Russian Journal of Bioorganic Chemistry, Vol. 30, No. 3, 2004, pp. 224–233. Translated from Bioorganicheskaya Khimiya, Vol. 30, No. 3, 2004, pp. 254–263.

Monomers for Oligonucleotide Synthesis with Linkers Carrying Reactive Residues: I. The Synthesis of Deoxynucleoside Derivatives with Methoxyoxalamide Groups in Heterocyclic Bases

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Received December 20, 2002; in final form, June 16, 2003

Abstract—A number of monomers for the standard phosphoamidite oligodeoxynucleotide synthesis that carry reactive methoxyoxalamide groups attached to the thymidine, 2'-deoxycytidine, and 2'-deoxyadenosine heterocyclic bases were prepared.

Key words: modified nucleosides, linkers carrying reactive groups, oligonucleotide synthesis

INTRODUCTION

The synthesis of modified oligodeoxyribonucleotides carrying various fluorescent groups, residues of intercalating residues, cationic lipids, etc. is currently an almost routine procedure.[2] Many modified oligonucleotides are commercially available. Some companies, e.g., Glen Research, purchase a variety of reagents for the synthesis of modified oligonucleotides [1]. However, solution of the problems of molecular biology associated with the directed effect on biopolymers steadily requires new nucleotide derivatives. In view of this, it is expedient to develop a maximally general method for their modification. One of the approaches to the solution of this problem is the precursor strategy based on the intercalation of active precursor groups into an oligonucleotide. It consists in the introduction of linkers carrying chemically active groups that are subsequently converted into the desired derivatives [2]. This approach is rather universal and also enables the use of methods of combinatorial chemistry for the synthesis of oligonucleotide derivatives.

In recent years, we have been engaged in the synthesis of artificial ribonucleases [3, 4]. The directed highly specific cleavage of ribonucleic acids by imidazole residues is possible when the synthetic ribonuclease contains an addressing part, an oligodeoxyribonucleotide complementary to a particular fragment of the ribonucleic acid. A striking example of successful use of linkers carrying reactive groups for the synthesis of highly specific artificial ribonucleases is the cleavage of phenylalanine tRNA by an oligodeoxyribonucleotide containing four catalytically active imidazole residues at its 5'-terminus [5]. These residues were introduced after the completion of the solid-phase synthesis of the addressing oligodeoxyribonucleotide carrying the reactive methoxyoxalylamide groups at its 5'-end.

One might expect that the introduction of catalytically active groups into the middle of the addressing oligonucleotide chain would enhance the cleavage efficiency of target due to a decreased binding of the reagent to hydrolysis products and would also enable varying the selectivity of artificial RNase [6]. In this case, the use of linkers containing reactive groups is promising since they enable the introduction of a set of catalytically active groups into the artificial RNase construct, with the synthesis of oligonucleotide addressing moiety of the construct remaining easy. The phosphoamidite reagents on the basis of nucleosides with properly modified heterocyclic bases are convenient to use for the automated oligonucleotide synthesis when inserting the linkers with terminal chemically active groups into various positions of oligodeoxyribonucleotide. The possibility of modifying various positions of purine and pyrimidine bases would allow a thorough study of the interaction of modified oligonucleotides with complementary nucleic acids, since the insertion sites of reactive groups would affect their position in both the small and large grooves of the complementary duplex [7].

This study is devoted to the synthesis of monomers for the phosphoamidite oligonucleotide synthesis that contain reactive methoxyoxalylamide groups attached to the heterocyclic bases of thymidine, 2'-deoxycytidine, and 2'-deoxyadenosine. A particular emphasis

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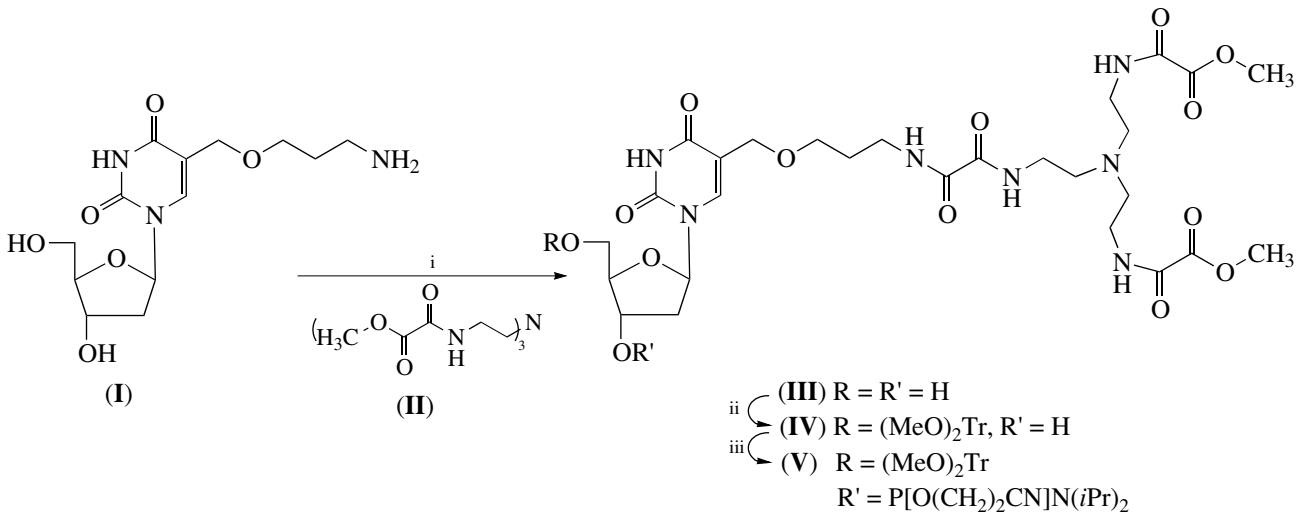
[2] Abbreviations: TBD, 1,5,7-triazabicyclo[4,4,0]dec-5-ene; TEA, triethylamine; Tetr, tetrazole; and Tri, 1,2,4-triazole.



Reagents: (i) (**II**)/TEA; (ii) $(MeO)_2TrCl$; (iii) $NC(CH_2)_2OP[N(iPr)_2]_2$/Tetr-$HN(iPr)_2$.

Scheme 1.

