ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2009, Vol. 35, No. 1, pp. 68–74. © Pleiades Publishing, Ltd., 2009.
Original Russian Text © S.V. Kochetkova, A.M. Varizhuk, N.A. Kolganova, E.N. Timofeev, V.L. Florent'ev, 2009, published in Bioorganicheskaya Khimiya, 2009, Vol. 35, No. 1, pp. 76–83.

Synthesis of 3'-Deoxy-3'-Carboxymethylnucleosides, Precursors of Oligonucleotides with an Amide Internucleoside Bond

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Received February 19, 2008; in final form, April 2, 2008

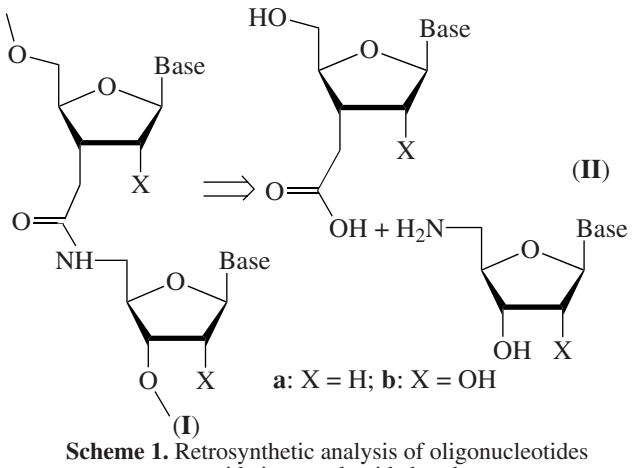
Abstract—An improved method for the synthesis of 3-deoxy-3-carboxymethyl nucleosides was suggested. Oxidation of 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-*D*-xylofuranose resulted in the 3-keto derivative, which was treated with triethylphosphonoacetate in the presence of sodium hydride to obtain the 3-deoxy-3-ethoxycarbonylmethylene derivative. Hydrogenation of the unsaturated compound proceeded strictly stereospecifically and gave the product with the *ribo*-configuration. Acetolysis of the resulting compound with AcOH–Ac_2O–CH_3SO_3H led to 1,2-di-*O*-acetyl-5-*O*-benzoyl-3-deoxy-3-ethoxycarbonylmethyl-*D*-ribofuranose, whose interaction with persilylated nucleic bases gave 3-deoxy-3-ethoxycarbonylmethylnucleosides in a total yield of 42–49% from the starting compound.

Key words: branched carbohydrates, nucleoside analogues, synthesis

DOI: 10.1134/S1068162009010099

INTRODUCTION

Therapy with synthetic oligonucleotide analogues (antisense therapy) is one of promising methods of treatment of virus and oncological diseases [1–4].[2] The idea of the method consists in the suppression of gene translation due to the specific binding of a synthetic oligonucleotide with the corresponding mRNA site. The high specificity of hybridization allows the selective suppression of expression of the genes involved in pathogenesis of disease. An antisense oligonucleotide should however possess specific characteristics. It should be stable to the action of endonucleases and at the same time specifically and strongly contact with the target mRNA sequence. Furthermore, it should penetrate easily through the cell membrane, possess low toxicity and, show no side effects. For the last 15 years a large number of oligonucleotide analogues modified at the nucleic base, the carbohydrate residue, and at the internucleotide bond (see [5–7]) have been synthesized. Currently the oligonucleotide analogues with the amide internucleoside bond attract the intent attention of researchers. Such analogues initially possess at least two characteristics of antisense oligonucleotides. They are stable to endonucleases; the absence of the negative charge promotes their easier penetration into cells. The analogues with the C3'–CH_2–CO–NH–C5' bond substituted for the phosphodiester bond (**I**) (Scheme 1) are of especial interest, as they form the more stable duplexes with complementary natural oligonucleotides, than the corresponding natural nucleotides (ΔMp = 0.5–0.9°C for modification depending on the sequence [8, 9]).



Scheme 1. Retrosynthetic analysis of oligonucleotides amide internucleotide bond.

As seen from Scheme 1, 3'-deoxy-3'-carboxymethyl nucleosides (**II**) are the key compounds for the synthesis of such oligonucleotide analogues. 2',3'-Dideoxy-3'-carboxymethyl nucleosides (**IIa**) were obtained 15 years ago [8, 10, 11]. However, ribonucleoside analogues (**IIb**), which are the precursors of modified oligoribonucleotides, are of essentially greater interest. Just the synthetic oligoribonucleotide analogues are recently considered as promising regulators of biological processes [12, 13].

Two methods for the synthesis of analogues (**IIb**) from 2',5'-di-*O*-protected ribonucleosides [14, 15] have

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[2] Abbreviations: BSAA, *N*,*O*-bis(trimethylsilyl)acetamide; Tf, trifluoromethanesulfonyl.

been published. The methods aforementioned have a number of disadvantages. In particular, this is the necessity of selective protection of nucleoside 2'- and 5'-hydroxyl groups, but the main disadvantage is that the methods are not general and do not allow the obtaining of all analogues with the natural bases, to say nothing of analogues with modified bases. The method of synthesis starting from the available compounds, which uses rather simple and reproducible techniques and allows the obtaining of a nucleoside analogues both with natural and modified bases, is preferred.

