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Design and synthesis of dinucleotide 5′-triphosphates with expanded functionality

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ABSTRACT

We propose the new approach to the synthesis of 5′-triphosphate derivatives of natural and modified dinucleotides with expanded functionality. Our strategy includes the combination of the solution phase synthesis of necessary dimers using the wide range of nucleic acids chemistry methods and the subsequent introduction of the triphosphate residue. A number of the new potential substrates for the template dependent synthesis of nucleic acids with expanded functionality are obtained, namely, 5′-triphosphates of dinucleotides containing the functionally active groups in heterocyclic bases, in carbohydrate–phosphate backbone, and the groups mimicking the residues of natural amino acids. The abilities of the proposed synthetic route are also demonstrated by the synthesis of 5′-triphosphates of dinucleotides with modified carbohydrate–phosphate backbone.

1. Introduction

The profound interest in the molecular biology investigations during the last decades gave the impetus for the development of synthetic methods in nucleic acids chemistry. Achievements and new approaches in this area are summarized in the reviews.[1,2] Chemical synthesis of modified oligonucleotides is routine procedure now due to the fast introduction of innovations appearing in scientific literature in commercial practice; the catalogs of leading companies dealing with the synthesis of oligonucleotides and their derivatives are regularly updated.[3–6] At the same time very little progress has been attained in the enzyme mediated modified nucleic acids synthesis. This situation is due for the most part to objective causes, namely, modified triphosphates ought to be effective non-terminating substrates of the corresponding enzymes. Recently, a number of works devoted to the investigation of substrate properties of a wide range of modified nucleoside 5′-triphosphates appeared.[7–10] Functionally active nucleic acids obtained by the chemical or enzymatic methods are the perspective tools of molecular biology used in the various areas of fundamental and applied research.[11–14] So, further development of the functionalized nucleic acid synthesis and of the available modification spectrum is an actual task. We believe that 5′-triphosphates of dinucleotides used as substrates for NA polymerases might be the solution of the problem (Fig. 1). One can suggest that such triphosphates would have more modification points non critical for substrate properties. Preliminary experiments showed that 5′-triphosphate dinucleotides are the substrates for some DNA-polymerases.[15] The substrate properties of the modified 5′-triphosphodinucleotides are currently under intensive investigation, the results were partially presented in the plenary lecture in International conference in Novosibirsk, Russia.[16]

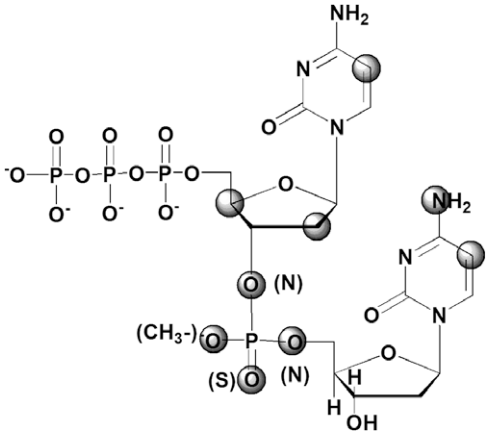

Figure 1. 5′-Triphospho-{2′-d-[cytidinyl(3′-phospho-5′)cytidine]}. The possible sites of ribose-phosphate backbone and heterocyclic base modifications are indicated.

A few methods for the synthesis of 5′-triphosphates of di- and oligonucleotides in solution[17,18] and on the solid support[19] are known, however the yields of the desired products did not exceed 50%. Furthermore, the quantity of the product is limited by the oligonucleotide synthesis scale in the case of the solid phase

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synthesis. The main problem in the synthesis of 5′-triphosphate derivatives of di- and oligonucleotides is the presence of an additional highly reactive center in the molecule – internucleotide phosphate group. The convenient method for the synthesis of 5′-triphosphates of di- and oligonucleotides in the solution with high yields starting from the corresponding deprotected 5′-monophosphates was recently proposed.[20] However, in general it is necessary to use protected oligonucleotides. Functional groups introduced in oligonucleotide usually also require protection both during the oligonucleotide synthesis and the triphosphate residue addition.

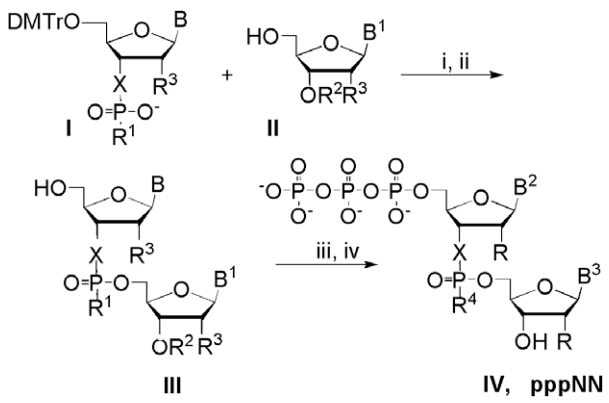
We propose the new approach to obtaining the 5′-triphosphate derivatives of natural and modified dinucleotides consisting of the necessary dimer synthesis in solution using all methods of oligonucleotide chemistry following the introduction of the triphosphate residue in the suitably protected oligomers. Our approach provides the availability of 5′-triphosphodinucleotides containing the wide range of modifications both in ribose–phosphate backbone and heterocyclic bases with high yield and in large quantity.