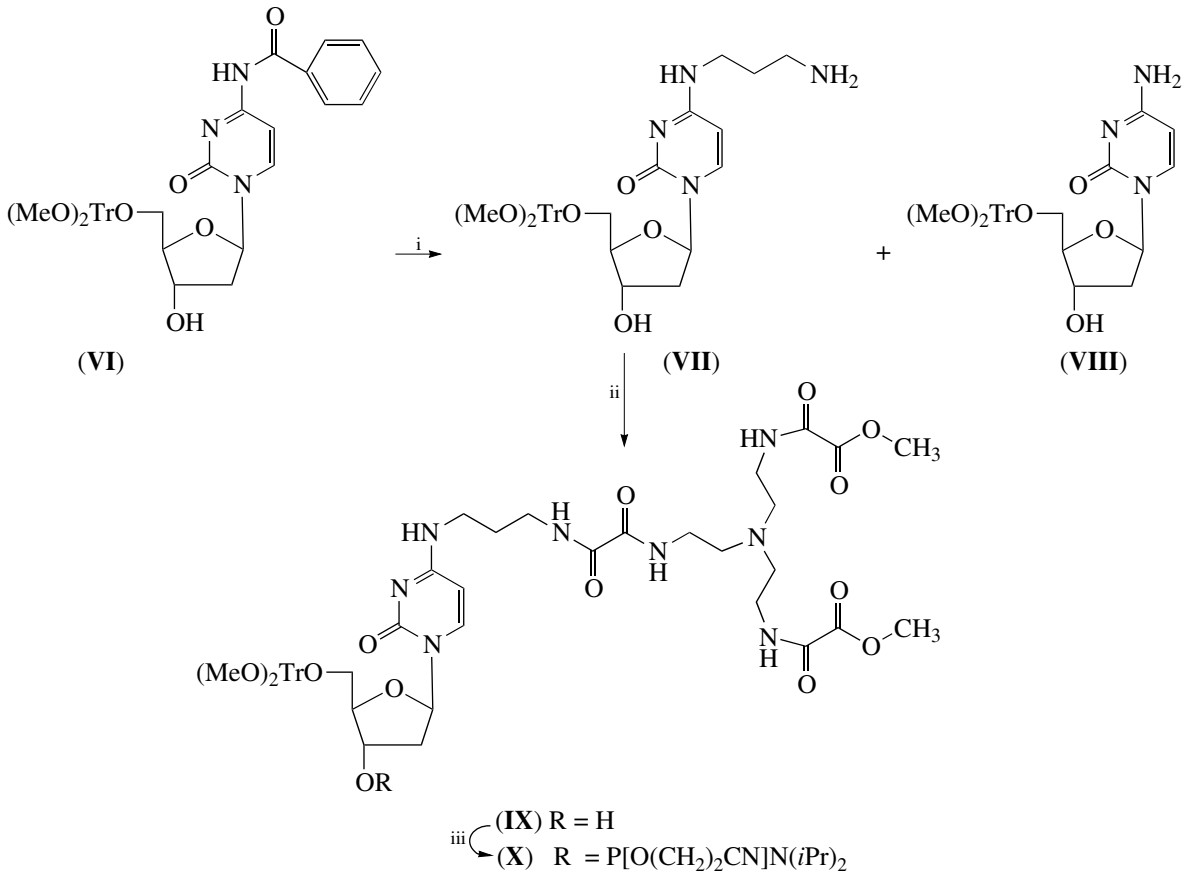
was placed on the use of the most readily available starting compounds and the choice of synthetic schemes providing the greatest yields of target compounds.

RESULTS AND DISCUSSION

2'-Deoxyuridine or its derivatives are usually used as starting compounds for the synthesis of thymidine analogues modified at the heterocyclic base [8, 9]. However, we used thymidine as a starting compound because of its commercial availability. The synthesis of thymidine derivatives containing 5-(2-aminoethyl)oxymethyl linker was reported in [10]. In a similar way, we obtained the thymidine derivative (**I**) containing a 5-(3-aminopropyl)oxymethyl residue (Scheme 1). The derivative with two methoxyoxalylamide groups was obtained in [11] by the successive treatment of the amino group of starting compound with dimethyl oxalate, tris(2-aminoethyl)amine, and again with dimethyl oxalate. We reduced the number of stages in the synthesis of this derivative by synthesizing tris(methoxyoxalylamidoethyl)amine (**II**). Reaction of the thymidine derivative (**I**) with (**II**) led to the formation of nucleoside (**III**) in 57% yield. After protecting the 5'-hydroxy group of deoxyribose and the subsequent phosphitylation of the nucleoside, phosphoamidite (**V**) suitable for the standard oligonucleotide synthesis was obtained (Scheme 1).

The introduction of an alkyl substituent into 4-amino group of 2'-deoxycytidine does not lead to its disability to form hydrogen bonds during complementary interactions [9]. The 2'-deoxycytidine analogues containing a monoalkylated exocyclic amino group can be synthesized from 2'-deoxyuridine by the reaction of its 4-triazolyl- or 4-thioderivatives with the corresponding amines [12, 13]. The search for the methods of synthesis of 2'-deoxycytidine derivatives using easily available starting compounds forced us to draw our attention to a simple method of one-step synthesis of N^4-(6-aminohexyl)-2'-deoxycytidine protected at its 5'-hydroxy group from N^4-benzoyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxycytidine (**VI**) by the transamination reaction [14]. This method allowed us to similarly synthesize N^4-(3-aminopropyl)-2'-deoxycytidine protected at 5'-hydroxy group (**VII**) (Scheme 2). The yield of the transamination reaction was relatively low (40%). However, the availability of starting compound (**VI**), which is a precursor of the phosphoamidite reagent for the standard oligonucleotide synthesis, and the possibility of regeneration of the starting nucleoside from the major by-product 5'-(4,4'-dimethoxytrityl)-2'-deoxycytidine (**VIII**) (Scheme 2) made this method of obtaining (**VII**) rather attractive. At the next stage of the synthesis, we introduced into the modified nucleoside (**VII**) a linker with reactive methoxyoxalylamide groups by analogy with the reaction of modification of the aminopropyl thymidine derivative (**I**) (Scheme 1i). The last stage of the synthesis consisted in the phosphitylation of 3'-hydroxy group of nucleoside (**IX**) to yield phosphoamidite (**X**) suitable for oligonucleotide synthesis.

The oligodeoxyribonucleotides containing 5-methyl-2'-deoxycytidine substituting for 2'-deoxycytidine are widely used in molecular biology for studying the complementary interactions of nucleic acids in duplexes and triplexes [15]. To examine the completeness and the specificity of cleavage of complementary nucleic acids by modified oligonucleotides containing 2'-deoxycytidine derivatives with various positions of



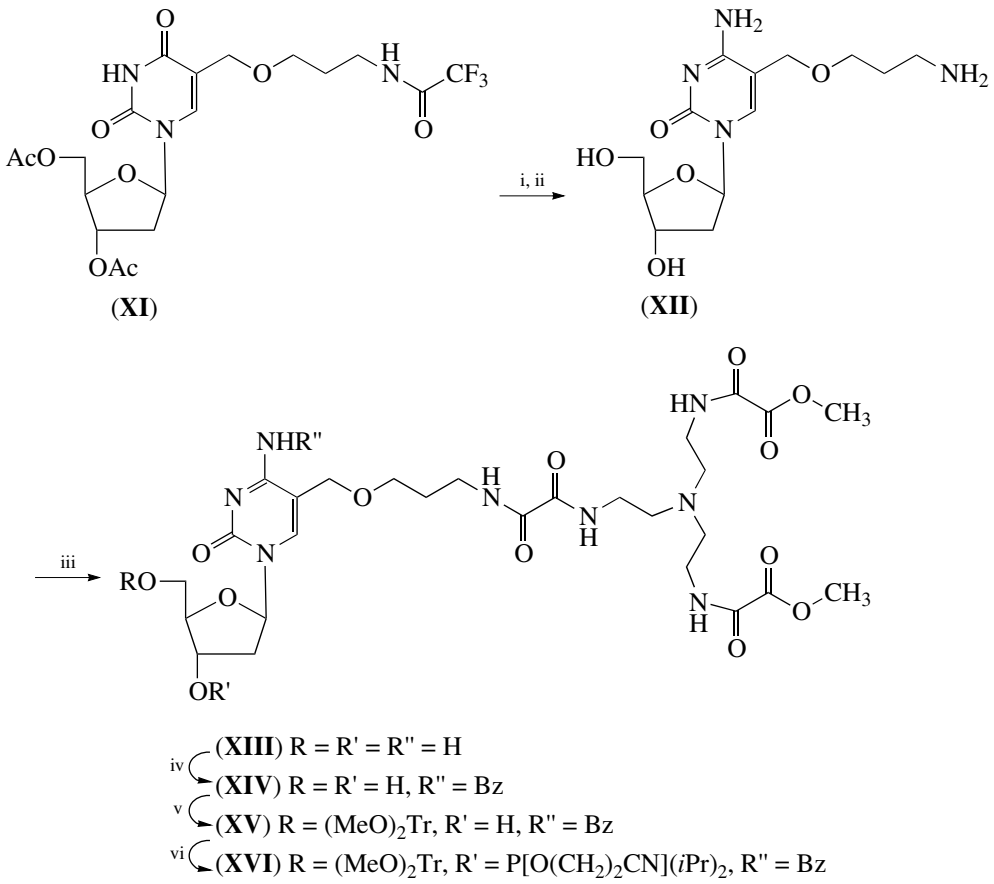
Scheme 2.

reactive groups, we synthesized (**XVI**) in which active functional groups were attached at the position 5 of the heterocyclic base of 2'-cytidine (Scheme 3).

