SHORT COMMUNICATION

EurJOC European Journal of Organic Chemistry

DOI: 10.1002/ejoc.201300261

Photochromism of Diarylethene-Functionalized 7-Deazaguanosines

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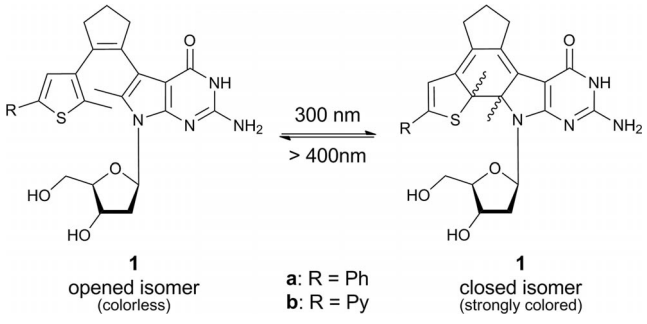
Keywords: Photochromism / Nucleosides / Biosensors / Cyclization

In a prior report we introduced a novel class of photochromic nucleosides (PCNs) that combine the structural features of adenine with the photochromic properties of diarylethenes. Herein, we translated this concept to the nucleoside guanosine, generating reversibly switching guanosine-like PCNs. These switches consist of a 7-deazaguanosine unit and a second aryl functionality, which are linked through a cyclopentene unit. Irradiation of the open-form isomer with light at 300 nm induces a pericyclic reaction that can be reversed with visible light. In addition to optical stimulation, these switches respond to light-independent stimuli, such as the presence of acid or complexation with metal ions.

Introduction

The incorporation of light-responsive modules into biopolymers has become a powerful strategy for the investigation and control of biological processes in vitro and in living cells. Distinct alterations of the chemical and photophysical properties of the photosensitive moiety can be triggered at the molecular level in a spatially and temporally resolved fashion by optical, and thus noninvasive, stimulation.[1] In the field of nucleic acids the photocontrol of DNA or RNA functions, such as transcription,[2] gene expression,[3] aptamer folding[4] or catalytic activity[5] has been demonstrated with photolabile protecting groups. However, the inherent limitation of this approach is irreversible cleavage of the "caged nucleosides". In contrast, bistable photochromic compounds such as azobenzenes and spiropyranes can be switched reversibly and, with their incorporation into nucleic acids, reversible modulation of very basic functions such as duplex[6] and triplex[7] stability have been demonstrated. However, these photochromic subunits do not share most structural motifs of the canonical nucleotides. Their application in highly structured nucleic acids, such as (desoxy)ribozymes, is constricted because their non-nucleosidic nature can lead to detrimental effects on the correct folding required for activity.[8] Furthermore, it would be desirable – from a spectroscopic point of view – to generate switches with distinct optical properties for the generation of multimodal systems with two or more independently switchable units.

We addressed these issues by designing novel photochromic compounds that mimic natural nucleosides.[9] These photochromic nucleosides (PCNs) were structurally related to adenosine. We continued to investigate the generality of this concept, with the goal of preparing PCNs of the other natural nucleosides. Herein, we present reversibly switching photochromic guanosine derivatives **1a** and **1b** that are based on a diarylethene scaffold (Scheme 1). The purine moiety of guanosine was derivatized with a cyclopentenethiophene unit to form a functional diarylethene. These compounds undergo a reversible pericyclic reaction, generating closed and opened isomers by irradiation with light of different wavelengths. Furthermore, they provide hydrogen donor and acceptor positions for Watson–Crick base pairing, as well as key structural features of nucleosides like ribose and purine moieties. Apart from the diarylethene-specific photoisomerization, these switches respond to light-independent stimuli, such as the presence of acid or complexation with metal ions.



Scheme 1. Photoisomerization of diarylethene-functionalized guanosine derivatives **1a** and **1b**.

