ORGANIC LETTERS

2011
Vol. 13, No. 6
1450–1453

Wavelength-Selective Uncaging of dA and dC Residues

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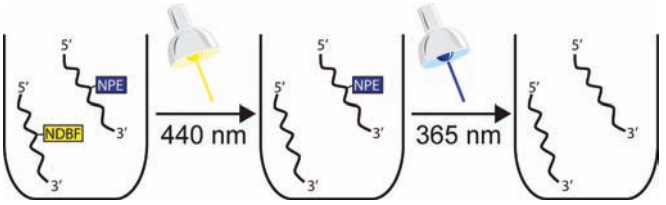
Received January 17, 2011

ABSTRACT



Nitrodibenzofuran (NDBF) groups are used as photolabile "caging" groups to temporarily mask the Watson−Crick interaction of dA and dC residues. They show improved masking capabilities and are photodeprotected 12 times more efficiently than 1-(*o*-nitrophenyl)-ethyl (NPE) caging groups in these positions. Furthermore, NDBF groups can be removed wavelength-selectively in the presence of NPE groups. This will allow more complex (un)caging strategies of oligonucleotides − beyond the usual irreversible triggering.

Caged compounds are substances which have been temporarily inactivated by attaching a photolabile protecting group. By irradiating these compounds with light of a suitable wavelength their biological activity can be restored.[1] Spatiotemporal control of biological functions becomes possible using established methods such as confocal microscope and laser technologies. Nucleic acids are a very important class of biomolecules which can be used for example in the regulation of gene expression or modulation of protein function using the aptamer approach. With caged oligonucleotides it is possible to photoregulate siRNA function, aptamer activity, nucleic acid folding, DNAzymes, or antisense activity, and they are even useful in PCR reactions.[2] Since nucleic acid activity relies in almost all cases on Watson−Crick base pairing it has become convenient to disrupt base pairing by connecting the photolabile group directly to the nucleobase.

Many different types of caging groups have been described, for example, coumarin and bromocoumarin derivatives,[3] the *p*-hydroxyphenacyl (pHP) group,[4] and 1-(*o*-nitrophenyl)-ethyl (NPE) or 2-(*o*-nitrophenyl)-propyl (NPP) groups.[5] An important issue in light-inducible systems is to optimize their photolysis efficiency in order to keep the amount of light needed as low as possible. The product $\phi \cdot \varepsilon$ of uncaging quantum yield (ϕ) and extinction coefficient (ε) is used to describe the efficiency of a caging group in dilute solutions. Although the NPE and NPP groups and their derivatives are not the most efficient caging groups, because of their low extinction coefficient at the typical uncaging wavelength of 365 nm, they have been used very often for caging oligonucleotides. This is

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10.1021/ol200141v © 2011 American Chemical Society
Published on Web 02/22/2011

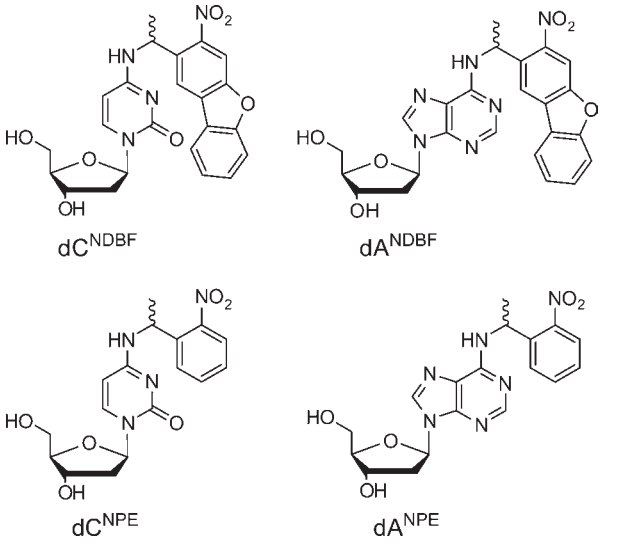

Figure 1. Caged nucleosides used in this study.

due to the drawback that the caging group has to be stable toward the basic cleavage conditions after solid phase synthesis. Even the "ultramild" conditions[6] for the final cleavage after a solid phase synthesis are incompatible with some caging groups that would otherwise have a preferred uncaging behavior.

