

Improved synthesis of oligonucleotides containing 2-thiouridine derivatives by use of diluted iodine solution

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Abstract—It is known that the 2-thiocarbonyl group of 2-thiouridine (s^2U) derivatives reacts easily with various oxidizing agents used in oligonucleotide synthesis to give a complex mixture. In this letter, we report an improved method for the synthesis of oligonucleotides containing s^2U derivatives. It turned out that the 2-thiocarbonyl group of oligonucleotides containing s^2U derivatives was stable in a 0.02 M solution of iodine in pyridine–THF–H₂O. These conditions were successfully applied to the synthesis of oligonucleotides containing s^2U derivatives on an automated DNA/RNA synthesizer. Moreover, no undesirable side reactions were detected so that these modified oligonucleotides could be obtained in markedly improved yields.
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1. Introduction

2-Thiouridine (s^2U) and its derivatives have been discovered from tRNAs.^{1–4} It is known that the thionation of the uracil ring of the 2-carbonyl group leads to the C3'-*endo* conformation because of steric repulsion between the 2-thiocarbonyl group and the 2'-hydroxy group.^{5,6} Thus, oligonucleotides containing its derivatives can form stable duplexes with the complementary RNAs.^{7–10} Moreover, s^2U and its derivatives proved to have an exact base-pair recognition ability.^{9,10} In a U–G wobble base pair, a hydrogen bond between the 2-carbonyl group of the uracil base and the amino group of the guanine base is formed. Since the ability of the sulfur atom as a donor of the hydrogen bonding is lower than that of the carbonyl oxygen atom, a 2-thiouracil-guanine wobble base pair is destabilized so that the base recognition ability of s^2U toward A significantly increases. Actually, our melting temperature analysis of oligoribonucleotides having s^2U and s^2Um revealed predominant base recognition ability of the 2-thiouridine derivatives over the uracil base.¹⁰ These properties of s^2U derivatives are favorable for antisense strategy as well as SNPs analysis.

It is known that various oxidizing agents react with the 2-thiocarbonyl group of 2-thiouridine derivatives.^{11–15} In oligonucleotides synthesis, an oxidation step was required for the phosphoramidite and *H*-phosphonate methods. In earlier papers, 2-thiothymidine (s^2T) was incorporated into DNA by the *H*-phosphonate and phosphoramidite method but no side reactions at the oxidation step using iodine were reported.^{16,17} However, Kuimelis and co-workers reported that, in incorporation of s^2T into oligonucleotides via the phosphoramidite method, side reactions occurred at the iodine oxidation step.¹⁸ In order to avoid these side reactions at the iodine oxidation step, they used the toluoyl group for protection of the base moiety.¹⁹

In the synthesis of RNA containing s^2U , it was reported that the 2-thiocarbonyl group of the 2-thiouracil base also reacted with iodine. Davis et al. proposed the use of a solution of *tert*-butyl hydroperoxide in CH₃CN as the oxidizing agent in place of a solution of iodine in pyridine–H₂O to avoid this problem.²⁰ Recently, Sochacka reported the reactivity of the 2-thiocarbonyl moiety of s^2U -loaded LCAA-CPG toward various oxidizing agents, which were prescribed for the usual solid-phase synthesis of oligonucleotides.¹⁴ They claimed that the 2-thiocarbonyl moiety underwent side reactions with *tert*-butyl hydroperoxide in CH₃CN and reported that the best result was obtained by the use of carbon

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tetrachloride-*N*-methylmorpholine in pyridine–water–acetonitrile. However, in our hands, this oxidation system did not give the desired oligonucleotides at all. Therefore, we reexamined suitable oxidizing agents for the synthesis of oligonucleotides containing s^2U derivatives.

2. Results and discussion

Oligonucleotides containing s^2U derivatives were synthesized in our previous studies.^{10,21} However, the yields of modified oligonucleotides were quite low (ca. 5–20%) when Davis' oxidizing agent was used. In order to improve the yield of oligonucleotides containing s^2U derivatives, several conditions for the oxidation step were examined.

