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THE CHEMICAL SYNTHESIS OF ANTICODONS $tRNA_1^{Lys}$ FROM E.COLI B AND $tRNA_3^{Lys}$ FROM RABBIT LIVER

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Abstract: The chemical synthesis of oligoribonucleotides mnm^5s^2UpUpU, mnm^5s^2UpU, mcm^5s^2UpUpU and mcm^5s^2UpU have been described.

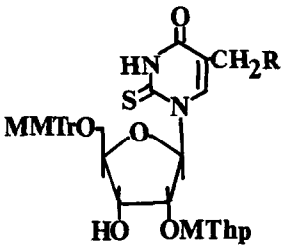
The synthetic oligonucleotides with the sequences related to the anticodons may serve as tools for the biophysical investigations of the interaction of modified nucleosides with backbone[1] and multifarious biochemical study e.g. post-transcriptional modification of the wobble nucleoside in the " anticodon substituted " tRNAs[2].

In this communication we present the utility of previously reported[3] derivatives of 5-methylaminomethyl-2-thiouridine(mnm^5s^2U) 1, 2 and 5-carbomethoxymethyl-2-thiouridine(mcm^5s^2U) 3 in the synthesis of the title oligoribonucleotides by the triester approach[4].

We have found[3], that the exo-amino function of 1 and 2 can be deprotected under the conditions reported for the removal of 2,2,2-trichloroethyl[5a] and o-chlorophenyl[5b] groups from the phosphate residue of fully protected oligomers. Therefore, 4 and 5 have been used for the synthesis of anticodon $tRNA_1^{Lys}$.

To obtain oligonucleotides with mcm^5s^2U as component, neutral or slightly acidic conditions are preferable for the deblocking operation ; 6 fulfils these requirements and was used for the synthesis of $tRNA_3^{Lys}$ from rabbit liver.

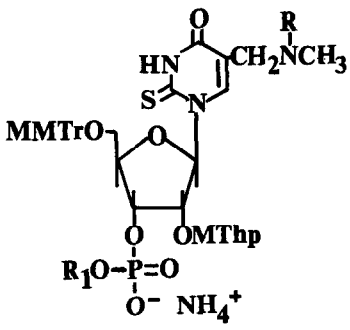
All phosphorylations and coupling reactions were achieved using an excess of 1-(mesitylenesulphonyl)-1H-1,2,4-triazole(MST)[6] as the activating agent (three fold excess for phosphorylation ; two fold excess for coupling reaction). Thus, 1, 2, and 3 were allowed to react with pyridinium 2,2,2-trichloroethyl phosphate or o-chlorophenyl phosphate(RT, 2 days) to give 4,5,and 6 in high isolated yields[7]. To obtain protected trimers 7 and 9 ,phosphates 4 and 6 (ammonium salt of 4 and triethylammonium salt of 6) were coupled with 0.5 molar excess of 2'- O-tetrahydropyranyluridylyl-(3'-5')-(2,2,2-trichloroethyl)-2', 3'-O-methoxymethylideneuridine[8](RT , 4 days). The reaction mixtures were quenched with ice-water,crude products were extracted with chloro-



1 R= –NCOOCH$_2$CCl$_3$ (CH$_3$)

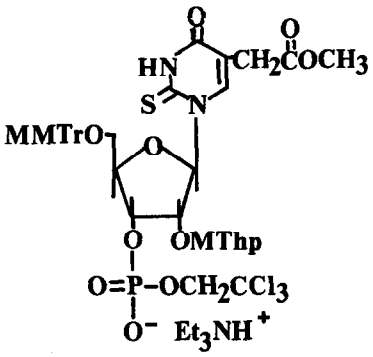
2 R= –NCOCF$_3$ (CH$_3$)

