Pyrimido[4,5-\textit{d}]pyrimidine-2,4,5,7-(1\textit{H}, 3\textit{H}, 6\textit{H}, 8\textit{H})-tetraone as a novel universal base

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ABSTRACT

A derivative of pyrimido[4,5-\textit{d}]pyrimidin-2,4,5,7-(1\textit{H},3\textit{H},6\textit{H},8\textit{H})-tetraone (PPT), which may form various keto-enol tautomers suitable for forming base pairs with all natural bases, and is thus expected to serve as a universal base, was synthesized. The ability of PPT to form base pairs with natural bases was evaluated by UV analysis.

INTRODUCTION

A universal base analogue forms a pseudo base pair with all natural bases, with little discrimination between types. A number of such analogues have been developed and investigated as biochemical tools.\textsuperscript{1} Most of these analogues act as hydrophobic spacers and stabilize the DNA duplex without hydrogen bonds. Analogues of 2'-deoxyinosine\textsuperscript{2} have also been investigated and found to behave in a similar manner. These analogues have aromatic properties and hydrogen-bonding ability and may therefore be expected to be useful as a universal base. However, 2'-deoxyinosine exhibits significant discrimination among natural bases and a wide range of melting temperatures are found when it is paired with natural bases within duplexes.

Here we report the investigation of pyrimido[4,5-\textit{d}]pyrimidin-2,4,5,7-(1\textit{H}, 3\textit{H}, 6\textit{H}, 8\textit{H})-tetraone (1, PPT) as a novel hydrogen-bonding universal base.

RESULTS AND DISCUSSION

PPT is expected to form pseudo base pairs with all natural bases via dynamic transformation. The keto-enol tautomerization at the C5 position, assisted by the carbonyl oxygen at the C4 position, allows the compound to pair with natural bases. Within the duplex, the rotation of N1 and the nucleotide backbone (R) around a bond axis results in a transformation between purine-type and pyrimidine-type rotamers (Figure 1). Consequently, PPT has extensive possibilities as a hydrogen-bonding universal base.

\begin{center}
\includegraphics[width=0.5\textwidth]{figure1.png}
\end{center}

\textit{Figure 1.} Dynamic transformation of PPT analogue.
The synthesis of 1-(2-tert-butylidiphenylsiloxyethyl)-PPT (2) for spectrometric analysis was carried out according to the reported procedure with small modifications (Scheme 1). The compound 1-(2-tert-butylidiphenylsiloxyethyl)-6-aminouracil, 3, was reacted with ethyl isocyanatoformate, giving 4 in 40% yield. Subsequently, the cyclization of 4 was carried out, using sodium ethoxide as a base catalyst, to provide 2 in 92% yield. The structure of 2 was identified by $^1$H and $^{13}$C NMR spectra and exact ESI-TOF MS analysis [calcd for C$_{33}$H$_{37}$N$_2$O$_5$Si (M+1) m/z 479.17, found m/z 479.21].

The UV analysis of the interaction between PPT and natural bases was undertaken by the ratio gradient method using a mixture of 2 and 1-methylated natural base, resulting in an obvious deviation (6-11% at 1:1 ratio) compared to calculations based on the Lambert-Beer law. This is evidence that PPT interacts with each natural base, which demonstrates its suitability as a universal base. In this analysis, mismatched pairs, for example A-C, A-G, C-T, and G-T, did not exhibit an obvious deviation compared to calculations based on the Lambert-Beer law.

CONCLUSION

We developed a derivative of pyrimido[4,5-d]pyrimidine-2,4,5,7-(1H,3H,6H,8H)-tetrone for use as a novel universal base. This artificial base may form various keto-enol tautomers suitable for forming base pairs with all natural bases. Evidence of formation of PPT-natural base complexes was obtained by UV analysis.

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REFERENCES


Scheme 1. Synthesis of 1-(2-tert-Butylidiphenylsiloxyethyl)-PPT (2).