Synthesis and Application of a ^{19}F-labeled Fluorescent Nucleoside as a Dual-mode Probe for i-Motif DNA Structures

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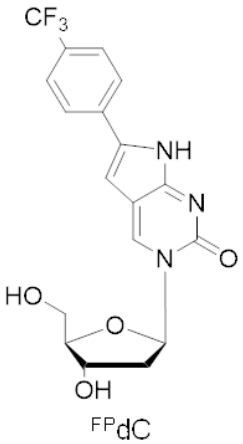
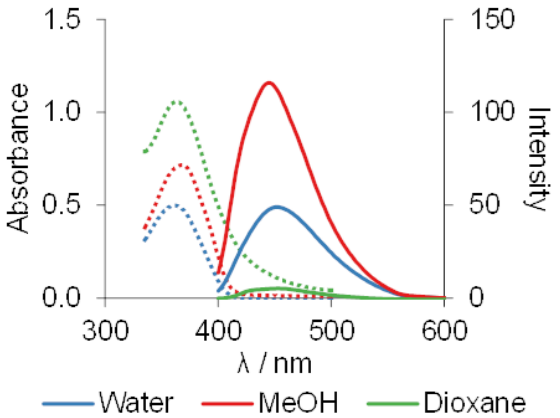
Emissive isomorphic nucleoside analogues are versatile tools for the investigation of DNA conformations due to their stable orientations from base-pairing and stacking interactions.[1] Furthermore, in contrast to conventional small molecules or protein dyes, emissive isomorphic nucleosides cause minimal disturbance to native DNA folding and interactions, allowing for a more accurate picture of nucleic acid structures.

Herein, we report the synthesis of a fluorine-labeled fluorescent cytosine analogue, ^{FP}dC, and its incorporation into i-motif-forming DNA sequences. DMTr-protected ^{FP}dC phosphoramidite was synthesized in eight steps and successfully utilized in solid-phase synthesis to obtain the desired oligonucleotides. Compared to previously reported fluorescent tricyclic cytosine derivatives, ^{FP}dC monomer presented a four-fold improvement in brightness (12 000) due to its high molar absorptivity (24 000 mol^{-1} dm^3 cm^{-1}) and quantum yield (0.50). When incorporated into oligonucleotides and upon formation of i-motif structures, significant changes in fluorescence intensity and lifetime, as well as ^{19}F NMR chemical shifts were observed. The changes in fluorescence intensity were observed to be highly reversible when the folding and unfolding of an i-motif structure was induced with Ag(I) ion and cysteine.



^{FP}dC



1) Hirashima, S.; Han, J. H.; Park, S.; Sugiyama, H. *Chem. Eur. J.* **2019**, *25*, 9913–9919.