Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 6549–6555
Bioorganic & Medicinal Chemistry

A facile and effective synthesis of dinucleotide 5′-triphosphates

Tatiana V. Abramova,[a,*] Svetlana V. Vasileva,[a] Inna Yu. Serpokrylova,[a] Hadar Kless[b] and Vladimir N. Silnikov[a]

[a]*Institute of Chemical Biology and Fundamental Medicine, Lavrent'ev Avenue, 8, Novosibirsk 630090, Russia*
[b]*NuAce Technologies, Ltd., 14 Moskovitz Street, Rehovot 76474, Israel*

Received 8 May 2007; revised 3 July 2007; accepted 10 July 2007
Available online 25 July 2007

Abstract—We report on the successful synthetic procedure for the conversion of 5′-monophosphorylated 2′-deoxydinucleotides into their 5′-triphosphate derivatives in satisfactory to excellent yields. The activation of the terminal phosphate group was achieved under the Mukaiyama conditions in the presence of a nucleophilic catalyst. The reaction conditions (solvent, counter ions, activation time and reagent excess) were optimized for all dinucleotides.

1. Introduction

Nucleoside 5′-triphosphates have important therapeutic and diagnostic applications. Natural and modified nucleoside 5′-triphosphates (dNTP or NTP) are extensively used in biochemistry and molecular biology,[1,2] and a wide range of them are commercially available. On the contrary, 5′-triphosphates of di- and oligonucleotides have had limited usage as scientific tools so far. These compounds, for example, are proved to be useful for studying protein synthesis elongation factor I binding to a ribosome[3] and mRNA binding centre of *Escherichia coli* ribosomes.[4] Developed in early 1990s, SELEX (selective evolution of ligands by exponential enrichment) technique and aptamers[5] are now the powerful methods which need as much functional diversity of the nucleoside triphosphates as possible. The use of 5′-triphosphate derivatives of dinucleotides as substrates in the template dependent enzymatic DNA synthesis may provide additional possibilities for the functional diversification of aptamers.[6]

A few good methods for the synthesis of monomer nucleoside 5′-triphosphates are known.[7] However, none of them is general and versatile enough to provide the appropriate yields in the synthesis of di- and oligonucleotide 5′-triphosphates. The methods of synthesis of di- and oligodeoxynucleotide 5′-triphosphates published recently[8,9] operate with the fully blocked oligomers, either on the solid support or in solution. The solid support oligonucleotide synthesis is not commercially justified in the case of the short oligomers, when a significant quantity of a product is necessary. The solution phase phosphotriester chemistry may provide large quantities of the short oligomers with minimal cost.[10] The 5′-monophosphorylated oligonucleotides obtained by this method may serve as a starting material for the 5′-triphosphate synthesis.

The development of a synthetic method that could provide the sufficient quantities of ribo- and deoxyribodinucleotide 5′-triphosphates with the reasonable yields and a good reproducibility was our main task in this study.

2. Results and discussion

The activation of the monoesterified terminal phosphate group that is necessary for the subsequent reaction with the nucleophile (pyrophosphate) can be achieved by several methods. We chose the reaction of the phosphate with triphenylphosphine/2,2′-dipyridyl disulfide (Ph_3P/$(PyS)_2$) redox pair, proposed by Mukaiyama,[11] as a starting point of our study. As demonstrated in a number of works, such activation causes side-reactions neither with the functional groups of heterocyclic nucleoside bases nor with internucleotide phosphodiester bond.[12,13] This is especially important in the case of

