ORGANIC LETTERS

2013
Vol. 15, No. 21
5500–5503

Four Levels of Wavelength-Selective Uncaging for Oligonucleotides

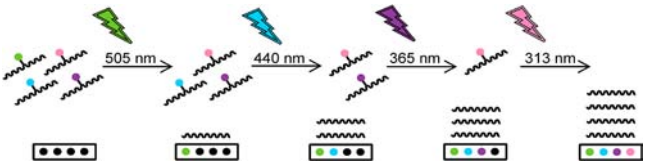
Alexandre Rodrigues-Correia, Xenia M. M. Weyel, and Alexander Heckel*

Institute for Organic Chemistry and Chemical Biology, Buchmann Institute for Molecular Life Sciences, Goethe-University Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany

heckel@uni-frankfurt.de

Received September 13, 2013

ABSTRACT



In this study the new nucleobase-caged nucleotides dT^{NpHP} and dT^{DEACM} are introduced. Together with two other caging groups (NDBF and NPP) this results in four layers of wavelength-selective uncaging for oligonucleotides, sequentially going from 505 to 440 nm, 365 nm, and finally to 313 nm for the photolysis reaction.

Regulation with light as an external trigger signal is a formidable way to control experiments because it is a highly selective cue and one can rely on a multitude of well worked-out technologies for the generation and manipulation of light. For coupling light to a microscopic effect there is a choice of three technologies:[1] one of them uses photolabile protecting groups which temporarily block a compound's activity.[2] This approach has been called "uncaging" and can be applied to a large amount of different types of molecules.[3] The second technology uses bistable photoswitches,[4] and the third big field uses engineered and natural systems which can be biologically expressed, such as for example channelrhodopsins. Uncaging is usually very straightforward and can achieve excellent ON/OFF ratios. This is due to the fact that uncaging usually yields the unmodified, active parent compound. However, uncaging is a one-way triggering concept. Photoswitching allows for much more sophisticated experiments because of the dual switching mode but is conceptually more difficult. This is due to the fact that no photoswitch can be transformed from one pure photoisomer to the other pure photoisomer. In addition to this inevitable mixture of states, a photoswitch is always present in the molecule and must be engineered into the molecule to be switched in a way so that the transition in properties has maximal influence on the activity. Otherwise one loses twice in the purity of the activity of the states that can be prepared. Despite these shortcomings of photoswitching, brilliant applications of this principle have been realized already, either by coupling all-or-nothing processes to the photoswitching event[5] or combining the power of multiple photoswitches.[6]

An equally versatile solution to more complicated photoregulation scenarios could be to realize different layers of triggering using the caging approach.[1] Attempts have been made to trigger (uncage) molecules selectively with light of different wavelengths. Pioneering work comes from the group of Bochet[7] while more recent work has been made for

(1) Brieke, C.; Rohrbach, F.; Gottschalk, A.; Mayer, G.; Heckel, A. *Angew. Chem., Int. Ed.* **2012**, *51*, 8446.

(2) Klán, P.; Šolomek, T.; Bochet, C. G.; Blanc, A.; Givens, R.; Rubina, M.; Popik, V.; Kostikov, A.; Wirz, J. *Chem. Rev.* **2013**, *113*, 119.

(3) (a) Gardner, L.; Deiters, A. *Curr. Opin. Chem. Biol.* **2012**, *16*, 1. (b) Puliti, D.; Warther, D.; Orange, C.; Specht, A.; Goeldner, M. *Bioorg. Med. Chem.* **2011**, *19*, 1023. (c) Drepper, T.; Krauss, U.; Meyer zu Berstenhorst, S.; Pietruszka, J.; Jaeger, K.-E. *Appl. Microbiol. Biotechnol.* **2011**, *90*, 23. (d) Lee, H.-M.; Larson, D. R.; Lawrence, D. S. *ACS Chem. Biol.* **2009**, *4*, 409.

(4) (a) Szymanski, W.; Beierle, J. M.; Kistemaker, H. A. V.; Velema, W. A.; Feringa, B. L. *Chem. Rev.* **2013**, *113*, 6114. (b) Fehrentz, T.; Schoenberger, M.; Trauner, D. *Angew. Chem., Int. Ed.* **2011**, *50*, 12156.