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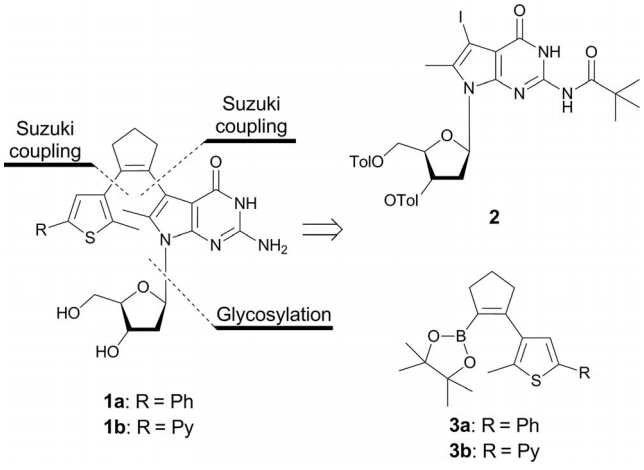
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201300261.

The photophysical and photochemical properties of diarylethene switches are known to depend strongly on the electronic properties of the associated heteroaryl units.[10] As a consequence, substitutions at the thiophene moiety that increase or decrease the electron density allow the spectral properties of the resulting diarylethenes to be controlled. With appropriate functionalization of these switches, this feature can be used for reversible in situ alteration of spectral properties, e.g., by protonation or complexation, thus allowing these switches to be addressed in a light-independent manner. Here, we demonstrate spectral tuning through the introduction of a pyridyl substituent, as well as acidichromism (i.e., pH-dependent switching) of diarylethene-functionalized nucleosides. Finally, we report the effect of metal ion complexation on the spectral properties of such switches.

Results and Discussion

The general synthetic approach to the guanosine-like PCNs **1a** and **1b** is depicted in Scheme 2. The basic scaffold of diarylethenes requires two – usually identical – heteroaryl units that are linked through a 1,2-cyclopentenyl linker to form the photoreactive hexatriene core. To obtain photochromic nucleosides, we took advantage of the variability of these heteroaryl units and introduced a novel glycosylated deazapurine derivative **2**, which is structurally related to the natural nucleoside guanosine. This fragment was linked to cyclopentene-thiophene conjugates **3a** and **3b** to resemble the photochromic structure. The eight-step synthesis started from 2,6-diaminopyrimidin-4-one and led to title compounds **1a** and **1b**. A detailed reaction scheme (Scheme S1) and the experimental procedures can be found in the Supporting Information.



Scheme 2. Retrosynthetic approach to guanosine-like PCNs.

The photochromic properties of compounds **1a** and **1b** were assessed in acetonitrile solutions at a concentration of 30 μM.[11] For ring closure, irradiation was performed with a 100 W Xenon lamp equipped with a monochromator at 300 nm or with a hand-held UV-lamp at 366 nm. The cycloreversion was triggered by a LED lamp emitting a broad spectrum in the visible range. The photoisomerization reaction was monitored by UV/Vis and HPLC analysis (see the Supporting Information).

Upon irradiation with UV-light, the initially colorless solutions of compounds **1a** and **1b** display a strong coloration (Figure 1), which is characteristic for diarylethenes that undergo ring closure. The color rapidly fades upon irradiation with visible light – due to cycloreversion – demonstrating that both compounds are fully functional diarylethenes. Figure 2 (a and b) show the evolution of absorbance spectra during photoisomerization. In both cases, irradiation with UV-light triggers the emergence of a broad absorption band in the visible range, with **1a** having its maximum at 505 nm, whereas the maximum of **1b** is located at 536 nm. The difference in their spectral properties is attributed to the thiophene substituents, which alter the electronic properties of the switches. The bathochromic shift of **1b** is due to a relative decrease of electron density in the thiophene moiety caused by the electron-deficient pyridyl substituent, as compared with the electron-rich phenyl substituent of **1a**. This illustrates the possibility of fine-tuning the spectral profile of the switches by changing the electronic properties of the heteroaryl moieties.