2. Results and discussion

We began the synthesis of target compounds from the construction of the nucleotide part of the molecule containing necessary modifications and functional groups. The choice of synthetic strategy was determined by the structure and the location of the modification. The synthesis of 2′-d-[cytidinyl-(3′-phospho-5′)-5-(aminoallyl)uridine] 5′-triphosphate is depicted in Scheme 1 as an example. Nucleotide part of the other triphosphates was formed by the similar approach. Monomer blocks **I** and **II** were synthesized by the various methods[21–23] or were commercially available (Table 1), then we carried out coupling reaction to produce suitably protected dimers **III** presumably using the phosphotriester chemistry (compounds **3**, **4**, **7**–**43**, Table 1).[24,25]

Some modifications of the target molecules are specified in Table 2. We employed 'locked' nucleosides, 2′-deoxy-2′-amino, 2′-OMe groups as the examples of carbohydrate modifications; adenine, cytosine and uracil containing aminoalkyl linkers as the examples of heterocyclic base modifications; methylphosphono- and phosphamide groups as the examples of phosphate modifications.

In the case of 3′-*N*-5′-*O*-phosphamide internucleotide bond and chimerical ribo-deoxyribodinucleotides (compounds **5** and **6** in Table 1) the phosphosphoramidite chemistry was found to be more convenient,[24] but the coupling reaction was carried out in solution instead of usually employed solid phase approach. Methylphosphonate derivatives (compounds **1** and **2** in Table 1) were synthesized by the phosphorodiimidazolide method.[26] Thus we constructed the nucleotide part of the target derivatives depending on its structure using a wide range of the oligonucleotide chemistry methods. Solution phase chemistry allows easily obtaining substances in large quantities (up to several grams). The careful purification of the dinucleotides with only the 5′-hydroxyl function free by the chromatography provided high yield during the introduction of the triphosphate residue.



Scheme 1. Synthesis of the 5′-triphosphates of modified dinucleotides as exemplified by obtaining the 2′-d-[cytidinyl-(3′-phospho-5′)-5-(aminoallyl)uridine] 5′-triphosphate. B = N^4-benzoylcytosine, B^1 = 5-(trifluoroacetamidoallyl)uracil, B^2 = cytosine, B^3 = 5-(aminoallyl)uracil, R^1 = *O*-*p*-chlorophenyl, R^2 = acetyl, R = R^3 = H, R^4 = O^-. (i) 2,4,6-Triizopropylbenzenesulfonylchloride, *N*-Me-imidazole, Py; (ii) HOAc/H_2O; (iii) $POCl_3$, Py, then $[(C_4H_9)_3N]_2H_2P_2O_7$ in acetonitrile, $N(C_4H_9)_3$; (iv) NH_3/H_2O.

The choice of the triphosphate residue introduction method was determined by the nucleotide structure and the presence and type of protective groups. Mainly we used modified Ludwig's method (Scheme 1).[27,28] We carried out the additional optimization of the reaction conditions and succeeded in increasing the yield of the target product (compound **IV**, Scheme 1) up to 80% after purification against 20% in the earlier article.[28] Purification of the 5′-triphosphodinucleotide derivatives was performed by two sequential reverse phase chromatographies, initially before the deprotection of the product and then after removing of the protective groups. This allowed us to easily separate the excess of the pyrophosphate anion from the target products.

In some cases the 5′-triphosphodinucleotide modification can be carried out post-synthetically. For example, we introduced the amino alkyl residue in the N^4-position of cytosine (compounds **29–33**, the Table 1) by the transamination catalyzed by the bisulfite anion analogously the published procedure[28]; 5′-triphosphothymidinyl-(3′-phospho-5′)-2′-deoxycytidine was used as a parent compound in this reaction. Azide group was used as a precursor of an amine function in 2′-amino-2′-deoxynucleosides; the reduction of the intermediate **9** by the H_2 over Pd/C similar to the published procedure[30] afforded the compound **10** (Table 1).

In order to the widening of the functional group repertoire present in the 5′-triphosphodinucleotides we subjected aliphatic amine groups in the modified 5′-triphosphodinucleotides further modification (compounds **3, 4, 7, 11, 17-39, 41, 43** in Table 1, Fig. 2). We introduced reporter or reactive groups (biotin, fluorescein, photo reactive *p*-azidotetrafluorobenzoyl residue), and the groups mimicking side radicals of natural amino acids. Natural amino acid character notation is used in Figure 2 and Table 1 to indicate the residues mimicking the corresponding amino acid side radicals. Necessary organic acids were first activated usually as *N*-hydroxysuccinimide esters in the presence of DCC analogously one of the numerous published procedures; activated derivatives without purification were used for the transformation of the triphosphate derivatives (see Supplementary data).[9,31] Sometimes we employed corresponding anhydrides, *p*-nitrophenyl and pentafluorophenyl esters if they were commercially available. In the lactic acid case we brought into use its methyl ester in anhydrous conditions though the reactivity of this substance was found to be rather low.