Much more complicated light-induction strategies could be devised, using the simple and efficient caging approach, if different caging groups could be addressed with different wavelengths within the same molecule. Even though caged compounds have been introduced over 30 years ago, very few examples for a successful implementation of such an approach exist. Pioneering spadework comes from the groups of Bochet, Branda, and Hagen.[7] Unfortunately these approaches are not applicable for caging nucleic acids. Hagen et al. used a thiocarbonate linkage which is not compatible with basic deprotection conditions, and Bochet et al. used light of 254 nm for deprotection which is not suitable for uncaging nucleic acids due to the generation of photodamage.

Recently Ellis-Davies et al. described a new analogue of the *o*-nitrobenzyl type protecting groups, having interesting uncaging properties, with a nitrodibenzofuran (NDBF) core.[8] Deiters et al. reported the successful installation of this caging group on the N^3 of a thymidine and analyzed the deprotection kinetics of NDBF-thymidine.[9] So far the successful incorporation of NDBF-caged nucleosides into an oligonucleotide has not been reported. Our initial attempts to incorporate a guanosine caged at

(6) Typical conditions are for example a 1:1 mixture of aequous ammonia and methanol for 4 h at room temperature.

(7) (a) Blanc, A.; Bochet, C. G. *Org. Lett.* **2007**, *9*, 2649. (b) Lemieux, V.; Gauthier, S.; Branda, N. R. *Angew. Chem., Int. Ed.* **2006**, *45*, 6820. (c) Kotzur, N.; Briand, B.; Beyermann, M.; Hagen, V. *J. Am. Chem. Soc.* **2009**, *131*, 16927.

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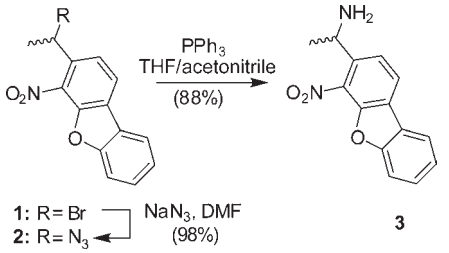
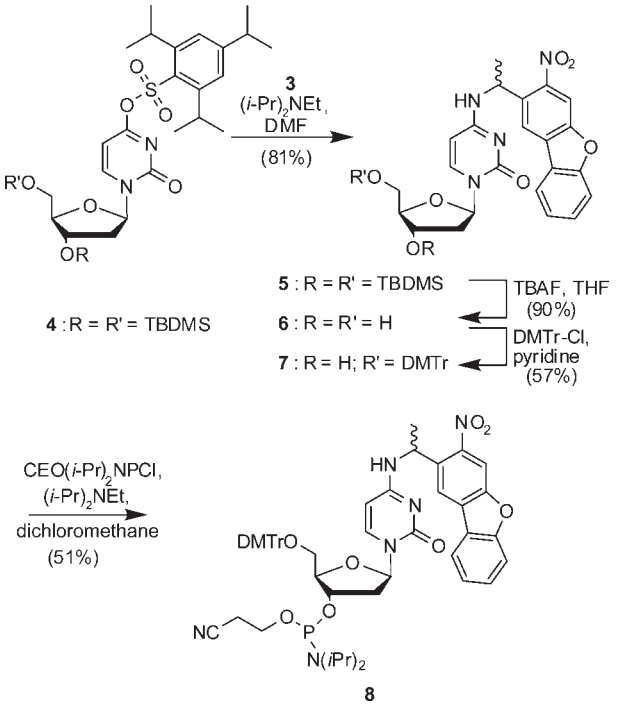
(9) Lusic, H.; Uprety, R.; Deiters, A. *Org. Lett.* **2010**, *12*, 916.

Scheme 1. Synthesis of 1-(3-Nitrodibenzofuran-2-yl)-ethanamine **3**



Scheme 2. Synthesis of $\mathbf{dC^{NDBF}}$ Phosphoramidite **8** (for synthesis of $\mathbf{dA^{NDBF}}$, see Supporting Information)



position O^6 with the NDBF group into a DNA oligomer failed due to the base lability of this moiety. Therefore we set out to prepare an NDBF-caged deoxycytidine and deoxyadenosine instead where the NDBF group is attached via the N^4 and N^6, respectively (Figure 1).

