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Novel benzyl rearrangements in electrospray ionization multistage tandem mass spectra of benzyl 2′,3′-didehydro-2′,3′-dideoxythymidin-5′-yl H-phosphonate

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Several alkyl 2′,3′-didehydro-2′,3′-dideoxythymidin-5′-yl H-phosphonates were synthesized and analyzed by electrospray ionization multistage tandem mass spectrometry (ESI − MS^n). Two kinds of novel benzyl rearrangement reactions were observed in ESI − MS^2 of $[M + H]^+$, $[M + Na]^+$ and $[M + K]^+$ of benzyl 2′,3′-didehydro-2′,3′-dideoxythymidin-5′-yl H-phosphonate. Results from tandem mass spectrometry, high-resolution mass spectrometry and control experiments showed that the benzyl migration could undergo a four-membered cyclic rearrangement reaction, and benzyl was essential in the process. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: rearrangement reaction; benzyl; migration; electrospray ionization tandem mass spectrometry; benzyl 2′,3′-didehydro-2′,3′-dideoxythymidin-5′-yl H-phosphonate

INTRODUCTION

Great efforts are currently being made all to discover all kinds of new drugs in order to control AIDS. 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T) is an inhibitor of HIV reverse transcriptase and was approved by the US Food and Drug Administration (FDA) for the treatment of AIDS.[1–3] It was found, however, that there were some problems with its toxic and side effects,[4,5] drug resistance[6] and so on. It is necessary to modify the structure of d4T to improve its therapeutic potential. In our previous work, its analogues such as AZT H-phosphonates,[7] amino acid thiophosphoramidates of nucleosides[8] and 3′,5′-dithymidine phosphoramidates[9] were also analyzed by electrospray ionization multistage tandem mass spectrometry (ESI-MS^n), and the fragmentation pathways were summarized. Since d4T H-phosphonate derivatives can be potential candidates as anti-HIV drugs, it is important to study their mass spectral fragmentation pathways, which is helpful in the analysis of other similar compounds. In this work, several d4T H-phosphonate derivatives (compounds **1**–**4** in Scheme 1) were synthesized and analyzed by ESI-MS^n, their fragmentation pathways were investigated and some new rearrangements were discovered.

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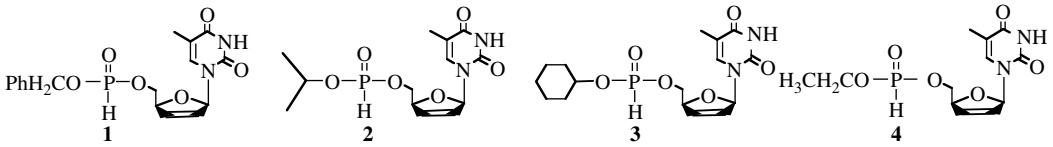
EXPERIMENTAL

Synthesis of alkyl 2′,3′-didehydro-2′,3′-dideoxythymidin-5′-yl H-phosphonate[11]

A 1 mmol amount of d4T was added to 10 mmol of PCl_3 in 10 ml of dichloromethane at −30 °C under a nitrogen atmosphere and stirred for 1 h at this temperature and for 6 h at room temperature. The solvent and excess of PCl_3 were removed under reduced pressure, then the residue was dissolved in 10 ml of dichloromethane. A 2.5 mmol amount of alcohol (different products from different alcohols) in 5 ml of dichloromethane was added dropwise to the solution at 0 °C and the reaction was completed within 30 min. A 2 mmol amount of triethylamine was added to the resulting solution, 10 min later the solvent was removed by rotary evaporation and the product was purified by column chromatography on silica gel using CH_2Cl_2–MeOH (20 : 1) as eluent.

