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Coumarin-Caged dG for Improved Wavelength-Selective Uncaging of DNA

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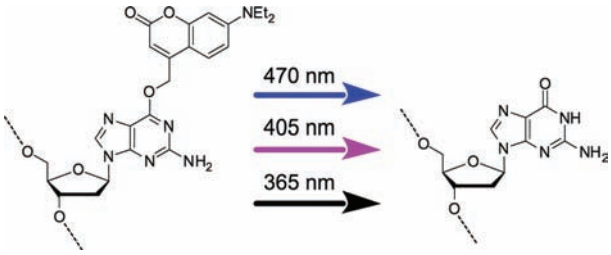
ABSTRACT



Herein we report on diethylaminocoumarin (DEACM) as a new photoremovable protecting group for 2′-deoxyguanosine in oligonucleotides. An oligonucleotide with O^6-DEACM-caged dG was synthesized and photochemically analyzed. The DEACM group shows superior photochemical properties at 405 nm with an uncaging efficiency ($\varepsilon \cdot \phi$) for deprotection that is 17 times higher than that for 2-(*o*-nitrophenyl)-propyl NPP caging groups in the same position. Wavelength-selective deprotection in the presence of NPP groups proceeds up to 80 times faster.

The masking of biologically active molecules with photolabile protecting groups (caging groups) has become an important technique for the investigation of biological processes. Through irradiation of biological samples with nondamaging light (> 360 nm) the activity of the caged compound can be restored in a very selective and mild manner with exact control of region and time, for example in a confocal microscope. Therefore this approach has been used successfully to regulate the activity of small molecules, peptides, proteins, and oligonucleotides with light.[1] Oligonucleotides are an interesting class of biomolecules as they can be used to control protein function and gene expression. Caged oligonucleotides have already been applied to photoregulate nucleic acid folding, transcription, siRNA function, antisense activity, DNAzymes, aptamer function, or DNA nanoarchitecture assembly.[2] The caging moiety can be introduced either statistically as a backbone modification[3] or during oligonucleotide solid phase synthesis (SPS) as a nucleobase-caged nucleotide building block.[4] The former method leads to a randomized caging pattern with the difficulty of incomplete (un)caging. The latter allows the protection of specific nucleobases, which are very effective caging sites as most functions of oligonucleotides rely on the Watson–Crick base pairing. This minimizes problems with incomplete on/off behavior and also the amount of light, that has to be used, and hence the potential photodamage.

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In the majority of the studies on caged DNA or RNA only examples of the class of *o*-nitrobenzyl-type caging groups such as 1-(*o*-nitrophenyl)-ethyl (NPE) or 2-(*o*-nitrophenyl)-propyl (NPP)[5] have been used although they exhibit only low photolytic efficiency around 360 nm. This is due to their straightforward synthesis and stability toward the aqueous basic cleavage conditions after SPS. Recently, our group reported on the incorporation of a novel *o*-nitrobenzyl-type caging group on dC and dA residues into DNA (see for example $\mathbf{dA}^{\mathbf{NDBF}}$ in Figure 1).[6] The nitrodibenzofuran (NDBF) chromophore had first been introduced by Ellis-Davies as part of a calcium chelator that released calcium photolytically up to 160 times more efficiently as other widely used calcium cages.[7] In our studies the uncaging efficiency ($\varepsilon \cdot \phi$) of $\mathbf{dA}^{\mathbf{NDBF}}$-containing oligonucleotides ($\phi = 0.13$) was 12 times higher than that for $\mathbf{dA}^{\mathbf{NPE}}$-containing oligonucleotides ($\phi = 0.14$) due to a much higher extinction coefficient at 365 nm. Also, due to the red-shifted absorption, at 440 nm selective uncaging of NDBF-protected oligonucleotides was achieved in the presence of NPE-caged analogues.

