Chemistry
A European
Journal

Chemistry Europe
European Chemical Societies Publishing

Accepted Article

Title: DNA Functionality with Photoswitchable Hydrazone Cytidine

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To be cited as: *Chem. Eur. J.* 10.1002/chem.202100742

Link to VoR: https://doi.org/10.1002/chem.202100742

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DNA Functionality with Photoswitchable Hydrazone Cytidine

Song Mao,[a,b] Zhihua Chang,[a,b] Ya Ying Zheng,[a,b] Alexander Shekhtman[a] and Jia Sheng*[a,b]

Abstract: A new family of hydrazone modified cytidine phosphoramidite building block was synthesized and incorporated into oligodeoxynucleotides to construct photoswitchable DNA strands. The *E*-*Z* isomerization triggered by the irradiation of blue light with a wavelength of 450 nm was investigated and confirmed by ^{1}H NMR and HPLC in the contexts of both nucleoside and oligodeoxynucleotides. The light activated *Z* form isomer of this hydrazone-cytidine with a six-member intramolecular hydrogen bond was found to inhibit DNA synthesis in the primer extension model by using *Bst* DNA polymerase. In addition, the hydrazone modification caused the misincorporation of dATP together with dGTP into the growing DNA strand with similar selectivity, highlighting the potential G to A mutation. This work provides a novel functional DNA building block and an additional molecular tool that have potential chemical biology and bio-medicinal applications to control DNA synthesis and DNA-enzyme interactions using cell friendly blue light irradiation.

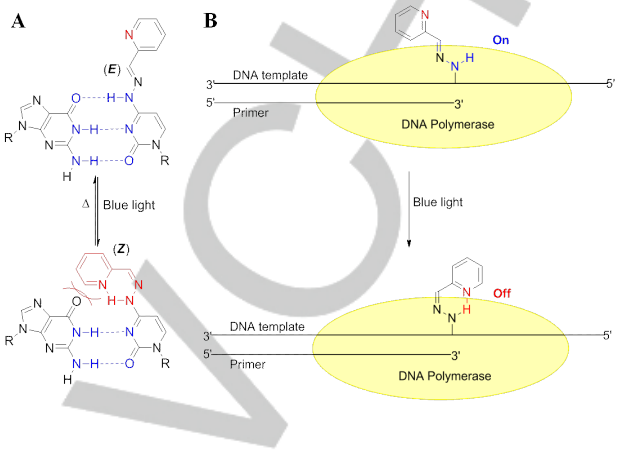

Figure 1. Schematic illustration of our photoswitchable hydrazone-DNA system and its application. (A) Light control of G:C base pair. (B) Regulation of DNA-Protein interaction and DNA synthesis.

Introduction

Deoxyribonucleic acid (DNA) carries the genetic information of living organisms and plays critical roles in numerous normal and diseased cellular functions and processes. In addition, it has tremendous application potentials in nanotechnology and advanced material science.[1] Since the elucidation of the double stranded helical structure of DNA,[2] immense work have been developed and utilized in the study of nucleic acid structure diversity and the impacts of the structural regulations on biological processes in the past few decades.[3] Other structures such as hairpin,[4] triplexes,[5] Holliday junction[6] and G-quadruplexes[7] have been demonstrated as natural approaches to regulate DNA functions. In addition, a variety of external stimuli, including temperature,[8] pH,[9] small molecule ligands,[10] metal ions[11] and light,[12] have been extensively developed to control DNA synthesis and structures thus to impact a wide range of biological processes such as DNA replication, RNA transcription, as well as the overall gene expression & repair. Among all the existing stimuli and triggers for chemical and biological manipulations of DNA, light seems to be an ideal and very attractive candidate because it is noninvasive and has a high level of spatial and temporal resolution for *in vivo* application.

In order to achieve the photoregulation of oligonucleotides, a wide range of photoswitches, a class of compounds that could undergo light-controlled reversible chemical conversions, have been incorporated into DNA and RNA oligonucleotides to control their structures and biological functions,[12f, 12h] owing to the well-developed nucleic acid solid phase synthesis in the past decades.[13] Among them, azobenzenes,[14] stilbenes,[12g] hemithioindigos and their derivatives[15] belong to the *cis-trans* isomerization category, while spiropyrans, diarylethenes, thiophenefulgides and their derivatives[16] have reversible open-and-closed states. Another regularly used DNA control approach is to use light sensitive caging groups to block the normal DNA structures and functions in some key positions and subsequently to release the inhibitory cages upon light irradiations for DNA recovery.[12d, 17] By taking advantage of the high modularity and synthetic accessibility of oligonucleotides, these photoregulatory functionalities could be installed or modified on nucleobases, sugar moiety and phosphate backbone of DNA. Despite the tremendous progress in DNA functionalities, however, the application of the photoregulation strategy is often limited by the use of UV light, which is toxic and damaging to DNA and the whole cells.[18] In this content, the development of novel photocontrollable chromophores that could use nonhazardous visible lights to trigger the photo conversions on DNA has recently emerged as a very important challenge.

