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Short communication

Electrospray ionization mass spectrum of *S*-Acyl-2-thioethyl phosphoramidate diester derivatives

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ABSTRACT

The fragmentation pathways of three *S*-Acyl-2-thioethyl (SATE) phosphoramidate diester derivatives were studied in detail on tandem electrospray ionization mass spectrometry (ESI-MS^n) in positive mode. A novel rearrangement of the SATE moiety was observed. The loss of 60 u from the SATE moiety was found in the ESI-MS^2.

1. Introduction

Acquired Immunodeficiency Syndrome (AIDS) has constituted a major threat to the health of human being. Among lots of researches seeking effective agents against HIV, AZT (3′-azido-2′,3′-dideoxythmidine) has emerged as the first RT inhibitor approved for the treatment of AIDS by FDA and one of the most promising and effective agents [1].

However, it should be noticed that AZT could also bring numerous undesirable side effects, while contributing to the fight against HIV. Some serious adverse effects include marrow toxicity, myopathy and hepatic abnormalities [2]. Moreover, AZT cannot inhibit virus production and infection of uninfected cells, though the replication of HIV could be inhibited effectively [3,4]. To overcome all those drawbacks and adverse effects of AZT, an increasing number of AZT prodrugs have been designed. The aim of prodrug includes penetration with parent compounds through membranes, release of AZT at the target site, and protection of AZT from harsh physiological environment [5–10].

Currently, most prodrugs of AZT have concentrated on the derivatization of the 5′-O position, expecting those conjugates could be hydrolyzed to AZT or AZT-MP [11]. To this end, SATE alkylamino 5′-nucleotidyl derivatives of AZT were synthesized. It is expected that the AZT-MP could be released with the SATE and alkylamino moiety hydrolyzed by carboxylesterase and phosphoramidase, respectively [12,13]. The electrospray ionization (ESI) mass spectra fragmentation pathways of those derivatives are discussed in details.

2. Experimental

2.1. Preparation of samples

All of the samples were synthesized as shown in Scheme 1. The activity of delivering AZTMP had also been tested [12].

2.2. Mass spectrometry

Mass spectra were collected on a Bruker Esquire-LC ion-trap mass spectrometer equipped with a gas nebulizer probe capable of analyzing ions up to m/z 6000. Samples were dissolved in methanol and ionized by electrospray ionization. The flow rate of samples and nitrogen as drying gas was 3 μL/min and 4 L/min, respectively. The capillary was typically held at 4 kV and the source temperature was maintained at 300 °C. The nebulizer pressure was 7 psi and the multistage mass spectrum was obtained by collision of ions with helium. The high-resolution tandem ESI-MS was conducted on the Bruker Apex-Qe-FTMS with the scanning range of 100–2000 Da.

