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Synthesis of 5'-Deoxy-5'-Ethoxycarbonylmethyl Nucleosides, the Precursors of Oligonucleotides with the Amide Internucleoside Bond C3'-NH-C(O)-CH_2-C5'

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Abstract—A method for the synthesis of 5'-deoxy-5'-ethoxycarbonylmethyl nucleosides has been developed. 3-*O*-benzyloxymethyl-1,2-*O*-isopropylidene-α-*D*-allofuranose was oxidized by sodium periodate to form a 5'-aldo derivative, which was converted by the reaction with triethylphosphonoacetate in the presence of sodium hydride into a 5-deoxy-5-ethoxycarbonylmethylene derivative. The hydration of the unsaturated compound gave 5-deoxy-5-ethoxycarbonylmethyl-1,2-*O*-isopropylidene-α-*D*-ribofuranose. After the benzylation of 3-hydroxyl, the removal of the isopropylidene group by heating with acetic acid, and the subsequent acetylation, 1,2-di-*O*-acetyl-3-*O*-benzyl-5-deoxy-5-ethoxycarbonylmethyl-*D*-ribofuranose was obtained, which reacted with persilylated nucleic acid bases to form 5'-deoxy-5'-ethoxycarbonylmethyl nucleosides.

Key words: nucleoside analogues, synthesis

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INTRODUCTION

Therapy with synthetic oligonucleotide analogues (antisense therapy) is a promising method of the treatment of viral and oncological diseases [1, 2].[2] The idea of the method consists of the suppression of gene translation through the specific binding of a synthetic oligonucleotide to the corresponding site of mRNA. The main mechanism of action of synthetic deoxyoligonucleotides is the attack on the resulting DNA–RNA hybrid of the intracellular endonuclease of RNase H, which destroys the ribo chain of the hybrid duplex, thereby preventing the translation [3, 4]. Therefore, the efforts of investigators have been directed at the synthesis of modified deoxyoligonucleotides. In the last 15 years, a great number of oligonucleotide analogues modified at the nucleic acid base, carbohydrate residue, and internucleotide bond have been synthesized (see reviews [5–7]).

With the discovery of RNA interference, the interest in chemically modified ribooligonucleotides greatly increased (see reviews [8–10]). RNA interference is a natural mechanism of regulation of gene expression in which short (20–30 nt) RNAs (siRNA, short interfering RNAs; miRNA, microRNA) play a key role. In view of this, the idea to use chemically modified analogues of siRNA and miRNA in the therapy of human diseases appears to be very promising [11–13].

Previously, we have synthesized 3'-deoxy-3'-carboxymethyl ribonucleosides, the precursors of oligonucleotides in which the phosphate bond is substituted for by the C3'–CH_2–CO–NH–C5' bond [14]. The present study is devoted to the synthesis of the precursors of oligonucleotides with a reverse sequence of atoms in the internucleoside bond C3'–NH–CO–CH_2–C5', namely, 5'-deoxy-5'-ethoxycarbonylmethyl ribonucleosides.

RESULTS AND DISCUSSION

The starting compound for the synthesis was 1,2 : 5,6-di-*O*-isopropylidene-α-*D*-allofuranose (**I**) [15] (see the scheme). After the protection of 3-hydroxyl by the benzyloxymethyl group [compound (**II**)], the 5,6-isopropylidene group was selectively removed by heating in 50% acetic acid to 50°C. The resulting diol (**III**) was oxidized by sodium periodate to aldehyde (**IV**). Aldehyde (**IV**) was converted into the unsaturated compound (**V**) by the Wadsworth–Horner–Emmons reaction [16]. The resulting olefin was hydrated at atmospheric pressure. In this case, simultaneously with the hydration of the double bond, the benzyloxymethyl protection [compound (**VI**)] is removed.

