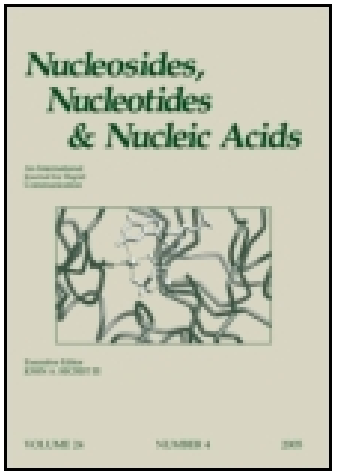

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6-Thioguanine in Peptide Nucleic Acids. Synthesis and Hybridization Properties

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6-THIOGUANINE IN PEPTIDE NUCLEIC ACIDS. SYNTHESIS AND HYBRIDIZATION PROPERTIES

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ABSTRACT: The synthesis of *N*-((2-amino-6-benzylthiopurine-9-yl)acetyl)-*N*-(2-tBoc-aminoethyl)glycine **4** and its incorporation into a peptide nucleic acid (PNA) oligomer are described. Introduction of a single 6-thioguanine residue (6sG) in the PNA of a 10-mer PNA:DNA heteroduplex resulted in a decrease in Tm of 8.5 °C. Furthermore, we observed a hypochromic and a bathochromic shift of 6 nm above 346 nm when the 6sG containing PNA was hybridized to its complementary DNA strand.

Peptide Nucleic Acid (PNA)[1] is a nucleic acid mimic having a *N*-(2-aminoethyl)glycine backbone with the nucleobases linked to the backbone via methylene carbonyl linkers. PNA-oligomers have been shown to form very stable complexes with complementary RNA and DNA under physiological conditions obeying the Watson-Crick hydrogen bonding rules[2-3]. It has been found that 6sG containing DNA oligomers hybridize less efficiently to complementary oligonucleotides[4-5] and the resulting duplex structure is significantly perturbed[6], presumably due to the much larger sulphur atom which causes sterical interference between the sulphur of 6sG and the exocyclic amino-group of cytosine[6]. It could be argued that the presumably more flexible polyamide backbone of PNA might be able to better accommodate structural changes and thus, the duplex perturbation should be reduced. Since the π-π^* absorbance band for the thio-guanine chromophore can be measured without interference from the other nucleobases 6sG may be used as an internal UV-probe in a PNA complex. In order to address these issues, we synthesized the oligomers TATAGGAATT and TATAGG^{S}AATT where the backbones are comprised of deoxyribose phosphate units (DNA) or aminoethylglycine

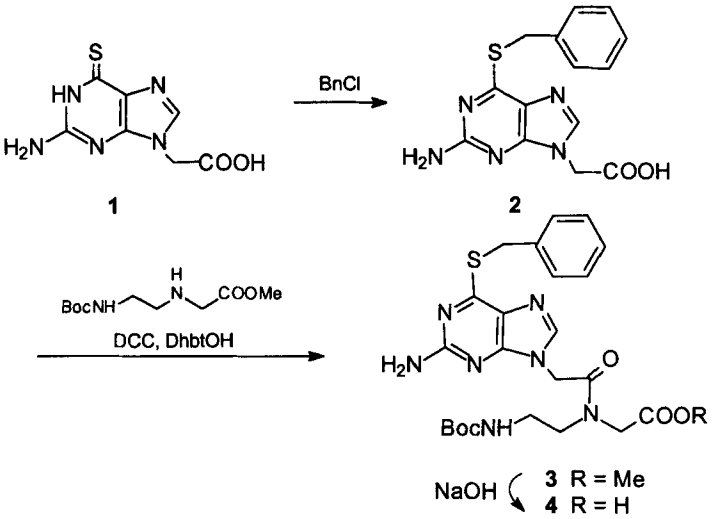

FIG. 1. Synthesis of N-((2-amino-6-benzylthiopurine-9-yl)acetyl)-N-(2-tBocaminoethyl)glycine **4**.

units (PNA). The 6sG PNA monomer was prepared in a standard way (FIG. 1) from 2-amino-9-(carboxymethyl)-6-purinethione[7] and built into the 10-mer PNA using previously reported coupling and cleavage procedures[8].

