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Facile synthesis of 2′-*O*-cyanoethyluridine by ring-opening reaction of 2,2′-anhydrouridine with cyanoethyl trimethylsilyl ether in the presence of $BF_3{\cdot}Et_2O$

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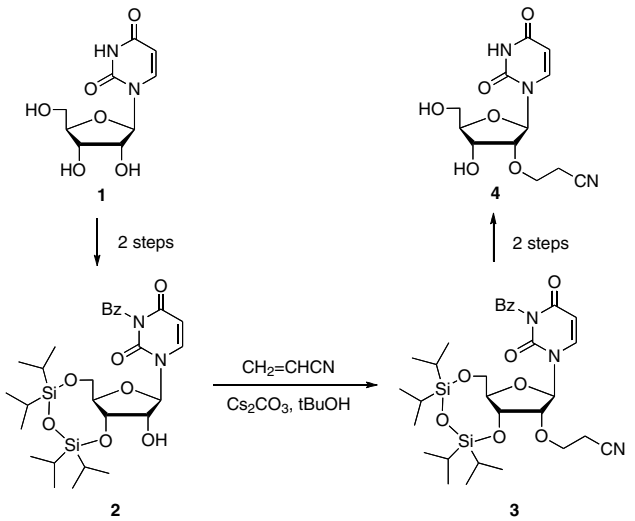
Abstract—In this Letter, a facile method for the synthesis of 2′-*O*-cyanoethyluridine, which is a key intermediate in the synthesis of fully and partially 2′-O-cyanoethylated oligoribonucleotides as well as unmodified oligoribonucleotides, was developed by the ring-opening reaction of 2,2′-anhydrouridine with 2-cyanoethyl trimethylsilyl ether in the presence of $BF_3{\cdot}Et_2O$ in dimethylacetamide. The 2′-*O*-cyanoethyluridine 3′-phosphoramidite derivative was converted into the 2′-*O*-cyanoethyl-4-*N*-acetylcytidine 3′-phosphoramidite derivative by a series of reactions involving displacement of the 4-(1*H*-1,2,4-triazol-1-yl)uridine derivative with ammonia followed by acetylation.

Chemically modified RNAs have proved to be useful tools for various studies related to molecular biology, gene detection, gene therapy, and bioindustry.[1–4] Modification of ribonucleoside sugar residues at the 2′-position has been extensively studied for enhancing the hybridization affinity of synthetic oligoribonucleotides to target DNA/RNA molecules as well as their nuclease resistance and cellular uptake.[5–12] It is desirable that RNA monomer building blocks of 2′-O-modified ribonucleoside derivatives can be synthesized by a series of simple and convenient short-step reactions.

In our laboratory, a general method for the synthesis of 2′-O-cyanoethylated RNA oligomers has recently been developed using the Michael reaction for the introduction of the cyanoethyl group into 2′-hydroxyl under mild conditions (Scheme 1).[13] It was found that 2′-*O*-cyanoethyl RNAs have higher hybridization affinity for DNA and RNA and more improved enzyme resistance than unmodified RNAs and 2′-*O*-methyl RNAs. Furthermore, we found that RNAs could be easily converted to unmodified RNAs by treatment with Bu_4NF in THF.[14] This means that the 2′-*O*-cyanoethylribonucleoside building blocks could be used as the common starting materials for the synthesis of not only 2′-O-cyanoethylated RNAs but also unmodified RNAs.



Scheme 1. Previous method for the synthesis of 2′-*O*-cyanoethyl uridine (**4**).

