Bioorganic Chemistry

Photoinduced Transcription by Using Temporarily Mismatched Caged Oligonucleotides**

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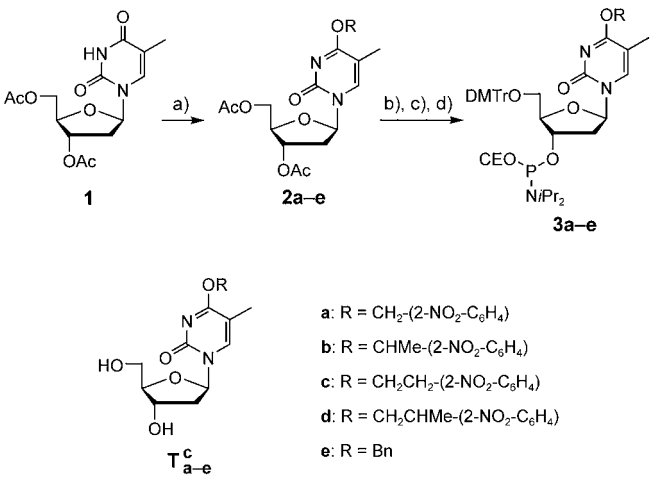
DNA and RNA are responsible for the storage and flow of information in nature. Additionally, and especially together with their analogues and derivatives, DNA and RNA can be used for a multitude of applications, such as regulation of gene expression (RNA interference,[1a] microRNA,[1b] riboswitches,[1c] antisense approach,[1d] DNAzymes[1e]), modulation of protein function (aptamers,[1f] DNA/RNA decoys[1g]), molecular diagnostics (microarrays[1h]), or as structural or functional building blocks for nanoscale material.[1i] In all of these applications the nucleobases play a central role. To develop a mechanism for the temporal and spatial control of the DNA or RNA function, we are investigating ways to temporarily block the recognition properties of the nucleobases. Since light has the advantage of being a highly orthogonal trigger signal we decided to use photolabile "protecting" groups for this purpose. The regulation of gene expression by light, for example, would be a valuable tool for the analysis of protein function. The strategy of masking the activity of a biological compound with a photolabile protecting group is called "caging" and, for example, caged neurotransmitters or Ca^{2+}-complexing agents have already been synthesized and investigated.[2] Likewise, in a pioneering investigation, adenosine triphosphate (ATP) caged with a photolabile 1-(2-nitrophenyl)ethyl group was used to study the Na/K-pump in human red blood cell ghosts.[3]

Herein, we report the synthesis of caged thymidine phosphoramidites and the use of caged oligonucleotides to control transcription by light. Photolabile protecting groups have been used before in oligonucleotide synthesis as temporary protecting groups[4] and photolabile protecting groups in the 2′-position in RNA can also inhibit ribozyme action[4b,5] but they do not prevent Watson–Crick base pairing. Also, nucleoside analogues and derivatives have been made which induce DNA strand breaks upon irradiation.[6]

The exocyclic O4 position of thymidine was chosen for the attachment of the photolabile group (caging group; Scheme 1). The O4-substituted derivatives of thymidine are conveniently accessible by the reaction of the suitably protected precursor **1** with triisopropylbenzenesulfonyl chloride and then with the respective alcohols of the caging groups (→**2a–e**). As photolabile protecting groups the commonly used 2-nitrobenzyl group and its faster deprotecting α-methyl-substituted derivative as well as the 2-nitrophenethyl group and its α-methyl-substituted derivative were chosen. The 2-nitrophenethyl group has been used by Pfleiderer et al.[7] and has the advantage of not forming a nitroso compound upon deprotection[8], a property that is important for cellular and in vivo studies. The benzylated derivative **2e** and its subsequent derivatives were also included in these studies as control compounds which are not deprotected by light. Deacetylation and DMTr-protection of the 5′-position afforded the protected 3′-OH-free intermediates which were converted under standard conditions into the phosphoramidites **3a–e**.



Scheme 1. Synthesis of the photoactivatable caged phosphoramidites **3a–e** as well the caged nucleosides $\mathbf{T^c_{a-e}}$. Reaction conditions: a) 1. *i*Pr$_3$C$_6$H$_2$SO$_2$Cl, DMAP, Et$_3$N; 2. respective alcohol, Et$_3$N; b) NH$_3$/MeOH (quant.); c) DMTrCl, pyridine; d) CEOP(Cl)N*i*Pr$_2$, DIEA. DMAP = N,N-dimethyl-aminopyridine, DMTr = dimethoxytrityl, DIEA = diisopropylethylamine, CE = (2-cyanoethyl), Bn = benzyl.

