CHEMISTRY AND TECHNOLOGY OF MEDICINAL COMPOUNDS AND BIOLOGICALLY ACTIVE SUBSTANCES

ХИМИЯ И ТЕХНОЛОГИЯ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ И БИОЛОГИЧЕСКИ АКТИВНЫХ СОЕДИНЕНИЙ

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RESEARCH ARTICLE

Study of the multiple incorporation of modified nucleotides into the growing DNA strand

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Abstract

Objectives. *This study investigated the substrate properties of the modified derivatives of triphosphates of purine and pyrimidine deoxynucleosides (5-propynyl-2'-deoxyuridine-5'-triphosphate, 5-propynyl-2'-deoxycytidine-5'-triphosphate, 5-methyl-2'-deoxycytidine-5'-triphosphate, and N^6-methyl-2'-deoxyadenosine-5'-triphosphate) during their simultaneous incorporation in enzymatic reactions (polymerase chain and primer extension reactions).*

Methods. *The real-time polymerase chain and primer extension reactions were used to study the substrate efficiency of modified deoxynucleotide triphosphates. Various pairwise combinations of modified derivatives were used; specially designed synthetic DNA fragments and libraries for the Systematic Evolution of Ligands by Exponential Enrichment technology were used as templates. Reactions were conducted using DNA polymerases: Taq, Vent (exo-), DeepVent (exo-), and KOD XL.*

Results. *In each case, a pair of compounds (modified dUTP + dCTP, dUTP + dATP, and dCTP + dATP) was selected to study the simultaneous incorporation into the growing DNA strand. The most effective combinations of nucleotides for simultaneous insertion were dU and dC, having 5-propynyl substitution. The Vent (exo-) DNA polymerase was found as the most effective for the modified substrates.*

Conclusions. *The selected compounds can be used for the enzymatic preparation of modified DNA, including aptamers with extended physicochemical properties.*
Keywords: *modified aptamers, modified nucleotides, primer extension reaction, real-time polymerase chain reaction*

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НАУЧНАЯ СТАТЬЯ

Изучение множественного встраивания модифицированных нуклеотидов в растущую цепь ДНК

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Аннотация

Цели. *Целью данной работы является изучение субстратных свойств модифицированных производных трифосфатов дезоксинуклеозидов пуриновой и пиримидиновой природы (5-пропинил-2'-дезоксиуридин-5'-трифосфат, 5-пропинил-2'-дезоксицитидин-5'-трифосфат, 5-метил-2'-дезоксицитидин-5'-трифосфат, N⁶-метил-2'-дезоксиаденозин-5'-трифосфат) при их одновременном встраивании в процессе ферментативных реакций (полимеразной цепной реакции и реакции удлинения праймера).*
Методы. *В работе для изучения субстратной эффективности модифицированных трифосфатов дезоксинуклеозидов использовали методы полимеразной цепной реакции в режиме реального времени и реакции удлинения праймера. Использовали различные попарные сочетания модифицированных производных, в качестве матриц применяли специальным образом сконструированные синтетические фрагменты ДНК и библиотеки для SELEX. Реакции проводили с применением ДНК-полимераз: Taq, Vent (exo-), DeepVent (exo-) и KOD XL.*
Результаты. *В каждом случае из исследуемых соединений выбирали пару соединений (модифицированные dUTP + dCTP, dUTP + dATP, dCTP + dATP) для изучения одновременного встраивания в растущую цепь ДНК. Найдены наиболее эффективные сочетания нуклеотидов для одновременного встраивания, а именно: dU и dC, имеющие 5-пропинильный заместитель. Также найдена наиболее эффективная (из протестированных) ДНК-полимераза: Vent (exo-).*
Выводы. *Выбранные соединения можно использовать для ферментативного получения модифицированных ДНК, в частности аптамеров с расширенными физико-химическими свойствами.*

Ключевые слова: *модифицированные нуклеотиды, модифицированные аптамеры, полимеразная цепная реакция в режиме реального времени, реакция удлинения праймера*

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INTRODUCTION

Modified nucleic acids are currently used in many fields: molecular biology [1], therapy [2], diagnostics [3], and analytical chemistry [4]. Because of their chemical diversity, modified nucleic acids can be used to introduce fluorescent labels into samples and select aptamers [5, 6]. One way to obtain modified nucleic acids is the enzymatic synthesis of DNA or RNA polymerases using modified 2-deoxynucleoside triphosphates (dNTPs) as substrates [7, 8]. Modifications using functional groups (e.g., analogs of amino acid side chains) allow increasing the affinity of aptamers to protein targets [9].

