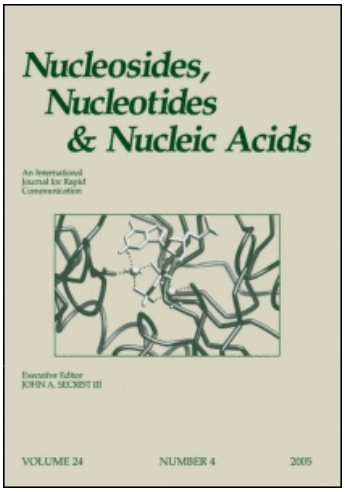


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# Fast Atom Bombardment and Tandem Mass Spectrometry for the Identification of Nucleoside Adducts with Phenyl Glycidyl Ether

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# FAST ATOM BOMBARDMENT AND TANDEM MASS SPECTROMETRY FOR THE IDENTIFICATION OF NUCLEOSIDE ADDUCTS WITH PHENYL GLYCIDYL ETHER

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## Abstract

The present study deals with the use of fast atom bombardment (FAB) in combination with constant neutral loss (CNL) scanning, high resolution mass spectrometry and tandem mass spectrometry (MS-MS) with collisionally activated decomposition (CAD), as complementary methods for the identification and structural analysis of phenyl glycidyl ether-nucleoside adducts. Selective detection of the parent ions of the modified nucleosides at the 1-10 ng level has been achieved by suitably designed CNL scans. The elemental composition of the adducts has been determined by accurate mass measurements. CAD-MS has been carried out on the $[M + H]^+$ and $[M - H]^-$ ions to derive structural data on the size and nature of the base, sugar and alkyl substituent. In some cases, information on the alkylation site has been obtained, which is very useful for distinguishing isomeric adducts.

## Introduction

Mono- and difunctional glycidyl ethers are widely used in the production of epoxy resins and to improve the processing and stability of industrial polymers. It is well known that these chemicals have mutagenic and carcinogenic properties, which are generally believed to be induced by covalent reaction with cellular DNA.[1] In order to understand these toxic effects at the molecular level, it is relevant to know how glycidyl ethers react with cellular nucleic acids and to identify their reaction products. One of the challenging aspects of characterizing nucleic acid adducts is

the determination of the alkylation site on the nucleic acid components. If sample size is adequate, these structural details can usually be established through a combination of spectroscopic methods, i.e. UV, NMR and mass spectrometry. However, the amount of nucleoside adducts isolated from modified polynucleotides is generally very small. Consequently, not all of the techniques, mentioned above, are equally efficacious. As mass spectrometry can provide both molecular weight and structural information on minute quantities of material, it is one of the most suitable tools for the characterization of isolated adducts.

This paper deals with the mass spectrometric characterization of covalently modified nucleosides formed in the reaction of phenyl glycidyl ether (PGE) with thymidine (Thy), 2'-deoxycytidine (dCyd), 2'-deoxyadenosine (dAdo) and 2'-deoxyguanosine (dGua). In view of the polar nature of the nucleoside adducts, FAB was used as the ionization method. The mass spectral techniques used included constant neutral loss linked scanning, high resolution mass spectrometry and tandem mass spectrometry in combination with collisionally activated decomposition. Experimental aspects of these techniques will be evaluated. Furthermore, the applicability of the FAB-MS-CAD-MS technique for the direct analysis of mixtures and for the differentiation between positional isomers will be documented.



## Experimental

### Instrumentation

All analysis were performed on a VG 70-SEQ hybrid mass spectrometer (VG Analytical Ltd., Manchester, U.K.), equipped with an Ion Tech saddle field atom gun. The instrument consists of a high resolution double focusing mass spectrometer with EB configuration (MS-I), followed by an RF-only quadrupole collision gas cell and a high performance quadrupole mass analyzer (MS-II). Xenon atoms with energies of approximately 8 keV and a beam flux of 1 mA were used as the ionizing beam. Positive and negative FAB spectra were recorded under control of the VG 11-250 J data system by repetitive scanning over the range 20-600 u, using a scan time of 2 s decade$^{-1}$. The analytes were dissolved in the matrix at the low microgram level. CNL scans were obtained by simultaneously scanning the electric and magnetic sector fields, and keeping the $B^2(1-E)/E^2$ ratio constant. In this scan mode only the ions which spontaneously loose a fragment of a well-defined mass in the first field free region (i.e. between the ion accelerating region and the electric sector) are recorded. Scan speed was 8 s decade$^{-1}$, and generally 10 scans were

