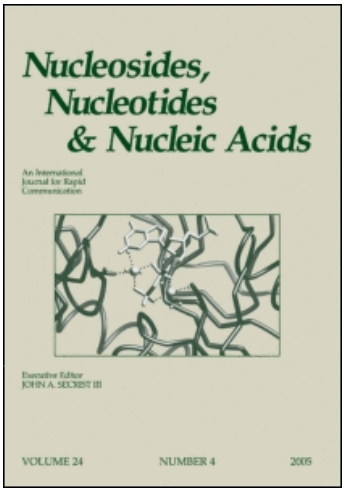

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The tRNA "Wobble Position" Uridines. IV. The Synthesis of 2'-O-Methyl-5-methoxycarbonylmethyluridine and its Derivatives

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THE tRNA "WOBBLE POSITION" URIDINES. IV[1] THE SYNTHESIS OF 2'-O-METHYL-5-METHOXYCARBONYLMETHYLURIDINE AND ITS DERIVATIVES

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Abstract. 2'-O-Methyl-5-methoxycarbonylmethyluridine (1) was synthesized via N^3,5',3'-O-protected intermediate 6. Nucleoside 1 was transformed to the next "wobble uridines", 2 and 3, by hydrolysis and ammonolysis, respectively.

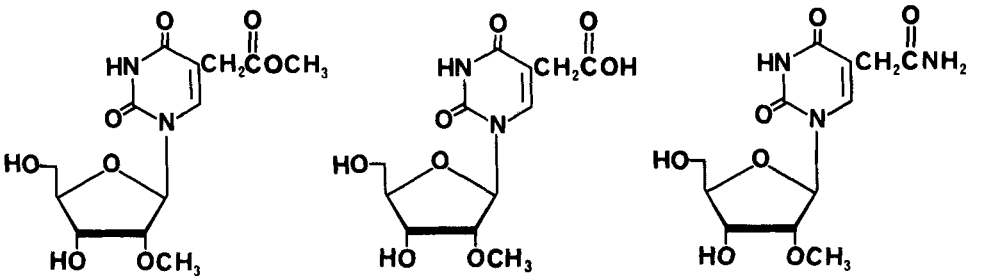
Introduction

The pyrimidine nucleosides modified with the 2-thio or 2'-O-CH_3 group stabilize the secondary structure of polynucleotides[2-6], as well as helix formed between complementary oligomers[4-6]. This stabilization has been ascribed to the shift towards ^3E (gg) anti conformers in conformational equilibrium of such modified units[6,7].

It is noteworthy that analogous arguments were used to explain restrictive pairing between several tRNA "wobble position" uridines ($^*U_{34}$) and the third codon letter[6-10]. In this context, the postranscriptional U_{34} 2'-O-methylation could be considered as a substitute of U_{34} 2-thiation, which has been selected by a cell to functionalize tRNA gene transcript. However, methylation seems to be less dependent on the conformation of the biopolymer[11].

The modified uridines 1, 2, 3 (SCHEME 1) have been reported as tRNA's sequence components[6], although one cannot rule out that the last two derivatives are artifacts produced during biopolymers isolation or sequencing processes. The series of nucleosides derived from

5-methoxycarbonylmethyluridine structure[1] represent models suitable to study an influence of heterobase and sugar moieties modifications on conformation of the modified uridines[10] and tRNAs anticodon loop fragments[12-14].



