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THE MODIFIED NUCLEOSIDES FROM THE " WOBBLE POSITION " OF tRNAs. THE SYNTHESIS OF 5-CARBOXYMETHYLAMINOMETHYLURIDINE AND 5-CARBOXYMETHYLAMINOMETHYL-2-THIOURIDINE

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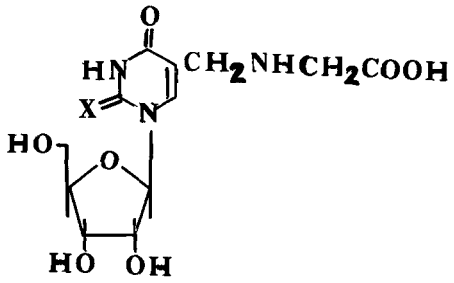
Abstract:The synthesis of 5-carboxymethylaminomethyluridine isolated from $tRNA_1^{Gly}$(B. subtilis)and its 2-thio analogue from $tRNA^{Lys}$(B. subtilis) have been described.

The modified nucleosides located at the first position of the anticodons of $tRNA_1^{Gly}$ and $tRNA^{Lys}$ from B. subtilis were determined as 5-carboxymethylaminomethyluridine(1)[1] and 5-carboxymethylaminomethyl-2-thiouridine(2)[2]. The proof of structure was mainly based on the mass spectrometry analysis of persilylated derivatives of isolated compounds and remarkable resemblance of their UV spectra to the respective spectral data of 5-methylaminomethyluridine and 5-methylaminomethyl-2-thiouridine. It is noteworthy , that 5-methylaminomethyluridine and 5-methylaminomethyl-2-thiouridine structurally are decarboxylated analogues of 1 and 2 and also have been found in the anticodons of tRNAs[3,4].

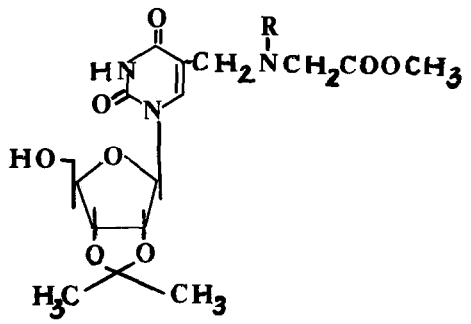
Now, we report the synthesis of nucleosides 1 and 2, to confirm previously reported structures[1,2], as well as the partially blocked derivatives 6 and 7 , as potential intermediates for the oligonucleotide synthesis.

As suitable substrates for the preparation of 1 and 2 2′, 3′-O-isopropylidene derivatives of 5-chloromethyluridine[5] and 5-chloromethyl-2-thiouridine[5] have been used.Thus,condensation of 2′,3′-O-isopropylidene-5-chloromethyluridine with an equimolar amount of N-benzylglycine methyl ester in DMF in the presence of triethylamine(RT, 12h)followed by separation of the product on the short column[6] gave pure compound 3 in 75% yield[7] [R_f 0.75[8a] ; ^{1}H NMR (CO_3COCD_3) δ ppm: 7.87(1H,s,-H6), 7.36(5H,m,aromatic), 5.95(1H,d,J=2 Hz,1′-H), 3.85(2H,s,-CH_2Ph), 3.70(3H,s,-OCH_3), 3.65(2H,s,-CH_2N-), 3.40(2H,s,-NCH_2COO-), 1.53, 1.32(6H,s,isopropylidene)]. Hydrogenolysis of 3 with 10% Pd on C(25°C, 1 atm, 5h) in methanol led to 4 contaminated with small amounts of impurities[9].



