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# Photochemical Control of RNA Structure by Disrupting π-Stacking

Marino J. E. Resendiz,[†] Arne Schön,[‡] Ernesto Freire,[‡] and Marc M. Greenberg*[,†]

[†]Department of Chemistry and [‡]Department of Biology, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218, United States

Supporting Information

**ABSTRACT:** Photolabile nucleotides that disrupt nucleic acid structure are useful mechanistic probes and can be used as tools for regulating biochemical processes. Previous probes can be limited by the need to incorporate multiple modified nucleotides into oligonucleotides and in kinetic studies by the rate-limiting step in the conversion to the native nucleotide. Photolysis of aryl sulfide **1** produces high yields of 5-methyluridine, and product formation is complete in less than a microsecond. Aryl sulfide **1** prevents RNA hairpin formation and complete folding of the preQ$_1$ class I riboswitch. Proper folding is achieved in each instance upon photolysis at 350 nm. Aryl sulfide **1** is a novel tool for modulating RNA structure, and formation of 5-methyluridine within a radical cage suggests that it will be useful in kinetic studies.

Nucleic acids, particularly RNA, undergo conformational changes in their secondary and tertiary structure.[1−3] Complex structural changes occur during RNA folding, which in the case of a riboswitch is triggered by a ligand.[4] There is continuing interest in developing methods for detecting and controlling nucleic acid folding in order to provide a better mechanistic understanding of these processes.[5,6] In addition, methods for controlling nucleic acid hybridization and structure are also under development as tools for regulating gene expression and other biochemical processes.[7−9] Photochemistry is a popular tool for triggering nucleic acid structural changes because it provides spatiotemporal control and can be used in conjunction with time-resolved detection methods. One approach utilizes a modified nucleotide(s) in which a photolabile group alters the nucleobase's ability to form base pairs thus destabilizing the biopolymer's folded structure.[10,11] Ultraviolet irradiation restores the native nucleotide's structure. The *o*-nitrobenzyl photoredox reaction is often employed in these systems. This venerable photochemical reaction proceeds in high chemical yields and is very useful in preparative nucleic acid chemistry.[12] However, the need in some instances to incorporate multiple photolabile nucleotides in a single oligonucleotide to impart structural control is a limitation.[13] Furthermore, time-resolved studies are limited by the time scale (up to 1 min) on which the leaving group (e.g., the native nucleotide) is released from an intermediate formed following irradiation.[14] We report a new photolabile molecule that disrupts nucleic acid structure by perturbing base stacking. This molecule is amenable to use in applications that require a shorter time scale because its photolysis produces a native nucleotide via a radical pair that undergoes disproportionation within a solvent cage.

When designing **1** we took advantage of the precedent for electron-rich aryl sulfides undergoing carbon−sulfur bond homolysis upon irradiation within the same region of the aforementioned *o*-nitrobenzyl derivatives.[15,16] The methyl group was included to ensure that a substituent in the dihydropyrimidine would adopt a pseudoaxial orientation thus ensuring base stacking disruption. Molecular mechanics energy minimization indicated that the 5*R*-diastereomer of **1** would adopt a conformation in which the aryl sulfide adopts a pseudoaxial position while maintaining the C3′-endo conformation of the ribose ring that is present in A-form RNA (Figure 1). This is consistent with the expected larger A-value

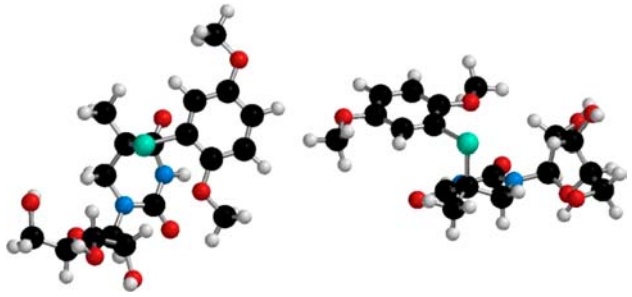

**Figure 1.** Energy minimized (Spartan) structure of photochemical substrate **1**. Two perspectives are shown to highlight different structural aspects of **1**.

of the methyl group and calculations on related dihydropyrimidines.[17] The 5*R*-diastereomer was examined because this was expected to be the major isomer upon sulfenylation (**3**) of the enolate of **2** (Scheme 1).[16,18] Indeed, a single isomer of **1** was obtained following deprotection of **3**, which was difficult to purify, and was carried on to the requisite phosphoramidite (**4**).

