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Monomers Containing 2'-*O*-Alkoxymethyl Groups as Synthons for the Oligonucleotide Synthesis by the Phosphotriester Method

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Abstract—A general scheme for the synthesis of ribonucleotides containing an alkoxymethyl group at the 2'-*O*-position of ribose and an *O*-nucleophilic catalytic 4-methoxy-1-oxido-2-picolyl phosphate-protecting group has been developed for the introduction into oligonucleotides during their solid-phase synthesis by the phosphotriester method. The scheme has been tested in the synthesis of monomers with 2'-*O*-modifying groups as examples: 2-azidoethoxymethyl, propargyloxymethyl, and 3,4-cyclocarbonatebutoxymethyl groups.

Keywords: phosphotriester method, azides, alkynes, aldehydes, conjugation, crosslink

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INTRODUCTION

Oligoribonucleotides containing residues of 2'-*O*-modified nucleotides are widely used at present in molecular biology for the regulation of gene expression. In particular, they are employed in the synthesis of small interfering RNAs (siRNAs) [1, 2] and antisense oligonucleotides [3, 4], the preparation of conjugates stabilizing the structure of NA, and for the covalent linking of reporter groups, e.g., fluorescent and spin labels, as well as labels for the electrochemical detection of NAs [5]. In addition, of great interest are conjugates of NA with compounds facilitating their entry into the cell, e.g., cholesterol or polycationic molecules [6], and with biopolymers accomplishing their targeted delivery, e.g., peptides and carbohydrates [7].

In the literature, conjugates of NAs with peptides coupled to the terminal 3'- and 5'-OH functions or the heterocyclic bases of nucleotides have been described [8]. In the first case, the number of peptides attached to NA and their position are restricted; in the second case, the thermal stability of duplexes formed by the modified chain with a complementary target sometimes decreases, due to the adverse effect of substituents on the hydrogen bonding between nitrogenous bases. Therefore, the use of 2'-modifying groups for this purpose seems to be a more promising trend.

One of the first known examples of this approach involved the formation of conjugates between peptides and oligonucleotides containing a 2'-amino group [9, 10]. However, this reaction proceeded ineffectively, and the resulting amide bond led to a destabilization of the duplex of the oligonucleotide with the complementary target [11]. Later, an approach was developed in which a group containing the aldehyde function was introduced into ribonucleotides, which enabled one to perform a highly effective conjugation with various organic molecules with the formation of the imino group, thiazolidine, oxime or hydrazine bonds [12, 13]. Besides, the oxo group can be converted into a reactive hydrazine group [14].

The introduction of the acetylene function into the 2'-*O*-modifying group made it possible to perform the [3 + 2]-dipolar cycloaddition reaction catalyzed by Cu(I) [15]. In recent years, attention of many investigators has also been given to the development of chemical methods of synthesis of cyclic [16–18] and cross-linked oligonucleotides [19–24], in particular, for studying the mechanism of DNA repair [21, 25] and the preparation of nanostructures [26].

The goal of this study was to develop methods for the synthesis of monomers containing an *O*-nucleophilic catalytic 4-methoxy-1-oxido-2-picolyl phosphate-protecting group and 2'-*O*-alkoxymethyl modifying groups for their introduction into the oligonucleotide chain by the phosphotriester method.

Abbreviations: DBU, diazabicyclo[5.4.0]undec-7-ene; DMTr, 4,4'-dimethoxytrityl; NIS, *N*-iodosuccinimide; TEA, triethylamine; TBAF, tetra-*n*-butylammonium fluoride; TfOH, trifluoromethanesulfoacid; TPS, 2,4,6-triisopropylbenzenesulfonyl chloride.