First, we checked the stability of the 2-thiocarbonyl groups toward *tert*-butyl hydroperoxide and iodine in solution. The s^2U derivative **1** was prepared by use of Vorbrüggen's method. When compound **1** was treated with a 0.1 M solution of *tert*-butyl hydroperoxide in CH_3CN , the 2-thiocarbonyl group of **1** was completely converted to a carbonyl group in 12 h to give the uridine

derivative **3**. Our result indicated that prolonged reaction time resulted in damage of the 2-thiocarbonyl group. Since the conditions used by Davis et al. required a relatively long reaction time (each oxidation required 12 min), it was likely that the desired oligonucleotides could not be obtained in good yields.

Contrary to this result, only the desulfurization product **2** was formed with a 0.1 M solution of iodine in pyridine– H_2O . To our surprise, however, it was found that the use of a one-fifth diluted solution of iodine, that is, a 0.02 M solution of iodine in pyridine– H_2O ,²² resulted in virtually no formation of **2** even after 24 h. Under this diluted condition, it was confirmed that compound **1** remained unchanged. These results are summarized in Table 1.

Based on these unexpected but satisfactory results, the diluted iodine solution was applied to the synthesis of oligonucleotides containing s^2U derivatives on an automated DNA/RNA synthesizer. In this study, we used 2'-*O*-methyl-2-thiouridine (s^2Um) instead of s^2U because the 2'-*O*-methylated nucleoside was easily handled. The s^2Um 3'-phosphoramidite derivative was synthesized according to our previous paper.^{10,21}

Table 1. Stability of 2-thiouridine derivatives under conditions using *tert*-butyl hydroperoxide or iodine

Conditions	Equiv ^a	Time (h)	Recovery of 1 (%)	Product (%) ^b	
				2	3
0.1 M <i>tert</i> -Butyl hydroperoxide in CH_3CN	10	12		nd ^c	70
0.1 M Iodine in pyridine– H_2O (9:1, v/v)	10	1		53	nd
0.02 M Iodine in pyridine– H_2O (9:1, v/v)	2	24	86	nd	nd

^a Equivalent of an oxidizing agent for compound **1**.

^b Isolated yield.

^c The abbreviation of 'nd' refers to not detected.

