Chemical synthesis of the 5-taurinomethyl(-2-thio)uridine modified anticodon arm of the human mitochondrial $tRNA^{Leu(UUR)}$ and $tRNA^{Lys}$

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ABSTRACT

5-Taurinomethyluridine (τm^5U) and 5-taurinomethyl-2-thiouridine (τm^5s^2U) are located at the wobble position of human mitochondrial (hmt) $tRNA^{Leu(UUR)}$ and $tRNA^{Lys}$, respectively. Both hypermodified units restrict decoding of the third codon letter to A and G. Pathogenic mutations in the genes encoding hmt-$tRNA^{Leu(UUR)}$ and hmt-$tRNA^{Lys}$ are responsible for the loss of the discussed modifications and, as a consequence, for the occurrence of severe mitochondrial dysfunctions (MELAS, MERRF). Synthetic oligoribonucleotides bearing modified nucleosides are a versatile tool for studying mechanisms of genetic message translation and accompanying pathologies at nucleoside resolution. In this paper, we present site-specific chemical incorporation of τm^5U and τm^5s^2U into 17-mers related to the sequence of the anticodon arms hmt-$tRNA^{Leu(UUR)}$ and hmt-$tRNA^{Lys}$, respectively employing phosphoramidite chemistry on CPG support. Selected protecting groups for the sulfonic acid (4-(*tert*-butyldiphenylsilanyloxy)-2,2-dimethylbutyl) and the exoamine function (-$C(O)CF_3$) are compatible with the blockage of the canonical monomeric units. The synthesis of τm^5s^2U-modified RNA fragment was performed under conditions eliminating the formation of side products of 2-thiocarbonyl group oxidation and/or oxidative desulphurization. The structure of the final oligomers was confirmed by mass spectroscopy and enzymatic cleavage data.

Keywords: human mitochondrial tRNA; modified ribonucleosides; 5-taurinomethyluridine; 5-taurinomethyl-2-thiouridine; phosphoramidite chemistry

INTRODUCTION

Mitochondria have a largely autonomic system of genetic message expression. The human mitochondrial genome encodes 13 proteins responsible for oxidative phosphorylation process (OXPHOS), two rRNAs, and 22 tRNAs (Florentz et al. 2003; Sissler et al. 2008). Knowledge about the detailed structure of mitochondrial tRNAs (mt-tRNAs) is still limited (Suzuki et al. 2011b). Research using specific chemical and enzymatic structure probes (Sissler et al. 2008) has shown that the secondary (2D) and tertiary (3D) arrangements of mt-tRNAs more or less deviate from the classical cloverleaf and L-shaped organization characteristic of their cytosolic counterparts (Helm et al. 2000; Jühling et al. 2012; Wende et al. 2014). mt-tRNAs are also significantly less prone to post-transcriptional modifications than cytosolic tRNAs (Watanabe 2010).

5-Taurinomethyluridine (τm^5U, **1**, Fig. 1A) and 5-taurinomethyl-2-thiouridine (τm^5s^2U, **2**, Fig. 1A) are present at the wobble position of human mitochondrial (hmt) tRNAs specific for Leu, Trp, and Lys, Glu, Gln, respectively (Kirino et al. 2004; Watanabe 2010). Like other $xm^5(s^2)U$-type wobble uridines, nucleosides **1**, **2** decode A and G as the third codon letter (Kurata et al. 2008). The genes encoding hmt-$tRNA^{Leu}$ and hmt-$tRNA^{Lys}$ are highly susceptible to point mutations. The most often occurring transitions A3243G, T3271C, and A8344G result in the absence of nucleosides **1**, **2** (Suzuki et al. 2011a,b). Deficiency of the taurine-modified uridines **1**, **2** is considered to be a key factor responsible for the severe mitochondrial diseases MELAS and MERRF (Suzuki et al. 2011a,b). In fact, the taurine residue linked to the atom C-5 by a methylene group has been found critical for G decoding (Kirino et al. 2004; Kurata et al. 2008), while the absence of both modifying functions (the C-5 substituent and the 2-thiocarbonyl group) results in a translational defect for the cognate codons UUA(G) and a disruption of protein biosynthesis (Suzuki et al. 2011a).

The abundance of mt-tRNAs is very poor as compared with their cytosolic homologues (Sissler et al. 2008) limiting

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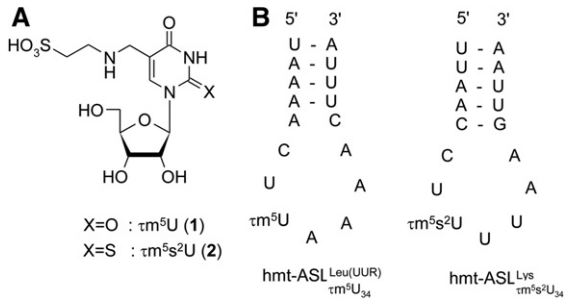

FIGURE 1. (*A*) The structure of 5-taurinomethyluridine (τm^5U, **1**) and 5-taurinomethyl-2-thiouridine (τm^5s^2U, **2**). (*B*) The sequence and secondary structure of the human mitochondrial $tRNA^{Leu(UUR)}$ and $tRNA^{Lys}$ anticodon stem and loop (hmt-$ASL^{Leu(UUR)}$ and hmt-ASL^{Lys}) bearing τm^5U and τm^5s^2U, respectively. The native sequence of hmt-$ASL^{Leu(UUR)}$ has pseudouridine (Ψ) at position 27, while in the sequence of hmt-ASL^{Lys} there are two pseudouridines (Ψ) at positions 27 and 28, and *N*-[(9-β-D-ribofuranosyl-9*H*-purin-6-yl)carbamoyl]-L-threonine (t^6A) at position 37.

their availability from biological material. hmt-$tRNA^{Leu(UUR)}$ molecule devoid of τm^5U (Kirino et al. 2004) as well as *Escherichia coli* $tRNA^{Leu(UUR)}$ analog bearing only this one modified unit (Kurata et al. 2008) have been synthesized using the molecular surgery technique. These "chimeric" tRNAs have been used in bioassays investigating their binding to programmed ribosomes and as elements of a cell-free translation system (Kirino et al. 2004; Kurata et al. 2008). An RNA fragment recombination technique and the 5′,3′-bisphosphate of τm^5U ($p\tau m^5Up$) have been applied for the preparation of an analog of the hmt-$tRNA^{Leu}$ anticodon arm sequence modified at the wobble position. To visualize the geometry of the codon-anticodon mini-helix, the construct was introduced into the A-site of *Thermus thermophilus* crystal 30S ribosomal subunit programmed with UUA(G) codon (Kurata et al. 2008). The 5′-half fragment of the molecule was observed to be dynamic, however, and the geometry of the wobble pair τm^5U-G was not established in detail (Kurata et al. 2008).

Chemically synthesized modified tRNA fragments, and in particular anticodon stem–loop hairpins (ASLs), have been widely utilized in model studies on the influence of post-transcriptional modification on molecule conformation/3D structure dynamics in solution (Duram et al. 2005; Cantara et al. 2012) as well as on conformation in the solid state (Weixlbaumer et al. 2007; Cantara et al. 2013). Recently, similar model studies on the impact of modification (f^5C) on the structure/dynamics of the anticodon arm domain have been extended to hmt-tRNA specific for Met (Lusic et al. 2008). Modified oligomers are also used as substrates for enzymatic ligation of RNA fragments to generate longer RNA constructs with site-specific location of the modified nucleoside(s) (Kurschat et al. 2005). In order to synthesize RNA oligomers including taurine-modified uridines **1**, **2**, appropriate protection for highly reactive functional groups, in particular the sulfonic acid residue, is required. An attempt to employ phenyl protection for the sulfonic acid residue of τm^5U and τm^5s^2U phosphoramidites has been reported (Ogata and Wada 2006). However, their use in oligoribonucleotide solid-phase synthesis was limited to RNA dimers $\tau m^5(s^2)UpU$ (Ogata and Wada 2008).

This paper presents a milligram scale chemical synthesis of τm^5U and τm^5s^2U-modified oligoribonucleotides (phosphoramidite chemistry on solid support) related to the sequence of the hmt-tRNAs anticodon arm domain specific for Leu and Lys, respectively (Fig. 1B). The 4-(*tert*-butyldiphenylsilanyloxy)-2,2-dimethylbutyl group (neoO-dPS) was selected as the most useful protection for sulfonic acid residue of taurine derivatives.

RESULTS AND DISCUSSION

The incorporation of nucleosides **1** and **2** into the RNA sequences using phosphoramidite chemistry on CPG support requires such protection of the highly reactive aliphatic amine function as well as the sulfonic acid residue that would be compatible with the blockage of canonical monomeric units. The aliphatic amine function was protected with a base-labile trifluoroacetyl group, following the methodology developed previously for the incorporation of $mnm^5(s^2)U$ into RNA oligomers (Malkiewicz and Sochacka 1983; Agris et al. 1995; Leszczynska et al. 2011, 2012).

The most appropriate method for the protection of alkyl or aryl sulfonic acids, including taurine, is their transformation into sulfonate esters (Roberts et al. 1997; Klán et al. 2002; Wrobel et al. 2002; Yan and Müller 2004; Avitabile et al. 2005; Seeberger et al. 2007; Hussain et al. 2008; Ali et al. 2009; Miller 2010). The masking of sulfonic acids as sulfonamides or ammonium salts has also been proposed (Klamann and Hofbauer 1953; Richman and Atkins 1974; Andrianov et al. 2004). The cleavage of most of the reported protecting groups employs, however, strongly acidic or basic conditions unsuitable for oligoribonucleotide synthesis. Substituted phenyl esters of taurine have been utilized for solid support synthesis of dimers with $\tau m^5(s^2)U$ at the 5′ end (Ogata and Wada 2006, 2008). However, this blockage has proven too labile to be used in the synthesis of longer, hypermodified RNA sequences (G Leszczynska and A Malkiewicz, unpubl.). In search of alternative protection for the sulfonic acid function, model *N*-Boc taurine esters have been synthesized (Fig. 2) and their stability/cleavage has been examined under the typical reaction conditions of RNA synthesis on solid support.