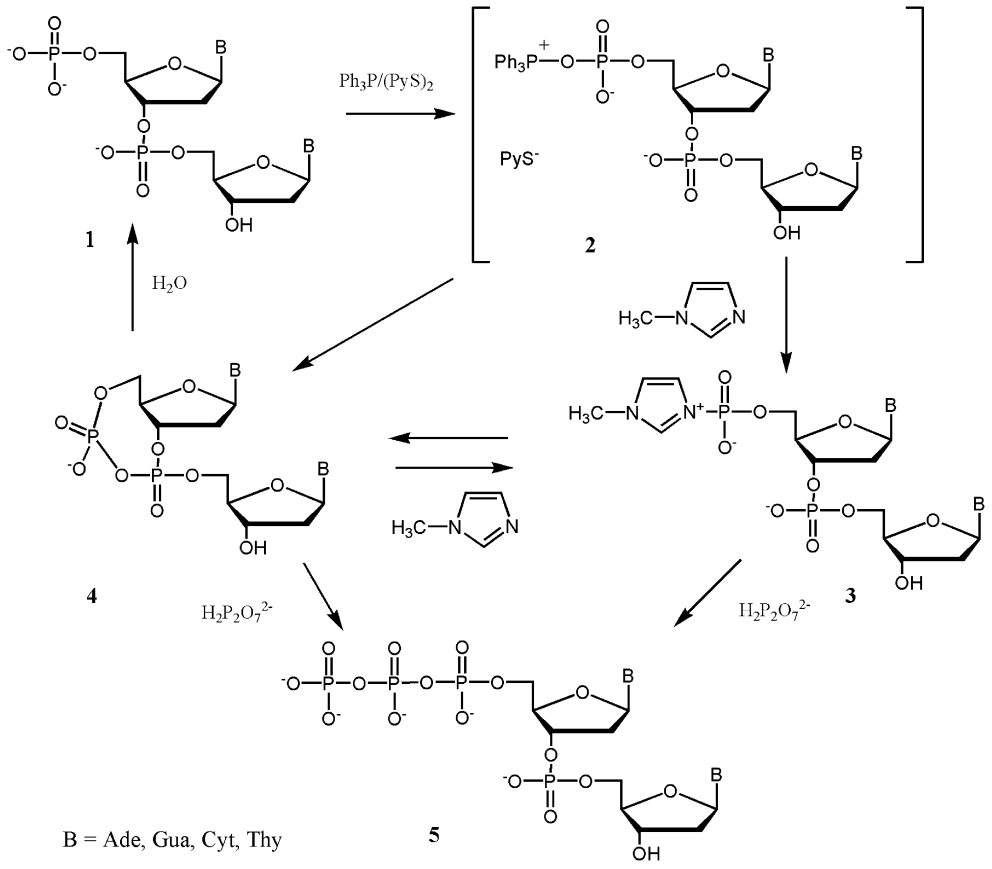
Keywords: Triphosphates; Oligonucleotides; Terminal phosphate activation.
* Corresponding author. Tel.: +7 383 3333762; fax: +7 383 3333677; e-mail: abramova@niboch.nsc.ru

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.07.002

oligoribonucleotides, where an activation of internucleotide phosphodiester bond may result in 3′ → 2′-isomerisation. The prolonged treatment (48 h) of oligouridylate with the $Ph_3P/(PyS)_2$ redox pair results only in 5% ribose modification by the 2-thiopyridine.[14] The employment of a nucleophilic catalyst (1-methylimidazole (1-MeIm) or 4-dimethylaminopyridine (DMAP)) combined with the ($Ph_3P/(PyS)_2$) redox activation gives rise to the considerable progress in the derivatization of the oligonucleotide terminal phosphate group,[15] presumably by the phosphamide bond formation. In the case of low nucleophilic or highly basic amines the addition of nucleophilic catalysts was essential to obtain the acceptable yields of the phosphamide derivatives.[16] As the pyrophosphate anion appears to be a poor nucleophile, we used a nucleophilic catalyst in our reaction.

Scheme 1 depicts the reaction pathway in the dinucleotide 5′-triphosphate synthesis as an example of a general reaction sequence in the preparation of oligonucleotide 5′-triphosphates. This scheme has been composed basing on ^{31}P NMR data recorded during the reaction, HPLC analysis of the reaction products and according to the literature data.[11,16–18] The reaction has been carried out in DMSO/DMF mixture through the following steps: activation of the terminal phosphate group in the presence of the nucleophilic catalyst followed by the addition of inorganic pyrophosphate to the reaction mixture without isolation of the reaction intermediates, and the removal of the excess of the activating reagents. A typical example of an anion exchange chromatography profile of the reaction mixture aliquot in the course of triphosphate synthesis is depicted in Figure 1. The assignment of the reaction products **1** and **5** (Scheme 1 and Fig. 1) was made after isolation of the compounds from the reaction mixture on the basis of their NMR characteristics and mass spectrum data. The assignment of the reactive intermediates **3** and **4** (Scheme 1 and Fig. 1) was made on the basis of observed correlation between integrated intensity of NMR signals and chromatographic peaks in the appropriate time. NMR data for the activated intermediates **3** and **4** formed in the course of the terminal phosphate group activation in dinucleotides were published earlier.[18]

