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Synthesis of a fluorescence resonance energy transfer-based probe containing a tricyclic nucleoside analog for single nucleotide polymorphism typing

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ABSTRACT

Here, we report the synthesis of a fluorescence resonance energy transfer (FRET)-based probe for single nucleotide polymorphism (SNP) typing. The probe contains a fluorescent tricyclic base, 8-amino-3-(2,3-dihydroxypropyl)imidazo[4′,5′:5,6]pyrido[2,3-*d*]pyrimidine, as a donor molecule and 7-diethylaminocoumarin-3-carboxylic acid as an acceptor molecule. FRET was observed between the donor and acceptor molecules on the probe. The identity of the target bases on DNA and RNA strands could be determined using the probe.

A single nucleotide polymorphism (SNP) is a single-base-pair difference in the DNA sequence of members of a species.[1,2] SNPs in the human genome are proven to be linked to diseases and individual differences in drug responses.[1,2] The development of accurate, rapid, and cost-effective methods for SNP typing is very important in pharmacogenomics[3] and for the realization of personalized medicine.[4–6]

Recently, we developed a novel method for SNP typing using a probe containing the fluorescent nucleoside analog 8-amino-3-(2,3-dihydroxypropyl)imidazo[4′,5′:5,6]pyrido[2,3-*d*]pyrimidine (**1**) (Fig. 1).[7–9] The fluorescence intensity of **1** is greater in more polar solvents, such as methanol and water, than in less polar solvents, such as chloroform. When a discriminating base **X** in the probe is complementary to the target base **Y**, the base pairing between **X** and **Y** makes the analog **1** flip outside of the DNA helix (Fig. 2A). This strengthens the fluorescence intensity of **1**. On the other hand, when the target base **Y** is mismatched with the discriminating base **X**, **1** intercalates into the DNA helix, which weakens the fluorescence intensity of **1**. Thus, the identity of the target nucleoside **Y** can be determined by comparing the fluorescence intensity of each duplex. However, since the fluorescence emission maximum of **1** is around 390 nm, we could not detect the SNPs in the target by visible colors.

In this Letter, we report the synthesis of a fluorescence resonance energy transfer (FRET)-based probe containing the analog **1** for SNP typing (Fig. 2B) to expand the fluorescence color range of the probe.

We selected 7-diethylaminocoumarin-3-carboxylic acid (**2**)[10] as an acceptor molecule of the FRET probe. The analog **1** has a fluorescence emission maximum at ~390 nm under 332-nm excitation while **2** has an ultraviolet (UV) absorption maximum at ~432 nm and a fluorescence emission maximum at ~456 nm. Thus, we envisioned that, if we could align **1** and **2** at the appropriate positions on DNA, FRET would be observed between these molecules.

We planned to synthesize the FRET probe by a post-synthetic modification method.[11] First, we synthesized oligonucleotides (ONs) containing the analog **1** and 5-aminomethyl-2′-deoxyuridine (**3**) by using phosphoramidites **5** and **6**.[8,12] Then, we tried to introduce the acceptor molecule **2** at the aminomethyl moieties on ONs via amide bonds. The sequences of ONs are depicted in Table 1. In the current study, we chose a *CYP2C9* sequence containing an A1075(C) mutation as a model sequence.[13,14] ON **1** has one nucleoside between the analogs **1** and **3**, while ON **3** has three nucleosides between analogs **1** and **3**. ON **1** and **3** were treated with the coumarin derivative **3** in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and *N*-hydroxysuccinimide in the buffer 1 M Tris–HCl (pH 8.5). Representative reversed-phase high performance liquid chromatography (RP-HPLC) profiles of reaction mixtures are shown in Figure S1. The

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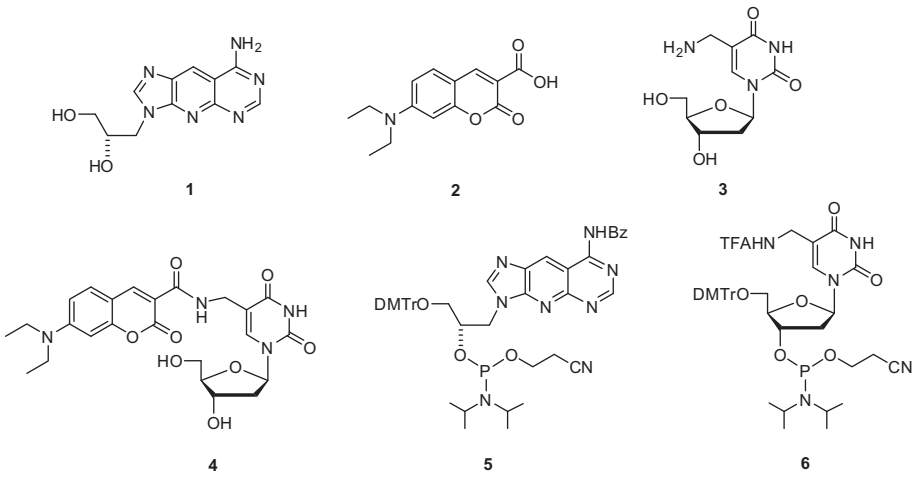

Figure 1. Structures of nucleoside analogs and fluorescent molecules. DMTr: 4,4′-dimethoxytrityl. Bz: benzoyl. TFA: trifluoroacetyl.