We began the synthesis of (**XVI**) from the conversion of 5',3'-diacetyl-5-(3-trifluoroacetamidopropyl)oxymethyl-2'-deoxyuridine (**XI**), an intermediate in the synthesis of (**I**), into a 2'-deoxycytidine derivative by the reaction with 1,2,4-triazole and phosphorus oxychloride as described in [16]. After treatment with ammonia, 5-(3-aminopropyl)oxymethyl-2'-deoxycytidine (**XII**) was obtained. The reaction of this nucleoside with (**II**) led to (**XIV**); in this case, the exocyclic amino group did not react. At the next stage of the synthesis, 4-amino group of the 2'-deoxycytidine moiety was blocked by benzoic anhydride as described in [17] to form (**XVI**). 5'-Hydroxy group of the modified nucleoside was protected, and the reaction product (**XV**) was phosphitylated to yield phosphoamidite (**XVI**), suitable for the oligonucleotide synthesis.

Phosphoamidite (**XX**) was synthesized (Scheme 4) to determine the effect of 5'-methyl group in the 2'-deoxycytidine analogue on the efficiency of complementary interactions of nucleic acids. It differs from monomer (**X**) only by the presence of methyl group in position 5 of its heterocycle. We synthesized (**XX**) by the successive treatment of 5'-*O*-(4,4'-dimethoxytrityl)thymidine (**XVII**) with trimethylchlorosilane and 1,2,4-triazole and phosphorus oxychloride. The intermediate triazolide was treated, without isolation, with 1,3-diaminopropane as described in [16]. After treatment with ammonia, N^4-(3-aminopropyl)-5'-*O*-(4,4'-dimethoxytrityl)-5-methyl-2'-deoxycytidine (**XVIII**) was obtained (Scheme 4). A linker with reactive methoxyoxalylamide groups was then introduced by the reaction with (**II**), and the modified nucleoside was phosphitylated to yield the target reagent (**XX**).

We have an interest in studying the cleavage of nucleic acids at pyrimidine nucleotides and, therefore, synthesized a purine nucleoside containing methoxyoxalylamide groups. 2'-Deoxyadenosine served as a starting compound for the modification. The key compound in the synthesis of 2'-deoxyadenosine derivatives modified at position 6 is 9-(2'-deoxy-β-*D*-ribofuranosyl)-6-chloropurine [18–20], which is obtained either by the chlorination of 2'-deoxyinosine [21] or by the deamination of 2'-deoxyadenosine by the action of



Reagents: (i) Tri/$POCl_3$; (ii) NH_3; (iii) (**II**)/TEA; (iv) $(C_6H_5CO)_2O$; (v) $(MeO)_2TrCl$; (vi) $NC(CH_2)_2OP[N(iPr)_2]_2$/Tetr-$HN(iPr)_2$.

Scheme 3.

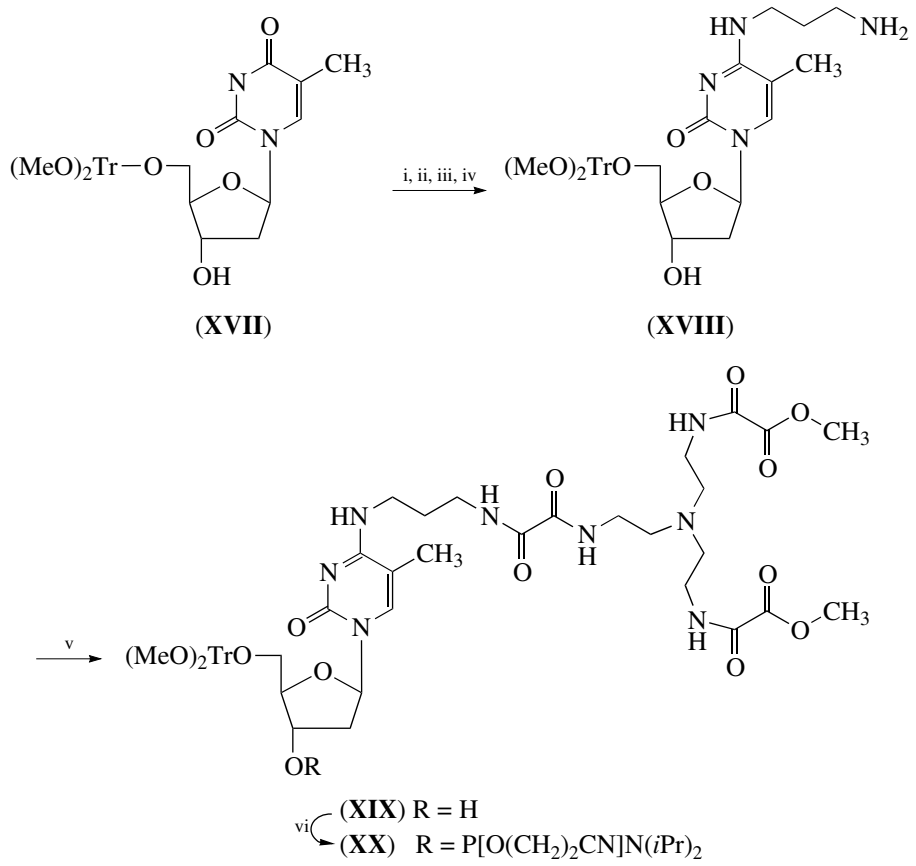
amyl nitrite in the presence of CCl_4 [22]. A drawback of these synthetic methods is a high sensitivity of 2'-deoxyadenosine derivatives to apurinization under acidic conditions, which reduces the reaction yield.

It was proposed to synthesize 6-modified 2'-deoxyadenosine derivatives through the formation of intermediate triazolides [23, 24]. We synthesized N^6-(3-aminopropyl)-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyadenosine (**XXIII**) by analogy with the method described in [24] (Scheme 5). After the introduction of a linker with methoxyoxalylamide groups by the reaction with (**II**) and phosphitylation of nucleoside (**XXIV**), the target phosphoamidite (**XXV**) was obtained.

Thus, we obtained synthons for oligodeoxyribonucleotide synthesis (**V**), (**X**), (**XVI**), (**XX**), and (**XXV**), which allow the introduction of reactive methoxyoxalamido groups into any position of oligodeoxyribonucleotide sequence. The synthesis of all the modified nucleosides requires no expensive starting compounds, is readily reproduced, and leads to target products in relatively high yields.