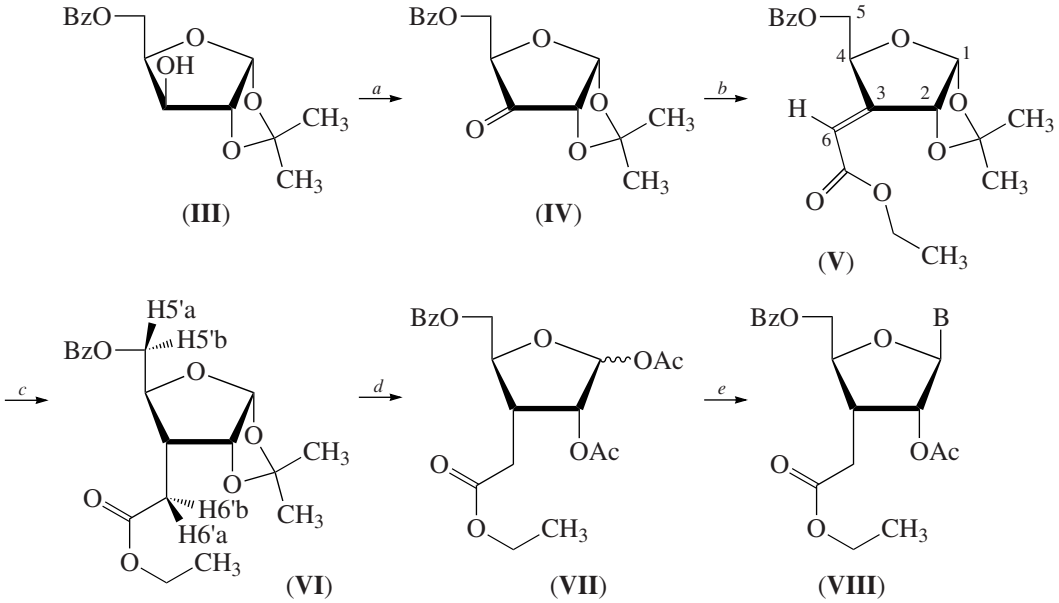
Asymmetric synthesis starting from the achiral precursors of 1,2-di-*O*-acetyl-3-deoxy-3-*tert*-butyldiphenylsilyloxyethyl-*D*-ribose, which was then converted to nucleoside analogues by the standard technique, was suggested in [16]. After removal of the silyl protective group, the hydroxyethyl group was oxidized to the carboxymethyl moiety. This method included 9–10 steps; the yields of nucleoside analogues [(**IIb**): B = Ura, Cyt, Ade, and Gua] were 15–19% from the starting achiral compounds.

The method [17, 18] based on the approach to the synthesis of branched sugars suggested earlier [19] has been reported. This method included oxidation of 5-*O*-protected 1,2-*O*-isopropylidene-α-*D*-xylofuranose, treating the resulting 3-ulose with [(ethoxycarbonyl)methylene]triphenylphosphorane, hydrogenation of the adduct, and opening of the isopropylidene cycle by treating with either HCl in methanol with the subsequent acetylation [17], or AcOH–Ac_2O–H_2SO_4 [18]. The resulting methyl-2-*O*-acetylriboside or 1,2-di-*O*-acetyl derivative were used for the synthesis of nucleoside analogues ((**IIb**): B = Thy) in 16% yield (5 steps) [17] or ((**IIb**): B = Ura, Thy, or Ade) in 22–26% yield (9 steps) [18] calculated for 5-*O*-protected 1,2-*O*-isopropylidene-α-*D*-xylofuranose.

We herein report the improved method for the synthesis of nucleoside analogues (**IIb**: B = Ura, Thy, Cyt, Ade, Gua) based on the approach afore-mentioned. The method allows the obtaining of nucleoside analogues in five steps in 42–49% yield starting from 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-*D*-xylofuranose.