The duplex thermal stability results presented in the TABLE show that the introduction of a single 6sG residue within a 10-mer PNA decreases the antiparallel PNA-DNA duplex stability by 8.5 °C which is somewhat more than the effect seen for the corresponding DNA-DNA duplexes (ΔTm = 6.0 °C). The introduction of 6sG has a lesser effect on the stability of the parallel complex (ΔTm = 5.5°C). A mismatch opposite to the s6G in the target strand gives a decrease in the Tm of 7.5 °C implying that the thioguanine moiety is still hydrogen bonded with some specificity to the DNA cytosine.

When the PNA oligomer containing 6sG was hybridized to the antiparallel DNA target, a hypochromic as well as a bathochromic shift (6 nm) was observed for the thiochromophore (λ_{max} = 346 nm), FIG. 2. This shift is attributed to stacking of 6sG between the neighbouring nucleobases. Although the Tm measurements indicate distortion of the helix at the 6sG:C basepair, this observation implies that the base stacking through the duplex is retained. The characteristic shift in absorbance and λ_{max} between unhybridized and hybridized 6sG containing PNA-oligomers may be exploited as a convenient method of quantitatively monitoring hybridization.

TABLE. Thermal stability of DNA:DNA and PNA:DNA duplexes. Buffer:100 mM NaCl, 1 mM EDTA, 10 mM $NaHPO_4$, 1 mM DTT, pH 7. The Tm values given are the inflection points of the melting profile obtained by monitoring the absorbance at 260 nm while heating the samples slowly from 5 to 90 °C. n.d.: not detected.

	5'-dATATCCTTAA-3'	5'-dAATTCCTATA-3'	5'-dAATTTCTATA-3'
5'-dTATAGGAATT-3'	n.d.	23.0°	<10°
5'-dTATAGG^{S}AATT-3'	n.d.	17.0°	<10°
H-TAT AGGAATT-LysNH_2	36.0°	47.5°	32.0°
H-TATAGG^{S}AATT-LysNH_2	30.5°	39.0°	31.5°

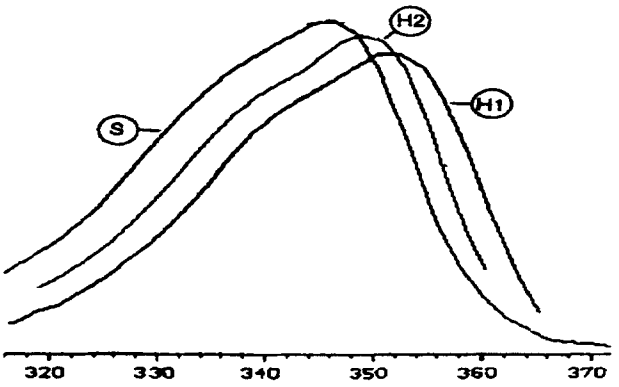

FIG. 2. UV absorption spectra of PNA H-TATAGG^{S}AATT-LysNH_2. S: single stranded, H1: Hybridized to 5'-dAATTCCTATA-3' and H2 hybridized to 5'-dAATTTCTATA-3'. The spectra were obtained at 10° C in the buffer specified in the TABLE.

SYNTHESIS

The PNA-monomers, Boc-A(Z)aegOH, Boc-C(Z)aegOH, Boc-G(Z)aegOH and BocTaegOH were purchased from Perseptive Biosystem (USA). The PNA-oligomers were assembled by a modified Merrifield[8] method and purified by reverse phase C18-HPLC. All PNA oligomers were verified by FAB^+MS or MALDI-TOF mass spectrometry (Kratos). The DNA-oligomers were synthesized by phosphoramidite chemistry on a 1.0 μmol scale using a MilliGen/Biosearch 8700 Nucleic Acid Synthesizer (USA). The phosphoramidites were purchased from Cruachem (UK) with the exception of s6-DNP-dG-CE phosphoramidite which was obtained from Glen Research (USA). The oligomers were deprotected in concentrated ammonia under standard automated DNA synthesis conditions with the exception of s6-DNP-dG containing oligomers which were

cleaved in the presence of 10% mercaptoethanol at room temperature for 48 h[9]. After cleavage and drying the oligomers were washed thoroughly with abs. ethanol.

