Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic Chemistry 32 (2004) 82–91
BIOORGANIC CHEMISTRY
www.elsevier.com/locate/bioorg

Structures of two new "minimalist" modified nucleosides from archaeal tRNA

Shaolian Zhou,[a] Devarasetty Sitaramaiah,[a] Kathleen R. Noon,[a] Rebecca Guymon,[a] Takeshi Hashizume,[a] and James A. McCloskey[a,b,*]

[a] *Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT 84112-5820, USA*
[b] *Department of Biochemistry, University of Utah, Salt Lake City, UT 84132-3201, USA*

Received 22 September 2003

Abstract

The wyeosine (or wye) family of tricyclic ribonucleosides from archaeal and eukaryal $tRNA^{Phe}$ constitutes one of the most complex and interesting series of posttranscriptional RNA modifications, and has been the object of numerous studies of their chemical and biological synthesis and distribution. We report the structures of two minimally elaborated wye derivatives from archaea, raising the known number of wye nucleosides to eight: 3,4-dihydro-6-methyl-3-β-D-ribofuranosyl-9*H*-imidazo[1,2-*a*]purine-9-one (symbol imG-14), and 3,4-dihydro-6,7-dimethyl-3-β-D-ribofuranosyl-9*H*-imidazo[1,2-*a*]purine-9-one (symbol imG2). Structures were determined primarily by mass spectrometry, and confirmed by comparison of physicochemical properties with those of chemically synthesized nucleosides. The nucleosides contain no amino acid side chains at C-7 (1*H*-imidazo[1,2-*a*]purine nomenclature) and are the only wye derivatives not methylated at N-4. These features suggest a minimal role for wye methyl groups and side chains in maintenance of anticodon stem–loop structures, and support the concept that archaeal tRNA nucleoside modification motifs are generally simpler than those of their counterparts in eukarya and bacteria.

Keywords: Nucleosides RNA modification; transfer RNA; mass spectrometry

* Corresponding author. Fax: 1-801-581-7457.
E-mail addresses: shaolian.zhou@covance.com (S. Zhou), sitaram_d@yahoo.com (D. Sitaramaiah), krnoon@umich.edu (K.R. Noon), rebecca.guymon@rna.pharm.utah.edu (R. Guymon), james.mccloskey@utah.edu (J.A. McCloskey).

0045-2068/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.bioorg.2003.09.005

1. Introduction

The posttranscriptionally modified nucleotides of transfer RNA display an exceptional diversity of modification motifs [1,2]. A number of these structures are shared within the three major evolutionary domains (Archaea, Bacteria, and Eukarya), while some are phylogenetically unique to each domain [1]. One of the most structurally interesting nucleoside families are the wyosine (or "wye") tricyclic nucleosides, found in eukaryal and archaeal tRNAs [3]. The earliest and perhaps best known member of the family is the hypermodified nucleoside wybutosine (**1**, Fig. 1), isolated from yeast tRNA [4,5]. The wye nucleosides are generally thought to occur specifically at position 37 in the anticodon loop of $tRNA^{Phe}$ [3,6]. All six previously reported wye nucleosides [1] are methylated at N-4 (N-3 in purine nomenclature), four of which possess C_4 [7] or C_7 [4,8,9] amino acid-based side chains. We report the structures of two "minimalist" wyosine tRNA nucleosides from archaea, designated imG-14 and imG2 (structures **2**, **3** in Fig. 1). Both nucleosides were first detected by liquid chromatography/electrospray ionization mass spectrometry (LC/MS) in unfractionated tRNA from the hyperthermophile *Pyrolobus fumarii* [10]. Nucleoside imG-14, also designated as unknown imG*, was also reported in tRNAs from six *Methanococci* [11], a lineage of marine euryarchaea, and from the

