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New Nonnucleoside Substrates for Terminal Deoxynucleotidyl Transferase: Synthesis and Dependence of Substrate Properties on Structure

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Abstract—*N*-(9-Fluorenylmethoxycarbonyl)-ω-aminoalkyl-, *N*-(9-fluorenylmethoxycarbonyl)-8-amino-3,6-dioxaoctyl, and *N*-[(9-fluorenylmethoxycarbonyl)-6-aminohexanoyl]-2-aminoethyl triphosphates were synthesized. All of them were shown to be the substrates of the calf thymus terminal deoxynucleotidyl transferase. Their substrate properties depend on the length and structure of the linker between the 9-fluorenylmethoxycarbonyl and triphosphate moieties.

Key words: nucleoside triphosphate analogues, substrate properties, terminal deoxynucleotidyl transferase

INTRODUCTION

Terminal deoxynucleotidyl transferase (EC 2.7.7.31) is a template-independent DNA polymerase playing an important role in the formation of human and animal immune response [1].[2] 2'-Deoxynucleoside 5'-triphosphates are the natural substrates for this enzyme. However, it has recently been shown that the presence of nucleoside fragment is not necessary for triphosphates to manifest substrate properties toward TDT [2]. In particular, some triphosphates containing bulky substituents instead of a nucleoside component are recognized by this enzyme, the efficiency of recognition depending on both the nature of the substituent replacing the nucleic base [3] and the linker nature [4]. We synthesized triphosphates (**Ia**)–(**Id**), (**II**), and (**III**), in which the bulky Fmoc residue mimicking the nucleoside moiety was attached to the triphosphate fragment via various linkers, in order to comprehensively study the effect of the linker length and structure on their substrate properties toward TDT.

RESULTS AND DISCUSSION

Compounds (**Ia**)–(**Ic**) were obtained by the reaction of the corresponding aminoalcohol with 9-fluorenylmethoxycarbonyl chloride followed by the Ludwig triphosphorylation [5] without the isolation of intermediate monophosphates (Scheme 1).

The preparation of triphosphate (**Id**) was started with the synthesis of 1-monomethoxytrityl-6-aminohexanol (Scheme 2). Hexane-1,6-diol was successively treated with monomethoxytrityl chloride and methansulfonyl chloride, azidated, and the azido group was reduced with 2-mercaptoethanol. After the introduction of Fmoc residue and deblocking the (MeO)Tr protection, the Ludwig triphosphorylation was carried out. Triphosphate (**III**) was prepared in a similar manner from triethylene glycol.

The amide bond in (**II**) is labile in the presence of phosphorus oxychloride, unlike the amide bond of the carbamate (urethane) group in (**I**) and (**III**). Therefore, we should use another scheme for its synthesis (Scheme 3). 6-Aminohexanoic acid was first treated with 9-fluorenylmethoxycarbonyl chloride and, then, activated with thionyl chloride and subjected to the interaction with the preliminarily silylated 2-aminoethyl phosphate. The resulting monophosphate was activated with CDI and treated with tributylammonium pyrophosphate (Scheme 3).

The synthesized Fmoc-containing triphosphates proved to be rather labile under the conditions of ion-exchange chromatography on DEAE cellulose, and, therefore, we developed the following algorithm for their isolation. The reaction mixture was diluted with water and passed through a Dowex 50 (NH_4^+) column. The resulting solution was concentrated, and the target product was isolated on a reversed-phase LiChroprep RP-18 column. Final yields of the triphosphates were 10–20% from the starting amino alcohol. All the Fmoc-containing triphosphates demonstrated characteristic UV spectra with λ_{max} 265 nm and ε 17000 M^{-1} cm^{-1}.

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[2] Abbreviations: CDI, 1,1'-carbonyldiimidazole; Fmoc, 9-fluorenylmethoxycarbonyl; TDT, terminal deoxynucleotidyl transferase.

