DNA Photoswitches

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Nucleoside-Based Diarylethene Photoswitches and Their Facile Incorporation into Photoswitchable DNA**

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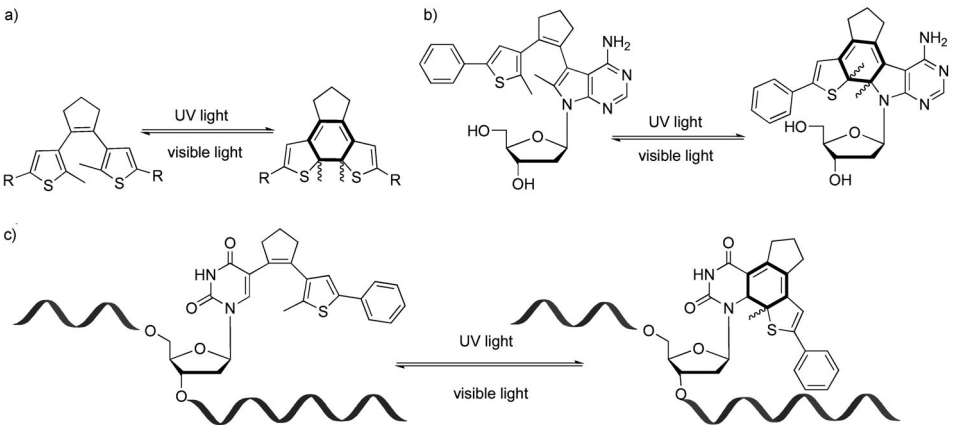
The artificial control of DNA structure and function is an attractive field in chemical and synthetic biology,[1] and light is a powerful and convenient trigger: it is non-invasive, provides high spatio-temporal resolution, and offers the option of orthogonality. DNA is reactive to light: the UV-light-induced cyclodimerization of pyrimidine nucleosides is an important type of DNA damage and has been studied intensively.[2] Interestingly, this chemical property has never been exploited for the construction of DNA-based reversible photoswitches, and all published work in this field is based on the covalent functionalization of DNA with small autonomous photoactive molecules.[3] Different classes of such photoactive molecules have been studied for DNA photoregulation;[4] azobenzenes have been used most frequently and were, for example, employed for modulating oligonucleotide duplex and triplex formation and DNA transcription.[5] Other substance classes studied in this context include arylvinyl derivatives,[6] spiropyranes,[3a] and recently also diarylethenes.[3a,7] While a certain influence of photoisomerization on the properties of the nucleic acid was always observed, these approaches share one common limitation: because an autonomous photoswitch was attached to the DNA, either as an appendage or substituting for nucleosides, the rearrangement of chemical bonds upon encountering a photon (i.e., the photochemical reaction) was strictly confined to this non-nucleosidic moiety.



Scheme 1. a) Isomerization of typical diarylethene photoswitches. b) A diarylethene photoswitch involving 7-deazadeoxyadenosine.[8] c) New photoswitchable DNA where a pyrimidine nucleotide is part of the photoswitch.

Recently, our lab reported a new type of diarylethene photoswitches in which one of the two aryl moieties was replaced by a nucleoside, namely 7-deaza-8-methyldeoxyadenosine (Scheme 1).[8] These photoswitches were synthesized in a convergent multi-step approach in which a substituted cyclopentenyl boronic ester was reacted with protected 7-iodo-8-methyl-7-deazadeoxyadenosine by Suzuki cross-coupling, followed by deprotection. Upon irradiation with light, these compounds were found to undergo a highly efficient, reversible, electrocyclic rearrangement and the switching wavelength could be tuned by the chemical nature of substituents. Switching was found to be near-quantitative in aprotic solvents, and the compounds retained the key properties of nucleotides, such as their capability to form Watson–Crick base-pairs. Unfortunately, the photoisomerization was found to proceed with low efficiency in aqueous solvents, and the demanding synthesis involved limited the application of these photoswitches to oligonucleotides.

