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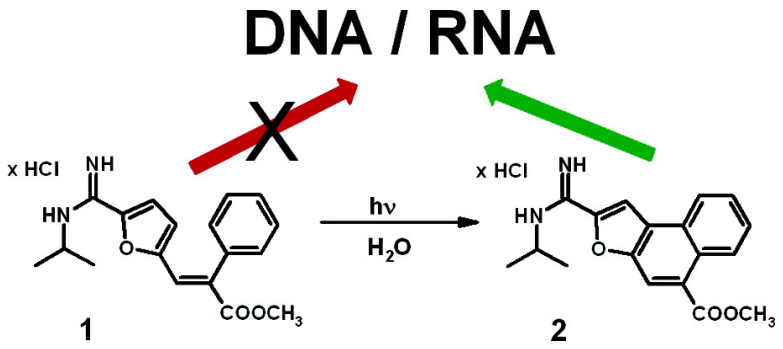
J. Am. Chem. Soc., **2005**, 127 (4), 1074-1075• DOI: 10.1021/ja0438866 • Publication Date (Web): 07 January 2005



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Published on Web 01/07/2005

Photoinduced Switch of a DNA/RNA Inactive Molecule into a Classical Intercalator

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The intercalation of planar aromatic or heteroaromatic molecules into the DNA/RNA is considered to be important for the therapeutic action of many antitumor agents.[1,2] One of the widely studied synthetic approaches to polycondensed aromatic compounds is the photoinduced dehydrocyclization of the 1,2-arenyl ethenes.[3] The two most important differences between reactant and product are the small aromatic surface and nonplanarity of the former, neither supporting intercalation into DNA, and the large, condensed, planar structure of the latter being favorable for intercalative binding.[2] These differences offer an intriguing possibility for a new concept of anticancer therapy based on the photoassisted transformation of an nonintercalating molecule into an intercalator which could be realized by local irradiation of tumor tissue containing nonintercalating acyclic reactant. This concept is essentially different from the one used in photodynamic therapy (PDT).[4,5] To the best of our knowledge, the only prior study of non-covalent interactions of photochromic compounds with DNA was performed in organic solvent/water mixtures and focused on utilization of DNA as the advanced matrix of functional materials.[6]

As a model of the photoinduced dehydrocyclization that should occur in aqueous media we have chosen the system presented in Scheme 1. According to our previous experience with the close analogues of *E*-3-(5-*N*- isopropylamidinium-2-furyl)-2-phenylacrylate (**1**), photoinduced transformation into methyl-2-(*N*-isopropyl)-amidinonaphtho[2,1-b]furan-5-carboxylate (**2**) should be irreversible and should proceed smoothly in protic solvents such as alcohols or water.[3] The amidinium group present in both **1** and **2** not only makes them readily soluble in water but also could actively participate in DNA/RNA interactions.[2] Conversion of larger amounts of **1** into **2**, necessary for a compound characterization, was done by irradiation with a high-pressure mercury arc lamp immersed into a Pyrex water-cooled coat (cut off light $\lambda < 300$ nm) in ethanol due to easier workup of crude product compared to aqueous solution.[7] Structures of both **1** and **2** were ascertained by detailed spectroscopic characterization and elemental analysis (Supporting Information). The electronic absorption spectra of **1** and **2** taken in water [**1**: λ_{max}/nm, (ϵ): 327, (26 540); **2**: 258, (17 630); 321, (15 570); 342, (15 520)] were found to be linearly concentration dependent up to 8×10^{-5} mol dm^{-3}, and the solutions were thermally stable up to 100 °C. In the pH range of 3.5−8.0, the absorption spectra of **1** and **2** do not change significantly, which is in agreement with the pK_a values of ∼8−9 reported for aromatic amidines.[8] Compounds **1** and **2** exhibit fluorescence emission with maxima at 422 and 419 nm, respectively, emission of the latter (Q(**2**) = 0.69) being more than 100 times stronger than emission of the former (Q(**1**) < 0.001; the value could be only estimated because of optical absorbance of sample higher than 0.05).