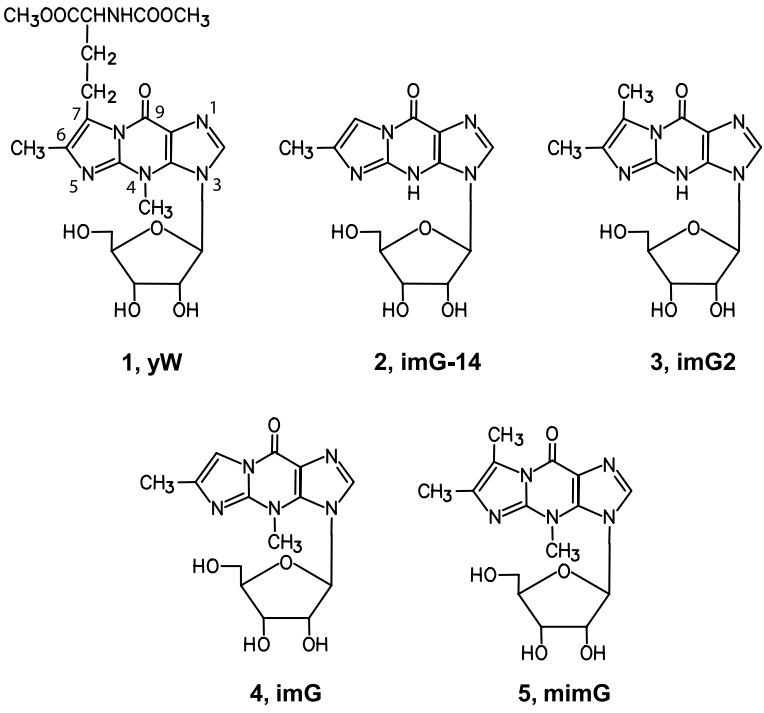

Fig. 1. Structures of wybutosine (**1**, yW) from yeast $tRNA^{Phe}$, and wye family nucleosides from archaeal tRNA: imG-14 (**2**), imG2 (**3**), imG (**4**), and mimG (**5**).

psychrotolerant archaeon *Methanococcides burtonii* [12]. imG2 was found in tRNA from the thermophile *Stetteria hydrogenophilia* tRNA [12]. These nucleosides contain no side chains, and are the first known members of this class not to be methylated at N-4, which bears on the issues of the biosynthesis and function of the wyosine methyl groups.

2. Materials and methods

2.1. Abbreviations and nomenclature

Symbol imG-14 ("imG minus 14") previously designated imG* [11]), common name, 4-demethylwyosine; systematic name, 3,4-dihydro-6-methyl-3-β-D-ribofuranosyl-9*H*-imidazo[1,2-*a*]purine-9-one. Symbol imG2; common name, isowyosine; systematic name, 3,4-dihydro-6,7-dimethyl-3-β-D-ribofuranosyl-9*H*-imidazo[1,2-*a*]purine-9-one. Structures, names and abbreviation symbols for other members of the wye nucleoside family can be found on the World Wide Web at: http://medlib.med.utah.edu/RNAmods/, under modified guanosines in tRNA.

2.2. Sources of tRNAs and sample preparation for mass spectrometry

Unfractionated tRNAs from *Methanococcus igneus*, *Methanococcus thermolithotrophicus* and *Stetteria hydrogenophila* were isolated in conjunction with earlier studies as described [11,12]. Unfractionated *Sulfolobus solfataricus* P2 tRNA was isolated by standard protocols [13] from cells cultured as previously described [14]. Enzymatic digestion of tRNA to nucleosides prior to mass spectrometry measurements was accomplished using nuclease P_1, venom phosphodiesterase I and bacterial alkaline phosphatase by a standard procedure [15], usually on a scale of 10–100 μg of tRNA.

2.3. Liquid chromatography/electrospray ionization mass spectrometry (LC/MS)

Nucleoside mixtures produced by tRNA hydrolysis (data in Fig. 2) were analyzed using a Quattro II triple quadrupole mass spectrometer (Micromass) with electrospray ionization (ESI), interfaced to a HP1090 liquid chromatograph with photodiode array detector (Hewlett–Packard), as earlier detailed [11]. Collision-induced dissociation (CID) mass spectra of protonated base ions (data in Fig. 3) were acquired using the same instrument, under the following conditions: electrospray ion source cone voltage for enhanced release of protonated base ions (*m/z* 190) from protonated nucleosides, 65 V; gas cell collision energy 25 eV.

