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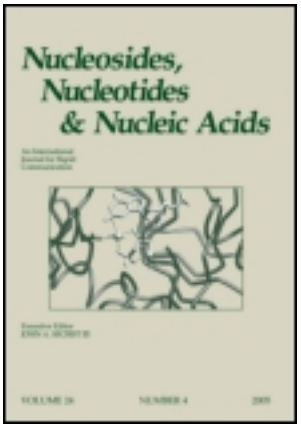

Nucleosides, Nucleotides and Nucleic Acids

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Synthesis of Novel Nucleoside 5′-Triphosphates and Phosphoramidites Containing Alkyne or Amino Groups for the Postsynthetic Functionalization of Nucleic Acids

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SYNTHESIS OF NOVEL NUCLEOSIDE 5′-TRIPHOSPHATES AND PHOSPHORAMIDITES CONTAINING ALKYNE OR AMINO GROUPS FOR THE POSTSYNTHETIC FUNCTIONALIZATION OF NUCLEIC ACIDS

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□ *A series of novel nucleoside 5′-triphosphates and phosphoramidites containing alkyne or amino groups for the postsynthetic functionalization of nucleic acids were designed and synthesized. For this purpose, the new 3-aminopropoxypropynyl linker group was used. It contains two alternative functional capabilities: an amino group for the reaction of amino–alkynyl-modified oligonucleotides with corresponding activated esters and an alkyne group for the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reaction. It was shown that a variety of methods of the attachment of the new linker can be used to synthesize a diversity of modified pyrimidine nucleosides.*

Keywords Modified nucleosides; triphosphate analogues; nucleoside synthesis; modified oligonucleotides

INTRODUCTION

Modified nucleoside triphosphates and phosphoroamidite synthons are of great importance for both enzymatic and chemical synthesis of modified DNA containing insertions of chemically active nucleotides. They can be then postsynthetically modified to design new biologically active or labeled oligonucleotide derivatives for the fundamental research or medical applications and probes for diagnostics and imaging.[1–6]

C5-amino-modified nucleosides are usually used for the incorporation of different functional groups into DNA (or RNA). The procedure includes

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the attachment of the protected allyl–amino linker to the C5 position of uridine or cytidine by conventional palladium-coupling chemistry by using halogenated nucleosides.[7–9] However, it was shown[10] that the removal of the protecting group leads to the formation of the bicyclic cytosine analogue. With uridine derivatives, this reaction can become particularly prominent. The propargylamine linker allows one to avoid the cyclization. Triphosphates containing this linker were shown to be substrates for a number of polymerases.[4, 11, 12] However, some steric hindrances may arise due to the small length of the propargylamine linker if the subsequent postsynthetic introduction of a large hydrophobic group is necessary.

At the same time, the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reaction introduced into the arsenal of bioorganic chemistry is widely used for the functionalization of biopolymers.[13–15] One publication out of many reports on the synthesis of alkyne modified nucleosides for "click" reactions showed exclusive cyclization of an *N*-disubstituted propargylamine analogue in the C5 position of uridine in the presence of Cu(I).[16]

We realized a need to create and utilize a universal linker to modify DNA (or RNA) containing amino or alkyne functional groups. In addition, this linker should be more extended and flexible than propargylamine in order to avoid the steric hindrance during the postsynthetic modification that follows.

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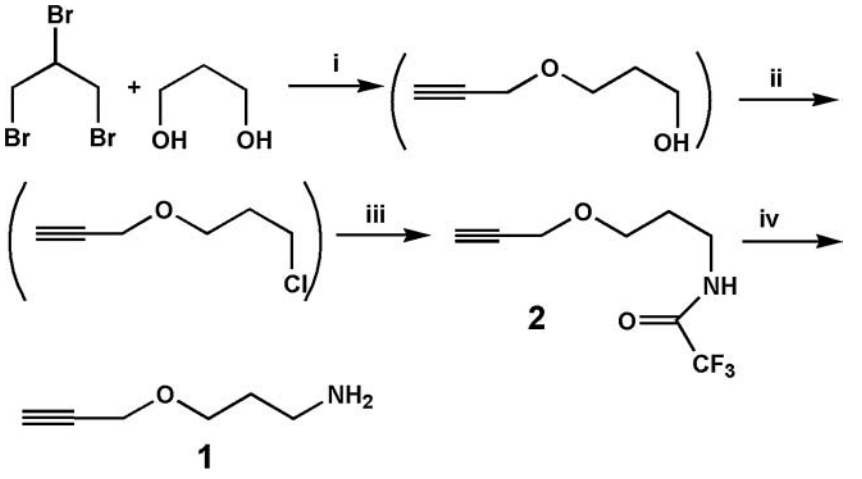
RESULTS AND DISCUSSION

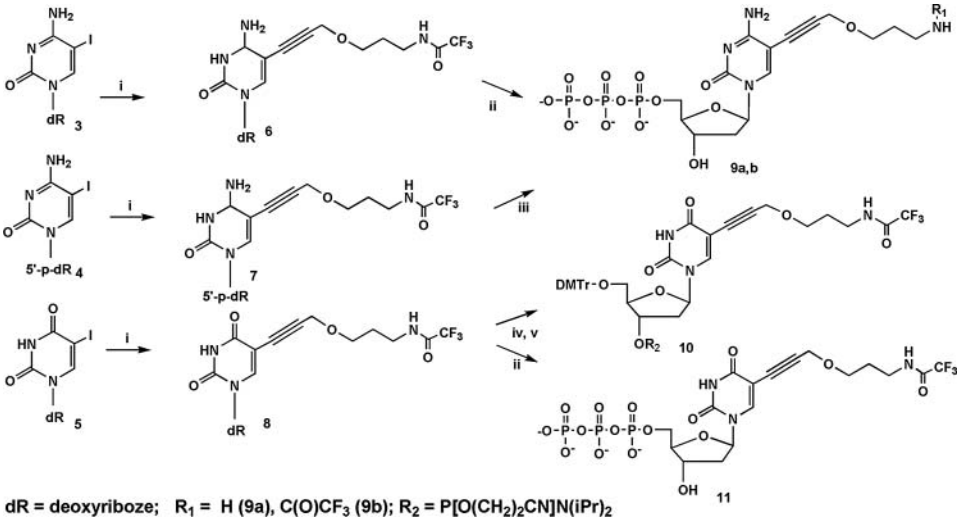
In our previous work, the new 3-aminopropoxypropynyl linker for DNA (or RNA) modification was proposed.[17] This linker (**1**, Scheme 1) is flexible and more hydrophilic due to the presence of oxygen; it has a more extended structure than propargylamine and probably causes less steric hindrance in the enzymatic synthesis and subsequent modifications of the amino or alkyne groups. It can be introduced both in the C5 position of pyrimidine nucleosides by the Sonogashira coupling reaction[18] and in the N^4 position using the reactivity of the amino group.