Mass spectrometry

Mass spectra were acquired using a Bruker ESQUIRE-LC ion trap mass spectrometer equipped with a gas nebulizer probe, capable of analyzing ions up to m/z 6000. Nitrogen was used as the drying gas at a flow-rate of 4 l min^{-1}, the nebulizer pressure was 7 psi and the electrospray needle was typically held at 4 kV. The heated capillary temperatures was 300 °C. Samples dissolved in methanol were ionized by ESI and infused continuously into the ESI chamber at a



Scheme 1. Structures of compounds **1**–**4**.

flow-rate of 0.6–0.7 ml h^{-1} by a Cole-Parmer 74 900 syringe pump. The scan range was generally from m/z 50 to 500. Five scans were averaged for each spectrum. The selected $[M + H]^+$, $[M + Na]^+$ and $[M + K]^+$ ions were analyzed by MS^n. The selected ion was first isolated and then fragmented through collisions with helium to yield tandem mass spectra. The fragmentation amplitude values were 0.5–1.0 V and the fragmentation time was 40 ms. High-resolution mass spectra of benzyl 2′,3′-didehydro-2′,3′-dideoxythymidin-5′-yl H-phosphonate were recorded on a Bruker APEX II Fourier transform ion cyclotron resonance (FT-ICR) MS instrument equipped with 4.7 T super-conduction magnets and an analytical electrospray source. The samples were generally dissolved in methanol–water (1 : 1, containing 2%AcOH). All samples were analyzed using ESI in the positive ion mode at a concentration of ~30 μM.

Calculations

The geometry was optimized at the B3LYP/6–31G* level using the Gaussian 98 program. The frequency calculation of stationary conformation was also carried out at the B3LYP/6–31G* level, and only real frequency values were obtained.

RESULTS AND DISCUSSION

Positive ESI mass spectra of **1**–**4** were determined as shown in Fig. 1, and three adduct ions $[M + H]^+$, $[M + Na]^+$ and $[M + K]^+$ were observed. Their ESI-MS^n fragmentation pathways were investigated by multistage technique. For example, multistage mass spectral fragmentation pathways of **1** are shown in Schemes 2–5.

ESI-MS^n fragmentation pathways of $[M + H]^+$ for compound 1

The ESI-MS^2 fragmentation pattern of the protonated molecule $[M + H]^+$ at m/z 379 of **1** can be summarized as shown in Scheme 2, corresponding to Fig. 1(B) and (C). The precursor ion $[M + H]^+$ at m/z 379 produced a fragment ion at m/z 253 for loss of thymine and a pair of complementary ions at m/z 207 and 171 were observed. There were, however, another two ions, **D** and **F**, at m/z 217 and 297, which were not so easily explained. In order to clarify their structures, ESI-MS^3 was performed. The two ions at m/z 91 and 127 were detected in the ESI-MS^3 of the ion at m/z 217, which might be benzyl ion and protonated thymine. High-resolution ESI-MS (Fig. 2) indicated that the exact mass of ion **D** was 217.0972, corresponding to elemental composition $C_{12}N_2O_2H_{13}$, which could be the conjugate with benzyl and thymine as shown in Scheme 3. The ion **F** at m/z 297 was also identified by tandem and high-resolution (the exact mass 297.1236 corresponding to $C_{17}N_2O_3H_{17}$) MS techniques, and

