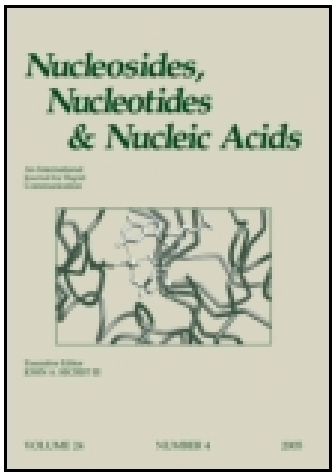
This article was downloaded by: [Simon Fraser University]
On: 10 November 2014, At: 10:10
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

4,5-BIS(ETHOXYCARBONYL)-[1,3]DIOXOLAN-2-YL AS A NEW ORTHOESTER-TYPE PROTECTING GROUP FOR THE 2′-HYDROXYL FUNCTION IN THE CHEMICAL SYNTHESIS OF RNA

Boleslaw Karwowski [a b], K. Seio [a c] & M. Sekine [a b]
[a] Department of Life Science, Tokyo Institute of Technology, Japan
[b] JST (Japan Science and Technology Corporation), Japan
[c] Frontier Collaborative Research Institute, Tokyo Institute of Technology, Japan
Published online: 15 Nov 2011.

To cite this article: Boleslaw Karwowski, K. Seio & M. Sekine (2005) 4,5-BIS(ETHOXYCARBONYL)-[1,3]DIOXOLAN-2-YL AS A NEW ORTHOESTER-TYPE PROTECTING GROUP FOR THE 2′-HYDROXYL FUNCTION IN THE CHEMICAL SYNTHESIS OF RNA, Nucleosides, Nucleotides and Nucleic Acids, 24:5-7, 1111-1114, DOI: 10.1081/NCN-200061895

To link to this article: http://dx.doi.org/10.1081/NCN-200061895

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):1111–1114, (2005)
Copyright © Taylor & Francis, Inc.
ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200061895

Taylor & Francis
Taylor & Francis Group

Downloaded by [Simon Fraser University] at 10:10 10 November 2014

4,5-BIS(ETHOXYCARBONYL)-[1,3]DIOXOLAN-2-YL AS A NEW ORTHOESTER-TYPE PROTECTING GROUP FOR THE 2′-HYDROXYL FUNCTION IN THE CHEMICAL SYNTHESIS OF RNA

Boleslaw Karwowski ▫ *Department of Life Science, Tokyo Institute of Technology, Japan and JST (Japan Science and Technology Corporation), Japan*

K. Seio ▫ *Department of Life Science, Tokyo Institute of Technology, Japan and Frontier Collaborative Research Institute, Tokyo Institute of Technology, Japan*

M. Sekine ▫ *Department of Life Science, Tokyo Institute of Technology, Japan and JST (Japan Science and Technology Corporation), Japan*

▫ *We wish to report 4,5-bis(ethoxycarbonyl)-[1,3]dioxolan-2-yl as a new protecting for the 2′-hydroxyl function. Our cyclic orthoester-type group is compatible with the DMTr strategy for oligonucleotide synthesis. This group was introduced to the 2′-hydroxyl group of appropriately protected nucleoside derivatives in good yields under mild acidic conditions. Post-synthetic conversion of the moiety of this protecting group with an amine resulted in formation of a new amide moiety that is more stable to acid deprotection in aqueous solution, but it can still be easily removed by treatment with acids in organic solvents. In this article, we also describe the stability of not only the original and modified protecting groups but also internucleotidic phosphate linkages of protected RNA intermediates under deprotection conditions.*

Keywords RNA Protecting Group, 2-Ethoxy-[1,3]Dioxolane-4,5-Dicarboxylic Acid Diethyl Ester, Acid Stability

INTRODUCTION

During the last decade, many chemical strategies have been developed for oligoribonucleotide synthesis.[1] The protecting group of the 2′-hydroxyl function is one of the most important subjects in RNA synthesis. Although a number of 2′-hydroxyl protecting groups have been explored, only a few have survived in the field of nucleoside and nucleotide chemistry, since very limited conditions have