signal averaged. The accurate mass data were obtained by carrying out a linear voltage scan at 10 000 resolution, and by using poly(ethylene) glycol as matrix and reference. Two reference peaks were used to establish the mass scale, whereas a third reference peak was used to verify the accuracy of the mass measurement. Data were collected for at least 10 scans in the multichannel analysis mode. Daughter ion spectra were obtained by collisionally activated decomposition (CAD) in the RF-only quadrupole gas cell, using argon as collision gas, and by scanning MS-II. For most CAD experiments a gas pressure of approximately 1-5 mTorr and collision energies below 100 eV were used, which result in an optimal daughter ion yield. Parent ions were selected at a resolution of 1000. Daughter ion spectra were acquired, using unit resolution at the quadrupole analyzer. Scans were of 5 s duration, and 10-15 scans were accumulated.



## Materials

The FAB matrices: glycerol (Gly), *m*-nitrobenzylalcohol (*m*-NBA), poly(ethylene glycol) and triethanolamine (TEA) were purchased from Janssen Chimica (Belgium). The adducts investigated in the present study were synthesized by reacting PGE (2,3-epoxypropylphenyl ether) with thymidine, 2'-deoxycytidine, 2'-deoxyadenosine and 2'-deoxyguanosine in methanol at 37 °C, for 24 or 48 hrs. The 2-hydroxy-3-phenoxypropyl (HPP) adducts formed (listed in Fig. 1), were isolated by centrifugal circular TLC (Kieselgel 60 PF 254, eluent : $CH_2Cl_2$/THF) and reversed phase HPLC (10 RP 18 or PRP-1 column, eluent : 0.01 M $HCOONH_4/CH_3OH$). A more detailed description of the synthesis, isolation and additional characterization of these adducts by UV and $^1$H-NMR spectroscopy has been published elsewhere.[2-4]

## Results and Discussion

The 7-HPP-dGua adduct has been selected as a representative compound to illustrate the general characteristics of the FAB mass spectra, which can be obtained for HPP-nucleoside adducts. In the positive ion spectrum (Fig. 2a), the characteristic ions correspond to the protonated molecule, $[M + H]^+$ (*m/z* 418), and a fragment ion at *m/z* 302, indicated as $RBH_2^+$, which represents the base part of the molecule. This ion results from protonation on the base moiety followed by transfer of a hydrogen atom from the sugar to the base part. Although the $[M + H]^+$ and $RBH_2^+$