By analogy with the protection of the t^6A carboxyl group (Boudou et al. 2000; Sundaram et al. 2000; Bajji and Davis 2002; Bajji et al. 2002; Eshete et al. 2007; Bilbille et al. 2009), variously substituted *N*-Boc taurine 2-phenylethyl esters (Fig. 2, **3a–3g**) removable via the β-elimination process (10% DBU/MeCN) have been prepared (Leszczynska et al. 2013). Several attempts to prepare 2-(trimethylsilyl)ethyl ester (**3j**) which could be removed by treatment with fluoride anions have not been successful (data not shown). As an

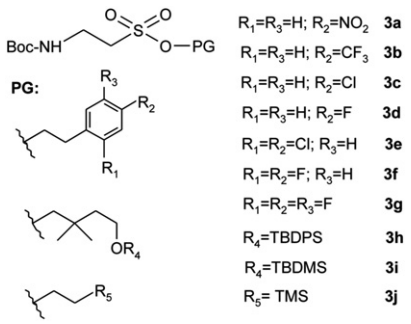

FIGURE 2. Protecting groups selected for sulfonic acid residue of taurine; TBDPS, *tert*-butyldiphenylsilyl; TBDMS, *tert*-butyldimethylsilyl; TMS, trimethylsilyl.

alternative, we have synthesized and tested 4-(*tert*-butyldiphenylsilanyloxy)-2,2-dimethylbutyl ester of *N*-Boc taurine (Fig. 2, **3h**; Seeberger et al. 2007) and its analog, 4-(*tert*-butyldimethylsilanyloxy)-2,2-dimethylbutyl ester (**3i**).

The esters **3a–3i** were stable in 8 M ethanolic ammonia (24 h, room temperature [rt]), offering a simple way for the simultaneous deblocking of base-labile protecting groups, e.g., 2-cyanoethyl, -tac, and the cleavage of oligomers from CPG support without the risk of amide formation. Among 2-arylethyl esters **3a–3g** only 2-(*p*-nitrophenyl)ethyl (**3a**), 2-(*p*-trifluoromethylphenyl)ethyl (**3b**), and 2-(2,4,5-trifluorophenyl)ethyl (**3g**) esters of *N*-Boc taurine were found to be easily deprotected under β-elimination conditions (10% DBU/MeCN, 40 min, 45°C). Both *p*-substituted phenylethyl esters **3a**, **3b** were, however, significantly unstable also in the presence of other tertiary amines, e.g., Et_3N or iPr_2NEt, which excluded their use in the synthesis of τm^5U and τm^5s^2U phosphoramidites. Examination of the stability of *N*-Boc taurine 4-(*tert*-butyldiphenylsilanyloxy)-2,2-dimethylbutyl ester (**3h**) as well as its analog, 4-(*tert*-butyldimethylsilanyloxy)-2,2-dimethylbutyl ester (**3i**), revealed their effective cleavage with a standard desilylating reagent, 1 M TBAF/NMP (24 h, rt) as well as 1 M TEAF/NMP (24 h, rt) and triethylamine trihydrofluoride (TEA•3HF/NMP, 24 h, rt). In contrast to neoO-dPS sulfonate ester **3h**, ester **3i** was unstable under detritylation conditions (3% TCA/DCM). The usefulness of neoO-dPS as a protecting group for sulfonic acid residue has been previously confirmed in the synthesis of variously *N*-substituted taurines (Seeberger et al. 2007). The removal of neoO-dPS was performed with a small excess of TBAF in THF via cleavage of the Si-O bond, and then spontaneous cyclization of the desilylated intermediate (Seeberger et al. 2007).

In summary, evaluation of the stability of various *N*-Boc taurine esters (Fig. 2) allowed for the selection of two protecting groups for the sulfonic acid residue, the 2-(2,4,5-trifluorophenyl)ethyl group and the 4-(*tert*-butyldiphenylsilanyloxy)-2,2-dimethylbutyl group. An attempt to introduce the 2-(2,4,5-trifluorophenyl)ethyl-protected τm^5U and τm^5s^2U phosphoramidites into RNA sequences resulted, however, in very low yields of the target oligoribonucleotides (data not shown). The use of strong basic DBU solution required for the 2-(2,4,5-trifluorophenyl)ethyl deprotection caused a Michael-type addition of 2,4,5-trifluorostyrene to RNA and the loss of the 2-thiocarbonyl moiety in the case of τm^5s^2U-modified oligomer. The use of scavengers of 2,4,5-trifluorostyrene did not increase the yields of synthesis.

Finally, the 4-(*tert*-butyldiphenylsilanyloxy)-2,2-dimethylbutyl group (neoO-dPS) was effectively used for the synthesis of $\tau m^5(s^2)U$ phosphoramidites **16a/16b** (Fig. 3) and then for their incorporation into target RNA sequences.

Initially, the synthesis of **16a/16b** involved the introduction of 5′-*O*-DMTr and 2′-*O*-TBDMS protecting groups after the installation of a taurine ester side chain at position C-5,1. However, the steric hindrance around the 5′-hydroxyl group, caused by the presence of a large substituent at the atom C-5, decreased the yield of dimethoxytritylation to 5% (data not shown). As an alternative strategy, a fully protected taurine skeleton was installed after 5′-*O*-DMTr and 2′-*O*-TBDMS protection of the sugar moiety (Fig. 3). 5-Hydroxymethyl-2′,3′-*O*-isopropylidene(-2-thio)uridine (**4a/4b**) was used for the preparation of 5-azidomethyl(-2-thio)uridine derivatives **6a/6b** (Seio et al. 1998). The treatment of **4a/4b** with an excess of trimethylsilyl chloride in 1,4-dioxane at 60°C gave 5-chloromethyl-2′,3′-*O*-isopropylidene(-2-thio)uridine **5a/5b**, which without purification was reacted with an excess of sodium azide in DMF at 60°C. In comparison with the synthesis of **6a**, a similar method of preparation of the 2-thio derivative **6b** was considerably less effective (40% vs. 70%).

The 2′,3′-*O*-isopropylidene group was removed from **6a/6b** by treatment with 50% aqueous (aq.) trifluoroacetic acid (Myerscough et al. 1992) to afford **7a/7b** in 90% yield. Subsequently, incorporation of the 5′-*O*-DMTr, and then 2′(3′)-*O*-TBDMS, protecting groups was performed according to the standard procedures (Damha and Ogilvie 1993). A mixture of 2′ and 3′ TBDMS isomers (**9a/9b** and **10a/10b**, respectively) was separated by column chromatography on silica gel only in the amount required for spectral analysis. The separation of 2′ and 3′ TBDMS isomers at this stage was not convenient because the alkaline conditions of the subsequent reaction led to spontaneous isomerization giving an equimolar mixture of 2′ and 3′ regiomers. Therefore, a mixture of azides **9a**, **10a** or their 2-thio analogous **9b**, **10b** was reduced to a suitable mixture of amines **11a**, **12a** or **11b**, **12b**, respectively, by treatment with triphenyl phosphine in anhydrous pyridine followed by 25% aq. ammonia (Seio et al. 1998). The resulting mixtures of regiomers **11a**, **12a** or **11b**, **12b** were effectively separated by flash chromatography. The storage of 3′ isomers **12a/12b** in methanol for a longer period of time resulted in their partial isomerization, enabling improvement of the overall yield of 2′-TBDMS derivatives **11a/11b**. Separated 5-aminomethyl(-2-thio)uridine **11a/11b** was then used as a donor in a Michael-type addition to 4-(*tert*-butyldiphenylsilanyloxy)-2,2-dimethylbutyl ethenesulfonate (**13**) (Baxter et al. 2000; Ogata and

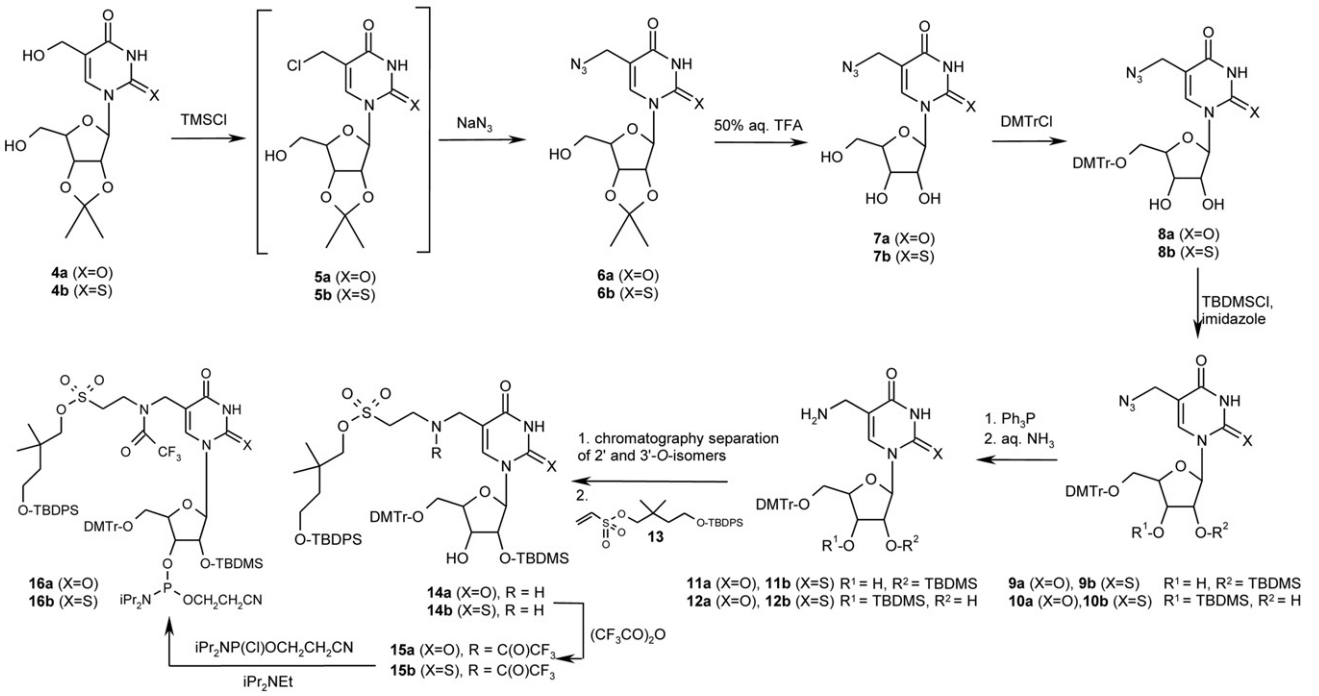

FIGURE 3. Chemical synthesis of τm^5U and τm^5s^2U phosphoramidites. TMSCl, trimethylsilyl chloride; TFA, trifluoroacetic acid; DMTrCl, 4,4′-dimethoxytrityl chloride; TBDMSCl, *tert*-butyldimethylsilyl chloride; TBDPS, *tert*-butyldiphenylsilyl group.