2.4. Chemical synthesis of nucleosides imG-14 and imG2

Syntheses for both compounds were carried out from guanosine by ring closure reaction to form a N1-N^2 two-carbon bridge, analogous to reported procedures

[16,17]. The preparation of imG-14 was first reported in 1976 using essentially the same method [16].

2.5. imG-14

Sodium hydride (7.12 mg, 0.86 mmol) as a 60% suspension in oil was added to a solution of guanosine (50 mg, 0.177 mmol) in anhydrous DMSO (0.6 ml) and the mixture stirred with exclusion of moisture for 1 h. The almost clear solution was treated with bromoacetone (25 mg, 0.186 mmol) for 2 h. The reaction mixture was then made basic by addition of concentrated NH_4OH (0.3 ml) and after 2 h at room temperature the dark red solution was concentrated under reduced pressure to 0.6 ml volume. The solution was diluted with acetone (0.3 ml) and added with stirring to a mixture of acetone (4 ml) and hexane (0.5 ml). After 5 h at 0 °C the oily precipitate was separated by decantation of the supernatant, washed with hexane with stirring and dried under reduced pressure, then dissolved in water (1 ml) and adsorbed on a portion of silica gel (1 g, 200–400 mesh) by repeated co-evaporation with 2-propanol. The dried gel was applied to a silica gel short column and the product was eluted with chloroform:methanol (6:2). Fractions containing the required product were evaporated to afford a white solid (40 mg). ^{1}H-NMR (Me_2SO-d_6): δ 2.42 [3H, s, C(6)-Me], 7.36 [1H, s, C(7)-H], 8.16 [1H, s, C(2)-H]; lit [16] (Me_2SO-d_6) 231, 285. HPLC/ESI-MS: *m/z* 322 (MH^+), 190; R_t 25.7 min, λ_{max} (HPLC) 229, 282 nm; lit [16] (H_2O) 231 and 285 nm.

2.6. imG2

The procedure used was identical to that described for imG-14 (above) except that 3-bromo-2-butanone (28 mg, 0.186 mmol) was used instead of bromoacetone, yielding 38 mg of white solid final product. ^{1}H-NMR (Me_2SO-d_6): δ 2.08 [3H, s, C(6)-Me], 2.56 [3H, s, C(7)-Me], 8.16 [1H, s, C(2)-H]. HPLC/ESI-MS: *m/z* 336 (MH^+), 204; R_t 30.7 min, λ_{max} (HPLC) 233 and 287 nm.

3. Results

3.1. Structure assignments for nucleosides imG-14 and imG2

The recognition of imG-14 and imG2 as new RNA nucleosides was made principally from their ESI mass spectra in combination with relative HPLC retention times. A summary of identification parameters, along with those for related nucleosides from archaeal tRNA (structures **4** and **5**, Fig. 1) is given in Table 1. The UV spectral characteristics of both nucleosides (Table 1) suggested that they were likely members of the tricyclic wyosine family [11,12]. The molecular mass of imG-14, 321, is unique to all known RNA nucleosides (tabulated but not mass-ordered at http://medlib.med.utah.edu/RNAmods) and is 14 Da lower than that of wyosine (imG), hence the original designation imG-14 [10] suggesting a possible structural difference

Table 1
Parameters for identification of wyosine derivatives from archaeal tRNA

Nucleoside[a]	λ_{max} (nm)	HPLC R_t (min)	Mass				
			M_r	MH^+	BH_2^+	MD^+	BD_2^+
imG-14	229, 282[b]	25.7[c]	321	322	190	327	193
imG2	233, 287[c]	30.7[c]	335	336	204	341	207
imG	235, 293[d]	27.7[d]	335	336	204	340	206
mimG	239, 301[e]	32.4[e]	349	350	218	354	220

[a] Structures shown in Fig. 1.
[b] Ref. [11].
[c] Ref. [12].
[d] Values derived from experiments reported in [11].
[e] Values derived from experiments reported in [12].

of one methyl group. In the case of unknown imG2, the molecular mass is the same as that of the known nucleosides imG and N^6-isopentenyladenosine (both 335), but its chromatographic elution time in reversed-phase HPLC is three min later than that of imG2 and five min earlier than that of N^6-isopentenyladenosine [18].