Photolysis (350 nm) of an aqueous solution of **1** (4 min) under aerobic conditions produced 5-methyluridine in 78% yield (85% mass balance). The yield of 5-methyluridine (MeU) was unaffected by β-mercaptoethanol (BME) at concentrations as high as 3.5 M, and increasing the BME to 7 M (50% by volume) only decreased the yield to 65%.[21] The inability of the thiol to compete with the disproportionation reaction suggests that 5-methyluridine is predominantly formed within a radical cage (Scheme 2). Importantly, if one assumes that BME reacts with the alkyl radical with a rate constant of at least $2.6 \times 10^6$

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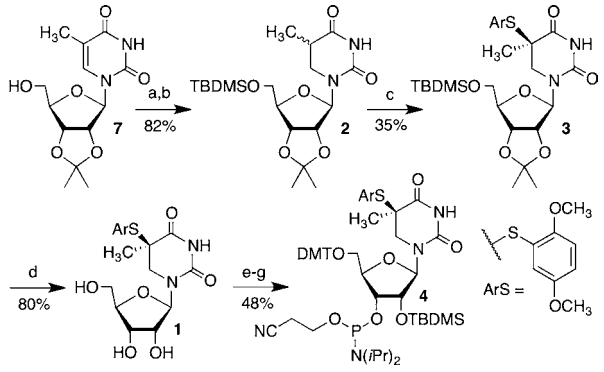
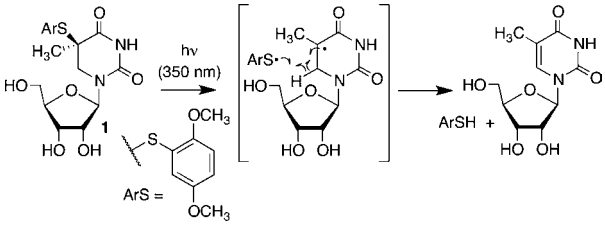
**Scheme 1**[a]



[a]Key: (a) $H_2$, $Rh/Al_2O_3$; (b) TDBMSCl, (c) LDA, disulfide; (d) TFA; (e) DMTCl; (f) TBDMSCl, $AgNO_3$, pyridine; (g) Phosphitylation.

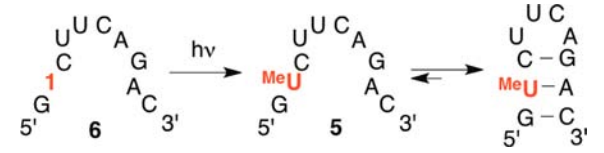
**Scheme 2**



$M^{-1}$ $s^{-1}$,[19] the lack of an effect of 3.5 M BME on the product yield indicates that 5-methyluridine is formed in less than a microsecond. This is several orders of magnitude more rapid than product release from the *o*-nitrobenzyl photochemical reaction and suggests that **1** could be used for probing conformational changes that occur on the microsecond time scale.[14]

The ability of **1** to disrupt secondary structure was initially examined in RNA hairpins (**5**–**8**) (Scheme 3). The hairpin

**Scheme 3**



sequences were chosen based upon a previous report in which the described systems could serve as a starting point such that incorporation of **1** would turn off hairpin formation.[20] Aryl sulfide **1** was introduced into the oligonucleotides (**6**, **8**) via standard solid phase synthesis methods using slight modifications for the coupling of **4** and subsequent deprotection of the biopolymers.[21] Oxidation was carried out using *t*-BuOOH in order to minimize sulfoxide and/or sulfide formation. The oligonucleotides containing **1** or MeU were separable from one another by denaturing and native polyacrylamide gel electrophoresis.[21] Photolysis for 10 min completely converted the modified oligonucleotides into ones that comigrated with the otherwise identical RNAs containing MeU.