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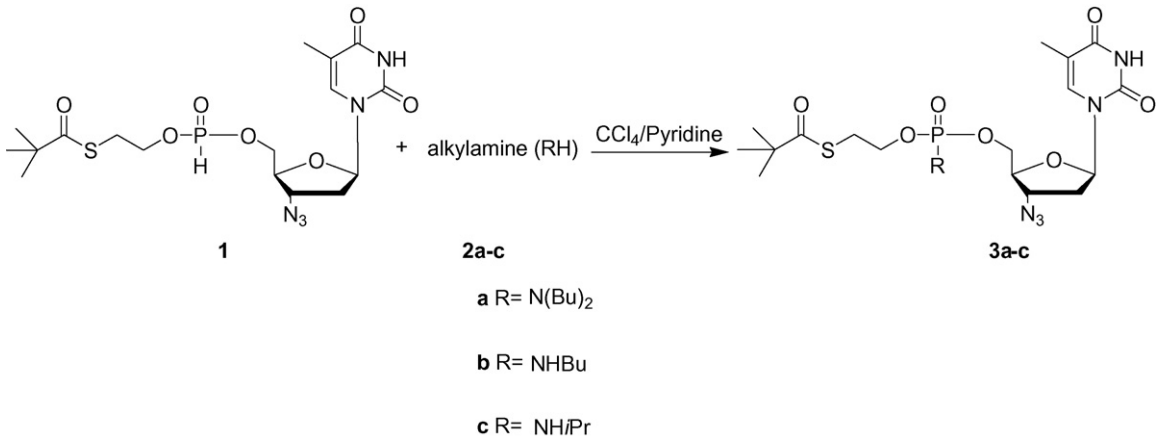
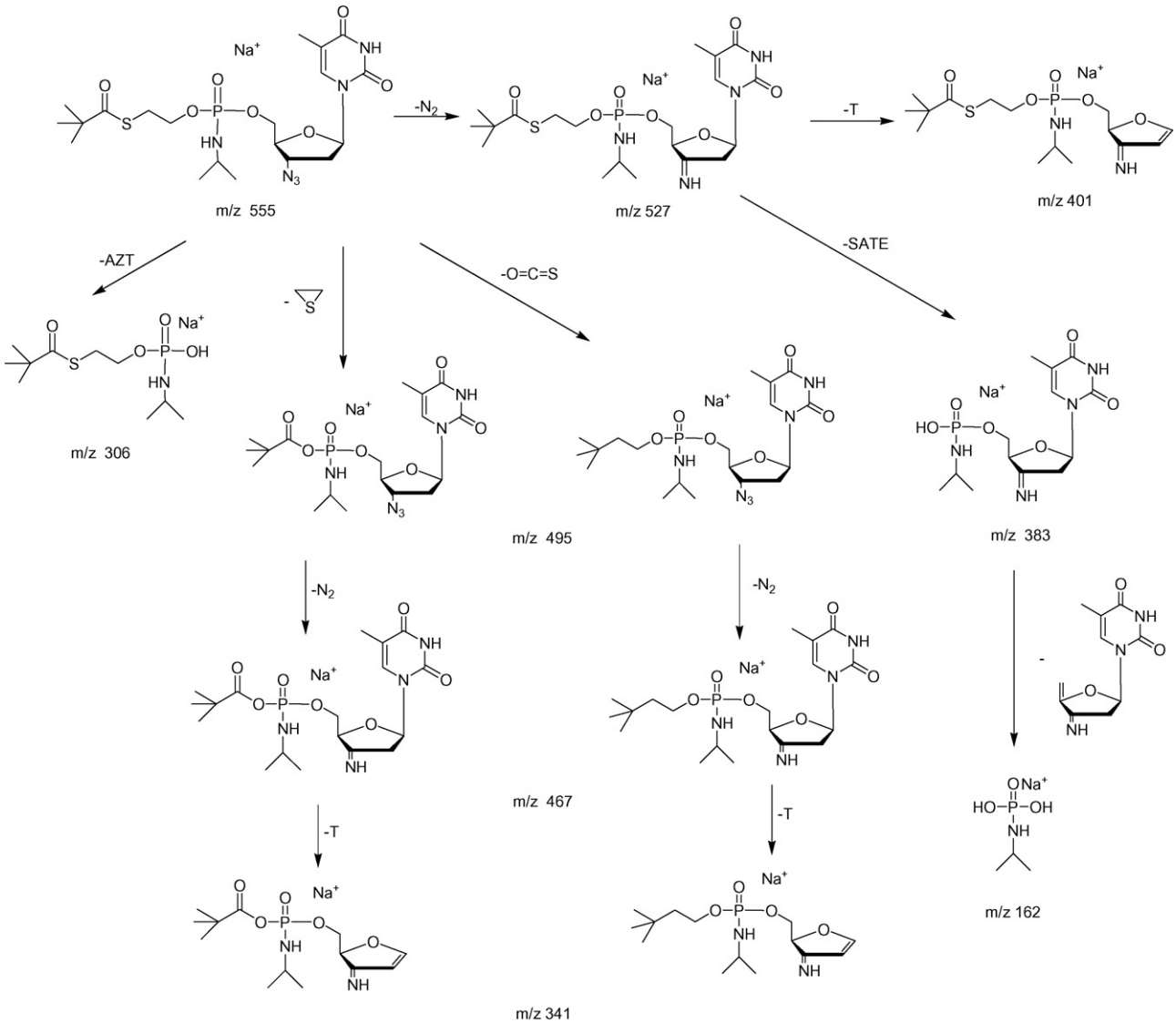


Scheme 1. Synthesis of *S*-Acyl-2-thioethyl phosphoramidate diester derivatives of AZT.



