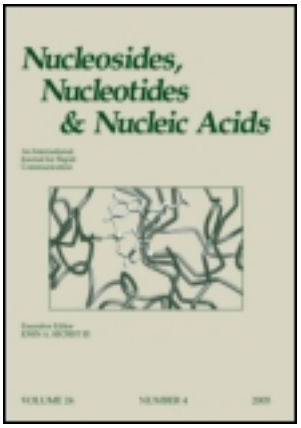

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Synthesis of Oligoribonucleotides Containing 2′-O-Methoxymethyl Group by the Phosphotriester Method

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SYNTHESIS OF OLIGORIBONUCLEOTIDES CONTAINING 2′-*O*-METHOXYMETHYL GROUP BY THE PHOSPHOTRIESTER METHOD

Vladimir A. Efimov, Andrey V. Aralov, and Oksana G. Chakhmakhcheva
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□ *An effective procedure for the synthesis of ribonucleotide monomers containing a 2′-O-methoxymethyl-modifying group was developed. These monomers were used for the synthesis of RNA fragments by the solid-phase phosphotriester method under O-nucleophilic intramolecular catalysis. The properties of 2′-O-methoxymethyl-containing oligoribonucleotides were examined.*

Keywords Oligoribonucleotides; synthesis; phosphotriester method; methoxymethyl group

INTRODUCTION

Chemical modification of natural oligonucleotides (ONs) is widely used to improve their physicochemical and biochemical properties. Because natural ONs are rapidly degraded by cellular nucleases, numerous attempts have been made to develop modified ONs having both increased affinity to the complementary DNA and RNA fragments and enhanced nuclease resistance. The modifications at the ribose 2′-position appear to be the most promising because they do not generally cause essential distortions in the ON structure. One example is 2′-F modification, which increases the hybridization affinity but, unfortunately, does not enhance the nuclease resistance in comparison with unmodified ONs.[1] Other types of 2′-modified ONs containing *O*-alkyl substitutions, such as methyl, methoxyethyl, and cyanoethyl groups, the introduction of which leads to enhanced hybridization affinity and nuclease resistance of ONs compared with natural ones, were also described.[2–5]

Earlier, it was shown that the rapid phosphotriester method of ON synthesis with the use of *O*-nucleophilic catalysis for the internucleotide bond formation is an alternative to the phosphoroamidite approach, and it was

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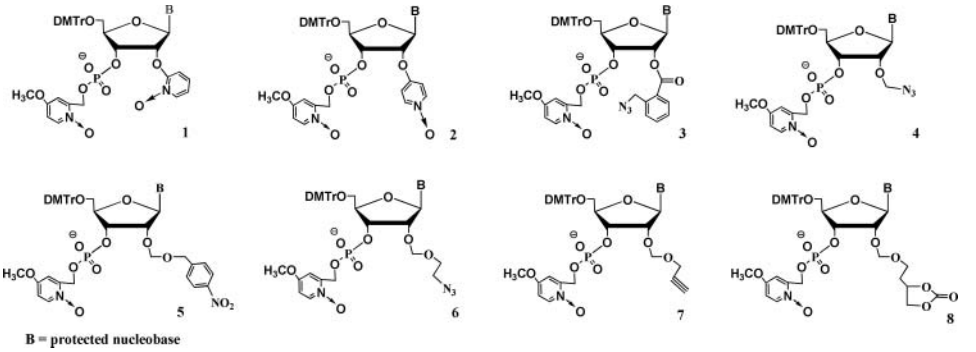


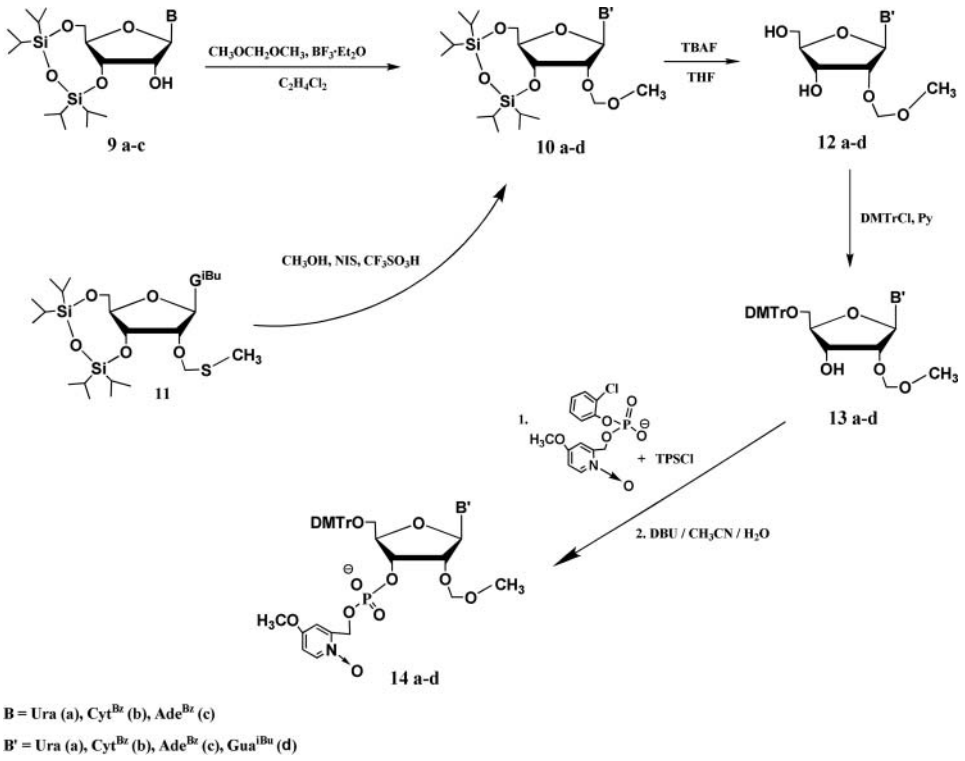
FIGURE 1 Monomers containing 2′-*O*-protecting and -modifying groups for rapid phosphotriester method of ON synthesis.