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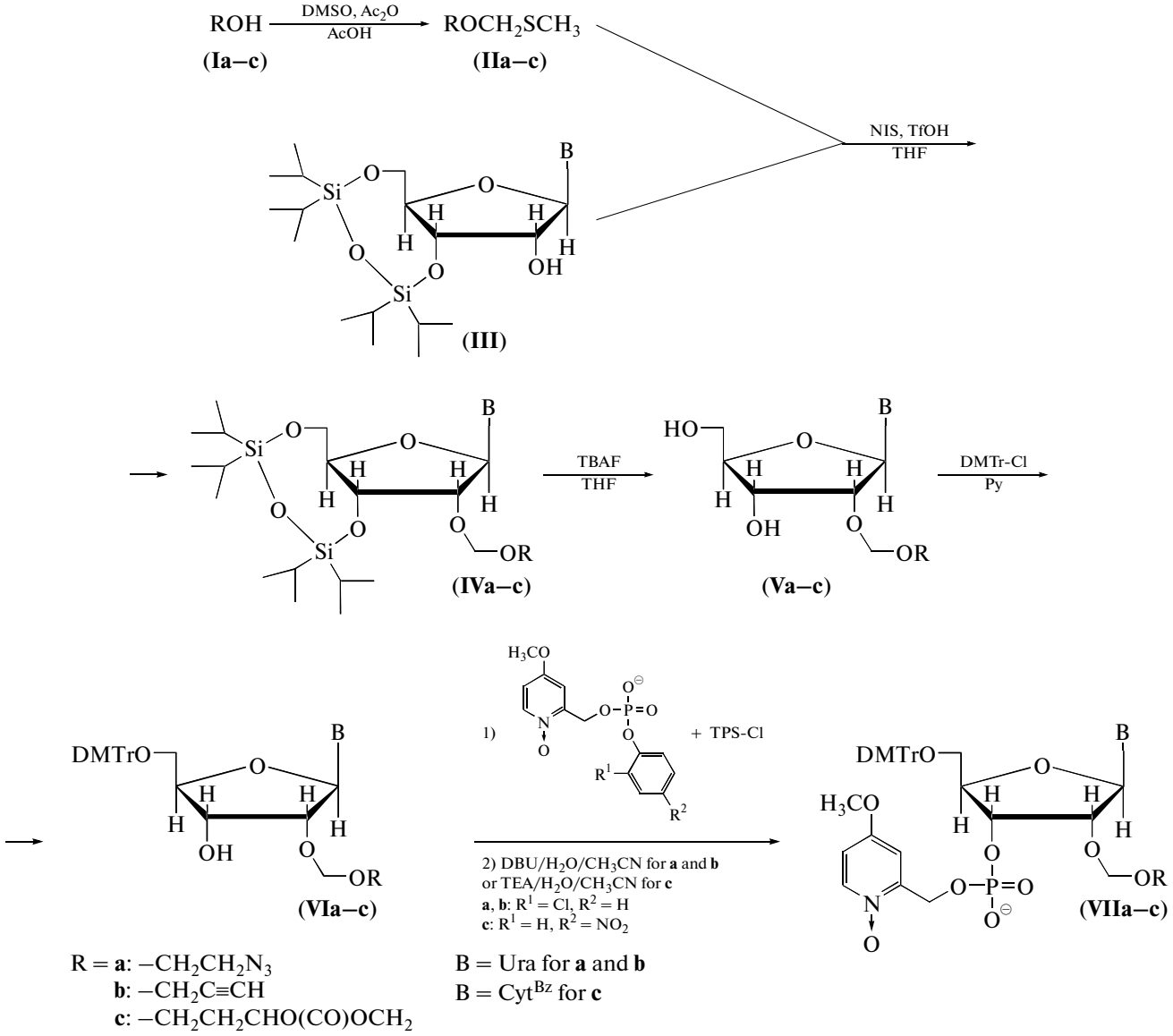


Scheme 1.

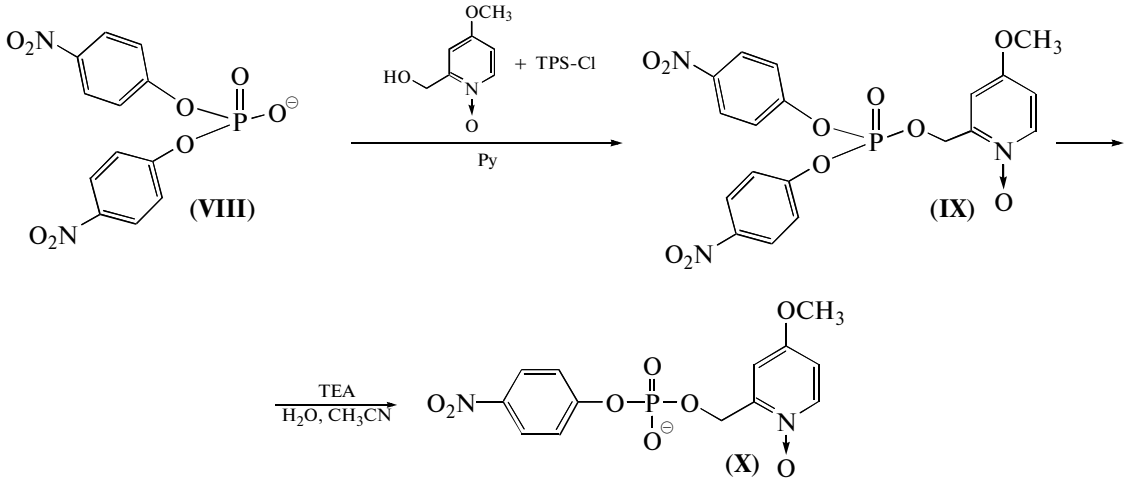
RESULTS AND DISCUSSION

Unfortunately, attempts of targeted introduction into the oligonucleotide chain of 3'-*O*-phosphoroamidites of nucleosides [27] bearing azido-containing groups at the 2'-position failed [15] since the azide group adversely affects the efficiency of internucleotide condensation due to the formation of iminophosphorane by the reaction of Staudinger [28]. An alternative approach, which enables the targeted introduction of azido-containing modifying and protective groups into oligonucleotides during the chain elongation is the phosphotriester method, that is based on the *O*-nucleophilic intramolecular catalysis at the stage of the internucleotide link formation [29–31]. We have earlier demonstrated this in the synthesis of 2'-*O*-azidomethyl- and 2'-*O*-(2-azidomethyl)benzoyl-containing oligonucleotides [29, 30].

As an extension of studies devoted to the development of the phosphotriester method for the synthesis of modified RNA fragments, we obtained monomers with 2'-*O*-alkoxymethyl groups containing a catalytic 4-methoxy-1-oxido-2-picolyl phosphate-protecting group (Scheme 1). Methylthiomethyl derivatives (**IIa**)–(**IIc**) were obtained by treating the alcohols (**Ia**)–(**Ic**) with a mixture of DMSO, acetic anhydride, and acetic acid, as described in [32]. 2'-*O*-Alkoxymethyl modifying groups were introduced by treating the 3',5'-*O*-protected derivative (**III**) with the appropriate *O*,*S*-acetal (**IIa**)–(**IIc**) in the presence of TfOH and NIS in dry THF. It should be noted that the synthesis of compound (**IVa**) was earlier described by Bobkov et al.; however, the method of its synthesis involved three stages with a total yield of as little as 50–55% [33], whereas our method made it possible to obtain this

compound in one stage with a yield of 78%. Subsequent removal of the silyl protecting group from compounds of the type (**IV**) by TBAF and the introduction of the 5'-*O*-dimethoxytrityl group led to 5'-*O*-DMTr-2'-*O*-modified derivatives (**VIa**)–(**VIc**).