3. Conclusion

We synthesized more than 40 modified and natural 5′-triphosphodinucleotides. Modifications introduced in the dinucleotides involve heterocyclic bases, carbohydrates and phosphate backbone. Other modifications may be available as they are compatible with the conditions of oligonucleotide synthesis and protective group removing. The general efficacy of our approach is determined most of all by method of the internucleotide bond formation. Phosphotriester chemistry is the method of choice in our approach because of better yields of solution phase coupling. Some physical–chemical characteristics, parent monomer blocks, type of the coupling reaction used and post-synthetic modifications are

Table 1
Modified 5′-triphosphodinucleotides — compounds **IV** (Scheme 1)

	pppNN	Monomers **I** and **II**, method of synthesis	Method of coupling of monomers **I** and **II**	Post-synthetic modification
1	pppd[G(pCH_3)A], 2′-d-[guanosinyl(3′-methylphosphono-5′)adenosine] 5′-triphosphate (diastereomer mixture)	5′-*O*-(DMTr)dG^{iBu}, dA^{Bz}-3′-*O*-(Ac)[a]	Phosphorodiimidazolide[26]	—
2	pppd[C(pCH_3)A], 2′-d-[cytidinyl(3′-methylphosphono-5′)-adenosine] 5′-triphosphate	5′-*O*-(DMTr)dC^{Ac}, dA^{Bz}-3′-(Ac)[a]	- « -	—
3	pppd[U$^{(5\text{-}Flu)}$pT], 2′-d-{5-[3-(fluoreceinthiocarbamoylamido)propyloxymethyl]uridinyl(3′-phospho-5′)thymidine} 5′-triphosphate	5′-*O*-(DMTr)-2′-dU$^{(5\text{-}MeOPrNHTfa)}$[b],[21] 5′-*O*-(*p*-ClPhP)dT-3′-*O*-(Lev)[c],[25]	Phosphotriester[25]	Fluorescein isothiocyanate[31]
4	pppd[U$^{(5\text{-}Bio)}$pT], 2′-d-{5-[3-(biotinamidopropyl)oxymethyl]uridinyl(3′-phospho-5′)thymidine} 5′-triphosphate	- « -	- « -	Biotin *p*-nitrophenyl ester[31]
5	pppd[T(3′NHp)C], 3′-d-3′-amido-2′-d-thymidinyl(3′-*N*-phospho-5′)-2′-d-cytidine 5′-triphosphate	5′-*O*-(DMTr)-3′-NH_2-3′-dT, reduction of the corresponding azide derivative,[23] 5′-*O*-[$CNC_2H_4OPN(iPr)_2$]dC^{Ac}–*O*-(Ac) similar to[21]	Phosphoramidite[24]	—
6	pppUpdT, uridinyl(3′-phospho-5′)-2′-d-thymidine 5′-triphosphate	5′-*O*-(DMTr)-2′-*O*-(TBDMS)-U-3′-*O*-[$CNC_2H_4OPN(iPr)_2$], dT-*O*-(Ac)[a]	- « -	—
7	pppd[CpU$^{(5\text{-}L\text{-}Bio)}$], 2′-d-[cytidinyl(3′-phospho-5′)-5-(biotin-6-amidocaproyl-6-amidocaproylamidoallyl)uridine] 5′-triphosphate	5′-*O*-(DMTr)dC^{Bz}-3′-*O*-(*p*-ClPhP), 2′-dU$^{(5\text{-}aaTfa)}$-3′-*O*-(Ac)[d] similar to[21,25]	Phosphotriester[25]	*N*-Hydroxysuccinimidyl biotin-6-amidocaproyl-6-amidocaproate[31]
8	pppd[U(2′-NH_2)pC], 2′-d-2′-aminouridinyl(3′-phospho-5′)-2′-d-cytidine 5′-triphosphate	5′-*O*-(DMTr)-2′-dU-2′-NH(Tfa)[22], 5′-*O*-(*p*-ClPhP)dC^{Bz}3′-*O*-(Lev)[25]	- « -	—
9	pppd[A(2′-apaN_3)pC], 2′-d-2′-azidoarabinoadenosinyl(3′-phospho-5′)-2′-d-cytidine 5′-triphosphate	5′-*O*-(DMTr)-2′-d-2′-araN_3-A^{Bz},[23] 5′-*O*-(*P*-ClPhP)dC^{Bz}-3′-*O*-(Lev)[25]	- « -	—
10	pppd[A(2′-araNH_2)pC], 2′-d-2′-aminoarabinoadenosinyl(3′-phospho-5′)-2′-d-cytidine 5′-triphosphate	- « -	- « -	Reduction of the corresponding azide derivative similar to[30]
11	pppd[GpU$^{(5\text{-}aa\text{-}Flu)}$], 2′-d-[guanosinyl(3′-phospho-5′)-5-(fluoresceinthiocarbamoylamidoallyl)uridine] 5′-triphosphate	5′-*O*-(DMTr)dG^{iBu}3′-*O*(-*p*-ClPhP), 2′-dU$^{(5\text{-}aaTfa)}$-3′-*O*-(Ac)[21,25]	- « -	Fluorescein isothiocyanate[31]
12	pppd[(LNA-C)pT], locked-cytidinyl(3′-phospho-5′)-2′-d-thymidine 5′-triphosphate	5′-*O*-(DMTr)(LockedC)^{Ac},[a] 5′-*O*-(*p*-ClPhP)dC^{Bz}3′-*O*-(Lev)[25]	- « -	—
13	pppd[TpA$^{(6\text{-}ae)}$],[e] 2′-d-[thymidinyl(3′-phospho-5′)-6-(2-aminoethyl)adenosine] 5′-triphosphate	5′-*O*-(DMTr)dT-3′-*O*-(*p*-ClPhP), dA$^{(6\text{-}aeTfa)}$-3′-*O*-(Ac)[21,25]	- « -	—