The synthesis of *N*-3-phthalimidopropyl propargyl ether was described in our previous publication.[17] The phthalimide protection of the amino group was removed, and the product was isolated as hydrochloride of **1** or trifluoroacetamide **2** depending on the subsequent use of the linker. Here we present a more convenient one-step way for the synthesis of *N*-(3-propynyloxypropyl)trifluoroacetamide **2** and then 3-propynyloxypropylamine **1**, without the phthalimide protection.

Sonogashira coupling was the Pd-catalyzed reaction of corresponding 5-iodo nucleosides with *N*-(3-propynyloxypropyl)trifluoroacetamide (**2**, Scheme 2). For the preparation of 5-iodo nucleosides, a number of described procedures was examined.[19–23] The best yields of 5-iodo-2′-deoxycytidine **3**



SCHEME 1 Synthesis of 3-aminopropoxypropynyl linker group: (i) KOH/t-BuOH; (ii) $SOCl_2$; (iii) $CF_3C(O)NH_2 + Cs_2CO_3$/DMF; (iv) $NaOH/H_2O$.



SCHEME 2 Synthesis of C5-substituted pyrimidine derivatives by Sonogashira coupling reaction: (i) $(Ph_3P)_4Pd$, CuI, DMF, Et_3N, *N*-(3-propynyloxypropyl)trifluoroacetamid **2**, argon, yield 58%–65%; (ii) $POCl_3/[MeO]_3PO/N(C_4H_9)_3$, 0°C, 10 minutes, $(Bu_4N)_2H_2P_2O_7$ in acetonitrile, $N(C_4H_9)_3$, 0°C, 10 minutes, TEAB 0.1 M (pH = 7), 2 hours, yield 54%; (iii) $N(C_4H_9)_3$ in acetonitrile, then 2,2′-dithiodipyridine/Ph_3P/*N*-Me-Im/DMF/DMSO, then $[(C_4H_9)_3N]_2H_2P_2O_7$ in acetonitrile, then NH_3/H_2O, yield 68%; (iv) DMTrCl, pyridine, DMAP; (v) $((iPr)_2N)_2PO(CH_2)_2CN$/tetrazole-$NH(iPr)_2/CH_2Cl_2$, yield 70%.

and 5-iodo-2′-deoxyuridine **5** were achieved in the presence of iodic acid, as described in the literature[19, 20] (up to 80% compared with 40%[21]). However, we were not able to reproduce the iodination reactions using procedures described in the literature.[22, 23] Deoxycytidine 5′-monophosphate, as was shown,[19] can also be iodinated in the presence of iodic acid. Because

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of their greater solubility than the deoxyribonucleosides in polar solvents, the best method for the isolation of product **4** is an anion exchange chromatography. As a result, **4** was obtained in a yield of up to 95%.

3-Trifluoroacetamidopropoxypropynyl was also successfully attached to the C5 position of cytidine, cytidine-5′-monophosphate, and uridine heterocyclic bases in 58%–65% yields (compounds **6**, **7**, and **8**, respectively, Scheme 2). Reduced yields of the desired products are probably caused by the formation of the furano[2,3-d]pyrimidine derivatives, which are common byproducts in the Pd-catalyzed reaction of corresponding 5-iodo uridine with alkines.[24] However, the formation of byproducts was not the subject of our study.

Another way of introducing the new linker into pyrimidine nucleoside is the use of the amino group reactivity. In this case, we obtained modified pyrimidine nucleosides bearing the alkyne group, which can be used for the functionalization of nucleic acids by the CuAAC reaction.

It was shown that the introduction of an alkyl substituent into the 4-amino group of 2′-deoxycytidine did not prevent the hydrogen bond formation during complementary interactions.[25] For the synthesis of the N^4-substituted 2′-deoxycytidine and 5-methyl-2′-deoxycytidine analogues, bisulfite-mediated transamination and the method of the replacement of O^4-benzoyl protection with alkyl amine were examined.[26, 27] Unfortunately, these reactions did not give acceptable yields of the desired product. Perhaps, this is due to a somewhat reduced reactivity of the amino group in 3-propynyloxypropylamine as hydrochloride compared with aliphatic diamines, which were used in the original method. Finally, the approach that utilizes triazolyl derivatives was chosen.[28, 29] The desired compounds were synthesized from 5′,3′-*O*-diacetyl-2′-deoxyuridine **14a** and 5′,3′-*O*-diacetyl-thymidine **14b** by the reaction of its 4-triazolyl-derivatives with 3-propynyloxypropylamine hydrochloride (Scheme 3). We obtained N^4-(3-propynyloxypropyl)-2′-deoxycytidine **16a** and N^4-(3-propynyloxypropyl)-5-methyl-2′-deoxycytidine **16b** in 70%–80% yields.

For the synthesis of pyrimidine derivatives bearing the linker with the alkyne functional group at the C5 position, we chose the convenient procedure recommended by the related literature.[30] The original method involves the reaction between protected 5-bromomethyl-2′-deoxyuridine and alcohol. The starting material, 3′,5′-*O*-diacetyl-5-bromomethyl-2′-deoxyuridine (**19**, Scheme 4), was synthesized according to the referenced method.[31] The treatment of **19** with propargyl alcohol in dry dimethylformamide (DMF) afforded 3′,5′-*O*-diacetyl-5-propargyloxymethyl-2′-deoxyuridine **20a**. This reaction occurred in 18 hours. After removing the protective groups, 5-propargyloxymethyl-2′-deoxyuridine **21a** was obtained in a 60% yield. The analogous treatment of **19** with 3-propynyloxypropylamine hydrochloride in the presence of triethylamine afforded 3′,5′-*O*-diacetyl-5-(3-propynyloxypropyl)aminomethyl-2′-deoxyuridine **20b**. The reaction mixture was analyzed by high performance

