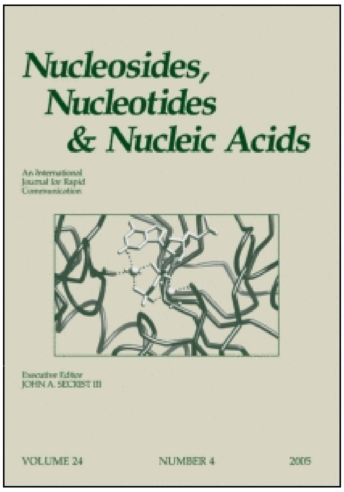

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THE CHEMICAL SYNTHESIS OF *E.coli* tRNA^{Lys} ANTICODON LOOP FRAGMENT AND ITS ANALOGUES

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ABSTRACT: *E.coli* tRNA^{Lys} anticodon loop fragment (Umnm^{5}s^{2}UUUt^{6}A) **1** and its analogues **2-6** were synthesized by the classical phosphotriester approach in solution. The preparation of suitably protected derivatives of N^6-threonylcarbamoyladenosine **18** is also described.

INTRODUCTION

The posttranscriptional tRNA modification is considered to be very important for the functionality of the biopolymer in various cellular processes[1-3]. The anticodon loop of tRNA^{Lys} *E.coli* contains 5-methylaminomethyl-2-thiouridine (mnm^{5}s^{2}U) at the position 34 (wobble position) and N^6-threonylcarbamoyladenosine (t^{6}A) at the position 37 , while *E.coli* tRNA^{Glu} is modified with mnm^{5}s^{2}U at the first anticodon letter and 2-methyladenosine (m^{2}A) at the position 3'-adjacent to anticodon[4]. Watanabe *et al.* have demonstrated that circular dichroism spectrum of *E.coli* tRNA^{Lys} exhibits a positive ellipticity derived from the tiocarbonyl chromophore, while tRNA^{Glu} *E.coli* shows a negative peak at the same wavelength that is characteristic of s^2 U$_{34}$ mononucleotide[5]. Since tRNA^{Glu} *E.coli* is known to have a typical tertiary structure, the conformation of tRNA^{Lys} *E.coli* anticodon loop appears to be different from those of most tRNAs[5]. Recently we have found, that CD spectrum of bovine tRNA^{Lys} (homologous to human tRNA^{Lys3})[6] closely resembles CD pattern of tRNA^{Lys} *E.coli*[7]. Human tRNA^{Lys3} is recruited by human

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This paper is dedicated to the late Professor Tsujiaki Hata

[§] phone (48-42) 31-31-41; fax (48-42) 36-55-30; e-mail ejsochac@ck-sg.p.lodz.pl immunodeficiency virus type-1 for priming reverse transcription and it has 5-methoxycarbonylmethyl-2-thiouridine (mcm^5s^2U) at the wobble position 34 and adenosine 37 modified to 2-methylthio-N^6-threonylcarbamoyladenosine (ms^2t^6A)[8]. Interestingly, *E.coli* $tRNA^{Lys}$ was shown to compete effectively with human $tRNA^{Lys3}$ in binding to RT (p66/p51), yet has only 62% sequence homology with human $tRNA^{Lys3}$, but its anticodon region is essentially identical[9]. Mammalian $tRNA^{Lys}$ is also responsible for a -1 frameshift required in the correct translation of polymerase at the *gag-pol* junction of many retroviral RNAs[10]. Correct translation of bacterial DNA polymerase γ subunit also requires a -1 frameshift and this is produced by the bacterial $tRNA^{Lys}$ [11]. One can expect that these different biological activities can be explained by an unique anticodon loop conformation of both mammalian and bacterial $tRNA^{Lys}$.

The synthetic oligoribonucleotide with the sequence of *E.coli* $tRNA^{Lys}$ anticodon loop $Umnm^5s^2UUUt^6A$ (**1**) and its structural analogues Us^2UUUt^6A (**2**), $UUUUt^6A$ (**3**), $Umnm^5s^2UUUA$ (**4**), Us^2UUUA (**5**), UUUUA (**6**) were obtained as model compounds to study an influence of nucleosides modification on the tertiary structure of anticodon loop of $tRNA^{Lys}$ species[7].