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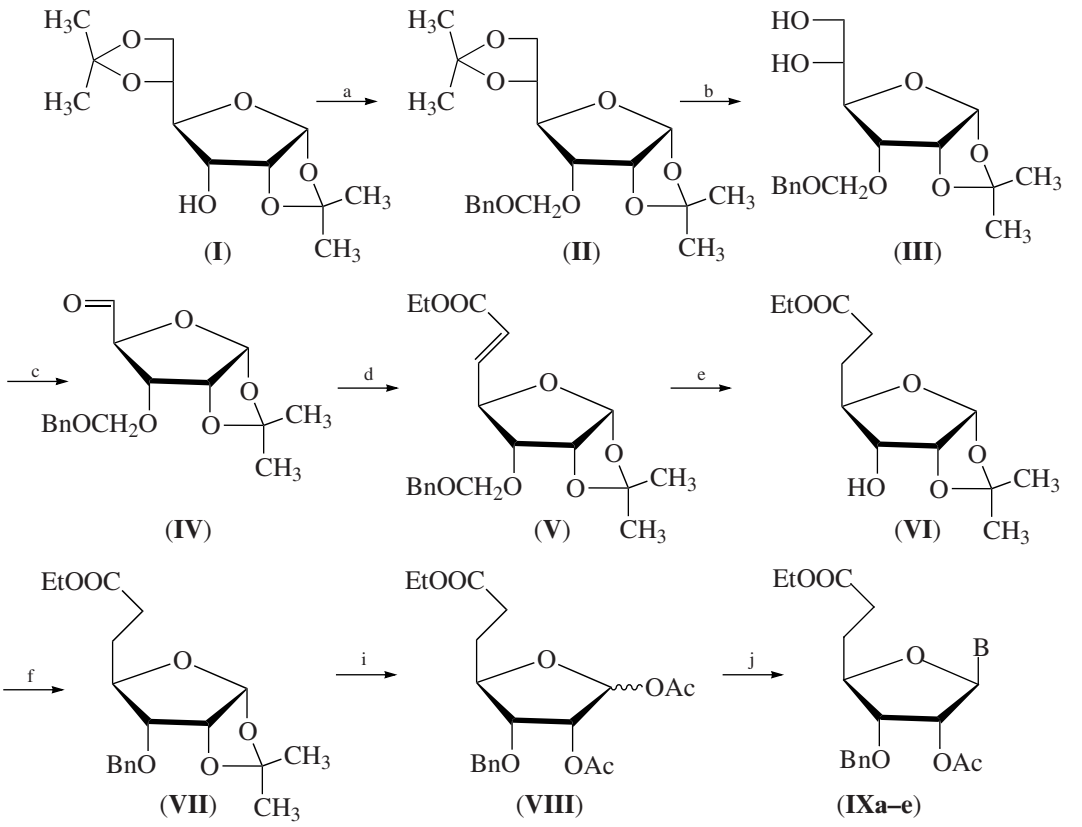
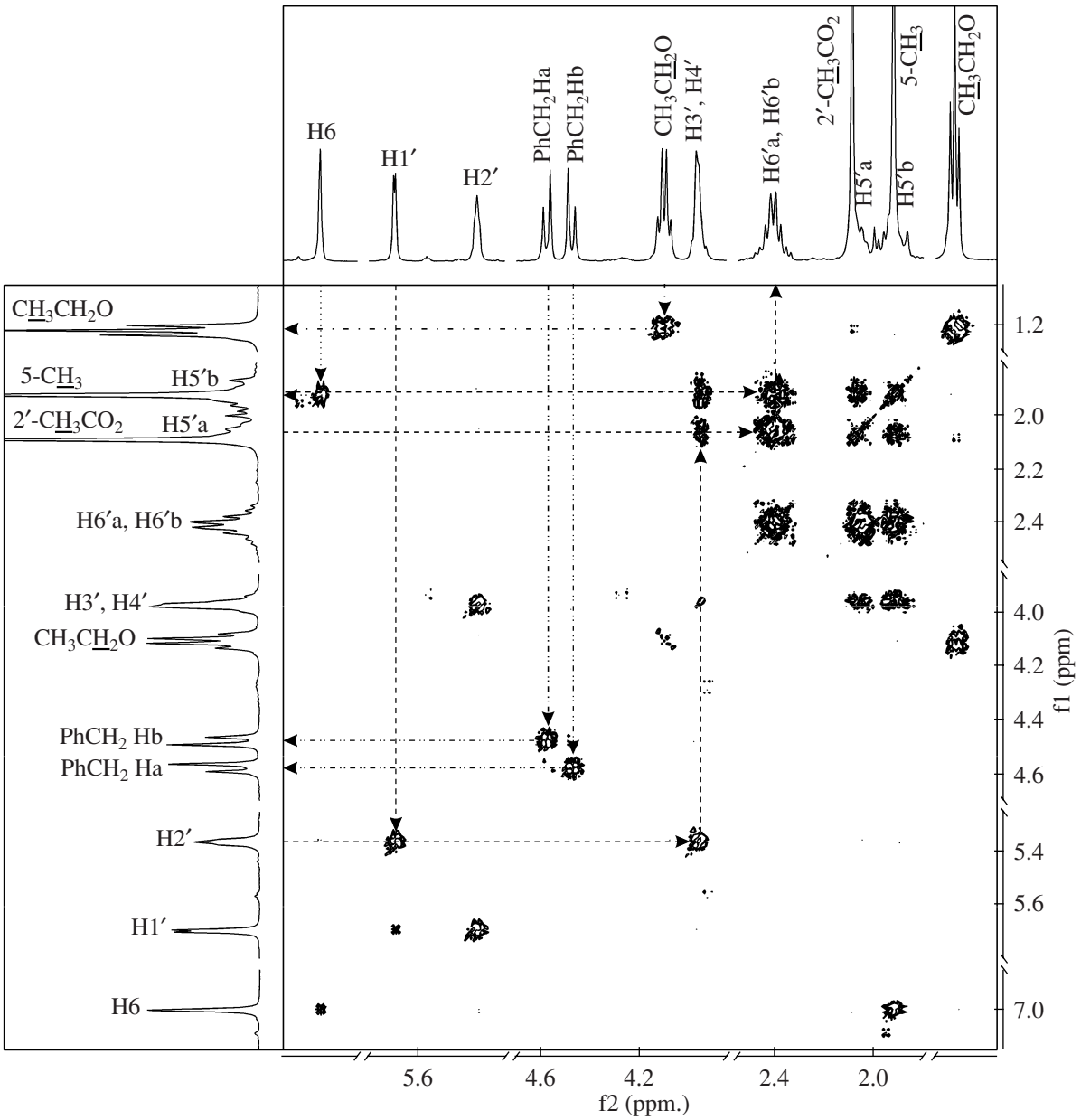
[2] Abbreviations: Bn, benzyl; BSA, *N*-*O*-bis(trimethylsilyl)acetamide; DIPEA, diisopropylethylamine; TBDMS, *tert*-butyldimethylsilyl; Tf, trifluoromethanesulfonate.