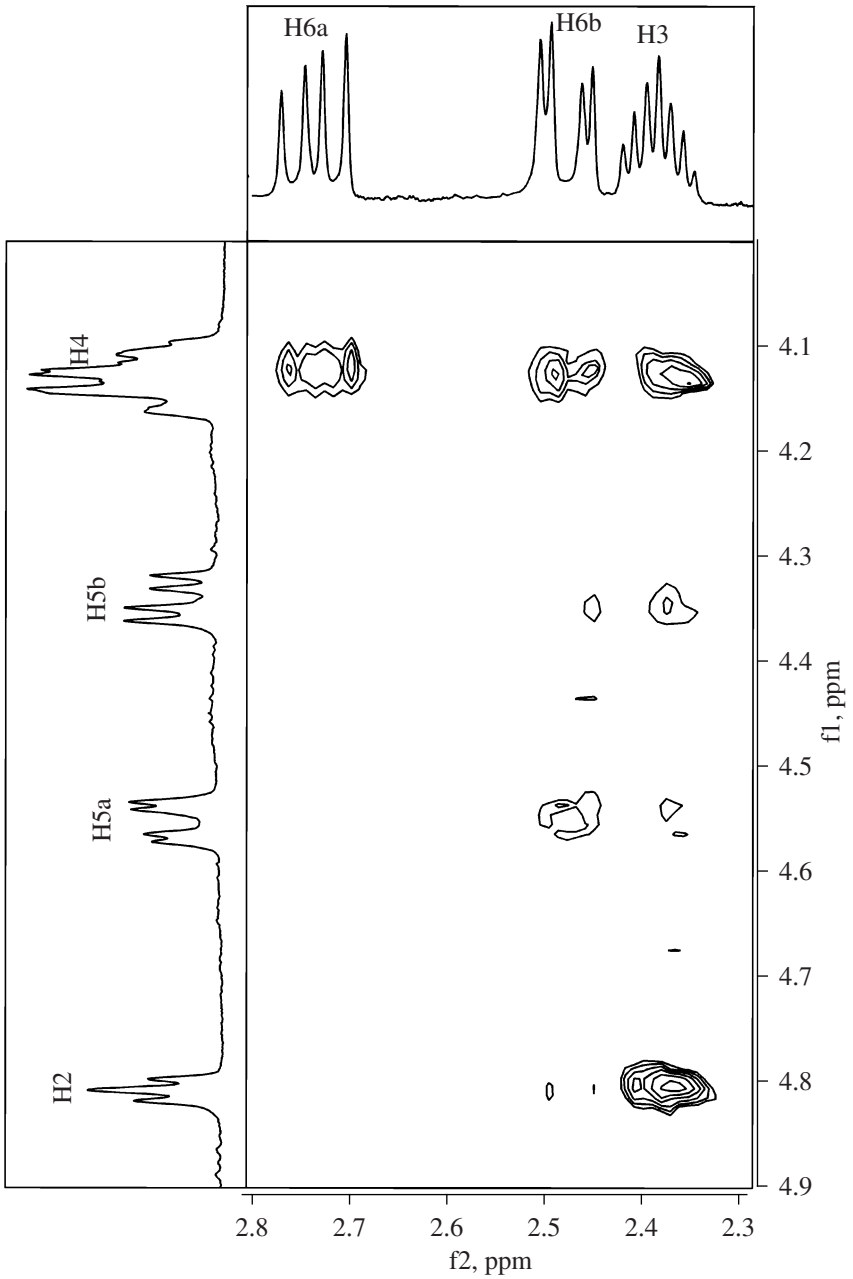
RESULTS AND DISCUSSION

5-*O*-Benzoyl-1,2-*O*-isopropylidene-α-*D*-xylofuranose (**III**) [20, 21] was the starting compound in the synthesis (Scheme 2). In [17, 18] compound (**III**) was oxidized with CrO_3–pyridine–Ac_2O [22]. Though this method results keto sugars in high yields, it is laborious enough and rather sensitive to the ratio of reagents and temperature. Oxidation of (**III**) with ruthenium tetroxide is described in CCl_4 [23]. This reaction however requires very large volumes of the solvent and the yield of ketone (**IV**) reaches only 55%. Oxidation with sodium periodate in the presence of catalytic amount of ruthenium dioxide in the two-phase chloroform–water system gives very good results [24]. The attempt to use this method toward xylose derivative (**III**) failed. It had been previously shown [25] that sugar derivatives hardly soluble in water can be oxidized in the system under consideration in the presence of the interphase transfer catalyst. Really, the addition of the catalytic amount of triethylbenzylammonium chloride to the reaction mixture resulted in the formation of ketone (**IV**) in 83% yield.



a – Thy; **b** – Ura; **c** – Cyt; **d** – Ade; **e** – *N*-AcGua

Scheme 2. Reagents and conditions: *a*, $NaIO_4$, RuO_2, Et_3BnNCl, chloroform/water; *b*, $(EtO)_2P(O)CH_2COOEt$, NaH, THF; *c*, H_2, $Pd(OH)_2$/C, ethanol; *d*, AcOH, Ac_2O, $MeSO_2OH$; *e*, nucleic bases, BSA, TMSOTf, acetonitrile



A fragment of the NOESY spectrum of 5-*O*-benzoyl-3-deoxy-3-ethoxycarbonylmethyl-1,2-*O*-isopropylidene-α-*D*-ribofuranose (**VI**), which displays the interaction of protons H2, H5a, H5b, and H4 with protons H6a, H6b, and H3. The signal of proton H4 overlaps with the signal of the CH_2 protons of the ethoxy group.

Ketone (**IV**) was converted to unsaturated compound (**V**) by the Horner–Wadsworth–Emmons reaction [26]. The resulting mixture of *E*- and *Z*-isomers (7 : 1, according to ^{1}H NMR spectroscopy data) was hydrogenated under atmospheric pressure to result in the only compound (**VI**) in the almost quantitative yield, whose *ribo*-configuration was corroborated by the NOESY-spectrum (see the figure).