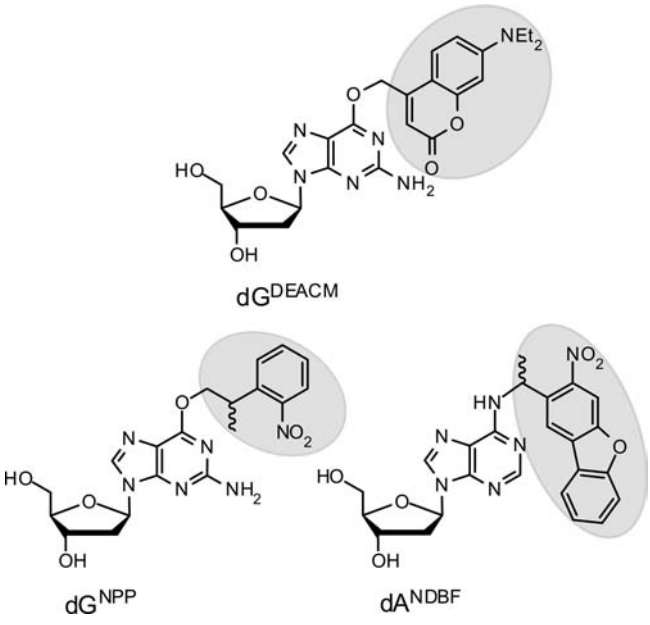

Figure 1. Newly introduced residue $\mathbf{dG}^{\mathbf{DEACM}}$ and the known residues $\mathbf{dG}^{\mathbf{NPP}}$ and $\mathbf{dA}^{\mathbf{NDBF}}$ for comparison.

This wavelength-selective photolysis of caging groups for independently addressing different functions in a biological system expands the scope of caging strategies significantly. After the fundamental work on this topic by the groups of Bochet[8] and Hagen,[9] only recently wavelength-selective deprotection of caged surfaces,[10] selective photoactivation of two protein kinase pathways,[11] dual wavelength control of Wip1 phosphatase activity,[12] and selective two-photon uncaging of glutamate and GABA for action potential modulation were reported.[13] Our $\mathbf{dA}^{\mathbf{NDBF}}$ and the analogous $\mathbf{dC}^{\mathbf{NDBF}}$ residues were the first nucleobase-caged nucleosides that offered the possibility of wavelength-selective uncaging oligonucleotides.

Among the coumarin-type protecting groups the (6-bromo-7-hydroxycoumarin-4-yl)methyl (Bhc)[14] and (7-diethylaminocoumarin-4-yl)methyl (DEACM)[15] groups exhibit the most interesting photochemical properties. Bhc possesses the highest one- and two-photon photolysis quantum yields of coumarin-type protecting groups, and DEACM shows the most red-shifted absorption maximum (around 390 nm), allowing efficient photocleavage at wavelengths over 400 nm. Bhc was already introduced on phosphate groups of oligonucleotides.[3b,16] Apart from that, only mononucleotides caged with these groups have been reported until now and none of these approaches is applicable for an SPS of oligonucleotides.[15,17] Due to the solvent-assisted photoheterolysis mechanism of the cleavage, it is discussed that coumarins are suitable cages only for acidic functional groups and not applicable for direct caging of nucleotide bases.[7,18] Therefore, hydroxyl or amino functions are typically caged via a carbonate/carbamate linkage, which would be hydrolyzed in the basic cleavage step of oligonucleotide SPS.

Being aware of the electron-withdrawing nature of the aromatic systems of nucleobases, we set out to prepare a deoxyguanosine caged directly on the O^6 position with DEACM (Figure 1). DEACM was chosen due to its high red-shifted absorption and because this group does not require protection groups for the SPS, in contrast to Bhc.

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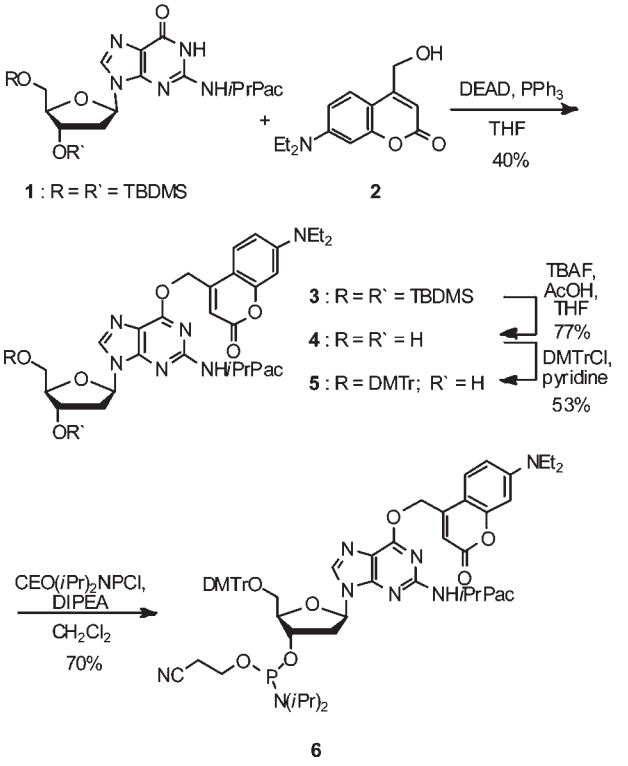
According to the literature[15b] DEACM-alcohol **2** was synthesized in two steps (see Supporting Information) and coupled to TBDMS- and isopropylphenoxyacetyl-(*i*PrPac) protected deoxyguanosine **1** under Mitsunobu conditions (→**3**). Silyl group deprotection with TBAF (→**4**), introduction of a dimethoxytrityl (DMTr) group at the 5′−OH (→**5**), and phosphitylation of the 3′−OH with 2-cyanoethoxy-*N,N*-diisopropylaminochlorophosphine (CEO(*i*Pr)$_2$NPCl) afforded the protected and coumarin-caged phosphoramidite **6**, which was directly used in the SPS (Scheme 1). The amidite of **dG^{NPP}** was synthesized as described before.[4a]