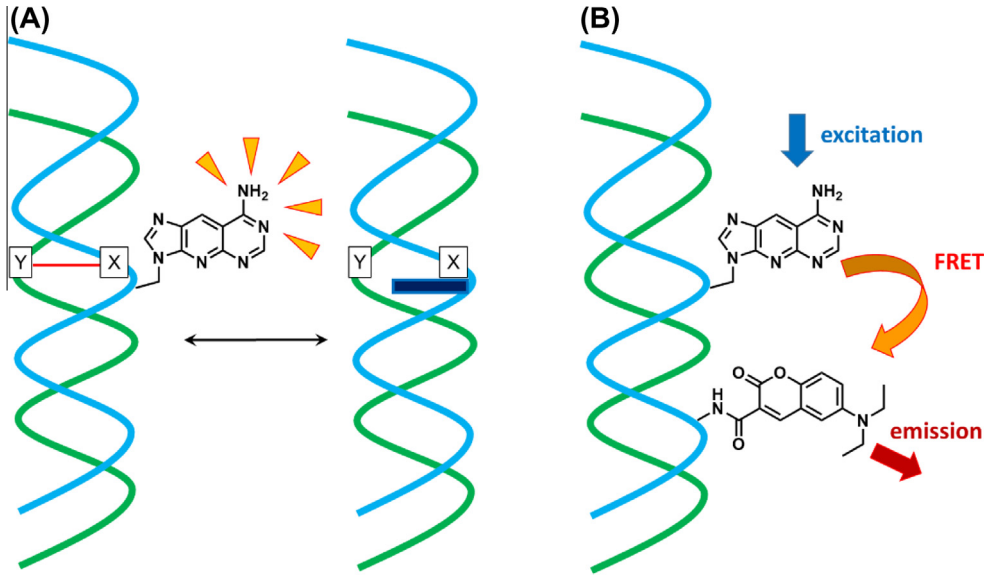

Figure 2. Principle of our SNP-typing method using the fluorescent tricyclic nucleoside analog.

Table 1
Oligonucleotide sequences. The underlined letters indicate discriminating bases. The italicized letters represent target bases

Abbreviation	Sequence
ON **1**	5′-d(GAA GGT CAA A**1**G **3**AT CTC T)-3′
ON **2**	5′-d(GAA GGT CAA A**1**G **4**AT CTC T)-3′
ON **3**	5′-d(GAA GGT CAA A**1**G TA**3** CTC T)-3′
ON **4**	5′-d(GAA GGT CAA A**1**G TA**4** CTC T)-3′
SdT	3′-d(CTT CCA GTT *T*CA TAG AGA)-5′
SdC	3′-d(CTT CCA GTT *C*CA TAG AGA)-5′
SdA	3′-d(CTT CCA GTT *A*CA TAG AGA)-5′
SdG	3′-d(CTT CCA GTT *G*CA TAG AGA)-5′
SrU	3′-r(CUU CCA GUU *U*CA UAG AGA)-5′
SrC	3′-r(CUU CCA GUU *C*CA UAG AGA)-5′
SrA	3′-r(CUU CCA GUU *A*CA UAG AGA)-5′
SrG	3′-r(CUU CCA GUU *G*CA UAG AGA)-5′
Helper	3′-d(CCA GTT CCA TAG AG)-5′

obtained ONs, **2** and **4**, were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS), and the observed molecular weights were in agreement with their structures.

Fluorescence emission spectra of the duplexes containing the analogs **1** and **4** under 339-nm excitation are shown in Figure 3. Figure 3A and C depict the results for DNA targets, while Figure 3B and D represent the results for RNA targets. Figure 3A and B exhibit the results for the ON **2** probe, whereas Figure 3C and D indicate the results for the ON **4** probe. In all the sequences, a fluorescence emission peak was not observed around 400 nm, an emission wavelength of the analog **1**, while fluorescence emission peaks were observed around 480 nm, an emission wavelength of the compound **2**. These results indicated that FRET took place between the analogs **1** and **4**.

Next, we compared the fluorescence intensities of the duplexes at 480 nm. As shown in Figure 4A and B, when ON **2** was used as a probe, the base-discriminating abilities of the probe were found to be low, for both DNA and RNA targets. Incorporation of the analog **4** into the position close to the analog **1** might disturb the local structure of the duplex around the analogs. Thus, we considered that the analog **1** could not intercalate into the DNA duplex. On the other hand, when the ON **4** was used as a probe, the fluorescence intensities were greatest when the duplexes were composed of matched sequences both for the DNA and RNA targets