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LETTERS TO THE EDITOR

An Azidomethyl Protective Group in the Synthesis of Oligoribonucleotides by the Phosphotriester Method

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Abstract—A rapid and effective method of an automatic oligoribonucleotide synthesis alternative to the phosphoramidite one was developed. This method is based on the phosphotriester approach to internucleotide bond formation under intramolecular *O*-nucleophilic catalysis and the use of an azidomethyl group for protection of a nucleotide 2'-hydroxyl function.

Key words: azidomethyl group, rapid phosphotriester method, oligoribonucleotides, synthesis

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INTRODUCTION

Natural and modified oligonucleotides are universal tools for the solution of a wide range of problems in molecular biology, gene engineering, biotechnology, and medicine.[2] Recently, synthetic RNA fragments, particularly, ribozymes and small double-stranded RNA interferences, which are widely used in functional genomics and regarded as potential drugs, have attracted the interest of numerous research groups. Therefore, the necessity of the development of effective methods of chemical synthesis of natural RNA fragments and their analogues with predetermined properties has increased.

Until now, the phosphoramidite method was the only well-developed method of oligoribonucleotide synthesis [1]. However, due to its chemical specific features it cannot be used for the synthesis of some modified oligonucleotides, particularly derivatives containing strong electrophilic centers (for example, azide or *N*-oxide groups) or stereospecific phosphorothioate analogues of nucleic acids.

The phosphotriester method involving *O*-nucleophilic intramolecular catalysis at the stage of internucleotide bond formation is an alternative approach to the oligoribonucleotide synthesis. Earlier, it was successfully used for the synthesis of natural oligodeoxyribonucleotides [2] as well as modified oligonucleotides containing 2'-azido- and 2'-amino-2'-deoxyuridine residues [3]. In addition, the potential of this method for preparation of stereospecific oligonucleotide phosphorothioate analogues was shown [4, 5].

Unlike deoxyribonucleotides, ribonucleotides contain additional 2'-OH groups, which considerably hampers synthesis of their oligomers and makes the choice of protective groups for this function one of the crucial steps of the oligoribonucleotide synthesis. A protective 2'-OH group must be stable both in the process of chain elongation and upon removal of other protective groups, and must be removed at the last step under conditions preventing degradation of the resulting oligoribonucleotide. For blocking 2'-OH groups in the phosphoramidite method, a tert-butyldimethylsilyl (TBDMS) [6], triisopropylsilyloxymethyl (TOM) [7], and bis(2-acetoxyethoxy)methyl (ACE) [8] groups are used. However, all of them suffer from some disadvantages which complicate the synthesis. In particular, bulky TBDMS and TOM groups raise spatial hindrances that results in an increase in internucleotide coupling time and yield reduction, whereas the synthesis of ANA phosphoroamidite derivatives is difficult and commercially unreasonable.

In the process of development of effective approaches to the synthesis of natural and modified oligoribonucleotides by the phosphotriester method under intramolecular *O*-nucleophilic catalysis, we screened various groups for the ribonucleotide 2'-OH hydroxyl protection that met the requirements of the phosphotriester approach. One of the selected groups was a 2'-*O*-azidomethyl one. The preparation and properties of 2'-*O*-azidomethyl nucleoside derivatives were described earlier [9], but this group was not used in the phosphoramidite and H-phosphonate methods of oligonucle-

