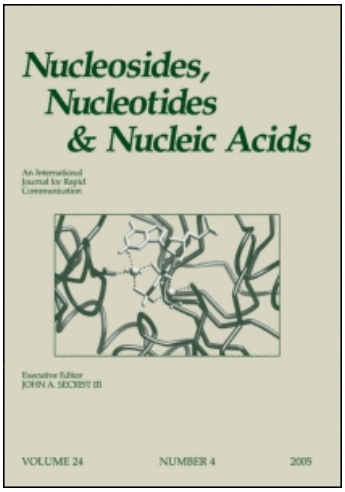

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The tRNA "WOBBLE POSITION" Uridines. III.[1] the Synthesis of 5-[S-Methoxycarbonyl (Hydroxy)Methyl] Uridine and its 2-Thio Analogue

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THE tRNA "WOBBLE POSITION" URIDINES. III.[1] THE SYNTHESIS OF 5-[S-METHOXYCARBONYL(HYDROXY)METHYL] URIDINE AND ITS 2-THIO ANALOGUE.

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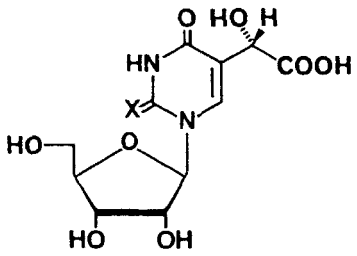
Abstract. The diastereoisomers 2a, 2b and their 2-thio analogues 4a and 4b were obtained by three-step transformation of uridine and 2-thiouridine, respectively. The absolute configuration at C-5[1] in 2a and 2b was established by CD, while for 4a and 4b the configurational assignment was based on the chemical correlation. The acids 1 and 3 were obtained by alkaline hydrolysis of 2a and 4a, respectively.

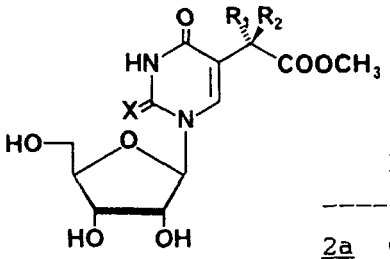
Introduction

An ability of $tRNA_1^{Gly}$ (Bombyx mori posterior silk gland) as well as $tRNA_2^{Gly}$ (of the same origin) for decoding within the glycine family codons is controversial[2,3]. Furthermore, either 5-[S-carboxy(hydroxy)methyl]uridine (1, chm^5U) or 5[S-methoxycarbonyl(hydroxy)methyl]uridine (2a, $mchm^5U$) have been identified in $tRNA_2^{Gly}$ "wobble position" (SCHEME 1)[4,5]. One can argue that at least three isoacceptor tRNAs are necessary to translate certain synonymous codons contexts on the fibroin mRNA. Indeed, in the case of specialized tissues, which produce predominantly one protein, the relationship between the frequency of synonymous codon usage and abundance of isoacceptor tRNAs has been clearly demonstrated[6,7,8]. However, the influence of the "wobble position" uridines on the mRNA-ribosome-tRNA complex binding energy and selection on this way from among competing tRNA

species remains not clear so far[8,9]. It seems that steric puckering of the modified uridines modulates dynamics of the "extended anticodon" region conformation[10-14].

SCHEME 1





1 X=O
3 X=S

	X	R_1	R_2
2a	O	OH	H
2b	O	H	OH
4a	S	OH	H
4b	S	H	OH

In continuation of our work on the preparation of models for the study of the conformation of tRNA modified units[14] and anticodon loop fragments[15] we present the synthesis of 1 and 2a as well as 3 and 4a, which can be considered as potential components of the biopolymer sequences[9].

