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Chemical Synthesis and Properties of 5-Taurinomethyluridine and 5-Taurinomethyl-2-thiouridine

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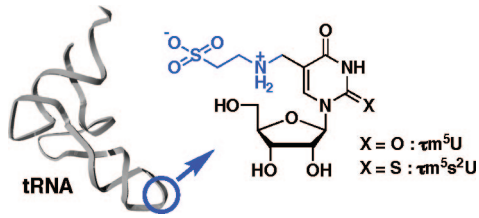

Unique taurine-containing uridine derivatives, 5-taurinomethyluridine (τm^5U) and 5-taurinomethyl-2-thiouridine (τm^5s^2U), which were discovered in mammalian mitochondrial tRNAs, exist at the first position of the anticodon. In this paper, we report the first efficient synthesis of τm^5U and τm^5s^2U and describe their physicochemical properties. These modified ribonucleosides were synthesized by the reaction of 5-substituted uridine derivatives with a tetrabutylammonium salt of taurine that is highly reactive and well-soluble in common organic solvents. UV and ^1H NMR spectrometric studies revealed the structural properties of the taurine-containing base moieties and the sugar conformations of these modified ribonucleosides.

A wide variety of base-modified ribonucleosides exist in RNA.[1] These modified nucleosides play crucial roles in specific RNA molecules to realize their inherent functions based on the unique structures. The proportion of the modified ribonucleosides in tRNA is higher than that in other RNA species.[2] Furthermore, a number of modified ribonucleosides are present at the first position of the anticodon (the "wobble" position)[3] and are involved in the codon−anticodon interaction.[4]

Unmodified uridine at the wobble position can recognize all four nucleobases at the third position of the codon as a result of its conformational flexibility. Hence, unmodified uridines at the wobble position are frequently found in tRNA species that recognize four synonymous codons present in *Mycoplasma* spp. and mitochondria.[5−8] On the other hand, uridines at the wobble position are often post-transcriptionally modified in several tRNAs. The 5-methyl uridine derivatives (xm^5U) are known as frequently occurring modified ribonucleosides.[9] In particular, 5-aminomethyl uridine derivatives (xnm^5U-type), that is, 5-methylaminomethyl-modified uridines ($mnm^5(s^2)U$)[9−11] and 5-carboxymethylaminomethyl-modified uridines ($cmnm^5(s^2)U$ and $cmnm^5Um$),[12−14] are found at the wobble position in several tRNAs of prokaryotic or eukaryotic organelle that are responsible for codons including purine bases (A or G) at the third position.[9]

With regard to the sugar conformations, s^2U derivatives are fixed in the C3′-*endo* form of ribose puckering as a result of the steric effect of the 2-sulfur atom and the 2′-oxygen atom.[6,15] As a result, the xnm^5s^2U modification generally tends to recognize adenine. However, these recognition characteristics of $xnm^5(s^2)U$ do not depend on the difference of the C^5-substituents. The chemical characteristics and structural features of the C^5-substituents of $xnm^5(s^2)U$, especially during the higher recognition of G, are unknown.

We previously isolated and identified two novel base-modified uridine derivatives, 5-taurinomethyluridine (τm^5U, Figure 1, **1a**) in tRNAs for leucine and 5-taurinomethyl-2-thiouridine (τm^5s^2U, Figure 1, **1b**) in tRNAs for lysine, glutamine, and glutamic acid from bovine and human mitochondrial tRNAs.[16] These modified nucleosides were found at the wobble position, which is considered to be responsible for precise codon recognition. In addition, the absence of these modified uridine derivatives causes

