounds	Molecular weight	Precursor ions (*m/z*)	Imine ions (*m/z*, relative intensity percentage)
1	361	362	196 (100)
2	333	334	196 (100)
3	305	306	196 (100)
5	378	379	241 (100)
6	363	364	226 (100)
7	367	368	230 (100)
8	351	352	214 (100)
9	347	348	210 (100)
10	327	328	190 (100)
11	297	298	160 (100)
12	325	326	188 (100)
14	299	300	162 (100)
16	339	340	202 (100)
17	334	335	197 (100)
18	359	360	222 (100)
19	339	340	202 (100)
20	305	306	168 (100)

In order to test the proposed hydrogen migration mechanism of α-amino phosphonates, compound **2** with a diethyl phosphate group was analyzed by ESI-MS/MS in combination with deuterium labeling. As shown in Fig. 3(A), two abundant product ions at *m/z* 218 and 161 were produced from the precursor $[M+Na]^+$ ion at *m/z* 356 by cleavage of the P–C bond. By comparison with the fragmentation pathways of compound **1**, no alkyl group loss was observed for compound **2**. In addition, compound **2** was labeled with deuterium via solution-phase hydrogen/deuterium exchange prior to ESI-FTICR-MS analysis. After labeling, the $[M+Na]^+$ ion of compound **2** was observed at *m/z* 357.1444 (theoretical mass 357.1454, relative error 2.8 ppm), a 1 u increase, which is consistent with the structure of compound **2** with one labile hydrogen atom. As shown in Fig. 3(B), the product ion at *m/z* 162.0394 (theoretical mass 162.0406, relative error 7.4 ppm) was also increased by 1 u from the product ion at *m/z* 161 without deuterium labeling (Fig. 3(A)), indicating that the deuterium atom of the precursor ion might be transferred to the phosphoryl group. Meanwhile, an imine ion was observed at *m/z* 218.0940 (theoretical mass 218.0946, relative error 2.7 ppm), suggesting that its structure was the same as that of the native product ion. In summary, cleavage of the P–C bond of the α-amino phosphonate occurs simultaneously with the intramolecular hydrogen atom migration.

To study the hydrogen migration pathway further, three α-amino phosphonates (compounds **9**, **10**, and **11**) without the active hydrogen atom were synthesized and studied by ESI-MS/MS (Table 1). As a representative, the ESI-MS/MS spectrum of the sodium adduct of compound **9** in positive ion mode is shown in Fig. 4. Only one product ion at *m/z* 210, corresponding to an imine ion, was generated from the precursor ion by a neutral loss of 160 Da through cleavage of the P–C bond. However, no rearrangement ion was detected, indicating that the methyl group in the molecule could not migrate to the phosphoryl group.

ESI-MS/MS analysis of $[M+H]^+$ ions of α-amino phosphonates

In comparison with the fragmentation pathways of $[M+Na]^+$ ions, the ESI-MS/MS spectra of the protonated α-amino phosphonates were systematically determined and summarized in Table 2. It was found that the fragmentation pathways of $[M+H]^+$ ions were simpler than those of the sodium adducts. As shown in Fig. 5, the precursor $[M+H]^+$ ion at *m/z* 362

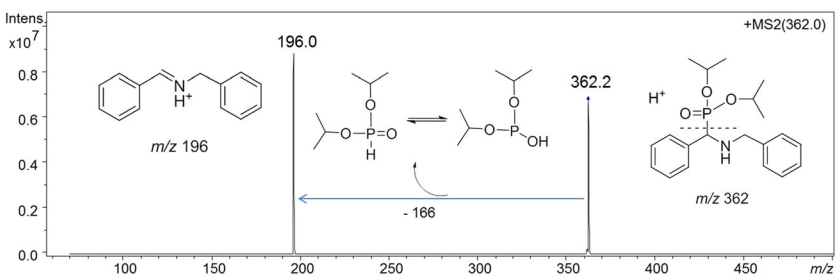


Figure 5. ESI tandem mass spectrum of $[M+H]^+$ ion of compound **1** at *m/z* 362 in positive ion mode.

fragmented to produce a product ion at *m/z* 196 by neutral loss of one molecule of diisopropyl H-phosphite (166 Da) through cleavage of the P–C bond with an active hydrogen-atom transfer. However, different from the fragmentation pathways of sodium adducts, the $[M+H]^+$ ion of the diisopropyl H-phosphite could not be detected for all the compounds investigated. It is postulated that the different coordination affinities of the sodium ion and the proton with the phosphoryl group in the gas phase might be the key factor responsible for the appearance of characteristic H-phosphite ions.

CONCLUSIONS

α-Amino phosphonates with different chemical structures were successfully synthesized and analysed by ESI-MS/MS in combination with deuterium labeling. An intramolecular hydrogen atom migration between the amino and phosphoryl groups was observed, with cleavage of the P–C bond through a five-membered-ring intermediate. The possible migration mechanism and structures of key product ions were confirmed by hydrogen/deuterium exchange and high-resolution FTICR-MS/MS. In view of these experimental results, selective cleavage of P–C bonds might be a general process for α-amino phosphonates under our mass spectrometry conditions. These characteristic fragmentation pathways might not only provide some insights into the basic chemistry of P–C bonds, but also have some potential applications in the structural determination of α-amino phosphonate analogues.

Acknowledgements

This work was supported by the Chinese National Natural Science Foundation (21305115, 21375113, and 21173178).

