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Tetrahedron Letters 45 (2004) 9529–9531
Tetrahedron Letters

Oligoribonucleotide synthesis by the use of 1-(2-cyanoethoxy)ethyl (CEE) as a 2′-hydroxy protecting group

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Received 30 September 2004; revised 20 October 2004; accepted 22 October 2004
Available online 11 November 2004

Abstract—A novel method for the synthesis of oligoribonucleotides using 1-(2-cyanoethoxy)ethyl (CEE) as a 2′-hydroxy protecting group has been developed. A CEE group was introduced to the 2′-position of *N*-acyl-3′,5′-*O*-silyl-protected ribonucleosides under acidic conditions in good yields. The 2′-*O*-CEE group was found to be stable in an aqueous or ethanolic ammonia and was quickly removed by treatment with anhydrous tetrabutylammonium fluoride (TBAF). A combination of the use of *N*-acyl and 2′-*O*-CEE protecting groups enabled a reliable and complete two-step deprotection, first with NH_3–EtOH, then with TBAF in THF, without cleavage of internucleotidic linkages.

Chemically synthesized oligoribonucleotides have become increasingly important since the discovery of small interfering RNAs (siRNAs).[1] The mammalian siRNAs consist of only 21–23 nucleotides,[2,3] and their chain lengths are suitable for chemical synthesis. Many research groups have successfully synthesized RNAs by various strategies.[4] Compared with DNA, each nucleotide unit of RNA contains an additional 2′-hydroxy group, which has to be protected regioselectively. 2′-*O*-TBDMS-Protection has been widely used for the oligoribonucleotide synthesis by the phosphoramidite approach.[5] However, 2′-*O*-TBDMS-protected monomers possess lower coupling efficiency compared with that of 2′-deoxyribonucleoside derivatives due to the steric hindrance of the TBDMS group. In recent years, new protecting groups have been reported for the 2′-hydroxy function, such as fluoride-labile silicon-based protecting groups[6,7] and acid-labile acetals[8–10] as well as orthoester-type protecting groups.[11] The acetal-type protecting groups have excellent features such as capability for the 2′-*O*-selective protection and lower steric hindrance. However, these protecting groups are somewhat unstable under the strong acidic conditions prescribed for the deprotection of the 5′-*O*-DMTr group.

It has been reported that the introduction of an electron-withdrawing group to an acetal function stabilizes the protecting group under acidic conditions.[8,9] Pfleidrer and co-workers have reported that the 1-(2-cyanoethoxy)ethyl (CEE) was an acid-stable 2′-hydroxy protecting group for uridine.[9] However, this stabilized acetal group requires drastic acidic conditions for complete removal. Therefore, the CEE group has not hitherto been applied for oligoribonucleotide synthesis. In this letter, we wish to report the application of a CEE group as the first example of a base-labile acetal-type of 2′-hydroxy protection group for oligoribonucleotide synthesis.

The 2′-*O*-CEE-protected ribonucleoside 3′-phosphoramidite building blocks **4a–d** were synthesized via the *N*-acyl-5′,3′-*O*-(tetraisopropyldisiloxan-1,3-diyl)-protected nucleosides **1a–d**. Compounds **1a,b** and **d** were allowed to react with vinyloxypropionitrile[12] in the presence of *p*-toluenesulfonic acid in 1,4-dioxane to give the 2′-*O*-CEE-protected nucleosides (**2a,b** and **d**) in 94–97% yields.[9] In the case of the *N*-2-phenoxyacetylguanosine derivative **1c**, a side reaction occurred under the same conditions. Therefore, the reaction was carried out in the presence of pyridinium *p*-toluenesulfonate at 55 °C to afford **2c** in an acceptable yield (75%). The selective deprotection of the 3′,5′-cyclic silyl ethers was achieved by treatment of **2** with 5 equiv of tetrabutylammonium fluoride (TBAF) and 6 equiv of acetic acid in THF.[9] The resulting intermediates bearing the free 5′-OH were

