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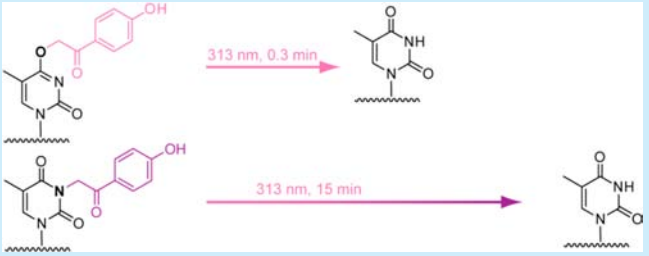
Selective Uncaging of DNA through Reaction Rate Selectivity

Alexandre Rodrigues-Correia, Diana Knapp-Bühle, Joachim W. Engels,* and Alexander Heckel*

Institute for Organic Chemistry and Chemical Biology, Goethe-University Frankfurt, Max-von-Laue-Strasse 9, 60438 Frankfurt, Germany

Supporting Information

ABSTRACT: The synthesis and use of the new nucleobase-caged nucleotides $\mathbf{dT^{pHP}}$ and $\mathbf{dT^{NDEACM}}$ is reported. Through a combination of time and wavelength selectivity four levels of selective uncaging with only two cages, and only two wavelengths, were obtained. The new residue $\mathbf{dT^{pHP}}$ can be uncaged at 313 nm without the formation of unwanted cyclic pyridine dimers.



The regulation of molecular processes with light is a very appealing approach because it allows an unrivaled level of control over the space, time, and extent to which such a process is favored or disfavored. The many commercially available light sources and established microscopy or endoscopy techniques add to the versatility of this principle. Possible ways of implementation include the two disciplines optogenetics and optochemical biology. The former, for example, uses genetically expressible light-sensitive ion channels, while the latter chemically introduces photolabile groups or reversible photoswitches to control the activity of diverse molecules.[1] The removal of photolabile groups leading to a change in activity is referred to as uncaging and is inherently irreversible but conceptually easy to implement. It often leads to excellent ON/OFF ratios. The use of photoswitches is often more complex to implement, also due to the fact that no photoswitch has a switching amplitude of 100%, but there are excellent examples documented in the literature.[2−4]

To make the uncaging approach more versatile and to allow more complex light-regulation scenarios, more and more studies deal with the establishment of methods to selectively address several uncaging processes in the same sample. Pioneering work from the group of Bochet coined the expression "chromatic orthogonality"[5a] for a set of caging groups where every individual one can be photolyzed at different wavelengths leaving the others intact, for example, using the kinetic isotope effect.[5b] More recently, Bochet and del Campo have studied the spectral windows for wavelength-selective uncaging using a set of seven caging groups and arrived at up to four different functional levels.[6] Specht and del Campo used the wavelength-selective principle for dual photoresponsive polymers.[7] The group of Ellis-Davies extended the principle of chromatic orthogonality to the domain of two-photon activatable caging groups.[8] Jullien et al. have recently introduced thiocoumarin derivatives for chromatically orthogonal uncaging.[9]

In our own work, we have shown that up to four levels of sequential uncaging for DNA are possible using four photolabile groups (DEACM, NDBF, NPP, and pHP),[10] which could be released sequentially going from 505 nm over 440 and 365 nm to 313 nm in the uncaging process.[11,12] While good selectivities could be obtained with this set, the reaction rate for the uncaging of the pHP-containing residue of this previous study ($\mathbf{dT^{NpHP}}$, see Figure 1) was in such a range that the desired photoprocess,

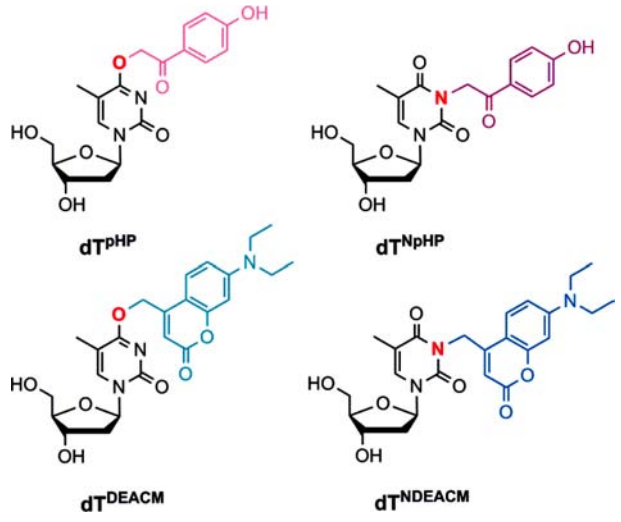


Figure 1. Caged nucleosides used in this study.

i.e., the uncaging, competed with the unwanted formation of cyclic pyridine dimers (CPDs), a known photodamage reaction.[13] Not all of these possible [2 + 2] cycloadditions are as likely to occur, but for example, the predominant ones are the formation of T<>T and T<>C dimers.[14] Hence, this approach was only applicable for sequences in which these motifs were avoided.