Scheme 2. Possible positive ESI-MS^2 fragmentation pathway of the Na^+ adduct at *m*/*z* 355.

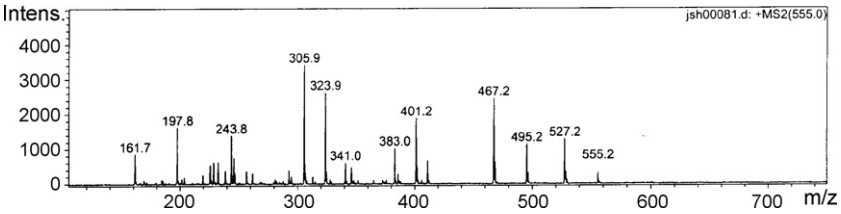


Fig. 1. ESI-MS^2 spectrum of the Na^+ adduct at m/z 555.

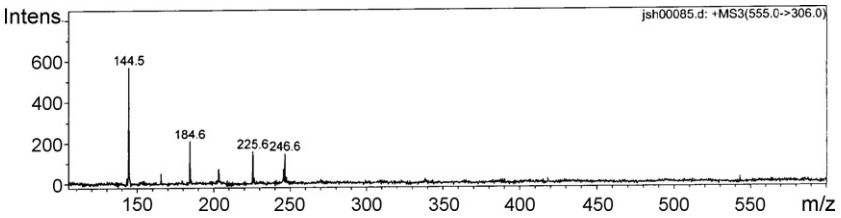


Fig. 2. ESI-MS^3 spectrum of the $[M\text{-}T\text{-}N_2+Na]^+$ ion at m/z 306.

Table 1
ESI mass spectra of compounds **3a–c**

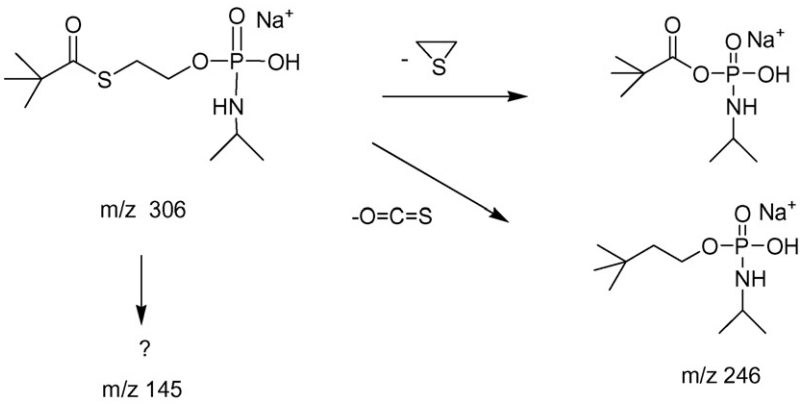
Compound	Positive ions (m/z) $[M+Na]^+$
3a	625
3b	569
3c	555

3. Results and discussion

The mass spectra of synthetic derivatives are shown in Table 1. Given that those derivatives display similar pathway of fragmentation, the following discussion would focus on compound **3c** as an example.

The positive ESI mass spectrum of **3c** mainly showed the form of Na^+ adduct. Fig. 1 is the ESI-MS^2 spectrum of the Na^+ adduct at m/z 555. As shown in Scheme 2, some characteristic fragmentation patterns of AZT derivatives are found, including losing N_2 to afford m/z 527 and losing T and N_2 to afford m/z 401 [14]. It is noteworthy that the alkylamine moiety is quite stable and no loss or change of this moiety is noticed. Also, the loss of 60 u as the characteristic fragmentation pattern of SATE moiety is observed. It has been found that under the FAB-MS condition, the SATE moiety could lose a neutral fragment of 60 u, which could be epithioethane or S=C=O [15]. We postulate that this mechanism could also be possible in ESI mass spectrum from the fragments m/z 495 observed.