REFERENCES

[1] M. Horiguchi, M. Kandatsu. Isolation of 2-aminoethane phosphonic acid from rumen protozoa. *Nature* **1959**, *184*, 901.
[2] E. D. Naydenova, P. T. Todorov, K. D. Troev. Recent synthesis of aminophosphonic acids as potential biological importance. *Amino Acids* **2010**, *38*, 23.
[3] K. S. Ju, J. R. Doroghazi, W. W. Metcalf. Genomics-enabled discovery of phosphonate natural products and their biosynthetic pathways. *J. Ind. Microbiol. Biotechnol.* **2014**, *41*, 345.
[4] J. S. Kittredge, E. Roberts. A carbon-phosphorus bond in nature. *Science* **1969**, *164*, 37.
[5] S. C. Fields. Synthesis of natural products containing a C-P bond. *Tetrahedron* **1999**, *55*, 12237.
[6] H. M. Seidel, S. Freeman, H. Seto, J. R. Knowles. Phosphonate biosynthesis: isolation of the enzyme responsible for the formation of a carbon-phosphorus bond. *Nature* **1988**, *335*, 457.
[7] E. D. Naydenova, P. T. Todorov, K. D. Troev. Recent synthesis of aminophosphonic acids as potential biological importance. *Amino Acids* **2010**, *38*, 23.
[8] O. I. Kolodiazhnyi. Asymmetric synthesis of hydroxyphosphonates. *Tetrahedron Asymmetry* **2005**, *16*, 3295.
[9] A. Mucha, P. Kafarski, L. Berlicki. Remarkable potential of the α-aminophosphonate/phosphinate structure motif in medicinal chemistry. *J. Med. Chem.* **2011**, *54*, 5955.
[10] D. Bonarska, H. Kleszczynska, J. Sarapuk. Antioxidative activity of some phenoxy and organophosphorous compounds. *Cell. Mol. Biol. Lett.* **2002**, *7*, 929.
[11] J. Grembecka, A. Mucha, T. Cierpicki, P. Kafarski. The most potent organophosphorus inhibitors of leucine aminopeptidase. Structure-based design, chemistry, and activity. *J. Med. Chem.* **2003**, *46*, 2641.
[12] J. Parrish, L. Tong, M. Wang, X. Chen, E. B. Lansdon, C. Cannizzaro, X. Zheng, M. C. Desai, L. Xu. Synthesis and biological evaluation of phosphonate analogues of nevirapine. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1493.
[13] G. Forlani, A. Occhipinti, L. Berlicki, G. Dziedziola, A. Wieczorek, P. Kafarski. Tailoring the structure of aminobisphosphonates to target plant P_5C reductase. *J. Agric. Food. Chem.* **2008**, *56*, 3193.
[14] V. Agarwal, S. A. Borisova, W. W. Metcalf, W. A. van der Donk, S. K. Nair. Structural and mechanistic insights into C-P bond hydrolysis by phosphonoacetate hydrolase. *Chem. Biol.* **2011**, *18*, 1230.
[15] S. C. Peck, W. A. van der Donk. Phosphonate biosynthesis and catabolism: a treasure trove of unusual enzymology. *Curr. Opin. Chem. Biol.* **2013**, *17*, 580.
[16] H. Seto, K. Tomohisa. Bioactive natural products with carbon-phosphorus bonds and their biosynthesis. *Nat. Prod. Rep.* **1999**, *16*, 589.
[17] J. W. McGrath, J. P. Chin, J. P. Quinn. Organophosphonates revealed: new insights into the microbial metabolism of ancient molecules. *Nat. Rev. Microbiol.* **2013**, *11*, 412.
[18] X. Gao, F. Ni, J. Bao, Y. Liu, Z. Zhang, P. X. Xu, Y. F. Zhao. Formation of cyclic acylphosphoramidates in mass spectra of N-monoalkyloxyphosphoryl amino acids using electrospray ionization tandem mass spectrometry. *J. Mass Spectrom.* **2010**, *45*, 779.
[19] X. Gao, Z. P. Zeng, P. X. Xu, G. Tang, Y. Liu, Y. F. Zhao. Comparison of the isomeric α-amino acyl adenylates and amino acid phosphoramidates of adenosine by electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 291.
[20] J. Chen, Y. Chen, Y. Jiang, H. Fu, Y. F. Zhao. Rearrangement of P-N and P-O bonds in mass spectra of N-diisopropyloxyphosphoryl amino acids/alcohols. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 1936.
[21] S. Cao, J. Zhang, J. Xu, X. Liao, Y. Zhao. Electrospray tandem mass spectrometric studies of all twenty N-phosphoryl amino acids. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 2237.
[22] Z. Zeng, P. Luo, Y. Jiang, Y. Liu, G. Tang, P. Xu, Y. Zhao. A novel hydrogen migration of dialkylphosphonic acid esters using electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 3314.
[23] H. Fang, M. J. Fang, C. J. Zhu, L. N. Liu, Y. F. Zhao. Study on [(4-substituted-benzoyl amino)-phenyl-methyl] phosphonic acid diisopropyl esters under electrospray ionization tandem mass spectrometric conditions. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 3629.
[24] Z. Zheng, Y. Liu, Z. Cai, H. Zhang, H. Fang, M. Fang, Y. Zhao. Electrospray ionization tandem mass spectrometry of phosphonate peptides. *Eur. J. Mass Spectrom.* **2010**, *16*, 503.
[25] Y. Gao, Z. Huang, R. Zhuang, J. Xu, P. Zhang, G. Tang, Y. Zhao. Direct transformation of amides into α-amino phosphonates *via* a reductive phosphination process. *Org. Lett.* **2013**, *15*, 4214.
[26] A. N. Pudovik, I. V. Konovalova. Addition reactions of esters of phosphorus(III) acids with unsaturated systems. *Synthesis* **1979**, *81*.
[27] F. W. McLafferty. Mass spectrometric analysis. Molecular rearrangements. *Anal. Chem.* **1959**, *31*, 82.