Wada 2006; Seeberger et al. 2007). This strategy of synthesis of a fully protected taurine skeleton is more effective than procedures involving 5-chloromethyl- or 5-formyluridine as substrates (Leszczynska et al. 2013) and in the case of β-amino acids it should be considered as the method of choice. To exclude the isomerization of 2′-*O*-TBDMS **11a/11b** to 3′-regiomers the reaction of ethenesulfonate **13** with **11a/11b** requires an aprotic solvent. Consequently, an equimolar amount of amine **11a/11b** and 4-(*tert*-butyldiphenylsilanyloxy)-2,2-dimethylbutyl ethenesulfonate (**13**) was mixed in DCM for 72 h at rt. The resulting material **14a/14b** was purified by column chromatography in 70% yield.

Following the previously reported procedure (Malkiewicz et al. 1983), the amine function of **14a/14b** was protected with trifluoroacetyl to afford **15a/15b** in 80% yield. Phosphitylation of **15a/15b** was performed with 2-cyanoethyl *N*, *N*-diisopropylaminochlorophosphoramidite under standard conditions (Damha and Ogilvie 1993) giving $\tau m^5(s^2)U$ phosphoramidites **16a/16b** in ~85% yield.

Fully protected phosphoramidites **16a/16b** were used for the synthesis of analogs of the anticodon arm domain of human mt-$tRNA^{Leu(UUR)}$ and mt-$tRNA^{Lys}$ modified with τm^5U and τm^5s^2U, respectively (Fig. 1B). The synthesis of oligomers was conducted manually on a 5-µmol scale using commercial tac-protected phosphoramidites of the canonical units and 5-(3,5-bis(trifluoromethyl)phenyl)-1*H*-tetrazole as the activator. The couplings were conducted in 8 molar excess of A, U, C, and G amidites for 8 min, while the modified units were used in 12 molar excess and coupled twice, each time using 6 molar excess of an amidite and 12-min coupling time. Coupling yields were in the range of 90%–95%.

It is generally known that the 2-thiocarbonyl group of 2-thiouridine derivatives reacts with various oxidizing reagents used in the oligoribonucleotide synthesis giving products of 2-thio → 2-oxo transformation and/or oxidative desulfurization ($s^2 \rightarrow H^2$) (Sochacka 2001; Okamoto et al. 2006). In model studies (Leszczynska et al. 2011, 2012), a 0.02 M iodine solution (8 equiv, 2 min) in THF-H_2O-pyridine (Okamoto et al. 2006) was selected as the most promising oxidizing agent, leading to very small amounts of side products of 2-thiocarbonyl group degradation. In contrast to diluted iodine solution, 0.25 M *t*BuOOH (8 equiv, 2 min) in toluene or acetonitrile gave considerable amounts of side products.

"Trityl-off" CPG-bound RNA was treated with Et_3N in CH_3CN, and then with 8 M ethanolic ammonia. The two-step deprotection procedure made it possible to avoid the reaction of heterobase residues with acrylonitrile generated during the deprotection of phosphate residue (Capaldi et al. 2003). For the simultaneous removal of the TBDMS and neoO-dPS protecting groups in τm^5U-modified RNA, Et_3N•3HF was effectively employed. An alternative use of 1 M Bu_4NF gave the desired product, but the yield of the oligomer was drastically reduced. In the case of τm^5s^2U-modified RNA, desilylation was performed with several reagents. We found that the only effective condition for the complete removal of neoO-dPS and TBDMS protections without observable degradation of the 2-thiocarbonyl function was the treatment with 1 M Et_4NF in NMP (24 h, rt). The use of

Et_3N•3HF caused a significant loss of the 2-thiocarbonyl function in the τm^5s^2U-modified oligomer. The excess of the desilylating reagent was deactivated by the addition of ethoxytrimethylsilane for Et_3N•3HF or phosphate buffer for Et_4NF. Crude products were purified by preparative IE-HPLC (Fig. 4A). The homogeneity and composition of synthetic oligoribonucleotides were verified by MALDI-TOF mass spectrometry (Supplemental Material) as well as RNA enzymatic digestion (Gehrke et al. 1982; Gehrke and Kuo 1989) to the expected mixture of nucleosides whose composition was tested by RP HPLC, and the data were compared with those recorded under identical conditions for modified nucleosides (Wada et al. 2002) as a reference (Fig. 4C,D).

CONCLUSIONS

The 4-(*tert*-butyldiphenylsilanyloxy)-2,2-dimethylbutyl (neoO-dPS) protecting group has been adopted for solid supported synthesis of oligoribonucleotides (phosphoramidite chemistry) bearing taurine-modified wobble uridines **1**, **2**. NeoO-dPS blockage is compatible with the protection of commercially available canonical monomeric units resistant to treatment with 8 M ethanolic ammonia and removable under mild, neutral conditions with fluoride anions. The usefulness of the discussed methodology was verified by the site-specific insertion of nucleosides **1**, **2** into the anticodon arm sequence of hmt-$tRNA^{Leu,\ Lys}$. The presented work enables effective chemical synthesis of hypermodified RNA sequences which can be used for model studies on the mechanism of decoding processes in mitochondria and their pathologies on a molecular level.

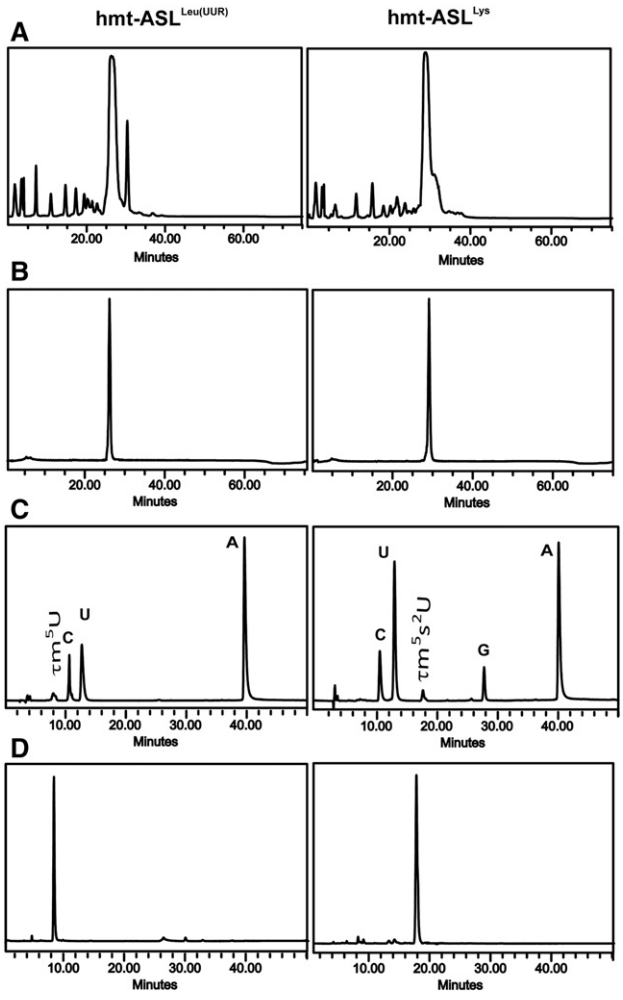

FIGURE 4. (*A*) Anion-exchange HPLC of crude, deprotected hmt-$ASL^{Leu(UUR)}$ (τm^5U_{34}) and hmt-ASL^{Lys} ($\tau m^5s^2U_{34}$). (*B*) Analytical injection of desalted and lyophilized oligoribonucleotides. (*C*) HPLC nucleoside composition of mt-ASLs. (*D*) References of τm^5U and τm^5s^2U injected in control experiments.

MATERIALS AND METHODS

NMR spectra were recorded on a Bruker Avance DPX 250 spectrometer at 250.0 (^{1}H), 62.9 (^{13}C), and 101.3 (^{31}P) MHz or a Bruker Avance II Plus 700 spectrometer at 700.0 (^{1}H) and 176.0 (^{13}C) MHz. Chemical shifts are reported in ppm relative to TMS (internal standard) for ^{1}H and ^{13}C, and 85% phosphoric acid (external standard) for ^{31}P. Chemical shifts are described as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), and bs (broad singlet). Coupling constants (*J*) are reported in hertz. IR spectra were recorded on a Bruker FT-IR ALPHA spectrometer equipped with a platinum ATR QuickSnap module. High-resolution mass spectra were obtained from a Finnigan MAT 95 spectrometer (FAB ionization) and Maldi SYNAPT G2-S HDMS (ESI ionization). MALDI-TOF spectra were recorded on an Applied Biosystems Voyager-Elite mass spectrometer. Thin layer chromatography was done on Merck $60F_{254}$ coated plates, and Merck silica gel 60 (mesh 230–400) was used for column chromatography. HPLC was performed with a Waters chromatograph interfaced with a 996 spectral diode array detector.

5-Azidomethyl-2′,3′-*O*-isopropylidene(-2-thio)uridine (6a/6b)

Nucleoside **4a**/**4b** (13.0 mmol, 1.0 equiv) was dissolved in 1,4-dioxane (128 mL), and then trimethylsilyl chloride (8 mL, 65 mmol, 5 equiv) was added. After being stirred for 4.5 h (**4a**)/7 h (**4b**) at 60° C, the mixture was cooled to rt, and anhydrous acetone (58 mL) was added. Stirring was continued for 1.5 h at rt. The mixture was then concentrated under reduced pressure and co-evaporated with anhydrous 1,4-dioxane. The resulting foam **5a**/**5b** was dissolved in DMF (118 mL) and treated with NaN_3 (5.1 g, 78.0 mmol, 6.0 equiv). The reaction mixture was stirred for 3 h at 60°C. NaCl precipitate was filtered off. The filtrate was concentrated under reduced pressure. The solid residue was dissolved in CH_2Cl_2/py (4:1, v/v; 58 mL) and washed with H_2O (23 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Pyridine was removed by co-evaporation with anhydrous toluene. The resulting foam was purified by column chromatography.

