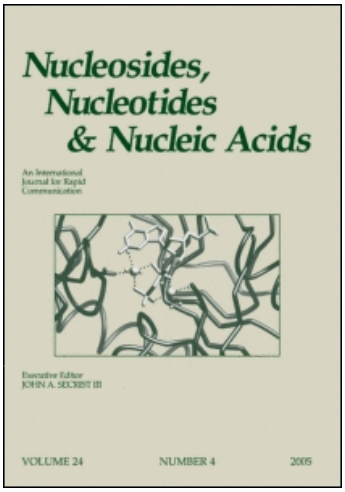

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Applications of LC/MS and Tandem Mass Spectrometry to the Characterization of Nucleosides in Mixtures

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APPLICATIONS OF LC/MS AND TANDEM MASS SPECTROMETRY TO THE CHARACTERIZATION OF NUCLEOSIDES IN MIXTURES

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Abstract: *Liquid chromatography-mass spectrometry (LC/MS) and tandem mass spectrometry (MS/MS) provide new approaches for structural studies of nucleosides, in the nanogram range, in mixtures. Examples are given of the use of LC/MS for rapid screening of synthesis reaction mixtures, and of MS/MS for the detection and characterization of nucleoside isomers in RNA hydrolysates.*

The structural characterization of nucleosides is a problem common to many fields, including synthetic chemistry (e.g., nucleoside analogs), pharmacology (metabolized products), and biochemistry (natural modification in RNA and DNA; xenobiotic modifications). The structure determination of new or unexpected products is particularly difficult in quantities below ~10^{-6} gm, and becomes acute in complex mixtures. Mass spectrometry has played a valuable role in the characterization of nucleosides and related compounds (reviewed in ref. 1) due mainly to its high sensitivity, the availability of various microscale approaches,[2] and the advantages which accrue from its direct combination with chromatography. In particular, the development of LC/MS and tandem mass spectrometry has provided new approaches to the microscale characterization of nucleosides in mixtures (reviewed in ref. 3).

As an extension of LC/MS- and MS/MS-based methods[1,3,4], some of which have been applied to structural problems involving natural nucleosides,[5] we comment here on two additional types of analyses: (1) use of LC/MS for rapid screening of synthesis reaction mixtures, and (2) initial characterization of nucleoside isomers by MS/MS, without chromatography, in hydrolysates of nucleic acids.