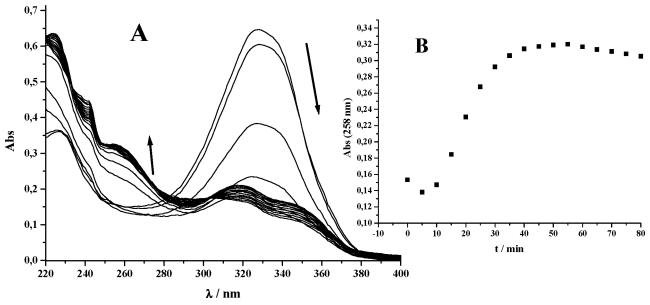
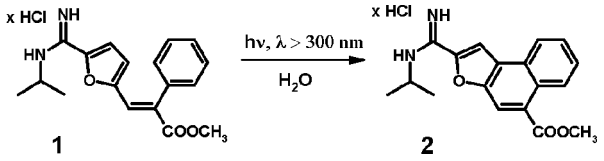


Figure 1. (A) Changes of UV/vis spectra of the aqueous solution of **1**, c(**1**) = 2.5×10^{-5} mol dm^{-3}, c(HCl) = 1×10^{-3} mol dm^{-3}, irradiated by a high-pressure Hg lamp (400 W) immersed into a Pyrex coat (cut off light $\lambda < 300$ nm). (B) Time-dependent increase at $\lambda_{max} = 258$ nm characteristic for **2**.

Scheme 1 Photoinduced Dehydrocyclization of **1** into **2** Occurring in Aqueous Media



Next, to prove that the photoinduced transformation of **1** into **2** can occur under biologically relevant conditions, the air-saturated aqueous solutions of **1** ($c = 2.5 \times 10^{-5}$ mol dm^{-3}) were irradiated with a high-pressure Hg lamp (400 W) immersed into a Pyrex water-cooled coat (cut off light $\lambda < 300$ nm). A significant difference in the UV/vis spectra of **1** and **2** ($\lambda_{max} = 258$ nm specific for **2**) has offered a simple and sensitive method to follow the progress of the reaction. The changes at $\lambda_{max} = 258$ nm were continuously followed using the through-flow quartz cell system (total volume $V = 2$ mL). Reactions undertaken in pure water (acidic due to dissolving **1** as HCl salt) and in diluted HCl ($c = 1 \times 10^{-3}$ mol dm^{-3}) were successful, giving rise to the UV maximum at $\lambda = 258$ nm characteristic of **2** (Figure 1A). The sharp maximum of **1** at $\lambda = 327$ nm changed into two peaks, with maxima at $\lambda =$ 321 and 342 nm, also agreeing well with the UV/vis spectrum of **2** (Figure 1A). In both solutions, cis−trans isomerization of **1** was the dominant reaction in the first 10 min (Figure 1B), followed by conversion of the cis isomer of **1** into product **2**. The reaction was completed in about 60 min (Figure 1B).

In the next set of experiments, the interactions of acyclic **1** and cyclic **2** with ct-DNA, polyA−polyU, and polyG−polyC were studied. Titrations of aqueous solutions of **2** with ct-DNA and ds-RNA polynucleotides resulted by significant batochromic and

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10.1021/ja0438866 CCC: $30.25

Table 1. Binding Constants (log K_s) and Ratios *n* ([Bound **2**]/[Polynucleotide Phosphate])[a] Calculated from the UV/Vis Titrations of **2** with ds-Polynucleotides at pH = 7.0 (Buffer Na−Cacodylate, I = 0.05 mol dm^{-3})

	ct-DNA	poly A–poly U	poly G–poly C
log K_s	4.9	4.3	4.9
n	0.22	0.22	0.10
H/%[c]	50	41	14
$\Delta\lambda_{342\ nm}$[d]	8 nm	8 nm	1 nm

[a] Accuracy of $n \pm 10-30\%$; consequently, log K_s values vary in the same order of magnitude. [b] Titration data were processed according to the Scatchard equation.[10] [c] Hypochromic effect; $H = [\mathrm{Abs}(\mathbf{2}) - \mathrm{Abs(complex)}]/\mathrm{Abs}(\mathbf{2}) \times 100$. [d] $\Delta\lambda_{342\ nm} = \lambda_{342\ nm}(\mathbf{2}) - \lambda_{342\ nm}$(complex).