Compound **6a** was purified on a silica gel column with 5% MeOH in $CHCl_3$ as eluent to obtain a white foam in 69% yield. Spectroscopic data were in agreement with those presented previously (Seio et al. 1998).

Compound **6b** was purified on a silica gel column with 2% MeOH in $CHCl_3$ as eluent to obtain a light yellow foam in 40% yield. TLC $R_f = 0.52$ ($CHCl_3$/MeOH, 9:1 v/v); ^{1}H NMR (700 MHz, $CDCl_3$): δ 1.37 (s, 3H), 1.62 (s, 3H), 3.91 (dd, 1H, $J = 2.80$ Hz, $J = 11.90$ Hz), 4.06 (dd, 1H, $J = 2.10$ Hz, $J = 11.90$ Hz), 4.15 (q, 2H, $J = 11.20$ Hz), 4.35–4.36 (m, 1H), 4.77 (dd, 1H, $J = 2.10$ Hz, $J = 5.60$ Hz), 4.90 (q, 1H, $J = 3.50$ Hz), 6.84 (d, 1H, $J = 2.80$ Hz), 8.15 (s, 1H), 10.40 (s, 1H); ^{13}C NMR (176 MHz, $CDCl_3$): δ 26.49, 28.24, 48.20, 62.78, 80.19, 86.56, 87.60, 95.17, 115.04, 115.54, 140.28, 160.45, 176.16; IR (ATR): 2106 cm^{-1}; HRMS (ESI): calcd for $C_{13}H_{17}N_5O_5NaS$ $[M + Na]^+$ 378.0848, found 378.0847.

5-Azidomethyl(-2-thio)uridine (7a/7b)

Nucleoside **6a**/**6b** (4.5 mmol, 1 equiv) was dissolved in 50% aq. trifluoroacetic acid (13 mL). After being stirred for 1.5 h at rt, anhydrous toluene (18 mL) was added, and the mixture was concentrated under reduced pressure. The solid residue was co-evaporated with anhydrous toluene and purified by column chromatography.

Compound **7a** was purified on a silica gel column with 12% MeOH in $CHCl_3$ as eluent to obtain a white foam in 93% yield. TLC $R_f = 0.18$ ($CHCl_3$/MeOH 9:1, v/v); ^{1}H NMR (700 MHz, DMSO-d_6): δ 3.61 (dd, 1H, $J = 3.50$ Hz, $J = 11.90$ Hz), 3.71 (dd, 1H, $J = 3.50$ Hz, $J = 11.90$ Hz), 3.91 (q, 1H, $J = 3.50$ Hz), 4.03 (t, 1H, $J = 4.90$ Hz), 4.09 (t, 1H, $J = 4.90$ Hz), 4.11 (s, 2H), 5.83 (d, 1H, $J = 4.90$ Hz), 8.13 (s, 1H); ^{13}C NMR (176 MHz, DMSO-d_6): δ 47.35, 61.10, 70.02, 73.89, 85.23, 88.36, 108.79, 140.53, 150.86, 163.25; IR (ATR): 2106 cm^{-1}; HRMS (FAB$^-$): calcd for $C_{10}H_{12}N_5O_6$ $[M - H]^-$ 298.0788, found 298.0788.

Compound **7b** was purified on a silica gel column with 8% MeOH in $CHCl_3$ as eluent to obtain a light yellow foam in 88% yield. TLC $R_f = 0.28$ ($CHCl_3$/MeOH 9:1, v/v); ^{1}H NMR (700 MHz, DMSO-d_6): δ 3.60–3.62 (m, 1H), 3.74–3.76 (m, 1H), 3.90–3.92 (m, 1H), 3.98 (q, 1H, $J = 5.60$ Hz), 4.05–4.06 (m, 3H), 5.08 (d, 1H, $J = 5.60$ Hz), 5.29 (t, 1H, $J = 4.90$ Hz), 5.43 (d, 1H, $J = 4.90$ Hz), 6.50 (d, 1H, $J = 3.50$ Hz), 8.36 (s, 1H), 12.81 (s, 1H); ^{13}C NMR (176 MHz, DMSO-d_6): δ 47.43, 60.12, 69.11, 74.98, 85.02, 93.28, 113.68, 140.30, 160.11, 176.32; IR (ATR): 2101 cm^{-1}; HRMS (ESI):calcd for $C_{10}H_{13}N_5O_5NaS$ $[M + Na]^+$ 338.0535, found 338.0524.

5-Azidomethyl-5′-*O*-(4,4′-dimethoxytrityl)(-2-thio) uridine (8a/8b)

Nucleoside **7a**/**7b** (3.5 mmol, 1 equiv) was dissolved in anhydrous pyridine (25 mL). 4,4′-Dimethoxytrityl chloride (1.4 g, 4.2 mmol, 1.2 equiv) was added, and the mixture was stirred at rt for 24 h. The reaction was quenched with H_2O (25 mL). The resulting solution was extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were washed with water (2 × 15 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product **8a/8b** was purified by column chromatography.

Compound **8a** was isolated by flash chromatography using 2% MeOH in $CHCl_3$ as eluent to obtain a white foam in 45% yield. TLC $R_f = 0.57$ ($CHCl_3$/MeOH 9:1, v/v); ^{1}H NMR (700 MHz, acetone-d_6): δ 3.41 (dd, 1H, $J = 2.80$ Hz, $J = 11.20$ Hz), 3.45–3.47 (m, 2H), 3.69 (d, 1H, $J = 14.00$ Hz), 3.79 (s, 6H), 4.15–4.16 (m, 1H), 4.43–4.45 (m, 2H), 5.97 (d, 1H, $J = 4.90$ Hz), 6.90–7.50 (m, 13H), 7.87 (s, 1H); ^{13}C NMR (176 MHz, acetone-d_6): δ 46.83, 54.67, 63.31, 70.50, 74.43, 83.58, 86.63, 89.39, 109.03, 113.19, 126.94, 127.89, 128.21, 130.13, 130.15, 135.52, 135.77, 139.42, 144.90, 150.34, 158.90, 158.91, 162.52; IR (ATR): 2089 cm^{-1}; HRMS (FAB$^-$): calcd for $C_{31}H_{30}N_5O_8$ $[M - H]^-$ 600.2094, found 600.2095.

Compound **8b** was isolated by flash chromatography using 1% MeOH in $CHCl_3$ as eluent to obtain a yellow foam in 74% yield. TLC $R_f = 0.63$ ($CHCl_3$/MeOH 9:1, v/v); ^{1}H NMR (700 MHz, acetone-d_6): δ 3.30 (d, 1H, $J = 14.00$ Hz), 3.49 (dd, 1H, $J = 2.10$ Hz, $J = 10.50$ Hz), 3.54 (dd, 1H, $J = 3.50$ Hz, $J = 10.50$ Hz), 3.65 (d, 1H, $J = 14.00$ Hz), 3.80 (s, 6H), 4.22–4.24 (m, 1H), 4.32 (d, 1H, $J = 7.00$ Hz), 4.45–4.47 (m, 1H), 4.48–4.51 (m, 1H), 4.81 (d, 1H, $J = 4.90$ Hz), 6.65 (d, 1H, $J = 2.80$ Hz), 6.92–7.51 (m, 13H), 8.01 (s, 1H), 11.46 (s, 1H); ^{13}C NMR (176 MHz, acetone-d_6): δ 46.73, 54.66, 62.44, 69.59, 75.06, 83.43, 86.66, 93.87, 113.22, 113.74, 127.00, 127.95, 128.23, 130.15, 130.18, 135.39, 135.68, 138.99, 144.79, 158.94, 158.96, 159.27, 176.30; IR (ATR): 2097 cm^{-1}; HRMS (ESI): calcd for $C_{31}H_{31}N_5O_7NaS$ $[M + Na]^+$ 640.1842, found 640.1842.

5-Azidomethyl-2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(4,4′-dimethoxytrityl)(-2-thio)uridine (9a/9b) and 5-azidomethyl-3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(4,4′-dimethoxytrityl)(-2-thio)uridine (10a/10b)

The 5′-DMT nucleoside **8a**/**8b** (1.7 mmol, 1.0 equiv) was dissolved in anhydrous pyridine (17 mL), then imidazole (0.34 g, 5.0 mmol, 3 equiv) and *tert*-butyldimethylsilyl chloride (0.31 g, 2.1 mmol, 1.2 equiv) were added. After being stirred for 24 h at rt, the reaction was quenched with H_2O (20 mL). The resulting solution was extracted with $CHCl_3$ (3 × 35 mL). The combined organic layers were washed with water (35 mL), dried over $MgSO_4$, and the solvent was removed under reduced pressure. An equimolar mixture of 2′- and 3′-TBDMS isomers was purified by column chromatography and separated only in an amount sufficient for spectral analysis.

Mixture of 2′- and 3′-TBDMS isomers (**9a**, **10a**) was purified on a silica gel column with 3% acetone in DCM as eluent to obtain a white foam in 82% yield. Compound **9a**: TLC $R_f = 0.69$ (DCM/acetone 9:1, v/v); ^{1}H NMR (700 MHz, acetone-d_6): δ 0.17 (s, 3H), 0.18 (s, 3H), 0.94 (s, 9H), 3.41 (d, 1H, $J = 14.00$ Hz), 3.44 (dd, 1H, $J = 2.8$ Hz, $J = 10.5$ Hz), 3.47 (dd, 1H, $J = 3.50$ Hz, $J = 10.50$ Hz), 3.64 (d, 1H $J = 13.30$ Hz), 3.80 (s, 6H), 3.89 (d, 1H, $J = 5.60$ Hz), 4.17–4.19 (m, 1H), 4.38–4.40 (m, 1H), 4.53 (t, 1H, $J = 4.90$ Hz), 5.99 (d, 1H, $J = 4.90$ Hz), 6.91–7.50 (m, 13H), 7.91 (s, 1H), 10.33 (s, 1H); ^{13}C NMR (176 MHz, acetone-d_6): δ −5.44, 17.87, 25.29, 46.75, 54.68, 63.22, 70.76, 76.11, 83.54, 86.78, 88.88, 109.19, 113.22, 127.02, 127.94, 128.17, 130.14, 135.40, 135.64, 139.05, 144.86, 150.24, 158.95, 158.97, 162.39; IR (ATR): 2100 cm^{-1}; HRMS (FAB$^-$): calcd for $C_{37}H_{44}N_5O_8Si$ $[M - H]^-$ 714.2959, found 714.2944. Compound **10a**: TLC $R_f = 0.48$ (DCM/acetone 9:1, v/v); ^{1}H NMR (700 MHz, $(CD_3)_2CO$): δ 0.05 (s, 3H), 0.12 (s, 3H), 0.87 (s, 9H), 3.37 (dd, 1H, $J = 3.50$ Hz, $J = 10.50$ Hz), 3.49–3.52 (m, 2H), 3.71 (d, 1H, $J = 13.30$ Hz), 3.77 (s, 1H), 3.80 (s, 6H), 4.13–4.14 (m, 1H), 4.40–4.42 (m, 1H), 4.51 (t, 1H, $J = 4.90$ Hz), 5.96 (d, 1H, $J = 4.90$ Hz), 6.91–7.50 (m, 13H), 7.91 (s, 1H), 10.29 (s, 1H); ^{13}C NMR (176 MHz, acetone-d_6): δ −4.61, −4.26, 18.81,

26.29, 47.83, 55.64, 63.91, 72.78, 75.31, 84.84, 87.71, 90.59, 109.98, 114.14, 114.16, 127.96, 128.85, 129.19, 131.11, 131.13, 136.42, 136.55, 140.32, 145.69, 151.25, 159.92, 163.43; IR (ATR): 2101 cm^{-1}.

