Supporting Information

A DNA-Based Two-Component Excitonic Switch Utilizing High-Performance Diarylethenes

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1. General Procedures

General Information

All chemicals were purchased in analytical grade and used without further purification. Reactions requiring the exclusion of moisture and/or oxygen were carried out under argon atmosphere using standard Schlenk techniques. Normal- and reverse-phase column chromatography were performed manually or automated by the use of an INTERCHIM Puriflash 420 purification system equipped with silica- or C18-Flash columns. HPLC was performed on an AGILENT 1100 series system with a diode array detector on a C18-H column (VDSpher Pur 100 C18- H, 250’4.6 mm, 5 µM) from VDS Optilab. NMR spectra were recorded on a VARIAN Mercury Plus 300 or VARIAN Mercury Plus 500 System. Mass spectrometry was performed with a BRUKER microTOF QII ESI system. Absorption spectra in the liquid phase were recorded on a Cary 100 Bio UV/VIS-spectrometer from Varian and the data were analyzed with the software CaryWinUV and OriginPro 9.1. Fluorescence spectra in the liquid phase were recorded on a Jasco FP-6500. For normal intensity irradiation experiments, mounted high-power LEDs from Thorlabs operated at 350-700 mA were used. High-intensity irradiation experiments were conducted using a light source from Asahi spectra (MAX-303, 300W operated at maximal power). Photophysical properties were measured in DMSO for the nucleoside DAES and in an aqueous phosphate buffer (10 mM, 0.1 M NaCl, pH 7) for the oligonucleotide DAES.

Determination of the photochromic properties

For measuring the absorbance spectra of the nucleosides, 2 mL of a 60 µM solution of the sample in water/ethanol (4/1) or DMSO was prepared and added to a quartz cuvette from Hellma (Model: 110-10-40, Macro). After the irradiation of the sample with a UV-light LED (310 nm LED: Thorlabs, Mounted High Power LED, operated at 350 mA with an output power of 2.6 mWcm⁻², or 340 nm LED: Thorlabs, Mounted High Power LED, operated at 700 mA with an output power of 7.9 mWcm⁻²) at defined time points, an absorbance spectrum was recorded with a Cary 100 Bio UV/VIS-spectrometer from Varian and the data was analyzed with the software CaryWinUV and OriginPro 9.1.

Determination of the fatigue resistance

For the measurement of the fatigue resistance under standard conditions 2 mL of a 60 µM solution of the sample in water/ethanol (4/1) was prepared and successively irradiated with UV- (310 nm LED: Thorlabs, Mounted High Power LED, operated at 350 mA, output power: 2.6 mWcm⁻²) and Vis-light (490 nm LED: Thorlabs, Mounted High Power LED, operated at 350 mA, output power: 15.2 mWcm⁻²) until the PSS⁷⁵ and PSS¹⁰⁰ were reached. In total 10 switching cycles were measured in a cuvette from Hellma (Model: 110-10-40, Macro).

For the measurement of the fatigue resistance under high intensity conditions a light source from Asahi spectra (Model: MAX-303, 300 W, operated at maximal power, output power at 320 nm: 10.9 mWcm⁻², output power at 530 nm: 20.5 mWcm⁻²) equipped with a collimator lens, light guide, and bandpass-filters (UV: 320 nm, Vis: 530 nm) was used to irradiate the sample. For the determination of the fatigue resistance, 800 µL of a 50 µM solution of the sample in DMSO was prepared and added to a quartz glass cuvette from Hellma (Model: 110-10-40, Macro). The sample was then successively irradiated with UV- (320 nm, 70 s) and Vis-light (530 nm 75 s) until the PSS⁷⁵ and PSS¹⁰⁰ were reached, thereby completing two consecutive switching cycles. An absorbance spectrum of each PSS was recorded with a Cary 50 Bio UV-VIS-spectrometer from Varian after two cycles. This procedure was repeated 20 times, whereby a total number of 40 cycles was completed. The absorption maximum in the visible wavelength range of the closed ring isomer was normalized and plotted versus the cycle number. The fatigue resistance of the all-optical excitonic switch was determined using the high intensity conditions (see above) in a 0.5 mL cuvette from Hellma containing 400 µL of a 50 µM solution of the sample in aqueous phosphate buffer (10 mM, 0.1 M NaCl, pH 7).

Determination of the thermal stability

For the determination of the thermal stability, 2 mL of a 60 µM solution of the sample in DMSO was prepared and added to a quartz glass cuvette from Hellma (Model: 110-10-40, Macro). The following measurements were performed in triplicate. Irradiation of the sample was performed with a UV-light LED (310 nm LED: Thorlabs, Mounted High Power LED, operated at 350 mA, or 340 nm LED: Thorlabs, Mounted High Power LED, operated at 700 mA) until the photostationary state (PSS⁷⁵) was reached. Then the absorption maximum at ~ 490 nm of the closed-ring isomer was recorded over 100 min at 90°C with a Cary 100 Bio UV/VIS-spectrometer from Varian. The recorded data was analyzed with the software Cary Win UV and OriginPro 9.1. Data processing included baseline correction and plotting the absorbance at ~ 490 nm of the closed isomer at a time t (A), divided by
the absorption measured at the beginning of the measurement \((A_o)\) versus time. The thermal half-lives were calculated by fitting the normalized absorbance at \(\sim 490\ \text{nm}\) with a 1\textsuperscript{st} order exponential decay equation from OriginPro 9.1.

**Determination of the switching efficiency**

For the determination of the switching efficiency, HPLC measurements were performed using an Agilent 1100 Series HPLC system equipped with a multi-wavelength detector (MWD). A C18 column from VDS optilab (VDSphar Pur 100 C18-H, 250*4.6 mm, 5 \(\mu\)m) was used with a flow rate of 1.0 mL/min and eluting with a gradient of buffer A and B (buffer A = 100 mM triethylammonium acetate in water, buffer B = 100 mM triethylammonium acetate in 80% acetonitrile). The absorbance was recorded at the isosbestic wavelength of the open and closed isomers of the photoswitch. The gradients used for the determination of the photostationary states (PSS\(^{10}\)) and the isosbestic wavelengths are summarized in Table S1. The switching efficiency was then determined by calculating the ratio of the peak areas of the open and closed isomer at the isosbestic point. Peaks that could not be separated completely on the HPLC were separated with the peak analyzer of OriginPro 2015.

**Table S1:** HPLC-gradients used for the determination of the photostationary states (PSS\(^{10}\)) and the isosbestic wavelengths of the corresponding compound.

| Gradient [% of B] | dU-Ph | dU-2Py | dU-Ph\'Bu | dU-Me-Ph | dU-Me-2Py | dU-Me-Ph\'Bu |
|------------------|-------|--------|-----------|----------|----------|-------------|
| 40-65            | 274   | 312    | 313       | 301      | 310      | 312          |
| 40-65            | 45-70 | 45-70  | 45-70     | 50-75    |          |              |