EXPERIMENTAL

The following reagents were used: thymidine and 2'-deoxyadenosine (NPO Vostok; Omutninsk, Russia); N^4-benzoyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxycytidine, 5'-*O*-(4,4'-dimethoxytrityl)thymidine (Group of oligonucleotide synthesis, Novosibirsk Institute of Bioorganic Chemistry, Russia); 1,2,4-triazole and 4,4'-dimethoxytrityl chloride (OKhP, Novosibirsk Institute of Organic Chemistry, Russia); trimethylchlorosilane (Fluka, Switzerland); phosphoryl chloride (Merck, Germany); and tris(2-aminoethyl)amine, *N*,*N*,*N'*,*N'*-tetraisopropyl-(2-cyano)ethyl phosphodiamidite, and dimethyl oxalate (Aldrich, USA). 5-Bromomethyl-5',3'-diacetyl-2'-deoxyuridine was synthesized as described in [10], 3-*N*-trifluoroacetylamidopropanol as described in [25], and 1,2-bis[(dimethylamino)methylene]hydrazine as described in [26]. Other reagents and solvents were Russian preparations of analytical and reagent grade. TLC was carried out on precoated Kieselgel 60 F_{254} plates (Merck, Germany) in 9 : 1 methylene chloride–methanol mixture unless otherwise indi-



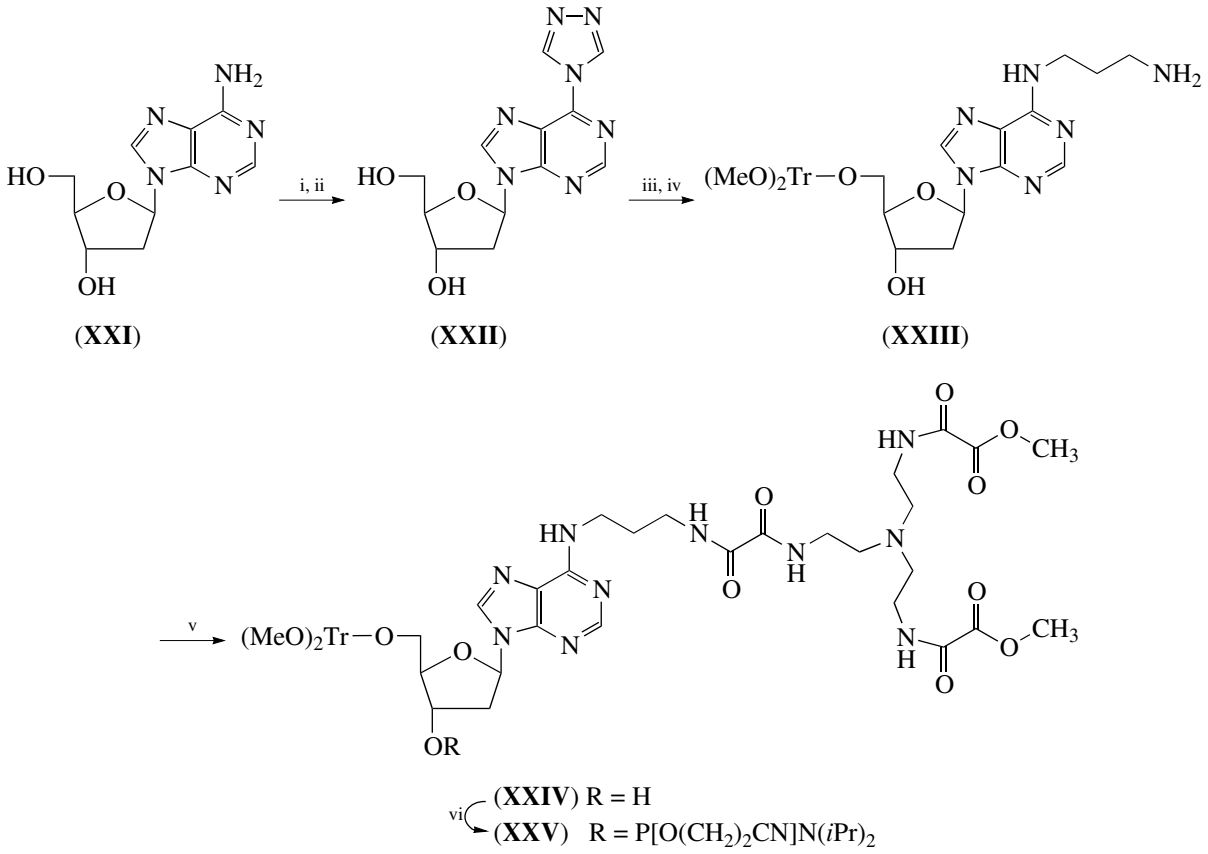
Reagents: (i) Me_3SiCl; (ii) Tri/$POCl_3$; (iii) $H_2N(CH_2)_3NH_2$; (iv) ammonia; (v) (**II**)/TEA; (vi) $NC(CH_2)_2OP[N(iPr)_2]_2$/Tetr-$HN(iPr)_2$.

Scheme 4.

cated. For column chromatography, Porasil Silica 125A, 55–100 μm (Waters, United States) was used. The compositions of eluents for column chromatography are given in volume percent. NMR spectra were recorded on an AM-400 spectrometer (Bruker, Germany); chemical shifts are given in ppm, and spin–spin coupling constants in Hz. Tetramethylsilane was used as an internal standard in ^1H NMR spectra, and 85% phosphoric acid as an external standard in ^{31}P NMR spectra.

5-(3-Aminopropyl)oxymethyl-2'-deoxyuridine (I). 5-Bromomethyl-5',3'-diacetyl-2'-deoxyuridine (0.5 g, 1.23 mmol) was dissolved in dry DMF (5 ml), and 3-*N*-trifluoroacetylamidopropanol (2 g, 11.7 mmol) was added. The reaction mixture was stirred for 16 h at room temperature and evaporated. The resulting oil was treated with concentrate ammonia (20 ml), and the mixture was kept for 3 h at 45°C. When the unblocking was over, the solution was evaporated. The residue was dissolved in water (40 ml) and applied onto a column packed with Dowex 50Wx2 (50 ml, H^+-form). The column was washed with water until the absence of UV absorption at 254 nm. The product was eluted with 1.2 M ammonia. The ammonia solution of (**I**) was evaporated, and the residue was coevaporated with acetonitrile. After drying in a vacuum, 5-(3-aminopropyl)oxymethyl-2'-deoxyuridine (**I**) was obtained; yield 0.28 g (0.89 mmol, 72%); R_f 0.45 (99 : 1 methanol–acetic acid); ^1H NMR (D_2O): 7.93 (1 H, s, H6), 6.37 (1 H, quasi-triplet, J 6.5, H1'), 4.53 (1 H, m, H3'), 4.34 (2 H, s, CH_2O), 4.11 (1 H, m, H4'), 3.96 (1 H, dd, ^2J 12.3, J 3.1, $H5'_a$), 3.86 (1 H, dd, J 4.5, $H5'_b$), 3.74 (2 H, t, J 5.9, OCH_2), 3.15 (2 H, t, J 6.8, CH_2NH_2), 2.52–2.37 (2 H, m, H2'), 2.00 (2 H, m, $CH_2CH_2CH_2$).

Tris(2-methoxyoxalylamidoethyl)amine (II). Tris(2-aminoethyl)amine (1.8 ml, 12 mmol) was dissolved in dry methanol (6 ml), and dimethyl oxalate (4.45 g, 40 mmol) in 20 ml of dry methanol was added to the stirred solution dropwise for 2 h. Stirring was continued for additional 2 h, and then the reaction mixture was refluxed for 10 min and evaporated. The residue was dissolved in methylene chloride (10 ml) and passed through a column of silica gel. After the elution



Reagents: (i) $Me_3SiCl/[(CH_3)_2NCH{=}N]_2$; (ii) MeOH; (iii) $(MeO)_2TrCl$; (iv) $H_2N(CH_2)_3NH_2$; (v) (**II**)/TEA; (vi) $NC(CH_2)_2OP[N(iPr)_2]_2$/Tetr-$HN(iPr)_2$.