RESULTS AND DISCUSSION

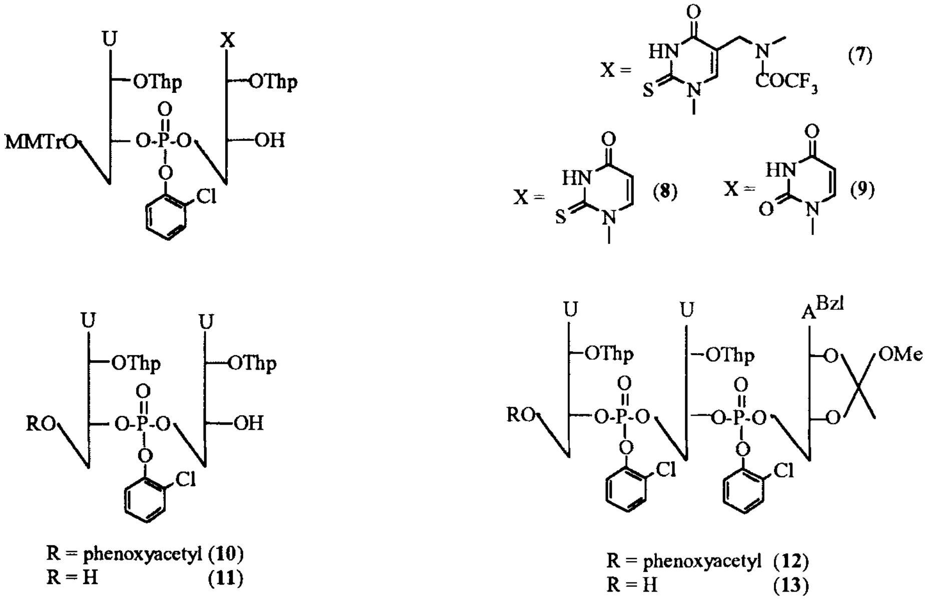
The multimilligram scale syntheses of fully protected pentamers **1a**-**6a** were accomplished by means of the classical phosphotriester approach in solution[12,13]. Oligoribonucleotides: $Umnm^5s^2UUUt^6A$ (**1a**), Us^2UUUt^6A (**2a**), and $UUUUt^6A$ (**3a**) were synthesized by block condensation of dimers **7**-**9** with uridine dinucleotide **11** and then with suitably protected t^6A derivative **18** , while the pentamers: $Umnm^5s^2UUUA$ (**4a**), Us^2UUUA (**5a**), UUUUA (**6a**) were obtained by coupling the same dimers **7**-**9** with the building block **13** as is outlined in FIG. 1.

5-Methylaminomethyl-2-thiouridine (mnm^5s^2U) was obtained as reported previously[14,15] and the trifluoroacetyl group was used for protection of an *exo* amino function of this nucleoside[15]. The 2'-hydroxyl functions of N-trifluoroacetyl-5-methylaminomethyl-2-thiouridine, 2-thiouridine, and uridine were protected with tetrahydropyranyl group (Thp) according to the Markiewicz procedure[14,16]. Monomethoxytritylation (MMTr) and phenoxyacetylation (PhAc) of 5'-hydroxyl group of nucleosides were carried out according to literature procedures[17,18].

The protected building blocks **Fully protected pentamers**

Umnm^{5}s^{2}U (7) **Umnm^{5}s^{2}UUUt^{6}A (1a)**
Us^{2}U (8) + UU (11) + t^{6}A (18) $\longrightarrow$ Us^{2}UUUt^{6}A (2a)
UU (9) **UUUUt^{6}A (3a)**

Umnm^{5}s^{2}U (7) **Umnm^{5}s^{2}UUUA (4a)**
Us^{2}U (8) + UUA (13) $\longrightarrow$ Us^{2}UUUA (5a)
UU (9) **UUUUA (6a)**



R = phenoxyacetyl (10)
R = H (11)

R = phenoxyacetyl (12)
R = H (13)

FIG. 1. Scheme of the Block Synthesis of Oligomers.