successfully applied to the synthesis of both natural and modified oligodeoxyribonucleotides.[6,7] Moreover, it was shown that this methodology can be used for the preparation of stereospecific phosphorothioate ON analogues.[8,9] To develop the efficient solid-phase phosphotriester method for the synthesis of natural and modified RNA fragments, we examined a number of protecting and modifying groups for 2′-hydroxyls, including (1-oxidopyridin-2-yl) **1**, (1-oxidopyridin-4-yl) **2**,[9] (2-azidomethyl)benzoyl **3**, azidomethyl **4**,[10–12] *p*-nitrobenzyloxymethyl **5**,[13] (2-azidoethoxy)methyl **6**, propargyloxymethyl **7**, and (3,4-cyclocarbonatebutoxy)methyl **8**[14] groups (Figure 1). In this paper, we describe the continuation of these studies and elaboration of schemes for the synthesis of RNA monomers containing the modifying 2′-*O*-methoxymethyl (MOM) group.

The methoxymethyl (MOM) protecting group found a wide application in organic synthesis for the protection of hydroxyl functions due to the simplicity of its introduction and stability in both strong alkaline and moderate acidic conditions, as well as under conditions used for the removal of silyl, alkoxyacetyl, and benzyl groups.[15–17] Earlier, the introduction of the MOM-group in various positions of deoxyribo- and ribonucleosides was described.[18] Also, the 2′-*O*-MOM-U-containing oligonucleotides synthesized by the H-phosphonate method were reported.[19] In this case, the 2′-*O*-MOM derivative was obtained from the 2′-*O*-methylthiomethyl nucleoside. Moreover, it was reported that the 2′-*O*-MOM-modified antisense oligonucleotide targeting survivin mRNA, LY2181308 (Eli Lilly and Co.), has completed a phase I trial in patients with advanced cancers,[20] and a phase II study in combination with docetaxel in hormone-refractory prostate cancer patients has started.[21]

RESULTS AND DISCUSSION

Our approach to the synthesis of 2′-*O*-MOM nucleotide derivatives **14a–d** is depicted in Scheme 1. Nucleoside derivatives **9a–c** containing



SCHEME 1 Preparation of 2′-O-MOM-modified monomers.

protected 3′- and 5′-OH groups and amino functions of heterocyclic bases (in the case of A and C) were treated with the excess of dimethoxymethane in the presence of boron trifluoride etherate ($BF_3 \cdot Et_2O$) for 2 hours. In the case of guanosine, the treatment of the corresponding 3′,5′-*O*-protected derivative of type **9** with dimethoxymethane in the presence of $BF_3 \cdot Et_2O$ led to the formation of a large amount of a fluorescent side product. Therefore, the synthesis of the 2′-*O*-MOM derivative **10d** was carried out starting from methylthiomethyl nucleoside **11** by the treatment with trifluoromethane sulfonic acid and *N*-iodosuccinimide (NIS) in a mixture of THF–methanol (25:1, v/v) for 20 minutes at –45°C, as it was described.[19,22] After the isolation of the fully protected nucleoside **10a–d**,3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl) group (TIPDS) was removed by the action of 1 M tetrabutylammonium fluoride (TBAF) in THF for 1 hour. The resultant derivatives **12** were 5′-*O*-dimethoxytritylated. Then, the compounds **13** were 3′-phosphorylated by the action of (2-chlorophenyl)-(1-oxido-4-methoxy-2-picolyl) phosphate in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) following the removal of 2-chlorophenyl group by the action of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in aqueous acetonitrile, as described.[11,12] The structures of intermediates and the target monomers **14a–d** were confirmed by mass-spectrometry and NMR spectroscopy methods.

