

Synthesis and Purification of 2'-O-Methyl RNA Containing Amide-linked RNA Modified with Pyrene at the 2'-Position

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Oligonucleotides containing 2'-pyrene modified uridine (Upy) exhibit the increase of pyrene monomer emission when hybridize with the complementary RNA^{1,2)}. The increase of fluorescence intensity is caused by the conformational change of the pyrene residue to the minor groove of the A-form duplex. On the other hand, amide-linked RNA is a modified RNA which consists of 3'-5' methyleneamide linkages in place of 3'-5' phosphodiester linkages. The sugar conformation is restricted to C3'-*endo* form. Thus, amide-linked RNA modified with pyrene at the 2'-position is expected to function as fluorescent probe for the complementary RNA. Previously, a DNA containing amide-linked dimer with pyrene at the 2'-position (dCATGUpy·adCTAC) was synthesized. It showed the increase of fluorescent intensity to 7-fold at 375 nm when hybridized with RNA³⁾. The small increase of the fluorescence intensity seems to be caused by the A-like structure of the duplex. Thus, we have synthesized a 9-mer of modified 2'-*O*-methyl RNA containing amide-linked uridine dimer with pyrene at the 2'-position (**1**) (Figure 1 (b)) to make typical A-form duplex with the complementary RNA for the enhancement of the increase of fluorescent intensity.

First, tetramer of 2'-*O*-methyl RNA having 5'-terminal amino group (H₂N-UOMeUOMeAOMeCOMe) was synthesized on CPG by using a 5'-terminal building block **2**. Next, 2'-pyrene modified building block **3** was assembled to the tetramer by the use of PyFOP as the coupling reagent in 69% yield. Successive chain elongation of 2'-*O*-methyl RNA gave the 9-mer on CPG in 38% overall coupling yield. After deprotection of the 9-mer, HPLC purification of the main product gave the pyrene-modified oligonucleotide in 53% recovery yield. The identification of the product as **1** is currently underway.

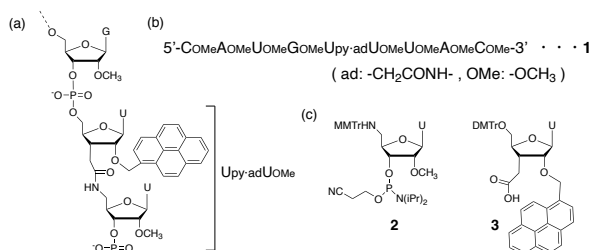


Figure 1. (a) Structure of pyrene-modified amide-linked uridine dimer region in 2'-*O*-methyl RNA. (b) Sequence of 2'-*O*-methyl RNA containing amide-linked RNA modified with pyrene (**1**). (c) Structure of the compounds **2** and **3**.

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