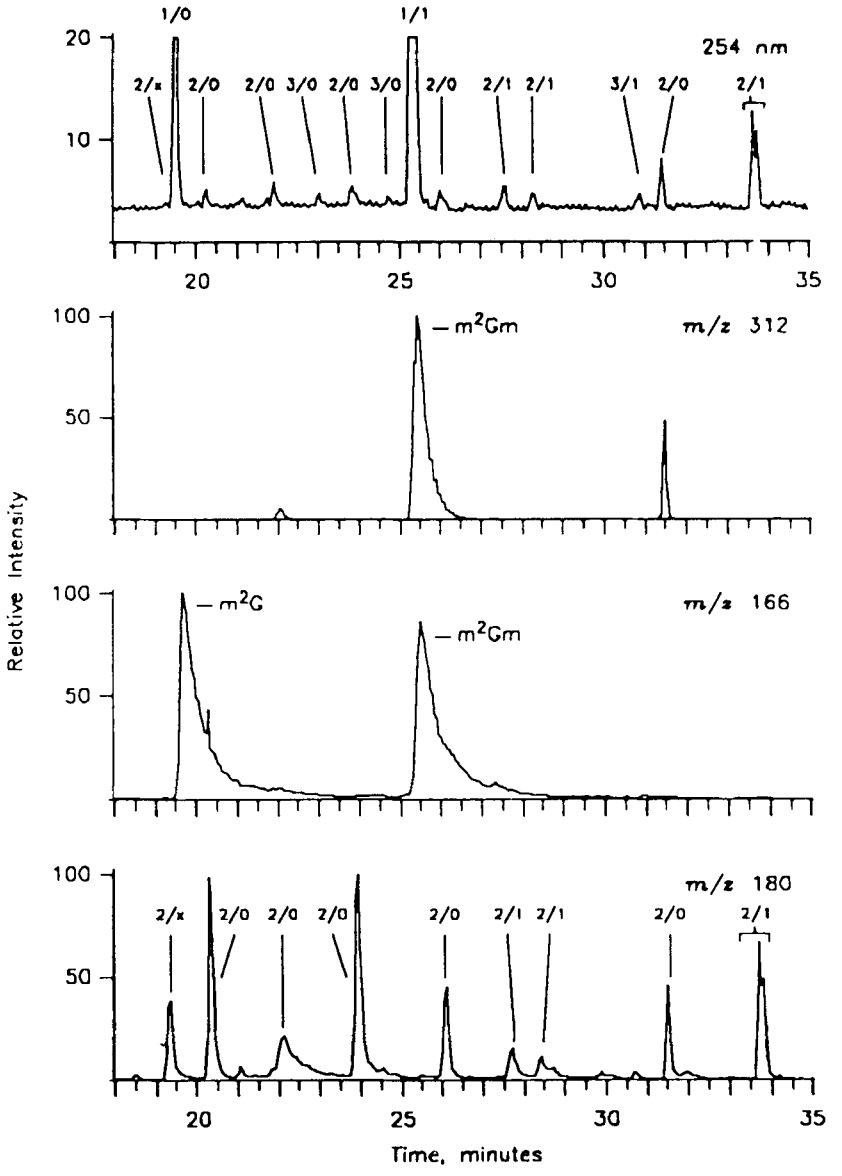

FIG. 1 LC/MS analysis of methylated products of N^2-methylguanosine ($SnCl_2$, CH_2N_2/glyme, DMF, 17 hrs, 23°C). Top: UV detection. Peak designations show numbers of methyl groups in base and sugar, respectively, determined from mass spectra; x = unknown number. Bottom panels: examples of mass channels, taken from full mass spectra recorded every 3 sec.; m/z 312, molecular (MH^+) ion showing overall incorporation of two methyls; m/z 166 and 180, base (BH_2^+) ions showing incorporation of one and two methyls, respectively, in the base. Instrument: non-commercial quadrupole, conditions previously described.[4]

