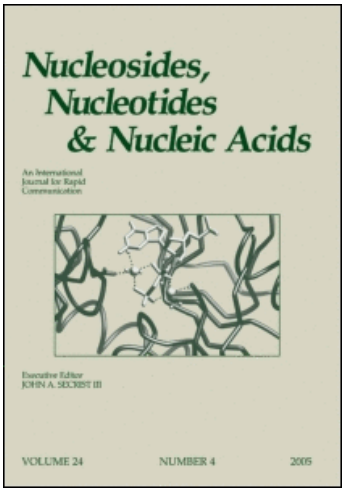

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

The Influences of A Nitrogen Atom Position in Dinucleoside 2-,3-,4-Pyridylphosphonates on Fragmentation Patterns in Electrospray Ionization Multistage Tandem Mass Spectra

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To cite this Article Ji, Sanhao , Ju, Yong , Fu, Hua , Zhao, Yufen , Johansson, Tommy and Stawinski, Jacek(2006) 'The Influences of A Nitrogen Atom Position in Dinucleoside 2-,3-,4-Pyridylphosphonates on Fragmentation Patterns in Electrospray Ionization Multistage Tandem Mass Spectra', Nucleosides, Nucleotides and Nucleic Acids, 25: 7, 771 — 784
To link to this Article: DOI: 10.1080/15257770600726067
URL: http://dx.doi.org/10.1080/15257770600726067

Nucleosides, Nucleotides, and Nucleic Acids, 25:771–784, 2006

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770600726067

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THE INFLUENCES OF A NITROGEN ATOM POSITION IN DINUCLEOSIDE 2-,3-,4-PYRIDYLPHOSPHONATES ON FRAGMENTATION PATTERNS IN ELECTROSPRAY IONIZATION MULTISTAGE TANDEM MASS SPECTRA

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□ *2-,3-,4-Pyridylphosphonates and their phosphonothioate congeners were analyzed by electrospray ionization multistage tandem mass spectrometry (ESI-MS^n). It was found that the fragmentation pathways of these compounds were not influenced to any detectable extent by the stereochemistry at the phosphorus centers but were sensitive to the position of a nitrogen atom in the pyridine ring of these compounds. Possible mechanisms for fragmentations of the investigated compounds are discussed in detail.*

Keywords Pyridylphosphonates; Pyridylphosphonothioates; Nitrogen atom; Pyridyl ring; Electrospray ionization spectrometry (ESI)

INTRODUCTION

Inspired by broad spectrum of biological activity of simple dialkyl pyridylphosphonates[1–6] and the fact that a pyridine ring is an essential constituent of various biologically important compounds (*e.g.*, vitamin B6, nicotinamide adenine dinucleotide phosphate), we have investigated nucleoside pyridylphosphonates as a new type of modification for nucleic acids.[7–9]

Received 26 May 2005; accepted 24 February 2006.

The authors acknowledge financial support from the Chinese National Natural Science Foundation (No. 20172033), the Chinese Education Ministry, and the Swedish Research Council.

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In honor and celebration of the life and career of John A. Montgomery.

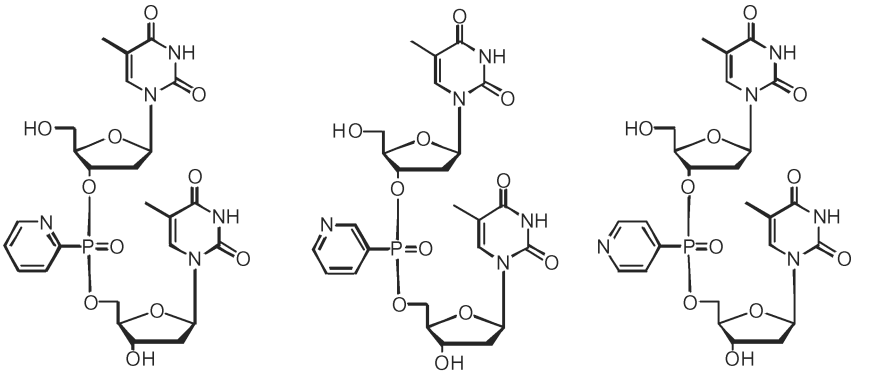
An attractive feature of pyridylphosphonates as potential therapeutics is that chirality at the phosphorus center (Rp and Sp diastereomers) and the site of attachment of the phosphonate function to the pyridyl ring (2-, 3-, and 4-pyridyl derivatives) can be used for tuning their chemical and biological properties. Our recent studies on pyridylphosphonate-modified oligonucleotides as possible antisense/antigene agents showed that the sense of chirality at the phosphorus center and the position of the nitrogen atom in the pyridyl ring of a pyridylphosphonate moiety are important factors governing stability of double- and triple-stranded complexes formed by these oligonucleotides.[10]

To get a deeper insight into chemistry of this class of compounds, we investigated ESI-MS fragmentation patterns of diastereomerically pure Rp and Sp dinucleoside pyridylphosphonates (2-, 3-, and 4-pyridyl derivatives) and their Rp and Sp pyridylphosphonothioate congeners (2- and 4-pyridyl derivatives).

EXPERIMENTAL

Synthesis

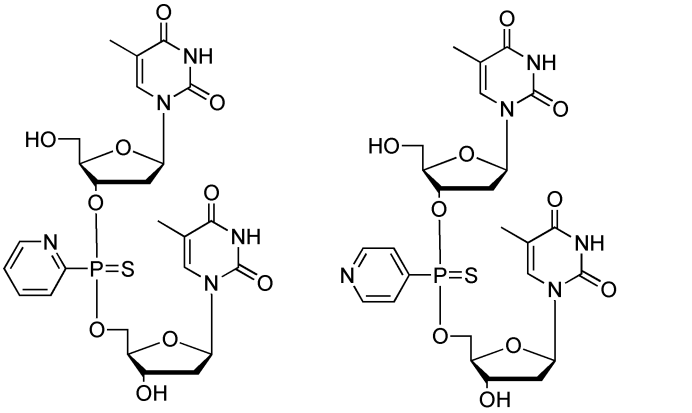
Diastereomerically pure Rp/Sp dinucleoside pyridylphosphonates **1**, **2**, **3**[7–9] (Scheme 1) and Rp/Sp dinucleoside pyridylphosphonothioates **4** and **5**[11] (Scheme 2), were synthesized according to published procedures.



