ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2011, Vol. 37, No. 2, pp. 254–258. © Pleiades Publishing, Ltd., 2011.
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LETTER
TO THE EDITOR

Methoxymethyl and (*p*-Nitrobenzyloxy)methyl Groups in Synthesis of Oligoribunucleotides by the Phosphotriester Method

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Received October 1, 2010; in final form, November 1, 2010

Abstract—An efficient method to synthesize monomer ribonucleotide synthons containing 2'-*O*-methoxymethyl and 2'-*O*-(*p*-nitrobenzyloxy)methyl groups is developed. These synthons are applied to the oligonucleotide phosphotriester method using *O*-nucleophilic intramolecular catalysis at the stage of the internucleotide bond formation. The former synthons may be used for the automatic synthesis of 2'-modified oligonucleotides; the latter synthons made be used for the synthesis of phosphotriester oligoribonucleotides in high yields.

Keywords: *oligoribinucleotides, phosphotriester method, O-nucleophilic catalysis, methoxymethyl and p-nitrobenzyloxymethyl groups*

DOI: 10.1134/S106816201102004X

The phosphotriester method of oligonucleotide synthesis with the use of *O*-nucleophilic catalysis at the stage of the internucleotide bond formation is an alternative to the phosphoroamidite approach. The well-developed phosphotriester method was successfully used for the synthesis of both native and modified oligonucleotides [1, 2]. Moreover, it was shown that this method can be used for the preparation of stereospecific phosphorothioate oligonucleotide analogs [3, 4].

To develop the efficient phosphotriester method of the synthesis of native and modified oligoribonucleotides, we examined a number of protecting and modifying groups [5–7]. In continuation of the study, we elaborated schemes of the synthesis of monomer synthons for the solid-phase synthesis of RNA fragments containing modifying 2'-*O*-methoxymethyl (MOM) and protecting 2'-*O*-*p*-nitrobenzyloxymethyl (4-NBOM) groups. The synthesis of oligoribonucleotides was performed using these synthons.

For the first time, the synthesis of uridine monomer containing the 4-NBOM group and its use for the synthesis of oligo(U) by the phosphoroamidite method was described by Gough et al. [8]. It was further shown that this protecting group along with (*o*-nitrobenzyloxy)-, [4-(*N*-methylamino)benzyloxy]-, and [4-(*N*-dichloroacetyl-(*N*-methylamino)benzyloxy]methyl groups can be applied for the efficient synthesis of RNA fragments by the phosphoroamidite method [9–11]. However, the synthesis of 2'-*O*-(4-NBOM)-uridine by the Gough method led to the formation of the hard-to-separate mixture of 2'-*O*- and 3'-*O*-(4-NBOM) derivatives and, thereby, to the low yield of the target compound.

We used the alternative scheme of regioselective introduction of the 4-NBOM group at the 2'-position (Scheme 1). At the first stage, 3'- and 5'-OH functions of nucleosides were blocked by the treatment with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDS-Cl_2) [12] to prepare derivatives (**IV**). At the same time, *p*-nitrobenzyl alcohol was successively converted into methyl ester of (*p*-nitrobenzyl)oxyacetic acid (**I**), (*p*-nitrobenzyl)oxyacetic acid (**II**), and acetoxymethyl-*p*-nitrobenzyl ester (**III**), which was used for the introduction of the 4-NBOM group into the 2'-position of nucleosides (**IV**).

Abbreviations: DBU, 1,8-diazabicyclo[5.4.0]undec-7-en; DMTr, 4,4'-dimethoxytrityl; MOM, methoxymethyl; 4-NBOM, *p*-nitrobenzyloxymethyl; NIS, *N*-iodosuccinimide; TPS, 2,4,6-triisopropylbenzene-sulfonyl; TBAF, tetrabutylammonium fluoride.