Mixture of 2′- and 3′-TBDMS isomers (**9b**, **10b**) was purified on a silica gel column with $CHCl_3$ as eluent to obtain a white foam in 86% yield. Compound **9b**: TLC $R_f = 0.53$ ($CHCl_3$/MeOH 99:1, v/v); ^1H NMR (700 MHz, acetone-d_6): δ 0.18 (s, 3H), 0.19 (s, 3H), 0.95 (s, 9H), 3.32 (d, 1H, $J = 14.00$ Hz), 3.49 (1H, dd, $J = 11.20$ Hz, $J = 2.10$ Hz), 3.52 (1H, dd, $J = 11.20$ Hz, $J = 3.50$ Hz), 3.61 (d, 1H, $J = 14.00$ Hz), 3.81 (s, 6H), 4.12 (d, 1H, $J = 5.60$ Hz), 4.25–4.27 (m, 1H), 4.42 (q, 1H, $J = 4.90$ Hz), 4.56 (t, 1H, $J = 4.9$ Hz), 6.78 (d, 1H, $J = 3.50$ Hz), 6.92–7.50 (m, 13H), 8.01 (s, 1H), 11.50 (s, 1H); ^{13}C NMR (176 MHz, acetone-d_6): δ −5.18, −5.15, 17.99, 25.43, 46.60, 54.67, 62.87, 70.43, 76.85, 83.54, 86.85, 93.15, 113.24, 114.11, 127.08, 127.97, 128.21, 130.18, 135.28, 135.52, 139.00, 144.75, 158.98, 159.01, 159.06, 176.60; IR (ATR): 2095 cm^{-1}; HRMS (ESI): calcd for $C_{37}H_{45}N_5O_7NaSiS$ $[M + Na]^+$ 754.2707, found 754.2704. Compound **10b**: TLC $R_f = 0.37$ ($CHCl_3$/MeOH 99:1, v/v); ^1H NMR (700 MHz, acetone-d_6): δ 0.02 (s, 3H), 0.10 (s, 3H), 0.83 (s, 9H), 3.31 (d, 1H, $J = 12.60$ Hz), 3.41 (dd, 1H, $J = 11.20$ Hz, $J = 3.50$ Hz), 3.60 (d, 1H, $J = 14.00$ Hz), 3.64 (dd, 1H, $J = 10.50$ Hz, $J = 2.10$ Hz), 3.81 (s, 6H), 4.11 (d, 1H, $J = 4.90$ Hz), 4.23–4.25 (m, 1H), 4.44–4.46 (m, 1H), 4.54–4.56 (m, 1H), 6.66 (d, 1H, $J = 2.10$ Hz), 6.92–7.50 (m, 13H), 8.12 (s, 1H), 11.45 (s, 1H); ^{13}C NMR (176 MHz, acetone-d_6): δ −5.63, −5.20, 17.73, 25.25, 46.74, 54.69, 62.04, 70.73, 75.01, 83.48, 86.78, 94.12, 113.19, 113.22, 113.69, 127.14, 127.93, 128.36, 130.25, 130.27, 135.29, 135.38, 138.92, 144.52, 159.05, 159.28, 176.24; IR (ATR): 2096 cm^{-1}.

5-Aminomethyl-2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(4,4′-dimethoxytrityl)(-2-thio)uridine (11a/11b)

A mixture of 2′- and 3′-TBDMS isomers **9a, 10a/9b, 10b** (1.36 mmol, 1.0 equiv) was dissolved in anhydrous pyridine (8.1 mL), and Ph_3P (0.64 g, 2.5 mmol, 1.8 equiv) was added. After being stirred for 24 h at rt, 25% NH_4OH (8.1 mL) was added. The solution was stirred for 1 h at rt and then extracted with $CHCl_3$ (3 × 30 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The solid residue was co-evaporated with anhydrous toluene and purified by column chromatography. 2D COSY NMR experiments were used to confirm the identity of 2′ isomers from the correlation of H3′ with 3′OH. To obtain additional quantities of the 2′ TBDMS isomer **11a/11b**, the 3′ isomer **12a/12b** was isomerized to an equimolar mixture of 2′ and 3′ isomers by stirring in methanol.

Compound **11a** was purified on a silica gel column with 4% MeOH in DCM as eluent to obtain a white foam in 65% yield. TLC $R_f = 0.52$ ($CHCl_3$/MeOH 9:1, v/v); ^1H NMR (700 MHz, DMSO-d_6): δ 0.04 (s, 3H), 0.06 (s, 3H), 0.85 (s, 9H), 2.99 (d, 1H, $J = 14.00$ Hz), 3.08 (d, 1H, $J = 14.00$ Hz), 3.24 (d, 2H, J = 3.50 Hz), 3.75 (s, 6H), 3.97–3.99 (m, 1H), 4.01–4.02 (m, 1H), 4.25 (t, 1H, $J = 4.90$ Hz), 5.07 (d, 1H, $J = 5.60$ Hz), 5.85 (d, 1H, $J = 4.90$ Hz), 6.90–7.41 (m, 13H), 7.55 (s, 1H); ^{13}C NMR (176 MHz, acetone-d_6): δ −5.46, −5.44, 17.88, 25.30, 46.58, 54.66, 63.74, 70.81, 75.77, 83.38, 86.47, 88.34, 113.14, 114.02, 126.75, 127.84, 128.12, 130.13, 130.16, 131.79, 131.84, 135.72, 136.79, 145.12, 150.48, 158.79, 162.77; HRMS (ESI): calcd for $C_{37}H_{48}N_3O_8Si$ $[M + H]^+$ 690.3211, found 690.3204.

Compounds **11b** was purified on a silica gel column with 1% MeOH in $CHCl_3$ as eluent to obtain a white foam in 60% yield. TLC $R_f = 0.46$ ($CHCl_3$/MeOH 9:1, v/v); ^1H NMR (250 MHz, DMSO-d_6): δ 0.05 (s, 3H), 0.07 (s, 3H), 0.86 (s, 9H), 2.99 (q, 2H, $J = 14.50$ Hz), 3.23–3.44 (m, 2H), 3.73 (s, 6H), 3.96–4.01 (m, 1H), 4.05–4.10 (m, 1H), 4.22–4.26 (m, 1H), 5.18 (d, 1H, $J = 5.50$ Hz), 6.67 (d, 1H, $J = 3.80$ Hz), 6.88–7.42 (m, 13H), 7.63 (s, 1H); ^{13}C NMR (176 MHz, acetone-d_6): δ −4.26, 18.91, 26.38, 47.44, 55.57, 64.62, 71.54, 77.75, 84.32, 87.41, 93.95, 114.05, 114.06, 119.88, 127.69, 128.77, 129.03, 131.04, 136.52, 136.61, 138.14, 145.96, 159.69, 159.71, 160.44, 176.86; HRMS (ESI): calcd for $C_{37}H_{48}N_3O_7SiS$ $[M + H]^+$ 706.2982, found 706.2973.

2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(4,4′-dimethoxytrityl)-*N*-[(1-β-D-ribofuranosyl-1*H*-(-2-thio)pyrimidin-5-yl)methyl]taurine 4-(*tert*-butyldiphenylsilanyloxy)-2,2-dimethylbutyl ester (14a/14b)

Nucleoside **11a/11b** (0.56 mmol, 1.0 equiv) was dissolved in DCM (1.8 mL) and cooled in an ice bath. Then, 4-(*tert*-butyldiphenylsilanyloxy)-2,2-dimethylbutyl ethenesulfonate (**13**, 0.25 g, 0.56 mmol, 1.0 equiv) was dissolved in DCM (275 µL) and added dropwise (total synthesis of ester **13** was previously described by Seeberger et al. 2007). The reaction mixture was stirred at rt for 72 h and then concentrated under reduced pressure. The resulting material was purified by column chromatography to obtain the pure compound **14a/14b**.

Compound **14a** was purified on a silica gel column with 1% MeOH in $CHCl_3$ as eluent to obtain a white foam in 70% yield. TLC $R_f = 0.45$ ($CHCl_3$/MeOH 98:2, v/v); ^1H NMR (700 MHz, C_6D_6): δ 0.27 (s, 3H), 0.38 (s, 3H), 0.92 (s, 6H), 1.03 (s, 9H), 1.30 (s, 9H), 1.64 (t, 2H, $J = 7.00$ Hz), 2.88–2.92 (m, 4H), 3.01 (d, 1H, $J = 13.30$ Hz), 3.33 (d, 1H, $J = 14.00$ Hz), 3.53–3.54 (m, 6H), 3.71–3.73 (m, 2H), 3.83 (t, 2H, $J = 7.00$ Hz), 3.91 (s, 2H), 4.25–4.26 (m, 1H), 4.45 (t, 1H, $J = 4.90$ Hz), 4.54 (t, 1H, $J = 4.20$ Hz), 6.29 (d, 1H, $J = 3.50$ Hz), 6.96–7.91 (m, 23H), 8.00 (s, 1H); ^{13}C NMR (176 MHz, C_6D_6): δ −5.24, −4.57, 18.00, 19.13, 23.99, 25.65, 26.90, 33.56, 40.94, 43.20, 45.62, 49.82, 54.73, 54.75, 60.55, 63.14, 70.75, 76.28, 77.37, 83.68, 87.10, 89.15, 112.93, 113.59, 113.61, 127.29, 128.19, 128.49, 129.85, 130.47, 130.49, 133.89, 135.65, 135.68, 135.79, 137.08, 145.12, 150.56, 159.19, 163.63; HRMS (ESI): calcd for for $C_{61}H_{82}N_3O_{12}Si_2S$ $[M + H]^+$1136.5158, found 1136.5187.