Aptamers are short, single-stranded DNA or RNA molecules that can interact with target molecules. The production of aptamers is performed using the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) technology. Modifying the aptamers' structure improves their physicochemical properties and binding to target molecules [10]. For introducing modifications, the enzymatic method is most often used (in most cases, the primer extension reaction is used) using modified dNTP derivatives. Producing aptamers containing different functional groups can increase their affinity for their targets because of the greater variety of types of aptamer–target interactions.

Early studies report that the simultaneous incorporation of nucleotides with different modifications can improve the properties of the resulting modified aptamers [11]. The main concern when using the enzymatic method is the modified derivatives' substrate compatibility with DNA polymerases.

In this paper, we evaluated the effectiveness of two enzymatic methods—polymerase chain reaction (PCR) and primer extension reaction (PEX)—and compared the effectiveness of different polymerases with no 3'–5' correcting exonuclease activity for the pairwise simultaneous incorporation of modified nucleotides in one growing DNA chain.

EXPERIMENTAL

Modified analogs of dNTPs: All compounds are manufactured by *TriLink BioTechnologies, Inc.* (San Diego, CA, USA): N-2016, N-2017, N-2025, and N-2026.

DNA templates: Synthetic DNA templates with a length of 49 nt were used for PEX, the sequences of which are given below:

M1U 5'-CT**AAAA**CTCT**AAA**CTCT**AA**CTCT**A**CT-*GGCTACCAGTATGGAGCTGACAG*-3'

M1A 5'-CT**TTTT**CTCT**TTT**CTCT**TT**CTCT**T**CT-*GGCTACCAGTATGGAGCTGACAG*-3'

M2UA 5'-CT**TATA**CTCT**ATA**CTCT**TA**CTCT**A**CT-*GGCTACCAGTATGGAGCTGACAG*-3'

M2AU 5'-CT**ATAT**CTCT**TAT**CTCT**AT**CTCT**T**CT-*GGCTACCAGTATGGAGCTGACAG*-3'

The synthetic templates used to study the incorporation of modified dCs have been studied earlier [12].

The nucleotides complementary to the ones under study are marked in bold. The primary areas are highlighted in italics. The sequence of the primer used is the following:

5'-CTGTCAGCTCCATACTGGTAGCC-3'

A combinatorial DNA library and corresponding primers were used for PCR [13].

DNA polymerases used: The following polymerases were used: Taq (*Thermo Scientific*, Waltham, MA, USA), Vent (*exo*-), Deep Vent (*exo*-) (*New England Biolabs*, Ipswich, MA, USA), and KOD XL (NovaTaq™, *Merck KGaA*, Darmstadt, Germany). Polymerases were used in reaction buffers and in concentrations recommended by the manufacturers.

Solid-phase synthesis of template oligonucleotides

The solid-phase synthesis of template oligonucleotides was conducted using an automatic synthesizer ABI 394 DNA/RNA (*Applied Biosystems*, Foster City, CA, USA) according to standard regulations on using commercial solvents and reagents.

Chromatographic purification of template oligonucleotides

For the chromatographic purification of oligonucleotides, a BDS Hypersil C18 (*Thermo Scientific*) column with a size of 250 × 4.6 mm and a particle size of 5 μm was used in the eluent system: buffer A contained 0.1-M TEAA, and buffer B is 50% acetonitrile in buffer A. Both buffers were prepared using Milli-Q and CH_3CN for high-performance liquid chromatography (ChromAR® HPLC, MACRON), filtered using a ZAPCAP-CR Nylon filter (0.22-μm pore size, 47-mm diameter; *Sigma-Aldrich*, St. Louis, MO, USA). The products were separated at a temperature of 25°C. The eluent feed rate is 1 mL/min. The detection was conducted at two wavelengths: λ_1 = 270 nm and λ_2 = 295 nm. BD Syringes 1 mL, without needle (*Becton Dickinson*, Franklin Lakes, NJ, USA). Replacement filters for Acrodisc® LC, 13-mm syringes with a 0.2-μm polyvinylidene fluoride (PVDF) membrane, HPLC certified (*PALL Corporation*, NY, USA).