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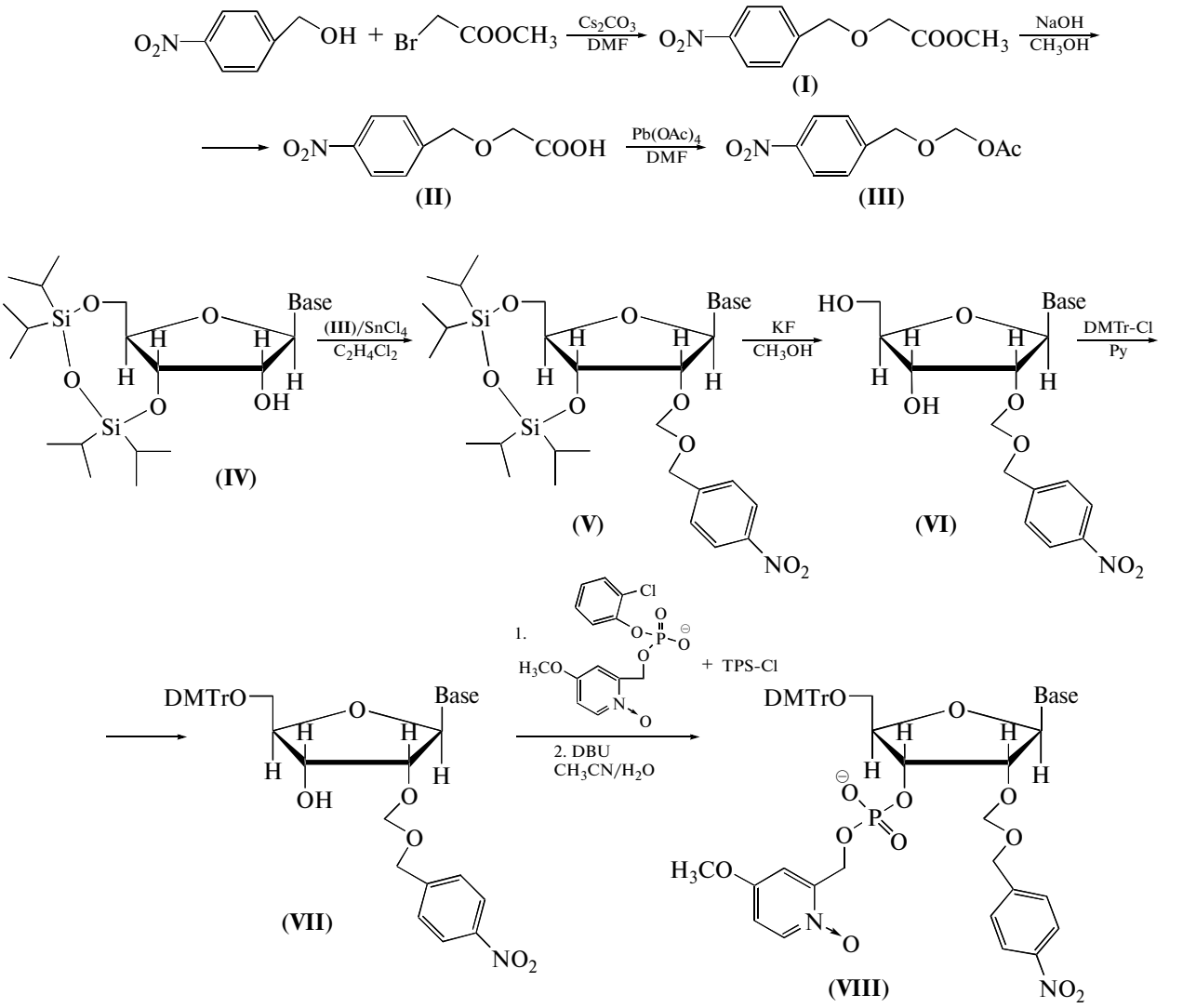