In the case of compounds (**VIa**) and (**VIb**), the introduction of a phosphate residue containing a 4-methoxy-1-oxido-2-picolyl group and the selective splitting of 2-chlorophenyl phosphoester was successfully carried out as described previously (treatment with DBU) [30]. However, the treatment of (**VIc**) in this way led to a quantitative removal of its 2'-*O*-cyclocarbonate group. We found that this group remains resistant to TEA in aqueous organic medium at least within 24 h. Therefore, phosphate group was introduced into the compound (**VIc**) using phosphodiester (**X**) obtained by the condensation of bis(4-nitrophenyl)phosphate (**VIII**) with 1-oxido-4-methoxy-2-pyridinemethanol in the presence of TPS-Cl followed by the selective removal of one 4-nitrophenyl group from the phosphotriester (**IX**) by TEA in an aqueous organic medium (Scheme 2). The phosphorylation of (**VIc**) by phosphodiester (**X**) in the presence of a condensation reagent followed by treatment with TEA in an aqueous organic medium gave monomer (**VIIc**). The introduction of the monomer (**VIIc**) into the oligonucleotide structure with the subsequent removal of the cyclocarbonate protecting group after the termination of chain elongation by the treatment with a base (in particular, DBU) followed by the periodate oxidation of the resulting diol system makes it possible to obtain oligonucleotides containing 2'-*O*-aldehyde groups.



Scheme 2.

At present, we use synthons (**VIIa**)–(**VIIc**), which contain a (2-azidoethoxy)methyl, a propargyloxymethyl, or a (3,4-cyclocarbonatebutoxy)methyl group at the 2'-*O*-position, in the solid-phase synthesis of modified oligonucleotides for the introduction of the corresponding modified units at different sites of the oligonucleotide chain. In addition, studies are carried out to determine the conditions for the preparation of conjugates of these modified oligonucleotides with other organic molecules and cross-linked NA fragments. Thus, by using 2'-*O*-alkoxymethyl modifying groups: the 2-azidoethoxymethyl and the propargyloxymethyl groups, after the introduction of the appropriate monomers into the oligonucleotide chain, it would be possible to obtain conjugates with other organic molecules, as well as cyclic single-strand and cross-linked double-strand NA fragments by the [3 + 2]-dipolar cycloaddition reaction catalyzed by Cu(I) ions [15, 19, 34, 35]. In turn, the (3,4-cyclocarbonatebutoxy)methyl group would enable one to generate a reactive aldehyde function [12–14], which can be useful in obtaining conjugates with organic molecules and cross-links with oligonucleotides containing the amino group.

EXPERIMENTAL

Solvents and reagents were purchased from commercial sources and were used without additional purification. 2-Azidoethanol (**Ia**) was obtained from 2-chloroethanol by the method described in [36]. NMR spectra (δ, ppm *J*, Hz) were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker DPX300 device (Germany) at a working frequency of 300 (^{1}H) and 121.5 MHz (^{31}P). Chemical shifts are given relative to tetramethylsilane (^{1}H) and H_3PO_4 (^{31}P). Mass spectra were measured in the linear mode with the registration of positive and negative ions on an Ultraflex II MALDI-TOF mass spectrometer (Bruker Daltonics). Column chromatography was carried out on Silicagel 60 (Merck). TLC was performed on Silicagel 60 F254 plates (Merck) using the following systems: (A) $CHCl_3$–hexane 1 : 1; (B) $CHCl_3$–CH_3OH–H_2O, 65 : 25 : 4;

(C) $CHCl_3$–CH_3OH, 9 : 1; and (D) $CHCl_3$–CH_3OH, 39 : 1. Derivatives (**IIa**)–(**IIc**) were detected on TLC plates by iodine vapors.

1,2-*O*-Cyclocarbonatebutane-1,2,4-triol (Ic). A mixture of 1,2,4-butanetriol (5.31 g, 50 mmol), diphenylcarbonate (10.71 g, 50 mmol), and sodium bicarbonate (1.01 g, 12.0 mmol) in anhydrous DMF (75 ml) was refluxed for 5 min. After cooling to room temperature, chloroform (150 ml) was added to the reaction mixture, and the target product was extracted with water (3 × 100 ml). Combined water extracts were evaporated to dryness, and the residue was dried by evaporation with acetonitrile (2 × 50 ml). The product was purified by chromatography on a silica gel column in a gradient of methanol (0–3%) in chloroform. Fractions containing compound (**Ic**) were evaporated to foam and dried in vacuo. Yield: 2.58 g (39%). R_f 0.55 (D); ^{1}H NMR (DMSO-d_6): 4.93–4.83 (1 H, m, C*H*O(CO)CH_2O), 4.68 (1 H, t, *J* 5.0, O*H*), 4.57 (1 H, dd, *J* 8.3, 7.9, CHO(CO)CH_αO), 4.19 (1 H, dd, *J* 8.3, 7.4, CHO(CO)CH_βO), 3.59–3.43 (2 H, m, OCH_2CH_2), 1.95–1.76 (2 H, m, OCH_2CH_2).

