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Synthesis of 6-(2-Thienyl)purine Nucleoside Derivatives That Form Unnatural Base Pairs with Pyridin-2-one Nucleosides

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Abstract—Unnatural bases, 2-amino-6-(2-thienyl)purine and 2-amino-6-(2-furanyl)purine, were newly designed to replace the previously developed purine analogue, 2-amino-6-(*N,N*-dimethylamino)purine, which specifically pairs with pyridin-2-one. These nucleoside derivatives were synthesized via the 6-substitution of 6-iodopurine nucleosides with tributylstannylthiophene or tributylstannylfuran. As compared with 2-amino-6-(*N,N*-dimethylamino)purine, 2-amino-6-(2-thienyl)purine reduced the interference in the stacking interactions with the neighboring bases in a DNA duplex and improved the efficiency of the enzymatic incorporation of the nucleoside triphosphate of pyridin-2-one opposite the unnatural base.

The development of unnatural nucleobase pairs would increase nucleic acid functionality and eventually lead to an expansion of the genetic alphabet.[1–4] We previously designed an unnatural base pair, 2-amino-6-(*N,N*-dimethylamino)purine (designated as **x**) and pyridin-2-one (designated as **y**) (Fig. 1a)[5,6] on the basis of the concepts of both the hydrogen-bond donor–acceptor pattern and the shape complementarity. The bulky 6-dimethylamino group of **x** efficiently prevents the non-cognate base pairing, such as **x**–T (or **x**–U) (Fig. 1b) and **x**–C, and the ribonucleoside triphosphate of **y** can be specifically incorporated into RNA opposite **x** of a DNA template by T7 RNA polymerase.[6] This transcription enables the synthesis of functional RNAs containing unnatural nucleosides at desired positions[6] and facilitates the expansion of the genetic code for the site-specific incorporation of unnatural amino acids into proteins. However, the dimethylamino group interferes with the stacking interactions between the unnatural and the neighboring base pairs in a DNA duplex. Accordingly, further applications, especially replication, of the **x**–**y** pair have remained restricted.

To reduce the steric hindrance between the unnatural and the neighboring base pairs, we replaced the 6-dimethylamino group of **x** with heterocycles, such as thiophene and furan (Fig. 1c). These heterocycles might function as bulky groups on the pairing surface as well as the 6-dimethylamino group of **x** (Fig. 1d). In addition, these heterocycles may increase the stacking stability with the neighboring base pairs in DNA duplexes as compared to the dimethylamino group of **x**, although there is a possibility that the heterocycle moieties deviate from the surface of the purine base. We synthesized these unnatural nucleosides and examined the thermal stability of the DNA duplexes and the incorporation into DNA involving the unnatural base pair between 2-amino-6-(2-thienyl)purine (**1a**) and **y**.

We first examined the photochemical synthesis of the 6-(2-thienyl)purine 2′-deoxyribonucleosides according to the literature.[7] Photoarylation was carried out with 2-isobutyrylamino-6-iodo-9-(2-deoxy-3,5-di-*O*-isobutyryl-β-D-ribofuranosyl)purine (**2**)[8,9] in nitrogen-purged thiophene (Scheme 1, Method A). After irradiation for 24 h,

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*N*2-isobutyrylamino-6-(2-thienyl)-9-(2-deoxy-3,5-di-*O*-isobutyryl-β-D-ribofuranosyl)purine (**3a**) was obtained in a 78% yield. After deprotection of the isobutyryl groups of **3a** [1 M NaOH in pyridine/MeOH/H_2O (17:6:1) for 4 h at rt], the product was purified by C18-HPLC. However, the ^1H NMR spectrum indicated contamination with a by-product, which was determined to be 2-amino-6-(2,2′-bithienyl)-9-(2-deoxy-3,5-di-*O*-acetyl-β-D-ribofuranosyl)purine (**5**) (see Scheme 1); the ratio of 2′-deoxyribonucleosides of **4a** to **5** was 9:1 (Fig. 2, upper chart). The by-product may result from the addition of thiophene to the radical intermediate of **3a** during irradiation.

Then, we examined the synthesis of **3a** by the palladium catalyzed coupling of **2** with tributylstannylthiophene and bis(triphenylphosphine)palladium(II) chloride (Scheme 1, Method B).[10] By this coupling in DMF for 2 h at 100 °C, **3a**[11] was obtained in a 94% yield, and no by-product was observed in the products after deprotection and HPLC purification (Fig. 2, lower chart). In addition, the 2′-deoxyribonucleoside of **1b** (**3b**) was synthesized by using tributylstannylfuran in a 98% yield. Similarly, the corresponding nucleosides without the 2-amino group, 6-(2-thienyl)-9-(2-deoxy-3,5-di-*O*-acetyl-β-D-ribofuranosyl)purine and 6-(2-furanyl)-9-(2-deoxy-3,5-di-*O*-acetyl-β-D-ribofuranosyl)purine, were also synthesized from 6-iodo-9-(2-deoxy-3,5-di-*O*-acetyl-β-D-ribofuranosyl)purine in 79 and 87% yields, respectively (Scheme 1).