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(1) Limbach, P. A.; Crain, P. A.; McCloskey, J. A. *Nucleic Acids Res.* **1994**, *22*, 2183–2196.
(2) Björk, G. R. In *tRNA: Structure, Biosynthesis, and Function*; ASM Press: Washington, DC, 1995; pp 165−205.
(3) Agris, P. F.; Vendeix, F. A.; Graham, W. D. *J. Mol. Biol.* **2007**, *366*, 1–13.
(4) Knight, R. D.; Freeland, S. J.; Landweber, L. F. *Nat. Rev. Genet.* **2001**, *2*, 49–58.
(5) Barrell, B. G.; Anderson, S.; Bankier, A. T.; de Bruijn, M. H. L.; Chen, E.; Coulson, A. R.; Drouin, J.; Eperon, I. C.; Nierlich, D. P.; Roe, B. A.; Sanger, F.; Schreier, P. H.; Smith, A. J. H.; Staden, R.; Young, I. G. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3164–3166.
(6) Bonitz, S. G.; Berlani, R.; Coruzzi, G.; Li, M.; Macino, G.; Nobrega, F. G.; Nobrega, M. P.; Thalenfeld, B. E.; Tzagoloff, A. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3167–3170.
(7) Andachi, Y.; Yamao, F.; Iwami, M.; Muto, A.; Osawa, S *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7398–7402.
(8) Inagaki, Y.; Kojima, A.; Bessho, Y.; Hori, H.; Ohama, T.; Osawa, S. *J. Biol. Chem.* **1995**, *251*, 486–492.
(9) Yokoyama, S.; Watanabe, T.; Murao, K.; Ishikura, H.; Yamazumi, Z.; Nishimura, S.; Miyazawa, T. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4905–4909.
(10) Yarian, C.; Marszalek, M.; Sochacka, E.; Malkiewicz, A.; Guenther, R.; Agris, P. F. *Biochemistry* **2000**, *39*, 13390–13395.
(11) Murphy, F.; Ramakrishnan, V.; Malkievicz, A.; Agris, P. F. *Nat. Struct. Biol.* **2004**, *11*, 1186–1191.
(12) Martin, R. P.; Sibler, A. P.; Gehrke, C. W.; Kuo, K.; Edmonds, C. G.; McCloskey, J. A.; Dirheimer, G. *Biochemistry* **1990**, *29*, 956–959.
(13) Andachi, Y.; Yamao, F.; Muto, A.; Osawa, S. *J. Mol. Biol.* **1989**, *209*, 37–54.
(14) Sakurai, M.; Ohtsuki, T.; Suzuki, T.; Watanabe, K. *FEBS Lett.* **2005**, *579*, 2767–2772.
(15) Testa, S. M.; Disney, M. D.; Turner, D. H.; Kierzek, R. *Biochemistry* **1999**, *38*, 16655–16662.
(16) Suzuki, T.; Suzuki, T.; Wada, T.; Saigo, K.; Watanabe, K. *EMBO J.* **2002**, *21*, 6581–6589.

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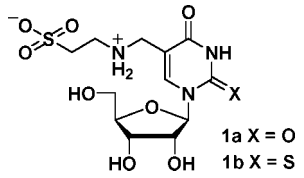


FIGURE 1. 5-Taurinomethyluridine (τm^5U, **1a**) and 5-taurinomethyl-2-thiouridine (τm^5s^2U, **1b**).

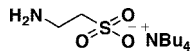


FIGURE 2. Taurine tetrabutylammonium salt.

mitochondrial encephalomyopathic diseases such as MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes)[17] and MERRF (myoclonus epilepsy associated with ragged-red fibers).[18]

When the nucleoside is located at the wobble position, τm^5U recognizes purine bases (A or G) as well as the other xnm^5U derivatives. In vitro biochemical studies revealed that the absence of 5-taurinomethyl modification decreased G recognition.[19,20]

As mentioned above, biological and biochemical studies of RNAs including these two modified uridines, τm^5U and τm^5s^2U, have advanced significantly in recent years, whereas the physicochemical studies on the same have not been successful because these modified ribonucleosides exist only in a small amount in vivo.