The synthesis of the NDBF caging group precursor started from the known compound **1** (see Supporting Information), which was converted into the azide **2** and then reduced to the amine **3** by a Staudinger reaction (see Scheme 1).

The amine was then coupled to a TBDMS-protected triisopropylbenzenesulfonyl activated deoxyinosine **11** and deoxyuridine **4**.[2d] Silyl group deprotection with TBAF, protection of 5′-OH with DMTr-Cl, and phosphorylation of the 3′-OH with 2-cyanoethoxy-*N,N*-diisopropylamino-chlorophosphine (CEO(*i*-Pr)$_2$NPCl) afforded the caged

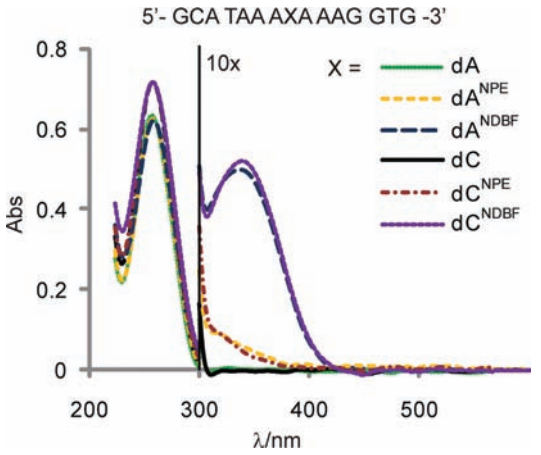


Figure 2. UV−vis spectra of DNA 15-mer strands (in PBS buffer). The area above 300 nm is scaled 10×.

and protected phosphoramidites **15** and **8** ready for use in solid phase synthesis (see Scheme 2 for synthesis of compound **8** and the Supporting Information for the synthesis of the deoxyadenosine analogue **15**).

The amidites used for synthesis of the NPE-containing strands were synthesized as described before.[1c,2d] The resulting UV/vis spectra of the oligonucleotides are depicted in Figure 2. It can be seen that the NDBF-containing oligonucleotides have a significantly higher extinction coefficient at 365 nm, a typical wavelength used for uncaging. Also pronounced absorption of visible light of wavelengths higher than 400 nm is detectable.

In the next step the uncaging quantum yields of these oligonucleotides in PBS buffer solution were determined using dimethoxynitrobenzene actinometry[10] (for details see Supporting Information). Table 1 shows the results of these experiments.

The uncaging quantum yields are all approximately in the same range. For the oligonucleotides with caged **dA** residues both protecting groups show almost the same quantum yield, whereas in the case of caged **dC** residues the quantum yield for the NPE group was even slightly higher than the one for the NDBF group. However, as pointed out before, for an evaluation of the uncaging efficiency it is important to consider the product $\phi \cdot \varepsilon$. Taking this into account the new NDBF derivatives perform 12 times better than the NPE derivatives.

The observation that NDBF-caged oligonucleotides have a considerable absorption above 400 nm in contrast to NPE-caged oligonucleotides led us to question if it was possible to deprotect the NDBF-caged oligonucleotide selectively in the presence of an intact NPE-caged oligonucleotide. We chose an LED with a wavelength of 440 nm (30 mW) for these experiments and irradiated a solution

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Table 1. Photochemical Properties (λ = 365 nm) of the Investigated Oligonucleotides (Error in Quantum Yields ±0.02 for **dC**$^{\mathbf{NPE}}$ and ±0.01 for the Other Oligonucleotides)

X	ϕ	ε [M^{-1} cm^{-1}]	$\varepsilon \cdot \phi$ [M^{-1} cm^{-1}]
dA$^{\mathbf{NPE}}$	0.14	684	97
dA$^{\mathbf{NDBF}}$	0.13	9801	1171
dC$^{\mathbf{NPE}}$	0.17	492	82
dC$^{\mathbf{NDBF}}$	0.10	9208	971

