Synthesis of novel modified uracil for dual-purpose: Quenching and photoswitching

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Novel azo-based uracil derivatives have been synthesized with a photoswitching and quenching properties. The advantage of these derivatives over the traditional azobenzene photochromophore is maintaining the hydrogen bonding networks with the complementary nucleosides. *Trans*-to-*cis* Photoisomerization is observed up on illumination at 365nm, this process can be reversed by illumination thermally.

Keywords: Quencher, photoisomerization, dimethylphenylazo, uracil

Introduction

Hybridization of nucleic acids is considered the cornerstone for many biomedical applications as antigene and antisense. Recently, much effort has been devoted to regulate nucleic acid hybridization using external stimuli as light, pH,[1] electric field[2] and heat.[3] Light is preferred over all other external stimuli due to many reasons such as efficiency, clean and does not produce any contaminants to the biological system under study.

Photoregulation of nucleic acids can be achieved via attaching a photoresponsive molecule to the single-stranded DNA. Azobenzene one of photochromic molecules that extensively has been widely used for photoregulation of nucleic acid through geometrical structural change from *trans* to *cis* isomer and reverse with response of optical excitation with light at suitable wavelength.[4-9] Irradiation with UV light led to *trans*-to-*cis* isomerization while irradiation with visible light or thermally recovers the *trans* isomer.
Incorporation of azobenzene to oligonucleotides was obtained via two approaches (i) as a side chain on the oligonucleotide phosphate backbone[10] or (ii) as a linker between two oligonucelotide segments in the main chain of an oligonucleotide.[11] In principle,

introduction of several azobenzene residues to oligonucleotides led to an increase in melting temperature difference ∇Tm corresponding to the photo-induced isomerization. Despite the efficiency of azobenzenes in reversible photoswitching of nucleic acid-related activities, a desire to overcomer the non-nucleosidic nature of the attached azobenzene moiety by designing azo-nuclobase analogue that able to maintain the H-binding ability and thusly enhance the stabilization of duplex formation.

4-(dimethylamino)azobenzene-4'-carboxylic acid (DABCYL), universal quencher, one of azobenzene derivatives that widely used as a quencher for nucleic acid detection using fluorescent probe technique. Here we report the synthesis of novel azo-based uracil derivatives with potential dual-purpose as photo trigger and/or as a quencher that mimics the structure of the universal quencher DABCYL. The modified uracil is designed to have 4-(dimethylamino)phenylazo moiety at C5 position due to at this position there is no interference with the hydrogen bonding sites of uracil and therefore retain the base pairing ability with adenine. During the progress of this work, two examples of azobenzene-modified 2'-deoxyuridine at C5 position have been reported.[12]

Results and discussions

We are interested in the synthesis of 4-(dimethylamino)phenylazo derivative as azobenzene analogue for several reasons: 1) 4-(dimethylamino)phenylazo moiety is able to induce a conformational changes by photoisomerization. 2) The presence of dimethylamino group which is susceptible to protonation facilitates the investigation of pH effect. 3) The structure of azo-based nucleoside is structurally mimics the universal quencher. 4-(dimethylamino)azobenzene-4`-carboxylic acid (DABCYL), therefore, may have potential quenching effect as shown in figure 1.

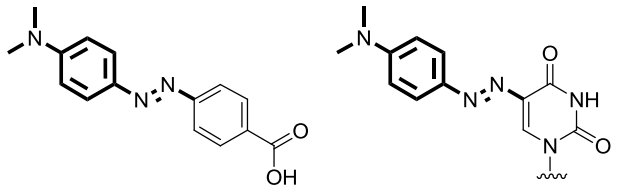
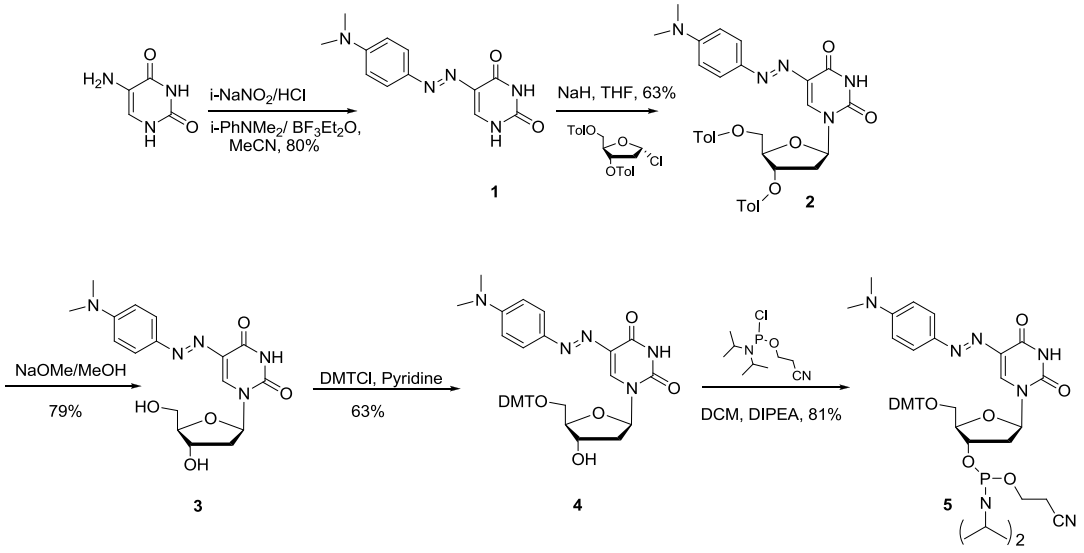