| | |
|---|---|
| 5'-G$^{Me}$UC UUC AGA C | **5** |
| 5'-G**1**C UUC AGA C | **6** |
| 5'-GAG UUC AC$^{Me}$U C | **7** |
| 5'-GAG UUC AC**1** C | **8** |

The effect of **1** on secondary structure was analyzed using CD spectroscopy. Molecule **5** displayed all of the spectroscopic features at 25 °C that are characteristic of an RNA hairpin (Figure 2A).[22,23] These include intense bands displaying

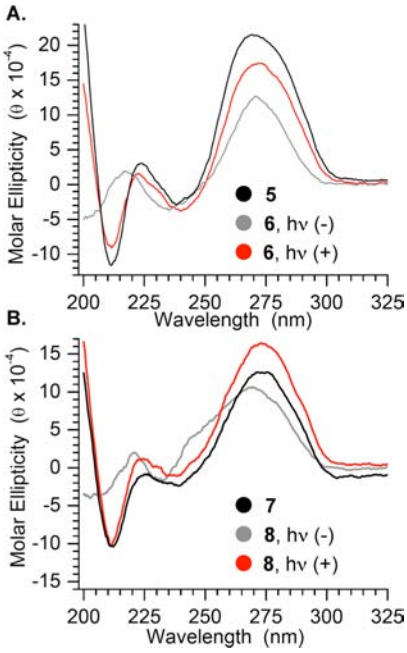



**Figure 2.** CD spectra of RNA oligonucleotides (65 $\mu$M) **5** and **6**. (A) **6** at 25 °C before and after h$\nu$ overlaid with **5**. (B) Comparable spectra for **7** and **8**.

positive ($\lambda_{max}$ = 272 nm) and negative ($\lambda_{max}$ = 212 nm) ellipticities and two weaker bands at (−) 240 and (+) 225 nm. Heating the sample resulted in hypochromicity of both of the signature bands at 272 and 212 nm, the analysis of which yielded a $T_m$ value of 43.1 ± 0.8 °C.[21] In contrast, CD spectra of **6**, which contains photolabile **1** instead of MeU, lacked the features that correspond to A-form RNA (Figure 2A), most notably the band at 212 nm. However, photolysis (20 min) of **6** yielded a product that exhibited a CD spectrum that contains the same bands as that obtained from **5** (Figure 2A). Further support for the successful photochemically induced transformation of **6** into **5** is gleaned from the $T_m$ (44.2 ± 1.1 °C) of the material produced upon photolysis of **6**, which is within experimental error of that measured for **5** (43.1 ± 0.8).[21] Comparing the CD spectra of **8** before and after photolysis with that of **7** shows the same overall effect (Figure 2B). Hence, a single molecule of aryl sulfide **1** disrupts hairpin formation but is readily converted to MeU, which promptly folds to yield CD spectra containing the same characteristic features as those produced from independently synthesized oligonucleotides containing MeU (**5**, **7**).

Successful modulation of secondary structure by **1** led us to examine its ability to control folding in a more complicated, biologically relevant molecule. The preQ$_1$ class I riboswitch specifically recognizes 7-aminomethyl-7-deazaguanine (preQ$_1$), which is an intermediate in queuosine biosynthesis. The aptamer region of this riboswitch contains only 34 nucleotides, and its folded structure from different species is well characterized.[24−28] In the absence of preQ$_1$ the receptor from *Fusobacterium nucleatum* contains a (5 bp) stem-loop region (Scheme 4).[24,27] This stem-loop is retained upon preQ$_1$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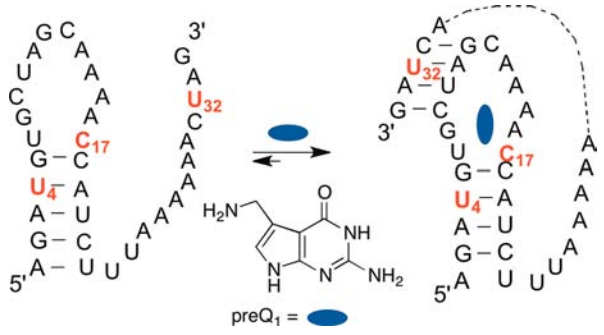
**Scheme 4**




binding, which also gives rise to pseudoknot formation. We examined the ability of **1** to modulate folding of the preQ$_1$ aptamer by substituting the dihydropyrimidine (in separate experiments) for uridines that are involved in pseudoknot ($U_{32}$) and stem formation ($U_4$).

