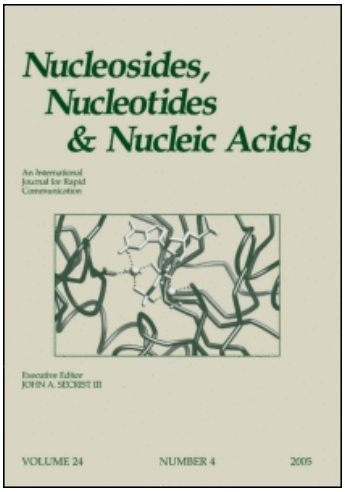

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Properties of O-β-D-Ribofuranosyl-(1''-2')-Adenosine-5''-O-Phosphate and Its Derivatives

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To cite this Article Rodionov, Andrei A. , Efimtseva, Ekaterina V. , Mikhailov, Sergey N. , Rozenski, Jef , Luyten, Ingrid and Herdewijn, Piet(2000) 'Synthesis and Properties of O-β-D-Ribofuranosyl-(1''-2')-Adenosine-5''-O-Phosphate and Its Derivatives', Nucleosides, Nucleotides and Nucleic Acids, 19: 10, 1847 — 1859
To link to this Article: DOI: 10.1080/15257770008045465
URL: http://dx.doi.org/10.1080/15257770008045465

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(10–12), 1847–1859 (2000)

SYNTHESIS AND PROPERTIES OF *O*-β-D-RIBOFURANOSYL-(1″- 2′)-ADENOSINE-5″-*O*-PHOSPHATE AND ITS DERIVATIVES

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ABSTRACT: The synthesis of *O*-β-D-ribofuranosyl-(1″-2′)-adenosine-5″-*O*-phosphate and its suitably protected derivative for oligonucleotide synthesis have been developed.

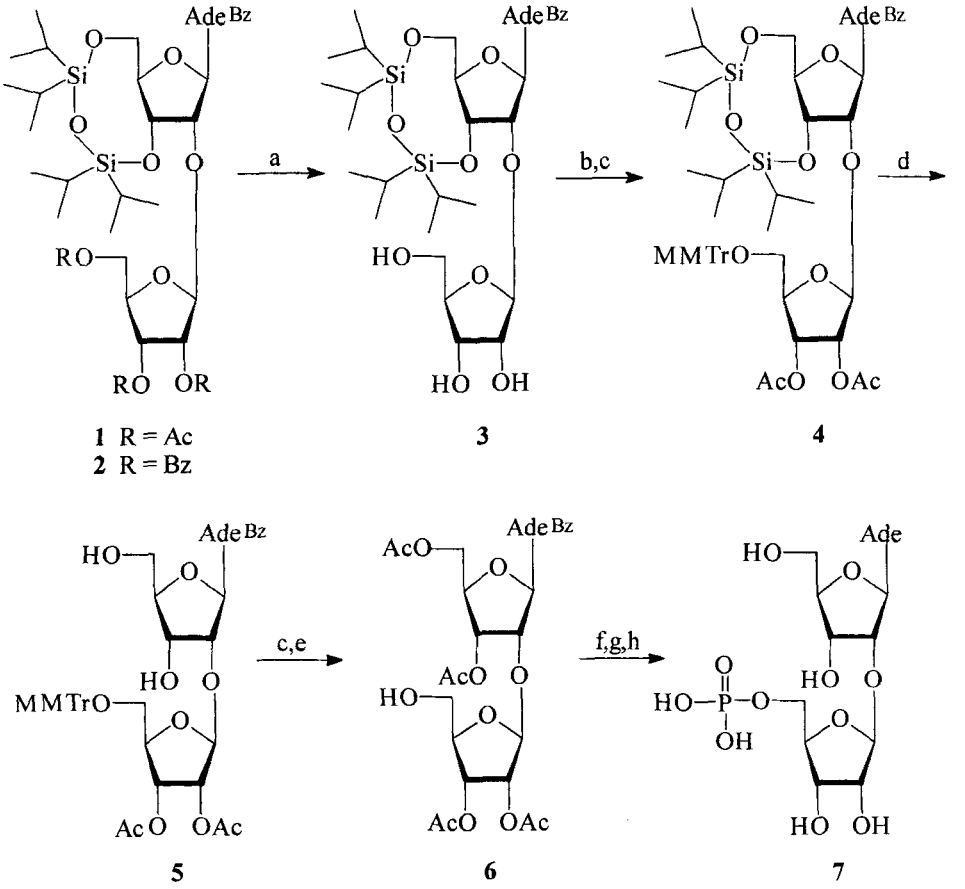
Recently a minor nucleoside was isolated from yeast methionine initiator tRNA and its structure was determined to be *O*-β-D-ribofuranosyl-(1″-2′)-adenosine-5″-*O*-phosphate[1-3]. We have developed a general route for preparation of *O*-β-D-ribofuranosyl-(1″-2′)-nucleosides (2′-*O*-β-D-ribofuranosyl-nucleosides)[4,5]. The selective blocking of five hydroxyl groups in these derivatives is an essential part in the preparation of the title compound. Here we present our results on the preparation of phosphate **7** (for the preliminary report see ref.[6]) and its protected derivative suitable for automated oligonucleotide synthesis.