Scheme 1. Synthesis of **dG^{DEACM}** Phosphoramidite **6**



Irradiation of **dG^{DEACM}**-containing oligonucleotides with light of 365 nm resulted in a clean and rapid photorelease of uncaged oligonucleotides (see Supporting Information), proving the good leaving group properties of O^6 of guanine.

Therefore UV/vis spectra of the resulting oligonucleotides were recorded at physiological conditions (PBS buffer, pH 7.4) and compared with spectra of **dA^{NPE}**- and **dA^{NDBF}**-containing oligonucleotides (Figure 2). The intensive absorption maxima around 260 nm are mainly caused by the nucleobases (for the absorption properties of the coumarin alcohol **2**, see the Supporting Information). At the typical uncaging wavelength of 365 nm the extinction coefficients of **dG^{DEACM}**-caged oligonucleotides compared to NDBF-containing oligonucleotides are slightly higher (see also Table 1). But both absorb significantly stronger than NPP- or NPE-modified DNA. The DEACM-containing oligonucleotide exhibits a very intensive red-shifted absorption band

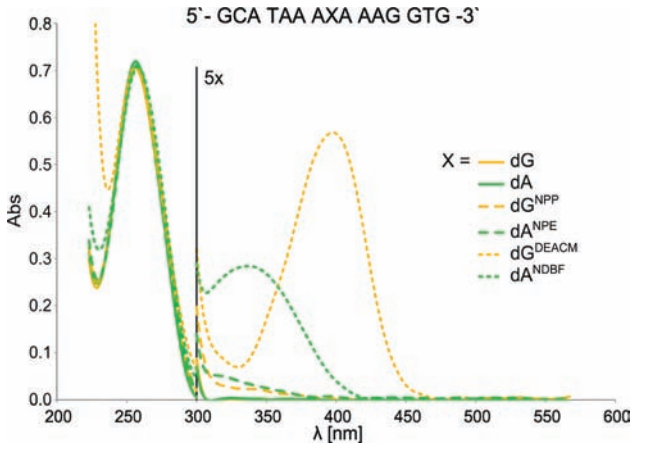


Figure 2. UV/vis spectra of 15-mer oligonucleotides (4 μM solution in PBS buffer). For the spectra above 300 nm a 20 μM solution was used.

with λ_{max} = 398 nm that belongs to $\pi-\pi^*$ transitions of the coumarin chromophore.[19] This absorption maximum shows a slight bathochromic shift compared to a single **dG^{DEACM}** nucleoside (λ_{max} = 392 nm) and free coumarin alcohol **2** with λ_{max} = 385 nm (see Supporting Information).

As a next step we determined the uncaging quantum yields of **dG^{NPP}**- and **dG^{DEACM}**-containing oligonucleotides using dimethoxynitrobenzene actinometry[20] (for details see Supporting Information). The results are shown in Table 1. The quantum yields for X = **dG^{DEACM}** are in the

Table 1. Photochemical Properties (Uncaging Quantum Yield ϕ and Molar Extinction Coefficient ε) of Investigated Oligodeoxynucleotides 5′-GCATAAAXAAAGGTG-3′ in PBS Buffer, pH 7.4 at Different Wavelengths λ

X	λ [nm]	ϕ	ε [M^{-1} cm^{-1}]	$\varepsilon\cdot\phi$ [M^{-1} cm^{-1}]
dG^{NPP}	365	0.16	625	97
dG^{DEACM}	365	0.01	15 318	178
dG^{DEACM}	405	0.06	27 321	1672
dA^{NDBF}	365	0.13	9801	1171

same range as those reported for γ-P-caged CTP (ϕ = 0.029)[15b] but significantly lower compared to caged cyclic nucleotides ($\phi \approx 0.25$).[15a,17a] In comparison with the *o*-nitrobenzyl-type caged oligonucleotides the quantum yields are also lower. However, to evaluate the uncaging efficiency the product $\phi\cdot\varepsilon$ is deciding factor. Therefore, due to the high extinction coefficients, **dG^{DEACM}** is still cleaved nearly two times more effectively at 365 nm than **dG^{NPP}**. In comparison **dA^{NDBF}** performed around 7 times

