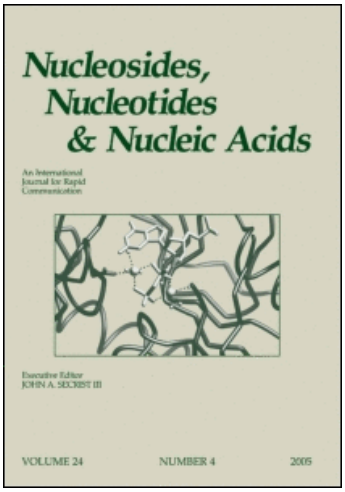

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Tandem Mass Spectrometry for Structure Assignments of Wye Nucleosides from Transfer RNA

Shaolian Zhou[ab]; Devarasetty Sitaramaiah[a]; Steven C. Pomerantz[a]; Pamela F. Crain[a]; James A. McCloskey[acd]
[a] Department of Medicinal Chemistry, University of Utah, Salt Lake City, Utah, USA [b] Covance Laboratories, Inc., Madison, Wisconsin, USA [c] Department of Biochemistry, University of Utah, Salt Lake City, Utah, USA [d] University of Utah, Salt Lake City, Utah, USA

Online publication date: 02 October 2004

To cite this Article Zhou, Shaolian , Sitaramaiah, Devarasetty , Pomerantz, Steven C. , Crain, Pamela F. and McCloskey, James A.(2004) 'Tandem Mass Spectrometry for Structure Assignments of Wye Nucleosides from Transfer RNA ', Nucleosides, Nucleotides and Nucleic Acids, 23: 1, 41 — 50
To link to this Article: DOI: 10.1081/NCN-120027816
URL: http://dx.doi.org/10.1081/NCN-120027816

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 1 & 2, pp. 41–50, 2004

Tandem Mass Spectrometry for Structure Assignments of Wye Nucleosides from Transfer RNA†

Shaolian Zhou,[1,#] Devarasetty Sitaramaiah,[1] Steven C. Pomerantz,[1] Pamela F. Crain,[1] and James A. McCloskey[1,2,*]

[1]Department of Medicinal Chemistry and [2]Department of Biochemistry, University of Utah, Salt Lake City, Utah, USA

ABSTRACT

The tricyclic wye nucleoside family of eight known members constitutes one of the most complex and interesting series of posttranscriptionally modified nucleosides in transfer RNA. The principal reaction paths represented in collision-induced dissociation mass spectra of wye bases and their analogs have been studied in order to determine those structural features that can be readily established by mass spectrometry. The main routes of fragmentation are determined by the presence vs. absence of an amino acid side chain at C-7 (1*H*-imidazo[1,2-*a*]purine nomenclature). The common methionine-related side chain is cleaved at two points, providing a ready means of establishing the presence and net level of side chain modification. For those molecules without a side chain, the initial reaction steps are characteristically controlled by the presence vs. absence of methyl at N-4, allowing determination of the methylation status of that site. In the latter case initial opening of the central (pyrimidine) ring, in analogy to the dissociation behavior of guanine, causes loss of

†In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

#Current address: Shaolian Zhou, Covance Laboratories, Inc., Madison, Wisconsin, USA.
*Correspondence: James A. McCloskey, University of Utah, School of Medicine, 50 N. Medical Drive, Salt Lake City, UT 84132, USA; E-mail: james.mccloskey@utah.edu.

DOI: 10.1081/NCN-120027816 1525-7770 (Print); 1532-2335 (Online)

identity of C-6/C-7 so that placement of a single methyl at either site is not possible. Subsequent complex reaction paths follow, which include loss of CO and sequential loss of two molecules of HCN.

Key Words: tRNA nucleosides; Wye nucleosides; Mass spectrometry; Collision-induced dissociation.

