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Reversibly Photoswitchable Nucleosides: Synthesis and Photochromic Properties of Diarylethene-Functionalized 7-Deazaadenosine Derivatives

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Abstract: Photochromic nucleosides were designed that combine the structural features and molecular recognition properties of nucleic acids with the light-sensitivity of diarylethenes. Target compounds **1a**−**c** consist of a 7-deazaadenosine unit that is linked to a thiophene as the second aryl functionality via a 1,2-cyclopentenyl linker. These nucleoside analogues undergo a reversible electrocyclic rearrangement, generating strongly colored closed-ring isomers upon irradiation with UV-light, while exposure to light in the visible range triggers the cycloreversion to the colorless opened-ring form. UV−vis spectroscopy, HPLC, and ^{1}H NMR measurements revealed recognition of complementary thymidine and up to 97% conversion to the thermally stable closed-ring isomers after illumination with UV-light. The required wavelength for ring closure was found to vary depending on the substituents attached to the thiophene moiety. In a first design step, we used this important feature of diarylethenes to shift the switching wavelength from initially 300 nm (**1a**) to 405 nm (**1cH**$^+$). In a second step, we generated a pair of orthogonal switches, differing enough in their respective switching wavelengths to be controlled independently in the same sample. Finally, a molecular switch was developed that showed both photochromism and acidichromism, thereby illustrating the possibility to gate the spectral properties to multiple stimuli. These new photochromic nucleosides represent useful building blocks for the generation of light-sensitive nucleic acids either by inducing conformational changes upon isomerization or by exploring the different spectral properties of the closed and opened isomers, for example, for use as reversible fluorescence quenchers.

Introduction

Light is a convenient and powerful trigger to control the reactivity of biomolecules.[1] Complex and compartimentalized environments like cells benefit not only from the noninvasive and noninterfering nature of light, but also from its spatial and temporal resolution. The artificial induction of photosensitivity in biomolecules that are per se unreactive to light relies in most of the cases on the covalent functionalization with small photoactive molecules.[1] They act as mediators to translate, by photocleavage or isomerization, an optical input into a chemical or physical signal that is transduced to the bioactive species of interest. Peptides, proteins, and nucleic acids[2] have been used in combination with various types of photochromic molecules, like fulgides,[3] spiropyranes,[4−6] and azobenzenes[7−11] to demonstrate this principle.

For nucleic acids, the irreversible control of biological activity,[12,13] structural features,[14,15] and catalytic properties[16,17] was demonstrated using photolabile protecting groups, whereas the reversible modulation of duplex[18−20] and triplex stability,[21]

(1) Goeldner, M.; Givens, R. *Dynamic Studies in Biology: Phototriggers, Photoswitches and Caged Biomolecules*; Wiley-VCH: Weinheim, 2005.
(2) Mayer, G.; Heckel, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4900–4921.
(3) Willner, I.; Rubin, S.; Wonner, J.; Effenberger, F.; Baeuerle, P. *J. Am. Chem. Soc.* **1992**, *114*, 3150–3151.
(4) Lion-Dagan, M.; Willner, I. *J. Photochem. Photobiol., A* **1997**, *108*, 247–252.
(5) Sakata, T.; Yan, Y.; Marriott, G. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 4759–4764.
(6) Weston, D. G.; Kirkham, J.; Cullen, D. C. *Biochim. Biophys. Acta* **1999**, *1428*, 463–467.
(7) Renner, C.; Moroder, L. *ChemBioChem* **2006**, *7*, 869–878.
(8) Volgraf, M.; Gorostiza, P.; Numano, R.; Kramer, R. H.; Isacoff, E. Y.; Trauner, D. *Nat. Chem. Biol.* **2006**, *2*, 47–52.
(9) Willner, I.; Rubin, S.; Riklin, A. *J. Am. Chem. Soc.* **1991**, *113*, 3321–3325.
(10) Woolley, G. A. *Acc. Chem. Res.* **2005**, *38*, 486–493.
(11) Zhang, F. Z.; Zarrine-Afsar, A.; Al-Abdul-Wahid, M. S.; Prosser, R. S.; Davidson, A. R.; Woolley, G. A. *J. Am. Chem. Soc.* **2009**, *131*, 2283–2289.
(12) Ando, H.; Furuta, T.; Tsien, R. Y.; Okamoto, H. *Nat. Genet.* **2001**, *28*, 317–325.
(13) Monroe, W. T.; McQuain, M. M.; Chang, M. S.; Alexander, J. S.; Haselton, F. R. *J. Biol. Chem.* **1999**, *274*, 20895–20900.
(14) Hobartner, C.; Silverman, S. K. *Angew. Chem., Int. Ed.* **2005**, *44*, 7305–7309.
(15) Mayer, G.; Krock, L.; Mikat, V.; Engeser, M.; Heckel, A. *ChemBioChem* **2005**, *6*, 1966–70.
(16) Chaulk, S. G.; MacMillan, A. M. *Nucleic Acids Res.* **1998**, *26*, 3173–8.
(17) Ting, R.; Lermer, L.; Perrin, D. M. *J. Am. Chem. Soc.* **2004**, *126*, 12720–1.
(18) Asanuma, H.; Shirasuka, K.; Yoshida, T.; Takarada, T.; Liang, X.; Komiyama, M. *Chem. Lett.* **2001**, 108–109.
(19) Asanuma, H.; Ito, T.; Yoshida, T.; Liang, X. G.; Komiyama, M. *Angew. Chem., Int. Ed.* **1999**, *38*, 2393–2395.
(20) Asanuma, H.; Liang, X. G.; Yoshida, T.; Komiyama, M. *ChemBioChem* **2001**, *2*, 39–44.
(21) Liang, X. G.; Asanuma, H.; Komiyama, M. *J. Am. Chem. Soc.* **2002**, *124*, 1877–1883.