Primer extension reaction

The reaction mixture contained natural dATP and dGTP (when studying the incorporation of modified deoxyadenosine to natural dGTP and dCTP) at a concentration of 0.2 mM each, as well as various combinations of the dNTPs indicated in Fig. 1; 1.5-U Taq-or 0.5-U Vent (*exo*-) DNA polymerase (the reaction buffer corresponded to the

applied polymerase); a primer for PEX; and one of the synthetic templates. The reaction was performed using a MiniCycler DNA amplifier (*MJ Research Inc.*, Hercules, CA, USA) according to the following program: 5 min at 95°C, 30 s at 65°C, and 40 min at 78°C.

Real-time PCR

We used a mixture similar to that used for the primer extension reaction and two flanking primers instead of one in the case of PEX. The dye EvaGreen (*Biotium*, Moscow, Russia) was added to the reaction mixture to visualize the process. Amplification was performed using an IQ5 device (*Bio-Rad Laboratories*, Inc., Hercules, CA, USA) according to the following program: after preheating at 95°C for 3 min, 40 cycles were performed at 95°C for 20 s, 66°C for 30 s, and 72°C for 40 s. After that, the final incubation was done at 72°C for 5 min.

Determination of chemical yield

The resulting PCR products were separated in a 4% agarose gel. Staining was performed using ethidium bromide. The amounts of the products were estimated from the optical density of the corresponding bands in the gel tracks using the ImageJ program (National Institutes of Health, USA).

RESULTS AND DISCUSSION

Previously, we studied the patterns of the simultaneous incorporation of pyrimidine nucleotides into DNA with complete replacement of the corresponding natural dNTPs [12]. Derivatives with less bulk functional groups have been shown to be better substrates for DNA polymerases. Probably, in the case of the use of electroneutral modifying groups, steric factors play the main role in the formation of a catalytically active “closed” conformation of DNA polymerase in the formation of a complex with a substrate [14, 15, 16].

In this study, we investigated the substrate properties of modified dNTPs (mod-dUTP, mod-dCTP, and mod-dATP) with extended sets of DNA polymerases. Modifications were introduced into the heterocyclic bases of compounds to provide structural differences and possibly create pairs with similar modifications. Nucleotides of purine and pyrimidine nature with similar modifications cannot be considered as complete analogs. Still, their comparison is of interest in identifying the patterns of influence of modifying groups.

The structures of the compounds used are shown in Fig. 1.

PCR and PEX were performed with complete substitution of dTTP, dCTP, and dATP using various pairwise combinations of the modified derivatives. DU + dC, dU + dA, and dC + dA pairs with different or identical modifications were created.

We used a combinatorial DNA library as a template for PCR, which we used earlier in preparing aptamers using SELEX [17], because the substrate