Deuterium labeling of each nucleoside was carried out by hydrogen–deuterium exchange on-line during LC/MS analysis of total tRNA digests [18,19]. These data, annotated in Fig. 2, were used to infer structural features related to the number of exchangeable hydrogens in each molecule (i.e., carbon-bound vs. N- or O-bound hydrogen atoms). For imG-14 a shift upon labeling of 5 *m*/*z* units in the protonated molecule corresponds to 4 Da in the neutral molecule (correcting for the proton of ionization, which is exchangeable [19]). A shift of 3 *m*/*z* units in the protonated base is equivalent to 2 Da in the neutral base. A mass shift of 2 is interpreted as resulting from exchange of the labile hydrogen atom transferred to the base (presumably at N-3) from ribose during fragmentation to form the protonated base ion [19], plus one additional exchangeable hydrogen. The second site can only be N-4 because hydrogens at C-6 or C-7 would not be labile under the conditions used. This leads to the unexpected inference that N-4 is not methylated, unlike all previously reported members of the wye nucleoside family. The single methyl attached to the tricyclic ring system therefore conceivably resides by default at either C-6 or C-7, the other known sites of ring substitution in the natural wye nucleoside family.

An independent test of methyl group placement in the imG-14 base was made from the mass spectrum produced by collision-induced dissociation of the protonated base ion, using tandem mass spectrometry, Fig. 3A. In particular, the absence of significant methyl loss from the base to form *m*/*z* 175 ion as the dominant first decomposition product is a hallmark of the absence of CH_3 at N-4 [20]. Instead, the expulsion of CO from C-9 (*m*/*z* 162) followed by successive losses of HCN (*m*/*z* 135, 108) is characteristic of the 4-demethylwyosine nucleus [20].

From both deuterium exchange and CID mass spectral data a structural candidate for imG-14 therefore emerged in which N-4 is unmethylated, and there is a single methyl group residing at C-6 or C-7. Based solely on the biological precedent of

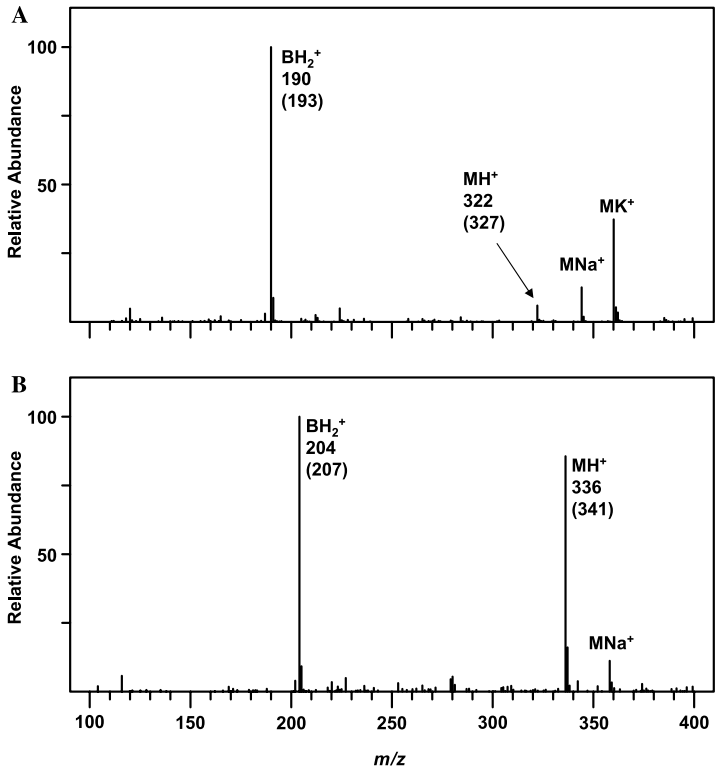

Fig. 2. Electrospray ionization mass spectra of nucleosides from enzymatic digests of unfractionated tRNAs. (A) imG-14, from *M. igneus*; (B) imG2 from *S. hydrogenophila*. Values in parentheses indicate the corresponding *m/z* values following hydrogen–deuterium exchange. MH^+, protonated molecule; BH_2^+, protonated base.

conserved methylation of C-6 in wyosine (see Fig. 1), the 6-monomethyl isomer was selected for chemical synthesis and direct comparison against the natural nucleoside.