INTRODUCTION

Members of the tricyclic ''wye'' family of ribonucleosides from transfer RNA constitute one of the most elaborate and extensive series (eight known members) of natural RNA nucleosides.[1,2] Their fluorescent properties, complex biosynthetic pathways[3] (still not fully established) and unusual chemical structures have made them attractive targets for numerous studies of their influence on anticodon structure and function in $tRNA^{Phe}$,[4] of their physico-chemical characteristics (e.g., Refs. [5,6]) and of their chemical synthesis.[7,8] Beginning with the pioneering microscale structure elucidation work of Nakanishi in 1970[9] and of Kasai and his collaborators (Ref. [10] which includes an extensive list of early publications), the basic structural features of the wye nucleosides were established: a tricyclic nucleus biologically derived from encoded guanine,[11,12] with H, CH_3, or methionine-related side chains at C-7, an invariant CH_3 at C-6, and until two recently reported new wye family members,[13] a CH_3 group at N-4 (N-3 in purine nomenclature). The numbering system used[14] follows that of 1*H*-imidazo[1,2-*a*]purine. Discussion of early nomenclature is given in

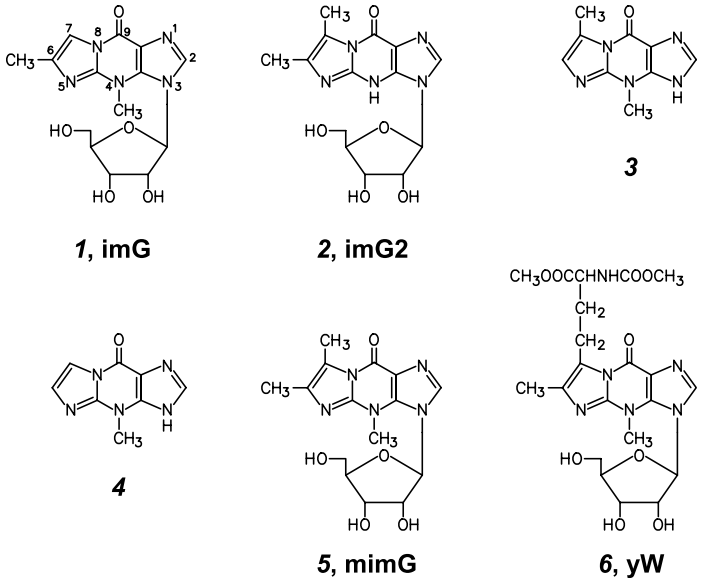

Figure 1. Structures of wye-related nucleosides and bases studied. Symbols are given for those that are known naturally as components of tRNA.

Refs. [10,14]. Chemical structures, systematic and common names and currently used symbols for tRNA nucleosides in the wye family can be found at: http://medlib.med.utah.edu/RNAmods.

We present the result of preliminary studies on the use of mass spectrometry (MS) for the rapid characterization of some structural features of the natural wye nucleoside family: the number and disposition of alkyl groups in the tricyclic nucleus, and the presence and nature of an amino acid side chain at C-7. MS-related methods are potentially advantageous due to inherent sensitivity of the measurement (nanogram-level quantities of nucleoside) and direct applicability to components of complex mixtures, such as in RNA hydrolysates or crude chemical reaction products, via conventional liquid chromatography-mass spectrometry (LC/MS). The compounds studied are the nucleosides and bases in Figure 1.

RESULTS AND DISCUSSION

The principal fragmentation paths of the wye bases represented in their mass spectra can be broadly divided into those associated with amino acid side chains at C-7 (e.g., compound **6**, Figure 1) and those without side chains (**1–5**). In either case when details of base structure are of interest a more informative spectrum will result if the base, rather than the nucleoside, is subjected to dissociation. When working with nucleosides the protonated base can be efficiently released from the protonated molecule in the electrospray ionization region of the mass spectrometer. The first mass analyzer (MS-1) of the tandem mass spectrometer is used to mass-select the protonated base ions of interest, which are then transmitted to a gas collision cell for dissociation, and acquisition of the resulting mass spectrum using the second mass analyzer, MS-2.[15] If the starting sample consists only of the base then the ion source dissociation step is not necessary.