The possible conformers for stereoisomers with the *ribo*-and the *xylo*-configuration were computed. The average values $(1/r)^6$, where r is the interproton distance were calculated for the 10 most stable conformers (Table 1).

The computation showed that the bicyclic system is conformationally rigid and the distances H2–H3 and H3–H4 are practically independent from the conformation of side chains, H2 and H3 being in the nearest position in the *ribo*-configuration and H3 and H4 being in the nearest position in the *xylo*-configuration. As seen from the figure, just the H2–H3 cross-peak is the most intensive. Furthermore, H4–H6a and H4–H6b cross peaks should be observed in the case of the *ribo*-config-

Table 1. The values $(1/r)^6$ for 5-*O*-benzoyl-3-deoxy-3-ethoxycarbonylmethyl-1,2-*O*-isopropylidene-α-*D*-ribo- and -α-*D*-xylofuranose averaged for the most stable conformers (the values of $(1/r)^6$ were normalized to unity for ease of comparison)

Configuration	The values of $(1/r)^6$ for proton pairs							
	H2–H3	H3–H4	H4–H6a*	H4–H6b*	H6a*–H5a*	H6a*–H5b*	H6b*–H5a*	H6b*–H5b*
ribo	1.00	0.22	0.23	0.56	0.05	0.05	0.19	0.11
xylo	0.39	1.00	0.05	0.05	0.27	0.20	0.25	0.21

Note: * 5a, *pro-S*-proton of the 5-CH_2 group; 6a, *pro-R*-proton of the 6-CH_2-group (the spatial arrangement of these protons is shown in Scheme 2).

uration, and they should not be observed in the *xylo*-configuration that is in a good agreement with the experimental spectrum. Absence of H6a–H5a and H6a–H5b cross peaks in the NOESY-spectrum is an additional confirmation of the *ribo*-configuration of the compound obtained.

The removal of the isopropylidene group and the obtaining of 2-*O*-acetyl derivative is the key stage of the synthesis. In the study [17] isopropylidene derivative (**VI**) was treated with 1% HCl in methanol and then acetylated without isolation. However, methyl-5-*O*-benzoyl-3-deoxy-3-methoxycarbonylmethyl-2-*O*-acetyl-*D*-ribofuranoside was obtained only in 41% yield.

Peterson et al. [18] demonstrated that acetolysis of 5-*O*-acetyl-3-deoxy-3-ethoxycarbonylmethyl-1,2-*O*-isopropylidene-α-*D*-ribofuranose with AcOH–Ac_2O–H_2SO_4 proceeds with a low yield (25%). The authors found that the substitution of the 5-*O*-methanesulfonyl group for 5-*O*-acetyl group essentially raises (up to 70%) the yield of acetolysis. Therefore, they introduced two additional steps in the synthetic scheme, namely, the substitution of the 5-*O*-methanesulfonyl group for 5-*O*-acetyl group and the reverse substitution of the 5-*O*-acetyl group for the 5-*O*-methanesulfonyl group after acetolysis. The total yield from the three steps was 51%.

We succeeded to increase the yield of diacetate (**VII**) up to 82% by the substitution of methanesulfonic acid for sulfuric acid on and by the choice of reagent ratio. The resulting diacetate (**VII**) was used for the synthesis of nucleoside analogues (**VIIIa**)–(**VIIIe**) by to some extend modified standard procedure [27].

The structure of all compounds obtained was confirmed with ^{1}H NMR spectra (Table 2). As can be seen, the spectrum of ketone (**IV**) has no signal of H3, and the signal of H1 is shifted downfield for 0.219 ppm as compared to its position in the spectrum of the starting compound (**III**) that is due to the effect of the carbonyl group anisotropy. Furthermore, the distant (through four bonds) coupling constant (J = 1.2 Hz) between H2 and H4 pointing to the sp^2-hybridization of the C3 atom is observed. The signal of H6 being a triplet due to the distant (allyl) coupling constant with H2 and H4 appears at 6.149 ppm in the spectrum of compound (**V**). The signal of H2 appears as a doublet of triplets (because of interaction with H4 and H6). Hydrogenation of the double bond in compound (**VI**) results in the appearance of expected upfield (2.390 ppm) signal of H3 and two signals H6a and H6b being doublets of doublets. The large heminal coupling constant (J = 16.61 Hz) is a specialty of the 6-CH_2 group. This specialty retains in all products obtained from (**VI**). The spectrum of diacetate (**VII**) contains no signal of CH_3 fragments of the isopropylidene group and two singlets of acetyl groups.

The spectra of the nucleosides (given in the Experimental) retain the specialties of the spectrum of diacetate and also contain the signals of the nucleic base.

Thus, the proposed method allows us to obtain the nucleoside analogues in five steps in a yield of 42–49% starting from 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-*D*-xylofuranose.