**Determination of the cyclization and cycloreversion quantum yields**

Quantum yields were measured on an updated Quantum yield determination setup (QYDS) by Megerle et al.\(^1\) Irradiation of the photoswitches was performed using UV-light LEDs (Nikkiso SMD mounted 300 nm LED, model: VPC173) and visible light LED (Osram Oslox SSL80 505 nm LED, model: LVCK7P-JYKZ). The LED output radiant power was calibrated against the output voltage of the solar cell by using a power meter from Coherent (model: PowerMax-USB PS19Q). The raw data measured with the QYDS were further processed with a Mathcad script provided by the Riedle group (LMU Munich). The calculation of the quantum yields is based on the "initial slope method". For this purpose, the power of the LED was reduced to such an extent that the formation of the product is linear. Data processing with the Mathcad script includes baseline correction, spectral decomposition of each spectrum into the substrate and product base spectrum, and calculation of the quantum yields by the number of incoming photons per second and wavelength. Conversion to the cyclization product was calculated using the extinction coefficients of both isomers. Hereby, the concentration changes of the two isomers are numerically simulated accounting for the spectral dependencies of molar absorptivities and LED light. The spectral composition of the PSS\(^{10}\) is a linear combination of the open- and closed-isomer, which makes it possible to calculate the spectrum of pure closed isomer if the composition of the PSS\(^{10}\) is known. The composition of the PSS\(^{10}\) was determined as described earlier, by the separation of both isomers on an analytical HPLC column and subsequent integration of the peaks at an isosbestic point. The spectrum of pure closed isomer can be calculated using the following equation, where \(X\) represents the portion of open form and \(Y\) the portion of the closed form in the photostationary state (PSS\(^{10}\)):

\[
E_{CF} = \frac{(E_{PS2} - E_{CF})X}{Y}
\]

**Film preparation and measurements in the solid phase**

For solid-phase measurements, glass substrates (Schott AF32 eco, 500 \(\mu\)m thickness) were cleaned by ultrasonication in acetone and 2-propanol (10 min each) and UV/ozone treatment (UV Ozone Cleaner E511, Ossila Ltd., 10 min). Films were prepared by drop-casting 3-12 \(\mu\)L of the solutions onto the glass substrates while the temperature was kept at 50 °C. The height profile of the films was determined by surface profilometry (Bruker DektakXT Stylus Profiler). Baseline-corrected absorption spectra were recorded on a Cary 6000i UV-vis-nIR spectrometer (Varian Inc.). In between the spectra acquisition, films were irradiated with a light source from Asahi spectra (Model: MAX-303, 300 W, operated at maximal power, output power at 320 nm: 10.9 mWcm\(^{-2}\), output power at 530 nm: 20.5 mWcm\(^{-2}\)) at 320 nm and 530 nm for 150 s, respectively.

Fluorescence spectra and emission intensities were measured with a Fluorolog-3 spectrophotometer (Horiba Jobin-Yvon GmbH) equipped with a xenon lamp (450 W). A double monochromator was used to select the excitation wavelength, and the emission was spectrally resolved with a grating (550 nm blaze, 1200 lines mm\(^{-1}\)) and detected by a PPD-900 photomultiplier. For switching
experiments, films were directly irradiated with the wavelength-filtered output of the xenon lamp at 340 nm and 470 nm, respectively, and the emission intensity at 500 nm was recorded.

**Measurement of the Fluorescence quantum yields**

For the determination of the fluorescence quantum yields, umbelliferone was used as a standard, which has a $\Phi_R$ of 0.62 in water. The fluorescence and absorbance spectra at 3 different concentrations (20 μM, 15 μM, 12μM) were measured for the reference (umbelliferone) as well as for the different nucleoside and oligonucleotide photoswitches. Whereas the $\Phi_R$ of dU-Me-PhBu was measured in a water/ethanol mixture of 9/1, all photochromic oligonucleotides were measured in buffered aqueous solution (Phosphate buffer 10 mM, 0.1 M NaCl, pH 7). The area under the peak of the fluorescence spectrum was then determined by origin2015 and plotted against the absorbance at the excitation wavelength (340 nm for the oligonucleotides and 328 nm for dU-Me-PhBu and the reference). After a linear fit, the slopes of both the reference and the probe were determined and used for the following equation:

$$\Phi_x = \Phi_{ref} \left( \frac{Grad_x}{Grad_{ref}} \right) \left( \frac{\eta_x^2}{\eta_{ref}^2} \right)$$

Here the subscript $Ref$ refers to the reference (umbelliferone) and $x$ to the probe (different photoswitches), $\Phi$ is the fluorescence quantum yield, Grad is the slope of the plot of integrated fluorescence intensity and absorbance and $\eta$ the refractive index of the solvents.

**CD-spectra and determination of the melting temperatures**

Melting temperatures and CD-spectra were determined for functionalized DNA duplexes prepared by annealing the acceptor strand with their complementary donor oligonucleotides prepared by solid-phase phosphoramidite chemistry. DNA duplexes containing no modified nucleotides served as control. The final concentration of double-stranded DNA for Tm measurement and CD-spectra was 10 μM in 400 μl phosphate buffer (10 mM, 0.1 M NaCl pH 7). Thermal denaturation studies were performed on a Cary 100 Bio UV/Visible spectrometer with a temperature controller (Varian) using cuvettes from X with a 1 mm path length. Melting temperatures (Tm values in °C) were obtained by plotting temperature versus $\Delta\varepsilon$, applying a differential curve fit.

**Determination of the FRET efficiencies**

Experimental FRET efficiencies were calculated based on the donor fluorescence given by the following equation.

$$E = 1 - \frac{IDA}{ID}$$

IDA and ID are the fluorescence intensities of the donor at 420 nm in the presence or absence of the acceptor. FRET efficiencies of the duplexes were measured in an aqueous phosphate buffer (10 mM, 0.1 M NaCl, pH 7) with a small excess (1.15 equiv) of the complementary strands.

**Calculation of the theoretical FRET efficiencies**

The theoretical FRET efficiencies were calculated according to Hirashima et al. $^3$ The FRET efficiency is given by:

$$E = \frac{1}{1 + \left( \frac{R}{R_0} \right)^2}$$

$R$ is defined as the distance between donor and acceptor. $R_0$ is the Förster radius, a distance in which the FRET efficiency is 50%. The Förster radius is calculated by:

$$R_0 = 0.211 \times \left[ J(\lambda) \kappa^2 n^{-4} \Phi_D \right]^{1/6}$$

$J(\lambda)$ is the donor/acceptor spectral overlap integral, i.e., the overlap between donor emission and acceptor absorption. $n$ is the refractive index of the medium and $\Phi_D$ is the fluorescence quantum yield of the donor. $\kappa^2$ is the orientation factor which takes the relative orientation of the transition dipole moments of the donor and acceptor into account.

The distance (R) between donor and acceptor in canonical right-handed B-DNA was calculated by:
\[ R = \sqrt{2a^2 \left[ 1 - \cos(n_{DA}\beta + \alpha) \right] + \left[ \delta(n_{DA} + 1) \right]^2} \]

\( a \) is the distance between fluorophore center and DNA helix center, 3 Å. \( b \) is the distance between two neighboring base pairs, 3.4 Å. \( \alpha \) (phase angle), angle between the transition dipole moments of the donor and acceptor in the perspective of a helical axis, when the neighboring base of the donor is the complementary base of the acceptor, 134.8°. \( \beta \) (helical rise angle), rotation angle of base pairs with respect to the helical axis, 34.3°. \( n_{DA} \) is the number of base pairs separating donor and acceptor.

The orientation factor was calculated by:

\[ \kappa = \cos(n_{DA}\beta + \alpha) - 3 \left( \frac{a \sin(n_{DA}\beta + \alpha)}{R_{DA}} \right)^2 \]

Using \( R \) and \( \kappa \), the theoretical FRET efficiencies were calculated.