Fully protected 2′-*O*-β-D-ribofuranosyladenosines **1** and **2** were prepared by condensation of N^6,3′,5′-*O*-protected adenosine with an excess of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose or 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in the presence of tin tetrachloride in dichloroethane (0°C, under nitrogen) according to the previously developed general method[4,5] (Scheme 1). The *O*-glycosylation reaction proceeded stereospecifically

This paper is dedicated to the memory of Professor Alexander Krayevsky.

with the formation of the β-glycosides. It should be mentioned that the yield of the disaccharide protected with *O*-benzoyl groups was higher (50% and 75% for **1** and **2** respectively).

Scheme 1



a. 0.1 M NaOMe; b. MMTrCl/Py; c. Ac_2O/Py; d. Bu_4NF/THF; e. 2% CF_3COOH in $CHCl_3$; f. $NC(CH_2)_2OPO_3H_2$/DCC/Py; g. NH_3/MeOH; h. 1M NaOH.

Treatment of **1** with 0.15 M MeONa in methanol for 10 min at 20°C gave **3** in 82 % yield. However, the deprotection of **2** proceeded more slowly and was accompanied by the formation of several by-products. The overall yields for these two steps using *O*-acetyl and *O*-benzoyl groups were comparable (41-44%). The 5′-hydroxyl group of the additional *O*-ribofuranosyl moiety in **3** was protected with a monomethoxytrityl group.

The conversion of **3** → **6** was achieved without difficulties using standard methods. The phosphorylation of **6** and subsequent deprotection gave **7** in good yield.

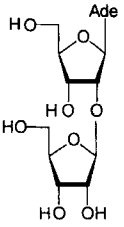
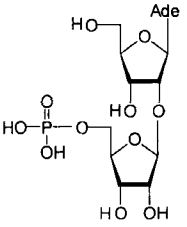
The structures of **1-7** were confirmed by 1D and 2D NMR spectroscopy (2D DQF-COSY[7]). Table 1 shows the ^{1}H chemical shifts and coupling constants of **7** and 2'-*O*-β-D-ribofuranosyladenosine[5]. The presence of the phosphate group in **7** was evident from characteristic downfield shift of H5'a and H5'b of the ribose moiety and their couplings with phosphorous ($J_{H,P}$) (Table 1). It may be concluded that introduction of the phosphate group slightly drives the conformation of additional *O*-ribofuranosyl moiety towards S-region.

It is stated in the literature[8,9] that the secondary phosphate ionization (pK 6.5) gives rise to a deshielding of the H-8 proton in 5'-purine nucleotides but not in 3'-nucleotides. We also observe at pH 9 and 11.5 that the H-8 proton shows a selective downfield shifts of 0.06 ppm compared with only a change of 0.02 ppm for the H-2 proton which probably indicates that the phosphate group is in a close proximity to H-8 proton. It should be mentioned that in 2'-*O*-β-D-ribofuranosylnucleosides exocyclic 5'-CH_2OH of additional 2'-*O*-ribose residue is located near heterocyclic base in solution and crystal[5].

Following a similar strategy, we have prepared *O*-β-D-ribofuranosyl-(1''-2')-adenosine-5''-*O*-phosphate synthon suitable for automated oligonucleotide synthesis (Scheme 2). For the protection of the additional phosphate residue p-nitrophenylethyl (NPE) group[10] was chosen. The 5'-monomethoxytrityl group in **4** was removed with trifluoroacetic acid in chloroform to give the nucleoside **8** which was condensed with bis(p-nitrophenylethyl)phosphate in the presence of 2,4,6-triisopropylbenzenesulphonyl chloride (TPSCl) and 1-methylimidazole to yield the phosphotriester **9** (72%).

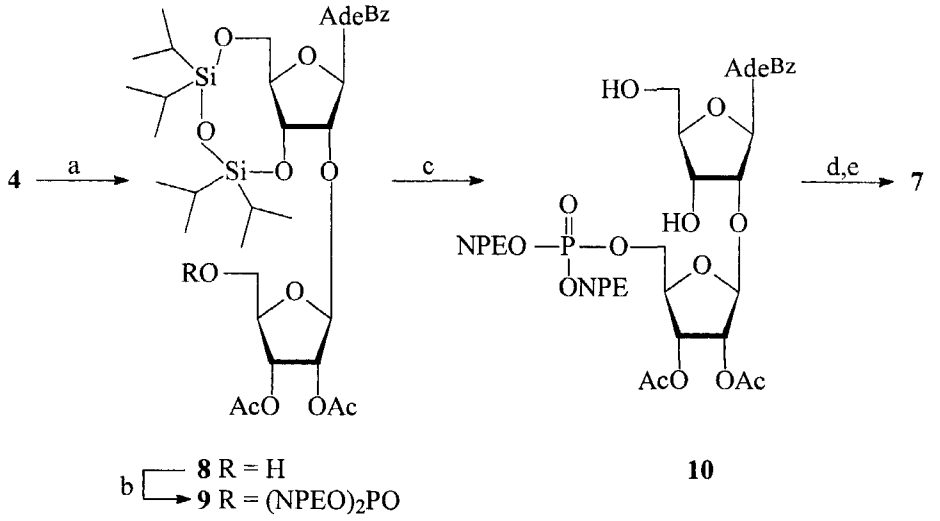
The first attempts to remove the 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl group as in the case of compound **4** were unsuccessful. The deblocking of fully protected triester **9** with tetrabutylammonium fluoride in tetrahydrofuran resulted in a low yield of **10** due to the partial cleavage of NPE groups during the product separation. Recently it was shown that 2-cyanoethyl protecting group on the phosphate is unstable under the desilylation conditions[11]. A modified deprotection procedure included trapping of residual tetrabutylammonium ions with Dowex-50 (Na^+-form)[12] before the silica gel column chromatography was used. Subsequent cleavage of the NPE groups in triester **10** using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dry pyridine followed by deacylation with ammonia in methanol gave free monophosphate **7** in high overall yield.