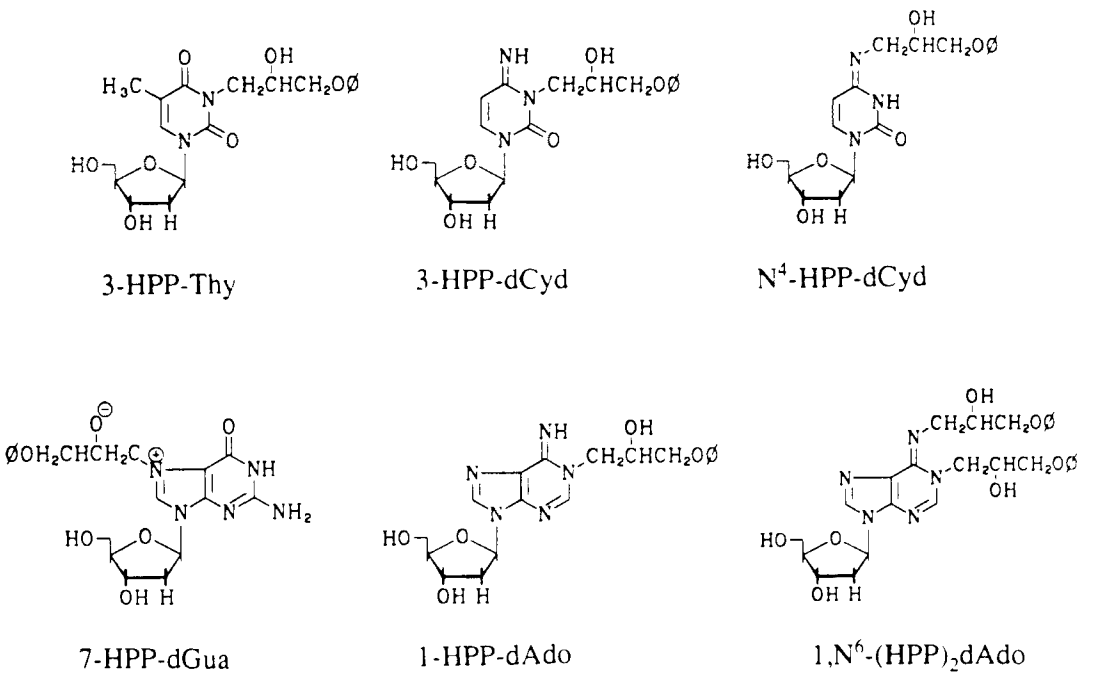


**Figure 1.** Structures of the HPP-nucleoside adducts studied.



ions are always easily discerned, it is often difficult to detect the other minor fragment ions of the HPP-adduct in the presence of background and matrix ions. The major background ions found in the FAB-MS spectrum correspond to protonated or cationized glycerol clusters. Most of the ions observed in the positive ion mode have their counterparts in the negative ion mode (Fig. 2b). The two diagnostic ions are the $[M - H]^-$ ion at *m/z* 416 and a fragment ion at *m/z* 300, denoted as $RB^-$, which is formed by cleavage of the base-sugar bond with negative charge retention on the base part of the molecule. As in the positive ion spectrum, there is a high contribution of background ions, which mainly correspond to deprotonated glycerol clusters. Comparison of the FAB-MS results obtained in the two ion modes shows that the signal intensity of the $[M + H]^+$ ions is always significantly higher than the signal intensity of the $[M - H]^-$ ions. The detection limit (S/N = 5) for the $[M + H]^+$ ions in the FAB mass spectra is around 100 ng. At lower concentrations, matrix interference becomes a major problem and the "peak-at-every-mass" phenomenon severely obscures the detection.

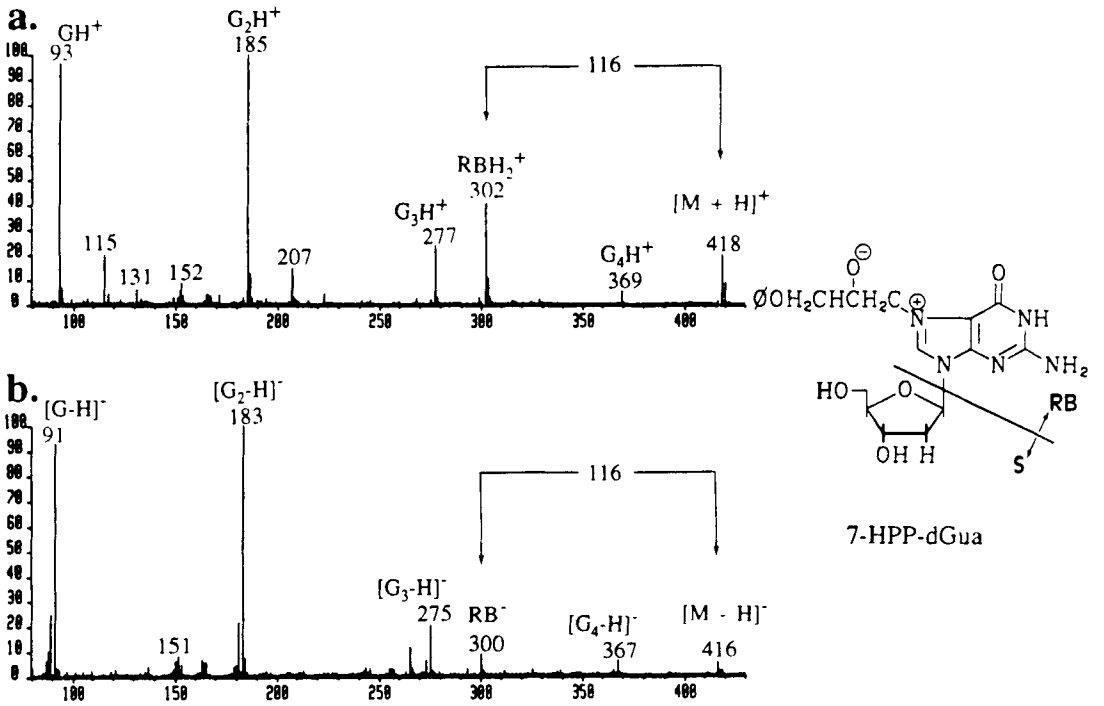


**Figure 2.** FAB mass spectra of a HPLC-fraction containing 7-HPP-dGua: (a) positive and (b) negative ion mode (matrix = glycerol).