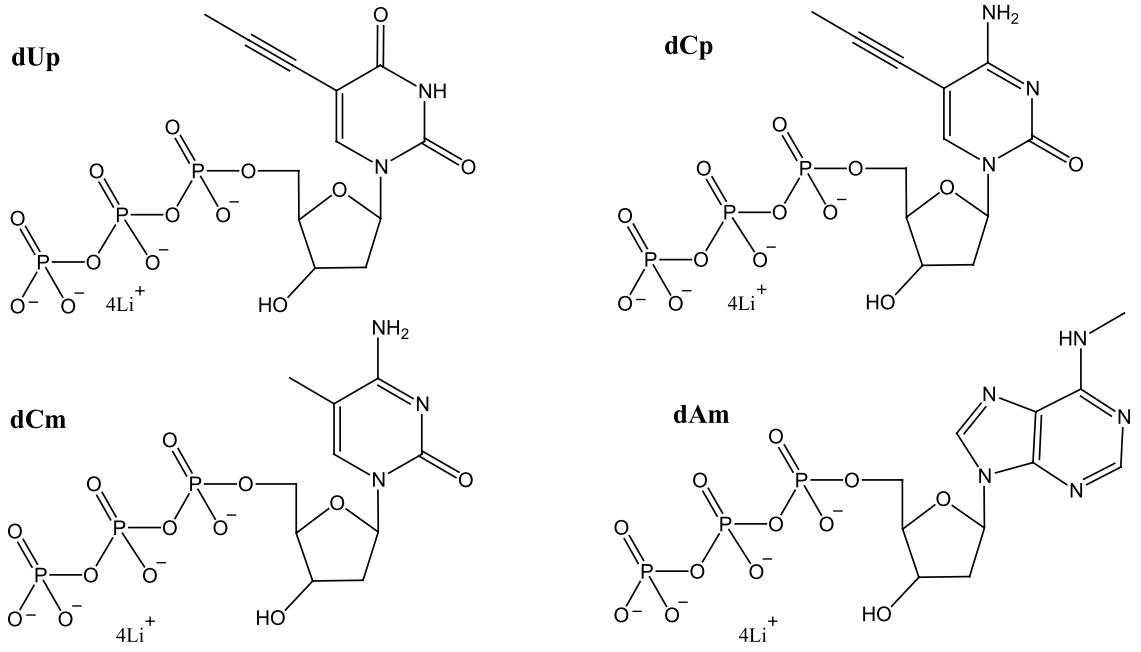

Fig. 1. Modified triphosphates of deoxyuridine, deoxycytidine, and deoxyadenosine.
dUp and dCp are derivatives containing 5-propynyl; dCm and dAm are derivatives containing a methyl group in the 5 and N^6 positions, respectively.

behavior of the modified substrates of polymerases with such template is of particular interest for producing aptamers with new properties (Fig. 2). The most complex substrate for all polymerases was dA with a methyl substituent (dAm). For this substrate, obtaining a product using only the Deep Vent polymerase was possible, but many incomplete products were obtained. When dC (dCm) and dA (dAm) with methyl substituents were simultaneously embedded, the formation of a full-size product was observed.

Using the Taq polymerase with none of the selected modified dNTPs, obtaining full-size products was impossible, except for the variant with a combination of propinyl dU (dUp) and methyl dA (dAm). Both full- and half-size products were formed. The KOD XL polymerase with none of the modified substrates used formed a full-length product, except for dUp, but even in this case, the formation of two products of different lengths was observed.

The Vent (*exo*-) polymerase in this experiment proved to be the most effective enzyme for all the selected combinations, except for dAm. Products could not be obtained using this substrate, but in combination with dCp and dUp, full-size products were reproducibly formed with high efficiency calculated from real-time PCR results.

The PCR analysis showed that the most complex substrates for all the polymerases used are modified derivatives with a methyl substituent. Interestingly, combining triphosphates with propinyl and methyl substituents resulted in a better substrate efficiency than those of derivatives with only a methyl substituent. It should also be noted that polymerases better perceive substrates that have a pyrimidine nature.

We synthesized artificial template oligonucleotides to study the individual and simultaneous integration of dU and dC into one growing DNA chain by the PEX method [12]. Additional ones (the sequences are indicated in the Experimental section) were constructed to the existing synthetic templates to study the patterns of embedding of modified deoxyadenins (nucleotides of purine nature), aimed at understanding the multiple sequential incorporation of modified dA both individually and in pairs with different modified nucleotides of the dU type.

The template M1A is designed to sequentially study the individual incorporation of modified dA 1, 2, 3, and 4 times during primer extension along the template chain. Conducting PEX allows evaluating the effectiveness of the multiple sequential incorporation of mod-dA. It can be seen that the spacer regions of the template do not contain complementary deoxyadenin nucleotides (dT). In addition, compared with the results of pairwise incorporation with dU (on the M2UA and M2AU templates), the spacer plots also do not contain dA.

The M2UA and M2AU templates are designed to study the multiple pairwise incorporation of dA and dU in different sequences, which is reflected in the names of the template oligonucleotides.

In PEX with synthetic templates (Fig. 3), the Taq and Vent (*exo*-) and Deep Vent polymerases could embed both deoxyuridine with a propyl substituent and deoxyadenosine with a methyl substituent.