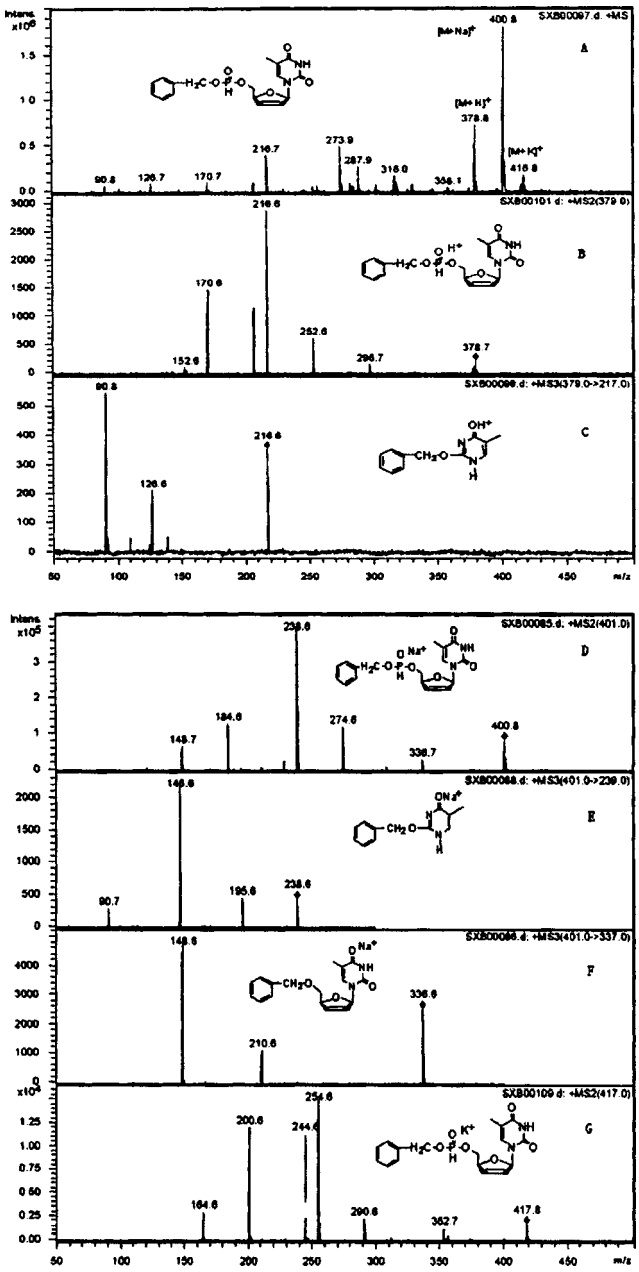
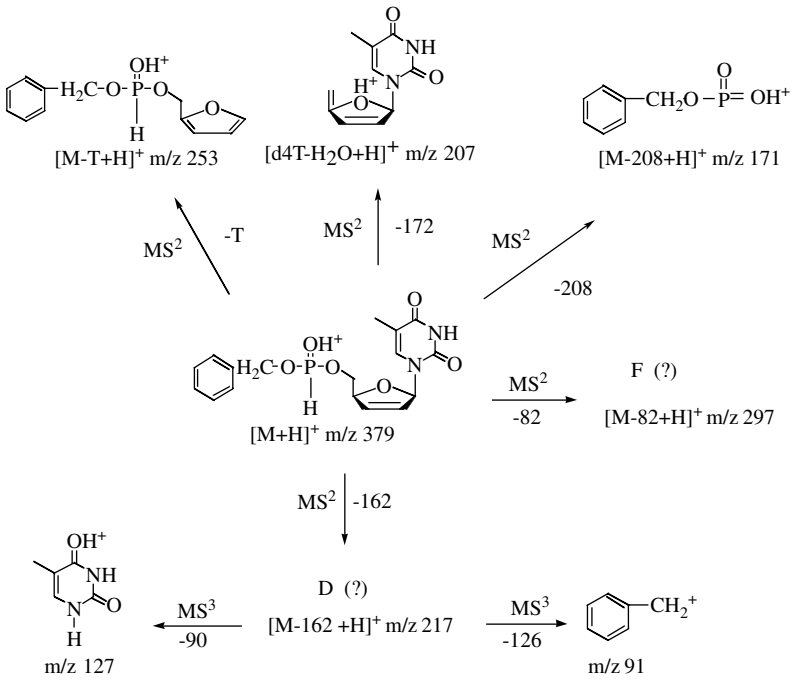