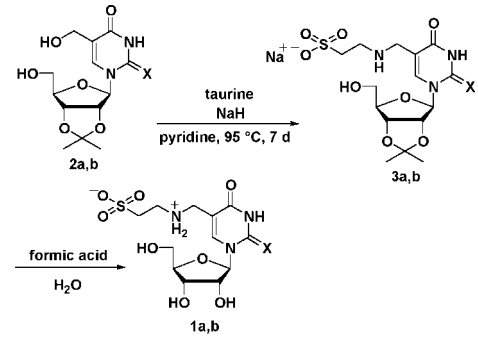
The chemical synthesis of these modified ribonucleosides will contribute to understanding their chemical characters and structural features. Furthermore, these synthetic nucleosides are also useful for biological researches because they can be used as substrates for many enzymatic reactions. From another point of view, taurine is abundant in mammalian plasma, but its exact functions in biochemical terms are still to be elucidated. In our previous paper, we have briefly described the preliminary results of the synthesis of these modified ribonucleosides.[21] In this paper, we wish to report an in-depth study of the synthesis of these modified ribonucleosides and elucidate some physicochemical properties.

Chemical Synthesis of τm^5U and τm^5s^2U. The first chemical synthesis of τm^5U and τm^5s^2U was achieved by the use of 5-hydroxymethyluridine derivatives (**2a,b**) as the starting material (Scheme 1).[21] In this reaction, NaH was employed as a base, and the taurine residue was introduced directly.

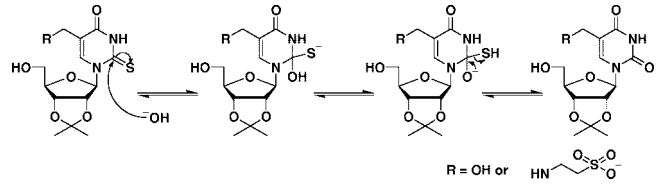
However, this reaction required high temperature and a long reaction time (95 °C, 7 days) because taurine is almost insoluble in the solvent. Therefore, during the synthesis of τm^5s^2U, about 10% desulfurization was observed since hydroxide ion attack at the C^2 resulted in the elimination of SH^- (Scheme 2).

For the efficient chemical synthesis of τm^5s^2U, the reaction should be carried out under sufficiently mild conditions to suppress the desulfurization. In this context, activation of both the nucleophile (taurine) and the electrophile (5-substituted uridine derivative) is necessary. A tetrabutylammonium salt of

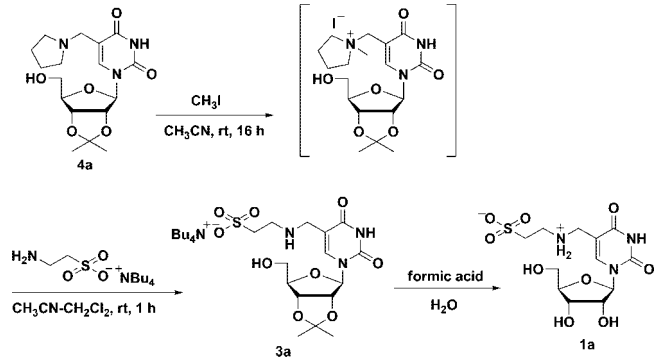
SCHEME 1. First Synthesis of τm^5U and τm^5s^2U



SCHEME 2. A Mechanism of Desulfurization



SCHEME 3



taurine (Figure 2) was found to have good solubility in polar as well as nonpolar organic solvents. In addition, the quaternary ammonium salt of taurine is highly nucleophilic compared to crystalline taurine because of the existence of the amino group in the free form.

