Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Red light-controlled polymerase chain reaction

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Content

Materials and methods

Commercially available chemicals of the best quality from Aldrich/Sigma/Fluka (Germany) were obtained and used without purification. Phosphoramidites and controlled pore glass (CPG) solid support were from Aldrich (Germany) and Link Technologies (UK). A LightCycler® 2.0 IVD (Roche, Germany) was used for qRT-PCR experiments. Synthesis of oligonucleotides was conducted on a K&A H-8 DNA /RNA synthesizer. ^{1}H- and ^{13}C-NMR spectra were measured on a Bruker Avance 400. MALDI-TOF mass spectra were recorded on a Shimadzu Axima mass spectrometer. The matrix mixture (2:1 v/v) was prepared from 2′,4′,6′-trihydroxyacetophenone (THAP, 0.3 M solution in acetonitrile) and diammonium citrate (0.1 M in water). Samples for mass spectrometry were prepared by the dried droplet method using a 1:2 probe/matrix ratio. Mass accuracy with external calibration was 0.1 % of the peak mass, that is ±7.0 at *m*/*z* 7.000. HPLC was performed at 65 °C, or at 70 °C with a Varian MethaTerm™ HPLC column temperature control, on a Shimadzu liquid chromatograph equipped with UV and fluorescence detectors and a Macherey–Nagel Nucleosil C18 250 × 4.6 mm column. Gradient of solution B (CH_3CN) in solution A [0.1 M aqueous (NEt_3H)(OAc)] was applied to purify conjugates. UV/Vis spectra were measured on a Lambda Bio+ UV/Vis spectrophotometer (Perkin Elmer) by using quartz glass cuvettes (Hellma GmbH, Germany) with a sample volume of 1 mL, respectively micro cuvettes with a sample volume of 100 µL (BRAND GmbH, Germany), if the absorbance of DNA was measured. Fluorescence spectra were acquired on a Varian Cary Eclipse fluorescence spectrophotometer using fluorescence cuvettes (Hellma GmbH, Germany) with a sample volume of 1 mL. Irradiation experiments were performed with an LED Array 672 (λ = 650 nm) from Cetoni GmbH (Germany).

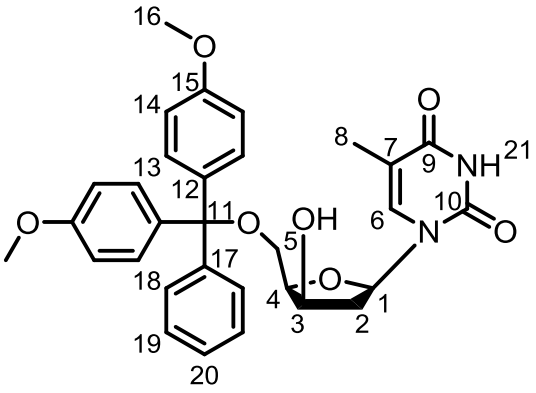
Syntheses of small molecules

1-[5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxy-*β*-D-furanosyl] thymidin

5'-*O*-(4,4'-dimethoxytrityl) thymidine (9.55 g, 17.55 mmol, 1.0 eq) and triethylamine (4.44 g, 43.87 mmol, 2.5 eq) was dissolved under nitrogen atmosphere at 0 °C in dry THF (80 mL). Mesyl chloride (3.02 g, 26.32 mmol, 1.5 eq) was added dropwise, the

ice bath removed and the resulting reaction mixture was stirred for 45 minutes at room temperature. Ethanol (40 ml) and $NaOH_{aq}$ (1.0 M, 50 mL) were added and the reaction stirred for 90 minutes under reflux at 85 °C. $NaOH_{aq}$ (10.0 M, 35 ml) is added to the reaction and stirred for additional 3 h under reflux. Then, an equivalent amount of $NaOH_{aq}$ (20 M, 35 mL) is added and the reaction stirred over night at 60 °C. After concentration under vacuum, water (100 mL) was added and the solution was neutralized with 1.0 M HCl. It was diluted with CH_2Cl_2 (50 mL). After separating the organic from the aqueous phase, the aqueous phase was extracted three times with CH_2Cl_2 (50 mL). The joined organic phases were washed with water and dried over anhydrous $MgSO_4$. Next, the solvent was removed under reduced pressure and the crude product was filtered through silica. After removing the solvent under reduced pressure **1** was obtained as a white powder (9.55 g,17.55 mmol, 100 %); thin layer chromatography (TLC) on SiO_2, eluent dichloromethane/methanol (20/1, v/v), R_f = 0.31.

^{1}H NMR (400.05 MHz, Acetone-*d6*)



δ = 10.00 (bs, 1H, H^{21}), 7.76 (s, 1H, H^6), 7.55 (d, ^3J = 7.30 Hz, 2H, H^{19}), 7.43-7.39 (m, 4H, H^{14}), 7.34-7.30 (m, 2H, H^{18}), 7.26-7.22 (m, 1H, H^{20}), 6.90 (d, ^3J = 8.80 Hz, 4H, H^{13}), 6.22 (dd, ^3J = 8.20, 2.40 Hz, 1H, H^1), 4.45 (m, 2H, H^5), 4.21-4.19 (m, 1H, H^3), 3.80 (s, 6H, H^{16}), 3.64-3.40 (m, 2H, H^2), 2.72-2.65 (m, 1H, H^4), 1.74 (s, 3H, H^8) ppm.

^{13}C {^{1}H} NMR (100.50 MHz, Acetone-*d6*)

δ = 164.01, 159.11, 151.02, 145.77, 137.57, 136.55, 136.39, 130.53, 128.57, 128.07, 127.02, 113.39, 109.28, 86.53, 85.28, 84.06, 72.54, 63.18, 55.02, 41.53, 12.27 ppm.

MS (HR-ESI$^+$)

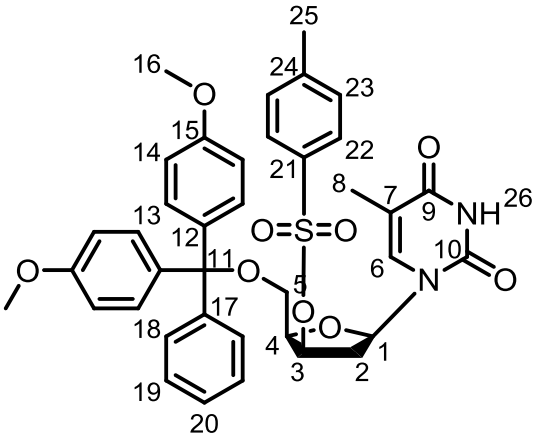
m/z = 567.2102 ([M+Na]$^+$) calculated for $C_{31}H_{32}O_7N_2$. *m/z* = 567.2121 ([M+Na]$^+$) found.