Results

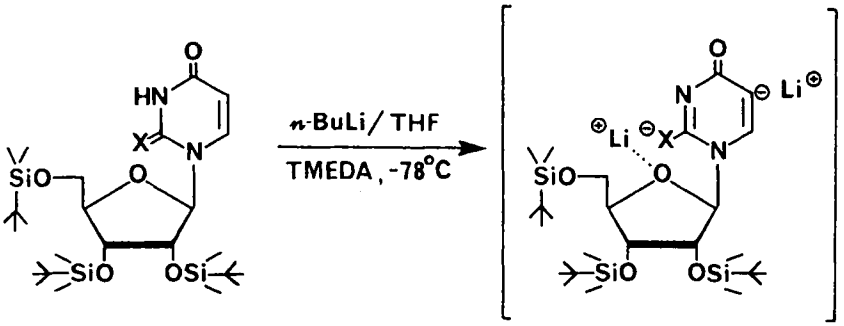
Kawakami et al.[5] have briefly reported a seven-step transformation of 5-bromo-2,4-di-*tert*-butoxypyrimidine to 1 and 2. However, neither the synthetic procedure nor spectroscopic and chromatographic data have been as yet described.

The recently reported method for the uridine regioselective 5-lithiation, controlled by *tert*-butyldimethylsilyl protection of the sugar moiety[16], allows us to simplify substantially the above mentioned approach.

Thus, treatment of 2',3',5'-tris-O-(*tert*-butyldimethylsilyl)uridine (5)[17] with n-butyl lithium in tetrahydrofuran

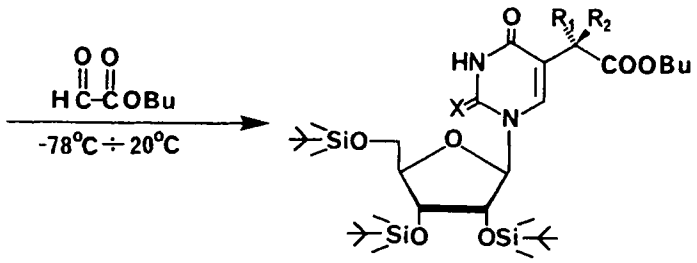
in the presence of tetramethylenediamine (TMEDA) formed dianion 7 which was then condensed at -78°C with freshly distilled n-butyl glyoxylate[18] to give 9a and 9b (SCHEME 2). Diastereoisomers 9a and 9b were separated from the reaction mixture in the ratio 2:1 by column chromatography on silica gel in ca. 30% yield.

SCHEME 2



5 X=O
6 X=S

7 X=O
8 X=S



	X	R_1	R_2
9a	O	OH	H
9b	O	H	OH
10a	S	OH	H
10b	S	H	OH

9a —(1N HCl/MeOH, RT. 24h)→ 2a

An analogous alkylation of 2',3',5'-tris-O-(*tert*-butyldimethylsilyl)-2-thiouridine (6) underwent in higher yield (40%) leading to diastereoisomers 10a and 10b in the ratio 1:1. The spectral data of both pairs of diastereoisomers 9a, 9b and 10a, 10b fully confirm the 5-substitution of persilylated uridine or 2-thiouridine with n-butoxycarbonyl (hydroxy)methyl group.

Treatment of individual diastereoisomers 9a, 9b, 10a and 10b with methanolic hydrogen chloride leads to the removal of *tert*-butyldimethylsilyl groups with simultaneous transesterification giving the title nucleosides 2a, 2b, 4a and 4b, respectively.

Separation of 2-oxo and 2-thio diastereoisomeric pairs is best accomplished with protected nucleosides for which more distinctive differences of R_f values were noticed (TABLE 1). Deprotected nucleosides 2a, 2b, 4a and 4b show significantly different HPLC retention times and can be identified by this technique (TABLE 1).

The ^1H NMR spectra of 2a, 2b, 4a and 4b have no diagnostic value for the determination of the absolute configuration at C-5[1] atom or for the identification of diastereoisomers within 2-oxo or 2-thio pairs, e.g. ^1H NMR spectrum of the equimolar mixture of 2a and 2b exhibits only minute differences of the chemical shifts for the corresponding H-6 and H-1' protons ($\Delta\delta_{H-6}$ = 6Hz, $\Delta\delta_{H-1'}$ = 1.2Hz at 300 MHz).

The absolute configuration at the asymmetric C-5[1] atom of 2a and 2b was established by comparison of their CD spectra with those of substituted S(+) methyl mandelates as well as S(+) methyl atrolactate[19,20]. Model compounds show a weak negative band at ca. 260 nm, which is assigned to the aromatic ^1L_b band, and a second, much stronger, positive band at ca. 220 nm, which is possibly due to the interaction between the carbonyl and aromatic ^1L_a transitions.