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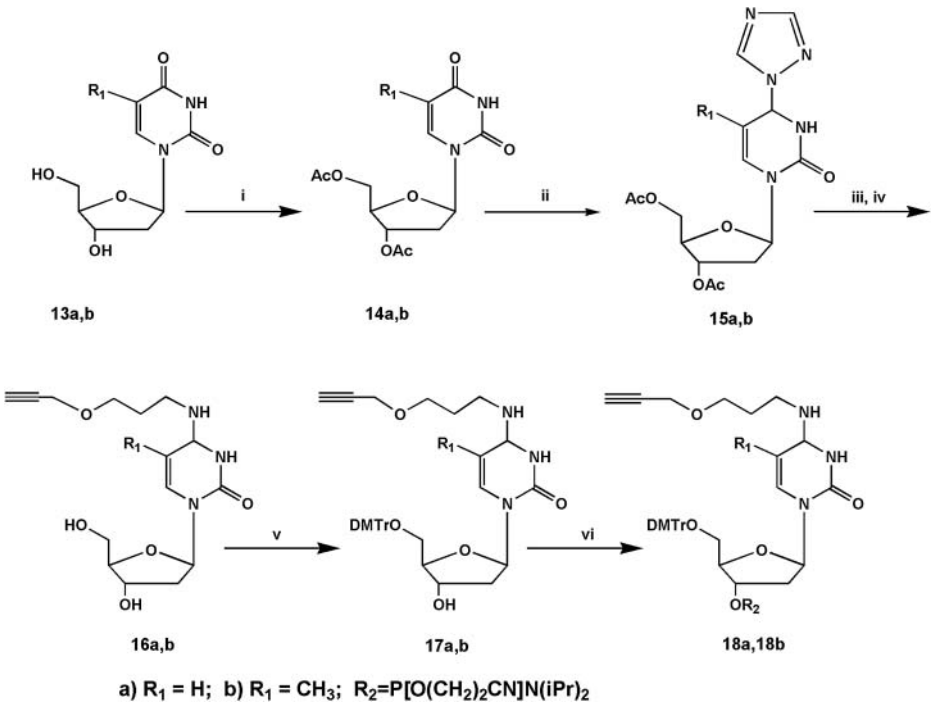

SCHEME 3 Synthesis of N^4-substituted pyrimidine derivatives bearing the alkyne functional group: (i) Ac_2O, pyridine, yield (a) 82%, (b)76%; (ii) $POCl_3$, Et_3N, 1,2,4-triazole, acetonitrile; (iii) 3-propynyloxypropylamine hydrochloride, Et_3N, acetonitrile; (iv) NH_3/H_2O, overall yield 72%; (v) DMTrCl, pyridine, DMAP; (vi) $((iPr)_2N)_2PO(CH_2)_2CN$/tetrazole-$NH(iPr)_2/CH_2Cl_2$, yield 69%–75%.

liquid chromatography (HPLC). The reaction time was 4–6 hours at room temperature. After deprotection of **20b** by the treatment with 24% aqueous ammonia, 5-(3-propynyloxypropyl)aminomethyl-2′-deoxyuridine **21b** was obtained in a 43% yield.

Thus, four N^4- and C5-modified pyrimidine nucleosides bearing the alkyne functional group (**16a**, **16b**, **21a**, and **21b**, Schemes 3 and 4) were obtained along with C5-alkynyl-modified uridine, cytidine, and 5′-monophospho-cytidine derivatives bearing the protected amino group (**6**, **7**, and **8**, Scheme 2). Each of these modified nucleosides may be converted into general-purpose triphosphates or phosphoramidite synthones for oligonucleotide synthesis. Since the attachment of the amino linker to the C5 position through the triple bond guarantees the preservation of the substrate specificity of corresponding triphosphates to polymerases, we converted compounds **6**, **7**, and **8** to triphosphates. Nucleosides **16a**, **16b**, **21a**, and **8** were converted into corresponding phosphoramidites synyhones.

5-(3-Fluoroacetamidopropoxyprop-1-ynyl)-5′-*O*-(4,4′-dimethoxytrityl)-3′-(2-cyanoethyl-*N*,*N*-diisopropylamino)-2′-deoxyuridine (**10**, Scheme 2), N^4-(3-propynyloxypropyl)-5′-*O*-(4,4′-dimethoxytrityl)-3′-(2-cyanoethyl-*N*,*N*-diisopropylamino)-2′-deoxycytidine, N^4-(3-propynyloxypropyl)-5-methyl-5′-

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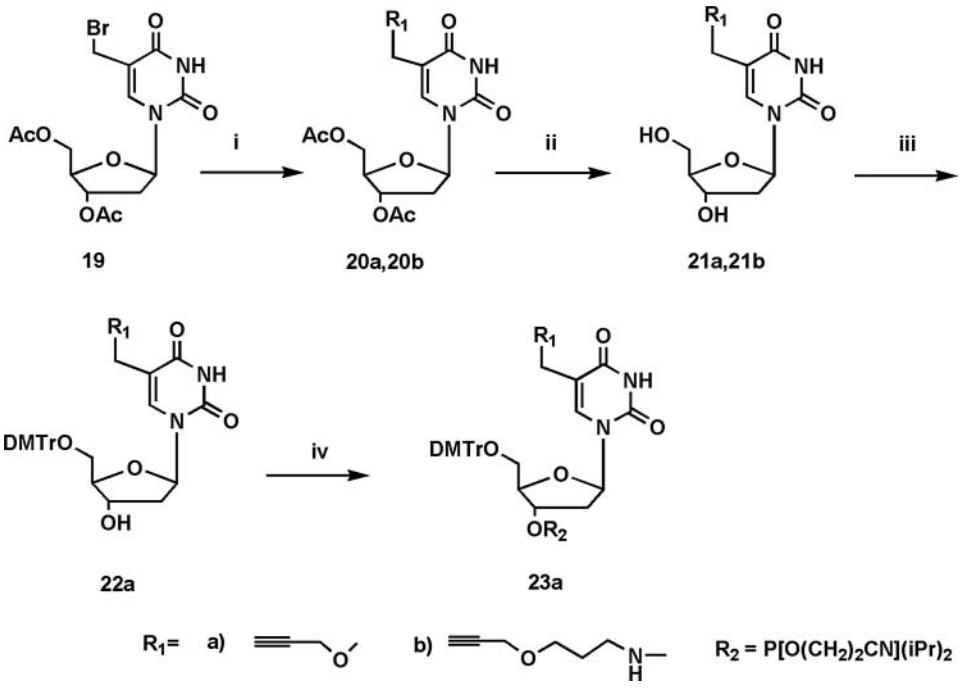

SCHEME 4 Synthesis of C5-substituted pyrimidine derivatives bearing the alkyne functional group: (i) (a) Propargyl alcohol, (b) 3-propynyloxypropylamine hydrochloride, Et_3N, DMF; (ii) NH_3/H_2O, overall yield 72%–43%; (iii) DMTrCl, pyridine, DMAP; (iv) $((iPr)_2N)_2PO(CH_2)_2CN$/tetrazole-$NH(iPr)_2/CH_2Cl_2$, yield 69%–75%.

