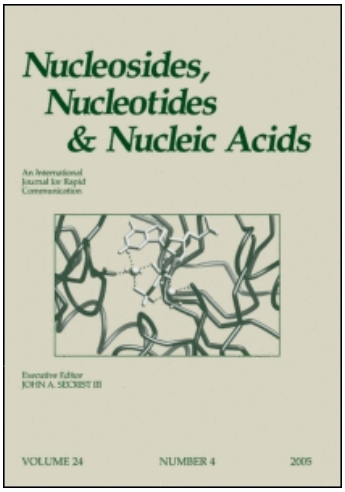

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Studies on Synthesis and Structure of *O*-β-D-Ribofuranosyl(1''→2')-ribonucleosides and Oligonucleotides

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STUDIES ON SYNTHESIS AND STRUCTURE OF *O*-β-D-RIBOFURANOSYL(1"→2')RIBONUCLEOSIDES AND OLIGONUCLEOTIDES*

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ABSTRACT: Minor nucleosides found in several eukaryotic initiator $tRNAs_i^{Met}$, *O*-β-D-ribofuranosyl(1"→2')adenosine and -guanosine (*Ar* and *Gr*), as well as their pyrimidine analogues, were obtained from *N*-protected 3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)ribonucleosides and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in the presence of tin tetrachloride in 1,2-dichloroethane. A crystal structure has been solved for 2'-*O*-ribosyluridine. The 3'-phosphoramidites of protected 2'-*O*-ribosylribonucleosides were prepared as the reagents for 2'-*O*-ribofuranosyloligonucleotides synthesis. *O*-β-D-Ribofuranosyl(1"→2')adenylyl(3'→5')guanosine (*ArpG*) was obtained and its structure was analysed by NMR spectroscopy.

INTRODUCTION

Recently two novel modified nucleotides were found in eukaryotic cytoplasmic initiator methionine tRNAs in position 64 [1,2]. These nucleotides were identified as β-anomers of *O*-ribosyl(1"→2')-5"-phosphates of adenosine (*Ar(p)*, **1**) and guanosine (*Gr(p)*, **2**) respectively.

*This paper is dedicated to the late Professor Tsujiaki Hata.

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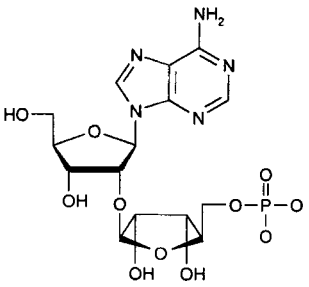
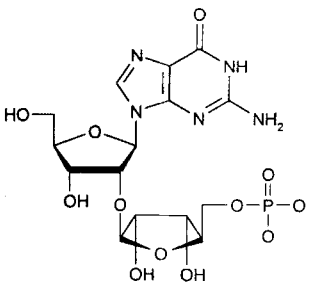
The 2'-*O*-phosphoribosylation was suggested to be a possible general feature of cytoplasmic $tRNAs_i^{Met}$ in plants and fungi that may control discrimination between their initiator *vs.* elongator function [3].

In order to obtain a better insight to this matter we undertook detailed studies on synthesis and structure of 2'-*O*-ribosyl nucleic acids and their components. We reported recently preliminary results of the studies on the synthesis of non-phosphorylated 2'-*O*-ribosyladenosine (*Ar* , **6a**) [4]. In this communication syntheses of ribosyl derivatives of four ribonucleosides (**6a-d**) are described.



1, *Ar(p)*



2, *Gr(p)*

Studies on ribosylated nucleosides and disaccharide nucleoside derivatives following the synthetic scheme starting with the tetraisopropyldisiloxane nucleoside substrates reported by us were recently followed by Mikhailov et al.[5,6].

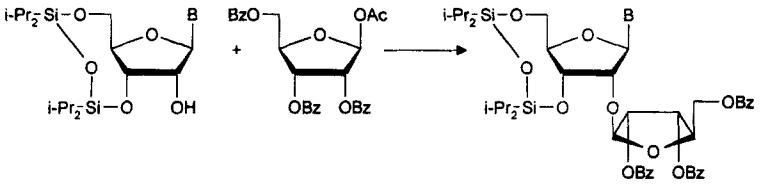
RESULTS AND DISCUSSION

Synthesis of 2'-*O*-ribosylribonucleosides

3',5'-*O*-Tetraisopropyldisiloxane (TIPDSi) derivatives of nucleosides proved to be useful intermediates in the synthesis of many groups of nucleic acids components [7-9]. We have found yet another application of the TIPDSi group in protecting of hydroxyl functions during acetalisation of 2'-hydroxyl group activated by tin tetrachloride [4,10].