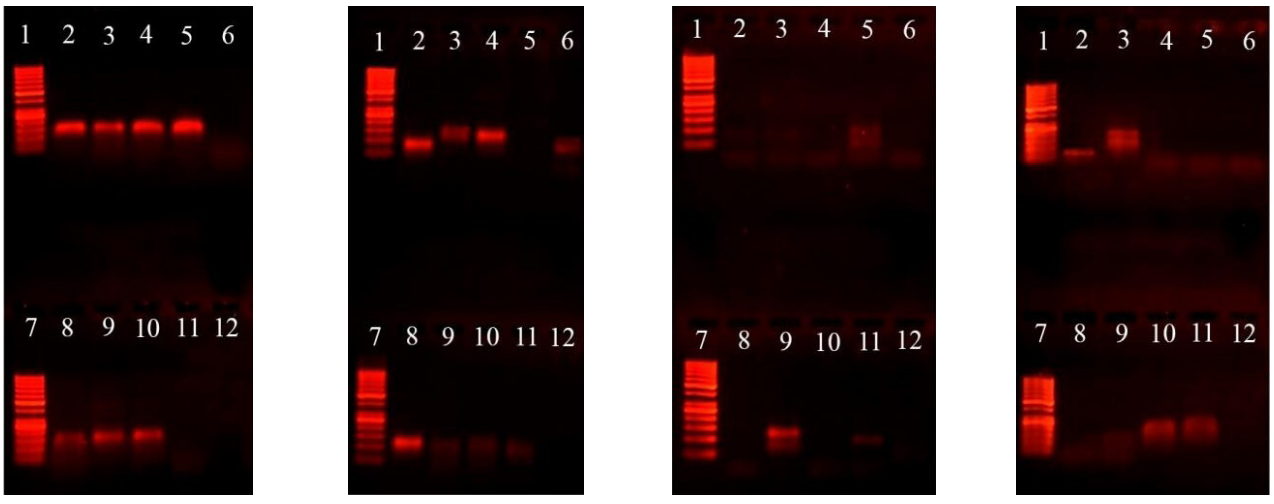

Fig. 2. Polymerase chain reaction electrophoretic analysis using different DNA polymerases and a DNA library.
In each figure: (1) and (7) are GeneRuler 50bp DNA ladder, (2) dT + dC, (3) dUp, (4) dCp, (5) dCm, (6) dAm, (8) dUp + dCp, (9) dUp + dAm, (10) dCp + dAm, (11) dCm + dAm, and (12) negative control.

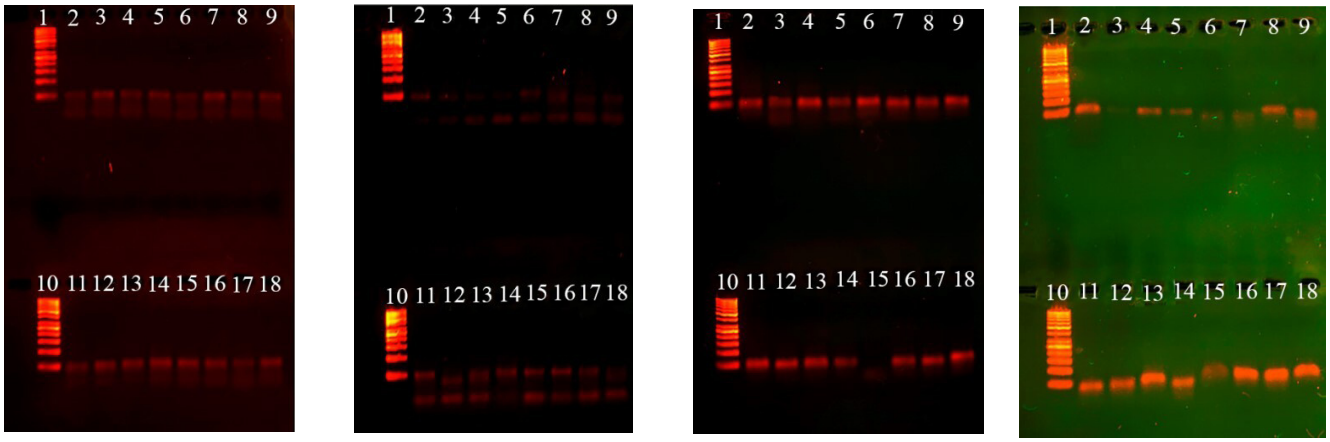

Fig. 3. PEX electrophoretic analysis with various DNA polymerases and specially designed templates. In each figure: (2–5) matrix M1U; (6–9) matrix M1A; (11–14) matrix M2UA; (15–18) matrix M2AU; (1) and (10) length marker for GeneRuler products 50 bp; (2, 6, 11, and 15) dT + dA; 3, 7, 12, and 16) dUp; (4, 8, 13, and 17) dAm; (5, 9, 14, and 18) dUp + dAm.