The synthesis of t^{6}A protected derivative **18** (FIG. 2.) was achieved using N-benzoyl-2',3'-*O*-methoxymethylideneadenosine as a starting material[19]. Treatment of nucleoside **14** with *tert*-butyldimethylchlorosilane and imidazole in pyridine and the subsequent removal of N-benzoyl group with 2.5 M ammonia in methanol afforded N^6-deprotected adenosine **15** in very good yield. Nucleoside **15** was reacted with phenoxycarbonyltetrazole in dioxane at 37 °C for 18 h[20] and the carbamate **16** was then isolated by silica gel column chromatography (88% yield). Reaction of L-threonine methyl ester or L-

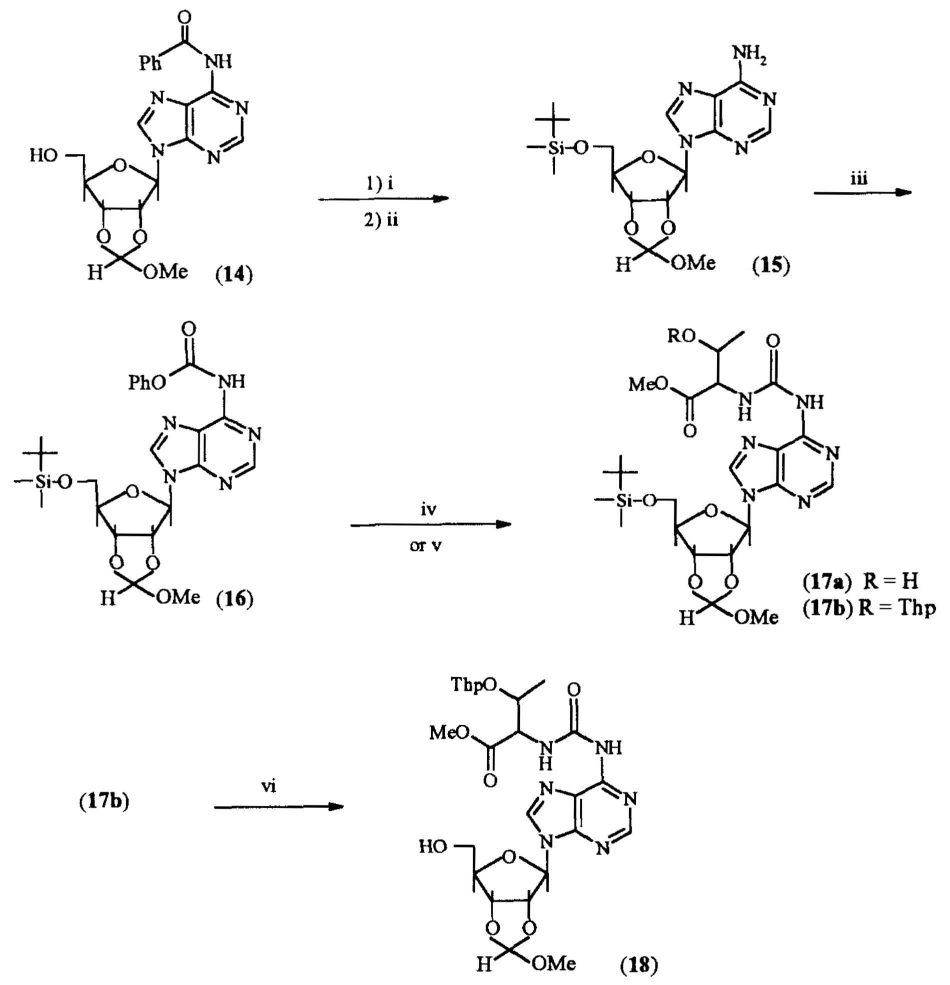


FIG. 2. Synthesis of the Protected t⁶A Component: i) TBDMSCl/imidazole/pyridine; ii) 2.5 M ammonia in methanol; iii) phenoxycarbonyltetrazole/dioxane; iv) L-threonine methyl ester; v) 2-*O*-tetrahydropyranyl-L-threonine methyl ester; vi) 1 M tetrabutylammonium fluoride in THF.

threonine-*O*-tetrahydropyranyl methyl ester with nucleoside **16** afforded compounds **17a** or **17b** in 86% and 57% yield, respectively. The use of L-threonine methyl ester with an unprotected hydroxyl function gives t^6A derivative **17a** in higher yield due to less steric hindrance during aminolysis. The free hydroxyl function of threonine residue of **17a** can be blocked with a suitable protecting group in the next step of the synthesis. Final removal of *tert*-butyldimethylsilyl group from nucleoside **17b** (1 M tetrabutylammonium fluoride in tetrahydrofuran) followed by column chromatography provided suitably protected t^6A derivative **18** in 95% yield.