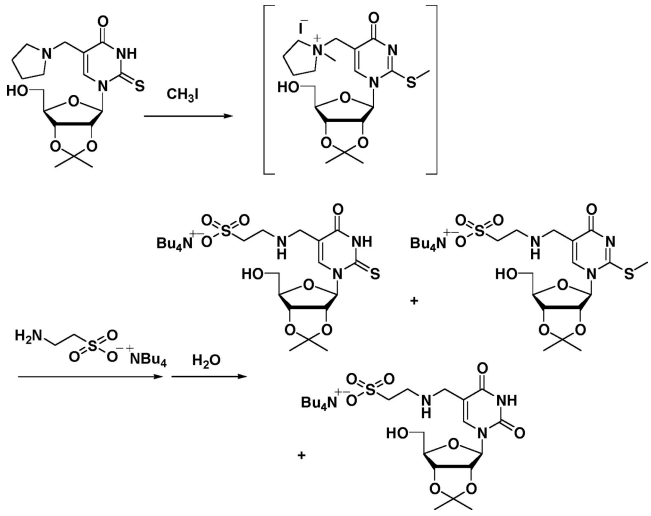
Next, a 5-pyrrolidinomethyluridine derivative (**4a**) was used as the starting material (Scheme 3).[22] The pyrrolidinyl group of **4a** was methylated by treatment with methyl iodide to afford the corresponding quaternary ammonium group, which is a good leaving group for the nucleophilic substitution at the 5-methylene carbon. The resulting intermediate was allowed to react with the tertabutylammonium salt of taurine in the mixture of CH_3CN and CH_2Cl_2. The reaction proceeded quickly at rt to give the desired τm^5U in 36% yield.

In a similar manner, we tried to synthesize the 2-thio derivative. However, a complex mixture was obtained under the same reaction conditions used for τm^5U. The major side reaction was desulfurization, which was confirmed by ^1H NMR spectroscopy.

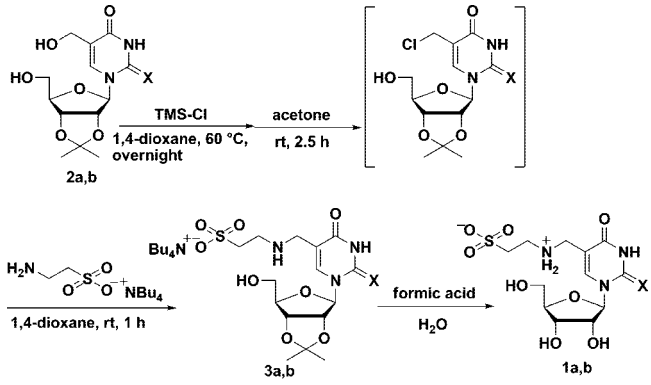
This side reaction was probably caused by the methylation of the 2-sulfur atom resulting in the 2-methylthio group becoming a good leaving group when a hydroxide ion or the primary amino group of taurine attacks on the C^2 atom (Scheme

(17) Yasukawa, T.; Suzuki, T.; Suzuki, T.; Ueda, T.; Ohta, S.; Watanabe, K. *J. Biol. Chem.* **2000**, *275*, 4251–4257.
(18) Yasukawa, T.; Suzuki, T.; Ishii, N.; Ueda, T.; Ohta, S.; Watanabe, K. *FEBS Lett.* **2000**, *467*, 175–178.
(19) Kirino, Y.; Yasukawa, T.; Ohta, S.; Akira, S.; Ishihara, K.; Watanabe, K.; Suzuki, T. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 15070–15075.
(20) Kurata, S.; Weixlbaumer, A.; Ohtsuki, T.; Shimazaki, T.; Wada, T.; Kirino, Y.; Takai, K.; Watanabe, K.; Ramakrishnan, V.; Suzuki, T. *J. Biol. Chem.* **2008**, *283*, 18801–18811.
(21) Wada, T.; Shimazaki, T.; Nakagawa, S.; Ohtsuki, T.; Kurata, S.; Suzuki, T.; Watanabe, K.; Saigo, K. *Nucleic Acids Symp. Ser.* **2002**, *2*, 11–12.
(22) (a) Jones, S. S.; Reese, C. B.; Ubasawa, A. *Synthesis* **1982**, 259–261. (b) Reese, C. B.; Sanghvi, Y. S. *J. Chem. Soc., Chem. Commun.* **1984**, *1*, 62–63.

SCHEME 4



SCHEME 5



4).[23] Since the chemoselective methylation of **4a** is difficult, we tried to use a more reactive substrate for the nucleophilic substitution at the 5-methylene group.

