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THE PROTECTED DERIVATIVES OF 5-METHYLAMINOMETHYL-2-THIOURIDINE AND 5-CARBOMETHOXYMETHYL-2-THIOURIDINE AS COMPONENTS FOR THE OLIGONUCLEOTIDE SYNTHESIS

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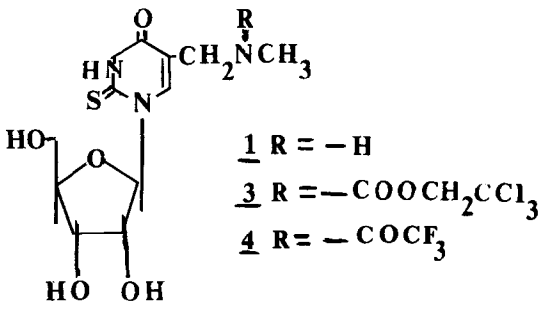
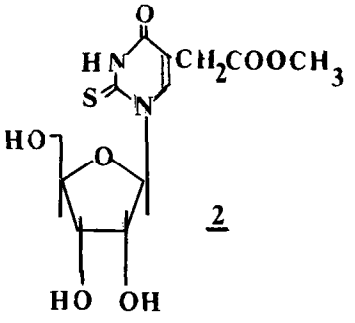
Abstract:The syntheses of protected derivatives of 5-methylaminomethyl-2-thiouridine(mnm^5s^2U) 11, 12 and 5-carbomethoxymethyl-2-thiouridine(mcm^5s^2U) 13 as well as their unprotected 3'- phosphates 14, 15 have been described.

Various modified nucleosides when present in the wobble position of the anticodons of tRNAs strongly influence the codon-anticodon interaction[1]. The restriction in the base pairing between 5-substituted 2-thiouridines 1,2 and the third letter of codons has been recently intensively investigated[2]. However, synthetic oligomers with the sequences related to the anticodons, which are promising for such study, have not been reported so far.

This communication deals with the synthesis of protected derivatives of 1 and 2 as terminal units for the chemical synthesis of oligoribonucleotides with mnm^5s^2U and mcm^5s^2U as components[3].



1 R = —H
3 R = —$COOCH_2CCl_3$
4 R = —$COCF_3$



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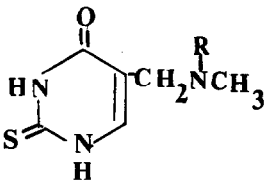
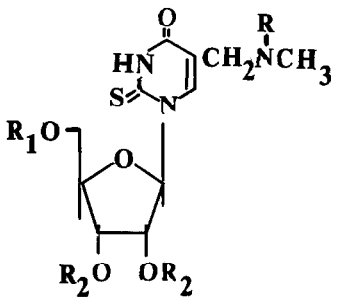
The trifluoroacetyl and 2,2,2-trichloroethoxycarbonyl protecting groups have been selected and used to protect exo-amino function of nucleoside 1. Both protecting groups have been introduced by two independent routes:(i) using suitably blocked derivatives of heterobases 6,7 ;(ii)by protecting of exo-amino function of 5-methylaminomethyl-2-thiouridine 1[4].

(i)Heterocyclic base 5[4]was reacted with 2,2,2-trichloroethylchloroformate or trifluoroacetic anhydride in pyridine solution to give 6

(m.p. 216-218°C, ethanol) or 7 m.p.(209-211°C, ethanol) in 75% and 79% yield respectively[5].





5 R = —H

6 R = —$COOCH_2CCl_3$

7 R = —$COCF_3$

8 R = —$COOCH_2CCl_3$
$R_1 = R_2 = -COC_6H_5$

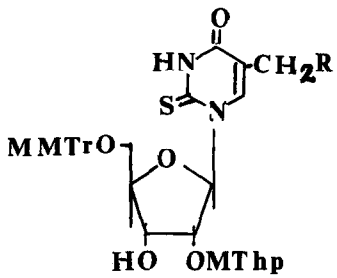
9 R = $-COCF_3$
$R_1 = R_2 = -COC_6H_5$

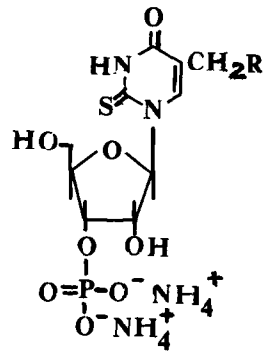
10 R = $-COOCH_2CCl_3$
$R_1 = -H$ $R_2 = >C(CH_3)(CH_3)$