Thus, 6-*N*-benzoyl-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)adenosine (**3a**) [7] was reacted with slight molar excess of 1-*O*-acetyl-2,3,5-*O*-tribenzoyl-β–D-ribofuranose (**4**) pre-activated with tin tetrachloride in 1,2-dichloroethane. The reaction was completed at

room temperature in 1 hr and after aqueous work-up and purification by silica gel chromatography fully protected 2'-*O*-ribosyladenosine (**5a**) was obtained in 62% yield [4]. The protecting groups were removed by overnight treatment with aqueous ammonia in pyridine at 50 °C and 2'-*O*-ribosyladenosine (**6a**) was obtained in ca 82% yield after purification by silica gel chromatography using a mixture of aqueous isopropanol and ammonia.



3a, B = Ade^{Bz}
3b, B = Cyt^{Bz}
3c, B = Gua$^{i\text{-}bu}$
3d, B = Ura

4

5a, B = Ade^{Bz}
5b, B = Cyt^{Bz}
5c, B = Gua$^{i\text{-}bu}$
5d, B = Ura

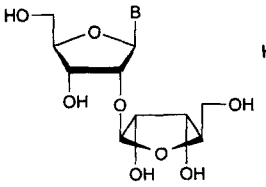
The same procedure was applied for the other TIPDSi derivatives of ribonucleosides (**3b**, **3c**) resulting in 2'-*O*-ribosylcytidine (**6b**) and 2'-*O*-ribosylguanosine (**6c**).

However, the results of synthesis of the uridine derivative **6d** were more complex than for other nucleosides. ^{1}H NMR spectrum of a chromatographically homogenous **6d** revealed a presence of a minor product (ca 20%) and for this compound the structure of 5'-ribosyl isomer (**7**) was proposed. In order to check this hypothesis appropriately 2',3'-*O*-TIPDSi protected uridine derivative (**8**) was prepared and reacted with **4** under the same conditions as before. The removal of the protecting groups from the reaction product **9** gave an expected 5'-isomer **7** as shown by NMR analysis. The isomer **7** was found to co-migrate with **6d** in all chromatographic system checked by us. Similarly, fully protected isomers **5d** and **9** have the same R_f values under different chromatographic conditions. This explains why the applied purification procedures lead to the impure product. However, the partial deprotection of the TIPDSi group from **5d** and **9** leads to intermediates that differ during a silica gel chromatography in isopropanol-ammonia-water systems. The chromatographic purification at this stage of the synthesis allowed to obtain pure **6d**.

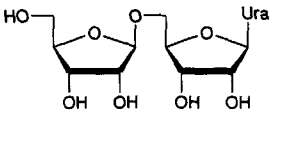
The formation of 5'-ribosyluridine (7) from **3d** can be explained by the reaction of isomerisation of the TIPDSi group from 3',5' to 2',3' positions under the acidic conditions (Lewis type acid) of the acetalisation reaction in the presence of tin tetrachloride. In our earlier studies of isomerisation of the TIPDSi group it was shown that under anhydrous acidic conditions 3',5'-*O*-TIPDSi ribonucleosides undergo the transformation to more stable 2',3'-isomers and that this reaction is especially facile for the uridine derivative **3d** [9,11]. The above isomerisation of the TIPDSi group takes place for guanosine derivative **3c** [9]. We did not study this process in details during the synthesis of **5c**. However, the formation of guanine analogue of **7** cannot be excluded as the yield of **5c** was moderate (40%). The chromatographic properties of the 5'-isomer of **5c** most probably differ from those of the main product **5c** otherwise similar difficulties could be encountered for guanine and uracil derivatives.

The above observations allow to conclude that the 5',3' to 2',3'-*O*-TIPDSi isomerisation is rather fast process as the formation of 2',5'-bis-*O*-ribosyl products was not detected. This is in agreement with the intramolecular mechanism of the 5',3' to 2',3'-*O*-TIPDSi isomerisation proposed previously [11].

The NMR spectra of fully protected derivatives (**5a-d**) corroborated their structures. The ^{1}H NMR spectra of deprotected 2'-*O*-ribosylribonucleosides (**6a-d**) were in agreement with our earlier collected data [4] and the data published by others [5]. ^{13}C NMR data are collected in TABLE 1.



6a, B = Ade
6b, B = Cyt
6c, B = Gua
6d, B = Ura



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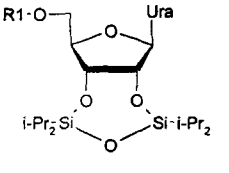


8, R1 = H
9, R1 = ribBz3

ribBz3 = 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl-1

X-ray and NMR analysis of 2'-*O*-ribosyluridine

2'-*O*-Ribosyluridine (**6d**) was obtained in crystalline form from water allowing to solve its structure by x-ray analysis. Thus, the labelling of the atoms and a thermal-ellipsoid representation of **6d** is shown in FIG. 1.

TABLE 1. ^{13}C NMR chemical shifts of compounds **6a-d** recorded in D_2O. Chemical shifts are referenced to internal dioxane ($\delta_{dioxane}$ = 67.4 ppm).