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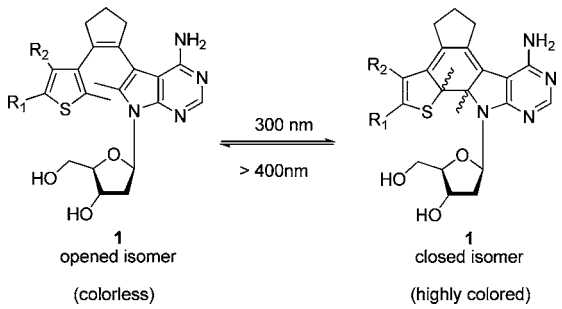
Scheme 1. Photoisomerization of Nucleosidic Diarylethenes



protein-mediated enzymatic activities related to nucleic acids,[22−25] binding of aptamers,[26] and the catalytic activity of DNAzymes[27,28] were achieved on the basis of bistable photoswitches like azobenzenes and spiropyranes. These latter reports, however, and similar experiments with azobenzene-derivatized Diels−Alderase ribozymes in our lab (Singer and Jäschke, unpublished data) also revealed the limitations of bistable artificial mediators that do not possess important properties of nucleobases: Even though *trans*-azobenzene readily undergoes stacking interactions,[29] it is obviously neither able to participate in hydrogen-bonding networks, nor does it sterically resemble a nucleobase. This often results in detrimental effects on structural features of nucleic acids. In particular for DNAzymes and ribozymes, the incorporation of such mediators leads to losses in catalytic activity even for the nominally active form,[28] likely by interfering with the active conformation.

Surprisingly, diarylethenes are still unexplored in the context of biopolymers despite their good photochromic performance, interesting spectral properties, and useful structural features.[30] This work intends to investigate their potential use in combination with nucleic acids. Toward this end, we designed a set of novel photoactive mediators that are structurally related to the nucleoside adenosine and capable to undergo a diarylethene-specific electrocyclic rearrangement. The proposed structures and the photoisomerization reaction are shown in Scheme 1.

The photochromic nucleosides presented in this work were designed to circumvent the above-mentioned limitations since they combine important properties of natural nucleic acids like Watson−Crick base pairing with the ability of reversible photoisomerization. In contrast to the work by Maeda, who showed light-dependent duplex stability[31] and reversible binding of a G-quadruplex to an aptamer[32] using stilbene-like guanosine derivatives with *cis*−*trans* isomerizable substituents attached to the 8-position,[33] the approach we present here relies on pericyclic isomerizations that directly involve the ring atoms of the nucleobase. This offers several advantages: First, diarylethenes are known to have good photochromic properties, such as negligible thermal relaxation and high fatigue resistance.[30] Second, the spectral tunability[34−36] allows to not only adapt their photophysical properties to specific needs, for example, higher switching wavelengths, but also to generate orthogonal switches that can be controlled independently. And third, in addition to the original purpose to induce conformational changes by photoisomerization, the strong absorption bands that occur in the closed-ring isomers can be exploited for conditional resonance energy transfers. This gives access to reversible fluorescence quenchers, as shown for other diarylethene−fluorophore conjugates.[37−40] Herein, we describe the synthesis and photochromism of light-sensitive adenosine derivatives and demonstrate their ability to form base pairs as well as the spectral tunability and orthogonality of different switches on the level of nucleoside monomers.

Experimental Section

Synthetic Procedures. Compounds **2** and **6a**−**c** were prepared as reported previously.[41−44] The synthesis and characterization of compounds **3**−**5** as well as **7a**−**c**, **8a**−**c**, **9a**−**c**, and the final switches **1a**−**c** are described in the Supporting Information.

Base Pairing Studies. Thymidine and 2′-deoxyadenosine were purchased from Sigma-Aldrich and used without further purification. ^{1}H NMR measurements were performed on a Varian Systems 500 MHz instrument at 25 °C. Single components were measured in 5 mM d^3-MeCN solutions and compared with the respective mixtures containing 5 mM of each component.