Hydrazones is a kind of well-developed chromophore that can undergo *E*/*Z* isomerizations in response to photochemical or thermal stimuli with high conversion yields to both states.[19] They have been widely used in medicinal[20] and adaptive materials developments.[21] Particularly, the pyridyl hydrazone unit, initially developed by Lehn and coworkers,[22] has attracted great attentions due to the fact that 2-pyridyl ring can form a six-membered intramolecular hydrogen bond with the amide N-H of the hydrazone in the *Z* form upon irradiation with blue light with the wavelength of ~450 nm.[23] Inspired by this property, we designed the 2-pyridyl hydrazone on the N4 position of cytidine

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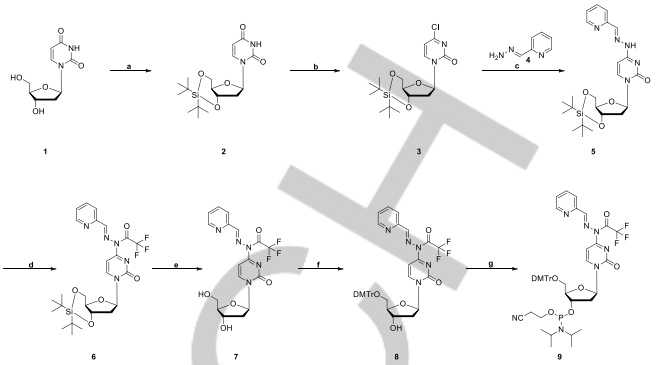
and incorporated it into oligodeoxynucleotides. We hypothesize that the formation of intramolecular H-bond with pyridine ring upon blue light irradiation could cause steric hindrance with the carbonyl group of guanosine and perturb the Watson-Crick C:G base pair (**Figure 1A**). Before light exposure, this hydrazone modified nucleoside keeps the stable *E* form and retains the normal Watson-Crick G:C pairing with three hydrogen bonds, which still could be recognized by enzymes. After light exposure, the 2-pyridyl hydrazone switches to the *Z* form and disrupts the base pair by the six-membered intramolecular hydrogen bonding, this incomplete G:C pair may not be recognized by enzymes. This *E*-*Z* conversion process can be controlled by light and heat. In addition, we also demonstrated that this light controlling mechanism can be used to affect DNA-protein interactions and further regulate DNA synthesis during gene replication (**Figure 1B)**.

Results and Discussion

Chemical synthesis of photoswitchable hydrazone phosphoramidite building block and its containing oligodeoxynucleotides

We started the synthesis of target photoswitchable hydrazone modified cytidine phosphoramidite from commercially available deoxyuridine **1** (**Scheme 1**). Silylation of the 5′- and 3′-hydroxy groups with di-*tert*-butylsilyl ditriflate gave silylated compound **2**, which was converted to compound **3** *via* Appel reaction in the presence of PPh_3 and CH_2Cl_2/CCl_4. The coupling of pyridine-2-aldehyde hydrazone **4** with **3** in the absence of base provided the hydrazone modified nucleoside **5**, which was further protected with the trifluoroacetyl group at N4 position to afford compound **6**. The trifluoroacetyl group, instead of the normally used acetyl group, was chosen because of its easier deprotection in the context of oligonucleotides. Subsequently, this compound was desilylated by using hydrogen fluoride in pyridine (HF•Py), followed by the tritylation step with trityl chloride (DMTrCl) at 5′-position to generate the key intermediate **8**, which was converted into the final photoswitchable hydrazone phosphoramidite **9** through a regular phosphitylation reaction for the solid-phase synthesis. This building block **9** was unstable in light and was therefore purified by quickly flushing over an aluminum foil covered SiO_2 column, dissolved in acetonitrile under an argon atmosphere, and immediately used in DNA synthesizer. This hydrazone phosphoramidite building block was well compatible with the regular solid-phase synthesis conditions, including trichloroacetic acid (TCA) and oxidative iodine treatments. The modified oligodeoxynucleotides were also found to be fully deprotected and stable under basic cleavage from solid-phase beads. As a demonstration, various strands of oligodeoxynucleotides containing this hydrazone chromophore were synthesized, purified and confirmed by Orbitrap MS, as shown in **Table 1**.



Scheme 1. Synthesis of photoswitchable hydrazon-deoxycytidine phosphoramidite **9**. Reagents and conditions: (a) $(t\text{-Bu})_2Si(OTf)_2$, imidazole, DMF; (b) PPh_3, CH_2Cl_2/CCl_4; (c) pyridine-2-aldehyde hydrazone **4**, DMF; (d) TFAA, DCM; (e) HF•Py, THF; (f) DMTrCl, Py; (g) $(i\text{-Pr}_2N)_2P(Cl)OCH_2CH_2CN$, $(i\text{-Pr})_2NEt$, 1-methylimidazole, CH_2Cl_2.

Table 1. DNA sequences containing hydrazone photoswitch.