SCHEME 1 Structures of compounds **1–3**.

Mass Spectrometry

The mass spectra were obtained 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 and



SCHEME 2 Structures of nucleoside 2- and 4-pyridylphosphonothioates.

pumped 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 temperature was 300°C. Samples dissolved in methanol were continuously pumped into the ESI resource at a flow rate of 0.6–0.7 ml h^{-1}. The scan range for primary mass spectra was generally from *m/z* 400 to 2000. The selected $[M+H]^+$ and $[M+Na]^+$ ions were analyzed by MS^n. The ions were 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. The high-resolution mass spectra of 2-pyridylphosphonate were recorded on a Bruker APEX spectrometer in positive ion mode.

RESULTS AND DISCUSSION

In positive ESI mass spectra of compounds **1–3** (Table 1) two type of quasi-molecular ions, $[M+H]^+$ and $[M+Na]^+$, were observed and their

TABLE 1 ESI-MS Data of Compounds **1–3**

Compounds	Precursor ions Na^+ adducts	Fragment ions and relative intensity percentage (in parentheses)
1	630	504 (4), 406 (100), 262 (12), 247 (22)
	504	378 (13), 262 (8), 247 (100)
	406	280 (13), 262 (100), 265 (6), 247 (36), 182 (24)
2	630	504 (21), 406 (33), 262 (10), 247 (100)
	504	378 (5), 262 (5), 247 (100)
	406	280 (53), 265 (33), 262 (100)
3	630	504 (11), 406 (18), 262 (6), 247 (100)
	504	378 (5), 262 (5), 247 (100)
	406	280 (72), 265 (27), 262 (100)

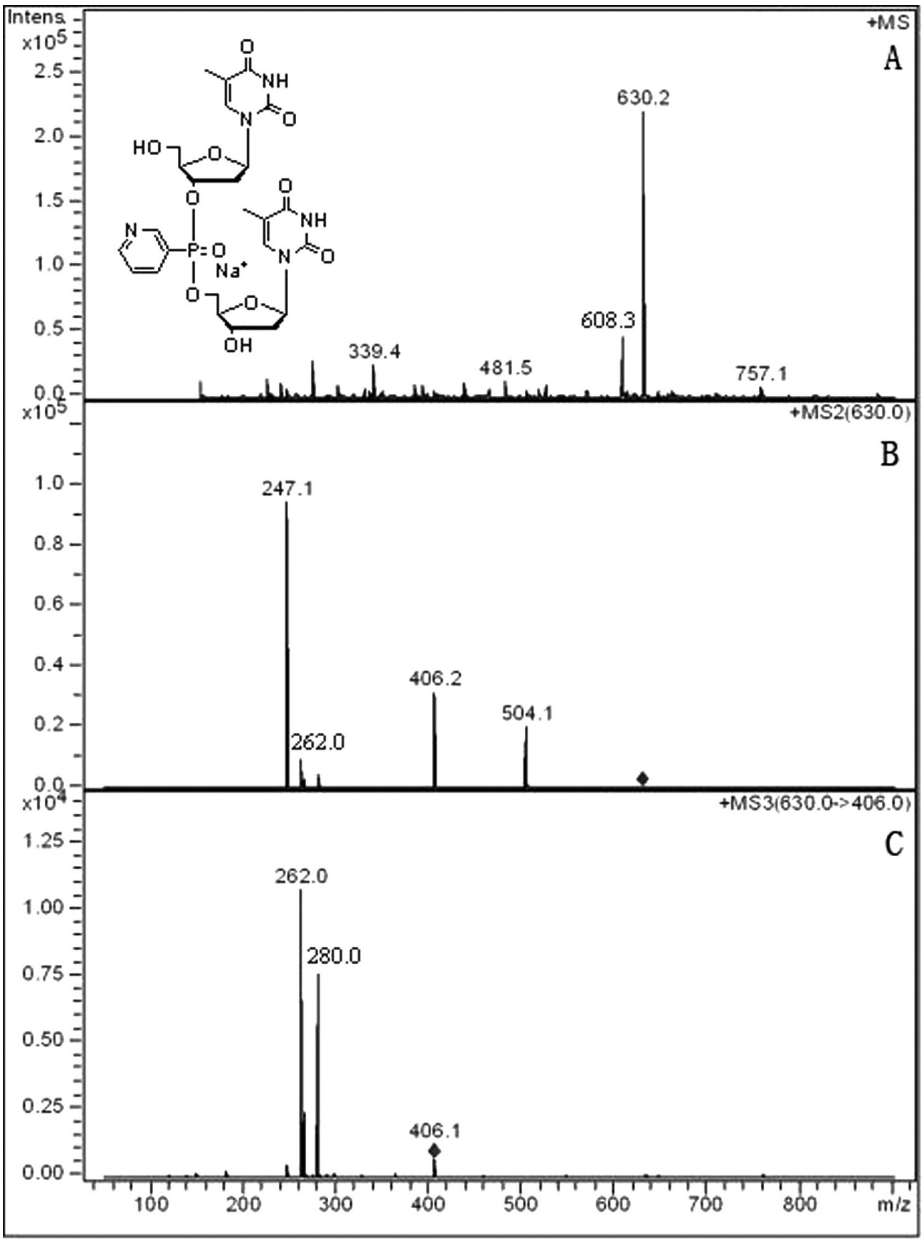
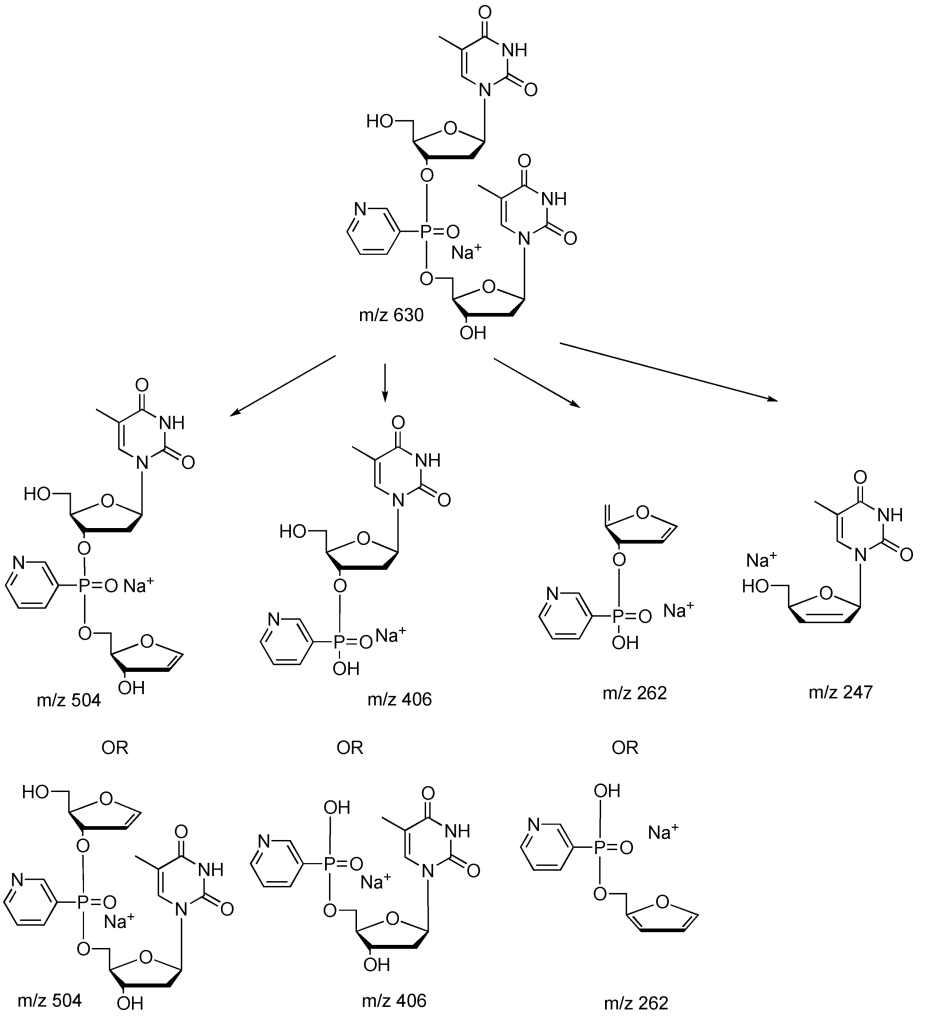


