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Synthesis and properties of novel 2′-*O*-alkoxymethyl-modified nucleic acids

Koichiro Arai, Naoki Uchiyama, Takeshi Wada *

Department of Medical Genome Sciences, The University of Tokyo, Bioscience Building 702, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8562, Japan

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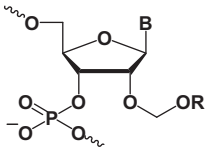
ABSTRACT

Novel 2′-*O*-modified oligoribonucleotides with alkoxymethyl skeletons were synthesized, and their ability to hybridize complementary nucleic acids and their nuclease resistance were analyzed. The hybridization ability was improved by introducing electron-withdrawing groups and the increases in melting temperature (T_m value) was particularly high for chlorine-substituted compounds. Nuclease resistance of these 2′-*O*-alkoxymethylated oligomers was lower than expected, but cyano substitution resulted in a higher nuclease resistance than 2′-*O*-methylation.

Numerous chemically modified nucleic acids have been developed to improve the chemical and biological properties of natural nucleic acids in drugs. In particular, 2′-*O*-modified nucleic acids, with modifications such as the introduction of 2′-*O*-methyl[1], 2′-*O*-methoxyethyl[2], and 2′-*O*-aminopropyl[3] groups, are known to exhibit high duplex stability and nuclease resistance. However, some 2′-*O*-modifications are often difficult to introduce mainly because the alkylation of nucleobases occurs as a side reaction during the synthesis. For this reason, the synthesis of ribonucleotide monomers in most of studies of 2′-*O*-modification has been limited to those bearing pyrimidine bases, and only few studies have been reported for the monomers bearing purine bases.[4]

The 2′-*O*-alkoxymethyl group is a skeleton often used as a 2′-OH protecting group.[5–8] Ribonucleotide monomer bearing this type of group generally exhibits high coupling efficiency in oligomer synthesis, and introduction of this group to the 2′-position is performed by using the same strategy for all nucleobases of with no side reactions. These properties are practically attractive as 2′-*O*-modifications; however, the simplest alkoxymethyl group, the ethoxymethyl group, was reported to destabilize a duplex when it was introduced to the 2′-position.[2,9] Several 2′-*O*-modified oligoribonucleotides with alkoxymethyl skeleton[10,11] have been reported; however, they either resulted in destabilization of duplexes[10] or no hybridization analysis was conducted.[11]

Recently, 2′-*O*-modified RNAs with electron-withdrawing groups at the 2′-position has been reported to demonstrate high duplex stability.[4,12] These results motivated us to study the substituent effect of electron-withdrawing groups in the 2′-*O*-alkoxymethyl skeleton. In this report, we describe the synthesis and properties of novel 2′-*O*-alkoxymethyl-modified oligoribonucleotides bearing electron-withdrawing groups.



A: 2′-*O*-EOM : R = CH_2CH_3
B: 2′-*O*-MCEM : R = CH_2CH_2Cl
C: 2′-*O*-DCEM : R = CH_2CHCl_2
D: 2′-*O*-TCEM : R = CH_2CCl_3
E: 2′-*O*-CEM : R = CH_2CH_2CN
F: 2′-*O*-DFEM : R = CH_2CHF_2
G: 2′-*O*-TFEM : R = CH_2CF_3

Figure 1. Structure of 2′-*O*-modifications. Abbreviations: EOM, 2′-*O*-ethoxymethyl; MCEM, 2′-*O*-(2-chloroethoxy)m ethyl; DCEM, 2′-*O*-(2,2-dichloroethoxy)methyl; TCEM, 2′-*O*-(2,2,2-trichlo rorethoxy)methyl; CEM, 2′-*O*-(2-cyanoethoxy)methyl; DFEM, 2′-*O*-(2,2-di fuluoroethoxy)methyl; TFEM, 2′-*O*-(2,2,2-trifluoroethoxy)methyl.