Dissociation Reactions in the Absence of a Side Chain

In this structural category the key features of mass spectra are clearly controlled by the presence or absence of methylation at N-4, as in the natural isomers **1** vs. **2** as shown in Figure 1. Facile loss of CH_3 (or CH_4) from N-4 of the protonated base is the major dissociation process (Figure 1A), which produces an odd-electron ion that can be stabilized by charge and radical delocalization through the nitrogen-rich tricyclic ring system. No information is available to establish whether elimination of CH_4 occurs by loss of H from the *m/z* 189 fragment ion or directly from the *m/z* 204 parent ion. By contrast the energetically unfavored loss of methyl from unsaturated carbons (C-6 or C-7) in Figure 2B and 2C is a minor process which cannot compete with other dissociation pathways involving ring opening. The distinction conferred by N-4 methylation is also clearly apparent in the mass spectrum of **4** (Figure 3) compared under the same experimental conditions with that of the natural 6-methyl isomer imG-14 (mass spectrum shown in Ref. [13]), the two structures differing only by location of the single methyl group. Compound **4** undergoes methyl loss as a major first reaction step, while the analogous $190^+ \rightarrow 175^+$ process in the isomer imG-14 is a very low abundance pathway.[13]

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

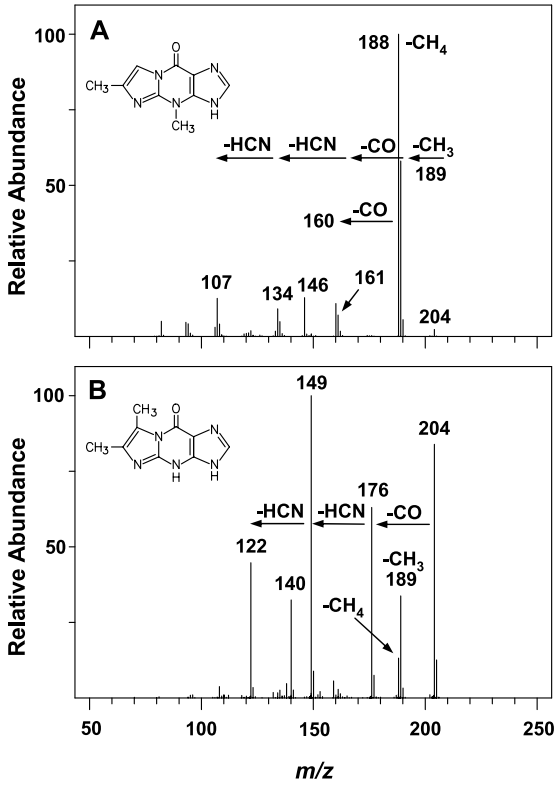

Figure 2. Mass spectra of the bases of (A), **1**, (B) **2**, following their gas phase production from the corresponding nucleoside.

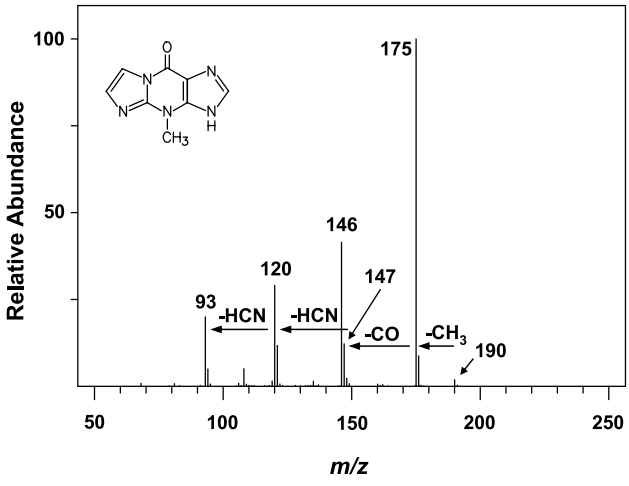

Figure 3. Mass spectrum of compound **4**.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

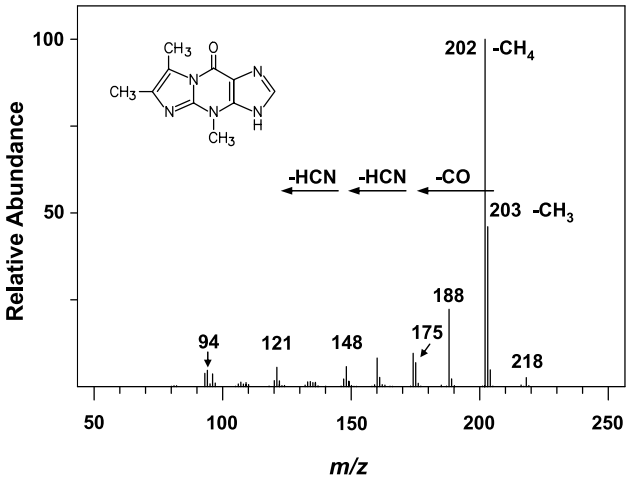

Figure 4. Mass spectrum of the base of compound **5** following its gas-phase production from the nucleoside.