Compound **14b** was purified on a silica gel column with 1% MeOH in $CHCl_3$ as eluent to obtain a white foam in 65% yield. TLC $R_f = 0.70$ ($CHCl_3$/MeOH 98:2, v/v); ^1H NMR (700 MHz, acetone-d_6): δ 0.17 (s, 3H), 0.19 (s, 3H), 0.95 (s, 9H), 0.96 (s, 6H), 1.05 (s, 9H), 1.66 (t, 2H, $J = 7.00$ Hz), 2.73 (t, 2H, $J = 7.00$ Hz), 2.79 (d, 1H, 14.00 Hz), 3.05–3.09 (m, 2H), 3.16 (d, 1H, $J = 14.00$ Hz), 3.49 (d, 2H, 2.80 Hz), 3.79 (s, 6H), 3.81 (t, 2H, $J = 7.00$ Hz), 3.92 (s, 2H), 4.04 (bs, 1H), 4.24–4.25 (m, 1H), 4.35–4.36 (m, 1H), 4.55 (t, 1H, $J = 4.20$ Hz), 6.86 (d, 1H, $J = 4.20$ Hz), 6.92–7.72 (m, 23H), 7.94 (s, 1H); ^{13}C NMR (176 MHz, acetone-d_6): δ −4.28, −4.18, 18.90, 19.67, 24.54, 24.55, 26.36, 27.29, 34.45, 41.70, 43.92, 46.03, 50.28, 55.64, 61.30, 64.08, 71.56, 77.70, 78.66, 84.54, 87.65, 93.71, 114.19, 118,79, 128.70, 128.91, 129.10, 130.66, 131.14, 134.49,

136.29, 136.34, 136.50, 138.21, 145.84, 159.84, 159.87, 160.51, 177.11; HRMS (ESI): calcd for $C_{61}H_{82}N_3O_{11}Si_2S_2$ $[M+H]^+$ 1152.4929, found 1152.4932.

2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(4,4′-dimethoxytrityl)-*N*-[(1-*β*-D-ribofuranosyl-1*H*-(-2-thio)pyrimidin-5-yl)methyl]-*N*-(trifluoroacetyl)taurine 4-(*tert*-butyldiphenylsilanyloxy)-2,2-dimethylbutyl ester (15a/15b)

Nucleoside **14a/14b** (0.52 mmol, 1.0 equiv) was dissolved in anhydrous pyridine (11 mL), cooled in an ice bath, and trifluoroacetic anhydride (211 µL, 1.56 mmol, 3.0 equiv) was added dropwise. The mixture was stirred at rt for 2 h. The reaction was quenched with 5% aq. $NaHCO_3$ (30 mL). The resulting solution was extracted with $CHCl_3$ (3 × 40 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Pyridine was removed by co-evaporation with anhydrous toluene, and the resulting foam was purified by column chromatography affording products **15a/15b** as rotamers about the $-NC(O)CF_3$ amide bond (two chemical shifts were observed for some ^1H and ^{13}C NMR resonances; secondary shifts in ^{13}C NMR spectra are given in parentheses).

Compound **15a** was purified on a silica gel column with 2% MeOH in $CHCl_3$ as eluent to obtain a white foam in 81% yield. TLC $R_f = 0.51$ ($CHCl_3$/MeOH 98:2, v/v); ^1H NMR (700 MHz, C_6D_6): δ 0.30 (s, 2.4H), 0.36 (s, 0.6H), 0.37 (s, 2.4H), 0.48 (s, 0.6H), 1.01 (s, 6H), 1.06 (s, 7.2H), 1.10 (s, 1.8H), 1.34 (s, 1.8H), 1.35 (s, 7.2H), 1.64 (t, 0.4H, $J = 7.00$ Hz), 1.71 (t, 1.6H, $J = 7.00$ Hz), 3.22–3.28 (m, 1H), 3.31–3.35 (m, 1H), 3.56–3.57 (m, 6H), 3.66 (dd, 0.8H, J = 11.20 Hz, $J = 4.20$ Hz), 3.71 (dd, 0.2H, J = 11.20 Hz, $J = 4.20$ Hz), 3.80 (d, 1H, $J = 14.00$ Hz), 3.84–3.87 (m, 1H), 3.90 (t, 2H, $J = 7.00$ Hz), 3.93 (d, 1H, $J = 14.00$ Hz), 4.09–4.13 (m, 2H), 4.20–4.24 (m, 1H), 4.35–4.40 (m, 2H), 4.46–4.48 (m, 1H), 4.56–4.59 (m, 1H), 6.20 (d, 0.2H, $J = 2.80$ Hz), 6.22 (d, 0.8H, $J = 4.20$ Hz), 7.00–7.96 (m, 23H), 8.34 (s, 1H); ^{13}C NMR (176 MHz, acetone-d_6): δ −3.21 (−3.18), −3.10 (−3.04), 20.22 (20.27), 21.11, 25.93, 25.95, 27.66 (27.69), 28.71 (28.73), 35.97, 43.17 (43.20), 43.82, 45.73, 47.24, 50.09, 56.98, 62.73, 65.85 (66.01), 72.67 (72.78), 78.19 (78.28), 80.55 (80.95), 85.34 (85.88), 88.77 (88.81), 91.46, 110.28, 115.49, 118.68 (q, $J = 287.23$ Hz), 129.10, 130.20, 130.60, 132.12, 132.58, 135.93, 137.79, 138.34, 144.22, 147.35, 152.35, 158.72 (q, $J = 35.73$ Hz), 161.12, 165.67; HRMS (ESI): calcd for $C_{63}H_{80}N_3O_{13}F_3NaSi_2S$ $[M+Na]^+$ 1254.4800, found 1254.4789.

Compound **15b** was purified on a silica gel column with 2% MeOH in $CHCl_3$ as eluent to obtain a white foam in 80% yield. TLC $R_f = 0.46$ ($CHCl_3$/MeOH 95:5, v/v); ^1H NMR (700 MHz, acetone-d_6): δ 0.15 (s, 2.4 H), 0.17 (s, 3H), 0.21 (s, 0.6H), 0.94 (s, 7.2H), 0.95 (s, 1.8H), 0.99 (s, 1.2H), 1.01 (s, 4.8H), 1.04 (s, 1.8H), 1.05 (s, 7.2H), 1.68 (t, 0.4H, $J = 7.00$ Hz), 1.70 (t, 1.6H, $J = 7.00$ Hz), 3.47 (dd, 0.8H, $J = 11.20$ Hz, $J = 2.10$ Hz), 3.50 (dd, 0.2H, $J = 11.20$ Hz, $J = 2.10$ Hz), 3.54 (dd, 0.2H, 11.20 Hz, $J = 4.90$ Hz), 3.62 (dd, 0.8H, $J = 11.20$ Hz, $J = 5.60$ Hz), 3.66–3.72 (m, 2H), 3.78–3.79 (m, 6H), 3.81–3.85 (m, 2H), 3.92 (s, 2H), 3.99–4.02 (m, 1H), 4.05–4.12 (m, 3H), 4.15–4.19 (m, 1H), 4.27–4.31 (m, 1H), 4.41–4.46 (m, 1H), 6.66 (d, 0.2H, $J = 2.10$ Hz), 6.69 (d, 0.8H, $J = 2.80$ Hz), 6.84–7.72 (m, 23H), 7.89 (s, 0.2H), 8.02 (s, 0.8H), 11.52 (s, 0.8H), 11.55 (s, 0.2H); ^{13}C NMR (176 MHz, acetone-d_6): δ −2.83, −2.78 (−2.61), 20.38 (20.45), 21.13, 25.95, 25.97, 27.86 (27.90), 28.73, 35.98, 43.20 (44.01), 45.93, 47.37 (48.15), 50.06, 56.97, 57.02, 62.74, 65.72 (66.13), 72.39 (72.60), 79.09 (79.20), 80.56 (81.04), 85.29 (85.88), 88.88 (88.92), 95.88 96.69, 115.03 (115.10), 115.52 (q, $J = 288.64$ Hz), 129.16 (129.40), 129.78 (129.84), 130.15–130.26 (m), 130.68, 131.54, 132.13, 132.60–132.68 (m), 135.93, 137.80, 138.22, 138.33, 142.43 (142.70), 144.20, 147.21 (147.48), 158.81 (q, $J = 35.20$ Hz), 162.47, 178.34 (178.51); HRMS (ESI): calcd for $C_{63}H_{80}N_3O_{12}F_3NaSi_2S_2$ $[M+Na]^+$ 1270.4572, found 1270.4581.

2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(4,4′-dimethoxytrityl)-*N*-[(1-*β*-D-ribofuranosyl-1*H*-(-2-thio)pyrimidin-5-yl)methyl]-*N*-(trifluoroacetyl)taurine 4-(*tert*-butyldiphenylsilanyloxy)-2,2-dimethylbutyl ester 3′-(cyanoethyl *N*,*N*-diisopropylphosphoramidite) (16a/16b)

The 5′-DMTr, 2′-TBDMS nucleoside **15a/15b** (0.15 mmol, 1.0 equiv) was dissolved in anhydrous DCM (928 µL) under Ar atmosphere, then diisopropylethylamine (102 µL, 0.59 mmol, 4.0 equiv) and 2-cyanoethyl *N*,*N*-diisopropylaminochlorophosphoramidite (65 µL, 0.30 mmol, 2.0 equiv) were added. The reaction mixture was stirred at rt for 3 h. The solution was diluted with DCM (12 mL) and washed with 5% aq. $NaHCO_3$ (8 mL) and water (2 × 8 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product **16a/16b** was purified by flash chromatography using petroleum ether:ethyl acetate (2:1 v/v). The material exists as a mixture of stereoisomers about phosphorus, wherein each is a rotamer about the $-NC(O)CF_3$ amide bond and two or more chemical shifts are observed for some of the ^1H and ^{31}P NMR resonances.