Both nucleosides 2a and 2b show CD spectra exhibiting positive Cotton effects within 260-280 nm and 195-210 nm spectral regions. CD maxima of the opposite signs (2a:$\Delta\epsilon$ =

TABLE 1. TLC and HPLC chromatographic data of the synthesized nucleosides

Nucleoside	TLC silica gel R_f (solvent system)*	TLC cellulose R_f (solvent system)*	HPLC* Retention time (min)
1	-	0.18(E)	6.62[c]
3	-	0.19(E)	8.01[b]
2a	0.13(C)	0.73(E)	3.50[a]
2b	0.11(C)	0.73(E)	5.00[a]
4a	0.34(C)	0.38(F)	10.00[a]
4b	0.27(C)	0.38(F)	16.50[a]
9a	0.11(A)	-	-
9b	0.17(A)	-	-
10a	0.32(A)	-	-
10b	0.47(A)	-	-

* Solvent systems and HPLC conditions are described in Experimental part.

+1.06, 2b:$\Delta\epsilon$ = -2.28), however, are observed for the 220 nm transition (FIGURE 1). In conclusion chirality of C-5[1] of 2a is proposed to be S. This observation is further supported by the ORD spectra of nucleosides 2a and 2b (FIGURE 1) when compared with those obtained by Kawakami et al.[5] for the analogues of 2a and 2b containing reduced side chain.

CD spectra of 2-thio derivatives 4a and 4b are almost identical with the spectrum of 2-thiouridine[21] in the 240-360 nm range, but they exhibit additional negative band at ca. 220 nm, which is "less negative" for 4a (FIGURE 1). Although it may suggest the S configuration at C-5[1] in 4a, we found a more reliable proof of the configuration of 2-thio analogues which is based on the chemical correlation. Thus, under the non-racemizing conditions (H_2O_2, pH 8.0, 20