Entry	DNA Sequences	Measured (calc.) m/z
ON1	5'-AATGC**C***GCACTG-3'	$[M+H^+]^+$ = 3734.7 (3734.7)
ON2	5'-GGACT**C***CTGCAG-3'	$[M+H^+]^+$ = 3750.7 (3750.7)
ON3	5'-T**C***GTACGA-3'	$[M+H^+]^+$ = 2513.5 (2513.5)
ON4	5'-CCGG**C***GCCGG-3'	$[M-H^+]^-$ = 3132.6 (3132.6)
ON5	5'-GAA**C***GCTATGAGGACATGGCAGCCTTC-3'	$[M+H^+]^+$ = 8413.5 (8413.5)
ON6	5'-AT**C***ACG-3'	$[M+H^+]^+$ = 1880.4 (1880.4)

E/Z isomerization studies in contexts of both nucleoside and DNA oligonucleotide

We first demonstrated the photoswitchable *E*/*Z* isomerization of this hydrazone cytidine by using a solution of 85 mM compound **5-*E*** in $CDCl_3$ irradiated with a 450 nm blue light and monitoring the conversion by ^1H NMR. As the spectra shown in **Figure 2A**, after 2 hours, 89% of isomer **5-*Z*** with a strong H-bond between imino proton of the hydrozone and the nitrogen of the pyridine at 14.58 ppm was obtained, which is consistent with our TLC study (**Figure S29**). Meanwhile, the characteristic imine proton peak of the ***5-E*** (peak 5) with chemical shift of 8.70 ppm gradually shifted to 7.35 ppm in ***5-Z***. The time-course ratios of *Z*:*E* at 10 min, 30 min and 60 min were also monitored respectively. We further investigated the back isomerization from **5-*Z*** to **5-*E*** under different temperatures. As a result, heating the samples at 37 ºC under dark for 24 h only gave 47:53 ratio of *E*:*Z* (**Figure S30**), while a maximum ratio of 67:33 was observed after heating the solution at 60 ºC for 48 h (**Figure S31**), indicating that this six-member intramolecular hydrogen bonding structure is quite stable once formed by blue light irradiation.

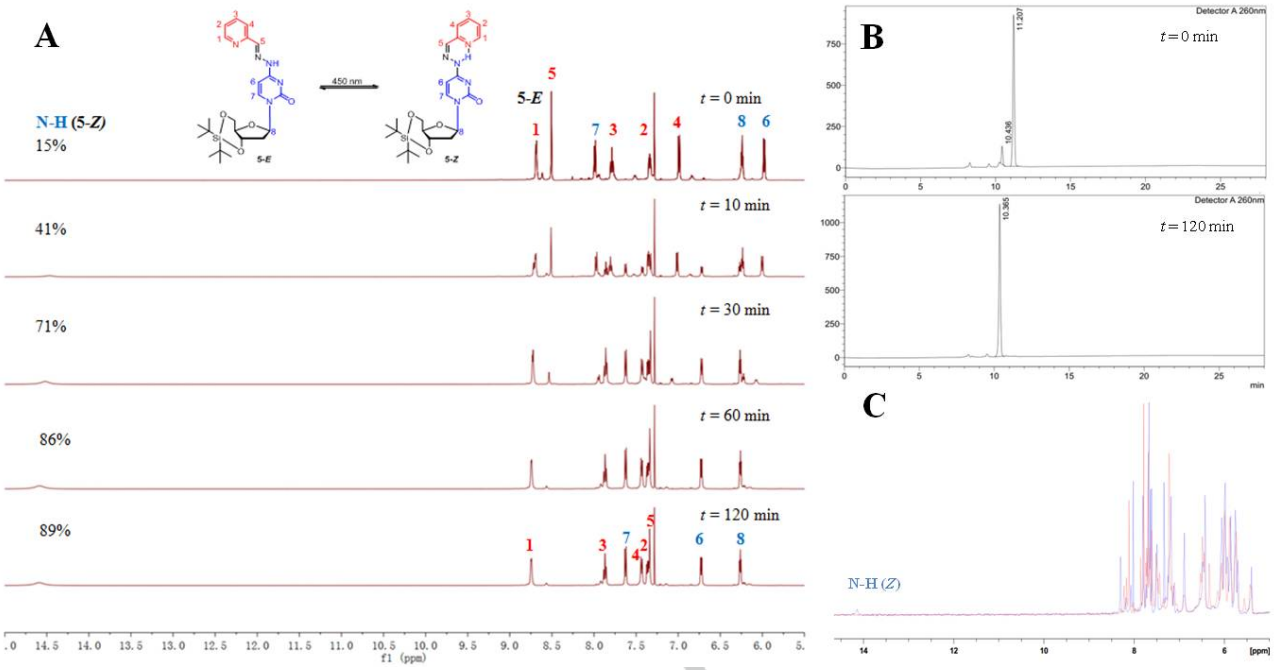


Figure 2. (A) 500 MHz ^{1}H NMR spectra of a 85 mM solution in $CDCl_3$ of **5-*E*** under 450 nm blue light irradiation for different time. (B) Analytical HPLC spectra of **ON6** 5′-AT**C***ACG-3′ before and after a 450 nm blue light irradiation, with the retention time of 11.2 and 10.4 mins respectively. (C) 600 MHz ^{1}H NMR spectra of a 100 μM of **ON6** 5′-AT**C***ACG-3 in 10 mM sodium phosphate buffer (pH = 6.5) with 50 mM NaCl containing 10% D_2O before (red) and after (blue) the blue light irradiation. Water gate pulse sequence was used for water suppression during ^{1}H NMR experiment[24].