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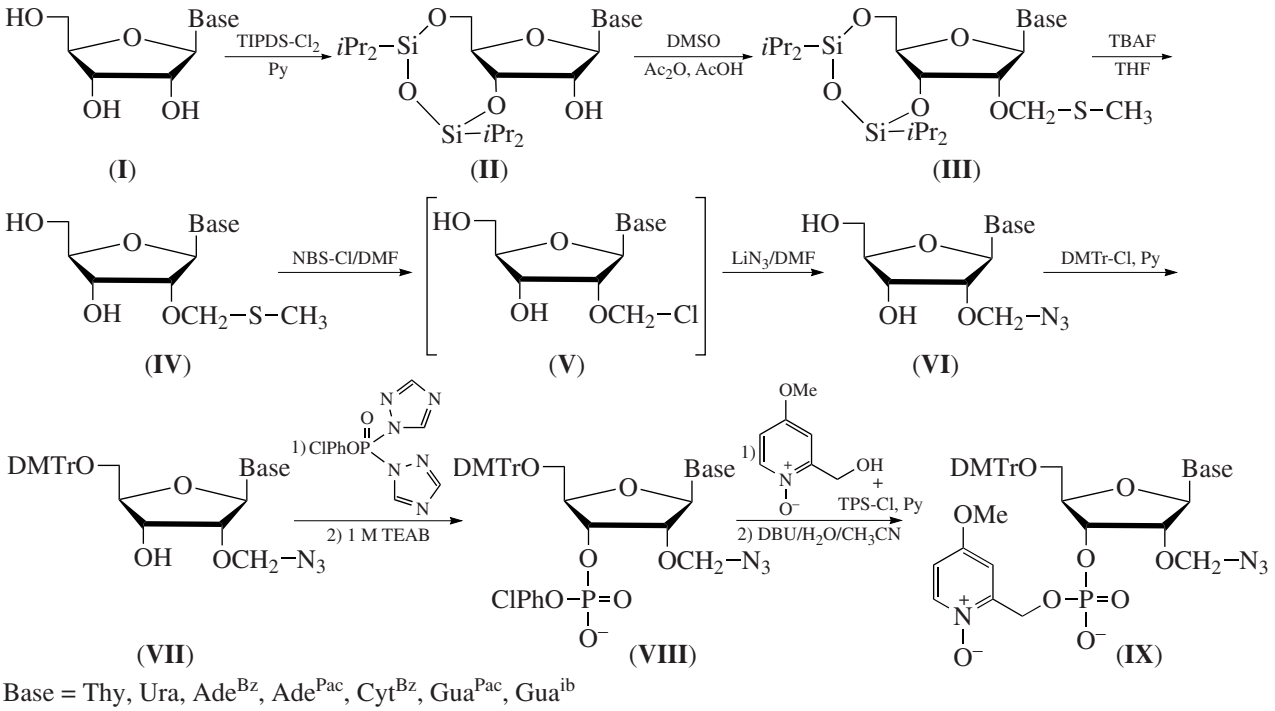
[2] Abbreviations: MTM, methylthiomethyl group; NBS-C1, 2-nitrobenzenesulfenyl chloride; TBAF, tetrabutylammonium fluoride; TIPDS-Cl_2, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane; TPS-C1, 2,4,6-triisopropylbenzenesulfonyl chloride; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene.

otide synthesis because of its capacity to oxidize the P(+III) to P(+V).

We developed a scheme for synthesis of monomeric synthons (**IX**) containing 2'-*O*-azidomethyl groups and *O*-nucleophilic catalytic 4-methoxy-1-oxido-2-picolyl phosphate protective groups (scheme). For the preparation of 2'-*O*-azidomethyl nucleoside derivatives (**VI**), 3'- and '5'-OH groups of nucleosides (**I**) were protected by treatment of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDS-Cl_2) in pyridine [10] and then *O*-methylthiomethyl groups (MTM) were introduced into the 2' positions of compounds (**II**) by the reaction with a mixture of dimethylsulfoxide and acetic anhydride in the presence of acetic acid. The described synthetic scheme included further conversion of MTM derivatives (**III**) into azidomethyl ones by successive treatment with sulfuryl chloride (or Br_2) and lithium azide solution in dimethylformamide [11, 12].

We found, however, that a side reaction of halogenation of the uracil and cytosine 5 positions took place in the presence of both Br_2 and sulfuryl chloride, and the yields of the halogenation products achieved 20–50%.



(**I**) (**II**) (**III**) (**IV**) (**V**) (**VI**) (**VII**) (**VIII**) (**IX**)

Scheme. Synthesis of monomeric ribonucleotide synthons with 2'-*O*-azidomethyl protective groups for the phosphotriester synthesis using *O*-nucleophilic intramolecular catalysis.