1-[5'-*O*-(4,4'-dimethoxytrityl)-3'-O-tosyl-2'-deoxy-*β*-D-furanosyl] thymidin **(1)**

Compound **1** (4.30 g, 7.90 mmol, 1.0 eq) and 4-toluenesulfonyl chloride (4.52 g, 23.70 mmol, 3.0 eq) were dissolved in dry pyridine (20 mL) under nitrogen atmosphere. The resulting mixture was stirred at room temperature for 24 hours. After concentration under vacuum the crude product was filtered through silica. After removing the solvent under reduced pressure **2** was obtained as a yellowish powder (4.10 g, 5.87 mmol, 74 %); thin layer chromatography (TLC) on SiO_2, eluent petroleum ether/ethylacetate (1/5, v/v), R_f = 0.43.

^{1}H NMR (400.05 MHz, Acetone-*d6*)

δ = 10.02 (bs, 1H, H^{26}), 7.67 (d, ^3J = 8.30 Hz, 2H, H^{22}), 7.48 (m, 2H, H^{23}), 7.40-7.28 (m, 10H, $H^{6,14,18,19,20}$), 6.91 (m, 4H, H^{13}), 6.21 (m, 1H, H^1), 5.29 (m, 1H, H^3), 4.39 (m, 1H, H^4), 3.83 (s, 6H, H^{16}), 3.50-3.21 (m, 2H, H^5), 2.88 (m, 2H, H^2), 2.44 (m, 3H, H^{25}), 1.68 (s, 3H, H^8) ppm.



^{13}C {^{1}H} NMR (100.50 MHz, Acetone-*d6*)

δ = 163.68, 159.20, 150.70, 150.12, 145.89, 145.41, 136.12, 135.98, 135.42, 133.83, 130.49, 128.46, 128.15, 129.91, 127.15, 124.10, 113.47, 110.00, 86.84, 84.47, 82.02, 80.24, 62.25, 55.06, 39.23, 21.11, 12.17 ppm.

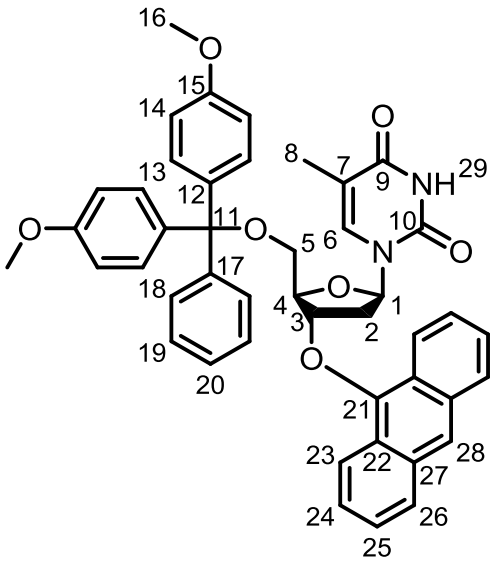
MS (HR-ESI$^+$)

m/z = 721.2190 ([M+Na]$^+$) calculated for $C_{38}H_{38}O_9N_2S$. *m/z* = 721.2168 ([M+Na]$^+$) found.

5'-*O*-(4,4'-dimethoxytrityl)-3'-*O*-(9-anthracenyl)-thymidine **(2)**

Anthrone (4.03 g, 20.77 mmol, 2.5 eq) and sodium hydride 60 % dispersion in mineral oil (0.83 g, 20.77 mmol, 2.5 eq) were dissolved in dry dimethyl sulfoxide (50 mL) under nitrogen atmosphere, stirring and at room temperature. Compound **2** (5.80

g, 8.31 mmol, 1.0 eq) was dissolved in dry dimethyl sulfoxide (20 mL) and added dropwise over 2 h to the red reaction mixture. The reaction mixture was allowed to stir for 24 h under nitrogen atmosphere. Then it was diluted with CH_2Cl_2 (50 mL) and washed with *aq.* saturated NaCl solution (100 mL). After separating the organic from the aqueous phase, the aqueous phase was extracted three times with CH_2Cl_2 (50 mL). The joined organic phases were washed with water and dried over anhydrous $MgSO_4$. Next, the solvent was removed under reduced pressure and the crude product was filtered through silica. After removing the solvent under reduced pressure, **3** was obtained as a yellow powder (5.30 g, 7.36 mmol, 89 %); thin layer chromatography (TLC) on SiO_2, eluent petroleum ether/ethylacetate (1/1, v/v), R_f = 0.35.



^{1}H NMR (400.05 MHz, Acetone-*d6*)

δ = 10.08 (bs, 1H, H^{29}), 8.41 (s, 1H, H^{28}), 8.38-8.36 (m, 2H, H^{26}), 8.12 (m, 2H, H^{23}), 7.60 (s, 1H, H^6), 7.57-7.54 (m, 4H, $H^{24.25}$), 7.25-7.10 (m, 9H, $H^{14,18,19,20}$), 6.80-6.75 (m, 5H, $H^{1,13}$), 5.52 (m, 1H, H^3), 4.65 (m, 1H, H^4), 3.77 (s, 6H, H^{16}), 3.35-3.24 (m, 2H, H^5), 2.95-2.52 (m, 1H, H^2), 1.49 (s, 3H, H^8) ppm.

^{13}C {^{1}H} NMR (100.50 MHz, Acetone-*d6*)

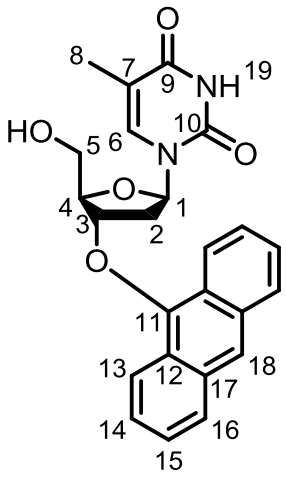
δ = 163.71, 159.15, 150.83, 149.23, 145.16, 135.76, 135.66, 132.88, 130.33, 130.26, 129.07, 128.27, 128.16, 125.25, 122.63, 113.44, 110.62, 86.99, 85.32, 85.21, 83.53, 63.95, 55.02, 40.86, 38.05, 11.68 ppm.

MS (HR-ESI$^+$)

m/z = 743.2728 ([M+Na]$^+$) calculated for $C_{45}H_{40}O_7N_2$. *m/z* = 743.2734 ([M+Na]$^+$) found.

3'-*O*-(9-anthracenyl) thymidine **(3)**

Compound **3** (0.24 g, 0.33 mmol, 1.0 eq) was dissolved in dry acetonitrile (5 mL) under nitrogen atmosphere. A solution of trichloroacetic acid in acetonitrile (0.26 M, 30.0 eq) was added. The reaction mixture was allowed to stir for 12 h under nitrogen atmosphere. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with *aq.* saturated $NaHCO_3$ solution (50 mL). After separating the organic from the aqueous phase, the aqueous phase was extracted three times with CH_2Cl_2 (10 mL). The joined organic phases were washed with water and dried over anhydrous $MgSO_4$. Next, the solvent was removed under reduced pressure and the crude product was filtered through silica. After removing the solvent under reduced pressure, **4** was obtained as a yellow powder (0.10 g, 0.24 mmol, 72 %); thin layer chromatography (TLC) on SiO_2, eluent petroleum ether/ethylacetate (1/2, v/v), R_f = 0.30.