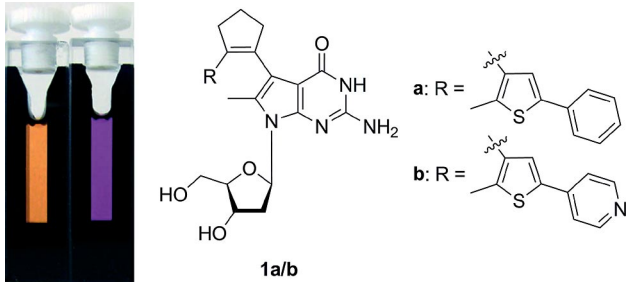


Figure 1. Solutions of **1a** (left cuvette) and **1b** (right cuvette) in acetonitrile after irradiation with UV-light.

Comparison of these data with our previous results on adenosine-like PCNs reveal that switches with identical thiophene substituents but different deazapurines are also subjected to a shift in their respective absorbance maxima. In conjunction with the phenylthiophene partner, exchange of the deazaadenosine by deazaguanosine leads to a bathochromic shift of 20 nm, whereas the pyridylthiophene-bearing PCNs display a difference of 31 nm.[9]

Upon irradiation of switches **1a** and **1b** with UV-light, the kinetic plots presented in Figure 2 (a and b) reach plateaus that reflect the photostationary states (PSS) of both compounds. The ratio between opened and closed form was investigated by HPLC, detecting both isomers at their isosbestic points for quantification (see the Supporting Information). After irradiation at 300 nm, the chromatograms of **1a** and **1b** show the formation of a novel peak with spectral properties that are identical to those of the closed-ring species. Ring closure at the PSS is achieved to 86 and 81% for **1a** and **1b**, respectively (see the Supporting Information). Exposure of the cyclized PCNs to visible light effectively induced cycloreversion, depicted in the kinetic plot by a rapid decrease of the absorbance at the maximum in

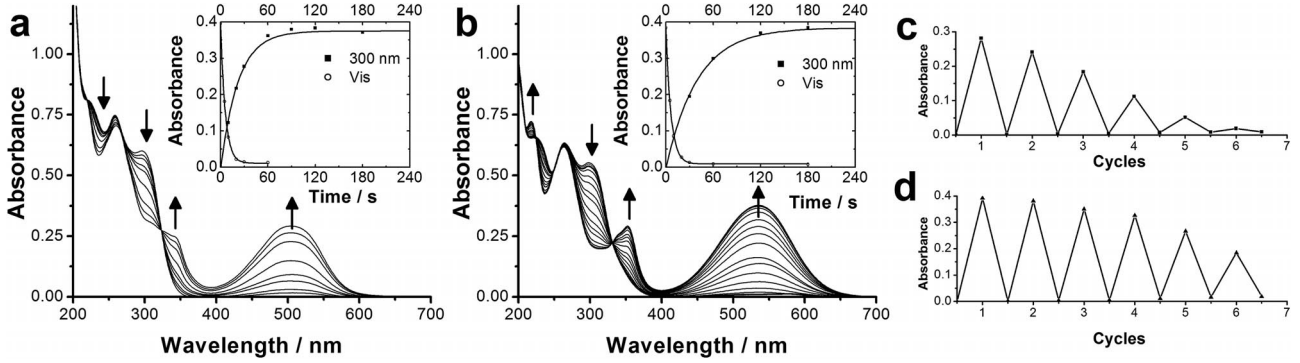


Figure 2. Absorption spectra evolution and time course of photoisomerization of compounds **1a** (a) and **1b** (b). Arrows indicate changes in the absorbance upon irradiation with UV-light (300 nm). Cycling between the opened and closed form (c, d) was performed by alternating illumination with 366 nm (UV-handheld lamp) and visible light. The decline in absorbance of **1a** (c) and, to a lesser extent, in **1b** (d) is attributed to degradation during the switching process.

the visible range. This demonstrates that the photoisomerization is reversible and can be repeated several times by altering the illumination wavelength. Monitoring several cycles by UV/Vis spectrophotometry, however, reveals a continuous decrease in the maximal absorbance intensity of the closed state (Figure 2, c and d), indicating that during these cycles the guanosine-like PCNs slowly degrade. Because the yellow colored by-product(s) formed in that process are not photochromic, the switching behavior is gradually lost. The photodegradation was not analyzed further. However, based on previous studies, it can be assumed that a stable conjugated, possibly aromatic compound, is generated that does not react back to the open form.[12] Notably, by-product formation could not be suppressed by oxygen depletion and the stability of the PCNs was dependent on the thiophene as well as the deazapurine hetereoaryl component. Direct comparison with adenosine-like PCNs shows that the deazaadenosine moiety confers a much higher photostability to the resulting PCNs, irrespective of the thiophene unit.[9]