The $[M + H]^+$ and $[M - H]^-$ ions of the HPP-nucleoside adducts spontaneously fragment by the expulsion of the deoxyribose moiety (minus one hydrogen), which corresponds to the loss of 116 mass units (cf. Fig. 2). By taking advantage of the specificity of this reaction, detection limits can be greatly improved. This is achieved by CNL linked scanning, which makes it possible to detect only those ions which loose a neutral fragment of a well-defined mass. The enhanced specificity and sensitivity obtained by monitoring the ions, which fragment by loss of 116 mass units, is demonstrated in the positive ion FAB-CNL spectrum of a HPLC fraction containing the 7-HPP-dGua adduct (Fig. 3). The only two significant peaks in the spectrum correspond to the $[M + H]^+$ ions of 7-HPP-dGua (*m/z* 418) and dGua (*m/z* 268), which is a minor sample constituent. Compared to the original FAB mass spectrum (cf. Fig. 2a), the chemical background in the neutral loss

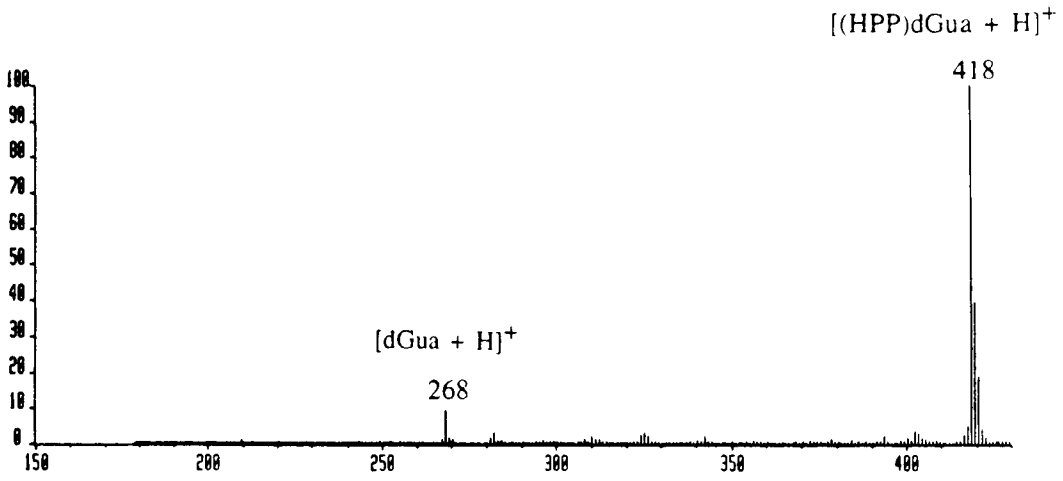


**Figure 3.** FAB-CNL spectrum (116 u) of a HPLC-fraction containing 7-HPP-dGua (matrix = glycerol)

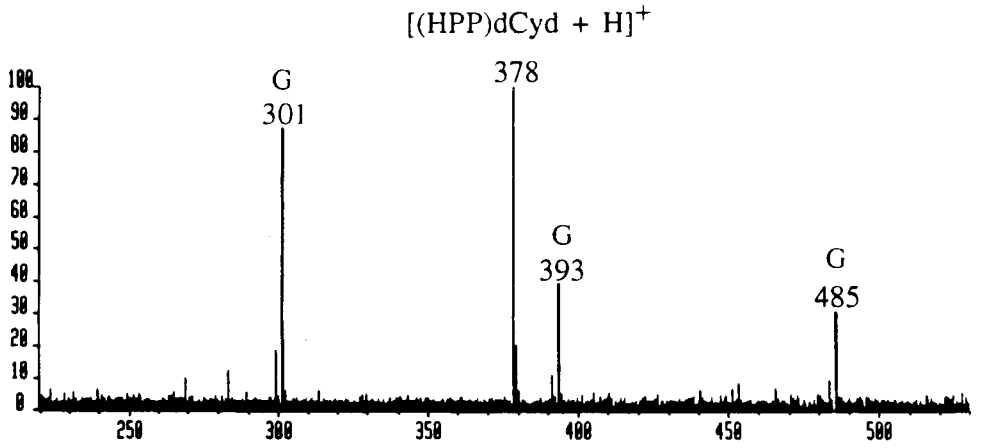


**Figure 4.** FAB-CNL spectrum (116 u) of a HPLC-fraction containing 5 ng of 3-HPP-dCyd (matrix = glycerol).

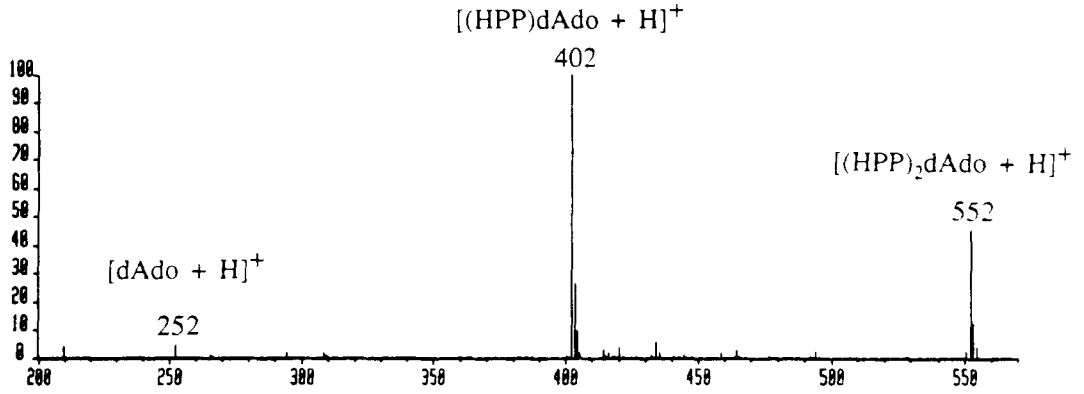


