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3-Nitro-3-deaza-2′-deoxyadenosine as a Versatile Photocleavable 2′-Deoxyadenosine Mimic

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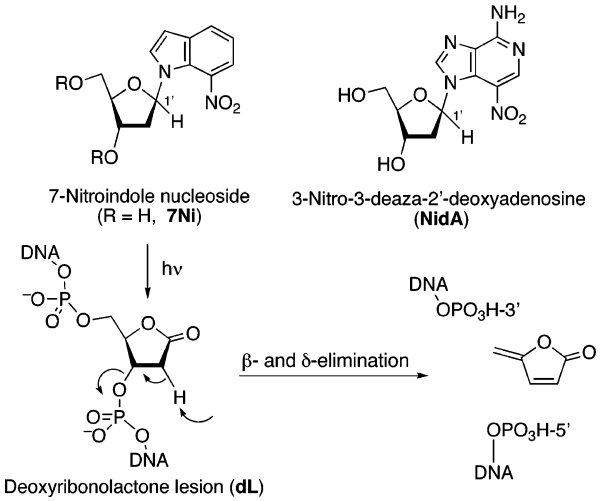
The photochemical manipulation of DNA is one of the most demanded technologies in the current context for development of new DNA-based nanotechnologies. Photochemical reactions are performed in reagent-free medium and allow spatial and temporal control. While many DNA-photocleaving agents are known,[1] only a few photocleavable "building blocks" of DNA have been developed.[2] Most are based on the photochemical properties of a *o*-nitrobenzyl group attached to rings devoid of hybridization properties. It is desirable to introduce new photochemical properties into well-designed modified DNA with minimum structural modifications and, in particular, preserving specific recognition capacity.

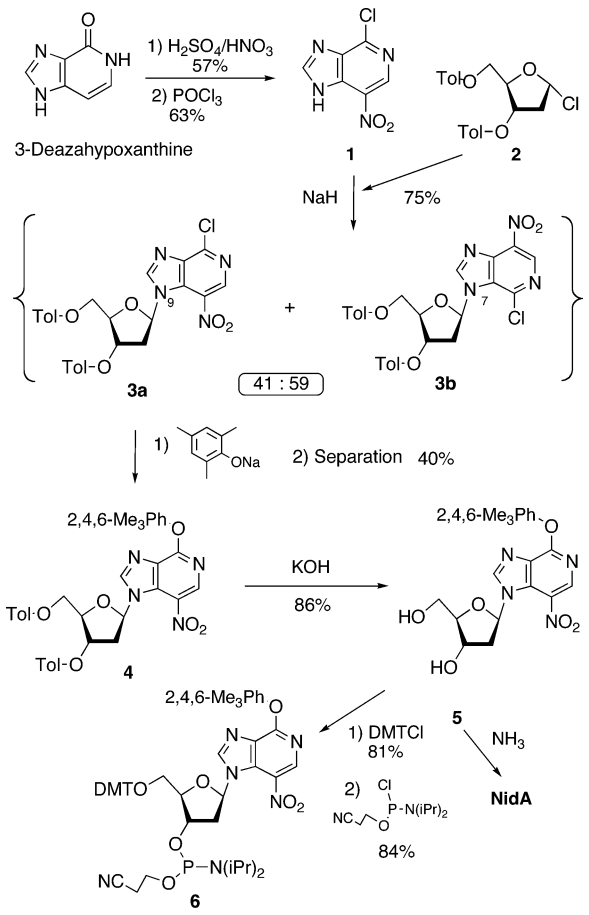
We report here a photocleavable 2′-deoxyadenosine mimic, 3-nitro-3-deaza-2′-deoxyadenosine (**NidA**, Scheme 1). The design

Scheme 1



of **NidA** is based on the properties of 7-nitroindole nucleoside **7Ni**. We reported **7Ni** as a precursor for introduction of the deoxyribonolactone lesion in oligonucleotides.[3] Irradiation of oligonucleotides containing the photosensitive nitroindole nucleoside **7Ni** triggers a radical process in which the excited nitro group induces intramolecular H-1′ abstraction leading to deoxyribonolactone. Subsequent mild basic or thermal treatment leads to total cleavage of the DNA strand (Scheme 1). Following this strategy, we studied the nucleoside analogue **NidA** in which the nitroindole moiety in **7Ni** was replaced by the isosteric 3-nitro-3-deazaadenine nucleus. The new nucleoside possesses the two recognition sites present in adenine, i.e., the H-acceptor internal N-1 nitrogen and the H-donor primary amino group, and is expected to possess both the cleavage properties of **7Ni** and the hydrogen bonding capacities of adenine. We report here on the synthesis of nucleoside **NidA**, its incorporation in oligonucleotides, and subsequent irradiation and piperidine treatment leading to strand cleavage. We also show that **NidA** in DNA possesses the hybridization properties of adenine.

Scheme 2



The strategy for incorporation of **NidA** is outlined in Scheme 2. 3-Deazahypoxanthine, prepared in three steps from 3,4-diaminopyridine,[4] was nitrated and chlorinated to afford 6-chloro-3-nitro-3-deazapurine **1**. Glycosylation of **1** with chloro sugar **2** using Robins' conditions[5] gave the hardly separable mixture of 9-N- and 7-N-isomers **3a** and **3b**, respectively, in a 41/59 ratio. It appeared that the most efficient strategy for preparing the modified oligonucleotide involved the incorporation of **NidA** as its 6-*O*-trimethylphenyl precursor **5**.[6] The mixture of **3a** and **3b** was thus treated with 2,4,6-trimethyl phenolate to give the mixture of the corresponding ethers from which the 9-N-derivative **4** was easily isolated by column chromatography. Basic hydrolysis of the toluoyl protections gave the key trimethylphenyl ether nucleoside **5**. We found that the aryl ether substituent was quantitatively replaced by $-NH_2$ to afford nucleoside **NidA** when **5** was treated with NH_3 in the conditions of final deprotection classically used in oligonucleotide synthesis. Accordingly, **5** was tritylated and phosphitylated to give phosphoramidite **6** that was used in solid-phase DNA synthesis.