In the absence of 4-CH_3 in the tricyclic nucleus the primary reaction path (Figure 2B) is initiated by expulsion of CO from C-9 in the center ring, followed by sequential loss of two molecules of HCN (*m/z* 149, 122). The latter processes are characteristic of purines, as in the spectra of adenine[16] and guanine,[17] but identification of specific atoms involved in HCN losses in the present study would require extensive isotopic labeling which was not carried out. In the more common case in which 4-CH_3 is present and initial dissociation is dominated by methyl loss, subsequent minor pathways are still evident involving sequential losses of CO and HCN as marked in Figures 2A, 3 and 4.

Interestingly, mass spectra from the structural analogs **1** (Figure 2A) and **3** (data not shown) are indistinguishable in all details. This is interpreted in terms of analogous unexpected similarity between the spectra of 1- and N^2-methylguanines[17] and supported by studies of dissociation of guanine in which all five nitrogen atoms were individually labeled by ^{15}N,[17] as follows. An initial reaction following collisional activation occurs (Figure 5) in which the N-8, C-9 bond is broken, followed by free rotation around the N-4, C-4a bond so that the structural distinction between N-5/N-8, and C-6/C-7 is lost prior to further dissociation. This has the possible consequence in the wye nucleoside series that the mass spectra are insensitive to C-6 vs. C-7 substitution, although the number of methyl groups present can be inferred from the mass of the base ion.

Dissociation Reactions in the Presence of a Side Chain

Four of the eight known natural wye nucleosides[1,13] are substituted at C-7 by amino acids derived from methionine (in the case of yeast)[18] or possibly lysine (in Vero cells).[19] The significant structural addition of a side chain, e.g., **6** vs. **1**, **2** and **5**, provides additional favorable fragmentation pathways that bear side chain structural

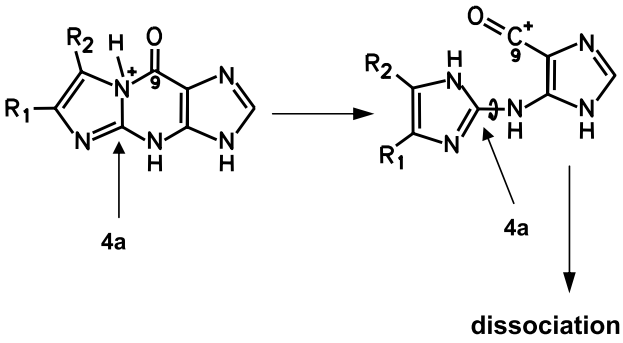

Figure 5. Proposed initial ring-opening reaction leading to loss of identities of C-6 and C-7 prior to dissociation. The site of protonation from electrospray ionization is shown for convenience as N-8.

information and overshadow the wye nucleus dissociation reactions, including the otherwise favored loss of 4-CH_3. Figure 6 shows the mass spectrum of the base of wybutosine from yeast tRNA, one of the earliest and best-known of the side chain-containing wye tRNA nucleosides.[9]

The mass spectrum is dominated by simple cleavage of the side chain between the beta and gamma amino acid carbons, Figure 7, a process also observed in electron ionization mass spectra of wye base derivatives.[9,14,20–22] The assignment shown is supported by measurement of exact mass: fd. *m/z* 216.0887; required for $C_{10}H_{10}N_5O$, 216.0885. This process is consistent with initial protonation of the side chain, producing a highly stabilized cation (*m/z* 216) in which the charge can be extensively delocalized through the heteroaromatic nucleus. The mass of this favored cleavage product in related molecules would reflect the number of methyl groups (but not their locations) in the conserved tricyclic nucleus.

