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# Nucleotide insertion and bypass synthesis of pyrene- and BODIPY-modified oligonucleotides by DNA polymerases†

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**The chromophores pyrene and bordipyrromethenylbenzene directly linked to the 5-position of uridine are tolerated and recognized as thymine derivatives by DNA polymerases in primer extension experiments.**

If fluorophores are attached to DNA bases for oligonucleotide labeling,[1] an alkyl chain linker is inserted between the chromophore and DNA base to allow the replication by DNA polymerases. However, the direct covalent attachment of chromophores to DNA bases yields unique optical properties, such as solvatochromism and exciplex-type emission[2] that are suitable for DNA probing. A critical issue about this direct linkage is the question if the canonical base recognition complementarity persists in DNA polymerase-catalyzed primer extension experiments.[3] For instance, fluorophore-labeled nucleosides and fluorosides can be applied as substrates for the DNA polymerase.[4]

Over the past years, we attached synthetically pyrene[5–7] or ethynylpyrene[8], for example, to DNA bases for electron transfer studies and as fluorescent probes for DNA. To gain more insight into the counterbase selectivity, we performed primer extension experiments with a representative set of modified oligonucleotides (Scheme 1). The templates contained 5-(pyren-1-yl)-2′-deoxyuridine (1PydU),[5] 5-(pyren-2-yl)-2′-deoxyuridine (2PydU),[7] 5-[4-(2,6-diethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a-4a-diaza-*s*-indacyl)phenyl]-2′-deoxyuridine (BodU) or 8-(pyren-1-yl)-2′-deoxyguanosine (PydG)[6] as single modifications. The length of the radioactively labeled primer was chosen such that the modified nucleotide in the template strand codes for the first nucleotide during primer extension. Single-base incorporations were performed with each of the four dNTPs exclusively to get information about the insertion selectivity opposite to the modified nucleotide. In addition, experiments employing all four dNTPs simultaneously were performed to study the elongation bypassing the modification site.

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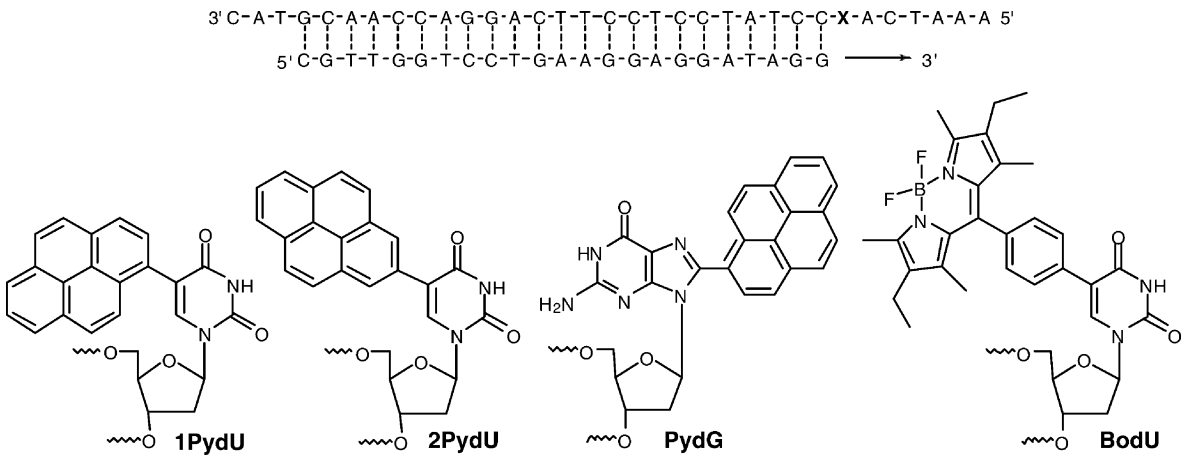
† Electronic supplementary information (ESI) available: Synthesis, spectra and characterization details; primer extension experiments; DNA polymerase activity determination; UV absorption spectra. See

First we investigated the Klenow fragment (exo-) of *E. coli* DNA polymerase I (KF-) in its propensity to insert a nucleotide opposite the modified DNA nucleobase. Gel electrophoretic analysis of the radiometric primer extension reactions revealed that the canonical bases are predominantly incorporated, that means A opposite to 1PydU, 2PydU and BodU, and C opposite to PydG (Fig. 1). Only minor amounts of misincorporation of G opposite to 2PydU and less opposite to 1PydU were observed. When all four dNTPs are present in the primer extension experiment, KF- is able to bypass all three types of uridine modifications (1PydU, 2PydU, and BodU) but not the modified guanosine (PydG). This is a remarkable result since the steric hindrance by the chromophores, especially by the bordipyrromethenylphenyl substituent, was expected to be significant.

Subsequently, human DNA polymerase β (Pol β), a member of the DNA polymerase X family involved in DNA repair, and DNA polymerase Dpo4, a representative of the Y-family, were examined (Fig. 1). In the single nucleotide insertion experiments both enzymes placed the canonical nucleotides opposite the modification sites, but Pol β was unable to incorporate any nucleotide opposite PydG. In contrast to KF-, a significant amount of misincorporation was not observed. However, both enzymes, Pol β and Dpo4, were only able to bypass the modified uridines (except 1PydU with Pol β) in experiments with all four dNTPs using higher polymerase concentrations and using an extended incubation time of 60 min. Even under these conditions, PydG could not be bypassed by any of the polymerases (Fig. S4–S5, ESI†). The reason for this might be that the pyrene at the 8-position induces the *syn*-conformation[5] of the nucleotide, yielding altered base pairing properties.