As an electron-withdrawing group, we introduced several halogen atoms into the 2′-*O*-alkoxymethyl skeleton (Fig. 1). We also investigated the 2′-*O*-CEM group, which was developed by Ohgi et al. , as a 2′-OH protecting group.[5,6]

We first synthesized various phosphoramidite monomers with electron-withdrawing substituted 2′-*O*-alkoxymethyl groups, according to a previous report on the 2′-OH protecting group[6] (Scheme 1). The introduction of alkoxymethyl group was performed under the acidic condition, using 2′-*O*-methylthiomethyl uridine and alcohols with electron-withdrawing group.

Next, 2′-*O*-modified oligoribonucleotides were synthesized using the phosphoramidite monomer **5**. The synthesis was conducted by an Expedite 8909 automated synthesizer (Applied Biosystems) using a standard protocol for 0.2 μmol scale synthesis of

* Corresponding author.
E-mail address: wada@k.u-tokyo.ac.jp (T. Wada).

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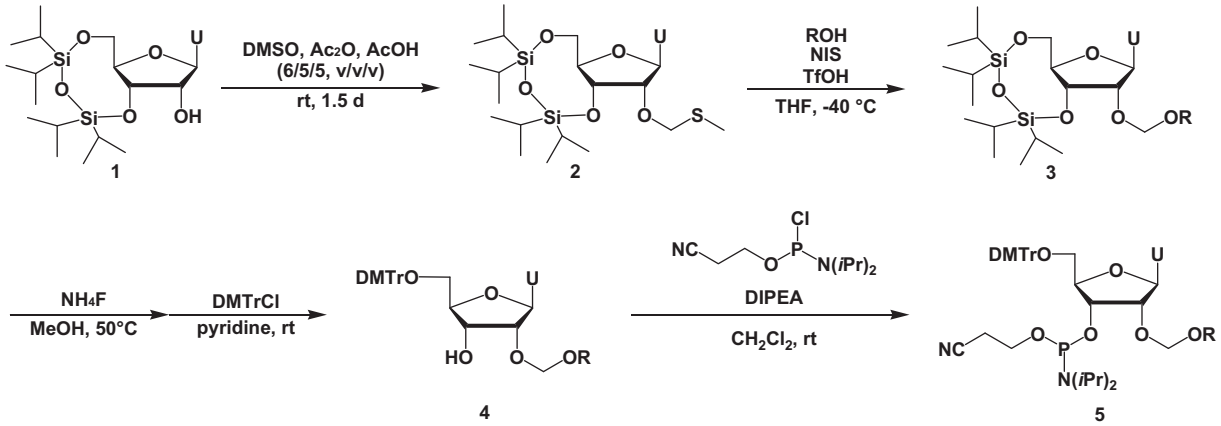

Scheme 1. General synthetic procedure for 2′-*O*-modified phosphoramidite monomers.

RNA. After the synthesis, oligoribonucleotides were cleaved from solid supports and purified by reversed-phase HPLC.

The hybridization properties of 2′-*O*-modified oligoribonucleotides against complementary RNAs were studied (Table 1). The hybridization affinity of 2′-*O*-alkokymethylated U_{12} for A_{12} was compared with that of unmodified U_{12} and 2′-*O*-methylated U_{12}. As shown in Table 1, the introduction of 2′-*O*-EOM without substitutions led to decrease in the T_m value, as previously reported[2,9] (A, Table 1). In contrast, oligoribonucleotides with electron-withdrawing substitution in the 2′-*O*-alkoxymethyl groups exhibited a higher T_m value than that of oligoribonucleotides with the 2′-*O*-EOM group (B–G, Table 1). This result suggests that the introduction of electron-withdrawing groups into the alkoxymethyl skeleton leads to the stabilization of hybridization with complementary RNA.