O-(4,4′-dimethoxytrityl)-3′-(2-cyanoethyl-*N*,*N*-diisopropylamino)-2′-deoxycytidine (**18a,b**, Scheme 3), and 5-propargyloxymethyl-5′-*O*-(4,4′-dimethoxytrityl)-3′-(2-cyanoethyl-*N*,*N*-diisopropylamino)-2′-deoxyuridine (**23a**, Scheme 4) were prepared following procedures described in the literature[32, 33] in yields of 70%, 69%, 76%, and 79%, respectively, from **8** and preprotected 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxynucleosides **17a,b** and **22a**. All phosphoramidite derivatives were characterized by ^1H, ^{31}P NMR, and mass spectroscopy. The data are summarized in Table 1 (supplementary material is available online).

Various methods were used for the synthesis of 5′-triphosphates of nucleoside derivatives **6**, **7**, and **8**, (Scheme 2). For the conversion of 5-(3-fluoroacetamidopropoxyprop-1-ynyl)-2′-deoxycytidin-5′-monophosphate **7** into its 5′-triphosphate derivative **9a**, we used the previously reported synthetic procedure.[34] Activation of the terminal phosphate group was achieved under Mukaiyama conditions in the presence of a nucleophilic catalyst (68% yield). We also used Ludwig's method[35] for the synthesis of 5′-triphosphates **9b** and **11** from unprotected 5-(3-trifluoroacetamidopropoxyprop-1-ynyl)-2′-deoxycytidine and 5-(3-trifluoroacetamidopropoxyprop-1-ynyl)-2′-deoxyuridine, respectively, in trimethylphosphate

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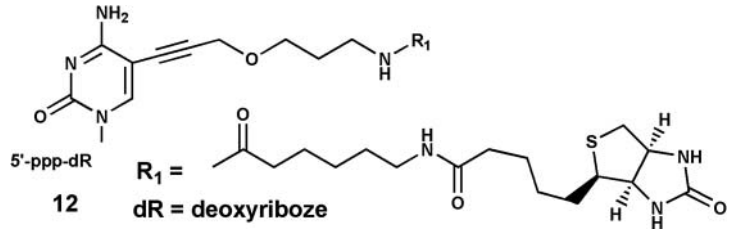

FIGURE 1 dCTP analogue 5-(biotinyl-6-amidocaproyl-3-amidopropoxyprop-1-ynyl)-2′-deoxycytidine-5′-triphosphate.

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(50% and 54% yields). It was shown that iodination of the C5 position in 2′-deoxycytidine 5′-phosphate occurred at a higher extent than that in 2′-deoxycytidine, so compound **4** was obtained in a much higher yield (up to 95%) as compared with compounds **3** and **5**. Furthermore, the formation of 5′-triphosphate **9a,b** from compound **7** by activation of the terminal phosphate group is carried out in a higher and reproducible yield as compared to the formation of the same triphosphate from a nucleoside derivative **6** using Ludwig's method. Therefore, the synthesis of modified triphosphates starting from a nucleotide seems to be the most attractive. Some reporter, photoreactive, and functional groups can be introduced after deprotection of the amino group in the synthesized amino–alkynyl-modified nucleotides. As an example of functionalization with corresponding activated esters, the deoxycytidine triphosphate (dCTP) analogue (**12**) containing the biotin residue covalently bound to the C5 position of the pyrimidine ring through the 6-amidocaproyl-3-amidopropoxyprop-1-ynyl arm (Figure 1) was synthesized.[36, 37] All 5′-triphosphate derivatives were characterized by ^{1}H, ^{31}P NMR, and mass spectroscopy. The data are summarized in Table 1 (supplementary material is available online).

CONCLUSION

The universal hydrophilic linker to modify DNA (or RNA) was proposed. As was shown, a variety of methods of the attachment of the new linker can be used to synthesize a number of pyrimidine nucleosides containing a functional alkyne or amino group. A series of modified 5′-triphosphates and phosphoramidites containing the new alkynyl–amino linker for the functionalization of nucleic acids was synthesized. The synthesis of amino–alkynyl-modified nucleotides was optimized due to both the new linker and the proposed way of its 5′-triphosphate synthesis through 5′-phosphate derivatives.

EXPERIMENTAL

The following reagents were used: 1,2,3-tribromopropane, cesium carbonate, trifluoroacetamide, thionylchloride, bis(tri-n-butylammonium)

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pyrophosphate, 2,2′-dithiodipyridine, 1,3-propanediol (Sigma-Aldrich, USA); 4-(N,N-dimethylamino)pyridine, triphenylphosphine (Fluka Chemie AG, Buchs, Switzerland); 4,4′-dimethoxytrityl chloride and 1,2,4-triazole (Chem-IMPEX, USA). Nucleosides, their protected derivatives, and 5′-monophosphorylated 2′-deoxycytidine were purchased from ChemGenes Corporation (USA). N-Hydroxysuccinimidyl biotinyl-6-amidocaproate was purchased from NanoTex-C (Novosibirsk, Russia). All other reagents were from Sigma-Aldrich (Milwaukee, WI, USA). Organic solvents were dried and purified by standard procedures. ^{1}H and ^{31}P NMR spectra were recorded on Bruker AV-400 and AV-300 spectrometers. The chemical shifts (δ) are reported in ppm relative to the residual solvent signals. In case of ^{31}P, an external standard of 85% H_3PO_4 was used. Coupling constant (J) values are estimated in hertz (Hz) and spin multiples are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), app.t (apparent triplet), and br (broad singlet). Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectra were run on Reflex III (Bruker Daltonics, Germany) in a positive detector mode with dihydroxybenzoic acid as a matrix. A 6410 Agilent LCMS triple quadrupole mass spectrometer [liquid chromatography–mass spectrometry (LC-MS) with an electrospray ionization (ESI) interface was also used for molecular weight measurements. Elemental analyses were performed on the Elemental Analyzer Model 1106 (Carlo Erba, Italy). UV-absorption spectra were recorded on a Specord M40 spectrophotometer (Carl Zeiss, Jena, Germany). The product yields (Schemes 1–3, Figure 1) were evaluated as described in the figure legends. Aliquots of the reaction mixtures were taken off at the appropriate time, diluted 10-fold with water or ethanol, centrifuged, and analyzed by analytical anion exchange HPLC or analytical reverse phase HPLC. The analytical anion exchange chromatography was performed on a Milichrom-4 chromatograph (Econova, Russia) using a 2.5 × 60 mm column packed with Polysil SA, 15 μm (Vector, Russia). A linear gradient (flow rate 50 μL/minute) from 0 to 0.8 M of K_2HPO_4/KH_2PO_4 (pH 7.0) was used. The peaks were integrated from chromatographic profiles generated by the chromatography system software. Preparative anion exchange chromatography was performed with Polysil SA, 15 μm (Vector, Russia), or DEAE Sephadex A-25, 40–120 μ, (Pharmacia Fine Chemicals, Sweden). Analytical reverse phase HPLC was performed using a Milichrom A-02 chromatograph (Econova, Russia) and a 2 × 75 mm column packed with ProntoSIL 120-5C18 AQ (Bischoff, Leonberg, Germany). Preparative reverse phase chromatography was performed on Polygoprep C_{18}, 50–100 mkm (Macherey-Nagel, Germany). Thin layer chromatography was performed using Alufolien Kieselgel 60 F_{254} plates (Merck, Darmstadt, Germany) in appropriate solvent mixtures and was visualized by UV irradiation, ninhydrin (for amine groups), or cystein/aqueous sulphuric acid (for nucleosides). All evaporations were performed under reduced pressure.