TABLE 1. ^{1}H NMR chemical shifts (ppm) and coupling constants (Hz) of **7** and 2′-*O*-β-D-ribofuranosyladenosine in D_2O at 27°C.

Compound			 **7**	
moiety → chemical shifts ↓	Ado	Rib	Ado	Rib
H-8	8.34 s		8.42s	
H-2	8.22 s		8.28 s	
H-1′	6.14 d	5.07 s	6.25 d	5.02 d
H-2′	4.82 dd	4.15 d	4.93 dd	4.20 dd
H-3′	4.57 dd	4.01 dd	4.63 dd	4.24 dd
H-4′	4.31 ddd	3.84 ddd	4.32 ddd	3.99 ddd
H-5′a	3.94 dd	3.34 dd	3.90 dd	3.73 ddd
H-5′b	3.86 dd	2.79 dd	3.87 dd	3.54 ddd
coupling constants ↓				
1′-2′	6.4	< 0.5	6.4	1.2
2′-3′	5.0	4.5	5.5	4.6
3′-4′	3.3	7.4	3.1	6.4
4′-5′a	2.6	3.7	2.7	4.3
4′-5′b	3.6	6.8	3.7	5.7
5′a-5′b	-13.0	-12.0	-12.8	-11.6
5′a-P				6.4
5′b-P				7.0

The structure of the compounds were supported by NMR spectroscopy and mass spectrometry. The chemical shifts were assigned using double resonance techniques and COSY experiments. Several conclusions were drawn from the ^{1}H NMR spectra analysis. In disaccharides **1**, **3**, **4**, **8** and **9** with 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl group the coupling constants $J_{1',2'}$ of both ribose and nucleoside moieties are less than 0.5 Hz. The removal of the silyl group and introduction of a bulky substitutent at the 5′-O-position of the additional ribofuranosyl residue resulted in significant conformational changes. The presence of monomethoxytrityl and bis-(p-nitrophenylethyl)phosphoryl groups in

Scheme 2



a. 2% CF_3COOH in $CHCl_3$; b. bis(p-nitrophenylethyl)phosphate/TPSCl/1-methylimidazole; c. Bu_4NF/THF; d. DBU/Py; e. NH_3/MeOH.

disaccharides **5** and **10,** respectively, increased the coupling constant $J_{1',2'}$ in the nucleoside moiety (7.0-7.2 Hz), whereas a less pronounced effect was observed in the ribofuranosyl residue ($J_{1',2'}$ = 2.2-2.4 Hz).

Using standard procedures, **10** was converted via its dimethoxytrityl derivative to the corresponding phosphoramidite. Its incorporation into oligonucleotides will be published shortly.

EXPERIMENTAL SECTION

NMR spectra were recorded using a Gemini 200 NMR spectrometer and a Varian 500 Unity spectrometer at 300 K. Chemical shifts were measured relative to the solvent signals. The coupling constants (*J*) are given in Hz. The signals were assigned using double resonance techniques and COSY experiments. The 2D DQF-COSY[7] consisted of 2048 datapoints in t_2 and 256 increments in t_1. The data were apodized with a shifted sine-bell square function in both dimensions and processed to a 2K x 1K matrix.

Mass spectrometry and exact mass measurements were performed on a quadrupole / orthogonal-acceleration time-of-flight tandem mass spectrometer (Q-Tof-2, Micromass,

Manchester, UK) equipped with a standard electrospray ionization (ESI) interface. Column chromatography was performed on silica gel (0.06-0.20 mm), TLC was carried out on Kieselgel 260 F (Merck) with detection by UV light using the following systems: chloroform (A); chloroform - ethanol, 98:2 (B); chloroform - ethanol, 96:4 (C); chloroform - ethanol, 85:15 (D); chloroform - methanol - triethylamine, 95:5:5 (E).