EXPERIMENTAL

Benzene, chloroform, acetonitrile, ethyl acetate, tetrahydrofuran, and acetic acid (all reagent grade) were from Khimmed (Russia); acetic anhydride (reagent grade) was from Reakhim (Russia); cytosine, thymine, adenine, and guanine were from Fluka (Switzerland); triethylphosphonoacetate, *N*,*O*-bis(trimethylsilyl)acetamide, trimethylsilyltrifluoromethanesulfonate, sodium hydride, ruthenium dioxide, and sodium periodate were from Aldrich (United States). Benzene and acetonitrile were dried by distillation from phosphorus pentoxide; tetrahydrofuran was dried by distillation from lithium aluminum hydride. 2-*N*-Acetylguanine was obtained by the method [28].

NMR spectra (δ, ppm, J, Hz) were registered in $CDCl_3$ (when it is not especially specified) on a Bruker AMXIII-400 spectrometer at a working frequency of 400 MHz.

Conformational computation was carried out by the Monte–Carlo method (Conformational Search option) using HyperChem 8.04 (HyperCube, Canada) program.

Column chromatography was carried out on Silica gel 60 (Merck, Germany). TLC was carried out on Silica gel 60 F_{254} (Merck, Germany) precoated palates in (A) 1 : 19 methanol–benzene, (B) 1 : 1 chloroform–hexane containing 3% isopropanol, (C) 1 : 19 isopropanol–hexane, (D) 1 : 19 ethanol–chloroform, and (E) 1 : 9 ethanol–chloroform.

5-*O*-Benzoyl-1,2-*O*isopropylidene-α-*D*-xylofuranose (III) was obtained from *D*-xylose in two steps

Table 2. The ^{1}H NMR data for (**III**)–(**VII**) (solutions in $CDCl_3$)

Protons	Chemical shifts, ppm (*J*, Hz)				
	(**III**)	(**IV**)*	(**V**)*	(**VI**)	(**VII**)
H1	5.947 d ($J_{1,2}$ 3.53)	6.166 d ($J_{1,2}$ 4.47)	5.958 d ($J_{1,2}$ 4.14)	5.865 d ($J_{1,2}$ 3.97)	6.116 br. s
H2	4.582 d ($J_{1,2}$ 3.53)	4.681 dd ($J_{1,2}$ 4.47, $^4J_{2,4}$ 1.2)	5.653 dt ($J_{1,2}$ 4.14, $^4J_{2,4(6)}$ 1.52)	4.819 pseudo t ($J_{1,2}$ 3.97, $J_{2,3}$ 4.51)	5.341 d ($J_{2,3}$ 4.73)
H3	4.174 d ($J_{3,4}$ 4.75)			2.390 m	2.967 m
H4	4.343–4.416 m	4.864 m	5.183 m	4.112 m	4.295 m
H5a	4.789 dd ($J_{4,5a}$ 9.29, $^2J_{5a,5b}$ 12.84)	4.536 dd ($J_{4,5a}$ 2.92, $^2J_{5a,5b}$ 12.19)	4.534 dd ($J_{4,5a}$ 3.11, $^2J_{5a,5b}$ 12.04)	4.558 dd ($J_{4,5a}$ 2.70, $^2J_{5a,5b}$ 12.26)	4.603 dd ($J_{4,5a}$ 2.16, $^2J_{5a,5b}$ 12.11)
H5b	4.343–4.416 m	4.434 dd ($J_{4,5b}$ 4.35, $^2J_{5a,5b}$ 12.19)	4.467 dd ($J_{4,5b}$ 4.65, $^2J_{5a,5b}$ 12.04)	4.336 dd ($J_{4,5b}$ 5.05, $^2J_{5a,5b}$ 12.26)	4.324 dd ($J_{4,5b}$ 4.89, $^2J_{5a,5b}$ 12.11)
H6a			6.149 t ($^4J_{6.2(4)}$ 1.81)	2.745 dd ($J_{3,6a}$ 9.89, $^2J_{6a,6b}$ 16.97)	2.595 dd ($J_{3,6a}$ 8.81, $^2J_{6a,6b}$ 16.44)
H6b				2.484 dd ($J_{3,6b}$ 4.38, $^2J_{6a,6b}$ 16.97)	2.511 dd ($J_{3,6b}$ 6.00, $^2J_{6a,6b}$ 16.44)
CH_3*i*Pr	1.494 s	1.419 s	1.384 s	1.507 s	
CH_3*i*Pr	1.314 s	1.363 s	1.331 s	1.321 s	
O**CH**$_2$CH$_3$			4.148 quart (*J* 7.09)	4.135 quart (*J* 7.13)	4.134 quart (*J* 7.14)
OCH$_2$**CH**$_3$			1.216 t (*J* 7.09)	1.246 t (*J* 7.13)	1.237 t (*J* 7.14)
1-OAc					1.950 s
2-OAc					2.107 s
o-H Bz	8.043 m	7.932 m	7.925 m	8.040 m	8.057 m
m-H Bz	7.445 m	7.532 m	7.534 m	7.432 m	7.433 m
n-H Bz	7.581 m	7.676 m	7.668 m	7.559 m	7.564 m

* Registered in DMSO-d_6.

according to the methods described in [20, 21] in a total yield of 67%. ^{1}H NMR spectral data are given in Table 2.