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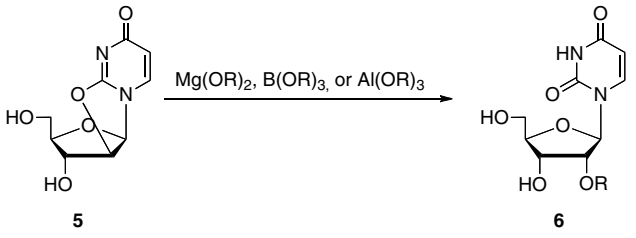
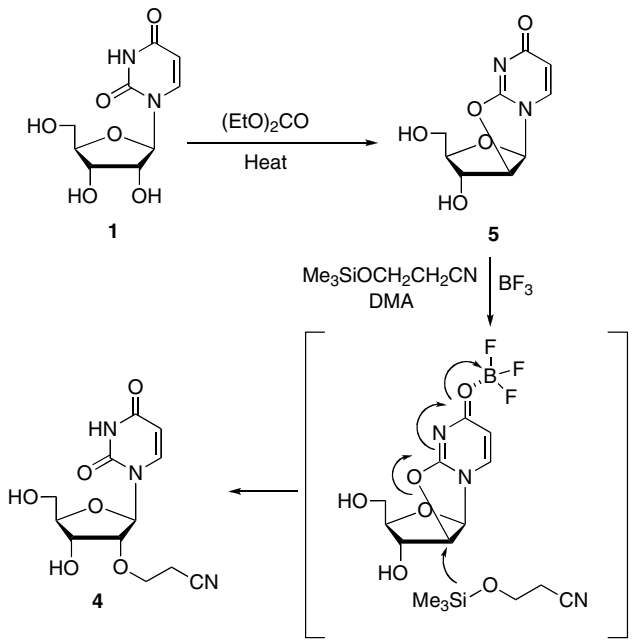
The key intermediate of 2′-*O*-cyanoethylribonucleoside derivative **3** was synthesized by the reaction of 2′-free 3′,5′-O-protected ribonucleoside derivatives **2** with acrylonitrile in *t*-BuOH in the presence of Cs_2CO_3. This strategy was used for all four ribonucleoside derivatives. However, for the uridine derivative, this strategy required a five-step reaction to obtain 2′-*O*-cyanoethyluridine from uridine (**1**), as shown in Scheme 1. In particular, Cs_2CO_3-promoted cyanoethylation needed pre-protection of the N^3 imido group of uridine. Therefore, the procedure for the protection/deprotection of its protecting group could not be avoided. Therefore, we focused on an alternate method for the synthesis of 2′-*O*-cyanoethyluridine **4** by a shorter route. McGee and Zhai reported a straightforward method for the synthesis of 2′-O-alkylated uridine derivatives **6** by the ring-opening reaction of 2,2′-anhydrouridine (**5**) with $Mg(OR)_2$, as shown in Scheme 2.[15] Later, Ross et al. reported that a similar reaction occurred when $B(OMe)_3$ was used as a metal alkoxide.[16] Reese[17] also reported that $Al(OCHCHOCH_3)_3$ was effective as a reagent for the introduction of the methoxyethoxy group into the 2′-position. On the other hand, Ishido and his co-workers reported that simple heating of uridine with diethyl carbonate quantitatively gave 2,2′-anhydrouridine (**5**), as shown in Scheme 3.[18]

If the ring-opening reaction of **5** with similar metal cyanoethoxides could be explored, only a two-step reaction would be required to synthesize 2′-*O*-cyanoethyluridine (**4**). However, it seemed difficult to use magnesium



Scheme 2. Ring-opening reaction of 2,2′-anhydrouridine (**5**) with metal alkoxides to give 2′-O-alkylated uridine derivatives **6**.



Scheme 3. A new route to compound **4** by the BF_3-promoted ring-opening reaction of **5**.

cyanoethoxide because the reaction medium was strongly basic and the cyanoethyl group might be easily eliminated from the once-formed 2′-*O*-cyanoethyluridine. No good methods exist for the synthesis of boron and aluminum. It was reported that the nitrile function is very sensitive to hydrogen halide gas in producing an adduct.[19] On the other hand, a trimethylsilyl ether of 2-cyanoethanol was used for the synthesis of (2-cyanoethyl)dichlorophosphine without the use of any base.[20] This silyl ether seemed to be better as a donor molecule of the 2-cyanoethyl group.

Therefore, 2-cyanoethyl trimethylsilyl ether was used as the nucleophile for the ring-opening reaction of compound **4**. We chose $BF_3{\cdot}Et_2O$ as the Lewis acid for the activation of the cyclic ether bond by coordination of the 4-carbonyl oxygen, which is the most basic site, as

Table 1. Reactions of 2,2′-anhydrouridine with 2-cyanoethyl trimethylsilyl ether in the presence of $BF_3{\cdot}Et_2O$

Entry	Solvent	Time (h)	Temp (°C)	TMS–CE ether (equiv)	Lewis acid	equiv	Yield of **4** (%)
1	Dioxane–HMPA (10:1)	15	120	2.5	$BF_3{\cdot}Et_2O$	2.5	10
2	Dioxane–HMPA (1:1)	15	120	2.5	$BF_3{\cdot}Et_2O$	2.5	17
3	Dioxane–HMPA (1:1)	15	120	5.0	$BF_3{\cdot}Et_2O$	2.5	29
4	HMPA	15	120	5.0	$BF_3{\cdot}Et_2O$	2.5	31
5	DMA	15	120	5.0	$BF_3{\cdot}Et_2O$	2.5	65
6	DMA	15	120	5.0	$BF_3{\cdot}Et_2O$	3.0	61
7	DMA[a]	15	120	5.0	BF_3Et_2O	3.0	51
8	DMA	5	120	5.0	$BF_3{\cdot}Et_2O$	2.5	51
9	DMA	5	120	5.0	$BF_3{\cdot}Et_2O$	3.0	63
10	DMF	15	120	5.0	$BF_3{\cdot}Et_2O$	2.5	25
11	Dioxane	15	Reflux	5.0	$BF_3{\cdot}Et_2O$	2.5	Trace
12	DMA	15	120	5.0	$AlCl_3$	2.5	0[b]
13	DMA	18	40	5.0	BCl_3	2.5	0[b]
14	DMA	15	140	5.0	$ZnCl_2$	2.5	0
15	DMA	15	140	5.0	$MgBr_2$	2.5	0