Figure 1: Comparison between dapcyl and the modified azo-based uracil

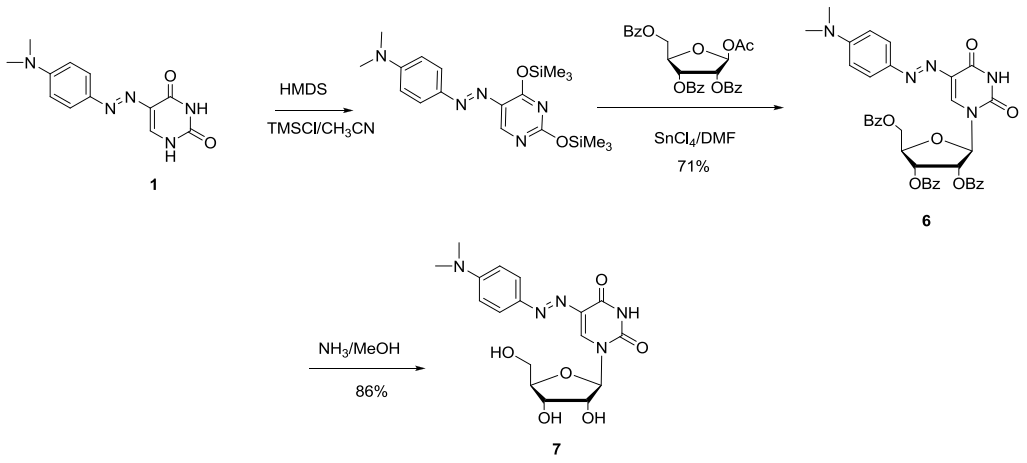
The synthesis of azo-based uracil **1**, was obtained by treatment of 5-aminouacil with $NaNO_2$ in the presence of HCl to afford the corresponding diazonium salt followed by coupling with dimethylaniline to give the desired product **1** in 80% yield. The synthetic approach to synthesize the phosphoramidite **5** for DNA synthesis purpose is depicted in scheme 1. Azo-modified uracil **1** then reacted with 1`α-chloro-3,5-di-*O*-p-toluoyl-1,2-dideoxy-α-D-ribofuranose under basic condition to give the corresponding deoxyriboside **2** followed by removal of the toluoyl protecting groups using sodium methoxide in methanol giving the azo-based 2-deoxyriboside **3** in 79% yield. Standard methods were used to convert the unprotected nucleosides to 5`-dimethoxytrityl-protected derivative by using dimethoxytrityl chloride (DMTCl) in pyridine giving **4** in 63% yield followed by conversion to the corresponding cyanoethyl phosphoramidite derivative **5** in 81 % yield.



Scheme 1. Schematic synthesis of deoxyuridine phosphoramidite possesses phenylazo moiety for photoswitching.

To get an access for RNA chemistry, ribonucleoside **7** was synthesized following Vorbrüggen procedure[13] starting with refluxing azo-modified uracil **1** with hexa-methyldisilazane (HMDS) and trimethylsilyl chloride (TMSCl) to form the corresponding silylated nucleoside followed by coupling with 1-*O*-acetyl-2,3,5-tribenzoyl ribofuranose in dimethylformamide (DMF) in the presence of $SnCl_4$ to afford **6** in 71 %.

Removal of benzoyl groups was carried out using ammonia in methanol to afford azo-based riboside **7** in 86 % yield.



Scheme 2. Schematic synthesis of photochromic riboside.

Photoisomerization studies

Azobenzene is well known to isomerize from its mostly thermally stable planar *trans*-isomer to *cis*-isomer upon exposure to UV-light irradiation (300nm ~ 400nm), and the reverse process takes place corresponding to irradiation with visible light (> 400nm) or thermally (Fig. 2). This process is completely reversible under UV and visible irradiations. From this vein, the synthesized nucleosides carrying azophenyl moiety is expected to follow the same behavior that azobenzene follows.

