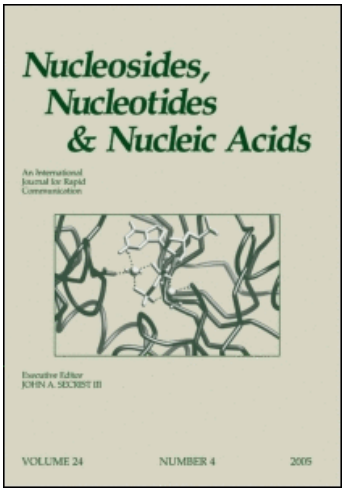

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Pyrene-Functionalized 2 '-Amino-α-L-LNA as Potential Diagnostic Probes

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PYRENE-FUNCTIONALIZED 2′-AMINO-α-L-LNA AS POTENTIAL DIAGNOSTIC PROBES

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□ *Oligodeoxyribonucleotides (ONs) containing two incorporations of 2′-N-(pyren-1-yl)acetyl-2′-amino-α-L-LNA monomer **Y** are sensitive probes for detection of single nucleotide polymorphisms (SNP) in DNA. In addition, the ability of ONs containing pyrene-functionalized 2′-amino-α-L-LNA monomers (**W-Z**) to stabilize duplexes with an abasic site is demonstrated.*

Keywords Single nucleotide polymorphisms; abasic sites; pyrene; 2′-amino-α-L-LNA

INTRODUCTION

Fluorescent probes that are sensitive to single base alterations in the target strand or abasic sites arising from DNA lesions are of great importance in DNA diagnostics for typing SNPs and detection of damaged DNA.[1,2] Herein, the propensity of N2′functionalized 2′-amino-α-L-LNA monomers to present intercalators precisely in the duplex core,[3] is used to detect single base mismatches in DNA targets and to form remarkably stable duplexes with strands containing a model abasic site **Φ** (Figure 1).

RESULTS AND DISCUSSION

An ON probe with two incorporations of 2′-*N*-(pyren-1-yl)acetyl-2′-amino-α-L-LNA monomer **Y** shows promising results for detection of single base alterations in DNA targets.[4] While the single stranded probe exhibits an excimer emission band ($\lambda_{max} \sim 480$ nm), hybridization to complementary DNA results in intercalation of both pyrene moieties and

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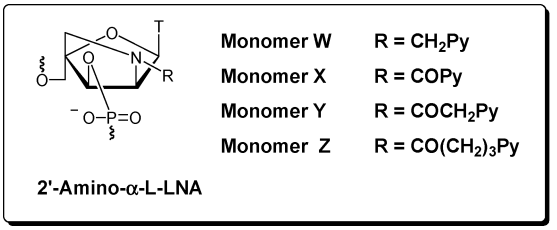

FIGURE 1 Structures of pyrene-functionalized 2′-amino-α-L-LNA monomers and model abasic site used in this study.

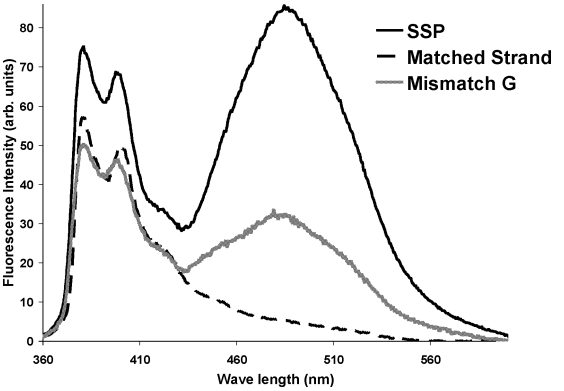

FIGURE 2 Steady state fluorescence spectra (concentration = 1.0 μM, at 5°C) of 5′-d(GCA **Y**A**Y** CAC) single-stranded probe (SSP), its duplex with complementary DNA and a DNA target with a mismatched **G** opposite to **A5**.

nearly complete reduction in excimer intensity. Interestingly, hybridization to DNA targets containing a 'single base mismatch' opposite of **Y4**, **A5**, **Y6**, and **C7** results in a significant increase in excimer band intensity (shown for one particular example in Figure 2). These observations indicate that introduction of mismatches in this region leads to conformational changes of the duplex in the vicinity of pyrene moieties, whereby the two pyrene moieties flip out, π-π stack and thereby form excimer.

TABLE 1 Thermal denaturation temperatures (T_m-values) of duplexes containing abasic sites[a]

	T_m (ΔT_m/mod)/°C				
Duplex	**B** = T	**B** = W	**B** = X	**B** = Y	**B** = Z
5′-d(GTG A**B**A TGC) 3′-d(CAC TA**Φ** ACG)	<5	31.0 (>+26.0)	32.0 (>+27.0)	28.5 (>+23.5)	30.0 (>+25.0)

[a] T_m-values measured as the maximum of the first derivative of the melting curve (A_{260} vs. temperature) recorded in medium salt buffer ($[Na^+]$ = 110 mM, $[Cl^-]$ = 100 mM, pH 7.0 (NaH_2PO_4/Na_2HPO_4)), using 1.0 μM concentrations of the two complementary strands. ΔT_m/mod is measured relative to the duplex between the unmodified DNA and the strand containing the abasic site. See Figure 1 for structure of monomers.

Furthermore, ONs that are singly modified with pyrene-functionalized 2′-amino-α-L-LNA monomers **W-Z** recognize DNA strands with abasic sites (**Φ**) in a +1 zipper position with exceptional stability (Table 1). This can, most likely, be explained by interaction of the pyrene moiety with nucleobases in a highly directional manner.

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4. Full experimental details will be published elsewhere.