To develop straight-forward access to truly photoswitchable DNA, we reconsidered our design approach; in contrast to 7-iodo-8-methyl-7-deazadeoxyadenosine, the 5-iodo-substituted pyrimidine nucleosides 5I-dU and 5I-dC represent oligonucleotide modifications readily available from commercial suppliers, and offer the desired reactivity for different cross-coupling reactions.[9] This could allow for the postsynthetic conversion of an iodo-modified oligonucleotide into a photoswitch, leading to the target compounds shown in Scheme 1c. We note that this design challenges some of the common design principles for diarylethene photoswitches:[10] neither unsubstituted nor substituted pyrimidines have ever been applied as components of diarylethene photoswitches, and our target compounds contain just one (rather than two) alkyl groups attached to the carbon atoms that form the new bond in the cyclization reaction, a feature that is thought to be important for the reversibility of photoswitching. This design was first tested on the nucleoside level employing 5-iodo-

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deoxyuridine and 5-iododeoxycytidine. The reaction of these compounds with 2-[2-methyl-5-phenylthien-3-yl]cyclopent-1-ene boronic acid pinacol ester,[8] under Suzuki–Miyaura cross-coupling conditions that were previously optimized,[11] gave the corresponding products in yields of 44–61% (Figure 1a).

The photochromic behavior of these compounds was observed on TLC plates under UV light during the preparation. Moreover, an intense orange to red color change occurred upon UV irradiation in chloroform. To test their performance in polar protic solvents, the compounds were dissolved to a concentration of 300 μM in water/ethanol 9:1, and irradiated at 254 nm. A rapid photochromic reaction took place in this solvent system, too, yielding a change from colorless to orange (**dU^{PS}**) or red (**dC^{PS}**; Figure 1a).

Because irradiation by 254 nm UV light is known to cause DNA damage, we tested whether irradiation at 366 nm would also induce the photochromic reaction, which turned out to be the case, and all further measurements were performed with irradiation at 366 nm. The UV absorption spectra of the closed-ring forms of **dU^{PS}** and **dC^{PS}** showed new maxima at 470 nm and 507 nm, respectively (Figure 1b; Supporting Information, Figure S1). In 30 μM solution, the photostationary state was reached after five minutes of irradiation (UV hand lamp, 8 W, 6 cm distance), and prolonging the irradiation did not lead to further spectral changes. The ring-opening reverse reaction occurred more rapidly and was complete after two minutes of irradiation by visible light (Intralux-600-1 lamp, low intensity light, 150 W, 6 cm distance). The process was highly reversible and consistent switching behavior was verified over seven cycles of irradiation of 60 μM solutions by UV light for ten minutes and visible light for ten minutes (Figure 1c, Figure S1b). For any practical application, it is important that the photoisomers are thermally stable and do not revert over time without irradiation. Surprisingly, we found a significant difference in the thermal stability between **dU^{PS}** and **dC^{PS}**. Although **dU^{PS}**