5-*O*-Benzoyl-1,2-*O*-isopropylidene-α-*D*-*erythro*-pentafuranos-3-ulose (IV). Water (50 ml), ruthenium dioxide (0.1 g), $NaIO_4$, (12.8 g, 60 mmol), K_2CO_3 (1.0 g, 7.2 mmol), and benzyltriethylammonium chloride (0.136 g, 0.6 mmol) were added to a solution of compound (**III**) (5.88 g, 20 mmol) in chloroform (50 ml) free of alcohol at stirring. The mixture was stirred for 24 h at a room temperature (TLC control in system A). Then isopropanol (10 ml) was added; the mixture was stirred for 10 min and then filtered through Celite, which washed with chloroform (3 × 10 ml) on the filter. The organic layer was separated; the aqueous layer was extracted with chloroform (2 × 30 ml). The combined chloroform extract was washed with saturated aqueous $NaHCO_3$ (50 ml) and water and dried with anhydrous Na_2SO_4; the solvent was evaporated in a vacuum. The residue was re-evaporated with 1 : 1 anhydrous benzene–anhydrous acetonitrile (4 × 10 ml) and dried in a vacuum to give 4.86 g (83%) of (**IV**) as hard oil; R_f 0.36 (A). For the ^{1}H NMR spectral data see Table 2.

5-*O*-Benzoyl-3-deoxy-3-ethoxycarbonylmetylene-1,2-*O*-isopropylidene-α-*D*-ribofuranose (V). A 60% suspension of sodium hydride in mineral oil (0.7 g, 17.6 mmol) and anhydrous THF (10 ml) were placed in a flask supplied by a condenser with a calcium chloride tube, a drop funnel and a thermometer. A solution of triethylphosphonoacetate (3.95 g, 17.6 mmol) in anhydrous THF (10 ml) was added dropwise to the mixture, so that the temperature did not exceed 5°C. After the addition was completed, the mixture was stirred for additional 20 min at 0–5°C. Then a solution of ketone (**IV**) (4.7 g, 16.1 mmol) in anhydrous THF (10 ml) was added (the temperature should not exceed 5°C). Cooling was removed; the mixture was stirred for1 h at room temperature and for 30 min at 50°C. The mixture was diluted with water (50 ml) and extracted with ethyl acetate (3 × 50 ml). The combined organic extract was washed with water and dried with anhydrous Na_2SO_4. The solvent was removed; the residue chromatographed on a Silica gel column (4 × 20 cm) in 1 : 1 chloroform–hexane containing isopropanol (a linear gradi-

ent from 1 to 5% isopropanol). The fractions containing the target product [the mixture of two isomers in a ratio of 1 : 7, R_f = 0.37 and 0.46 (B)], were combined; the solvent was removed in a vacuum to give 5.8 g (91%) of (**V**) as hard oil. The ^{1}H NMR spectral data are given in Table 2.

5-*O*-Benzoyl-3-deoxy-3-ethoxycarbonylmethyl-1,2-*O*-isopropylidene-α-*D*-ribofuranose (VI). $Pd(OH)_2$/C (0.57 g) was added to a solution of compound (**V**) (5.7 g, 15.7 mmol) in ethanol (30 ml); the mixture was hydrogenated under atmospheric pressure and room temperature for 3 days. The mixture filtered through Celite, which was then washed on the filter with ethanol (3 × 10 ml). The solvent was removed in a vacuum to give 5.6 g (98%) of (**VI**) as hard oil, R_f = 0.46 (B). The ^{1}H NMR spectral data are given in Table 2.

1,2-Di-*O*-acetyl-5-*O*-benzoyl-3-deoxy-3-ethoxycarbonylmethyl-*D*-ribofuranose (VII). A mixture of glacial acetic acid (60 ml) and acetic anhydride (15 ml) was stirred for 30 min at room temperature. Then isopropylidene derivative (**VI**) (5.5 g, 15.1 mmol) was added. The resulting solution was cooled with ice; methanesulfonic acid (6 ml) was slowly flowed at vigorous stirring. Cooling was removed; the reaction mixture was stirred at room to temperature for 24 h, poured slowly to the mixture of water and ice (300 ml), and stirred for 45 min. The mixture was extracted with chloroform (3 × 50 ml); the extracts were washed with saturated aqueous $NaHCO_3$ (3 × 50 ml) and water, dried with anhydrous Na_2SO_4, and evaporated. The residue was chromatographed on a Silica gel column (4 × 20 cm) in 1 : 1 chloroform–hexane containing isopropanol (a linear gradient from 1 to 5% isopropanol). The fractions containing the target product [R_f = 0.36 (B)] were combined; the solvent was evaporated to give 5.1 g (83%) of (**VII**) as hard oil, R_f = 0.36 (B). The ^{1}H NMR spectral data are given in Table 2.