14	pppd[ApA$^{(6\text{-}ae)}$], 2′-d-[adenosinyl(3′-phospho-5′)-6-(2-aminoethyl)adenosine] 5′-triphosphate	5′-*O*-(DMTr)dA^{Bz}-3′-*O*-(*p*-ClPhP), dA$^{(6\text{-}aeTfa)}$(Ac)[21,25]	- « -	—
15	pppd[CpA$^{(6\text{-}ae)}$], 2′-[d-cytidinyl(3′-phospho-5′)-6-(2-aminoethyl)adenosine] 5′-triphosphate	5′-*O*-(DMTr)dC^{Bz}-3′-*O*-(*p*-ClPhP), dA$^{(6\text{-}aeTfa)}$(Ac)[21,25]	- « -	—
16	pppd[GpA$^{(6\text{-}ae)}$], 2′-d-[guanosinyl(3′-phospho-5′)-6-(2-aminoethyl)adenosine] 5′-triphosphate	5′-*O*-(DMTr)dG^{iBu}-3′-*O*-(*p*-ClPhP), dA$^{(6\text{-}aeTfa)}$-3′-*O*-(Ac)[21,25]	- « -	—
17	pppd[TpA$^{(6\text{-}ae\text{-}H)}$], 2′-d-{thymidinyl(3′-phospho-5′)-6-[2-(urocanoylamido)ethyl]adenosine} 5′-triphosphate	5′-*O*-(DMTr)dT-3′-*O*-(*p*-ClPhP), dA$^{(6\text{-}aeTfa)}$-3′-*O*-(Ac)[21,25]	- « -	*N*-Hydroxysuccinimidyl urocanoate[31]
18	pppd[TpA$^{(6\text{-}ae\text{-}D)}$], 2′-d-{thymidinyl(3′-phospho-5′)-6-[2-(succinylamido)ethyl]adenosine} 5′-triphosphate	- « -	- « -	Succinic anhydride similar to[31]
19	pppd[TpA$^{(6\text{-}ae\text{-}K)}$], 2′-d-[thymidinyl(3′-phospho-5′)-6-[2-(6-aminocaproylamido)ethyl]adenosine} 5′-triphosphate	- « -	- « -	*N*-Hydroxysuccinimidyl 6-(trifluoroacetylamido)hexanoate,[31] ammonia treatment
20	pppd[TpA$^{(6\text{-}ae\text{-}V)}$], 2′-d-{thymidinyl(3′-phospho-5′)-6-[2-(isobutyrylamido)ethyl]adenosine} 5′-triphosphate	- « -	- « -	Isobutyric anhydride similar to[31]
21	pppd[TpA$^{(6\text{-}ae\text{-}R)}$], 2′-d-{thymidinyl(3′-phospho-5′)-6-[2-(4-guanidinebutyrylamido)ethyl]adenosine} 5′-triphosphate	- « -	- « -	*N*-Hydroxysuccinimidyl 4-guanidinobutyrate[31]
22	pppd[TpA$^{(6\text{-}ae\text{-}W)}$], 2′-d-{thymidinyl(3′-phospho-5′)-6-[2-(indole-3-acetylamido)ethyl]adenosine} 5′-triphosphate	- « -	- « -	Pentafluorophenyl indole-3-acetate similar to[31]
23	pppd[TpA$^{(6\text{-}ae\text{-}M)}$], 2′-d-{thymidinyl(3′-phospho-5′)-6-[2-(2-methylthioacetylamido)ethyl]adenosine} 5′-triphosphate	- « -	- « -	*N*-Hydroxysuccinimidyl 2-methylthioacetate[31]
24	pppd[TpA$^{(6\text{-}ae\text{-}T)}$], 2′-d-{thymidinyl(3′-phospho-5′)-6-[2-(2-hydroxypropionylamido)ethyl]adenosine} 5′-triphosphate	- « -	- « -	Methyl lactate (racemic mixture), pyridine
25	pppd[TpA$^{(6\text{-}ae\text{-}C)}$], 2′-d-{thymidinyl(3′-phospho-5′)-6-[2-(3-mercaptopropionylamido)ethyl]adenosine} 5′-triphosphate	- « -	- « -	Bis-*N*-hydroxysuccinimidyl 3,3′-dithiodipropionate;[31] precipitation in the presence of 2-mercaptoethanol
26	pppd[TpA$^{(6\text{-}ae\text{-}N)}$], 2′-d-{thymidinyl(3′-phospho-5′)-6-[2-(amidosuccinylamido)ethyl]adenosine} 5′-triphosphate	- « -	- « -	*N*-Hydroxysuccinimidyl monoamidosuccinate[31]
27	pppd[TpA$^{(6\text{-}ae\text{-}Y)}$], 2′-d-{thymidinyl(3′-phospho-5′)-6-[2-(3-(*p*-hydroxyphenyl)propionylamido)ethyl]adenosine} 5′-triphosphate	- « -	- « -	*N*-Hydroxysuccinimdyl 3-(*p*-hydroxyphenyl)propionate[31]
28	pppd[TpA$^{(6\text{-}ae\text{-}F)}$], 2′-d-{thymidnyl(3′-phospho-5′)-6-[2-(phenylacetylamido)ethyl]adenosine}-5′-triphosphate	- « -	- « -	*N*-Hydroxysuccinimidyl phenylacetate[31]
29	pppd[TpC$^{(4\text{-}ae)}$], 2′-d-[thymidinyl(3′-phospho-5′)-4-(2-aminoethyl)cytidine] 5′-triphosphate	5′-*O*-(DMTr)dT[a], 5′-*O*-(*p*-PhP)dC^{Bz}-*O*-(Lev)[25]	- « -	HSO_3^-, EDA·2HCl[29]
30	pppd[TpC$^{(4\text{-}ae\text{-}V)}$], 2′-d-{thymidinyl(3′-phospho-5′)-4-[2-(isobutyrylamido)ethyl]cytidine} 5′-triphosphate	- « -	- « -	HSO_3^-, EDA·2HCl;[29] isobutyric anhydride similar to[31]