In the present study, we propose to solve two problems: with the introduction of the new residue $\mathbf{dT^{pHP}}$ the uncaging process

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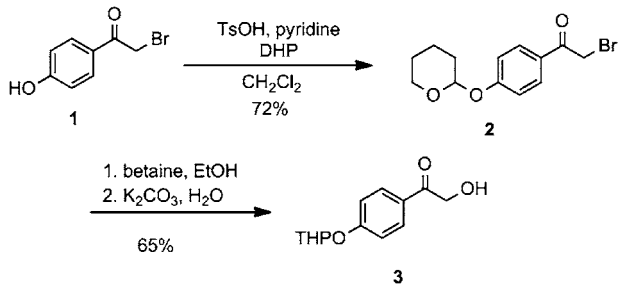
at 313 nm is greatly accelerated so that it happens much faster than the formation of CPDs, and we also propose using reaction rate differences to obtain selective uncaging since the use of wavelength selectivity seems to be close to reaching the currently possible limit of diversification. Figure 1 gives an overview of the residues used in this study. Each of the two caging groups pHP and DEACM was attached to either the N^3- or O^4-position of dT.

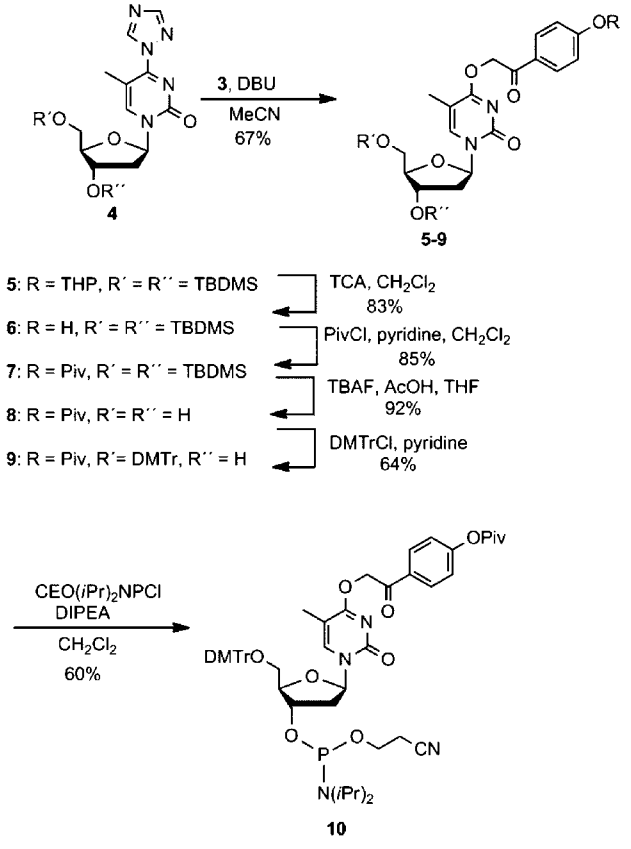
For the incorporation of a $\mathbf{dT^{pHP}}$ residue in an oligonucleotide solid-phase synthesis, we started from the known compound **1** which was protected with a tetrahydropyranyl (THP) group (→ **2**). Then the protected caging group precursor **3** was synthesized via hydrolysis of compound **2** using betaine (Scheme 1). Alcohol

Scheme 1. Synthesis of Protected pHP 3



3 was reacted with the TBDMS-protected triazole-activated deoxythymidine **4** (→ **5**, Scheme 2). Deprotection of the THP group with trichloroacetic acid (TCA, → **6**) and protection of the