^{1}H NMR (400.05 MHz, Acetone-*d6*)

δ = 10.00 (bs, 1H, H^{19}), 8.42 (s, 1H, H^{18}), 8.40-8.38 (m, 2H, H^{16}), 8.13-8.11 (m, 2H, H^{13}), 7.86 (s, 1H, H^{6}), 7.59-7.53 (m, 4H, ^{H14,15}), 6.83 (dd, $^{3}J_{H-H}$ = 8.70, 5.70 Hz, 1H, H^{1}), 5.30 (m, 1H, H^{3}), 4.61 (bs, 1H, OH), 4.38 (m, 1H, H^{4}), 3.83-3.72 (m, 2H, H^{5}), 2.81-2.40 (m, 2H, H^{2}), 1.82 (s, 3H, H^{8}) ppm.

^{13}C {^{1}H} NMR (100.50 MHz, Acetone-*d6*)

δ = 163.75 (C^{9}), 150.99 (C^{10}), 149.32 (C^{11}), 136.32 (C^{6}), 132.91 (C^{17}), 128.96 (C^{16}), 126.09 (C^{14}), 126.03 (C^{15}), 125.38 (C^{12}), 123.26 (C^{18}), 122.74 (C^{13}), 110.36 (C^{7}), 86.18 (C^{3}), 85.87 (C^{1}), 85.25 (C^{4}), 62.75 (C^{5}), 38.00 (C^{2}), 12.10 (C^{8}) ppm.

MS (HR-ESI^{+})

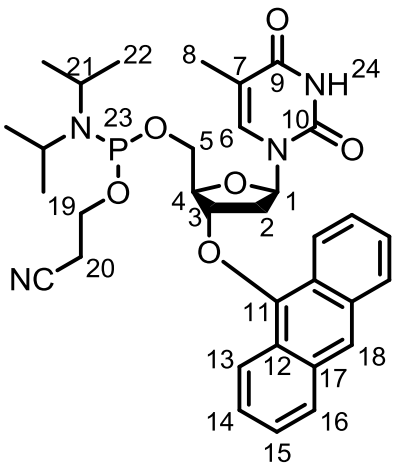
m/z = 441.1421 ([M+Na]$^{+}$) calculated for $C_{24}H_{22}O_5N_2$. *m/z* = 441.1424 ([M+Na]$^{+}$) found.

3'-*O*-(9-anthracene) thymidin-3'-[(2-cyanoethyl)-(*N,N*-diisopropyl)]-phosphoramidite **(4)**

Compound **4** (0.10 g, 0.24 mmol, 1.0 eq) and *N,N*-diisopropylethylamine (0.12 g, 0.16 mL, 0.96 mmol, 4.0 eq) were dissolved in dry CH_2Cl_2 (5 mL) under argon atmosphere, stirring and at room temperature. 2-Cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.08 g, 0.36 mmol, 1.5 eq) was added dropwise to the reaction mixture. The reaction mixture was allowed to stir for 2 h under argon atmosphere. Then it was diluted with dry CH_2Cl_2 (10 mL) and washed with *aq.* saturated $NaHCO_3$ solution (20 mL). After separating the organic from the aqueous phase, the aqueous phase was extracted three times with CH_2Cl_2 (10 mL). The joined organic phases were washed with water and dried over anhydrous $MgSO_4$. Next, the solvent was removed under reduced pressure and the crude product was filtered through silica. After removing the solvent under reduced pressure, **5** was obtained as a yellow powder (0.10 g, 0.16 mmol, 66 %). Thin layer chromatography (TLC) on SiO_2, eluent petroleum ether/ethylacetate (1/1, v/v) and 1% triethylamine (v/v), R_f = 0.32.

^{1}H NMR (400.13 MHz, Acetone-*d6*)

δ = 10.03 (bs, 1H, H^{24}), 8.44-8.39 (m, 3H, $H^{16,18}$), 8.14-8.12 (m, 2H, H^{13}), 7.71-7.54 (m, 5H, $H^{6,14,15}$), 6.86-6.83 (m, 1H, H^{1}), 5.34-5.31 (m, 1H, H^{3}), 4.70-4.64 (m, 1H, H^{4}), 4.08-3.06 (m, 8H, $H^{5,19,20,21}$), 3.84-3.72 (m, 2H, H^{22}), 2.67-2.52 (m, 2H, H^{2}), 1.87 (m, 3H, H^{8}), 1.17-0.89 (m, 12H, H^{22}) ppm.



^{31}P NMR (121.49 MHz, Acetone-*d6*)

δ = 149.66, 148.92 ppm.

MS (HR-ESI$^+$)

m/z = 641.2499 ([M+Na]$^+$) calculated for $C_{33}H_{39}O_6N_4P$. *m/z* = 641.2489 ([M+Na]$^+$) found.

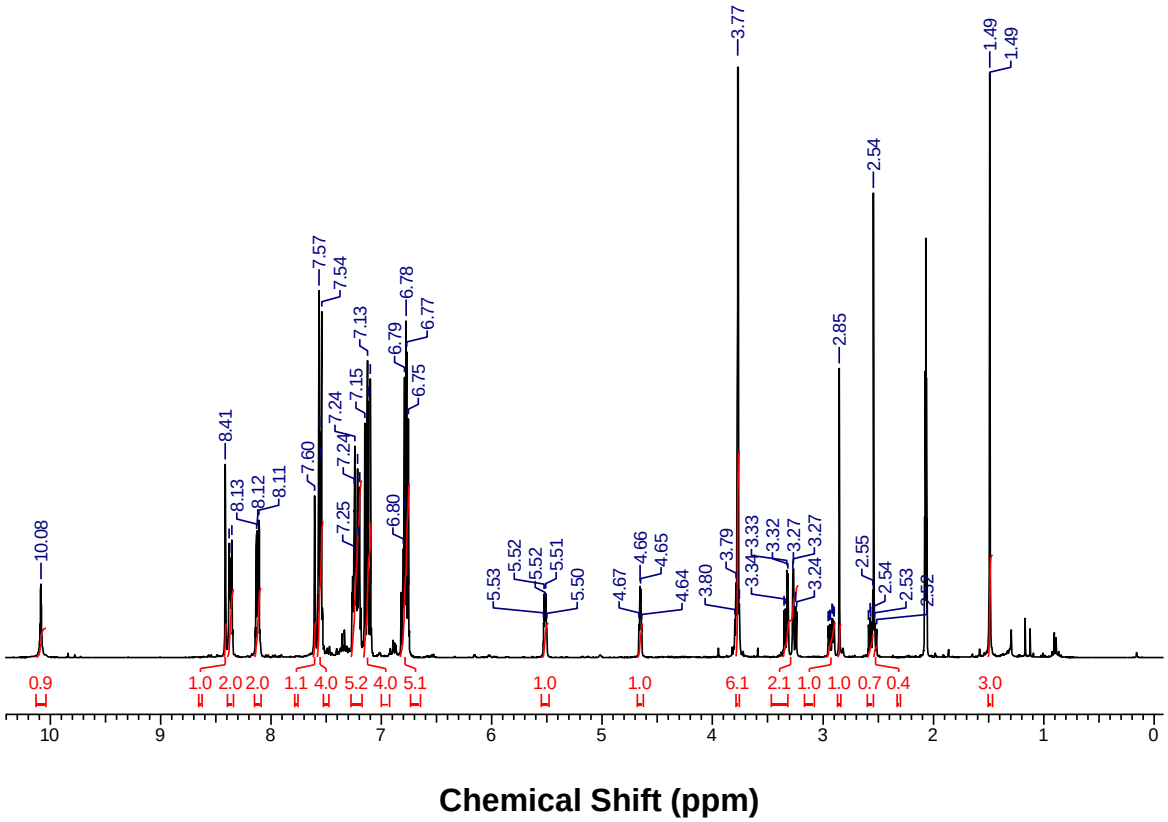


Figure **S1**: ^{1}H NMR spectrum of compound **2**.

