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An efficient approach for conversion of 5-substituted 2-thiouridines built in RNA oligomers into corresponding desulfured 4-pyrimidinone products

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ABSTRACT

An efficient approach for the desulfuration of C5-substituted 2-thiouridines (R5S2U) bound in the RNA chain exclusively to 4-pyrimidinone nucleoside (R5H2U)-containing RNA products is proposed. This post-synthetic transformation avoids the preparation of a suitably protected H2U phosphoramidite, which otherwise would be necessary for solid-phase synthesis of the modified RNA. Optimization of the desulfuration, which included reaction stoichiometry, time and temperature, allowed to transform a set of ten R5S2U-RNAs into their R5H2U-RNA congeners in ca. 90% yield.

Transfer RNAs are unique among natural RNAs due to the presence of modified nucleosides.[1,2] Those located in the tRNA anticodon tune the translation process through highly accurate codon–anticodon recognition. Among them there are C5-substituted 2-thiouridines (R5S2U) present in the first (wobble) position of the anticodon in transfer RNAs specific for lysine ($tRNA^{Lys3}$), glutamic acid ($tRNA^{Glu}$) and glutamine ($tRNA^{Gln}$). Nucleosides containing thiocarbonyl function may be sensitive to oxidizing conditions. It was reported that thiouridines in tRNA chain get desulfured in the presence of oxidative agents, although detailed structures of the products were not determined.[3] Other studies reported that in 100 mM H_2O_2 2-thiouridines were oxidized to uridines and converted into another, unidentified product, which retained the ring structure and did not have a disulfide bond.[4] However, when S2U nucleoside is oxidized in 'organic' non-aqueous conditions, for example, in the presence of *trans*-2-phenylsulfonyl-3-phenyloxaziridine (PSO)[5] or *m*-chloroperbenzoic acid (*m*CPBA),[6] it undergoes selective transformation to the 4-pyrimidinone nucleoside (H2U), which is a uridine analog deprived of the C2 oxygen and N3 hydrogen atoms (Scheme 1). The loss of the sulfur atom was also observed in aqueous solutions. First effective desulfuration of 2-thiouridine located in a DNA pentamer 5′-TdA(S2U)dGdC-3′ was carried out with PSO in water/acetonitrile solution.[5] The product contained a H2U unit (identified by MALDI-TOF mass spectrometry), so at the nucleoside and oligonucleotide levels the desulfuration of 2-thiouridine predominantly yielded the H2U analog. In further studies we applied conditions mimicking an oxidative stress in a cell (100 mM H_2O_2) and found that, either alone or in an RNA chain, S2U is desulfured to uridine and 4-pyrimidinone nucleoside (Scheme 1).[7] The products ratio depended on pH,[8] C5-substituent and concentration of the oxidant (Bartos et al., will be published elsewhere). Importantly, the reactions carried out either in aqueous H_2O_2 or $KHSO_5$ (oxone) at pH 6.6 furnished mainly H2U, while uridine was a major product at pH 7.6.

Conformational and crystallographic studies demonstrate that S2U predominantly exists in the C3′-*endo* sugar ring conformation,[9] typical for ribonucleosides/ribonucleotides, while H2U preferentially adopts the C2′-*endo* sugar puckering, characteristic for the 2′-deoxyribo congeners.[10] Moreover, due to the altered pattern of hydrogen bonding, the H2U nucleoside exerts different base pairing. Thus, oxidative modification of S2U-tRNA would substantially affect the codon–anticodon interactions, as well as recognition of tRNA by proteins involved in cellular processes. Data obtained from UV-melting[7] and DSC experiments[11] indicate that the affinity of H2U-RNA to its Watson–Crick complement is much

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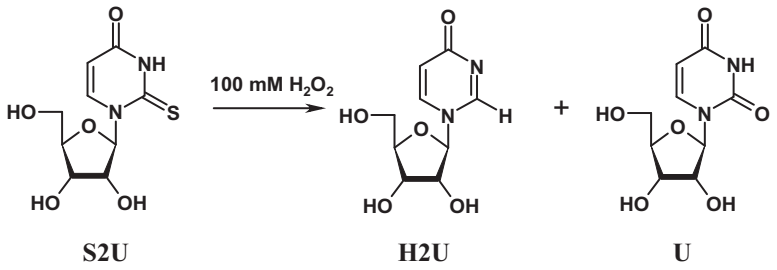

Scheme 1. Transformation of 2-thiouridine (S2U) to 4-pyrimidinone nucleoside (H2U) and uridine (U).

smaller than that of S2U-RNA. Additionally, H2U-RNA under basic conditions decomposes to the abasic form, with eventual strand scission.[7] Therefore, it should be clarified whether the S2U→H2U transformation occurs in vivo resulting in the tRNA chain cleavage within the damaged anticodon wobble site. For that purpose, a reliable access to H2U-RNA models is urgently needed.

