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DEVELOPMENT OF A METHOD FOR THE SYNTHESIS OF CPG-OLIGONUCLEOTIDES PROMISING FOR IMMUNOTHERAPY OF CANCER

M.-A. I. Idilov

Idilov M.-A.I.
Biotechnological company «MDinc», Moscow, Russia
E-mail: idilov@mdinc.ru

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This article describes a new method for the synthesis of 2`-O-methoxymethyl monomers applicable for the efficient automated synthesis of 2`-O-modified oligoribonucleotides used for immunotherapy of oncological diseases localized in the gastrointestinal tract.

Introduction

Cancer is the second leading cause of death in the world. Over 9.5 million people died from cancer in 2019. One of the most common methods of cancer treatment is chemotherapy – treatment of a disease by administering a chemotherapeutic agent, which is a poison or toxin, to the patient's body, attacking the cells of a malignant tumor, but also healthy cells of the patient. Due to the lack of selectivity, today one of the important tasks of modern oncology and pharmacology is the development of compounds that inhibit the growth of tumors, selectively affecting cancer cells, without exerting a negative effect on the body. Despite the large number of ongoing studies, the question of finding and using effective anticancer drugs capable of selectively affecting cancer remains open [1, 2].

The main targets of the action of new generation drugs should be specific components of cancer cells necessary for their existence and reproduction. In normal somatic cells, there is a mechanism for controlling proliferation, due to the gradual shortening of the terminal sections of chromosomes, the so-called telomeres, in each cycle of cell division. Cancer cells have the ability to bypass this mechanism and thereby acquire the property of immortality – unlimited reproductive potential [3, 4].

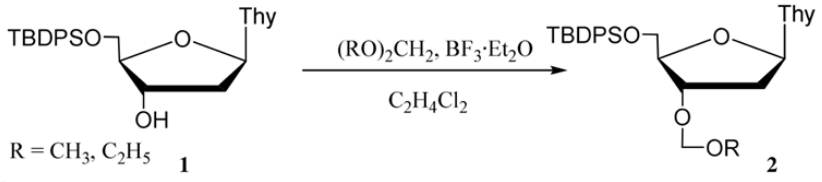
A special group of compounds currently being investigated as a potential drug for anticancer therapy are short nucleotide sequences (oligonucleotides) complementary to the messenger RNA of a protein involved in the development of the disease and capable of inhibiting the translation of the messenger RNA of this protein [5].

Synthesis of ribonucleotide monomers

For the study, it was necessary to develop a method for the synthesis of 2'-O-modified oligoribonucleotides complementary to the immune checkpoints of T-lymphocytes and T-killers of the human body [6].

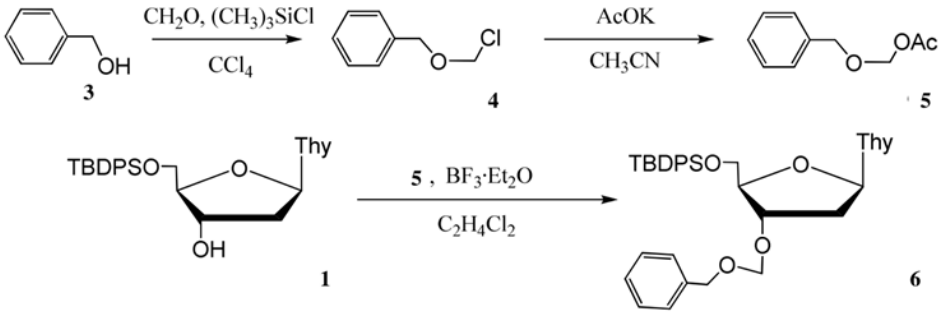
Compound (2) was synthesized in order to select the conditions for the removal of the 3'-hydroxyl function that do not lead to cleavage and migration of internucleotide bonds. For this, 5'-O- (tert-butyldiphenylsilyl) thymidine in the presence of boron trifluoride etherate was treated with dimethoxymethane.

When compound (2) was treated with a 1M LiI solution in acetonitrile in the presence of 0.01M HCl, it was found that this leads to a significant removal of the 3`-O-methoxymethyl group, on the basis of which it was concluded that the treatment of 2`-O-methoxymethyl oligoribonucleotides hydrochloric acid will not lead to their degradation [7].



In the course of the work, the main task was to reduce the cost of synthesis, therefore, for an objective assessment of the choice of the thymidine derivative, it was decided, in addition to 5′,3′-O-protected thymidine derivatives containing a methoxymethyl group, to synthesize two compounds containing an ethoxymethyl and benzyloxymethyl group and, by comparing the time and resources spent on the synthesis, determine the compound that will be used as a monomer for further synthesis.