Compound **16a** was isolated as a white foam in 83% yield. TLC $R_f = 0.38$ (benzene, DCM, Et_3N 7:2:1 v/v/v); ^1H NMR (700 MHz, C_6D_6): δ 0.17–0.27 (m, 6H), 0.97–1.02 (m, 9H), 1.07–1.12 (m, 18H), 1.22–1.26 (m, 9H), 1.75–1.80 (m, 2H), 3.43–3.58 (m, 2H), 3.62–3.80 (m, 6H), 3.85–3.96 (m, 9H), 4.00–4.16 (m, 5H), 4.19–4.29 (m, 3H), 4.35–4.46 (m, 1H), 4.52–4.65 (m, 1H), 5.94–6.09 (m, 1H), 6.95–7.02 (m, 3H), 7.30–7.52 (m, 16H), 7.77–7.90 (m, 4H), 8.02–8.09 (m, 1H), 10.43 (s, 1H); ^{31}P NMR (101.25 Hz, C_6D_6): δ 149.62, 150.18, 150.61, 150.81; HRMS (ESI): calcd for $C_{72}H_{97}N_5O_{14}F_3NaSi_2SP$ $[M+Na]^+$ 1454.5879, found 1454.5863.

Compound **16b** was isolated as a light yellow foam in 92% yield. TLC $R_f = 0.70$ (benzene, DCM, Et_3N 7:2:1 v/v/v); ^1H NMR (250 MHz,C_6D_6): δ 0.20–0.30 (m, 6H), 0.98–1.03 (m, 9H), 1.05–1.12 (m, 18H), 1.18–1.31 (m, 9H), 1.74–1.80 (m, 2H), 3.54–3.81 (m, 7H), 3.86–3.95 (m, 9H), 3.97–4.06 (m, 2H), 4.08–4.11 (m, 2H), 4.14–4.17 (m, 3H), 4.20–4.29 (m, 2H), 4.43–4.54 (m, 1H), 4.57–4.66 (m, 1H), 6.90–7.04 (m, 4H), 7.24-.67 (m, 16H), 7.77–7.81 (m, 4H), 8.11–8.17 (m, 1H), 11.63 (s, 1H); ^{31}P NMR (101.25 Hz, C_6D_6): δ 149.80, 150.75, 150.95, 151.24; HRMS (ESI): calcd for $C_{72}H_{97}N_5O_{13}F_3NaSi_2S_2P$ $[M+Na]^+$ 1470.5650, found 1454.5636.

Oligonucleotide synthesis

Oligoribonucleotides were synthesized manually on a 5-µmol scale using slightly modified Sproat's procedure (Sproat 2005). Commercially available monomeric units A, C, U, and G were protected with DMTr and TBDMS on the 5′- and 2′-hydroxy functions, re-

spectively, and the exocyclic amine functions of A, C, and G were masked with 4-*tert*-butylphenoxyacetyl (tac) (Proligo). Typical rA (tac)-succinyl-CPG (Proligo) support and 0.1 M acetonitrile solutions of monomeric units were used. A, U, C, and G amidites were coupled in 8 molar excess for 8 min in the presence of Activator 42 (0.25 M solution of 5-(3,5-bis(trifluoromethyl)phenyl)-1*H*-tetrazole in CH_3CN), while modified units were used in 12 molar excess and coupled twice, each time using 6 molar excess of amidite and 12 min coupling time. Capping was performed with tac anhydride (Fast protection Cap A:Cap B 1:1.1 v/v) for 2 min. A 0.02 M iodine solution in THF-H_2O-pyridine (90.5:0.45:9.05 v/v/v; 8 equiv) was used as an oxidizing agent for 2 min for each oxidation step.

RNA deprotection and purification

The "trityl-off" CPG-bound RNA was transferred from the column to a screw cap glass vial, and 6.5 mL of Et_3N/CH_3CN 1:1 v/v was added. The solution was stirred for 25 min, and then the solvent was removed. The support-bound RNA was washed with acetonitrile, dried in vacuum for 30 min, and treated with 8.5 mL of 8 M ethanolic ammonia at rt for 8 h. The supernatant was removed and the support was washed with an additional 3 mL of anhydrous ethanol. The combined washings were evaporated on a Speed-Vac concentrator.

Desilylation of hmt-$ASL^{Leu(UUR)}$(τm^5U_{34}) was conducted with $Et_3N{\bullet}3HF$/NMP (1:1, v/v, 2 mL) at rt for 24 h. The reaction was quenched by addition of ethoxytrimethylsilane (4 mL), and crude RNA was precipitated using *t*-butyl methyl ether (10 mL). RNA was collected by centrifugation, washed with *t*-butyl methyl ether (2 × 10 mL), and purified using IE-HPLC.

hmt-ASL^{Lys}($\tau m^5s^2U_{34}$) was dissolved in 5 mL of 1 M TEAF in NMP and stirred. Desilylation was conducted at rt for 24 h and then quenched by the addition of 0.05 M Na_2HPO_4–NaH_2PO_4 buffer solution (pH 7.6). Crude RNA was desalted on a column packed with Sephadex G-25 (elution with 20% aqueous ethanol), monitored by UV detection at 260 nm. The RNA-containing eluate was lyophilized and then purified.

Fully deprotected RNAs were purified by anion-exchange HPLC on a Waters AP-2 column packed with TSK SuperQ-5PW resin. For elution, we used a linear gradient of NaBr (50–650 mM) in sterile 20 mM Na_2HPO_4–NaH_2PO_4 buffer solution (pH 7.5), containing EDTA (50 μM) and 10% CH_3CN; flow 9 mL/min. Fractions containing the desired product were collected, concentrated, and desalted on a column packed with Sephadex G-25. The desalted RNAs were lyophilized to obtain 120 OD_{260} of hmt-$ASL^{Leu(UUR)}$ (τm^5U_{34}) and 100 OD_{260} of hmt-ASL^{Lys}($\tau m^5s^2U_{34}$). The oligomers were analyzed by MALDI-TOF (Supplemental Material).

RNA enzymatic digestion and HPLC analysis of nucleoside composition

The nucleoside composition of oligoribonucleotides was confirmed by enzymatic hydrolysis of RNAs to nucleosides using nuclease P_1 and alkaline phosphatase (Gehrke et al. 1982; Gehrke and Kuo 1989). The resulting nucleoside mixtures were analyzed with a C18 column (ODS2, 4.6 mm × 250 mm), with a linear gradient of buffer A (10 mM KH_2PO_4; pH 5.3) and buffer B (20% methanol in 10 mM KH_2PO_4; pH 5.1) with a flow of 0.75 mL/min. As shown in Figure 4C, HPLC elutions monitored at 264 nm indicate that hmt-$ASL^{Leu(UUR)}$ and hmt-ASL^{Leu} contain τm^5U (9.51 min) and τm^5s^2U (18.04 min), respectively. The peaks were compared with reference samples of the modified units in separate control experiments (Fig. 4D).

SUPPLEMENTAL MATERIAL

Supplemental material is available for this article.

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REFERENCES

Agris PF, Malkiewicz A, Kraszewski A, Everett K, Nawrot B, Sochacka E, Jankowska J, Guenther R. 1995. Site-selected introduction of modified purine and pyrimidine ribonucleosides into RNA by automated phosphoramidite chemistry. *Biochimie* **77:** 125–134.

Ali AM, Hill B, Taylor SD. 2009. Trichloroethyl group as a protecting group for sulfonates and its application to the synthesis of a disulfonate analog of the tyrosine sulfated $PSGL\text{-}1_{43–50}$ peptide. *J Org Chem* **74:** 3583–3586.

Andrianov AK, Marin A, Chen J, Sargent J, Corbett N. 2004. Novel route to sulfonated polyphosphazenes: single-step synthesis using "noncovalent protection" of sulfonic acid functionality. *Macromolecules* **37:** 4075–4080.

Avitabile BG, Smith CA, Judd DB. 2005. Pentafluorophenyl sulfonate ester as a protecting group for the preparation of biaryl- and heterobiaryl sulfonate esters. *Org Lett* **7:** 843–846.

Bajji AC, Davis DR. 2002. Synthesis of the $tRNA^{Lys,3}$ anticodon stem–loop domain containing the hipermodified ms^2t^6A nucleoside. *J Org Chem* **67:** 5352–5358.

Bajji AC, Sundaram M, Myszka DG, Davis DR. 2002. An RNA complex of the HIV-1 A-loop and $tRNA^{Lys,3}$ is stabilized by nucleoside modifications. *J Am Chem Soc* **124:** 14302–14303.

Baxter NJ, Rigoreau LJM, Laws AP, Page MI. 2000. Reactivity and mechanism in the hydrolysis of β-sultams. *J Am Chem Soc* **122:** 3375–3385.

Bilbille Y, Vendeix FAP, Guenther R, Malkiewicz A, Ariza X, Vilarrasa J, Agris PF. 2009. The structure of the human $tRNA^{Lys3}$ anticodon bound to the HIV genome is stabilized by modified nucleosides and adjacent mismatch base pairs. *Nucleic Acids Res* **37:** 3342–3353.

Boudou V, Langridge J, Van Aerschot A, Hendix C, Millar A, Weiss P, Herdewijn P. 2000. Synthesis of the anticodon hairpin $tRNA_f^{Met}$ containing *N*-{[9-(β-D-ribofuranosyl)-9*H*-purin-6-yl]carbamoyl}-L-threonine (=N^6-{{[(1*S*,2*R*)-1-Carboxy-2-hydroxypropyl]amino}carbonyl} adenosine, t^6A). *Helv Chim Acta* **83:** 152–161.

Cantara WA, Bilbille Y, Kim J, Kaiser R, Leszczynska G, Malkiewicz A, Agris PF. 2012. Modifications modulate anticodon loop dynamics and codon recognision of *E.coli* $tRNA^{Arg1,2}$. *J Mol Biol* **416:** 579–597.

Cantara WA, Murphy FV IV, Demirci H, Agris PF. 2013. Expanded use of sense codons is regulated by modified cytidines in tRNA. *Proc Natl Acad Sci* **110:** 10964–10969.