[a] Ethylenecyanhydrine was used as a nucleophile.
[b] In these cases, 2′-chloro-2′-deoxyuridine was obtained as the exclusive product, as described in the text.

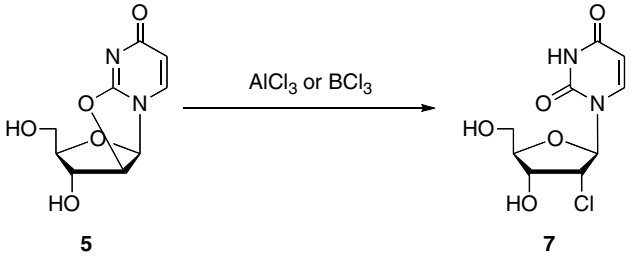
illustrated in Scheme 3. The Si–F bond is known to have a strong bond energy (552.7 kcal);[21] even if the trimethylsilyl fluoride is generated in the reaction medium, it would remain intact with other functional groups of the uridine molecule.

Considering this, we examined a variety of conditions to obtain the desired product **4**. These results are shown in Table 1. It was found that this ring-opening reaction required an elevated temperature of 120 °C and polar solvents such as HMPA and *N*,*N*-dimethylacetamide (DMA). As a result, we could obtain the desired product in 65% yield using 5.0 equiv of 2-cyanoethyl trimethylsilyl ether and 2.5 equiv of $BF_3{\cdot}Et_2O$ in DMA at 120 °C for 15 h.[26] This yield is satisfactory compared with the total yield (ca. 45–50%) of the same product starting from uridine reported previously.[22] The use of $AlCl_3$ and BCl_3 as Lewis acids did not give the desired product **4** but 2′-chloro-2′-deoxyuridine (**7**) as the exclusive product in 95% and 94% yields, respectively, as shown in Scheme 4.

In the synthesis of fully or partially 2′-O-cyanoethylated RNAs as well as unmodified RNAs using N-protected 2′-*O*-cyanoethyl-5′-*O*-dimethoxytritylribonucleoside 3′-phosphoramidites, it is desirable if these monomer building blocks could be synthesized from a common starting material. It is well known that for a pyrimidine series of ribonucleoside derivatives, uridine derivatives can be converted into cytidine derivatives by in situ phosphorylation followed by the displacement of the resulting 4-O-substituted intermediates with 1*H*-1,2,4-triazole.[23] The 4-(1*H*-1,2,4-triazol-1-yl)uridine derivative can be converted into the 4-amino derivative by treatment with ammonia, which can be further converted into the 4-N-protected species by acylation.[24]

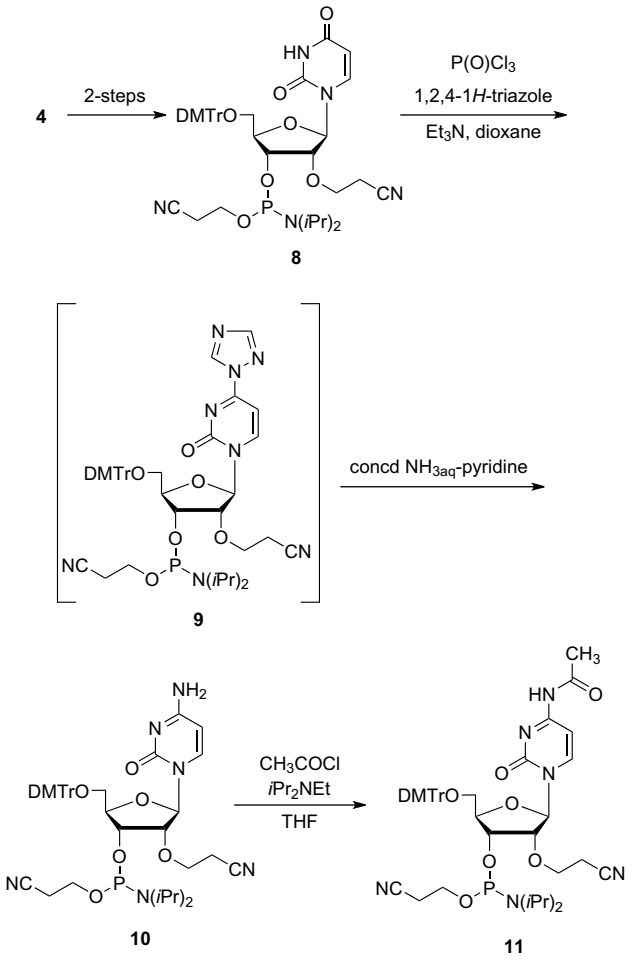
Therefore, we studied the conversion of the uridine unit **8** obtained from compound **4** into the cytidine unit **11** through intermediate **10** by the above-mentioned strategy, as shown in Scheme 5. Thus, the cytosine unit **10** could be synthesized in 80% yield from compound **8**. The desired cytidine 3′-phosphoramidite derivative **11** was easily obtained in 85% yield by acylation of **10** with acetyl chloride.[26]