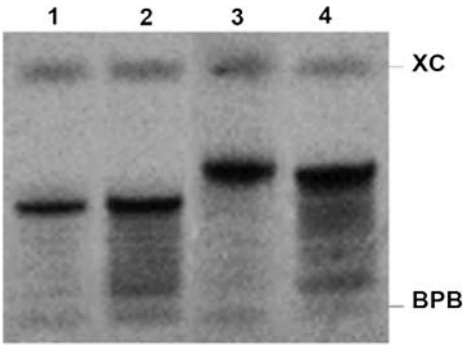


FIGURE 2 Analysis of 2′-*O*-MOM-modified ONs by a denaturing 15% PAGE before (1, 3) and after (2, 4) the treatment with 1 M solution of LiI in acetonitrile–water (20:1, v/v) in the presence of 0.01 M HCl for 5 hours at room temperature. Lines (1, 2)—U_{15} and (3, 4)— 5′- r(CGAUCUCAUCACCUCUCCAU). Visualization was by UV-shadowing at 254 nm.

Monomers **14a–d** were applied to the solid phase synthesis of 15–25-mer oligoribonucleotides. The synthesis was carried out on the standard CPG support using TPSCl as a condensing agent for the internucleotide bond formation, as was described earlier.[6,12] The yields at the chain elongation steps were 98–99%. After the synthesis was completed, ONs bound to the support were treated with 1M lithium iodide in acetonitrile (5 hours at room temperature) for deprotection of internucleotide phosphate residues.[11] Removal of oligonucleotides from the support and deprotection of heterocyclic bases were performed by the action of conc. ammonia. Then, ONs carrying modifying 2′-*O*-MOM groups were isolated by gel filtration, and their homogeneity was examined by gel-electrophoresis (Figure 2), or reversed phase chromatography.

It is known that the MOM-group can be removed by boiling in aqueous organic solutions in the presence of catalytic amounts of CBr_4 or by the action of $ZnBr_2$ in the presence of a mercaptan.[23] Unfortunately, we have found that these conditions are not appropriate for removal of MOM-groups from ONs in view of their degradation. Our attempts to remove the 2′-*O*-MOM groups from the synthesized ONs with 30% aqueous trifluoroacetic acid[17,24] led also to a significant degradation of the oligomer chain. We found that the treatment of MOM-containing nucleosides **12a–d** with 1M LiI in acetonitrile–water (20:1, v/v) in the presence of 0.01M HCl for 2–3 hours resulted in practically quantitative removal of the 2′-*O*-MOM protecting group. However, in the case of treatment of MOM-containing ONs, in addition to the removal of MOM groups, the partial degradation of the oligomer chain still occurred under these conditions (Figure 2).

We have found that the presence of 2′-*O*-MOM groups had no significant influence on the specificity of ONs binding to the complementary RNA fragments and the stability of formed duplexes (the increase of T_m was on average about 0.2°C per one unit). For comparison, Bizdena et al. reported

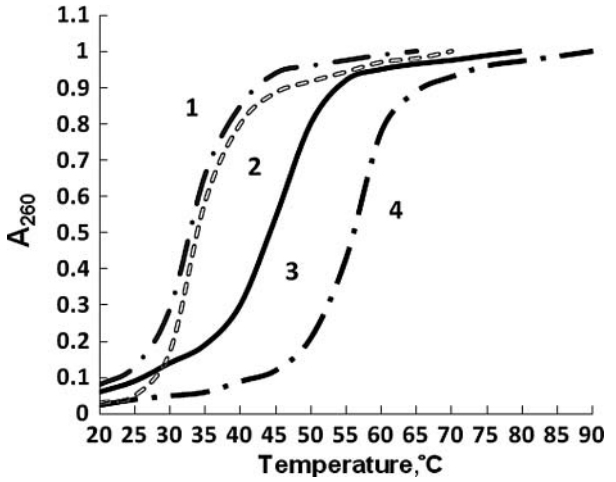


FIGURE 3 Normalized melting curves of duplexes formed by 2′-*O*-MOM-modified ONs U_{15} (1, 2) and 5′- r(CGAUCUCAUCACCUCUCCAU) (3, 4) with the complementary oligodeoxyribonucleotides (1, 3) and oligoribonucleotides (2, 4).

that the 2′-*O*-MOM modification even caused a slight destabilization of duplexes with RNA.[19] At the same time, we have found that complexes of 2′-*O*-MOM-containing ONs with DNA had the lower T_m values than those of complexes formed by natural oligoribonucleotides with the same sequences (destabilization was ~0.3°C per unit) (Figure 3). The introduction of a single nucleotide mutation in DNA and RNA targets led to the decrease of melting temperatures of corresponding duplexes by 10–15°C, depending on the position of the mutation (data not shown).

The results obtained in the analysis of 2′-*O*-MOM-modified ONs' stability revealed that these oligomers are resistant to the action of RNA-specific nucleases (RNase A and RNase T1) as well as to the action of S1 nuclease, mung bean nuclease, and exonuclease III (data not shown). At the same time, snake venom and bovine spleen phosphodiesterases were able to cleave 2′-*O*-MOM-modified oligoribonucleotides, but their resistance to these enzymes was higher in comparison with the resistance of natural ONs (Figure 4).