All phosphorylation, coupling, and deprotection reactions were accomplished under conventional conditions used for oligoribonucleotide synthesis by phosphotriester approach[12,13,21-27]. Thus, 2-chlorophenyl phosphorodichloridate in the presence of 1,2,4-triazole[24] was used as phosphorylating agent and the appropriate phosphates were obtained in 80-95% yields. All coupling reactions were accomplished in the presence of mesitylenesulphonyl tetrazole[25,26] to give the products in 40-75% yields. The use of shortest possible coupling reaction times (TLC analysis) and the optimum concentration of components (0.01-0.05 M) allowed to decrease the scope of side reactions. The fully protected pentamers **1a-6a** were purified on silica gel columns and precipitated from hexane. Their structures were confirmed by ^{31}P NMR and liquid secondary ion mass spectrometry analysis.

For simultaneous removal of 2-chlorophenyl groups from internucleotide bonds and hydrolysis of methyl ester from threonine residue of t^6A unit, the fully protected oligomers **1a-3a** were treated with 0.1 M sodium hydroxide in pyridine / water (1:1) solution for 3 h at room temperature[27]. Oligomers **4a-6a** were reacted with concentrated aqueous ammonia in pyridine to deprotect the internucleotide bonds as well as to remove N-benzoyl group from the adenosine unit[24]. The removal of acid labile groups (monomethoxytrityl, tetrahydropyranyl, and methoxymethylidene) from all pentamers (**1a-6a**) was achieved under acidic conditions (0.015 M HCl in water / methanol 4:1)[21-23]. The deblocked products were separated by paper chromatography (Whatman 3MM in 2-propanol / ammonia / water 11:2:7). Further purification was carried out by reverse phase HPLC[22]. The homogeneity and structures of oligoribonucleotides **1-6** were confirmed by HPLC, LSIMS data and enzymatic digestion followed by HPLC analysis of nucleoside composition[28] (FIG.3).

EXPERIMENTAL

TLC analysis was performed on silica gel and cellulose plates (Merck, DC-Fertigplatten Kieselgel 60F-254 and Cellulose F plates). Silica gel 60F 230-400 mesh, (Merck) was used for flash column chromatography. Paper chromatography was made using Whatman 3MM paper. The following solvent systems were used: A - chloroform /

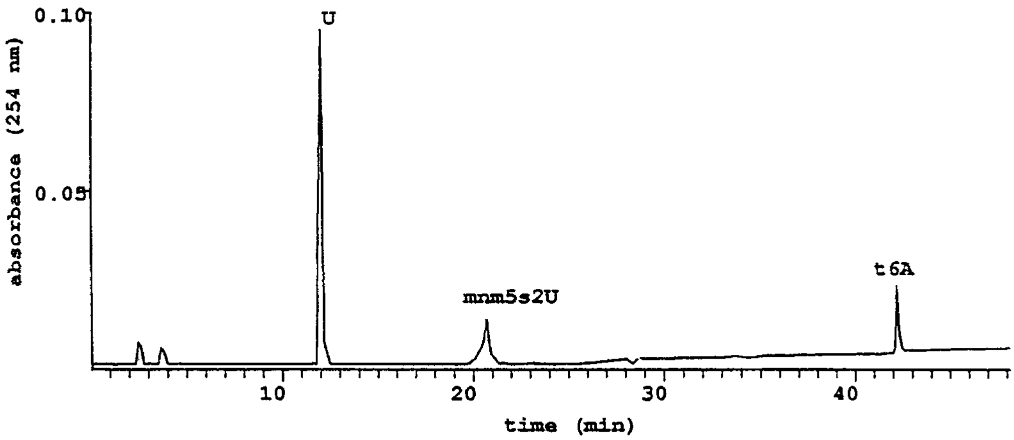


FIG.3. HPLC Elution Profile of Nucleosides after Enzymatic Digestion of Umnm^{5}s^{2}UUUt^{6}A (1)