3 R= –COOCH$_3$

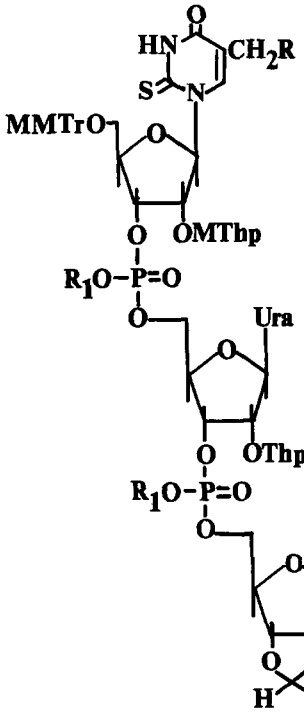


4 R = –COOCH$_2$CCl$_3$
R$_1$ = –CH$_2$CCl$_3$

5 R = –COCF$_3$
R$_1$ = –oClPh



6

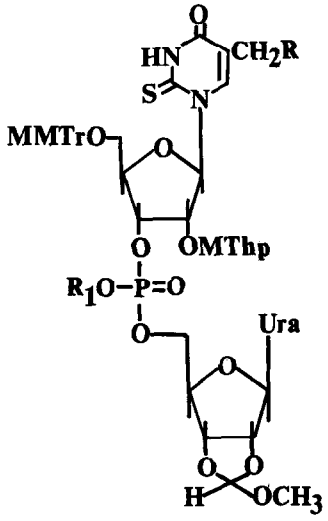


7 R = –NCOOCH$_2$CCl$_3$ (CH$_3$)
R$_1$ = –CH$_2$CCl$_3$

8 R = –NHCH$_3$
R$_1$ = –NH$_4^+$ salt

9 R = –COOCH$_3$
R$_1$ = –CH$_2$CCl$_3$

10 R = –COOCH$_3$
R$_1$ = –Et$_3$NH$^+$ salt



11 R = –NCOCF$_3$ (CH$_3$)
R$_1$ = –oClPh

12 R = –NHCH$_3$
R$_1$ = –NH$_4^+$ salt

MMTr – monomethoxytrityl
MThp – methoxytetrahydropyranyl
Thp – tetrahydropyranyl
Ura – uracil

form, finally separated and purified on short column[9] to give analytically pure 7 and 9 as mixtures of diastereoisomers(7: yield 75% ; R_f 0.14[10] ; ^{31}P NMR[11] δ = -3.73,-3.83,-4.57,-4.83 ppm; 9: yield 67% ; R_f 0.25[10]; ^{31}P NMR[11] δ = -3.90,-3.98,-4.31,-4.49 ppm).

The 4 and 6 were condensed with 2',3'-O-methoxymethylideneuridine[12] following the presented procedure to give fully protected dimers mnm^5s^2UpU 13 and mcm^5s^2UpU 14 in 65-70% yield(13: R_f 0.21,0.17[10]; ^{31}P NMR[11] δ = -3.93,-4.23 ppm; 14: R_f 0.27,0.25[10]; ^{31}P NMR[11] δ = -3.60,-4.67 ppm).

The trimers 7,9 and dimers 13,14 were deprotected in the following order: (i) with Zn/acetylacetone in pyridine(RT,8h)[5a] and partially deblocked oligomers were chromatographed on TLC preparative plates in isopropanol:conc.ammonia:water-7:1:2 for 7,13 and isopropanol:water-7:3 for 9,14; (ii) Acid labile groups were removed by treating partially deprotected oligomers with 0.01n HCl(RT,7h)[4]. Totally deprotected oligomers mnm^5s^2UpUpU(15), mcm^5s^2UpUpU(16), mnm^5s^2UpU(17), mcm^5s^2UpU(18), were purified on DEAE-32 column using TEAB buffer for a gradient elution(0.05-0.5M), next by paper chromatography(Whatman 3MM in n-propanol: ammonia:water-11:2:7 for 15,17 and isopropanol:water-7:3 for 16,18)and lyophylised to give fluffy solids. Spectral data are showed in the Table I.

Table I

	Yield %	R_f /TLC/	^{31}P NMR[c]	EV[d]	A_{280}/A_{260}/pH/
15	42	0.42[a]	-1.01; -1.27	0.47	0.78/2/ 0.67/12/
16	51	0.53[b]	-0.4 + +1.20	0.70	0.75/2/ 0.68/12/
17	45	0.57[a]	-1.01	0.18	1.00/2/ 0.78/12/
18	48	0.62[b]	-0.41	0.45	0.92/2/ 0.7o/12/

Merck cellulose 60 F_{254} plates were used for TLC in systems: a/ n-propanol: ammonia:water-11:2:7; b/ isopropanol:water-7:3
c/ in water; H_3PO_4 as external standard
d/ electrophoretical mobility reffered to 3'Up in phosphate buffer (pH 7.5)

Deprotection of oligomers with Zn/acetylacetone system lead to products slightly contaminated with metal, which can falsify chemical and biochemical activity test[13]. To overcome this disadvantage compound 5 has been tested as component for the oligonucleotide synthesis. Thus, 5 was condensed with 2',3'-O-methoxymethylideneuridine under the previously described conditions to give 11 in 68% yield(R_f 0.18 , 0.22[10]; ^{31}P NMR[11] δ = -8.06, -8.37 ppm). Simultaneous deprotection of amine and phosphate functions was achieved with

0.1n NaOH in dioxane:water-4:1 according to Reese procedure[5b]. Removal of acid labile groups with 0.01n HCl(RT ,7h)and purification according to the discussed previously procedure gave dimer 13 in the yield comparable with that, obtained by the former way. Homogeneity of all synthesized oligonucleotides was confirmed by spectral data, EV , chromatography / Table I / as well as by complete digestion with T_2 nuclease.

Using conventional Nirenberg-Leder filter assay[14] it has been found that 70S ribosomes from E. coli are more active in the binding $tRNA^{Phe}$ in the presence of 15,16, than programmed by $(Up)_3U$[15].

Acknowledgment

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