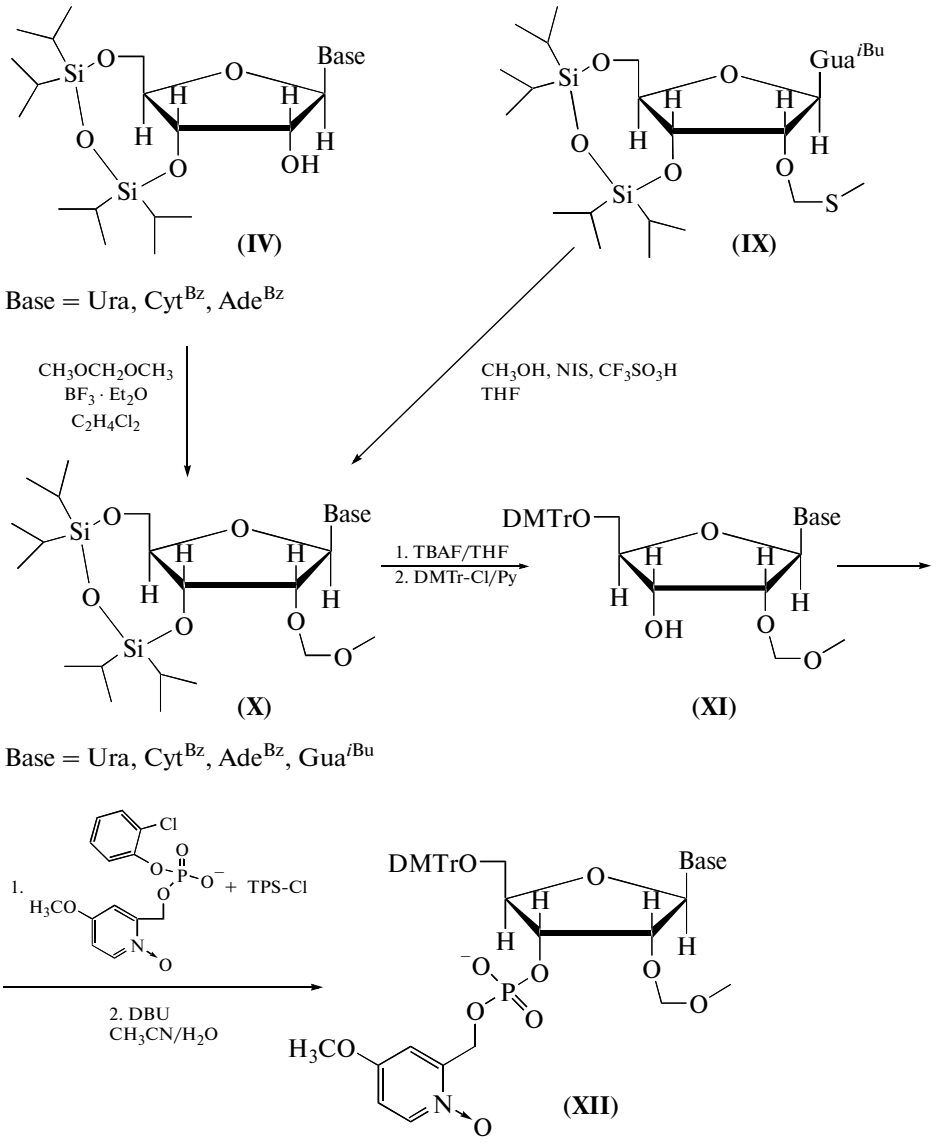
Scheme 1.

The 3'- and 5'-functions of resultant compounds (**V**) should be deprotected. It was previously shown that ammonium fluoride in methanol may be used for this purpose [10]. We found that the 2'-*O*-(4-NBOM) group was also not touched by potassium fluoride in methanol; on the other hand, it can be easily removed from compounds (**V**) along with the TIPDS group by treatment with tetrabutylammonium fluoride (TBAF) in THF. The further introduction of the 4,4'-dimethoxytrityl protecting group at the 5'-hydroxyl function of nucleosides (**VI**) and the preparation of 3'-phosphate derivatives (**VIII**) containing 4-methoxy-1-oxide-2-picolyl *P*-protecting group were carried out according to [6, 7].

The methoxymethyl protecting group found wide application in both classical organic synthesis and nucleoside and nucleotide chemistry for the protection of hydroxyl functions due to the simplicity of its introduction and stability in both strong alkaline and moderate acidic conditions, as well as under conditions used for the removal of silyl, alkoxyacetyl, and benzyl groups [13–15].

2'-*O*-MOM nucleotide derivatives (**XII**) were synthesized according to successive reactions presented in Scheme 2. Uridine, cytidine, and adenosine derivatives (**IV**) with protected 3'- and 5'-hydroxyls and amino groups of heterocyclic bases (in the cases of Cyt and Ade) were treated with an excess of dimethoxymethane in dichloroethane in the presence of boron trifluoride etherate at room temperature for 2 h. Completely protected nucleosides (**X**) were isolated, and TIPDS groups were removed with 1 M

TBAF in THF for 1 h followed by tritylation of the resultant MOM derivatives. The prepared compound (**XI**) was treated with (4-chlorophenyl)-(1-oxido-4-methoxy-2-picolyl)phosphate in the presence of TPS-Cl and then with DBU in aqueous acetonitrile [6, 7] to introduce the phosphoric acid residue containing the 4-methoxy-1-oxido-2-picolyl *P*-protected group at the 3'-hydroxyl group. In the case of guanosine derivative, the treatment of nucleoside (**IV**) with dimethoxymethane in the presence of boron trifluoride etherate led to the formation of a large amount of the fluorescent side product. In this case, therefore, the synthesis of the 2'-*O*-MOM derivative (**X**) was carried out starting from the methylthiomethyl derivative (**IX**) (its preparation can be seen in [16]) by its treating with trifluoromethane sulfonic acid and NIS in a THF–methanol mixture (25 : 1) for 20 min at –40°C. In all cases, the structures of the intermediate compounds and target monomer synthons (**VIII**) and (**XII**) were confirmed by mass spectrometry and NMR spectroscopy methods.