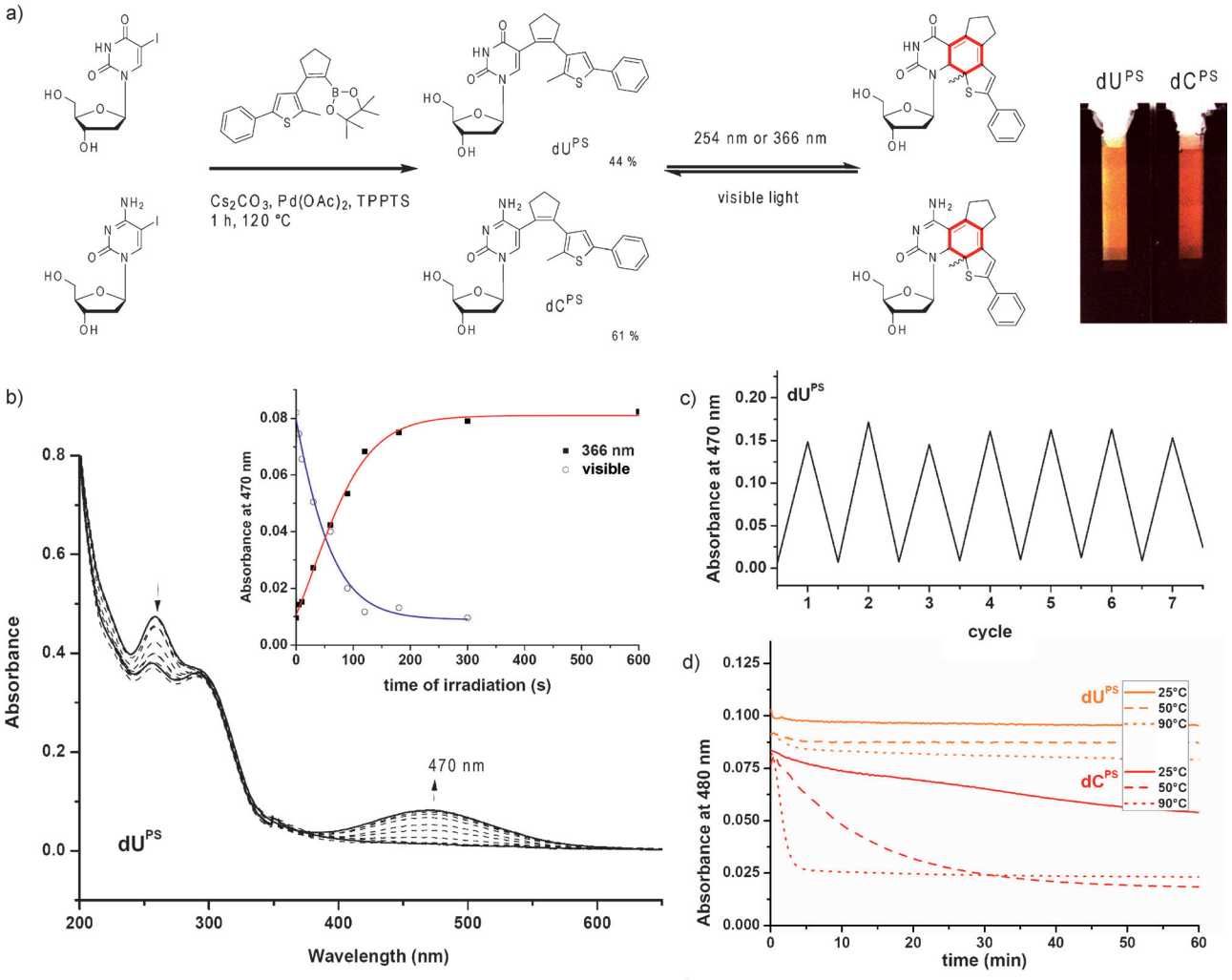


Figure 1. a) Aqueous Suzuki–Miyaura cross-coupling of 5-iododeoxyuridine and 5-iododeoxycytidine with boronic acid pinacol ester and photoisomerization reaction of the nucleosides **dU^{PS}** and **dC^{PS}**. TPPTS = triphenylphosphine-3,3′,3″-trisulfonic acid trisodium salt. b) Absorption spectrum of a **dU^{PS}** solution after irradiation by 366 nm UV light (band at 470 nm increases) and subsequent irradiation with visible light (band at 470 nm decreases). Inset: time trace at the absorbance maximum. c) Reversibility of the photoisomerization measured in a solution of **dU^{PS}** (60 μM) over seven cycles of 10 min irradiation with 366 nm and 10 min with visible light. d) Thermal stability of the closed-ring form measured in solutions of **dU^{PS}** and **dC^{PS}** (30 μM) over 60 min at 25 °C, 50 °C, and 90 °C.

showed high stability and displayed very little degradation even when stored for 60 minutes at 90 °C, **dC^{PS}** reverted to the open-ring form rapidly already at room temperature (Figure 1 d).

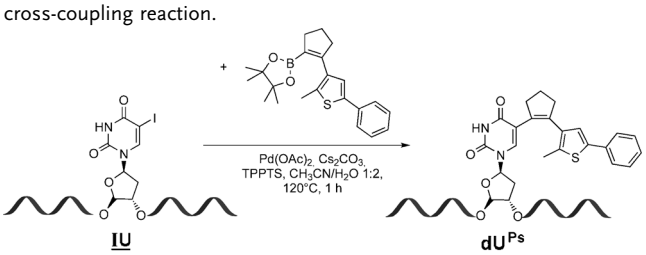
By combining ^{1}H NMR spectroscopy and UV/Vis spectrometry, the photostationary states (i.e., the amount of opened- and closed-ring forms) after UV irradiation were determined to contain 83 % closed-ring isomer for **dU^{PS}** (Figure S2) and 98 % for **dC^{PS}** (Figure S3). The ring-opening reaction upon visible light irradiation was found to be quantitative (less than 0.5 % closed-ring form, Figure S4).