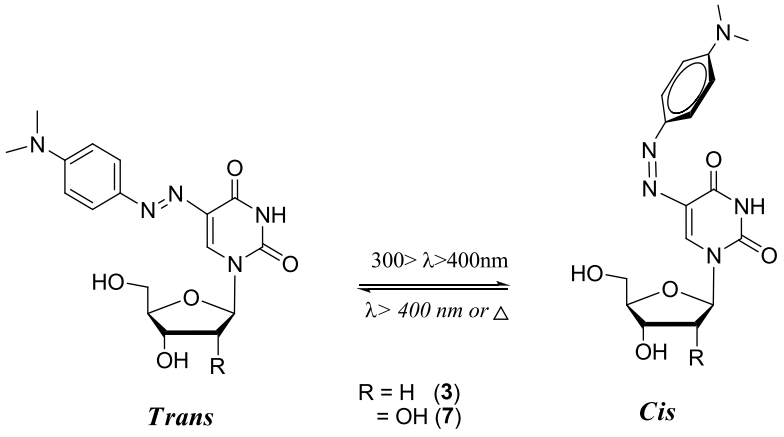


Figure 2. Schematic representation of reversible *trans-cis* photoisomerization of the photochromic nucleoside **3** and **7**.

To assess the photochromic behavior of the synthesized nucleosides **3** and **7** by monitoring the changes that may occur at their absorption with response of photoirradiation. As shown in figure 3, the absorption spectrum of *trans* isomer has a major peak centered at 454 nm (in dichloromethane for **3**) and at 443 nm (in acetonitrile for **7**) attributed to strong π-π* transition which overlapped with the weak peak attributed to weak n-π* transition. Upon irradiating with monochromic light at 365 nm, a decrease in the intensity of the absorption band was observed along with the irradiation time due to *trans*-to-*cis* isomerization, while the reverse *cis*-to-*trans* isomerization was achieved via thermal relaxation.

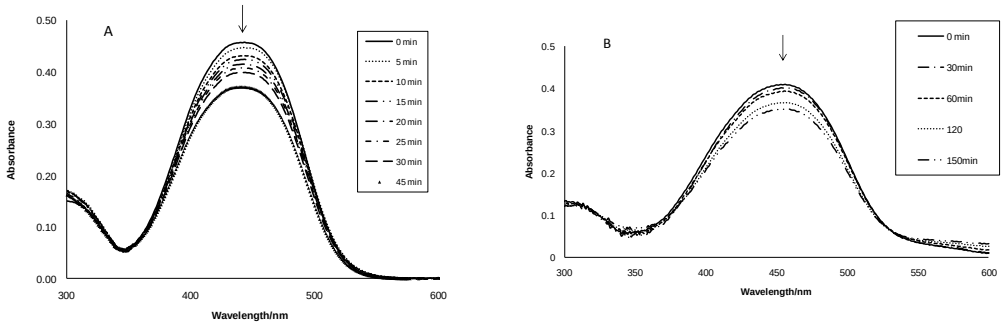


Figure 3. Absorption spectra of a) **3** in dichloromethane and b) for **7** in acetonitrile at 10μM concentrations for *trans-cis* photoisomerization upon illumination with UV-Light at 366nm.

To assess the quenching ability of the synthesized azo-based uracil **1**, a preliminary, qualitative study to quench the emission signals of fluorescein and pyrene luminophores has been made**.** As shown from the absorption spectra of **1** in ethanol, modified uracil **1** has absorption range 350-525 nm with maximum wavelength 436 nm, which is expectedly overlap with emission spectra of fluorophores used in the study. A solution of fluorophores in ethanol was titrated with aliquots of modified uracil **1**, as illustrated in

(Fig. 4), upon addition of small portions of **1** to the fluorophore, a subsequent quenching of fluorescent signal was observed.