Scheme 5.

with 5% methanol in methylene chloride, the target fractions were evaporated, and the residue was triturated with ether. The crystalline product was dried in a vacuum to yield 3.9 g of tris(2-methoxyoxalylamidoethyl)amine (9.8 mmol, 80%); R_f 0.55; mp. 95–97°C; ^{1}H NMR ($CDCl_3$): 7.46 (3 H, br t, *J* 5.6, NH), 3.88 (9 H, s, OCH_3), 3.39 (6 H, quasi-quartet, CH_2CH_2NH), 2.69 (6 H, t, *J* 5.8, $CH_2CH_2N<$).

A general procedure for the introduction of a linker carrying two methoxyoxalylamide groups. A solution of nucleoside modified with the aminopropyl linker (1 mmol) and of triethylamine (3 mmol) in methanol (4 ml) was added in portions for 4 h to a solution of (**II**) (2.5 mmol, 1.01 g) in dry methanol (10 ml). The reaction mixture was stirred for 3 h and evaporated. The target products were purified by chromatography on silica gel, eluting the product by a gradient of methanol (from 0%) in methylene chloride (the final concentration of methanol in the eluting solution depended on a particular compound; see below). The fractions with target product were evaporated, and it was precipitated from methylene chloride by a tenfold volume of hexane, if the next stage of the synthesis was the phosphitylation. In another case, the chromatographic fractions were evaporated, and the residual foam product was dried.

5-{3-[*N*,*N*-Di(2-methoxyoxalylamidoethyl)aminoethyl]amidooxalylamidopropyl}oxymethyl-2'-deoxyuridine (III) was obtained from (**I**) by the above-described procedure. The final concentration of methanol in the eluting solution was 25%. (**III**): yield 57%; R_f 0.37 (4 : 1 methylene chloride–methanol); ^{1}H NMR (C_5D_5N): 13.27 (1 H, br s, H3), 9.29 *J* 5.8, $CH_2NHC(O)$), 9.25 *J* 4.8, $CH_2NHC(O)$), 9.14 *J* 5.5, $CH_2NHC(O)$), 8.48 (1 H, s, H6), 6.98 (1 H, quasi-triplet, *J* 6.5, H1'), 5.01 (1 H, m, H3'), 4.45 (1 H, m, H4'), 4.41 (1 H, d, *J* 11.9, CH_aH_bO), 4.31 (1 H, d, *J* 11.9, CH_aH_bO), 4.22 (1 H, dd, ^2J 12.0, *J* 3.0, $H5'_a$), 4.14 (1 H, dd, *J* 3.0, $H5'_b$), 3.74 (6 H, c, OCH_3), 3.62–3.48 (10 H, m, $CH_2CH_2CH_2NH$, OCH_2CH_2, and $CH_2CH_2N<$), 2.78 (6 H, m, $CH_2CH_2N<$), 2.69 (2 H, m, H2'), 1.91 (2 H, m, $CH_2CH_2CH_2$).

A 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 end of reaction (2–3 h), the reaction mixture was evaporated, and the residue was dissolved in methylene chloride and applied onto a silica gel column. The 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-{3-[*N*,*N*-Di-(2-methoxyoxalylamidoethyl)aminoethyl]amidooxalylamidopropyl}oxymethyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyuridine (IV) was obtained from (**III**) as described above. The product was precipitated from a solution in a 4 : 1 methylene chloride–pyridine mixture by a tenfold volume of hexane, and the precipitate was washed with hexane to give (**IV**); yield 65%; R_f 0.21; ^{1}H NMR (C_5D_5N): 13.30 (1 H, s, H3), 9.24 (2 H, t, *J* 4.8, $CH_2NHC(O)$), 9.19 (1 H, t, *J* 5.8, $CH_2NHC(O)$), 9.12 (1 H, t, *J* 5.6, $CH_2NHC(O)$), 8.04 (1 H, s, H6), 7.76–7.08 [9 H, m, $(MeO)_2Tr$], 6.98 (4 H, m, $(MeO)_2Tr$), 6.92 (1 H, quasi-triplet, *J* 6.5, H1'), 4.92 (1 H, m, H3'), 4.50 (1 H, m, H4'), 4.21 (1 H, d, *J* 11.2, CH_aH_bO), 4.36 (1 H, d, *J* 11.2, CH_aH_bO), 3.74 (6 H, s, OCH_3), 3.70 (6 H, s, OCH_3), 3.66 (2 H, m, H5'), 3.54 (10 H, m, $CH_2CH_2CH_2NH$, OCH_2CH_2, and $CH_2CH_2N<$), 2.78 (6 H, m, $CH_2CH_2N<$), 2.67 (2 H, m, H2'), 1.85 (2 H, m, $CH_2CH_2CH_2$).

N^4-(3-Aminopropyl)-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxycytidine (VII). TBD (1.3 g, 9.4 mmol) and 1,3-diaminopropane (3.5 ml, 42 mmol) were added to a solution of (**VI**) (2 g, 3.16 mmol) in DMF (10 ml). The reaction mixture was heated at 60°C for 16 h and evaporated. The residue was dissolved in methylene chloride (100 ml) and washed with 1 M NaOH (50 ml) and water (5 × 50 ml). The organic layer was dried with anhydrous Na_2SO_4 and evaporated. The residue was dissolved in methylene chloride and applied onto a silica gel column. The product was eluted in a gradient of methanol concentration in methylene chloride (0 ⟶ 20%) supplemented with 10% triethylamine. The fractions with target product were evaporated, the product (**VII**) was precipitated from the solution in methylene chloride with a tenfold volume of hexane; yield 0.74 g (1.26 mmol, 40%); R_f 0.13 (7 : 2 : 1 methylene chloride–methanol–triethylamine); ^{1}H NMR ($CDCl_3$): 7.56 (1 H, d, *J* 7.8, H6), 7.42–7.03 [9 H, m, $(MeO)_2Tr$], 6.73 [4 H, d, *J* 8.6, $(MeO)_2Tr$], 6.15 (1 H, quasi-triplet, *J* 6.0, H1'), 5.62 (1 H, d, H5), 4.38 (1 H, m, H3'), 4.00 (1 H, m, H4'), 3.66 (6 H, s, OCH_3), 3.38 (2 H, quasi-triplet, *J* 5.5, $NHCH_2CH_2$), 3.29 (2 H, m, H5'), 2.95 (2 H, t, *J* 5.0, $CH_2CH_2NH_2$), 2.38 (1 H, m, $H2'_a$), 2.05–1.78 (3 H, m, $H2'_b$, $CH_2CH_2CH_2$).