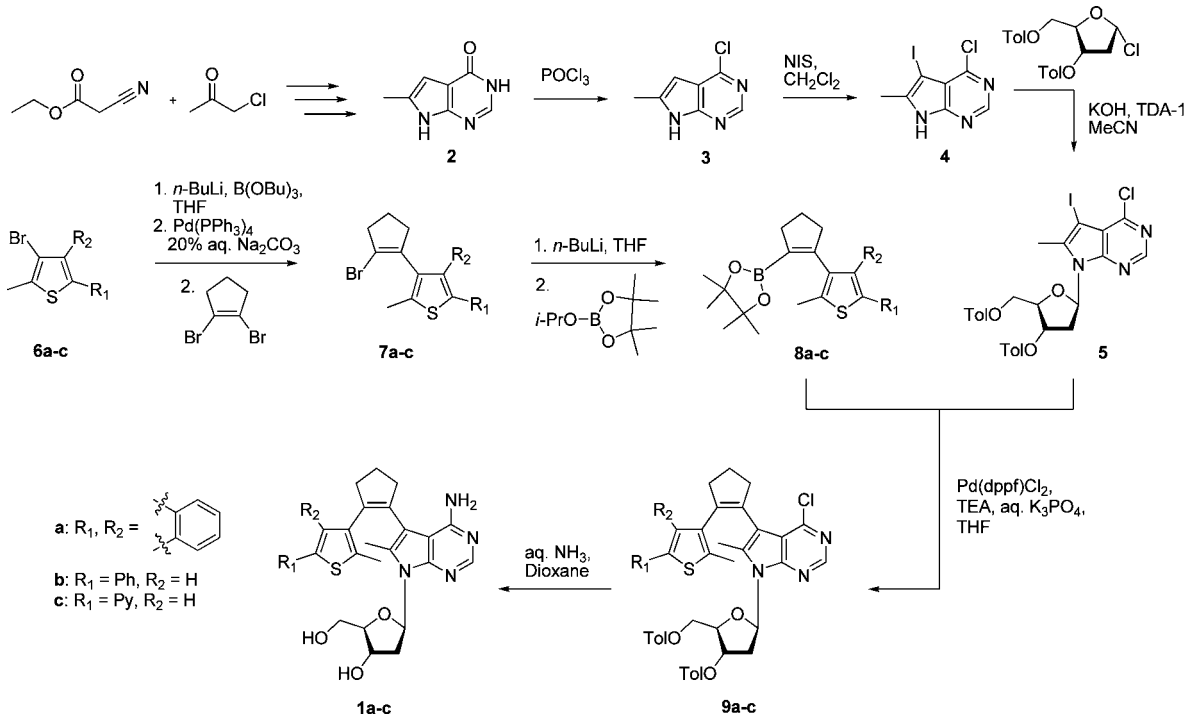
Photoisomerization. The photochromic properties of compounds **1a**−**c** were assessed in 30 μM acetonitrile solutions at 25 °C, using either a standard hand-held UV-lamp (254 nm, 366 nm) or a 100 W xenon lamp at variable wavelength (monochromator) as light source for ring closure. The cycloreversion was induced with a white LED lamp, having a broad emission spectrum in the visible range, or with the above-mentioned 100 W xenon lamp, if monochromatic light was used. The reactions were conducted in 1 mL quartz cuvettes and monitored by recording the UV−vis absorbance on a Cary 100 Bio UV−vis spectrometer.

Results and Discussion

Synthesis Strategy. The versatility of heteroaryl substituents in diarylethenes[45,46] has prompted us to investigate purine-