methanol 95:5; B - chloroform / methanol 90:10; C - chloroform / methanol 85:15; D - chloroform / methanol 85:15; E - 2-propanol / 25% aq. ammonium / water 7:1:2; F - 2-propanol / 25% aq. ammonium / water 11:2:7. HPLC was accomplished with Spherisorb C-18 column (25 cm x 4.6 mm) in a gradient elution (0-40% acetonitrile in 0.1 M ammonium acetate, 0-40 min.) at a flow rate of 1 ml/min.

The NMR spectra were recorded on the Bruker DPX-250 spectrometer. The chemical shift values are expressed relative to tetramethylsilane (internal) for ^{1}H spectra or phosphoric acid (85%, external) for ^{31}P spectra (key: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, b=broad and d=double). LSIMS mass spectra and high resolution LSIMS measurements were taken on the Finnigan MAT 95 mass spectrometer.

5'- *O-tert*-Butyldimethylsilyl-2',3'-*O*-methoxymethylidene adenosine (15). 2',3'-*O*-Methoxymethylidene-N-benzoyladenosine (14) (1.85 g, 4.5 mmol) in pyridine (8 ml) was treated with *tert*-butyldimethylsilyl chloride (1g, 6.6 mmol) and imidazole (0.87 g, 12.8 mmol) at room temperature. After 2 h, the reaction mixture was decomposed with water (10 ml) and dichloromethane (20 ml). The aqueous layer was extracted with dichloromethane (2 x 10 ml) which was combined with the organic layer. After evaporation of the solvent the residue was coevaporated twice with toluene and chromatographed on a silica gel using 0-2% methanol in chloroform as the eluent. 5'-*O-tert*-

Butyldimethylsilyl-2',3'-*O*-methoxymethylidene-N-benzoyladenosine, obtained in 91% yield (2.15 g, R_f=0.61 system A, R_f=0.88 system B), was subsequently treated with 2.5 M ammonia in methanol at room temperature. After 24 h, TLC analysis showed complete removal of the benzoyl group (R_f=0.53 system B). Evaporation of solvent and purification of the oily residue on silica gel (0-5% methanol in chloroform) gave 1.6 g of pure **15** (yield 92%). LSIMS (nba): 424 $(M+H)^+$, 446 $(M+Na)^+$; ^1H NMR ($CDCl_3$) δ (ppm): 8.33 and 8.32 (two s, 1H, H-8), 7.97 and 7.95 (two s, 1H, H-2), 6.27 and 6.12 (two d, J=2.5 Hz, 1H, H-1'), 6.03 and 5.95 (two s, 1H, O-CH-O), 5.65 (bs, 2H, NH_2), 5.41 (m, 1H, H-2'), 5.04 (m, 1H, H-3'), 4.46 and 4.34 (two m, 1H, H-4'), 3.80 (m, 2H, H-5',H-5"), 3.44 and 3.34 (two s, 1H, O-CH_3), 0.84 (s, 9H, *tert*-butyl-Si), 0.02 (s, 6H, CH_3-Si).

5'-*O*-*tert*-Butyldimethylsilyl-2',3'-*O*-methoxymethylidene-N^6-phenoxycarbonyladenosine (16). Phenoxycarbonyltetrazole (570 mg, 3 mmol) was added to the stirred solution of **15** (423 mg, 1 mmol) in anhydrous dioxane (10 ml). After 18 h at 37 °C the solvent was evaporated and the product was separated from an excess of phenoxycarbonyltetrazole by silica gel column chromatography (chloroform was used as the eluent). The pure phenyl carbamate derivative **16** was obtained in 88% yield (480 mg).TLC (R_f=0.76 system B); LSIMS (nba): 544 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ (ppm): 8.93 (bs, 1H, NH), 8,78 (s, 1H, H-8), 8.17 and 8.19 (two s, 1H, H-2),7.56 (m. 5H, phenyl), 6.35 and 6.19 (two d, J=3.0 Hz, 1H, H-1'), 6.05 and 5.97 (two s, 1H, O-CH-O), 5.42 (m, 1H, H-2'), 5.12 and 5.04 (two dd, 1H, J=2.5 Hz, H-3'), 4.49 and 4.39 (two m, 1H, H-4'), 3.83 (m, 2H, H-5',H-5"), 3.46 and 3.35 (two s, 3H, O-CH_3), 0.86 (s, 9H, *tert*-butyl-Si), 0.02 (s, 6H, CH_3-Si).