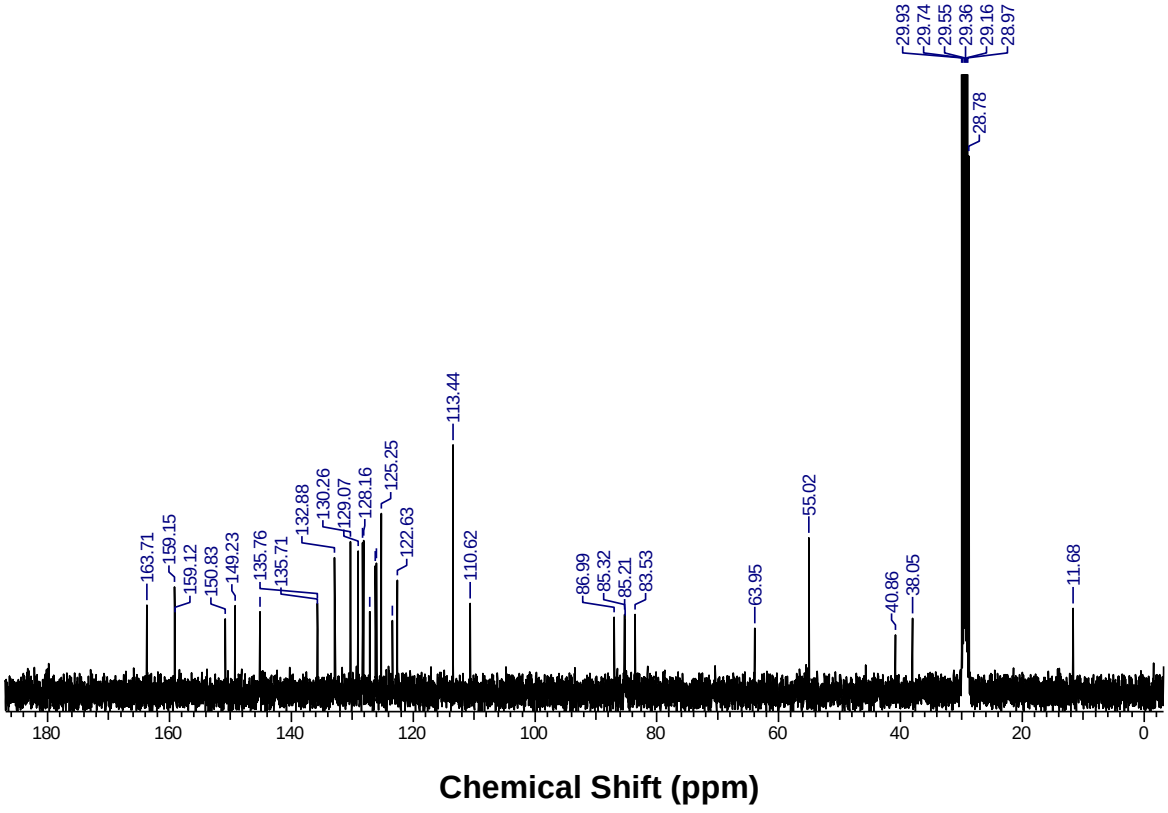


Figure **S2**: ^{13}C NMR spectrum of compound **2**.

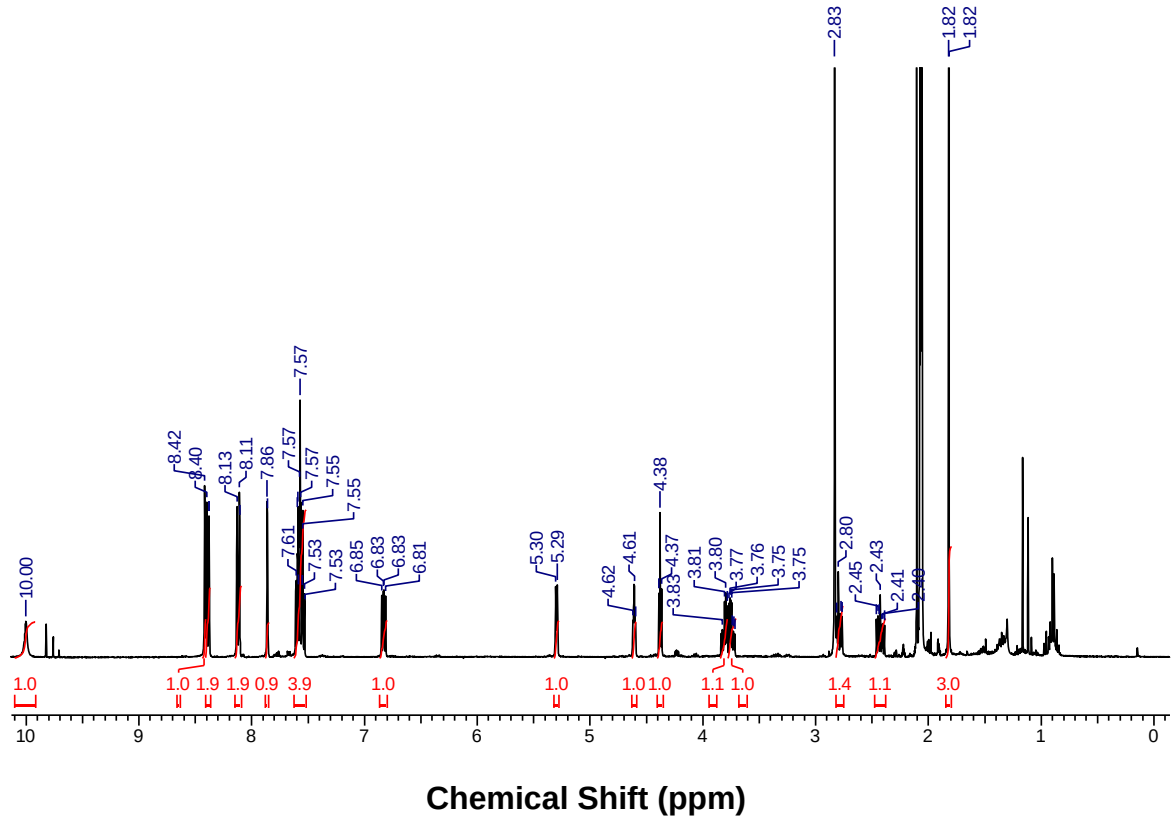


Figure **S3**: ^{1}H NMR spectrum of compound **3**.