(a) $BnOCH_2Cl$, DIPEA, methylene chloride; (b) 50% AcOH, 50°C; (c) $NaIO_4$; (d) $(EtO)_2P(O)CH_2COOEt$, NAH, THF; (e) H_2, $Pd(OH)_2/C$, ethanol; (f) BnBr, NaH, THF; (i) 80% AcOH, boiling, then Ac_2O, pyridine; (j) nucleic acid bases, BSA, Me_3Si-Otf, CH_3CN.

The choice of a new protecting group for 3-hydroxyl was dictated by the following requirements: first, it should be stable under acidic conditions (the next stage of synthesis) and, second, because the resulting nucleosides has to be used in the oligonucleotide synthesis, the protecting group should enable one to clearly discriminate between 2- and 3-hydroxyl groups of sugar. The benzyl group best satisfies these conditions. After the benzylation of (**VI**), the completely protected derivative (**VII**) was subjected to acid hydrolysis (boiling in 80% acetic acid) followed by acetylation. The resulting diacetate (**VIII**) was used for the synthesis of nucleosides analogues (**IXa**)–(**IXe**) by the standard method [17] with some modifications.

The structures of all compounds synthesized were confirmed by ^1H NMR spectra. The spectra of compounds (**I**), (**III**), and (**VI**), which contain hydroxy groups, were measured in DMSO-d_6, which made it possible not only to show the presence of free hydroxyls by the occurrence of their signals (signals were identified from their disappearance after the addition of D_2O to the sample), but also to determine their position in the molecule. The spectrum of aldehyde (**IV**) showed a characteristic signal of H5 at 9.676 ppm. The double bond in compound (**V**) made itself evident in the appearance of two signals of olefin protons at 6.960 (H5) and 6.156 ppm (H6), both having the form of a doublet of doublets. This is related to both the interaction of protons with each other ($J_{5,6}$ 15.71 Hz) and with H4 ($J_{4,5}$ 5.19, $^4J_{4,6}$ 1.46). Based on the spin–spin coupling constants, it can be assumed that the double bond has an *E* configuration. The hydration of the double bond in (**V**) leads to the disappearance of olefin proton signals and the appearance of signals of H5a and H5b (1.899 and 1.637 ppm) and 6-CH_2 (2.365 ppm) in the expectedly high field of the spectrum. In the spectrum of diacetate (**VIII**), the signals of two methyls of the isopropylidene group are absent, and two singlets of the acetyl groups (at 1.568 and 1.337 ppm) appear. Interestingly, the diastereotropism of methylene protons of the $OC\underline{H}_2CH_3$ group manifests itself only in the spectrum of diacetate. The signal of the CH_2 group in the spectra of preceding compounds is a quartet, whereas



A fragment of the COSY-DQF spectrum of 2'-*O*-acetyl-3'-*O*-benzyl-5'-deoxy-5'-ethoxycarbonylmethyl-5-ribosylthymine (**IXa**), which illustrates the interaction of protons of the carbohydrate cycle (dashed line), thymine (dotted line), ethoxygroup (dot-and-dash line), and the methylene group of benzene (two dots-and-dash line).

in the spectrum of diacetate, it is a complex multiplet. In the spectra of nucleosides, the characteristic features of the spectrum of diacetate are retained, and the signals of the nucleic acid base appear (see the figure).

The 2D spectrum of compound (**IXa**) (see the figure) enables one to unambiguously assign the signals to the corresponding protons of the molecule and identify signals that overlap in the 1D spectra. Thus, the peak at 4 ppm contains two signals, H3' (is identified from the cross peak with H2') and H4' (cross peaks with H5'a and H5'b). It is seen that the H5'a signal overlaps with the signal of methyl of the 2'-acetyl group, and the H5'b signal overlaps with the signal of 5-CH_3.

Thus, we developed a convenient method for the synthesis of all four 5'-deoxy-5'-ethoxycarbonylmethyl ribonucleosides.

EXPERIMENTAL

The following preparations were used: benzene, chloroform, methylene chloride, acetonitrile, ethyl acetate, tetrahydrofuran, acetic acid, and pyridine (all of

extra purity grade) (Khimmed, Russia); acetic anhydride of extra purity grade (Reakhim, Russia); cytosine, thymine, adenine, and guanine (Fluka, Switzerland); and triethylphosphonoacetate, benzyloxymethylchloride, benzyl bromide, BSA, trimethylsilyl trifluoromethanesulfonate, sodium hydride, sodium periodate, and DIPEA (Aldrich, United States). Benzene, acetonitrile, and methylene chloride were dried by distillation over phosphorus pentoxide; tetrahydrofurane was dried over lithium alumohydride; and pyridine, over calcium hydride. 2-*N*-Acetylguanine was obtained as described in [18].

NMR spectra (δ, ppm, spin–spin coupling constant, Hz) were recorded on a Bruker AMXIII-400 spectrometer with a working frequency of 400 MHz. The spectra were processed using the program MestReNova, version 5.3.0 (Mestrelab Research SL). For compounds containing hydroxy groups (spectrum in DMSO-d_6), the multiplicity of signals of nonexchanging protons that interact with the proton of the hydroxy group was determined after the addition of ^2H_2O to the sample.