An alternative route to synthesize 5-aminomethyluridine derivatives involves a 5-chloromethyluridine intermediates.[24] A 5-hydroxymethyluridine derivative (**2a**) was treated with trimethylsilyl chloride (TMS-Cl) in dioxane to form the 5-chloromethyluridine intermediate. During this reaction, a partial removal of the 2′,3′-*O*-isopropylidene protecting group was observed by TLC monitoring of the reaction mixture. Upon adding acetone to the reaction mixture and stirring for 2.5 h, the missing 2′,3′-protection was recovered. After removal of the excess amount of TMS-Cl and solvents, a tetrabutylammonium salt of taurine was allowed to react with the 5-chloromethyluridine derivative in dioxane at rt for 1 h. After workup and purification, the desired τm^5U was obtained in 30% yield (Scheme 5). Applying the same reaction conditions to the 2′,3′-*O*-protected 5-hydroxymethyl-2-thiouridine, τm^5s^2U was obtained in 16% yield. Noteworthy, no detectable desulfurization was observed by the reverse-phase HPLC (RP-HPLC) analysis of the crude product.

Liquid chromatography/mass spectrometry (LC/MS) spectrometry analyses of the products revealed that these chemically synthesized nucleosides were in accord with those isolated from

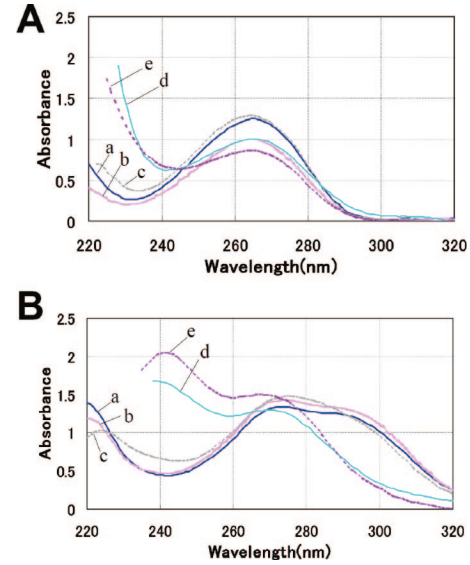


FIGURE 3. pH dependence of the UV spectra of (A) τm^5U and (B) τm^5s^2U: (a) 0.1 M HCl (pH 1.0), (b) 0.1 M sodium citrate buffer (pH 5.0), (c) 0.1 M HEPES-KOH (pH 7.4), (d) 0.1 M sodium carbonate buffer (pH 9.0), and (e) 0.1 M NaOH (pH 13.0).

bovine mitochondria tRNAs. The nucleosides were eluted at the same retention time, and the CID fragment patterns and NMR spectra of those were identical.[16] These nucleosides (**1a**, **1b**) are stable as uridine or 2-thiouridine and are soluble in H_2O and common aqueous buffers but insoluble in alcohols such as EtOH.

UV Absorption Spectra of τm^5U and τm^5s^2U. The UV spectra of τm^5U and τm^5s^2U were measured in the pH range of 1.0−13.0 (Figure 3). The UV spectra for the pH range 1.0−7.4 were nearly the same for both. The values of λ_{max} for τm^5U and τm^5s^2U were at 265 and 274 nm, respectively, and the value of λ_{max} for U was at 260 nm. These properties of the nucleosides are similar to those of T (5-methyluridine). The modification of an electron-donating group such as a methyl or a methylene group at the C^5 position of the uracil ring increases the π-electron density of the pyrimidine. Therefore, the C^5 substitution resulted in a shift of the value of λ_{max} to a longer wavelength. Simultaneously, these data showed that the 2-thio substitution of uridines resulted in a larger shift of the value of λ_{max} to long wavelengths than the substitution at the C^5 position.

In addition to the distortion due to 2-thio substitution, a prominent peak was observed at 241 nm under alkaline conditions, which is a characteristic feature of 2-thiouridine derivatives.

As the pH was increased to around 9.0, the behavior of both τm^5U and τm^5s^2U changed and their λ_{min} values showed a significantly shallow profile. The results suggest that the deprotonation/protonation of these uracils occurs when the pH is around 9.0. Such a low pK_a of protonation/deprotonation is possible because of the presence of a positively charged group in the 5-substitiuent.