FIGURE 1 Mass spectra of **2**: (A) full MS spectrum; (B) MS^2 of sodium adduct ion at *m/z* 630; (C) MS^3 of the sodium adduct ion at *m/z* 406 (for the assignment, see Schemes 3 and 4; diamond denotes mass of the precursor ion).

fragmentation pathways were investigated by multistage technique ESI-MS^n. The spectral data for compounds **1–3** are summarized in Table 1, while in Figure 1 and Scheme 3, an example of multistage mass spectral fragmentation pathways for 3-pyridylphosphonate **2** is shown.

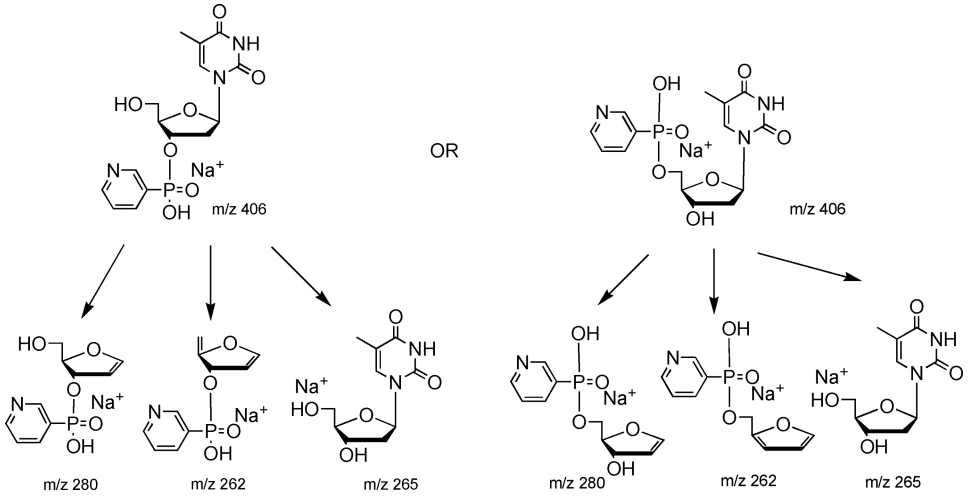


SCHEME 3 Proposed fragmentation pathways of positive ion at *m/z* 630 in ESI-MS^2 spectrum of 3-pyridylphosphonate 2.

Preliminary experiments on 3- and 4-pyridylphosphonates (compounds **2** and **3**, respectively) revealed that the positive and negative fragmentation pathways of these compounds were completely identical. Moreover, neither positive nor negative ESI-MS could tell the compounds with Rp configuration from the corresponding Sp diastereomers. For this reason, all data in this article refer to Rp diastereomers of the investigated compounds **1–5** as the fragmentation patterns were indistinguishable from those of the Sp diastereomers. Also, since fragmentation patterns of $[M+H]^+$ and $[M+Na]^+$ ions were usually similar, data in the tables give masses of Na^+ adducts, unless stated otherwise.

