Transformation of Some rRNA “Wobble Uridines” to their 2-Thioanalogues

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Introduction

The “wobble position” uridines of rRNA (U*4) 5-carboxymethylaminomethyluridine (cmcmU) (1) and its 2-thioanalogue (s^2cmcmU) 1a recognize A as the “third letter” of codons, while 5-carboxymethoxyuridine (cmoU or V) (3) and its methyl ester (mcmoU or mV) 4 are able to “read” A, G, and U [1, 2, 11]. This phenomenon has been explained as arising from the “rigid” or “flexible” character of the “wobble position” inside 3'-stacked arrangement of rRNA anticodon loop, although different arguments have been presented [3–8].

Recently published results clearly demonstrate the formation of Mg^{2+}-rRNA^{Phe} anticodon loop complex, which influences the equilibrium between the 5'-stacked and 3'-stacked conformations of the loop, modulating the multi-step codon—anticodon recognition process [9, 10]. Indeed, much data have been collected to confirm the ability of several purine nucleotides located at the 37 position of rRNA, (pyW, pt^-A, pmR^-A) as well as pI to chelate Mg^{2+} (Mn^{2+}) ions [11, 12]. In contrast, modified uridines from the “first position” of rRNA anticodons (U*4) were examined only marginally in this respect [13]. However, the Mg^{2+} ion mediated interaction of U*4 with the backbone or ribosome architecture can be considered basing on the previously reported findings and suggestions [3, 4, 8, 14, 15].

The ability of nucleosides to form ternary complexes with Me^{2+}-peptides decreases from 6-keto-purines to uridine [16]. Nevertheless, several substituent modifications for U*4 e.g. 1, 1a and 3 have the potential to alter this arrangement.

SCHEME 1

Accumulation of knowledge about conformational dynamics of U*4 containing oligomers under physiological conditions could be relevant to discuss several controversial aspects of molecular mechanism of...
rRNA interaction with other partners of the ribosomal translation process.

As a part of our programme directed toward the synthesis of oligomers with modified units [17–19] we have developed the procedures for the preparation of the nucleosides 2, 2a and 4, 4a using 5 and 6 as suitable intermediates, respectively.

Results and Discussion

Although spectral and X-ray analysis data of 3 and 4 have been reported [20–23], the syntheses of these compounds have not been published in details. However, the reaction of 5-hydroxy-2'-deoxyuridine sodium salt with iodoacetic acid (molar ratio 1:3) leading to 2'-deoxy analogue of the nucleoside 3 has been described [23]. According to this method the sodium salt of 5-hydroxyuridine (7) [24] was transformed to the N3-isomer 9 as a sole product, whose structure was univocally confirmed by spectral data and positive test for the free enol function. On the other hand the condensation of the monoanion of 8 with ethyl iodoacetate in ethanol/water or DMF readily produced 6, which was isolated by means of column chromatography in ca. 50% yield.

The synthesis of the nucleoside 5 was based upon two-step procedure consisting of trifluoroacetylation of the ester 14 followed by the selective deprotection of the intermediate 15 [19].

The relatively stable ester 19 has been prepared in moderate yield by aminolysis of the readily available 11 or 12 with tert-butylglycinate [19, 25]. However, methyl glycinate had to be excluded as the intermediate for the analogous synthesis of the nucleoside 14, due its known tendency to cyclisation into diketopiperazine under reaction conditions and the easy formation of N,N-disubstituted products during the alkylation processes. In order to avoid the above side reactions the chloride 11 was condensed with a slight excess of N-benzylglycine methyl ester in DMF and in the presence of triethylamine to give the N-protected ester 13 in good yield.

The hydrogenolysis of 13 in the presence of 10% Pd/C yielded the debenzylated compound 14 in almost quantitative yield. However, in contrast to N-benzyl protected derivative 13, which can be stored for weeks at room temperature, 14 dissolved in usual solvents is subject to side reactions. They involve the glycine residue and result in the formation of mixture of less polar products (as shown by t.l.c.), which are not sensitive to the ninhydrine test.

Thus, the crude hydrogenolysis product was immediately trifluoroacetylated with an excess of trifluoroacetyl anhydride in pyridine [17, 19], to give the N,5'-O-disubstituted compound 15. This was selectively deprotected at the 5'-hydroxy function by treatment with a 5% solution of sodium hydrogen carbonate (10 min, 0 °C). The N-trifluoroacetylated uridine 5 was eluated from a silica gel column as the
first fraction absorbing UV light and its structure was confirmed by spectral data. It is noteworthy that, the $^1$H NMR spectrum of 5 exhibits doublet of doublets of $H_1'$ and doublet of $H_6$ signals in several solvents (deuterated: acetone, dimethyl sulphoxide, methanol), which overlap after addition of deuterated chloroform. It seems that, an equilibrium between conformers of uridines or uracils bearing N-acylated exo-amine function is a common phenomenon in this class of compounds [19, 26–28].