The next most abundant product ion, *m/z* 204 corresponds to the unexpected net loss of the entire side chain, occurring concomitantly with the transfer of hydrogen from the neutral side chain moiety which is lost. This assignment is supported by exact mass measurement: fd. *m/z* 204.0905; required for $C_9H_{10}N_5O$, 204.0885. This process is envisioned as occurring from a nucleus-protonated precursor, although a mechanism originating with an amino acid side chain protonated species may also be possible. The constrained nature of thus far known wye nucleoside structures is such that an ion pair equivalent to *m/z* 216/204 will characteristically occur 12 mass units apart (or 12.000 if exact mass measurements are made). Presence of two carbomethoxy groups in the side chain is reflected in the sequential elimination of the two molecules of CH_3OH ($377^+ \rightarrow 345^+ \rightarrow 313^+$). These minor but structurally diagnostic assignments are supported by measurements of exact mass, made by LC/MS/MS using quadrupole/time-of-flight tandem mass analyzers: fd. *m/z* 345.1285; required for CH_3OH loss, 345.2422; fd. *m/z* 313.1119, required for CH_3OH loss, 313.1049.

The molecular weight of the nucleoside (as determined by the MH^+ ion) in all probable wye-like structures will readily indicate the presence vs. absence of a side chain, with the mass (or elemental composition from exact mass measurement) of the

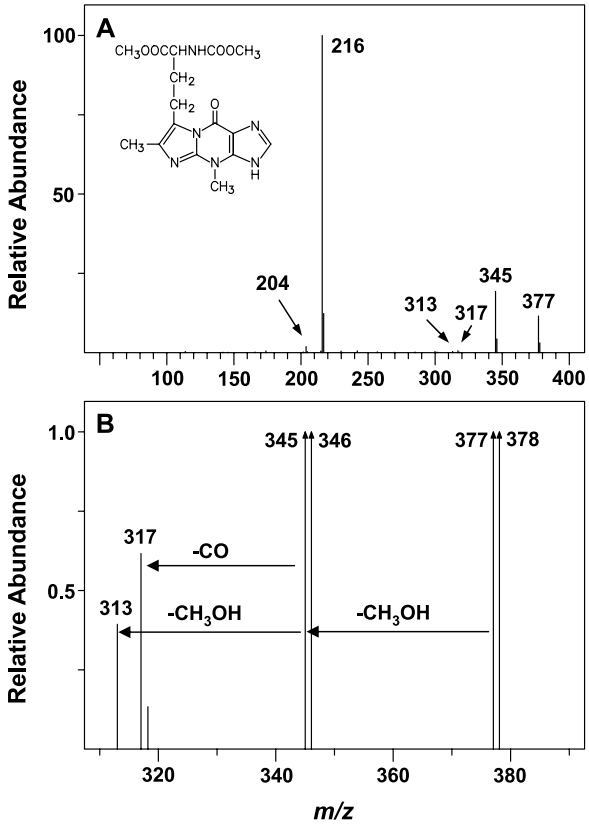

Figure 6. (A). Mass spectrum of the base of compound **6** acquired by LC/MS/MS from a nucleoside digest of brewer's yeast $tRNA^{Phe}$ in which the base was released from the nucleoside in the ionization region. (B). Enlarged portion of the *m/z* 310–380 region showing successive losses of CH_3OH.

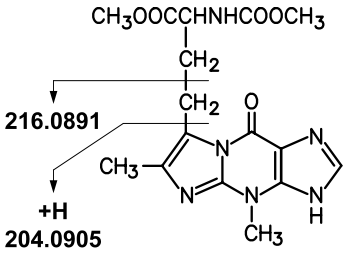

Figure 7. Side chain cleavage reactions in the base of nucleoside **6**. The proton of ionization is not shown.

side chain being determined by difference using the *m/z* 216/204-type ions. However because of the dominance of side chain-induced fragmentation in the mass spectrum it appears unlikely that the disposition of alkyl groups in the wye nucleus could be established using single stage MS/MS.

EXPERIMENTAL

Materials

Compounds **2** and **5** were prepared in this laboratory in conjunction with other studies,[13,23] respectively). Compounds **1**, **3**, and **4** were prepared by chemical synthesis and were gifts from Dr. H. Kasai, University of Occupational and Environmental Health, Japan. Brewer's yeast $tRNA^{Phe}$ was purchased from Sigma (R 4018).

