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An intramolecular benzyl rearrangement of 1-(*N*-benzyloxycarbonylamino)alkylphosphonate diesters under electrospray ionization conditions

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The mass spectrometric behavior of eleven 1-(*N*-benzyloxycarbonyl(Cbz)amino)alkylphosphonate diesters was studied under positive ion electrospray ionization (ESI) conditions. Their fragmentation pathways are depicted and supported by tandem mass spectrometry. Besides the common eliminations of ether, benzyl alcohol, phosphite and an ether plus benzyl alcohol from molecular ions, the title compounds show a tendency to undergo an interesting intramolecular benzyl rearrangement to yield benzylphosphonate ions. The fragmentation patterns do not depend on the substituent attached to the α-carbon atom. Copyright © 2003 John Wiley & Sons, Ltd.

Phosphonate esters are recognized as an important class of enzyme inhibitors either as transition-state analogues[1–4] or as nonhydrolyzable phosphate surrogates.[5] They are also widely used in the design of transition-state analogues as haptens for the production of catalytic antibodies with esterase or amidase activity.[6–10] To date, a number of synthetic methods for the preparation of 1-aminoalkylphosphonate esters have been reported.[11–16]

The mass spectra of aminoalkylphosphonic acids have been studied either by derivatization[17–19] or by chemical ionization (CI).[20] Yuan *et al.* investigated the electron impact mass spectrometry (EI-MS) of diethyl and diisobutyl 1-(*N*-benzyloxycarbonyl(Cbz)amino)arylphosphonates, and discussed their fragmentation patterns in detail.[21] In Yuan's report,[21] the main pathway of the fragmentation of these compounds involves the cleavage of the C–P bond in competition with debenzylation that is the predominant process in all cases providing the fragment ion at *m/z* 91 as the base peak (Scheme 1). In addition to the fragments given in Scheme 1, the fragment ions at *m/z* 109 and 81 are also formed for all diethyl phosphonate compounds with discernible abundances. These may be due to the process showed in Scheme 2. In the present paper, eleven dimethyl and diethyl 1-(*N*-Cbz-amino)alkylphosphonates were studied by positive ion electrospray ionization mass spectrometry in conjunction with tandem mass spectrometry (ESI-MS^n) and they showed different fragmentation mechanisms from the previous report.[21]

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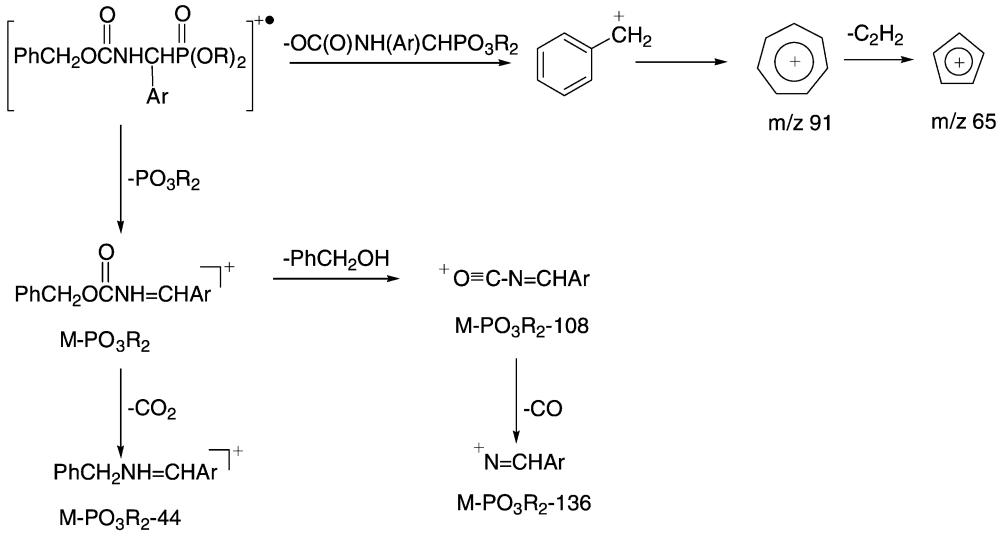
MATERIALS AND METHODS