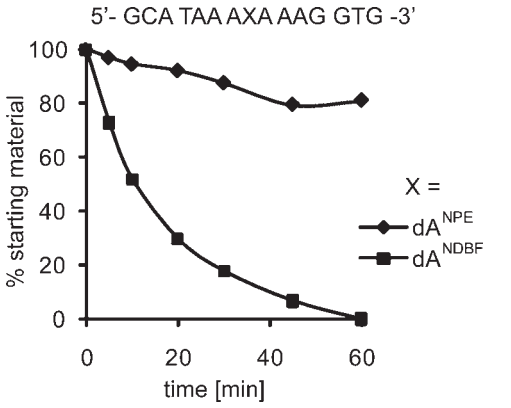

Figure 3. Uncaging kinetics of simultaneous deprotection of a solution containing a **dA**$^{\mathbf{NDBF}}$ and a **dA**$^{\mathbf{NPE}}$-caged oligonucleotide at 440 nm (each 2 μM in 1× PBS, 30 mW LED power).

containing the same amounts of the NDBF- and the NPE-caged oligonucleotide. The irradiated solution was afterward analyzed by RP-HPLC. Figure 3 shows that a wavelength-selective uncaging is indeed possible with a selectivity in the range of 1 order of magnitude (for the corresponding dC-containing oligonucleotides, see Supporting Information).

In addition to these improved photochemical properties we were also interested in how efficiently a DNA duplex can be destabilized by our new NDBF-caged nucleosides compared to NPE-caged nucleosides. Therefore we performed melting point studies (see Figure 4) and observed that 15-mer duplexes are destabilized significantly higher by incorporation of NDBF-caged nucleosides than by NPE-caged nucleosides. Compared to the native duplex, incorporation of a **dC**$^{\mathbf{NPE}}$ led to a destabilization of 8 °C, while incorporation of a **dC**$^{\mathbf{NDBF}}$ destabilized the duplex by 16.1 °C. The same trend was found for dA-containing oligonucleotides (**dA**$^{\mathbf{NPE}}$: 6.2 °C; **dA**$^{\mathbf{NDBF}}$: 11.7 °C).

In conclusion we have shown that NDBF-protected nucleosides can be incorporated into an oligonucleotide and are stable toward basic cleavage conditions. NDBF-caged oligonucleotides can be deprotected about 12 times more efficiently than NPE-caged analogues owing to their higher extinction coefficient at 365 nm. Furthermore NDBF-caged residues destabilize a DNA duplex significantly

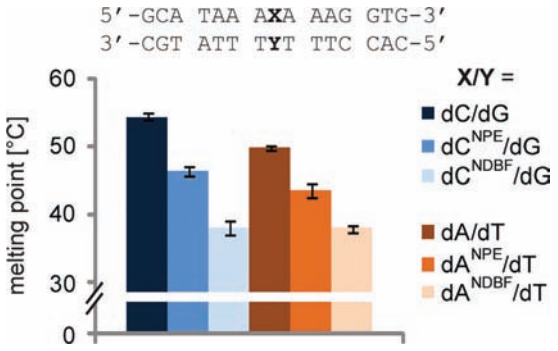

Figure 4. Melting points of DNA duplexes with different (caged) residues **X** (concentration of each oligonucleotide 1 μM in 1× PBS buffer).

better than NPE-caged residues. Additionally, NDBF-caged oligonucleotides can be deprotected completely at 440 nm while an NPE-caged analogue is almost unaffected by irradiation with this wavelength at the same time, which gives the possibility for orthogonal uncaging. This gives rise to more complex caging strategies.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft (Emmy Noether Fellowship HE 4597/1-1 to A.H. and Cluster of Excellence Macromolecular Complexes EXC 115) and by the Alexander von Humboldt Foundation (fellowship to K.B.J.). A.H. gratefully acknowledges the generous donation of silyl protecting group precursors by Wacker. The authors thank Angelika Schneider and Dragica Podgorski (students of Goethe University Frankfurt) for their help in the synthesis of the phosphoramidites.

Supporting Information Available. Experimental procedures, technical details, and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.