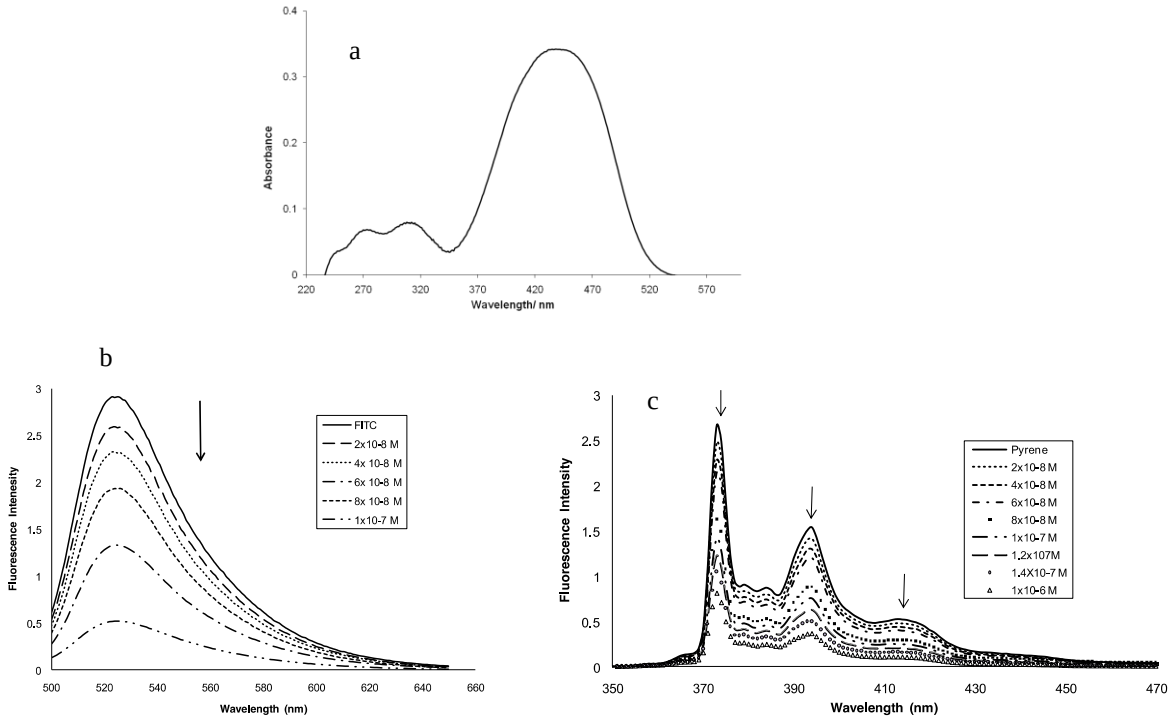


Figure 4-: a) Absorption spectrum of compound **1**, quenching of b) fluorescein (with excitation wavelength 488 nm) and c) pyrene (with excitation wavelength 330 nm) emission signals upon addition of various amount of azo-uracil **1** in ethanol.

pH-Sensitivity

Azobenzenes especially aminoazobenzene and dimethylaminoazobenzene attracted much attention due to their ability to change colour in solution with change of the pH. It is well established that protonation of aminoazobenzene derivatives led to the formation of two tautomeric azonium and ammonium forms.[14] As illustrated in figure 5, Azonium ion is obtained by protonation on azo-nitrogen leading to delocalized of the lone-pair electrons of the aromatic ring and as consequence a red shift in absorption maximum was occurred. Ammonium ion is obtained by protonation at amino-nitrogen resulting in prevention the conjugation of electrons lone-pairs and thusly the UV-Vis spectrum looks similar to that of unsubstituted azobenzene due to diminish the effect of amino substituent.

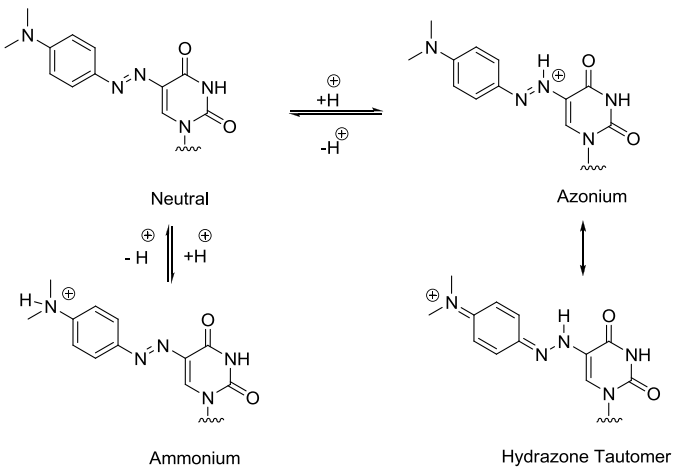


Figure 5. Molecular structure of dimethylaminoazophenyl(deoxy)uridine and its protonated forms. Sugar unit was removed for simplicity

The presence of an azo- and dimethylamino groups in the synthesized nucleosides promoting us to study the effect of acid. We investigated the effect of trifluoroacetic acid solution (0.005 M) in dichloromethane and ethanol on the nucleosides **3** and **7** respectively. Upon protonation, the band at λ_{max} 411 nm, which is attributed to neutral *trans*-dimethylaminoazobenzene, has been red shifted to λ_{max} 535 nm attributed to the azonium ion with appearance of new absorption band at λ_{max} 318 nm attributed to ammonium ion of *trans*-dimethylaminoazobenzene.

Similarly, as can be seen in figure **6**, upon addition of TFA solution to deoxyriboside **3** in dichloromethane leads to a decrease in the π-π absorption band at λ_{max} 455 which is attributed to neutral form with an increase in π-π absorption band centered at 618 nm and 350 nm attributed to azonium and ammonium ions respectively. There are two isosbestic points were observed at 376, 507 nm indicate that compound **3** exists either as non-protonated or protonated species in tautomeric equilibrium. While, upon titrating of 7 with small aliquots of TFA in ethanol, the π-π absorption band for neutral form at λ_{max} 436 nm was decreased with appearance of new absorption bands centered at 623nm and 342 nm attributed to the protonated forms azonium and ammonium ion respectively. These results are in excellent agreement with the aforementioned study. Surprisingly, subjecting solutions of **3**, **7** in halogenated solvents as dichloromethane or chloroform to

TFA leads to color change from yellow to deep blue color while using solvent ethanol, methanol, ethyl acetate instead of halogenated solvents this color change was not observed.