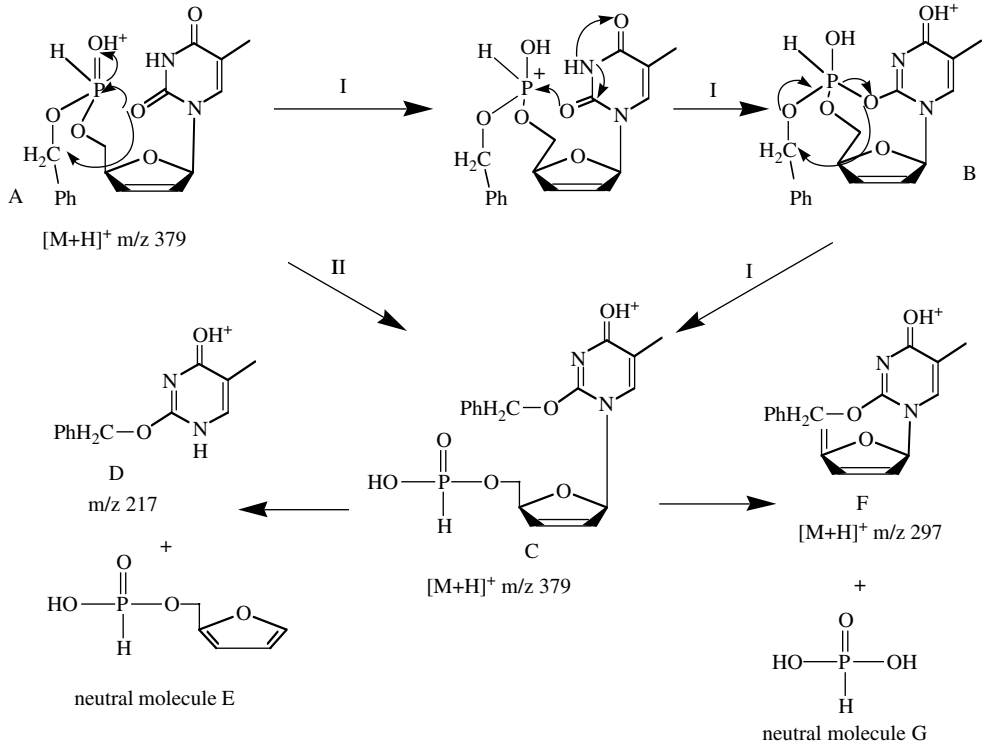