ESI-MS^n Fragmentation Pathways of $[M+Na]^+$ for 3- and 4-Pyridylphosphonates

The ESI-MS^2 fragmentation pattern of the molecular ion $[M+Na]^+$ at *m/z* 630 of **2** is shown in Scheme 3 and Figure 1. This ion produced a fragment at *m/z* 504 for the loss of thymine, and also three other ions at *m/z* 406, 262, and 247. Fragment at *m/z* 406, which turned out to be instrumental in differentiating isomeric 2-, 3-, and 4-pyridylphosphonates, was formed most likely through an elimination mechanism, which split the dinucleotide skeleton of these compounds into two parts. The subsequent ESI-MS^3 fragmentation of the ion at *m/z* 406 resulted in formation of ions at *m/z* 280 (the expulsion of thymine base), at *m/z* 265 (due to loss of a pyridylphosphonate moiety), and at *m/z* 262 (two eliminations with the loss of a thymine and a hydroxyl function) (Scheme 4). Similar fragmentation patterns were observed when 4-pyridylphosphonate **3** was subjected to ESI-MS^n analysis (see Table 1).



SCHEME 4 Proposed fragmentation pathways of positive ion at *m/z* 406 in ESI-MS^3 spectrum of 3-pyridylphosphonate 2.

Some parts of the pathways shown in Schemes 3 and 4 are reminiscent of the fragmentation of natural dinucleoside.[12]

ESI-MS^n Fragmentation Pathways of $[M+Na]^+$ for 2-Pyridylphosphonate 1

The only structural difference between compounds **1** and **2** is the position of a nitrogen atom in the pyridine ring of the pyridylphosphonate moieties. Table 1 shows that 2-pyridylphosphonate **1** produced the same type of fragments in ESI-MS^2 as observed for the isomeric pyridylphosphonates

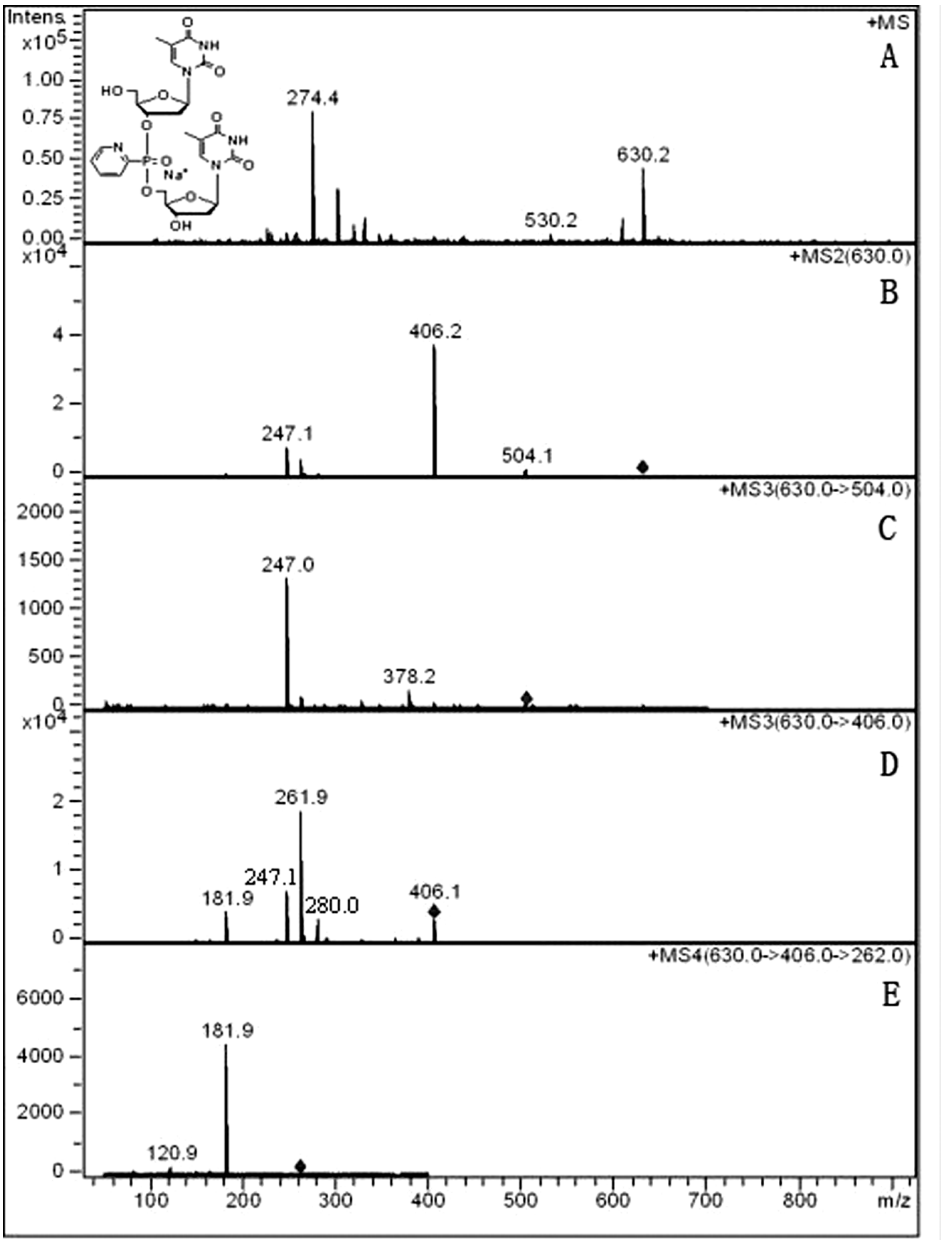
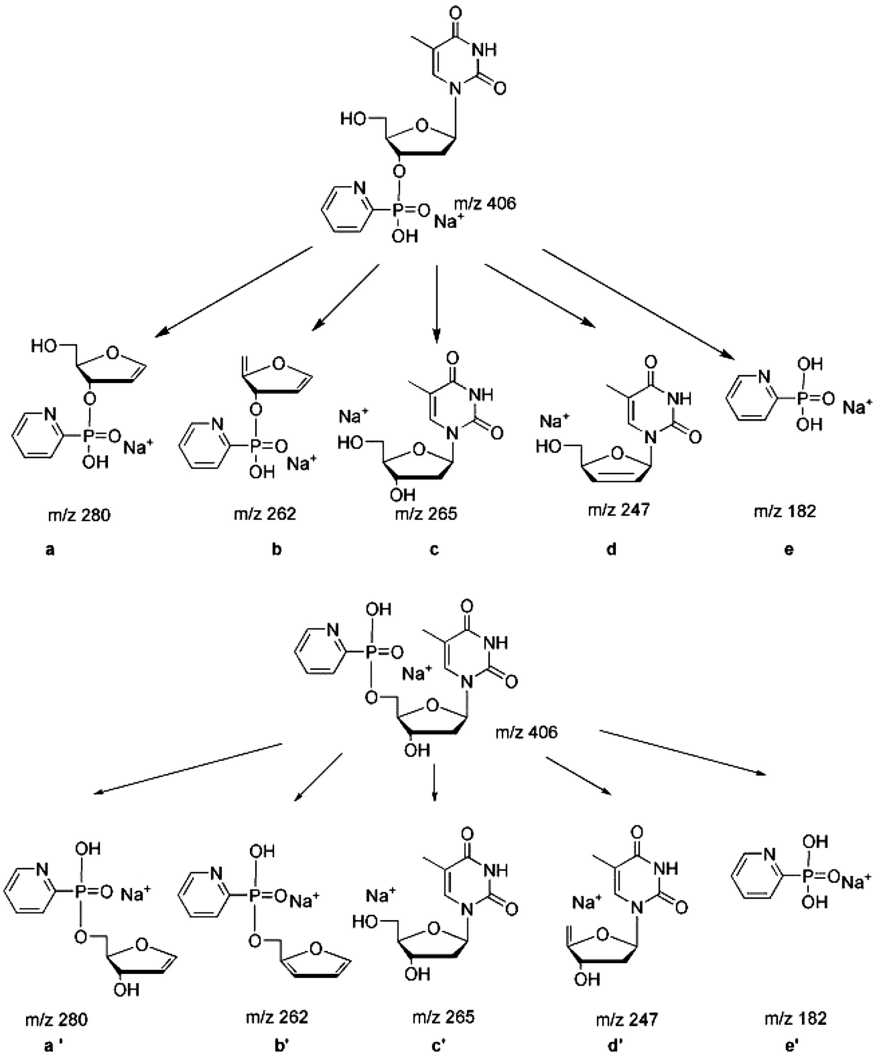