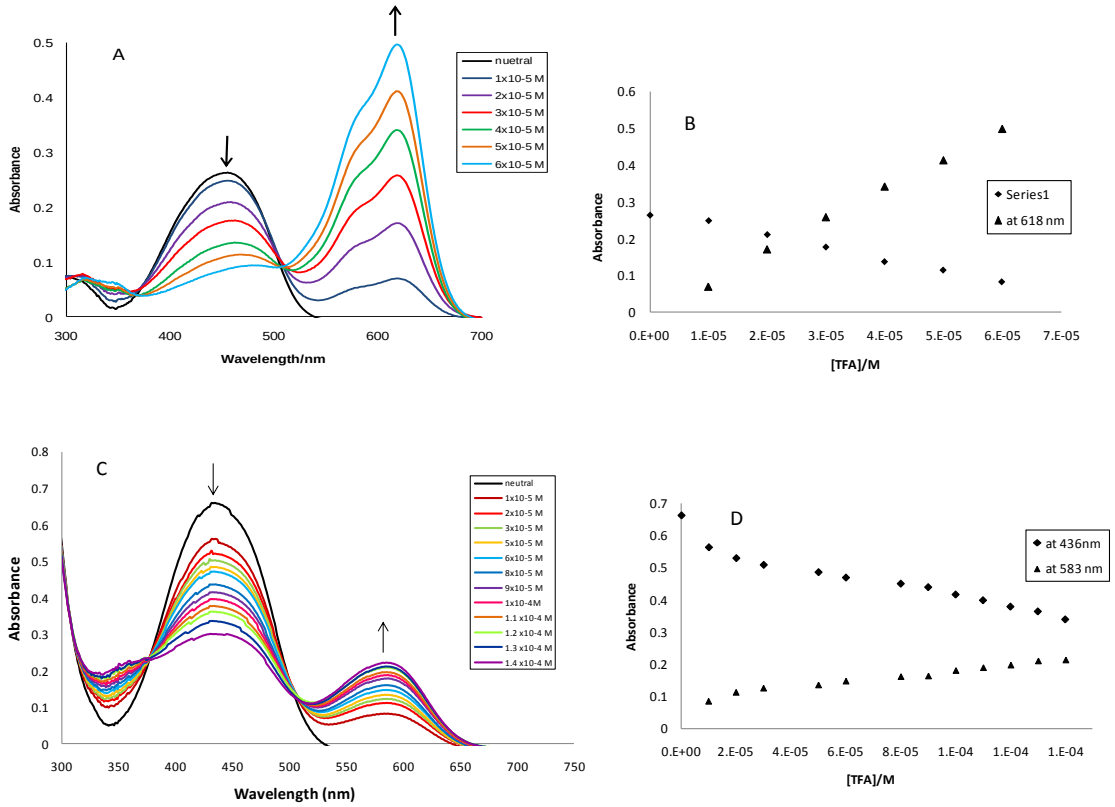


Figure 6. Changes in UV-Vis spectra of A) 3 at 6.2 μM concentration and C) 7 at 13.3 μM concentration upon addition of increasing concentrations of TFA, arrows indicate the progressive decrease of absorption band for neutral form and the growth of protonated form. B, D) Variation of absorbance vs TFA concentration for the respective absorption bands.

Conclusion

We have successfully designed and synthesized novel photochromic azo-based nucleosides with potential photoswitching and quenching properties for DNA and RND chemistry with potential duplex stabilization by retaining the ability of formation of hydrogen bond between the modified nucleobase and the complementary nucleobase,. These nucleosides showed ability to undergo *trans*-to *cis* isomerization upon irradiation with monochromic

light at 365nm and reversibly the *trans* configuration was recovered thermally in the dark. In addition, it showed halochromic effect upon acid treatment. Therefore, the synthesized photochromic nucleosides may be contribute in photregulation of nucleic acids.

Materials and Methods

General consideration. All solvents and chemicals were commercially available and were used as purchased. Reactions were monitored by thin layer chromatography (TLC) on pre-coated 0.2 mm Merck Kieselgel 60 TLC plates. Silica gel 60 F254 (Merck) plates and visualized with an ultraviolet light source at 254 nm. Silica gel (Merck Kieselgel 60, 15-40 mm) was used for column chromatography. ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded at a Varian Mercury Plus spectrometer (400.09 MHz, 100.61MHz and 242.9 MHz respectively) at room temperature. Chemical shifts are reported in parts per million (δ), were measured from tetramethylsilane (0 ppm) and are referenced to the residual proton in the deuterated solvent: $CDCl_3$ (7.26 ppm), DMSO-*d*6 (2.48 ppm), CD_3OD (4.87 and 3.31 ppm) for ^{1}H NMR and $CDCl_3$ (77.0 ppm), DMSO-*d*6 (39.5 ppm) and CD_3OD (49.1) for ^{13}C NMR. Multiplicities are described as s (singlet), d (doublet), t (triplet), m (multiplet), and br s (broad singlet). Coupling constants (*J*) are reported in Hertz (Hz). High resolution mass spectra (HRMS) were obtained using electrospray ionization time of flight methods (ESI-TOF).