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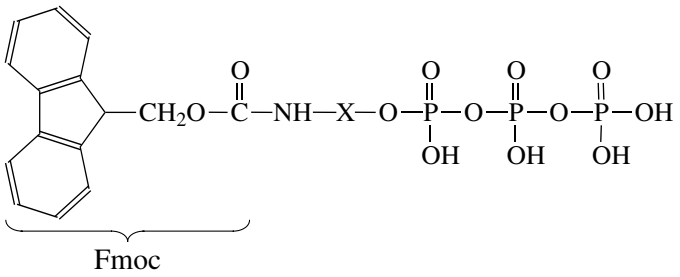


(**Ia**): X = $-(CH_2)_2-$ (**II**): X = $-(CH_2)_5C(O)NH(CH_2)_2-$

(**Ib**): X = $-(CH_2)_3-$ (**III**): X = $-(CH_2)_2[O(CH_2)_2]_2-$

(**Ic**): X = $-(CH_2)_4-$

(**Id**): X = $-(CH_2)_6-$

The structures and purities of the compounds synthesized were confirmed by TLC, ^1H, and ^{31}P NMR spectroscopy.

The substrate properties of the triphosphates were studied in the primer elongation reaction catalyzed by TDT (Scheme 4). In the course of the reaction, the substituted phosphate residue was cleaved from the studied triphosphate and linked to the 3'-hydroxyl group of 5'-^{32}P-labeled oligodeoxynucleotide under the TDT catalysis. The reaction products were analyzed by electrophoresis in 20% denaturing polyacrylamide gel.

All the synthesized triphosphates demonstrated substrate properties toward TDT and could serve donors of the substituted phosphate residue in the oligodeoxynucleotide elongation reaction. The substrate properties of (**Ia**)–(**Ic**) were similar to those of dTTP: 50% primer elongation was observed at the triphosphate concentrations of 1–2 μM [figure, data for (**Ib**) not shown]. The activity of (**Id**) was markedly lower: the primer utilization did not exceed 30% at its concentration of 5 μM. At the same triphosphate concentration, the incorporation of the substituted monophosphate released from triphosphate (**II**) was no more than 10%, whereas the degree of the primer incorporation with (**III**) was lower (data not given).

To conclude, the substrate properties toward TDT of the synthesized triphosphates (**Ia**)–(**Ic**) containing short alkyl linkers did not differ from one another and showed practically the same reactivity as dTTP. In the case of longer linkers containing up to six carbon atoms (**Id**) and more [(**II**) and (**III**)], the substrate properties of the compounds were considerably worse. The triphosphates bearing the alkyl linkers proved to be the most effective TDT substrates. The studied triphosphates can be arranged according to their elongation efficiency in the following order: dTTP ≈(**Ia**) ≈ (**Ib**) > (**Ic**) > (**Id**) > (**II**) > (**III**).

EXPERIMENTAL

2-Aminoethanol, 4-aminobutanol, triethyl phosphate, tributylamine, triethylene glycol, thionyl chloride, CDI, 2-mercaptoethanol, hexamethyldisilazane, and *N*,*O*-bis(trimethylsilyl)acetamide were from Fluka; 9-fluorenylmethoxycarbonyl chloride, 3-aminopropanol, 2-aminoethyl phosphate, phosphorus oxychloride, and DMF were from Aldrich. Sodium cacodylate, dithiothreitol, EDTA salt, acrylamide, formamide and calf thymus TDT (15 U/μl, Amersham) were used in enzymatic reactions. Oligonucleotide was obtained from Sintol company (Russia).

Adsorption column chromatography was carried out on LiChroprep RP-18 (25–40 μm) and Kieselgel (63–100 μm) (Merck). For ion-exchange column chromatography, Dowex 50 WX8 in NH_4^+-form (Fluka) was used. TLC was carried out on Kieselgel 60 F_{254} precoated plates (Merck, Germany); the elution system was 6 : 4 : 1 dioxane–water–25% aqueous ammonia.