EXPERIMENTAL

Solvents and reagents were obtained from commercial suppliers and were used without further purification. ^{1}H- and ^{31}P-NMR spectra were obtained with a DPX-300 spectrometer (Bruker, Germany). Chemical shifts are given in p.p.m. relative to tetramethylsilane (^{1}H) or H_3PO_4 (^{31}P). Mass spectra were recorded using an Ultraflex II MALDI-TOF (Bruker Daltonics, Germany) mass spectrometer. TLC was carried out on Merck Silica Gel 60 F_{254} plates in $CHCl_3$–CH_3OH–H_2O (65:25:4, v/v/v; solvent A), $CHCl_3$–CH_3OH

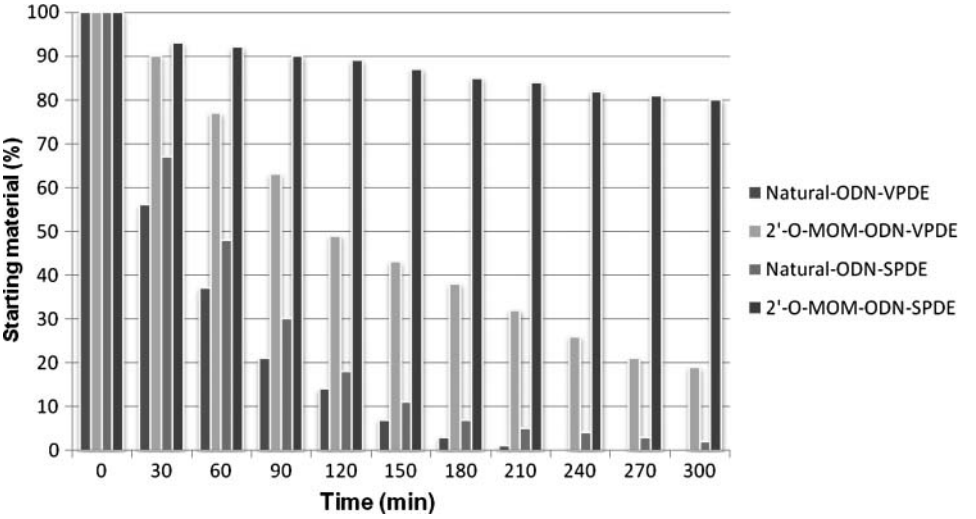


FIGURE 4 Time course of degradation of 2′-*O*-MOM-U_{15} and natural U_{15} by venom (VPDE) and spleen (SPDE) phosphodiesterases. The digestions of ONs (0.02 μmol) with VPDE were performed in 200 μL of 20 mM Tris-HCl (pH 8.0)/50 mM NaCl/10 mM $MgCl_2$, and assays for SPDE stability were performed in 200 μL of 30 mM NaOAc (pH 6.0)/10mM $MgCl_2$ at 37°C. At appropriate periods, aliquots of the reaction mixture were taken, and an enzyme was deactivated by heating at 100°C for 2 minutes. The mixtures were then analyzed by 20% denaturing PAGE and the relative density of ON bands on the gel was estimated using the UVP imaging system.

(9:1, v/v; solvent B), and $CHCl_3$–CH_3OH (39:1, v/v; solvent C). Silica gel column chromatography was performed using Merck silica gel 60. Synthesis of ONs was performed on a 1 μmol scale using Applied Biosystems Synthesizer 381A and universal CPG support (Glen Research), as described.[11] Deprotection and purification of 2′-*O*-MOM-containing ONs were performed, as described earlier.[13] PAGE of ONs was performed in the presence of 7 M urea in 0.1 M Tris–borate/EDTA buffer (pH 8.3). Nuclease stability of 2′-*O*-MOM-ON was examined essentially, as described.[11] Absorbance (260 nm) versus temperature curves of duplexes formed by ONs (3 μM solutions of each ON) were measured using a Pharmacia Ultrospec 2000 UV-VIS spectrophotometer equipped with a heated sample holder and a Peltier temperature control accessory in a buffer containing 0.1 M NaCl, 0.02 M tris-HCl (pH 7.5), and 0.01 M $MgCl_2$.

3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(methoxymethyl)nucleosides (10)

The 3′,5′-*O*-protected nucleoside of type **9a–c** (1.0 mmol) was dissolved in 1,2-dichloroethane (5 mL), then dimethoxymethane (20 mmol), molecular sieves 4 Å (0.4 g), and $BF_3{\cdot}Et_2O$ (4.0 mmol) were added. The reaction mixture was stirred at room temperature for 2 hours and filtered. The

filtrate was diluted with chloroform (15 mL) and washed with 1M TEAB (10 mL).