Following the Mitsunobu procedure [29] 5 and 6 were reacted with ethyl azadicarboxylate in the presence of triphenyl-phosphine and triethylamine to afford the 2,5'-anhydronucleosides 16 and 10, isolated in ca. 70% yield by means of silica gel column chromatography with chloroform-acetone system as the eluant [30] (Scheme 2, 3).

The reaction of 2',3'-O-isopropylidene-2,5'-anhydouridine with hydrogen sulphide in the presence of pyridine or triethylamine has been frequently used to introduce sulphur into 2-position of uridine [31]. However, neither structurally complex molecules of the type represented by 16 and 10 were tested, nor mechanism of this reaction was studied in detail [31].
In general, 2',3'-isopropylidene-2,5'-anhydrouridines under treatment with hydrogen sulphide—tertiary amine undergo the two competitive processes: i) 2-thiation by addition-elimination reaction involving enone function (pathway a), ii) substitution with sulphhydryl group at the C5' atom [31] (pathway b, Scheme 4). Ueda et al. [32] synthesized 2',3'-isopropylidene-2-thiouridine in ca. 90% yield in the reaction of suitable anhydroneucleoside with a large excess of liquid hydrogen sulphide-pyridine 1:1 (V/V). Nevertheless, the compounds 16 and 10 when subjected to the same transformation exhibit enhanced “leaving group tendency” of their 5-substituted uracil residues and the desired 2-thiouridines 17 and 18 were isolated from the multi-components mixtures by extensive silica gel chromatography in ca. 50% and 30% yield, respectively.

Acid catalysed methanolysis led to the deprotection of the cis diol function and the esterification of 6 and 18 to produce 5-methoxycarbonylmethoxyuridine (4) and its 2-thioanalogue 4a in high yield. The nucleosides 5 and 17 were selectively deblocked to 5-[(N-trifluoroacetyl)methoxycarbonylmethyl]uridine (2) and its 2-thioanalogue 2a under treatment with 50% acetic acid. The spectral data and chromatographic mobilities of samples 2, 2a and 4, 4a were in agreement with those, recorded for the authentic specimens synthesized by other independent methods [19, 33].

### Experimental

Melting points are uncorrected. 1H NMR spectra: Tesla B.S. 487 C spectrometer, tetramethylsilane was used as the internal standard. UV spectra: Specord UV-VIS spectrometer. Electron impact mass spectra (MS) at 15 eV.: G.S.M.S. LKB 2091 instrument, abbrev. B-heterobase; s-sugar. Thin layer chromatography (t.l.c.) was performed on silica gel plates 60 F254 (Merck) using the following systems (V/V): chloroform—methanol 95:5 (A); chloroform—acetone 2:1 (D); isopropanol—ammonia—water 7:1:2 (E); chloroform—acetone—ethyl acetate 5:1:1 (F). Silica gel F60 (230–400 mesh) was used for column chromatography.

Evaporations were carried out under reduced pressure and bath temperature below 40 °C.

1. **2',3'-O-Isopropylidene-5-hydroxyuridine (8)**

A mixture of 5-hydroxyuridine (3.8 g) [24], 2,2-dimethoxypropane (10.45 g) and p-toluene sulphonic acid (0.36 g) was stirred two days at room temperature. The solution was neutralized with triethylamine to pH = 7, excess of ketal was evaporated and oily residue chromatographed on silica gel column with chloroform—methanol 97:3 (V/V) to give 8 (3.2 g, 84%) m.p. 217–219 °C (from chloroform/petroleumether) Rf = 0.39 (C).

UV (H2O): pH = 2, λ max(ε) = 283 (6780); pH = 12, λ max(ε) = 218 (12000), 307 (5400).

NMR (CD3SOCD3): δ 7.39 (s., H, H6), 5.98 (d., J = 2.2 Hz, H, H'1), 1.51, 1.53 (d., s., 6H, -(CH3)2—C—). MS: m/e 300 (4%, M+), 285 (10%, M—CH3), 173 (40%, s.).