**Preparation of the modified oligonucleotides**

Donor and acceptor strands were synthesized by solid-phase phosphoramidite chemistry with an automated oligonucleotide synthesizer (H6, V.01.02, K&A Laborgeraete GbR) operated in the DMT-off mode. As solid support, 5' - DMT-dG(tac)-Suc-CPG; 500 A and 5' - DMT-dC(tac)-Suc-CPG; 500A was used. DNA fast deprotection phosphoramidites were dissolved in dry acetonitrile with a concentration of 0.1 M. The modified phosphoramidites were dissolved in dry acetonitrile with a concentration of 0.1 M. A standard 1 μmol DNA protocol was used. After the synthesis, the oligonucleotides were cleaved off the solid support by treatment with 25% aqueous ammonia at rt for 12 h, followed by repeated extraction with water and lyophilization. Purification by HPLC using a semi-preparative Luna C18 column (5 μm, 250*15.0 mm) from Phenomenex and repeated lyophilization afforded the final oligonucleotides.
2. Supporting tables and figures

Table S1: Sequences and MS analysis of donor and acceptor oligonucleotides. The modification sites are marked with a red X for phosphoramidite (7) and Y for the tC phosphoramidite. The oligonucleotides were analyzed by mass spectrometry (ESI-TOF, high resolution, negative mode).

| Donor | Sequence | calc. mass | meas. mass |
|-------|----------|------------|------------|
| D11   | 5’-CTC CCT ATT AXT ATT ATT GGG TCC ACC CAC-3’ | 10302.6248 | 10302.5454 |
| D14   | 5’-CTC CCT ATT AXT ATT ATT GGG TCC ACC CAC-3’ | 10302.6248 | 10302.5325 |
| D17   | 5’-CTC CCT ATT AXT ATT ATT GGG TCC ACC CAC-3’ | 10302.6248 | 10302.5605 |
| D20   | 5’-CTC CCT ATT AXT ATT ATT AXT GGG TCC ACC CAC-3’ | 10302.6248 | 10302.5459 |

| Acceptor | Sequence | calc. mass | meas. mass |
|----------|----------|------------|------------|
| A10      | 5’-GAG GGA TAA TAA TAA TAA CCC Y AGG TGG GTG-3’ | 10404.7795 | 10404.4789 |
| A11      | 5’-GAG GGA TAA TAA TAA TAA TAA C Y AGG TGG GTG-3’ | 10404.7795 | 10404.4117 |
| A12      | 5’-GAG GGA TAA TAA TAA TAA TAA CCC Y CCC AGG TGG GTG-3’ | 10404.7795 | 10404.4955 |

Table S2: Possible combinations of donor and acceptor strands yielding FRET duplexes that differ in the number (n=1-12) of nucleotides between donor and acceptor chromophore.

| n=   | D20 | D17 | D14 |
|------|-----|-----|-----|
| A12  | 1   | 4   | 7   |
| A11  | 2   | 5   | 8   |
| A10  | 3   | 6   | 9   |

Table S3: Melting temperatures and FRET efficiencies (measured and calculated) of all possible FRET duplexes (n=1-12).

| n=   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|------|---|---|---|---|---|---|---|---|---|----|----|----|
| $T_m$ (°C) | 63.5 | 61.8 | 60.7 | 62.6 | 62.6 | 61.0 | 63.0 | 62.1 | 60.5 | 62.9 | 61.5 | 61.8 |
| FRET eff. (%) measured | 90 | 60 | 41 | 47 | 57 | 42 | 22 | 9 | 5 | 17 | 16 | 15 |
| FRET eff. (%) calculated | 95 | 80 | 31 | 0 | 35 | 38 | 20 | 8 | 0 | 6 | 8 | 6 |
Figure S1. HPLC-based determination of the PSS$^{\text{UV}}$ of singly and doubly methylated deoxyuridine-based DAEs. HPLC traces of irradiated samples were recorded at the isosbestic wavelength (black line) of each compound and 490 nm (red line) visualizing the closed isomer only. Compounds dU-Ph and dU-Me-Ph were irradiated with 300 nm UV-light. Compounds dU-2Py, dU-Me-2Py, dU-Ph-Bu, and dU-Me-Ph-Bu were irradiated with 340 nm UV-light. a) HPLC traces of dU-Ph. b) HPLC traces of dU-Me-Ph. c) HPLC traces of dU-2Py. d) HPLC traces of dU-Me-2Py. e) HPLC traces of dU-Ph-Bu. f) HPLC trace of dU-Me-Ph-Bu.
Figure S2. Determination of the cyclization quantum yields of singly methylated 2'-deoxy-uridine photoswitches. a) Measurement of dU-Ph. Left: Time course of the isomer concentrations during the determination of the quantum yield with 300 nm UV-light. Right: Shutter file of the measurement. b) Measurement of dU-2Py. Left: Time course of the isomer concentrations during the determination of the quantum yield with 340 nm UV-light. Right: Shutter file of the measurement. c) Measurement of dU-PhBu. Left: Time course of the isomer concentrations during the determination of the quantum yield with 340 nm UV-light. Right: Shutter file of the measurement. The Shutter file shows the irradiation times and the power measured by the solar cell.
Figure S3. Determination of the cyclization quantum yields of doubly methylated 2'-deoxy-uridine photoswitches. a) Measurement of dU-Me-Ph. Left: Time course of the isomer concentrations during the determination of the quantum yield with 300 nm UV-light. Right: Shutter file of the measurement. The Shutter file shows the irradiation times and the power measured by the solar cell. b) Measurement of dU-Me-2Py. Left: Time course of the isomer concentrations during the determination of the quantum yield with 340 nm UV-light. Right: Shutter file of the measurement. The Shutter file shows the irradiation times and the power measured by the solar cell. c) Measurement of dU-Me-PbBu. Left: Time course of the isomer concentrations during the determination of the quantum yield with 340 nm UV-light. Right: Shutter file of the measurement. The Shutter file shows the irradiation times and the power measured by the solar cell.
Figure S4. Determination of the cycloreversion quantum yields of singly methylated 2'-deoxy-uridine photoswitches. a) Measurement of dU-Ph. Left: Time course of the isomer concentrations during the determination of the quantum yield with 300 nm UV-light. Right: Shutter file of the measurement. The Shutter file shows the irradiation times and the power measured by the solar cell. b) Measurement of dU-2Py. Left: Time course of the isomer concentrations during the determination of the quantum yield with 340 nm UV-light. Right: Shutter file of the measurement. The Shutter file shows the irradiation times and the power measured by the solar cell. c) Measurement of dU-PhtBu. Left: Time course of the isomer concentrations during the determination of the quantum yield with 340 nm UV-light. Right: Shutter file of the measurement. The Shutter file shows the irradiation times and the power measured by the solar cell.
Figure S5. Determination of the cycloreversion quantum yields of doubly methylated 2'-deoxy-uridine photoswitches. a) Measurement of dU-Me-Ph. Left: Time course of the isomer concentrations during the determination of the quantum yield with 300 nm UV-light. Right: Shutter file of the measurement. The Shutter file shows the irradiation times and the power measured by the solar cell. b) Measurement of dU-Me-2Py. Left: Time course of the isomer concentrations during the determination of the quantum yield with 340 nm UV-light. Right: Shutter file of the measurement. The Shutter file shows the irradiation times and the power measured by the solar cell. c) Measurement of dU-Me-Ph/Bu. Left: Time course of the isomer concentrations during the determination of the quantum yield with 340 nm UV-light. Right: Shutter file of the measurement. The Shutter file shows the irradiation times and the power measured by the solar cell.
Figure S6. Comparison of the reversibility measurements of a) dU-Ph and b) dU-Me-Ph performed under standard irradiation conditions. A 50 µM solution of the compound in water/ethanol (4/1) was irradiated alternatingly with UV-light (310 nm LED: Thorlabs, Mounted High Power LED, operated at 350 mA, output power: 2.6 mW cm$^{-2}$) and Vis-light (490 nm Mounted High Power LED, operated at 350 mA, output power: 15.2 mW cm$^{-2}$) until the PSS$_{UV}$ and PSS$_{Vis}$ were reached. The absorption peak in the visible wavelength range was normalized and plotted. Under these conditions, both compounds show no signs of fatigue.
Figure S7. Reversibility measurements of a) dU-Ph, b) dU-Me-Ph, c) dU-2Py, d) dU-Me-2Py, e) dU-PhBu and f) dU-Me-PhBu under high-irradiation intensity conditions. A 50 µM solution of the compound in DMSO was irradiated alternatingly with UV-light (320 nm, output power: 10.9 mWcm⁻², 60s) and Vis-light (530 nm, output power: 20.5 mWcm⁻², 70s) until the PSSUV and PSSVis were reached. 20 out of 40 absorption spectra are shown to illustrate the changes in the spectrum. The inset shows the absorption peak in the visible wavelength range which was normalized and plotted. Singly methylated photoswitches show the appearance of a new absorption band at 400 nm indicative of the formation of a byproduct. The same cannot be observed for the doubly methylated photoswitches. Overall, it can be concluded that doubly methylated photoswitches can withstand the high-intensity irradiation in DMSO better than the singly methylated photoswitches.
LC-MS-based analysis of the byproduct formation of dU-2Py (open form) upon alternating irradiation with UV-and Vis-light under high power irradiation conditions in DMSO at room temperature. 