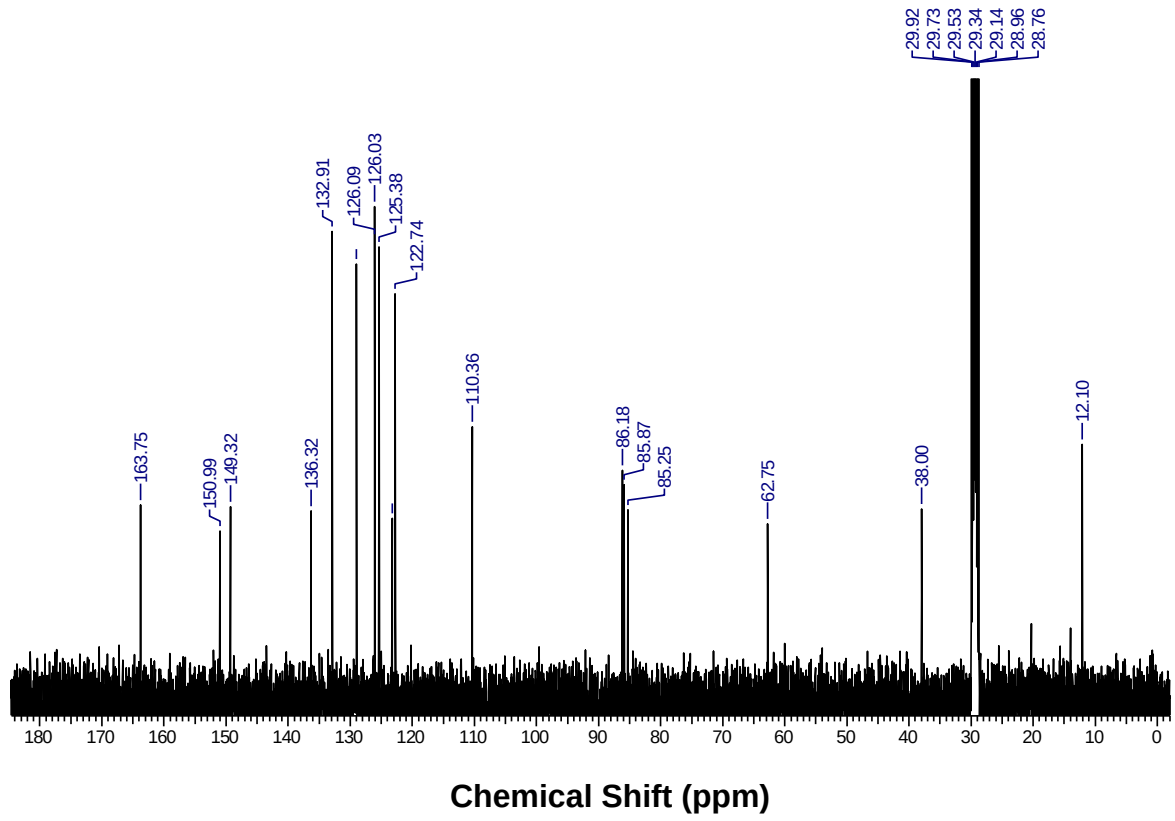


Figure **S4**: ^{13}C NMR spectrum of compound **3**.

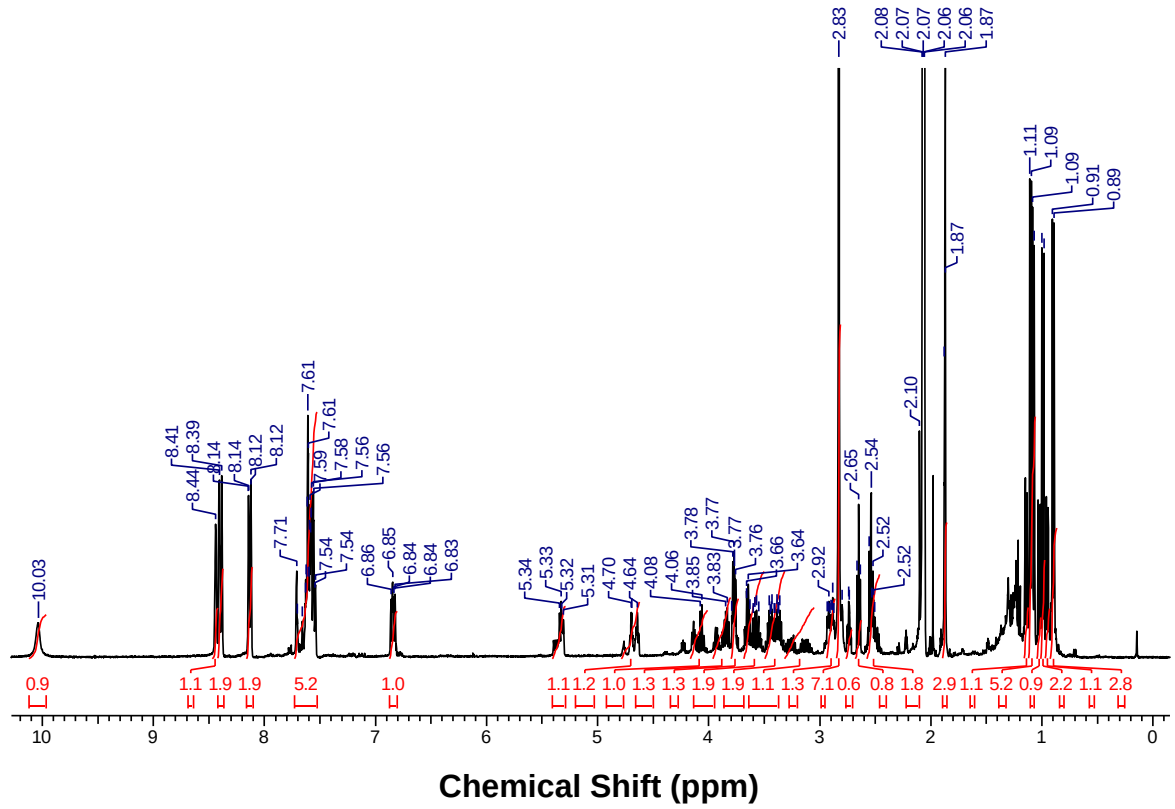


Figure **S5**: ^{1}H NMR spectrum of compound **4**.