Substances were purified by flash chromatography on silica gel [19]. Silica gel 60, 40–63 μm (Merck, Germany) was used. TLC was carried out on Silica gel 60 F_{254} plates (Merck, Germany) in systems of ethanol–chloroform 1 : 49 (system A); isopropanol–hexane 1 : 19 (system B); isopropanol–hexane 3 : 47 (system C); isopropanol–hexane 1 : 49 (system D); methanol–chloroform 1 : 49 (system E); and methanol–chloroform 1 : 19 (system F).

1,2,5,6-Di-*O*-isopropylidene-α-*D*-allofuranose (I) was obtained from 1,2,5,6-di-*O*-isopropylidene-α-*D*-glucofuranose as described in [15]. ^{1}H NMR (DMSO-d_6): 5.657 (1 H, d, $J_{1,2}$ 3.63, H1), 5.046 (1 H, d, *J* 7.13, 3-OH), 4.456 (1 H, t, *J* 4.21, H2), 4.228 (1 H, dt, $J_{4,5}$ 7.15, $J_{5,6}$ 2.75, H5), 3.929 (1 H, dd, $J_{3,4}$ 9.06, $J_{4,5}$ 7.15, H4), 3.828 (2 H, m, H6a, H6b), 3.743 (1 H, dd, $J_{2,3}$ 4.62, $J_{3,4}$ 9.06, H3), 1.446 (3 H, c, CH_3), 1.323 (3 H, s, CH_3), 1.275 (3 H, c, CH_3), 1.267 (3 H, s, CH_3).

3-*O*-Benzyloxymethyl-1,2,5,6-di-*O*-isopropylidene-α-*D*-allofuranose (II). DIPEA (7.24 g; 9.59 ml, 0.056 mol) and then benzyloxymethyl chloride (7.52 g, 6.59 ml, 0.048 mol) were added with stirring to a solution of 10.4 g (0.04 mol) of diisopropylidene allose (**I**) in 40 ml of absolute methylene chloride. The mixture was refluxed for 5 h and left overnight under stirring. Water (40 ml) was added and the mixture was stirred for 30 min. The organic layer was separated and the aqueous layer was extracted by methylene chloride. The combined organic extracts were washed with water, a 10% $KHSO_4$ solution, and water, dried over anhydrous Na_2SO_4, and evaporated. Purification on a column of silica gel and elution with isopropanol : hexane 2 : 49 yielded 13.3 g (87.4%) of the crystalline substance (**II**); yield; R_f 0.8 (A), mp 46–47°C (from cyclohexane). ^{1}H NMR (DMSO-d_6): 7.39–7.26 (5 H, m, Ph), 5.715 (1 H, d, $J_{1,2}$ 3.61, H1) 4.818 (1 H, d, $^2J_{a,b}$ 6.78, Ha PhC$\underline{H}_2$O), 4.797 (1 H, d, $^2J_{a,b}$ 6.78, Hb PhC$\underline{H}_2$O), 4.687 (1 H, d, $^2J_{a,b}$ 11.55 Ha OC$\underline{H}_2$O), 4.662 (1 H, t, *J* 4.01, H2), 4.572 (1 H, d, $^2J_{a,b}$ 11.5, Hb OC$\underline{H}_2$O), 4.221 1 H, dt, $J_{4,5}$ 3.62, $J_{5,6}$ 6.75, H5), 3.992 (1 H, dd, $J_{3,4}$ 8.89, $J_{4,5}$ 3.62 H4), 3.958 (1 H, dd, $J_{5,6a}$ 6.78, $J_{6a,6b}$ 8.08, H6a), 3.912 (1 H, dd, $J_{2,3}$ 4.12, $J_{3,4}$ 8.89, H3), 3.805 (1 H, dd, $J_{5,6b}$ 6.73, $J_{6a,6b}$ 8.08, H6b), 1.463 (3 H, s, CH_3), 1.324 (3 H, c, CH_3), 1.263 (3 H, c, CH_3), 1.259 (3 H, s, CH_3).

3-*O*-Benzyloxymethyl-1,2-*O*-isopropylidene-α-*D*-allofuranose (III). Water (30 ml) was added to a solution of compound (**II**) (12.7 g, 33.4 mmol) in acetic acid (30 ml) and the suspension was heated with stirring to 50°C until a homogeneous solution formed (0.5 h, control TLC). The solution was evaporated and the residue was evaporated several times with water and then with absolute benzene. Chromatography on a silica gel column (ethanol–chloroform 2 : 49) yielded 9.1 g (80%) of crystalline substance (**III**); mp 62–63°C (from a cyclohexane–ethyl acetate mixture 30 : 1), R_f 0.34 (A). ^{1}H NMR (DMSO-d_6): 7.39–7.26 (5 H, m, Ph), 5.697 (1 H, d, $J_{1,2}$ 3.64, H1), 4.822 (1 H, d, *J* 4.79 5-OH), 4.793 (1 H, d, $^2J_{a,b}$ 6.76, Ha PhC$\underline{H}_2$O), 4.733 (1 H, d, $^2J_{a,b}$ 6.76, Hb PhC$\underline{H}_2$O), 4.683 (1 H, d, $^2J_{a,b}$ 10.89, Ha OC$\underline{H}_2$O), 4.624 (1 H, t, *J* 4.07, H2), 4.537 (1 H, d, $^2J_{a,b}$ 10.89, Hb OC$\underline{H}_2$O), 4.498 (1 H, d, *J* 5.61, 5-OH), 4.091 (1 H, dd, $J_{2,3}$ 4.44, $J_{3,4}$ 8.81, H3), 4.007 (1 H, dd, $J_{3,4}$ 8.81, $J_{4,5}$ 2.58, H4), 3.700 (1 H, m, H5), 3.467 (1 H, dd, $J_{5,6a}$ 6.02, $J_{6a,6b}$ 11.92, H6a), 3.363 (1 H, dd, $J_{5,6b}$ 7.48, $J_{6a,6b}$ 11.92, H6b), 1.457 (3 H, c, CH_3), 1.257 (3 H, s, CH_3).