Keywords: Oligoribonucleotide; Chemical synthesis; Protecting group.
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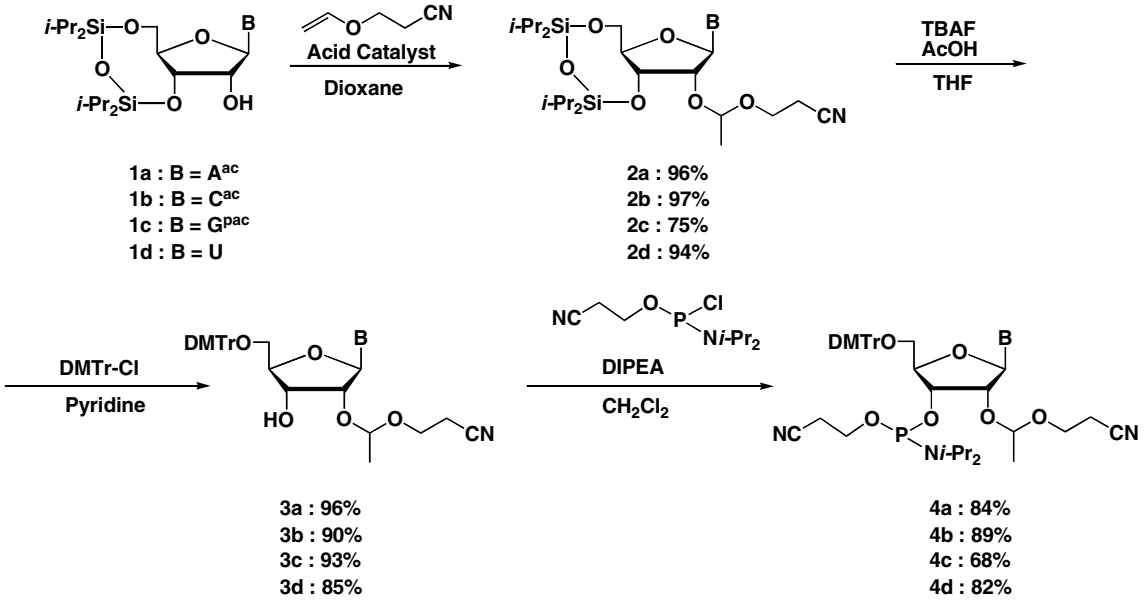
doi:10.1016/j.tetlet.2004.10.151



Scheme 1.

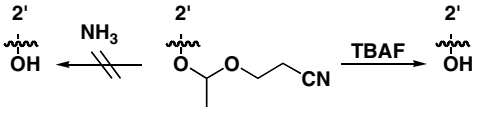
treated with DMTr-Cl in pyridine to give the 2′-*O*-CEE-5′-*O*-DMTr-ribonucleosides **3** in 85–96% yields (two steps), which were further phosphitylated with *O*-(2-cyanoethyl)-*N,N*′-diisopropyl-aminophosphorochloridite in the presence of diisopropylethylamine (DIPEA) to give the phosphoramidite building blocks **4a**–**d** in 68–89% yields (Scheme 1).

Next, the stability of the 2′-*O*-CEE group was examined by using 2′-*O*-CEE-5′-*O*-DMTr-uridine **3d** as a model compound (Table 1). The CEE group was completely retained for at least 30 h at rt in an aqueous or ethanolic ammonia, which are prescribed for the deprotection of *N*-acyl and phosphate protecting groups. On the other hand, the CEE group was gradually removed by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) via a β-elimination mechanism. Finally, it was found that the CEE group was easily removed by treatment with TBAF in dry THF within 1 min. When the reaction was carried out in the presence of excess water, the CEE group was deprotected much slower than under anhydrous conditions. In addition, the CEE group was stable even in the presence of a large excess amount of $Et_3N{\cdot}3HF$ as a fluoride ion source. These results indicate that the rate of the deprotection reactions does not simply depend on the basicity of the reagents in water, but is more probably affected by their basicity in aprotic solvents. Quaternary ammonium fluorides such as TBAF have already been reported to be useful reagents for various base-catalyzed reactions in polar aprotic solvents, and their basicity is controllable by the addition of a protic solvent such as water.[13] Thus, the CEE would be deprotected by treatment with anhydrous TBAF as a base via a β-elimination process.