SCHEME 1

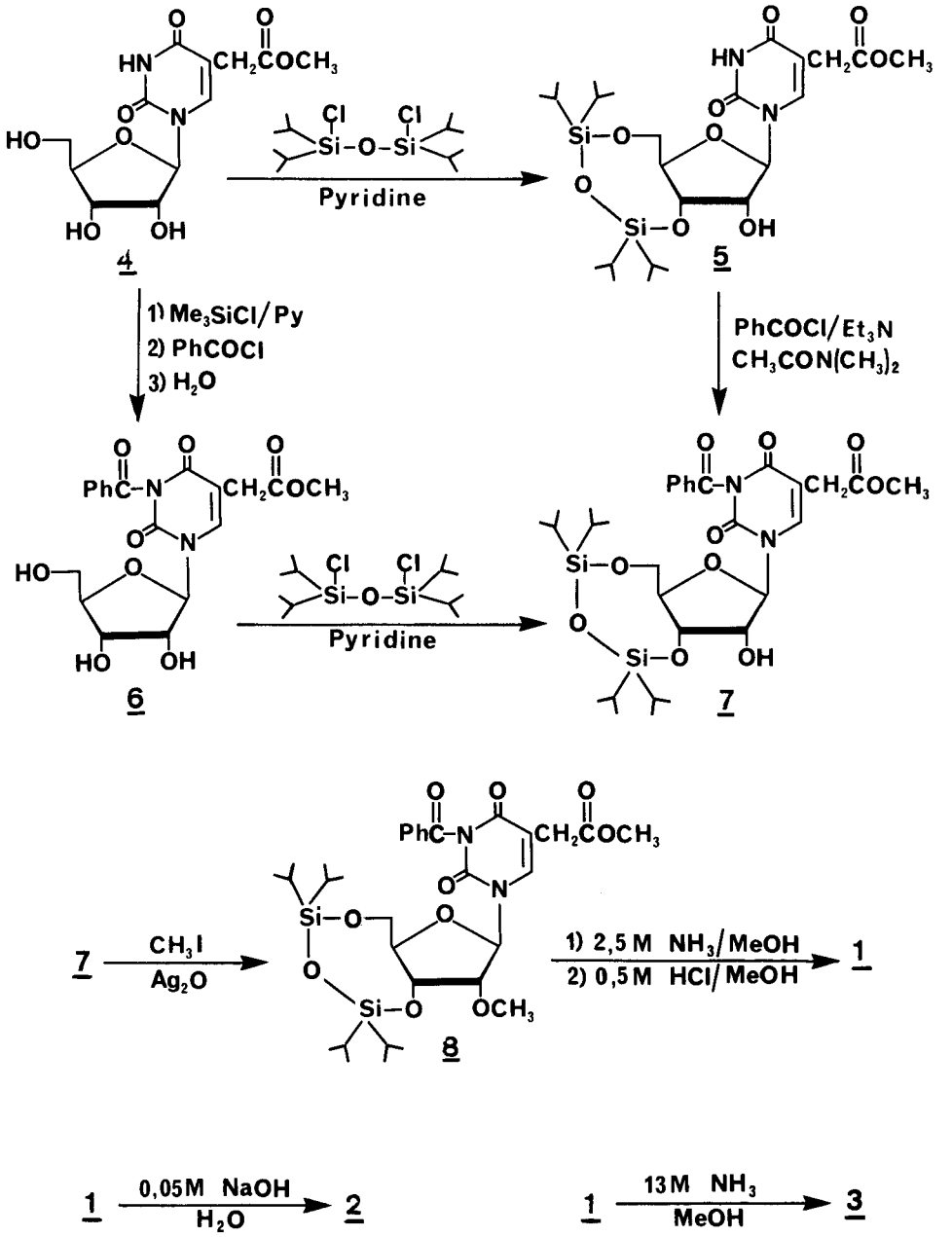
We present here the synthesis of the title compounds 1-3. Our synthetic method enables also their efficient ^{13}C-labelling at the 2'-OCH_3 function.

Results and discussion

The readily available 5-methoxycarbonylmethyluridine (4)[10] was used as a starting material. Efficient regioselective methylation of uridine diol function with methyl iodide or diazomethane has not been reported so far[15,16]. The alkylation of 5',3'-O-tetraisopropyldisiloxane-1,3-diyluridine activated at 2'-OH group[15] or N^3-protected[4,16,19] seems to be the method of choice.