To investigate the duplex-destabilizing effect of the caged thymidine monomers $\mathbf{T^c_{a-e}}$ (Scheme 1; **c** = caged) they were included in 25-mer-oligodeoxynucleotides in the positions indicated in Table 1 and thermal denaturation experiments were performed using dA_{25} as the counter strand. As the results in Table 1 show, the introduction of just one caged nucleotide leads to a significant reduction in the melting temperature (up to 5 °C). This effect can be increased if more

Table 1: Results of thermal denaturation experiments of the given oligonucleotides with dA_{25} as counter strand.[a]

X	5′-T$_8$TTTTXTTTTT$_8$–3′	5′-T$_8$TTTXXXTTTT$_8$-3′	5′-T$_8$XTTTXTTTXT$_8$-3′
$\mathbf{T^c_a}$	50.3 (51.1)	47.3 (46.9)	41.2 (44.3)
$\mathbf{T^c_b}$	48.6 (53.2)	44.2 (51.1)	32.3 (51.9)
$\mathbf{T^c_c}$	48.9 (53.0)	44.9 (51.7)	34.7 (51.8)
$\mathbf{T^c_d}$	48.5 (54.0)	44.5 (52.7)	32.3 (53.2)
$\mathbf{T^c_e}$			33.8 (34.2)

[a] Melting temperatures in °C before and (in parenthesis) after irradiation of the sample at 366 nm for 30 min (error ca. 0.7 °C). With unmodified T_{25} a melting temperature of 53.8 (54.0) °C was obtained.

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caged nucleotides are included, especially if they are not cumulated around one position within the oligonucleotide. Branched caging groups result in a slightly increased destabilization of the duplex compared to their nonbranched analogues (see for example the entries for the oligomers containing $\mathbf{T}^{c}_{a}$ und $\mathbf{T}^{c}_{b}$). With the incorporation of only three caged nucleotides a maximum decrease in melting temperature of 21.5 °C was obtained. In a second series of experiments the melting temperatures were determined after irradiation of the samples for 30 min at 366 nm. As can be seen in Table 1, only the oligonucleotides with the caged $\mathbf{T}^{c}_{d}$ showed the same melting temperature, after irradiation, as the unmodified T_{25} oligomer used as control compound. These findings suggest that the caged nucleotides can be thought of as being "temporary mismatches". Including the benzylated thymidine $\mathbf{T}^{c}_{e}$ in an oligonucleotide leads to a decrease in melting temperature which remained unchanged upon irradiation with light.

To study the deprotection kinetics 4 nmol of each of the oligonucleotides shown in Table 1 was irradiated at 366 nm and the formation of the resulting uncaged T_{25} was detected by HPLC (Figure 1). As can be seen, in the case of the

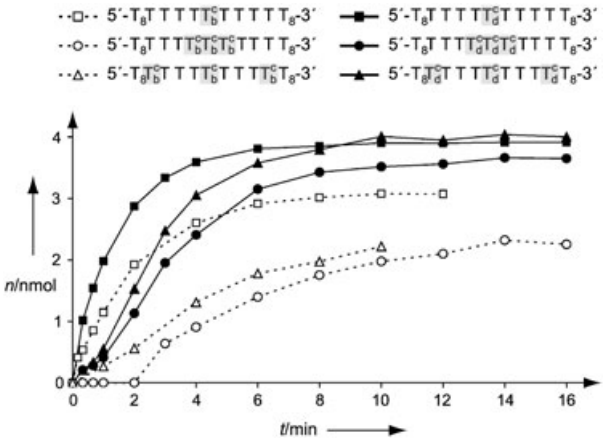

Figure 1. Amount of uncaged oligonucleotide during the photodeprotection (366 nm). In every experiment 4 nmol of the caged oligonucleotide was used and the product formation was detected by HPLC.

photodeprotection of the oligomers containing the caged $\mathbf{T}^{c}_{b}$ the reaction reached a stationary state in which, as well as the product, an as yet unidentified byproduct was present which did not react further upon irradiation. In the case of the oligomers with the caged $\mathbf{T}^{c}_{d}$ however, the deprotection was not only faster under the irradiation conditions used but the yield of resulting uncaged oligonucleotide was almost quantitative within error limits. The oligomers with the caged $\mathbf{T}^{c}_{a}$ and $\mathbf{T}^{c}_{c}$ were also subjected to this test. However, especially with several caged nucleotides in the same oligomer, the deprotection was very slow and the yields were poor. In conclusion, the caged $\mathbf{T}^{c}_{d}$ showed the best properties with regard to perturbation of duplex structure and ease of photodeprotection.