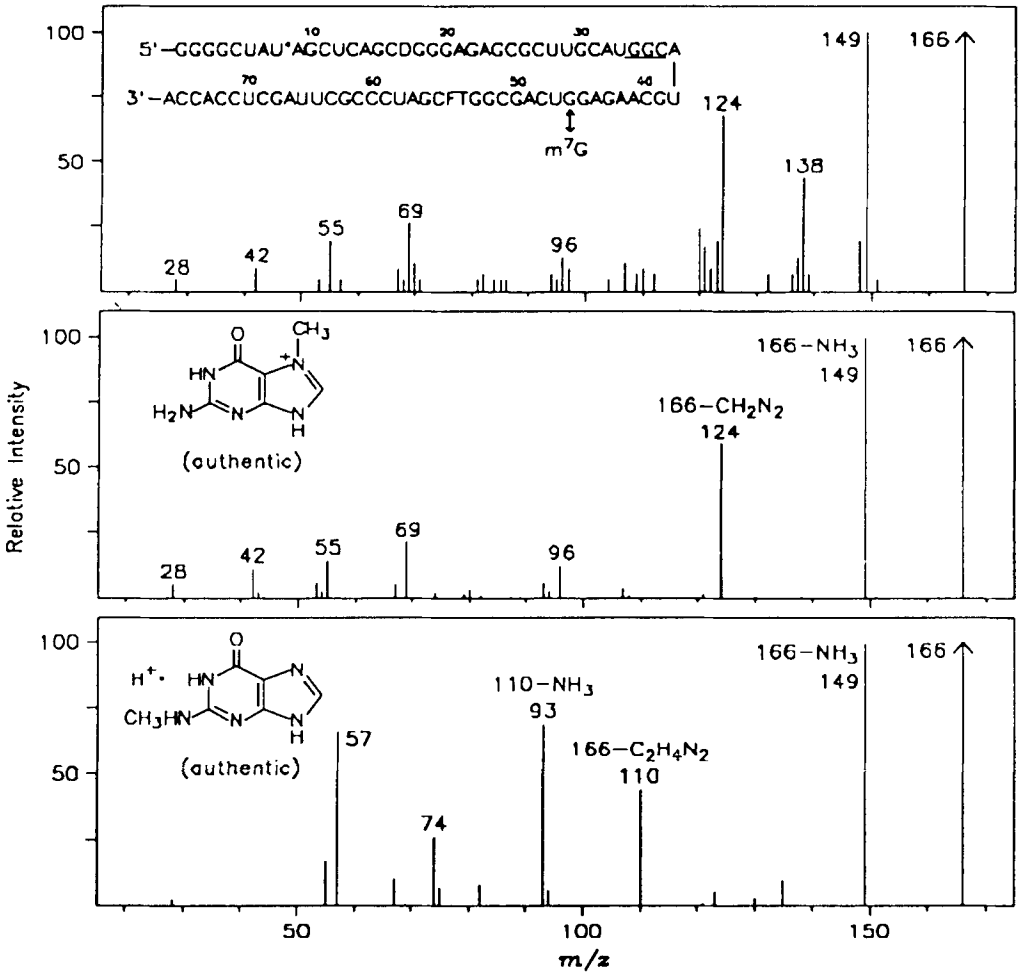

FIG. 2 FAB-MS/MS analysis of nucleosides produced by enzymatic digestion[4] of *E. coli* $tRNA^{Ala}_{GGC}$ corresponding to 5 μg of tRNA. Top: product ions from CID of m/z 166 (<25 ng of nucleoside); inset, published sequence.[9] Bottom panels: product ions from CID of m/z 166 (BH_2^+) from authentic m^7G (center) and m^2G (bottom). Instrument: VG 70-SEQ; dithioerythritol:dithiothreitol (4:1) matrix; Xe FAB gas; Kr collision gas, 30 eV collision energy.

Screening of Reaction Mixtures by Thermospray LC/MS

Although less sensitive than GC/MS, thermospray LC/MS is applicable to nucleosides in amounts of about 10 ng or greater per HPLC injection (scanned spectra), or 2 ng for selected ion monitoring,[4] and does not require derivatization for enhancement of volatility. With appropriate reversed phase HPLC procedures,[6] these characteristics are advantageous for rapid screening of reaction mixtures when the elution position of the component of interest is not known, either as a guide for subsequent isolation, or for identification of reaction products.

Figure 1 shows a typical example, in which N^2,2'-O-dimethylguanosine is sought as a product from reaction of diazomethane with N^2-methylguanosine. The 19.5 min. eluant is readily identified as starting material by its mass spectrum (not shown), and the 25.4 min. eluant as a candidate for the desired product (verified by collection, trimethylsilylation, and EI mass spectrometry[7]). Mass spectrometry provides significant enhancement of signal compared with UV detection (compare UV and m/z 180 channels), with considerable structural information as dictated by choice of mass channels. In appropriate cases an LC column can be omitted, and the reaction solution directly injected.

Characterization of Nucleoside Isomers in Mixtures by MS/MS

MS/MS can be used, often without the necessity of chromatography, for recognition of modified nucleosides in crude enzymatic digests of RNA.[5,8] Figure 2 shows the detection of 7-methylguanosine in a hydrolysate of isoaccepting tRNA, where it occurs at trace levels at position-46 by partial replacement of guanosine.[9] Its differentiation from other isomers, for example N^2-methylguanosine, is clearly made by both mass and abundance of ions in the m/z 42-124 range. As a cautionary note, it should be recognized that mixtures of isomers having the same precursor mass, if present, will give overlapping CID spectra whose interpretation may be ambiguous. The MS/MS approach is preferred over LC/MS when nucleotides or other highly polar products, which do not exhibit good thermospray mass spectra, are present in the mixture.

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