Due to rather low stability of methoxycarbonylmethyl function under alkaline conditions, the methoxyethoxymethyl (MEM) group[17] removable by triphenylmethyl fluoroborate, was tested for N^3-protection of 4. However, in the test experiment we were unable to deblock N^3-MEM-U according to the reported procedure[17].

Thus, N^3-benzoyl protected derivative 7 was synthesized as the target intermediate in our approach (SCHEME 2). The reaction of 4 with Markiewicz reagent[18] afforded 5',3'-



SCHEME 2

cyclic silyl ether derivative 5 in 88% yield. Treatment of 5 with benzoyl chloride in the presence of triethylamine in N,N-dimethylacetamide solution[4] gave the required compound 7 in 56% yield only due to the formation of dibenzoylated (N^3,02') by-product.

Alternatively, 5-methoxycarbonylmethyluridine (4) was sequentially treated with trimethylchlorosilane and benzoyl chloride in anhydrous pyridine followed by the mild hydrolysis to remove trimethylsilyl groups. Standard work-up and purification of crude product by column chromatography gave N^3-benzoylated nucleoside 6 in 83% yield. Compound 6 can be almost quantitatively converted to N^3,5',3'-O-protected derivative 7 by treatment with 1,1,3,3-tetraisopropyl-1,3-dichlorosilane under standard conditions[18].

Nucleoside 7 was methylated with an excess of methyl iodide (15 equiv.) in the presence of silver oxide (50 equiv.) in dry acetone at room temperature or with methyl iodide (25 equiv.) and small excess of silver oxide (3 equiv.) in benzene at 40°C for 6 hours. The second variant of methylation was more efficient and allows us to obtain 8 in 85% yield.

Debenzoylation of 8 under controlled conditions, warranting the stability of methyl ester function (2.5 M NH_3/MeOH), followed by the removal of silyl protection group with 0.5 M HCl/MeOH, afforded 2'-O-methyl-5-methoxycarbonylmethyluridine 1 in 79% yield.

Nucleoside 1 can serve as a useful precursor for two other 2'-O-methylated modified nucleosides: acid 2 and amide 3. Thus, mild alkaline hydrolysis of 1 with 0.05 M NaOH/H_2O for 2 hours gave, after neutralization and crystallization, nucleoside 2 in 86.5% yield (mp. 122-123°C from methanol). Prolonged treatment of 1 with 13 M NH_3/MeOH (3 days, room temperature) afforded quantitatively amide 3 in crystalline form (mp. 219-220°C from methanol)[20].

Structures of all compounds described in this paper were confirmed by ^1H NMR, UV and MS spectra.

Experimental

Melting points are uncorrected. ^{1}H NMR spectra: Tesla BS 487 (60 MHz), TMS as internal standard; Bruker MSL 300 (300 MHz) spectrometer, TMS and DSS as external standards. UV spectra: Specord UV-VIS spectrometer. Electron impact mass spectra (MS) at 70 eV: GCMS LKB-2091 instrument, abbrev. B - heterobase, s - sugar moiety. Thin layer chromatography (tlc) was performed on silica gel plates 60 F_{254} (Merck) using the following systems (v/v): chloroform-methanol 95:5 (A); chloroform-methanol 9:1 (B); chloroform-methanol 85:15 (C); chloroform-methanol 8:2 (D); isopropanol-water 7:3 (E). Silica gel 60 F (230-400 mesh) was used for column chromatography. Evaporations were carried out under reduced pressure and bath temperature below 40°C.