N^4-{3-[*N*,*N*-Di-(2-methoxyoxalylamidoethyl)aminoethyl]amidooxalylamidopropyl}-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxycytidine (IX) was obtained from compound (**VII**) according to the above-described procedure. The final concentration of methanol in the eluting solution was 12%. Yield of (**IX**) 55%; R_f 0.18; ^{1}H NMR ($CDCl_3$): 8.23 (1 H, t, *J* 6.0, $CH_2NHC(O)$), 8.10 (3 H, m, $CH_2NHC(O)$), 7.75 (1 H, d, *J* 6.8, H6), 7.45–7.18 [9 H, m, $(MeO)_2Tr$], 6.81 [4 H, d, *J* 8.6, $(MeO)_2Tr$], 6.32 (1 H, quasi-triplet, *J* 5.8, H1'), 5.71 (1 H, br t, $NHCH_2$), 5.39 (1 H, d, H5), 4.49 (1 H, m, H3'), 4.05 (1 H, m, H4'), 3.78 (6 H, s, OCH_3), 3.77 (6 H, s, OCH_3), 3.60–3.24 (12 H, m, H5, $NHCH_2CH_2CH_2$, $CH_2CH_2CH_2NH$ and $CH_2CH_2N<$), 2.72–2.52 (6 H, m, $CH_2CH_2N<$), 2.35 (1 H, m, $H2'_a$), 2.21 (1 H, m, $H2'_b$), 1.79 (2 H, m, $CH_2CH_2CH_2$).

5-(3-Aminopropyl)oxymethyl-2'-deoxycytidine (XII). 3-*N*-Trifluoroacetylamidopropanol (4 g, 23.4 mmol) was added to a solution of 5-bromomethyl-3',5'-diacetyl-2'-deoxyuridine (1 g, 2.46 mmol) in DMF (10 ml). The reaction mixture was stirred for 16 h at room temperature and evaporated. The oily residue was dissolved in methylene chloride (50 ml) and washed with water (3 × 25 ml). The organic layer was dried with Na_2SO_4, evaporated, dried by coevaporation with anhydrous acetonitrile, and dissolved in 6 ml of anhydrous acetonitrile. Separately, phosphoryl chloride (0.95 ml, 10 mmol) and triethylamine (7 ml, 50 mmol) were successively added to a suspension of 1,2,4-triazole (3.45 g, 50 mmol) in dry acetonitrile (60 ml) under cooling with ice and stirring. Stirring was continued for 30 min, and then the nucleoside solution in acetonitrile was added to the reaction mixture. Cooling was terminated, and the reaction mixture was stirred for 1.5 h at room temperature and evaporated. The residue was dissolved in methylene chloride (100 ml), washed with 5% $NaHCO_3$ (50 ml) and water (50 ml). The organic layer was dried with Na_2SO_4 and evaporated, the residue was dissolved in 20 ml of dioxane, and concentrate ammonia (20 ml) was added. The reaction mixture was kept for 16 h at room temperature and evaporated. The residue was dried by coevaporation with acetonitrile and triturated with ether to give (**XII**); yield 0.47 g (1.5 mmol, 60%); R_f 0.26 (99 : 1 methanol–acetic acid); ^{1}H NMR (D_2O): 8.04 (1 H, s, H6), 6.33 (1 H, t, *J* 6.5, H1'), 4.51 (1 H, m, H3'), 4.48 (2 H, s, CH_2O), 4.17 (1 H, m, H4'), 3.95 (1 H, dd, ^2J 12.5, *J* 3.0, H5'), 3.84 (1 H, dd, *J* 4.1, H5'), 3.70 (2 H, t, *J* 5.8, OCH_2CH_2), 3.18 (2 H, t, *J* 7.2, $CH_2CH_2NH_2$), 2.55 (1 H, m, $H2'_a$), 2.40 (1 H, m, $H2'_b$), and 2.09 (2 H, m, $CH_2CH_2CH_2$).

5-{3-[*N*,*N*-Bis(2-methoxyoxalylamidoethyl)aminoethyl]amidooxalylamidopropyl}oxymethyl-2'-deoxycytidine (XIII) was obtained from (**XII**) by the above-described procedure, using 35% methanol as the final eluting solvent; yield 58%; R_f 0.18 (4 : 1 methylene

chloride–methanol); ^{1}H NMR ($(CD_3)_2SO$): 8.64 (3 H, br t, $CH_2NHC(O)$), 8.45 (1 H, br t, $CH_2NHC(O)$), 7.69 (1 H, s, H6), 6.14 (1 H, quasi-triplet, *J* 6.4, H1'), 4.22 (1 H, m, H3'), 4.16 (2 H, s, CH_2O), 4.11 (1 H, m, H4'), 3.79 (6 H, s, OCH_3), 3.62 (4 H, m, H5', OCH_2CH_2), 3.41–3.11 (8 H, m, $CH_2CH_2CH_2NH$ and $CH_2CH_2N<$), 2.60 (6 H, m, $CH_2CH_2N<$), 2.22–1.88 (2 H, m, H2'), and 1.72 (2 H, m, $CH_2CH_2CH_2$).

***N*^{4}-Benzoyl-5-{3-[*N*,*N*-bis(2-methoxyoxalylamidoethyl)aminoethyl]amidooxalylamidopropyl}oxymethyl-2'-deoxycytidine (XIV).** Benzoic anhydride (0.181 g, 0.8 mmol) was added to a solution of 0.6 g (0.87 mmol) of (**XIII**) in DMF (4 ml). The reaction mixture was kept for 16 h at room temperature and treated with additional benzoic anhydride (18 mg). After 1 h at 45°C, the mixture was evaporated. The residue was triturated with ether (3 × 5 ml) and dried in a vacuum to give (**XIV**); yield 0.4 g (0.5 mmol, 58%); R_f 0.45 (4 : 1 methylene chloride–methanol); ^{1}H NMR (C_5D_5N): 9.35–9.07 (4 H, m, $CH_2NHC(O)$), 8.53 (2 H, m, Bz), 8.47 (1 H, s H6), 7.50 (3 H, m, Bz), 6.85 (1 H, quasi-triplet, *J* 6.1, H1'), 5.02 (1 H, m, H3'), 4.60 (1 H, d, *J* 12.0, CH_aH_bO), 4.48 (1 H, d, *J* 12.0, CH_aH_bO), 4.46 (1 H, m, H4'), 4.26 (1 H, dd, ^2J 12.0, *J* 3.0, H5'), 4.09 (1 H, dd, *J* 2.9, H5'), 3.78 (8 H, m, OCH_3, OCH_2CH_2), 3.68–3.42 (8 H, m, $CH_2CH_2CH_2NH$ and $CH_2CH_2N<$), 2.88–2.55 (8 H, m, $CH_2CH_2N<$ and H2'), and 1.96 (2 H, m, $CH_2CH_2CH_2$).

***N*^{4}-Benzoyl-5-{3-[*N*,*N*-bis(2-methoxyoxalylamidoethyl)aminoethyl]amidooxalylamidopropyl}oxymethyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxycytidine (XV)** was obtained from (**XIV**) by the above-described procedure, using 10% methanol as the final eluting solvent; yield 0.27 g (0.27 mmol, 62%); R_f 0.28; ^{1}H NMR ($CDCl_3$): 8.20 (2 H, d, *J* 6.9, Bz), 7.91 (2 H, m, H6, CH_2NHCO), 7.68 (2 H, quasi-triplet, *J* 6.5, $CH_2NHC(O)$), 7.58–7.12 (13 H, m, $MeO)_2Tr$, $CH_2NHC(O)$ and Bz), 6.80 (4 H, d, $(MeO)_2Tr$), 6.35 (1H, quasi-triplet, *J* 6.4, H1'), 4.55 (1 H, m, H3'), 4.13 (2 H, s, CH_2O), 4.05 (1 H, m, H4'), 3.81 (6 H, s, OCH_3), 3.76 (8 H, m, OCH_3, OCH_2CH_2), 3.60–3.13 (10 H, m, H5, $CH_2CH_2CH_2NH$ and $CH_2CH_2N<$), 2.79–2.36 (8 H, m, $CH_2CH_2N<$ and H2'), 1.68 (2 H, m, $CH_2CH_2CH_2$).