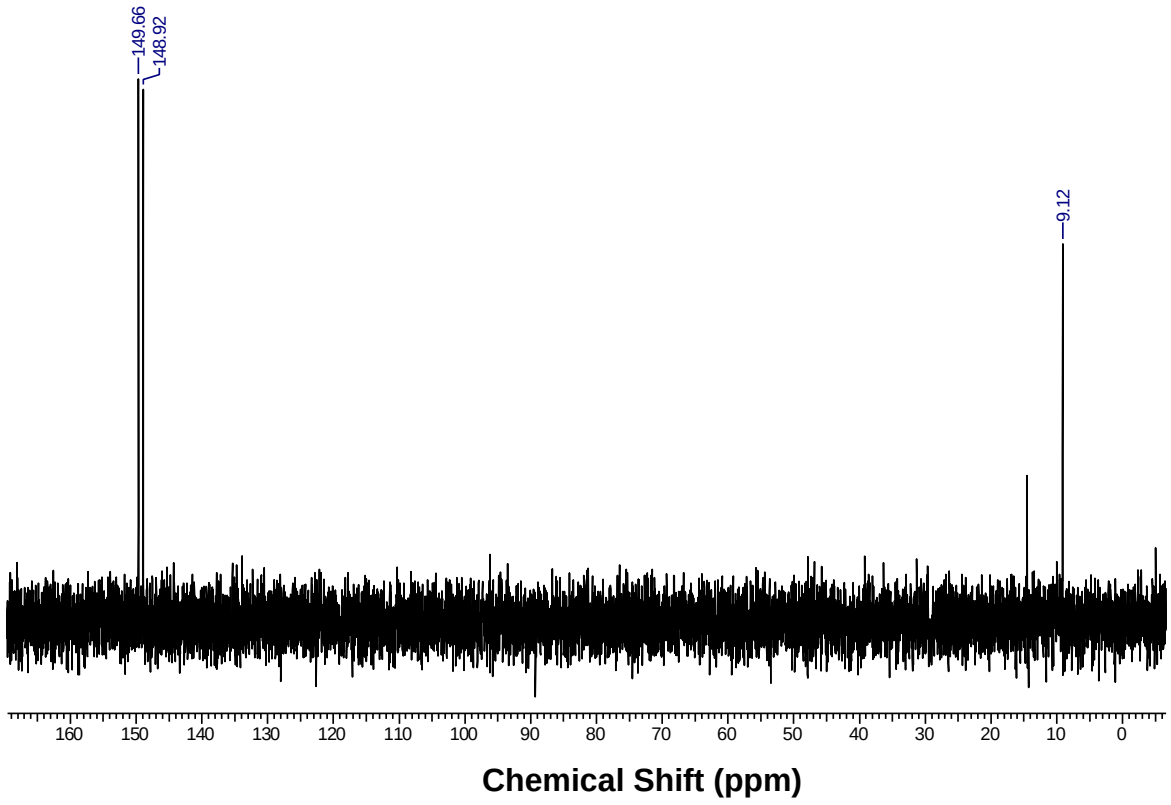


Figure **S6**: ^{31}P NMR spectrum of compound **4**.

Singlet oxygen mediated cleavage of compound 3

The decomposition of compound **3** was examined by UV/Vis-, fluorescence and ^{1}H-NMR spectroscopy, furthermore via ESI mass spectrometry.

For the UV/Vis and fluorescence examination a 1 mM stock solution of compound **3** in methanol (1 mL) with 0.1 equivalent of InPPa (**P-OH**) was irradiated with LED Array 672 (λ = 650 nm, 100 % intensity) for 15 and 30 minutes. For UV/Vis spectroscopy 100 µl of the stock solution were diluted in 900 µl methanol and measured with a Varian Cary 100 Bio. In the UV/Vis spectrum of compound **3**, characteristic peaks are observed in the region between 370 and 410 nm. These spectral features disappear in the presence of photogenerated $^{1}O_2$. For fluorescence spectroscopy (λ_{ex} = 368 nm) 10 µl of the stock solution were diluted in 990 µl methanol. In the fluorescence spectrum of compound **3**, characteristic peaks are observed in the region between 380 and 440 nm. These spectral features disappear also in the presence of photogenerated $^{1}O_2$, indicating that compound **3** reacts with $^{1}O_2$.

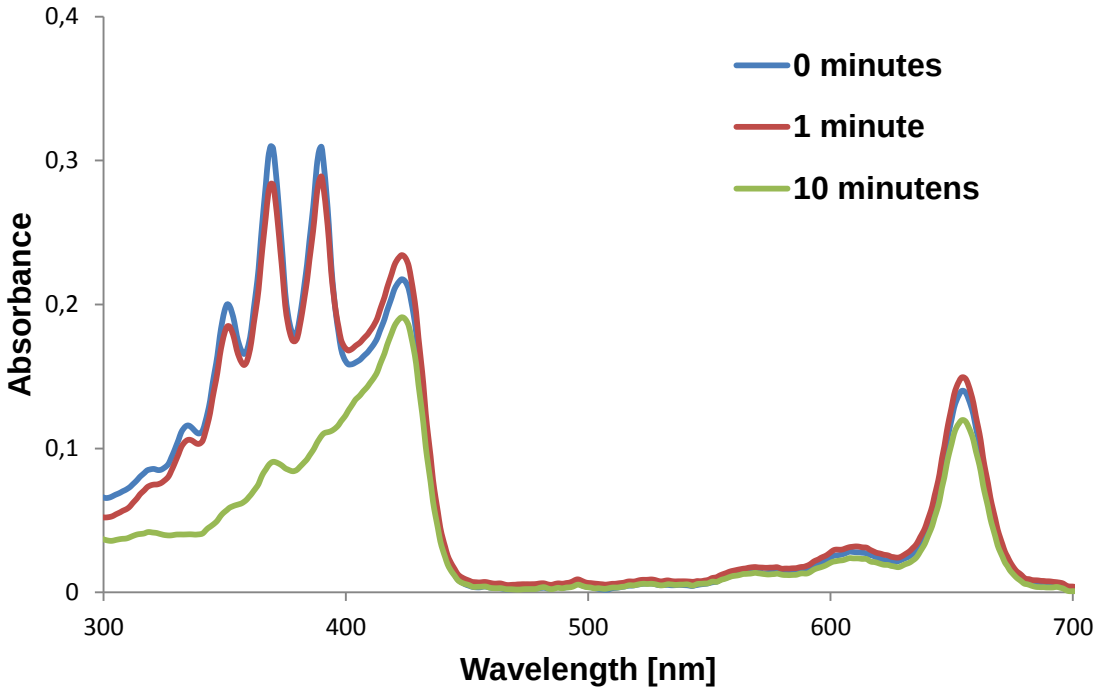


Figure **S7**: UV-visible absorbance of modified nucleoside **3** (1 mM) and In(III)(pyropheophorbide-a)chloride (**PS**, 100 µM) in methanol were irradiated with light (650 nm, 0.29 W) for 0, 1 and 10 min. The mixtures were diluted with methanol 10-fold to acquire UV-visible absorbance spectra.