Figure 1. Mass spectra of benzyl 2′,3′-didehydro-2′,3′-dideoxythymidin-5′-yl H-phosphonate: (A) all MS; (B) MS^2 of $[M + H]^+$; (C) MS^3 of the rearrangement ion at *m/z* 217; (D) MS^2 of $[M + Na]^+$; (E) MS^3 of rearrangement ion at *m/z* 239; (F) MS^3 of the rearrangement ion *m/z* 337; (G) MS^2 of $[M + K]^+$.

its possible structure is shown in Scheme 3. In view of these experimental results, a possible mechanism was proposed (Scheme 3). Considering Scheme 3, there were two possible pathways. In one route the oxygen of thyminyl in **A** could directly attack the benzyl to produce **C**, and in the other

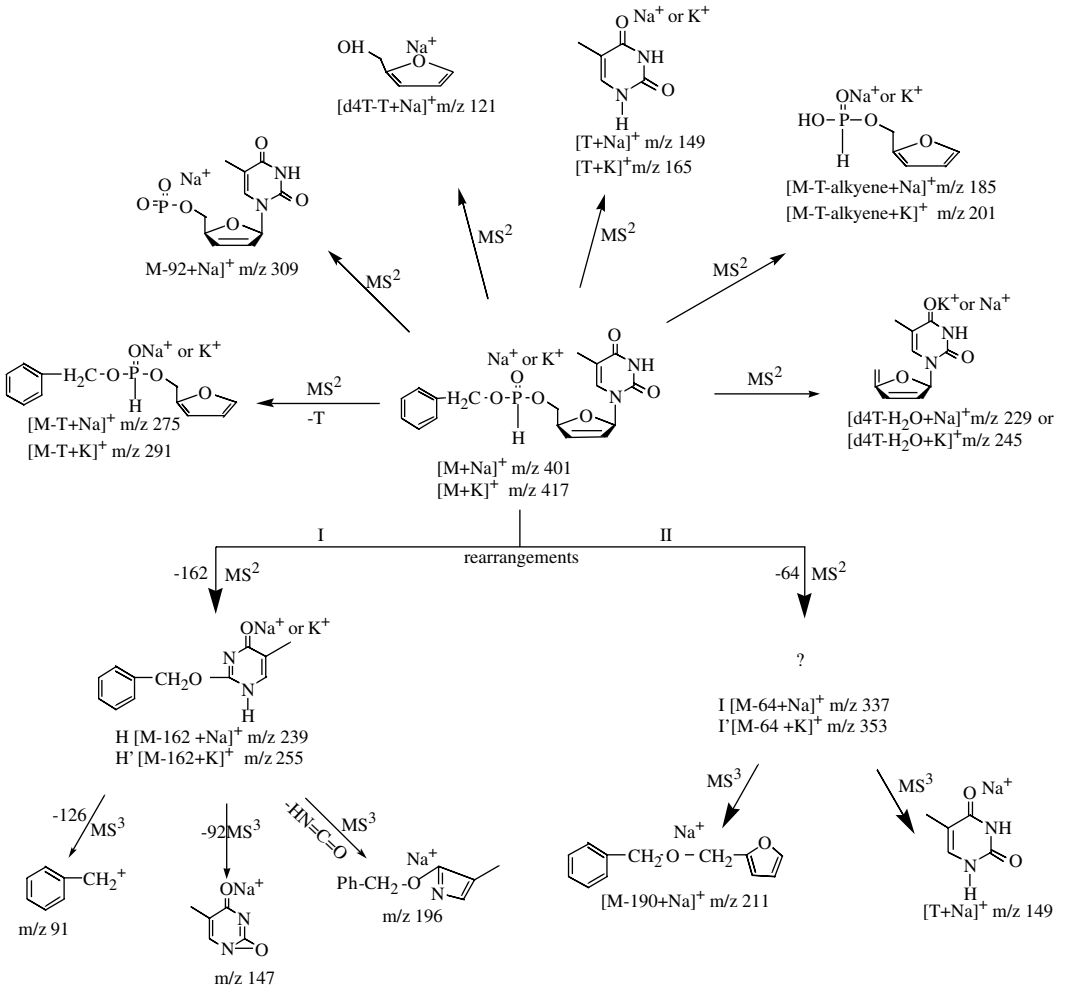


Scheme 2. Proposed fragmentation pathways of $[M + H]^+$ for benzyl 2′,3′-didehydro-2′,3′-dideoxythymidin-5′-yl H-phosphonate (corresponding to Fig. 1(B) and (C)).

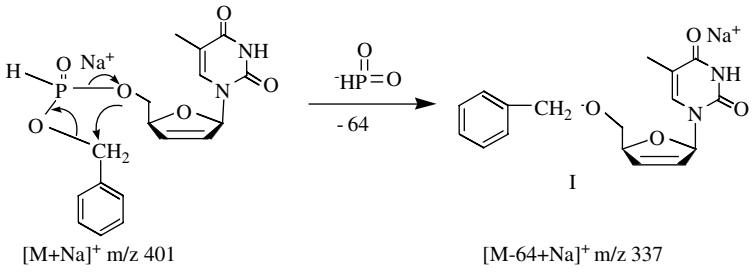


Scheme 3. Possible formation mechanism of rearrangement ions at *m/z* 217 and 297 from the precursor ion $[M + H]^+$ at *m/z* 379 in mass spectrum of benzyl 2′,3′-didehydro-2′,3′-dideoxythymidin-5′-yl H-phosphonate.

the carbonyl of the amide of thyminyl in **A** could attack the phosphorus nucleophilically to lead to pentacoordinated phosphorane intermediate **B**, the subsequent four-membered cyclic rearrangement of benzyl led to **C** and ordinary fragmentation of **C** yielded ions **D** at m/z 217 and **F** at m/z 297. The distance between benzyl and thyminyl in the protonated molecule **A** is long, so the formation mechanism of the ions **D** and **F** might involve a pentacoordinated phosphorane intermediate as shown in Scheme 3. In fact, the pentacoordinated phosphorus intermediates have been trapped[11] in previous synthetic organic chemistry, and some stable pentacoordinated phosphoranes containing



Scheme 4. Proposed fragmentation pathways of $[M + Na]^+$ and $[M + K]^+$ for benzyl 2′,3′-didehydro-2′,3′-dideoxythymidin-5′-yl H-phosphonate (corresponding to Fig. 1(D)–(G)).