(22) Asanuma, H.; Tamaru, D.; Yamazawa, A.; Liu, M. Z.; Komiyama, M. *ChemBioChem* **2002**, *3*, 786–789.
(23) Liu, M. Z.; Asanuma, H.; Komiyama, M. *J. Am. Chem. Soc.* **2006**, *128*, 1009–1015.
(24) Matsunaga, D.; Asanuma, H.; Komiyama, M. *J. Am. Chem. Soc.* **2004**, *126*, 11452–11453.
(25) Yamazawa, A.; Liang, X. G.; Asanuma, H.; Komiyama, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 2356–2357.
(26) Kim, Y. M.; Phillips, J. A.; Liu, H. P.; Kang, H. Z.; Tan, W. H. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 6489–6494.
(27) Keiper, S.; Vyle, J. S. *Angew. Chem., Int. Ed.* **2006**, *45*, 3306–3309.
(28) Liu, Y.; Sen, D. *J. Mol. Biol.* **2004**, *341*, 887–892.
(29) Liang, X. G.; Asanuma, H.; Kashida, H.; Takasu, A.; Sakamoto, T.; Kawai, G.; Komiyama, M. *J. Am. Chem. Soc.* **2003**, *125*, 16408–16415.
(30) Irie, M. In *Molecular Switches*; Feringa, B. L., Ed.; Wiley-VCH: Weinheim; New York, 2001; p 37−62.
(31) Ogasawara, S.; Maeda, M. *Angew. Chem., Int. Ed.* **2008**, *47*, 8839–8842.
(32) Ogasawara, S.; Maeda, M. *Angew. Chem., Int. Ed.* **2009**, *48*, 6671–6674.
(33) Ogasawara, S.; Saito, I.; Maeda, M. *Tetrahedron Lett.* **2008**, *49*, 2479–2482.
(34) Pu, S.; Yang, T.; Li, G.; Xu, J.; Chen, B. *Tetrahedron Lett.* **2006**, *47*, 3167–3171.
(35) Pu, S. Z.; Zheng, C. H.; Le, Z. G.; Liu, G.; Fan, C. B. *Tetrahedron* **2008**, *64*, 2576–2585.
(36) Uchida, K.; Matsuoka, T.; Kobatake, S.; Yamaguchi, T.; Irie, M. *Tetrahedron* **2001**, *57*, 4559–4565.
(37) Fukaminato, T.; Sasaki, T.; Kawai, T.; Tamai, N.; Irie, M. *J. Am. Chem. Soc.* **2004**, *126*, 14843–14849.
(38) Giordano, L.; Jovin, T. M.; Irie, M.; Jares-Erijman, E. A. *J. Am. Chem. Soc.* **2002**, *124*, 7481–7489.
(39) Yan, S. F.; Belov, V. N.; Bossi, M. L.; Hell, S. W. *Eur. J. Org. Chem.* **2008**, 2531–2538.
(40) Zhao, H.; Al-Atar, U.; Pace, T. C. S.; Bohne, C.; Branda, N. R. *J. Photochem. Photobiol., A* **2008**, *200*, 74–82.
(41) Davoll, J. *J. Chem. Soc.* **1960**, 131–138.
(42) Gilat, S. L.; Kawai, S. H.; Lehn, J. M. *Chem.−Eur. J.* **1995**, *1*, 275–284.
(43) Irie, M.; Lifka, T.; Kobatake, S.; Kato, N. *J. Am. Chem. Soc.* **2000**, *122*, 4871–4876.
(44) Katritzky, A. R.; He, H.-Y.; Long, Q.; Cui, X.; Level, J.; Wilcox, A. L. *ARKIVOC* **2000**, *1*, 240–251.
(45) Irie, M. *Chem. Rev.* **2000**, *100*, 1685–1716.

Scheme 2. Synthesis of Photochromic Nucleosides **1a**−**c**



related nucleosidic structures as aryl subunit. To meet the structural requirements of diarylethenes, however, the purine scaffold has to be modified in two positions. First, the nitrogen atom at position 7 (purine numbering is used throughout the manuscript) has to be replaced by a carbon to allow the desired electrocyclic reaction. Second, a methyl group is introduced at the 8-position to avoid directly attached hydrogen atoms in the cyclohexadiene ring formed upon cyclization, thereby preventing an irreversible oxidative aromatization and other side reactions.[45]

Taking these considerations into account, we designed the protected 8-methyl-7-deazapurine nucleoside **5**, a precursor for an adenosine analogue, as aryl subunit in the diarylethene scaffold. The second part of this scaffold consists of the corresponding thiophene−cyclopentene conjugate. Here, we introduced several combinations of substitutions in the thiophene moiety, in order to investigate their effect on the photochromic properties in the final product. Cyclopentene was chosen as connection between both aryl functionalities because it is known to enhance the photochromic performance of diarylethenes in polar solvents[47,48] and due to its well-established compatibility with diarylethene photoswitching.[49] Since the protected nucleoside analogue **5** is not compatible with the more commonly used coupling procedures that involve lithiation with *n*-BuLi, the assembly of the unsymmetrical diarylethene is performed as described by Belser et al.,[50] with a Suzuki coupling under relatively mild conditions, followed by deprotection and amination in the 6-position. This convergent strategy, using several substituted thiophene−cyclopentene conjugates as coupling partners, allows to rapidly synthesize a variety of unsymmetric diarylethenes that differ in their photochromic properties.

Synthesis of Compound 5. The synthetic procedure is based on the formation of 6-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine **2**, shown in Scheme 2. This synthesis was performed in 5 steps as described by Davoll[41] with an overall yield of 17%, starting from 2-cyanoacetoxyethyl ester and chloroacetone, involving pyrimidine formation with thiourea, cyclization to a 7-deazapurine, and dethiolation with Raney-Nickel. Chlorination was performed with $POCl_3$ to yield compound **3** (83%) which was subsequently iodinated in the 7 position with *N*-iodosuccinimide (89%). Toluoyl-protected β-D-nucleoside **5** was obtained in 60% yield by liquid−solid phase-transfer glycosylation with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride[51] according to a general glycosylation protocol by Seela and co-workers.[52,53]

Synthesis of Compounds 8a−**c.** The second part consists of the thiophene−cyclopentene conjugates, carrying different substitutions at the thiophene moiety. We synthesized three different thiophene bromides **6a**−**c**, depicted in Scheme 2, following published procedures[42−44] and converted them in a one-pot reaction, first to the respective boronic esters using *n*-BuLi and tributylborate, followed by direct conjugation via Suzuki coupling with 1,2-dibromocyclopentene (1.5 equiv) in the presence of 5 mol % palladium, sodium carbonate, and water. Under these conditions, the reactions afforded a series of monosubstituted cyclopentenes (**7a**−**c**) in 40−44% yield with only 5−10% of symmetric diarylethenes formed as byproducts that were separated by column chromatography. A direct conversion of these compounds to the respective boronic esters with tributylborate and immediate coupling with the protected iodonucleoside **5** in a one-pot reaction was not successful.