Synthesis of t^{6}A derivatives 17a, 17b and 18. A mixture of compound **16** (271 mg, 0.5 mmol) and L-threonine methyl ester (200 mg, 1.5 mmol) were stirred in anhydrous dioxane (5 ml). After 18 h at 37 °C, TLC analysis in system B revealed no starting material **16** in the reaction mixture. The solvent was then evaporated and the residue chromatographed over silica gel using 0-2% methanol in chloroform to give 250 mg (86%) of **17a**. TLC (R_f=0.25 system B); LSIMS (nba): 583 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ(ppm): 10.13 (d, J=9.5 Hz, 1H, a-NH), 8.59 (s, 1H, N^{6}H), 8.22 (s, 1H, H-8), 8.18 (s, 1H, H-2), 6.33 and 6.18 (two d, J=2.5 Hz, 1H, H-1'), 6.04 and 5.97 (two s, 1H, O-CH-O), 5.34 (m, 1H, H-2'), 5.07 and 5.01 (two dd, J=2.3 Hz, 1H, H-3'), 4.68 and 4.65 (two d, J=2.75

Hz, 1H, -CH-)4.45 (m, 2H, H-4' and -CH-), 3.86 (m, 2H, H-5',H-5"), 3.84 (s, 3H, $COOCH_3$), 3.45 and 3.35 (two s, 1H, O-CH_3), 1.33 and 1.30 (two s, 3H, -CH_3) 0.82 (s, 9H, *tert*-butyl-Si), 0.02 (s, 6H, CH_3-Si).

When methyl ester of *O*-tetrahydropyranyl-L-threonine was used for aminolysis of **16** (5 days, 37 °C), the t^6A derivative **17b** was isolated by means of silica gel column chromatography (0-3% methanol in chloroform) in 57% yield. TLC (R_f=0.35 system B); LSIMS (nba): 667 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ(ppm): 10.16 (m, 1H, a-NH), 8.51 (s, 1H, N^6H), 8.19 (m, 2H, H-8, H-2), 6.32 and 6.17 (two m, 1H, H-1'), 6.03 and 5.96 (two d, 1H, O-CH-O), 5.32 (m, 1H, H-2'), 5.01 (m, 1H, H-3'), 4.87 - 4.30 (m, 4H, H-4', -CH-CH-, Thp-acetal proton), 3.90 - 3.68 (m, 5H, H-5',H-5", $COOCH_3$), 3.45 and 3.35 (two d, 3H, O-CH_3), 1.80 - 1.51 (m, 6H, Thp-methylene protons), 1.37 and 1.26 (two d, 3H, -CH_3) 0.80 (s, 9H, *tert*-butyl-Si), 0.02 (s, 6H, CH_3-Si).

The solution of **17b** in anhydrous THF (4 ml) was treated with 1 M tetrabutylammonium fluoride in THF (1.1 ml) for 0.5 h at room temperature. Then 30 ml of chloroform and 10 ml of pyridine were added. The solution was washed with 2% aqueous sodium hydrogen carbonate, dried over magnesium sulphate and the solvents were evaporated in *vacuo*. The residue was coevaporated with toluene and subjected to flash chromatography on silica gel (0-5% methanol in chloroform). The pure t^6A derivative **18** was obtained in 95% yield (290 mg). TLC (R_f=0.18 system B); LSIMS (nba): 553 $(M+H)^+$, HRMS *m/z* 553.2232 (553.2258 calc. for $C_{23}H_{33}N_6O_{10}$); ^1H NMR ($CDCl_3$) δ (ppm): 10.25 (m, 1H, α-NH), 9.16 and 9.07 (two s, 1H, N^6H), 8.54 (m, 1H, H-8), 8.32 (m, 1H, H-2), 6.31 and 6.01 (two m, 1H, H-1'), 6.07 and 5.99 (two s, 1H, O-CH-O), 5.35 - 5.10 (m, 3H, H-2', H3', Thp-acetal proton), 4.79 - 4.57 (m, 3H, H-4', -CH-CH-), 3.98 - 3.79 (m, 4H, H-5',H-5", O-CH_2), 3.79 (s, 3H, $COOCH_3$), 3.48 and 3.36 (two s, 1H, O-CH_3), 1.81 - 1.57 (m, 6H, Thp-methylene protons), 1.40 and 1.27 (two d, 3H, -CH_3)