Scheme 5. Possible formation mechanism of the ion at *m/z* 337 from the precursor ion $[M + Na]^+$ *m/z* 401 in the mass spectrum of benzyl 2′,3′-didehydro-2′,3′-dideoxythymidin-5′-yl H-phosphonate.

amino acid residues were synthesized and determined by MS in our previous work.[12–15] In addition, we modeled structure of benzyl 2′,3′-didehydro-2′,3′-dideoxythymidin-5′-yl H-phosphonate with Gaussian 98,[16] and the results showed that the distance (3.4 Å) between P and the oxygen of carbonyl in thymine was shorter than that (4.9 Å) between benzyl and the same oxygen, and the charge on P atom is positive (+1.128), but negative (−0.169) on the carbon of benzyl (Scheme 6 and Table 1). Hence the protonated benzyl 2′,3′-didehydro-2′,3′-dideoxythymidin-5′-yl H-phosphonate in MS might form a pentacoordinated phosphorane intermediate as shown in Scheme 3.

Table 1. The Mulliken charge and distance of some atoms in compound **1**

Charge		Distance (Å)	
C20	−0.168 776	O15—P17	3.405 065
P17	1.128 242	O15—C20	4.971 416
O15	−0.615678		

ESI-MS^n fragmentation pathways of $[M + Na]^+$ and $[M + K]^+$ for compound 1

The ESI-MS^2 fragmentation patterns of the Na^+ and K^+ adduct ions $[M + Na]^+$ at m/z 401 and $[M + K]^+$ at m/z 417

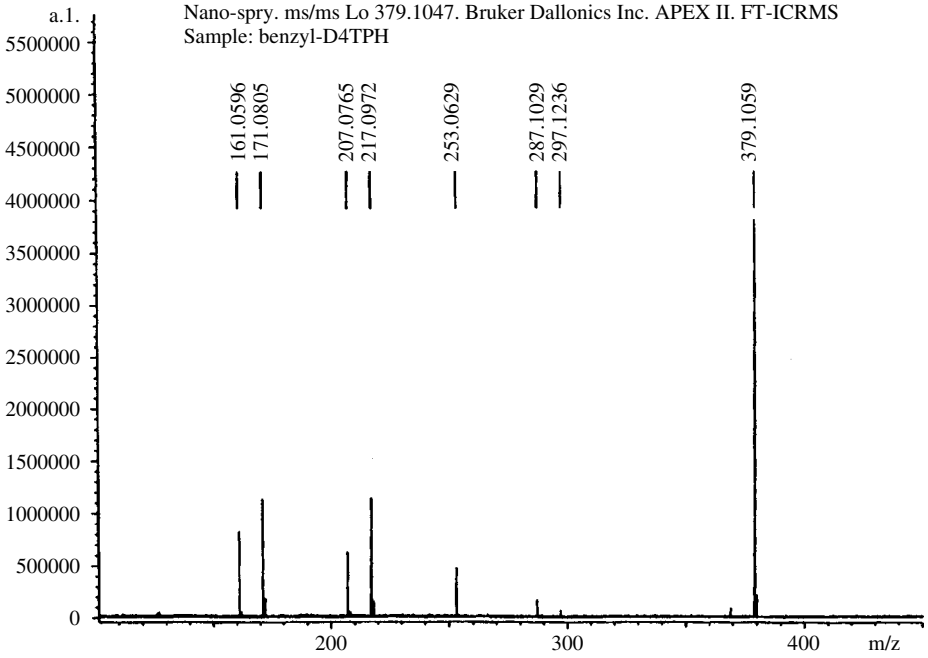


Figure 2. High-resolution MS^2 of benzyl 2′,3′-didehydro-2′,3′-dideoxythymidin-5′-yl H-phosphonate $[M + H]^+$.