(46) Tian, H.; Yang, S. J. *Chem. Soc. Rev.* **2004**, *33*, 85–97.
(47) Kobatake, S.; Terakawa, Y.; Imagawa, H. *Tetrahedron* **2009**, 6104–6108.
(48) Zou, Y.; Yi, T.; Xiao, S.; Li, F.; Li, C.; Gao, X.; Wu, J.; Yu, M.; Huang, C. *J. Am. Chem. Soc.* **2008**, *130*, 15750–1.
(49) de Jong, J. J. D.; Lucas, L. N.; Hania, R.; Pugzlys, A.; Kellogg, R. M.; Feringa, B. L.; Duppen, K.; van Esch, J. H. *Eur. J. Org. Chem.* **2003**, 1887–1893.
(50) Kuhni, J.; Adamo, V.; Belser, P. *Synthesis* **2006**, 1946–1948.
(51) Hoffer, M. *Chem. Ber.* **1960**, *93*, 2777–2781.
(52) Seela, F.; Peng, X. H. *Synthesis* **2004**, 1203–1210.
(53) Seela, F.; Westermann, B.; Bindig, U. *J. Chem. Soc., Perkin Trans. 1* **1988**, 697–702.

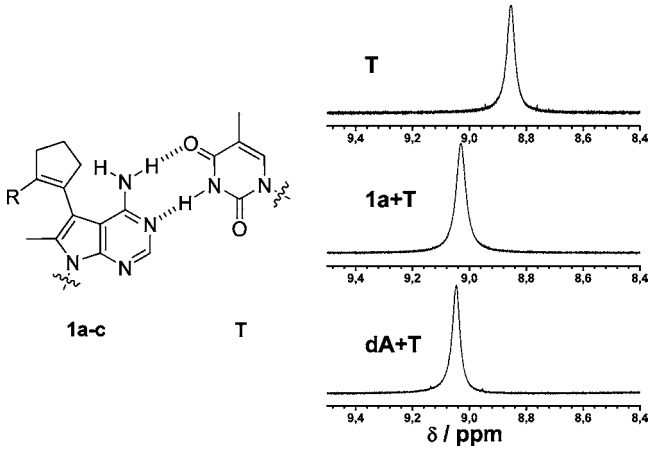


Figure 1. (Left panel) Putative binding mode between **1a**−**c** and **T**. (Right panel) Partial NMR spectra showing the downfield chemical shift of the imine proton in thymidine (**T**) upon addition of **1a** or 2′-deoxyadenosine (**dA**).

Therefore, the monoarylated cyclopentenes were converted to the pinacolatoboronic esters **8a**−**c** before proceeding to the coupling reaction.

Synthesis of Photochromic Nucleosides 1a−**c.** The final switches could be obtained by reacting the boronic esters **8a**−**c** with the protected iodonucleoside **5** via Suzuki coupling, using 1,1′-bis(diphenylphosphino)ferrocene-palladium(II)dichloride in the presence of potassium phosphate in a THF−water mixture. The initially very low yields of this reaction could surprisingly be improved by addition of 5 equiv of triethylamine. Under these conditions, coupling products **9a**−**c** could be obtained in 50−85% yields. The final deprotection and amination in the 6-position were performed in one step by incubation in dioxane/aqueous ammonia for 12 h at 120 °C in an autoclave, yielding the unsymmetrical diarylethenes **1a**−**c**. The HPLC chromatograms of all switches showed single peaks after column chromatography (Supporting Information Figure S1).

Base Pairing Properties. The association of complementary nucleoside monomers can be monitored by ^{1}H NMR in aprotic solvents.[54,55] The formation of hydrogen bonds between nucleobases leads to a distinct downfield chemical shift of involved exocyclic amine and endocyclic imine protons. To study the ability of compounds **1a**−**c** to form Watson−Crick-like base pairs, we performed ^{1}H NMR measurements in the presence of thymidine (**T**) monomers in deuterated acetonitrile using the chemical shift of **T**-NH as a probe for the formation of hydrogen bridges. Significant downfield shifts of up to 0.18 ppm (90 Hz) could be observed for the imine proton of **T** upon addition of **1a** as shown in Figure 1. Comparison of the measured chemical shifts to free thymidine and a 2′-deoxyadenosine−thymidine mixture clearly indicates an association of **1a** with the complementary thymidine monomer under these conditions. Similar shifts of **T**-NH are observed in the presence of **1b** and **1c**, whereas the addition of **5** shows no effect, since it lacks the required amino functionality for base pairing (Supporting Information Figure S2). Additionally, a weaker downfield shift of the exocyclic amine in the deazapurine moieties of all switches (Supporting Information Table S1) suggests a Watson−Crick-like binding as shown in Figure 1 (left panel).[55]

(54) Katz, L.; Penman, S. *J. Mol. Biol.* **1966**, *15*, 220–231.
(55) Shoup, R. R.; Miles, H. T.; Becker, E. D. *Biochem. Biophys. Res. Commun.* **1966**, *23*, 194–201.