2. **3-Carboxymethyl-5-hydroxyuridine (9)**

To the solution of 5-hydroxyuridine (260 mg, 1 mmol) in 0.5 N sodium hydroxide (2 ml) iodoacetic acid (558 mg, 3 mmol) in water (2 ml) was added dropwise and the reaction mixture was left for 16 h. The solution was acidified with 2 N hydrochloric acid and water was evaporated. The residue was precipitated by addition of ethanol, it was filtered off and washed with ice-cold ethanol to give 9 (175 mg, 54%) Rf = 0.25 (E).

NMR (CD3SOCD3): 7.70 (s., H, H6), 6.13 (d., J = 5 Hz, H, H'1), 4.2 (br.s., 2H, −CH2—N—).

UV (H2O): pH = 2, λ max = 283; pH = 12, λ max = 307.

MS: m/e 141 (3.5%, B—CO2).

3. **2',3'-O-Isopropylidene-5-ethoxycarbonylmethoxyuridine (6)**

To the solution of 8 (3 g, 10 mmol) in the mixture of 55% aqueous ethanol (50 ml) and 1 M solution of sodium hydroxide (10 ml) ethyl iodoacetate (2.35 ml, 20 mmol) was added. The reaction was left for 3 h at room temperature, the solution neutralized with acetic acid to pH = 7 and water was evaporated. The residue was dissolved in chloroform, the solution extracted with ice-cold water (2×10 ml) and dried. Chloroform was evaporated and the residue chromatographed on silica gel column with chloroform—methanol 97:3 (V/V) to give 6 (1.85 g, 84%) m.p. 132–133 °C (from methanol/hexane) Rf = 0.61 (C).

UV (H2O): pH = 2, λ max(ε) = 277 (7800); pH = 12, λ max(ε) = 214 (15200), 278 (6000).

NMR (CDCl3): δ 7.67 (s., H, H6), 5.77 (br.s., H, H'1), 4.59 (s., 2H, −CH2—O—), 4.21 (q., J = 7 Hz,
2H, -CH₂-CH₃). 1.57, 1.37 (d.s., 6H, (CH₃)₂-C-). 1.27 (t., J = 7 Hz, 3H, -CH₃).

MS: m/e 371 (8%, M-CH₃), 214 (100%, B+H), 141 (46%, B-COOCH₃).

4. 2',3'-O-Isopropylidene-5-chloromethyluridine (11)

2',3'-O-Isopropylidene-5-hydroxyuridine (8) was transformed to the title chloride 11 using the reported method [34]. Rᵥ = 0.48 (C).

NMR (CDCl₃): δ 8.18 (s., H, H₆), 5.93 (d., J = 1.4 Hz, H, H₇'), 4.95 (s., 2H, -CH₂-), 1.53, 1.33 (d.s., 6H, (CH₃)₂-C-).

The crude product (it was ca. 95% pure based on NMR spectrum) was used for the further reaction.

5. 2',3'-O-Isopropylidene-5-(N-benzyl)methoxycarbonylmethylaminomethyluridine (13)

To the stirred solution of methyl N-benzylglycinate (710 mg, 4 mmol) and triethylamine (0.55 ml, 4 mmol) in anhydrous acetonitrile (7 ml) was added. The reaction mixture was stirred for 16 h at room temperature, the precipitate filtered off, it was washed with DMF (2×5 ml) and the filtrates were combined. The solvent was evaporated, the residue dissolved in chloroform (100 ml). The solution extracted with water (2×15 ml) and dried. Chloroform was evaporated and the residue chromatographed on silica gel column with linear gradient of chloroform–methanol (99:1 to 97:3 V/V) to give 13 (1.05 g, 75%) Rᵥ = 0.49 (A).

NMR (CDCl₃): δ 8.02 (br.s., H, H₆), 5.87 (d., J = 7 Hz, H, H₇'), 4.56 (q., 2H, -CH₂-), 3.77 (s., 3H, -OCH₃), 1.53, 1.32 (d.s., 6H, (CH₃)₂-C-).

Analysis for C₂₇H₄₀N₄O₆ (475.48)
Calcd C 58.09 H 6.14 N 8.83
Found C 57.61 H 6.07 N 8.33.

6. 2',3'-O-Isopropylidene-5-[(N-trifluoroacetyl)methoxycarbonylmethylaminomethyl]uridine (5)

The nucleoside 13 (1.05 g, 2.2 mmol) was dissolved in methanol (60 ml) and hydrogenated for 5 h under 10% Pd/C catalyst (100 mg) at room temperature and under normal pressure. The catalyst was filtered off and the solvent evaporated. The residue was co-evaporated with pyridine (2×15 ml, bath temperature 27 °C), dissolved in the same solvent (40 ml) and treated with trifluoroacetic anhydride (2.5 ml) for 16 h at 4 °C. The solution was concentrated to ca. 10 ml, it was stirred for 10 min with ice-cold 5% solution of sodium hydrogen carbonate (100 ml) and the mixture extracted with chloroform (3×70 ml).