To obtain the guanosine derivative **10d**, 3′,5′-*O*-(1,1,3,3-tetraiso propyldisiloxane-1,3-diyl)-2′-*O*-(methylthiomethyl)guanosine **11** (1.0 mmol) prepared, as described,[25] was dissolved in dry THF/CH_3OH (25:1, v/v, 5 mL). After the addition of molecular sieves 4 Å (0.4 g), CF_3SO_3H (1.6 mmol) was added dropwise at −45°C. Then, NIS (1.6 mmol) in dry THF (1 mL) was added, and the resulting mixture was stirred for 20 min at −45°C. The reaction was stopped by the addition of triethylamine (TEA) (5.0 mmol), and the mixture was filtered, diluted with ethylacetate (20 mL), and washed with saturated aqueous solution of $Na_2S_2O_3$ (20 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness.

The desired products **10a–d** were isolated by silica gel column chromatography using a gradient of chloroform (50–100%) in *n*-hexane. The fractions containing the derivatives **10** were evaporated to a gum and dried in vacuo.

***3′,5′-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-O-(methoxymethyl)uridine*(10a)**

The yield was 87%. R_f (solvent C) = 0.55. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.69 (1H, br s, Ura-N*H*), 7.88 (1H, d, J = 8.2 Hz, Ura-*H*-6), 5.76 (1H, s, *H*-1′), 5.66 (1H, dd, J = 8.2 Hz, J = 1.8 Hz, Ura-*H*-5), 4.96 (1H, d, J = 6.6 Hz, OCH_αO), 4.86 (1H, d, J = 6.6 Hz, OCH_βO), 4.29–4.14 (4H, m, *H*-2′, *H*-3′, *H*-4′, H_α-5′), 3.99 (1H, dd, J = 13.6 Hz, J = 2.3 Hz, H_β-5′), 3.46 (3H, s, OCH_3), 1.12–0.95 (28H, m, *i*Pr-*H*). MS: 553.28, calc. for $C_{23}H_{42}N_2NaO_8Si_2^+$ $(M+Na)^+$ 553.24.

***N*^{4}-Benzoyl-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-O-(methoxymethyl)cytidine (10b)**

The yield was 86%. R_f (solvent C) = 0.60. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.36 (1H, d, J = 7.6 Hz, Cyt-*H*-6), 7.95–7.47 (6H, m, Bz-*H*-Ar, Cyt-*H*-5), 5.86 (1H, s, *H*-1′), 5.10 (1H, d, J = 6.5 Hz, OCH_αO), 4.88 (1H, d, J = 6.5 Hz, OCH_βO), 4.31 (1H, d, J =13.6 Hz, H_α-5′), 4.25–4.19 (3H, m, *H*-2′, *H*-3′, *H*-4′), 4.01 (1H, dd, J = 13.6 Hz, J = 1.5 Hz, H_β-5′), 3.48 (3H, s, OCH_3), 1.14–0.94 (28H, m, *i*Pr-*H*). MS: 634.37, calc. for $C_{30}H_{48}N_3O_8Si_2^+$ $(M+H)^+$ 634.30.

***N*^{6}-Benzoyl-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-O-(methoxymethyl)adenosine (10c)**

The yield was 84 %. R_f (solvent C) = 0.65. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 9.09 (1H, s, Ade-N*H*), 8.79 (1H, s, Ade-*H*-8), 8.32 (1H, s, Ade-*H*-2), 8.10–7.49 (5H, m, Bz-*H*-Ar), 6.14 (1H, br s, *H*-1′), 4.98 (1H, d, J = 6.5 Hz, OCH_αO), 4.95 (1H, d, J = 6.5 Hz, OCH_βO), 4.78–4.69 (1H, m, *H*-3′), 4.54–4.51 (1H, m, *H*-2′), 4.29–4.16 (2H, m, *H*-4′, H_α-5′), 4.07–4.04 (1H, m, H_β-5′), 3.32 (3H, s, OCH_3), 1.14–0.90 (28H, m, *i*Pr-*H*). MS: 658.28, calc. for $C_{31}H_{48}N_5O_7Si_2^+$ $(M+H)^+$ 658.31.

***N^2-Isobutyryl-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-O-(methoxymethyl)guanosine* (10d)**

The yield was 81%. R_f (solvent C) = 0.30. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 12.08 (1H, br s, Gua-N*H*), 8.95 (1H, br s, Gua-N*H*), 7.99 (1H, s, Gua-*H*-8), 5.87 (1H, s, *H*-1′), 4.92 (1H, d, J = 6.5 Hz, OCH_αO), 4.82 (1H, d, J = 6.5 Hz, OCH_βO), 4.49 (1H, dd, J = 9.4 Hz, J = 4.6 Hz, *H*-3′), 4.26–4.19 (2H, m, *H*-2′, H_α-5′), 4.15–4.10 (1H, m, *H*-4′), 4.00 (1H, dd, J = 13.4 Hz, J = 2.5 Hz, H_β-5′), 3.39 (3H, s, OCH_3), 2.68 (1H, sept, J = 6.9 Hz, *i*Bu-C*H*), 1.28–1.21 (6H, m, *i*Bu-CH_3), 1.10–0.96 (28H, m, *i*Pr-*H*). MS: 640.32, calc. for $C_{28}H_{50}N_5O_8Si_2^+$ $(M+H)^+$ 640.32.

