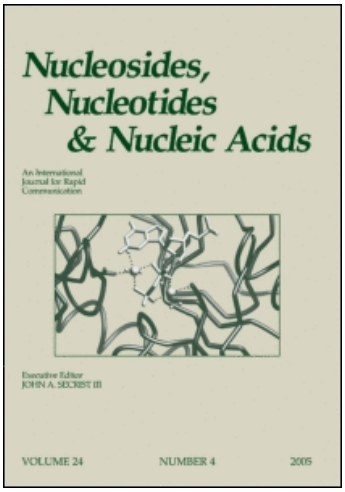

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N-Dimethylaminomethylene-O-trialkylsilyl Derivatives of Nucleosides for Chromatography and Mass Spectrometry

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N-DIMETHYLAMINOMETHYLENE-O-TRIALKYLSILYL DERIVATIVES OF NUCLEOSIDES FOR CHROMATOGRAPHY AND MASS SPECTROMETRY.

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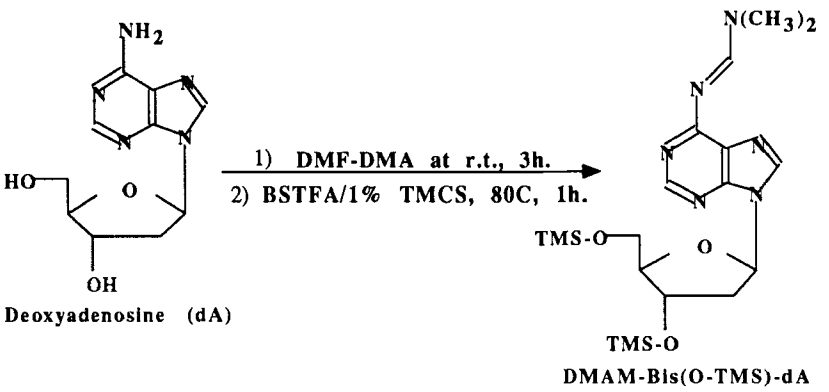
Abstract. Dimethylformamide dimethyl acetal (DMF-DMA) reacts with nucleosides under mild conditions to give N-dimethylaminomethylene (N-DMAM) derivatives. Silylation provides the DMAM-O-trialkylsilyl mixed derivatives which have good chromatographic and mass spectral properties.

INTRODUCTION.

N-Dimethylaminomethylene derivatization has found uses in the study of the spectroscopic properties of nucleosides and nucleotides[1]. Until recently, however, little was known about the chromatographic and mass spectral properties of these compounds. We have reported a mixed derivatization procedure which produced N-dimethylaminomethylene-O-tert-butyldimethylsilyl (N-DMAM-O-TBDMS) derivatives of 2'-deoxynucleosides for analysis by combined liquid chromatography - mass spectrometry (LC-MS)[2,3]. This procedure has now been extended to the trimethylsilyl derivatives for gas chromatography - mass spectrometry (GC-MS).

Recent advances in LC-MS has made it possible to use the technique for the analysis of many polar compounds. To determine the advantages that the N-DMAM derivatives may have over the underivatized compounds in LC-MS, the chromatographic separation of the nucleosides and these derivatives has been studied. The results are reported here.



Scheme 1

METHODS.

Derivatization. Briefly, the N-DMAM derivatives were prepared by allowing the substrate and the reagent to stand in dry DMF at room temperature for 3 hours. Water (100-fold molar excess) was added and the sample was evaporated to dryness under a stream of nitrogen. The sample was then silylated for GC-MS or for LC-MS, Scheme 1.

Analytical Methods. GC-MS was performed in the CI mode on a Hewlett-Packard HP5988 quadrupole system. The CI reagent gas was ammonia. HPLC separations were performed on either Spectra Physics SP8000 liquid chromatograph or Hewlett-Packard HP1090 system, both equipped with variable wavelength UV detectors. EIMS and LC-MS analyses were carried out on VG7070 double focusing instrument.

RESULTS AND DISCUSSION.

The underivatized nucleosides were earlier separated using phosphate buffer/methanol (94:6, v/v)[3,4]. We have now separated both the deoxy- and ribonucleosides in one analysis using ammonium acetate buffer/methanol, see Fig. 1. This buffer is more compatible with LC-MS than phosphate buffers and has been used for the analysis of polar compounds. Uridine and deoxycytidine are not completely resolved. This may present some problems in LC-MS analysis. When the compounds are reacted with DMF-DMA, the adenine, cytosine, and guanine nucleosides form the N-DMAM derivatives, Scheme 1. Methylation of the other pyrimidine nucleosides does not take place under the mild conditions of the reaction. HPLC analysis, Fig. 2, indicates that all the nucleosides, derivatized and underivatized, are now have excellent resolved.