Deuterium labeling measurements on unknown nucleoside imG2, Fig. 2B, showed four exchangeable hydrogens in the neutral molecule and two in the neutral base. Through reasoning similar to that presented above for imG-14, these results led to a likely structural candidate in which N-4 is unsubstituted, thus requiring that the two methyls (required by the mass of the base) reside at C-6 and C-7. This candidate was chemically synthesized for comparison against natural imG2.

Mass spectra, HPLC retention times and UV spectra (from HPLC photodiode array detection) acquired in back-to-back LC/MS comparisons of natural (see Table 1) and synthetic nucleosides (see synthesis descriptions), and showed that imG-14 and imG2 were experimentally indistinguishable from their respective synthetic counterparts. Because the proposed structure of imG-14 was less certain from preliminary data than that of imG2, the protonated base of synthetic imG-14 was subjected to collision-induced dissociation. The resulting mass spectrum, showing the fragmentation pattern of the tricyclic heteroaromatic nucleus adds an additional

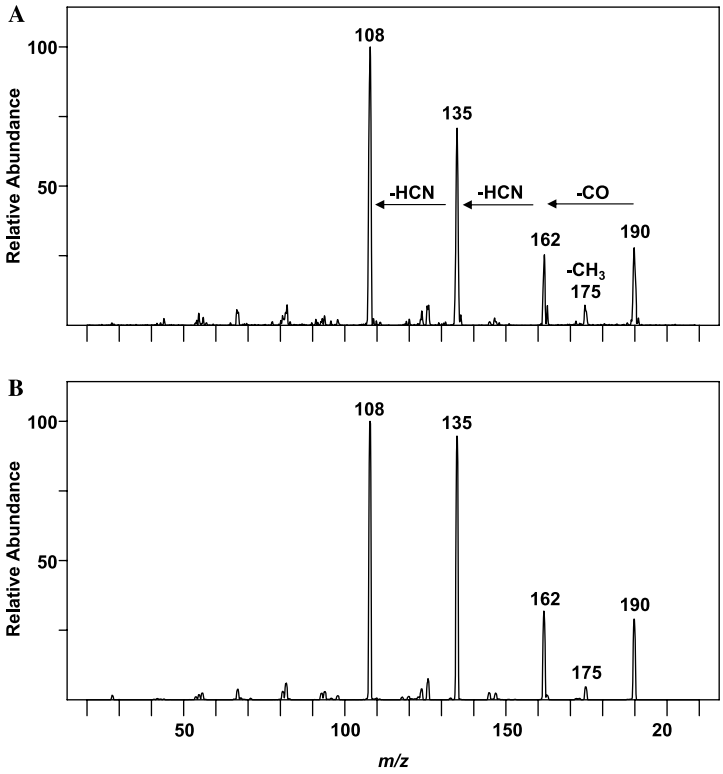

Fig. 3. Mass spectra of the protonated base (*m/z* 190) of imG-14 resulting from collision-induced dissociation. (A) Natural nucleoside from enzymatic digest of unfractionated *M. thermolithotrophicus* tRNA; (B) chemically synthesized nucleoside.

and significant dimension of comparison with the natural compound, particularly with respect to the methylation status of N-4. The resulting mass spectrum, Fig. 3B, is experimentally indistinguishable from that of the tRNA component in Fig. 3A. Together, these data confirm the structures of natural imG-14 and imG2 nucleosides as those shown in Fig. 1, in which neither nucleoside possesses a side chain, nor a methyl at N-4 in the central ring.