(*continued on next page*)

Table 1 (*continued*)

	pppNN	Monomers **I** and **II**, method of synthesis	Method of coupling of monomers **I** and **II**	Post-synthetic modification
31	pppd[TpC$^{(4\text{-ae-R})}$], 2′-d-{thymidinyl(3′-phospho-5′)-4-[2-(4-guanidinobutyrylamido)ethyl]cytidine} 5′-triphosphate	- « -	- « -	HSO_3^-, EDA·2HCl;[29] *N*-hydroxysuccinimidyl 4-guanidinobutyrate[31]
32	pppd[TpC$^{(4\text{-ae-C})}$], 2′-d-{thymidinyl(3′-phospho-5′)-4-[2-(3-mercaptopropionylamido)ethyl]cytidine} 5′-triphosphate	- « -	- « -	HSO_3^-, EDA·2HCl;[29] bis-*N*-hydroxysuccinimidyl 3,3′-dithiodipropionate;[31] precipitation in the presence of 2-mercaptoethanol
33	pppd[TpC$^{(4\text{-ae-H})}$], 2′-d-{thymidinyl(3′-phospho-5′)-4-[2-(urocanoylamido)ethyl]cytidine} 5′-triphosphate	- « -	- « -	HSO_3^-, EDA·2HCl;[29] *N*-hydroxysuccinimidyl urocanoate[31]
34	pppd[CpA$^{(6\text{-ae-W})}$], 2′-d-{cytidinyl(3′-phospho-5′)-6-[2-(indole-3-acetylamido)ethyl]adenosine} 5′-triphosphate	5′-*O*-(DMTr)dC^{Bz}-3′-*O*(-*p*-ClPhP), dA$^{(6\text{-aeTfa})}$-3′-*O*-(Ac)[21,25]	- « -	Pentafluorophenyl indole-3-acetate similar to[31]
35	pppd[CpA$^{(6\text{-ae-Y})}$], 2′-d-{cytidinyl(3′-phospho-5′)-6-[2-(3-(*p*-hydroxyphenyl)propionylamido)ethyl]adenosine} 5′-triphosphate	- « -	- « -	*N*-Hydroxysuccinimidyl 3-(*p*-hydroxyphenyl) propionate[31]
36	pppd[U$^{(5\text{-aa-Y})}$pT], 2′-d-{5-[(3-(*p*-hydroxyphenyl)propionylamido)allyl]uridinyl(3′-phospho-5′)thymidine} 5′-triphosphate	5′-*O*-(DMTr)-2′-dU$^{(5\text{-aa-Tfa})}$,[21] 5′-*O*(-*p*-ClPhP)dT-3′-*O*-(Lev)[25]	- « -	*N*-Hydroxysuccinimdyl 3-(*p*-hydroxyphenyl) propionate[31]
37	pppd[U$^{(5\text{-aa-M})}$pT], 2′-d-{5-[(2-methylthioacetylamido)allyl]uridinyl(3′-phospho-5′)thymidine} 5′-triphosphate	- « -	- « -	*N*-Hydroxysuccinimdyl 2-methylthioacetate[31]
38	pppd[U$^{(5\text{-aa-D})}$pT], 2′-d-{5-[(succinylamido)allyl]uridinyl(3′-phospho-5′)thymidine} 5′-triphosphate	- « -	- « -	Succinic anhydride similar to[31]
39	pppd[U$^{(5\text{-aa-T})}$pT], 2′-d-{5-[(2-hydroxypropionylamido)allyl]uridinyl(3′-phospho-5′)thymidine} 5′-triphosphate	- « -	- « -	Methyl lactate (racemic mixture), pyridine
40	pppd[U$^{(5\text{-aa})}$pT], 2′-d-[5-(aminoallyl)uridinyl(3′-phospho-5′)thymidine] 5′-triphosphate	- « -	- « -	—
41	pppd[U$^{(5\text{-l-Bio})}$pT], 2′-d-{5-[(biotin-6-amidocaproyl)amidoallyl]uridinyl(3′-phospho-5′)thymidine} 5′-triphosphate	- « -	- « -	*N*-Hydroxysuccinimidyl biotin-6-amidocaproate[31]
42	pppd[CpU$^{(5\text{-aa})}$], 2′-d-[cytidinyl(3′-phospho-5′)-5-(aminoallyl)uridine] 5′-triphosphate	5′-*O*-(DMTr)dC^{Bz}-3′-*O*(-*p*-ClPhP), 2′-dU$^{(5\text{-aaTfa})}$-3′-(Ac)[d] similar to[21,25]	- « -	—
43	pppd[CpU$^{(5\text{-l-Bio})}$], 2′-d-{cytidinyl(3′-phospho-5′)-5-[(biotin-6-amidocaproylamido)allyl]uridine} 5′-triphosphate	- « -	- « -	*N*-Hydroxysuccinimidyl biotin-6-amidocaproate[31]