Since KF- was capable of bypassing DNA template modifications when all four dNTPs were present, we measured the activity of the enzyme on the respective templates in comparison to the unmodified template (Table 1). We employed an assay previously established to measure DNA polymerase activity on non-natural DNA primer template complexes.[9] The data show that the chemical modifications significantly impair bypass efficiency. These effects are most pronounced when PydG was used. The C5 modifications at pyrimidines are somewhat better tolerated as has been observed with other modifications before.[10]

Finally, we examined the absorption (Fig. S1–S3, ESI†) and fluorescence properties (Fig. 2) of the chromophore-uridine modified template–primer duplex in comparison with the synthetic full-length duplex. Additionally, an oligonucleotide was synthesized that contained the primer sequence and an additional A as counterbase to the chromophore-modified




**Scheme 1** DNA template and primer sequence (X = 1PydU, 2PydU, PydG, BodU).

uridine. Remarkably, with both 1PydU and 2PydU the emissions increase from the template–primer duplex to the full-length duplex. Interestingly, elongation with one A opposite to 1PydU or 2PydU is not sufficient to obtain this fluorescence enhancement. The BodU modification behaves completely independently of whether a primer extension has occurred or not. For all three modifications, the observed fluorescence changes are accompanied by changes in the emission maxima. This is typical for solvatochromic fluorophores. The exciplex-type emission reacts to changes in the stacking situation from the single-strand to the full-length duplex.

**Table 1** Activities of KF- on unmodified and modified templates[a]

| Oligonucleotide | [fmol dNTP incorporation per fmol pol per min] |
|---|---|
| Non-modified | 11660 ± 722.4 |
| 2PydU | 1395 ± 71.8 |
| 1PydU | 457.5 ± 17.3 |
| PydG | 146.2 ± 6.6 |
| BodU | 1402 ± 78.5 |

[a] Data were obtained from multiple individual measurements. Instead of the modified building block a dA residue was present in the template strand. pol = DNA polymerase.

In summary, we have shown that the DNA polymerase-catalyzed nucleotide incorporation opposite to attached pyrene and bordipyrromethenylbenzene at the 5-position of uridine follows Watson–Crick selectivity. KF- is also able to bypass the modification site during further elongation. These observations make these kinds of fluorescent labels promising tools for *in vivo* experiments in cell biology.

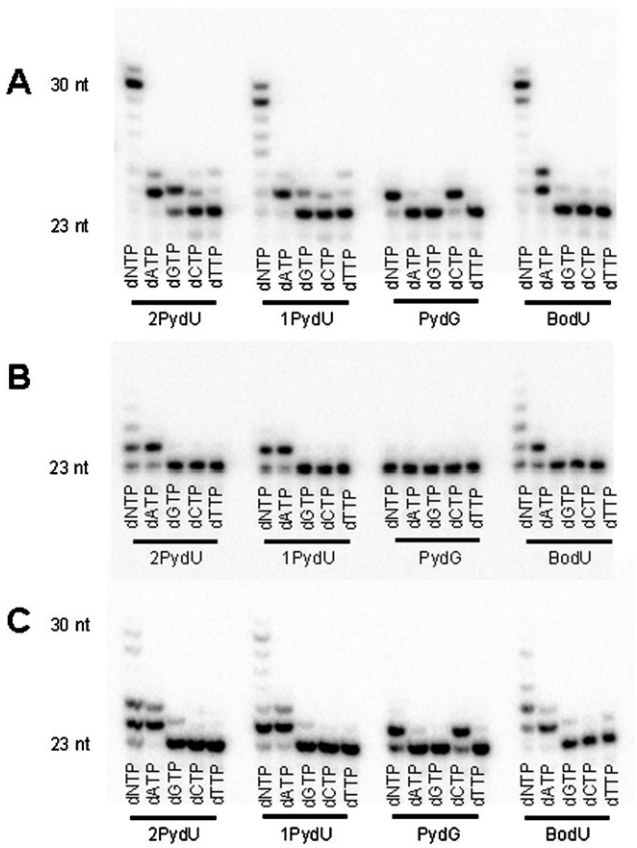



**Fig. 1** Gel electrophoretic analysis of primer extension experiments with 1PydU-, 2PydU-, BodU- and PydG-modified templates: (A): Klenow fragment (exo-) of *E. coli* DNA polymerase I, (B) human DNA polymerase β, (C) Dpo4 DNA polymerase. All reactions contained 200 μM of the respective dNTP and were incubated for 30 min at 37 °C. For more experimental details see the ESI†.

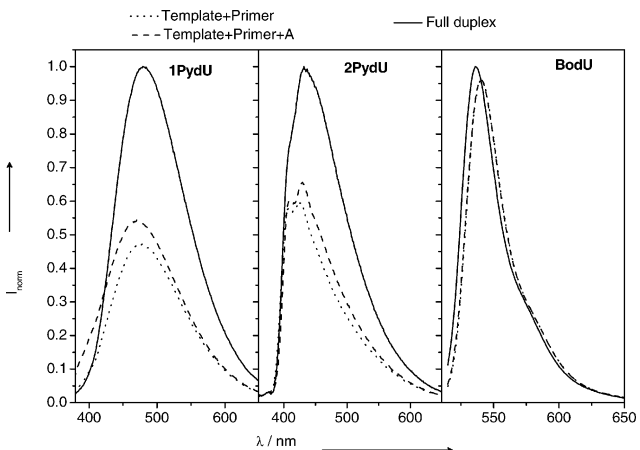



**Fig. 2** Fluorescence spectra of 2.5 μM duplex in 10 mM Na-$P_i$-buffer, pH 7, 250 mM NaCl, 20 °C, excitation at 360 nm (1PydU), 340 nm (2PydU), 510 nm (BodU).

## Notes and references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