N^6-Benzoyl-9-[3,5-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-*O*-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]adenine (1). To a cooled solution (0°C) under nitrogen of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (1.27 g, 4 mmol) in 1,2-dichloroethane (25 ml), tin tetrachloride (0.59 ml, 5 mmol) was added and the solution was kept at 0°C for 10 min. After addition of N^6-benzoyl-9-(3,5-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl)adenine (1.23 g, 2 mmol), the resulting solution was kept at 0°C for 7 h. The reaction mixture was diluted with chloroform (25 ml), 10% aqueous solution of sodium bicarbonate (30 ml) was added and the suspension was stirred at 20°C for 20 min. The suspension was filtered through Hyflo Super Cel, the organic layer was separated, washed with water (20 ml), dried over Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography on silica gel (50 g). Elution with system A gave **1** as a foam. Yield 871 mg (50%). R_f 0.44 (B). +ESI MS: calcd. for $C_{40}H_{57}N_5O_{13}Si_2 + H^+$ 872.3569; found 872.3587. ^{1}H NMR ($CDCl_3$): 8.97 brs (1H, NH), 8.75 s (1H, H-8), 8.26 s (1H, H-2), 8.02 d (2H, Bz), 7.61 t (1H, Bz), 7.53 t (2H, Bz), 6.10 s (1H, H-1' Ado), 5.52 s (1H, H-1' Rib), 5.45 dd (1H, $J_{3',2'}$ = 5.0, $J_{3',4'}$ = 6.3, H-3' Rib), 5.43 d (1H, H-2' Rib), 4.89 dd (1H, $J_{3',2'}$ = 4.6, $J_{3',4'}$ = 9.3, H-3' Ado), 4.72 d (1H, H-2' Ado), 4.41 dd (1H, $J_{5'a,4'}$ = 2.6, $J_{5'a,5'b}$ = -10.7, H-5'a Rib), 4.33 ddd (1H, $J_{4',5'b}$ = 6.6, H-4' Rib), 4.30 dd (1H, H-5'b Rib), 4.21 dd (1H, $J_{5'a,4'}$ = 1.5, $J_{5'a,5'b}$ = - 13.4, H-5'a Ado), 4.13 ddd (1H, $J_{4',5'b}$ = 2.2, H-4' Ado), 4.03 dd (1H, H-5'b Ado), 2.09 s (3H, Ac), 2.07 s (3H, Ac), 2.04 s (3H, Ac), 1.11-1.03 m (28H, iPr). ^{13}C NMR ($CDCl_3$): 170.47, 169.64, 169.28 and 164.53 (C=O), 152.81 (C-2), 150.78 (C-6), 149.32 (C-4), 141.67 (C-8), 132.72, 128.86, and 127.82 (Bz), 123.80 (C-5), 105.48 (C-1', Rib), 88.82 (C-1', Ado), 81.52, 79.08 (C-4'), 78.67, 74.77 (C-2'), 71.57, 69.88 (C-3'), 64.93 (C-5', Rib), 59.93 (C-5', Ado), 20.78, 20.47 and 20.38 (Ac), 17.42, 17.34, 17.28, 17.19, 17.04, 16.89, 13.37, 12.95, 12.84 and 12.71 (iPr).

N^6-Benzoyl-9-[3,5-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-*O*-(β-D-ribofuranosyl)-β-D-ribofuranosyl]adenine (3).

Method A. A solution of nucleoside **1** (744 mg, 0.89 mmol) in 0.15 M sodium methylate in dry methanol (24 ml) was kept for 10 min at 20°C and then 10% acetic acid in methanol was added to pH 7.0. The resulting solution was concentrated *in vacuo* to dryness and the residue was partitioned between ethyl acetate (50 ml) and water (20 ml), the organic layer was washed with water (20 ml), dried over Na_2SO_4 and evaporated to dryness. The residue was applied to a column of silica gel (30 g). The column was washed with system A (200 ml) and then eluted with system B to give **3** as a foam. Yield 550 mg (82%). R_f 0.14 (C).

Method B. A solution of N^6-Benzoyl-9-[3,5-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-*O*-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]adenine (**2**)[5] (1.66 g, 1.56 mmol) in 0.15 M sodium methylate in dry methanol (42 ml) was kept for 40 min at 20°C and then 10% acetic acid in methanol was added to pH 7.0. After the usual work up, the residue was purified by column chromatography on silica gel (50 g). The column was washed with system A (300 ml) and then eluted with system B to give **3** as a foam. Yield 680 mg (58%). +ESI MS: calcd. for $C_{34}H_{51}N_5O_{10}Si_2 + H^+$ 746.3252; found 746.3250. ^1H NMR ($CDCl_3$): 9.28 brs (1H, NH), 8.76 s (1H, H-8), 8.43 s (1H, H-2), 8.03 d (2H, Bz), 7.60 t (1H, Bz), 7.52 t (2H, Bz), 6.11 s (1H, H-1' Ado), 5.33 s (1H, H-1' Rib), 4.76 dd (1H, $J_{3',2'}$ = 4.6 Hz, $J_{3',4'}$ = 7.6, H-3' Rib), 4.50 dd (1H, $J_{3',2'}$ = 4.2, $J_{3',4'}$ = 9.2, H-3' Ado), 4.47 d (1H, H-2' Ado), 4.29 d (1H, $J_{5'a,5'b}$ = - 13.2, H-5'a Ado), 4.17 m (2H, H-2' Rib, H-4' Ado), 4.03 m (3H, H-4',5'a Rib, H-5'b Ado), 3.76 d (1H, $J_{5'b,5'a}$ = -10.7, H-5'b Rib), 1.14-0.95 m (28H, iPr). ^{13}C NMR ($CDCl_3$): 164.58 (C=O), 152.17 (C-2), 149.89 (C-6), 149.66 (C-4), 140.55 (C-8), 132.73, 128.72, and 127.85 (Bz), 123.65 (C-5), 106.11 (C-1', Rib), 89.17 (C-1', Ado), 83.79, 81.67 (C-4'), 76.62, 75.49 (C-2'), 68.98, 68.91 (C-3'), 60.21 (C-5', Rib), 59.42 (C-5', Ado), 17.32, 17.25, 17.15, 17.07, 16.89, 13.27, 12.78, 12.78 and 12.57 (iPr).