3-*O*-Benzyloxymethyl-1,2-*O*-isopropylidene-α-*D*-*ribo*-pentadialdo-1,4-furanose (IV). Sodium periodate (8.56 g, 40 mmol) and water (5 ml) were added to a solution of derivative (**III**) (9.1 g, 26.7 mmol) in ethyl acetate (50 ml). The mixture was stirred at room temperature overnight (control TLC in a system of isopropanol–hexane 1 : 19), filtered, and the residue on the filter was washed with ethyl acetate (2 × 20 ml). The filtrate was washed with water, dried over anhydrous Na_2SO_4, and evaporated. The yield of product (**IV**) as a dense oil was 8.1 g (98.4%); R_f 0.41 (B). ^{1}H NMR ($CDCl_3$): 9.676 (1 H, d, $J_{4,5}$ 1.82, H5), 7.38–7.26 (5 H, m, Ph), 5.842 (1 H, d, $J_{1,2}$ 3.31, H1), 4.891 (1 H, d, $^2J_{a,b}$ 7.08, Ha PhC$\underline{H}_2$O), 4.850 (1 H, d, $^2J_{a,b}$ 7.08, Hb PhC$\underline{H}_2$O), 4.713 (1 H, d, $^2J_{a,b}$ 11.57, Ha OC$\underline{H}_2$O), 4.664 (1 H, pseudo s, t, *J* 3.71, H2), 4.598 (1 H, d, $^2J_{a,b}$ 11.57, Hb OC$\underline{H}_2$O), 4.442 (1 H, dd, $J_{3,4}$ 9.44, $J_{4,5}$ 1.82, H4), 4.066 (1 H, dd, $J_{2,3}$ 4.32, $J_{3,4}$ 9.44, H3), 1.601 (3 H, c, CH_3), 1.353 (3 H, s, CH_3).

3-*O*-Benzyloxymethyl-5-deoxy-1,2-*O*-isopropylidene-5-ethoxycarbonylmethylene-α-*D*-ribofuranose (V). A 60% suspension of sodium hydride (1.26 g, 31.6 mmol) in mineral oil and dry THF (15 ml) were placed in a retort equipped with a magnetic stirrer, a dropping funnel, and a thermometer. The mixture was cooled in an ice bath, and triethylphosphonoacetate (7.08 g, 6.27 ml, 31.6 mmol) in dry THF (21 ml) was added dropwise with stirring so that the temperature was no higher than 5°C. The mixture was stirred for another 20 min at 0–5°C. Then, a solution of aldehyde

(**IV**) (8.1 g, 26.3 mmol) in dry THF (50 ml) was added and care was taken that the temperature was no higher than 5°C. The temperature was brought to room temperature and the mixture was stirred for 90 min. During stirring, a resin-like sediment of sodium diethylphosphate was formed. The mixture was diluted with water (100 ml) and extracted with ethyl acetate. Organic extracts were washed with a 10% sodium bicarbonate solution and water, dried over anhydrous Na_2SO_4, evaporated, and the residue was chromatographed on a silica gel column. Elution with isopropanol–hexane 3 : 97 yielded 8.2 g (82.4%) of (**V**) as a dense oil; R_f 0.38 (B). ^{1}H NMR ($CDCl_3$): 7.36–7.26 (5 H, m, Ph), 6.960 (1 H, dd, $J_{4,5}$ 5.19, $J_{5,6}$ 15.71, H5), 6.156 (1 H, dd, $^4J_{4,6}$ 1.46, $J_{5,6}$ 15.71, H6), 5.782 (1 H, d, $J_{1,2}$ 3.52, H1), 4.902 (1 H, d, $^2J_{a,b}$ 6.99, Ha PhC$\underline{H}_2$O), 4.820 (1 H, d, $^2J_{a,b}$ 6.99, Hb PhC$\underline{H}_2$O), 4.744 (1 H, d, $^2J_{a,b}$ 11.43, Ha OC$\underline{H}_2$O), 4.646 (1 H, pseudo s, t, *J* 3.92, H2), 4.626 (1 H, ddd, $J_{4,3}$ 9.26, $J_{4,5}$ 5.19, $^4J_{4,6}$ 1.46, H4), 4.577 (1 H, d, $^2J_{a,b}$ 11.43, Hb OC$\underline{H}_2$O), 4.182 (2 H, q, *J* 7.12, OC$\underline{H}_2$CH$_3$), 3.713 (1 H, dd, $J_{2,3}$ 4.19, $J_{3,4}$ 9.26, H3), 1.604 (3 H, c, CH_3), 1.337 (3 H, s, CH_3), 1.266 (3 H, t, *J* 7.12, OCH$_2$C$\underline{H}_3$).