Scheme 2. Synthesis of $\mathbf{dT^{pHP}}$ Phosphoramidite 10 for the Introduction of $\mathbf{dT^{pHP}}$ Residues in a DNA Solid-Phase Synthesis



liberated phenol with pivaloyl chloride afforded compound **7**. Silyl group deprotection with TBAF (→ **8**), followed by protection of 5′-OH with DMTr-Cl (→ **9**) and phosphitylation of the 3′-OH with 2-(cyanoethoxy)-*N*,*N*-diisopropylaminochlorophosphine ($CEO(i\text{-}Pr)_2NPCl$) afforded the fully protected caged phosphoramidite **10**. The synthesis of the phosphoramidite to incorporate a $\mathbf{dT^{NDEACM}}$ residue is described in the Supporting Information. The two other phosphoramidites for the incorporation of $\mathbf{dT^{NpHP}}$ and $\mathbf{dT^{DEACM}}$ residues were obtained as previously described.[10]

The building blocks $\mathbf{dT^{NpHP}}$, $\mathbf{dT^{NDEACM}}$, and $\mathbf{dT^{DEACM}}$ were incorporated into oligonucleotides by solid phase synthesis using standard coupling protocols. The sequences are shown for example in Figure 2. In the case of the building block $\mathbf{dT^{pHP}}$ it was

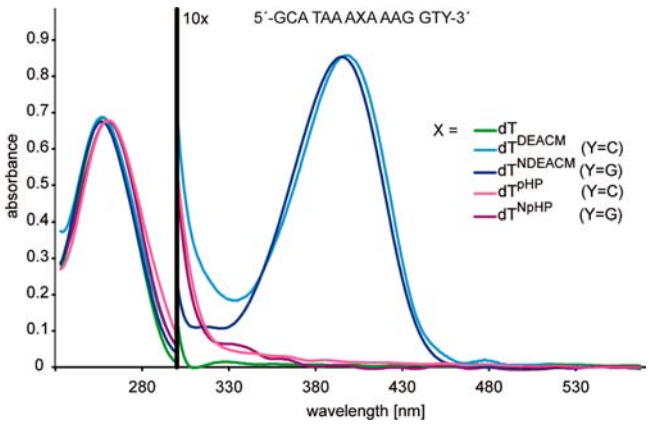


Figure 2. UV/vis spectra of the indicated DNA 15-mer strands in PBS buffer. The wavelength region above 300 nm is scaled 10×.

necessary to use different conditions for the synthesis of the oligonucleotide because pHP covalently bound to the O^4 position was not stable even using ultramild basic workup at the end of the solid-phase synthesis. Here, we applied the base-free solid-phase synthesis strategy of Sekine in which *N*-unprotected phosphoramidites are used.[15] The solid support contained a silyl linker which could be cleaved with $Et_3N{\cdot}3HF$. Using this protocol, it was possible to obtain an oligonucleotide with a $\mathbf{dT^{pHP}}$ residue with good yields. For further details, we refer to the Supporting Information.

In the case of a middle $\mathbf{dT^{pHP}}$ residue the 3′-end was a dC residue. This was due to the fact that the special solid support for the Sekine strategy could only be obtained with a dC residue. For comparison reasons, the O^4-caged $\mathbf{dT^{DEACM}}$-containing sequence was also prepared with a terminal dC, while the other two sequences contained a terminal dG as in our previous work.[11,12]

Figure 2 gives an overview of the UV/vis absorption spectra of all oligonucleotides used in this study. As expected, it can be seen that there is no significant difference to the absorption spectra if a cage is covalently bound to the N^3 or O^4 position of deoxythymidine.

Figure 3 shows HPLC traces of a mixture of the four caged oligonucleotides indicated in Figure 2 with and without irradiation. The left-most peak results from uracil, which was used as an internal standard. It turned out that under our conditions the N^3-caged sequences eluted earlier than the O^4-caged ones and the pHP-containing ones earlier than the DEACM-containing ones. At first, upon irradiation at 420 nm the $\mathbf{dT^{DEACM}}$-containing oligonucleotide was cleanly uncaged in 1

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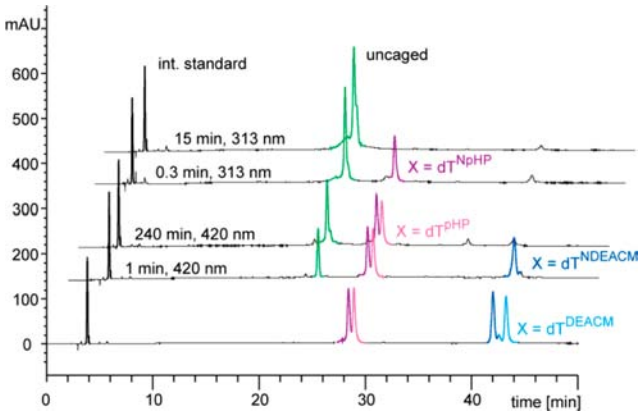