N^6-Benzoyl-9-[3,5-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-*O*-(2,3,-di-*O*-acetyl-5-*O*-monomethoxytrithyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]adenine (4). A solution of nucleoside **3** (518 mg, 0.7 mmol) and monomethoxytrityl chloride (260 mg,

0.84 mmol) in dry pyridine (3 ml) was stirred for 16 h at 20°C. Acetic anhydride (0.4 ml, 4.20 mmol) was added. The reaction mixture was kept for 2 h at 20°C. Methanol (0.3 ml) was added and after 30 min the mixture was evaporated *in vacuo*. The residue was dissolved in chloroform (30 ml), washed with saturated sodium bicarbonate solution (15 ml) and water (2x10 ml). The organic layer was dried over Na_2SO_4, evaporated *in vacuo*, co-evaporated with toluene (2x10 ml) and purified by column chromatography in system A to give **4** as a foam. Yield 640 mg (83%). R_f 0.65 (B). +ESI MS: calcd. for $C_{58}H_{71}N_5O_{13}Si_2$ + H^+ 1102.4665; found 1102.4735. ^1H NMR ($CDCl_3$): 8.98 brs (1H, NH), 8.59 s (1H, H-8), 8.02-7.15 m (18H, H-2, Bz, Ph), 6.74 d(2H, Ph), 5.76 s (1H, H-1' Ado), 5.73 dd (1H, $J_{3',2'}$ = 3.3 Hz, $J_{3',4'}$ = 6.9, H-3' Rib), 5.54 s (1H, H-1' Rib), 5.53 d (1H, H-2' Rib), 5.11 dd (1H, $J_{3',2'}$ = 4.8, $J_{3',4'}$ = 8.8, H-3' Ado), 4.87 d (1H, H-2' Ado), 4.27 ddd (1H, $J_{4',5'a}$ = 4.8, $J_{4',5'b}$ = 3.7, H-4' Rib), 4.05 m (3H, H-4',5'a,5'b Ado), 3.73 s (3H, OMe), 3.36 dd (1H, $J_{5'a,5'b}$ = - 10.3, H-5'a Rib), 3.23 dd (1H, H-5'b Rib), 2.09 s (3H, Ac), 2.03 s (3H, Ac), 1.11-1.04 m (28H, iPr). ^{13}C NMR ($CDCl_3$): 169.75, 169.42 and 164.56 (C=O), 158.70 (Ph), 152.63 (C-2), 150.60 (C-6), 149.42 (C-4), 142.44 (C-8), 135.06, 132.75, 130.45, 128.90, 128.47, 128.38, 127.81 and 127.02 (Bz, Ph), 123.36 (C-5), 113.03 (Ph), 105.45 (C-1', Rib), 88.84 (C-1', Ado), 86.17 (C-O), 81.22, 79.82 (C-4'), 78.64, 74.97 (C-2'), 71.36, 69.96 (C-3'), 62.95 (C-5', Rib), 59.85 (C-5', Ado), 55.15 (OMe), 20.46 and 20.37 (Ac), 17.33, 17.28, 17.18, 17.00, 16.82, 13.23, 12.95, 12.84 and 12.60 (iPr).

***N*^{6}-Benzoyl-9-[2-*O*-(2,3,-di-*O*-acetyl-5-*O*-monomethoxytrithyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]adenine (5).** Nucleoside **4** (324 mg, 0.29 mmol) was dissolved in tetrahydrofuran (4 ml) and a solution of tetrabutylammonium fluoride trihydrate (280 mg, 0.88 mmol) in tetrahydrofuran (3 ml) was added and kept for 15 min at 20°C. The mixture was evaporated, evaporated with chloroform (2x10 ml) to dryness and applied to a column with silica gel (20 g). The column was washed with system A (300 ml) and then eluted with system B to give **5** as a foam. Yield 230 mg (92%). R_f 0.32 (C). +ESI MS: calcd. for $C_{46}H_{45}N_5O_{12}$ + H^+ 860.3143; found 860.3137. ^1H NMR ($CDCl_3$): 9.11 brs (1H, NH), 8.77 s (1H, H-8), 8.01-7.28 m (18H, H-2, Bz, Ph), 6.88 d (2H, Ph), 5.90 d (1H, $J_{1',2'}$ = 7.0, H-1' Ado), 5.42 dd (1H, $J_{3',2'}$ = 5.0, $J_{3',4'}$ = 5.2, H-3' Rib), 5.35 dd (1H, $J_{2',1'}$ = 2.2, H-2' Rib), 5.01 dd (1H, $J_{2',3'}$ = 4.8, H-2' Ado), 4.81 d (1H, H-1'