Mass Spectrometry

Electrospray ionization mass spectra were acquired using either a liquid chromatography-mass spectrometry (LC/MS/MS) system consisting of a Quattro II (Micromass) triple quadrupole mass spectrometer, interfaced to an HP 1090 (Hewlett-Packard) liquid chromatograph and Phenomenex Luna C-18 reversed phase column (data in Figure 2B and Figure 6), or using a Micromass Q-Tof-2 instrument and direct sample infusion (data in Figures 2A, 3 and 4). The mass spectrum of the base of **6** (Figure 6) was acquired by LC/MS/MS from a nucleoside digest of brewer's yeast $tRNA^{Phe}$ prepared by standard nuclease digestion protocols.[24]

All mass spectra were produced by collision-induced dissociation of protonated base ions, mass-selected by MS-1 and dissociated in an argon gas collision cell at 20-25 eV energy. In the case of nucleosides **1**, **2**, **5**, and **6**, protonated base ions were released using elevated cone voltage in the electrospray ionization region prior to the mass selection, as described.[15] The assignments for ions *m/z* 204, 216, 313 and 345 from **6** were supported by exact mass measurements in a separate experiment by LC/MS/MS of a brewer's yeast $tRNA^{Phe}$ digest using the Q-Tof-2 instrument interfaced to a Waters CapLC liquid chromatograph; nucleoside **6**, R_t 36.5 min, MH^+ *m/z* 509, BH_2 *m/z* 377.[25]

ACKNOWLEDGMENTS

This work was supported by grant GM29812 from the National Institutes of Health. The authors thank Hiroshi Kasai, and Takeshi Hashizume for generous gifts of synthetic bases and nucleosides.

REFERENCES

1. Motorin, Y.; Grosjean, H. Appendix 1: chemical structures and classification of posttranscriptionally modified nucleosides in RNA. In *Modification and Editing of*

RNA; Grosjean, H., Benne, R., Eds.; ASM Press: Washington, DC, 1998; 543–549.
2. McCloskey, J.A. Appendix: modified nucleosides from RNA. In *RNA*; Söll, D., Nishimura, S., Moore, P.B., Eds.; Elsevier: Amsterdam, 2001; 309–316.
3. Garcia, G.A.; Goodenough-Lashua, D.M. Mechanisms of RNA-modifying and -editing enzymes. In *Modification and Editing of RNA*; Grosjean, H., Benne, R., Eds.; ASM Press: Washington, DC, 1998; 135–168.
4. Curran, J.F. Modified nucleosides in translation. In *Modification and Editing of RNA*; Grosjean, H., Benne, R., Eds.; ASM Press: Washington, DC, 1998; 493–516.
5. Glemarec, C.; Wu, J.-C.; Remaud, G.; Bazin, H.; Oivanen, M.; Lönnberg, H.; Chattopadhyaya, J. Structure and reactivity of wyosine (Y-nucleoside) and its derivatives. Chemical, kinetic and spectroscopic studies. Tetrahedron **1988**, *44*, 1273–1290.
6. Nordlund, T.M.; Rigler, R.; Glemarec, C.; Wu, J.-C.; Bazin, H.; Remaud, G.; Chattopadhyaya, J. Fluorescence properties of Y-nucleoside derivatives. Nucleosides Nucleotides **1988**, *7*, 805–811.
7. Funamizu, M.; Terahara, A.; Feinberg, A.M.; Nakanishi, K. Total synthesis of *dl*-Y base from yeast phenylalanine transfer ribonucleic acid and determination of its absolute configuration. J. Am. Chem. Soc. **1971**, *93*, 6706–6708.
8. Itaya, T.; Morisue, M.; Takeda, M.; Kumazawa, Y. Synthesis of 7-methyl-3-β-D-ribofuranosylwye, the putative structure for the hypermodified nucleoside isolated from archaebacterial transfer ribonucleic acids. Chem. Pharm. Bull. **1990**, *38*, 2656–2661.
9. Nakanishi, K.; Furutachi, N.; Funamizu, M.; Grunberger, D.; Weinstein, I.B. Structure of the fluorescent Y base from yeast phenylalanine transfer ribonucleic acid. J. Am. Chem. Soc. **1970**, *92*, 7617–7619.
10. Kasai, H.; Goto, M.; Ikeda, K.; Zama, M.; Mizuno, Y.; Takemura, S.; Matsuura, S.; Sugimoto, T.; Goto, T. Structure of wye (Yt base) and wyosine (Yt) from *Torulopsis utilis* phenylalanine transfer ribonucleic acid. Biochemistry **1976**, *15*, 898–904.
11. Li, H.J.; Nakanishi, K.; Grunberger, D.; Weinstein, I.B. Biosynthetic studies of the Y base in yeast phenylalanine tRNA. Incorporation of guanine. Biochem. Biophys. Res. Commun. **1973**, *55*, 2183–2196.
12. Thiebe, R.; Poralla, K. Origin of the nucleoside Y in yeast $tRNA^{Phe}$. FEBS Lett. **1973**, *38*, 27–28.
13. Zhou, S.; Sitaramaiah, D.; Noon, K.R.; Guymon, R.; Hashizume, T.; McCloskey, J.A. Structures of two new ''minimalist'' modified nucleosides from archaeal tRNA. Bioorg. Chem., **2004**. *in press*.
14. Blobstein, S.; Grunberger, D.; Weinstein, I.B.; Nakanishi, K. Isolation and structure determination of the fluorescent base from bovine liver phenylalanine transfer ribonucleic acid. Biochemistry **1973**, *12*, 188–193.
15. Crain, P.F. Detection and structure analysis of modified nucleosides in RNA by mass spectrometry. In *Modification and Editing of RNA*; Grosjean, H., Benne, R., Eds.; ASM Press: Washington, DC, 1998; 47–57.
16. Nelson, C.C.; McCloskey, J.A. Collision-induced dissociation of adenine. J. Am. Chem. Soc. **1992**, *114*, 3661–3668.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