NMR spectra were registered on a Bruker AMX III-400 (Bruker, Germany) instrument operating at the working frequency of 400 MHz for ^1H and 162 MHz for ^{31}P nuclei (with P–H decoupling). Chemical shifts are given in ppm; coupling constants, in Hz; tetramethylsilane and sodium 3-(trimethylsilyl)-1-propanesulfonate were internal references for organic solvents and water solutions, respectively; for ^{31}P NMR, 85% phosphoric acid was used as an external standard.

***N*-(9-Fluorenylmethoxycarbonyl)-4-aminobutyl triphosphate (Ic).** A solution of 4-aminobutanol (186 μl, 2 mmol) was successively treated with a solution of Na_2CO_3 (860 mg, 8 mmol) in water (8 ml) and a solution of *N*-(9-fluorenylmethoxycarbonyl chloride (400 mg, 1.6 mmol) in 1,4-dioxane (800 μl). The reaction mixture was stirred for 18 h at room temperature, the precipitated *N*-(9-fluorenylmethoxycarbonyl)-4-aminobutanol was filtered and washed with water to give 446 mg (93%) of the product; ^1H NMR ($CDCl_3$): 7.76 and 7.57 (4 H, 2 d, *J* 7.5, H1, H4, H5, and H8 of

$$H_2N–X–OH \xrightarrow{\text{Fmoc-Cl}} \text{Fmoc-NH–X–OH} \xrightarrow[\text{2) } (Bu_3NH)_2H_2P_2O_7]{\text{1) } POCl_3} \textbf{(Ia)–(Ic)}$$

Scheme 1.

$$HO(CH_2)_6OH \xrightarrow{\text{(MeO)Tr-Cl}} HO(CH_2)_6O(MeO)Tr$$

$$\xrightarrow[\text{3) } HO(CH_2)_2SH]{\text{1) MsCl 2) } NaN_3} H_2N(CH_2)_6O(MeO)Tr \xrightarrow[\text{2) } H^+]{\text{1) Fmoc-Cl}} \text{Fmoc-NH}(CH_2)_6OH \xrightarrow[\text{2) } (Bu_3NH)_2H_2P_2O_7]{\text{1) } POCl_3} \textbf{(Id)}$$

Scheme 2.

$$H_2N(CH_2)_5COOH \xrightarrow{\text{Fmoc-Cl}} \text{Fmoc-NH}(CH_2)_5COOH$$

$$\xrightarrow[\text{2) } H_2N(CH_2)_2OP(O)(OH)_2]{\text{1) } SOCl_2} \text{Fmoc-NH}(CH_2)_5CONH(CH_2)_2O\text{–}\underset{\displaystyle OH}{\overset{\displaystyle O}{\overset{\|}{P}}}\text{–OH} \xrightarrow[\text{2) } (Bu_3NH)_2H_2P_2O_7]{\text{1) CDI}} \textbf{(II)}$$

Scheme 3.

$$[5'\text{-}^{32}P](pdN)_{13}pdN + \text{ppp-L-Fmoc} \longrightarrow [5'\text{-}^{32}P](pdN)_{13}pdNp\text{-L-Fmoc} + \text{pp},$$

where L is linker.

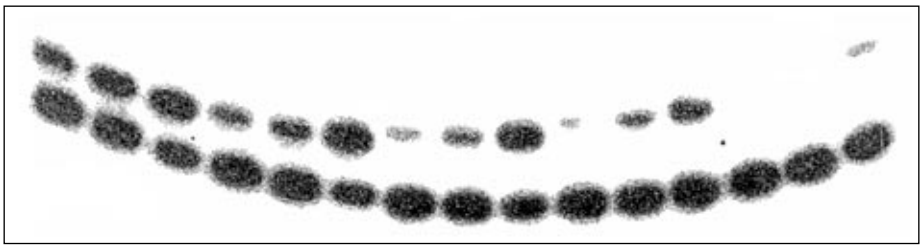
Scheme 4.