In our earlier reports we have described the synthesis of a suitably protected H2U phosphoramidite monomer[5] and its incorporation into an RNA chain.[7,11] That approach is not straightforward because H2U-RNA is unstable in basic environment, even during routine RP-HPLC purification employing only slightly alkaline triethylammonium bicarbonate eluent (pH 7.5). Here we report an optimized method for the S2U-RNA→H2U-RNA transformation after the model RNA oligomers containing different hypermodified 5-substituted 2-thiouridines (R5S2U) were already assembled and deprotected.

For optimization of the transformation we selected ten RNAs (Table 1) containing 2-thiouridine or its C5-substituted derivatives (Fig. 1), naturally occurring in tRNAs. The modified nucleosides (generally denoted X) were built into the RNA hairpin structure (with the R5S2U modification in the position corresponding to the wobble position, Table 1, oligomers **1**–**6**, **8**), into a U_{11} oligomer (**7**), or into the RNA chain coding the passenger strands of siRNA (oligomers **9**, **10**). These oligomers were synthesized according to already described procedures.[11–14] Illustrative procedures for synthesis of the R5S2U-RNA oligomers are given in Supplementary material.

To prepare all solutions intended to contact with R5S2U-RNAs, sterile water (0.1% DEPC-treated, and then autoclaved) was used. Desulfuration was done with aqueous solutions of $KHSO_5$ (oxone®), as this oxidizing agent is easy to handle and one can precisely control its ratio (5–20-fold molar excess, 2 mM solution) to the R5S2U-

Table 1
Sequences, calculated molecular weights (M.W.) and mass spectrometry data for RNA oligonucleotides used

No.	X	Oligonucleotide sequence	M.W. (g/mol)	MALDI-TOF MS (*m*/*z*)
1	S2U	5′-CGGCU**X**UUAACCGA-3′	4423	4418.9
2	mcm5S2U	5′-CGGCU**X**UUAACCGA-3′	4495	4490.9
3	τm5S2U	5′-UUAACCU**X**UUAAGUUAA-3′	5472	5468.0
4	mcm5S2U	5′-CCAGUUGACU**X**UUAAUCAAUUGG-3′	7354	7352.9
5	mnm5S2U	5′-CCAGUUGACU**X**UUAAUCAAUUGG-3′	7325	7324.6
6	S2U	5′-CCAGUUGACU**X**UUAAUCAAUUGG-3′	7282	7280.0
7	mnm5S2U	5′-UUUUUU**X**UUUU-3′	3366	3361.4
8	S2U	5′-UCACGCU**X**UCACCGUGG-3′	5355	5350.0
9	m5S2U	5′-UUACCAACCACAACACUG**X**TT-3′	6583	6583.3
10	S2Um	5′-UUACCAACCACAACACUG**X**TT-3′	6583	6583.5

The structures of nucleosides denoted X are given in Figure 1. The *m*/*z* values correspond to $[M-H]^-$ ions.

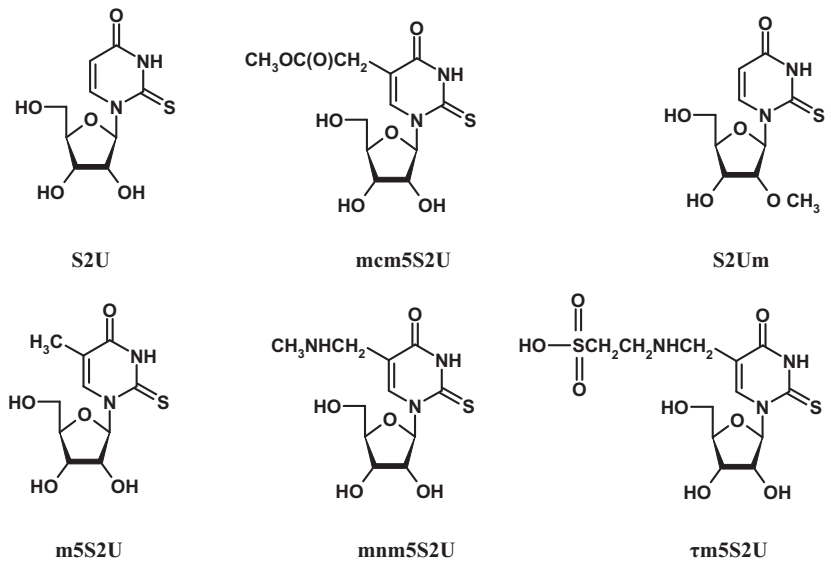

Figure 1. Modified C5-substituted 2-thiouridines used in these studies.