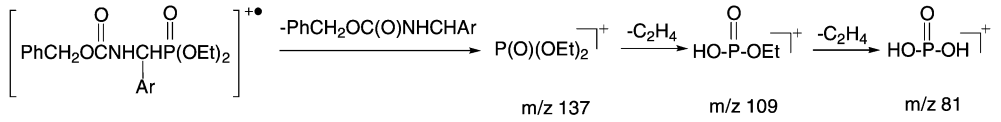
Dimethyl and diethyl 1-(*N*-Cbz-amino)alkylphosphonates were prepared by the method described in the literature.[18] The mass spectra were acquired using a Bruker ESQUIRE-LC™ ESI ion trap spectrometer equipped with a gas nebulizer probe, capable of analyzing ions up to *m/z* 6000. The experiments were operated in positive mode as follows: nitrogen was used as a drying gas at a flow rate of 4 L/min; nebulizer pressure 7 psi; capillary voltage 4 kV; heated capillary temperature 300°C. The samples dissolved in methanol were ionized by ESI and continuously infused into the ESI chamber at a flow rate of 4 μL/min using a Cole-Parmer 74900 syringe pump (Cole-Parmer Instrument Company). The scan range of the ions is *m/z* 50–500 and cutoff mass 50 was used during ion accumulation. The ions of the mass-to-charge (*m/z*) ratio of interest were isolated and fragmented by collision with helium to obtain MS^n spectra. The fragmentation amplitude values were 0.5–1.0 V and the fragmentation time was 40 ms. All of the sodium adduct ions were yielded from endogenous sources.

RESULTS AND DISCUSSION

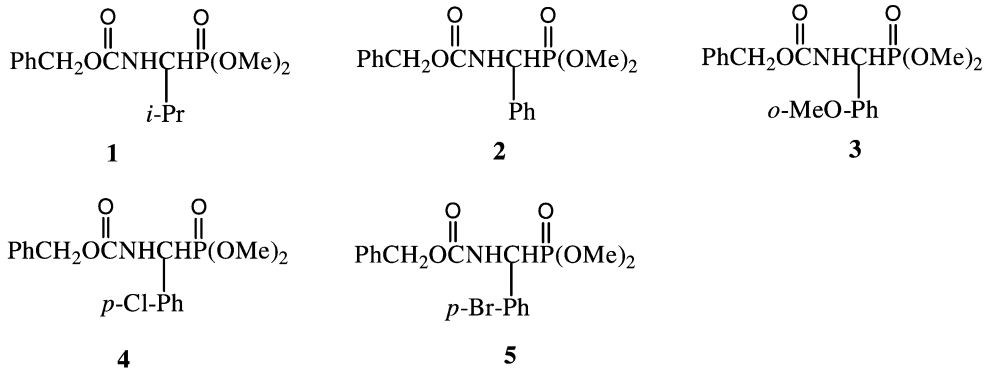
The mass spectra can be arranged into two different groups according to the substituent alkoxyl groups attached to the phosphorus atom (Schemes 3 and 6). The most important fragment ions of dimethyl and diethyl 1-(*N*-Cbz-amino)alkylphosphonates are listed in Tables 1 and 2, respectively. The peaks are arranged in columns according to their compositions or to the nature of the fragments lost. Both dimethyl



Scheme 1. EI-MS fragmentation pathways of diethyl and diisobutyl 1-(*N*-Cbz-amino)arylphosphonates.



Scheme 2. Another EI-MS fragmentation pathway of diethyl 1-(*N*-Cbz-amino)arylphosphonates.



Scheme 3. Dimethyl 1-(*N*-Cbz-amino)alkylphosphonates **1**–**5**.

and diethyl 1-(*N*-Cbz-amino)alkylphosphonates gave molecular ion sodium adducts with high intensity and had a tendency to form fragment ion sodium adducts in tandem mass spectra. We tried to increase the abundance of protonated ions ($[M+H]^+$) by adding acetic acid to the methanolic solution of compound **1**. However, even when the concentration of acetic acid was increased up to 50%, the relative intensity of the $[M+H]^+$ ion was only 0.03%.