Address correspondence to Dr. Boleslaw Karwowski, CEA Grenoble, LAN/SCIB/DRFMC, 17 Avenue, Des Martyrs, Grenoble, F-38054, France; Fax: 33-4-38-78-50-90; E-mail: karwowski@cea.fr

Order reprints of this article at www.copyright.rightslink.com

been required for chemical transformation. For the projected reaction, a protecting group should fulfill the following requirements:

1. The reagent can be easily prepared.
2. No new chiral centers should be generated.
3. The protecting group should be stable during the projected reaction.
4. Removal of the protecting group must be highly selective without damage of the other functional groups.[2]

The methoxymethylidene group is well known as the protecting group of ribonucleoside *cis*-2′,3′-diol derivatives.[3] From the other point of view, the methanol is protected by orthoester-type backbone structures. This idea led us to investigate a new method for the protection of nucleoside 2′-hydroxyl groups with such orthoester structures. In the literature there are only two examples of the orthoester-type as protecting groups for RNA synthesis,[4,5] but they have several drawbacks such as low yields of starting materials and the use of a highly cost co-reagent.[5]

RESULTS AND DISCUSSION

Our attention was paid to cyclic orthoesters which are commercially available. Compounds **1** and **2** (Figure 1), could be used as introducing reagents for orthoester functions, as shown in so that they did not generate new chiral centres.

We checked the stability of **3** and **4** under weakly acidic conditions (1-*H*-tetrazole), which are necessary for activation of nucleoside phosphoramidites during the oligonucleotide synthesis by the phosphoramidite strategy. However, both **3** and **4** were found to be unstable. Because compounds **3** and **4** were not suitable for the solid-phase synthesis of RNA, our attention was focused on orthoester derivatives of the D- or L-isomer of tartaric acid. This compound is suitable for the synthesis of chiral olefins and L-dioxolane nucleosides,[6] the promising stability of this compound under different conditions led us to study a new methodology for the protecting group. The starting material 2-ethoxy-[1,3]dioxolane-4,5-dicarboxylic acid diethyl ester (D- or L-form) was obtained in excellent yield from the tartaric acid L or D isomer and triethoxyethane.

Since the backbone structures of compounds **6** and **7** were used in our new strategy for oligonucleotide synthesis, we decided to test their stability under acidic conditions which are necessary for deprotection of the DMT group from the

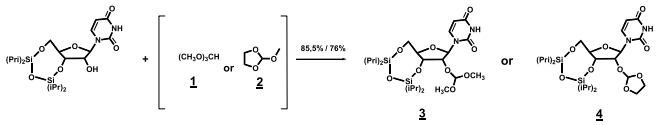


FIGURE 1 Synthesis of nucleotides with orthoester function as a potential protecting group.

Downloaded by [Simon Fraser University] at 10:10 10 November 2014

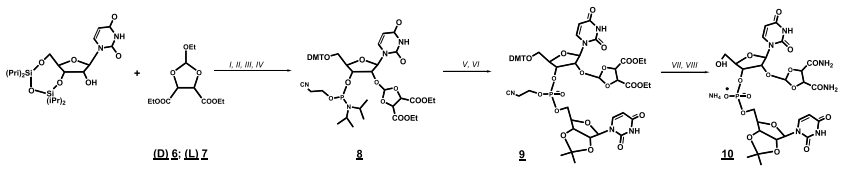


FIGURE 2 Synthesis of dinucleotide UpU via 5'-O-DMT-2'-O-[4,5-bis(ethoxycarbonyl)-[1,3]dioxolan-2-yl]uridine as a substrate. (*I*) H^+, (*II*) TBAF, (*III*) DMTrCl, (*IV*) (Cl)P(N-^{i}Pr$_2$)-O(CH$_2$)$_2$CN, (*V*) 1-*H*-tetrazol, 2,3-isopropylideneuridine, (*VI*) I_2/H_2O/Pyridine, (*VII*) NH_3aq, (*VIII*) H^+.