Figure 3. HPLC chromatograms before and after irradiation at different times and wavelengths of four caged oligonucleotides and uracil as internal standard. (70 μL, 300 pmol, PBS buffer pH 7.4) The labeled peaks result from the oligonucleotides with the indicated central residues.

min leaving the other caged oligonucleotides unreacted within error limits (see the Supporting Information). For the deprotection of the **dT**^{NDEACM}-containing oligonucleotide an extended period of time at the same wavelength as before (420 nm) was needed. In this uncaging step, >90% of the **dT**^{pHP}-containing oligonucleotide remained intact while the **dT**^{NpHP}-containing one remained unreacted within error limits. The durations of irradiation required in this case will certainly not allow triggering kinetic events but will be useful for addressing space and dose. In the next step, the uncaging wavelength was changed to 313 nm. Again the new O^4-caged residue **dT**^{pHP} was uncaged significantly faster than the previously introduced N^3-caged **dT**^{NpHP} residue, which remained intact to more than 90%. Continuing to irradiate at 313 nm led to the uncaging of the remaining caged oligonucleotide. Thus, we were able to obtain four levels of sequential uncaging using only two caging groups and a mixture of time- and wavelength-selective uncaging.

From the results of the HPLC studies we determined the respective uncaging quantum yields. The results are summarized in Table 1. They also reflect the significant difference in uncaging

Table 1. Photochemical Properties (Uncaging Quantum Yield ϕ and Molar Extinction Coefficient ε) of the Caged Oligonucleotides of Figure 2 at the Indicated Wavelength λ in PBS Buffer (pH = 7.4)

X	λ (nm)	ϕ	ε (M^{-1} cm^{-1})	$\varepsilon\phi$ (M^{-1} cm^{-1})
dT^{pHP}	313	0.016	21290	330
dT^{NpHP}	300	0.001	14480	11
dT^{DEACM}	405	0.014	17550	247
dT^{NDEACM}	365	6×10^{-5}	10689	0.7
dT^{NDEACM}	313	7×10^{-5}	10345	0.7

an oxygen position compared to uncaging a nitrogen position with ratios of the uncaging efficiency ($\varepsilon\phi$) of ~30 for pHP and ~350 for DEACM.

We were wondering if the set of caged oligonucleotides used in this study could also be used for orthogonal uncaging. In our previous studies,[11,12] this was not possible because in the caging group series DEACM, NDBF, NPP, and pHP uncaging of any group also led to uncaging of all preceding groups in the indicated order. This allowed only for sequential wavelength-selective uncaging.

Figure 4 shows, however, that with **dT**^{pHP} and **dT**^{NDEACM} it was possible for the first time to establish orthogonal uncaging of

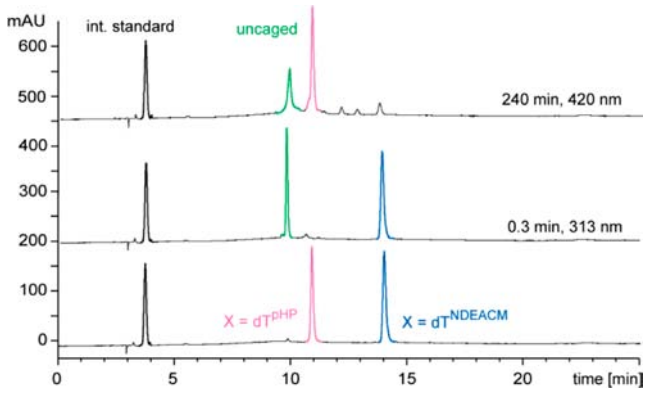

Figure 4. HPLC chromatograms before and after irradiation at different wavelengths using uracil as internal standard (70 μL, 300 pmol, PBS buffer pH 7.4). The labeled peaks result from the oligonucleotides with the indicated central residues.

oligonucleotides because upon irradiation at 420 nm the **dT**^{NDEACM}-containing oligonucleotide was cleanly uncaged leaving 95% of the **dT**^{pHP}-containing oligonucleotide intact, whereas at 313 nm the **dT**^{pHP} residue was quantitatively uncaged leaving 99% of the **dT**^{NDEACM} residue intact. Again, this could only be realized using the extended irradiation period necessary for uncaging of the **dT**^{NDEACM} residue which will not allow for triggering kinetic experiments.