After demonstrating the function of these photoswitches at the nucleoside level, the next task was to develop a synthetic route to photoswitch-modified DNA, preferably also using Suzuki cross-coupling, because this would allow for the use of the same intermediates. To our knowledge, there is only one example of the Suzuki cross-coupling of oligonucleotides, namely the reaction of short (2-mer to 15-mer) 8-Br-dG-modified oligonucleotides with various phenyl boronic acids and one benzothiophene boronic acid.[12] Neither Suzuki cross-coupling of other halogenated oligonucleotides nor the attachment of more sterically demanding boronic acids has been reported. Application of the conditions elaborated by Omumi et al. (70 °C for 24 hours with Na_2CO_3 as a base in aqueous solution)[12] to our problem (5-iodinated pyrimidine oligonucleotides, combined with sterically demanding boronic acid esters) however, did not yield detectable amounts of product. We therefore tested conditions developed for the derivatization of sensitive nucleoside triphosphates with a variety of boronic acids ($CsCO_3$, water/acetonitrile mixture, argon atmosphere, 120 °C, 60 minutes).[13] The reactions were performed on a seven nanomole scale and the products immediately purified by HPLC (Figures S5,S6). Nine different modified oligonucleotides (15- and 19-mers, Table 1; Table S1) bearing one or two photoswitchable groups were prepared by this method and the yields of the isolated products were between 16 % and 35 %. Cross-coupling at terminal positions (3′ or 5′) was generally more efficient than at internal ones. All products were characterized by ESI mass spectrometry (Figures S7,S8). Dehalogenation is known to be the major side reaction under conditions of steric hindrance,[14] and we could confirm for several oligonucleotides that the major undesired product was indeed the dehalogenated oligonucleotide (Table 1).

The photoswitch-modified oligonucleotides were then tested for photochromicity. Solutions of single-stranded oligonucleotides **15mer-dU^{PS}1**–**15mer-dC^{PS}3** (25 μM) were irradiated for five minutes at 366 nm, and then their UV/Vis spectra were measured (Figure 2). All prepared oligonucleotides exhibited photoswitching properties. Compared to the free nucleosides, the absorbance maxima of **dU^{PS}** and **dC^{PS}** incorporated into DNA showed a bathochromic shift from 470 nm to 477 nm for **dU^{PS}** and from 507 nm to 540 nm for **dC^{PS}**.

These absorbance maxima did not depend on the oligonucleotide sequence or position of the modification within the oligonucleotide, however, the absolute absorbance was highest when the photoswitch was incorporated at the 5′-end, while it was lower for 3′-terminal incorporation and lowest for internal incorporation, suggesting that the location of the photostationary state after UV irradiation varies with the structural context. As part of a DNA oligonucleotide, the diarylethene chromophore showed highly reversible photoswitching (Figure 2 b) and high thermal stability (Figure 2 c).

Table 1: Sequences of oligonucleotide substrates for Suzuki–Miyaura cross-coupling reaction.



Oligo	Substrate[a]	Yield[b]	Dehalogenation[c]
15mer-dU^{PS}1	5′-AGCAACA**IU**CGATCGG-3′	25 %	
15mer-dC^{PS}1	5′-AGCAACA**IC**CGATCGG-3′	22 %	
15mer-dU^{PS}2	5′-**IU**GCAACATCGATCGG-3′	26 %	
15mer-dC^{PS}2	5′-**IC**GCAACATCGATCGG-3′	34 %	
15mer-dU^{PS}3	5′-AGCAACATCGATCG**IU**-3′	25 %	
15mer-dC^{PS}3	5′-AGCAACATCGATCG**IC**-3′	35 %	
19mer-dU^{PS}1	5′-TCTAATACGACTCAC**IU**ATA-3′	20 %	45 %
19mer-dU^{PS}2	5′-TCTAATACGACTCACTA**IU**A-3′	19 %	48 %
19mer-dU^{PS}3	5′-TCTAATACGACTCAC**IU**A**IU**A-3′	16 %	30 %, 45 %

[a] Substrate of the Suzuki-Miyaura cross-coupling reaction. [b] Yield of the isolated product. [c] Yield of the isolated product of dehalogenation.