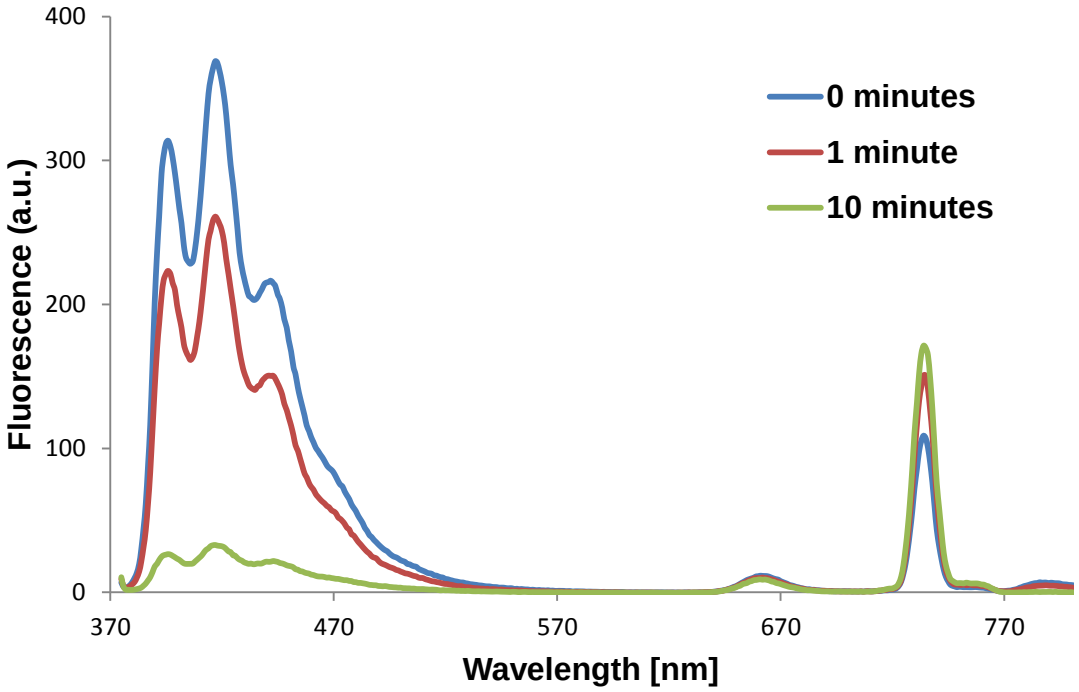


Figure **S8**: Fluorescence spectra of modified nucleoside **3** (1 mM) and **PS** (100 µM) in methanol were irradiated with light (650 nm, 0.29 W) for 0, 1 and 10 min. The mixtures were diluted with methanol 100 fold to acquire fluorescence spectra (λ_{ex}= 368 nm, B). a.u. – arbitrary units.

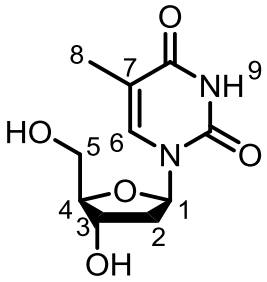
For monitoring cleavage of compound **3** in the presence of $^1O_2$ by ^{1}H-NMR spectroscopy a solution of compound **3** (5 mM) with **PS** (0.1 eq) was prepared in $CDCl_3$ containing 1% DMSO-*d6* (v/v) with 1% trifluoroacetic acid (v/v). To generate $^1O_2$ the resulting mixture was exposed to red light (λ = 650 nm, 0.29 W) for 30 minutes.

In the presence of 1% trifluoroacetic acid (v/v) compound **3** decomposes to thymidine (**5**) and anthraquinone (**6**) (Figure **S9**).

NMR parameters of these products:

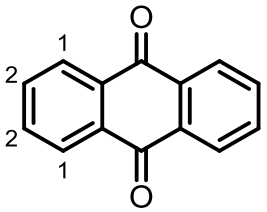
^{1}H NMR (400.13 MHz, $CDCl_3$) of compound **5**

δ = 7.55 (s, 1H, H^6), 6.14 (t, $^3J_{H\text{-}H}$ = 6.57 Hz, 1H, H^1), 4.65–4.61 (m, 1H, H^3), 4.09–4.06 (m, 1H, H^4), 4.00-3.86 (m, 2H, H^5), 2.48-2.35 (m, 2H, H^2), 1.95 (s, 3H, H^8) ppm.



^{1}H NMR (400.13 MHz, $CDCl_3$) of compound **6**

δ = 8.35-8.32 (m, 4H, H^1), 7.84-7.81 (m, 4H, H^2) ppm.