***N*^{4}-(3-Aminopropyl)-5'-*O*-(4,4'-dimethoxytrityl)-5-methyl-2'-deoxycytidine (XVIII).** Trimethylchlorosilane (0.47 ml, 3.7 mmol) was added to a stirred solution of 5'-*O*-(4,4'-dimethoxytrityl)thymidine (**XVII**) (1 g, 1.84 mmol) in dry pyridine (5 ml). After 30 min, the reaction mixture was evaporated, the oil was dissolved in methylene chloride (100 ml), and the solution was washed with 5% $NaHCO_3$ (50 ml) and water (50 ml). The organic layer was dried with Na_2SO_4, evaporated, coevaporated with toluene to remove pyridine (3 × 10 ml), dissolved in dry acetonitrile (5 ml), and mixed with triethylamine (0.5 ml). Separately, phosphoryl chloride (0.69 ml, 7.36 mmol) and triethylamine (5.15 ml, 36.8 mmol) were successively added to an ice-cooled suspension of 1,2,4-triazole (2.54 g, 36.8 mmol) in dry acetonitrile (50 ml). The suspension was stirred for 30 min, treated with the nucleoside solution in acetonitrile, and then stirred for 1.5 h at room temperature, and evaporated. The residue was dissolved in methylene chloride (100 ml); the solution was washed with 5% $NaHCO_3$ (50 ml) and water (50 ml), dried with Na_2SO_4, and evaporated. The residue was dissolved in acetonitrile (20 ml), and 1,3-diaminopropane (1.54 ml, 18.4 mmol) was added. After 1 h, 10 ml of concentrate ammonia was added to the reaction mixture, and it was kept overnight at room temperature and evaporated. The residue was dissolved in methylene chloride (100 ml), washed with 0.1 M NaOH (30 ml) and water (5 × 50 ml), dried with Na_2SO_4, and evaporated. The residue was dissolved in methylene chloride (5 ml), and the product (**XVIII**) was precipitated with hexane; yield: 0.9 g (1.5 mmol, 81%); R_f 0.14 (7 : 2 : 1 methylene chloride–methanol–triethylamine); ^{1}H NMR ($CDCl_3$): 7.58 (1 H, s, H6), 7.42–7.11 [9 H, m, $(MeO)_2Tr$], 6.80 [4 H, d, *J* 8.0, $(MeO)_2Tr$], 6.43 (1 H, quasi-triplet, *J* 6.1, H1'), 4.50 (1 H, m, H3'), 4.04 (1 H, m, H4'), 3.77 (6 H, s, OCH_3), 3.72 (1 H, m, $NHCH_2$), 3.62 (2 H, m, $NHCH_2CH_2$), 3.44 (1 H, dd, ^2J 10, *J* 3.0, H5'), 3.30 (1 H, dd, *J* 2.9, H5), 2.87 (2 H, t, *J* 5.9, $CH_2CH_2NH_2$), 2.54 (1 H, m, $H2'_a$), 2.20 (1 H, m, $H2'_b$), 1.70 (2 H, m, $CH_2CH_2CH_2$), and 1.46 (3 H, s, CH_3).

***N*^{4}-{3-[*N*,*N*-Bis(2-methoxyoxalylamidoethyl)aminoethyl]amidooxalylamidopropyl}-5'-*O*-(4,4'-dimethoxytrityl)-5-methyl-2'-deoxycytidine (XIX)** was obtained from (**XVIII**) by the above-described procedure, using 12% final concentration of methanol in the eluting solution; yield 41%; R_f 0.18; ^{1}H NMR ($CDCl_3$): 8.05 (1 H, t, *J* 6.1, $CH_2NHC(O)$), 7.70(3 H, m, $CH_2NHC(O)$), 7.61 (1 H, s, H6), 7.52–7.15 [9 H, m, $(MeO)_2Tr$), 6.80 [4 H, d, *J* 8.2, $(MeO)_2Tr$), 6.43 (1 H, quasi-triplet, *J* 6.4, H1'), 5.94 (1 H, br t, *J* 6.1, $NHCH_2CH_2CH_2$), 4.50 (1 H, m, H3'), 4.04 (1 H, m, H4'), 3.82 (6 H, s, OCH_3), 3.77 (6 H, s, OCH_3), 3.58–3.27 (12 H, m, H5, $NHCH_2CH_2CH_2$, $CH_2CH_2CH_2NH$, and $CH_2CH_2N<$), 2.75–2.60 (6 H, m, $CH_2CH_2N<$), 2.52 (1 H, m, $H2'_a$), 2.22 (1 H, m, $H2'_b$), 1.84 (2 H, m, $CH_2CH_2CH_2$), and 1.54 (3 H, s, CH_3).

***N*^{6}-(1,2,4-Triazole-4-yl)-2'-deoxyadenosine (XXII).** Trimethylchlorosilane (0.5 ml, 4 mmol) and 1,2-bis[(dimethylamino)methylene]hydrazine (1.11 g, 7.8 mmol) were added to a solution of 2'-deoxyadenosine (**XXI**) (0.5 g, 2 mmol) in dry pyridine (5 ml). The reaction mixture was refluxed for 5 h and kept for 16 h at room temperature. An additional quantity of trimethylchlorosilane (0.5 ml) was added to the reaction mixture, and after 20 min the solution was diluted with methylene chloride (20 ml) and extracted with 1 N HCl

(3 × 20 ml). The organic layer was dried with Na_2SO_4, evaporated, and then three times coevaporated with toluene to remove pyridine. The residue was dissolved in methanol (10 ml) and refluxed for 6 h. After recrystallization from methanol, (**XXII**) was obtained; yield 0.38 g (1.25 mmol, 62%); R_f 0.14; ^{1}H NMR (D_2O): 9.60 (2 H, s, H3'', 5'', 8.88 (1 H, s, H8), 8.76 (1 H, s, H2), 6.64 (1 H, quasi-triplet, *J* 6.6, H1'), 4.18 (1 H, m, H4'), 3.81 (2 H, m, H5'), 2.93 (1 H, m, $H2'_a$), 2.69 (1 H, m, $H2'_b$).

***N*^{6}-(3-Aminopropyl)-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyadenosine (XXIII).** Dimethoxytrityl protecting group was introduced into (**XXII**) by the above-described procedure. The chromatographic fractions with the target product were evaporated, the residue was dissolved in pyridine (10 ml), and 1,3-diaminopropane (0.85 ml, 10 mmol) was added. The reaction mixture was heated for 8 h at 70°C and evaporated, and the residue was dissolved in methylene chloride (50 ml) and washed with water (3 × 25 ml). The organic layer was dried with Na_2SO_4 and evaporated. The product (**XXIII**) was precipitated from its solution in methylene chloride with a tenfold volume of hexane; yield 0.56 g (0.91 mmol, 73%); R_f 0.11 (7 : 2 : 1, methylene chloride–methanol–tryethylamine); ^{1}H NMR ($CDCl_3$): 8.28 (1 H, s, H8), 7.85 (1 H, s, H2), 7.73–7.09 [9 H, m, $(MeO)_2Tr$), 6.78 [4 H, d, *J* 8.9, $(MeO)_2Tr$), 6.39 (1 H, quasi-triplet, *J* 6.5, H1'), 6.31 (1 H, br s, N*H*CH_2), 4.67 (1 H, m, H3'), 4.10 (1 H, m, H4'), 3.76 (8 H, m, OCH_3, NHC*H*$_2$$CH_2$), 3.39 (2 H, m, H5'), 2.85 (2 H, t, *J* 6.5, $CH_2CH_2NH_2$), 2.78 (1 H, m, $H2'_a$), 2.52 (1 H, m, $H2'_b$), 1.79 (2 H, m, $CH_2CH_2CH_2$).