3.2. Occurrence of nucleoside imG2 in S. solfataricus tRNA

During the course of structure assignment for imG2, data from electrospray ionization LC/MS analysis of *S. solfataricus* P2 tRNA were retrospectively examined, and indicated the presence of imG2, based on retention time and mass parameters shown in Table 1. An appropriate mass shift was evident from deuterium labeling carried out in a separate analysis. imG2 is a minor component in this tRNA, and the strength of its UV spectrum was insufficient for determination of λ_{max} values. The modified nucleoside content of *S. solfataricus* tRNA was reported previously

[18] without recognition of the occurrence of imG2. This difference is attributed to use of thermospray ionization in the earlier work, which is significantly less sensitive for detection of minor nucleosides than is the case using electrospray ionization.

4. Discussion

Assignment of the structures of tRNA nucleosides imG-14 and imG2 raises the number of known wyosine family structures [21] to eight. Four are phylogenetically unique to eukarya [1,21], one is shared by eukarya and archaea (imG), and three are unique to archaea (imG-14, imG2, mimG, Fig. 1). None are known in bacterial tRNA, supporting earlier observations that archaeal tRNA nucleoside modification motifs are in general much more similar to those in eukarya than in bacteria [11,22]. Although imG2 has thus far been found only in tRNA of thermophilic crenarchaeotes, imG-14 is found in both the major archaeal kingdoms (euryarchaea and crenarchaea), in organisms grown at a range of temperatures between 4 °C [12] and 106 °C [10]. The eukaryal wye nucleosides typically have amino acid side chains substituted at C-7 that are related to methionine, and until the present finding all wyosine family members were reported as methylated at N-4 (N-3 in purine nomenclature). Absence of an amino acid C_6 or C_7 side chain in the archaeal nucleosides suggests the lack of an important functional role for the side chain. Also no structural role for the side chain is evident from the structure of yeast $tRNA^{Phe}$ in which wybutosine is present, derived from x-ray crystallography [23,24]. The functional importance of the wyosine derivatives specifically adjacent to the 3′ end of the anticodon (position 37) derives mainly from their influence in stabilization of the anticodon stem–loop by vertical base stacking of residues 34–38, in which the tricyclic ring has strong influence [25,26]. From a biological perspective, anticodon region modifications in general [27,28], and wyebutosine-37 in particular [29], are believed to be involved in maintenance of translational reading frame.

Within the wyosine family, nucleoside imG-14 may represent the "minimal" wye structure, assuming that the 6-CH_3, which is ubiquitous to all eight wye structures is introduced as part of an obligatory C_2 or C_3 unit used to effect ring closure from guanosine (for review of wye nucleoside biosynthesis see [30]). The structure of imG-14 supports the conclusion that methyls at N-4 and C-7 are neither required for function nor a necessary byproduct of wye nucleus biosynthesis although the presence of one or more methyl groups on the base presumably contributes to stacking stabilization [31]. The same reasoning is reflected in the structure of wyosine from yeast (the 4,6-dimethyl isomer of imG2), which also has no C-7 substitutuent. Earlier wye base biosynthesis studies employing ^{13}C-labeling from [methyl-^{13}C]SAM using NMR [32] and mass spectrometry [33] are consistent with the initial conclusion that the third wye nucleus ring (at least in eukarya) is formed from 1-methylguanosine precursor [34], although this pathway would appear to be inherently inefficient, because it requires removal of all three hydrogens from the guanine N-1 CH_3 group to ultimately form the tricyclic nucleus. It has been noted, however, that the details of the complex wye biosynthesis pathway may differ according to phylogenetic origin

[30]. Neither of the previous NMR and MS measurements could establish the site of ^{13}C in the bridge specifically as C-7 (as would be required from 1-[methyl-^{13}C]guanosine precursor), vs. C-6. The 6-CH_3 group was determined to not be SAM-derived [32].