5-Deoxy-1,2-*O*-isopropylidene-5-ethoxycarbonylmethyl-α-*D*-ribofuranose (VI). $Pd(OH)_2$/C (0.8 g) was added to a solution of unsaturated compound (**V**) (8.1 g, 21.4 mmol) in ethanol (40 ml) and the mixture was hydrated at room temperature and atmospheric pressure for 24 h. The mixture was filtered through cellite, cellite on the filter was washed with ethanol (3 × 20 ml), and the solvent was evaporated. The yield of compound (**VI**) was 5.5 g (98.7%) as a dense oil; R_f 0.32 (C). ^{1}H NMR (DMSO-d_6): 5.627 (1 H, d, $J_{1,2}$ 3.69, H1), 5.040 (1 H, d, *J* 6.92, 3-OH), 4.429 (1 H, pseudo s, t, *J* 4.11, H2), 4.048 (2 H, q, *J* 7.11, OC$\underline{H}_2$CH$_3$), 3.685 (1 H, dt, $J_{3,4}$ 8.61, $J_{4,5a}$ 4.21, $J_{4,5b}$ 8.22, H4), 3.486 (1 H, dd, $J_{2,3}$ 4.11, $J_{3,4}$ 8.61, H3), 2.365 (2 H, m, H6a, H6b), 1.899 (1 H, m, H5a), 1.637 (1 H, m, H5b), 1.421 (3 H, s, CH_3), 1.250 (3 H, s, CH_3), 1.175 (3 H, t, *J* 7.11, OCH$_2$C$\underline{H}_3$).

3-*O*-Benzyl-5-deoxy-1,2-*O*-isopropylidene-5-ethoxycarbonylmethyl-α-*D*-ribofuranose (VII). A suspension of NaH (1.12 g, 28 mmol) was added with stirring and cooling to a solution of compound (**VI**) (5.21 g, 20 mmol) in dry THF (20 ml). After the termination of hydrogen release, the suspension was stirred for an additional 10 min and benzyl bromide (4.1 g, 2.85 ml, 24 mmol) was added. The mixture was stirred at room temperature for 4 h, acetic acid (2 ml) was added, and extraction with chloroform was carried out. Chloroform extracts were washed with a saturated $NaHCO_3$ solution and water, dried over anhydrous Na_2SO_4, and chromatographed. The purification of the product on a column of silica gel and elution with isopropanol–hexane 1 : 49 yielded 4.9 g (69.9%) of compound (**VII**) as a dense oil; R_f 0.56 (D). ^{1}H NMR ($CDCl_3$): 7.39–7.26 (5 H, m, Ph), 5.687 (1 H, d, $J_{1,2}$ 3.80, H1), 4.763 (1 H, d $^2J_{a,b}$ 11.92, Ha PhC$\underline{H}_2$O), 4.543 (1 H, t, *J* 4.05, H2), 4.530 (1 H, d, $^2J_{a,b}$ 11.92, Hb PhC$\underline{H}_2$O), 4.095 (2 H, q, *J* 7.14, OC$\underline{H}_2$CH$_3$), 4.018 (1 H, dt, $J_{3,4}$ 8.90, $J_{4,5a}$ 4.01, $J_{4,5b}$ 8.38, H4), 3.413 (1 H, dd, $J_{2,3}$ 4.36, $J_{3,4}$ 8.90, H3), 2.394 (2 H, m, H6a, H6b), 2.054 (1 H, m, H5a), 1.767 (1 H, m, H5b), 1.568 (3 H, s, CH_3), 1.337 (3 H, s, CH_3), 1.222 (3 H, t, *J* 7.14, OCH$_2$C$\underline{H}_3$).

3-*O*-Benzyl-5-deoxy-1,2-di-*O*-acetyl-5-ethoxycarbonylmethyl-*D*-ribofuranose (VIII). Water (10 ml) was added to a solution of compound (**VII**) (4.8 g, 13.7 mmol) in acetic acid (40 ml) and the solution was heated under stirring and weak boiling until the termination of the reaction (0.5 h). The solution was evaporated and the residue was reevaporated with water to remove acetic acid and then with absolute pyridine (4 × 5 ml). The residue was dissolved in absolute pyridine (30 ml), acetic anhydride (5.61 g, 7.17 ml, 55 mmol) was added, and the mixture was stirred overnight at room temperature. Water (2 ml) was added, and the solution was kept for 30 min and evaporated. The residue was dissolved in chloroform (100 ml), washed with a saturated $NaHCO_3$ solution, and dried over anhydrous Na_2SO_4, after which the solvent was evaporated. The chromatography on a silica gel column and elution with isopropanol–hexane 1 : 49 yielded 5 g (92.5%) of compound (**VIII**) as a dense oil; R_f 0.48 (D). ^{1}H NMR ($CDCl_3$): 7.39–7.26 (5 H, m, Ph), 6.088 (1 H, s, H1), 5.279 (1 H, d, $J_{2,3}$ 4.04, H2), 4.604 (1 H, d, $^2J_{a,b}$ 11.34, Ha PhC$\underline{H}_2$O), 4.450 (1 H, d, $^2J_{a,b}$ 11.34, Hb PhC$\underline{H}_2$O), 4.105 (2 H, m, OC$\underline{H}_2$CH$_3$), 4.068 (1 H, m, H4), 3.937 (1 H, dd, $J_{2,3}$ 4.04, $J_{3,4}$ 7.82, H3), 2.381 (2 H, m, H6a, H6b), 2.105 (3 H, c, 2-CH_3CO), 2.056 (3 H, s, 1-CH_3CO), 2.018 (1 H, m, H5a), 1.853 (1 H, m, H5b), 1.232 (3 H, t, *J* 7.14, OCH$_2$C$\underline{H}_3$).

