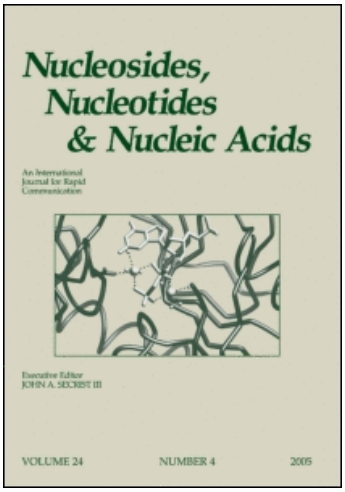

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Solid-Phase Synthesis of Modified RNAs Containing Amide-Linked Oligoribonucleosides at Their 3'-End and Their Application to siRNA

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SOLID-PHASE SYNTHESIS OF MODIFIED RNAs CONTAINING AMIDE-LINKED OLIGORIBONUCLEOSIDES AT THEIR 3′-END AND THEIR APPLICATION TO siRNA

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□ *siRNAs against luciferase mRNA were modified with amide-linked oligoribonucleosides (amide-linked RNA) at their 3′-overhangs. Tm values of the modified siRNAs increased compared with that of the unmodified siRNA. These results indicate that the modified overhangs increase the thermodynamic stability of the siRNAs. The modified overhangs improved stability of siRNAs against degradation by nuclease S1 and 50% mouse plasma. Furthermore the modified siRNAs reduced the target gene expression in a similar manner to the unmodified siRNA in cultured cells. These results suggest that the overhang modifications are tolerated for the siRNA activity.*

Keywords modified RNAs; amide-linked RNA; siRNA activity

INTRODUCTION

Short interfering RNAs (siRNAs)[1] are required to have the properties of nuclease resistance and sufficient activity to inhibit the target gene expression for the effective biological and therapeutic applications. Several chemical modifications of the siRNAs have been reported for these requirements.[2] It has been suggested that backbone-modified RNA with 3′-5′ methyleneamide linkages (amide-linked RNA)[3–6] has conformationally restricted furanose rings to C3′-*endo* which is characteristic of A-type duplex of RNA.[3] We reported modified RNAs containing trimer to hexamer of amide-linked RNA at the 3′-terminal parts, which formed A-type duplexes with the complementary RNA.[6] The amide-linked RNA segments also exhibited rigid conformational property.[6] From these observations, we expected that the modification of 3′ overhang regions of siRNAs by

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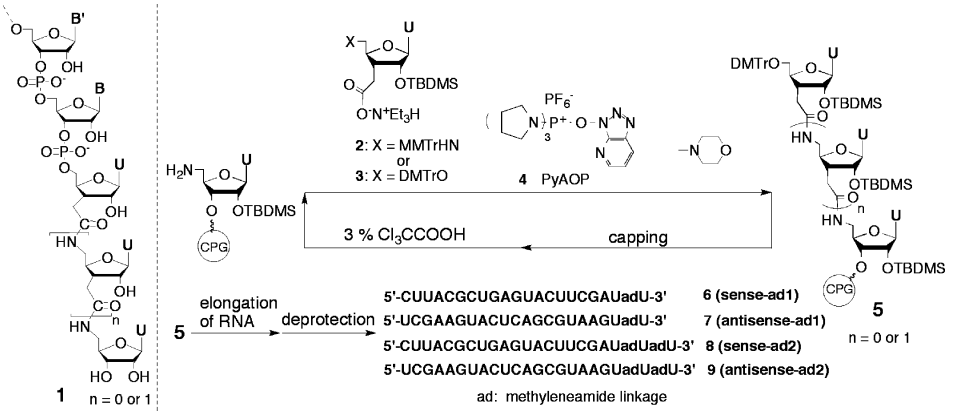
amide-linked RNA increases the thermodynamic stability of the siRNAs by the stacking effect of the 3′-overhangs. The amide-linked RNA segments are also anticipated to increase the nuclease resistance of siRNAs. Here we report the synthesis of modified RNAs containing dimer or trimer of amide-linked RNA at the 3′-ends (**1**) by solid-phase approach. We also investigate the thermodynamic stability, nuclease resistance and inhibitory effect of the target gene expression by the siRNAs containing dimer or trimer of amide-linked RNA segments at the 3′-overhangs.