Fmoc), 7.39 and 7.31 (4 H, 2 m, *J* 7.2, H2, H3, H6, and H7 of Fmoc), 4.87 (1 H, br. s, NH), 4.40 (2 H, d, *J* 6.8, CH_2 of Fmoc), 4.21 (1 H, t, *J* 6.8, H9 of Fmoc), 3.66 (2 H, m, CH_2O), 3.23 (2 H, m, CH_2N), and 1.55–1.45 [4 H, m, $(CH_2)_2$].

Phosphorus oxycloride (133 μl, 1.43 mmol) was added to a solution of *N*-9-(fluorenylmethoxycarbonyl)-4-aminobutanol (175 mg, 0.59 mmol) in triethyl phosphate (1 ml) cooled to 0°C. The reaction mixture was kept for 18 h at 5°C and tributylamine (1 ml, 4.3 mmol) and 0.8 mM bis(tributylammonium) pyrophosphate (3 ml, 2.4 mmol) in DMF were added under vigorous stirring. The mixture was stirred for 3 h at room temperature, applied onto a Dowex 50 column (2 × 4 cm), and eluted with 50% aqueous methanol (50 ml). The solvents were removed in a vacuum, and the residue was chromatographed on a LiChroprep RP-18 column (2 × 18 cm) in a gradient of methanol (0 ⟶ 20%) in water to give 40 mg (12%) of (**Ic**); R_f 0.44; ^{1}H NMR (D_2O): 7.89 and 7.64 (4 H, 2 d, *J* 7.5, H1, H4, H5, and H8 of Fmoc), 7.40 and 7.48 (4 H, 2 t, *J* 7.5, H2, H3, H6, and H7 of Fmoc), 4.64 (2 H, d, *J* 6.9, CH_2 of Fmoc), 4.20 (1 H, t, *J* 6.9, H9 of Fmoc), 3.94 (2 H, m, CH_2O), 3.02 (2 H, m, CH_2N), and 1.55–1.23 [4 H, m, $(CH_2)_2$]; ^{31}P NMR (D_2O): –10.30 (1 P, d, *J* 19.3, P^γ), –10.38 (1 P, d, *J* 20.3, P^α), –22.79 (1 P, dd, P^β).

***N*-(9-Fluorenylmethoxycarbonyl)-2-aminoethyl triphosphate (Ia)** was obtained from 2-aminoethanol according to the procedure described for (**Ic**); yield 40 mg (11%); R_f 0.41; ^{1}H NMR (D_2O): 7.83 and 7.69 (4 H, 2 d, *J* 7.5, H1, H4, H5, and H8 of Fmoc), 7.42 and 7.36 (4 H, 2 m, H2, H3, H6, and H7 of Fmoc), 4.35 (2 H, d, *J* 6.5, CH_2 of Fmoc), 4.24 (1 H, t, H9 of Fmoc), 4.06 (2 H, m, CH_2O), and 3.44 (2 H, m, CH_2N); ^{31}P NMR (D_2O): –8.20 (1 P, d, *J* 19.8, P^γ), –8.79 (1 P, d, *J* 20.3, P^α), and –20.73 (1 P, dd, P^β).

***N*-(9-Fluorenylmethoxycarbonyl)-3-aminopropyl triphosphate (Ib)** was obtained from 3-aminopropanol according to the procedure described for (**Ic**);



μM	0.2	0.5	1	0.2	0.5	2	0.2	0.5	2	1	2	5	1	2	5
Compound	**dTTP**			(**Ia**)			(**Ic**)			(**Id**)			(**II**)		

Electrophoregram of the products of the TDT-catalyzed elongation reaction of the single-stranded oligodeoxynucleotide.