The use of a milder reagent, 2-nitrobenzenesulfenyl chloride (NBS-C1), at this stage provided a solution. Since NBS-C1 cannot interact with free hydroxyl groups in the absence of bases, we removed a nucleoside 3',5'-protective TIPDS group with 0.5 M TBAF in tetrahydrofurane (20°C, 5 min) and the resulting 2'-*O*-MTM derivatives (**IV**) were treated in dimethylformamide with NBS-C1 (1.6 eq, 20°C, 5 min), followed by in situ treatment with lithium azide (5 eq, 0°C, 5 min).

For preparation of purine nucleoside derivatives (**VI**), NBS-C1 was added in the presence of 1 eq of trifluoromethanesulfonic acid to avoid the side interaction of intermediate 2'-*O*-chloromethyl derivative with the purine base *N*-3 atom to give cyclonucleoside. The yields at the stage of (**IV**) conversion into azidomethyl derivative (**VI**) were 85–90% for pyrimidine ribonucleosides, 80% for rA, and 75% for rG. The subsequent introduction of a 4,4'-dimethoxytrityl group for the protection of the nucleoside 5'-hydroxyl groups and preparation of 3'-phosphate derivatives (**IX**) containing catalytic 4-methoxy-1-oxido-2-picolyl phosphate protective groups were performed as described in [2, 5]. The structures of the obtained monomeric synthons were confirmed by mass spectrometry and NMR spectroscopy data. The efficacy of the automatic solid phase oligoribonucleotide synthesis by the phosphotriester method using a 2'-*O*-azidomethyl protective group was demonstrated with several oligonucleotides as an

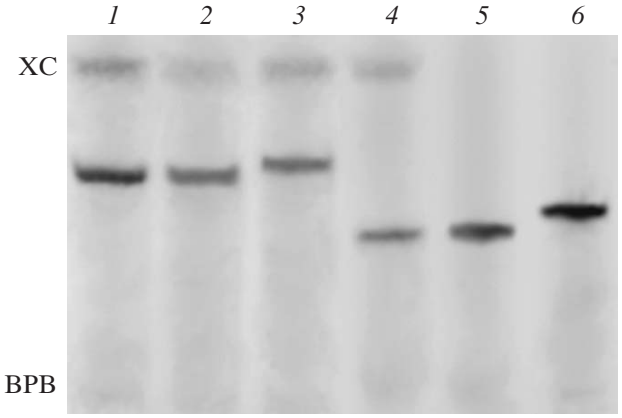


Fig. 1. Analysis of deprotected oligoribonucleotides 5'CGCUCUCGUCGCUCUCCAUGU (lanes *1–3*) and U_{15} (lanes *4–6*) by electrophoresis in 15% denatured polyacrylamide gel. *1,4*, reference oligonucleotides obtained by the phosphoramidite method; *2, 5*, fully deprotected oligonucleotides obtained by the phosphotriester method; *3, 6*, oligonucleotides bearing 2'-*O*-azidomethyl groups. A photo was taken in reflected UV light at 254 nm. (XC, xylenecyanol FF; BPB, bromophenol blue).

example. The synthesis was performed on the standard CPG support as described in [2]. The reaction of internucleotide bond formation was carried out in a 3 : 1 acetonitrile–pyridine mixture in the presence of monomers (**IX**) (0.05 M) and TPS-Cl (0.15 M) for 3 min. The yields at the stages of internucleotide couplings exceeded 98%. After the chain elongation was completed, the oligonucleotides linked to the support were treated with piperidine for 10 h at room temperature, or with 1 M lithium iodide in acetonitrile (20°C, 5 h) to deblock phosphate residues. Then, removal of protecting groups from heterocyclic bases with simultaneous cleavage of oligomer from the support was performed by the action of concentrated ammonia.

Azidomethyl groups were removed from 2'-hydroxyls of oligonucleotide by the action of 0.1 M triphenylphosphine solution in acetonitrile–water or pyridine–water (9 : 1, v/v, 20°C, 2 h).