Dimethyl 1-(*N*-Cbz-amino)alkylphosphonates

All of the compounds **1**–**5** show similar fragmentation pathways under positive ion ESI conditions. There are four dominant fragmentation patterns in the spectra of these five compounds (Scheme 4). One is the cleavage of the C–P bond to generate a pair of complementary ions, the dimethylphosphite sodium adduct $[HP(O)(OMe)_2+Na]^+$ at *m/z* 133 and $[M+Na-110]^+$ in which the two components competed for the Na^+ cation. Isocyanate derivative $[M+Na-108]^+$ ions could be obtained from $[M+Na]^+$ through the elimination of a benzyl alcohol. By subsequent expulsion of the dimethyl ether, $[M+Na-154]^+$ species were observed in the further isolation and fragmentation of the $[M+Na-108]^+$ ion (MS^3 experiment). Another process to yield $[M+Na-154]^+$ ions is due to the loss of both benzyl alcohol and dimethyl ether simultaneously from $[M+Na]^+$.

The most interesting ion is the common ion of all five compounds **1**–**5** at *m/z* 223, whose formation could be explained by an intramolecular benzyl migration. The study of the rearrangements of organic cations in the gas phase has been an active field of research for more than five decades.[22–26] Benzyl group migration from the urethane moiety to nitrogen in CID of protonated Cbz-protected amino acids during FAB-MS has been verified.[27] For Cbz-blocked

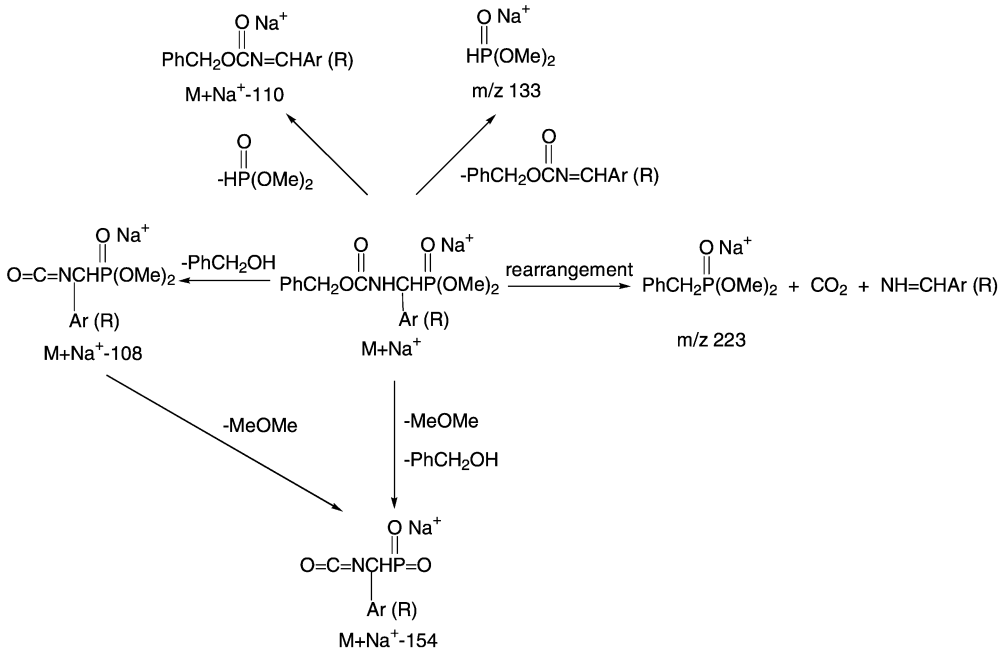
Table 1. Fragment ions observed in the tandem mass spectra of sodium adducts of compounds **1–5**

	Precursor ions	Fragment ions and relative intensity percentage (in parentheses)				
Compounds	$M+Na^+$	$M+Na^+-108$	$M+Na^+-110$	$M+Na^+-154$	$PhCH_2P(O)(OMe)_2+Na^+$	$HP(O)(OMe)_2+Na^+$
1	338 (100)	230 (92)	228 (27)	184 (29)	223 (26)	133 (11)
2	372 (12)	264 (92)	262 (52)	218 (100)	223 (21)	133 (16)
3	402 (30)	294 (28)	292 (100)	248 (33)	223 (3)	N/A
4	406 (33)	298 (100)	296 (48)	252 (94)	223 (40)	133 (34)
5	452 (81)	344 (97)	342 (100)	298 (85)	223 (48)	133 (21)