The consideration of imG-14 and imG2 as "minimalist" versions of nucleoside modification in archaeal tRNA is in keeping with the known relatively simple modification motifs observed in archaea, particularly in comparison with related nucleosides from eukaryal tRNA. For example, the extended family of 5-substituted uridines (mostly occurring in the first position of the tRNA anticodon) that contain multiple side chain modifications and are common to bacteria and eukarya, are rare in archaea [11,35]. The 7-deazaguanosine nucleoside queuosine with its unusual cyclopentenediol side chain [36] common to eukarya and bacterial tRNAs (and further elaborated by glycosylation in higher eukarya [37]) is formed by a related transglycosylase enzyme in archaea [38,39] at a different sequence site as the structurally simpler 7-formamidino nucleoside archaeosine [40] in a wide range of archaeal tRNAs. Finally, the numerous structural variations in the characteristic N^6-modified adenosines commonly found at position 37 in tRNA are far more extensive in eukarya and bacteria than in archaea where it is most often limited to N^6-threonylcarbamoyladenosine (t^6A).

Acknowledgments

This work was supported by Grant GM29812 from the National Institute of General Medical Sciences. The authors are indebted to D.E. Graham and G.J. Olsen, University of Illinois, Urbana, and M. Thomm, Christian Albrechts Universität Kiel, for unfractionated tRNAs used as sources of natural nucleosides for comparison with synthetic compounds. We thank E. Bruenger for assistance with isolation of *S. solfataricus* tRNA, S.C. Pomerantz for figure drawings and P.F. Crain for a critical reading of the manuscript.

References

[1] J. Rozenski, P.F. Crain, J.A. McCloskey, Nucleic Acids Res. 27 (1999) 196–197.
[2] J.A. McCloskey, in: D. Söll, S. Nishimura, P.B. Moore (Eds.), RNA, Elsevier, Amsterdam, 2001, pp. 309–316.
[3] P. Auffinger, E. Westhof, in: H. Grosjean, R. Benne (Eds.), Modification and Editing of RNA, ASM Press, Washington, DC, 1998, pp. 569–576.
[4] K. Nakanishi, N. Furutachi, M. Funamizu, D. Grunberger, I.B. Weinstein, J. Am. Chem. Soc. 92 (1970) 7617–7619.
[5] M. Funamizu, A. Terahara, A.M. Feinberg, K. Nakanishi, J. Am. Chem. Soc. 93 (1971) 6706–6708.
[6] M. Sprinzl, C. Horn, M. Brown, A. Ioudovitch, S. Steinberg, Nucleic Acids Res. 26 (1998) 148–153.
[7] Y. Kuchino, H. Kasai, Z. Yamaizumi, S. Nishimura, E. Borek, Biochim. Biophys. Acta 565 (1979) 215–218.
[8] K. Nakanishi, S. Blobstein, M. Funamizu, N. Furutachi, G. Van Lear, D. Grunberger, K.W. Lanks, I.B. Weinstein, Nat. New Biol. 234 (1971) 107–109.