(5) (a) Stein, M.; Middendorp, S. J.; Carta, V.; Pejo, E.; Raines, D. E.; Forman, S. A.; Sigel, E.; Trauner, D. *Angew. Chem., Int. Ed.* **2012**, *51*, 10500. (b) Fehrentz, T.; Schoenberger, M.; Trauner, D. *Angew. Chem., Int. Ed.* **2011**, *50*, 12156.

(6) (a) Nishioka, H.; Liang, X.; Kato, T.; Asanuma, H. *Angew. Chem., Int. Ed.* **2012**, *51*, 1165. (b) Zhou, M.; Liang, X.; Mochizuki, T.; Asanuma, H. *Angew. Chem., Int. Ed.* **2010**, *49*, 2167.

(7) (a) Bochet, C. G. *Pure Appl. Chem.* **2006**, *78*, 241. (b) Bochet, C. G. *Synlett* **2004**, 2268.

10.1021/ol402657j © 2013 American Chemical Society
Published on Web 10/10/2013

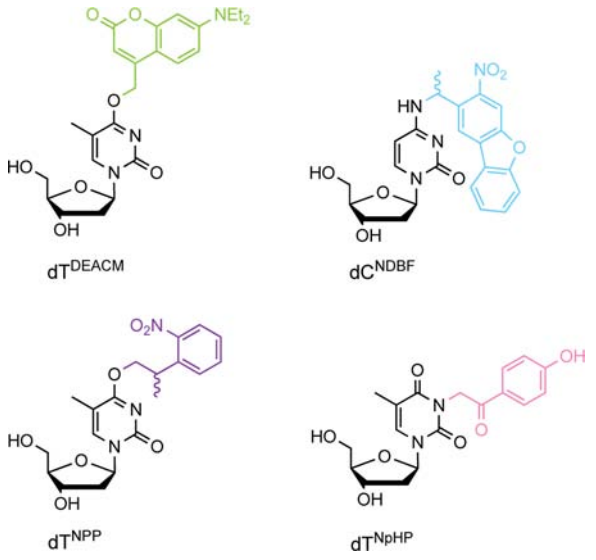
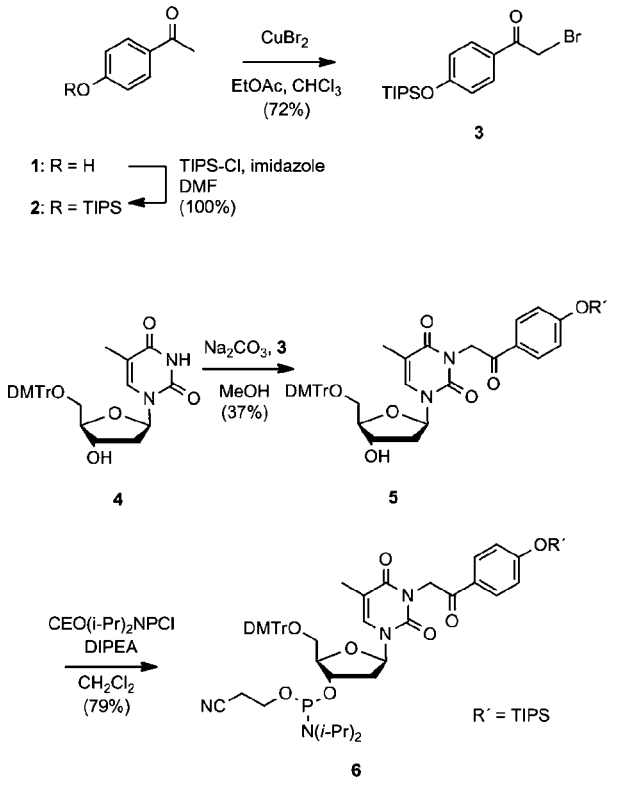


Figure 1. Caged nucleosides used in this study.