[a] Monomers are commercially available.
[b] Tfa—trifluoroacetyl.
[c] *O*-*p*-ClPhP—*p*-chlorophenylphosphate.
[d] aa—aminoallyl.
[e] ae—2-aminoethyl.

Table 2
Modifications of 5′-triphosphodinucleotides (Scheme 1, compounds **IV**)

Ribose–phosphate backbone	Heterocyclic bases
X = NH, O, S	4-*N*-(Aminoalkyl)cytosine (including 5-Me derivatives)
R = H, OH, N_3, NH_2 (including arabino configuration), OCH_3	5-(Aminoallyl)uracil
R^4 = O, S, CH_3	5-(Aminopropyl)-hydroxymethyluracil
'Locked' nucleosides	6-*N*-(Aminoalkyl)adenine

summarized in Table 1 and 3 (Supplementary data). We find our approach very efficient and capable to produce 5′-triphosphophate derivatives of dinucleotides with high yields and in any quantities independently of the structure of the target compounds.

4. Experimental

4.1. General

Nucleosides, their protected derivatives and some modified protected nucleosides were purchased from ChemGenes Corporation (USA) and NanoTex-C (Novosibirsk, Russia). The other reagents were from Sigma-Aldrich, Inc. (USA). Organic solvents were dried and purified by standard procedures. NMR spectra were acquired on Bruker AM-400 and AV-300 instruments (Bruker, Germany) in appropriate deuterated solvents at 30 °C. Chemical shifts (δ) are reported in ppm relative to TMS signals. In the case of ^{31}P an external standard of 85% H_3PO_4 was used. Coupling constants *J* are reported in Hertz. MALDI-TOF mass spectra were run on Reflex III (Bruker Daltonics, Germany) in positive detector mode with dihydroxybenzoic acid as a matrix.

Preparative silica gel chromatography was performed on Kieselgel 55–100 μm (Merck, Germany). Preparative reverse phase chromatography was performed on Porasil C_{18}, 55–100 mkm, (Waters, USA). Semi-preparative reverse phase HPLC was performed using the Bruker ChromStar Chromatography system (Bruker, Bremen, Germany) and a 1 × 25 cm column packed with LiChroprep C18 (15–25 μm) (Merck, Darmstadt, Germany). Thin layer chromatography was performed using Alufolien Kieselgel 60 F_{254} plates (Merck, Darmstadt, Germany) in the appropriate solvent mixtures and visualised by UV irradiation, ninhydrin (amine groups) or cystein/aqueous sulfuric acid (nucleoside). All evaporations were performed under reduced pressure. The analytical anion-exchange chromatography was performed on Milichrom-4 chromatograph (Econova, Novosibirsk, Russia) using a 2.5 × 60 mm column packed with Polisil SA, 15 μm (Vector, Novosibirsk, Russia). A linear gradient (flow rate 100 μl/min) from 0 to 0.4 M of K_2HPO_4/KH_2PO_4 (pH 7.0) in 30% aqueous acetonitrile was employed.

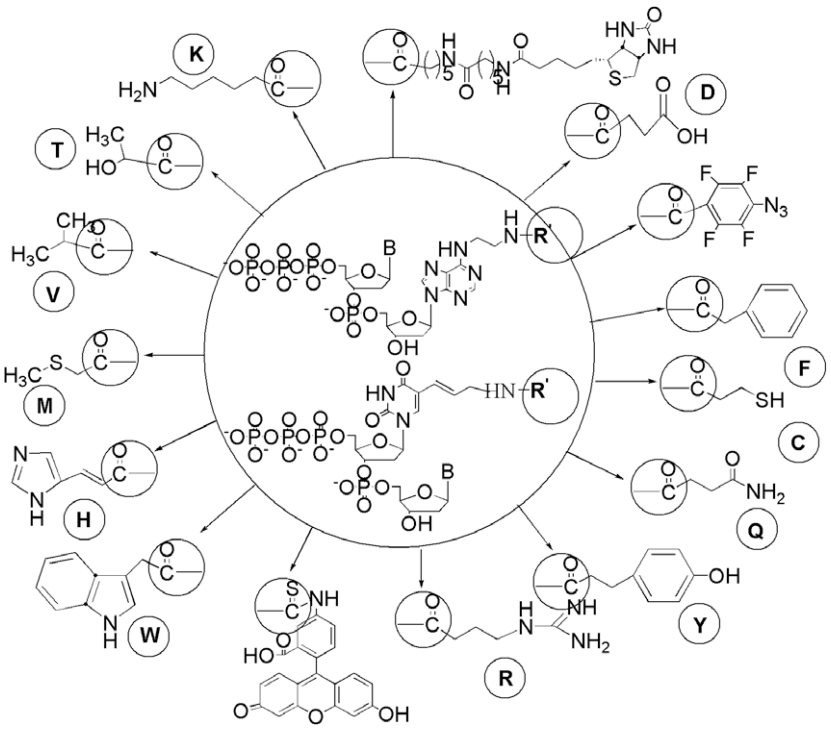


Figure 2. Post-synthetic modification of the 5′-triphosphodinucleotides containing the aliphatic amine group joined to heterocyclic bases. Residues mimicking natural amino acid side radicals are designated by the corresponding character notation.