Compound	6a	6b	6c	6d
C-2	153.03	158.06	154.68	152.62
C-4	149.14	166.72	152.02	167.20
C-5	119.81	97.54	117.42	103.47
C-6	156.26	142.86	159.73	142.92
C-8	141.63	-	139.02	-
C-1'	87.70	89.14	87.29	88.86
C-2'	79.03	79.30	78.75	79.30
C-3'	69.81	69.31	69.76	69.27
C-4'	87.21	85.53	86.86	85.51
C-5'	62.23	61.76	62.21	61.59
C-1"	106.84	107.40	106.95	107.67
C-2"	75.12	75.25	75.15	75.25
C-3"	71.67	71.67	71.86	71.56
C-4"	83.49	83.74	83.58	83.78
C-5"	63.54	63.92	63.84	63.74

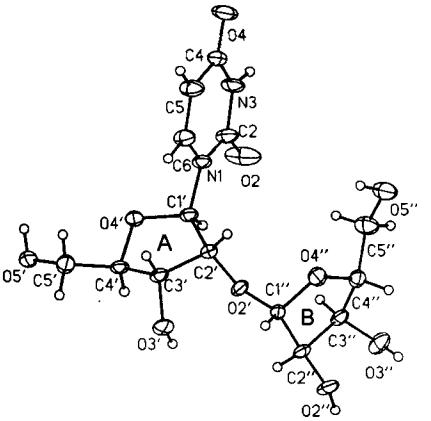


FIG. 1. Molecular structure of 2'-*O*-ribosyluridine (**6d**).

The bond distances and angles in the uracil moiety and in the furanose rings are in good agreement with the averaged values reported earlier. The ribose ring A is in C4'-exo ($_4$E) and ribose ring B is in C2''-exo ($_2$E) conformation. In terms of pseudorotation [12] the ribose conformation is characterised by P_A = 54.2(6)°, τ_A = 36.3(4)°, P_B = -16.0(8)°, τ_B = 38.7(6)°. The glicosidic torsion angle, which describes the relative orientation of the base with respect to the sugar, is in the anti region with χ = -114.7(3)° [torsion angle O(4')-O(1')-N(1)-C(2)]. The torsion angles ϕ_{OC} = O(5')-C(5')-C(4')-C(3') and ϕ_{OO} = O(5')-C(5')-C(4')-O(4') (-177.1(2), 68.0(3)° and -178.3(3), 63.7(3)° for ribose ring A and B respectively) indicate that the side chains are in *-ap* (*gauche, trans*) conformations. The three dimensional network of hydrogen bonds is characterised in TABLE 2.

The coupling constants of the sugar protons (TABLE 3) were interpreted in terms of pseudorotational parameters and population of N- and S-conformers using PSEUROT 6.0 program [13]. It was found that in solution uridine sugar exists in equilibrium between N- and S-type (P_N = 26, P_S = 141 $\varphi_N = \varphi_S$ = 36, %S = 52). In the case of 2'-*O*-ribosyl a high preference of 92% for N-type puckering (P_N = -13, φ_N = 36) was found.

The conformation of the glycosidic bond was established on the basis of NOEs. Strong NOEs of H-6 with H-3' and H-2' are observed that indicates *high-anti* conformation with a large portion of N-type puckering [14].

The sum of coupling constants $\Sigma J_{4'5'}+J_{4'5''}$ was used to derive the population of exocyclic torsion angle γ [15].

For the sugar of uridine moiety it was calculated that the population of *+sc* conformer is 52%. A large fraction (63%) of *-sc* and *ap* conformers are present in 2'-*O*-ribosyl moiety.

Synthesis of 3'-phosphoramidites of ribosylribonucleosides and their use in the preparation of *ArpG*

Phosphoramidites of 2'-*O*-ribosylribonucleosides had to be obtained and used in the synthesis of oligonucleotides containing modified ribosylribonucleoside units in order to get an insight into their structure and chemical properties. Previously we reported a synthesis of the dimer *ArpG* using the 5'-phosphoramidite of appropriately protected

TABLE 2. The geometry of hydrogen bonds in **6a**.

D-H···A	D-H [Å]	H···A [Å]	D···A [Å]	D-H···A angle[°]
N(3)-H(3)····O(2") (i)	0.79(5)	2.16(5)	2.943(4)	175(4)
O(3')-H3O'····O4 (ii)	0.89(4)	1.89(4)	2.762(8)	169(4)
O(3')-H3O'····O' (iii)	0.89(4)	2.44(4)	2.758(3)	101(3)
O(5')-H5O'····O5" (iv)	0.83(4)	1.94(4)	2.760(6)	175(4)
O(2")-H2O"····O5' (v)	0.85(4)	1.85(4)	2.695(6)	172(4)
O(3")-H3O"····O3' (vi)	0.82(6)	2.07(5)	2.813(6)	151(5)
O(5")-H5O"····O2 (vii)	0.87(7)	2.02(6)	2.782(4)	146(6)

Symmetry codes: (i) x, y, -1+z; (ii) 1+x, y, 1+z; (iii) x, y, z; (iv) -1-x, -1/2+y, -2-z; (v) -x, 1/2+y, -1-z; (vi) -1-x, 1/2+y, -1-z; (vii) -1+x, y, z.

