A ribonucleoside with pyrimido[4,5-d]pyrimidine-2,4,5,7-(1H,3H,6H,8H)-tetraone as a nucleobase, which universally binds to natural nucleosides

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ABSTRACT

A ribonucleoside with pyrimido[4,5-d]pyrimidine-2,4,5,7-(1H,3H,6H,8H)-tetraone (PPT) changes the structure through dynamic transformation as shown in Figure 1. Thus, when keto-enol tautomerization at the C-5 position, assisted by the carbonyl oxygen at the C-4 position, takes place in PPT, this compound is allowed to pair with all natural nucleobases, \textit{i.e.}, adenine, cytosine, guanine, and thymine, through hydrogen bonds. Within the duplex, the rotation of the backbone (R) around an N-1 bond axis results in a transformation between purine-type and pyrimidine-type rotamers. Accordingly, oligonucleotides including the nucleoside with PPT as a base (PPT-nucleoside) are of great interest. In creation of such oligonucleotides, it is essential to prepare a nucleoside with PPT as a building block and is important to know whether the resulting nucleoside has a high potentiality as a universal nucleoside. Thus, we prepared the PPT-ribonucleoside 7 and investigated its binding affinity to four kinds of natural 2’-deoxyribonucleosides.

RESULTS AND DISCUSSION

The PPT-nucleoside, 7, was prepared according to the procedure exhibited in Scheme 1. Reaction of the protected ribofuranose 1 (100 mM) and the silylated pyrimidine derivative 2 (200 mM) using trimethylsilyl triflate (TMSOTf) (120 mM) as a catalyst\textsuperscript{2} was carried out at 30 °C for 24 h in acetonitrile containing molecular sieves (MS) 4A as a moisture scavenger to give the N(1)-nucleoside product 3 in a 94% yield. In this reaction, a small amount of the N(3)-nucleoside product (<5%) was obtained. The N-dimethylformamidime (dmf) and tri-O-benzoyl protecting groups of 3 were removed by treatment with a 30% methanamine/ethanol solution and then the resulting product (100 mM) was converted to the tri-\textit{O}-\textit{tert}-butyldimethylsilyl derivative 4 by exposure to a mixture of \textit{tert}-butyldimethylsilyl chloride (TBDMS-Cl) (600 mM) and imidazole (900 mM) in DMF at 60 °C for 3 h. The overall yield of 4 from 3 was 93%. Subsequently, 4 (100 mM) was condensed with ethyl isocyanatoformate (150 mM)\textsuperscript{3} in dichloromethane (25 °C, 5 h) to afford 5 in 90% yield. This compound 5 (10 mM) was treated with sodium ethoxide (11 mM) in ethanol at 60 °C for 16 h to construct the PPT ring via intramolecular condensation, and, finally, all O-TBDMSC protectors of the resulting product were removed by treatment with tetrabutylammonium fluoride (TBAF) in THF at 25 °C for 12 h to get the target product 7 in a 53% overall yield in these two steps. Since the pKa of 7 is 2.4 (H2O), 7 was obtained as a tetrabutylammonium salt: \textit{1}H NMR (400 MHz, DMSO-\textit{d}_6) \textit{δ} 0.93 (t, \textit{J} = 7.3 Hz, 12H), 1.30 (m, \textit{J} = 7.2 Hz, 8H), 1.51–1.59 (m, 8H), 3.13–3.17 (m, 8H), 3.34–3.46 (m, 1H), 3.55–3.66 (m, 2H), 4.17–4.22 (m, 1H), 4.50–4.56 (m, 2H), 4.68

![Figure 1](https://example.com/image1.png) Expected base pairs of a PPT compound and natural nucleosides.
Scheme 1  Synthesis of PPT-ribonucleoside 7: (a) TMSOTf, MS 4A, acetonitrile, 30 °C, 24 h; (b) 30% methylamine/ethanol solution, 25 °C, overnight; (c) TBDMS-Cl, imidazole, DMF, 60 °C, 3 h; (d) ethyl isocyanatoformate, dichloromethane, 25 °C, 5 h; (e) sodium ethoxide, ethanol, 60 °C, 16 h; (f) TBAF, THF, 25 °C, 12 h.

(d, J = 7.0 Hz, 1H), 4.89 (d, J = 4.9 Hz, 1H), 6.49 (s, 1H), 9.42 (s, 1H), 10.2 (s, 1H) ppm; 13C NMR (100 MHz, DMSO-d6) δ 139.7, 19.7, 23.5, 58.0, 63.1, 70.5, 71.9, 84.7, 86.4, 88.7, 151.1, 157.7, 159.8, 162.4, 163.3 ppm; HRMS (ESI+) m/z 327.0724 (calcd for C_{11}H_{19}N_4O_8 [M − H]^+ m/z 327.0582).

The UV analysis of the interaction between the PPT-nucleoside and four natural deoxyribonucleosides, i.e., deoxyadenosine, deoxyctytidine, deoxyguanosine, and thymidine, was undertaken by the ratio gradient method with a 1:1 mixture of 6 and a 3',5'-bis(tert-butylidimethylsilyl) derivative of the deoxyribonucleoside, resulting in an obvious deviation (6-9%) compared to calculations based on the Lambert-Beer law. This result indicated that 7 interacts with each natural nucleoside, though it is not clear whether interaction takes place through hydrogen bonding or other modes.

CONCLUSION

We prepared a ribonucleoside with PPT as a base and revealed that this artificial base may serve as a universal nucleoside. Thus, it is highly attractive to investigate utility of oligonucleotides containing 7. The synthesis of these compounds is now in progress.

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REFERENCES

4. The pKa values of some PPT derivatives will be reported in another paper.