We next examined whether similar hydrogen bonded *Z* form isomer can be formed in DNA contexts. As the example, a short 6-mer DNA **ON6 (Table 1)** 5′-AT**C***ACG-3′ with the hydrazone modification in the middle position was evaluated by analytical HPLC and ^{1}H NMR. In the initial state, a mixture of *E*:*Z* isomers with the ratio of 91:9 was determined by analytical HPLC (**Figure 2B**). Upon 2 h irradiation with a 450 nm blue light, over 99% of *Z* isomer was ideally achieved. And the ^{1}H NMR spectrum (**Figure 2C**) in the aqueous solution, 10 mM sodium phosphate buffer (pH = 6.5) with 50 mM NaCl containing 10% D_2O, showed the hydrazone N-H imino proton signal of *Z* isomer at 14.13 ppm, indicating the intramolecular H-bond between the imino proton of the hydrozone and the nitrogen of the pyridine can also form and is more dominant in the oligodeoxynucleotides than in the nucleoside form. In addition, the hydrogen bonded *Z* form isomer in DNA is quite stable. No isomerization of *Z* back to *E* was observed from the analytical HPLC profiles after heating the solution at 37 ºC for one hour under dark condition (**Figure S32-S33**).

Control of DNA synthesis with hydrazone photoswitch in primer extension reactions

DNA replication occurs in all living organisms and acts as the most essential part for biological inheritance. Chemical modifications on DNA polymerase[25] and DNA nucleotides[11g, 26] have been developed for controlling DNA synthesis. Having demonstrated the intramolecular H-bond *Z* isomer can be formed in both nucleoside and oligodeoxynucleotides, we further set out to investigate the potential impacts of this hydrazone modification on DNA synthesis by conducting the template directed primer extension reactions as the DNA replication model. As shown in **Figure 3**, the 5′-end of DNA primer was labeled with fluorescent FAM group and the 27nt-long modified DNA (**ON5, Table 1**) was designed as the template with hydrazone on the starting site of the replication reaction, which represents a direct and effective way to explore the enzymatic compatibility and coding property of the modified residue in this biological process. The yields and fidelity of DNA synthesis with different base pairing substrates in the presence of two different example DNA polymerases, the Bacillus stearothermophilus (*Bst*) DNA polymerase[27] and *DreamTaq* DNA polymerase,[28] were quantitated by fluorescence gel images with single-nucleotide resolution.

Primer extension reactions

DNA primer 5'-FAM- CCATGTCCTCATAGC
DNA template 3'-CTTCCGACGGTACAGGAGTATCG**C***AAG-5'

C*: either C or hydrazone modified C

Figure 3. Primer extension reaction.

When the *Bst* DNA polymerase was used in this system, the primer extension reaction completed with high yield of 27nt-full length product in the presence of all the natural dNTPs with native template (lane Nat, natural template with all four dNTPs, in **Figure 4A**), while the template with hydrazone modification before light exposure can also give the full-length product with moderate yield and some uncompleted short DNA products (lane M, hydrazone-modified template before blue light irradiation with all four dNTPs, in **Figure 4A**). Strikingly, after irradiating the template with a 450 nm blue light for 2 hours, the DNA synthesis was fully shut down without the formation of any full-length product (lane M-hv, hydrazone-modified template after 2 h blue light irradiation with all four dNTPs, in **Figure 4A**), indicating the resulting *Z* isomer with intramolecular H-bond dramatically inhibited the polymerase activity in this DNA synthesis. Next, the *DreamTaq* DNA polymerase was examined in the same system. As expected, the full-length product was obtained in similar yield as the *Bst* case in the absence of light stimulus (lane M in **Figure 4B**), although some over synthesized DNA bands were observed, similar as in the native conditions (lane Nat). After the blue light irradiation, although the overall yield of full-length product was significantly decreased, in comparison to the *Bst* case, a much lower level of synthesis impedance of this isomerization was detected (lane M-hv in **Figure 4B**). It is known that the *DreamTaq* DNA polymerase has higher flexibility and substrate compatibility, which might result in lower replication fidelity for target DNA synthesis. Indeed, we further investigated the single nucleotide incorporation under these conditions and found that the hydrazone modification dramatically decreased the dGTP incorporation efficiency and increased the dATP incorporation to the same level as dGTP during the first nucleotide synthesis in the presence of either DNA polymerase (lanes A and G in **Figure 5** and **S35**), implying a G to A mutation in the synthesized DNA. This process is not associated with the light irradiation (**Figure 5B** and **5C**, **S34B** and **S34C**).