TABLE 3. Values of $^3J_{HH}$ coupling constants of compounds *Ur* (**6a**) and *ArpG* (**15**) [Hz]

	Ur		ArpG		
	Ur	2'-*O*-ribosyl	Ar	pG	2'-*O*-ribosyl
J1'2'	5.2	<1	6.3	4.8	<1
J2'3'	5.3	4.9	5.0	5.2	4.5
J3'4'	4.9	7.3	2.5	5.1	7.5
J4'5'	3.4	3.4	2.3	2.8	3.6
J4'5"	5.2	6.7	2.8	5.1	7.2
J5'5"	-12.2	-12.1	-13.0	n. a.	-12.0

n. a. - not available

guanosine [4]. In the present study the preparation of 3'-phosphoramidites of all 2'-*O*-ribosylribonucleosides (**13a-d**) is described.

Fully protected crude ribosylribonucleosides (**5a-d**) obtained from the acetalisation reaction were first treated with tetra-*n*-butylammonium fluoride (TBAF) to remove the TIPDSi group. Then, crude desilylated partially protected ribosylribonucleosides **11a-d** were reacted with 4,4'-dimethoxytrityl chloride (DMTCl) in pyridine. The 5'-*O*-dimethoxytrityl derivatives **12a-d** were obtained after a silica gel chromatography in high overall yields. The phosphitylation of **12** with bis-*N*,*N*-diisopropylamino-2-cyanoetoxyphosphine [11] in the presence of tetrazole gave desired phosphoramidites of ribosylribonucleosides **13a-d**.

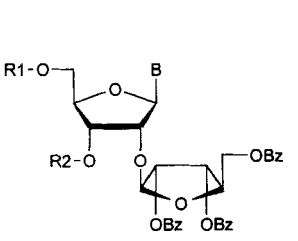
Thus, the dimer, *O*-β-D-ribofuranosyl(1"→2')adenylyl(3'→5')guanosine (*ArpG*, **15**), was synthesised by the phosphoramidite method [16] in solution using **13a** and 2-*N*-

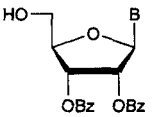
isobutyryl-2',3'-di-*O*-benzoylguanosine [4] (**14**) in the presence of acetonitrile. The oxidation with iodine in aqueous pyridine, purification by silica gel chromatography and final deprotection gave desired product **15** in 43% yield.

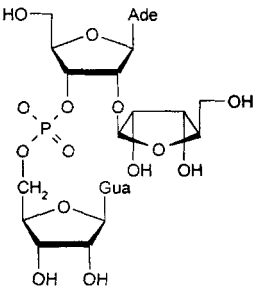
The stability of **15** in alkaline conditions indicates that hydroxyl functions of 2'-*O*-ribosyl residue cannot participate, as expected, in intramolecularly geared cleavage of internucleotide linkages. Therefore, 2'-*O*-ribosylation of RNA can be regarded from a chemical point of view as having the similar influence on the stability of internucleotide chains as e. g. 2'-*O*-methylation.

NMR analysis of *ArpG* (15)

Conformational characteristics of sugar rings of **15** was analysed on the basis of spin coupling constants (TABLE 3). It was found that for adenosine sugar S-type conformer (P_S = 151 φ = 38, %S = 68) is predominant while sugar of guanosine residue shows a preference for N-type puckering (P_N = 35, φ = 34) to slightly greater extent (%N = 54) than S-type (P_S = 167, φ = 34). 2'-*O*-Ribosyl exists almost exclusively as N-type (P_N = -11, φ = 38, %N = 94).







11a, B = Ade^{Bz}, R1 = R2 = H
11b, B = Cyt^{Bz}, R1 = R2 = H
11c, B = Gua$^{i\text{-}bu}$, R1 = R2 = H
11d, B = Ura, R1 = R2 = H

14, B = Gua$^{i\text{-}bu}$

15

12a, B = Ade^{Bz}, R1 = DMT, R2 = H
12b, B = Cyt^{Bz}, R1 = DMT, R2 = H
12c, B = Gua$^{i\text{-}bu}$, R1 = DMT, R2 = H
12d, B = Ura, R1 = DMT, R2 = H

DMT = 4,4'-dimethoxytrityl

13a, B = Ade^{Bz}, R1 = DMT, R2 = P(III)
13b, B = Cyt^{Bz}, R1 = DMT, R2 = P(III)
13c, B = Gua$^{i\text{-}bu}$, R1 = DMT, R2 = P(III)
13d, B = Ura, R1 = DMT, R2 = P(III)

P(III) = P(OCH2CH2CN)N(i-Pr2)

The analysis of sum of coupling constants $\Sigma J_{4'5'}+J_{4'5''}$ points out to the existence of a predominately +*sc* conformer for γ angle of adenosine (88%) and guanosine (55%)

sugar rings. A large fraction (70%) of *-sc* and *ap* conformers is observed for the 2'-*O*-ribosyl moiety.