The extract was dried, the solvent evaporated and the residue was chromatographed on silica gel column with chloroform–acetone–ethyl acetate 5:1:1 (V/V) to give 5 (450 mg, 42%) Rᵥ = 0.34 (B); 0.56 (C).

NMR (CDCl₃): δ 8.02 (br.s., H, H₆), 5.87 (d., J = 7 Hz, H, H₇'), 4.55 (s., 2H, -CH₂-), 3.75 (s., 3H, -CH₃), 1.55, 1.35 (d.s., 6H, (CH₃)₂-C-).

MS: m/e 481 (2.5%, M⁺), 466 (1%, M-CH₃), 384 (4%, M-COOF₃), 309 (60%, B+H), 249 (17%, B-COOCH₃), 212 (100%, B-COOF₃), 173 (30%, s.).

7. 2',3'-O-Isopropylidene-2,5'-anhydro-5-(ethoxycarbonylmethoxy)uridine (10)

To the stirred solution of 6 (772 mg, 2 mmol), triphenylphosphine (786 mg, 3 mmol) and triethylamine in anhydrous acetonitrile (5 ml), ethyl azadicarboxylate (522 mg, 3 mmol) in anhydrous acetonitrile (4 ml) was added dropwise. After 3 h water (1 ml) was added, the solvent was evaporated and the oily residue was extracted with chloroform (2×70 ml). The extract was washed with water, dried, chloroform evaporated and the residue chromatographed on silica gel column with chloroform–acetone 4:1 (V/V) to give 10 (664 mg, 90%) Rᵥ = 0.34 (D).

UV (ethanol): λ max (e) = 262 (10,500).

NMR (CDCl₃): δ 7.36 (s., H, H₆), 5.37 (s., H, H₇'), 4.77 (s., 2H, -OCH₂-), 4.50 (q., J = 7 Hz, 2H, -CH₂CH₃), 1.41, 1.35 (d.s., 6H, (CH₃)₂-C-), 1.27 (t., J = 7 Hz, 3H, -CH₃).

MS: m/e 368 (12%, M⁺), 353 (15%, M-CH₃), 295 (100%, M-COOCH₃), 141 (6%, B-COOCH₃).

8. 2',3'-O-Isopropylidene-2,5'-anhydro-5-[(N-trifluoroacetyl)methoxycarbonylmethylaminomethyl]uridine (16)

The compound 5 (1.87 g, 3.9 mmol) was transformed to the title anhydronucleoside 16 using the same procedure as described for the preparation of 10. The mixture of products was chromatographed on silica gel column with linear gradient of chloroform–acetone (4:1 to 3:1 V/V) to give 16 in 80% yield (1.45 g) Rᵥ = 0.26 (D).

UV (ethanol): λ max (e) = 257 (9800).

NMR (CDCl₃): δ 7.73 (s., H, H₆), 5.46 (br.s., H, H₇'), 3.77 (s., 3H, -OCH₃), 1.53, 1.36 (d.s., 6H, (CH₃)₂-C-).

MS: m/e 463 (10%, M⁺), 448 (15.3%, M-CH₃), 390 (4%, M-CH₇COOCH₃), 366 (14%, M-COOF₃).
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9. 2',3'-O-Isopropylidene-5-ethoxycarbonyl-3'-methoxy-2-thiouridine (18)

A solution of the nucleoside 10 (368 mg, 1 mmol) in pyridine (10 ml) was prepared in 100 ml glass tube and cooled to -75 °C. Liquid hydrogen sulphide (10 ml) was added and the vessel closed in the steel capsule for 5 days at room temperature. After decomposition an excess of hydrogen sulphide was removed by the passage of nitrogen through the solution and the solvent was evaporated. The residue was co-evaporated consecutively with ethanol, toluene and chromatographed on silica gel column with chloroform—acetone 4:1 (V/V) to give 18 (132 mg, 83%).

UV (H2O): pH = 12, λ max = 249, 273.
NMR (CDCl3): δ 7.96 (s., H, H), 6.69 (d., J = 2 Hz, H, H'), 4.50 (s., 2H, -O-CH2-), 4.16 (q., J = 7 Hz, 2H, -CH3-CH3), 1.49, 1.29 (d.s., 6H, (CH3)2-C-), 1.22 (t., J = 7 Hz, 3H, -CH3).
MS: m/e 402 (3%, M+), 387 (5%, M-CH3), 230 (100%, B+H), 173 (12%, s.).