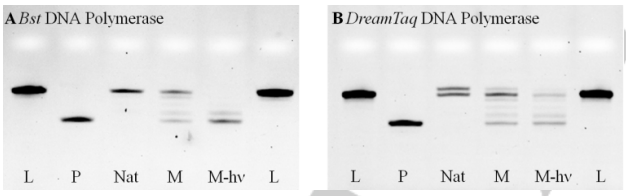


Figure 4. Fluorescent gel images of standing-start primer extension reactions for *Bst* **(A)** and *DreamTaq* **(B)** DNA polymerase using native and hydrazone-modified DNA templates. Lanes: L, reference DNA 27-mer ladder; P, primer; Nat, natural template with all four dNTPs; M, hydrazone-modified template before blue light irradiation with all four dNTPs; M-hv, hydrazone-modified template after 2 h blue light irradiation with all four dNTPs.

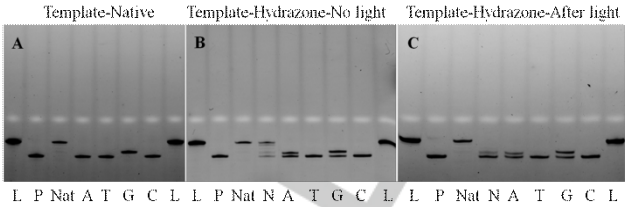


Figure 5. Fluorescent gel images of primer extension reactions with *Bst* DNA polymerase using native (**A**), hydrazone-modified DNA templates before (**B**) and after (**C**) blue light irradiation. Lanes: L, reference DNA 27-mer ladder; P, primer; Nat, natural template with all four dNTPs as positive controls in each gel; A, T, G, and C, reactions in the presence of the respective dNTP only; N, reactions in the presence of all the four dNTPs.

Conclusions

In conclusion, the hydrazone modified cytidine phosphoramidite building block was synthesized and successfully incorporated into the oligodeoxynucleotides by using solid phase synthesis to construct photoswitchable DNA strands. The *E-Z* isomerization triggered by the irradiation of blue light with a wavelength of 450 nm was investigated and confirmed by ^{1}H NMR and HPLC in the contexts of both nucleoside and oligodeoxynucleotides. The light activated *Z* form isomer of this hydrazone-cytidine with a six-member intramolecular hydrogen bond was found to inhibit DNA synthesis in our primer extension model in the presence of *Bst* DNA polymerase. When using the *DreamTaq* DNA polymerase, this photoswitchable hydrazone modification partially impeded the DNA synthesis to a lower extent than the *Bst* one. In addition, this hydrazone modified cytidine in the template could cause the misincorporation of dATP together with dGTP into the growing DNA strand with similar selectivity. This work provides a novel functional DNA building block and an additional molecular tool that can be exploited for the control of DNA synthesis and DNA-enzyme interactions using cell friendly blue light irradiation.

Experimental Section

Materials and general procedures of synthesis

Anhydrous solvents were used and redistilled using standard procedures. All solid reagents were dried under a high vacuum line prior to use. Air sensitive reactions were carried out under argon. RNase-free water, tips and tubes were used for RNA purification and thermodynamic studies. Analytical TLC plates pre-coated with silica gel F254 (Dynamic Adsorbents) were used for monitoring reactions and visualized by UV light. Flash column chromatography was performed using silica gel (32-63 μm). All ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker 500 MHz spectrometer. Chemical shift values are in ppm. ^{13}C NMR signals were determined by using APT technique. High-resolution MS were achieved by ESI at University at Albany, SUNY. Irradiation of isomerization *E* to *Z* was carried out using a 12W PAR38 Blue LED flood lamps from ABi LED lighting.

Synthesis of photoswichable hydrazone phosphoramidite

3',5'-O-di-tert-butylsilylene-2'-deoxyuridine **2**. To a solution of deoxyuridine **1** (5.3 g, 23.2 mmol) in DMF (60 mL) was added di-*tert*-butylsilyl-bis(trifluoromethanesulfonate) (9.6 mL, 25.5 mmol) at 0 °C. After 30 min of stirring at room temperature, imidazole (4 g, 58 mmol) was added. The resulting mixture was stirred for another 1 h at room temperature. After removal of DMF under high vacuum, the residue was dissolved in ethyl acetate (300 mL), washed with water (200 mL), 5% $NaHCO_3$ (200 mL) and brine (200 mL), and dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by silica gel chromatography to give compound **2** (7.8 g, 21.2 mmol, 91% yield) as a white solid. TLC R_f = 0.5 (50% ethyl acetate in hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.26 (s, 1H), 7.26 (d, *J* = 8.5 Hz, 1H), 6.19 (dd, *J* = 7.5, 3.5 Hz, 1H), 5.78 (dd, *J* = 8.0, 1.0 Hz, 1H), 4.44 (dd, *J* = 9.5, 5.0 Hz, 1H), 4.21-4.15 (m, 1H). 4.01-3.97 (m, 1H), 3.72-3.67 (m, 1H), 2.43-2.33 (m, 2H), 1.06 (s, 9H), 1.00 (s, 9H).