Before examining the impact of **1** on the aptamer we investigated the effect of MeU substitution on folding. No difference was detected in the CD spectra of **9**–**11**, indicating that substituting MeU for uridine does not alter aptamer folding (Figure 3).[21,29] For instance, in the absence of preQ$_1$

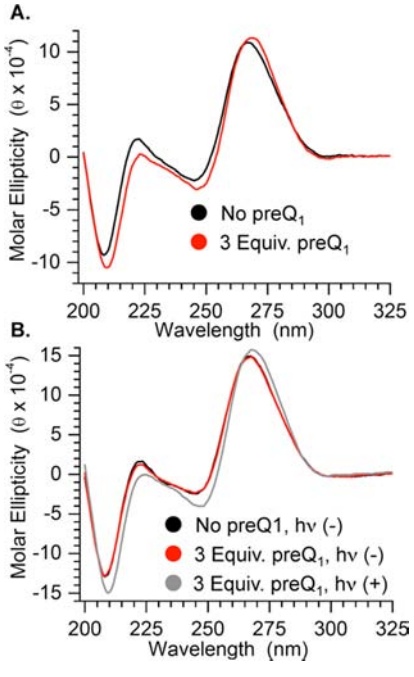



**Figure 3.** CD spectroscopy analysis of the effect of **1** on preQ$_1$ folding. (A) **11** with and without preQ$_1$ (3 equiv). (B) **13** with and without preQ1 (3 equiv) before h$\upsilon$ and with preQ$_1$ after h$\upsilon$. [RNA oligonucleotides] = 10 $\mu$M. Comparable spectra for **9**, **10**, and **12** are in the Supporting Information.

the CD spectrum of **11** is consistent with hairpin loop formation (Figure 3A). Addition of preQ$_1$ results in an increase in the intensity of the main bands, which is consistent with formation of a pseudoknot.[30,31] A slight bathochromic shift was also observed upon the addition of preQ$_1$ in each instance.

| | |
|---|---|
| 5′-AGA UGU GCU AGC AAA ACC AUC UUU AAA AAA CUA G | **9** |
| 5′-AGA $^{Me}$UGU GCU AGC AAA ACC AUC UUU AAA AAA CUA G | **10** |
| 5′-AGA UGU GCU AGC AAA ACC AUC UUU AAA AAA C$^{Me}$UA G | **11** |
| 5′-AGA **1**GU GCU AGC AAA ACC AUC UUU AAA AAA CUA G | **12** |
| 5′-AGA UGU GCU AGC AAA ACC AUC UUU AAA AAA C **1**A G | **13** |

The lack of an effect of preQ$_1$ on the CD spectra of RNA molecules containing **1** in place of either $U_4$ (**12**)[21] or $U_{32}$ (**13**, Figure 3B) indicates that the dihydropyrimidine prevents pseudoknot but not stem-loop formation. However, the dihydropyrimidine significantly destabilizes the stem region. In the absence of preQ$_1$ the (UV-melting) $T_m$ of **12** was 12 °C lower (46.2 ± 0.3 °C) than that of **10** (58.5 ± 0.3 °C). Photochemical conversion of **1** to MeU, which is complete by gel electrophoresis within 6 min, is accompanied by the anticipated change in **13**'s CD spectrum upon pseudoknot formation in the presence of preQ$_1$ (Figure 3B).[21]

In summary, we have developed an unnatural nucleotide (**1**) that modulates secondary and tertiary nucleic acid structure. The molecule is converted in high yield to 5-methyluridine upon photolysis via a process that occurs in less than a microsecond. We anticipate that **1** will be a useful tool in studying RNA folding and in developing photoregulated nucleic acid molecules.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Experimental procedures. Mass spectra of all oligonucleotides. Sample autoradiograms showing photochemical conversion of **1** in oligonucleotides. CD spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Corresponding Author

mgreenberg@jhu.edu

### Notes

The authors declare no competing financial interest.

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