FIGURE 2 Mass spectra of 2-pyridylphosphonate **1**: (A) full MS spectrum; (B) MS^2 of the sodium adduct ion at *m/z* 630; (C) MS^3 of the ion at *m/z* 504; (D) MS^3 of the ion at *m/z* 406; (E) MS^3 of the ion at *m/z* 262 (for the assignment, see Scheme 5; diamond denotes mass of the precursor ion).

2 and **3**, but with richer fragmentation pattern at *m/z* 406 in the ESI-MS^3 (Figure 2). Besides ions **a**, **b**, and **c**, common for all pyridylphosphonates **1–3**, additional fragments, **d** and **e**, at *m/z* 247 and *m/z* 182, were found for 2-pyridyl derivative **1** (Scheme 5). High-resolution ESI-MS indicated

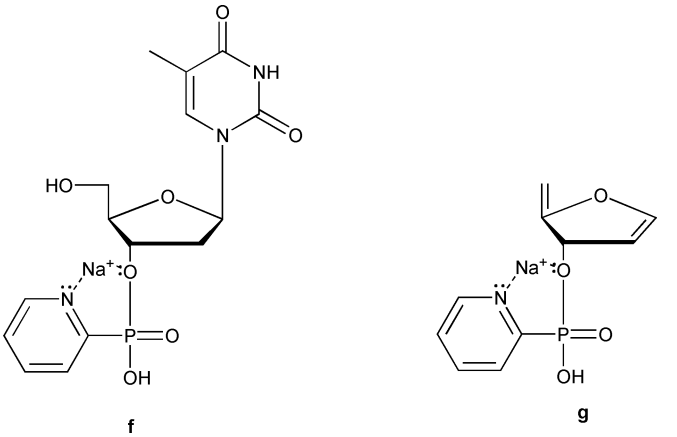


SCHEME 5 Positive ion of ESI-MS^3 spectrum of the ion at *m/z* 406 of 2-pyridylphosphonate **1**.

that the exact mass of ion **d** was 247.0402, corresponding to elemental composition $C_{10}H_{12}N_2O_4Na^+$. The mass of ion **e** at *m/z* 182 was also determined by tandem and high-resolution MS techniques (the exact mass 182.0050 corresponding to $C_5H_6NO_3PNa^+$). Possible structures for ions **d** and **e** are shown in Scheme 5.

Comparing ESI-MS spectra of isomeric pyridylphosphonates **1–3**, it seems that 2-pyridyl derivative **1** shows a distinctive abundance differences, which most likely is due to the position of a nitrogen atom in the pyridine ring of this compound. Since 2-pyridylphosphonates can form chelate complexes with metal cations, it was tempting to assume that this phenomenon

might open a new low-energy fragmentation pathway for the Na^+ adduct ion at *m/z* 406 derived from **1**. Although mechanistic details remain highly hypothetical, it is speculated that chelation of Na^+ facilitates the departure of a 2-pyridyphosphonate residue from intermediate **f** (Scheme 6). The sodium ion could share lone pair electrons on the nitrogen atom and oxygen atom to form the key intermediate **f**, while for 3- and 4-pyridylphosphonate, such a structure cannot be formed. This may also explain why the intensity of ion *m/z* 406 that results from *m/z* 630 is stronger for 2-pyridylphosphonate **1** than for the isomeric 3- or 4-pyridyl counterparts (Table 1).