A General Method for the Synthesis of Methylthiomethyl Derivatives (**IIa**)–(**IIc**)

A solution of 1 mmol of the corresponding alcohol (**Ia**)–(**Ic**) in DMSO (2.8 ml) was treated for 48 h with acetic anhydride (0.57 ml, 6 equivalents) and acetic acid (0.95 ml, 16.6 equivalents) at room temperature. Then a saturated aqueous $NaHCO_3$ solution was added at room temperature under stirring to the reaction mixture until the gas liberation terminated, and the product was extracted with ethyl acetate (2 × 15 ml). The organic layer was separated, washed with a saturated aqueous NaCl solution (3 × 30 ml), and dried over Na_2SO_4. The solvent was distilled in vacuo, and the product was purified by chromatography on a silica gel column in a gradient of chloroform (0–15%) in hexane in the case of derivatives (**IIa**)–(**IIb**) and in a gradient of chloroform (0–50%) in hexane in the case of (**IIc**). Fractions containing product (**II**) were evaporated to oil and dried by an oil pump.

Methylthiomethyl ester of 2-azidoethanol (IIa). Yield: 59%; R_f 0.50 (A). ^{1}H NMR ($CDCl_3$): 4.58 (2 H, s, OCH_2S), 3.98–3.91 (1 H, m, OCH^αCH_2N$_3$), 3.86–3.82 (1 H, m, OCH^βCH_2N$_3$), 3.51–3.43 (2 H, m, OCH_2CH_2N$_3$), 2.09 (3 H, s, SCH_3).

Methylthiomethyl ester of 2-propin-1-ol (IIb). Yield: 61%; R_f 0.50 (A). ^{1}H NMR ($CDCl_3$): 4.52 (2 H, s, OCH_2S), 4.41 (2 H, d, ^4J 2.3, OCH_2C≡), 2.43 (1 H, t, ^4J 2.3, C*H*≡), 2.08 (3 H, s, SCH_3).

Methylthiomethyl ester of 1,2-*O*-cyclocarbonatebutane-1,2,4-triol (IIc). Yield: 64%; R_f 0.20 (A). ^{1}H NMR ($CDCl_3$): 4.92–4.81 (1 H, m, C*H*O(CO)CH_2O), 4.61 (2 H, d, *J* 0.9, OCH_2S), 4.55 (1 H, dd, *J* 8.6, 8.0, CHO(CO)CH^αO), 4.18 (1 H, dd, *J* 8.6, 7.4, CHO(CO)CH^βO), 3.74–3.60 (2 H, m, OCH_2CH_2), 2.13 (3 H, s, SCH_3), 2.13–1.97 (2 H, m, OCH_2CH_2).

A General Method of Introducing 2'-O-Modifying Groups into Nucleosides

Molecular sieves (4 Å, 0.4 g) and 2.0 mmol of the corresponding methylthiomethyl ester (**IIa**)–(**IIc**) were added to a solution of 5',3'-*O*-(tetraisopropyldisiloxane-1,3-diyl)nucleoside (**III**) (1 mmol) in dry THF (4 ml). Then TfOH (2.0 mmol) and a 1.0 M solution of NIS (2.0 ml) in dry THF were added under stirring and cooling to –40°C. The mixture was stirred at –40°C for 30 min, the reaction was terminated by the addition of TEA (5.0 mmol), and the reaction solution was filtered, diluted with ethyl acetate (20 ml), and washed with a saturated aqueous sodium thiosulfate solution (2 × 10 ml). The organic layer was evaporated to oil, and the residue was purified by chromatography on a silica gel column in a gradient of chloroform (50–100%) in hexane. Fractions containing the target compound (**IV**) were evaporated to foam, and the residue was dried in vacuo.

5',3'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2'-*O*-[(2-azidoethoxy)methyl]uridine (IVa). Yield: 78%; R_f 0.75 (D); ^{1}H NMR ($CDCl_3$): 9.11 (1 H, s, N*H* Ura), 7.87 (1 H, d, *J* 8.3, *H*6 Ura), 5.75 (1 H, s, *H*1'), 5.67 (1 H, d, *J* 8.3, *H*5 Ura), 5.00 (2 H, dd, *J* 8.3, 7.2, OCH_2O), 4.25 (1 H, d, *J* 13.8, H^α5'), 4.24–4.19 (2 H, m, *H*2', *H*3'), 4.13 (1 H, dd, *J* 9.3, 2.2, *H*4'), 3.98 (1 H, dd, *J* 13.8, 2.2, H^β5'), 3.93–3.88 (1 H, m, OCH^αCH_2N$_3$), 3.81–3.76 (1 H, m, OCH^βCH_2N$_3$), 3.49–3.40 (2 H, m, OCH_2CH_2N$_3$), 1.12–0.94 (28 H, m, *H* Pr^i-groups); MS, *m/z:* 608.38 $[M + Na]^+$, calculated for $C_{24}H_{43}N_5NaO_8Si_2^+$ *M* 608.25; 624.34 $[M + K]^+$, calculated for $C_{24}H_{43}KN_5O_8Si_2^+$ 624.23.

