Synthesis and properties of oligonucleotides having a chemically stable 2-(trimethylsilyl)benzoyl group

Ken Yamada, Haruhiko Taguchi, Akihiro Ohkubo, Kohji Seio, and Mitsuo Sekine

Department of Life Science, Tokyo Institute of Technology, 4259 Nagatsuta, Midoriku, Yokohama 226-8501

ABSTRACT

Acyl groups such as benzoyl and phenoxyacetyl have been used as a fundamental tool for protection of reactive hydroxyl and amino groups in chemical synthesis of DNA and RNA oligomers. It is well known that such protecting groups can be easily cleaved under basic conditions. Here we report 2-(trimethylsilyl)benzoyl (TMSBz), i.e., a chemically stabilized benzoyl group that is substituted with a trimethylsilyl group at its 2-position. To the best of our knowledge, the TMSBz group is the most resistant to basic conditions as an acyl group in nucleic acid chemistry.

INTRODUCTION

Acyl groups in a variety of biological compounds containing the structure of esters or amides serve as important functions, as exemplified by aminacylated tRNAs, acetyl CoA, and acetylated histones. In the current organic synthesis, acyl groups have also been utilized as protecting groups for chemical conversions and as transient masking groups for generation of genuine drugs in prodrug strategy (1). Various aspects of their usefulness led us to consider if acyl groups could be used to provide a new insight into nucleic acid chemistry by changing their ubiquitous properties. It was reported that ester derivatives of nucleosides such as 5’-O-benzoylthymidine and 3’-O-acetylthymidine are easily cleaved under basic conditions such as concentrated ammonia and 0.1 M NaOH that were used for the solid-phase synthesis of oligonucleotides. Therefore, it has long been recognized that ester skeletons could not be used for designing new functions in artificial nucleic acids because of their base-lability. To utilize such ester skeletons more effectively in this field, we designed sterically hindered acyl groups of esters. One is 2-(trimethylsilyl)benzoyl (TMSBz), i.e., a benzoyl group by substituted with a trimethylsilyl group at its 2-position.

Trialkysilyl groups of organosilicon compounds have unique properties toward nucleophiles containing electron-rich atoms such as anionic alkoxide and fluoride ions. Peterson olefination is an example of the reactions where a silyl group coordinates with a hydroxyl anion located at its β-position and the resulting transient pentacoordinate silicate intermediate decomposes to give a sterically-controlled olefin (2). It might be expected that, when a trimethylsilyl group could be introduced into the 2-position of the benzoyl group in benzoic acid esters, a Si-O interaction between the benzoyl oxygen and the TMS group might occur. In such a case, the silyl group might work as a Lewis acid, while the carbonyl oxygen might work as a Lewis base. As a result, such an interaction would activate the carbonyl group and accelerate hydrolysis reaction. Otherwise, the steric hindrance of the TMS group might affect the inhibition of hydrolysis. Therefore, we have recently studied to test if the TMS group attached to the benzoyl group at its 2-position could show the above-mentioned activation or stabilization of esters consisting of a TMSBz group and thymidine. Consequently, we found the TMSBz group exhibited the latter property, i.e., rather base-resistance. This result led us to study the potential possibility of the TMSBz group as a new and stable functional group in oligonucleotide chemistry.

On the other hand, it is known that, acyl groups of 2’-O- or 3’-O-acylated ribonucleoside derivatives tend to migrate to give mixtures containing their position-isomers (3). For example, it was reported that conversion of 5’-O-(4,4’-dimethoxytrityl)-2’-O-levminyluridine to its 3’-phosphoramidite derivative resulted in 2’-3’ isomerization of the levminyl group so that the product was obtained in low yield (4). To overcome the inherent property of acyl-type protecting groups, we also considered that the TMSBz group could be used as a new 2’-hydroxyl protecting group to prohibit such 2’-3’ migration because of high stability of the TMSBz group.

In this paper, we report the synthesis of thymidine and uridine derivatives masked with the TMSBz group and oligonucleotides incorporating 5’-O- or 3’-O-TMSBz-thymidine and their promising base-resistant properties.