4-chloro-1-((4aR,6R,7aS)-2,2-di-tert-butyltetrahydro-4H-furo[3,2-d][1,3,2]dioxasilin-6-yl)pyrimidin-2(1H)-one **3**. A mixture of compound **2** (3.7 g, 10 mmol) and PPh_3 (6.5 g, 25 mmol) in CH_2Cl_2 (60 mL) and CCl_4 (60 mL) was refluxed for 3 h at 70 °C. After cooling down to room temperature, the solvent was removed under reduced pressure. The residue was purified by flash silica gel chromatography to give compound **3** (1.5 g, 3.9 mmol, 39% yield) as a white solid. TLC R_f = 0.8 (50% ethyl acetate in hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, *J* = 7.0 Hz, 1H), 6.42 (d, *J* = 7.0 Hz, 1H), 6.05 (dd, *J* = 2.5, 6.5 Hz, 1H), 4.49 (dd, *J* = 5.0, 9.5 Hz, 1H), 4.05-3.99 (m, 1H), 3.83-3.77 (m, 1H), 2.48-2.44 (m, 2H), 1.02 (s, 9H), 0.99 (s, 9H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 166.8, 152.9, 142.8, 105.2, 86.5, 78.7, 73.9, 67.3, 39.2, 27.4, 27.1, 22.7, 20.1.

1-((4aR,6R,7aS)-2,2-di-tert-butyltetrahydro-4H-furo[3,2-d][1,3,2]dioxasilin-6-yl)-4-((E)-2-(pyridin-2-ylmethylene)hydrazinyl)pyrimidin-2(1H)-one **5**. A mixture of compound **3** (1.5 g, 3.8 mmol) and compound **4** (460 mg, 3.8 mmol) in DMF (5 mL) was stirred overnight at room temperature. The solution was diluted with DCM (200 mL) and washed with water (100 mL) and brine (100 mL), and dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by silica gel chromatography to give compound **5** (1.4 g, 3.0 mmol, 79% yield) as a yellow solid. TLC R_f = 0.3 (5% methanol in dichloromethane). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (d, *J* = 4.5 Hz, 1H), 8.51 (s, 1H), 7.99 (d, *J* = 7.5 Hz, 1H), 7.81-7.77 (m, 1H), 7.35-7.33 (m, 1H), 6.99 (d, *J* = 8.5 Hz), 6.26-6.22 (m, 1H), 5.98 (d, *J* = 8.5 Hz), 4.46 (dd, *J* = 9.5, 5.0 Hz, 1H), 4.25-4.19 (m, 1H), 4.04-3.99 (m, 1H), 3.72-3.66 (m, 1H), 2.40-2.36 (m, 2H), 1.09 (s, 9H), 1.03 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.2, 153.1, 152.8, 149.6, 148.7, 136.8, 134.8, 124.6, 122.2, 101.4, 83.6, 77.9, 74.9, 67.4, 38.4, 27.4, 27.1, 22.7, 22.1. HRMS (ESI-TOF) $[M+H]^+$ = 472.2398 (calc. 472.2380). Chemical formula: $C_{23}H_{33}N_5O_4Si$.

(E)-N-(1-((4aR,6R,7aS)-2,2-di-tert-butyltetrahydro-4H-furo[3,2-d][1,3,2]dioxasilin-6-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-2,2,2-trifluoro-N'-(pyridin-2-ylmethylene)acetohydrazide **6**. To a solution of compound **5** (942 mg, 2 mmol) in DCM (20 mL) was added TFAA (1.4 mL, 10 mmol) at 0 °C. The resulting solution was stirred overnight at room temperature. The reaction was quenched with sat. $NaHCO_3$ and extracted with DCM. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by silica gel chromatography to give compound **6** (700 mg, 1.2 mmol, 60% yield) as a yellow solid. TLC R_f = 0.6 (50% ethyl acetate in hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64-8.60 (m, 1H), 7.80-7.66 (m, 2H), 7.37-7.30 (m, 1H), 7.01-6.93 (m, 2H), 6.10-5.96 (m, 2H), 4.43-4.38 (m, 1H), 4.18-4.09 (m, 1H), 3.99-3.93 (m, 1H), 3.64-3.58 (m, 1H). 2.31-2.28 (m, 1H), 2.25-2.20 (m, 1H), 1.06 (d, 9H), 0.98 (d, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.0, 151.4, 150.1, 147.5, 145.5, 137.1, 135.7, 124.9, 124.7, 115.8 (q, *J* = 282.0 Hz), 94.3, 83.4, 77.9, 75.6, 74.8, 67.3, 38.4, 27.4, 27.1, 22.7, 20.1. HRMS (ESI-TOF) $[M+H]^+$ = 568.2203 (calc. 568.2252). Chemical formula: $C_{25}H_{32}F_3N_5O_5Si$.