Capaldi DC, Gaus H, Krotz AH, Arnold J, Carty RL, Moore MN, Scozzari AN, Lowery K, Cole DL, Ravikumar VT. 2003. Synthesis of high-quality antisense drugs. Addition of acrylonitrile to phosphorothioate oligonucleotides: adduct characterization and avoidance. *Org Process Res Dev* **7:** 832–838.

Damha MJ, Ogilvie KK. 1993. Oligoribonucleotide synthesis. The silyl-phosphoramidite method. *Methods Mol Biol* **20:** 81–114.

Duram PC, Bajji AC, Sundaram M, Kumar RK, Davis DR. 2005. Structural effects of hypermodified nucleosides in the *Escherichia coli* and human $tRNA^{Lys}$ anticodon loop: the effect of nucleosides s^2U, mcm^5U, mcm^5s^2U, mnm^5s^2U, t^6A and ms^2t^6A. *Biochemistry* **44:** 8078–8089.

Eshete M, Marchbank MT, Deutscher SL, Sproat B, Leszczynska G, Malkiewicz A, Agris PF. 2007. Specificity of phage display selected peptides for modified anticodon stem and loop domains of tRNA. *Protein J* **26:** 61–73.

Florentz C, Sohm B, Tryoen-Tóth P, Pütz J, Sissler M. 2003. Human mitochondrial tRNAs in health and disease. *Cell Mol Life Sci* **60:** 1356–1375.

Gehrke CW, Kuo KC. 1989. Ribonucleoside analysis by reversed-phase high-performance liquid chromatography. *J Chromatogr* **471:** 3–36.

Gehrke CW, Kuo KC, McCune RA, Gerhardt KO. 1982. Quantitative enzymatic hydrolysis of tRNAs: reversed-phase high-performance liquid chromatography of tRNA nucleosides. *J Chromatogr B Biomed Sci Appl* **230:** 297–308.

Helm M, Brulé H, Friede D, Giegé R, Pütz J, Florentz C. 2000. Search for characteristic structural features of mammalian mitochondrial tRNAs. *RNA* **6:** 1356–1379.

Hussain M, Ahmed V, Hill B, Ahmed Z, Taylor SD. 2008. A re-examination of the difluoromethylenesulfonic acid group as a phosphotyrosine mimic for PTP1B inhibition. *Bioorg Med Chem* **16:** 6764–6777.

Jühling E, Pütz J, Florentz C, Stadler PF. 2012. Armless mitochondrial tRNAs in Enoplea (Nematoda). *RNA Biol* **9:** 1161–1166.

Kirino Y, Yasukawa T, Ohta S, Akira S, Ishihara K, Watanabe K, Suzuki T. 2004. Codon-specific translational defect caused by a wobble modification deficiency in mutant tRNA from a human mitochondrial disease. *Proc Natl Acad Sci* **101:** 15070–15075.

Klamann D, Hofbauer G. 1953. Reduktive Spaltungen von Sulfamiden. *Chem Ber* **86:** 1246–1252.

Klán P, Pelliccioli AP, Pospíšil T, Wirz J. 2002. 2,5-dimethylphenacyl esters: a photoremovable protecting group for phosphates and sulfonic acids. *Photochem Photobiol Sci* **1:** 920–923.

Kurata S, Weixlbaumer A, Ohtsuki T, Shimazaki T, Wada T, Kirino Y, Takai K, Watanabe K, Ramakrishnan V, Suzuki T. 2008. Modified uridines with C5-methylene substituents at the first position of the tRBA anticodon stabilize U·G wobble pairing during decoding. *J Biol Chem* **283:** 18801–18811.

Kurschat WC, Muller J, Wombacher R, Helm M. 2005. Optimizing splinted ligation of highly structured small RNAs. *RNA* **11:** 1909–1914.

Leszczynska G, Pięta J, Sproat B, Malkiewicz A. 2011. Chemical synthesis of an RNA sequence containing 2-thiocytidine (s^2C): the DY647 labelled anticodon stem and loop sequence of *Staphylococcus aureus* $tRNA^{Arg}$ (s^2C_{32}, mnm^5U_{34}, t^6A_{37}). *Tetrahedron Lett* **52:** 4443–4447.

Leszczynska G, Pięta J, Leonczak P, Tomaszewska A, Malkiewicz A. 2012. Site-specific incorporation of 5-methylaminomethyl-2-thiouridine and 2-thiouridine(s) into RNA sequences. *Tetrahedron Lett* **53:** 1214–1217.

Leszczynska G, Leonczak P, Dziergowska A, Malkiewicz A. 2013. mt-tRNA components: synthesis of (2-thio)uridines modified with blocked glycine/taurine moieties at C-5,1. *Nucleosides Nucleotides Nucleic Acids* **32:** 599–616.

Lusic H, Gustilo EM, Vendeix FA, Kaiser R, Delaney MO, Graham WD, Moye VA, Cantara WA, Agris PF, Deiters A. 2008. Synthesis and investigation of the 5-formylcytidine, modified anticodon stem and loop of the human mitochondrial tRNAMet. *Nucleic Acids Res* **36:** 6548–6557.

Malkiewicz A, Sochacka E. 1983. The protected derivatives of 5-methylaminomethyl-2-thiouridine and 5-carbomethoxymethyl-2-thiouridine as components for the oligonucleotide synthesis. *Tetrahedron Lett* **24:** 5387–5390.

Miller SC. 2010. Profiling sulfonate ester stability: identification of complementary protecting groups for sulfonates. *J Org Chem* **75:** 4632–4635.

Myerscough PM, Fairbanks AJ, Jones AH, Bruce I, Choi SS, Fleet GWJ, Al-Daher SS, di Bello IC, Winchester B. 1992. Inhibition of α-mannosidases by seven carbon sugars: synthesis of some seven carbon analogues of mannofuranose. *Tetrahedron* **48:** 10177–10190.

Ogata T, Wada T. 2006. Chemical synthesis of RNA including 5-taurinomethyluridine. *Nucleic Acids Symp Ser* **50:** 9–10.

Ogata T, Wada T. 2008. Chemical synthesis of RNA including 5-taurinomethyluridine and 5-taurinomethyl-2-thiouridine. *Nucleic Acids Symp Ser* **52:** 323–324.

Okamoto I, Seio K, Sekine M. 2006. Improved synthesis of oligonucleotides containing 2-thiouridine derivatives by use of diluted iodine solution. *Tetrahedron Lett* **47:** 583–585.

Richman JE, Atkins TJ. 1974. Nitogen analogs of crown ethers. *J Am Chem Soc* **96:** 2268–2270.

Roberts JC, Gao H, Gopalsamy A, Kongsjahju A, Patch RJ. 1997. Neopentyl ester protecting groups for arylsulfonic acids. *Tetrahedron Lett* **38:** 355–358.

Seeberger S, Griffin RJ, Hardcastle IR, Golding BT. 2007. A new strategy for the synthesis of taurine derivatives using the 'safety-catch' principle for the protection of sulfonic acids. *Org Biomol Chem* **5:** 132–138.

Seio K, Wada T, Sakamoto K, Yokoyama S, Sekine M. 1998. Chemical synthesis and properties of conformationally fixed diuridine monophosphates as building blocks of the RNA turn motif. *J Org Chem* **63:** 1429–1443.

Sissler M, Lorber B, Messmer M, Schaller A, Pütz J, Florentz C. 2008. Handling mammalian mitochondrial tRNAs and aminoacyl-tRNA synthetases for functional and structural characterization. *Methods* **44:** 176–189.

Sochacka E. 2001. Efficient assessment of modified nucleoside stability under conditions of automated oligonucleotide synthesis: characterization of the oxidation and oxidative desulfurization of 2-thiouridine. *Nucleosides Nucleotides Nucleic Acids* **20:** 1871–1879.

Sproat BS. 2005. RNA synthesis using 2′-*O*-(*tert*-butyldimethylsilyl) protection. *Methods Mol Biol* **288:** 17–31.

Sundaram M, Crain PF, Davis DR. 2000. Synthesis and characterization of the native anticodon domain of *E. coli* $tRNA^{Lys}$: simultaneous incorporation of modified nucleosides $mnmm^5s^2U$, t^6A, and pseudouridine using phosphoramidite chemistry. *J Org Chem* **65:** 5609–5614.

Suzuki T, Nagao A, Suzuki T. 2011a. Human mitochondrial diseases caused by lack of taurine modification in mitochondrial tRNAs. *WIREs RNA* **2:** 376–386.

Suzuki T, Nagao A, Suzuki T. 2011b. Human mitochondrial tRNAs: biogenesis, function, structural aspects, and diseases. *Annu Rev Genet* **45:** 299–329.

Wada T, Shimazaki T, Nakagawa S, Otuki T, Kurata S, Suzuki T, Watanabe K, Saigo K. 2002. Chemical synthesis of novel taurine-containing uridine derivatives. *Nucleic Acids Res Suppl* **2:** 11–12.

Watanabe K. 2010. Unique feature of animal mitochondrial translation systems. The non-universal genetic code, unusual features of the translational apparatus and their relevance to human mitochondrial diseases. *Proc Jpn Acad Ser B Phys Biol Sci* **86:** 11–39.

Weixlbaumer A, Murphy FV IV, Dziergowska A, Malkiewicz A, Vendeix FAP, Agris PF, Ramakrishnan V. 2007. Mechanism for expanding the decoding capacity of transfer RNAs by modification of uridines. *Nat Struct Mol Biol* **14:** 498–502.

Wende S, Platzer EG, Jühling F, Pütz J, Florentz C, Stadler PF, Mörl M. 2014. Biological evidence for the world's smallest tRNAs. *Biochimie* **100:** 151–158.

Wrobel J, Rogers J, Green D, Kao W. 2002. Use of an isopropyl ester moiety as a sulfonic acid protecting group in a greatly improved synthesis of an arylsulfonic acid-based follicle stimulating hormone antagonist. *Synth Commun* **32:** 2695–2704.

Yan L, Müller CE. 2004. Preparation, properties, reactions, and adenosine receptor affinities of sulfophenylxanthine nitrophenyl esters: toward the development of sulfonic acid prodrugs with peroral bioavailability. *J Med Chem* **47:** 1031–1043.

Chemical synthesis of the 5-taurinomethyl(-2-thio)uridine modified anticodon arm of the human mitochondrial tRNA $^{Leu(UUR)}$ and tRNA ^{Lys}

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