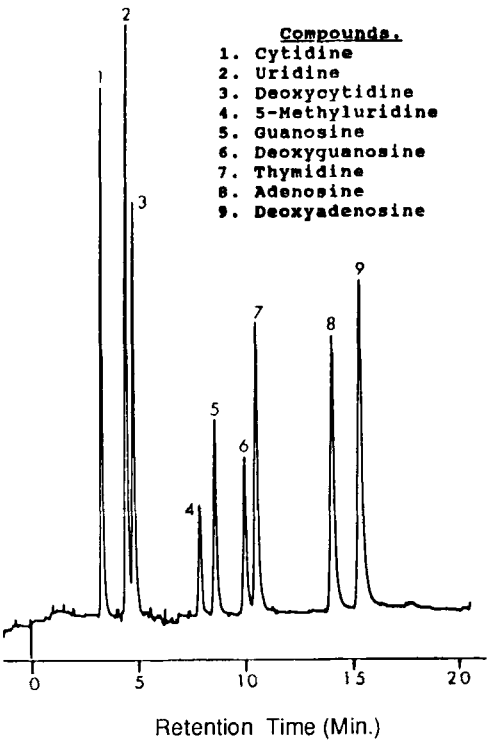


FIG. 1: RP-HPLC Analysis of Underivatized Nucleosides. Column: Octadecylsilane(ODS); 0.1M NH_4OAc + MeOH(0-12% @ 0.8%/min); Flow: 0.8mL/min. Chart speed: 0.5cm/min.

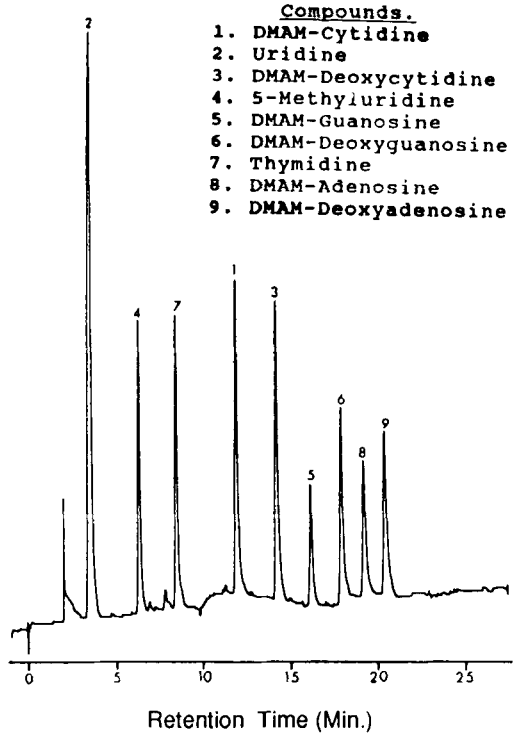
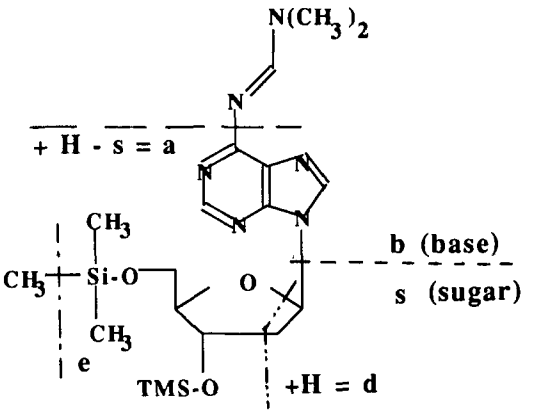


FIG. 2: RP-HPLC of DMAM-Derivatized Nucleosides. ODS; 0.1M NH_4OAc+MeOH (0-25% @ 1%/min.); Flow: 1mL/min. Chart speed: 0.5cm/min.

TABLE 1: CORRELATION OF m/z VALUES FOR FRAGMENT IONS OF TMS AND TBDMS DERIVATIVES OF DMAM-rA AND DMAM-dA.[a]

Fragment Ion	m/z		
	DMAM-dA		DMAM-rA
	TMS	TBDMS	TBDMS
M^+	450(4)	534(24)	664(-)
$[M-Me]^+$	435(1)	519(-)	N.O.
$[M-t-Bu]^+$	N.O.	477(68)	607(80)
$[M-Me-bH]^+$	245(1)	329(-)	N.O.
$[M-tBu-bH]^+$	N.O.	287(19)	417(2)
$[b+H+CHO]^+$	219(27)	219(46)	219(100)
$[b+H+C_2H_3]^+$	217(16)	217(100)	N.O.
$[b+H+C_8H_{17}OSi]^+$	N.O.	N.O.	347(33)
$[b+2H]^+$	191(9)	191(62)	191(60)
$[b+H]^+$	190(81)	190(83)	190(10)
$[b+H-Me]^+$	175(100)	175(73)	175(20)

[a]Ion intensities in brackets. N.O. means not observed.



Fragmentation Behavior of
DMAM-Bis(O-TMS)-dA

Scheme 2

The HPLC analysis, Fig. 2, shows a single peak for each nucleoside. However, GC-MS analysis of the N-DMAM-O-TMS 2'-deoxynucleosides showed two products for 2'-deoxy-adenosine indicating that both isomers of the N-DMAM-nucleosides are formed. Deoxycytidine and deoxyguanosine derivatives do not elute from the GC column probably because they are too polar. Both the N-DMAM and N-DMAM-O-TBDMS derivatives have excellent liquid chromatographic properties. The N-DMAM-O-TBDMS/-O-TMS derivatives also have excellent mass spectral properties; they have intense ions which are indicative of the nucleobases, Table 1. The DMAM group tends to direct the charge to the base. The data in Table 1 seem to support the fragmentation behavior shown in Scheme 2.

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