5',3'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2'-*O*-(propargyloxymethyl)uridine (IVb). Yield: 80%; R_f 0.70 (D); ^{1}H NMR ($CDCl_3$): 8.74 (1 H, s, N*H* Ura), 7.88 (1 H, d, *J* 8.3, *H*6 Ura), 5.75 (1 H, s, *H*1'), 5.67 (1 H, dd, *J* 8.3, *J* 1.9, *H*5 Ura), 5.10 (1 H, d, *J* 7.0, OCH^αO), 5.03 (1 H, d, *J* 7.0, OCH^βO), 4.38 (2 H, d, ^4J 2.3, OCH_2C≡), 4.26 (1 H, d, *J* 13.7, H^α5'), 4.21 (1 H, dd, *J* 9.6, *J* 4.4, *H*3'), 4.18 (1 H, d, *J* 4.4, *H*2'), 4.14 (1 H, dd, *J* 9.6, 1.7, *H*4'), 3.98 (1 H, dd, *J* 13.7, 1.7, H^β5'), 2.41 (1 H, t, ^4J 2.3, C*H*≡), 1.13–0.93 (28 H, m, *H* Pr^i-groups); MS, *m/z:* 577.38 $[M + Na]^+$, calculated for $C_{25}H_{42}N_2NaO_8Si_2^+$ 577.24.

***N*^{4}-Benzoyl-5',3'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2'-*O*-[(3,4-*O*-cyclocarbonatebutoxy)methyl]cytidine (IVc).** Yield: 82%; R_f 0.60 (D); ^{1}H NMR ($CDCl_3$): 8.73 (1 H, br s, N*H* Cyt), 8.36 (1 H, d, *J* 7.6, *H*6 Cyt), 7.92–7.48 (6 H, m, *H* Bz, *H*5 Cyt), 5.80

(1 H, d, *J* 1.6, *H*1'), 5.05–4.80 (3 H, m, OCH_2O, C*H*O(CO)CH_2O), 4.65–4.62 (1 H, m, CHO(CO)CH_αO), 4.34–4.13 (5 H, m, *H*2', *H*3', *H*4', H_α5', CHO(CO)CH^βO), 4.06–3.89 (1 H, m, H^β5'), 3.76–3.65 (2 H, m, OCH_2CH_2), 2.11–1.97 (2 H, m, OCH_2CH_2), 1.14–0.94 (28 H, m, *H* Pr^i-groups); MS, *m/z:* 734.32 $[M + H]^+$, calculated for $C_{34}H_{52}N_3O_{11}Si_2^+$ 734.31; 756.30 $[M + Na]^+$, calculated for $C_{34}H_{51}N_3NaO_{11}Si_2^+$ 756.30; 772.28 $[M + K]^+$, calculated for $C_{34}H_{51}KN_3O_{11}Si_2^+$ 772.27.

A General Method of Desilylation of Nucleosides

A 1 M TBAF solution (2.2 ml, 2.2 mmol) in dry THF was added to a solution of 1 mmol 2'-*O*-protected 5',3'-*O*-(tetraisopropyldisiloxane-1,3-diyl)nucleoside derivative of type (**IV**) in dry THF (5 ml). After 2 h, the solution was evaporated to oil, and the product was purified by chromatography on a silica gel column in a gradient of methanol (0–7%) in chloroform. Fractions containing the target compound (**V**) were combined and evaporated to dryness, and the residue was dried in vacuo.

2'-*O*-[(2-Azidoethoxy)methyl]uridine (Va). Yield: 88%; R_f 0.30 (C). ^{1}H NMR (DMSO-d_6): 11.28 (1 H, s, N*H* Ura), 7.92 (1 H, d, *J* 8.3, *H*6 Ura), 5.90 (1 H, d, *J* 5.2, *H*1'), 5.64 (1 H, d, *J* 8.3, *H*5 Ura), 5.18 (1 H, d, *J* 5.5, O*H*3'), 5.10 (1 H, t, *J* 5.1, O*H*5'), 4.76 (2 H, s, OCH_2O), 4.17 (1 H, t, *J* 5.0, *H*2'), 4.13 (1 H, dd, *J* 5.0, 9.9, *H*3'), 3.90–3.87 (1 H, m, *H*4'), 3.69–3.55 (4 H, m, *H*5', OCH_2CH_2N$_3$), 3.46–3.35 (2 H, m, OCH$_2$CH_2N$_3$); MS, *m/z:* 366.26 $[M + Na]^+$, calculated for $C_{12}H_{17}N_5NaO_7^+$ 366.10; 382.23 $[M + K]^+$, calculated for $C_{12}H_{17}KN_5O_7^+$ 382.08.

2'-*O*-(Propargyloxymethyl)uridine (Vb). Yield: 74%; R_f 0.25 (C); ^{1}H NMR (DMSO-d_6): 11.28 (1 H, s, N*H* Ura), 7.92 (1 H, d, *J* 8.3, *H*6 Ura), 5.88 (1 H, d, *J* 5.0, *H*1'), 5.64 (1 H, dd, *J* 8.3, 2.2, *H*5 Ura), 5.20 (1 H, d, *J* 5.5, O*H*3'), 5.11 (1 H, t, *J* 5.0, O*H*5'), 4.80 (1 H, d, *J* 6.9, OCH^αO), 4.77 (1 H, d, *J* 6.9, OCH^βO), 4.20 (2 H, d, ^4J 2.5, OCH_2C≡), 4.14 (1 H, t, *J* 5.0, *H*2'), 4.11 (1 H, dd, *J* 5.0, 9.9, *H*3'), 3.90–3.87 (1 H, m, *H*4'), 3.68–3.64 (1 H, m, H^α5'), 3.59–3.55 (1 H, m, H^β5'), 3.35 (1 H, t, ^4J 2.5, C*H*≡); MS, *m/z:* 335.24 $[M + Na]^+$, calculated for $C_{13}H_{16}N_2NaO_7^+$ 335.09; 351.21 $[M + K]^+$, calculated for $C_{13}H_{16}KN_2O_7^+$ 351.06.