Proportions of Ribose Puckering from ^{1}H NMR. We calculated the conformation of the ribose rings of τm^5U and τm^5s^2U from the ^{1}H NMR spectra[25] and compared them to those calculated from unmodified and 5-carboxymethylami-

(23) Martin, J. C.; Jeffrey, G. A.; McGee, D. P. C.; Tippie, M. A.; Smee, D. F.; Matthews, T. R.; Verheyden, J. P. H. *J. Med. Chem.* **1985**, *28*, 358–362.
(24) Malkiewicz, A.; Sochacka, E.; Ahmed, A. F. S.; Yassin, S. *Tetrahedron Lett.* **1983**, *24*, 5395–5398.
(25) Sierzputowska-Gracz, H.; Sochacka, E.; Malkiewicz, A.; Kuo, K.; Gehrke, C. W.; Agris, P. F. *J. Am. Chem. Soc.* **1987**, *109*, 7171–7177.

TABLE 1. Calculated Ribose Puckering from the Coupling Constants[a]

	$J_{1'2'}$ (Hz)	$J_{3'4'}$ (Hz)	%[C3′-*endo*]
τm^5U	4.0	6.1	60[b]
$cmnm^5U$	4.5	5.4	55[b]
U	4.6	5.2	53[b]
τm^5s^2U	2.0	*c*	80[d]
$cmnm^5s^2U$	2.1	6.0	74[b]
s^2U	2.5	6.0	71[b]

[a] Measured by 600 MHz ^{1}H NMR. [b] %[C3′-*endo*] = $J_{3'4'}/(J_{1'2'} + J_{3'4'}) \times 100$ [c] Not detected. [d] %[C3′-*endo*] = $(10 - J_{1'2'})/10 \times 100$

nomethyl uridines. The conformation of s^2U derivatives is generally fixed in the C3′-*endo* form of ribose puckering due to the large van der Waals radius of the 2-sulfur atom. The fractional populations of the C3′-*endo* form for both τm^5U and τm^5s^2U were determined as 60% and 80%, respectively, whereas for U it was 53%. These results suggest that taurinomethyl modification would involve the ribose conformation to some extent.

We succeeded in the chemical synthesis of τm^5U and τm^5s^2U under milder conditions by the use of a tetrabutylammonium salt of taurine, which is sufficiently reactive and well-soluble in many organic solvents. Some of the structural properties of τm^5U and τm^5s^2U were revealed by UV and ^{1}H NMR spectroscopy. The chemical synthesis of these nucleosides including these modified bases will contribute to further studies of τm^5U and τm^5s^2U concerning the ability and mechanism of codon−anticodon recognitions. These nucleosides will be useful not only for physicochemical analyses, but also as substrates for the synthesis of RNA oligomers by an enzymatic or chemical approach. The chemical synthesis of RNA oligomers including these modified ribonucleosides is now underway.

Experimental Section

5-Taurinomethyluridine (1a). Method A (Scheme 3). To a solution of 2′,3′-*O*-isopropylidene-5-pyrrolidinomethyluridine (**4a**, 36.7 mg, 0.1 mmol) in dry CH_3CN (1 mL) under an argon was added dropwise CH_3I (62 μL, 1 mmol). After the mixture was stirred at rt for 16 h, CH_3I and CH_3CN were removed under reduced pressure. The mixture was dissolved in CH_3CN (1 mL), and tetrabutylammonium 2-aminoethanesulfonate (0.3 mmol) was added in dry CH_2Cl_2 (1 mL). After being stirred at rt for 1 h, the mixture was concentrated under reduced pressure, and the excess amount of taurine was removed by reverse-phase chromatography using a column of SEPABEADS SP700 (15 × 40 mm) with MeOH/H_2O (2:3, v/v) as an eluent. The fractions containing **3a** was collected and concentrated under reduced pressure, and 10% of the mixture was purified by RP-HPLC. The resulting **3a** was treated with 90% formic acid aqueous solution at rt for 12 h. The solution was then passed through a column of Dowex 50Wx8 (H^+ form, 5 × 20 mm) and concentrated to dryness under reduced pressure to give **1a** (3.62 μmol, 36%[26] from **4a**) as a colorless amorphous solid.