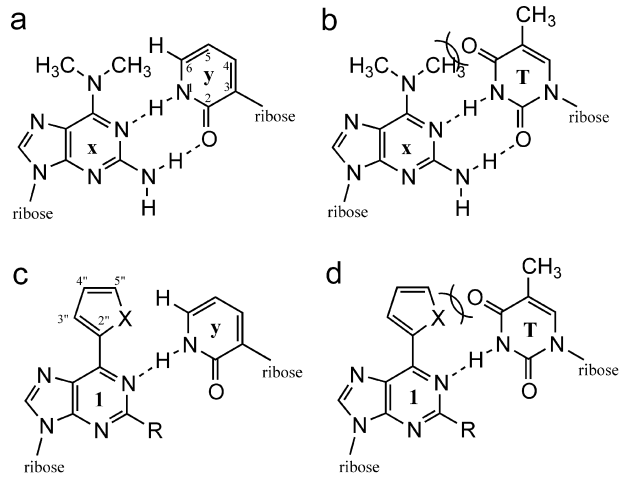
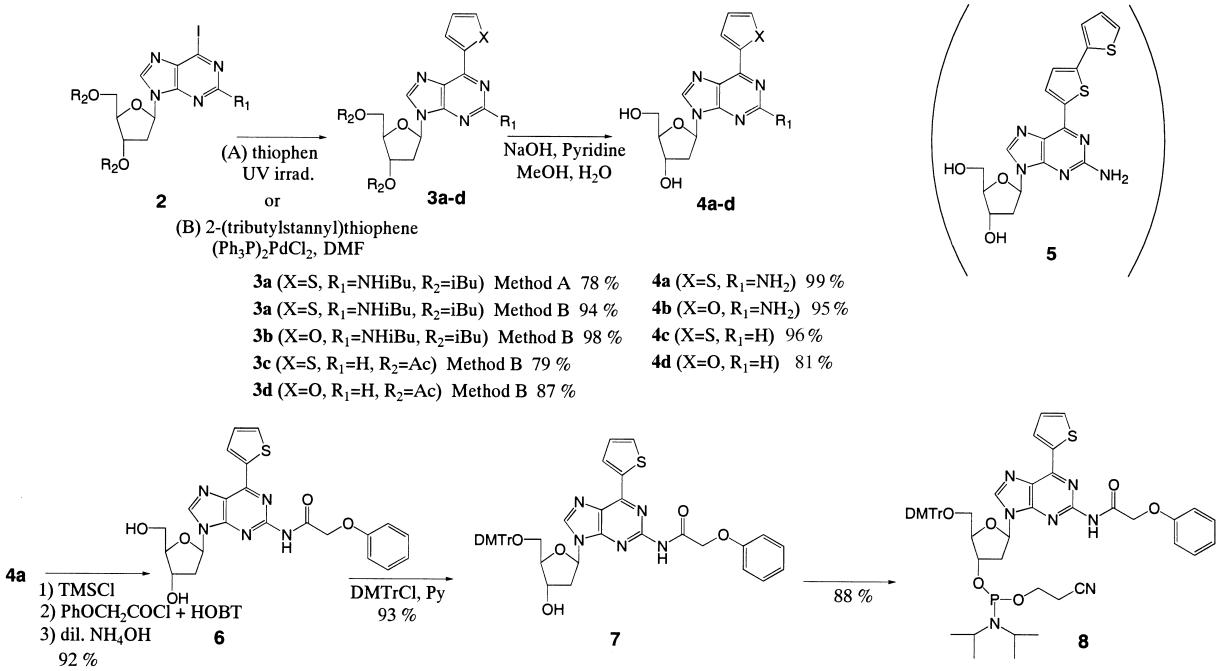


Figure 1. Unnatural and non-cognate base pairs. Compound **1a**: X = S and R = NH_2, **1b**: X = O and R = NH_2, **1c**: X = S and R = H, and **1d**: X = O and R = H.

The 2′-deoxyribonucleoside of 2-amino-6-(2-thienyl)purine (**1a**) was converted to the corresponding amidite (**8**) for DNA synthesis (Scheme 1). The furanyl derivatives gradually decomposed under the basic conditions used in DNA synthesis; nucleoside **4d** decomposed in a concentrated ammonia solution at room temperature. Thus, we synthesized DNA fragments containing **1a**. For the protection of the 2-amino group of **1a** during DNA synthesis, a phenoxycarbonyl group[12] was used, because the isobutyryl group was too stable as the protecting group. The deprotection of fully-protected DNA fragments was carried out by a conventional method (concentrated NH_4OH at rt for 1 h and then 55 °C for 6 h).

To assess the potential of the unnatural **1a**–**y** pair, we examined the thermal stability of the DNA duplex and the single-nucleotide insertion[13] by the exonuclease-deficient Klenow fragment of *Escherichia coli* DNA polymerase I. As expected, the thermal denaturation experiments showed the increased stability of the DNA duplex (12-mer, 5′-CGCAT**1a**GTTACC-3′/5′-GGTAA-



Scheme 1.