RESULTS AND DISCUSSION

The building block of 5′-N-(4-methoxytrityl)amino-2′-O-(*tert*-butyldimethylsilyl)-3′-carboxymethyl-3′,5′-dideoxyuridine[6] (**2**) was synthesized in 9 steps from 2′, 5′-bis-O-(*tert*-butyldimethylsilyl)uridine. Oxidation of 3′-hydroxyl group of the 2′, 5′-silylated uridine using DMSO/Ac_2O gave 2′, 5′-bis-O-(*tert*-butyldimethylsilyl)-3′-ketouridine[7] in 78% yield. In this reaction we observed by-products one of which was presumably caused by the migration of 2′-*tert*-butyldimethylsilyl group of the 2′, 5′-silylated uridine. The building block **2** was then prepared from the 3′-ketouridine derivative via 5′-amino-2′-O-(*tert*-butyldimethylsilyl)-3′-methoxycarbonylmethyl-3′, 5′-dideoxyuridine which was synthesized by the slightly modified procedure of Robins et al.[8]

We prepared dimer and trimer of amide-linked oligouridine derivatives by solid-phase synthesis using **2** and 5′-O-(4,4′-dimethoxytrityl)-2′-O-(*tert*-butyldimethylsilyl)-3′-carboxymethyl-3′-deoxyuridine (**3**)[8] as shown in Scheme 1. Coupling reaction was performed with PyAOP in 82% of the average coupling yield. Successive chain elongation of RNA by phosphoramidite



SCHEME 1 Structure of amide-linked RNA at 3′-terminal region of a modified RNA (**1**) and a scheme for the solid-phase synthesis of the modified RNAs.

TABLE 1 Sequences, abbreviations, and normalized luciferase activities of modified siRNAs containing amide-linked RNA

Sequence		Abbreviation	Normalized Luc./RL. (%)[a]
5′-CUUACGCUGAGUACUUCGAUadU-3′ 3′-UadUGAAUGCGACUCAUGAAGCU-5′	(6) (7)	siRNA-ad1	10.2 ± 1.0
5′-CUUACGCUGAGUACUUCGAUadUadU-3′ 3′-UadUadUGAAUGCGACUCAUGAAGCU-5′	(8) (9)	siRNA-ad2	6.0 ± 1.5
5′-CUUACGCUGAGUACUUCGAUU-3′ 3′-UUGAAUGCGACUCAUGAAGCU-5′		siRNA-control	10.8 ± 0

ad: methyleneamide linkage, upper chain: sense sequence, lower chain: antisense sequence.
[a]A549 Cells were transfected with pGL3 and pRL luciferase vectors and 20 nM siRNA. Ratios of firefly luciferase activity (Luc.) to *Renilla* luciferase activity (RL.) were normalized to the vector control experiment without siRNA.

method gave modified RNAs **6**∼**9**. The modified RNAs were annealed to obtain modified siRNAs targeting firefly luciferase as shown in Table 1.

Thermodynamic stability of the siRNAs was examined using UV melting temperature analysis. Tm values of siRNA-ad1 and siRNA-ad2 increased by 0.8°C and 2.0°C respectively compared to that of siRNA-control (69.5°C). These results suggest that the amide-linked RNA segments increase the thermodynamic stability of the siRNA.

To examine the nuclease resistance of the modified siRNAs, the siRNAs were incubated with nuclease S1. The modified siRNAs were observed as the principal constituents after 120 minutes whereas siRNA-control was degraded within 15 minutes. Therefore, the amide-linked RNA segments were proved to be resistant to nuclease S1. When incubated with 50% mouse plasma, the modified siRNAs remained at 1 hour whereas siRNA-control was degraded. These results suggest that the amide-linked RNA segments enhance the exonuclease resistance of the siRNAs.

To evaluate the compatibility of amide-linked RNA segments with RNAi activity, the siRNAs were examined for their ability to inhibit firefly luciferase in cultured A549 cells expressing both firefly and *Renilla* luciferases. As shown in Table 1, siRNA-ad1 and siRNA-ad2 effectively inhibited firefly luciferase activity similarly to the unmodified siRNA (siRNA-control). These results suggest that the amide-linked RNA segments were compatible with the RNAi activity.

CONCLUSION

In conclusion, the amide-linked RNA segments at 3′-overhangs of siRNA were found to increase the thermodynamic stability and the nuclease resistance of the siRNA. Furthermore the amide-linked RNA segments proved to be tolerated for the siRNA activity.

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