In order to optimize the conditions of the dinucleotide 5′-triphosphate synthesis we investigated the dependence of the product yield on the phosphate group counter ion, activation time, the type of nucleophilic catalyst and the duration of the reaction with the pyrophosphate. In our hands, we did not detect the heterocyclic base modification during the reaction of dinucleotide 5′-phosphate with the activating reagents. The replacement of 2,2′-dipyridyl disulfide with 2,2′-bis(4,6-dimethylpyrimidyl) disulfide or diphenyl disulfide did not contribute much to the reaction efficiency.



Scheme 1. Synthesis of deoxydinucleotide 5′-triphosphates.

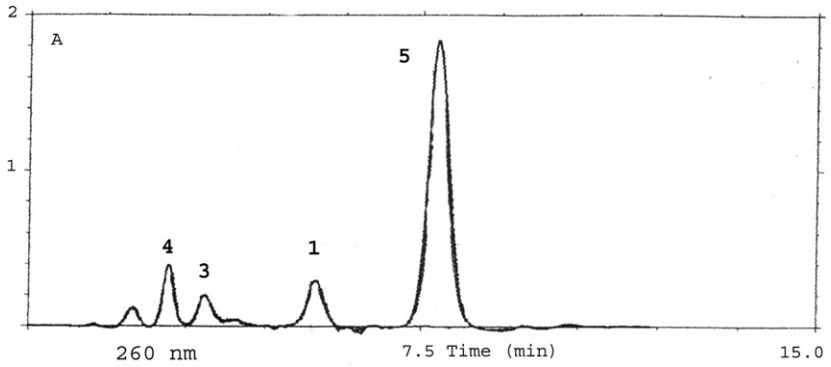


Figure 1. A typical anion exchange chromatography profile of the reaction mixture aliquot. Conditions: activation 5 min; reaction time with pyrophosphate 15 min; 20-fold excess of 1-MeIm; 5-fold excess of the activating reagents and inorganic pyrophosphate; dinucleotide (pppd(CT)) concentration is 0.1 M. Chromatography details are given in Section 4.

The extent of the internucleotide bond activation depended greatly on the type of phosphate salt used. The treatment of either cetyltrimethylammonium or triethylammonium salt of 5′-Tr-TpT for 2 h under the Mukaiyama conditions in the absence of nucleophilic catalysts resulted in the accumulation of the signal at −14 ppm, corresponding to the symmetrical tetrasubstituted pyrophosphate. Signal assignment was made according to the literature data.[17] As shown in Figure 2, the pyrophosphate accumulation rate was four times higher in the case of the triethylammonium counter ion, than the cetyltrimethylammonium one. Obviously, the side-reactions caused by the activation of internucleotide phosphates would result in the lowered yield of the target product under the oligonucleotide 5′-triphosphate formation conditions. Figure 3 represents the dependence of pppd(CT) and pppd(CG) yields on the salt type of the starting compound. As can be seen from the diagram, the yield of the 5′-triphosphate product is proportional to the size and the hydrophobicity of the counter cation.

The next step of our study was to find the optimal time of the activation step. Figure 4 depicts the dependence of the dinucleotide 5′-triphosphate yield upon the time of activation. In fact, the activation was completed in 5–7 min at 0.1–0.5 M concentrations of pd(NN) and 5- to 10-fold excess of the activating pair. At the same time, the side reaction progress at internucleotide phosphate in the cetyltrimethylammonium salts of deoxyribodinucleotide did not exceed 3–5% (Fig. 2). In the case of ribodinucleotide cetyltrimethylammonium salts the side-reactions originated from internucleotide phosphodiester activation were negligible. Even a prolonged treatment of the ribodinucleotide cetyltrimethylammonium salt with 5- to 10-fold excesses of $Ph_3P/(PyS)_2$ did not lead to the internucleotide phosphodiester activation, as could be detected by ^{31}P NMR spectroscopy (data not shown).