**Figure S8.**

- **a)** HPLC trace recorded at 355 nm of dU-2Py before the irradiation with UV-light. A single peak at a retention time of 12 min with a mass of dU-2Py ([M+H]+) is evident.
- **b)** HPLC trace recorded at 355 nm of dU-2Py (open form) after 40 consecutive irradiation cycles with 320 nm UV-light (output power: 10.9 mWcm⁻², 60 s) and 520 nm Vis-light (output power: 20.5 mWcm⁻², 90 s). A second peak at a retention time of 16 min with a slightly lower mass (dU-2Py-Ox) was observed. The lower mass fits a loss of two protons (dU-2Py-2H) and could be explained by an oxidation reaction of dU-2Py. Integration of the two peaks reveals a byproduct formation of approx. 20% after 40 switching cycles.
- **c)** HPLC trace recorded at 400 nm of dU-2Py (open form) after 40 consecutive irradiation cycles. The oxidized form of dU-2Py which was formed as the main byproduct shows a strong absorbance at 400 nm. At the recorded wavelength, another byproduct becomes visible with a mass of 484.1525 which could be explained by the formation of an N-oxide. However, this byproduct was only observed in small amounts.
- **d)** Proposed solvent-assisted reaction mechanism of the main byproduct (dU-2Py-Ox) formation of dU-2Py after 40 switching cycles in DMSO. Oxidation of the closed form, promoted by the solvent DMSO which acts as an oxidant, is proposed. The oxidation yields an additional C-C double bond at the cyclopentene bridge and disrupts the elongated π-system of the closed isomer, thereby explaining the strong absorbance at 400 nm. This oxidation also explains the absence of the byproduct formation in doubly methylated photoswitches since position 6 of the nucleobase required for the oxidation is blocked by the methyl group, thus preventing this side reaction.
- **e)** Mechanism for the main byproduct formation observed by Higashiguchi et al. in which the thiophene utilizes an extended valence shell of sulfur that allows cyclization between the 2- and 4'-positions. Subsequent dehydrogenation produces the byproduct (dU-2Py-Ox-2), releasing the extended valence shell of sulfur. Due to the close proximity of the aryl substituent of the thiophene and the sugar moiety of the nucleoside, and the observed absorption spectrum of dU-2Py-Ox-1 we consider the mechanism leading to dU-2Py-Ox-1 most accurate.
- **f)** Absorption spectrum and structure of dU-2Py-OF.
- **g)** Absorption spectrum and structure of dU-2Py-CF.
- **h)** Absorption spectrum and structure of dU-2Py-Ox-1.
LC-MS-based analysis of the byproduct formation of dU-Me-2Py and dU-2Py in DMSO or a mixture of water/ethanol (4/1).

a) HPLC trace recorded at 260 nm of dU-Me-2Py before the irradiation with UV-light. A single peak at a retention time of 24 min with a mass of dU-2Py (504.1552, [M+Na]⁺) is evident. b) HPLC trace recorded at 260 nm of dU-Me-2Py (open form) after exposing a 75 μM solution of dU-Me-2Py dissolved in DMSO at room temperature to 320 nm UV light for 2 h, using a high-intensity light source (xenon lamp, output power at 320 nm: 10.9 mWcm⁻²). A second peak at a retention time of 33 min with a greater mass of 582.1732 was observed. The greater mass fits to a DMSO adduct of the photoswitch ([M+DMSO+Na]⁺). Integration of the two peaks reveals a byproduct formation of approx. 30%. c) HPLC trace recorded at 260 nm of dU-2Py dissolved in DMSO at room temperature to 320 nm UV light for 2 h, using a high-intensity light source (xenon lamp, output power at 320 nm: 10.9 mWcm⁻²). A second peak at a retention time of 33 min with a slightly lower mass of 488.1245 was observed. The lower mass fits a loss of two protons (dU-2Py - 2H) d) HPLC trace recorded at 260 nm of dU-2Py before the irradiation with UV-light. A single peak at a retention time of 22 min with a mass of dU-2Py (490.1415, [M+Na]⁺) is evident. e) HPLC trace recorded at 355 nm of dU-2Py (open form) after exposing a 75 μM solution of dU-2Py dissolved in DMSO at room temperature to 320 nm UV light for 2 h, using a high-intensity light source (xenon lamp, output power at 320 nm: 10.9 mWcm⁻²). No significant amounts of byproduct are detectable. In the absence of DMSO both photoswitches exhibit strong fatigue resistance. g) Structures, chemical formulas and calculated MS data are shown.
Figure S10: Comparison of the fatigue resistance of a) dU-Me-Ph/Bu and b) bis(trifluoromethyl)benzene-substituted dithienylethene. A 50 µM solution in DMSO of the nucleosidic and non-nucleosidic DAE was prepared and was switched under high-irradiation intensity conditions UV-light (320 nm, output power: 10.9 mWcm$^{-2}$) and Vis-light (530 nm, 20.5 mWcm$^{-2}$) for 40 cycles. An absorption spectrum of the closed form before and after the 40 irradiation cycles was recorded. The addition of bis(trifluoromethyl)benzene groups at the periphery of a DAE was found to significantly increase their fatigue resistance. Therefore, the bis(trifluoromethyl)benzene-substituted dithienylethene was used as a literature-known standard for the comparison of the fatigue behavior under the same irradiation conditions with the best-performing nucleosidic DAE. Both DAEs show a decrease of their absorption peak in the visible wavelength range of 8-9%.
Figure S11: Thermal stability measurements of singly and doubly methylated deoxyuridine-based DAEs. A 60 M solution of the photoswitch in DMSO was irradiated with UV-light (310 nm LED operated at 350mA for phenyl residue, 340 nm LED for pyridyl and tert-butyl ester phenyl operated at 700 mA) until the PSS$_{UV}$ was reached. The solution was heated to 90°C and the absorption at 490 nm was measured over 1h. All measurements were performed in triplicate. The absorption was normalized and plotted against the time. Thermal half-lives were calculated by fitting the absorption with an exponential decay function. One measurement for each sample is shown. a) dU-Ph. b) dU-Me-Ph. c) dU-2Py. d) dU-Me-2Py. e) dU-Ph-tBu. f) dU-Me-Ph-tBu.
Figure S12. Normalized absorption and emission spectra of nucleosidic donor and acceptor chromophores. 

a) Normalized absorption (dotted line) and emission spectra of dU-Me-Ph/Bu before (black line) and after irradiation with UV light (blue line).

b) Normalized absorption (dotted line) and emission (green line) spectra of the acceptor. The dotted yellow line at 340 nm shows the preferred area for excitation, while the second dotted yellow line at 420 nm shows where the energy transfer between donor and acceptor takes place.