10. 2',3'-O-Isopropylidene-5-

The anhydronucleoside 16 (1.26 g, 2.7 mmol) was reacted with hydrogen sulphide according to the procedure described for the preparation of 18. The mixture of products was chromatographed with chloroform—acetone as the eluant (2:1 to 3:1 V/V) to give 17 (710 mg, 53%) Rf = 0.56 (C).

UV (ethanol): λ max (ε) = 278 (13,900).
NMR (CDCl3): δ 8.30 (br.s., H, H), 6.82 (d., J = 2 Hz, H, H'), 4.45 (s., 2H, -CH2-N-), 4.25 (s., 2H, -N-CH2-), 3.75 (s., 3H, -OCH3), 1.55, 1.30 (d.s., 6H, (CH3)2-C-).
MS: m/e 497 (1.5%, M+), 482 (15%, M-CH3), 365 (100%, B+41) (35), 325 (19%, B-H), 228 (11%, B-COCH3), 173 (10%, s.).

11. 5-Methoxycarbonyl-2-thiouridine (4)

The nucleoside 6 (193 mg, 0.5 mmol) was treated with 1 M solution of hydrochloride in anhydrous methanol (20 ml) for two days at room temperature. The solvent was evaporated, the residue co-evaporated with methanol (3×30 ml) and crystallized to give 4 (136 mg, 83%) m.p. 159-160 °C (from methanol—diethyl ether) Rf = 0.33 (B).

UV (H2O): pH = 2, λ max (ε) = 278 (7700); pH = 12, λ max (ε) = 217 (14,600), 279 (5800).

NMR (CD3SOCD3): δ 8.01 (s., H, H), 6.08 (d., J = 3.9 Hz, H, H'), 4.86 (s., 2H, -OCH2-), 3.9 (s., 3H, -CH3).
MS: m/e 332 (1.2%, M+), 200 (76%, B+H), 133 (10%, s.).

12. 5-Methoxycarbonyl-2-thiouridine (4a)

The nucleoside 18 (90 mg, 0.22 mmol) was transformed to 4a using the procedure described for the preparation of 4. 70 mg (89%) m.p. 165-166 °C (from methanol) Rf = 0.58 (C).

UV (H2O): pH = 2, λ max (ε) = 282 (8300); pH = 12, λ max (ε) = 249 (15,500), 272 (9800).
NMR (CD3OD): δ 8.17 (s., H, H), 6.58 (d., J = 1.5 Hz, H, H'), 4.69 (s., 2H, -OCH2-), 3.76 (s., 3H, -CH3).
MS: m/e 348 (1.5%, M+), 256 (0.7%, B+H) (35), 216 (100%, B+H).

13. 5-[(N-Trifluoroacetyl)methoxycarbonylmethylaminomethyl]uridine (2)

The nucleoside 5 (370 mg, 0.77 mmol) was refluxed with 50% acetic acid (5 ml) for 1-2 h and the solvent was evaporated. The residue was co-evaporated with methanol and crystallized to give 2 (280 mg, 75%) m.p. 186-187 °C (from ethanol) Rf = 0.35 (A); 0.56 (B).

UV (ethanol): λ max = 278.
NMR (CD3CN): δ 8.07, 7.96 (d.s., H, H), 5.82 (br.s., H, H'), 4.42 (s., 2H, -CH2-N-), 4.20 (s., 2H, -N-CH2-), 3.75 (s., 3H, -OCH3).
MS: m/e 441 (10%, M+), 345 (8.4%, M-COCH3), 309 (11%, B), 236 (14%, B-COCH3), 212 (100%, B-COCH3).

14. 5-[(N-Trifluoroacetyl)methoxycarbonylmethylaminomethyl]2-thiouridine (2a)

The nucleoside 17 (497 mg, 1 mmol) was transformed to 2a according to the procedure described for 2. 345 mg (76%) m.p. 201-202 °C (from methanol) Rf = 0.69 (B).

UV (ethanol): λ max = 228, 278; λ min = 248.
NMR (CD3COCD3): δ 8.43, 8.37 (d.s., H, H), 6.6, 6.48 (d., J = 2 Hz, 3.3 Hz, H, H'), 4.47 (br.s., 2H, -CH2-N-), 3.75 (s., 3H, -OCH3).
MS: m/e 457 (0.7%, M+), 365 (1.1%, B+41) (35).

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[30] The nucleosides 5 and 6 as well as their respective 2,5'-anhydro derivatives 16 and 10 give the same $R_f$ values in commonly used chloroform/methanol systems.