However, an unexpected ion at m/z 145 was found in ESI-MS^3 spectrum of the $[M\text{-}AZT+Na]^+$ ion at m/z 306 (Fig. 2, Fig. 3



Scheme 3. Positive ESI-MS^3 fragmentation pathway of the ion at m/z 306.

Table 2
ESI-MS^n spectra of compound **3a–c**

Compounds	Precursor ions	Fragment ions and relative intensity percentage [m/z (%)]
3a	625 $[M+Na]^+$	597(73), 565(10), 537(100), 471(89), 453(10), 411(13), 244(10), 232(72)
	471 $[M\text{-}N_2\text{-}T+Na]^+$	411(36), 232(100), 145(60)
3b	569 $[M+Na]^+$	541(49), 509(44), 481(47), 415(68), 324(100), 320(74), 244(41), 229(26), 198(70), 176(64)
	320 $[M\text{-}N_2\text{-}T+Na]^+$	260(13), 246(15), 185(10), 145(100)
3c	555 $[M+Na]^+$	527(38), 495(33), 467(72), 401(55), 383(30), 324(76), 306(100), 244(41), 198(48), 162(25)
	306 $[M\text{-}AZT+Na]^+$	246(26), 226(28), 185(37), 145(100)

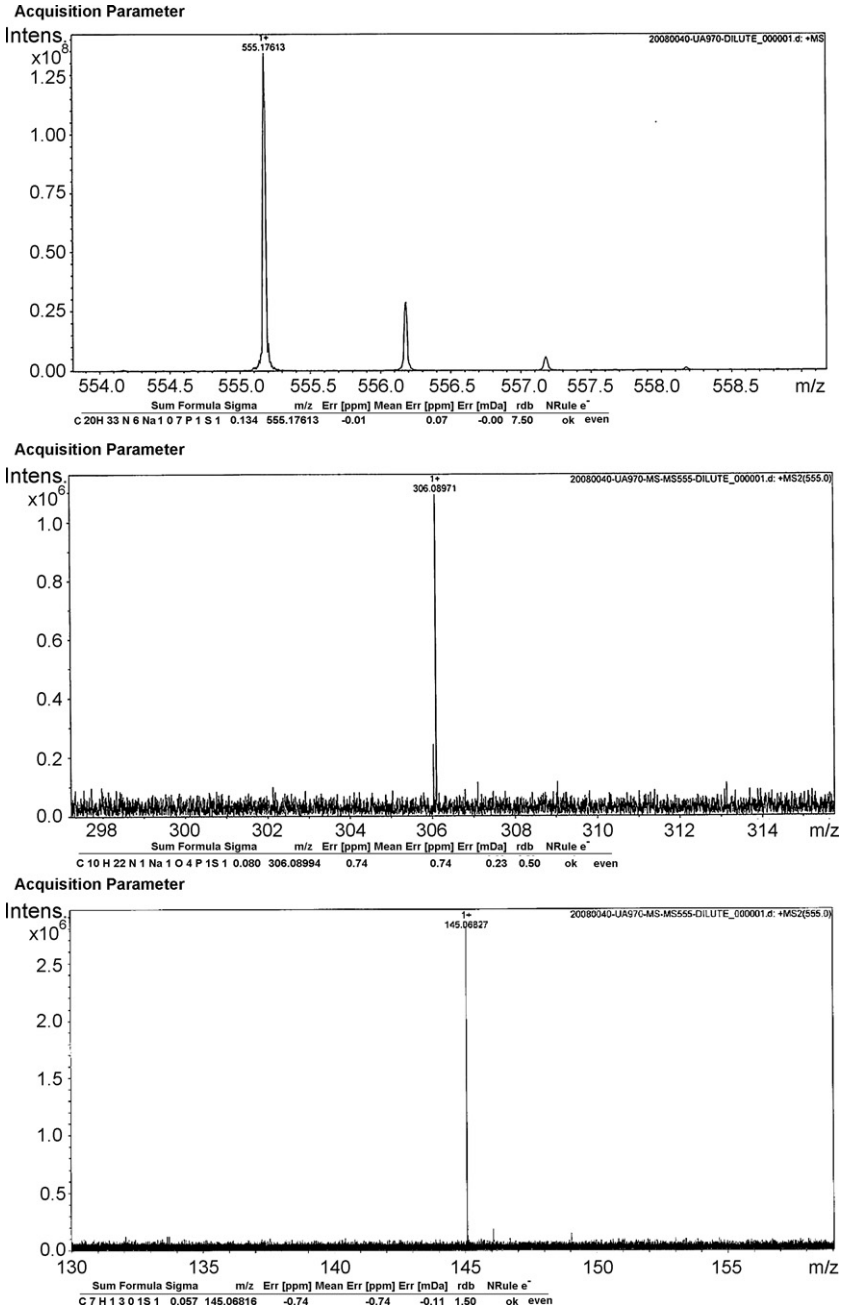
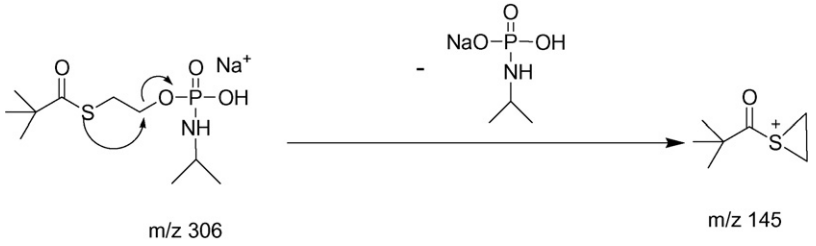