Rib), 4.64 dd (1H, $J_{3',4'}$ = 1.1, H-3' Ado), 4.29 d (1H, H-4' Ado), 4.04 ddd (1H, $J_{4',5'a}$ = 3.6, $J_{4',5'b}$ = 4.2, H-4' Rib), 3.96 d (1H, $J_{5'a,5'b}$ = - 13.2, H-5'a Ado), 3.73 s (3H, OMe), 3.70 d (1H, H-5'b Ado), 3.32 dd (1H, $J_{5'a,5'b}$ = - 10.6, H-5'a Rib), 3.23 dd (1H, H-5'b Rib), 2.05 s (3H, Ac), 2.02 s (3H, Ac). ^{13}C NMR ($CDCl_3$): 169.90, 164.53 (C=O), 158.92 (Ph), 152.12 (C-2), 150.36 (C-6), 149.78 (C-4), 141.42 (C-8), 134.79, 132.94, 130.48, 128.96, 128.47, 128.11, 127.87 and 127.32 (Bz, Ph), 124.16 (C-5), 113.39 (Ph), 106.62 (C-1', Rib), 89.72 (C-1', Ado), 86.93 (C-O), 86.83, 81.88 (C-4'), 81.04, 76.36 (C-2'), 75.00, 72.02 (C-3'), 71.14 (C-5', Rib), 63.01 (C-5', Ado), 55.21 (OMe), 20.43 (Ac)

N^6-Benzoyl-9-[3,5-di-*O*-acetyl-2-*O*-(2,3-di-*O*-acetyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]adenine (6). Acetic anhydride (0.13 ml, 1.37 mmol) was added to a solution of nucleoside **5** (198 mg, 0.23 mmol) in dry pyridine (3 ml). The reaction mixture was kept for 2 h at 20°C and then evaporated *in vacuo*. The residue was dissolved in chloroform (30 ml), washed with saturated sodium bicarbonate solution (15 ml) and water (2x10 ml). The organic layer was dried over Na_2SO_4, evaporated *in vacuo*, and co-evaporated with toluene (2x10 ml). The residue was dissolved in 2% trifluoroacetic acid in chloroform (15 ml) and kept for 10 min at 20°C. The solution was diluted with chloroform (20 ml), washed with saturated sodium bicarbonate solution (10 ml) and water (2x10 ml). The organic layer was dried over Na_2SO_4, evaporated to dryness and purified by column chromatography. The column was washed with system A and then elution with system B gave **6** as a foam. Yield 114 mg (74%). R_f 0.52 (C). +ESI MS: calcd. for $C_{30}H_{33}N_5O_{13}$ + H^+ 672.2153; found 672.2151. ^{1}H NMR ($CDCl_3$): 9.20 d (1H, $J_{N,H}$ = 9.5, NH), 8.76 s (1H, H-8), 8.28 s (1H, H-2), 8.03 d (2H, Bz), 7.61 t (1H, Bz), 7.52 t (2H, Bz), 6.40 d (1H, $J_{1',2'}$ = 2.7, H-1' Ado), 5.39 dd (1H, $J_{3',2'}$ = 4.9, $J_{3',4'}$ = 8.1, H-3' Rib), 5.29 d (1H, H-2' Rib), 5.25 dd (1H, $J_{3',2'}$ = 5.4, $J_{3',4'}$ = 7.1, H-3' Ado), 4.94 s (1H, H-1' Rib), 4.84 dd (1H, H-2' Ado), 4.49 m (2H, H-4', 5'a Ado), 4.37 dd (1H, $J_{5'b,4'}$ = 5.0, $J_{5'b,5'a}$ = - 13.2, H-5'b Ado), 4.12 ddd (1H, $J_{4',5'a}$ = 2.7, $J_{4',5'b}$ = 2.9, H-4' Rib), 3.77 dd (1H, $J_{5'a,5'b}$ = -12.9 Hz, H-5'a Rib), 3.54 dd (1H, H-5'b Rib), 2.19 s (3H, Ac), 2.14 s (3H, Ac), 2.09 s (3H, Ac), 2.07 s (3H, Ac). ^{13}C NMR ($CDCl_3$): 170.60, 170.42, 170.08, 169.69 and 164.89 (C=O), 152.54 (C-2), 150.99 (C-6), 150.02 (C-4), 141.34 (C-8), 133.39, 132.97, 128.87, and 128.02 (Bz), 123.65 (C-5), 105.50 (C-1', Rib), 88.78 (C-1', Ado), 81.61, 78.88 (C-4'),

78.21, 74.63 (C-2'), 70.14, 69.02 (C-3'), 62.13 (C-5', Rib), 60.07 (C-5', Ado), 20.67, 20.43 and 20.34 (Ac).