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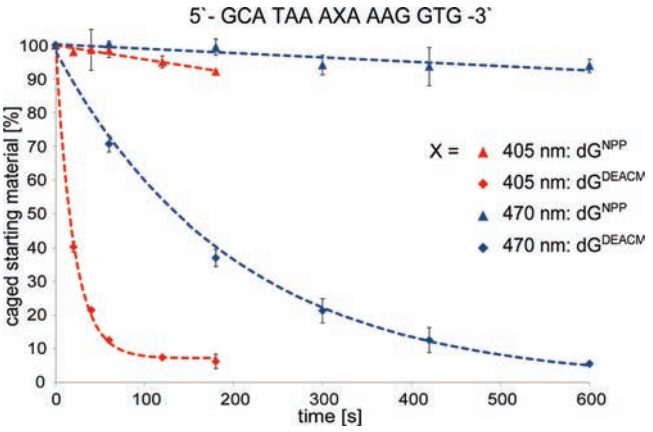

Figure 3. Uncaging kinetics of simultaneous deprotection of a solution containing a $\mathbf{dG^{DEACM}}$- and $\mathbf{dG^{NPP}}$-caged oligonucleotide (10 μM each, PBS buffer).

better at this wavelength. Using 405 nm for irradiation of $\mathbf{dG^{DEACM}}$ the uncaging efficiency for deprotection was 17 times higher than that for $\mathbf{dG^{NPP}}$.

This was then used for wavelength-selective uncaging studies, in which the time course of the uncaging of equimolar mixtures of $\mathbf{dG^{DEACM}}$- and $\mathbf{dG^{NPP}}$-containing oligonucleotides was determined by RP-HPLC analysis (for details, see Supporting Information). Using an LED with a wavelength of 405 nm (160 mW) $\mathbf{dG^{DEACM}}$ is cleaved with a selectivity of 18:1 over a time course of 3 min, while using an LED with 470 nm (850 mW) a selectivity of 16:1 is achieved for the uncaging of $\mathbf{dG^{DEACM}}$ over 10 min (Figure 3). This wavelength-selective deprotection proceeds around 30 and 10 times faster than in our previous study using $\mathbf{dA^{NDBF}}$/$\mathbf{dA^{NPE}}$-containing samples.[6] From analysis of the initial slope of the curves in Figure 3, deprotection of $\mathbf{dG^{DEACM}}$ was observed to proceed 80 times faster at 405 nm than that for $\mathbf{dG^{NPP}}$, while at 470 nm a factor of 40 times was obtained.

In addition we tested the duplex destabilization properties of DEACM- or NPP-caged dG, performing melting point measurements (Table 2). Both protecting groups caused a significant destabilization of a 15-mer duplex,

Table 2. Comparison of Melting Points of the Respective Oligonucleotides Containing Caged Residues (**X**)[a]

5′-GCA TAA AXA AAG GTG-3′
3′-CGT ATT TCT TTC CAC-5′

X	T_m [°C]	ΔT_m [°C]
dG	50.7	–
$\mathbf{dG^{NPP}}$	40.4	−10.3
$\mathbf{dG^{DEACM}}$	39.4	−11.3

[a] The concentration of each oligonucleotide was 1 μM in PBS buffer.

with $\mathbf{dG^{NPP}}$ leading to a destabilization of 10.3 °C and with $\mathbf{dG^{DEACM}}$ decreasing the melting point by 11.3 °C.

In conclusion we have shown that DEACM is a suitable photolabile protecting group for nucleobases in oligonucleotides. An O^6-caged deoxyguanosine phosphoramidite was synthesized and stable under oligonucleotide solid phase synthesis conditions. Compared to NPP-caged analogues uncaging at 405 nm proceeds 80 times faster for DEACM-caged oligonucleotides. Also deprotection can still be performed at 470 nm to diminish near UV irradiation damage on biological probes. Therefore $\mathbf{dG^{DEACM}}$ represents a useful expansion of the set of selectively cleavable caged nucleotide building blocks that permits efficient wavelength-selective uncaging for more sophisticated light regulation strategies in oligonucleotide applications.

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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.