1. 5',3'-(Tetraisopropyldisiloxane-1,3-diyl)-5-methoxycarbonylmethyluridine (5)

To the solution of 5-methoxycarbonylmethyluridine (4) (949 mg, 3 mmol) in dry pyridine (15 ml) 1,1,3,3-tetraisopropyl-1,3-dichlorosiloxane (1.1 g, 3.45 mmol) was added. After 3 h at 20°C, tlc (B) showed a complete conversion to a product (higher R_f). Water (5 ml) was added and the reaction mixture was extracted with chloroform (3 x 20 ml). The organic layers were pooled, dried ($MgSO_4$) and evaporated to dryness. The residue was co-evaporated with toluene (3 x 20 ml), to remove traces of pyridine and the product 5 (1.47 g) was isolated in 88% yield by silica gel chromatography (chloroform:methanol 95:5 as eluent). R_f = 0.59 (A); 0.80 (B). ^{1}H NMR ($CDCl_3$/TMS) δ ppm: 7.9 (s, 1H, H6), 5.7 (s, 1H, H1'), 4.4-3.9 (m, 5H, sugar protons), 3.7 (s, 3H, $COOCH_3$), 3.4 (s, 2H, CH_2COO), 1.1 (bs, 28H, isopropyl protons).

2. N^3-benzoyl-5-methoxycarbonylmethyluridine (6)

To 949 mg (3 mmol) of 5-methoxycarbonylmethyluridine (4) dried by coevaporation with pyridine and redissolved in the same solvent (15 ml), 2.6 ml (30 mmol) of trimethylchlorosilane was added. After stirring 2 h at

ambient temperature the mixture was cooled to 0°C and 1.05 ml (9 mmol) of benzoyl chloride was slowly dropped. The mixture was allowed to warm to 25°C and stirred additionally for 5 h. The reaction was terminated by the addition of water (5 ml) at 0°C. After stirring for further 3 h at room temperature the mixture was extracted with chloroform (5 x 20 ml). The organic layers were pooled, dried ($MgSO_4$) and concentrated to dryness. The residue was chromatographed on silica gel column (chloroform:methanol 9:1) to give 1.05 g (83% yield) of 6 as a foam. R_f = 0.11 (A); 0.30 (B).
^1H NMR (CD_3OD/TMS) δ ppm: 8.2-7.3 (m, 6H, H6 and benzoyl protons), 6.9 (d, 1H, $J_{1',2'}$ = 4Hz, H1'), 4.3-3.1 (m, 5H, H2', H3', H4', H5', H5"), 3.7 (s, 3H, $COOCH_3$), 3.4 (s, 2H, CH_2COO).

3. N^3-benzoyl-5',3'-(tetraisopropyldisiloxane-1,3-diyl)-5-methoxycarbonylmethyluridine (7)

a) Benzoyl chloride (0.3 ml, 2.5 mmol) was added to the stirred solution of 5 (1.3 g, 2.3 mmol) in N,N-dimethylacetamide (10 ml) and triethylamine (0.4 ml, 2.9 mmol). After 3 h the solution was concentrated in vacuo and partitioned between ethyl acetate and water. The organic layer was washed with water, dried ($MgSO_4$) and evaporated to dryness. Chromatography of the residue on the column of silica gel (chloroform:methanol 98:2 as an eluent) gave 0.85 g (56% yield) of 7 as a foam.

b) To the stirred solution of 6 (1.05 g, 2.5 mmol) in anhydrous pyridine (15 ml) 1,1,3,3-tetraisopropyl-1,3-dichlorosiloxane (0.93 g, 2.9 mmol) was added. The stirring was continued for 3 h at room temperature and then the reaction was terminated by the addition of water. The mixture was extracted with chloroform (3 x 50 ml); the organic layers were combined, dried ($MgSO_4$) and evaporated to dryness in vacuo. The residue was coevaporated three times with toluene to remove traces of pyridine and chromatographed on silica gel column (chloroform:methanol 98:2) to give 1.45 g (88% yield) of 7. R_f = 0.91 (A); 0.94 (B).