Because the **dC^{PS}** photoswitch was found not to be thermally stable in its closed form, we decided to focus our studies on the properties of double-stranded DNA (dsDNA) containing **dU^{PS}** nucleotides. The properties of the modified dsDNA before and after irradiation by 366 nm UV light were compared with those of unmodified DNA. First, the influence of the modification on duplex stability was analyzed by thermal denaturation analysis (Figure S9, Table S2). One internal modification (oligonucleotide **15mer-dU^{PS}1**) caused a decrease in the melting temperature (T_m) of 2.3 °C, both in the opened- and closed-ring form, compared to the unmodified duplex, while 3′- or 5′-terminal modifications were found to have a negligible effect on the stability in the open-ring form. On the other hand, these modifications were found to have a stabilizing effect after ring closure ($\Delta T_m = 0.9$ °C for 5′-terminal and $\Delta T_m = 1.6$ °C for 3′-terminal incorporation). To investigate the effect of the photoswitch modification on the overall helical structure, CD spectra were recorded prior to and following UV irradiation (Figure S9). The spectra were almost identical to unmodified DNA when the modification was terminal. Only when the **dU^{PS}** nucleotide was in center of the dsDNA, an apparent shift to a more A-like (i.e., RNA-like) conformation was observed in comparison with natural

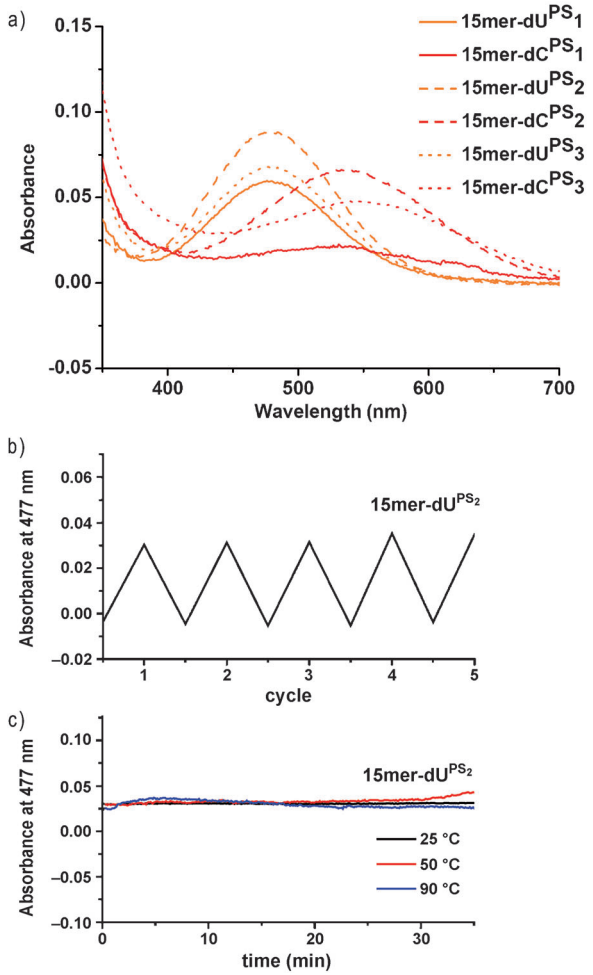


Figure 2. a) UV spectra of single-stranded oligonucleotides **15 mer-dU^{PS}1**–**15 mer-dC^{PS}3** after irradiation by 366 nm UV light for 5 min. b) Reversibility the photoisomerization measured in solutions of **15 mer-dU^{PS}2** (10 μM) over five cycles of 10 min irradiation with 366 nm light and 10 min with visible light. c) Thermal stability of the closed-ring form of **15 mer-dU^{PS}2** (10 μM solution) measured over 35 min at 25 °C, 50 °C, and 90 °C.