Synthesis

5-((4-(dimethylamino)phenyl)diazenyl)uracil **1**

To a cold solution of 5-aminouracil (1.0 g, 7.87 mmol) in hydrochloric acid 1N (15 mL), a solution of 6.9% $NaNO_2$ (6 mL) was added dropwise over a period of 15 min. a buff solid was precipitated. The reaction mixture was stirred for further 30 min for complete precipitation. Solid was collected by filtration, wash with iced water and dry under vacuum to give 5-diazouracil (1.04 g, 84%) and used for the next step without further purification. to a suspension of 5-diazouracil (0.5 g, 3.2 mmol) in anhydrous acetonitrile (50 mL), borontrifluoride etherate (0.41 mL, 3.2 mmol) and dimethylaniline (0.38 mL, 3.2 mmol) were added to the mixture and was stirred for 2 h. The separated solid was

filtered and refluxed in EtOH (100 mL) for 1 h The solid was collected and dried to afford (1.38 g, 80%) of **1**. ^{1}H NMR (DMSO-d6) δ : 11.42 (s, 1H), 11.28 (s, 1H), 7.66 (s, 1H), 7.61 (d, *J* = 8.9 Hz, 2H), 6.79 (d, *J* = 8.7 Hz, 2H), 3.01 (s, 6H); ^{13}C NMR (DMSO-d6) δ : 161.8, 152.7, 151.3, 143.5, 130.7, 129.5, 124.9, 112.2, 47.6. HRMS (ESI): calcd. for C_{12} H_{13} N_5O_2 $[M]^+$ 259.1069 found 259.1062.

5-((4-(dimethylamino)phenyl)diazenyl)2′-deoxyuridine-3′,5′-di-(p-toluoyl) ester **2**

To a suspension of 5-((4-(dimethylamino)phenyl)diazenyl)uracil **1** (0.44 g, 1.72 mmol) in dry THF (20 mL), NaH (60% dispersion in mineral oil, 82 mg, 3.44 mmol) was added and stirred at r.t. for 2 h. A solution of 1`α-Chloro-3`,5`-di-O-toluoyl-2`-deoxyribose (0.69 g, 2.23 mmol) in dry THF (5 mL) was added to the mixture in one portion and the reaction mixture was stirred for 24 h under inert atmosphere. Solvent was evaporated under vacuum and the residue was dissolved in dichloromethane (30 mL), was washed with saturated aqueous sodium Na_2CO_3, brine and dried over anhydrous Na_2SO_4. The solution was filtered, concentrated, and the residue was subjected to purification by flash chromatography with CH_2Cl_2/CH_3OH (20:1, v/v) as eluent to give bis-toluoyl ester **2** as red solid. The product was obtained as a mixture of two isomers (0.65 g, 63%). ^{1}H NMR (DMSO-d_6) δ: 11.78 (br s, 1H), 8.02 (s, 1H), 7.92-7.89 (m, 2H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.68 (d, *J* = 8.2 Hz, 1H), 7.50 (d, *J* = 8.9 Hz, 1H), 7.37-7.33 (m, 3H), 7.16 (d, *J* = 8.2 Hz, 1H), 6.91 (d, *J* = 8.2 Hz, 1H), 6.75 (d, *J* = 8.9 Hz, 1H), 6.69 (d, *J* = 9.3 Hz, 1H), 6.32 (m, 1H), 5.57 (d, *J* = 5.8,1H), 5.01 (m, 1H), 4.57 (m, 1H), 4.41 (m, 1H), 3.02 (s, 6H), 2.97 (m,1H), 2.61 (m, 1H), 2.37 (s, 3H), 2.26 (s, 3H); ^{13}C NMR ($CDCl_3$) δ : 166.1, 165.7, 152.2, 144.3, 143.5, 129.8, 129.6, 129.1, 126.5, 126.6, 126.3,125.7, 111.2, 87.9, 77.3, 74.6, 64.1, 40.1, 27.96. HRMS (ESI): calcd. 611.2380 for $C_{33}H_{33}N_5O_7$ $[M]^+$ found 611.2391.

5-((4-(dimethylamino)phenyl)diazenyl)2'-deoxyuridine **3**

To a cold solution of the toluoyl ester **2** (0.5 g, 0.82 mmol) in dry CH_2Cl_2 (5 mL), NaOMe solution (0.2 M in MeOH) (9.8 mL, 1.96 mmol) was added dropwise over 15 min. The reaction mixture was stirred at r.t. for 1 h. An aqueous solution of 5% NH_4Cl was added to the mixture till the pH was 8 and the mixture was extracted with

ethylacetate (3 x 30 mL). The combined organics were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford red oil residue. The residue was subjected to flash chromatography using hexane/ CH_2Cl_2 (70:30, v/v) as the eluent to yield the deoxyriboside **3** as red powder (0.25 g, 79%). ^{1}H NMR (CD_3OD) δ: 8.50 (s, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 6.77(d, *J* = 7.8 Hz, 1H), 6.33 (t, *J* = 6.5 Hz, 1H), 4.43 (m, 1H), 3.96 (m, 1H), 3.83 (m, 1H), 3.75 (m, 1H), 3.20 (dd, *J* = 3.3 and 7.3 Hz, 1H), 3.03 (s, 6H), 2.40 (m, 2H); ^{13}CNMR (CD_3OD): δ 163.5, 154.3, 151.5, 131.2, 126.4, 112.7, 89.3, 87.4, 72.1, 62.7, 41.8, 40.5. HRMS (ESI): calcd. 375.1543 for $C_{17}H_{21}N_5O_5$ $[M]^+$ found 375.1535.