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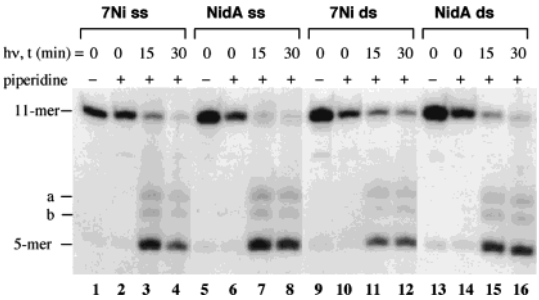

Figure 1. PAGE analysis of irradiation (λ > 320 nm, pH 8, time and treatment indicated) of 5′-^{32}P-labeled 11-mer containing **NidA 7 (**0.5 μM) (lanes 1−4, single stranded, and lanes 9−12, double stranded) and of 5′-^{32}P-labeled 11-mer containing **7Ni 8** (0.5 μM) (lanes 5−8, single stranded, and lanes 13−16, double stranded). Bands a and b are assigned to secondary products resulting from piperidine and DTT (present in labeling buffer) addition on lactone in **9** as observed previously.[8]

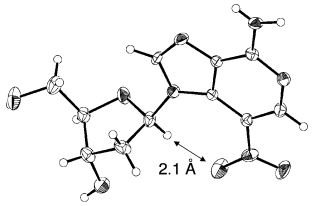

Figure 2. Perspective view of the crystallographic structure of **NidA** showing the distance between the oxygen of the nitro group and the anomeric hydrogen.

The 11-mer 5′-d(CGCAC-**NidA**-CACGC)-3′ **7** was thus prepared using standard conditions.[7] ES-MS analysis of the oligonucleotide confirmed its structure, which additionally proved the introduction of the NH_2 amino group in the **NidA** moiety during the last step of the synthesis (see Supporting Information).

Oligonucleotide **7** was irradiated at λ > 320 nm in dilute aqueous solution at room temperature. After 60 min, the **NidA**-containing oligonucleotide was transformed into the deoxyribonolactone-containing oligonucleotide 5′-d(CGCAC-**dL**-CACGC)-3′ **9** that was characterized by ES-MS (see Supporting Information). Mild piperidine treatment of **9** led to total cleavage of the modified strand (Figure 1). Quite interestingly, PAGE analysis shows that the photoinduced cleavage process is also efficient in the double-stranded state (T facing **NidA** in the complementary strand). It also reveals that the process is at least as efficient as that observed for the previously reported **7Ni**-containing oligonucleotide 5′-d(CGCAC-**7Ni**-CACGC)-3′ **8**.

The efficiency of the photoreaction may be attributed to the highly favorable conformation of the **NidA** nucleotide that presents, at least in the solid state, an anti conformation in which the H-1′ to be abstracted is located at a 2.1 Å distance from the oxygen of the nitro group (Figure 2).

Hybridization properties of **NidA** were examined by measuring the melting temperatures (T_m) of duplex 5′-d(GCGTG-**X**-GTGCG)-3′/5′-d(CGCAC-**Y**-CACGC)-3′**10** (Figure 3) X = T, C; Y = A, **NidA**. Very similar T_m values were determined respectively for the matched duplexes T:A and T:**NidA** (T_m = 59 and 60 °C, respectively) and for the mismatched duplexes **9** C:A and C:**NidA** (T_m = 46 and 46 °C, respectively). CD spectra of the A- and **NidA**-containing oligomers were also similar (see Supporting Information).

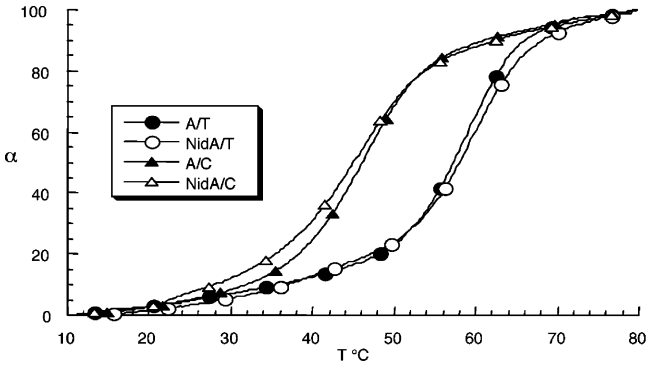

Figure 3. Melting curves of duplex **10** [5 μM]. pH 7 in PO_4 buffer (10 mM), [EDTA] = 1 mM, [NaCl] = 100 mM. α and T_m values were determined according to ref 9.

These results show that the nucleoside analogue **NidA** constitutes a remarkable photocleavable 2′-deoxyadenosine mimic. It can be efficiently prepared and introduced into any preselected site in oligonucleotides. Photocleavage is quantitative and rapid, both in single and double strands. Its hybridization properties are very similar to those of the natural adenosine nucleoside. Enzymatic incorporation in DNA is currently under way.

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Supporting Information Available: Detailed synthetic procedures for **6** and **7**, ES-MS and HPLC analyses of **7** and **8**, and CD spectrum of duplex **10** (PDF), as well as X-ray crystallographic data in CIF format for **NidA**. This material is available free of charge via the Internet at http://pubs.acs.org.

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