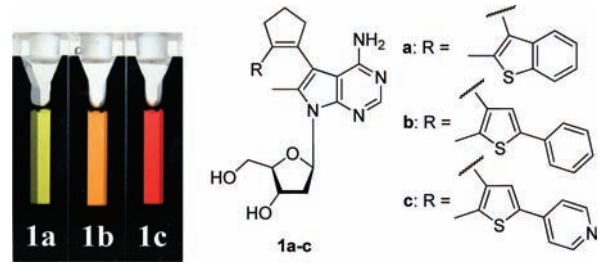


Figure 2. Solutions of **1a**−**c** in acetonitrile after irradiation with UV-light (250−370 nm).

Photochromic Properties. Nucleosides **1a**−**c** should exhibit a diarylethene-specific reversible photoisomerization (Scheme 1), generating a closed-ring isomer upon irradiation with UV-light. Cycloreversion to the opened form is expected to occur at a higher wavelength. Photoisomerization was monitored by UV−vis spectroscopy, HPLC, and ^{1}H NMR. Overall, the following data show that all three compounds undergo the anticipated electrocyclic rearrangement in a reversible manner, establishing a set of photochromic nucleosides with different photochemical properties. Exposing the colorless solutions of compounds **1a**−**c** to UV-light (250−370 nm) leads to a strong coloration, ranging from yellow to red (Figure 2), depending on the thiophene substitution. The corresponding changes in the absorption spectra during irradiation are shown in Figure 3 for all three switches. They share the emergence of a broad absorption band between 400 and 600 nm with maxima at 450 nm (**1a**), 485 nm (**1b**), and 505 nm (**1c**), which is typical for closed isomers of diarylethenes.[45] In all cases, the color is stable over days at 20 °C, as derived from absorbance traces of closed-ring isomers at different temperatures (Supporting Information Figure S6), but rapidly fades upon irradiation with light in the visible range. The coloration−decoloration cycle can be repeated many times, as shown in Figure 3e, even though minor deterioration occurs, indicated by the slightly decreasing absorption maximum in the closed state. The conversion to the closed isomers in the photostationary state for all switches was determined by HPLC (Supporting Information Figure S3, and Table S2) and ^{1}H NMR (Supporting Information Figure S4) in the case of **1c**, and is listed in Table 1, along with their thermal stabilities and absorption maxima. The largest switching factor was observed for compound **1c** with 97% conversion to the closed isomer after irradiation at 300 nm. However, in the case of **1a**, the photostationary state is already reached at 60% conversion. Thus, single-point attachment of aryl and heteroaryl substitutions as in **1b**/**1c** seems to be more favorable with respect to cyclization yields than a benzo fusion as in **1a**. Additionally, the synthetic approach to **1b**/**1c** allows an easier variation of the substituents and the resulting electronic properties.

These three examples also illustrate the possibility to tune spectral properties of diarylethenes by simply altering substituents on the thiophene moiety. Decreasing the electron density on the thiophene shifts the absorption maximum in the closed state to a more bathochromic region and also increases the maximum wavelength for ring closure from 300 nm (**1a**) to 405 nm (**1cH**$^+$) as can be seen in Figure 3d. This feature of diarylethenes permits the fine-tuning of the photochromic properties by design. In the current case, the shift to higher cyclization wavelengths allows avoidance of short-wavelength UV-irradiation, which is known to cause damage to nucleic