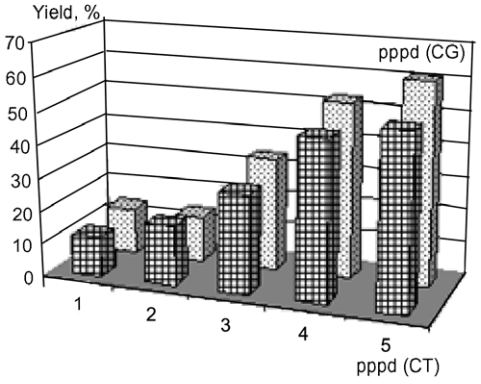


Figure 3. The pppd(CG) and pppd(CT) yield depending on the starting dinucleotide salt type **1**, Li^+; **2**, NH_4^+; **3**, Et_3NH^+; **4**, n-Bu_3NH^+; **5**, $C_{19}H_{42}N^+$. Conditions: activation 7 min; reaction time with pyrophosphate 15 min; 5-fold excess of the activating reagents, 1-MeIm and inorganic pyrophosphate; dinucleotide concentration is 0.1 M.

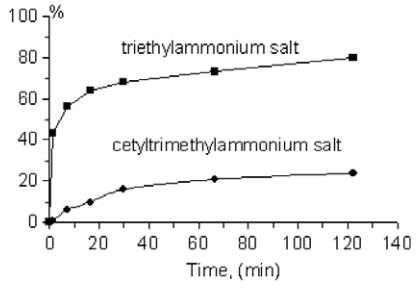


Figure 2. Kinetics of the $(TrTpT)_2$ symmetric pyrophosphate formation depending on the initial dinucleoside phosphate salt type. Conditions: 10-fold excess of the activating reagents; dinucleotide concentration is 0.5 M; the nucleophilic catalyst is absent.

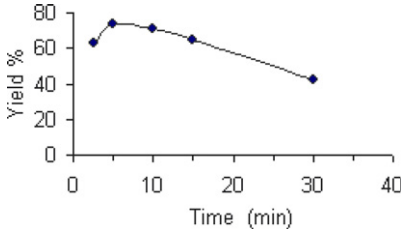


Figure 4. The pppd(CG) yield depending on the activation time. Conditions: reaction time with pyrophosphate 15 min; 20-fold excess of 1-MeIm; 5-fold excess of the activating reagents and inorganic pyrophosphate; dinucleotide concentration is 0.1 M.

As shown in a number of works, the addition of a nucleophilic catalyst such as 4-dimethylaminopyridine or 1-methylimidazole at the activation step results in the formation of reactive zwitterionic compounds[18] (compound **3**, Scheme 1). The formation of the zwitterionic intermediate is especially important in the reaction with negatively multicharged inorganic pyrophosphate because such zwitterionic intermediates have less electrostatic repulsion with inorganic pyrophosphate. In our work the final choice of 1-MeIm among a number of nucleophilic catalysts mainly resulted from a higher solubility of 1-MeIm-salt of pyrophosphate. This property enabled us to carry out the reaction at 0.1–0.5 M nucleotide concentrations, with the excesses of both the inorganic pyrophosphate and the nucleophilic catalyst. The requirement of the high excess of the nucleophilic catalyst was demonstrated in a series of experiments (Fig. 5). As seen in Figure 5, a quantitative yield was not achieved even at the high excess of 1-MeIm. The chromatographic profiles of all reaction mixtures treated with water after the activation step contained peaks corresponding to the mono charged molecules (Fig. 1, peaks **3** and **4**). The intensity of the peak referred by us as cyclopyrophosphate **4**[17] increased with the decrease of 1-MeIm concentration in the reaction mixture. When water was added to the reaction mixture at the activation step, more reactive 1-MeIm derivative **3** was rapidly transformed into the initial dinucleotide **1**, while the compound **4** was less reactive. The reaction mixtures treated with water for 24 h revealed a graduate decrease of the cyclophosphate **4** peak intensity, with the concomitant increase of the initial dinucleotide. A replacement of the pyrophosphate with morpholine (50-fold excess) at the second reaction step led to the rapid (5–7 min) formation of corresponding morpholidate with more than 90% yield. This fact points to the formation of phosphamides through both the intermediate **3** and the intramolecular cyclophosphate **4**. Lower yields of 5′-triphosphates in comparison with the morpholidates appear to be caused by the weaker nucleophility of inorganic pyrophosphate in comparison with morpholine in our conditions.