N^6-Benzoyl-9-[3,5-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-*O*-(2,3-di-*O*-acetyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]adenine (8). The nucleoside **4** (925 mg, 0.84 mmol) was dissolved in 2% trifluoroacetic acid in chloroform (30 ml) and kept for 10 min at 20°C. The solution was diluted with chloroform (50 ml), washed with saturated sodium bicarbonate solution (30 ml) and water (2x30 ml). The organic layer was dried over Na_2SO_4, evaporated to dryness and purified by column chromatography in system A to give **8** as a foam. Yield 540 mg (78%). R_f 0.32 (B). +ESI MS calcd. for $C_{38}H_{55}N_5O_{12}Si_2$ + H^+ 830.3464; found 830.3422. ^{1}H NMR ($CDCl_3$): 9.18 brs (1H, NH), 8.78 s (1H, H-8), 8.43 s (1H, H-2), 8.03 d (2H, Bz), 7.63 t (1H, Bz), 7.53 t (2H, Bz), 6.17 s (1H, H-1' Ado), 5.74 dd (1H, $J_{3',2'}$ = 4.5, $J_{3',4'}$ = 8.1, H-3' Rib), 5.50 d (1H, H-2' Rib), 5.40 s (1H, H-1' Rib), 4.51 dd (1H, $J_{3',2'}$ = 4.1, $J_{3',4'}$ = 9.0, H-3' Ado), 4.48 d (1H, H-2' Ado), 4.33 d (1H, $J_{5'a,5'b}$ = - 13.4, H-5'a Ado), 4.24 m (3H, H-4' Ado, H-4',5'a Rib), 4.05 dd (1H, $J_{5'b,4'}$ = 2.4, H-5'b Ado), 3.71 dd (1H, $J_{5'b,4'}$ = 5.4, $J_{5'b,5'a}$ = -12.9, H-5'b Rib), 2.10 s (3H, Ac), 2.08 s (3H, Ac), 1.12-0.95 m (28H, iPr). ^{13}C NMR ($CDCl_3$): 169.90, 169.45 and 164.71 (C=O), 152.36 (C-2), 150.11 (C-6), 149.75 (C-4), 140.58 (C-8), 132.91, 128.93, and 127.96 (Bz), 123.92 (C-5), 104.41 (C-1', Rib), 89.05 (C-1', Ado), 81.89, 81.67 (C-4'), 77.00, 74.91 (C-2'), 69.11, 68.99 (C-3'), 59.91 (C-5', Rib), 59.34 (C-5', Ado), 20.49 and 20.43 (Ac), 17.18, 16.91, 16.72, 13.30, 12.75 and 12.54 (iPr).

N^6-Benzoyl-9-[3,5-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-*O*-(2,3-di-*O*-acethyl-5-*O*-bis(p-nitrophenylethyl)phosphorylyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]adenine (9). A mixture of nucleoside **8** (517 mg, 0.62 mmol) and the triethylammonium salt of bis(p-nitrophenylethyl)phosphate (463 mg, 0.93 mmol) was coevaporated with dry pyridine (3 x 5 ml) and finally dissolved in pyridine (5 ml). Then 1-methylimidazole (428 mg, 5.49 mmol) and TPSCl (555 mg, 1.83 mmol) were added. The reaction mixture was stirred for 16 h at 20°C, and then concentrated *in vacuo*. The residue was dissolved in chloroform (50 ml), washed with 0.06 M phosphate buffer pH 7.0 (2x30 ml). The organic layer was dried over Na_2SO_4, evaporated *in vacuo*, co-evaporated with toluene (2x10 ml) and purified by column chromatography. The column was washed with

ether (300 ml) and eluted with system A to give **9.** Yield 540 mg (72%). R_f 0.32 (B). ^1H NMR ($CDCl_3$): 9.05 brs (1H, NH), 8.80 s (1H, H-8), 8.16 s (1H, H-2), 8.14-7.33 m (13H, Bz, Ph), 6.03 s (1H, H-1' Ado), 5.49 s (1H, H-1' Rib), 5.44 dd (1H, $J_{3',2'}$ = 4.9, $J_{3',4'}$ = 6.1, H-3' Rib), 5.38 d (1H, H-2' Rib), 4.97 dd (1H, $J_{3',2'}$ = 4.6, $J_{3',4'}$ = 9.0, H-3' Ado), 4.71 d (1H, H-2' Ado), 4.28-3.93 m (10H, H-4',5' Ado, H-4',5' Rib, CH_2O), 3.02 m (4H, CH_2), 2.09 s (3H, Ac), 2.04 s (3H, Ac), 1.10-0.98 m (28H, iPr).