(E)-2,2,2-trifluoro-N-(1-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-N'-(pyridin-2-ylmethylene)acetohydrazide **7**. To a solution of compound **6** (567 mg, 1 mmol) in THF (20 mL) at 0 °C was added a solution of hydrogen fluoride-pyridine complex (hydrogen fluoride ~70%, pyridine ~30%, 0.2 mL) in pyridine (1.5 mL). After 1 h at 0 °C, the reaction was complete and pyridine (5 mL) was added. After concentration under reduced pressure, the residue was purified by silica gel chromatography to give compound **7** (330 mg, 0.77 mmol, 77% yield) as a white solid. TLC R_f = 0.3 (10% methanol in dichloromethane). ^{1}H NMR (500MHz, CD_3OD) δ 8.59-8.56 (m, 1H), 7.92-7.87 (m, 1H), 7.77-7.68 (m, 2H), 7.45-7.41 (m, 1H), 7.11 (d, *J* = 9.5 Hz, 1H), 6.19-6.11 (m, 1H), 6.0 (dd, *J* = 8.0, 1.0 Hz, 1H), 4.39-4.35 (m, 1H), 3.90-3.85 (m, 1H), 3.78-3.68 (m, 2H), 2.21-2.07 (m, 2H). ^{13}C NMR (125 MHz, CD_3OD) δ 152.3, 151.3, 149.5, 148.1, 146.0, 137.4, 136.9, 124.8, 124.7, 116.0 (q, *J* = 283.9 Hz), 92.7, 87.4, 84.6, 75.8, 71.0, 61.6, 39.4. HRMS (ESI-TOF) $[M+H]^+$ = 428.1188 (calc. 428.1182). Chemical formula: $C_{17}H_{16}F_3N_5O_5$.

(E)-N-(1-((2R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxytetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-2,2,2-trifluoro-N'-(pyridin-2-ylmethylene)acetohydrazide **8**. To a solution of compound **7** (300 mg, 0.7 mmol) in dry pyridine (5 mL) was added 4,4'-Dimethoxytrityl chloride (DMTrCl, 475 mg, 1.4 mmol) under dark. The resulting solution was stirred overnight at room temperature under dark. The reaction was quenched with methanol (1 mL) and stirred for another 5 min. After concentration concentrated under vacuum, the residue was purified by silica gel chromatography to give compound **8** (412 g, 0.56 mmol, 81% yield) as a white solid. TLC R_f = 0.5 (50% ethyl acetate in dichloromethane). ^{1}H NMR (500 MHz, CD_3OD) δ 8.56-8.53 (m, 1H), 7.88-7.82 (m, 1H), 7.55 (dd, *J* = 7.0, 1.5 Hz, 1H), 7.45-7.38 (m, 3H), 7.33-7.29 (m, 6H), 7.27-7.22 (m, 1H), 7.06 (d, *J* = 11.0 Hz, 1H), 6.90-6.87 (m, 4H), 6.16-6.07 (m, 1H), 5.59 (dd, *J* = 12.0, 8.0 Hz, 1H), 4.51-4.47 (m, 1H), 4.17-4.10 (m, 1H), 3.96-3.92 (m, 1H), 3.78 (s, 6H), 3.53-3.46 (m, 1H), 3.42-3.35 (m, 1H), 2.29-2.14 (m, 2H). ^{13}C NMR (125 MHz, CD_3OD) δ 172.1, 158.9, 152.2, 149.5, 148.3, 145.8, 144.7, 137.4, 136.9, 135.5, 135.3, 130.1, 130.0, 128.0, 127.5, 126.7, 124.8, 124.6, 115.9 (q, *J* = 283.9 Hz), 112.9, 92.5, 86.7, 86.1, 84.7, 75.8, 70.7, 65.8, 63.2, 60.2, 54.4, 39.7. HRMS (ESI-TOF) $[M+H]^+$ = 730.2509 (calc. 730.2489). Chemical formula: $C_{38}H_{34}F_3N_5O_7$.

(2R,3S,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(2-oxo-4-((E)-2-(pyridin-2-ylmethylene)-1-(2,2,2-trifluoroacetyl)hydrazinyl)pyrimidin-1(2H)-yl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite **9**. To a solution of compound **8** (170 mg, 0.24 mmol) in DCM (5 mL) was added N,N-di-*iso*-propylethylamine (0.15 mL, 1.2 mmol), 1-Methylimidazole (0.041 mL, 0.24 mmol)and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.11 mL, 0.72 mmol). The resulting solution was stirred overnight at room temperature under argon gas. The reaction was quenched with water and extracted with ethyl acetate. After drying the organic layer over Na_2SO_4 and concentration. The residue was purified by flash silica gel chromatography to give compound **9** (147 mg, 0.16 mmol, 67% yield) as a white solid. TLC R_f = 0.5 (50% ethyl acetate in hexane). ^{31}P NMR (202 MHz, $CDCl_3$) δ 149.00, 148.60. HRMS (ESI-TOF) $[M+H]^+$ = 930.3608 (calc. 930.3567). Chemical formula: $C_{47}H_{51}F_3N_7O_8P$.