In conclusion, we have established a more straightforward route to the key intermediate **4** for the synthesis of the uridine phosphoramidite building block **8** using a new ring-opening 2′-O-cyanoethylation and an effective conversion of **9** to the cytidine unit **11**. The improved procedure[26] for the synthesis of the pyrimidine ribonucleoside 3′-phosphoramidite derivatives would significantly reduce the overall burden on the synthesis of the 2′-O-cyanoethylated and unmodified RNA derivatives.



5 **7**

Scheme 4. Formation of 2′-chloro-2′-deoxyuridine (**7**).



Scheme 5. Conversion of the uridine 3′-phosphoramidite building block **8** to the cytidine 3′-phosphoramidite building block **11**.

Acknowledgments

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26. *Experimental procedure for the synthesis of 2′-O-cyanoethyluridine* (**4**): 2,2′-Anhydrouridine (452 mg, 2 mmol) was coevaporated three times each with pyridine, toluene and dissolved in DMA (4 ml) under argon atmosphere. To the solution were added 2-cyanoethyl trimethylsilyl ether (1432 mg, 10 mmol) and BF_3·OEt (628 μl, 5 mmol). The mixture was stirred at 120 °C for 15 h, and then MeOH was added. The solution was mixed with NH-silica gel, and the resulting mixture was evaporated in vacuo. The powder was subjected on a silica gel column. Elution was performed with $CHCl_3$–MeOH (95:5, v/v) to give 2′-*O*-cyanoethyluridine (**4**) (383 mg, 65%) as a white solid. *Synthesis of cytidine monomer building block* **11**. 2′-*O*-(2-Cyanoethyl)-5′-*O*-(4,4′-dimethoxytrityl)uridine 3′-[2-cyanoethyl (*N*,*N*-diisopropyl)phosphoramidite] (**8**) (250 mg, 0.313 mmol) was coevaporated three times each with pyridine, toluene and dissolved in dry CH_3CN (10 ml) under argon atmosphere. To the solution were added triethylamine (1 ml, 7.2 mmol), 1,2,4-1*H*-triazole (486 mg, 7 mmol) and $POCl_3$ (57 μl, 0.625 mmol). The mixture was stirred at room temperature for 12 h and then diluted with ethyl acetate. The solution was extracted twice with aqueous 5% Na_2CO_3 solution. The organic layer was dried over Na_2SO_4 and filtered. The solution was evaporated under reduced pressure. The residue was dissolved with pyridine–concd NH_3 (4:1, v/v, 15 ml). After being stirred at room temperature for 2 h, the mixture was evaporated under reduced pressure. The residue was chromatographed on an NH-silica gel column with $CHCl_3$–MeOH (99:1, v/v) to give 2′-*O*-(2-cyanoethyl)-5′-*O*-(4,4′-dimethoxytrityl)cytidine 3′-[2-cyanoethyl (*N*,*N*-diisopropyl)phosphoramidite] (**10**) (200 mg, 80%) as a white foam. *Compound* **10** (180 mg, 0.225 mmol) was coevaporated five times each with pyridine, toluene and dissolved in dry THF (5 ml) under argon atmosphere. To the solution were added ethyldiisopropylamine (77 μl, 0.45 mmol) and acetyl chloride (20 μl, 0.27 mmol). After being stirred at room temperature for 2 h, the mixture was diluted with $CHCl_3$. The solution was washed with brine and aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4 and filtered. The solution was evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with $CHCl_3$–MeOH (98:2, v/v) containing 0.5% triethylamine to give 4-*N*-acetyl-2′-*O*-(2-cyanoethyl)-5′-*O*-(4,4′-dimethoxytrityl)cytidine 3′-(*N*,*N*-diisopropyl)phosphoramidite (**11**) as a white foam (160 mg, 85%).