When using the KOD XL polymerase, products that were somewhat different in mobility were formed, presumably not fully corresponding to the theoretical length of the full-size product. This may be due to the mobility of the modified products in the gel.

It is shown that under the conditions used and for the pairs of modified substrates under study, PEX leads to the more efficient formation of target products than that of PCR. This is due to the slower incorporation kinetics of the modified substrates than those of natural oligonucleotides. Therefore, the advantage of PEX is the length of the voluion time (significantly higher than that in PCR). The results correlate well with previously obtained data [12] and the world practice of using PEX for producing modified aptamers [18].

CONCLUSIONS

The substrate efficiency of the modified compounds used in this study depends on the chemical nature of the modification (massive or compact substituents) and the nucleotide used (purine or pyrimidine bases) and varies for different DNA polymerases. In addition, the enzymatic reaction used significantly affects the formation of full-size modified products. Thus, because of its long elongation time (elongation), the primer extension reaction has an advantage over PCR.

Of the tested compounds, modified dU and dC (i.e., pyrimidine nucleotides) combined with the Vent (*exo*-) DNA polymerase showed the greatest efficiency.

As a result, we obtained DNA modified simultaneously by pairs of nucleotide derivatives of both purine and pyrimidine types. These studies are important for producing DNA with multiple modifications, including a new generation of aptamers.

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Authors' contribution

O. S. Volkova – conducting research, preparation of the manuscript;

A.V. Chudinov – academic advising;

S.A. Lapa – design of experiments, editing the manuscript.

The authors declare no conflicts of interest.

REFERENCES

1. Lee K., Rafi M., Wang X., Aran K., Feng X., Lo Sterzo C., *et al*. *In vivo* delivery of transcription factors with multifunctional oligonucleotides. *Nat. Mater.* 2015;14(7):701–706. https://doi.org/10.1038/nmat4269

2. Smith C.I.E., Zain R. Therapeutic oligonucleotides: state of the art. *Annu. Rev. Pharmacol. Toxicol.* 2019;59:605–630. https://doi.org/10.1146/annurev-pharmtox-010818-021050

3. Wandtke T., Wozniak J., Kopinski P. Aptamers in diagnostics and treatments of viral infections. *Viruses.* 2015;7(2):751–780. https://doi.org/10.3390/v7020751

4. Peinetti A.S., Cerertti H., Mizrahi M., Gonzales G.A., Ramires S.A., Requejo F., *et al*. Confined gold nanoparticles enhance the detection of small molecules in label free impedance aptasensors. *Nanoscale.* 2015;7:7763–7769. https://doi.org/10.1039/C5NR01429H

5. Faltin B., Zengerle R., von Stetten F. Current methods for fluorescence-based universal sequence-dependent detection of nucleic acids in homogenous assays and clinical applications. *Clin.Chem.* 2013;59 (11):1567–1582. https://doi.org/10.1373/clinchem.2013.205211

6. Maier K.E., Levy M. From selection hits to clinical leads progress in aptamer discovery. *Mol. Ther. Methods & Clin. Develop.* 2016;5:16014. https://doi.org/10.1038/mtm.2016.14

7. Hocek M. Synthesis of base-modified 2′-deoxyribonucleoside triphosphates and their use in enzymatic synthesis of modified DNA for applications in bioanalysis and chemical biology. *J. Org. Chem.* 2014;79(21):9914–992. https://doi.org/10.1021/jo5020799

8. Kutyavin I.V. Use of base-modified duplex-stabilizing deoxynucleoside 5'-triphosphates to enhance the hybridization properties of primers and probes in polymerase chain reaction. *Biochemistry.* 2008;47(51):13666–13673. https://doi.org/10.1021/bi8017784