We did not succeed in increasing the 5′-triphosphate yield by prolongation of the time of reaction between the activated intermediates **3** and **4** and pyrophosphate. When the reaction proceeded longer than 15–20 min, a lower yield of the target product was observed (Fig. 6)

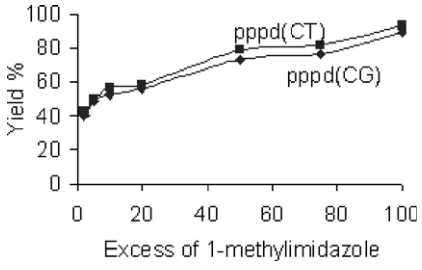


Figure 5. The pppd(CG) and pppd(CT) yield depending on 1-MeIm excess. Conditions: activation 7 min; reaction time with pyrophosphate 15 min; 5-fold excess of the activating reagents and inorganic pyrophosphate; dinucleotide concentration is 0.1 M.

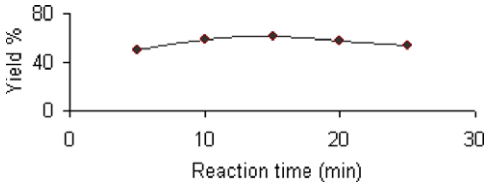


Figure 6. The pppd(CT) yield depending on a duration of the reaction with pyrophosphate. Conditions: activation 7 min; 20-fold excess of 1-MeIm; 5-fold excess of the activating reagents and inorganic pyrophosphate; dinucleotide concentration is 0.1 M.

and poly charged compounds appeared in the reaction mixture (HPLC data, not shown). Similar results were obtained when increasing the inorganic pyrophosphate excess.

Apart from 1-MeIm excess, increasing the reagent concentrations promoted the 1-MeIm intermediate **3** formation. Solubility of nucleotide species is a limiting factor in this case. It is possible to achieve 0.1–0.5 M nucleotide concentration using DMSO/DMF = 2:1 mixture as a solvent, depending on the oligonucleotide length and the primary structure.

The isolation of the nucleotide reaction products was very simple. After the reaction was complete the mixture was diluted 10-fold with 4% $LiClO_4$ in acetone. The 5′-triphosphorylated product precipitated quantitatively, together with the excess of inorganic pyrophosphate. The traces of the nucleophilic catalyst and activating reagents were eliminated completely by washing the precipitate by acetone and diethyl ether. The main nucleotide impurity was the starting oligonucleotide.

Purification of the 5′-triphosphate oligonucleotide derivatives was performed by two sequential anion exchange chromatographies. The yields of the purified 5′-triphosphate deoxydinucleotides are summarized in Table 1, 10–40 mg of each item was obtained. The product structure was confirmed by ^{1}H and ^{31}P NMR and mass spectroscopy. The data for some compounds are summarized in Table 2.