For adenosine residue NOE was observed from H-8 to H-2'. This is consistent with *anti* orientation about the glycosidic bond and preferred S-type conformation of the sugar ring. In case of guanosine residue NOEs were observed from H-8 to H-2' and H-3'. This corresponds to a *high-anti* conformation of glycosidic bond.

CONCLUSIONS

Methods of chemical synthesis of 2'-*O*-ribosylribonucleosides and their introduction into oligonucleotide chains were developed. Oligoribonucleotides containing 2'-*O*-ribosylribonucleoside units are stable under alkaline conditions. Analysis of X-ray and NMR data for 2'-*O*-ribosyl nucleoside derivatives indicates a preferred conformation of N-type (C2'-exo) for 2'-*O*-ribosyl moiety.

EXPERIMENTAL

General methods

All the solvents used in the reaction were purified and dried according to the earlier published procedures. 1,3-Dichlorotetraisopropyldisiloxane [7] ($TIPDSiCl_2$) (Ifotam Ltd. Łódź, Poland), trimethylsilyl chloride (TMSCl) (POCh, Poland), benzoyl chloride (POCh), tin tetrachloride (Aldrich), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (Pharma Waldhof, Germany) were used directly. Tetra-*n*-butylammonium fluoride (TBAF) was prepared from tetra-*n*-butylammonium hydroxide (Fluka, Switzerland) according to ref.[17] and was stored as 1 M solution in tetrahydrofuran in a plastic bottle. 3',5'-*O*-Tetraisopropyldisiloxane *N*-protected nucleosides (**3a-3d**) were obtained as previously described. ^{1}H NMR spectra were recorded on a Varian Unity 300 spectrometer operating at 299.9 MHz. Chemical shifts (ppm) were measured using tetramethylammonium chloride (TMA; δ = 3.18 ppm) for D_2O otherwise tetramethylsilane (TMS) was used as an internal standard. ^{13}C NMR spectra were recorded at frequency 75.4 MHz. ^{13}C chemical shifts were referenced to TMS. Dioxane was used as reference (δ = 67.4 ppm) for D_2O solutions. UV-VIS spectra were measured on Beckman DU-65 spectrophotometer. Thin layer chromatography was performed on: (i) E. Merck pre-coated plates silica gel 60 HF_{254} in the following solvent systems: S1 - chloroform-methanol (8:2); S2 - chloroform-methanol (9:1); S3 - chloroform-methanol (95:5); S4 - *n*-hexane-chloroform-methanol (20:75:5); S5 - isopropanol-concentrated aqueous ammonia-water (7:1:2); S6 acetone-*n*-hexane-triethylamine (45:45:10); S7 acetone-water (7:3); (ii) Machery-Nagel cellulose plates in the solvent C - isobutyric acid-25% aqueous ammonia-water (50:1.1:28.9). All solvent ratios were by volume. Short column chromatography was performed on silica gel Merck H 60. The organic extracts were dried with anhydrous sodium sulphate.

X-ray Analysis

6d was crystallised from water by slow cooling (from 60° to 21 °C) using the hot box method.

The crystals belong to the space group $P2_1$ with a = 4.97(2), b = 16.18(2), c = 10.13(4) Å and β = 106.40(1)°. Diffraction data were collected on the 180 mm Mar Research image plate detector using synchrotron radiation from the EMBL beamline X31 at the DORIS storage ring, DESY, Hamburg. The wavelength of the radiation was 0.7 Å. The crystal was rotated around the axis approximately parallel to the crystal a* axis.

The range of reciprocal space of 180° was covered by 90 images of Δφ = 2.0°. Additional 37 (Δφ = 4°) and 30 (Δφ = 5°) images were collected at the low resolution data and to cover the data that were overloaded on the previous images respectively. The data consisted of 8842 raw measurements which reduced to 1292 unique reflections. Programs DENZO and SCALE PACK [18] were used for data reduction and scaling.

The structure was solved by direct methods [19], 1287 reflections with I≥2δ(I) were used to refine it by full matrix least squares using SHELXL program [20].

All hydrogen atoms were found on a difference Fourier map and refined with isotropic thermal parameters. Anisotropic thermal parameters were applied for all non-hydrogen atoms. The refinement converged to R = 2.98%.

General Procedure of the Synthesis of *N*-Protected 3',5'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2'-*O*-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)nucleosides (5a-5d). A solution of tin tetrachloride in anhydrous 1,2-dichloroethane (0.4 M, 11.25 mL) was added with a syringe to **4** (1 g, 1.98 mmole) in a 20 ml sealed glass serum bottle. After 0.5 hr this solution was added to the solution in anhydrous 1,2 dichloroethane (0,4 M) of: **3a** (923 mg, 1.5 mmole); **3b** (883 mg, 1.5mmole); **3c** (891 mg, 1.5 mmole); **3d** (729 mg, 1,5 mmole). The TLC analysis showed that the reaction went to completion in 0.5 hr: for **5a** R_f (S2) 0.81, R_f (S3) 0.73 and R_f (S4) 0.61; for **5b** R_f (S4) 0.84; for **5c** R_f (S3) 0.43; for **5d** R_f (S3) and R_f (S4) 0.65, 0.6.