Among the oligoribonucleotides with electron-withdrawing substitution in the 2′-*O*-alkoxymethyl groups, oligoribonucleotides with chloro-substituted 2′-*O*-MCEM and 2′-*O*-DCEM groups exhibited similar T_m values to that of oligoribonucleotides with the 2′-*O*-methyl group (B, C, H, Table 1, Fig. 2). This result is unexpected because the electron-withdrawing effect of chlorine atoms is weaker than that of fluorine atoms or the cyano group. From these results, the stabilizing effect caused by the 2′-*O*-MCEM and 2′-*O*-DCEM groups is believed to be derived from another factor, such as hydrophobicity of chlorine atoms. On the basis of molecular modeling study, the mono- and disubstituted 2′-*O*-ethoxymethyl groups were located along the minor groove of the A-type duplex and fairly fitted to the relatively hydrophobic groove with van der Waals contact. These structures involving hydration would be important for stabilization of the duplex. In addition, the electron-withdrawing substitution in the ethoxy group may affect the conformational fixation of this group by the gauche effect. Interestingly, oligoribonucleotides with the trichloro-substituted 2′-*O*-TCEM group did not demonstrate high affinity to the complementary RNA (D, Table 1). This observation suggests that higher-order substitution could lead to destabilization of the duplex because of the bulkiness of the 2′-*O*-modification.

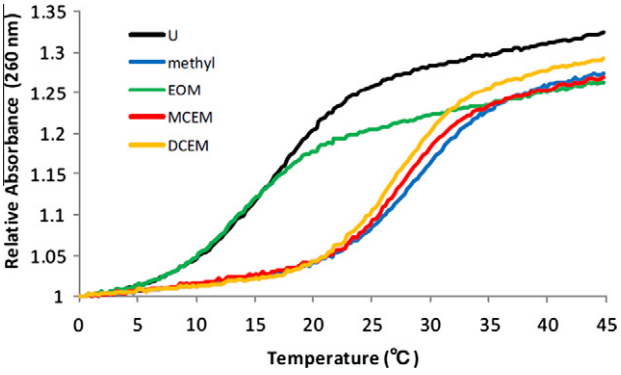

Figure 2. Melting curves of the 2′-*O*-modified oligoribonucleotides with complementary RNA; sequence and conditions are same as Table 1.

The hybridization properties of the 2′-*O*-MCEM- and 2′-*O*-DCEM-modified U_{12} against the complementary DNA dA_{12} were also studied (Fig. 3). In these cases, no significant increase in the T_m value was observed by the introduction of 2′-*O*-MCEM and 2′-*O*-DCEM groups. These results indicate that the duplex stabilizing effect of the 2′-*O*-MCEM and 2′-*O*-DCEM modifications is specific to RNA. This is

Table 1
Hybridization properties of 2′-*O*-modified RNA against complementary RNA relative to unmodified RNA[a]

entry	U*[b]	ΔT_m (°C)	ΔT_m/mod.(°C)
	U	0	0
A	2′-*O*-EOM-U	−2.5	−0.2
B	2′-*O*-MCEM-U	+11.0	+1.0
C	2′-*O*-DCEM-U	+10.8	+1.0
D	2′-*O*-TCEM-U	−0.8	−0.1
E	2′-*O*-CEM-U	+2.8	+0.3
F	2′-*O*-DFEM-U	+3.5	+0.3
G	2′-*O*-TFEM-U	+4.5	+0.4
H	2′-*O*-Me-U	+12.1	+1.1

[a] Conditions: 10 mM sodium phosphate buffer (pH 7.0), 100 mM NaCl, 0.1 mM EDTA, at 260 nm, 2.0 μM oligoribonucleotides and complementary RNA.
[b] Sequence: 5′-U*U*U*U*U*U*U*U*U*U*U*U-3′.

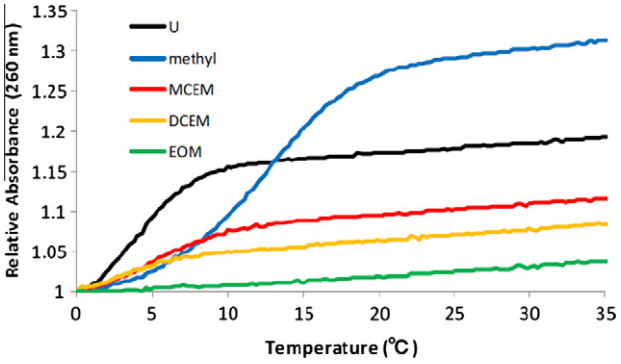

Figure 3. Melting curves of the 2′-*O*-modified oligoribonucleotides with complementary DNA; sequence and conditions are same as Table 1.