3. Conclusion

The approach presented in this paper allows the preparation of the 5′-triphosphate derivatives of di- and oligonucleotides in ribo- and deoxyriboseries in good yields starting from 5′-monophosphorylated oligomers. The reaction conditions were investigated. The yield of 5′-triphosphate derivatives was found to have a bell-shaped dependence on the reaction time at both steps (activa-

Table 1. The yield of some deoxydinucleotide 5′-triphosphates obtained according to Scheme 1

Dinucleotide	pppd(AA)	pppd(TA)	pppd(TC)
Yield (%)	61	54	88
Dinucleotide	ppp(TT)	pppd(GG)	pppd(CC)
Yield (%)	92	56	87

Table 2. NMR and mass spectra data for some products

	^{31}P, δ (ppm)[a]	^{1}H, δ (ppm)[b]	$[M+H]^+$
ppp(TT)	1.23 (s, 1P, P_{TT}); −7.17 (d, 1P, P_γ, $J_{\beta\gamma}$ 20); −9.10 (d, 1P, P_α, $J_{\alpha\beta}$ 19.5); −20.21 (t, 1P, P_β)	7.82 (s, 1H, H6); 7.79 (s, 1H, H6); 6.04 (app. t, 2H, H1′, *J* 8); 4.31–4.09 (m, 4H, H5′H5″); 2.72–2.38 (m, 4H, H2′2″); 2.01 (s, 3H, CH_3); 1.99 (s, 3H, CH_3)	786.64
pppd(GG)	−0.68 (s, 1P, P_{GG}); −5.83 (d, 1P, P_γ, $J_{\beta\gamma}$ 19); −10.28 (d, 1P, P_α, $J_{\alpha\beta}$ 19.5); −20.06 (t, 1P, P_β)	8.09 (s, 1H, H8); 8.05(s, 1H, H8); 6.31 (app. t, 1H, H1′); 6.19 (dd, 1H, H1′, *J* 6.0, 8.2); 4.36–4.21 (m, 4H, H5′H5″); 3.00–2,90 (m, 1H, H2′); 2.73–2.50 (m, 3H, H2′2″)	835.65
pppd(AA)	−2.77 (s, 1P, P_{AA}); −10.01 (d, 1P, P_γ, $J_{\beta\gamma}$ 18); −12.60 (d, 1P, P_α, $J_{\alpha\beta}$ 17.8); −23.42 (t, 1P, P_β)	8.34 (s, 1H, H8); 8.27 (s, 1H, H8); 8,03 (s, 1H, H2); 7. 93 (s, 1H, H2); 6.29 (app. t, 1H, H1′, *J* 7.0); 6.18 (dd, 1H, H1′, *J* 5.9, 9.1); 4.28–3.88 (m, 6H, H5′5′, H4′); 2.79–2.62 (m, 2H, H2′2″); 2.59–2.44 (m, 2H, H2′2″)	804.66
pppd(TA)	−1.28 (s, 1P, P_{TA}); −6.30 (d, 1P, P_γ, $J_{\beta\gamma}$ 20.1); −11.36 (d, 1P, P_α, $J_{\alpha\beta}$ 19.2); −21.67 (t, 1P, P_β)	8.42 (s, 1H, H8); 8.15 (s, 1H, H2); 7.45 (s, 1H, H6); 6.41 (app. t, 1H, H1′, *J* 6.8); 6.31 (dd, 1H, H1′, *J* 5.9, 11); 4.29–3.98 (m, 6H, H5′5″, H4′); 2.91–2.79 (m, 2H, H2′2″); 2.61–2.53 (m, 2H, H2′2″); 1.88 (s, 3H, CH_3)	795.66
pppd(TC)	−0.47 (s, 1P, P_{TC}); −9.80 (d, 1P, P_γ, $J_{\beta\gamma}$ 19.2); −10.98 (d, 1P, P_α, $J_{\alpha\beta}$ 19.2); −22.12 (t, 1P, P_β)	8.08 (d, 1H, H6(C), *J* 7.1); 7.88 (s, 1H, H6(T)); 6.45 (m, 2H, H1′); 6.30 (d, 1H, H5); 4.38–4,19 (m, 6H, H5′5″, H4′); 2.70–2.42 (m, 4H, H2′2″); 2.08 (s, 3H, CH_3)	771.64
pppd(CC)	−0.59 (s, 1P, P_{CC}); −8.53 (d, 1P, P_γ, $J_{\beta\gamma}$ 20.0); −10.62 (d, 1P, P_α, $J_{\alpha\beta}$ 19.0); −21.51 (t, 1P, P_β)	7.95 (d, 1H, H6, *J* 7.2); 7.91 (d, 1H, H6, *J* 7.5); 6.31 (app. t, 1H, H1′, *J* 6.0); 6.24 (app. t, 1H, H1′, *J* 6.1); 6.16 (d, 1H, H5); 6.12 (d, 1H, H5); 4.42–4.05 (m, 6H, H5′5″, H4′); 2.61–2.25 (m, 4H, H2′2″)	756.64

[a] Spectra were recorded in D_2O in P–H decoupled mode.
[b] Spectra were recorded in D_2O.

tion and nucleophilic substitution) and the excess of the pyrophosphate.