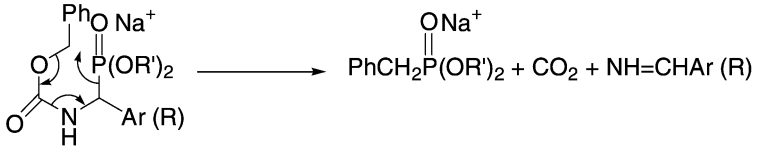
Table 2. Fragment ions observed in tandem mass spectra of sodium adducts of compounds **6–11**

	Precursor ions	Fragment ions and relative intensity percentage (in parentheses)							
Compounds	$M+Na^+$	$M+Na^+-28$	$M+Na^+-74$	$M+Na^+-108$	$M+Na^+-138$	$M+Na^+-182$	$M+H^+-182$	$PhCH_2P(O)(OEt)_2+Na^+$	$HP(O)(OEt)_2+Na^+$
6	366 (28)	338 (80)	292 (61)	258 (100)	228 (15)	184 (72)	162 (22)	251 (19)	161 (17)
7	400 (34)	372 (83)	326 (44)	292 (100)	262 (29)	218 (79)	196 (27)	251 (13)	161 (21)
8	414 (33)	386 (13)	340 (8)	306 (100)	276 (5)	232 (33)	210 (1)	251 (3)	161 (1)
9	430 (53)	402 (16)	356 (16)	322 (87)	292 (100)	248 (60)	226 (16)	251 (4)	161 (2)
10	434 (36)	406 (100)	360 (37)	326 (85)	296 (23)	252 (54)	230 (20)	251 (18)	161 (41)
11	480 (77)	452 (100)	406 (36)	372 (60)	342 (22)	298 (43)	276 (23)	251 (38)	161 (51)

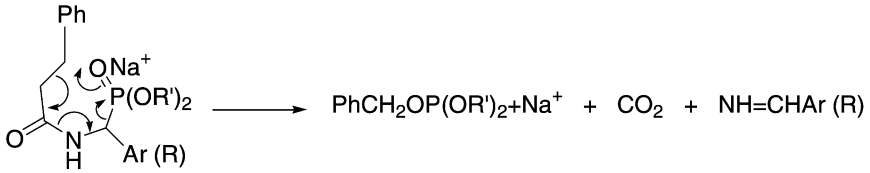
dipeptides, two competing pathways with the migration of the benzyl group of the urethane either to the terminal or to the amidic nitrogen have been documented.[28] The mechanism has been proposed as a four-membered ring transition state or as a seven-membered cyclic transition state. In the former, N-terminal nitrogen attacks the benzylic carbon as a nucleophile, and, in the latter, an amide nitrogen plays the same role.[27,28] Recently, a novel gas-phase substituted benzyl rearrangement from a imidazole nitrogen to a amine nitrogen atom in a farnesyl tranferase inhibitor has been reported and a intramolecular nucleophilic displacement mechanism was proposed for the rearrangement.[29] More recently, a benzyl transference from oxygen to the amidic nitrogen atom of a growth hormone secretagogue has also been reported by Qin;[30] this phenomenon was explained in terms of the proposed ion-neutral-complex mechanism. The studies by Mandelbaum's group on protonated dibenzyl derivatives containing benzyl–oxygen, –sulfur and –nitrogen bonds demonstrated that the extent of the benzyl rearrangement process from a heteroatom to a aromatic carbon atom strongly depends on the nature of the benzyl–heteroatom bonds,[31] which appear to decrease in the order O > S > N. It



Scheme 4. Fragmentation patterns of sodium adducts of compounds **1–5**.

