Synthesis and Purification of Antisense Oligonucleotide Containing Amide-linked RNA at Both Ends

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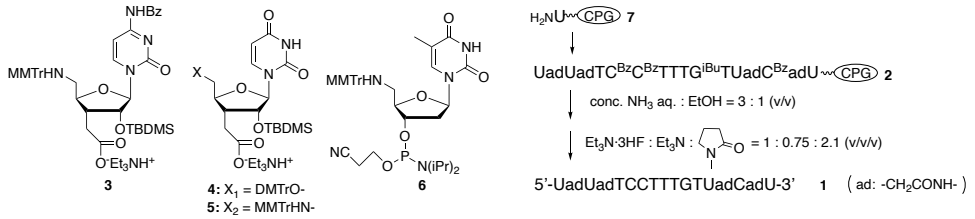
Chemical modification is required for antisense oligonucleotides to increase the nuclease resistance and to form stable duplex with the target mRNA. Amide-linked RNA is a modified RNA which has 3'-5' methyleneamide linkages as internucloside structure.[1] It has properties of nuclease resistance and formation of A-form duplex with the complementary RNA.[2] Thus we designed a gapmer type of antisense oligonucleotide having amide-linked RNA segments at the both ends of DNA, 5'-UadUadTCCTTTGTUadCadU-3' (ad: methyleneamide linkage) (**1**), to cleave the target mRNA by RNase H activity. We have previously reported the chain elongation of **1** on solid support to give **2**.[3] The amide-linked RNA regions were constructed by condensation of the building blocks (**3**)-(**6**). PyFOP was used as the coupling agent to activate (**3**)-(**5**). The overall coupling yield of **2** was 78% from the 3'-terminal uridine (**7**). Herein we report the deprotection of **2** and purification of **1**.

First, **2** was treated with the mixture of conc. NH_3 aq. and EtOH (3:1 v/v) at 21°C for 48 h for the cleavage of the 13-mer from solid support, and deprotection of cyanoethyl group, benzoyl group and isobutyryl group on the 13-mer. Next, removal of *tert*-butyldimethylsilyl group on 2'-hydroxy group of amide-linked RNA segments of the 13-mer was performed by the mixture of $Et_3N{\cdot}3HF$: Et_3N : 1-methyl-2-pyrrolidone (1 : 0.75 : 2.1 v/v/v) at 65°C for 3.5 h. After the purification by the use of Sep-Pak C18 and Nap-10 gel filtration, the crude product of **1** was obtained in 77% recovery yield from **2**. Then, isolation of **1** was accomplished by the use of C18 reversed phase HPLC as the main product in 48.7% isolation yield from the crude product. In conclusion, the overall deprotection and isolation yield of **1** was 37% from **2**. The composition analysis of the deoxyribonucleosides of **1** is currently underway.



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