1 X=O

2 X=S



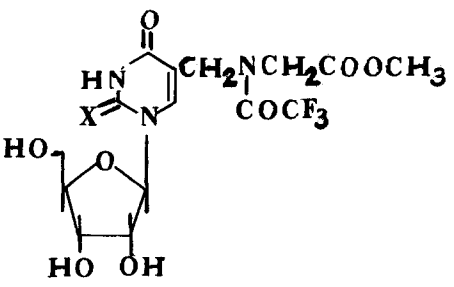
3 R= —CH_2Ph

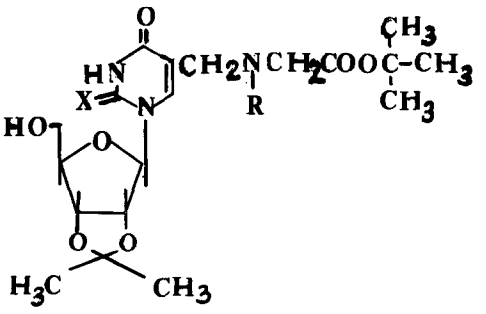
4 R= —H

5 R= —$COCF_3$

Unexpectedly, compound 4 partially decomposed on the silica gel column or TLC plates and even slightly acidic conditions accelerated the formation of less polar compound, giving negative ninhydrin test.This observation prompted us to transform crude 4 to the derivative which would be more stable for the chromatography operations.On the base of our previous findings[10] amide 5 was expected to be stable and useful for the oligonucleotide synthesis[11]. Thus, ester 4 was reacted with an excess of trifluoroacetic anhydride in pyridine (RT ,12h) to give 5′-O,N-diacylated derivative of 4.Hydrolysis of 5′-O-trifluoroacetyl group with 5 % sodium bicarbonate in water solution, followed by separation of the reaction mixture on the silica gel column afforded pure 5 in 80% yield [R_f 0.34[8a], 0.56[8b] ;MS m/z=481 $M^{+\cdot}$ (3.4%); ^{1}H NMR(CD_3COCD_3) δ ppm: 7.85, 7.80(1H,double singlet, H-6)[12] , 5.98,5.93 (1H,double doublet, J=2 Hz, 1′-H), 3.75(3H,s,-OCH_3), 1.53, 1.35(6H,s,isopropylidene)]. Compound 5 was heated with 50 % acetic acid(45 min,100°C) to give 6 in quantitatively yield m.p. 186-187°C, methanol; MS m/z=441 $M^{+\cdot}$ (3.5%).

The treatment of 2′, 3′-O-isopropylidene-5-chloromethyl-2-thiouridine with glycine t-butyl ester hydrochloride in DMF in the presence of triethylamine (RT, 10h) afforded 8 in 30% yield[13]. Crude 8 was trifluoroacetylated and the reaction mixture was worked up according to the procedure described for 5. Compound 9 was obtained in 25% yield after purification on the silica gel column [R_f 0.57[8a], 0.70[8b] ; ^{1}H NMR(CD_3COCD_3) δ ppm: 8.38, 8.30 (1H,double singlet, H-6), 7.08 , 6.98 (1H, double doublet , J=3Hz, 1′-H), 3.65 (2H,s,-CH_2N-), 3.40 (2H,s,-NCH_2COO-), 1.55, 1.32(6H,s,isopropylidene), 1.43(9H,s,t-butyl)]. Refluxing 9 with 0.5n HCl in dry methanol led to the simultaneous deprotection of hydroxyl functions of ribose moiety and transestrification to give 7 [m. p. 201-202°C, methanol; MS m/z=457 $M^{+\cdot}$ (0.3%), m/z=365 B+41(11.2%)[14]] in 90% yield.





6 X=O

7 X=S

8 R = -H X = S

9 R = $-COCF_3$ X = S

10 R = $-COCF_3$ X = O

Using the same reaction sequence 2′, 3′-O-isopropylidene-5-chloromethyluridine was converted to compound 10 and than to 6 , identical by TLC , MS and ^{1}H NMR with the nucleoside obtained by the previous way.

Alkali labile protecting groups of 6 and 7 were removed by treatment with $Ba(OH)_2$ (saturated solution in water)[15]. The reaction mixture was neutralised with sulphuric acid , the precipitate was filtered off and the filtrate passed through Dowex 50W(H^+ form) column. Nucleosides 1 and 2 were eluted with 2% aqueous ammonia and succesively purified on Sephadex G-10 (1% aqueous ammonia) and by paper chromatography (Whatman 3MM, n-propanol:conc.ammonia: water-11:2:7). Finally, pure samples were lyophilised and 1,2 precipitated (ethanol saturated with HCl/ether) as hydrochlorides:

1: R_f 0.21[8c], 0.50[8d], 0.13[16a], 0.47[16b]; $(\alpha)_D^{20}$ = -5.4° (c=1, H_2O); UV λ nm, (ε): pH2 λ_{min}=234 (700), λ_{max}=267 (9100); pH12 λ_{min}=243 (3000), λ_{max}=265 (5600); ^{1}H NMR (D_2O-DCl) δ ppm: 8.70 (1H,s,H-6), 6.40 (1H,d,J=3.5 Hz, 1′-H). 4.63 (2H,s, $-CH_2NH-$), 4.52 (2H,s, $-NHCH_2-$).