**Figure 5.** FAB-CNL spectrum (116 u) of a HPLC-fraction containing 1-HPP-dAdo and $1,N^6$-$(HPP)_2$dAdo (matrix = glycerol).



spectrum is very low and the S/N ratios for the $[M + H]^+$ ions are improved. In this way, the detection limit obtained for the $[M + H]^+$ ions of the HPP-nucleoside adducts is reduced to 1 ng. This improved sensitivity is also evident from the FAB-CNL spectrum obtained for a sample containing only 5 ng of the 3-HPP-dCyd adduct (Fig. 4). The signal corresponding to the $[M + H]^+$ ion is clearly detected at *m/z* 378, with a S/N ratio of 30:1. The FAB-CNL spectrum obtained for pure glycerol (not shown) indicates that the other signals observed at *m/z* 301, 393 and 405, originate from the glycerol matrix. CNL scanning can also be successfully applied as a screening method for the identification of different modified nucleosides present in a mixture. For example, the FAB-CNL spectrum obtained on a HPLC fraction, isolated from the reaction mixture of PGE with dAdo, shows the presence of three different components (Fig. 5). The two most prominent ions at *m/z* 402 and 552 correspond to the $[M + H]^+$ ions of mono- and bis-alkylated dAdo, respectively. The minor ion at *m/z* 252 can be attributed to the $[M + H]^+$ ion of dAdo itself, which appears to be present in the HPLC fraction.

To check the elemental composition of the HPP-nucleoside adducts, we have determined the accurate mass of the $[M + H]^+$ ions formed by FAB. The high resolution data, listed in Table 1, indicate that for all the adducts the elemental composition could be determined with an accuracy better than 1 mmu.