Synthesis of the oligodeoxynucleotides **containing hydrazone photoswitch**

All the oligodeoxynucleotides were chemically synthesized at 1.0 μmol scales by solid phase synthesis using the Oligo-800 synthesizer. The photoswitchable hydrazone phosphoramidite was dissolved in acetonitrile to a concentration of 0.2 M. I_2 (0.02 M) in THF/Py/H_2O solution was used as an oxidizing reagent. Coupling was carried out using 5-ethylthio-1H-tetrazole solution (0.25 M) in acetonitrile for 12 min, for both native and modified phosphoramidites. About 3% trichloroacetic acid in methylene chloride was used for the 5′-detritylation. Synthesis was performed on control-pore glass (CPG-500) immobilized with the appropriate nucleoside through a succinate linker. All the reagents used are standard solutions obtained from ChemGenes Corporation. All the oligonucleotides were prepared in DMTr-on form. After synthesis, the oligodeoxynucleotides were cleaved from the solid support and fully deprotected with 1:1 v/v ammonium hydroxide solution (28% NH_3 in H_2O) and Methylamine (40% w/w aqueous solution) at 65 °C for 2.5 h. After removal the ammonium and methylamine under Speed-Vac concentrator, all DNA samples in aqueous solution were further purified by reverse HPLC.

The DNA oligocleotides were analyzed and purified by reverse HPLC. Purification was carried out using a 21.2x250 mm, 10 μm Ultimate XB-C18 column at a flow rate of 6 mL/min. Buffer A contained 5% acetonitrile in 20 mM triethylammonium acetate (TEAAc, pH 7.1), while buffer B contained 50% acetonitrile in 20 mM triethylammonium acetate (TEAAc, pH 7.1). The DMTr-on oligonulceotides were eluded and purified in a linear gradient reaching 100% buffer B in 20 min. The collected fractions were lyophilized and the purified compounds were re-dissolved in fresh water. The 5′-DMTr deprotection was performed in a 3% trichloroacetic acid solution and desalted on Sep-Pac C18 columns, and re-concentrated the samples. ON6 was further purified in a linear gradient reaching 100% buffer B in 40 min by the 21.2*250 mm, 10 μm Ultimate XB-C18 column at a flow rate of 6 mL/min. Analytical HPLC was performed on a 4.6x250 mm, 5 μm Ultisil XB-C18 column at a flow rate of 1 mL/min, in a linear gradient reaching 100% buffer B in 20 min. Orbitrap MS is used to characterize all DNA samples.

Primer extension assays

The primer extension reactions were performed with *Bst* DNA polymerase (ThermoFisher), and *DreamTaq* DNA polymerase (ThermoFisher) in 10 μL total solution containing 1X corresponding buffer: 20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, and 0.1% Triton X-100 at pH 8.8@ 25 °C, the 5′-FAM-labeled primer was hybridized with each template (before and after 2 h irradiation with a 450 nm blue light of ON5 was used here) at a molar ration of 1:2 and first incubated for 30 min at room temperature. Final reaction mixtures contained DNA template (10 μM), DNA FAM-primer (5 μM) and dNTP (2 mM). After addition of each DNA polymerases: *Bst Pol.* (8 U) and *DreamTaq Pol.* (5 U), the mixtures were incubated at 37 °C for 30 min. The reactions were quenched with stop solution [98% formamide, 0.05% xylene cyanol (FF), and 0.05% bromophenol blue], heated to 90 °C for 5 min and then cooled to 0 °C at ice-bath. Reactions were analyzed by 15% PAGE 8 M urea at 250 V for 1-1.5 h. The fluorescent and UV gel imaging were done on a Bio-Rad Gel XR+ imager. Lanes: L, reference DNA 27-mer ladder; P, primer; Nat, natural template with all four dNTPs; M, hydrazone-modified template before blue light irradiation with all four dNTPs; M-hv, hydrazone-modified template after 2 h blue light irradiation with all four dNTPs.

Acknowledgements

We thank NSF (CHE-1845486, MCB-1715234 and CHE-1726724) and University at Albany, State University of New York for the financial support.

Conflicts of interest

There are no conflicts to declare.

Keywords: DNA function• Solid phase synthesis • Photo-regulation • DNA synthesis• Hydrazone photoswitch

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10.1002/chem.202100742

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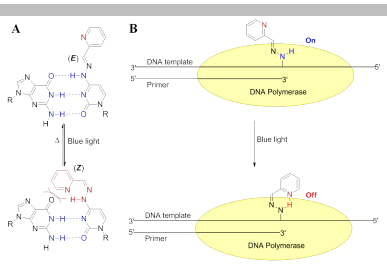
FULL PAPER

The synthesis of hydrazone modified cytidine phosphoramidite and oligodeoxynucleotides containing this modification has been presented. The *E-Z* isomerization triggered by the irradiation of a 450 nm blue light was investigated in the contexts of both nucleoside and oligodeoxynucleotide. The control of DNA synthesis *in vitro* was achieved by using the blue light in the primer extension reactions in the presence of DNA polymerases.



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DNA Functionality with Photoswitchable Hydrazone Cytidine