To studying the use of caged photoactivatable nucleotides in biological systems we tried to temporarily block transcription (Figure 2). An assay was used in which a 68-nucleotide (nt) antisense strand with a T7-promoter served as the template. The T7-promoter region was complemented with the shorter oligonucleotides $\mathbf{S_1}$–$\mathbf{S_6}$ to for a double strand

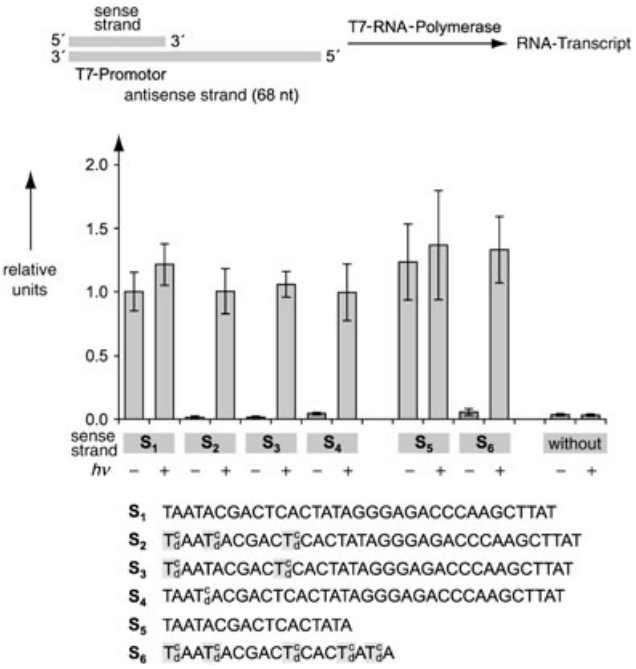

Figure 2. Relative amounts of transcription product formed after 1 h with or without initial irradiation (1 h, 366 nm) of the sample before starting the transcription by addition of the T7 RNA polymerase.

and allow the T7 RNA polymerase to recognize the promoter region. The amount of transcription product after 1 h of incubation was determined by phosphorimaging (α-^{32}P-guanosine triphosphate (GTP) was used for body labeling). The three oligonucleotides $\mathbf{S_2}$, $\mathbf{S_3}$, and $\mathbf{S_4}$ contain, respectively, three, two, and one caged nucleotide in the T7 promoter region. Unlike with the control oligonucleotide $\mathbf{S_1}$, in the presence of these unmodified oligonucleotides no transcription takes place. This result is not because the two DNA-strands in the experiment are not associated to one another since, for example, $\mathbf{S_4}$ and the antisense strand show a melting temperature of 72.0 °C in the transcription buffer. It must rather be due to a severe perturbation of the local duplex structure owing to the caging group which prevents the RNA polymerase from recognizing the promoter region.[9] After irradiation of the respective samples (before the addition of the T7 RNA polymerase) the same amount of transcription product was formed as in the control reaction with the uncaged oligomer $\mathbf{S_1}$. Transcription could also be triggered by irradiating the samples containing the intact caged sense strands after 1 h of incubation with T7 RNA polymerase in which no product had been formed. The shorter oligomers $\mathbf{S_5}$ and $\mathbf{S_6}$ behaved similarly.[10] However, the results shown in Figure 2 demonstrate that it is not necessary to heavily destabilize the entire duplex to prevent transcription and that the incorporation of one or two caged nucleotides is quite enough.

Other groups have also studied the regulation of gene expression by light.[11] However in their approaches entire plasmids or mRNA strands were subjected to benzylating conditions in which the photolabile groups were attached presumably to backbone phosphates. In contrast to the strategy presented herein, this approach does not lead to a well-defined product but rather to a statistical distribution of caged positions and introduces more modifications than necessary. In the other investigations the full transcriptional activity could not be restored.

In conclusion we have demonstrated that caged thymidines can be used as temporary mismatches to inhibit a function, transcription in this case. They can be introduced in oligomers at any desired and well-defined position through their easily accessible phosphoramidites **3a**–**e**. With long-wavelength UV light (366 nm) the photodeprotection of the caged $\mathbf{T}^{\mathbf{c}}_{\mathbf{d}}$ occurs fast, with high yield, and without formation of nitroso compounds. One or two caged nucleotides at the right position can be enough to completely inhibit a function.

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