2-Amino-9-(carboxymethyl)-6-purinethione (1) was prepared according to Nerstrøm et al[7]. ^{1}H NMR (DMSO-d$_6$) δ 4.79 (s, 2 H, CH_2), 6.95 (s, 2 H, NH_2), 7.87 (s, 1 H, H-8), 11.99 (s, 1 H, COOH), the NH-1 proton was not observed. MS (FAB$^+$) m/z 167 $(M + H)^+$.

2-Amino-6-benzylthiopurine-9-ylacetic acid (2). To a solution of 2.27 g (10 mmol) of 2-amino-9-(carboxymethyl)-6-purinethione in 30 ml of DMF was added 2.3 g (19 mmol) of benzyl chloride. The solution was stirred at 70 °C for four hours, and the solvent was removed under reduced pressure. Recrystallization from ethanol gave 2.79 g, 88% of light yellow crystals. ^{1}H NMR (DMSO-d$_6$) δ 4.59 (s, 2 H, CH_2CO), 4.87 (s, 2 H, CH_2S), 7.23-7.49 (m, 5 H, aromates), 8.07 (s, 1 H, H-8). MS (FAB$^+$) m/z 315 $(M + H)^+$.

Methyl-*N*-((2-amino-6-benzylthiopurine-9-yl)acetyl)-*N*-(2-tBocaminoethyl)-glycinate (3). A solution of 2.55 g (8.1 mmol) of **2** in 30 ml of DMF was added 1.8 g (1.1 eq.) of DCC, 1.45 g (1.1 eq.) of DhbtOH and 1.6 g (1.2 eq.) of methyl N-(2-tBocaminoethyl)glycinate.[10] The solution was stirred for 2 h at room temperature. The reaction mixture was filtered, 50 ml of DCM was added, and the solution was extracted with dilute aqueous $NaHCO_3$ (3x50 ml), dilute aqueous $KHSO_4$ (2x50 ml), and brine (1x50 ml). The organic phase was dried with $MgSO_4$, the solvent evaporated, and the residue recrystallized from absolute ethanol to afford 2.3 g, 54% of the target compound. ^{1}H NMR (DMSO-d$_6$) δ 1.37 and 1.38 (s, 9 H, Boc) 3.1-3.5 (m, 4 H, CH_2), 3.61 and 3.75 (s, 2 H, CH_3O), 4.56 (s, 2 H, CH_2S), 4.70 and 4.71 (s, 2 H, CH_2CO), 4.90 and 5.07 (s, 2 H, CH_2CO), 6.55 (s, 2 H, NH_2), 6.75 and 7.01 (m, 1 H, BocNH), 7.22-7.47 (m, 5 H, arom.), 7.77 (s, 1 H, H-8). MS (FAB$^+$) m/z 530 $(M + H)^+$.

***N*-((2-amino-6-benzylthiopurine-9-yl)acetyl)-*N*-(2-tBocaminoethyl)glycine (4)**. Compound **3**, 1.3 g (2.5 mmol) was hydrolyzed in 25 ml of 2N NaOH/THF (2:1) for 2 h at room temperature. The solution was acidified with 2N HCl to pH 2.5 whereby the target compound precipitated, yield 0.95 g, 74%. ^{1}H NMR (DMSO-d$_6$) δ 1.36 and 1.37 (s, 9H, Boc), 3.3-3.6 (m, 4H, CH_2), 3.99 and 4.30 (s, 2H, CH_2CO), 4.57 (s, 2H, CH_2S), 4.89 and 5.06 (s, 2H, CH_2CO), 6.55 (s, 2H, NH_2), 6.74 and 6.97 (m, 1H, BocNH), 7.18-750 (m, 5H, arom.), 7.77 and 7.78 (s, 1H, H-8). MS (FAB$^+$) m/z502 $(M + H)^+$.

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