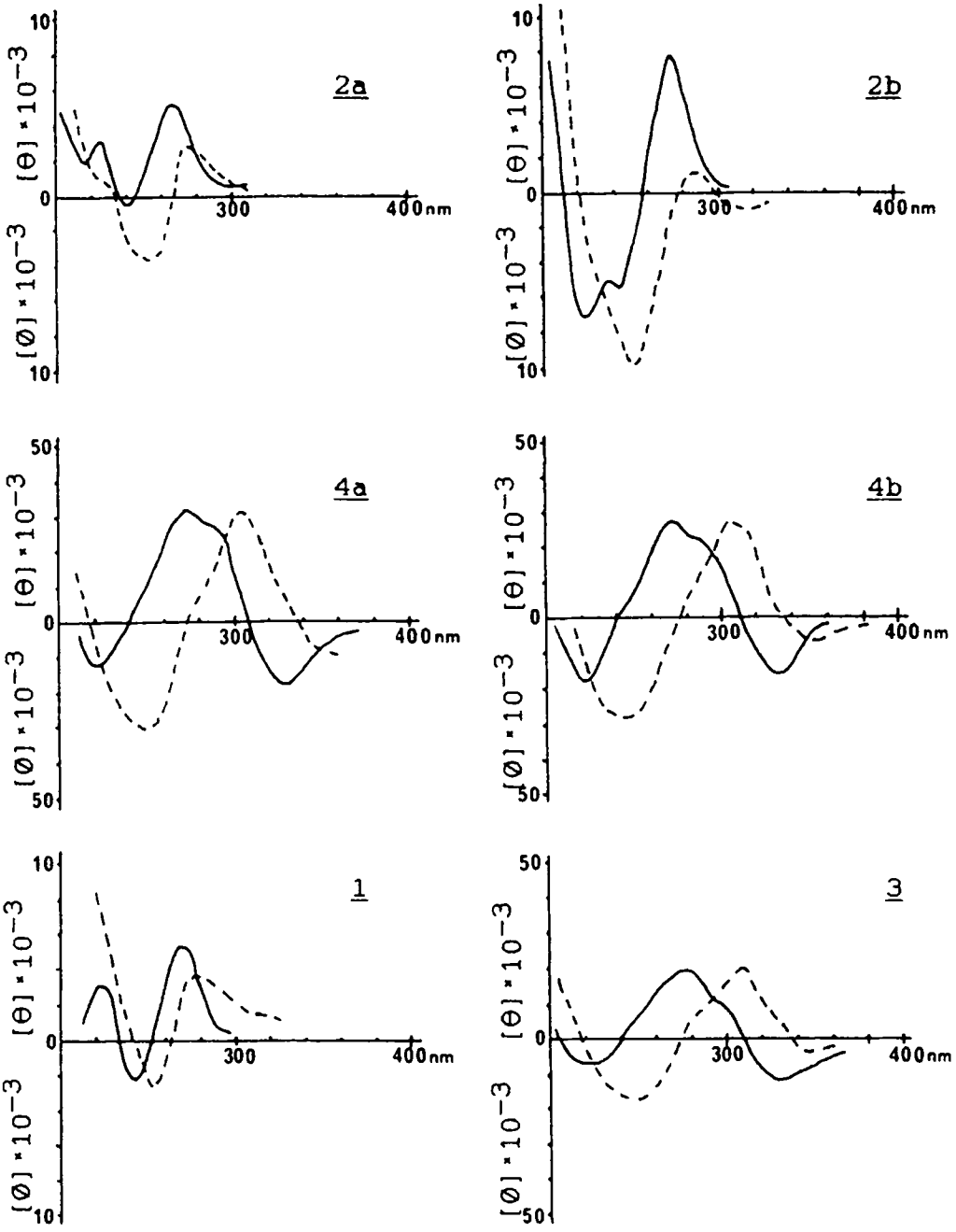
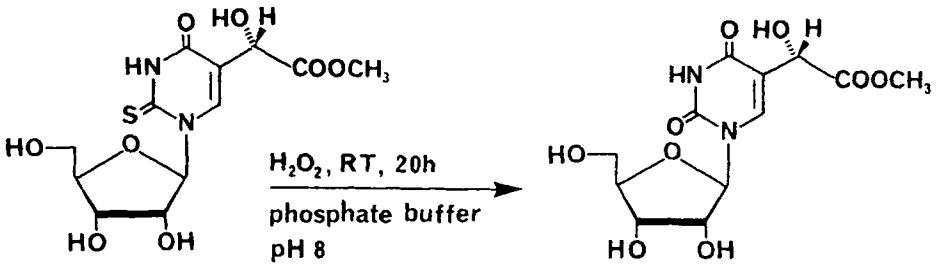


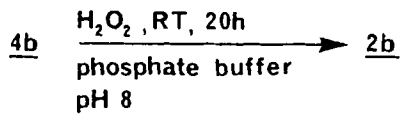
FIGURE 1. CD (————) and ORD (------) spectra of modified uridines and their 2-thio analogues.

h)[22] 4a and 4b were transformed exclusively to 2a and 2b, respectively, as it was demonstrated by the HPLC analysis of the crude products (SCHEME 3). Consequently, 2a and 4a have the S absolute configuration at C-5[1], while 2b and 4b are their C-5[1] epimers.

SCHEME 3



4a 2a



Finally, diastereoisomeric esters 2a and 4a were hydrolyzed under non-racemizing conditions[5,23] (0.01N $NaOH/H_2O$, RT, 48h) into the nucleosides 1 and 3, respectively. The purification of the free acids was accomplished by paper chromatography. Compounds 1 and 3 were characterized by ^1H NMR, CD and ORD (FIGURE 1), UV and HPLC (TABLE 1). Comparison of our spectral data for 1 with those reported earlier by Kawakami et al. for the natural product[4,5] shows their identity.

Experimental

^1H NMR spectra: TESLA BS 467 (60 MHz) - TMS was used as the internal standard and BRUKER MSL-300 (300 MHz) - TMS was

used as the external standard. Electron impact mass spectra (MS) at 70 eV: GS MS LKB 2091 instrument and field desorption mass spectra (FD MS) at 15 eV: Varian-Mat 711.

CD and ORD spectra: JASCO J-20 Automatic Recording Spectropolarimeter, room temperature, 10 mm cell.