5-((4-(dimethylamino)phenyl)diazenyl)2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)uridine **4**

The free nucleoside **3** (0.2 g, 0.54 mmol) was dried by coevaporation with dry pyridine (3 x 5 mL) and then was dissolved in dry pyridine (10 mL). A solution of 4,4´-Dimethoxy-tritylchloride (0.269 g, 0.69 mmol) in dry pyridine (5 mL) and DIPEA (7.0 mmol, 1 mL) was added and the mixture was stirred at r.t. for 3 h. The reaction mixture was quenched by the addition of methanol (2 mL), was diluted with CH_2Cl_2 (75 mL) and then was washed with saturated aqueous solution of $NaHCO_3$ (3 x 50 mL). The organics were combined, dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure to give red oil. The crude product was purified by flash column chromatography using CH_2Cl_2/ MeOH/ Et_3N (85: 10: 5, v/v) as elution solvent afforded the tetritylated derivative **4** as red solid (0.28 g, 63%). ^{1}H NMR ($CDCl_3$) δ: 8.25 (s, 1H), 7.65 (d, *J* = 8.9 Hz, 2H), 7.38 (d, *J* = 7.4Hz, 2H), 7.27 (d, *J* = 7.4 Hz, 4H), 7.22 (d, *J* = 7.0 Hz, 2H), 7.14 (m, 2H), 6.78 (d, *J* = 8.5 Hz, 3H), 6.51 (d, *J* = 8.5 Hz, 2H), 6.18 (m, 1H), 4.57 (m, 1H), 4.39 (m, 1H), 3.69 (s, 6H), 3.20 (m, 1H), 3.25 (m, 1H), 2.90 (s, 6H), 2.80 (m, 1H) 2.47 (m, 1H); ^{13}C NMR ($CDCl_3$) δ: 161.4, 158.3, 152.0, 149.7, 144.5, 143.3, 135.6, 135.39, 130.4, 129.8 , 127.9, 127.7, 126.6, 125.0, 113.0, 111.1, 89.1, 86.2, 71.9, 64.1, 55.0, 45.6, 40.0. HRMS (ESI): calcd. 678.2928 for $[M+H]^+$ $C_{38}H_{40}N_5O_7$ found 678.2906.

5-((4-(dimethylamino)phenyl)diazenyl)2′-deoxy-3′-(2-cyanoethyldiisopropylphosphor-amidite)-5′-O-(4,4′-dimethoxytrityl)uridine **5**

To a solution of DMT-deoxyuridine **4** (0.16 g, 0.24 mmol) in dry CH_2Cl_2 (5 mL), DIPEA (340 μL, 2.4 mmol), 2-cyanoethyl-(*N*,*N*'-diisopropylamino) chlorophosphite (105 μL, 0.47 mmol) was added. The reaction mixture was stirred at r.t. under ambient atmosphere for 3 h until deemed complete by TLC. Solvent was removed and the resultant residue was dissolved in CH_2Cl_2 (25 mL), washed with aqueous solution of 5% $NaHCO_3$ (25 mL), brine (20 mL) and was dried over anhydrous Na_2SO_4. After removal of solvent the crude product was subjected to flash chromatography using hexane/ ethylacetate/ Et_3N (80:15: 5 to 50:45:5, v/v) to yield the phosphoramidite derivative **5** (0.169 g, 81%). ^{1}H NMR ($CDCl_3$) δ: as two diastereomers: 8.19 and 8.17 (2s, 1H), 7.77 (d, *J* = 7.6 Hz, 2H), 7.38 (d, *J* = 7.6 Hz, 2H), 7.21 (m, 7H), 6.79 (d, *J* = 6.7Hz, 4H), 6.63 (d, *J* = 6.5 Hz, 2H), 6.29 (m, 1H), 4.80 (m, 1H), 4.54-4.46 (m, 2H), 3.75 (s, 6H), 3.57-3.48 (m, 2H), 3.03 (s, 6H), 3.38 (m,1H), 3.11 (m, 1H), 2.85 (m, 2H), 2.38-2.17 (m, 2H), 1.20 (s, 12H); ^{13}C NMR ($CDCl_3$) δ : 160.8, 158.5, 152.3 149.5, 144.3, 143.5, 135.5, 129.9, 128.0, 126.9, 125.1, 113.2, 111.2, 88.7, 85.5, 74.1, 67.1, 55.0, 43.0, 40.2, 32.2, 29.6, 26.3, 24.4, 23.38; ^{31}P NMR ($CDCl_3$): δ two diastereomeric peaks at 149.2 and 148.6. HRMS (ESI): calcd. 878.3880 for $C_{46}H_{55}N_8O_8P$, found 878.3889.