The introduction of the ethoxymethyl group was carried out by analogy with the introduction of the methoxymethyl group according to Scheme 1 using diethoxymethane. To obtain a compound containing a benzyloxymethyl group, the synthesis was carried out in several stages.

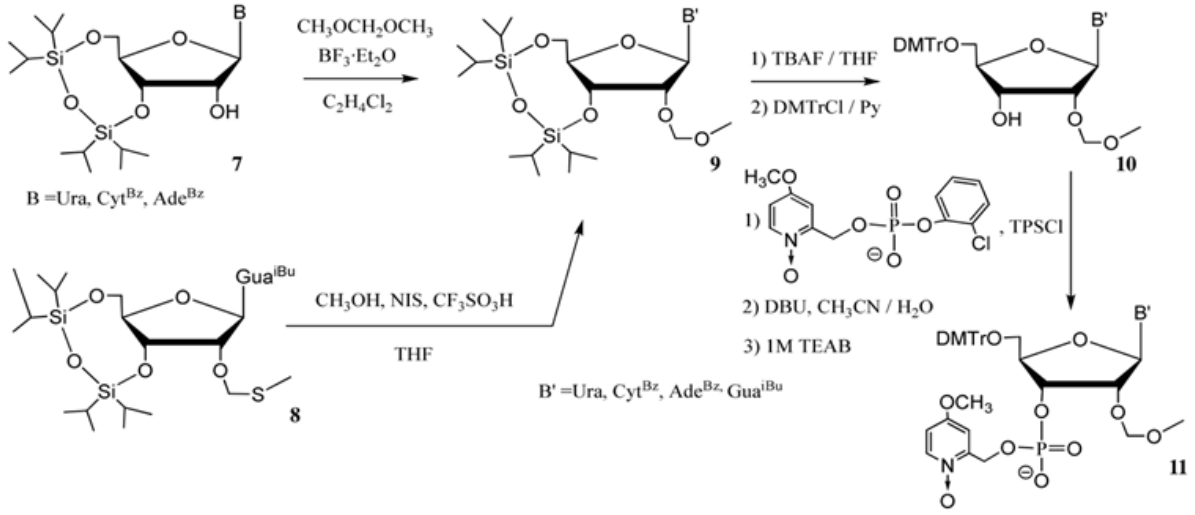


At the first stage, benzyl alcohol (3) was treated for 2 hours with paraform and trimethylchrosilane in carbon trichloride, the resulting a-chloroether (4) was boiled with potassium acetate in acetonitrile, as a result of which acetoxymethylbenzylether (5) was obtained. Further, (1) was condensed with (5) in the presence of boron trifluoride etherate, as a result of which

compound (6) – 5´,3´-O-protected thymidine derivative was obtained, containing a benzyloxymethyl group in the 3 'position [8].

The benzyloxymethyl and ethoxymethyl groups were also removed by treatment with LiI in the presence of 0.01M HCl. Through the experiment, it was found that the rate of their removal turned out to be lower than the rate of removal of the methoxymethyl group, which is associated with its smaller size and in connection with which it was chosen for further synthesis.

To remove completely blocked nucleosides (9), derivatives of uridine, cytidine and adenosine (7) were treated with an excess of dimethoxymethane for 2 hours in the presence of boron trifluoride etherate in 1,2-dichloroethane.



To remove the TIPDS group and obtain methoxymethyl derivatives, we decided to treat the isolated nucleosides (9) with a 1M solution of TBAF and THF for 1 hour. The processing time and the concentration of reagents were determined empirically.

Methoxymethyl derivatives were dimethoxytritylated, and then, the obtained compounds (10) were treated with (4-chlorophenyl)-(1-oxide-4-methoxy-2-picolyl) phosphate and 2,4,6-triisopro-pylbenzenesulfonyl chloride (TPSCl), after which the resulting compound was treated with diazabicycloundecene (DBU) in aqueous acetonitrile.

We found that guanosine derivatives, due to the degradation of bonds between nucleosides, upon treatment with dimethoxymethane formed a large amount of a fluorescent by-product (40%), and therefore, in this case, the synthesis of a 2`-O-methoxymethyl derivative (9) we was carried out by treating the methylthiomethyl derivative (8) with trifluoromethanesulfonic acid and nickel sulfide in a mixture of tetrahydrofuran and methanol (25:1) for half an hour.