yield 22 mg (7%); R_f 0.42; ^{1}H NMR (D_2O): 7.83 and 7.61 (4 H, 2 d, *J* 7.5, H1, H4, H5, and H8 of Fmoc), 7.41 and 7.33 (4 H, 2 m, *J* 7.2, H2, H3, H6, and H7 of Fmoc), 4.69 (2 H, d, *J* 6.9, CH_2 of Fmoc), 4.24 (1 H, t, H9 of Fmoc), 3.82 (2 H, m, CH_2O), 3.03 (2 H, m, CH_2N), and 1.65–1.53 [4 H, m, $(CH_2)_2$]; ^{31}P NMR (D_2O): –9.74 (1 P, d, *J* 20.3, P^γ), –10.48 (1 P, d, *J* 19.3, P^α), and –22.65 (1 P, dd, P^β).

***N*-(9-Fluorenylmethoxycarbonyl)-8-amino-3,6-dioxaoctyl triphosphate (III).** Monomethoxytrityl chloride (2.7 g, 8.9 mmol) was added to a solution of triethylene glycol (10 g, 66.5 mmol) in pyridine (50 ml), and the reaction mixture was kept for 18 h at 37°C. The solution was diluted with aqueous $NaHCO_3$, and the product was extracted with chloroform. The organic extracts were evaporated, and the residue was dissolved in pyridine (20 ml) and cooled to 0°C. Methanesulfonyl chloride (1.38 ml, 17.8 mmol) was added, and the reaction mixture was kept for 18 h at 5°C, diluted with aqueous $NaHCO_3$, and extracted with carbon tetrachloride. The extract was evaporated, and the residue was dissolved in DMF (30 ml). Sodium azide (1.16 g, 17.8 mmol) was added, and the reaction mixture was stirred for 5 h at 90°C and evaporated in a vacuum. The residue was diluted with water (30 ml) and extracted with carbon tetrachloride (3 × 20 ml). Organic extracts were evaporated, the residue was dissolved in DMF (30 ml), and mercaptoethanol (3 ml) and 25% ammonia (0.5 ml) were added. The reaction mixture was kept for 18 h at room temperature and applied onto a Dowex 50 column (3 × 6 cm). The column was washed with methanol (50 ml) and eluted with a mixture of methanol (90 ml) and 25% ammonia (10 ml). The solvents were evaporated, and the residue was coevaporated with pyridine (2 × 20 ml), dissolved in pyridine (20 ml), and 9-fluorenylmethoxycarbonyl chloride (2.3 g, 8.9 mmol) was added. After 18 h at room temperature, the reaction mixture was diluted with aqueous $NaHCO_3$ (30 ml) and extracted with carbon tetrachloride (3 × 20 ml). The extract was evaporated, coevaporated with toluene (2 × 20 ml), and 80% acetic acid (50 ml) was added. After 18 h at room temperature, the reaction mixture was evaporated, and the residue was applied onto a silica gel column (2 × 25 cm, 63–100 μm) and eluted with 3% methanol in chloroform to give *N*-(9-fluorenylmethoxycarbonyl)-8-amino-3,6-dioxaoctan-1-ol; yield of 440 mg (13%); ^{1}H NMR ($CDCl_3$ + CD_3OD): 7.76 and 7.60 (4 H, 2 d, *J* 7.5, H1, H4, H5, and H8 of Fmoc), 7.39 and 7.31 (4 H, 2 t, *J* 7.5, H2, H3, H6, and H7 of Fmoc), 4.41 (2 H, d, *J* 6.9, CH_2 of Fmoc), 4.21 (1 H, t, *J* 6.9, H9 of Fmoc), 3.70–3.56 (10 H, m, $CH_2OCH_2CH_2OCH_2CH_2O$), and 3.39 (2 H, m, CH_2N).