Table 1. Deprotection conditions and stability of 2′-*O*-CEE-5′-*O*-DMTr-uridine (**3d**)

Entry	Conditions[a]	Half-life (min)
1	0.5 M DBU/MeCN	240
2	0.5 M TBAF/THF	<1
3	TEA·3HF	Stable[b]
4	25% NH_3aq	Stable[b]
5	25% NH_3aq–EtOH (3:1, v/v)	Stable[b]
6	2 M NH_3/EtOH	Stable[b]

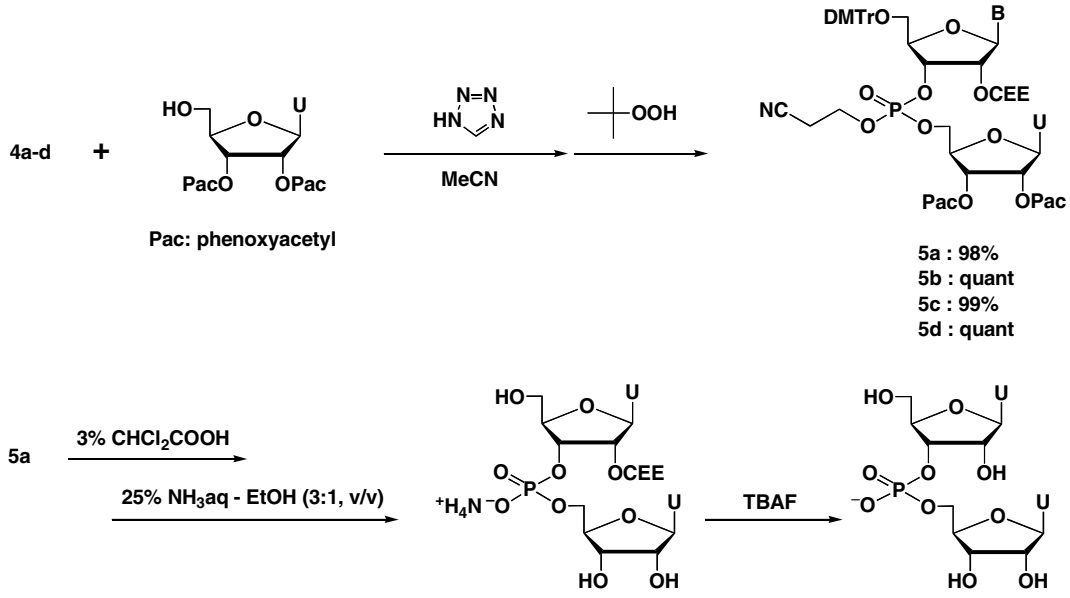
[a] **3d** (0.01 M) at rt.
[b] No degradation was observed for at least 30 h.



The phosphoramidites **4a**–**d** were condensed with 2′,3′-*O*-bis-phenoxyacetyluridine in the presence of 1*H*-tetrazole in acetonitrile by a conventional procedure. After successive oxidation with *tert*-butylhydroperoxide and purification by silica-gel column chromatography, the fully protected dimers **5a**–**d** were obtained in excellent yields (Scheme 2).

The 5′-*O*-DMTr group of **5a** was removed by treatment with 3% dichloroacetic acid (DCA) in CH_2Cl_2. Next, the base-labile protecting groups except for the 2′-*O*-CEE group were removed by treatment with 25% NH_3aq–EtOH (3:1, v/v). Finally, the 2′-*O*-CEE group was deprotected with anhydrous 1 M TBAF in THF at rt for 6 h to give UpU in 78% yield (three steps). The HPLC analysis of the crude product showed neither migration nor cleavage of the internucleotidic linkage (Fig. 1).

In conclusion, the use of the 2′-*O*-CEE group together with *N*-acyl and 2-cyanoethyl phosphate protecting groups enabled a two-step deprotection procedure, first with ammonia, then with TBAF. Thus, 2′-*O*-CEE protection is expected to be compatible with 2′-*O*-TBDMS



Scheme 2.

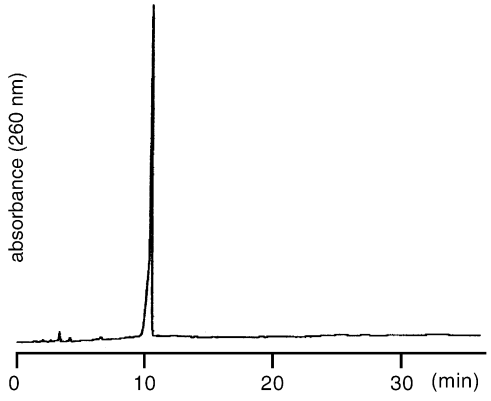


Figure 1. RP–HPLC analysis of the crude mixture of UpU (linear gradient: 0–15% MeCN/0.1 M TEAA in 30 min).

chemistry. Work on the solid-phase synthesis of oligoribonucleotides containing four kinds of nucleobases is now in progress.

Acknowledgements

This work was supported by a Grant-in-Aid for scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan. We are indebted to Professor K. Saigo for helpful suggestions.

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