Table 2. Melting Temperatures (ΔT_m/°C) of ct-DNA and Poly A−Poly U at Different Ratios *r* ([**2**]/[Polynucleotide Phosphate]), at pH = 7.0 (Buffer Na−Cacodylate, I = 0.05 mol dm^{-3})

r =	0.1	0.2	0.3
ct-DNA	1.0	2.0	2.5
poly A−poly U	1.0	1.2	1.6

hypochromic effects at $\lambda > 300$ nm in the UV/vis spectra of **2** (Table 1), both observed changes being characteristic for intercalation.[9] The appearance of isosbestic points in all UV/vis titrations strongly supported the presence of only two spectroscopically active species, namely, the free **2** and only one type of the **2**/DNA or **2**/RNA complex. Binding constants (K_s) and ratios *n* calculated from titrations (Table 1) are also in good agreement with those previously reported for a number of heteroaromatic small molecules that bind to ds-DNA and RNA by intercalation.[2,9]

Addition of all used polynucleotides efficiently quenched emission of **2**, the resultant complexes being nonfluorescent. Since fluorescence quenching can often happen for unexpected reasons (e.g., traces of transition metals in the solution), it is advisable to analyze emission changes in the complete spectra (range $\lambda = 400-500$ nm) by the multivariate least-squares program SPECFIT[11] to see if any other process was present except the compex formation observed by UV/vis experiments. Because of technical limitations of the SPECFIT program, the values of ratio *n* had to be fixed; we chose the values of *n* obtained in UV/vis titrations. Binding constants calculated from fluorescence titrations were found to be in the same order of magnitude as those obtained by UV/vis titrations. In contrast, the addition of the studied DNA and RNA ds-polynucleotides to the solutions of the opened analogue **1** under the same conditions failed to induce any measurable changes in the UV/vis and fluorescence spectra of **1**.

Addition of **2** to ct-DNA and polyA−polyU stabilized their double helices toward thermal denaturation (Table 2). The ΔT_m values do not increase proportionally with the increase of ratio $r_{[\mathbf{2}]/[\text{polynucleotide phosphate}]}$, which suggests saturation of dominant binding sites between $n = 0.2$ and 0.3, the values of ratio *n* that agree well not only with those obtained from UV/vis titrations (Table 1) but also with those common for intercalation.[9] Values of ΔT_m obtained for **2** (Table 2) are somewhat lower than those observed for classical intercalators;[2] however, such a result is not surprising because of the smaller aromatic surface of **2** compared to that of, for example, ethidium bromide or acridines. Under the same experimental conditions, thermal melting experiments were also performed with **1** but no effect on T_m values of either DNA or RNA polynucleotide was observed. Compounds **1** and **2** were also tested for the antiproliferative effect on a panel of six human cell lines, five of which were derived from five cancer types (Table 3). The observed results clearly demonstrate 1 order of magnitude superior action of **2** compared to **1**.

Table 3. In Vitro Growth Inhibition of Tumor Cells and Normal Human Fibroblasts (WI 38)

	IC_{50} (μM)[a]					
compd	HeLa	MCF-7	MiaPaCa-2	Hep-2	SW 620	WI 38
1	39 ± 43	78 ± 21	52.6 ± 0.9	60 ± 27	43 ± 45	50.3 ± 7
2	4.6 ± 0.7	5.4 ± 0.01	6 ± 0.3	5 ± 0.7	4.3 ± 0.2	7.2 ± 1.9

[a] IC_{50}; concentration that causes a 50% reduction of cell growth.

Results of UV/vis, fluorescence titrations, and thermal melting experiments strongly support intercalative binding of **2** to ds-polynucleotides. Additional support comes from the observation that analogue **1** failed to give any measurable interactions with ds-DNA and ds-RNA, thus pointing to the conclusion that the presence of the large condensed aromatic surface of **2** plays the essential role in binding to DNA/RNA. The differences in the interactions of **1** and **2** with polynucleotides are also reflected in the significantly more pronounced antiproliferative effects of **2** compared to **1** (Table 3).

In conclusion, the described experiments taken together present the conceptual basis for development of new photoinduced anticancer therapy based on the photochemical transformation on an nonintercalating molecule into the intercalating one. The conversion lasts only 60 min and is irreversible, thus leaving intercalating molecules of product to act further without outer excitation; this is a significant difference compared to common PDT therapy. An additional advantage of this concept lies in the fact that the photoinduced dehydrocyclization of the 1,2-arenyl ethenes is the widely studied reaction, offering thus access to a large number of already known or new molecules with selected properties compatible with physiologically relevant conditions. Studies of new systems capable of undergoing photoinduced dehydrocyclizations in water close to neutral pH and by visible light irradiation are in progress.

Acknowledgment. We thank the Ministry of Science, Education and Sport of the Republic of Croatia for financial support of this research. We are grateful to Dejana Carić from the Faculty of Agronomy, University of Zagreb, Croatia, for help in determination of relative fluorescence quantum yields (Q).

Supporting Information Available: Spectroscopic characterization and elemental analysis of novel compounds **1** and **2**. Detailed description of methods and material used in this work. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0438866