Scheme 1. Synthesis of the Phosphoramidite **6** for the Introduction of dT^{NpHP} Residues in a DNA Solid Phase Synthesis



example in the groups of del Campo[8] and Ellis-Davies.[9] In the field of oligonucleotides our group has shown that sequential uncaging is possible using caging groups with distinct light absorption properties. For example NPP-caged residues (NPP = 2-(*o*-nitrophenyl)propyl), which are usually uncaged at 365 nm, stay intact upon photolysis of NDBF-caged residues (NDBF = 1-(3-nitrodibenzofuran-1-yl)ethyl) at 440 nm[10] or upon photolysis of DEACM-caged residues (DEACM = (7-diethylaminocoumarin-4-yl)methyl) at 405 or 470 nm (Figure 1).[11] In this study the opposite is investigated: going to lower wavelengths and arriving altogether at four layers of wavelength-selective uncaging for oligonucleotides.

The new photolabile group used here is the pHP group (*p*-hydroxyphenacyl) due to its pronounced absorption at 300−350 nm. The synthesis of a phosphoramidite building block for the introduction of an N^3-pHP-caged thymidine into an oligonucleotide solid-phase synthesis is shown in Scheme 1: Starting from commercially available *p*-hydroxyacetophenone (**1**) a TIPS-protection (→**2**) and then a bromination of the methyl group were performed to arrive at the caging group precursor (**3**) in good yields. This compound was used to alkylate DMTr-protected thymidine (**4**) exclusively on the N^3-position (→**5**). Phosphitylation afforded the protected phosphoramidite **6** in good overall yields. The phosphoramidite building blocks for the incorporation of dC^{NDBF} and dT^{NPP} were obtained according to established protocols.[10,12] For the synthesis of the phosphoramidite for the incorporation of dT^{DEACM} we refer to the Supporting Information.

These building blocks were then used in oligonucleotide solid phase syntheses in which standard coupling protocols could be used in every step. As a sequence the one depicted in Figure 2 was chosen because of the body of data that had already been acquired in previous systematic studies using other caging groups.[13] A comparison of the UV/vis absorption spectra of the oligonucleotide containing the new dT^{NpHP} residue along with the spectra of control sequences containing the other nucleosides shown in Figure 1 as well as an unmodified dT residue are shown in Figure 2. It can be seen that each of the oligonucleotides has its characteristic absorption profile in the region beyond 300 nm.

In our previous studies up to now only a combination of two caging groups was used (NDBF and NPE or DEACM and NPP).[10,11] Figure 3 now shows HPLC traces of a mixture of four oligonucleotides with one of the residues of Figure 1 in the respective central position. At first at 505 nm the DEACM-caged oligonucleotide is selectively uncaged. It turned out that the choice of this wavelength was necessary for a good discrimination between DEACM and NDBF. After 90% uncaging of DEACM 80% of the NDBF-caged oligonucleotide remained unreacted and the other two oligonucleotides were nearly completely intact (Supporting Table 2). A subsequent irradiation at 440 nm selectively uncaged the NDBF-containing sequence

(8) (a) San Miguel, V.; Bochet, C. G.; del Campo, A. *J. Am. Chem. Soc.* **2011**, *133*, 5380. (b) Cui, J.; Miguel, V. S.; del Campo, A. *Macromol. Rapid Commun.* **2013**, *34*, 310.

(9) Kantevari, S.; Matsuzaki, M.; Kanemoto, Y.; Kasai, H.; Ellis-Davies, G. C. R. *Nat. Methods* **2010**, *7*, 123.

(10) Schäfer, F.; Joshi, K. B.; Fichte, M. A. H.; Mack, T.; Wachtveitl, J.; Heckel, A. *Org. Lett.* **2011**, *13*, 1450.

(11) Menge, C.; Heckel, A. *Org. Lett.* **2011**, *13*, 4620.

(12) Kröck, L.; Heckel, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 471.

(13) Rodrigues-Correia, A.; Koeppel, M. B.; Schäfer, F.; Joshi, K. B.; Mack, T.; Heckel, A. *Anal. Bioanal. Chem.* **2011**, *399*, 441.