Phosphorus chloride (133 μl, 1.43 mmol) was added to a solution of *N*-(9-fluorenylmethoxycarbonyl)-8-amino-3,6-dioxaoctan-1-ol (220 mg, 0.59 mmol) in triethyl phosphate (1 ml) cooled to 0°C. The reaction mixture was kept for 18 h at 5°C, and a mixture of tributylamine (1 ml, 4.3 mmol) and 0.8 mM bis(tributylammonium) pyrophosphate (3 ml, 2.4 mmol) in DMF was added under vigorous stirring. After a 3-h stirring at room temperature, the reaction mixture was applied onto a Dowex 50 column (2 × 4 cm), and the column was washed with 50% aqueous methanol (50 ml). The solvents were evaporated, and the residue was chromatographed on a LiChroprep RP-18 column (2 × 18 cm) in a gradient of methanol (0 ⟶ 20%) in water to give 40 mg (11%) of the target product; R_f 0.36; ^{1}H NMR (D_2O): 7.80 and 7.59 (4 H, 2 d, *J* 7.5, H1, H4, H5, and H8 of Fmoc), 7.41 and 7.33 (4 H, 2 t, *J* 7.5, H2, H3, H6, and H7 of Fmoc), 4.47 (2 H, m, CH_2 of Fmoc), 4.18 (1 H, m, H9 of Fmoc), 4.02 (2 H, m, CH_2OP), 3.64–3.38 (8 H, m, $CH_2OCH_2CH_2OCH_2$), and 3.11 (2 H, m, CH_2N); ^{31}P NMR (D_2O): –9.76 (1 P, d, *J* 19.3, P^γ), –10.49 (1 P, d, *J* 19.3, P^α), and –22.49 (1 P, t, P^β).

***N*-(9-Fluorenylmethoxycarbonyl)-6-aminohexyl triphosphate (Id)** was synthesized from hexane-1,6-diol by the procedure described for compound (**III**); yield of 28 mg (8%); R_f 0.45; ^{1}H NMR (D_2O): 7.94 and 7.71 (4 H, 2 d, *J* 7.5, H1, H4, H5, and H8 of Fmoc), 7.53 and 7.45 (4 H, 2 t, *J* 7.5, H2, H3, H6, and H7 of Fmoc),

4.69 (2 H, d, *J* 6.8, CH_2 of Fmoc), 4.34 (1 H, t, H9 of Fmoc), 3.89 (2 H, m, CH_2O), 3.03 (2 H, m, CH_2N), and 1.63–1.19 [8 H, m, $(CH_2)_4CH_2N$]; ^{31}P NMR (D_2O): –10.15 (1 P, d, *J* 19.3, P^{γ}), –10.24 (1 P, d, *J* 20.3, P^{α}), and –22.67 (1 P, dd, P^{β}).

***N*-[(9-Fluorenylmethoxycarbonyl)aminohexanoyl]-2-aminoethyl triphosphate (II).** A solution of *N*-9-fluorenylmethoxycarbonyl chloride (400 mg, 1.6 mmol) in dioxane (800 μl) was added to a solution of 6-aminohexanoic acid (260 mg, 2 mmol) in aqueous Na_2CO_3 (860 mg, 8 mmol, 8 ml), and the mixture was kept for 18 h at room temperature. The reaction mixture was diluted with water to 100 ml and extracted with ether (3 × 20 ml). The aqueous fraction (pH 10) was adjusted to pH 5 with 1 M HCl, the precipitate was filtered, washed with water, and dried to give 721 mg (93%) of *N*-(9-fluorenylmethoxycarbonyl)aminohexanoic acid; ^1H NMR ($CDCl_3$): 7.75 and 7.58 (4 H, 2 d, *J* 7.5, H1, H4, H5, and H8 of Fmoc), 7.53 and 7.45 (4 H, 2 t, *J* 7.5, H2, H3, H6, and H7 of Fmoc), 4.87 (1 H, br. s, NH), 4.39 (2 H, d, *J* 6.5, CH_2 of Fmoc), 4.21 (1 H, t, H9 of Fmoc), 3.19 (2 H, m, CH_2N), 2.35 (2 H, m, CH_2CO), and 1.64–1.36 [6 H, m, $(CH_2)_3CH_2N$].