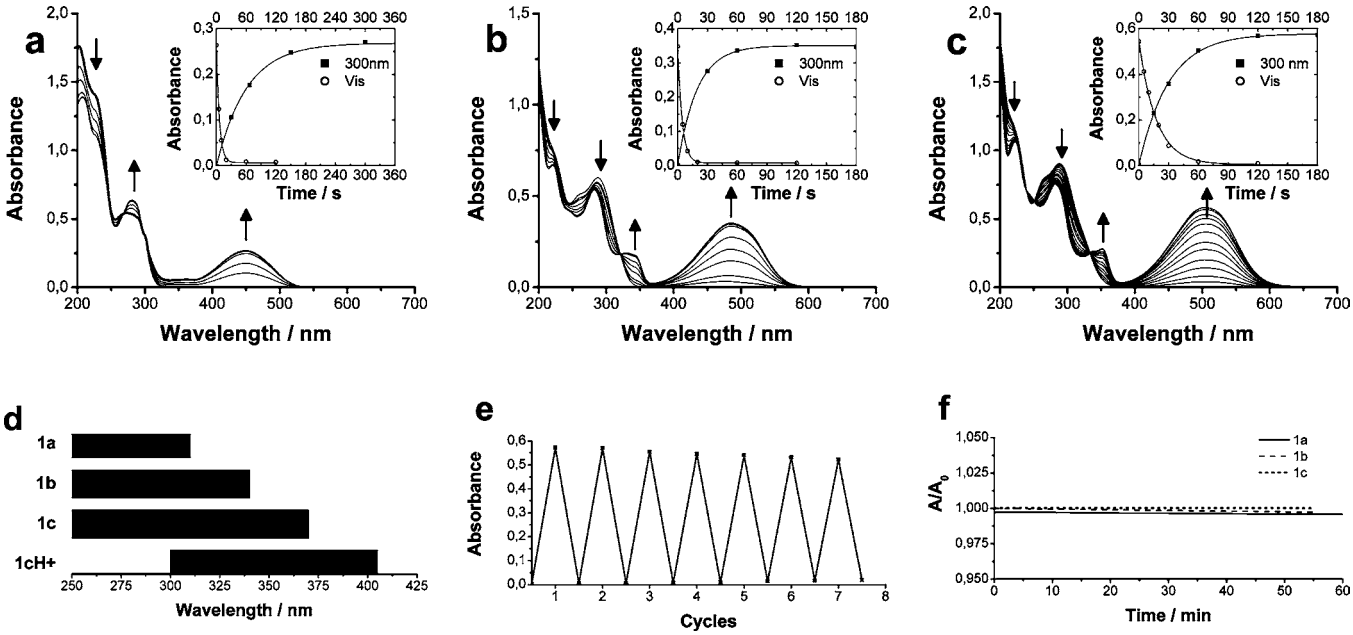


Figure 3. Absorption spectra evolution and time course of photoisomerization for compounds **1a** (a), **1b** (b) and **1c** (c). Arrows indicate changes upon irradiation with UV-light (300 nm). (d) Wavelength range to switch compounds **1a**−**c** to their respective closed state derived from kinetic investigations at different wavelengths (Supporting Figure S5). (e) Cycling between closed and opened state of **1c** by alternating illumination with 366 nm and visible light. (f) Thermal stabilities of **1a**−**c** in acetonitrile at 20 °C (for data at 40 and 60 °C see Supporting Figure S6).

Table 1. Spectral and Photochromic Parameters of Compounds **1a**−**c**

compound	λ_{max} (closed state)/nm	thermal stability/$t_{1/2}$[a]	conversion[b]
1a	450	>100 h	0.60
1b	485	>100 h	0.87
1c	505	>1000 h	0.97
1cH^{+}[c]	573	60 h	

[a] Measured at 20 °C. [b] In the photostationary state under irradiation with 300 nm. [c] Protonated form of **1c** in 3 mM trifluoroacetic acid (TFA).

acids[56] and, furthermore, offers the possibility to use standard laser equipment in fluorescence microscopes for the switching process. Moreover, this approach allows generation of switches that can be controlled independently from each other, given that their switching wavelengths are well separated. To demonstrate this principle, we used a mixture of **1a** and **1c** and specifically switched their photostationary states (PSS) by sequential illumination with different wavelengths (Figure 4).

First, both compounds were switched simultaneously to the closed state and back, using 300 nm, followed by visible light. Illumination at 334 nm then exclusively turned compound **1c** into the closed state, while **1a** remained unaffected until the wavelength was lowered to 300 nm. Subsequent irradiation of both compounds with 550 nm light converted 76% of **1c** into the opened-ring form. Finally, both compounds are synchronized in their initial opened states by illumination with visible light. It is thus possible to switch the PSS of a very similar set of photochromic nucleosides in a simultaneous (Figure 4, 300 nm and vis) as well as independent (Figure 4, 334 and 550 nm) manner at the same time by exploiting their different spectral properties.

The introduction of further reactivities expands the scope of diarylethenes beyond photochromism. In the case of compound

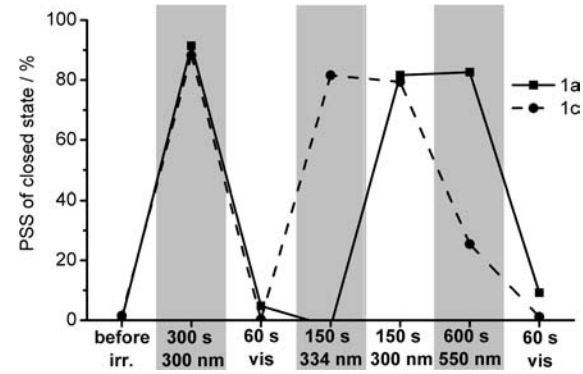


Figure 4. Orthogonal switching of a mixture of compound **1a** and **1c** by sequential illumination at different wavelengths. The PSS of the individual switches were determined by UV-spectroscopy.