2,4-bis-S,O-trimethylsilyl derivatives of heterobases 6, or 7 were condensed with 1-O-acetyl-2,3,5-O-tribenzoyl-D-ribofuranose in acetonitrile solution in the presence of $SnCl_4$[6] to give fully blocked 8 and 9 in 70-80% yield[7]. Selective ammonolysis of 8 (half saturated methanolic ammonia, 24h, RT) gave product 3 [yield 89% ; m.p. 186-188°C, ethanol; TLC R_f 0.20[7a], 0.51[7b], 0.60[7c]; MS m/z=477 $M^{+\cdot}$(1.2%); ^{1}H NMR DMSO-d_6 δ ppm: 8.12(1H,s,H-6), 6.59(1H,d,J=2Hz, 1'-H), 4.81(2H,s,$-OCH_2CCl_3$), 3.96(2H,s,$-CH_2N-$), 2.91(3H,s,$-NCH_3$)].
The ammonolysis of 9 under mild conditions (10% ammonia in methanol , 0°C) gave fully deprotected nucleoside 1. Amide 4 was obtained by the methanolysis of benzoyl groups with sodium methoxide (0°C, reaction was continuously monitored by TLC) in 20% yield m.p. [192-194°C ethanol; TLC R_f 0.17[7a], 0.46[7b] ; MS m/z=399 $M^{+\cdot}$(1.5%); ^{1}H NMR (DMSO-d_6) δ ppm:8.20(1H,broad singlet,H-6), 6.56 (1H,d,J=3 Hz,1'-H), 3.95(2H,s,$-CH_2N-$), 2.82, 2.96(3H, double singlet, $-NCH_3$)][8].

(ii) The reaction of nucleoside 1 with an excess of trifluoroacetic anhydride in pyridine solution , followed by selective removal of trifluoroacetyl groups from sugar moiety with 10% sodium bicarbonate gave 4 (in 81% yield), identical (TLC, UV, and ^{1}H NMR) with the specimen obtained by the route(i). Reaction of 2', 3'-O-isopropylidene derivative of 1 with 2,2,2-trichloroethyl-chloroformate in pyridine , followed by the separation of products on silica gel column afforded 10 in 71% yield. The 10 under treatment with 20% acetic acid(45 min, 100°C) gave 3 in quantitatively yield.

Fully blocked derivatives of 5-methylaminomethyl-2-thiouridine 11, 12 and 5-carbomethoxymethyl-2-thiouridine 13 were obtained in 55-60% total yield as follows:





11 R = $-N(CH_3)COOCH_2CCl_3$

12 R = $-N(CH_3)COCF_3$

13 R = $-COOCH_3$

14 R = $-NHCH_3$

15 R = $-COOCH_3$

MMTr — monomethoxytrityl
MThp — methoxytetrahydropyranyl

(i) Protection of 3′and 5′- hydroxyl function of 2,3,4 with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine by the general method introduced by Markiewicz[9]; (ii) Ketalization of 2′-hydroxyl function of silylated compounds with 4-methoxy-5,6-dihydro-2H-pyran in dioxane in the presence of p-toluenesulphonic acid[10], followed by the removal of 3′, 5′- silyl block with fluoride anion[11] and purification on silica gel column[7]; (iii) Final protection of 5′-hydroxyl function with monomethoxytrityl group according to the known method[12].

We have found that the protecting groups of exo-amino function of 11 and 12 could be removed under the conditions reported by Wiewiórowski[13] and Reese[14] for the removal of 2,2,2-trichloroethyl(Zn/acetylacetone/pyridine) and o-chlorophenyl(0,1 n NaOH dioxane:water-4:1) from the phosphate residue of fully blocked oligonucleotides.

3′-Phosphates 14 and 15 were obtained by phosphorylation of nucleosides 12 and 13 with methyl dichlorophosphate under the reported conditions[15], followed by removal of protecting groups: (i) trifluoroacetyl with 0.1n NaOH in dioxane:water-4:1(RT, 7h)[14]; (ii) acid labile groups(MMTr, MThp) with 0.01n HCl(RT, 7h)[10]. Final purification of crude products was achieved by means of DEAE cellulose column using TEAB buffer for a gradient elution (0.05-0.4M) and paper chromatography(Whatman 3MM) to give pure 3′-phosphates: 14: TLC R_f=0.40[16a]; electrophoretical mobility 0.63[17]; MS of silylated derivative m/z=743 M^{5+}(0.5%), m/z=728 M-15(1.2%), ^{31}P NMR δ 2.63 ppm (H_2O, H_3PO_4 as reference).
15: TLC R_f=0.64[16b]; electrophoretical mobility 0.87[17], MS of silylated derivative m/z=772 M^+(0.4%); ^{31}P NMR δ 2.56 ppm (H_2O, H_3PO_4 as reference).

Acknowledgment

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References and footnotes

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7. All nucleoside derivatives were purified by means of short column chromatography on silica gel H, Merck , using methanol - chloroform mixture for a gradient elution(0-10%)
Merck silica gel 60 F_{254} plates have been used for TLC in the solvent systems: a/ chloroform:methanol-90:10,b/chloroform:methanol-80:20, c/ isopropanol:conc.ammonia:water-7:1:2.
8. Analogous observation of the doubling signals in ^{1}H NMR spectrum of N-acetyl derivative of 5-methylaminomethyl-2-thiouridine has been done by Vorbrüggen H. in ref . 4 .
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17. A relative mobility in respect to 3'Up in 0.05M phosphate buffer(pH 7.5).

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