***N*^{6}-Benzoyl-9-[2-*O*-(2,3-di-*O*-acethyl-5-*O*-bis(p-nitrophenylethyl)phosphorylyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]adenine (10).** The protected triester **9** (540 mg, 0.45 mmol) was dissolved in tetrahydrofuran (5 ml) and a solution of tetrabutylammonium fluoride trihydrate (420 mg, 1.33 mmol) in tetrahydrofuran (5 ml) was added. After 15 min at 20°C water (10 ml) and Dowex-50 (Na^+-form) (1 ml) were added and the mixture was stirred for 30 min at 20°C. The resin was filtered off and washed with ethyl acetate. The filtrate was washed with 0.06 M phosphate buffer pH 7.0 (2x30 ml). The organic layer was dried over Na_2SO_4, evaporated to dryness and applied to a column of silica gel (50 g). The column was washed with system A (300 ml) and then eluted with system B to give **10** as a foam. Yield 258 mg (59%). R_f 0.38 (C). +ESI MS: calcd. for $C_{42}H_{44}N_7O_{18}P + H^+$ 966.2558; found 966.2535. ^1H NMR ($CDCl_3$): 9.07 brs (1H, NH), 8.78 s (1H, H-8), 8.19 s (1H, H-2), 8.15-7.36 m (13H, Bz, Ph), 6.06 d (1H, $J_{1',2'}$ = 7.2, H-1' Ado), 5.20 dd (1H, $J_{3',2'} = J_{3',4'}$ = 5.1, H-3' Rib), 5.13 dd (1H, $J_{2',1'}$ = 2.4, H-2' Rib), 5.10 dd (1H, $J_{2',3'}$ = 4.9, H-2' Ado), 4.84 d (1H, H-1' Rib), 4.60 d (1H, H-3' Ado), 4.33-4.20 m (5H, H-5'a Rib, CH_2O), 3.96 m (3H, H-4' Ado, H-4',5'b Rib), 3.76 m (2H, H-5' Ado), 3.07 m (4H, CH_2), 2.06 s (3H, Ac), 2.02 s (3H, Ac). ^{13}C NMR ($CDCl_3$): 169.68 (C=O), 151.99 (C-2), 150.09 (C-6), 149.42 (C-4), 144.69 (Ph), 141.43 (C-8), 132.97, 129.83, 128.94, 127.87 (Bz, Ph), 123.79 (C-5), 106.31 (C-1', Rib), 89.37 (C-1', Ado), 87.11 (C-4' Ado), 81.26 (C-2' Ado), 80.34 d (C-4'Rib), 74.83 (C-2' Rib), 72.15 (C-3' Rib), 70.26 (C-3' Ado), 67.50 m (CH_2OP), 66.22 d (C-5', Rib), 63.23 (C-5', Ado), 36.33, 36.27 ($PhCH_2$), 20.41, 20.34 (Ac).

***O*-β-D-Ribofuranosyl-(1''-2')-adenosine-5''-*O*-phosphate** (7).

Method A. A mixture of nucleoside **6** (95 mg, 0.14 mmol) and a 0.28 ml 1M solution of

β-cyanoethyl phosphate in pyridine was evaporated *in vacuo* and dried by coevaporations with dry pyridine (2x10ml). The residue was dissolved in 3 ml of pyridine, *N,N'*-dicyclohexylcarbodiimide (230 mg, 1.12 mmol) was added and the mixture was stored at 20°C for 4 days. After addition of water (3 ml), the precipitated dicyclohexyl urea was filtered off and washed with 10 ml of 20 % aqueous pyridine. The combined filtrates were washed with chloroform (2 x 20 ml), concentrated *in vacuo*, evaporated with toluene (3x10 ml) and applied on a column of silica gel (20 g). The column was washed with system B (300 ml) and then eluted with system E. Fractions containing the product were collected and evaporated *in vacuo* to dryness. The residue was dissolved in 5M ammonia in methanol (3 ml) and kept for 3 days at 20°C and then concentrated *in vacuo* to dryness. The residue was partitioned between chloroform (10 ml) and water (20 ml), the water layer was washed with chloroform (2 x 10 ml). The aqueous layer was concentrated *in vacuo*. The residue was dissolved in 1N NaOH (2 ml) and kept for 20 min at 20°C, then 10% aqueous acetic acid was added to pH 7.0. The solution was diluted with water (30 ml) and then applied to a column of DEAE-cellulose (200 ml, HCO_3^--form). The column was washed with water (500 ml), a 0.05 M solution of NH_4HCO_3 and eluted with 0.1 M solution of NH_4HCO_3. The UV-absorbing fractions were combined, evaporated *in vacuo*, and coevaporated with water (5 x 10 ml). The residue was dissolved in 20 ml of water and freeze-dried. Monophosphate **7** was obtained as its ammonium salt. Yield 0.046 mmol (32%).

Method B. A solution of triester **10** (48 mg, 0.05 mmol) in 0.5 M DBU in dry pyridine (4 ml) was stored for 24 h at 20°C, neutralized with acetic acid (0.11 ml, 2 mmol), and evaporated.The residue was dissolved in 5M ammonia in methanol (3 ml) and kept for 3 days at 20°C and then concentrated *in vacuo*, dissolved in water (30 ml) and washed with chloroform (2 x 10 ml). The aqueous layer was purified on DEAE-cellulose. Yield 0.04 mmol (80 %). UV (pH 7-13): λ_{max} 261 nm (ε 14000); (pH 1): λ_{max} 260 nm (ε 13500). +ESI MS: calcd. for $C_{15}H_{22}N_5O_{11}P + H^+$ 480.1132; found 480.1146; . calcd. for $C_{15}H_{22}N_5O_{11}P + Na^+$ 502.0951; found 502.0963; ^1H NMR (D_2O): See Table 1. ^{13}C NMR (D_2O): 156.54 (C-2), 153.31 (C-6), 149.15 (C-4), 142.6 (C-8), 119.99 (C-5), 108.20 (C-1', Rib), 88.40 (C-1', Ado), 86.70 (C-4', Ado), 82.67 (C-4', Rib), 80.20, 75.20 (C-2'), 71.50, 70.50 (C-3'), 65.70 (C-5', Rib), 62.68 (C-5', Ado). ^{31}P NMR (161.98 MHz) (D_2O): 1.92 ppm.

Financial support from RFBR, INTAS, NATO and KUL Research Council is gratefully acknowledged.

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