SCHEME 6 Possible intermediates for the formation of ions **d** and **e** in Scheme 5.

Also, the precursor Na^+ adduct ion *m/z* 262, generated from 2-pyridylphosphonate derivative, collapsed to the ion *m/z* 182 (Figure 2E), while for the corresponding 3- and 4-pyridylphosphonates, no such a fragmentation pathway for *m/z* 262 was observed. This, again, can be explained by invoking an intermediate of type **g** (Scheme 6) for the fragmentation of *m/z* 262 derived from the 2-pyridylphosphonate derivative.

In this context, it was interesting to observe that the corresponding protonated ions (*m/z* 384) derived from **1–3** all showed similar fragmentation patterns (Figure 3). Why the sodium-adduct, but not the protonated ion, undergoes a characteristic fragmentation in the instance of 2-pyridylphosphonate **1** is unclear, although influence of Na^+ on the fragmentation pathway was previously reported.[13]

ESI-MS^n Fragmentation Pathways of $[M+Na]^+$ for Pyridylphosphonothioates 4 and 5

To substantiate our observation on the influence of a position of nitrogen atom in the pyridine ring on the fragmentation pattern of pyridylphosphonates **1–3**, ESI-MS^n spectra of 2-pyridyl and 4-pyridylphosphonothioates (**4** and **5**, Scheme 2) were investigated. The ESI-MS^2 spectra of these two

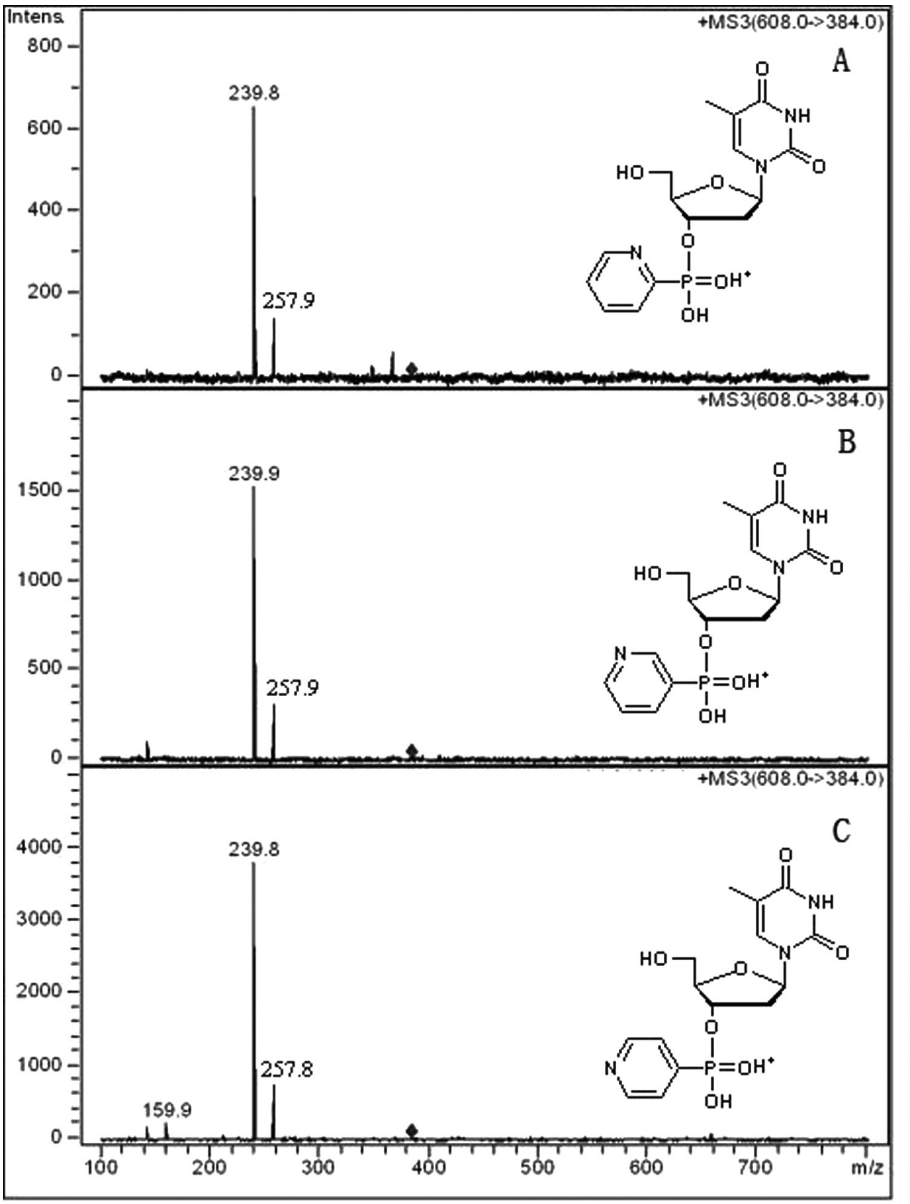


FIGURE 3 Positive mass spectra of compound **1–3:** (A) ESI-MS^3 of $[M+H]^+$ of **1** at *m/z* 384; (B) ESI-MS^3 of $[M+H]^+$ of **2** at *m/z* 384; (C) ESI-MS^3 of $[M+H]^+$ of **3** at *m/z* 384 (diamond denotes mass of the precursor ion).

compounds were found to be completely identical, while that of ESI-MS^3 for *m/z* 422 showed significant differences (Table 2). This was consistent with the observed fragmentation pattern of compound **1** and seemed to support the view that the presence of a phosphonyl group (as in compound