As important as the uncaging properties are the properties in the destabilization of duplex formation of the caged residues. These can be assessed, for example, by measuring the melting temperatures of the duplexes. Table 2 gives an overview of the

Table 2. Comparison of the Melting Temperatures of the Investigated Oligonucleotides Containing Caged Residues. The concentration of the duplex was 1 μM in PBS buffer

5′-GCA TAA AXA AAG GTY-3′ 3′-CGT ATT TAT TTC CAZ-5′		
X	T_M (°C)	ΔT_M (°C)
dT (Y = G, Z = C)	48.9	
dT (Y = C, Z = G)	48.9	
dT^{pHP} (Y = C, Z = G)	39.8	9.1
dT^{NpHP} (Y = G, Z = C)	39.4	9.5
dT^{DEACM} (Y = C, Z = G)	42.8	6.1
dT^{NDEACM} (Y = G, Z = C)	35.4	13.5

results we obtained. In comparison to our previous results, thermal destabilization by ca. 10 °C (as obtained with both pHP-containing residues) is very good while the measured 13.5 °C in the case of X = **dT**^{NDEACM} is among the best destabilization values ever obtained in our studies.[16] Interestingly, in this case there was a significant difference between placing the DEACM group at the O^4 or at the N^3 of deoxythymidine.

As mentioned above, in our previous study we found that the uncaging of the **dT**^{NpHP} residue happened at a reaction rate that was similar to the formation of CPDs at the required uncaging wavelength of 313 nm which limited the application to certain sequences in which T<>T and C<>T dimers had to be avoided (not all CPDs form at the same reaction rate[14]). Figure 5 shows that the reaction rate for uncaging of the new **dT**^{pHP} residue is now enhanced so that the formation even of the most easily

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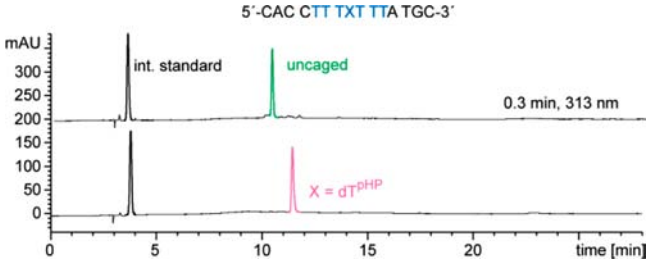

Figure 5. HPLC chromatograms before and after irradiation at 313 nm of the indicated sequence and uracil as internal standard (70 μL, 200 pmol, PBS buffer pH 7.4).[17]

formed T<>T dimers are no longer an issue: the additional caged oligonucleotide whose sequence is shown in Figure 5, containing several sequential deoxythymidines, was cleanly uncaged without the formation of [2 + 2] photoproducts.[17]

In conclusion, we have presented two new nucleobase-caged residues **dT^{pHP}** and **dT^{NDEACM}**. While the latter can be used with regular solid-phase synthesis protocols for the former, the base-free and protecting group-free protocols of Sekine had to be used. Both residues show significantly different uncaging reaction rates compared to their respective O^4- and N^3-caged counterparts. This made it possible to obtain four levels of sequential uncaging in the same reaction solution using only two caging groups and irradiating at two wavelengths for different amounts of time. The new **dT^{pHP}** residue can now be quantitatively uncaged at 313 nm at reaction times which do not lead to undesired [2 + 2] cycloaddition side products. Using the two residues **dT^{pHP}** and **dT^{NDEACM}**, it was even possible to orthogonally uncage the one or the other, albeit at the expense of losing time-resolution due to the long uncaging times needed for the latter residue. These new methods will increase the versatility of the tool set of nucleobase-caged oligonucleotides and will enable more complex light-regulation scenarios in the future. Future experiments will show if this approach can be used for biological applications.

■ ASSOCIATED CONTENT

Supporting Information

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.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: joachim.engels@chemie.uni-frankfurt.de.
*E-mail: heckel@uni-frankfurt.de.

Notes

The authors declare no competing financial interest.

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(10) DEACM = 7-(diethylamino)coumarin-4-yl)methyl, NDBF = NDBF = 1-(3-nitrodibenzofuran-1-yl)ethyl, NPP = 2-(*o*-nitrophenyl) propyl), pHP = *p*-hydroxyphenacyl

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(17) See our previous publication (ref 11) for examples for the detection of cyclic pyrimidine dimers by RP-HPLC.