General Procedure for the Synthesis of 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(methoxymethyl)nucleosides (13)

The derivative **10** (1.0 mmol) was dissolved in dry THF (5 mL) and treated with 1 M solution of TBAF (2.2 mL) in dry THF for 1 hour. After the evaporation, crude 2′-*O*-(2-methoxymethyl) nucleoside **12** was dissolved in chloroform and purified by silica gel column chromatography in a gradient of methanol (0–6%) in chloroform. The following 5′-*O*-protection of 2′-*O*-methoxymethyl nucleosides **12a–d** was performed by the action 4,4′-dimethoxytritylchloride (1.2 equiv.) in dry pyridine during 1.5 hours. The reaction was stopped by the addition of 0.5 M TEAB (20 mL), and the crude product was extracted with chloroform (2 × 20 mL). The combined organic fractions were evaporated to a gum. The residue was dissolved in chloroform, and a 5′-*O*-dimethoxytrityl-2′-*O*-methoxymethyl nucleoside **13** was isolated by silica gel column chromatography using a gradient of methanol (0–3%) in chloroform containing 0.1% of TEA. The fractions containing the desired derivative **13** were evaporated to a gum and dried in vacuo.

***5′-O-(4, 4′-Dimethoxytrityl)-2′-O-(methoxymethyl)uridine* (13a)**

Yield 82%. R_f (solvent B) = 0.55. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.33 (1H, br s, Ura-N*H*), 7.71 (1H, d, J = 8.1 Hz, Ura-*H*-6), 7.41–7.22 (9H, m, DMTr-*H*-Ar), 6.93–6.87 (4H, m, DMTr-*H*-Ar), 5.88 (1H, d, J = 3.8 Hz, *H*-1′), 5.31 (1H, d, J = 8.1 Hz, Ura-*H*-5), 5.26 (1H, d, J = 5.6 Hz, O*H*-3′), 4.73 (1H, d, J = 6.6 Hz, OCH_αO), 4.69 (1H, d, J = 6.6 Hz, OCH_βO), 4.28–4.21 (2H, m, *H*-2′, *H*-3′), 4.03–3.97 (1H, m, *H*-4′), 3.75 (6H, s, DMTr-OCH_3), 3.32 (1H, dd, J = 11.0 Hz, J = 4.5 Hz, H_α-5′), 3.27 (3H, s, OCH_3), 3.24 (1H, dd, J = 11.0 Hz, J = 2.8 Hz, H_β-5′). MS: 613.23, calc. for $C_{32}H_{34}N_2NaO_9^+$ $(M+Na)^+$ 613.22.

***N^4-Benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-O-(methoxymethyl)cytidine* (13b)**

Yield 79%. R_f (solvent C) = 0.45. ^{1}H-NMR (300 MHz, DMSO-d_6): 11.24 (1H, br s, Cyt-N*H*), 8.35 (1H, d, J = 7.3 Hz, Cyt-*H*-6), 8.03–7.48 (5H, m, Bz-*H*-Ar), 7.32–6.79 (9H, m, DMTr-*H*-Ar), 7.15 (1H, d, J = 7.3 Hz, Cyt-*H*-5), 6.96–6.89 (4H, m, DMTr-*H*-Ar), 5.89 (1H, d, J = 1.9 Hz, *H*-1′), 5.29 (1H, d, J = 7.3 Hz, O*H*-3′), 4.91 (1H, d, J = 6.4 Hz,

OCH_αO), 4.77 (1H, d, J = 6.4 Hz, OCH_αO), 4.39–4.31 (1H, m, *H*-3′), 4.18–4.15 (1H, m, *H*-2′), 4.12–4.06 (1H, m, *H*-4′), 3.76 (6H, br s, DMTr-OCH_3), 3.43–3.35 (2H, m, *H*-5′), 3.32 (3H, s, OCH_3). MS: 732.20, calc. for $C_{40}H_{43}N_3NaO_9^+$ $(M+Na)^+$ 732.29.

***N*^{6}-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(methoxymethyl)adenosine (13c)**

Yield 68%. R_f (solvent C) = 0.50. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.21 (1H, br s, Ade-N*H*), 8.67 (1H, s, Ade-*H*-8), 8.60 (1H, s, Ade-*H*-2), 8.07–7.51 (5H, m, Bz-*H*-Ar), 7.39–7.16 (9H, m, DMTr-*H*-Ar), 6.87–6.80 (4H, m, DMTr-*H*-Ar), 6.23 (1H, d, J = 5.1 Hz, *H*-1′), 5.42 (1H, d, J = 5.8 Hz, O*H*-3′), 4.96–4.91 (1H, m, *H*-2′), 4.72 (1H, d, J = 6.7 Hz, OCH_αO), 4.64 (1H, d, J = 6.7 Hz, OCH_βO), 4.53–4.46 (2H, m, *H*-3′), 4.17–4.11 (1H, m, *H*-4′), 3.72 (6H, d, J = 0.5 Hz, DMTr-OCH_3), 3.29–3.24 (2H, m, *H*-5′), 3.11 (3H, s, OCH_3). MS: 718.26, calc. for $C_{40}H_{40}N_5O_8^+$ $(M+H)^+$ 718.29.