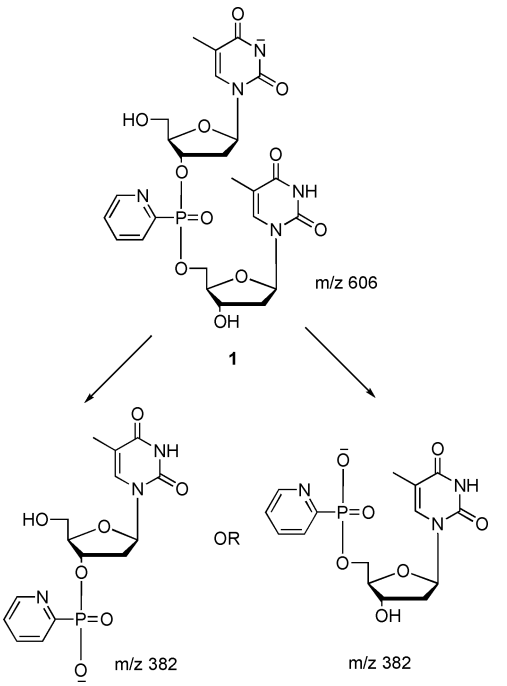
TABLE 2 ESI-MS Data of Compounds **4–5**

Compounds	Precursor ion Na^+ adducts	Fragment ions and relative intensity percentage (in parentheses)
4	646	520 (5), 422 (100), 265 (14), 247 (28)
	422	296 (10), 278 (16), 265 (100), 247 (14), 182 (11)
5	646	520 (34), 422 (16), 265 (13), 247 (100)
	422	296 (5), 265 (100)

1) or phosphonothioate group (as in compound **4**) in the 2-position of the pyridyl ring labilized the 3′-O-P linkage and apparently opened a new fragmentation pathway for the 2-pyridyl derivatives.

Negative Ion Mass Spectrometry of Pyridylphosphonates 1–3 and Pyridylphosphonothioates 4–5

Comparing to positive ion mass spectra, negative ion mass spectroscopy usually produces fewer fragment ions. In all spectra of pyridylphosphonates **1–3**, the major fragments observed were those at *m/z* 382 corresponding to nucleoside pyridylphosphonate anions (*e.g.*, for compound **1**, see Scheme 7), and which were formed from $[M\text{-}H]^-$ ions (*e.g.*, for compound



SCHEME 7 Proposed fragmentation pathways of $[M - H]^-$ for the 2-pyridylphosphonate (**1**) (arrow denotes mass of the precursor ion).

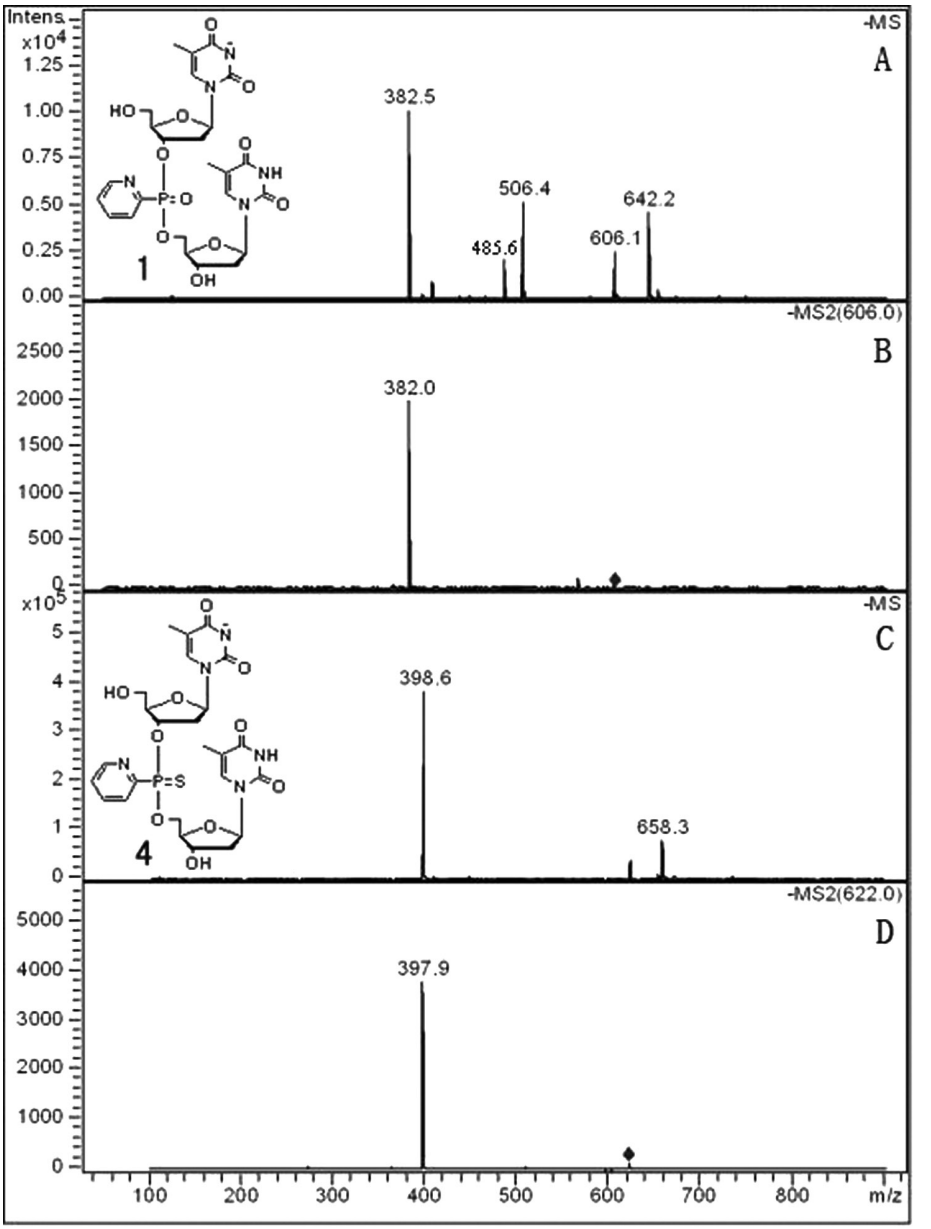


FIGURE 4 Negative mass spectra of compound **1** and **4:** (A) negative ion ESI-MS of **1**; (B) negative ion ESI-MS/MS of **1** at *m/z* 606; (C) negative ion ESI-MS of **4**; (D) negative ion ESI-MS/MS of **4** at *m/z* 622 (diamond denotes mass of the precursor ion).