The general procedure for obtaining nucleoside analogues (VIIIa)–(VIIIe). BSAA (1.1 mmol for each NH group of the base) was added to a suspension of a nucleic base (2.5 mmol) in anhydrous acetonitrile (5 ml) at stirring; the mixture was heated at 50°C up to dissolution of the precipitate (0.5–1 h). A solution of diacetate (**VII**) (0.82 g, 2 mmol) in anhydrous acetonitrile (4 ml) and then TMSOTf (0.56 g, 0.45 ml, 2.5 mmol) were added to the resulting solution. The mixture was refluxed for 4 h, cooled, poured to cold saturated aqueous $NaHCO_3$ (50 ml) at stirring, stirred for 30 min, and extracted with chloroform (3 × 20 ml). The extracts were washed with water (20 ml) and dried with anhydrous Na_2SO_4. The solvent was removed in a vacuum; the residue was chromatographed on a Silica gel column (3 × 20 cm).

2'-*O*-Acetyl-5'-*O*-benzoyl-3'-deoxy-3'-ethoxycarbonylmethylribosylthymine (VIIIa) was obtained from thymine (0.32 g, 2.5 mmol) and BSAA (1.12 g, 1.3 ml, 5.5 mmol). Elution with chloroform–ethanol (a linear gradient from 2 to 6% ethanol).Yield 0.77 g (81%) as firm foam, R_f = 0.36 (D). ^{1}H NMR: 8.725 (1 H, s, H3), 8.042 (2 H, m, *o*-H Bz), 7.588 (1 H, m, *p*-H Bz), 7.454 (2 H, m, *m*-H Bz), 7.116 (1 H, m, J = 1.17, H6), 5.808 (1 H, d, $J_{1',2'}$ = 2.60, H1'), 5.514 (1 H, dd, $J_{1',2'}$ = 2.60, $J_{2',3'}$ = 6.85, H2'), 4.717 (1 H, dd, $J_{4',5'a}$ = 2.41, $^2J_{5'a,5'b}$ = 12.62, H5'a), 4.473 (1 H, dd, $J_{4',5'b}$ = 4.07, $^2J_{5'a,5'b}$ = 12.62, H5'b), 4.245 (1 H, m, H4'), 4.117 (2 H, quart, J = 7.14, O**C**H_2CH$_3$), 2.994 (1 H, m, H3'), 2.595 (1 H, dd, $J_{3',6'a}$ = 8.81, $^2J_{6'a,6'b}$ = 16.44, H6'a), 2.511 (1 H, dd, $J_{3',6'b}$ = 6.00, $^2J_{6'a,6'b}$ = 16.44, H6'b), 2.114 (3 H, s, 2'-OCOCH$_3$), 1.658 (3 H, d, J = 1.31, 5-CH$_3$), 1.237 (3 H, t, J = 7.14, OCH$_2$**C**H_3).

2'-*O*-Acetyl-5'-*O*-benzoyl-3'-deoxy-3'-ethoxycarbonylmethyluridine (VIIIb) was obtained from uracil (0.28 g, 2.5 mmol) and BSAA (1.12 g, 1.3 ml, 5.5 mmol). Elution with chloroform–ethanol (a linear gradient from 2 to 6% ethanol). Yield 0.67 g (73%) as firm foam, R_f = 0.39 (D); ^{1}H NMR: 8.441 (1 H, s, H3), 8.025 (2 H, m, *o*-H Bz), 7.603 (1 H, m, *p*-H Bz), 7.467 (2 H, m, *m*-H Bz), 7.430 (1 H, d, $J_{5,6}$ = 8.24, H6), 5.784 (1 H, d, $J_{1',2'}$ = 1.67, H1'), 5.527 (1 H, dd, $J_{1',2'}$ = 1.67, $J_{2',3'}$ = 5.40, H2'), 5.522 (1 H, d, $J_{5,6}$ = 8.24, H5), 4.703 (1 H, dd, $J_{4',5'a}$ = 2.37, $^2J_{5'a,5'b}$ = 12.67, H5'a), 4.517 (1 H, dd, $J_{4',5'b}$= 3.99, $^2J_{5'a,5'b}$ = 12.67, H5'b), 4.269 (1 H, m, H4'), 4.120 (2 H, quart, J = 7.13, O**C**H_2CH$_3$), 2.929 (1 H, m, H3'), 2.582 (1 H, dd, $J_{3',6'a}$ = 8.21, $^2J_{6'a,6'b}$ = 16.61, H6'a), 2.496 (1 H, dd, $J_{3',6'b}$ = 6.23, $^2J_{6'a,6'b}$ = 16.61, H6'b), 2.124 (3 H, s, 2'-OCOCH$_3$), 1.225 (3 H, t, J 7.13, OCH$_2$**C**H_3).

