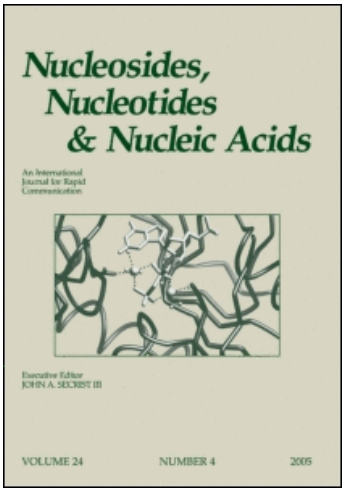

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Convenient Synthesis of Oligodeoxynucleotides Containing 2′-Deoxy-6-thioinosine

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ABSTRACT

A facile synthesis of oligodeoxynucleotides (ODN) containing 2′-deoxy-6-thioinosine (dI^{6S}) based on the convertible nucleoside *O*6-phenyl-2′-deoxyinosine is presented. After standard solid-phase DNA synthesis and removal of the cyanoethyl protecting groups with DBU treatment with aqueous sodium hydrogen sulfide introduces the sulfur functionality, deprotects the other nucleobases and cleaves the ODN from the solid support in a one-pot reaction. In addition, the extinction coefficient of 2′-deoxy-6-thioinosine is determined by enzymatic fragmentation of the resulting ODN in the presence of adenosine deaminase.

RESULTS AND DISCUSSION

DNA containing sulfur-substituted nucleobases is of interest because it enables a wide variety of further regioselective DNA modifications. These include conversion of 2′-deoxy-6-thioinosine (dI^{6S}) to various functionalised base analogues in DNA,[1] formation of interstrand disulfide bonds between dI^{6S} and 4-thiothymidine or

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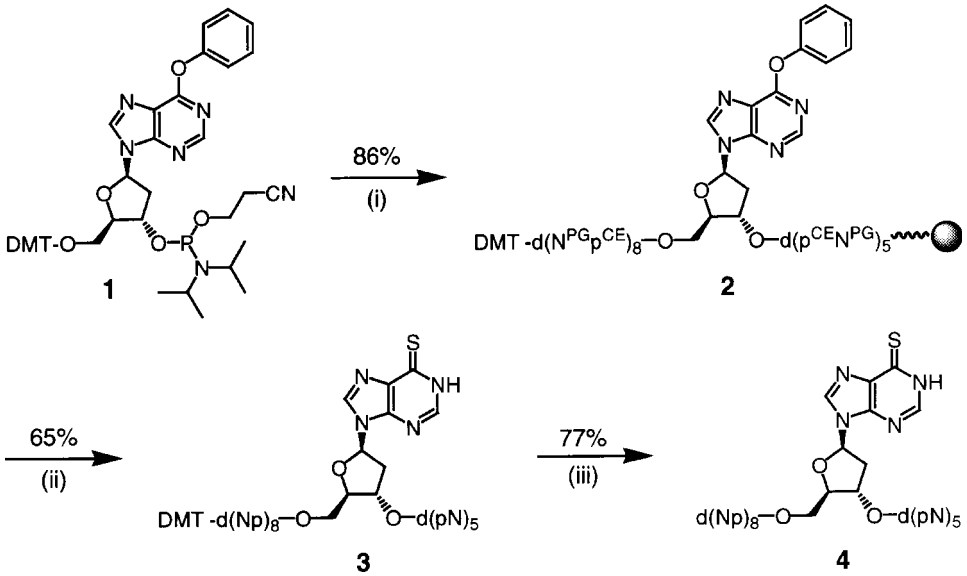
2′-deoxy-4-thiouridine[2] and binding of metal complexes to DNA.[3] Unfortunately, no phosphoramidite is commercially available for the direct incorporation of dI^{6S} into oligodeoxynucleotides (ODN) and it has to be synthesised from 6-chloropurine or 2′-deoxyinosine.[1c,4] Herein, we report a new convenient synthesis of ODN containing dI^{6S} using the convertible nucleoside approach.[5]

The commercially available convertible *O*6-phenyl-2′-deoxyinosine phosphoramidite **1**[5] was incorporated into the fully protected 14mer ODN **2** (5′-GCCG-CTCG**X**TGCCG-3′ with **X** = *O*6-phenyl-2′-deoxyinosine) by solid-phase DNA synthesis using fast deprotectable phosphoramidites for the natural nucleotides[a] (Scheme). Removal of the phosphate protecting cyanoethyl groups from the solid-phase bound ODN **2** with DBU prevents nucleophilic strand breakage in the following step. Treatment with aqueous NaSH leads to the concurrent introduction of sulfur via nucleophilic substitution of the phenoxy group, deprotection of the other nucleobases and cleavage from the solid support. Excess of NaSH is removed by gel filtration before RP-HPLC purification[b]. After detritylation of ODN **3** and a second RP-HPLC purification ODN **4** containing dI^{6S} is obtained in good overall yield (43%)[c].

The UV-absorption spectrum of ODN **4** exhibited an additional band at 324 nm which is characteristic for dI^{6S} (data not shown).[1c,4c] The presence of dI^{6S} was further demonstrated by enzymatic fragmentation followed by RP-HPLC analysis and coinjection with synthetic material[d](Figure left). However, calculation of the nucleotide composition from the chromatogram requires the extinction coefficient of dI^{6S} which is not given in the literature. Therefore, enzymatic fragmentation of ODN **4** was also performed with added adenosine deaminase (ADA) which hydrolyses 6-substituted purine nucleosides to the corresponding inosine derivatives.