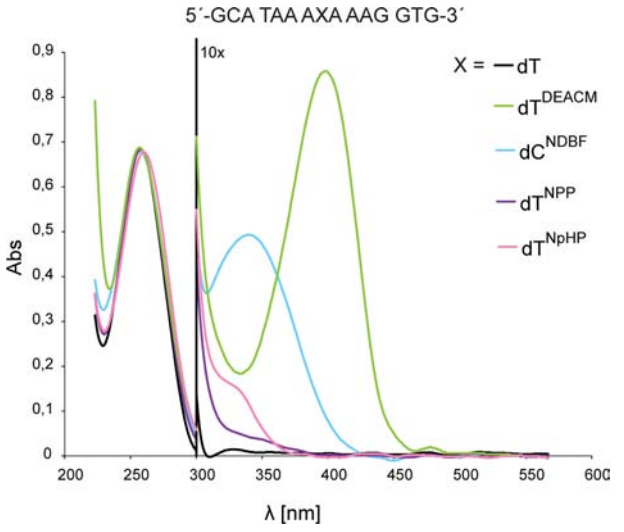


Figure 2. UV/vis absorption spectra of DNA 15-mer strands (4 μM solution in PBS buffer). For the spectra above 300 nm a 40 μM solution was used.

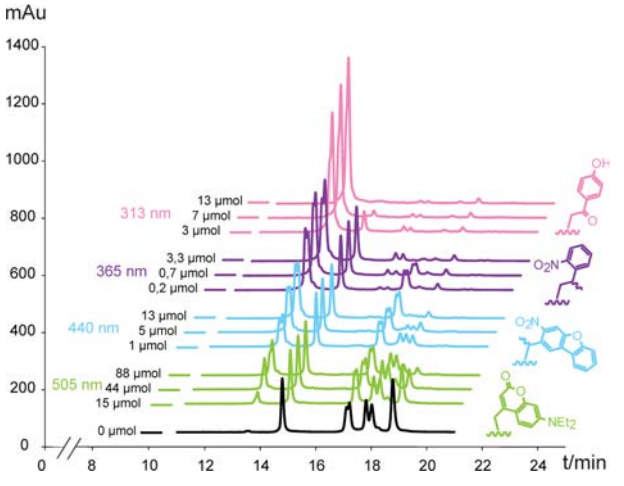


Figure 3. HPLC chromatograms after irradiation at a different wavelength and time. Each sample (60 μL) contained the four oligonucleotides 5′-GCA TAA AXA AAG GTG-3′ (X = dT^{DEACM}, dC^{NDBF}, dT^{NPP}, and dT^{NpHP}) at 3 μM concentration in PBS buffer. For better comparison the amount of photons is indicated since the power of the light sources varied considerably.

(which appears as two peaks in the HPLC trace due to the additional stereogenic center in the caging group). When 94% of the NDBF-containing oligonucleotide were uncaged, 93% of the NPP-containing one remained. Again the pHP-containing oligonucleotide was left untouched. Then irradiation at 365 nm cleaved off the NPP-group. Also here the NPP-caged oligonucleotide had given two peaks in the HPLC trace, albeit not as well resolved as in the case of NDBF. When 94% of the NPP-containing oligonucleotide had reacted, the pHP-containing one was still quantitatively present. Finally, irradiation at 313 nm led to a clean deprotection of the pHP-caged oligonucleotide. After the entire procedure essentially only one HPLC peak remained resulting from the unmodified oligonucleotides.

Table 1 gives an overview of the photochemical parameters. While the molar extinction coefficient of dT^{NpHP} is relatively high, the quantum yield for the uncaging process, unfortunately, is not. This results in the relatively low product $\varepsilon \cdot \varphi$ which can be used to compare caging groups. For a good discrimination between the DEACM and the NDBF group, it was necessary to use 505 nm light where the absorption of dT^{DEACM} is already relatively low and to irradiate longer. pHP shows the best selectivity in the entire series as the pHP-caged oligonucleotide remained untouched during all previous uncaging procedures in the series of Figure 3 (see also again Supplementary Table 2).