High-performance liquid chromatography (HPLC) was carried out on Laboratorni Pristroje (Praha) equipped with a UV VIS Detector LCD 2563. Separon SGX C_{18} 5 μm glass column (150 x 3.3 mm), 2.5% acetonitrile in water, flow rates: 1 mL/min (a), 0.6 mL/min (b). LiChrosorb RP-2 (Merck) 5 μm stainless steel column (250 x 4.6 mm), 5% acetonitrile in water, flow rate 0.66 mL/min (c).

Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} (Merck) plates in the solvent systems (v/v): A: chloroform/methanol 97/3, B: ethyl ether/hexane 2/1, C: chloroform/methanol 85/15 or on cellulose F (Merck) plates: E: isopropanol/water 7/3, F: n-butanol/water (saturated solution).

Silica gel F_{60} (230-400 mesh) (Merck) was used for column chromatography and 3MM Whatman paper was applied for paper chromatography.

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

2',3',5'-tris-O-(tert-butyldimethylsilyl)-2-thiouridine (6)

To the solution of 2-thiouridine (2 mmol, 520 mg) and imidazole (28 mmol, 1.90 g) in anhydrous DMF (20 mL) tert-butyldimethylsilyl chloride (14 mmol, 2.11 g) was added. The reaction was carried out at room temperature for 24 h and the solvent was evaporated under reduced pressure. The residue was co-evaporated with n-butanol, toluene and purified on silica gel short column (solvent system B) to give 6 as a foam (1.08 g, 90% yield). R_f = 0.82 (B); ^1H NMR $CDCl_3$ (δ, ppm): 11.23 (br s., 1H, NH), 8.36 (d, $J_{5,6}$ = 8 Hz, 1H, H-6), 6.35 (d, $J_{1',2'}$ = 2 Hz, 1H, H-1'), 5.93 (d, $J_{5,6}$ = 8 Hz, 1H, H-5), 4.23-3.56 (m, 5H, H-2',3',4',5',5"), 0.90 (m, 45H, TBDMS-protons); MS m/z (%): 587 (1.64, M^+·-15), 545 (100.00, M^+·-57), 474 (22.53), 261 (21.83), 185 (32.62).

5-[n-butoxycarbonyl(hydroxy)methyl]-2',3',5'-tris-O-(*tert*-butyldimethylsilyl)uridine (9a) and (9b)

To the solution of 2',3',5'-tris-O-(*tert*-butyldimethylsilyl)uridine (5)[17] (1 mmol, 586.2 mg) and TMEDA (2.5 mmol, 377 μL) in anhydrous THF (10 mL) cooled down to -78°C n-butyl lithium in hexane (2.5 mmol) was added (argon atmosphere). The solution was stirred for 1.5 h and freshly distilled n-butyl glyoxylate[18] (2 mmol, 240μL) in THF (2 mL) was added at -78°C. The mixture was stirred for 1 h at -78°C, then warmed to room temperature and kept overnight. Acetic acid (2.5 mmol, 142.5 μL) was added and the solvent removed under reduced pressure. The residue was dissolved in chloroform (20 mL), washed with cold 2% sodium bicarbonate and dried ($MgSO_4$). After evaporation of solvent the residue was chromatographed on the silica gel column (gradient of chloroform/methanol 100% to 99% v/v) to give two diastereoisomers 9a and 9b as foams. 9a (150 mg, 21.6% yield): R_f = 0.11 (A), ^1H NMR $CDCl_3$ (δ, ppm): 9.30 (br s, 1H, NH), 7.66 (s, 1H, H-6), 5.93 (d, $J_{1',2'}$ = 5 Hz, 1H, H-1'), 4.61 (s, 1H, H-C-5[1]), 4.20-3.56 (m, 7H, H-2',3',4',5',5", CH_2OCO), 1.60-1.03 (m, 7H, $CH_2CH_2CH_3$), 0.76 (m, 45H, TBDMS-protons); 9b (76 mg, 10.6% yield): R_f = 0.17 (A), ^1H NMR $CDCl_3$ (δ, ppm): 7.85 (s, 1H, H-6), 6.03 (d, $J_{1',2'}$ = 5 Hz, 1H, H-1'), 4.81 (s, 1H, H-C-5[1]), 4.35-3.76 (m, 7H, H-2',3',4',5',5", CH_2OCO), 1.65-1.08 (m, 7H, $CH_2CH_2CH_3$), 0.90 (m, 45H, TBDMS-protons).