^{1}H NMR ($CDCl_3$/TMS) δ ppm: 7.94-7.46 (m, 6H, H6 and benzoyl protons), 5.73 (d, 1H, $J_{1',2'}$ = 1.02 Hz, H1'), 4.42 (dd, 1H, $J_{3',2'}$ = 5.23 Hz, $J_{3',4'}$ = 8.52 Hz, H3'), 4.25 (d, 1H, $J_{2',3'}$= 5.23 Hz, H2'), 4.18 (dd, 1H, $J_{5',5''}$ = 12.74 Hz, $J_{5',4'}$ = 2.74 Hz, H5'), 4.08 (dt, 1H, $J_{4',3'}$ = 8.25 Hz, $J_{4',5'}$ = 2.74 Hz, $J_{4',5''}$ = 2.94 Hz, H4'), 4.02 (dd, 1H, $J_{5'',5'}$ = 12.74 Hz, $J_{5'',4'}$ = 2.94 Hz, H5''), 3.69 (s, 3H, $COOCH_3$), 3.35 (AB system, 2H, CH_2COO), 2.91 (d, 1H, J = 1.5 Hz, 2'-OH), 1.1-0.99 (m, 28H, isopropyl protons).

4. N^3-Benzoyl-5',3'-(tetraisopropyldisiloxane-1,3-diyl)-2'--O-methyl-5-methoxycarbonylmethyluridine (8)

To the solution of 7 (1.33 g, 2 mmol) in anhydrous benzene (15 ml) methyl iodide (3.1 ml, 50 mmol) and freshly precipitated silver oxide (1.4 g, 6 mmol) were added. The reaction mixture was stirred at 40°C for 6 h, then filtered through the short layer (1 cm) of celite. The filtrate was concentrated in vacuo and the residue chromatographed on a column of silica gel (chloroform:methanol 99:1) to give 1.15 g of 8 (85% yield) as a white foam. R_f = 0.96 (A); 0.98 (B). ^{1}H NMR ($CDCl_3$/TMS) δ ppm: 7.88-7.42 (m, 6H, H6 and benzoyl protons), 5.67 (s, 1H, H1'), 4.21-3.72 (m, 5H, H2', H3', H4', H5', H5''), 3.61 (s, 3H, $COOCH_3$), 3.53 (s, 3H, 2'-OCH_3), 3.30 (AB system, 2H, CH_2), 1.05-0.9 (m, 28H, isopropyl protons).

5. 2'-O-Methyl-5-methoxycarbonylmethyluridine (1)

The solution of 8 (677 mg, 1 mmol) in 2.5 M NH_3/MeOH (10 ml) was stirred at room temperature for 2 h. The reaction mixture was concentrated to dryness, coevaporated three times with methanol and the residue was dissolved in 0.5 M HCl/MeOH (10 ml). After being stirred overnight at room temperature the solvent was removed in vacuo. The residue was coevaporated several times with methanol and then chromatographed on a column of silica gel using linear gradient of chloroform:methanol (98:2 to 90:10) to give 260 mg of 1 (79% yield). R_f = 0.33 (C); 0.48 (D); 0.71 (E).

^{1}H NMR (CD_3OD/TMS) δ ppm: 8.07 (s, 1H, H6), 5.95 (d, 1H, $J_{1',2'}$ = 3.49 Hz, H1'), 4.53 (dd, 1H, $J_{3',2'}$ = 5.21 Hz, $J_{3',4'}$ = 6.17 Hz, H3'), 3.97 (dt, 1H, $J_{4',3'}$ = 6.17 Hz, $J_{4',5'}$ = 2.70 Hz, $J_{4',5''}$ = 2.90 Hz, H4'), 3.89 (dd, 1H, $J_{5',5''}$ = 12.40 Hz, $J_{5',4'}$ = 2.70, H5'), 3.85 (dd, 1H, $J_{2',1'}$ = 3.49 Hz, $J_{2',3'}$ = 5.21 Hz, H2'), 3.75 (dd, 1H, $J_{5'',5'}$ = = 12.40 Hz, $J_{5'',4'}$ = 2.90 Hz, H5''), 3.69 (s, 3H, $COOCH_3$), 3.52 (s, 3H, OCH_3), 3.30 (s, 2H, CH_2).
UV (EtOH) λ (nm), (ϵ) : λ_{max} = 210 (11500), λ_{min} = 235 (2700), λ_{max} = 266 (11300).
MS 70 eV, m/z (rel. int.): 330 (3.1), $M^{+}\cdot$; 185 (63.9), B+2H; 184 (33.6), B+H; 147 (35.2), s.