**Table 1. Accurate mass data for the $[M + H]^+$ ions of the HPP-nucleoside adducts.**

| Compound | Ionic Species | Elemental Composition | Calculated Mass | Observed Mass | Error (in mmu) |
|---|---|---|---|---|---|
| HPP-dCyd | $[M + H]^+$ | $C_{18}H_{24}N_3O_6$ | 378.1665 | 378.1668 | + 0.3 |
| HPP-Thy | $[M + H]^+$ | $C_{19}H_{25}N_2O_7$ | 393.1662 | 393.1661 | - 0.1 |
| HPP-dAdo | $[M + H]^+$ | $C_{19}H_{24}N_5O_5$ | 402.1777 | 402.1771 | - 0.6 |
| HPP-dGua | $[M + H]^+$ | $C_{19}H_{24}N_5O_6$ | 418.1727 | 418.1731 | + 0.4 |
| $(HPP)_2$-dAdo | $[M + H]^+$ | $C_{28}H_{34}N_5O_7$ | 552.2458 | 552.2449 | - 0.9 |



The application of FAB-MS to the structural characterization of the HPP-adducts is hindered by the limited number of fragment ions and the presence of a high chemical background, which obscures low abundant fragments. Additional problems arise when dealing with mixtures of modified nucleosides, for which it is very difficult to sort out which fragment ions are associated with which molecular ion species. FAB-MS-CAD-MS is a technique that can overcome these often-encountered drawbacks of FAB-MS. The application of this technique involves that the $[M + H]^+$ or $[M - H]^-$ ions of interest are selected by MS-I, induced to decompose by collisional activation and that a spectrum of the daughter ions is obtained by scanning MS-II. In this way, chemical noise is largely eliminated, stable ions are induced to fragment, and parent-daughter relationships can be determined when mixtures are present. With respect to sensitivity, daughter ion spectra of the $[M + H]^+$ or $[M - H]^-$ ions of the HPP-adducts could already be obtained on sample quantities in the order of 25 ng.

The daughter ion spectrum obtained for the $[M + H]^+$ ions of the 3-HPP-Thy adduct is given in Figure 6a. Fragmentations leading to the major ions in the spectrum are indicated. Cleavage of the glycosidic bond gives rise to an ion at *m/z* 117, corresponding to the sugar part, as well as to an ion at *m/z* 277, which corresponds to the protonated base. Further fragmentation of the latter ion results in fragments at *m/z* 259, 183, 165 and 127, which can be explained by the loss of water, phenol, water and phenol, and PGE, respectively. The ion at *m/z* 133