Scheme 1.

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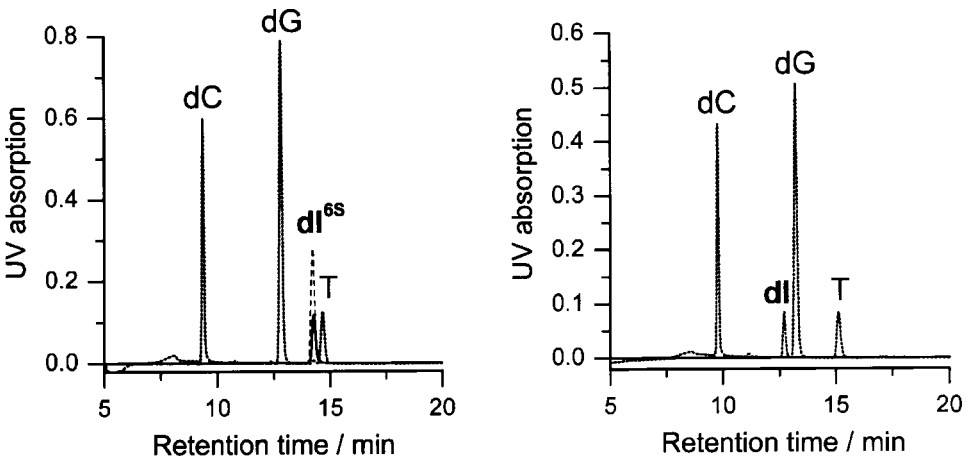

Figure 1.

Robins and Basom[6] reported that dI^{6S} is not a substrate for ADA, but we found that dI^{6S} is hydrolysed in the presence of large amounts of ADA. The obtained chromatogram shows a new signal at 12.8 min (254 nm) for 2′-deoxyinosine (dI) and the signal at 14.2 min (332 nm) for dI^{6S} has disappeared (Figure right). This indicates that ADA has completely converted dI^{6S} to dI. Since the ODN **4** does not contain dA, that would be the preferred substrate for ADA, all dI present in the fragmentation experiment should derive from dI^{6S}. Using the published extinction coefficients for dI[7a] and for dC, dG and T[7b] at 254 nm the experimental nucleotide composition was calculated and found to be in good agreement with the theoretical nucleotide composition (given in brackets): $dI = dI^{6S}$ 1.0 (1), dC 6.2 (6), dG 4.9 (5) and T 1.9 (2). Since the amount of dI relative to the other nucleosides dC, dG and T in this experiment should be the same as the amount of dI^{6S} relative to dC, dG and T in the fragmentation experiment in the absence of ADA (Figure left), the extinction coefficient of dI^{6S} can be calculated. The obtained extinction coefficient of dI^{6S} at 332 nm is $(13\pm0.5)\cdot10^3$ L mol^{-1} cm^{-1}.

In conclusion, we have developed a new and convenient synthesis of ODN containing 2′-deoxy-6-thioinosine based on the incorporation of the convertible nucleoside *O*6-phenyl-2′-deoxyinosine into DNA and subsequent reaction with sodium hydrogen sulfide. In addition, the extinction coefficient of 2′-deoxy-6-thioinosine was determined by enzymatic fragmentation of the resulting ODN and conversion to 2′-deoxyinosine in the presence of adenosine deaminase.

NOTES

a. Fast deprotectable *t*-butylphenoxyacetyl (TAC) protected phosphoramidites and coupling reagents including TAC anhydride as capping reagent were purchased from Proligo. The *O*6-phenyl-dI phosphoramidite **1** was from Glen Research. Solid-phase DNA synthesis was performed on an ABI

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DNA Synthesizer 392 in a 1 μmol scale (0.77 μmol effective loading determined from the first DMT release). After synthesis the DMT-protected ODN **2** (86% yield) was left on the solid-phase.

b. The CPG column was opened and the solid-phase bound ODN **2** was incubated with DBU (1 mL, 0.3 M) in anhydrous CH_3CN at room temperature for 1 h. The solid phase was washed with CH_3CN, CH_3CN containing triethylamine (1%) and CH_3CN (1 mL each). The resulting material was treated with aqueous NaSH (1 mL, 3 M, Fluka) at 55°C for 8 h. After centrifugation the solvent was removed and the solid-phase washed with water (200 μL). The combined aqueous solution was desalted by gel filtration (NAP-5 column, Amersham Biosciences) and subjected to standard C-18 RP-HPLC to give ODN 3 (65% yield).

c. After standard detritylation of ODN **3** with acetic acid (80%) (*Evaluating and Isolating Synthetic Oligonucleotides*, Applied Biosystems, Inc., **1992**, A6-A8), a second RP-HPLC purification and desalting on a NAP-5 column ODN **4** was obtained in 77% yield.

d. Enzymatic fragmentation of the ODN **4** (0.3 OD_{260}) was performed with phosphodiesterase I from *Crotalus adamanteus* (0.024 units, Sigma) and alkaline phosphatase from calf intestine (8.7 units, Roche) in buffer (100 μL, 10 mM KH_2PO_4, 10 mM $MgCl_2$, pH 7) at 37°C for 20 h. In an additional fragmentation experiment adenosine deaminase VI from calf intestinal mucosa (2 units, Sigma) was added to the mixture given before. The resulting nucleosides were analysed by C-18 RP-HPLC monitoring the UV absorbance at 254 nm and 332 nm. The synthetic sample of dI^{6S} used for the coinjection was prepared according to reference 4c.

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