5-[n-butoxycarbonyl(hydroxy)methyl]-2',3',5'-tris-O-(*tert*-butyldimethylsilyl)-2-thiouridine (10a) and (10b)

Following the procedure for the synthesis of 9a and 9b 2',3',5'-tris-O-(*tert*-butyldimethylsilyl)-2-thiouridine (6) (1 mmol, 602 mg) was transformed to the nucleosides 10a and 10b. 10a (155 mg, 21% yield): R_f = 0.32 (A), ^1H NMR $CDCl_3$ (δ, ppm): 10.06 (br s, 1H, NH), 7.90 (s, 1H, H-6), 6.86 (d, $J_{1',2'}$ = 5 Hz, 1H, H-1'), 4.73 (br s, 1H, H-C-5[1]), 4.30-3.53 (m, 7H, H-2',3',4',5',5", CH_2OCO), 1.63-1.03 (m, 7H, $CH_2CH_2CH_3$), 0.86 (br s, 45H, TBDMS-protons); 10b (131 mg,

18% yield) R_f = 0.47 (A), ^1H NMR $CDCl_3$ (δ, ppm): 9.70 (br s, 1H, NH), 7.93 (s, 1H, H-6), 6.73 (d, $J_{1',2'}$ = 5 Hz, 1H, H-1'), 4.70 (br s, 1H, H-C-5[1]), 4.26-3.50 (m, 7H, H-2',3',4',5',5", CH_2OCO), 1.58-1.00 (m, 7H, $CH_2CH_2CH_3$), 0.81 (br s, 45H, TBDMS-protons).

5-[S-methoxycarbonyl(hydroxy)methyl]uridine (2a)

The persilylated ester 9a (0.238 mmol, 171 mg) was dissolved in 1N HCl/MeOH (3 mL) and kept overnight at room temperature. The solvent was evaporated and the residue several times co-evaporated with methanol. Crude product was finally purified on Whatman 3MM paper (solvent system E) to give 2a (75 mg, 95% yield) as a foam. R_f = 0.13 (C), R_f = 0.73 (E), ^1H NMR CD_3OD (δ, ppm): 8.13 (s, 1H, H-6), 5.92 (d, $J_{1',2'}$ = 4.6 Hz, 1H, H-1'), 4.98 (s, 1H, H-C-5[1]), 4.22-4.14 (m, 2H, H-2',3'), 4.02 (dt, $J_{4',3'}$ = 4.3 Hz, $J_{4',5'}$ = 2.9 Hz, 1H, H-4'), 3.85 (dd, $J_{4',5'}$ = 2.9 Hz, $J_{5',5"}$ = 12.2 Hz, 1H, H-5'), 3.75 (dd, $J_{4',5"}$ = 3.0 Hz, $J_{5',5"}$ = 12.2 Hz, 1H, H-5"), 3.73 (s, 3H, CH_3); UV (nm) pH 7 (H_2O) λ_{max} = 267 (ϵ = 6000), λ_{min} = 234 (ϵ = 630), pH 12 (NaOH) λ_{max} = 267; HPLC T_{ret} = 3.5 min (a); FD MS m/z: 273 ($M^+ \cdot$-59).

5-[R-methoxycarbonyl(hydroxy)methyl]uridine (2b)