4.2. A general procedure for the phosphotriester condensation of the monomers I and II and the removal of the DMTr group (Scheme 1)

Monomers **I** (nucleotide component, 0.12 mmol) and **II** (nucleoside component, 0.1 mmol) were dissolved in anhydrous pyridine (1 mL). Triisopropylbenzenesulfonyl chloride (91 mg, 0.36 mmol) and the 1-methylimidazole (60 μL, 0.72 mmol) were added to the reaction mixture. After 1 h, the reaction was stopped by the addition of several drops of 5% aqueous $NaHCO_3$, and the reaction mixture was evaporated. The residue was distributed between 20 mL of dichloromethane and 20 mL of 5% aqueous $NaHCO_3$. The organic layer was separated, dried with Na_2SO_4, filtered and evaporated several times with water to remove traces of pyridine. The residue was dissolved in 80% aqueous acetic acid (5 mL). After 2 h, the reaction mixture was chilled in an ice bath; the solution was carefully neutralized with the concentrated aqueous ammonia till pH 7.0. The reaction mixture was evaporated; the dry residue was suspended in acetonitrile and filtered. The precipitate was washed with acetonitrile and methylene chloride. The filtrate was evaporated; the residue was dissolved in methylene chloride (2 mL). The target product was purified by silica gel chromatography. Elution was performed with a linear gradient of acetone in dichloromethane (the final concentration of acetone in the eluting solution depended on a particular compound **III**, usually 50–70%). Appropriate fractions were pooled and evaporated to give the suitably protected dimer **III** as a dry powder. The yield was 75–80%.

4.3. A general procedure for the phosphorodiimidazolide condensation

The solution of the 5′-(*O*-DMTr)-protected nucleoside with the free 3′-OH group (0.1 mmol) in pyridine (0.1 ml) was added gradually to the solution of methyphosphonodichloride (16 mg, 0,12 mmol) and imidazole (41 mg, 0.6 mmol) in dry acetonitrile with stirring. After 2 h, the solution of the 3′-(*O*-Ac)-protected nucleoside with the free 5′-OH group (0.1 mmol) in pyridine (0.1 mL) and tetrazole (21 mg, 0.3 mmol) were added to the reaction mixture. After 1 h, the reaction was stopped by the addition of several drops of 5% aqueous $NaHCO_3$, and the reaction mixture was subjected to the standard treatment as described above. The yield was 50–60%.

4.4. A general procedure for the phosphoroamidite condensation in solution

Thoroughly dried phosphoramidite component of the reaction (0.05 mmol), nucleoside component (0.05 mmol) and tetrazole (14 mg, 0.2 mmol) were dissolved in dry acetonitrile (0.5 mL). The reaction mixture was stirred for 30 min, then a 1% iodine solution in pyridine/H_2O = 98:2 (1 mL) was added. After 15 min, several drops of 5% aqueous $Na_2S_2O_3$ were added, and the reaction mixture was subjected to the standard treatment as described above. The yield was 50–60%.

4.5. A general procedure for the introduction of the 5′-triphosphate group into the dinucleotides

The appropriately protected dinucleoside with only the 5′-hydroxyl function free (compound **III**, Scheme 1, 0.1 mmol) was dissolved in anhydrous pyridine (1 mL) and the solution was chilled in an ice bath. Phosphorous oxychloride (0.018 mL, 0.2 mmol) was then added to the solution. The reaction mixture was stirred for 20 min and then was transferred to the mixture of 0.5 M solution of the bis(tetra-*n*-butylammonium) pyrophosphate in acetonitrile (1 mL) and tributylamine (1 mmol, 0.23 mL) under vigorous stirring. The cooling was removed and the reaction mixture was stirred 30 min at room temperature. 1 M TEAB buffer pH 7.5 (30 mL) was then added. After 1 h, the reaction mixture was carefully evap-

orated. The residue was dissolved in water and the product was purified by the reverse phase chromatography in the gradient of ethanol in water 0–50%. Appropriate fractions were pooled and evaporated. The resulting protected 5′-triphosphodinucleotide was subjected to the standard deprotection protocols (ammonia treatment for the acyl, 2-cyanoethyl and *p*-chlorophenyl protective groups, F^- treatment for the silyl protective group if necessary). After the deprotection was complete, the target 5′-triphosphodinucleotides (compounds **IV**, Scheme 1, Table 1) were purified by the reverse phase HPLC with the gradient of acetonitrile in water (the final concentration of acetonitrile in the gradient mixture depended on the hydrophobicity of the target product, usually 10–30%) in the presence of 0.1 M TEA–HOAc, pH 7.0. Appropriate fractions were pooled and evaporated. Compounds **IV** were precipitated as lithium salts as described earlier,[20] yield 0.05–0.08 mmol (50–80%).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.09.029.