c) Determination of the relative fluorescence quantum yield of dU-Me-Ph/Bu.
Figure S13: Trityl histograms for the synthesis of donor and acceptor oligonucleotides by phosphoramidite-based solid-phase synthesis. a) Synthesis of donor strand D11. The phosphoramidite (7) was incorporated at position 23. b) Synthesis of donor strand D14. The phosphoramidite (7) was incorporated at position 20. c) Synthesis of donor strand D17. The phosphoramidite (7) was incorporated at position 17. d) Synthesis of donor strand D20. The phosphoramidite (7) was incorporated at position 14. e) Synthesis of acceptor strand A10. The phosphoramidite of tC was incorporated at position 10. f) Synthesis of acceptor strand A11. The phosphoramidite of tC was incorporated at position 11. g) Synthesis of acceptor strand A12. The phosphoramidite of tC was incorporated at position 12.
Figure S14: CD spectra for all possible combinations n=1-12 (a-l) of FRET duplexes, measured in aqueous phosphate buffer (10 mM, 0.1 M NaCl, pH 7). All CD spectra indicate the formation of the classic B-form of the DNA.
Figure S15: Determination of the melting temperatures for all possible combinations (n=1-12) of FRET duplexes. The melting curves were extracted from the CD spectra and obtained by plotting duplex DNA content versus temperature.
Figure S16: Overview of the photophysical properties of the donor strands (D11-D20, a-d) in a duplex with a fully complementary unmodified strand. Equimolar concentrations of the donor strand and the unmodified complementary strand (50 \( \mu \text{M} \) in aqueous phosphate buffer, 10 mM, 0.1 M NaCl, pH 7) were mixed. Hybridization was initiated by incubation at 95°C for 5 min and snap cooling on ice. Absorption spectra were recorded in a Cuvette from Starna (Model: 28-F-Q). Absorption spectra of the open form (OF, black line), PSS\(^{35}\) after irradiation with UV light (blue line), and the calculated closed form (CF, red line) are shown. The upper right inset of each panel shows the reversibility measurement of the corresponding duplex over 40 cycles under high-intensity irradiation conditions (irradiation with 320 nm UV light for 90 s (output power: 10.9 mWcm\(^{-2}\) and 530 nm Vis light for 100 s, output power: 20.5 mWcm\(^{-2}\)). Thermal stability measurements of the closed form at 50°C are shown in the lower inset. A temperature of 50°C was used for this measurement to keep the duplex intact.
Figure S17. HPLC-based determination of the PSSUV of duplex donor strands (D11-D20, a-d). Before irradiation, equimolar concentrations of the corresponding donor strand and a fully complementary unmodified oligonucleotide were mixed in a phosphate-buffered aqueous solution (10 mM, 0.1 M NaCl, pH 7). Incubation at 95°C for 5 min and subsequent snap cooling on ice yielded the final duplexes. HPLC traces of irradiated samples were recorded at the isosbestic wavelength (black line) of each compound and 490 nm (red line) visualizing the closed isomer only. All duplex donor strands were irradiated with 340 nm UV-light (Thorlabs, Mounted High Power LED, operated at 700 mA) for 15 min. All HPLC traces show a sharp peak at a retention time of 12 min which corresponds to the single-stranded unmodified oligonucleotide. Open and closed forms are indicated in the diagram. Full conversion (96-98%) can be observed for all duplex donor strands.
Figure S18: Fluorescence measurements of duplex donor stands (a) D11, (b) D14, (c) D17, (d) D20. The duplex was generated by mixing the corresponding donor strand with 1.15 eq. of a fully complementary oligonucleotide in phosphate-buffered aqueous solution (10 mM, 0.1 M NaCl, pH 7), followed by incubation at 95°C for 5 min and subsequent snap cooling on ice. A slight excess of the unmodified complementary strand was used to prevent distortion of the measurement originating from un-hybridized single-stranded donor oligonucleotides. The absorption (dotted black line) and emission spectra (black line) of the open form and the emission of the PSSUV (blue line) are shown. Irradiation and excitation were carried out with 340 nm UV-light (Thorlabs, Mounted High Power LED, operated at 700 mA).

Figure S19: Determination of the fluorescence quantum yields of all donor strands in a duplex with a fully complementary unmodified oligonucleotide in aqueous phosphate buffer (10 mM, 0.1 M NaCl, pH 7). The relative quantum yields were measured in comparison to the reference (umbelliferone). Fluorescence and absorbance spectra at 3 different concentrations were measured, affording the slopes of the integrated fluorescence intensities vs. absorbance.
Figure S20: Absorbance and emission spectra of all acceptor strands in a duplex with a fully complementary unmodified oligonucleotide. The extinction coefficients were determined in triplicate by measuring the corresponding duplex acceptor in 3 different concentrations (10, 15, and 25 μM) mean values are shown in the absorption spectra (left panel). The fluorescence of the double-stranded acceptors was measured in the presence of 1.15 eq of the complementary strand (right panel). The absorbance (black line) and emission (green line) after excitation at 385 nm are shown. 

a) Absorption spectra of A10 measured in aqueous phosphate buffer (10 mM, 0.1 M NaCl, pH 7). 

b) Absorption spectra of A11 measured in aqueous phosphate buffer (10 mM, 0.1 M NaCl, pH 7). 

c) Absorption spectra of A12 measured in aqueous phosphate buffer (10 mM, 0.1 M NaCl, pH 7). 

d) Fluorescence spectra of A10 measured in aqueous phosphate buffer (10 mM, 0.1 M NaCl, pH 7). 

e) Fluorescence spectra of A11 measured in aqueous phosphate buffer (10 mM, 0.1 M NaCl, pH 7). 

f) Fluorescence spectra of A12 measured in aqueous phosphate buffer (10 mM, 0.1 M NaCl, pH 7).
Figure S21: a) Fluorescence spectra of all possible duplexes n=1-12 containing donor and acceptor moieties. The duplexes were generated by mixing the corresponding donor strand with 1.15 eq. of the acceptor strand in phosphate-buffered aqueous solution (10 mM, 0.1 M NaCl, pH 7), followed by incubation at 95°C for 5 min and snap-cooling on ice. Experimental FRET efficiencies based on the donor fluorescence were extracted and are summarized in Table S3. The FRET efficiencies show the expected orientation dependence. Combinations with n=1-3 nucleotides distance between donor and acceptor moieties showed the highest energy transfer efficiencies and were further investigated. b) Extended irradiation with 340 nm UV-light led to the formation of the PSS\textsuperscript{UV} and also resulted in a decreased fluorescence intensity of the all-optical excitonic switch at 500 nm, which corresponds to the emission of the acceptor. High ON/OFF contrasts of by 45% for n=1, 49% for n=2 and 56% for n=3 were observed. c) As a control experiment, acceptor A12 in a duplex with an unmodified complementary strand was irradiated under the same conditions (340 nm UV-light, Thorlabs, Mounted High Power LED, operated at 700 mA) for 15 min. No bleaching of the acceptor fluorescence was detectable.
Figure S22: Determination of the photochromic properties of the all-optical excitonic switch with n=1 in the solid phase. a) Absorbance spectra of donor strand D20 in a duplex with a fully complementary unmodified oligonucleotide. The absorption of the open form (OF, black line) and the PSS$^{UV}$ (blue line) are shown. b) Reversibility measurement of donor strand D20 in a duplex with a fully complementary unmodified oligonucleotide. The absorption maximum in the visible wavelength range of the closed form was plotted. Alternating irradiation under high intensity conditions with UV-light (320 nm, 10.9 mWcm$^{-2}$, 120 s) and Vis-light (505 nm, 20.5 mWcm$^{-2}$, 100 s) for 15 cycles showed no signs of fatigue. c) Profilometric analysis of a film of the donor strand D20 in a duplex with a fully complementary unmodified oligonucleotide. Drop-casting of the DNA duplex in an aqueous phosphate buffer resulted in the formation of a ring-shaped film on the surface of the glass substrate. The inset shows a picture of the solid phase on the glass substrate. d) Control experiment for the operation of the all-optical excitonic switch with n=1 in the solid phase. The absorption of the acceptor strand A12 in a duplex with a fully complementary unmodified oligonucleotide (black line) and the 470 nm excitation beam of the fluorometer (green line) are shown. The 470 nm excitation beam of the fluorometer does not overlap with the absorption band of the acceptor, thereby ruling out direct excitation. Furthermore, the 470 nm excitation beam of the fluorometer is not detectable at 500 nm, which was used to monitor the fluorescence of the all-optical excitonic switch.
3. Experimental Procedures
The boronic acid pinacolate esters (8g) was synthesized according to the literature procedure. The tC phosphoramidite was synthesized according to the literature procedure by Sandig et al.

