Synthesis and properties of oligonucleotides containing 2'-O-methyl-2-thiouridines

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ABSTRACT
New methods to synthesize 2'-O-methyl-2-thiouridine and its phosphoramidite building block for incorporation into oligonucleotides were developed. Oligonucleotides containing 2'-O-methyl-2-thiouridines were expected to be favorable as antisense agents in several respects, i.e., nuclease resistance, stable RNA duplex formation, and exact base recognition. Therefore, to make them clear, we synthesized oligonucleotides having 2'-O-methyl-2-thiouridine and analyzed their properties in detail.

INTRODUCTION
In transfer RNA (tRNA), various modified nucleosides have been discovered to date (1). It is generally thought that those modified nucleosides play an important role in stabilizing their original structures and controlling their functions. It was reported that uridine derivatives having the 2-thioketo group at the first letter of anticodon (the wobble position) improve the base pair recognition toward adenosine of the third letter of codon triplet and stabilize the codon-anticodon mini-helix structure (2). These selectivity and thermal stability are favorable properties for the antisense techniques (3). Oligonucleotides having 2-thiouridine(s) have been synthesized chemically and enzymatically, and were reported to form thermodynamically stable RNA duplexes (4,5). The thermal stability in a duplex of (poly-2-thiouridylate)2 is due mainly to the stacking effect between the 2'-thioketo group and the N1 atom of a downstream 2-thiouridine. Another important effect for stabilizing the RNA duplex is also sugar puckering predominance of the C3'-endo conformation, because the 2'-O-methyl group is larger than the 2'-hydroxyl group itself. As described above, the C3'-endo preference at the nucleoside level results in formation of more stable RNA duplex. Although 2'-O-methyl-2-thiouridine (1, see Figure 1) was previously found in the thermophilic bacteria and synthesized by McCloskey et al., their method needed to separate the 2'- and 3'-O-methylated isomers by HPLC purification (8). Therefore, in this study, to avoid such a bothersome separation procedure, the 5'- and 3'-hydroxyl groups of uridine was masked with the 1,1,3,3-tetraisopropyldisiloxane-l,3-diyl group, and selective methylation of the unprotected 2'-hydroxyl group was performed. Moreover, we developed a phosphoramidite building block 2 for incorporation of this modified nucleoside into oligoribonucleotides, and also studied various properties of 2'-O-methyl-2-thiouridine itself as well as oligonucleotides having 2'-O-methyl-2-thiouridine.

RESULTS AND DISCUSSION
2-Thiouridine (3, see scheme 1) synthesized by Vorbrüggen's method was used as a starting material (9). For selective 2'-O-methylation, the 3'- and 5'-hydroxyl groups were protected with the 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS) group described above. Surprisingly, subsequent benzoylation of the base moiety resulted in appearance of two sets of signals in 1H NMR spectrum of the product, although it was suggested by TLC analysis that a pure material was obtained. These two sets of signals were thought to be regioisomers or rotamers, because they changed into a single set of signals at high temperatures (over 60 °C) and the initial spectrum was recovered reversibly upon cooling to room temperature.

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in benzene gave a complex mixture because both the 2-thioketo group and the Ag₂O have "soft" acid and base characters, so that they reacted with each other. Therefore, a simple methylation, which involves combination of NaH and CH₃I in DMF, was used. Consequently, the fully-protected 2'-O-methyl-2-thiouridine derivative 5 could be obtained. Deprotection of the TIPDS and benzoyl groups gave the desired nucleoside 1 in a satisfactory yield from 3.

In the usual phosphoramidite procedure, the phosphite intermediate was treated with iodine-H₂O as the oxidizing reagent. However, the 2-thioketo group was reported to react with iodine having the "soft" character (10). In order to avoid decomposition of the 2-thioketo group, a protective group on the base moiety was needed (11). Therefore, the TIPDS group of the fully-protected compound 5 was selectively removed by treatment with TBAF-AcOH in THF, so that the benzoyl group remained in fact. Unexpectedly, in the successive 5'-O-dimethoxytritylation, the benzoyl group at the base moiety was partially eliminated. Therefore, the benzoyl group was completely deblocked by ammonia treatment to give the base-free 2'-O-methyl-2-thiouridine derivative. The lack of a protective group on the base moiety of 2'-O-methyl-2-thiouridine does not allow the use of the iodine-H₂O treatment in the phosphoramidite procedure, but phosphite intermediates could also be oxidized by treatment with t-BuOOH instead of iodine-H₂O (12). With this idea in mind, the phosphoramidite 2 was successfully synthesized in the usual way.

The phosphoramidite building block 2 and N³,2',3'-tribenzoyleuridine were condensed in the presence of 1H-tetrazole, and the resulting phosphite intermediate was oxidized in situ with t-BuOOH. All the protective groups of the product were removed successively by treatments with aqueous ammonia, 80% AcOH, and TBAF to give 2sUmpU. Analysis of the vicinal coupling constant of 2'-O-methyl-2-thiouridine indicated sugar puckering predominance of the N-type, i.e. C³'-endo conformation (%)N = 70%). Although this result is similar to that of 2-thiouridine reported by Davies (13), the 5'-upstream ribose of the RNA dimer described above has a very small J₁₂' value (< 1Hz), compared with that of 2sUmpU (J₁₂' = 1.6 Hz) (14). Such a smaller J₁₂' value revealed that the 5'-upstream ribose was nearly perfectly fixed in the N-type conformation.

Several oligoribonucleotides having 2'-O-methyl-2-thiouridine were synthesized by the usual phosphoramidite method except for the iodine-H₂O oxidation step. All the protective groups were removed by the usual workup. The crude oligomers obtained were purified by anion exchange HPLC, and these oligoribonucleotides were used in the following experiments. In order to study structural and thermodynamic properties of oligoribonucleotides containing 2'-O-methyl-2-thiouridine, we measured their CD spectra. The CD data indicated that the global structure of RNA duplexes bearing 2'-O-methyl-2-thiouridine is an A-type structure as seen in the natural RNA duplex. Therefore, MD simulation of RNA duplexes was started from an A-type RNA duplex having 2'-O-methyl-2-thiouridine. The 2-thioketo group did not affect its whole structure during the simulation.

REFERENCES