4. Experimental

4.1. General

N,*N*-Dimethylformamide (DMF), dimethylsulfoxide (DMSO), acetone, acetonitrile and diethyl ether were purified and dried according to standard procedures. 4-(*N*,*N*-Dimethylamino)pyridine (DMAP), triphenylphosphine, 2,2′-dipyridyl disulfide and $LiClO_4$ were purchased from Fluka AG (Switzerland). Cetyltrimethylammonium bromide was purchased from Merck (Germany). Bis(tri-*n*-butylammonium) pyrophosphate, poly(U) and RNAse A were purchased from Sigma (U.S.A.). 5′-Monophosphorylated deoxyoligonucleotides were synthesized according to the published procedure.[10] 2,2′-Bis(4,6-dimethylpyrimidyl) disulfide $(PyrS)_2$ and diphenyl disulfide were prepared according to the literature.[19] ^1H and ^{31}P NMR spectra were recorded on Bruker AM-400 and AC-200 spectrometers. The chemical shifts (δ) are reported in ppm relative to the residual solvent signals. In the case of ^{31}P an external standard of 85% H_3PO_4 was used. Coupling constants J are reported in Hertz. Mass spectra were recorded on a Reflex III instrument (Brucker Daltonics, Bremen, Germany) in the positive detector mode with dihydroxybenzoic acid as matrix. UV-absorption spectra were recorded on Specord M40 (Karl Zeiss, Yena, Germany). The molar extinction coefficients of oligonucleotides at 260 nm were estimated according to Ref. 20.

Different salt types of dinucleotides were obtained by passing the oligonucleotide solution in aqueous ethanol through the column packed with Dowex 50 W × 2 (Serva, U.S.A.) in the appropriate salt form.

Studies on the product yield (Figs. 1–6) were carried out at conditions mentioned in figures legends. Aliquots of the reaction mixtures were taken off at appropriate time or depending on the reagent excess, diluted 10-fold with water, centrifuged and analyzed by an analytical anion exchange HPLC. The analytical anion exchange chromatography was performed on Milichrom-4 chromatograph (Econova, Russia) using a 2.5 × 60 mm column packed with Polisil SA, 15 μm (Vector, Russia). A linear gradient (flow rate 50 μL/min) from 0 to 0.8 M of K_2HPO_4/KH_2PO_4 (pH 7.0) was employed. The peaks were integrated from chromatographic profiles by the Chromatography system software.

4.2. Synthesis of the 5′-triphosphate derivatives of dinucleotides in DMF/DMSO solution—general procedure

The reaction mixture containing cetyltrimethylammonium or trialkylammonium salt of dinucleotide 5′-monophosphate (0.05 mmol), Ph_3P (0.066 g, 0.25 mmol), $(PyS)_2$ (0.055 g, 0.25 mmol) (or either $(PhS)_2$, $(PyrS)_2$) and 1-methylimidazole (0.2 mL, 2.5 mmol) in DMF/DMSO (1:2, 10 mL) was incubated for 7 min at room temperature. After incubation, 0.5 M solution of bis(tri-*n*-butylammonium) pyrophosphate in CH_3CN (0.5 mmol, 1 mL) was added. The reaction mixture was incubated for 20 min at room temperature, and dinucleotide 5′-triphosphate was precipitated by the addition of 6% $LiClO_4$ in acetone (100 mL). The precipitate was washed by acetone and ether. Purification of the oligonucleotide derivatives was performed by anion exchange HPLC using a column (4.6 × 250 mm) packed with Polisil SA (Vector, Russia) and Waters 600E chromatography system equipped with Waters 484 tunable absorbance detector (U.S.A). A linear gradient (2 mL/min) of $(NH_4)_2SO_4$ concentration (0–0.8 M) in 20% aqueous methanol was used. Fractions containing the product were combined and diluted 10-fold with water, then applied to a column (3 × 9 cm) packed with DEAE Sephadex A-25 (Pharmacia, Sweden). Elution was performed with a linear gradient of 300 mL each of water and 1 M NH_4HCO_3. Appropriate fractions were pooled and evaporated. The residue was coevaporated several times with aqueous ethanol to remove traces of buffer. 5′-Triphosphates of dinucleotides were 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. All 5′-triphosphate derivatives were characterized by ^1H, ^{31}P NMR and mass spectroscopy. The data for some derivatives are summarized in the Table 2.