Then the reaction mixture was partitioned between saturated aqueous $NaHCO_3$ and dichloromethane. The precipitate was formed in an interphase and the mixture was centrifuged to separate layers. The organic layer was dried and the residue after concentration under reduced pressure was purified by silica gel chromatography using following solvent mixtures: firstly, dichloromethane (33 to 50%) in *n*-hexane and secondly, methanol (for **5a, 5b** 0.1 to 0.6%; for **5c** 0.1 to 1%; for **5d** 0.1 to 0.8%) in *n*-hexane-dichloromethane (1:1) to give as a white foam: **5a**, 981 mg, 0.93 mmole, 62% yield; **5b**, 1.23 g, 1.15 mmole, 76.7% yield; **5c**, 682 mg, 0.73 mmole, 40% yield; **5d**, 1.13 g, 1.22 mmole, 71% yield.

5a: FAB-MS: *m/z* $M+H^+$ 1058.4, calc. for $C_{55}H_{64}N5O_{13}Si_2$: 1058.47; ^1H NMR ^1H NMR (CD_3CN): δ (ppm) 8.19 (s, 1, H-2), 7.38 (s, 1, H-8), 8.04-7.36 (m, 20, 4xBz), 6.12 (s, 1, H-1'), 5.89 (t, 1, H-3"), 5.83 (d, 1, H-2"), 5.82 (s, 1, H-1"), 5.04-4.97 (m, 2, H-2',3'), 4.80-4.55 (m, 3, H-4",5") 4.12-3.97 (m, 3, H-4',5'), 1.05 (m, 28, TIPDSi).

5b: 1H NMR ($CDCl_3$): δ (ppm) 8.10-7.28 (m, 21, 4xBz,H-6), 5.98 (s, 1, H-1'), 5.94 (m, 1, H-3"), 5.90 (s, 1, H-1"), 5.82 (d, 1, H-2"), 5.69 (d, 1, H-5), 4.86-4.75 (m, 3, H-4",5"), 4.49 (d, 1, H-2'), 4.29-4.23 (m, 2, H-3',4'), 4.18 (d, 1, H-5'), 3.97 (d, 1, H-5'), 1.11-0.96 (m, 28, TIPDSi).

5c: ^{1}H NMR ($CDCl_3$): δ (ppm) 9.90(s, 1, N-H), 8.05 (s, 1, H-8), 8.00-7.26 (m, 15, 3xBz), 6.14 (t, 1, H-3"), 5.89 (d, 1, H-2"), 5.85 (s, 1, H-1'), 5.76 (s, 1, H-1"), 4.89 (m, 1, H-5"), 4.78 (m, 1, H-4"), 4.66-4.56 (m, 2, H-5",3'), 4.29-4.25 (m, 2, H-2',4'), 4.75 (d, 1, H-5'), 4.20 (d, 1, H-5'), 2.89 (m, 1, CH-ibu), 1.35, 1.25 (d, 6, CH_3-ibu), 1.12-0.96 (m, 28, TIPDSi).

5d: ^{1}H NMR ($CDCl_3$): δ (ppm) 8.10-7.28 (m, 21, 4xBz, H-6), 5.98 (s, 1, H-1'), 5.94 (m, 1, H-3"), 5.90 (s, 1, H-1"), 5.82 (d, 1, H-2"), 5.69 (d, 1, H-5), 4.86-4.75 (m, 3, H-4",5"), 4.49 (d, 1, H-2'), 4.29-4.23 (m, 2, H-3',4'), 4.18 (d, 1, H-5'), 3.97 (d, 1, H-5'), 1.11-0.96 (m, 28, TIPDSi).

General Procedure of Removal of Protecting Groups to Give *O*-β-D-Ribofuranosyl(1"→2')ribonucleosides (6a-d). **5a** (525 mg, 0.5 mmole), **5b** (483 mg, 0.5 mmole), **5c** (547 mg, 0.5 mmole), **5d** (500 mg, 0,5 mmole) was dissolved in THF (0.9 mL) and TBAF/THF (1 M, 1.1 mL) was added. The TLC analysis showed that the desilylation was completed in ca 20 min.: for desilylated **5a** R_f (S2) 0.44; for desilylated **5b** R_f (S3) 0.25; for desilylated **5c** R_f (S2) 0.47; for desilylated **5d** R_f (S3) 0.38. Then pyridine (1.5 mL) and concentrated aqueous ammonia (1 mL) were added and the tightly closed bottle was kept at 50 °C overnight. Then, the reaction mixture was concentrated and the traces of pyridine were removed by co-evaporation with ethanol. The residue was purified by silica gel chromatography with solvent mixtures containing 5% of concentrated aqueous ammonia and isopropanol (90 to 75%) with water (5 to 20%). The fractions containing the pure product were concentrated, dissolved in water and desalted by passing through G-25 Sephadex column (1.5x15 cm) and lyophilised: **6a**, 171 mg, 82.8 % yield, R_f (S5) 0.6 and R_f (C) 0.77; **6b**, R_f (S5) 0.32, 107 mg, 52.7 % yield; **6c**, R_f (S5) 0.43, 190 mg, 80 % yield.