Fig. 3. (a) HR-ESIMS spectrum of (c). (b) Tandem HR-ESIMS spectrum of Na^+ adduct at *m*/*z* 555. (c) Tandem HR-ESIMS spectrum of $[M\text{-}T\text{-}N_2+Na]^+$ ion at *m*/*z* 306.

and Scheme 3). Moreover, this ion also appeared in the ESI-MS^3 spectrum of other compounds (Table 2). It indicates that the ion at *m*/*z* 145 may be arisen from the rearrangement of SATE moiety. We propose that the sulfur could attack the carbon of the thioethyl group, losing a moiety of 161 u, to form the ion at *m*/*z* 145 which could be described as 2-*t*Bu-1thia-THF (Scheme 4). Tandem high-resolution ESI-MS of **3c** indicated that the exact mass of the ion at *m*/*z* 145 was 145.06827, corresponding to the



Scheme 4. Proposed rearrangement mechanism for the ion at *m*/*z* 145.

Table 3
High-resolution mass spectral data for the main ions of **3c**[a]

Ion species	Theoretical mass (m/z)	Measured mass (m/z)	Relative error (ppm)
$[M+Na]^+$	555.17613	555.17613	0
$[M\text{-}AZT+Na]^+$	306.08994	306.08971	0.74
$[M\text{-}AZT\text{-}161+Na]^+$	145.06816	145.06827	0.74

[a] Obtained in the positive ion mode.

formula $C_7H_{13}OS$ (calculated 145.06816, relative error 0.74 ppm) (Table 3).

4. Conclusions

The electrospray ionization mass spectra of three *S*-Acyl-2-thioethyl phosphoramidate diester derivatives were studied in positive mode. The loss of 60 u from SATE moiety was found in positive electrospray ionization tandem mass spectrometry. A rearrangement giving the ion at m/z 145 was observed from the ESI-MS^3 spectrum of the sodium adduct exclusively. These characteristic fragmentation patterns of SATE group will help in the study of the decomposition of those derivatives *in vivo*.

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