N^4-Benzoyl-2'-*O*-[(3,4-*O*-cyclocarbonatebutoxy)methyl]cytidine (Vc). Yield: 85%; R_f 0.20 (C); ^{1}H NMR (DMSO-d_6): 11.28 (1 H, br s, N*H* Cyt), 8.54 (1 H, dd, *J* 7.6, 3.0, *H*6 Cyt), 8.03–7.48 (5 H, m, *H* Bz), 7.34 (1 H, br d, *J* 7.6, *H*5 Cyt), 5.87 (1 H, br s, *H*1'), 5.28–5.20 (2 H, m, O*H*3', O*H*5'), 4.92–4.77 (3 H, m, OCH_2O, C*H*O(CO)CH$_2$O), 4.62–4.54 (1 H, m, CHO(CO)CH^αO), 4.21–4.09 (3 H, m, *H*2', *H*3', CHO(CO)CH^βO), 3.97–3.92 (1 H, m, *H*4'), 3.85–3.77 (1 H, m, H^α5'), 3.70–3.58 (3 H, m, OCH_2CH$_2$, H^β5'), 2.02–1.93 (2 H, m, OCH$_2$CH_2); MS, *m/z:* 492.20 $[M + H]^+$, calculated for $C_{22}H_{26}N_3O_{10}^+$ 492.16; 514.19 $[M + Na]^+$, calculated for $C_{22}H_{25}N_3NaO_{10}^+$ 514.14; 530.16 $[M + K]^+$, calculated for $C_{22}H_{25}KN_3O_{10}^+$ 530.12.

A General Method of the Tritylation of Nucleosides

A 2'-*O*-protected derivative of type (**V**) (1 mmol) was dried by evaporation with pyridine after which dry pyridine (5 ml) and DMTr-Cl (0.41 g; 1.2 equivalent) were added. The reaction mixture was allowed to stand for 2 h at room temperature, the reaction was terminated by adding 1 M TEAB (5 ml), and the product was extracted with chloroform (2 × 20 ml). Combined organic fractions were evaporated to oil and evaporated again with toluene to remove pyridine traces. The product was separated by chromatography on a silica gel column in a gradient of methanol (0–2%) in chloroform containing 0.1% TEA. Fractions containing the product (**VI**) were combined, evaporated to dryness, and dried in vacuo.

5'-*O*-(4,4'-Dimethoxytrityl)-2'-*O*-[(2-azidoethoxy)methyl]uridine (VIa). Yield: 84%; R_f 0.65 (D); ^{1}H NMR (DMSO-d_6): 11.34 (1 H, s, N*H* Ura), 7.72 (1 H, d, *J* 8.1, *H*6 Ura), 7.41–7.23 (9 H, m, Ar DMTr), 6.92–6.89 (4 H, m, Ar DMTr), 5.87 (1 H, d, *J* 3.2, *H*1'), 5.33 (1 H, d, *J* 8.1, Ura *H*5), 5.29 (1 H, d, *J* 5.8, O*H*3'), 4.82 (2 H, s, OCH_2O), 4.28–4.24 (2 H, m, *H*2', *H*3'), 4.01–3.98 (1 H, m, *H*4'), 3.75 (6 H, s, OCH_3 DMTr), 3.73–3.65 (2 H, m, OCH_2CH_2N$_3$), 3.46–3.37 (2 H, m, OCH$_2$CH_2N$_3$), 3.31 (1 H, dd, *J* 10.7, 4.4, H^α5'), 3.26 (1 H, dd, *J* 10.7, 2.6, H^β5'); MS, *m/z:* 668.27 $[M + Na]^+$, calculated for $C_{33}H_{35}N_5NaO_9^+$ 668.23; 684.25 $[M + K]^+$, calculated for $C_{33}H_{35}KN_5O_9^+$ 684.21.

5'-*O*-(4,4'-Dimethoxytrityl)-2'-*O*-(propargyloxymethyl)uridine (VIb). Yield: 88%; R_f 0.60 (D); ^{1}H NMR (DMSO-d_6): 11.38 (1 H, d, *J* 2.0, N*H* Ura), 7.72 (1 H, d, *J* 8.1, *H*6 Ura), 7.41–7.21 (9 H, m, *Ar* DMTr), 6.94–6.87 (4 H, m, *Ar* DMTr), 5.83 (1 H, d, *J* 3.4, *H*1'), 5.36 (1 H, d, *J* 6.0, O*H*3'), 5.28 (1 H, dd, *J* 8.1, 2.2, *H*5 Ura), 4.86 (1 H, d, *J* 7.0, OCH^αO), 4.82 (1 H, d, *J* 7.0, OCH^βO), 4.30–4.19 (4 H, m, *H*2', *H*3', OCH_2C≡), 4.02–3.96 (1 H, m, *H*4'), 3.74 (6 H, s, OCH_3 DMTr), 3.42 (1 H, t, ^4J 2.5, C*H*≡), 3.34–3.16 (2 H, m, *H*5'); MS, *m/z:* 637.18 $[M + Na]^+$, calculated for $C_{34}H_{34}N_2NaO_9^+$ 637.22; 653.16 $[M + K]^+$, calculated for $C_{34}H_{34}KN_2O_9^+$ 653.19.