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Synthesis of *N*-(3-Propynyloxypropyl)Trifluoroacetamide (2)

Potassium hydroxide [100 g (1.78 mol)] was dissolved in 500 mL of tert-butanol containing 122 g (1.6 mol) of 1,3-propanediol. The mixture was intensively stirred under reflux followed by the dropwise addition of 153 g (0.55 mol) of 1,2,3-tribromopropane. The reaction mixture was stirred at 80°C for 36 hours and then allowed to cool to room temperature. Precipitated KBr was filtered off and washed twice with tert-butanol. The wash liquor was added to the filtrate and evaporated under reduced pressure at 60°C, and the resulting oil was cooled and filtered. Thionyl chloride (300 mL) was slowly added to crude 3-hydroxypropyl propargyl ether. The reaction mixture was then stirred for 18 hours at room temperature and then refluxed for 6 hours. Next, the mixture was filtered, and the filtrate was evaporated under reduced pressure and distilled *in vacuo*. The fraction boiling up to 66°C (13 torr) was collected and redistilled. The crude 3-chloropropyl propargyl ether (29.2 g) was collected at 68°C–78°C under 13 torr. Trifluoroacetamide (11.3 g, 0.1 mol), 13.25 g (0.1 mol) of crude 3-chloropropyl propargyl ether, and 32.6 g (0.1 mol) of caesium carbonate was suspended in 100 mL DMF and stirred for 6 days at room temperature. The reaction mixture was cooled to −12°C, and the salts were filtered off. DMF was evaporated, and the residue was distilled in vacuo. The fraction boiling at 45°C (0.5 torr) was collected and redistilled. The yield of target product **2** was 6.97 g (13.2% calc. for 1,2,3-tribromopropane), b.p. 90°C (0.5 torr).

Synthesis of 3-Propynyloxypropylamine (1)

N-(3-propynyloxypropyl)trifluoroacetamide **2** [150 mg (0.72 mmol), Scheme 1] was dissolved in 1 mL of 1 M NaOH and incubated for 2 hours at room temperature. The reaction mixture was evaporated to 0.2 mL *in vacuo*, and the amine was extracted with ether. After the ether was evaporated, the yield of target amine **1** was 58%. An analytical sample was obtained as a picrate, m.p. (for picrate) 128–130 (decomposition). 3-Propynyloxypropylamine hydrochloride was obtained for subsequent syntheses.

Synthesis of C5-Substitued Pyrimidine Derivatives by Sonogashira Coupling Reaction: 5-(3-Fluoroacetamidopropoxyprop-1-ynyl)-2′-Deoxycytidine (6), 5-(3-Fluoroacetamidopropoxyprop-1-ynyl)-2′-Deoxycytidin-5′-Phosphate (7), 5-(3-Fluoroacetamidopropoxyprop-1-ynyl)-2′-Deoxyuridine (8)

5-Iodo-derivative (**3** or **4** or **5**, Scheme 2; 1.0 mmol) was dissolved in degassed, anhydrous DMF (8 mL). Copper (I) iodide (0.030 g, 0.1 mmol) was added, and the reaction mixture was stirred under an inert atmosphere

in the absence of light until copper (I) iodide was dissolved. Freshly distilled triethylamine (0.3 mL, 2.0 mmol) was added to the reaction mixture followed by the addition of *N*-(3-propynyloxypropyl)trifluoroacetamide **2** (1.13 g, 10 mmol) and *tetrakis*(triphenylphosphine)-palladium(0) (0.116 g, 0.1 mmol). The reaction mixture was stirred for 18 hours in the absence of light at room temperature under an inert atmosphere. Methanol (8.0 mL), dichloromethane (8.0 mL), and the excess of Dowex AG1×4 (bicarbonate form) were then added to the reaction mixture and the resulting suspension was stirred for 30 minutes, filtered, and concentrated by evaporation. The resulting brown oil was then suspended in an ethanol–water mixture, and the desired products **6** and **8** were isolated by reverse-phase chromatography in yields of 64%–65%. Crude product **7** was purified by anion exchange chromatography on DEAE Sephadex A-25 using a linear gradient of NH_4HCO_3 ($0 \rightarrow 1$ M) in 20% ethanol in a yield of 58%.

Synthesis of 5′-Triphosphate Derivatives by Ludwig's Method[35]: 5-(3-Trifluoroacetamidopropoxyprop-1-ynyl)-2′-Deoxycytidin-5′-Triphosphate (9b) and 5-(3-Trifluoroacetamidopropoxyprop-1-ynyl)-2′-Deoxyuridin-5′-Triphosphate (11)

The synthesis of **9b** and **11** was carried out from unprotected nucleosides (**6** and **8**, respectively; 0.23 mmol). Purification of the 5′-triphosphate derivatives was performed by anion exchange chromatography using a column packed with Polisil SA. A linear gradient of NaCl concentration ($0 \rightarrow 1$ M) in 0.1% AcOH was used. Fractions containing the product were combined, diluted 10-fold with water, and applied to a column packed with DEAE Sephadex A-25. Elution was performed in the linear gradient of NH_4HCO_3 (concentration, $0 \rightarrow 1$ M) in 20% ethanol. Appropriate fractions were pooled and evaporated. The residue was co-evaporated several times with aqueous ethanol to remove buffer traces. 5′-Triphosphates **9b** and **11** were precipitated as trilithium salts by the addition of a 10-fold volume of 4% $LiClO_4$ in acetone to aqueous solutions of the products. Yields of **9b** and **11** were 50% and 54%, respectively.