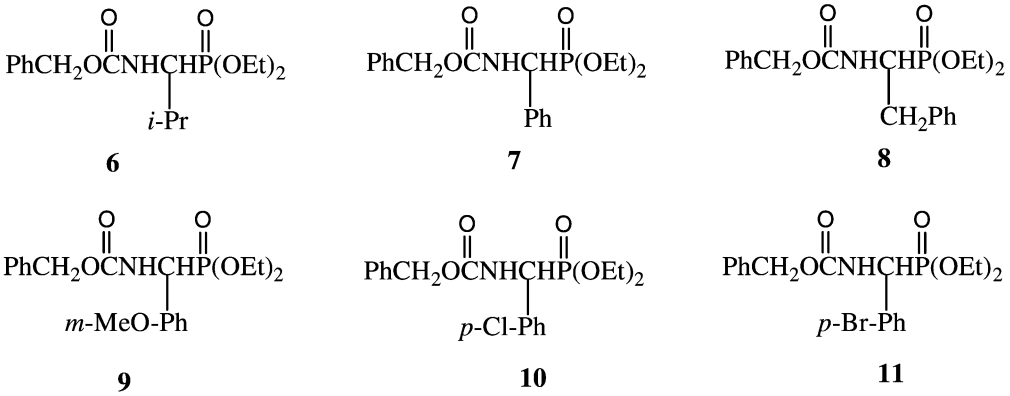


(a) six-membered ring transition state



(**b**) seven-membered ring transition state

Scheme 5. Proposed rearrangement fragmentation of compounds **1**–**11**. (a) Six-membered ring transition state and (b) seven-membered ring transition state.



Scheme 6. Diethyl 1-(*N*-Cbz-amino)alkylphosphonates **6**–**11**.

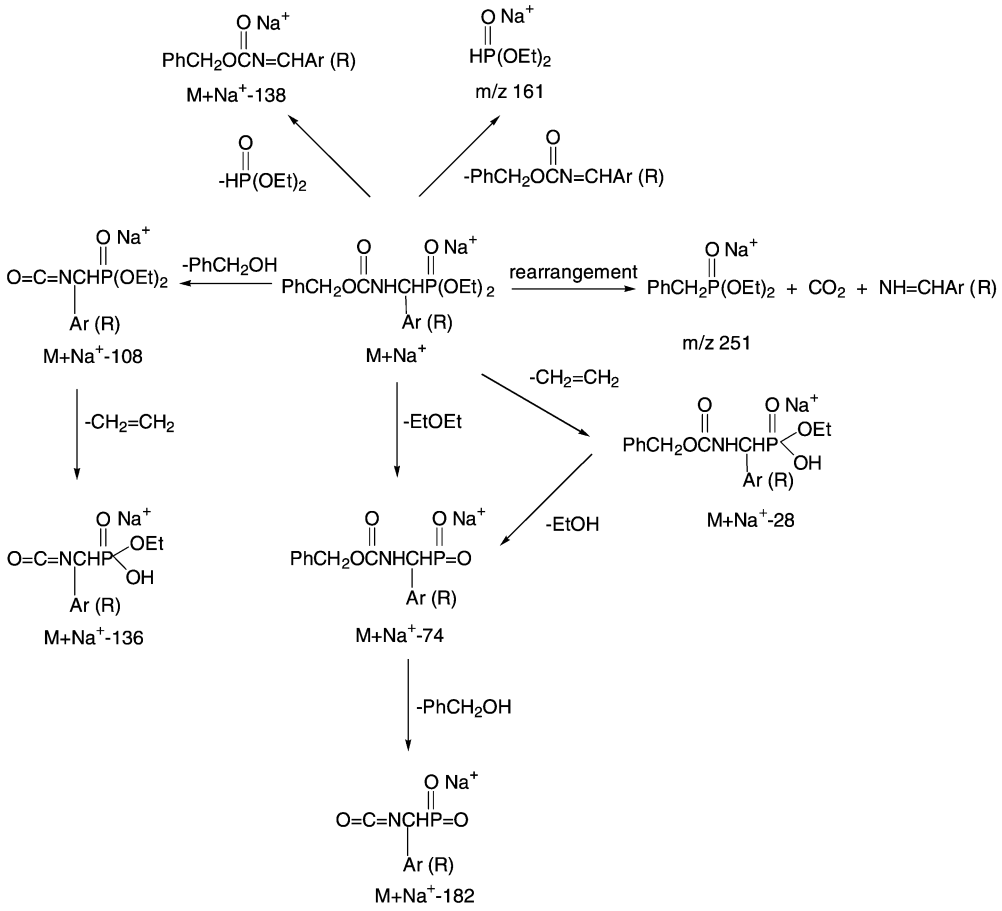
could be explained in terms of the energies of the benzyl–XH^+ bond heterolytic cleavages, which have been shown to increase in the order O < S < N.[31]