1c, pH-sensitivity is mediated by the pyridyl-substituent and induces acidichromism, that is, the reversible change of spectral properties upon acidification. We, therefore, tested the effect of adding trifluoroacetic acid (TFA) to the closed isomer of **1c**, using **1b** as control since it lacks the nitrogen atom required for protonation. Only in the case of **1c** the addition of TFA leads to a strong bathochromic shift of the absorption band in the visible range, changing the apparent color from red to violet (Figure 5), whereas **1b** shows no changes (Supporting Information Figure S7). For the opened isomer, the switching wavelength for ring closure is furthermore increased from 370 nm (**1c**) to 405 nm (**1cH**$^{+}$; Figure 3d). The original properties of **1c** can be restored by addition of triethylamine, proving this effect to be entirely reversible (Supporting Information Figure S7). Thus, multiaddressable switches can be easily constructed that gate the output signal to different types of stimuli.

Conclusion

Current approaches for reversible switching of nucleic acid-related activities, mainly relying on azobenzene, suffer from

(56) Beukers, R.; Eker, A. P. M.; Lohman, P. H. M. *DNA Repair* **2008**, *7*, 530–543.

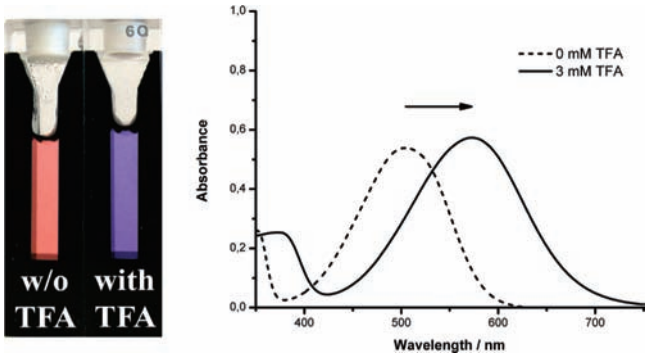


Figure 5. Acidichromism of compound **1c**. Protonation of the pyridyl-residue with TFA bathochromically shifts the absorption maximum by 68 nm.

drawbacks arising from the non-nucleosidic nature of the covalently attached photoswitches. In particular, highly structured DNA or RNA would benefit from mediators that combine base pairing properties with photoactivity. Diarylethenes are well-known photochromes and tolerate a variety of arylic subunits in their core structure. Herein, we designed photochromic nucleosides based on the scaffold of diarylethenes by introducing 7-deazaadenine as one of the two aryl moieties. On the basis of the data presented here, these compounds form functional switches that undergo the typical light-driven electrocyclization, yielding sterically and photophysically distinct closed and opened isomers. Even though the individual binding constants of these compounds to thymidine were not determined, the NMR data presented here clearly indicate an association between the complementary monomers, which is a key feature for the generation of light-responsive nucleic acids. Furthermore, they display good photochromic properties in terms of photoconversion, repeatability of switching, and thermal stability. The intrinsic properties of diarylethenes expand the scope of photochromic nucleosides by providing features not accessible by other approaches: First, the tunability of switching wavelengths not only allows to adapt the photochromic properties to specific needs but also gives access to orthogonal switches (see Figure 4). Second, these properties can be linked to other external signals, like the presence of acid (see Figure 5), complexation,[57] or redox state. Finally, the strong absorption band of the closed isomers in the visible range suggests a considerable quenching potential of attached fluorescent dyes as it has been shown for other diarylethene−fluorophore conjugates.[37−40] This could be exploited for conditional resonance energy transfer to reversibly switch fluorescence on and off, a very useful property for bioimaging applications like OLID[58,59] or super-resolution microscopy.[60−62] Future experiments will focus on the incorporation of these compounds into oligonucleotides and the investigation of the impact of photoisomerization on duplex stability and catalytic activity of nucleic acids.

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Supporting Information Available: Full experimental section. This material is available free of charge via the Internet at http://pubs.acs.org.

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(57) Roberts, M. N.; Nagle, J. K.; Finden, J. G.; Branda, N. R.; Wolf, M. O. *Inorg. Chem.* **2009**, *48*, 19–21.
(58) Marriott, G.; Mao, S.; Sakata, T.; Ran, J.; Jackson, D. K.; Petchprayoon, C.; Gomez, T. J.; Warp, E.; Tulyathan, O.; Aaron, H. L.; Isacoff, E. Y.; Yan, Y. L. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 17789–17794.
(59) Mao, S.; Benninger, R. K. P.; Yan, Y. L.; Petchprayoon, C.; Jackson, D.; Easley, C. J.; Piston, D. W.; Marriott, G. *Biophys. J.* **2008**, *94*, 4515–4524.
(60) Heilemann, M.; van de Linde, S.; Mukherjee, A.; Sauer, M. *Angew. Chem., Int. Ed.* **2009**, *48*, 6903–6908.
(61) Hell, S. W. *Science* **2007**, *316*, 1153–1158.
(62) Huang, B.; Bates, M.; Zhuang, X. W. *Annu. Rev. Biochem.* **2009**, *78*, 993–1016.