2'-*O*-Acetyl-5'-*O*-benzoyl-3'-deoxy-3'-ethoxycarbonylmethylcytidine (VIIIc) was obtained from cytosine (0.28 g, 2.5 mmol) and BSAA (1.68 g, 2 ml, 8.3 mmol). Elution with chloroform–ethanol (a linear gradient from 5 to 12% ethanol). Yield 0.77 g (84%) as firm foam, R_f = 0.27 (E); ^{1}H NMR: 8.025 (2 H, m, *o*-H Bz), 7.595 (1 H, d, $J_{5,6}$ = 7.41, H6), 7.586 (1 H, m, *p*-H Bz), 7.465 (2 H, m, *m*-H Bz), 5.828 (1 H, d, $J_{1',2'}$ = 1.91, H1'), 5.705 (1 H, d, $J_{5,6}$ = 7.41, H5), 5.569 (1 H, dd, $J_{1',2'}$ 1.91, $J_{2',3'}$ 5.97 = 1.91, $J_{2',3'}$ = 5.97, H2'), 4.677 (1 H, dd, $J_{4',5'a}$ = 2.46, $^2J_{5'a,5'b}$ = 12.61, H5'a), 4.525 (1 H, dd, $J_{4',5'b}$ = 4.39, $^2J_{5'a,5'b}$ = 12.61, H5'b), 4.283 (1 H, m, H4'), 4.087 (2 H, quart, J = 7.13, O**C**H_2CH$_3$), 2.884 (1 H, m, H3'), 2.551 (1 H, dd, $J_{3',6'a}$ = 7.86, $^2J_{6'a,6'b}$ = 16.66, H6'a), 2.437 (1 H, dd, $J_{3',6'b}$ = 6.26, $^2J_{6'a,6'b}$ = 16.66, H6'b), 2.113 (3 H, s, 2'-OCOCH$_3$), 1.229 (3 H, t, J 7.13, OCH$_2$**C**H_3).

2'-*O*-Acetyl-5'-*O*-benzoyl-3'-deoxy-3'-ethoxycarbonylmethyladenosine (VIIId) was obtained from adenine (0.34 g, 2.5 mmol) and BSAA (1.68 g, 2 ml, 8.3 mmol). Elution with chloroform–ethanol (a linear gradient from 1 to 6% ethanol). Yield 0.67 g (69%) as firm foam, R_f = 0.34 (D); ^{1}H NMR: 8.249 (1 H, s, H2), 7.949 (1 H, s, H8), 7.938 (2 H, m, *o*-H Bz), 7.551 (1 H, m, *p*-H Bz), 7.404 (2 H, m, *m*-H Bz), 5.998 (1 H, d, $J_{1',2'}$ = 1.61, H1'), 5.923 (1 H, dd, $J_{1',2'}$ = 1.61, $J_{2',3'}$ = 5.92, H2'), 4.694 (1 H, dd, $J_{4',5'a}$ = 2.63, $^2J_{5'a,5'b}$ = 12.48, H5'a), 4.694 (1 H, dd, $J_{4',5'b}$ = 4.74, $^2J_{5'a,5'b}$ = 12.48, H5'b), 4.381 (1 H, m, H4'), 4.129 (2 H, quart, J 7.13, O**C**H_2CH$_3$), 3.529 (1 H, m, H3'), 2.679 (1 H, dd, $J_{3',6'a}$ = 8.58,

$^2J_{6'a,6'b}$ = 16.52, H6'a), 2.602 (1 H, dd, $J_{3',6'b}$ = 6.02, $^2J_{6'a,6'b}$ = 16.52, H6'b), 2.144 (3 H, s, 2'-OCOC**H**$_3$), 1.226 (3 H, t, *J* 7.13, OCH$_2$**CH**$_3$).

2'-*O*-Acetyl-5'-*O*-benzoyl-3'-deoxy-3'-ethoxycarbonylmethyl-2-*N*-acetylguanosine (VIIIe) was obtained from *N*-2-acetylguanine (0.48 g, 2.5 mmol) and BSAA (1.68 g, 2 ml, 8.3 mmol). Elution with chloroform–ethanol (a linear gradient from 1 to 5% ethanol). Yield 0.78 g (72%) as firm foam, R_f = 0.22 (D); ^{1}H NMR: 7.838 (1 H, s, H8), 7.786 (2 H, m, *o*-H Bz), 7.555 (1 H, m, *p*-H Bz), 7.368 (2 H, m, *m*-H Bz), 5.930 (1 H, dd, $J_{1',2'}$ = 0.84, $J_{2',3'}$ = 5.28, H2'), 5.855 (1 H, d, $J_{1',2'}$ = 0.84, H1'), 4.759 (1 H, dd, $J_{4',5'a}$ = 3.58, $^2J_{5'a,5'b}$ = 12.39, H5'a), 4.595 (1 H, dd, $J_{4',5'b}$ = 3.23, $^2J_{5'a,5'b}$ = 12.39, H5'b), 4.315 (1 H, m, H4'), 4.124 (2 H, quart, *J* 7.13, O**CH**$_2$CH$_3$), 3.841 (1 H, m, H3'), 2.581 (2 H, m, H6'a, H6'b), 2.177 (3 H, s, 2-NCOC**H**$_3$), 2.151 (3 H, s, 2'-OCOC**H**$_3$), 1.248 (3 H, t, *J* 7.13, OCH$_2$**CH**$_3$).

ACKNOWLEDGMENTS

Work was supported by the Russian Foundation for Basic Research (project no. 08-04-01078).

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