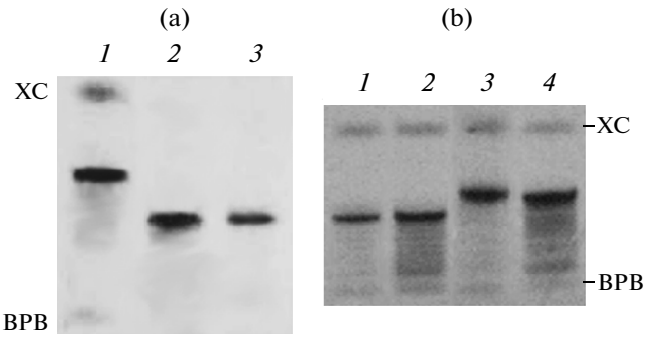
Scheme 2.

Monomers (**VIII**) and (**XII**) were used for the solid phase synthesis of 15–25-mer oligoribonucleotides. The synthesis was carried out on the standard CPG-support as described in [7, 17] using TPS-Cl as a condensing agent for the formation of the internucleotide bond. The yields at the chain elongation steps were

98–99%. After the synthesis was completed, the oligonucleotides bound to the support were treated with 1-M potassium iodide in acetonitrile (5 h at room temperature) to deblock phosphate residues and then with concentrated ammonia to cleave oligonucleotides from the support and to remove protecting groups from heterocyclic bases. *p*-Nitrobenzyloxymethyl groups were removed from 2'-OH oligonucleotide functions by the treatment with TBAF in THF. Oligonucleotides were then isolated by gel filtration on columns with Sephadex G-25. Homogeneity of the products were examined by electrophoresis in polyacrylamide gel (figure a) and reverse-phase chromatography. Oligonucleotides with the same sequences obtained by the phosphoroamidite method using commercial monomers were used as control compounds. Our results showed the reasonable efficiency of using the (4-NBOM)-protecting group for the protection of 2'-hydroxyls during the oligoribonucleotide synthesis by the phosphotriester method.

It should be noted that attempts to remove the 2'-*O*-MOM group from the synthesized oligoribonucleotides with acidic reagents, e.g., 30% aqueous trifluoroacetic acid [15, 18] or by the recently proposed method based on the treatment with $ZnBr_2$ in the presence of mercaptan [19], led to a significant degradation of the oligomer chain. We found that the treatment of MOM-containing nucleosides with 1-M LiI in a acetonitrile–water mixture (20 : 1) in the presence of 0.01-M HCl for 2–3 h resulted in an almost quantitative removal of the 2'-*O*-MOM protecting group. In case of oligoribonucleotides, however, in addition to the removal of MOM groups, the partial degradation of the chain still occurred under these conditions (figure b).

The presence of 2'-*O*-MOM groups was not shown to significantly influence the specificity of the binding of oligonucleotides to complementary RNA fragments and the stability of formed duplexes (the increase of T_m was on average about 0.2°C per one unit). However, complexes of 2'-*O*-MOM-containing oligonucleotides with DNA had the lower T_m values than those of complexes formed by native oligoribonucleotides of the same sequences (destabilization was ~0.3°C per unit). The introduction of single nucleotide substitutions in DNA and RNA targets led to the decrease of melting temperatures of corresponding duplexes by 10–15°C, depending on the position of the mutation. This suggests the possibility of using 2'-*O*-MOM-modified oligoribnucleotides and their stereospecific phosphorothioate analogs prepared by the phosphotriester method for various molecular-biological studies in the future.



Electrophoregrams in 15% denatured PAAG: (a) is oligonucleotide U_{15}, prepared with the use of 2'-*O*-(4-NBOM) protecting group before (*1*) and after (*2*) its removal, and the same deprotected oligonucleotide prepared by the phosphoroamidite method (*3*); (b) is oligonucleotides U_{15} (*1, 2*) and 5'-CGAUCUCAUCACCUCUCCAU (*3, 4*) prepared with the use of 2'-*O*-MOM protecting group before (*1, 3*) and, after (*2, 4*), its removal with 1-M LiI in a acetonitrile–water mixture (20 : 1) in the presence of 0.01-M HCl. The photograph was taken in reflected UV light at 254 nm (XC, xylene cyanol FF; BPB, bromophenol blue).