A general method of obtaining the nucleoside analogues (IXa)–(IXe). BSA was added with stirring to a suspension of a nucleic acid base in absolute acetonitrile and the mixture was heated to 60°C until the residue was dissolved (0.5–1 h). A solution of diacetate (**VIII**) in absolute acetonitrile followed by trimethylsilyltriflate was added to the solution. The mixture was heated under weak boiling for 4 h, cooled, and poured under stirring into a cold saturated $NaHCO_3$ solution (50 ml). The mixture was stirred for 30 min and extracted with chloroform. The extracts were washed with water and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was chromatographed on a column of silica gel.

2'-*O*-Acetyl-3'-*O*-benzyl-5'-deoxy-5'-ethoxycarbonylmethyl-5-methyluridine (IXa) was obtained from thymine (0.41 g, 3.3 mol), BSA (1.52 g, 1.83 ml, 7.5 mmol) in absolute acetonitrile (10 ml), diacetate (**VIII**) (1 g, 2.5 mmol) (a solution in 5 ml of absolute acetonitrile), and trimethylsilyltriflate (0.72 g, 0.58 ml, 3.3 mmol). Elution with methanol–chloroform 1 : 99 yielded 1.1 g (95.6%) of nucleoside (**IXa**) as a solid foam; R_f 0.74 (E). ^{1}H NMR ($CDCl_3$): 8.488 (1 H, s, br, H3), 7.39–7.26 (5 H, m, Ph), 7.004 (1 H, br d, *J* 1.14, H6), 5.694 (1 H, d, $J_{1',2'}$ 3.39, H1'), 5.355 (1 H, dd, $J_{1',2'}$ 3.39, $J_{2',3'}$ 4.88, H2'), 4.581 (1 H, d, $^2J_{a,b}$ 11.28, Ha PhC$\underline{H}_2$O), 4.478 (1 H, d, $^2J_{a,b}$ 11.28, Hb PhC$\underline{H}_2$O), 4.106

(2 H, q, *J* 7.14, Ha OC$\underline{H}_2$CH$_3$), 4.00–3.92 (2 H, m, H3', H4'), 2.409 (2 H, m, H6'a, H6'b), 2.095 (3 H, s, 2'-CH$_3$COO), 2.059 (1 H, m, H5'a), 1.926 (3 H, s, br, *J* 1.14, 5-CH$_3$), 1.914 (1 H, m, H5'b), 1.277 (3 H, t, *J* 7.14, OCH$_2$C$\underline{H}_3$).

2'-*O*-Acetyl-3'-*O*-benzyl-5'-deoxy-5'-ethoxycarbonylmethylcytidine (IXb) was obtained from cytosine (0.16 g, 1.42 mmol) in absolute acetonitrile (6 ml), BSA (1 g, 1.2 ml, 4.9 mmol), diacetate (**VIII**) (0.43 g, 1.1 mmol) (a solution in 4 ml of absolute acetonitrile), and trimethylsilyltriflate (0.32 g, 0.26 ml, 1.42 mmol). Elution with methanol–chloroform 1 : 24 yielded 0.44 g (90.6%) of nucleoside (**IXb**) as a solid foam; R_f 0.39 (F). ^{1}H NMR (CDCl$_3$): 7.39–7.26 (5 H, m, Ph), 7.348 (1 H, d, $J_{5,6}$ 7.42, H6), 5.798(1 H, d, $J_{5,6}$ 7.42, H5), 5.719 (1 H, d $J_{1',2'}$ 2.06, H1'), 5.494 (1 H, dd, $J_{1',2'}$ 2.06, $J_{2',3'}$ 5.29, H2'), 4.589 (1 H, d, $^2J_{a,b}$ 11.23, Hb PhC$\underline{H}_2$O), 4.411 (1 H, d, $^2J_{a,b}$ 11.23, Hb PhC$\underline{H}_2$O), 4.106 (2 H, m, OC$\underline{H}_2$CH$_3$), 3.995 (1 H, dt, $J_{3',4'}$ 8.17, $J_{4',5'a}$ 4.04, $J_{4',5'b}$ 8.35, H4'), 3.876 (1 H, dd, $J_{2',3'}$ 5.29, $J_{3',4'}$ 8.17, H3'), 2.438 (2 H, m, H6'a, H6'b), 2.096 (3 H, s, 2'-CH$_3$COO), 2.056 (1 H, m, H5'a), 1.923 (1 H, m, H5'b), 1.222 (3 H, t, *J* 7.08, OCH$_2$C$\underline{H}_3$).