9. Rohloff J.C., Gelinas A.D., Jarvis T.C., Ochsner U.A., Schneider D.J., Gold L., Janjic N. Nucleic Acid Ligands with Protein-like Side Chains: Modified Aptamers and Their Use as Diaognostic and Therapeutic Agents. *Mol. Ther. Nucleic Acids.* 2014;3(10):e201. https://doi.org/10.1038/mtna.2014.49

10. Tolle F., Mayer G. Dressed for success – applying chemistry to modulate aptamer functionality. *Chem. Sci.* 2013;4(1):60–67. https://doi.org/10.1039/c2sc21510a

11. Gawande B.N., Rohloff J.C., Carter J.D., von Carlowitz I., Zhang C., Schneider D.J., Janjic N. Selection of DNA aptamers with two modified bases. *Proc. Natl. Acad. Sci.* 2017;114(11):2898–2903. https://doi.org/10.1073/pnas.1615475114

12. Chudinov A.V., Shershov V.E., Pavlov A.S., Volkova O.S., Kuznetsova V.E., Zasedatelev A.S., Lapa S.A. Simultaneous incorporation of modified dU and dC derivativesin the growing DNA chain using PEX and PCR. *Bioorg. Khimiya = Bioorg. Chemistry.* 2020;46(5):546–549 (in Russ.). https://doi.org/10.31857/S0132342320050061

13. Lapa S.A., Romashova K.S., Spitsyn M.A., Shershov V.E., Kuznetsova V.E., Guseinov T.O., Zasedateleva O.A., Radko S.P., Timofeev E.N., Lisitsa A.V., Chudinov A.V. Preparation of modified combinatorial DNA libraries via emulsion PCR with subsequent strand separation. *Mol. Biol.* 2018;52(6):854–864. https://doi.org/10.1134/S0026893318060110

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14. Berman A.J., Kamtekar S., Goodman J.L., Lázaro de Vega M., Blanco L., Salas M., Steitz T.A. Structures of phi29 DNA polymerase complexed with subsreate: the mechanism of translocation in B-family polymerases. *EMBO J.* 2007;26(14):3494–3505. https://doi.org/10.1038/sj.emboj.7601780

15. Betz K, Malyshev DA, Lavergne T, Welte W, Diederichs K, Dwyer TJ, Ordoukhanian P, Romesberg FE, Marx A. KlenTaq polymerase replicates unnatural base pairs by inducing a Watson-Crick geometry. *Nat Chem Biol*. 2012;8(7):612–614. https://doi.org/10.1038/nchembio.966

16. Hollenstein M. Nucleoside triphosphates – building blocks for the modification of nucleic acids. *Molecules*. 2012;17(11):13569–13591. https://doi.org/10.3390/molecules171113569

17. Lapa S.A., Shershov V.E., Krasnov G.S., Volkova O.S., Kuznetsova V.E., Radko S.P., Zasedatelev A.S., Chudinov A.V. Method of terminal dissociation for the selection of DNA-aptamers. *Bioorg. Khimiya = Bioorg. Chemistry.* 2020;46(4):411–417 (in Russ.). https://doi.org/10.31857/S0132342320040156

18. Gold L., Ayers D., Bertino J., Bock C., Bock A., Brody E.N., Carter J., Dalby A.B., Eaton B.E., Fitzwater T., Flather D., Forbes A., Foreman T., Fowler C., Gawande B., Goss M., Gunn M., Gupta S., Halladay D., Heil J., Heilig J., Hicke B., Husar G., Janjic N., Jarvis T., Jennings S., Katilius E., Keeney T.R., Kim N., Koch T.H., Kraemer S., Kroiss L., Le N., Levine D., Lindsey W., Lollo B., Mayfield W., Mehan M., Mehler R., Nelson S.K., Nelson M., Nieuwlandt D., Nikrad M., Ochsner U., Ostroff R.M., Otis M., Parker T., Pietrasiewicz S., Resnicow D.I., Rohloff J., Sanders G., Sattin S., Schneider D., Singer B., Stanton M., Sterkel A., Stewart A., Stratford S., Vaught J.D., Vrkljan M., Walker J.J., Watrobka M., Waugh S., Weiss A., Wilcox S.K., Wolfson A., Wolk S.K., Zhang C., Zichi D. Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS One.* 2010;5(12):e15004. https://doi.org/10.1371/journal.pone.0015004

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