EI-GC/MS of the TMS derivative of **6a** (*m/z* and *r. i.*) 334, 0.84; 512, 9.53; 523, 0.05; 598, 0.30.

In the case of **6d,** desilylated **5d** was purified by silica gel chromatography with isopropanol-ammonia-water mixture: desilylated **5a** R_f (S5) 0.74, desilylated **9** R_f (S5) 0.64. Then the deprotection with ammonia in pyridine was performed as for **6a-c**. Otherwise **6d** was contaminated with an isomer **7**. Thus, **6d** to give pure main product was crystallised from water: R_f (S5) 0.56, 170 mg, 0.43 mmole, 83% yield.

For ^{1}H and ^{13}C NMR data see RESULTS & DISCUSSION and TABLE 1.

2',3'-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-5'-*O*-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)uridine (9). The solution of tin tetrachloride in anhydrous 1,2-dichloroethane (0,4 M; 7.5 ml) was added with syringe to **4** (660 mg; 1.3 mmoles) in 20 mL sealed glass serum bottle and was left at room temperature for 0.5 hr and then added to **8** (470 mg; 1 mmole) partially dissolved in 1,2-dichloroetane. The solution became homogenous. The TLC analysis showed that the reaction went to completion in 1.5 hr: R_f of the product **9** (S4) 0.6. Then reaction mixture was partitioned between water and dichloromethane. The organic layer was dried and concentrated under reduced pressure and residue was purified by silica gel chromatography in dichloromethane- methanol mixture (up 0.5%) to give **9** (550 mg; 61% yield).

***O*-β-D-Ribofuranosyl(1"→5')uridine (7).** **9** (0.2 mmoles; 200 mg) was dissolved in THF (0.36 mL) and TBAF/THF (1M; 0.44 mL) was added. The TLC analysis showed,

that the desilylation was completed after 15 min R_f (S3) 0.32, R_f (S5) 0.83. Next, the crude was purified by silica gel chromatography in dichloromethane-methanol (up to 3%). Then to the pure compound pyridine (1.5 mL) and concentrated aqueous ammonia (1.5 mL) were added and the tightly closed bottle was kept at 50 °C overnight. Next, the reaction mixture was concentrated and traces of pyridine were removed by co-evaporation with ethanol. The residue was purified by RP-2 silica gel column chromatography in water to give **7**, R_f (S5) 0.48, R_f (S7) 0.97 (25 mg; 32% yield); ^{1}H NMR (D_2O):δ(ppm): 7.81(d, 2, J = 5.4Hz, H-6), 5.89 (s, 1, H-1'), 5.87 (d, 1, J = 5.4, H-5), 5.05 (s, 1, H-1'), 4.32 (t, 1, J = 5.3, H-2'), 4.27-4.17 (m, 3, H-3', H-3", H-4'), 4.1 (m,2, H-51', H-2"), 4.04 (m, 1, H-4"), 3.8 (m, 1, H-51"), 3.69 (m ,1,H-52) 33.60 (m, 1, H-52"). ^{13}C NMR (D_2O) :δ(ppm): 167.10 (C-4), 152.31, (C-2), 142.41 (C-6), 102.92 (C-5), 102.02 (C-1"), 89.21 (C-1'), 86.12 (C-4'), 84.87 (C-4"), 79.30 (C-2'), 72.25 (C-2"), 70.51 (C-3"), 69.57 (C-3"), 62.28 (C-5"), 61.16 (C-5').

5'-*O*-Dimethoxytrityl-2'-*O*-(tri-2,3,5-*O*-benzoylribosyl-1)nucleoside 3'-*O*-(2-cyanoethyl)-*N,N*-diisopropylphosphoramidites (13a-d). Crude **5** (from reaction 0.6 mmoles **3**) was dissolved in THF (1.1 mL) and TBAF/THF (1M; 1.35 mL) was added. When the TLC analysis showed that the reaction went to completion (15 min) the reaction mixture was partitioned between $NaHCO_3$ and dichloromethane. The organic extracts were dried and concentrated under reduced pressure. The residue was dried by co-evaporation with anhydrous pyridine and dissolved in pyridine (3 mL) and DMTCl (256 mg) was added. TLC analysis showed complete the reaction of tritylation in 2 hr. The reaction mixture was partitioned between $NaHCO_3$ and dichloromethane. The combined extracts were dried and concentrated. The residue was purified by silica gel chromatography using methanol (0.5%) in dichloromethane as a solvent to give pure **12**: for **12a,** 68% yield, R_f (S3) 0.7, R_f (S_6) 0.57; for **12b,** 70% yield, R_f (S3) 0.72, R_f (S_6) 0.67; for **12c,** 86% yield, R_f (S2) 0.53; for **12d,** 61% yield, R_f (S3) 0.59.