Synthesis, unblocking and purification of 2`-O-modified oligoribonucleotides

The synthesis was carried out by the phosphotriester method on an Applied Biosystems synthesizer, model 381A. As resins, we used universal CPG carriers from GR. To assess the efficiency of chain extension, a spectrophotometric measurement of the concentration of the carbocation formed during the reaction was carried out at wavelengths of 478 and 498 nm. The chain elongation cycle was 7.5 minutes and is shown in Table 1.

Table 1. Oligonucleotide chain elongation cycle

Stage	Reagents	Time, min
Detritylation	3% dichloroacetic acid in dichloromethane	1.0
Flushing	Acetonitrile	1.0
Flushing	Acetonitrile-pyridine (3:1, v / v)	0.5
Capping	0.05M monomeric synthon; 0.15M TPSCl in acetonitrile-pyridine mixture (3:1, v / v)	3.0
Flushing	Acetonitrile-pyridine (3:1, v / v)	0.5
Capping	Acetic anhydride - 1-methylimidazole - acetonitrile (1:1:8, v / v)	0.5
Flushing	Acetonitrile	1.0

Upon completion of the chain extension, the P-protective 1-oxide-4-methoxy-2-picolyl group was removed: the process of unblocking of phosphate residues was carried out by treating the oligonucleotide with 1M LiI solution in acetonitrile at room temperature for 3 hours.

2`-O-modified oligoribonucleotides were obtained from the laboratory "Lumiprobe" on a universal CPG-carrier, and for their cleavage from the carrier and unblocking of acyl protecting groups, the product was treated with a mixture of 28% aqueous ammonia and ethanol (3:1) for 4 hours at temperature 60 °C. The N-azidomethylbenzoyl group from the heterocyclic bases of oligoribonucleotides was removed by reaction with 30% trifluoroacetic acid; however, this led to partial chain degradation, which is reflected in the electrophoregram (Fig. 1).

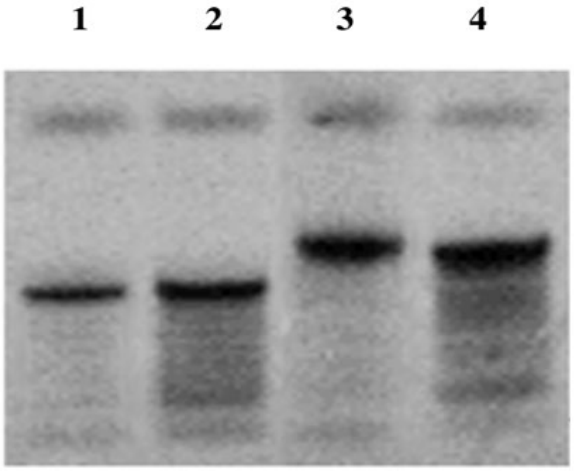


Fig. 1. Electrophoregram of oligonucleotides before (1,3) and after (2,4) their treatment in denaturing 15% solution

As you know, during electrophoresis, DNA fragments migrate in the gel under the influence of an electric field. In this case, the negatively charged sugar-phosphate backbone of the molecule moves towards the positively charged anode. Long molecules migrate in the gel more slowly, and therefore, after the separation of the DNA molecule, when the molecule is visualized using the fluorescent dyes xylencyanol and bromophenol blue in UV rays, shorter molecules that have undergone chain degradation will be located higher than long ones that are not subjected to this the phenomenon of a molecule, as demonstrated in the photograph (Fig. 1) in reflected UV light at 254 nm [9, 10].

The total yield of the product and the sequence of oligonucleotides are presented in Table 2.

Table 2. Sequences and yields of synthesized oligonucleotides

Sequence	Output at the stage of condensation, %	Final output, %
r(UUUUUUUUUUUUUUU)	99.2	56
r(AUGGUGACCGACGCCA)	98.5	51
r(CGCUCUCGUCGCUCUCCAUGU)	97.4	47
r(AAGAAGAGCCUGGAGCCCAUCU)	98.1	50
r(AGAUGGGCUCCAGGCUCUUCUU)	97.6	48
r(GCUCUCGUCGCUCUCCAUG)dTT	98.3	49
r(CAUGGAGAGCGACGAGAGC)dTT	97.7	55
r(UUUUUUUUUUUUUUU)	99.1	59
r(CGAUCUCAUCACCUCUCCAU)	98.9	57

Conclusion

The described method of synthesis, which implies the use of new phospholating reagents containing an O-nucleophilic catalytic P-protective group, can increase the product yield (by 14%) and reduce the synthesis time by half. Also, the synthesized prototypes are complementary to human DNA, which makes them promising for further research as a medicinal component of cancer immunotherapy.

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