The nucleoside 9b (0.15 mmol, 109 mg) was treated with 1N HCl/MeOH (2.5 mL) according to procedure described for 2a, to give 2b (48 mg, 95% yield). R_f = 0.11 (C), R_f = 0.73 (E), ^1H NMR CD_3OD (δ, ppm): 8.06 (s, 1H, H-6), 5.81 (d, $J_{1',2'}$ = 4.[2] Hz, 1H, H-1'), 4.87 (s, 1H, H-C-5[1]), 4.12-4.04 (m, 2H, H-2',3'), 3.92 (dt, $J_{4',5'}$ = 2.9 Hz, $J_{3',4'}$ = 4.1 Hz, 1H, H-4'), 3.76 (dd, $J_{4',5'}$ = 2.7 Hz, $J_{5',5"}$ = 12.1 Hz, 1H, H-5'), 3.64 (dd, $J_{4',5"}$ = 3.0 Hz, $J_{5',5"}$ = 12.1 Hz, 1H, H-5"), 3.63 (s, 3H, CH_3); UV (nm) pH 7 (H_2O): λ_{max} = 266 (ϵ = 5200), λ_{min} = 235 (ϵ = 570), pH 12 (NaOH): λ_{max} = 266; HPLC T_{ret} = 5.0 min (a); FD MS m/z: 333.3 ($M^+ \cdot$+1).

5-[S-methoxycarbonyl(hydroxy)methyl]-2-thiouridine (4a)

The compound 10a (0.34 mmol, 250 mg) was transformed to the title nucleoside 4a using the same procedure as

described for the preparation of 2a. 4a (111 mg, 94% yield): R_f = 0.34 (C), R_f = 0.38 (F), ^{1}H NMR CD_3OD (δ, ppm): 8.66 (s, 1H, H-6), 6.79 (d, $J_{1',2'}$ = 2.5 Hz, 1H, H-1'), 5.15 (s, 1H, H-C-5[1]), 4.41 (dd, $J_{1',2'}$ = 2.5 Hz, $J_{2',3'}$ = 4.8 Hz, 1H, H-2'), 4.33 (dd, $J_{2',3'}$ = 4.8 Hz, $J_{3',4'}$ = 7.0 Hz, 1H, H-3'), 4.26 (dt, $J_{3',4'}$ = 7.0 Hz, $J_{4',5'}$ = 2.4 Hz, 1H, H-4'), 4.14 (dd, $J_{4',5'}$ = 2.4 Hz, $J_{5',5''}$ = 12.4 Hz, 1H, H-5'), 3.98 (dd, $J_{4',5''}$ = 2.61 Hz, $J_{5',5''}$ = 12.4 Hz, 1H, H-5''), 3.92 (s, 3H, CH_3), UV (nm) pH 7 (H_2O): λ_{max} = 282 (ϵ = 10250), pH 12 (NaOH): λ_{max} = 242, λ_{max} = 276, λ_{min} = 260, A_{242}/A_{260} = 1.38, A_{276}/A_{260} = 1.19, HPLC T_{ret} = 10.0 min (a), FD MS m/z 349.0 ($M^+ \cdot$+1).

5-[R-methoxycarbonyl(hydroxy)methyl]-2-thiouridine (4b)

The persilylated ester 10b (0.27 mmol, 196 mg) was transformed to 4b according to the procedure described for 2a. 4b (88 mg, 94% yield): R_f = 0.27 (C), R_f = 0.38 (F), ^{1}H NMR CD_3OD (δ, ppm): 8.67 (br s, 1H, H-6), 6.78 (d, $J_{1',2'}$ = 2.4 Hz, 1H, H-1'), 5.16 (s, 1H, H-C-5[1]), 4.40 (dd, $J_{1',2'}$ = 2.4 Hz, $J_{2',3'}$ = 4.8 Hz, 1H, H-2'), 4.33 (dd, $J_{3',2'}$ = 4.8 Hz, $J_{3',4'}$ = 7.1 Hz, 1H, H-3'), 4.26 (dt, $J_{3',4'}$ = 7.1 Hz, $J_{4',5'}$ = 2.4 Hz, 1H, H-4'), 4.16 (dd, $J_{4',5'}$ = 2.3 Hz, $J_{5',5''}$ = 12.4 Hz, 1H, H-5'), 3.98 (dd, $J_{4',5''}$ = 2.4 Hz, $J_{5',5''}$ = 12.4 Hz, 1H, H-5''), 3.93 (s, 3H, CH_3), UV (nm) pH 7 (H_2O): λ_{max} = 280 (ϵ = 9200), pH 12 (NaOH): λ_{max} = 243, λ_{max} = 276, λ_{min} = 260, A_{243}/A_{260} = 1.36, A_{276}/A_{260} = 1.20, HPLC T_{ret} = 16.5 min (a), FD MS m/z 349.0 ($M^+ \cdot$+1).