5-iodo-6-methyluracil

In a round-bottom flask, 6-methyluracil (5 g, 39.65 mmol, 1.00 eq), iodine (6.04 g, 23.79 mmol, 0.60 eq), and cerium(IV) ammonium nitrate (10.87 g, 19.82 mmol, 0.50 eq) was suspended in 200 mL acetonitrile. The reaction mixture was heated to 90 °C for 6 h. After cooling to 0 °C, the colorless precipitate was filtered, washed twice with cold acetonitrile, and dried under reduced pressure. 5-iodo-6-methyluracil (7.32 g, 29.34 mmol, 74%) was obtained as a colorless solid. Recorded spectra are in accordance with the literature. 1H-NMR (300 MHz, DMSO-d6) δ = 11.27 (s, 2H), 2.27 (s, 3H), 2.06 (td, J = 7.2, 6.6, 2.4 Hz, 2H), 2.75 (s, 3H), 2.41 (s, 3H), 2.38 (s, 3H). 13C NMR (75 MHz, DMSO-d6) δ 161.50, 154.57, 150.72, 71.58, 39.52, 23.94. MS (HR-ESI, pos.): meas. m/z = 274.9283, calc. 274.9288 for C6H5IN2O4Na [M+Na]+.

5-iodo-6-methyl-2′-deoxyuridine-5′,3′-paramethylbenzoate

In a dried Schlenk flask under argon atmosphere, 5-iodo-6-methyluracil (2.50 g, 9.92 mmol, 1.00 eq) was suspended in hexamethyldisilazane (20 mL, 99.20 mmol, 10.00 eq) and trimethylsilyl chloride (4.31 g, 39.68 mmol, 4.00 eq) was added carefully at rt. The mixture was heated to 120 °C and stirred for 6 h until a clear solution was formed. The solvent was evaporated under reduced pressure and co-evaporated three times with anhydrous DCM. The residue was dissolved in 25 mL anhydrous DCM and Hoffer’s chlorosugar (4.63 g, 11.90 mmol, 1.20 eq) was added portion-wise and the mixture was stirred at rt for 24 h. A saturated aqueous NaHCO3 solution (100 mL) was added and the mixture was filtered. The aqueous layer was extracted with DCM for 3 times and the combined organic layers were dried over anhydrous MgSO4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (5:2, cyclohexane/ethyl acetate) affording 5-iodo-6-methyl-2′-deoxyuridine-5′,3′-paramethylbenzoate (1.52 g, 2.48 mmol, 25%) as a colorless foam. 1H NMR (300 MHz, Methanol-d4) δ 7.95 – 7.84 (m, 5H), 7.30 – 7.17 (m, 5H), 6.38 (t, J = 7.3 Hz, 1H), 5.54 (q, J = 7.4 Hz, 0H), 4.99 – 4.92 (m, 1H), 4.59 – 4.50 (m, 2H), 2.96 (td, J = 7.2, 6.6, 2.4 Hz, 2H), 2.75 (s, 3H), 2.41 (s, 3H), 2.38 (s, 3H). 13C NMR (75 MHz, Methanol-d4) δ 167.84, 167.51, 162.05, 156.95, 151.93, 150.45, 145.57, 144.73, 130.74, 130.70, 130.23, 130.14, 128.29, 128.14, 88.62, 83.38, 76.94, 65.28, 37.42, 27.21, 26.21, 21.14. MS (HR-ESI, pos.): meas. m/z = 627.0574, calc. 627.0599 for C26H25IN2O2Na [M+Na]+.

5-iodo-6-methyl-2′-deoxyuridine

In a microwave tube, 5-iodo-6-methyl-2′-deoxyuridine-5′,3′-paramethylbenzoate (200 mg, 0.33 mmol, 1.00 eq) was dissolved in methanol and aqueous ammonia (25 m%, 3 mL, 33 mmol, 100.00 eq) was added carefully. The mixture was stirred in the microwave (150 W, 60 °C, 20 bar) for 3 h until a clear solution was formed. Water (5 mL) and DCM (10 mL) were added and the aqueous layer was separated. The aqueous layer was washed twice with DCM (10 mL) and the solvent was evaporated under reduced pressure. The crude product was purified using silica gel column chromatography (20:1, DCM/Methanol + 0.2% NEt3). 5-iodo-6-methyl-2′-deoxyuridine (101 mg, 0.27 mmol, 83%) was obtained as colorless solid. 1H NMR (300 MHz, DMSO-d6) δ 11.61 (s, 1H), 6.11 (t, J = 7.6 Hz, 1H), 5.23 (d, J = 6.1 Hz, 1H), 4.68 (t, J = 4.6 Hz, 1H), 4.19 – 4.07 (m, 1H), 4.07 – 3.99 (m, 1H), 3.58 – 3.50 (m, 1H), 3.43 – 3.35 (m, 1H), 2.66 (s, 3H), 2.43 (t, J = 7.5 Hz, 2H). MS (HR-ESI, pos.): meas. m/z = 390.9764, calc. 390.9761 for C13H13IN2O2Na [M+Na]+.
General procedure A for the synthesis of the doubly methylated photoswitches (dU-Me-Ph, dU-Me-2Py, dU-Me-Ph+iBu)

In a microwave vial under argon atmosphere, 5-iodo-6-methyl-2'-deoxyuridine (1.00 eq), boronic acid pinacolate ester (8%) (1.30 eq), Pd(dppf)Cl₂ (0.05 eq), and Cs₂CO₃ (3 eq) were dissolved in a degassed mixture of DMSO/water (9/1). The mixture was stirred at 100 °C for 6 h. Then, water and ethyl acetate were added to the reaction mixture and the layers were separated. The aqueous layer was extracted with ethyl acetate (three times). The combined organic layers were dried over MgSO₄, filtered and the solvent was evaporated under reduced pressure. The crude product was absorbed on silica gel and purified by flash column chromatography affording dU-Me-R.