12 (0.4 mmole) and tetrazole (0.36 mmole, 0.9 equiv) were dried under reduced pressure during ca 12 hr. Bis(*N,N*-diisopropylamino)(2-cyanoetoxy)phosphine (1.1 equiv; 0.45 mmole; 0.143 mL) was added to **12** dissolved in anhydrous dichloromethane (3 mL) under argon atmosphere. Next, tetrazole was added in small portions under argon atmosphere. The TLC analysis showed the completion of the phosphitylation in 4 hr. The reaction mixture was purified by silica gel column chromatography with the solvent mixture: n-hexane 67%-37%; ethyl acetate 40%-60%; triethylamine 3% gave pure products after lyophilisation from benzene: **13a**; yield 80%; R_f (S3) 0.76; (S6) 0.64; ^{31}P NMR (ppm) 150.608; 150.280. **13b**; yield 78%; R_f (S6) 0.75; ^{31}P NMR (ppm) 151.341; 149.585. **13c**; yield 63%; R_f (S6) 0.43; ^{31}P NMR (ppm) 149.998; 150.898. **13d**; yield 63%; R_f (S7) 0.30; ^{31}P NMR (ppm) 151.002; 149.918.

Dimer *ArpG* (15). 13a (60 mg, 44 μmole, 1 equiv) and **14** (49 mg, 88 μmole, 2 equiv) with tetrazole (15.5 mg, 220 μmole, 5 equiv) were dried during 3 hr in separate flasks. Then, dry acetonitrile (300 μL for **15a** and 800 μL for **14**) and molecular sieves (3A) were added and the solutions were left at room temperature during 2 hr. Then the solution of **14** and tetrazole was added to the solution of **13a**. The TLC analysis showed the completion of condensation after 1.5 hr. The resultant P^{III} dimer was oxidised with the 5% solution of iodine in pyridine-water (98:2) (0.50 ml). After 20 min. saturated aqueous Na_2SO_3 (1.4 mL) was added. The reaction mixture was partitioned between saturated

$NaHCO_3$ and dichloromethane. The organic layer was dried, concentrated under reduced pressure and the crude fully protected dimer *ArpG* was purified by silica gel short column chromatography (70-230 mesh) using methanol (2%) in dichloromethane as solvent: 25 mg; 0.014 mmole; R_f (S3) 0.52). Then, pyridine (2 mL) and concentrated aqueous ammonia (4 mL) were added to fully protected dimer product and tightly closed bottle was kept at 50 °C overnight. The TLC analysis showed that reaction was completed: R_f (S5) 0.55. Then the reaction mixture was concentrated and the traces of pyridine were removed by co-evaporation with ethanol. Next 80 % aqueous acetic acid (2 mL) was added to crude of reaction. After 0.5 hr the TLC analysis showed that the detritylation was completed: R_f (S5) 0.37. Crude of reaction was dissolved in pyridine-water-ethanol (0.5 mL of each) and was purified on the plate for preparative chromatography (Merck preparative TLC plate with silica gel 60 F_{254}, 20x20 cm) in isopropanol-ammonia-water (7:1:2) during 9hr. Then silica gel band with **15** was eluted with water. The extracts were concentrated under reduced pressure and product was desalted by Sephadex G-25 column to give fully deprotected dimer **15** (173 A_{260} o. d. units, 6 μmoles). The overall yield after deprotection, TLC purification and the Sephadex column was 43%.

^{1}H NMR (D_2O): δ(ppm) 8.26 (s, 1, H-8Ade), 8.17 (1, s, H-2Ade), 7.97 (s, 1, H-8Gua), 6.00 (d, 1, H-1'Ade), 5.86 (d, 1, H-1'Gua), 5.06 (s, 1, H-1"), 4.78 (m, 3, H-2'Gua, H-2'Ade, H-3'Ade), 4.52(t, 1, H-3'Gua), 4.31 (m, 2 ,H-4'-Ade, H-4'Gua), 4.16 (m, 3, H-51Gua, H-52Gua, H2"), 3.93(m, 1, H-3") 3.82 (m, 1, H-4"), 3.68 (m, 2, H-51Ade, H-52Ade,), 3.26 (m, 1, H-51"), 2.67 (m, 1, H-52"). ^{13}C NMR (D_2O): δ(ppm) 167.10 (C-4Ade), 159.45 (C-6Gua), 154.52 (C-2Gua), 152.31 (C-2Ade), 152.24 (C-4Gua), 142.41 (C-6Ade), 138.32 (C-8Gua), 117.16 (C-5Gua), 102.92 (C-5Ade), 89.21 (C-1'Ade), 88.42(C-1'Gua), 86.12 (C-4'Ade), 84.87 (C-4"), 84.03 (C-4'Gua), 79.30 (C-2'Ade), 74.11 (C-2'Gua), 72.25 (C-2"), 70.93 (C-3'Gua), 70.51 C-3"), 69.57 (C-3'Ade), 66.23 (C-5'Gua), 62.28 (C-5"), 61.16 (C-5'Ade). ^{31}P NMR (D_2O): δ(ppm) -0.85.

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