N^4-Benzoyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-[(3,4-*O*-cyclocarbonatebutoxy)methyl]cytidine (VIc). Yield: 93%; R_f 0.50 (D); ^{1}H NMR (DMSO-d_6): 11.30 (1 H, br s, N*H* Cyt), 8.39 (1 H, dd, *J* 7.6, 1.5, *H*6 Cyt), 8.03–7.49 (5 H, m, *H* Bz), 7.45–7.24 (9 H, m, *Ar* DMTr), 7.14 (1 H, d, *J* 7.6, *H*5 Cyt), 6.96–6.89 (4 H, m, *Ar* DMTr), 5.85 (1 H, br s, *H*1'), 5.34 (1 H, dd, *J* 7.2, 3.2, O*H*3'), 4.99–4.81 (3 H, m, OCH_2O, C*H*O(CO)CH_2O), 4.63–4.56 (1 H, m, CHO(CO)CH^{α}O), 4.41–4.32 (1 H, m, *H*3'), 4.22–4.15 (2 H, m, *H*2', CHO(CO)CH^{β}O), 4.11–4.05 (1 H, m, *H*4'), 3.76 (6 H, s, OCH_3 DMTr), 3.73–3.64 (2 H, m, OCH_2CH_2), 3.43–3.33 (2 H, m, *H*5'), 2.05–1.96 (2 H, m, OCH$_2$CH_2); MS, *m/z:* 816.27 $[M + Na]^+$, calculated for $C_{43}H_{43}N_3NaO_{12}^+$ 816.27; 832.22 $[M + K]^+$, calculated for $C_{43}H_{43}KN_3O_{12}^+$ 832.25.

Triethylammonium salt of (4-nitrophenyl)-(1-oxido-4-methoxy-2-picolyl)phosphate (X). A mixture of 1-oxido-4-methoxy-2-pyridinemethanol monohydrate (0.52 g, 3.0 mmol) and pyridinium bis(4-nitrophenyl)phosphate (**VIII**) (1.12 g, 3.3 mmol) was evaporated with pyridine to remove moisture traces, the residue was dissolved in dry pyridine (15 ml), and TPS-Cl (1.51 g, 5 mmol) was added. The reaction mixture was allowed to stand at room temperature for 15 min, diluted with $CHCl_3$ (30 ml), and washed with water (2 × 20 ml). The organic layer was evaporated to oil, dissolved in 20 ml of an acetonitrile–water mixture (9 : 1, v/v), and TEA was added (2.8 ml, 20 mmol). The reaction mixture was held for 2 h at room temperature and evaporated to oil. The target product was purified by chromatography on a silica gel column in a gradient of methanol (5–20%) in chloroform. Fractions containing the substance were combined, evaporated to dryness, and dried in vacuo. Yield: 0.87 g (81%); R_f 0.30 (B); ^{1}H NMR ($CDCl_3$): 8.14–8.08 (2 H, m, *H* Ar), 8.07 (1 H, d, *J* 7.2, *H*6 picolyl), 7.43–7.37 (2 H, m, *H* Ar), 7.09 (1 H, d, *J* 3.4, *H*3 picolyl), 6.71 (1 H, dd, *J* 7.2, 3.4, *H*5 picolyl), 5.22 (2 H, d, *J* 7.2, P-OCH_2), 3.77 (3 H, s, OCH_3 picolyl), 3.07 (6 H, q, *J* 7.3, $^+$HNEt$_3$-CH_2), 1.32 (9 H, t, *J* 7.3, $^+$HNEt$_3$–CH_3). ^{31}P NMR ($CDCl_3$): -5.32. MS, *m/z:* 354.92 $[M - TEA\text{-}H^+]^-$, calculated for $C_{13}H_{12}N_2O_8P^-$ 355.03.

A General Method of the Synthesis of Monomers (**VIIa**)–(**VIIc**)

Nucleosides (**VIa**)–(**VIc**) were phosphorylated as described previously [32], except that, in the case of derivative (**VIc**), triethylammonium (4-nitrophenyl)-(1-oxido-4-methoxy-2-picolyl)phosphate (**X**) was used as a phosphorylation agent. The *p*-chlorophenyl group was removed from the phosphate group by treatment with DBU in an aqueous organic solvent, and in the case of the phosphotriester containing a 4-nitrophenyl phosphate-protecting group, by treatment with 20 equivalents of TEA in an acetonitrile–water mixture 10 : 1. The product was purified by chromatography on a silica gel column in a gradient of methanol (0–15%) in chloroform containing 0.1% TEA. Fractions containing the target compound were combined, evaporated to dryness, and the residue was dried in vacuo.