5-[S-carboxy(hydroxy)methyl]uridine (1)

Nucleoside 2a (0.06 mmol, 20 mg) was dissolved in 0.01N NaOH/H_2O (10 mL) and kept 48 h at room temperature. The reaction mixture was acidified with 0.1 N HCl to pH 2 and concentrated under reduced pressure. The residue was purified by paper chromatography (Whatman 3MM, solvent system E) to give 12 mg of 1 (60% yield) as a foam. R_f = 0.18 (E), ^{1}H NMR CD_3OD (δ, ppm): 8.13 (s, 1H, H-6), 5.92 (d,

$J_{1',2'}$ = 4.5 Hz, 1H, H-1'), 5.02 (H-C-5^1 signal covered by H_2O signal), 4.22-4.14 (m, 2H, H-2',3'), 4.02 (dt, $J_{4',5'}$ = 2.8 Hz, $J_{3',4'}$ = 4.3 Hz, 1H, H-4'), 3.85 (dd, $J_{4',5''}$ = 2.8 Hz, $J_{5',5''}$ = 12.2 Hz, 1H, H-5'), 3.74 (dd, $J_{4',5''}$ = 3.2 Hz, $J_{5',5''}$ = 12.2 Hz, 1H, H-5''). UV (nm) pH 7 (H_2O): λ_{max} =260 (ϵ = 6000), λ_{min} = 236 (ϵ = 850), HPLC T_{ret} = 6.62 min (c).

5-[S-carboxy(hydroxy)methyl]-2-thiouridine (3)

The nucleoside 4a (0.057 mmol, 20 mg) was transformed to 3 according to the procedure described for the preparation of 1. 15 mg (79% yield); R_f = 0.19 (E), ^1H NMR CD_3OD (δ, ppm): 8.48 (s, 1H, H-6), 6.60 (d, $J_{1',2'}$ = 2.5 Hz 1H, H-1'), 5.00 (H-C-5^1 signal covered by H_2O signal), 4.22 (dd, $J_{1',2'}$ = 2.5 Hz, $J_{2',3'}$ = 4.8 Hz, 1H, H-2'), 4.14 (dd, $J_{2',3'}$ = 4.8 Hz, $J_{3',4'}$ = 7.0 Hz, 1H, H-3'), 4.07 (dt, $J_{3',4'}$ = 7.0 Hz, $J_{4',5''}$ = 2.2 Hz, 1H, H-4'), 3.96 (dd, $J_{4',5'}$ = 2.2 Hz, $J_{5',5''}$ = 12.4 Hz, 1H, H-5'), 3.80 (dd, $J_{4',5''}$ = 2.6 Hz, $J_{5',5''}$ = 12.4 Hz, 1H, H-5''). UV (nm) pH 7 (H_2O): λ_{max} = 221 (ϵ = 7300) λ_{max} = 278 (ϵ = 7600), λ_{min} = 246 (ϵ = 2840), pH 12 (NaOH): λ_{max} = 242, λ_{max} = 273, λ_{min} = 260, A_{242}/A_{260} = 1.28 A_{273}/A_{260} = 1.11, HPLC T_{ret} = 8,01 min (b).

Transformation of 4a to 2a and 4b to 2b

To 4a (3.6 mg) dissolved in phosphate buffer (0.05 M sodium phosphate, pH 8.0, 191 μL) H_2O_2 (30% solution, 2.27 μL) was added. The reaction was completed after 20 h. The crude reaction mixture was co-chromatographed (HPLC) with authentic samples of 2a, 2b and 1. The ester 2a was identified as the sole product of the $S^2 \longrightarrow O^2$ transformation at the substrate C_2 atom. An analogous reaction of 4b leads to the formation of 2b as a sole product of the substrate $C_{(2)}S \longrightarrow C_{(2)}O$ transformation.

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23. Under described conditions ester 2a hydrolyzes exclusively to acid 1 (T_{Ret} = 6.62 min[c]), whereas ester 2b gaves only 5-[R-carboxy(hydroxy)methyl]uridine (T_{Ret} = 6.02 min[c]).

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