2'-deoxy-6-methyl-5-(2-(2-methyl-5-phenylthiophen-3-yl)cyclopent-1-en-1-yl)-uridine

The doubly methylated 2'-deoxyuridine-based photoswitchable nucleoside dU-Me-Ph was prepared according to general procedure A. Purification by flash column chromatography (silica gel, DCM/MeOH, 20/1-10/1) afforded dU-Me-Ph as a colorless solid with a yield of 56%. Due to the formation of syn- and anti-conformers of the compound, a set of two signals is observed, which is most dominant for the glycosidic proton (zoom-in shown in NMR in appendix). ¹H NMR (500 MHz, Methanol-d₄) δ 7.52 – 7.46 (m, 2H), 7.33 (dt, J = 7.7, 1.7 Hz, 2H), 7.22 (td, J = 7.3, 1.4 Hz, 1H), 7.04 (d, J = 7.4 Hz, 1H), 6.00 – 5.87 (m, 1H), 4.28 – 4.15 (m, 2H), 3.65 (dd, J = 12.0, 2.9 Hz, 1H), 3.51 (dd, J = 12.1, 4.2, 2.4 Hz, 1H), 3.03 – 2.91 (m, 2H), 2.67 – 2.65 (m, 1H), 2.59 – 2.45 (m, 2H), 2.45 – 2.36 (m, 1H), 2.24 (s, 3H), 2.14 – 2.07 (m, 2H), 2.06 (s, 3H). ¹³C NMR (126 MHz, Methanol-d₄) δ 164.63, 157.08, 154.86, 154.39, 152.12, 151.57, 151.29, 141.55, 140.67, 140.60, 129.94, 129.91, 128.28, 128.23, 126.21, 126.14, 124.57, 124.47, 113.17, 113.09, 88.62, 88.40, 88.36, 88.33, 62.99, 62.85, 38.90, 38.77, 38.70, 38.50, 37.96, 37.86, 23.99, 23.96, 17.88, 17.73, 14.40. MS (HR-ESI, pos.): meas. m/z = 503.1616, calc. 503.1611 for C₂₆H₂₅N₂O₂SNa [M+Na]⁺.

2'-deoxy-6-methyl-5-(2-(2-methyl-5-2-pyridylthiophen-3-yl)cyclopent-1-en-1-yl)-uridine

The doubly methylated 2'-deoxyuridine based photoswitchable nucleoside dU-Me-2Py was prepared according to general procedure A. Purification by flash column chromatography (silica gel, DCM/MeOH, 20/1-10/1) afforded dU-Me-2Py as a colorless solid with a yield of 49%. % Due to the formation of syn- and anti-conformers of the compound, a set of two signals is observed, which is most dominant for the glycosidic proton. ¹H NMR (500 MHz, DMSO-d₆) δ 11.33 (dd, J = 19.7, 8.8 Hz, 1H), 8.47 (d, J = 4.8 Hz, 1H), 7.82 – 7.72 (m, 2H), 7.48 (d, J = 11.4 Hz, 1H), 7.22 (t, J = 5.9 Hz, 1H), 6.05 – 5.80 (m, 1H), 5.18 – 5.03 (m, 1H), 4.62 – 4.53 (m, 1H), 4.27 – 3.92 (m, 2H), 3.61 – 3.45 (m, 1H), 2.99 – 2.79 (m, 2H), 2.69 – 2.55 (m, 1H), 2.46 – 2.24 (m, 3H), 2.15 (s, 3H), 2.03 – 1.97 (m, 2H), 1.96 (d, J = 8.6 Hz, 3H). ¹³C NMR (126 MHz, DMSO-d₆) δ 161.82, 161.72, 151.65, 150.68, 150.27, 149.98, 149.29, 148.92, 148.76, 148.51, 140.23, 137.35, 137.16, 137.00, 136.52, 136.19, 136.09, 133.91, 133.75, 125.81, 122.60, 118.10, 111.08, 110.85, 85.89, 85.76, 70.24, 70.01, 61.92, 61.34, 61.16, 37.39, 36.95, 36.80, 22.32, 17.05, 16.99, 14.11, 14.06. MS (HR-ESI, pos.): meas. m/z = 504.1583, calc. 504.1564 for C₂₆H₂₆N₂O₂SNa [M+Na]⁺.
Tert-butyl-2'-deoxy-6-methyl-5-(2-(2-methyl-5-phenylthiophen-3-yl)cyclopent-1-en-1-yl)-uridine-benzoate

The doubly methylated 2'-deoxyuridine based photoswitchable nucleoside dU-Me-PhtBu was prepared according to general procedure A. Purification by flash column chromatography (silica gel, DCM/MeOH, 20/1-10/1) afforded dU-Me-PhtBu as a colorless solid with a yield of 42%. Due to the formation of syn- and anti-conformers of the compound, a set of two signals is observed, which is most dominant for the glycosidic proton. 

\[ ^1H\text{ NMR} (500\text{ MHz, Methanol-} d_4) \delta 7.92 (\text{d, } J = 6.8\text{ Hz, } 2\text{H}), 7.59 (\text{d, } J = 8.5\text{ Hz, } 2\text{H}), 7.21 (\text{d, } J = 6.2\text{ Hz, } 1\text{H}), 6.08 - 5.87 (m, 1\text{H}), 4.28 - 4.12 (m, 2\text{H}), 3.64 (dd, J = 12.0, 3.0 Hz, 1\text{H}), 3.58 - 3.43 (m, 1\text{H}), 3.05 - 2.90 (m, 2\text{H}), 2.72 - 2.65 (m, 1\text{H}), 2.58 - 2.51 (m, 1\text{H}), 2.48 - 2.38 (m, 1\text{H}), 2.26 (d, J = 2.5 Hz, 3\text{H}), 2.10 (d, J = 7.9 Hz, 2\text{H}), 2.06 (s, 3\text{H}), 1.60 (s, 9\text{H}).\]

\[ ^{13}C\text{ NMR} (126\text{ MHz, Methanol-} d_4) \delta 167.03, 166.98, 152.03, 151.59, 151.32, 140.32, 140.16, 139.71, 138.07, 137.74, 137.43, 137.20, 135.31, 135.24, 133.27, 131.43, 131.07, 131.05, 126.24, 126.17, 125.80, 125.74, 112.98, 88.62, 88.38, 88.33, 82.31, 72.78, 72.59, 62.98, 62.85, 38.87, 37.66, 28.43, 23.97, 17.90, 17.76, 14.48.\]

\[ \text{MS (HR-ESI, pos.): meas. } m/z = 603.2138, \text{ calc. } 603.2135 \text{ for C}_{31}\text{H}_{36}\text{N}_{2}\text{O}_{7}\text{S} \text{Na} [\text{M+Na}]^+.\]

In a dried Schlenk flask under argon atmosphere, 5-ido-6-methyl-2'-deoxyuridine (4) (360 mg, 0.98 mmol, 1.00 eq), 4,4'-dimethoxytrityl chloride (364.49 mg, 1.08 mmol, 1.10 eq) and 4-(dimethylamino)-pyridine (5.99 mg, 0.05 mmol, 0.05 eq) were dissolved in anhydrous pyridine and stirred at rt for 16 h. NaHCO₃ (10 mL, sat. aqueous solution) was added and the mixture was stirred at rt for 10 min. The crude mixture was diluted by the addition of 25 mL of DCM and the aqueous layer was extracted three times with DCM. The combined organic layers were washed with brine, dried over anhydrous MgSO₄ and the solvent was removed under reduced pressure. The yellow crude product was purified using silica gel column chromatography (50/1, DCM/MeOH + 0.2% NEt₃) and 5-ido-6-methyl-5'-dimethoxytrityl-2'-deoxyuridine (5) (247 mg, 0.38 mmol, 39%) was obtained colorless foam. 