There are two possible molecular structures for the ion at *m/z* 223. One is the dimethyl benzylphosphonate sodium adduct $[PhCH_2P(O)(OMe)_2+Na]^+$, which might be formed by undergoing an intramolecular six-membered ring rearrangement and fragmentation (Scheme 5a). Another possible isomer which had the same elemental composition is the benzyl dimethyl phosphite sodium adduct $[PhCH_2OP(OMe)_2+Na]^+$ (Scheme 5b), which is formed by benzyl transfer to the oxygen atom through a seven-membered cyclic transition state. We tried to identify the structure of the ion at *m/z* 223 by comparison of fragmentation pathways of the ion at *m/z* 223 with the mass spectra of authentic samples of dimethyl benzylphosphonate and benzyl dimethyl phosphite. Unfortunately, the fragment ion peaks in further MS of the ion at *m/z* 223 were too low to be distinguished from the noise; we could not identify its structure through comparing its fragmentation with the MS/MS spectra of the authentic synthetic compounds. However, based on general organic chemistry knowledge, the six-membered ring transition state is more favorable than the seven-membered one, so we assumed that the ion at *m/z* 223 is a dimethyl benzylphosphonate ion formed through Scheme 5a. The AM1 semi-empirical calculations for heats of formation of dimethyl benzylphosphonate (−172.61 kcal/mol) and benzyl dimethyl phosphite (−160.75 kcal/mol) give alternative evidence to support the structure of dimethyl benzylphosphonate. The third piece of indirect evidence is that gained in our group's studies on the ESI-MS of aminoacylbenzylamines; elimination of formamide was observed and attributed to a transference of the benzyl group from amidic nitrogen to the N-terminal amino group. It was found that this kind of rearrangement preferred five- and six- (not seven-) membered ring transition states.[32] The generality of this rearrangement is also supported by the observation of a fragment ion at *m/z* 251 for compounds **6**–**11** (see Table 2).

MS/MS spectra of the $[M+H]^+$ ion of compound **1** show some differences from the corresponding $[M+Na]^+$ ions. The ions at *m/z* 206, 200 and 162 for compounds **1** are $[M+H-110]^+$, the protonated dimethyl benzylphosphonate ion, and $[M+H-154]^+$, respectively. However, isocyanate fragment ions $[M+H-108]^+$ and $[HP(O)(OMe)_2+H]^+$ were not observed from protonated molecular ions, while, in the MS/MS of $[M+Na]^+$, these two ions have discernible intensities.

Diethyl 1-(*N*-Cbz-amino)alkylphosphonates

Fragment ions originating from the sodium adducts of compounds **6**–**11** were determined by MS/MS or MS^3 and are summarized in Table 2. Scheme 7 shows the main



Scheme 7. Fragmentation patterns of sodium adducts of compounds **6**–**11**.

fragmentation pathways of these compounds. The C–P bond dissociates to generate the diethylphosphite sodium adduct at *m/z* 161 and its complementary sodium adduct ion $[M+Na-138]^+$. The ions $[M+Na-108]^+$ are produced from $[M+Na]^+$ through the loss of a benzyl alcohol, and can further expulse an ethylene to yield $[M+Na-136]^+$ species. As shown in Scheme 7, $[M+Na-74]^+$ results from $[M+Na]^+$ by the loss of a diethyl ether. It can also be obtained by first eliminating an ethylene to produce $[M+Na-28]^+$, subsequently undergoing an ethanol elimination process. The fragmentation of the $[M+Na-74]^+$ ions produces $[M+Na-182]^+$ by loss of a benzyl alcohol, which was supported by the results from ESI-MS/MS/MS. It is interesting to note that only protonated ions ($[M+H-182]^+$) could be observed for compounds **6–11**.

Similarly to compounds **1–5**, an intramolecular six-membered ring rearrangement and fragmentation process of compounds **6–11** yields an ion at *m/z* 251, to which the structure $[PhCH_2P(O)(OEt)_2+Na]^+$ is assigned.

In conclusion, eleven 1-(*N*-Cbz-amino)alkylphosphonate diesters were studied using positive ion electrospray ionization mass spectrometry. Their fragmentation pathways were rationalized and supported by tandem mass spectrometry. Besides the eliminations of ether, benzyl alcohol, phosphite and an ether plus benzyl alcohol from molecular ions, benzylphosphonate ions originating from an intramolecular benzyl rearrangement were observed. The fragmentation patterns do not depend on the substituent attached to the α-carbon atom, demonstrating that the substitution on the α-C plays a minor role in the fragmentation pathways.