2-Aminoethyl phosphate (52 mg, 0.3 mmol) was refluxed in *N*,*O*-bis(trimethylsilyl)acetamide (3 ml) for 10 h. The reagent was evaporated, and the residue was dissolved in a mixture of hexamethyldisilazane (1 ml) and DMF (1 ml). A solution obtained by the addition of thionyl chloride (220 μl, 3 mmol) to a solution of *N*-(9-fluorenylmethoxycarbonyl)aminohexanoic acid in dichloromethane (5 ml) was stirred for 3 h at room temperature, evaporated, and added to a solution of the silylated 2-aminoethyl phosphate. The reaction mixture was stirred for 12 h at room temperature, and evaporated. After the dissolution of residue in DMF (5 ml), CDI (324 mg, 2 mmol) was added, the mixture was stirred for 5 h, and 0.23 mM bis(tributylammonium) pyrophosphate (3.5 ml, 0.8 mmol) in DMF was added. After 3-h stirring, the mixture was applied onto a Dowex 50 column (2 × 4 cm) and eluted with 50% methanol (50 ml). The solvents were evaporated, and the residue was chromatographed on a LiChroprep RP-18 column (2 × 18 cm) in a gradient of methanol (0 ⟶ 20%) in water to give 18 mg (14%) of (**Id**); R_f 0.46; ^1H NMR (D_2O): 7.67 and 7.47 (4 H, 2 d, *J* 7.5, H1, H4, H5, and H8 of Fmoc), 7.53 and 7.45 (4 H, 2 t, *J* 7.5, H2, H3, H6, and H7 of Fmoc), 4.21 (2 H, d, *J* 6.8, CH_2 of Fmoc), 4.04 (1 H, t, H9 of Fmoc), 3.86 (2 H, m, CH_2O), 3.25 (2 H, m, CH_2CH_2OP), 2.85 (2 H, m, CH_2NHCO_2), 2.19 [2 H, m, CH_2CO], and 1.53–1.10 [6 H, m, $(CH_2)_3CH_2N$]; ^{31}P NMR (D_2O): –10.24 (1 P, d, *J* 20.3, P^{γ}), –10.64 (1 P, d, *J* 19.3, P^{α}), and –22.67 (1 P, dd, P^{β}).

The substrate activity of the synthesized compounds. The labeled oligonucleotide was obtained by the procedure described earlier [3]. The reaction mixture (10 μl) containing 0.02 μM [5'-^{32}P]-labeled 14-membered oligodeoxynucleotide, 0.2 U of TDT, 100 mM sodium cacodylate (pH 7.2), 2 mM $CoCl_2$, 0.1 mM dithiothreitol, and the substrates at various concentrations was incubated for 10 min at 37°C, terminated with formamide (5 μl) containing 0.5 mM EDTA and 0.1% Bromophenol Blue and Xylene Cyanole. The products were separated in 15% denaturing polyacrylamide gel. The gels were exposed to a Kodak RX roentgen film. For the quantitative determination, the autoradiograph was scanned on a Molecular Dynamics 300A Computing densitometer.

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REFERENCES

1. Baltimore, D., *Nature*, 1974, vol. 248, pp. 409–411.
2. Arzumanov, A.A., Victorova, L.S., and Jasko, M.V., *Nucleosides, Nucleotides & Nucleic Acids*, 2000, vol. 19, pp. 1787–1793.
3. Arzumanov, A.A., Victorova, L.S., Jasko, M.V., Yesipov, D.S., and Krayevsky, A.A., *Nucleic Acids Res.*, 2000, vol. 28, pp. 1276–1281.
4. Khandazhinskaya, A.L., Jasko, M.V., Shirokova, E.A., and Kukhanova, M.K., *Collection Symposium Series*, 2002, vol. 5, pp. 344–347.
5. Ludwig, I., *Acta Biochim. Biophys. Acad. Sci. Hung.*, 1981, vol. 16, pp. 131–133.