***N*^{2}-Isobutyryl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(methoxymethyl)guanosine (13d)**

Yield 68%. R_f (solvent B) = 0.50. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 12.10 (1H, br s, Gua-N*H*), 11.60 (1H, br s, Gua-N*H*), 8.11 (1H, s, Gua-*H*-8), 7.37–7.18 (9H, m, DMTr-*H*-Ar), 6.85–6.79 (4H, m, DMTr-*H*-Ar), 6.01 (1H, d, J = 5.6 Hz, *H*-1′), 5.32 (1H, d, J = 5.6 Hz, O*H*-3′), 4.72 (1H, d, J = 6.7 Hz, OCH_αO), 4.72–4.68 (1H, m, *H*-2′), 4.63 (1H, d, J = 6.7 Hz, OCH_βO), 4.39–4.33 (1H, m, *H*-3′), 4.10–4.04 (1H, m, *H*-4′), 3.72 (6H, d, J = 0.7 Hz, DMTr-OCH_3), 3.33–3.26 (1H, m, H_α-5′), 3.20–3.14 (1H, m, H_β-5′), 3.14 (3H, s, OCH_3), 2.76 (1H, sept, J = 6.9 Hz, *i*Bu-C*H*), 1.12,1.11 (6H, d, J = 6.9 Hz, *i*Bu-CH_3). MS: 700.35, calc. for $C_{37}H_{42}N_5O_9^+$ $(M+H)^+$ 700.29.

General Method for the Synthesis of Ribonucleotide Monomers (14) Containing 2′-*O*-MOM Modifying Groups

To introduce the phosphate residue bearing the *O*-catalytic protective group, the compound **13** (1.0 mmol) was allowed to react with (2-chlorophenyl)-(1-oxido-4-methoxy-2-picolyl) phosphate (2.0 mmol)[11] in dry pyridine/CH_3CN (1:4, v/v, 10 mL) in the presence of TPSCl (3.0 mmol) for 15 minutes. The reaction was terminated by the addition of 1 M TEAB (20 mL). The solution obtained was extracted with chloroform (2 × 20 mL), and the combined organic fractions were evaporated to dryness. To remove the traces of pyridine, the residue was co-evaporated with toluene. The gum obtained was treated with 10 mL of 0.25 M solution of DBU in CH_3CN-H_2O (9:1, v/v) for 16 hours at room temperature. After the following treatment with 0.5 M TEAB (10 mL), the reaction mixture was extracted with chloroform (2 × 15 mL). The combined organic fractions were washed with 0.5 M TEAB (3 × 20 mL) and evaporated to dryness. The product was isolated by silica gel column chromatography using 0–15% gradient of methanol in chloroform with 1% of TEA. The fractions containing the desired compound **14** were concentrated by evaporation to foam and dried in vacuo.

***5′-O-(4,4′-Dimethoxytrityl)-2′-O-(methoxymethyl)uridine 3′-O-(1-Oxido-4-methoxy-2-picolyl)phosphate* (14a)**

Yield 72%. R_f (solvent A) = 0.30. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 11.95 (1H, br s, Ura-N*H*), 8.06 (1H, d, J = 7.2 Hz, picolyl-*H*-6), 7.71 (1H, d, J = 8.2 Hz, Ura-*H*-6), 7.38–7.16 (10H, m, DMTr-*H*-Ar, picolyl-*H*-3), 6.85–6.77 (4H, m, DMTr-*H*-Ar), 6.70 (1H, dd, J = 7.2 Hz, J = 3.4 Hz, picolyl-*H*-5), 6.14 (1H, d, J = 6.6 Hz, *H*-1′), 5.16 (1H, d, J = 8.2 Hz, Ura-*H*-5), 5.16–5.10 (2H, m, P-OCH_2), 5.04–4.98 (1H, m, *H*-3′), 4.91 (1H, d, J = 6.7 Hz, OCH_αO), 4.63 (1H, d, J = 6.7 Hz, OCH_βO), 4.62–4.57 (1H, m, *H*-2′), 4.47–4.42 (1H, m, *H*-4′), 3.80 (3H, s, picolyl-OCH_3), 3.78 (6H, d, J =0.9 Hz, DMTr-OCH_3), 3.60–3.41 (1H, dd, J = 10.7 Hz, J = 2.3 Hz, H_α-5′), 3.35–3.48 (1H, m, H_β-5′), 3.29 (3H, s, OCH_3). ^{31}P-NMR (121.5 MHz, $CDCl_3$) δ = 0.10. MS: 806.28, calc. for $C_{39}H_{41}N_3O_{14}P^-$ $(M)^-$ 806.23.