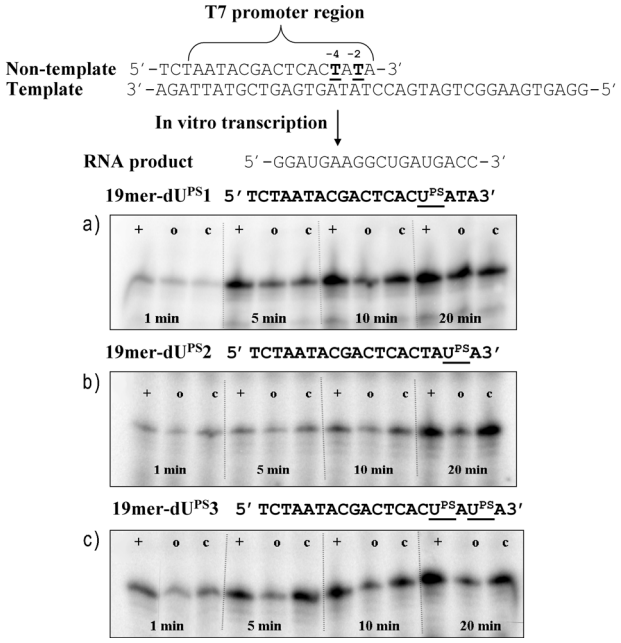


Figure 3. Denaturing polyacrylamide gel electrophoresis of in vitro transcription reactions performed on photoswitch-modified dsDNA a) **19 mer-dU^{PS}1**, b) **19 mer-dU^{PS}2**, or c) **19 mer-dU^{PS}3** without any irradiation, labeled "o", and after 10 min irradiation by 366 nm UV light, labeled "c". Transcription from an unmodified promoter, labeled "+".

DNA. In all cases, the UV-induced DNA conformational changes were small.

Photoregulation of fundamental biological processes like replication or transcription could give new opportunities for cell manipulation or bionanotechnology.[15] To investigate the influence of the diarylethene photoswitch on transcription, we incorporated one or two photoswitch moieties into the T7 promoter region of a partially double-stranded DNA template. Three different modified promoters were prepared with **dU^{PS}** nucleotides in positions −2 and/or −4 of the non-template strand. Each of these constructs was subjected to transcription with or without prior irradiation at 366 nm for ten minutes and compared with transcription from an unmodified template. Aliquots were withdrawn after 1, 5, 10, and 20 minutes and analyzed by polyacrylamide gel electrophoresis (Figure 3). The presence of the bulky photoswitch modification was not found to be detrimental to transcription; in all cases, the polymerase synthesized full-length product. Generally, the photoswitchable promoters in their closed-ring forms gave higher amounts of product compared to the opened-ring form. Clear positional effects could be observed: a single modification at position −4 had only a small effect (Figure 3a), while at position −2 (Figure 3b) and in the doubly modified promoter (Figure 3c) significant inactivation by the open-ring modified DNA was observed, but UV irradiation fully restored activity. A more comprehensive modification analysis could reveal other promoter positions sensitive to photoswitchting and ultimately result in higher switching factors.

We report herein the first example of photoswitchable oligonucleotides in which a structural constituent of DNA itself, namely the nucleobase, is involved in the rearrangement of chemical bonds, changes hybridization, bond order, and geometry, and therefore forms an active part of the photoswitch. The atomic positions involved are exactly the same that take part in the UV-induced cyclodimerization photo-damage reaction of DNA (C5 and C6 of the pyrimidines). A simple one-step method was presented for the conversion of iodo-modified oligonucleotides into highly specific, fully reversible photoswitches based on Suzuki chemistry. This new approach, combined with the well-known acceptance of iodo-modified nucleotides by DNA polymerases[16] and the recent development of mild and biocompatible cross-coupling conditions,[17] may lead to new applications of reversible DNA photoswitches in biology, microscopy, and nanotechnology.

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[1] a) P. W. K. Rothemund, *Nature* **2006**, *440*, 297; b) S. M. Douglas, H. Dietz, T. Liedl, B. Hogberg, F. Graf, W. M. Shih, *Nature* **2009**, *459*, 414; c) D. Y. Zhang, A. J. Turberfield, B. Yurke, E. Winfree, *Science* **2007**, *318*, 1121; d) A. Jäschke, *FEBS Lett.* **2012**, *586*, 2106.

[2] a) S. Kiontke, Y. Geisselbrecht, R. Pokorny, T. Carell, A. Batschauer, L. O. Essen, *EMBO J.* **2011**, *30*, 4437; b) B. D. Zoltowski, A. T. Vaidya, D. Top, J. Widom, M. W. Young, B. R. Crane, *Nature* **2011**, *480*, 396; c) H. W. Park, S. T. Kim, A. Sancar, J. Deisenhofer, *Science* **1995**, *268*, 1866.