6. 2'-O-Methyl-5-carboxymethyluridine (2)

The nucleoside 1 (100 mg, 0.3 mmol) was dissolved in 0.05 M NaOH/H_2O. After 2 h at room temperature tlc (D) showed a complete hydrolysis of ester. The solution was neutralized with Dowex-50W X-4 (H^+ form), the resin was filtered off, washed with methanol and water. The combined filtrates were evaporated to dryness and the residue was crystallized from methanol to give 82 mg of 2 (86.5% yield) - mp. 122-123°C. R_f = 0.16 (D); 0.63 (E).
^{1}H NMR (D_2O/DSS)) δ ppm: 7.92 (s, 1H, H6), 5.99 (d, 1H, $J_{1',2'}$ = 3.79 Hz, H1'), 4.35 (dd, 1H, $J_{2',3'}$ = 5.35 Hz, $J_{3',4'}$ = 6.33 Hz, H3'), 4.10 (dt, 1H, $J_{4',5'}$ = 2.78 Hz, $J_{4',5''}$ = 3.96 Hz, $J_{4',3'}$ = 6.33 Hz, H4'), 4.05 (dd, 1H, $J_{2',3'}$ = 5.35 Hz, $J_{2',1'}$ = 3.79 Hz, H2'), 3.94 (dd, 1H, $J_{5',5''}$ = 12.91 Hz, $J_{5',4'}$ = 2.78 Hz, H5'), 3.81 (dd, 1H, $J_{5'',5'}$ = 12.91 Hz, $J_{5'',4'}$ = 3.96 Hz, H5''), 3.52 (s, 3H, OCH_3), 3.35 (s, 2H, CH_2).
UV (EtOH) λ (nm) (ϵ): λ_{max} = 212 (9400), λ_{min} = 235 (1800), λ_{max} = 269 (10200).
MS 70 eV, m/z (rel.int.): 316 (0.3), $M^{+}\cdot$; 272 (10.3), $M-CO_2$; 171 (16.8), B+2H; 147 (45.8), s; 126 (19.5), $B+H-CO_2$; 125 (10.0), $B-CO_2$.

7. 2'-O-Methyl-5-carbamoylmethyluridine (3)

The nucleoside 1 (100 mg, 0.3 mmol) was dissolved in 13 M NH_3/MeOH solution (15 ml) and the reaction mixture was allowed to stand at room temperature for 3 days. The solvent was removed and the residue was coevaporated with methanol and crystallized to give 90 mg (95% yield) of amide 3 (mp. 219-220°C from methanol). R_f = 0.05 (D); 0.56 (E).

^1H NMR (D_2O/DSS) δ ppm: 7.87 (s, 1H, H6), 5.96 (d, 1H, $J_{1',2'}$ = 3.52 Hz, H1'), 4.31 (dd, 1H, $J_{2',3'}$ = 5.73 Hz, $J_{3',4'}$ = 5.83 Hz, H3'), 4.05 (m, 2H, H2', H4'), 3.90 (dd, 1H, $J_{5',5''}$ = 12,96 Hz, $J_{5',4'}$ = 2.52 Hz, H5'), 3.77 (dd, 1H, $J_{5'',5'}$ = 12.96 Hz, $J_{5'',4'}$ = 3.62 Hz, H5''), 3.49 (s, 3H, OCH_3), 3.30 (s, 2H, CH_2).

UV (EtOH) λ (nm) (ϵ): λ_{max} = 210 (8100), λ_{min} = 235 (1200), λ_{max} = 268 (8800).

MS 70 eV, m/z (rel.int.): 315 (6.0), $M^{+\cdot}$; 170 (78.8), B+2H; 169 (18.8), B+H; 147 (44.6) s.

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