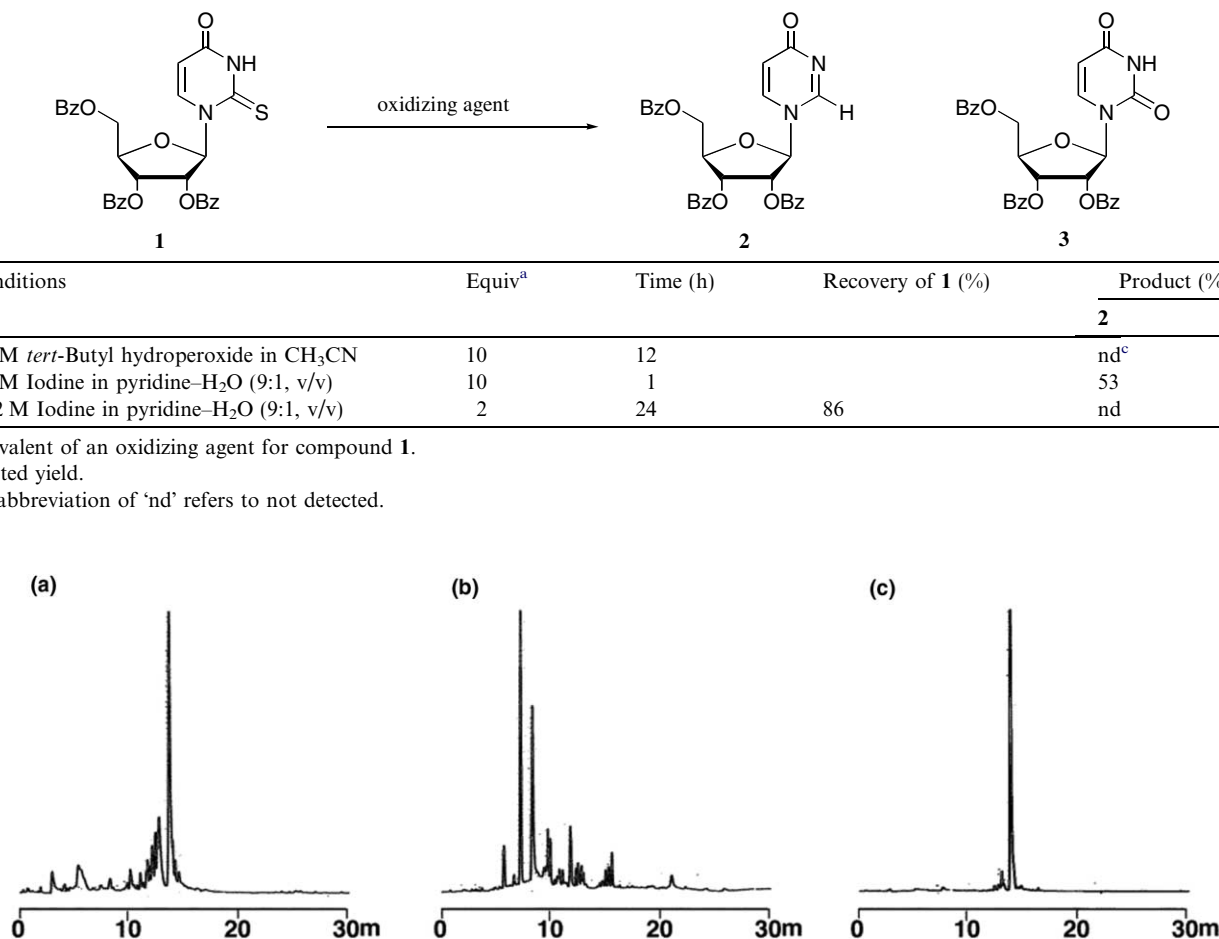


Figure 1. RP-HPLC Profiles of 5'-dTTTTTTS²UmT-3': (a) *tert*-BuOOH– CH_3CN 60 s, (b) *tert*-BuOOH– CH_3CN 15 s, (c) I_2 –pyridine– H_2O –THF 15 s.

Table 2. Yields and MALDI-TOF mass analysis of oligonucleotides containing s²Um

Oligonucleotide ^a	Calcd [M–H] [–]	Found	Yield (%)
dTTTTTTTXXT	2705.4	2705.8	53
dCGTTTTXXTTTTGC	3941.6	3941.9	31
dCGTTTTXXXXTTTTGC	4005.6	4004.8	30
dCGTTXXXXXXTTGC	4069.5	4071.1	19

^a = s²Um.

When *tert*-butyl hydroperoxide in CH₃CN was used as the oxidizing agent, many undesired products were observed as expected (Fig. 1a). Moreover, when the time for the oxidation was reduced at every oxidation step, little desired oligonucleotides could be formed because of incomplete oxidation (Fig. 1b). On the other hand, with the use of a 0.02 M solution of iodine in pyridine–H₂O–THF²² as the oxidizing agent, it was found that the synthesis of oligonucleotides containing s²Um could be carried out in good yields (ca. 20–50%) without any detectable side reaction products (Fig. 1c and Table 2). In the synthesis of the oligonucleotide derivative containing five modified bases, the main reason of the low yield (19%) is due to the low coupling efficiency of the s²Um phosphoramidite unit because of the steric hindrance of the 2'-*O*-methyl group.

In conclusion, it turned out that the 2-thiouracil base is very sensitive to the concentration of iodine so that there is a clear-cut difference in reactivity between 0.1 and 0.02 M solutions of iodine in pyridine–THF–water. It was unclear why such pronounced change of its reactivity was observed. Since iodine can form polyiodine or polyiodide complexes with other iodine (I₂) or iodide (I[–]) molecules,²³ it is likely that such polymeric species tend to be more easily formed at higher concentrations and might have more reactivity than the monomeric iodine. It should be noted that our finding is of great importance since the 2-thiouracil base has proved to have an excellent base-recognition ability, especially, only to the adenine moiety.^{9,10} The improved method described here would provide a practical and reproducible method for the synthesis of 2-thiouracil-containing oligonucleotides.

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