The resulting oligoribonucleotides were isolated by gel filtration as described previously [2, 5] and their homogeneity was checked by electrophoresis in polyacrylamide gel (Fig. 1) and reverse phase chromatography. Oligonucleotides with the same base sequences were obtained for comparison by the phosphoramidite method using commercially available monomers. In addition, the efficacy of hybridization of the synthesized oligoribonucleotides before and after removal of the 2'-*O*-azidomethyl group with complementary DNA and RNA fragments was examined (Fig. 2). It was shown that the presence of 2'-*O*-azidomethyl groups in oligoribonucleotides did not significantly affect the stability of duplexes, although slightly destabilized them (~0.3°C per unit). To summarize, we developed a highly effective phosphotriester-based oligoribonucleotide

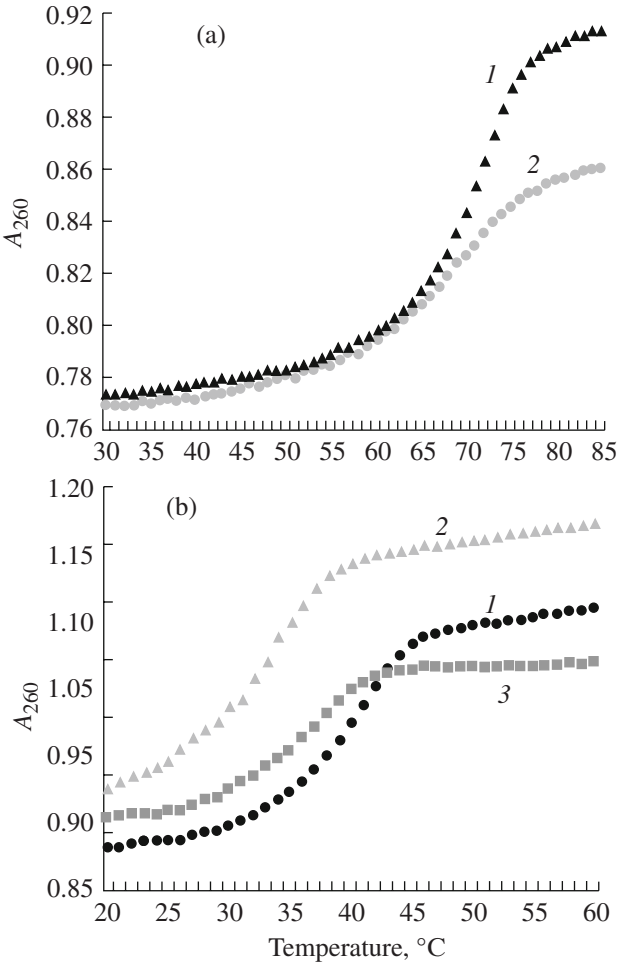


Fig. 2. Comparison of hybridization ability of oligonucleotides (a) 5'CGCUCUCGUCGCUCUCCAUGU and (b) U_{15} before and after removal of 2'-*O*-azidomethyl groups. Oligonucleotide solutions (1 μM) were annealed with the same volumes of complementary nucleic acid fragments (1 μM) in 0.01 M Tris–HCl (pH 7.5)/0.01 M $MgCl_2$/0.1 M NaCl at 90°C for 2 min, slowly cooled to 20°C and then heated at a rate of 0.5°C/min. Duplex melting curves formed by template DNAs and fully deprotected oligonucleotides (curves *1*) or oligonucleotides bearing 2'-*O*-azidomethyl groups (curves *2*); and by template RNA and exhaustively deprotected U_{15} (curve *3*, Fig. b) are shown.

synthesis using monomeric synthons containing 2'-*O*-azidomethyl protective groups. It is noteworthy that this method provided acceleration of the oligoribonucleotide elongation process when compared with the phosphoramidite method due to the reduction of internucleotide coupling time from 12 to 3 min. The developed method opens up prospects for the effective solid phase synthesis of stereospecific phosphorothioate oligoribonucleotide analogues. In addition, single incorporations of synthons containing 2'-*O*-azidomethyl group into oligonucleotides can be used for postsynthetic modifications of the latter by the introduction of various labels and functional groups via an azido function.

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