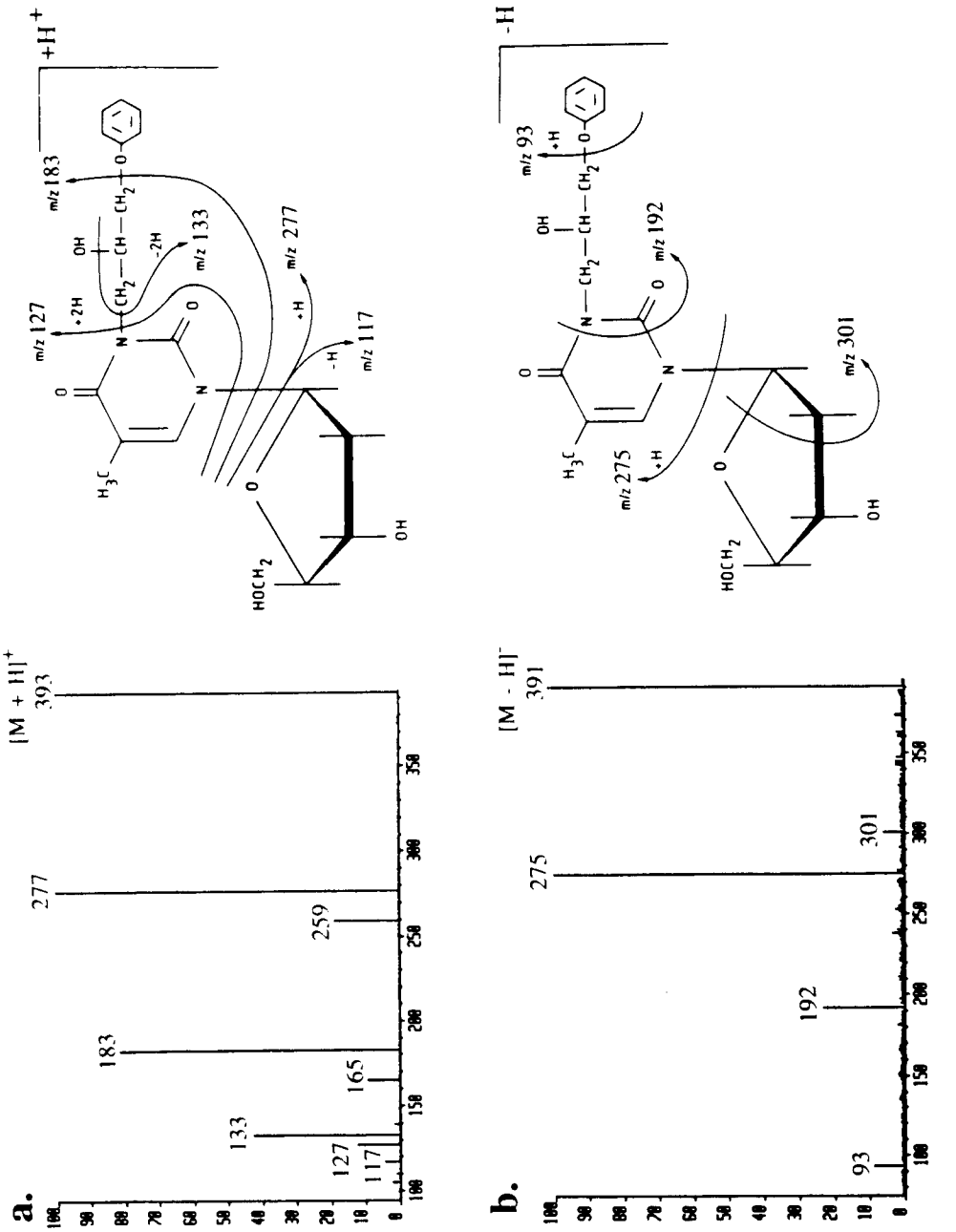


**Figure 6.** Daughter ion spectra of (a) $[M + H]^+$ (*m/z* 393) and (b) $[M - H]^-$ (*m/z* 391) of 3-HPP-Thy, obtained by CAD at $E_{coll}$ = 60 eV and argon gas pressure = 0.3 Pa.

corresponds to the $[HPP - H_2O]^+$ ion, which is a characteristic fragment of the side chain. The daughter ion spectrum obtained for the $[M - H]^-$ ions of the 3-HPP-Thy adduct is given in Figure 6b. The fragment at *m/z* 301 is formed by the expulsion of $C_3H_6O_3$ from the sugar part, whereas the ion at *m/z* 275 corresponds to the $RB^-$ ion, resulting from the loss of the complete deoxyribose moiety (minus one hydrogen). The daughter ion at *m/z* 192 can be rationalized by a retro-Diels-Alder (RDA) rearrangement in the pyridine ring with retention of the negative charge on the right side of the molecule. The formation of this ion indicates that alkylation took place at the N-3 position. The ion at *m/z* 93 corresponds to the phenolate anion, which is a characteristic fragment of the HPP substituent. Comparison of the CAD spectra obtained for the $[M + H]^+$ and $[M - H]^-$ ions demonstrates very nicely that both spectra provide "complementary" structural information. A similar fragmentation behaviour has been found for the HPP-adducts of the other nucleosides studied (results not shown).

In the case of mixtures, MS-MS is a powerful tool for separating the molecular ion species from all other ions, making it feasible to study a particular component's structure unambiguously. The FAB-MS-CAD-MS technique has been successfully applied for the structural analysis of the three different components present in the HPLC fraction isolated from the reaction mixture of PGE with dAdo (cf. Fig. 5). The daughter ion spectra of the $[M + H]^+$ ions at *m/z* 552, 402 and 252 yield structurally informative fragment ions, which are consistent with the structures of $1,N^6$-$(HPP)_2$dAdo, 1-HPP-dAdo and dAdo, respectively.[2]