Synthesis of 5′-Triphosphate from 5′-Monophosphate Derivative: 5-(3-Aminopropoxyprop-1-ynyl)-2′-Deoxycytidin-5′-Triphosphate (9a)

The synthesis was carried out by the procedure described in the literature[34] from 5-(3-aminopropoxyprop-1-ynyl)-2′-deoxycytidin-5′-phosphate **7** (0.140 g, 0.16 mmol). 5′-Triphosphate was precipitated by the addition of 6% $LiClO_4$ in acetone (100 mL). The precipitate was washed with acetone and ether, purified by reverse phase chromatography on Polygoprep C_{18},

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with a gradient of acetonitrile (0 → 10%) in water in the presence of 0.1 M TEA–AcOH (pH 7.0). Appropriate fractions were pooled and evaporated. The resulting protected 5′-triphosphate was subjected to the standard deprotection protocols (concentrated aqueous ammonia for the trifluoroacetic protective groups). After the deprotection, target 5′-triphosphate **9a** was purified by anion exchange chromatography using a column packed with DEAE Sephadex A-25. Elution was performed with a linear gradient of 300 mL each of 20% EtOH and 1 M NH_4HCO_3 in 20% EtOH. Appropriate fractions were pooled and evaporated. The residue was co-evaporated several times with aqueous ethanol to remove traces of buffer. 5′-Triphosphate **9a** was precipitated as trilithium salts by the addition of a 10-fold volume of 4% $LiClO_4$ in acetone to the aqueous solutions of the products in a yield of 0.1 mmol (68%).

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Synthesis of 5-(Biotinyl-6-Amidocaproyl-3-Amidopropoxyprop-1-ynyl)-2′-Deoxycytidin-5′-Triphosphate (12)

N-Hydroxysuccinimidyl biotinyl-6-amidocaproate (0.25 mmol) was dissolved in 1.5 mL of dimethyl sulfoxide (DMSO) and added to the solution of 5-(3-aminopropoxyprop-1-ynyl)-2′-deoxycytidine-5′-triphosphate **9a** or **9b** (0.025 mmol; previously deprotection was carried out by 24% aqueous ammonia) in 1.5 mL of 1 M aqueous $NaHCO_3/Na_2CO_3$ (pH 9.0). After 1.5 hours, the product was precipitated by the addition of a 10-fold volume of 2% $LiClO_4$ in acetone, washed with acetone, and dried. The target compound was purified by reverse phase chromatography on Polygoprep C_{18} in a gradient of acetonitrile (0 → 30%) in water in the presence of 0.1 M TEA-HOAc (pH 7.0). The yield of **12** was 70%.

Synthesis of N^4-Substitued Pyrimidine Derivatives: N^4-(3-Propynyloxypropyl)-2′-Deoxycytidine (16a), N^4-(3-Propynyloxypropyl)-5-Methyl-2′-Deoxycytidine (16b)

The synthesis was carried out as described in the literature[28] from 5′,3′-*O*-diacetyl-2′-deoxyuridine **14a** and 5′,3′-*O*-diacetyl-thymidine **14b** (0.194 mmol each), respectively, and 3-propynyloxypropylamine hydrochloride (3.88 mmol; Scheme 3). After the subsequent treatment with concentrated aqueous ammonia (30 mL), the reaction mixture was evaporated and the residue was purified by reverse phase chromatography on Polygoprep C_{18} in a gradient of ethanol (0 → 20%) in water. The target fractions were pooled and evaporated. The yields of **16a** and **16b** were 70 and 80%, respectively.

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Synthesis of C5-Substitued Pyrimidine Derivatives Containing a Functional Alkyne Group: 5-Propargyloxymethyl-2′-Deoxyuridine (21a), 5-(3-Propynyloxypropyl)aminomethyl-2′-Deoxyuridine (21b)

5′,3′-*O*-diacetyl-5-bromomethyl-2′-deoxyuridine (**19**, Scheme 4; 0.400 g, 0.98 mmol), synthesized as described in the literature[31], was dissolved in dried DMF (4 mL). Propargyl alcohol (0.550 g, 9.8 mmol) or 3-propynyloxypropylamine hydrochloride (9.8 mmol) and triethylamine (1.37 mL, 9.8 mmol) were added, and the reaction mixture was stirred for 16 hours at room temperature and concentrated by evaporation. The residue was dissolved in chloroform (20 mL) and washed with water (2 × 20 mL) and 5 M sodium chloride (20 mL). The organic phase was dried under anhydrous sodium sulfate and evaporated. Concentrated aqueous ammonium (15 mL) was then added to the residue, the reaction mixture was stirred for 16 hours at room temperature and concentrated by evaporation. The resulting oil was then suspended in an ethanol-water mixture and the desired products (**21a** and **21b**, Scheme 4) were purified by reverse phase chromatography in a gradient of ethanol in water 0 → 50%. Appropriate fractions were pooled and evaporated. The yields of **21a** and **21b** were 60% and 43%, respectively.

General Procedure for the Introduction of the 4,4′-Dimethoxytrityl Protecting Group

The corresponding nucleoside (1 mmol) was dissolved in dry pyridine (5 mL), and 4,4′-dimethoxytrityl chloride (1.15 mmol) was added at stirring. After the reaction was completed (2–3 hours), the reaction mixture was evaporated and the residue was dissolved in methylene chloride and applied onto a silica gel column. Elution with a gradient of methanol (0% → 12%) in methylene chloride supplemented with 1% pyridine led to the target product fractions. They were evaporated, and the product was precipitated from the solution in methylene chloride by a tenfold volume of hexane. 5′-*O*-(4,4′-dimethoxytrityl)-N^4-(3-propynyloxypropyl)-2′-deoxycytidine (**17a**), 5′-*O*-(4,4′-dimethoxytrityl)-N^4-(3-propynyloxypropyl)-5-methyl-2′-deoxycytidine (**17b**), and 5′-*O*-(4,4′-dimethoxytrityl)-5-propargyloxymethyl-2′-deoxyuridine (**22a**) were obtained in yields of 45%, 53%, and 43%, respectively.