***N*^{4}*-Benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-O-(methoxymethyl)cytidine 3′-O-(1-Oxido-4-methoxy-2-picolyl)phosphate* (14b)**

Yield 83%. R_f (solvent A) = 0.40. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.20 (1H, d, J = 7.4 Hz, Cyt-*H*-6), 8.04 (1H, d, J = 7.2 Hz, picolyl-*H*-6), 7.92–7.45 (5H, m, Bz-*H*-Ar), 7.43–7.19 (11H, m, DMTr-*H*-Ar, Cyt-*H*-5, picolyl-*H*-3), 6.86–6.79 (4H, m, DMTr-*H*-Ar), 6.69 (1H, dd, J = 7.2 Hz, J = 3.4 Hz, picolyl-*H*-5), 6.23 (1H, d, J = 4.3 Hz, *H*-1′), 5.10 (2H, ddd, J = 31.2 Hz, J = 17.0 Hz, J = 7.7 Hz, P-OCH_2), 5.01–4.93 (1H, m, *H*-3′), 4.96 (1H, d, J = 6.5 Hz, OCH_αO), 4.75 (1H, d, J = 6.5 Hz, OCH_αO), 4.59–4.54 (1H, m, *H*-2′), 4.50–4.45 (1H, m, *H*-4′), 3.79 (6H, d, J = 0.6 Hz, DMTr-OCH_3), 3.78 (3H, s, picolyl-OCH_3), 3.63–3.53 (2H, m, *H*-5′), 3.33 (3H, s, OCH_3). ^{31}P-NMR (121.5 MHz, $CDCl_3$) δ = −0.03. MS: 909.26, calc. for $C_{46}H_{46}N_4O_{14}P^-$ $(M)^-$ 909.28.

***N*^{6}*-Benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-O-(methoxymethyl)adenosine 3′-O-(1-Oxido-4-methoxy-2-picolyl)phosphate* (14c)**

Yield 84%. R_f (solvent A) = 0.40. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 9.12 (1H, br s, Ade-N*H*), 8.71 (1H, s, Ade-*H*-8), 8.14 (1H, s, Ade-*H*-2), 8.05 (1H, d, J = 7.3 Hz, picolyl-*H*-6), 8.04–7.47 (5H, m, Bz-*H*-Ar), 7.41–7.15 (10H, m, DMTr-*H*-Ar, picolyl-*H*-3), 6.79–6.73 (4H, m, DMTr-*H*-Ar), 6.70 (1H, dd, J = 7.3 Hz, J = 3.6 Hz, picolyl-*H*-5), 6.35 (1H, d, J = 7.4 Hz, *H*-1′), 5.24–5.06 (4H, m, *H*-2′, *H*-3′, P-OCH_2), 4.88 (1H, d, J = 6.8 Hz, OCH_αO), 4.63–4.59 (1H, m, *H*-4′), 4.53 (1H, d, J = 6.8 Hz, OCH_βO), 3.78 (3H, s, picolyl-OCH_3), 3.75 (6H, s, DMTr-OCH_3), 3.51–3.45 (2H, m, *H*-5′), 3.03 (3H, s, OCH_3). ^{31}P-NMR (121.5 MHz, $CDCl_3$) δ = 0.06. MS: 933.32, calc. for $C_{47}H_{46}N_6O_{13}P^-$ $(M)^-$ 933.29.

***N*^{2}*-Isobutyryl-5′-O-(4,4′-dimethoxytrityl)-2′-O-(methoxymethyl)guanosine 3′-O-(1-Oxido-4-methoxy-2-picolyl)phosphate* (14d)**

Yield 72%. R_f (solvent A) = 0.25. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 12.05 (1H, br s, Gua-N*H*), 10.78 (1H, br s, Gua-N*H*), 8.02 (1H, d, J = 7.2 Hz, picolyl-*H*-6), 7.74 (1H, s, Gua-*H*-8), 7.40–7.14 (10H, m, DMTr-*H*-Ar, picolyl-*H*-3), 6.76–6.70 (5H, m, DMTr-*H*-Ar, picolyl-*H*-5), 6.02 (1H, d, J = 6.3 Hz,

H-1′), 5.42–5.34 (1H, m, *H*-3′), 5.21 (2H, d, $J = 7.0$ Hz, P-OCH_2), 5.10–5.05 (1H, m, *H*-2′), 4.84 (1H, d, $J = 6.7$ Hz, OCH_αO), 4.58–4.52 (2H, m, *H*-4′, OCH_βO), 3.76 (3H, s, picolyl-OCH_3), 3.75 (6H, d, $J = 1.7$ Hz, DMTr-OCH_3), 3.47–3.38 (1H, m, H_α-5′), 3.28 (1H, dd, $J = 10.8$ Hz, $J = 4.1$ Hz, H_β-5′), 3.12 (3H, s, OCH_3), 2.55 (1H, sept, $J = 6.9$ Hz, *i*Bu-C*H*), 1.14, 1.07 (6H, d, $J = 6.9$ Hz, *i*Bu-CH_3). ^{31}P-NMR (121.5 MHz, $CDCl_3$) $\delta = 0.54$. MS: 915.43, calc. for $C_{44}H_{48}N_6O_{14}P^-$ $(M)^-$ 915.30.

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