[9] H. Kasai, Z. Yamaizumi, Y. Kuchino, S. Nishimura, Nucleic Acids Res. 6 (1979) 993–999.
[10] J.A. McCloskey, X.-H. Liu, P.F. Crain, E. Bruenger, R. Guymon, T. Hashizume, K.O. Stetter, Nucleic Acids Symp. Ser. 44 (2000) 267–268.
[11] J.A. McCloskey, D.E. Graham, S. Zhou, P.F. Crain, M. Ibba, J. Konisky, D. Söll, G.J. Olsen, Nucleic Acids Res. 29 (2001) 4699–4706.
[12] K.R. Noon, R. Guymon, P.F. Crain, J.A. McCloskey, M. Thomm, M. Lim, R. Cavicchioli, J. Bacteriol. 185 (2003) 5483–5490.
[13] M. Buck, M. Connick, B.N. Ames, Anal. Biochem. 129 (1983) 1–13.
[14] K.R. Noon, E. Bruenger, J.A. McCloskey, J. Bacteriol. 180 (1998) 2883–2888.
[15] P.F. Crain, Methods Enzymol. 193 (1990) 782–790.
[16] H. Kasai, M. Goto, K. Ikeda, M. Zama, Y. Mizuno, S. Takemura, S. Matsuura, T. Sugimoto, T. Goto, Biochemistry 15 (1976) 898–904.
[17] G. Golankiewicz, W. Folkman, Nucleic Acids Res. 11 (1983) 5243–5255.
[18] S.C. Pomerantz, J.A. McCloskey, Methods Enzymol. 193 (1990) 796–824.
[19] C.G. Edmonds, S.C. Pomerantz, F.F. Hsu, J.A. McCloskey, Anal. Chem. 60 (1988) 2314–2317.
[20] S. Zhou, D. Sitaramaiah, S.C. Pomerantz, P.F. Crain, J.A. McCloskey, Nucleosides Nucleotides Nucleic Acids 22 (2003), in press.
[21] Y. Motorin, H. Grosjean, in: H. Grosjean, R. Benne (Eds.), Modification and Editing of RNA, ASM Press, Washington, DC, 1998, pp. 543–549.
[22] C.G. Edmonds, P.F. Crain, R. Gupta, T. Hashizume, C.H. Hocart, J.A. Kowalak, S.C. Pomerantz, K.O. Stetter, J.A. McCloskey, J. Bacteriol. 173 (1991) 3138–3148.
[23] G.J. Quigley, A. Rich, Science 194 (1976) 794–806.
[24] B. Hingerty, R.S. Brown, A. Jack, J. Mol. Biol. 124 (1978) 523–534.
[25] R.W. Adamiak, P. Górnicki, Prog. Nucleic Acid Res. Mol. Biol. 32 (1985) 27–74.
[26] D.R. Davis, in: H. Grosjean, R. Benne (Eds.), Modification and Editing of RNA, ASM Press, Washington, DC, 1998, pp. 85–102.
[27] J. Urbonavicius, Q. Qian, J.M. Durand, T.G. Hagervall, G.R. Björk, EMBO J. 20 (2001) 4863–4873.
[28] J. Urbonavicius, G. Stahl, J.M.B. Durand, S.N. Ben Salem, Q. Qian, P.J. Farabaugh, G.R. Björk, RNA 9 (2003) 760–768.
[29] B.A. Carlson, J.F. Mushinski, D.W. Henderson, S.Y. Kwon, P.F. Crain, B.J. Lee, D.L. Hatfield, Virology 279 (2001) 130–135.
[30] G.A. Garcia, D.M. Goodenough-Lashua, in: H. Grosjean, R. Benne (Eds.), Modification and Editing of RNA, ASM Press, Washington, DC, 1998, pp. 135–168.
[31] P.O.P. Ts'o, in: P.O.P. Ts'o (Ed.), Basic Principles in Nucleic Acid Chemistry, Academic Press, New York, 1974, pp. 453–584.
[32] C. Smith, P.G. Schmidt, J. Petsch, P.F. Agris, Biochemistry 24 (1985) 1434–1440.
[33] J.A. McCloskey, G.R. Björk, E.B. Lindstrom, J.M. Peltier, Nucleic Acids Res. Symp. Ser. 35 (1996) 277–278.
[34] L. Droogmans, H. Grosjean, EMBO J. 6 (1987) 477–483.
[35] A.J. Wittwer, L. Tsai, W.M. Ching, T.C. Stadtman, Biochemistry 23 (1984) 4650–4655.
[36] H. Kasai, Z. Ohashi, F. Harada, S. Nishmura, N.J. Oppenheimer, P.F. Crain, J.G. Liehr, D.L. von Minden, J.A. McCloskey, Biochemistry 14 (1975) 4198–4208.
[37] H. Kasai, K. Nakanishi, R.D. Macfarlane, D.R. Torgerson, Z. Ohashi, J.A. McCloskey, H.J. Gross, S. Nishimura, J. Am. Chem. Soc. 98 (1976) 5044–5046.
[38] M. Watanabe, M. Matsuo, S. Tanaka, H. Akimoto, S. Asahi, S. Nishimura, J.R. Katze, J.A. McCloskey, P.F. Crain, T. Hashizume, N. Okada, J. Biol. Chem. 272 (1997) 20146–20151.
[39] D. Iwata-Reuyl, Bioorg. Chem. 31 (2003) 24–43.
[40] J.M. Gregson, P.F. Crain, C.G. Edmonds, R. Gupta, T. Hashizume, D.W. Phillipson, J.A. McCloskey, J. Biol. Chem. 268 (1993) 10076–10086.