[3] a) S. Barrois, C. Beyer, H. A. Wagenknecht, *Synlett* **2012**, 711; b) C. Beyer, H. A. Wagenknecht, *Synlett* **2010**, 1371.

[4] C. Brieke, F. Rohrbach, A. Gottschalk, G. Mayer, A. Heckel, *Angew. Chem.* **2012**, *124*, 8572; *Angew. Chem. Int. Ed.* **2012**, *51*, 8446.

[5] a) H. Asanuma, T. Ito, T. Yoshida, X. Liang, M. Komiyama, *Angew. Chem.* **1999**, *111*, 2547; *Angew. Chem. Int. Ed.* **1999**, *38*, 2393; b) A. A. Beharry, G. A. Woolley, *Chem. Soc. Rev.* **2011**, *40*, 4422; c) H. Ito, X. Liang, H. Nishioka, H. Asanuma, *Org. Biomol. Chem.* **2010**, *8*, 5519; d) T. Stafforst, D. Hilvert, *Angew. Chem.* **2010**, *122*, 10195; *Angew. Chem. Int. Ed.* **2010**, *49*, 9998.

[6] a) S. Ogasawara, M. Maeda, *Angew. Chem.* **2008**, *120*, 8971; *Angew. Chem. Int. Ed.* **2008**, *47*, 8839; b) S. Ogasawara, M. Maeda, *Angew. Chem.* **2009**, *121*, 6799; *Angew. Chem. Int. Ed.* **2009**, *48*, 6671.

[7] A. Mammana, G. T. Carroll, J. Areephong, B. L. Feringa, *J. Phys. Chem. B* **2011**, *115*, 11581.

[8] M. Singer, A. Jäschke, *J. Am. Chem. Soc.* **2010**, *132*, 8372.

[9] a) T. Kottysch, C. Ahlborn, F. Brotzel, C. Richert, *Chem. Eur. J.* **2004**, *10*, 4017; b) L. Wicke, J. W. Engels, *Bioconjugate Chem.* **2012**, *23*, 627.

[10] M. Irie, *Chem. Rev.* **2000**, *100*, 1685.

[11] P. Capek, H. Cahová, R. Pohl, M. Hocek, C. Gloeckner, A. Marx, *Chem. Eur. J.* **2007**, *13*, 6196.

[12] A. Omumi, D. G. Beach, M. Baker, W. Gabryelski, R. A. Manderville, *J. Am. Chem. Soc.* **2011**, *133*, 42.

[13] a) H. Cahová, L. Havran, P. Brazdilova, H. Pivonkova, R. Pohl, M. Fojta, M. Hocek, *Angew. Chem.* **2008**, *120*, 2089; *Angew. Chem. Int. Ed.* **2008**, *47*, 2059; b) H. Macíčková-Cahová, R. Pohl, P. Horakova, L. Havran, J. Spacek, M. Fojta, M. Hocek, *Chem. Eur. J.* **2011**, *17*, 5833; c) V. Raindlová, R. Pohl, M. Sanda, M. Hocek, *Angew. Chem.* **2010**, *122*, 1082; *Angew. Chem. Int. Ed.* **2010**, *49*, 1064; d) J. Riedl, R. Pohl, L. Rulisek, M. Hocek, *J. Org. Chem.* **2012**, *77*, 1026.

[14] D. R. Hannah, E. C. Sherer, R. V. Davies, R. B. Titman, C. A. Laughton, M. F. G. Stevens, *Bioorg. Med. Chem.* **2000**, *8*, 739.

[15] a) M. Liu, H. Asanuma, M. Komiyama, *J. Am. Chem. Soc.* **2006**, *128*, 1009; b) X. Liang, R. Wakuda, K. Fujioka, H. Asanuma, *FEBS J.* **2010**, *277*, 1551.

[16] T. Yokochi, D. M. Gilbert, *Curr. Protoc. Cell Biol.* **2007**, *Chapter 22*, Unit 22 10.

[17] a) C. D. Spicer, B. G. Davis, *Chem. Commun.* **2011**, *47*, 1698; b) R. M. Yusop, A. Unciti-Broceta, E. M. Johansson, R. M. Sanchez-Martin, M. Bradley, *Nat. Chem.* **2011**, *3*, 239.