Oligoribonucleotide Syntheses. Suitably protected nucleosides: 2'-*O*-tetrahydropyranyl derivatives of uridine, 2-thiouridine, N-trifluoroacetyl-5-methylaminomethyl-2-thiouridine, and 5'-*O*-monomethoxytrityl-2'-*O*-tetrahydropyranyluridine, 5'-*O*-phenoxyacetyl-2'-*O*-tetrahydropyranyl uridine, as well as 2',3'-*O*-methoxymetylidene-N-benzoyladenosine were prepared according to previously reported procedures[12-19].

General Procedure for Phosphorylation. The suitably protected nucleoside or oligomeric unit with free 3'-hydroxyl function was rendered anhydrous by coevaporation with pyridine and dissolved in anhydrous pyridine (0.4-0.5 M solution). 1,2,4-Triazole (3.4 equiv.) and 2-chlorophenyl phosphodichloridate (1.7 equiv.) were added to the reaction mixture at room temperature. After 0.5 - 1 h (TLC analysis, system B), cold 50% aqueous pyridine and 0.1 M triethylammonium bicarbonate (TEAB) (2:1 v/v) were added to the cooled (0° C) reaction mixture. After 15 min. the product was extracted with chloroform and the organic layer was washed with 0.1 M TEAB, dried over magnesium sulphate and concentrated under reduced pressure. The residue was coevaporated twice with toluene, dissolved in small amount of chloroform and precipitated with an excess of hexane. The obtained phosphates (80-95% yield) were used directly for the condensation reactions without further purification.

General Method for Condensation. 3'-*O*-2-Chlorophenylphosphate of 2',5'-*O*,N-protected nucleoside (or oligonucleotide) (1 mol equiv.) and an appropriate nucleoside (or oligonucleotide) containing free 5'-hydroxyl function (1.2 mol equiv.) were dissolved in anhydrous pyridine (0.05 - 0.01 M solution) and the solution was treated with mesitylenesulphonyl tetrazole (2.0-2.5 mol equiv.) for 0.5-2 h at room temperature. Aqueous pyridine (50 %) was added to the reaction mixture and after 15 min, the product was extracted with chloroform. The organic layer was washed with 0.1 M TEAB, then with water, dried over magnesium sulphate and concentrated under reduced pressure. The residue was coevaporated three times with toluene and purified by silica gel column chromatography (0-5% methanol in chloroform) to give, after precipitation from hexane, the fully protected oligomers: Umnm^{5}s^{2}UUUt^{6}A (**1a**) 43 mg, overall yield for condensation of **7** ,**11** and **18** - 27%; LSIMS (nba) 2978.1 (M-H)$^-$; ^{31}P NMR (CDCl$_3$); -7.25 ppm (m); Us^{2}UUUt^{6}A (**2a**) 35 mg, overall yield for condensation of **8**, **11** and **18** - 31% ; LSIMS (nba) 2839.3 (M-H)$^-$; ^{31}P NMR (CDCl$_3$); -7.21 ppm (m); UUUUt^{6}A (**3a**) 33 mg, overall yield for condensation of **9**, **11** and **18** - 49% ; LSIMS (nba) 2823.4 (M-H)$^-$; ^{31}P NMR (CDCl$_3$); -7.26 ppm (m); Umnm^{5}s^{2}UUUA (**4a**) 47 mg, overall yield for condensation of **7** and **13** - 57% ; LSIMS (nba) 2839.1 (M-H)$^-$; ^{31}P NMR (CDCl$_3$); -7.36 ppm (m); Us^{2}UUUA (**5a**) 29 mg, overall yield for condensation of **8** and **13** - 66%; LSIMS (nba) 2700.4 (M-H)$^-$; ^{31}P NMR (CDCl$_3$); -7.23 ppm (m); UUUUA (**6a**) 80 mg, overall yield for condensation of **9** and **13** - 69% ; LSIMS (nba) 2684.3 (M-H)$^-$; ^{31}P NMR (CDCl$_3$); -7.11 ppm (m).