\[ ^1H\text{ NMR} (300\text{ MHz, Chloroform-d}) \delta 7.41 (\text{dd, } J = 8.3, 1.3\text{ Hz, } 2\text{H}), 7.31 (d, J = 8.9\text{ Hz, } 5\text{H}), 7.26 - 7.17 (m, 2\text{H}), 6.82 (d, J = 1.6\text{ Hz, } 2\text{H}), 6.79 (d, J = 1.6\text{ Hz, } 2\text{H}), 6.16 (dd, J = 8.6, 4.9\text{ Hz, } 1\text{H}), 4.67 - 4.57 (m, 1\text{H}), 3.85 (q, J = 5.7\text{ Hz, } 1\text{H}), 3.78 (s, 6\text{H}), 3.46 (d, J = 5.9\text{ Hz, } 1\text{H}), 3.35 (dd, J = 9.9, 5.4\text{ Hz, } 1\text{H}), 2.89 - 2.78 (m, 1\text{H}), 2.72 (s, 3\text{H}), 2.19 (ddd, J = 14.2, 8.7, 6.1\text{ Hz, } 1\text{H}).\]

\[ ^{13}C\text{ NMR} (126\text{ MHz, Chloroform-d}) \delta 158.67, 144.82, 135.95, 135.94, 130.23, 130.32, 128.32, 127.98, 127.02, 113.29, 96.50, 86.67, 85.14, 77.16, 64.10, 55.39, 51.05, 38.65, 29.86, 26.21.\]

\[ \text{MS (HR-ESI, pos.): meas. } m/z = 693.1078, \text{ calc. } 693.1068 \text{ for C}_{31}\text{H}_{31}\text{N}_{2}\text{O}_{7}\text{Na} [\text{M+Na}]^+.\]
5-(tert-butyl-cyclopent-1-en-1-yl-5-methylthiophen-2-ylbenzoate)-6-methyl-5′-dimethoxytrityl-2′-deoxyuridine (6)

In a dried Schlenk flask under argon atmosphere, 5-iodo-6-methyl-5′-dimethoxytrityl-2′-deoxyuridine (5) (140 mg, 0.21 mmol, 1.00 eq), tert-butyl-4-((5-iodo-4-(2-(4,4,5,5-tetramethyl-1,3,2-di-oxaborolan-2-yl)cyclopent-1-en-1-yl)thiophen-2-yl)benzoate (116.87 mg, 0.25 mmol, 1.20 eq), cesium carbonate (204.09 mg, 0.63 mmol, 3.00 eq) and Pd(dppe)Cl2 (15.28 mg, 0.02 mmol, 0.1 eq) were dissolved in a degassed DMSO/water (9/1) mixture and heated to 100 °C for 4 h. Brine and ethyl acetate were added to the mixture and phases were separated. The aqueous layer was extracted three times with ethyl acetate and the combined organic layers were dried over magnesium sulfate, filtered and the solvent was evaporated under reduced pressure. The dark brown crude product was dried for several hours under vacuum and subsequently purified by silica gel column chromatography (50/1, DCM/MeOH + 0.2% NEt3).

5-(tert-butyl-cyclopent-1-en-1-yl-5-methylthiophen-2-ylbenzoate)-6-methyl-5′-dimethoxytrityl-2′-deoxyuridine (6) (81.50 mg, 0.91 mmol, 44%) was obtained as a yellow solid. Comment to the NMR spectra: Due to the formation of syn- and anti-conformers of the compound, a set of two signals is observed, which is most dominant for the glycosidic proton (δ 6.15-6.2).

1H NMR (300 MHz, Chloroform-d) δ 7.96 – 7.90 (m, 2H), 7.52 – 7.47 (m, 2H), 7.42 – 7.36 (m, 2H), 7.31 – 7.27 (m, 4H), 7.24 – 7.18 (m, 2H), 7.04 (d, J = 4.2 Hz, 1H), 6.79 (d, J = 8.7 Hz, 4H), 6.15 – 6.02 (m, 1H), 5.74 (dd, J = 8.8, 4.3 Hz, 1H), 4.66 – 4.46 (m, 1H), 3.77 (s, 6H), 3.49 – 3.40 (m, 1H), 3.36 – 3.23 (m, 1H), 3.10 – 2.96 (m, 1H), 2.91 – 2.83 (m, 1H), 2.67 (m, 1H), 2.24 (s, 3H), 2.11 (s, 2H), 2.01 (s, 3H), 1.60 (s, 9H).

13C NMR (75 MHz, Chloroform-d) δ 165.55, 161.58, 158.63, 149.39, 149.28, 144.85, 139.42, 139.27, 138.11, 138.04, 136.13, 136.00, 130.22, 130.16, 128.32, 128.25, 127.94, 127.01, 126.98, 124.92, 124.81, 124.74, 124.54, 123.28, 113.23, 123.25, 112.09, 86.67, 85.14, 81.19, 77.16, 73.32, 64.59, 55.37, 38.05, 28.38, 17.68, 14.61. MS (HR-ESI, pos.): meas. m/z = 905.3425, calc. 905.3442 for C31H28N2O2SNa [M+Na]+.

5-(tert-butyl-cyclopent-1-en-1-yl-5-methylthiophen-2-ylbenzoate)-6-methyl-3′-(cyanoethoxy)(diisopropylamino)phosphaneyl-5′-dimethoxytrityl-2′-deoxyuridine (7)

In a dried Schlenk flask under argon atmosphere, 5-(tert-butyl-cyclopent-1-en-1-yl-5-methylthiophen-2-ylbenzoate)-6-methyl-5′-dimethoxytrityl-2′-deoxyuridine (6) (75 mg, 84.93 µmol, 1.00 eq) and Hünig’s base (16.47 mg, 127.40 µmol, 1.50 eq) were dissolved in 5 mL anhydrous DCM and cooled to 0 °C. A solution of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (19.10 mg, 80.68 µmol, 0.95 eq) in anhydrous DCM (1 mL) was added dropwise and the mixture was stirred at rt for 1 h. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (10/1, cyclohexane/acetone + 0.2% NEt3), which afforded 5-(tert-butyl-cyclopent-1-en-1-yl-5-methylthiophen-2-ylbenzoate)-6-methyl-3′-(cyanoethoxy)(diisopropylamino)phosphaneyl-5′-dimethoxytrityl-2′-deoxyuridine (7) (75 mg, 69.64 µmol, 82%) as pale-yellow oil.

1H NMR (500 MHz, Chloroform-d) δ 7.95 – 7.87 (m, 2H), 7.54 – 7.49 (m, 1H), 7.46 (t, J = 8.2 Hz, 1H), 7.43 – 7.39 (m, 2H), 7.35 – 7.27 (m, 4H), 7.25 – 7.16 (m, 3H), 7.06 (dd, J = 20.7, 4.9 Hz, 1H), 6.76 (t, J = 9.4 Hz, 4H), 6.35 – 5.75 (m, 1H), 4.70 – 4.47 (m, 1H), 4.13 – 3.87 (m, 1H), 3.77 (t, J = 5.2 Hz, 6H), 3.62 – 3.43 (m, 4H), 3.33 (d, J = 5.1 Hz, 2H), 3.10 – 2.99 (m, 1H), 2.89 (dt, J = 12.9, 5.8 Hz, 2H), 2.73 – 2.65 (m, 1H), 2.51 (dt, J = 16.3, 6.3 Hz, 1H), 2.41 – 2.31 (m, 2H), 2.24 (d, J = 10.1 Hz, 3H), 2.09 – 2.03 (m, 4H), 1.60 (s, 9H), 1.34 – 1.22 (m, 4H), 1.11 (dt, J = 8.8, 4.1 Hz, 7H), 1.04 (dd, J = 12.0, 6.8 Hz, 4H), 0.98 – 0.92 (m, 3H).

31P NMR (202 MHz, Chloroform-d) δ 149.01, 148.88, 148.82, 148.74. MS (HR-ESI, pos.): meas. m/z = 1105.4477, calc. 1105.4421 for C61H52N2O12P2SnNa [M+Na]+.
4. NMR spectra

$^1$H-NMR of dU-Me-Ph:

13C-NMR of dU-Me-Ph:
$^1$H-NMR of dU-Me-2Py:

$^{13}$C-NMR of dU-Me-2Py:

$^1$H-NMR of dU-Me-Ph'Bu:
$^{13}$C-NMR of dU-Ph'Bu-Me$_2$: 

[Chemical structure image]

[1H-NMR spectrum image]
$^1$H-NMR of compound (5):

$^{13}$C-NMR of compound (5):
$^1$H-NMR of compound (6):

$^1$H-NMR of compound (7):
$^{31}$P-NMR of compound (7):

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