2'-*O*-Acetyl-3'-*O*-benzyl-5'-deoxy-5'-ethoxycarbonylmethyladenosine (IXc) was obtained from adenine (0.16 g, 1.2 mmol) in absolute acetonitrile (5 ml), BSA (0.84 g, 1 ml, 4.1 mmol), diacetate (**VIII**) (0.36 g, 0.91 mmol) (a solution in 4 ml of absolute acetonitrile), and trimethylsilyltriflate (0.27 g, 0.22 ml, 1.2 mmol). Elution with methanol–chloroform 1 : 49 yielded 0.23 g (53.7%) of nucleoside (**IXc**) as a solid foam; R_f 0.55 (F). ^{1}H NMR (CDCl$_3$): 8.318 (1 H, s, H2), 8.054 (1 H, s, H8), 7.39–7.26 (5 H, m, Ph), 5.997 (1 H, d, $J_{1',2'}$ 3.05, H1'), 5.854 (1 H, dd, $J_{1',2'}$ 3.05, $J_{2',3'}$ 5.25, H2'), 4.631 (1 H, d, $^2J_{a,b}$ 11.36, Ha PhC$\underline{H}_2$O), 4.548 (1 H, d, $^2J_{a,b}$ 11.36, Hb PhC$\underline{H}_2$O), 4.131 (1 H, m, H4'), 4.077 (2 H, m, OC$\underline{H}_2$CH$_3$), 4.445 (1 H, dd, $J_{2',3'}$ 5.25, $J_{3',4'}$ 6.88, H3'), 2.37 (2 H, m, H6'a, H6'b), 2.111 (3 H, s, 2'-CH$_3$COO), 2.082 (1 H, m, H5'a), 1.987 (1 H, m, H5'b), 1.196 (3 H, t, *J* 7.08, OCH$_2$C$\underline{H}_3$).

2'-*O*-Acetyl-3'-*O*-benzyl-5'-deoxy-5'-ethoxycarbonylmethyl-6-*N*-benzoyladenosine (IXd) was obtained from 6-*N*-benzoyladenine (0.11 g, 0.45 mmol) in absolute acetonitrile (3 ml), BSA (0.21 g, 0.25 ml, 1.03 mmol), diacetate (**VIII**) (0.16 g, 0.41 mmol) (a solution in 2 ml of absolute acetonitrile), and trimethylsilyltriflate (0.018 g, 0.015 ml, 0.08 mmol). Elution with methanol–chloroform 1 : 99 yielded 0.2 g (85.9%) of nucleoside (**IXd**) as a solid foam; R_f 0.69 (F). ^{1}H NMR (CDCl$_3$): 8.768 (1 H, s, H2), 8.054 (1 H, s, H8), 8.019 (2 H, m, *o*-H Bz), 7.605 (1 H, m, *n*-H Bz), 7.521 (2 H, m, *m*-H, Bz), 7.39–7.26 (5 H, m, Ph), 6.065 (1 H, d, $J_{1',2'}$ 3.05, H1'), 5.875 (1 H, dd, $J_{1',2'}$ 3.05, $J_{2',3'}$ 5.36, H2'), 4.644 (1 H, d, $^2J_{a,b}$ 11.35, Ha PhC$\underline{H}_2$O), 4.565 (1 H, d, $^2J_{a,b}$ 11.35, Hb PhC$\underline{H}_2$O), 4.454 (1 H, pseudo s, t, *J* 6.36, H3'), 4.165 (1 H, m, H4'), 4.082 (2 H, m, OC$\underline{H}_2$CH$_3$), 2.375 (2 H, m, H6'a, H6'b), 2.12 (3 H, s, 2'-CH$_3$COO), 2.102 (1 H, m, H5'a), 2.007 (1 H, m, H5'b), 1.199 (3 H, t, *J* 7.13, OCH$_2$C$\underline{H}_3$).

2'-*O*-Acetyl-3'-*O*-benzoyl-5'-deoxy-5'-ethoxycarbonylmethyl-2-*N*-acetylguanosine (IXe) was obtained from 2-*N*-acetylguanine (0.184 g, 0.95 mmol) in absolute acetonitrile (5 ml), BSA (0.67 g, 0.81 ml, 3.3 mmol), diacetate (**VIII**) (0.34 g, 0.86 mmol) (a solution in 4 ml of absolute acetonitrile), and trimethylsilyltriflate (0.22 g, 0.18 ml, 1 mmol). Elution with methanol–chloroform 1 : 49 yielded 0.33 g (72.6%) of nucleoside (**IXe**) as a solid foam; R_f 0.56 (E). ^{1}H NMR (CDCl$_3$): 7.662 (1 H, s, H8), 7.39–7.26 (5 H, m, Ph), 5.93 (1 H, d, $J_{1',2'}$ 5.76, H1'), 5.582 (1 H, pseudo s, t, *J* 5.36, H2'), 4.542 (2 H, s, br, Ha, Hb PhC$\underline{H}_2$O), 4.359 (1 H, m, H3'), 4.229 (1 H, m, H4'), 4.126 (2 H, m, OC$\underline{H}_2$CH$_3$), 2.424 (2 H, m, H6'a, H6'b), 2.291 (3 H, s, 2-CH$_3$CON), 2.058 (3 H, c, 2'-CH$_3$COO), 2.09 (1 H, m, H5'a), 2.005 (1 H, m, H5'b), 1.251 (3 H, t, *J* 7.11, OCH$_2$C$\underline{H}_3$).

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