5-((4-(dimethylamino)phenyl)diazenyl)(2,3,5-Tri-O-benzoyl-b-D-ribofuranosyl)uridine **6**

A suspension of 5-((4-(dimethylamino)phenyl)diazenyl)uracil **1** (0.26 g, 1 mmol) in of 1,1,1,3,3,3-hexamethyldisilazane (HMDS) (20 mL) and of trimethylsilyl chloride (1 mL) was refluxed until a clear solution was obtained. The solution was allowed to cool, and excess HMDS was removed under reduced pressure. The silylated uracil derivatives were dissolved in CH_3CN (5 mL) and the solution was burged with nitrogen for 15 min prior to addition of $SnCl_4$ (10% excess) (127 μL, 1.2 mmol) to the reaction mixture with vigorous stirring. A solution of 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-dribofuranose (0.56 g, 1.1 mmol) in CH_2Cl_2 (20 mL) was added dropwise to the reaction mixture. The solution was stirred at r.t. for 6 h at which the reaction came to completion as determined by TLC. The reaction mixture was quenched by aqueous saturated $NaHCO_3$ solution (100 ml) and was extracted with CH_2Cl_2 (3 x 50 ml) and was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure leaving brown residue. The crude product was purified by flash column chromatography using elution with a gradient of hexane/

CH_2Cl_2 (from 100:0 to 60:40, v/v). Compound **6** was isolated as a red solid (0.5 g, 71%). ^{1}H NMR (CDCl3) δ: 9.86 (br s, 1H), 8.05 (s, 1H), 7.96 (d, *J* = 7.4, 2H), 7.91 (d, *J* = 7.0, 2H), 7.70 (d, *J* = 7.4, 3H), 7.49 (m, 4H), 7.32 (m, 6H), 6.66 (d, *J* = 7.0, 2H), 6.29(m, 1H), 4.82 (m, 1H), 4.72 (m, 3H), 3.06 (m, 1H), 3.03 (s, 6H); ^{13}C NMR (CDCl3) δ: 166.2, 165.3, 160.6, 152.7, 149.4, 143.4, 133.8, 133.4, 129.9, 129.8, 128.5, 125.5, 111.2, 88.6, 80.9, 74.2, 71.5, 64.2, 40.2. HRMS (ESI): calcd. for C_{38} H_{34} N_5O_9 $[M+H]^+$ 704.2357 found 704.2358.

5-((4-(dimethylamino)phenyl)diazenyl)uridine **7**

Compound **6** (0.4 g, 0.57 mmol) was dissolved in saturated solution of ammonia in methanol (20 mL) and was stirred for 4 h at r.t. The solution was evaporated under pressure to give the crude product which was purified by flash column chromatography using CH_2Cl_2/MeOH (90: 10,v/v) yielding 0.19 g (86%). ^{1}H NMR (DMSO-d6) δ: 7.73 (s, 1H), 7.64 (d, *J* = 8.9, 2H), 6.78 (d, *J* = 8.9, 2H), 6.14 (m, 1H), 5.07 (m, 1H), 4.90 (m, 1H), 4.62 (m, 1H) (Exchangeable 3OH), 4.52 (m, 1H), 4.12 (d, *J* = 5.4, 1H), 3.69 (m, 1H), 3.62 (m, 2H), 3.44 (m, 1H), 3.01 (s, 6H); ^{13}C NMR (DMSO-d6) δ: 160.8, 152.5, 143.2, 124.8, 111.9, 94.9, 84.9, 71.4, 70.6, 63.7, 62.79, 40.5. HRMS (ESI): calcd. for $C_{17}H_{21}N_5NaO$ $[M+Na]^+$ 414.1390 found 414.1382.

UV-Vis measurments:

All UV-Vis spectra were measured with a Varian Cary 300 Bio spectrophotometer using quartz cuvette cells of 1 mm of optical path.

Photoisomerization.

Trans-to-*cis* photoisomerization of nucleosides **3** and **7** were monitored by UV-Vis spectroscopy. A solution of the nucleosides at 1.06 x10^{-5} M concentration (**3** in dichloromethane) and (**7** in acetonitrile) were irradiated with UV-light at room temperature using a UVGL-58 hand held lamp (6 watt, 366 nm) at A distance of approximately 5 cm. The spectral change for absorption maximum peak at 455 nm (for **3**) and 436 nm (for **7**) were monitored with the time course of irradiation. While the *cis*-to-*trans* isomerization was achieved and confirmed by retrieving the maximum absorption

bands at 455 nm (for **3**) and 436 nm (for **7**) thermally by leaving the samples at the dark at room temperature.

Quenching analysis

Effect of acids

The spectral change of the synthesized photochromic nucleosides at concentration $6.2x10^{-6}$ M (in dichloromethane for **3**) and $1.5x\ 10^{-5}$ M (in ethanol for **7**) were monitored upon addition of small aliquots of TFA solution (0.005 M).

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