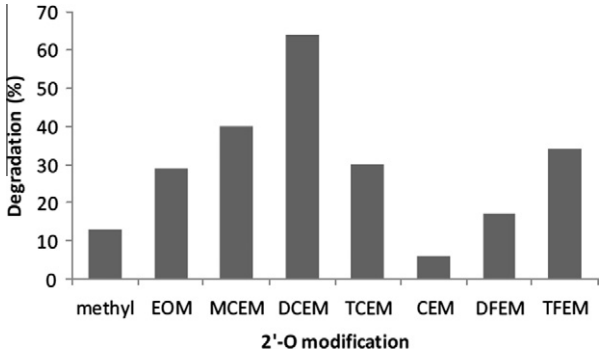

Figure 4. Relative nuclease resistance of oligoribonucleotides with 2′-*O*-modifications. [a]Sequence: 5′-U∗U∗U∗U∗U∗U∗U∗U∗U∗U∗U-3′. [b]Procedure: 10 μM oligoribonucleotides were digested with snake venom phosphodiesterase (4×10^{-4} units/ml) for 2 h in 50 mM Tris–HCl (pH 8.5), 72 mM NaCl and 14 mM $MgCl_2$.

contrast to the observed effect of the 2′-*O*-methyl modification, which demonstrated high affinity to the complementary strand in both RNA and DNA (Figs. 2 and 3). From these results, it could be stated that the 2′-*O*-MCEM or 2′-*O*-DCEM modification stabilizes the duplex in a manner different from that of the 2′-*O*-methyl modification.

Nuclease resistance of the 2′-*O*-modified oligoribonucleo-tides was tested by using snake venom phosphodiesterase (SVPD) (Fig. 4). Oligoribonucleotides were treated with the nuclease for 2 h and the degradation ratio was estimated by reversed phase HPLC analysis. Surprisingly, most of the oligoribonucleotides with 2′-*O*-alkoxy-methyl modifications were less resistant to the nuclease than the oligoribonucleotides with 2′-*O*-methyl modification, although the former groups are bulkier than the methyl group. It is also noteworthy that the degradation ratio increased when additional halogen atoms were substituted, except for the 2′-*O*-TCEM group. This result suggests that the introduction of halogen atoms, especially chlorine atoms, could lead to the reduction of nuclease resistance. In previous studies[12,13], benzyl groups reduced nuclease resistance when introduced into the 2′-position. From these results, it was implied that hydrophobic substitutions could possibly cause a reduction in nuclease resistance. In contrast, 2′-*O*-CEM-substituted oligoribonucleotides demonstrated higher nuclease resistance than the 2′-*O*-methyl oligoribonucleotides. A similar improvement in nuclease resistance was observed in the 2′-*O*-cyanoethyl modification[4], which suggests that the introduction of the cyano group into the 2′-modification could lead to a higher nuclease resistance.

In conclusion, we have synthesized and analyzed the properties of oligoribonucleotides bearing a novel 2′-*O*-modification with alkoxymethyl skeletons. Hybridization affinity of the oligoribonucleotides with electron-withdrawing substituted 2′-*O*-alkoxymethyl groups was higher than that of unmodified 2′-*O*-EOM oligoribonucleotides. The increases in T_m value were especially high in the 2′-*O*-MCEM and 2′-*O*-DCEM substitutions, in which the duplex stabilizing effect was specific to RNA. Nuclease resistance of the 2′-*O*-alkoxymethylated oligoribonucleotides, except for the 2′-*O*-CEM-substitutedoligoribonucleotides, was unexpectedly lower than that of 2′-*O*-methylated oligoribonucleotides. Further investigation of the substitution and synthesis of oligoribonucleotides bearing other nucleobases are in progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.09.003.

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