RESULTS AND DISCUSSION

2-(Trimethylsilyl)benzoic acid was synthesized as a reagent for introduction of a TMSBz group into hydroxyl groups of nucleoside derivatives, as shown in Figure 1.

Figure 1 Synthesis of 2-(trimethylsilyl)benzoic acid.
Lithiation of benzaldehyde gave an ortho-lithiated species, which, in turn, was allowed to react with TMSCl to give 2′-(trimethylsilyl)benzaldehyde. 2′-(Trimethylsilyl)benzoic acid was obtained in good yield after oxidation of 2′-trimethylsilylated-benzaldehyde (5).

The 5′-hydroxyl group of thymidine was selectively substituted with a TMSBz group by Mitsunobu reaction without protection of the 3′-hydroxyl group, whereas introduction of the TMSBz group into the 3′-hydroxyl group of thymidine and the 4-amino group of a deoxy cytidine derivative was achieved by using 2′-(trimethylsilyl) benzoyl chloride. (Figure 2)

![Figure 2. Nucleic acid derivatives substituted by TMSBz group.](image)

To clarify the hydrolytic properties of this unique benzoyl group, we examined the stability of 5′′-O-TMSBz-thymidine under basic conditions that are often used for the solid-phase synthesis of oligonucleotides. It was found that the TMSBz group showed very high stability against organic bases. When 5′′-O-TMSBz-thymidine was treated with ammonia, the TMSBz group was not cleaved after 24 h, while the 2-methylbenzoyl group was completely removed from the corresponding 5′′-O-acylated thymidine derivative after 24 h.

This result led us to clarify if the 2′′-O-TMSBz group of 2′′-O-TMSBz-uridine migrates to the 3′-hydroxyl group under basic conditions. Substitution of the TMSBz group into the 2′′-hydroxyl group of 3′′′-O-(1,1,3,3-tetraisopropylidilsoxane-1,3-diyl)uridine was achieved by using 2′-(trimethylsilyl)benzoyl chloride. The resulting product was deprotected by use of a fluoride reagent. In this deprotection, acyl migration from the 2′′-hydroxyl group to the 3′′′-hydroxyl group was not detected in 1H-NMR. It was also found that the TMSBz group did not migrate to the 3′′′-hydroxyl group in basic solvents such as pyridine derivatives. Taking this advantage into an account, we successfully synthesized 2′′-O-TMSBz-uridine 3′′′-phosphoroanidite building block using collidine as the base at the 3′′′-phosphitylation step. This is the first report about synthesizing 2′′-O-acylated ribonucleoside 3′′′-phosphoroamidite compounds without acyl migration.

Next, we synthesized several oligodeoxynucleotides incorporating 5′′-O-TMSBz-thymidine or 3′′-O-TMSBz-thymidine at their 5′′- and 3′′-termini using key intermediates of a 5′′-O-TMSBz-thymidine 3′′-phosphoroamidite derivative and a 3′′-O-TMSBz-thymidine 5′′-phosphoroamidite derivative, respectively. Modified oligodeoxynucleotides having these acylated thymidine residues proved to be isolated as stable materials. Exonucleases are enzymes that degradeate DNAs or RNAs from the 5′′- or 3′′-terminus. In connection with antisense/antigen strategies, many researchers developed a wide variety of chemically modified oligonucleotide to enhance their nuclease resistance. Therefore, we tested the enzyme resistance of our modified oligodeoxynucleotides toward snake venom phosphodiesterase and spleen phosphodiesterase. As the result, these modified oligonucleotides showed significant resistance to these enzymes.

**CONCLUSION**

We have developed TMSBz group as a base-resistant acyl group. Substitution with a bulky group at the 2-position markedly enhanced the original stability of the unmodified benzoyl group against organic bases. It should be noted that the acyl migration could be prevented upon introduction of the TMSBz group into the 2′′-hydroxyl group of uridine. Oligodeoxynucleotide modified by the TMSBz group at their termini also showed improved exonuclease resistance. The hydrophobic property of this acyl group would be useful with respect of membrane permeability in antisense/antigen strategies.

**REFERENCES**


*Corresponding author. E-mail: sekine.m.ab@m.titech.ac.jp