Acknowledgment

This work was supported by The Russian Fund for Fundamental Research, Grant No. 07-04-00990a and The Foundation for Assistance to Small Innovative Enterprises, Program "Start" N4924r/7340.

References and notes

1. Eckstein, F.; Thomson, J. B. *Methods Enzymol.* **1995**, *262*, 189.
2. Kuwahara, M.; Nagashima, J.-I.; Hasegawa, M.; Tamura, T.; Kitagata, R.; Kazuo, H.; Hososhima, S.-I.; Kasamatsu, T.; Ozaki, H.; Sawai, H. *Nucleic Acids Res.* **2006**, *34*, 5383.
3. Allende, J. E.; Allende, C. C.; Simoncsits, A.; Tomasz, J. *J. Biol. Chem.* **1975**, *250*, 2056.
4. Gimautdinova, O. I.; Karpova, G. G.; Komarova, N. I.; Frolova, S. B. *Russ. J. Bioorg. Chem.* **1985**, *11*, 499.
5. Breaker, R. R.; Joyce, G. F. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12268.
6. Kless, H. WO 01/16366, 2001; *Chem. Abstr.* **2001**, *134*, 217979.
7. Burgess, K.; Cook, D. *Chem. Rev.* **2000**, *100*, 2047.
8. Lebedev, A. V.; Koukhareva, I. I.; Beck, T.; Vaghefi, M. M. *Nucleos. Nucleot. Nucl. Acids* **2001**, *20*, 1403.
9. Abramova, T. V.; Bakharev, P. A.; Vasilyeva, S. V.; Silnikov, V. N. *Tetrahedron Lett.* **2004**, *45*, 4361.
10. Abramova, T. V.; Komarova, N. I.; Mundus, D. A.; Pereboeva, O. S. *Izv. Sib. Otd. Akad. Nauk, Ser. Khim.* **1990**, *5*, 45.
11. Mukaiyama, T. *Phosphorus Sulphur* **1976**, *1*, 371.
12. Mukaiyama, T. *Angew. Chem.* **1976**, *88*, 111.
13. Mishenina, G. F.; Samukov, V. V.; Shubina, T. N. *Russ. J. Bioorg. Chem.* **1979**, *5*, 886.

14. Nakaku, H.; Shimada, Y.; Nakayama, Y.; Hata, T. *Nucleic Acids Res.* **1976**, *3*, 1233.
15. Grimm, G. N.; Boutorine, A. S.; Helene, C. *Nucleos. Nucleot. Nucl. Acids* **2000**, *19*, 1943.
16. Knorre, D. G.; Alekseyev, P. V.; Gerasimova, Yu. V.; Silnikov, V. N.; Maksakova, G. A.; Godovikova, T. S. *Nucleos. Nucleot.* **1998**, *17*, 397.
17. Lebedev, A. V.; Rezvukhin, A. I. *Nucleic Acids Res.* **1984**, *12*, 5547.
18. Godovikova, T. S.; Zarytova, V. F.; Khalimskaya, L. M. *Russ. J. Bioorg. Chem.* **1986**, *12*, 475.
19. Field, L.; Lawson, J. E. *J. Am. Chem. Soc.* **1958**, *80*, 838.
20. Cantor, C. R.; Tinoco, I. J. *J. Mol. Biol.* **1965**, *13*, 65.