General Procedure for Obtaining Phosphoroamidite Reagents for Oligonucleotide Synthesis

2-Cyanoethyl-*N,N,N′,N*-tetraisopropylphosphoroamidite (4.5 mL, 14 mmol, and, after 2 hours, 2.2 mL, 6.9 mmol) was added portionwise under stirring to the solution of the protected nucleoside (10 mmol) and diisopropylammonium tetrazolide (900 mg, 4.47 mmol) in dichloromethane

(50 mL). After 3–7 hours (thin layer chromatography monitoring), the reaction mixture was evaporated to dryness, covered with hexane, and left overnight. The hexane was then decanted, and the residue was chromatographed on a silica gel column. Elution with a gradient of methanol ($0 \rightarrow 10\%$) in dichloromethane and the evaporation of the target fractions gave the product, which was precipitated from dichloromethane by hexane and dried in a vacuum.

5-(3-Fluoroacetamidopropoxyprop-1-ynyl)-5′-*O*-(4,4′-dimethoxytrityl)-3′-(2-cyanoethyl-*N,N*-diisopropylamino)-2′-deoxyuridine (**10**), N^4-(3-propynyloxypropyl)-5′-*O*-(4,4′-dimethoxytrityl)-3′-(2-cyanoethyl-*N,N*-diisopropylamino)-2′-deoxycytidine (**18a**), N^4-(3-propynyloxypropyl)-5-methyl-5′-*O*-(4,4′-dimethoxytrityl)-3′-(2-cyanoethyl-*N,N*-diisopropylamino)-2′-deoxycytidine (**18b**), and 5-propargyloxymethyl-5′-*O*-(4,4′-dimethoxytrityl)-3′-(2-cyanoethyl-*N,N*-diisopropylamino)-2′-deoxyuridine (**23a**) were obtained with yields of 70%, 79%, 69%, and 76%, respectively.

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REFERENCES

1. Gavriliu, D.; Fossey, C.; Fontaine, G.; Benzaria, S.; Ciurea, A.; Delbederi, Z.; Lelong, B.; Ladurée, D.; Aubertin, A.; Kirn, A. Synthesis and antiviral activity of C-5 substituted analogues of D_4T bearing methylamino- or methyldiamino-linker arms. *Nucleosides Nucleotides Nucleic Acids.* **2000**, 19, 1017–1031.
2. Herdewijn, P. Heterocyclic modifications of oligonucleotides and antisense technology. *Antisense Nucleic Acid Drug Dev.* **2000**, 10, 297–310.
3. Hobbs, F.W. Palladium-catalyzed synthesis of alkynylamino nucleosides. A universal linker for nucleic acids. *J. Org. Chem.* **1989**, 54, 3420–3422.
4. Battersby, T.; Ang, D.; Burgstaller, P.; Jurczyk, S.; Bowser, M.; Buchanan, D.; Kennedy, R.; Benner, S. Quantitative analysis of receptors for adenosine nucleotides obtained via in vitro selection from a library incorporating a cationic nucleotide analog. *J. Am. Chem. Soc.* **1999**, 121, 9781–9789.
5. Shinozuka, K.; Kohgo, S.; Ozaki, H.; Sawai, H. Multi-functionalization of oligodeoxynucleotide: a facile post-synthetic modification technique for the preparation of oligodeoxynucleotides with two different functional molecules. *Chem. Commun.* **2000**, 1, 59–60.
6. Nomura, Y.; Ueno, Y.; Matsuda, A. Site-specific introduction of functional groups into phosphodiester oligodeoxynucleotides and their thermal stability and nuclease-resistance properties. *Nucleic Acids Res.* **1997**, 25, 2784–2791.
7. Langer, P.; Waldroptt, A.; Ward, D. Enzymatic synthesis of biotin-labeled polynucleotides: novel nucleic acid affinity probes. *Proc. Natl. Acad. Sci. USA* **1981**, 78, 6633–6637.
8. Khan, S.; Grinstaff, M. The alkylation of iodouridine by a heterogeneous palladium catalyst. *J. Org. Chem.* **1999**, 64, 1077–1078.
9. Matulic-Adamic, J.; Daniher, A.; Karpeisky, A.; Haeberli, P.; Sweedler, D.; Beigelman, L. Functionalized nucleoside 5′-triphosphates for in vitro selection of new catalytic ribonucleic acids. *Bioorg. Med. Chem. Lett.* **2000**, 10, 1299–1302.
10. Shibata, T.; Buurma, N.; Brazier, J. 7,8-Dihydropyrido[2,3-*d*]pyrimidin-2-one; a bicyclic cytosine analogue capable of enhanced stabilisation of DNA duplexes. *Chem. Commun.* **2006**, 33, 3516–3518.
11. Jager, S.; Rasched, G.; Kornreich-Leshem, H.; Engeser, M.; Thum, O.; Famulok, M. A versatile toolbox for variable DNA functionalization at high density. *J. Am. Chem. Soc.* **2005**, 127, 15071–15082.
12. Roychowdhury, A.; Illangkoon, H.; Hendrickson, C.L.; Benner, S.A. 2′-deoxycytidines carrying amino and thiol functionality: synthesis and incorporation by vent (exo-) polymerase. *Org. Lett.* **2004**, 6, 489–492.
13. Amblard, F.; Cho, J.H.; Schinazi, R.F. Cu(I)-catalyzed huisgen azide–alkyne 1,3-dipolar cycloaddition reaction in nucleoside, nucleotide, and oligonucleotide chemistry. *Chem. Rev.* **2009**, 109, 4207–4220.