17. Gregson, J.M.; McCloskey, J.A. Collision-induced dissociation of protonated guanine. Int. J. Mass Spectrom. Ion Process. **1997**, *165/166*, 475–485.
18. Munch, H.-J.; Thiebe, R. Biosynthesis of the nucleoside Y in yeast $tRNA^{Phe}$: incorporation of the 3-amino-3-carboxypropyl group from methionine. FEBS Lett. **1975**, *51*, 257–258.
19. Pergolizzi, R.G.; Engelhardt, D.L.; Grunberger, D. Incorporation of lysine into Y base of phenylalanine tRNA in Vero cells. Nucleic Acids Res. **1979**, *6*, 2209–2216.
20. Kasai, H.; Goto, M.; Takemura, S.; Goto, T.; Matsuura, S. Structure and synthesis of a fluorescent Y-like base from *Torulopsis utilis* tRNA. Tetrahedron Lett. **1971**, *29*, 2725–2728.
21. Feinberg, A.M.; Nakanishi, K.; Barciszewski, J.; Rafalski, A.J.; Augustyniak, H.; Wiewiórowski, M. Isolation and characterization of peroxy-Y base from phenylalanine transfer ribonucleic acid of the plant *Lupinus luteus*. J. Am. Chem. Soc. **1974**, *96*, 7797–7800.
22. Kasai, H.; Yamaizumi, Z.; Kuchino, Y.; Nishimura, S. Isolation of hydroxy-Y base from rat liver $tRNA^{Phe}$. Nucleic Acids Res. **1979**, *6*, 993–999.
23. McCloskey, J.A.; Crain, P.F.; Edmonds, C.G.; Gupta, R.; Hashizume, T.; Phillipson, D.W.; Stetter, K.O. Structure determination of a new fluorescent tricyclic nucleoside from archaebacterial tRNA. Nucleic Acids Res. **1987**, *15*, 683–693.
24. Crain, P.F. Preparation and enzymatic hydrolysis of RNA and DNA for mass spectrometry. Methods Enzymol. **1990**, *193*, 782–790.
25. Pomerantz, S.C.; McCloskey, J.A. Analysis of RNA hydrolyzates by LC/MS. Methods Enzymol. **1990**, *193*, 796–824.

Received August 19, 2003
Accepted September 15, 2003

Request Permission or Order Reprints Instantly!