Synthesis of a benzo[a]pyrene-modified oligonucleotide and its application to the fluorescent colorimetric detection of single base alterations of RNA sequences

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The analytical methods for detection of single base alterations of RNA sequences are essential for diagnosis and treatment of serious diseases such as cancers. Previously, we reported facile detection of single base alterations of RNA sequences using a pyrene-modified oligonucleotide, OMUpy2.[1] OMUpy2 contains two 2'-*O*-pyrenylmethyluridine (Upy) units in the middle of 2'-*O*-methyl RNA sequences. OMUpy2 produces strong fluorescence only in the presence of complementary RNAs. In addition, OMUpy2 is capable of discrimination between RNAs and DNAs.

In this work, we report the synthesis and photophysical properties of a novel fluorescent oligonucleotide, OMCbpyUpy where a 2'-*O*-benzo[a]pyrenylmethylcytidine (Cbpy) unit and a Upy unit are consecutively introduced (**Figure 1a**). The oligonucleotides with a Cbpy unit may be useful for biological applications such as RNA imaging with confocal laser microscopy since benzo[a]pyrene exhibits an absorption band corresponded to canonical laser wavelength. The Cbpy phosphoramidite unit was synthesized in four steps from cytidine in 18% total yield and successfully incorporated into the 2'-*O*-methyl RNA sequences via solid-phase synthesis (**Figure 1b**). The fluorescence spectra of **OMCbpyUpy1** were measured at an excitation wavelength of 405 nm (**Figure 1c**). **OMCbpyUpy1** showed strong fluorescent signals at 500 nm in the presence of **Kras G12D**. The fluorescent intensity at 500 nm of **OMCbpyUpy1** in the presence of **Kras G12D** was 28-fold higher than that in the absence of **Kras G12D**. On the other hand, **OMCbpyUpy1** showed weak fluorescent signals at 500 nm in the presence of **Kras WT**. It is notable that the fluorescent intensity at 500 nm of the **OMCbpyUpy1** in the presence of **Kras WT** was one-sixth that of the **OMCbpyUpy1** in the presence of **Kras G12D**. Furthermore, the emission colors of these duplexes were clearly distinguished by naked eyes under UV irradiation. These results suggest that **OMCbpyUpy1** is applicable to facile analytical methods for detection of single base alterations of RNA sequences.

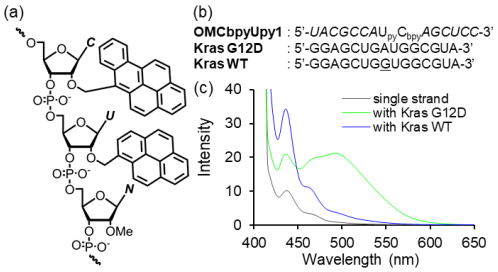

Figure 1. (a) Chemical structure of OMCbpyUpy. (b) The sequences of the oligonucleotides used in this work. Italic and capital letters represent 2'*O*-Me RNAs and RNAs, respectively. (c) Fluorescence spectra of **OMCbpyUpy1** in the presence of **Kras G12D** and **Kras WT** and in the absence of RNAs.

1) A. Mahara, *et al*., *Angew. Chem. Int. Ed*., **2002**, *41*, 3648-3650.

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