In addition to light, the cycloreversion of diarylethenes can be triggered thermally. We therefore tested the thermal stability of closed-ring isomers and monitored the absorbance changes at different temperatures (Figure S4). The closed isomers of both switches are stable at 20 °C for hours, whereas quantitative light-independent isomerization occurs at 60 °C within 20 min (see the Supporting Information). Even though thermal relaxation is generally an undesired feature of diarylethenes, it could be beneficial in the case of PCNs because a set of switches with different thermal stabilities could be addressed in an orthogonal light-independent way, simply by changing the temperature.

The photochromic and photophysical properties of PCNs strongly depend on the heteroaryl moieties, as demonstrated here and in our earlier report.[9] Alteration of the electronic properties of the thiophene unit in situ would provide a unique additional feature because this would allow these switches to be addressed with alternative stimuli in addition to light or thermal energy. The pyridyl substituent of compound **1b** is an example of a multi-addressable switch because it can be protonated in the presence of acid or complexed with an appropriate metal ion. Figure 3 shows the impact of protonation by trifluoroacetic acid (TFA) and complexation with Cu^{2+} on the spectral properties of the closed isomer. In both cases, the absorption maximum is significantly redshifted, with the copper-induced shift being more pronounced (165 nm) than that for protonation by TFA (89 nm). However, both protonation and complexation reduce the thermostability of the closed form, causing it to relax into its opened form at ambient temperature. Again, this property could be exploited to orthogonally address several PCNs.

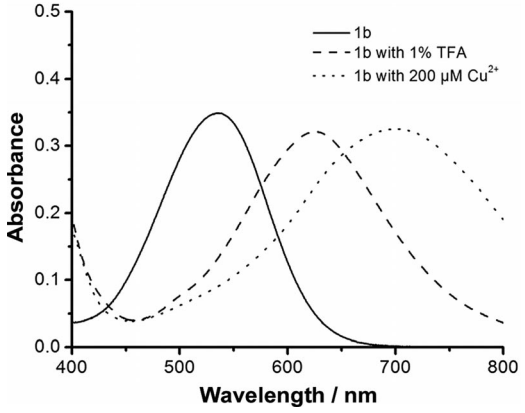


Figure 3. Bathochromic shift of the absorbance maximum of closed **1b** in acetonitrile (30 μM) upon addition of TFA or $CuSO_4$.

Conclusions

We have developed photochromic nucleosides based on glycosylated deazaguanine in combination with substituted thiophenes as heteroarylic subunits. These compounds form switches that undergo diarylethene-specific light-induced cyclization and cycloreversion reactions. Overall, their photochromic properties are defined by the electronic features of their heteroaryl subunits. We have shown that this can be exploited to generate switches with distinct spectral properties by changing the thiophene subunit. Alternatively,

multi-addressable PCNs with functionalized thiophene moieties can be built that are sensitive to other stimuli such as the presence of acid or metal ions. Further development of such switches could be useful for the generation of light-sensitive nucleic acids and may expand the current toolset of reversibly switchable modules for biological applications.

Supporting Information (see footnote on the first page of this article): Experimental procedures, chemical synthesis (Scheme S1), HPLC analysis of purity and photostationary states (Figures S1 and S2), thermal relaxation of closed isomers (Figure S3), and NMR spectra.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (DFG) (Ja 794/3). M. S. acknowledges a LGFG fellowship from the Federate State of Baden-Württemberg. A. N. acknowledges a research fellowship from the DFG (NI 1341/1-1). The authors thank Heiko Rudy and Tobias Timmermann for technical support.

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Received: February 19, 2013
Published Online: April 15, 2013