References and notes

1. Lönnberg, H. *Annu. Rep. Prog. Chem. Sect. B* **2001**, *97*, 177.
2. Vaijayanthi, B.; Kumar, P.; Ghosh, P. K.; Gupta, K. C. *Indian J. Biochem. Biophys.* **2003**, *40*, 377.
3. Sproat, B. S. *J. Biotechnol.* **1995**, *41*, 221.
4. Efimov, V. A.; Chakhmakhcheva, O. G. *Collect. Czech. Chem. Commun.* **2006**, *71*, 929.
5. Efimov, V. A.; Chakhmakcheva, O. G.; Wickstrom, E. *Nucleosides Nucleotides Nucleic Acids* **2005**, *24*, 1853.
6. Summerton, J. *Lett. Pept. Sci.* **2003**, *10*, 215.
7. Ohbayashi, T.; Kuwahara, M.; Hasegawa, M.; Kasamatsu, T.; Tamura, T.; Sawai, H. *Org. Biomol. Chem.* **2005**, *3*, 2463.
8. 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.
9. Li, Z.; Bai, X.; Ruparel, H.; Kim, S.; Turro, N. J.; Ju, J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 414.
10. Schoetzau, T.; Langner, J.; Moyroud, E.; Roehl, I.; Vonhoff, S.; Klussmann, S. *Bioconjugate Chem.* **2003**, *14*, 919.
11. Schubert, J.; Kurreck, J.. In *Handbook of Experimental Pharmacology*; Springer-Verlag: Berlin Heidelberg, 2006; Vol. 173. p 261.
12. Ordoukhanian, F.; Joyce, G. F. *J. Am. Chem. Soc.* **2002**, *124*, 12499.
13. Kwok, P.-Y.; Chen, X. *Curr. Issues Mol. Biol.* **2003**, *5*, 43.
14. Khodyreva, S. N.; Lavrik, O. I. *Curr. Med. Chem.* **2005**, *12*, 641.
15. Kless, H. WO 01/16366, 2001; *Chem. Abstr.* **2001**, *134*, 217979.
16. Abramova, T. V.; Vasileva, S. V.; Kasatkina, N. S.; Belousova, E. A.; Lebedeva, N. A.; Lavrik, O. I.; Silnikov, V. N. Proceedings of the International Conference 'Physical Chemical Biology'. July 30–August 3, 2006, Novosibirsk, Russia, 60–61.
17. Tomasz, J.; Simoncsits, A.; Kajtar, M.; Krug, R. M.; Shatkin, A. J. *Nucleic Acids Res.* **1978**, *5*, 2945.
18. Seto, Y.; Abe, K.; Itoh, M.; Sawai, H. *Bioconjugate Chem.* **2002**, *13*, 303.
19. Lebedev, A. V.; Koukhareva, I. I.; Beck, T.; Vaghefi, M. M. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1403.
20. Abramova, T. V.; Vasileva, S. V.; Serpokrylova, I. Yu.; Kless, H.; Silnikov, V. N. *Bioorg. Med. Chem.* **2007**, *15*, 6549.
21. Abramova, T. V.; Vasil'eva, S. V.; Ivanova, T. M.; Shishkin, G. V.; Silnikov, V. N. *Russ. J. Bioorg. Chem.* **2004**, *30*, 224.
22. Vasil'eva, S. V.; Abramova, T. V.; Ivanova, T. M.; Shishkin, G. V.; Sil'nikov, V. N. *Russ. J. Bioorg. Chem.* **2004**, *30*, 234.
23. Vasileva, S. V.; Krasnousova, E. E.; Donina, A. A.; Abramova, T. V.; Zhdanova, L. G.; Kovalenko, S. P.; Silnikov, V. N. *Russ. Chem. Bull.* **2006**, *55*, 1677.
24. Brown, D. M.. In *Methods in Molecular Biology*; Agrawal, S., Ed.; Humana Press: Totowa, NJ, 1993; Vol. 20, pp 1–80.
25. Abramova, T. V.; Komarova, N. I.; Mundus, D. A.; Pereboeva, O. S. *Izv. Sib. Otd. Akad. Nauk, Ser. Khim.* **1990**, *5*, 45.
26. Miller, P. S.; Reddy, M. R.; Murakami, A.; Blake, K. R.; Lin, S.-B.; Agris, C. H. *Biochemistry* **1986**, *25*, 5092.
27. Ludwig, J. *Acta Biochim. Biophys. Acad. Sci. Hung.* **1981**, *16*, 131.
28. Abramova, T. V.; Bakharev, P. A.; Vasilyeva, S. V.; Silnikov, V. N. *Tetahedron Lett.* **2004**, *45*, 4361.
29. Wlassoff, W. A.; Dobrikov, M. I.; Safronov, I. V.; Dudko, R. Y.; Bogachev, V. S.; Kandaurova, V. V.; Shishkin, G. V.; Dymshits, G. M.; Lavrik, O. I. *Bioconjugate Chem.* **1995**, *6*, 352.
30. Aurup, H.; Williams, D. M.; Eckstein, F. *Biochemistry* **1992**, *31*, 9636.
31. Sinha, N. D.; Striepeke, S. In *Oligonucleotides and Analogues: A Practical Approach*; Eckstein, F., Ed.; IRL Press at Oxford University Press: Oxford, New York, Tokyo, 1991; pp 185–210.