14. Moses, J.E.; Moorhouse, A.D. The growing applications of click chemistry. *Chem. Soc. Rev.* **2007**, 36, 1249–1262.
15. Meldal, M.; Tornoe, C.W. Cu-catalyzed azide–alkyne cycloaddition. *Chem. Rev.* **2008**, 108, 2952–3015.
16. Sirivolu, V.R.; Chittepu, P.; Seela, F. DNA with branched internal side chains: synthesis of 5-tripropargylamine-dU and conjugation by an azide–alkyne double click reaction. *Chem. Bio. Chem.* **2008**, 9, 2305–2316.
17. Vasileva, S.; Konevetz, D.; Budilkin, B.; Abramova, T.; Kasatkina, N.; Silnikov, V. A new linker group for functionalization of nucleic acids. *Nucleic Acids Symp. Ser.* **2008**, 52, 385–386.
18. Lee, S.E.; Sidorov, A.; Gourlain, T.; Mignet, N.; Thorpe, S.J.; Brazier, J.A.; Dickman, M.J.; Hornby, D.P.; Grasby, J.A.; Williams, D.M. Enhancing the catalytic repertoire of nucleic acids: a systematic study of linker length and rigidity. *Nucleic Acids Res.* **2001**, 29, 1565–1573.
19. Chang, P.K.; Welch, A.D. Iodination of 2′-deoxycytidine and related substances. *J. Med. Chem.* **1963**, 6, 428–430.
20. Chang, P.K. Iodination of 2′-deoxycytidine and related substances. A reinvestigation of the structures of the by-product, $C_9H_{10}I_2N_2O_5$, and its derivatives. *J. Org. Chem.* **1965**, 30, 3913–3915.
21. Lipkin, D.; Howard, F.B.; Nowotny, D.; Sano, M. The iodination of nucleosides and nucleotides. *J. Biol. Chem.* **1963**, 238, PC2249–PC2251.
22. Kumar, R.; Wiebe, L.I.; Knaus, E.E. A mild and efficient methodology for the synthesis of 5-halogeno uracil nucleosides that occurs via a 5-halogeno-6-azido-5,6-dihydro intermediate. *Can. J. Chem.* **1994**, 72, 2005–2010.
23. Asakura, J.; Robins, M.J. Cerium (IV)-mediated halogenation at C-5 of uracil derivatives. *J. Org. Chem.* **1990**, 55, 4928–4933.
24. a) Robins, M.; Barr, P.J. Nucleic acid related compounds. 39. Efficient conversion of 5-iodo to 5-alkynyl and derived 5-substituted uracil bases and nucleosides. *J. Org. Chem.* **1983**, 48, 1854–1862 b) McGuigan, C.; Yarnold, C.J.; Jones, G.; Velazquez, S.; Barucki, H.; Brancale, A.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J. Potent and selective inhibition of varicella-zoster virus (VZV) by nucleoside analogues with an unusual bicyclic base. *J. Med. Chem.* **1999**, 42, 4479–4484.
25. Meyer, R.B., Jr. Incorporaion of modified bases into oligonucleotides. *Methods Mol. Biol.* **1994**, 26, 73–91.
26. Hovinen, J. A simple synthesis of N^4-(6-aminohexyl)-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)cytidine. *Nucleosides Nucleotides Nucleic Acids.* **1998**, 17, 1209–1213.
27. Wlassoff, W.; Dobrikov, M.; Safronov, I.; Dudko, R.; Lavrik, O.I. Synthesis and characterization of (d)NTP derivatives substituted with residues of different photoreagents. *Bioconjugate Chem.* **1995**, 6, 352–360.
28. McGee, D.P.C.; Sebesta, D.; O'Rourke, S.; Martinez, R.; Jung, M.; Pieken, W. Novel nucleosides via intramolecular functionalization of 2,2′-anhydrouridine derivatives. *Tetrahedron Lett.* **1996**, 37, 1995–1998.
29. a) Divakar, K.; Reese, C. J. 4-(1,2,4-triazol-1-yl)- and 4-(3-nitro-1,2,4-triazol-1-yl)-1-(β-D-2,3,5-tri-O-acetylarabinofuranosyl)pyrimidin-2(1H)-ones. Valuable intermediates in the synthesis of derivatives of 1-(β-D-arabinofuranosyl)cytosine (ara-C). *Chem. Soc., Perkin Trans. I*, **1982**, 1171–1176; b) Roget, A.; Bazin, H.; Teoule, R. Synthesis and use of labelled nucleoside phosphoramidite building blocks bearing a reporter group: biotinyl, dinitrophenyl, pyrenyl and dansyl. *Nucleic Acids Res.* **1989**, 17, 7643–7651.
30. Levina, A.; Tabatadse, D.; Khalimskaya, L.; Prichodko, T.; Shishkin, G.; Alexandrova, L.; Zarytova, V.P. Oligonucleotide derivatives bearing reactive and stabilizing groups attached to C5 of deoxyuridine. *Bioconjugate Chem.* **1993**, 4, 319–325.
31. Barwolff, D.; Langer, P. 2/-deoxy-5(hydroxymethyl)uridine and 2′-deoxy-5-formyluridine selective bromination of the 5-methyl group of 5-methylpyrimidine nucleosides, in *Nucleic Acid Chemistry*, ed. L.B. Townsend, R.S. Tipson, Wiley, New York, 1978, vol. 1, pp. 359–366.
32. Vasiléva, S.; Abramova, T.; Ivanova, T.; Shishkin, G.; Silńikov, V. Monomers for oligonucleotide synthesis with linkers carrying reactive residues: II. The synthesis of phosphoamidites on the basis of uridine and cytosine and containing a linker with methoxyoxalamide groups in position 2′. *Russ. J. Bioorg. Chem.* **2004**, 30, 234–241.
33. Abramova, T.; Vasiléva, S.; Ivanova, T.; Shishkin, G.; Silńikov, V. Monomers for oligonucleotide synthesis with linkers carrying reactive residues: I. The synthesis of deoxynucleoside derivatives with methoxyoxalamide groups in heterocyclic bases. *Russ. J. Bioorg. Chem.* **2004**, 30, 224–233.

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34. Abramova, T.; Vasileva, S.; Serpokrylova, I.; Kless, H.; Silnikov, V. A facile and effective synthesis of dinucleotide 5′-triphosphates. *Bioorg. Med. Chem.* **2007**, 15, 6549–6555.
35. a) Ludwig, J. A new route to nucleoside 5′-triphosphates. *Acta Biochim. Biophys. Acad. Sci. Hung.* **1981**, 16, 131–133; b) Ludwig, J. In *Biophosphates and Their Analogues Synthesis, Structure, Metabolism and Activity*, ed. K.S. Bruzik, W.J. Stec, Elsevier, Amsterdam, 1987, pp. 201–204.
36. Li, Z.; Bai, X.; Ruparel, H.; Kim, S.; Turro, N.J.; Ju, J. A photocleavable fluorescent nucleotide for DNA sequencing and analysis. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, 100, 414–419.
37. Sinha, N.D.; Striepeke, S. In *Oligonucleotides and Analogues: A Practical Approach*, ed. F. Eckstein, IRL Press at the Oxford University Press, Oxford, New York, and Tokyo, 1991, pp. 185–210.

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