Table 1. Photochemical Properties of the Investigated Oligonucleotides 5′-GCA TAA AXA AAG GTG-3′ in PBS Buffer (pH = 7.4)[a]

X	λ	φ	ε [M^{-1} cm^{-1}]	$\varepsilon \cdot \varphi$ [M^{-1} cm^{-1}]
dT^{DEACM}	405	0.014	17 550	247
dC^{NDBF}	365	0.100	9208	971
dT^{NPP}	365	0.229	513	118
dT^{NpHP}	300	0.001	14 480	11

[a] The molar extinction coefficient is given for the wavelength indicated in the second column.

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 TYT TTC CAC-5′			
X	Y	T_M [°C]	ΔT_M [°C]
dT	dA	48.9	–
dT^{DEACM}	dA	42.8	6.1
dT^{NPP}	dA	39.3	9.6
dT^{NpHP}	dA	39.4	9.5

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

However, not only the uncaging efficiency is of importance but also the extent by which a new caged residue destabilized DNA duplex formation. This was assessed by comparing melting temperatures for the different strands used in this study (Table 2). It can be seen that with a ΔT_M of 9.5 °C the new residue dT^{NpHP} shows a duplex destabilization which is as good as the best nucleobase-caged thymidine residues known so far (for a more complete survey see ref 13).

The question remains whether irradiation at 313 nm is a viable method in light of the possible photodamage of the DNA to be uncaged. Out of the possible photodamage[14] to

(14) Mouret, S.; Baudouin, C.; Charveron, M.; Favier, A.; Cadet, J.; Douki, T. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 13765.

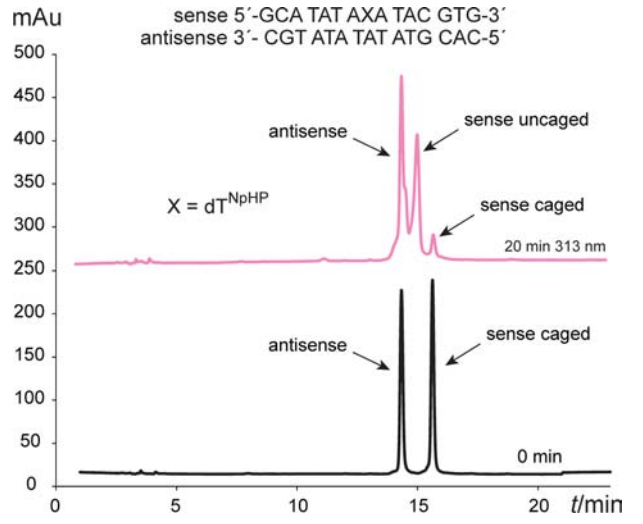


Figure 4. HPLC chromatograms before and after 20 min of irradiation at 313 nm of the indicated caged DNA double strand.

DNA the formation of T < > T dimers occurs most easily. Indeed, if the dT^{NpHP} residue was introduced into the counterstrand of the oligonucleotide of the one used in Figure 3, significant photodamage after 10 min of irradiation of this single strand was found (Supplementary Figure 4). However, by adjusting the sequence and omitting adjacent T residues this can be easily avoided. Figure 4 shows the result of an uncaging experiment of a DNA duplex of modified sequence. It can be seen that even after 20 min of uncaging only the single strands are obtained.

In summary, we have shown for the first time that four levels of wavelength selectivity in the uncaging of DNA are possible using a set of four caging groups with distinct absorption properties. In this process we have introduced the new residue dT^{DEACM} for comparison reasons.

We realized that the sequence of the oligonucleotides might need adjustment to avoid unintended photoreactions. This maximum number of four layers of uncaging was also the result of a different study by del Campo et al. in which functional groups on glass surfaces were released by uncaging at wavelengths between 275 and 435 nm.[8] Also due to the fact that at 313 nm other biomolecules can undergo photoreactions we believe that this new caged residue is probably less suitable for intracellular applications but rather has its justification in the application for example in light-triggering of DNA nanoarchitectures or DNA-based sensors where the sequences can be chosen more freely. However, it is potentially possible to realize 16 different hybridization scenarios with appropriate counterstrands in different locations on a surface in successive uncaging and hybridization steps.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft (HE 4597/3-1).

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.

The authors declare no competing financial interest.