ACKNOWLEDGMENTS

The authors are grateful to M.S. Smirnova (Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences) for participation at different stages of the project.

REFERENCES

1. Efimov, V., Buryakova, A., Dubej, I., Polushin, N., Chakhmakhcheva, O., and Ovchinnikov, Yu., *Nucleic Acid Res.*, 1986, vol. 14, pp. 6526–6540.
2. Polushin, N., Smirnov, I., Verentchikov, A., and Coull, J., *Tetrahedron Lett.*, 1996, vol. 37, pp. 3227–3230.
3. Almer, H., Szabo, T., and Stawinski, J., *Chem. Commun.*, 2004, pp. 290–291.
4. Efimov, V.A., Molchanova, N.S., and Chakhmakhcheva, O.G., *Nucleosides, Nucleotides and Nucleic Acids*, 2007, vol. 26, pp. 1087–1093.
5. Efimov, V.A., Aralov, A.V., Fedunin, S.V., Klykov, V.N., and Chakhmakhcheva, O.G., *Bioorg. Khim.*, 2009, vol. 35, pp. 270–273 [*Russ. J. Bioorg. Chem.* (Engl. Transl.), 2009, vol. 35, pp. 250–253].
6. Efimov, V.A., Aralov, A.V., Klykov, V.N., and Chakhmakhcheva, O.G., *Nucleosides, Nucleotides and Nucleic Acids*, 2009, vol. 28, pp. 846–865.
7. Efimov, V.A., Aralov, A.V., Grachev, S.A., and Chakhmakhcheva, O.G., *Bioorg. Khim.*, 2010, vol. 36, pp. 681–687 [*Russ. J. Bioorg. Chem.* (Engl. Transl.), 2010, vol. 36, pp. 628–633].
8. Gough, G.R., Miller, T.S., and Mantick, N.A., *Tetrahedron Lett.*, 1996, vol. 37, pp. 9981–9982.
9. Schwartz, M.E., Breaker, R.R., Asteriadis, G.T., de Bear, J.S., and Gough, G.R., *Bioorg. Med. Chem. Lett.*, 1992, vol. 2, pp. 1019–1024.

10. Cieślak, J., Kauffman, J.S., Kolodziejski, M.J., Lloyd, J.R., and Beaucage, S.L., *Org. Lett.*, 2007, vol. 9, pp. 671–674.

11. Cieslak, J., Grajkowski, A., Kauffman, J.S., Duff, R.J., and Beaucage, S.L., *J. Org. Chem.*, 2008, vol. 73, pp. 2774–2783.

12. Markiewicz, W.T., Biala, E., and Kierzek, R., *Bull. Polish Acad. Sci., Chem.*, 1984, vol. 32, pp. 433–451.

13. Greene, T.W. and Wuts, P.G.M., *Protective Groups in Organic Synthesis*, 4th ed., New York: Wiley, 1999.

14. Groziak, M.P. and Koohang, A., *J. Org. Chem.*, 1992, vol. 57, pp. 940–944.

15. Tanaka, H., Hayakawa, H., and Miyasaka, T., *Chem. Pharm. Bull.*, 1981, vol. 29, pp. 3565–3572.

16. Zavgorodny, S., Polianski, M., Besidsky, E., Kriukov, V., Sanin, A., Pokrovskaya, M., Gurskaya, G., Lonnberg, H., and Azhayev, A., *Tetrahedron Lett.*, 1991, vol. 32, pp. 7593–7596.

17. Efimov, V., Buryakova, A., Dubej, I., Polushin, N., Chakhmakhcheva, O., and Ovchinnikov, Yu., *Nucleic Acids Res.*, 1986, vol. 14, pp. 6526–6540.

18. Eskandari, R., Jayakanthan, K., Kuntz, D.A., Rose, D.R., and Pinto, B.M., *Bioorg. Med. Chem.*, 2010, vol. 18, pp. 2829–2835.

19. Han, J.H., Kwon, Y.E., Sohn, J.-H., and Ryu, D.H., *Tetrahedron*, 2010, vol. 66, pp. 1673–1677.