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REFERENCES

1. Karanewsky DS, Badia MC, Cushman DW, Deforrest JM, Dejneka T, Lee VG, Petrillo EW. *J. Med. Chem*. 1990; **33**: 1459.
2. Morgan BP, Scholtz JM, Ballinger MD, Zipkin ID, Bartlett PA. *J. Am. Chem. Soc*. 1991; **113**: 297.
3. Kaplan AP, Bartlett PA. *Biochemistry* 1991; **30**: 8165.
4. Chen SJ, Liu CH, Kwon DS, Walsh CT, Coward JK. *J. Med. Chem*. 1997; **40**: 3842.
5. Biller SA, Forster C, Gordon EM, Harrity T, Scott WA, Ciosek CP. *J. Med. Chem*. 1988; **31**: 1869.
6. Jacobs JW, Schultz PG, Powell M, Sugasawara R. *J. Am. Chem. Soc*. 1987; **109**: 2174.
7. Pollack SJ, Jacobs JW, Schultz PG. *Science* 1986; **234**: 1570.
8. Tramontano A, Janda KD, Lerner RA. *Science* 1986; **234**: 1566.
9. Jacobsen JR, Schultz PG. *Proc. Natl. Acad. Sci. USA* 1994; **91**: 5888.
10. Hirschmann R, Smith AB, III, Taylor CM, Benkovic PA, Taylor SD, Yager KM, Sprengeler PA, Benkovic SJ. *Science* 1994; **265**: 234.

11. Xu JX, Yu L. *Chin. J. Synth. Chem. (Hecheng Hua Xue)* 1999; **7**: 153, and references cited therein.
12. Green D, Patel G, Elgendy S, Baban JA, Claeson G, Kakar VV, Deadman J. *Tetrahedron* 1994; **50**: 5099.
13. Green D, Patel G, Elgendy S, Baban JA, Claeson G, Kakar VV, Deadman J. *Tetrahedron* 1993; **34**: 6917.
14. Xu JX, Ma Y, Duan LF. *Heteroat. Chem*. 2000; **11**: 417.
15. Xu JX, Fu NY. *J. Chem. Soc., Perkin Trans. 1* 2001; 1223.
16. Xu JX, Wei M. *Synth. Commun*. 2001; **31**: 1498.
17. Karison KA. *Biochem. Biophys. Res. Commun*. 1970; **39**: 847.
18. Ruppel ML, Suba LA, Marvel JT. *Biomed. Mass Spectrom*. 1976; **3**: 28.
19. Huber JW. *J. Chromatogr*. 1978; **152**: 220.
20. Constantin E, Neuzil E, Traldi P. *Org. Mass Spectrom*. 1986; **21**: 431.
21. Yuan C, Chen S, Wang G. *Phosphorus, Sulfur, Silicon* 1991; **60**: 97.
22. Stevenson DP, Hipple JA. *J. Am. Chem. Soc*. 1942; **64**: 1588.
23. Brown P, Djerassi C. *Angew. Chem., Int. Ed. Engl*. 1967; **6**: 477.
24. McLafferty FW. *Org. Mass Spectrom*. 1980; **15**: 114.
25. Kingston EE, Shannon JS, Lacey MJ. *Org. Mass Spectrom*. 1983; **18**: 183.
26. Splitter JS, Turecek F. *Applications of Mass Spectrometry to Organic Stereochemistry*. VCH: New York, 1994; chap. 16.
27. Danieli B, Rubino FM, Cremonesi A. *Org. Mass Spectrom*. 1989; **24**: 225.
28. Mammoliti E, Sindona G, Uccella N. *Org. Mass Spectrom*. 1992; **27**: 495.
29. Qin XZ. *J. Mass Spectrom*. 2001; **36**: 911.
30. Qin XZ. *J. Am. Soc. Mass Spectrom*. 2002; **13**: 371.
31. Mandelbaum A, Edelson-Averbukh M. *J. Chem. Soc., Perkin Trans 2* 2000; 989.
32. Chen J, Jiang Y, Fu H, Chen Y, Cheng CM, Zhao YF. *Rapid Commun. Mass Spectrom*. 2002; **15**: 1489.