1, A and B of Figure 4). These fragmentation patterns were observed irrespective of the collision energy applied and indicated rather high stability of the produced pyridylphosphonate anions. As expected, pyridylphosphonothioates **4** and **5** showed similar fragmentation pathways in a negative

ionization mode as compounds **1–3** (*e.g.*, for compound **4**, see C, D of Figure 4), most likely also due to stability of the corresponding nucleoside pyridylphosphonothioate ions formed.

CONCLUSION

Fragmentation patterns of Rp and Sp diastereomers of pyridylphosphonates **1–3** and pyridylphosphonothioates **4–5**, appeared to be identical in ESI-MS^n, both in positive and negative ionization mode, and thus this technique cannot be used to distinguish compounds with opposite sense of chirality at the phosphorus center. In positive ESI mass spectra of compounds **1–5**, various ions fragments were formed, while in the negative ionization mode, the main fragmentation pathway led to the formation of the corresponding nucleoside pyridylphosphonate and pyridylphosphonothioate ions. Among the investigated pyridylphosphonates and pyridylphosphonothioates, those bearing 2-pyridylphosphonate (compound **1**) or 2-pyridylphosphonothioate moiety (compound **4**) gave distinctive fragmentation patterns of molecular ions $[M+Na]^+$, enabling differentiation of these compounds from their isomeric 3- or 4-pyridyl counterparts. This phenomenon, however, was not observed for the molecular ions of type $[M+H]^+$.

REFERENCES

1. Protopopova, G.V.; Reidalova, L.I.; Pavlenko, A.F.; Sologub, L.S.; Kukhar, V.P.; Petrenko, V.S. Insecticidal and Nematocidal Activity of Dialkyl 2,3,5,6-Tetrachloro-4-Pyridylphosphonates. *Fiziologicheski Aktivnye Veschchestva* **1980**, 12, 16–18.
2. Kukhar, V.P.; Cherepenko, T.I.; Pavlenko, A.F. Relation between the Biological Activity of Polychloropyridine Derivatives and Chemical Structure. *Dopovidi Akademii Nauk Ukrainskoi RSR, Seriya B* **1982**, 60–63.
3. Akiba, K.; Tsuzuki, K. Industrial Fungicide, Japan Kokai Tokkyo Koho, Jpn, Pat. No. 61221102, 1986.
4. Malhotra, S.K.; Evoy, I.L. Diphenoxmethylpyridines having herbal properties, Dow Chemical, U.S. Pat. No. 4606757, 1986.
5. Erion, M.D.; Vanpoelje, P. A combination of fructose-1,6-biphosphatate (FBPase) inhibitors and insulin sensitizers for the treatment of diabetes, PCT Int. Appl., WO Pat. No. 0038666, 2000.
6. Dang, Q.; Kasibhatla, S.R.; Reddy, K.R.; Erion, M.D.; Reddy, M.R.; and Agarwal, A. Preparation of heteroaromatic phosphonates as fructose-1,6-biphosphatase inhibitors, PCT Int. Appl., WO Pat. No. 0014095, 2000.
7. Kers, A.; Stawinski, J. A New Type of Nucleotide Analogue with 4-Pyridylphosphonate Internucleotide Linkage. *Tetrahedron Letters* **1999**, 40, 4263–4266.
8. Johansson, T.; Kers, A.; Stawinski, J. 2-Pyridylphosphonates: A New Type of Modification for Nucleotide Analogues. *Tetrahedron Letters* **2001**, 42, 2217–2220.
9. Johansson, T.; Stawinski, J. Synthesis of Dinucleoside Pyridylphosphonates Involving Palladium(0)-Catalysed Phosphorus-Carbon Bond Formation as a Key Step. *Chemical Communication* **2001**,, 2564–2565.
10. Zmudzka, K.; Johansson, T.; Wojcik, M.; Janicka, M.; Nowak, M.; Stawinski, J.; Nawrot, B. Novel DNA Analogues with 2-, 3-, and 4-Pyridylphosphonate Internucleotide Bond: Synthesis and Hybridization Properties. *New Journal of Chemistry* **2003**, 27, 1698–1705.

11. Johansson, T.; Stawinski, J. Nucleoside H-Phosphonates. Part 19: Efficient Entry to Novel Nucleotide Analogues with 2-Pyridyl- and 4-Pyridylphosphonothioate Internucleotide Linkages. *Tetrahedron* **2004**, 60, 389–395.
12. Phillips, D.R.; McCloskey, J.A. A Comprehensive Study of the Low Energy Collision-Induced Dissociation of Dinucleoside Monophosphates. *International Journal of Mass Spectrometry and Ion Proceses* **1993**, 128, 61–82.
13. Chen, Y.; Sun, X.B.; Fu, H.; Liu, X.H.; Zhao, Y.F. Novel Benzyl Rearrangements in Electrospray Ionization Multistage Tandem Mass Spectra of Benzyl 2′,3′-Didehydro-2′,3′-Dideoxythymidin-5′-yl H-Phosphonate. *Journal of Mass Spectrometry* **2004**, 39, 730–735.