Table 2. MS/MS data for d4T *O*-alkyl-H-phosphonates

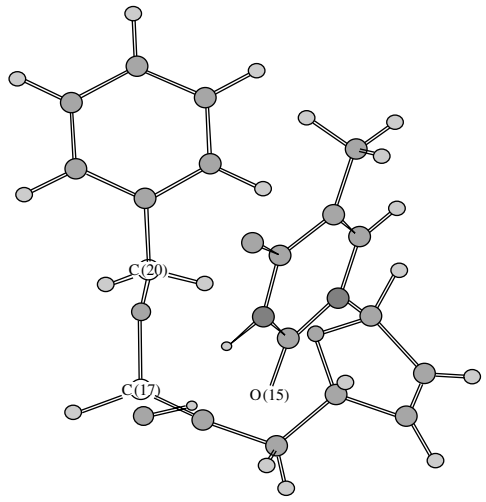
No compound	Precursor ions, *m/z*	Fragment ions, *m/z* (relative abundance, %)					Rearrangement ions[a]	
		$[M - T]^+$	$[d4T - H_2O]^+$	$[M - alkyene]^+$	$[M - T - alkyene]^+$	$[T]^+$	**I**	**II**
1 Benzyl	$[M + H]^+$ 379	253 (22)	207 (40)	—	—	—	297 (5)	
	$[M + Na]^+$ 401	275 (30)	229 (7)	—	185 (33)	149 (17)	217 (100)	211 (2)
	$[M + K]^+$ 417	291 (15)	245 (16)	—	201 (80)	165 (20)	239 (100)	337 (8)
							255 (100)	353 (9)
2 Isopropyl	$[M + H]^+$ 331	205 (81)	207 (100)	—	—	—	—	—
	$[M + Na]^+$ 353	227 (6)	—	311 (37)	185 (100)	149 (6)	—	—
	$[M + K]^+$ 369	267 (50)	245 (1)	327 (50)	201 (68)	165 (7)	—	—
3 Cyclohexyl	$[M + H]^+$ 371	245 (100)	207 (31)	—	—	—	—	—
	$[M + Na]^+$ 393	267 (1)	—	311 (74)	185 (100)	149 (1)	—	—
	$[M + K]^+$ 409	283 (9)	—	327 (94)	201 (100)	165 (5)	—	—
4 Ethyl	$[M + H]^+$ 317	191 (19)	207 (100)	—	—	—	—	—
	$[M + Na]^+$ 339	213 (77)	229 (3)	—	185 (1)	149 (100)	—	—
	$[M + K]^+$ 355	229 (100)	245 (2)	—	201 (1)	165 (3)	—	—

[a] Fragment ions **I** come from the ions from the rearrangement reaction in Scheme 3 whereas fragment ions **II** arise from the rearrangement reaction in Scheme 5.

were also investigated, as shown in Scheme 4, corresponding to Fig. 1(D)–(G). For example, the precursor ion $[M + Na]^+$ at *m/z* 401 gave the expected product ions at *m/z* 309, 275, 229, 185, 149 and 121, whose structures are shown in Scheme 4; however, ions at *m/z* 239 and 337 are unknown. ESI-MS^3 of the ion at *m/z* 239 yielded fragment ions at *m/z* 91 corresponding to benzyl ion and *m/z* 196 and 147; these results indicated that the structure of the ion at *m/z* 239 could be **H**, which is an analog of **D**, and its formation mechanism is similar to that of **D**. ESI-MS^3 fragmentation pathways of ion at *m/z* 337 were also investigated, yielding ions at *m/z* 211 and 149, the latter corresponding to the sodium adduct of thymine. The ESI-MS^n results showed that a four-membered cyclic migration of benzyl from precursor ion $[M + Na]^+$ occurred, as shown in Scheme 5. Hence the structure of the ion at *m/z* 337 could be **I**. The fragmentation pathways of $[M + K]^+$ for **1** and the MS^n rearrangements are similar to those of $[M + Na]^+$.

When benzyl was replaced with other alkyls such as isopropyl, cyclohexyl or ethyl, the rearrangement reactions could not be observed in ESI-MS^n (Table 2) because benzyl prefers to form a cation in mass MS, in contrast to alkyls, so benzyl is necessary for this kind of rearrangement.

In conclusion, two kinds of rearrangement ions were found in ESI-MS^n of benzyl 2′,3′-didehydro-2′,3′-dideoxythymidin-5′-yl H-phosphonate: the conjugate ion containing



Scheme 6. The stationary conformation of **1**. The geometry was optimized at the B3LYP/6−31G* level using the Gaussian 98 program. The frequency calculation of the stationary conformation was also carried out at the B3LYP/6−31G* level, and only real frequency values were obtained.

benzyl and furan rings came from the four-membered cyclic benzyl migration; and the complex ion with benzyl and thymine first formed a pentacoordinated phosphorane intermediate and then the four-membered cyclic rearrangement occurred. These novel rearrangements might be valuable for structure analysis of other analogs.

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