General procedures for oligoribonucleotides deprotection. Deprotection of the fully protected pentamers **1a** - **3a**. The pentamer (5 μmol) was treated with 5 ml of 0.1 M sodium hydroxide in pyridine / water (1:1 v/v) for 3 h at room temperature, the solution was passed slowly through a column of Dowex-50 X (pyridinium salt) and the column was washed with pyridine / water (1:1). The eluent and washings were combined and evaporated under reduced pressure. After coevaporation with toluene the oligoribonucleotide was dissolved in 30 ml of 0.015 M HCl in water / methanol (4:1 v/v) and left at room temperature for 30 h. Then the 0.1 M aqueous ammonia was added to neutralize the solution and the solvents were evaporated in *vacuo*. The residue was purified on Whatman 3MM papers (solvent system F). After lyophilization 3.5 mg of **1** (41.3%), 3.7 mg of **2** (44.8%), and 5.1 mg of **3** (62.3%) were obtained. HPLC: R_t= 13.37 for **1**; R_t= 33.21 for **3**; LSIMS (glycerol): 1694 $(M-H)^-$, 1716 $(M+Na-2H)^-$ for **1**; 1651 $(M-H)^-$, 1673 $(M+Na-2H)^-$ for **2**; 1635 $(M-H)^-$, 1657 $(M+Na-2H)^-$ for **3**.

Deprotection of the fully protected pentamers **4a** - **6a**. A fully protected oligomer (5 µmol) was dissolved in 5 ml of concentrated aqueous ammonia / pyridine (2:1 v/v). The reaction mixture was kept at room temperature for 48 h, then at 37 °C for 24 h. The solution was concentrated to dryness in *vacuo*, the residue was dissolved in water and washed with diethyl ether. The aqueous layer was evaporated to dryness and the residue was subjected to the removal of acid labile groups (MMTr, Thp, methoxymetylidene) according to the procedure described above. Purification of the product on Whatman 3MM papers (solvent system F) afforded 3.1 mg of **4** (40%), 2.8 mg of **5** (36.6%),and 3.0 mg of **6** (38.6%). HPLC: R_t= 8.54 for **4**; R_t= 15.73 for **6**; LSIMS (glycerol): 1549 $(M-H)^-$, 1561 $(M+Na-2H)^-$ for **4**; 1529 $(M+Na-2H)^-$ for **5**; 1512 $(M+Na-2H)^-$, 1528 $(M+K-2H)^-$ for **6**.

Enzymatic digestion and compositional analysis of oligoribonucleotides 1-6. 20 µg of each pentamer was treated with nuclease P1 and alkaline phosphatase and the mixture of nucleosides was analyzed by HPLC (Supelcosil LC-18S column, 250x4.6 mm). Elution was done using two solvents system: A: 0.01M KH_2PO_4, pH 5.4; B: 0.01M KH_2PO_4 :methanol - 80:20 as follows: 0-7 min 0% B, 7-15 min 10% B, 15-20 min 25% B, 20-27 min 60% B, 27-45 min 100% B. Authentic samples of U, s^2U, mnm^5s^2U, and t^6A were used as references. Hydrolysis of all pentamers **1-6** led to composition of nucleosides in the expected molar ratio (3.27 U : 1.0 mnm^5s^2U : 1.1 t^6A for **1**, 3.21 U : 1.0 s^2U : 1.08 t^6A for **2**, 4.17 U : 1.0 t^6A for **3**, 3.18 U : 1.0 mnm^5s^2U : 1.08 A for **4**, 3.23 U : 1.1 s^2U : 1.0 A for **5**, 4.12 U : 1.0 A for **6**)

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