Another interesting application of the FAB-MS-CAD-MS technique concerns the differentiation of isomeric adducts, i.e. 3-HPP-dCyd and $N^4$-HPP-dCyd, isolated as alkylation products from the reaction of PGE with dCyd. As it was not possible to differentiate both positional isomers on the basis of FAB-MS data alone, we have evaluated whether differentiation was possible by means of the CAD spectra of the $[M + H]^+$ or $[M - H]^-$ ions. The daughter ion spectra of the $[M + H]^+$ ions of both isomers are rather similar (not shown). The only remarkable difference relates to the signal intensity of the fragment ion at *m/z* 112, which is formed by the combined loss of the sugar moiety and PGE. The differences between the daughter ion spectra obtained for the $[M - H]^-$ ions are more pronounced (Fig. 7). Completely different fragment ions are observed for each of the isomers. The fragment at *m/z* 183 in the CAD spectrum of the N-3 alkylated isomer can be explained by a RDA rearrangement in the pyridine ring, with retention of the negative charge on

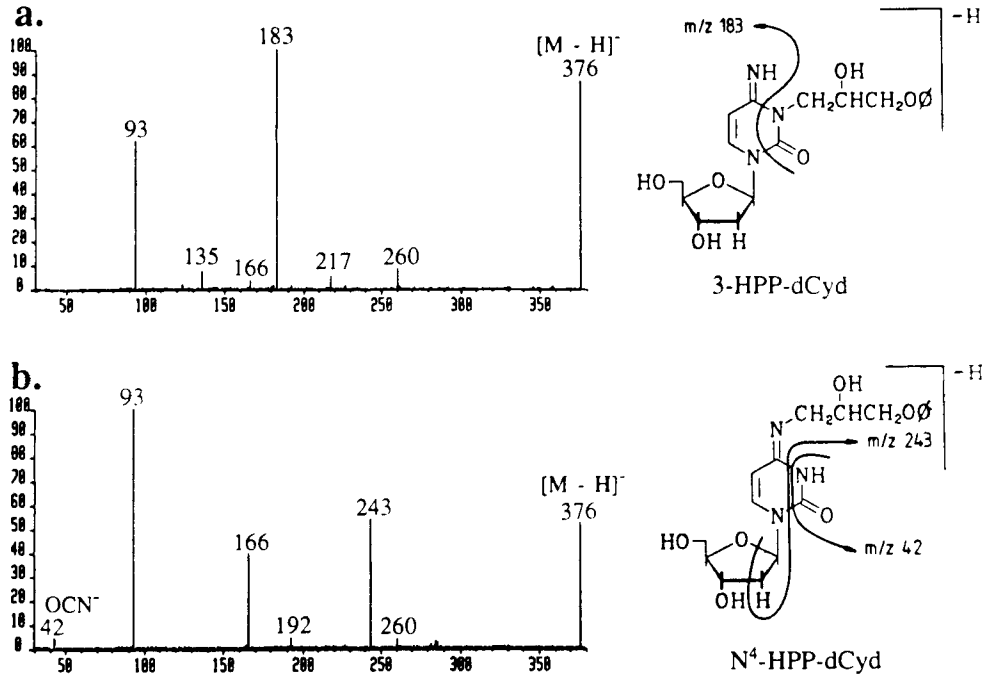


**Figure 7.** Daughter ion spectra of $[M - H]^-$ (*m/z* 376) of (a) 3-HPP-dCyd and (b) $N^4$-HPP-dCyd, obtained by CAD at $E_{coll}$ = 40 eV and argon gas pressure = 0.3 Pa.



the left side of the molecule. The formation of this ion gives proof that alkylation took place at the N-3 position. In the CAD spectrum of the N-4 alkylated isomer, the detection of the isocyanate anion (*m/z* 42) indicates that the alkylation did not take place at N-3. In addition, a characteristic ion is observed at *m/z* 243, which can be explained by the combined loss of isocyanic acid and a specific part of the sugar (i.e. $C_3H_6O_3$), and which points to alkylation at N-4.

## Conclusion

From the data presented in this paper it is evident that MS-MS is a very valuable tool for the direct mass spectral characterization of HPP-nucleoside adducts. Constant neutral loss scanning (116 u) makes it feasible to screen mixtures for the presence of deoxynucleosides and derivatives thereof, with enhanced selectivity and sensitivity. Daughter ion spectra of both $[M + H]^+$ and $[M - H]^-$

ions provide a wealth of complementary structural information. FAB-MS-CAD-MS can also be successfully applied to the direct analysis of mixtures and to differentiate isomeric adducts.

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