Triethylammonium salt of 5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-[(2-azidoethoxy)methyl]uridine 3'-*O*-(1-oxido-4-methoxy-2-picolyl)phosphate (VIIa). Yield: 79%; R_f 0.45 (B); ^{1}H NMR ($CDCl_3$): 9.35 (1 H, br s, N*H* Ura), 8.08 (1 H, d, *J* 7.2, *H*6 picolyl), 7.73 (1 H, d, *J* 8.2, *H*6 Ura), 7.37–7.19 (10 H, m, *Ar* DMTr, *H*3 picolyl), 6.84–6.77 (4 H, m, *Ar* DMTr), 6.71 (1 H, dd, *J* 7.2, 3.4, *H*5 picolyl), 6.11 (1 H, d, *J* 5.6, *H*1'), 5.21–5.07 (3 H, m, P-OCH_2, *H*5 Ura), 5.05–4.94 (2 H, m, OCH^{α}O, *H*3'), 4.77 (1 H, d, *J* 7.0, OCH^{β}O), 4.58–4.53 (1 H, m, *H*2'), 4.43–4.39 (1 H, m, *H*4'), 3.79 (3 H, s, OCH_3 picolyl), 3.77 (6 H, d, *J* 1.4, OCH_3 DMTr), 3.72–3.67 (2 H, m, OCH_2CH_2N$_3$), 3.57–3.47 (2 H, m, OCH$_2$CH_2N$_3$), 3.37–3.32 (2 H, m, *H*5'), 3.03 (6 H, q, *J* 7.3, CH_2 $^+$HNEt$_3$), 1.29 (9 H, t, *J* 7.3, CH_3 $^+$HNEt$_3$); ^{31}P NMR ($CDCl_3$): -0.14. MS, *m/z:* 861.12 $[M - TEA\text{-}H^+]^-$, calculated for $C_{40}H_{42}N_6O_{14}P^-$ 861.25.

Triethylammonium salt of 5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-(propargyloxymethyl)uridine 3'-*O*-(1-oxido-4-methoxy-2-picolyl)phosphate (VIIb). Yield: 80%; R_f 0.45 (B); ^{1}H NMR ($CDCl_3$): 9.30 (1 H, br s, N*H* Ura), 8.08 (1 H, d, *J* 7.2, *H*6 picolyl), 7.70 (1 H, d, *J* 8.3, *H*6 Ura), 7.36–7.17 (10 H, m, *Ar* DMTr, *H*3 picolyl), 6.84–6.77 (4 H, m, *Ar* DMTr), 6.71 (1 H, dd, *J* 7.2, 3.4, *H*5 picolyl), 6.13 (1 H, d, *J* 6.1, *H*1'), 5.22–5.07 (3 H, m, P-OCH_2, *H*5 Ura), 5.04–4.94 (2 H, m, OCH^{α}O, *H*3'), 4.85 (1 H, d, *J* 7.2, OCH^{β}O), 4.63–4.55 (1 H, m, *H*2'), 4.45–4.39 (1 H, m, *H*4'), 4.20 (2 H, ddd, *J* 24.8, 16.0, ^{4}*J* 2.1, OCH_2C≡), 3.79 (3 H, s, OCH_3 picolyl), 3.77 (6 H, br s, OCH_3 DMTr), 3.57–3.43 (2 H, m, *H*5'), 3.04 (6 H, q, *J* 7.3, CH_2 $^+$HNEt$_3$), 2.35 (1 H, t, ^{4}*J* 2.1, C*H*≡), 1.30 (9 H, t, *J* 7.3, CH_3 $^+$HNEt$_3$); ^{31}P NMR ($CDCl_3$): -0.21. MS, *m/z:* 830.08 $[M - TEA\text{-}H^+]^-$, calculated for $C_{41}H_{41}N_3O_{14}P^-$ 830.23.

Triethylammonium salt of N^4-benzoyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-[(3,4-*O*-cyclocarbonatebutoxy)methyl]cytidine 3'-*O*-(1-oxido-4-methoxy-2-picolyl) phosphate (VIIc). Yield: 83%; R_f 0.40 (B); ^{1}H NMR ($CDCl_3$): 8.36 (1 H, d, *J* 7.4, *H*6 Cyt), 8.04 (1 H, d, *J* 7.2, *H*6 picolyl), 7.92–7.46 (5 H,m, *H* Bz), 7.43–7.19 (9 H, m, *Ar* DMTr), 7.16 (1 H, d, *J* 3.2, *H*3 picolyl), 7.02 (1 H, br s, *H*5 Cyt), 6.86–6.79 (4 H, m, *Ar* DMTr), 6.70 (1 H, dd, *J* 7.2, 3.2, *H*5 picolyl), 6.05 (1 H, d, *J* 1.6, *H*1'), 5.18–4.84 (6 H, m, *H*3', P-OCH_2, OCH_2O, C*H*O(CO)CH$_2$O), 4.63–4.47 (2 H, m, *H*2', CHO(CO)CH_{α}O), 4.44–4.36 (1 H, m, *H*4'), 4.23–4.11 (1 H, m, CHO(CO)CH_{β}O), 3.92–3.67 (2 H, m, OCH_2CH_2), 3.79 (6 H, s, OCH_3 DMTr), 3.76 (3 H, s, OCH_3 picolyl), 3.77–3.50 (2 H, m, *H*5'), 3.03 (6 H, q,

J 7.3, $CH_2{}^+HNEt_3$), 2.13–1.98 (2 H, m, OCH_2CH_2), 1.28 (9 H, t, *J* 7.3, $CH_3{}^+HNEt_3$); ^{31}P NMR ($CDCl_3$): -0.14, -0.18. MS, *m/z*: 1009.47 [*M* – TEA-H^+]$^-$, calculated for $C_{50}H_{50}N_4O_{17}P^-$ 1009.29.

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