***N*^{6}-{3-[*N*,*N*-Bis(2-methoxyoxalylamidoethyl)aminoethyl]amidooxalylamidopropyl}-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyadenosine (XXIV)** was obtained from (**XXIII**) by the above-described procedure, using 12% final concentration of methanol in the eluting solution; yield 50%; R_f 0.26; ^{1}H NMR ($CDCl_3$): 8.28 (1 H, s, H8), 7.96 (1 H, s, H2), 7.74 (1 H, t, *J* 6.0, CH_2N*H*C(O)), 7.69 (2 H, m, CH_2N*H*C(O)), 7.73–7.09 (10 H, m, $(MeO)_2Tr$, CH_2N*H*C(O)), 6.77 [4 H, d, *J* 8.9, $(MeO)_2Tr$), 6.40 (1 H, quasi-triplet, *J* 6.5, H1'), 6.32 (1 H, br t, N*H*CH_2), 4.63 (1 H, m, H3'), 4.10 (1 H, m, H4'), 3.82 (6 H, s, OCH_3), 3.77 (8 H, m, OCH_3, NHC*H*$_2$$CH_2CH_2$), 3.48–3.23 (10 H, m, H5', $CH_2CH_2CH_2NH$, $CH_2CH_2N<$), 2.85–2.45 (8 H, m, H2', $CH_2CH_2N<$), 1.95 (2 H, m, $CH_2CH_2CH_2$).

A general method of the synthesis of phosphoamidite reagents for oligonucleotide synthesis. *N*,*N*,*N*',*N*'-Tetraisopropyl-(2-cyano)ethyl phosphodiamidite (4.5 ml, 14.2 mmol) was added to a stirred solution of a protected nucleoside carrying a free 3'-hydroxy group (10 mmol) and diisopropylammonium tetrazolide (0.9 g, 4.47 mmol) in a freshly distilled methylene chloride (50 ml). The course of reaction was monitored by TLC. After 2 h, an additional quantity of phosphitylating reagent (2.2 ml, 6.9 mmol) was added. After 3 to 7 h, the reaction mixture was evaporated, the residue was treated with hexane (50 ml), and the mixture was kept overnight. Hexane was then decanted, and the residue was dissolved in methylene chloride (20 ml) and chromatographed on silica gel. Elution with a gradient of methanol in methylene chloride (0 to 10%) gave the target fractions; the product was precipitated from methylene chloride with hexane; ^{31}P NMR (CD_3CN): 149.37 and 149.10 for (**V**); 149.01 for (**X**); 149.21 for (**XVI**); 148.88 for (**XX**); and 149.06 and 148.92 for (**XXV**).

ACKNOWLEDGMENTS

The study was supported by the Russian Foundation for Basic Research (projects nos. 02-04-48664a and 01-03-32439a).

REFERENCES

1. *Glen Research Corporation: Products for DNA Research*, 1999, pp. 36–41.
2. Ferentz, A.E. and Verdine, G.L., *Nucleosides Nucleotides*, 1992, vol. 11, pp. 1749–1763.
3. Yurchenko, L., Silnikov, V., Godovikova, T., Shishkin, G., Toulme, J.-J., and Vlassov, V., *Nucleosides Nucleotides*, 1997, vol. 16, pp. 1721–1725.
4. Beloglazova, N.G., Sil'nikov, V.N., Zenkova, M.A., and Vlassov, V.V., *FEBS Lett.*, 2000, vol. 481, pp. 277–280.
5. Beloglazova, N.G., Polushin, N.N., Sil'nikov, V.N., Zenkova, M.A., and Vlassov, V.V., *Dokl. Ross. Akad. Nauk*, 1999, vol. 369, pp. 827–830.
6. Komiyama, M. and Sumaoka, J., *Curr. Opin. Chem. Biol.*, 1998, vol. 2, pp. 751–757.
7. Travick, B.N., Daniher, A.T., and Bashkin, J.K., *Chem. Rev.*, 1998, vol. 98, pp. 939–960.
8. Ruth, J.L., *Oligonucleotides and Analogues: A Practical Approach*, Eckstein, F., Ed., New York: Oxford Univ. Press, 1991, pp. 255–282.
9. Meyer, R.B., *Methods in Molecular Biology*, 1994, vol. 26, pp. 73–91.
10. Levina, A.S., Tabatadse, D.R., Khalimskaya, L.M., Prichodko, T.A., Shishkin, G.V., Alexandrova, L.A., and Zarytova, V.P., *Bioconjugate Chem.*, 1993, vol. 4, pp. 319–325.
11. Polushin, N.N., *Nucleic Acids Res.*, 2000, vol. 28, pp. 3125–3133.
12. Divakar, K.J. and Reese, C.B., *J. Chem. Soc., Perkin Trans. I*, 1982, pp. 1171–1176.
13. Roget, A., Bazin, H., and Teoule, R., *Nucleic Acids Res.*, 1989, vol. 17, pp. 7643–7649.
14. Hovinen, J., *Nucleosides Nucleotides*, 1998, vol. 17, pp. 1209–1213.
15. Herdewijn, P., *Antisens and Nucl. Acids Drug Dev.*, 2000, vol. 10, pp. 297–310.
16. Horn, T. and Urdea, M.S., *Nucleic Acids Res.*, 1989, vol. 17, pp. 6959–6967.

17. Bhat, V., Ugarcar, B.G., Sayeed, V.A., Grimm, K., Kosora, N., Domenico, P.A., and Stocker, E., *Nucleosides Nucleotides,* 1989, vol. 8, pp. 179–183.
18. Gmeiner, W., Luo, W., Pon, R.T., and Lown, J.W., *Bioorg. Med. Chem. Lett.*, 1991, vol. 1, pp. 487–490.
19. Czernecki, S., Viswanadham, G., and Valery, J.M., *Nucleosides Nucleotides*, 1998, vol. 17, pp. 2087–2091.
20. Barends, J., van der Linden, J.B., van Delft, F.L., and Koomen, G.-J., *Nucleosides Nucleotides*, 1999, vol. 18, pp. 2121–2126.
21. Robins, V.J. and Basom, G.L., *Can. J. Chem.*, 1973, vol. 51, pp. 3161–3169.
22. Acedo, M., Fabrega, C., Avino, A., Goodman, M., Fagan, P., Wemmer, D., and Eritja, R., *Nucleic Acids Res.*, 1994, vol. 22, pp. 2982–2989.
23. Miles, R.W., Samano, V., and Robins, M.J., *J. Am. Chem. Soc.*, 1995, vol. 117, pp. 5951–5957.
24. Godzina, P., Adrych-Rozek, K., and Marckiewich, W.T., *Nucleosides Nucleotides*, 1999, vol. 18, pp. 2397–2414.
25. Lokhov, S.G., Podyminogin, M.A., Sergeev, D.S., Silnikov, V.N., Kutyavin, I.V., Shishkin, G.V., and Zarytova, V.F., *Bioconjug. Chem.*, 1992, vol. 3, pp. 414–419.
26. Bartlett, R.K. and Hamphrey, I.R., *J. Chem. Soc.*, 1967, pp. 1664–1667.