2: R_f 0.27[8c], 0.52[8d], 0.18[16a], 0.50[16b]; $(\alpha)_D^{20}$ = - 7.4° (c=1, H_2O); UV λ nm, (ε): pH 2 λ_{min}=242 (6900), λ_{max}=272 (14300), pH 12 λ_{max}=244 (16800), λ_{min}=261 (13000), λ_{max}=272 (13700); ^{1}H NMR (D_2O-DCl) δ ppm: 8.90 (1H,s,H-6), 6.98 (1H,d,J=2.5 Hz, 1′-H), 4.62 (2H,s, $-CH_2NH-$), 4.48 (2H,s, $-NHCH_2-$).

The mass spectra of persilylated derivatives of 1, 2 and their electrophoretical mobility were in excellent agreement with those reported previously[1,2].

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References and footnotes

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5. Ikeda K., Tanaka S., Mizuno Y., Chem. Pharm. Bull., 23,2958 (1975)
6. Merck Kieselgel H was used for the short column chromatography, using methanol-chloroform mixture for the gradient elution (0-10%)
7. All new compounds gave satisfactory elemental analysis.
8. Merck silica gel 60 HF_{254} pre-coated plates have been used for TLC in solvent systems : a/ chloroform:methanol-90:10 , b/ chloroform:methanol-85:15, c/ isopropanol:conc.ammonia:water-7:1:2, d/ n-propanol:conc. ammonia:water-11:2:7
9. Alternatively, 2', 3'-O-isopropylidene-5-aminomethyluridine , readily available from 2', 3'-O-isopropylidene-5-chloromethyluridine, was condensed with equimolar amount of methyl chloroacetate (RT,7h,DMF as solvent) to give 4 in 30% yield after purification on the silica gel column in chloroform:acetone-5:2 ; ^{1}H NMR CD_3COCD_3 δ ppm:7.80 (1H,s,H-6), 5.95 (1H,d,J=2 Hz,1'-H), 3.67 (3H,s,-OCH_3), 1.55, 1.35 (6H,s,isopropylidene)
10. Trifluoroacetyl group has been used to protect exoamino function of mnm^5s^2U for the preparation of oligonucleotides - Małkiewicz A., Sochacka E., Manuscript submitted to this Journal.
11. Manuscript submitted for publication.
12. Duplication of H-1', H-6 NMR signals of uridines and H-6 of uracil bearing exoamido function has been observed: Vorbrüggen H., Królikiewicz K., Liebigs Ann. Chem., 1980 1438 ; De N.C., Mittelman A., Jenkins E.E., Crain P.F., McCloskey J.A., Chheda G.B., J.Carbohyd., Nucleosides, Nucleotides, 7,113 (1980); Małkiewicz A., Sochacka E., Manuscript submitted to this Journal.
13. Prepared according to general method [Armstrong W., Eckstein F., Nucleic Acids Res.,Spec.Publ., No1, 97 (1975)] 2',3'-O-isopropylidene-5-formyl-2-thiouridine [^{1}H NMR $CDCl_3$ δ ppm:10.00 (1H,s,CHO), 9,00 (1H,s,H-6), 7.85 (1H.d,J=1.8 Hz,1'-H), 1.63, 1.35 (6H,s,isopropylidene)] was condensed in methanol with t-butyl ester of glycine in the presence of $NaCNBH_3$ and catalytical amount of formic acid to give 8 in 28% yield ; ^{1}H NMR (CD_3COCD_3) δ ppm:7.95 (1H,s,H-6), 7.03 (1H,d,J=2.5 Hz, 1'-H), 1.55 , 1.32 (6H,s,isopropylidene), 1.43 (9H,s,t-butyl).
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16. Merck cellulose 60 F_{254} plates in a/ isopropanol:conc.ammonia:water-7:1:2, b/ n-propanol:conc.ammonia:water-11:2:7

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