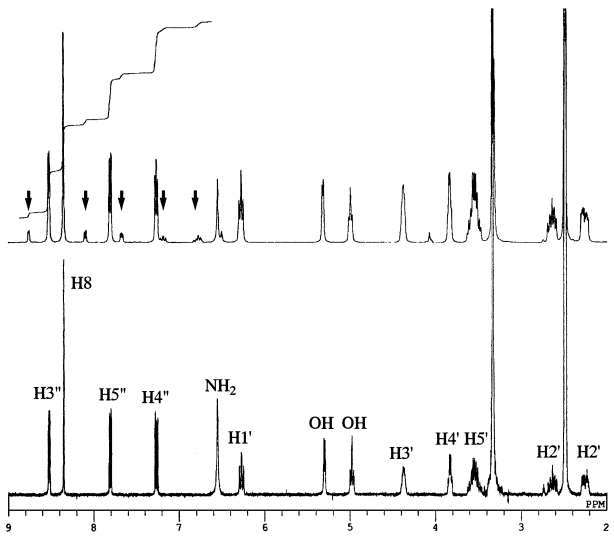


Figure 2. ^{1}H NMR spectrum (DMSO-d_6) of **4a** synthesized by method A (upper) and method B (lower). The integration of the aromatic region is shown. The peaks assigned to by-product **5** are indicated by the arrows in the upper chart.

Table 1. Steady-state kinetic parameters for insertion of single nucleotides into a template-primer duplex by the exonuclease-deficient Klenow fragment

dN'TP

primer 5'-^{32}P-CTCACTATAGGGAACGCCAG

template 3'-GAGTGATATCCCTTGCGGTCNTCACGCC

Template base (N)	Nucleotide triphosphate (N′)	K_M (μM)	V_{max} (% min^{-1})	Efficiency (V_{max}/K_M)
y	**1a**	130 (30)	1.2 (0.1)	9.2×10^3
T	**1a**	270 (70)	0.64 (0.13)	2.4×10^3
C	**1a**	1000 (100)	3.8 (0.6)	3.8×10^3
G	**1a**	n.d.	n.d.	
A	**1a**	150 (40)	0.33 (0.04)	2.2×10^3
y	**x**	440 (210)	1.2 (0.3)	2.7×10^3
T	**x**	550 (160)	2.7 (0.4)	4.9×10^3
C	**x**	1700 (200)	1.1 (0.1)	6.5×10^2
G	**x**	110 (40)	0.07 (0.01)	6.4×10^2
A	**x**	220 (50)	0.33 (0.05)	1.5×10^3

Assay was carried out at 37 °C for 7–20 min using 5 μM template–primer duplex, 20 or 50 nM enzyme, and 0.03–3 mM dNTP in a solution (10 μL) containing 50 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM DTT, and 0.05 mg/mL bovine serum albmin.
n.d. No inserted products were detected after incubation for 20 min with 3 mM dNTP. Parenthetic values are standard deviations.

CyATGCG-3′) containing the **1a**–**y** pair ($T_m = 43.4$ °C) as compared with that containing the **x**–**y** pair ($T_m = 39.5$ °C). As shown in Table 1, the enzymatic incorporation efficiency of triphosphate **y** into DNA opposite **1a** of the template was also increased and was higher than those of the non-cognate pairing with natural substrates. On the other hand, the incorporation of **y** opposite **x** was less efficient than the incorporation of T opposite **x**. Thus, our results show that the improvement of the stacking stability of the DNA duplex containing the unnatural base pair concurrently increased the incorporation efficiency of the unnatural base pairing. Although the incorporation specificity of the **1a**–**y** pairing is still insufficient for practical use in replication, the transcription specificity of the **1a**–**y** pairing was improved as compared to that of the **x**–**y** pairing (data not shown). Transcription–translation experiments for the incorporation of unnatural amino acids into proteins using the novel codon–anticodon interaction involving the **1a**–**y** pair are in progress.

References and Notes

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11. Compound **4a**: ^{1}H NMR (270 MHz, DMSO-d_6): δ 2.24 (ddd, 1H, $J = 13$, 6.1, 3.1 Hz), 2.64 (ddd, 1H, $J = 13$, 7.6, 6.4 Hz), 3.39 (m, 2H), 3.84 (m, 1H), 4.38 (m, 1H), 4.98 (t, 1H, $J = 5.4$ Hz), 5.30 (d, 1H, $J = 4.0$ Hz), 6.27 (dd, 1H, $J = 7.6$, 6.1 Hz), 6.56 (bs, 2H), 7.26 (dd, 1H, $J = 5.0$, 3.7 Hz), 7.80 (dd, 1H, $J = 5.0$, 1.2 Hz), 8.35 (s, 1H), 8.52 (dd, 1H, $J = 3.7$, 1.2 Hz). Electron spray mass spectrum: M + 1 (positive) = 333.90 (found), 334.099 (calcd), M + $HCOO^-$ (negative) = 377.76 (found), 378.089 (calcd).
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