5′-hydroxyl function. Compound **6** was stable in a dry solution of DCA and in acetic acid–water solution.

This data allowed us to make efforts for the synthesis of the nucleoside derivatives with a [1,3]dioxolane-4,5-dicarboxylic acid diethyl ester skeleton as a protecting group. Because it is possible to selectively remove the DMT group from the 5′-O position by DCA in CH_2Cl_2 we decided to synthesize the phosphoramidite derivative of 5′-O-DMT-2′-O-[4,5-Bis(ethoxycarbonyl)-[1,3]dioxolan-2-yl]uridine. This compound was easily obtained by the reaction of an appropriately protected uridine derivative with the phosphoramidite unit in 60% yield with the isomeric ratio of 65:35 (Figure 2). We also checked the utility of compound **8** for creation of the internucleotidic phosphorus bond by synthesis of the dinucleotide UpU.

During the creation of the internucleotide bond, we did not find any unexpected signals in ^{31}P NMR analysis. The crude product was analyzed by RP HPLC. This result is promising for future studies because, with the help of this diamide function, the change of this hydrophilic property enables us to purify them easily. The difference in the retention time between **10** and the dinucleotide with a DMT protecting group is big enough to purify each of them. It is an opposite situation to the current RNA synthesis with the *t*-butyldimethylsilyl 2′-protecting group where we did not observe a significant difference in the retention time.

CONCLUSION AND OUTLOOK

The new protecting group of the orthoester-type described in this poster could be widely used in nucleic acid chemistry as well as carbohydrate chemistry. We found that the 4,5-bis(ethoxycarbonyl)-[1,3]dioxolan-2-yl 2′-OH protecting group is stable under acidic conditions with moderate pH values. Fortunately, during our study, we never observed the loss of this group. It is promising for future research that our strategy is compatible with the DMT group which is most widely used in the oligonucleotide synthesis. The stability of this group changed during the synthesis from ester type **9** to amide-type **10**. Additionally, the purification of crude postsynthetic oligonucleotides should be fast and easy. Future work will continue to implement this strategy for the solid-phase synthesis of RNA.

Downloaded by [Simon Fraser University] at 10:10 10 November 2014

REFERENCES

1. Reese, C.B. The chemical synthesis of oligo- and poly-nucleotides: a personal commentary. Tetrahedron **2002**, *58*, 8893.
2. Sekine, M.; Hata, T. Cyclic orthoester functions as new protecting groups in nucleosides. J. Am. Chem. Soc. **1983**, *105*, 2044–20049.
3. Green, L.P.D.; Ravindranathan, R.; Reese, C.B.; Saffihill, R. The synthesis of oligoribonucleotides–VIII: the preparation of ribonucleoside 2′,5′-bisketals. Tetrahedron **1970**, *26*, 1031–1041.
4. Sandstrom, S.; Kwiatkowski, A.; Chattopadyaya, J. Chemical synthesis of a pentaribonucleoside tetraphosphate constituting the 3′-acceptor stem sequence of *E. coli* tRNAIle using 2′-O-(3-methoxy-1,5-dicarbomethoxypentan-3-yl)-ribonucleoside building blocks. Application of a new achiral and acid-labile 2′-hydroxyl protecting group in tRNA synthesis. J. Acta Chem. Scand., B **1985**, *39*, 273–280.
5. Scaringe, S.A. RNA oligonucleotide synthesis via 5′-silyl-2′-orthoester chemistry. Methods **2001**, *23*, 206–217.
6. Branalt, J.; Kvarnstrom, I.; Classon, B.; Samuelsson, B. Synthesis of [4,5-bis(hydroxymethyl)-1,3-dioxolan-2-yl]nucleosides as potential inhibitors of HIV. J. Org. Chem. **1996**, *61*, 3599–3603.

Downloaded by [Simon Fraser University] at 10:10 10 November 2014