Method B (Scheme 5). 2′,3′-*O*-Isopropylidene-5-hydroxymethyluridine (**2a**, 0.157 g, 0.5 mmol) was dried by repeated coevaporation with dry 1,4-dioxane and dissolved in dry 1,4-dioxane (5 mL). To the solution under an argon was added dropwise TMS-Cl (0.31 mL, 2.5 mmol). After being stirred at 60 °C overnight, the solution was cooled to rt, and dry acetone (5 mL) was added. After the mixture was stirred for 2.5 h, TMS-Cl and acetone were removed under reduced pressure. To the mixture was added tetrabutylammonium 2-aminoethane sulfonate (1.5 mmol) in dry 1,4-dioxane (2 mL). After stirring at rt for 1 h, the mixture was concentrated under reduced pressure and 2.5% of the mixture was purified in a manner similar to Method A to give **1a** (3.72 μmol, 30%[26] from **2a**). UV (H_2O) $\lambda_{max} = 265$ nm ($\varepsilon_{265} = 1.0 \times 10^4$, $\varepsilon_{260} = 1.0 \times 10^4$); ^{1}H NMR (300 MHz, D_2O) δ 8.22 (1H, s), 5.90 (1H, d, $J = 3.3$ Hz), 4.33 (1H, dd, $J = 4.5$ Hz, 4.2 Hz), 4.23 (1H, dd, $J = 5.7$ Hz, $J = 5.4$ Hz), 4.16 (1H, m), 4.08 (2H, s), 3.90 (2H, m), 3.49 (2H, t, $J = 6.6$ Hz), 3.30 (2H, t, $J = 6.6$ Hz); ^{13}C NMR (75.45 MHz, D_2O) δ 167.3, 154.1, 146.0, 107.3, 92.6, 87.0, 76.8, 71.8, 63.2, 49.2, 47.0, 45.4; HRMS (ESI) *m*/*z* calcd for $C_{12}H_{18}N_3O_9S^-$ $[M - H]^-$ 380.0764, found 380.0768.

5-Taurinomethyl-2-thiouridine (1b). Method C (Scheme 5). This compound was obtained from **2b** in a manner similar to the synthesis of **1a** as described in Scheme 5 to give **1b** (1.95 μmol, 16%[26] from **2b**) as a colorless amorphous solid, except for the purification by RP-HPLC after the treatment with 90% formic acid aqueous solution. UV (H_2O) $\lambda_{max} = 274$ nm ($\varepsilon_{274} = 1.5 \times 10^4$, $\varepsilon_{260} = 1.0 \times 10^4$); ^{1}H NMR (300 MHz, D_2O) δ 8.48 (1H, s), 6.51 (1H, s), 4.41 (1H, s), 4.20 (2H, m), 4.10 (2H, s), 3.98 (2H, m), 3.50 (2H, t, $J = 6.6$ Hz), 3.30 (2H, t, $J = 6.6$ Hz); ^{13}C NMR (75.45 MHz, D_2O) δ 180.0, 170.7, 141.9, 117.8, 96.3, 86.3, 77.7, 71.0, 62.5, 52.4, 47.9, 45.7; HRMS (ESI) *m*/*z* calcd for $C_{12}H_{18}N_3O_8S_2^-$ $[M - H]^-$ 396.0535, found 396.0517.

UV Absorption Measurements of τm^5U and τm^5s^2U. The UV spectra were measured at several pH values: 0.1 M HCl, 0.1 M sodium citrate buffer (pH 3.0), 0.1 M HEPES-KOH (pH 7.4), 0.1 M sodium carbonate buffer (pH 9.0), and 0.1 M NaOH. Background was measured with each buffer.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **1a** and **1b**. RP-HPLC profiles of **1a** and **1b**. Preparation of tetrabutylammonium 2-aminoethanesulfonate. General information for the experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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(26) The yields of the products were estimated by ^{1}H NMR using the integration value of CH_3CN as an internal standard.