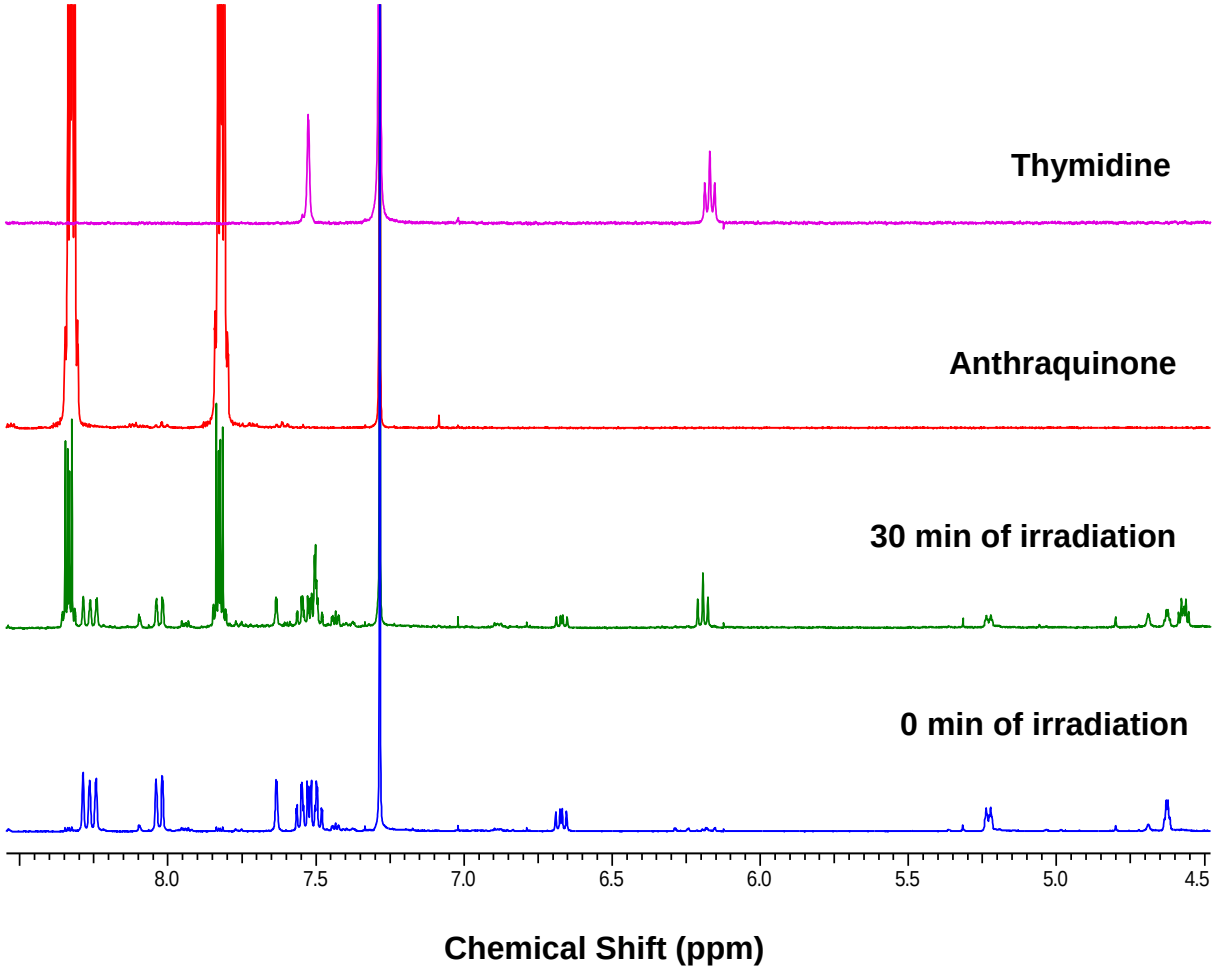


Figure **S9**. ^{1}H NMR spectra of a mixture of **4** (5 mM) and **PS** (0.1 eq) in $CDCl_3$ containing additionally 1% DMSO-*d6* (v/v) and 1% trifluoroacetic acid (v/v), which was irradiated with red light (650 nm. 0.29 W) for the time periods indicated on the plot. Pure anthraquinone and thymidine ^{1}H-NMR spectra are given for comparison.

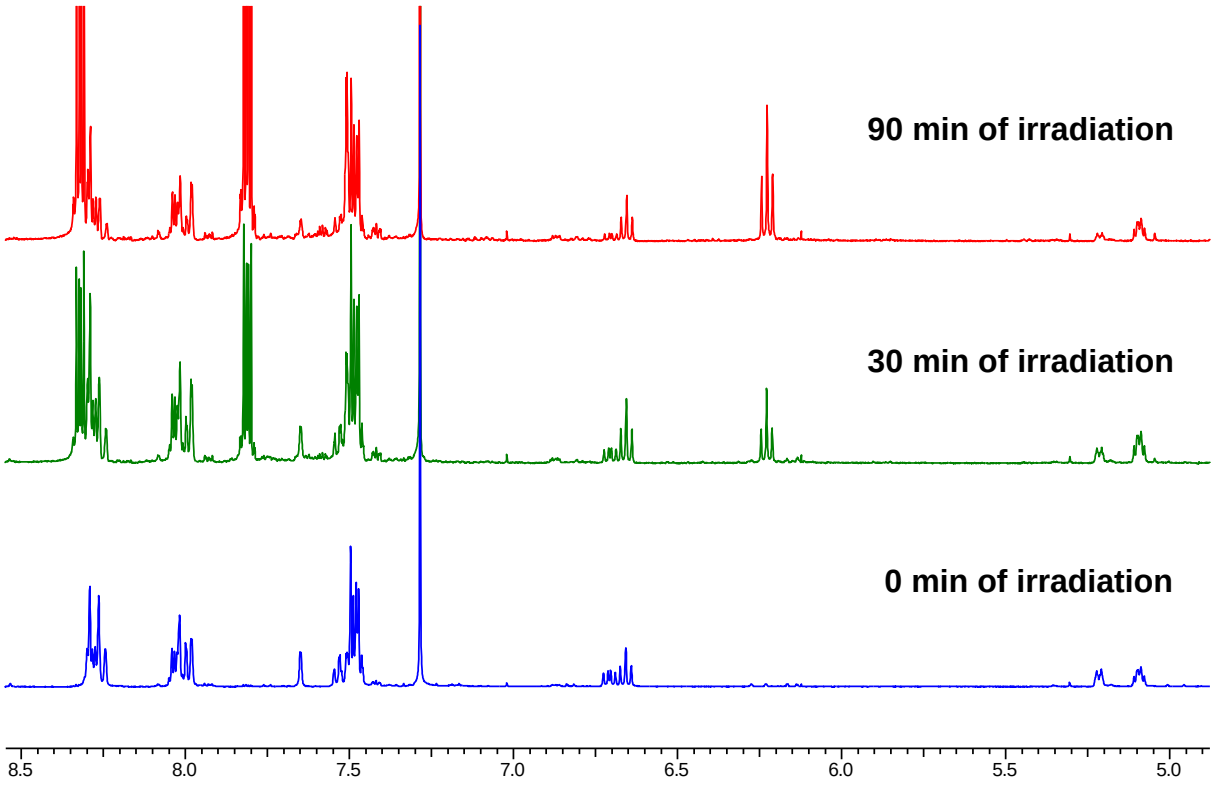
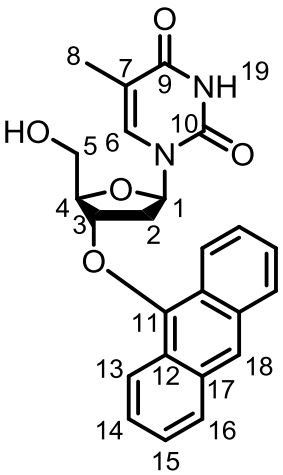


Figure **S10**. Competitive cleavage of compound **3** (5 mM) and previously reported 5'-*O*-(9-anhracenyl) thymidine (**7**) (5 mM) dissolved in dry $CDCl_3$, which was conducted in the presence of **P**~OH (0.1 eq) and 1% DMSO-*d6* (v/v). To generate $^1O_2$ the mixture was exposed to red light (λ = 650 nm, 0.29 W) for 30 minutes.

NMR parameters of the educts:

^{1}H NMR (400.05 MHz, $CDCl_3$)

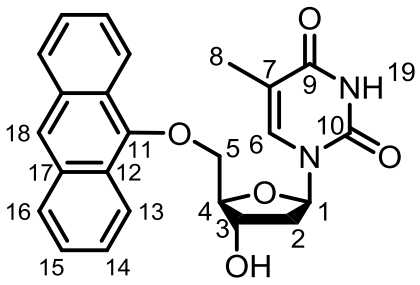
δ = 8.72 (bs, 1H, H^{19}), 8.26 (m, 3H, $H^{16,18}$), 8.02 (m, 2H, H^{13}), 7.64 (s, 1H, H^6), 7.55-7.46 (m, 4H, ^{H14,15}), 6.67 (dd, $^3J_{H-H}$ = 8.70, 5.70 Hz, 1H, H^1), 5.22 (m, 1H, H^3), 4.60 (m, 1H, H^4), 3.86-3.60 (m, 2H, H^5), 2.83-2.35 (m, 2H, H^2), 1.92 (s, 3H, H^8) ppm.



Compund **3**

^{1}H NMR (400.05 MHz, $CDCl_3$)

δ = 8.75 (bs, 1H, H^{19}), 8.30-8.28 (m, 3H, $H^{16,18}$), 8.05-8.02 (m, 2H, H^{13}), 7.99 (s, 1H, H^6), 7.51-7.47 (m, 4H, $H^{14,15}$), 6.66 (t, $^3J_{H-H}$ = 6.70 Hz, 1H, H^1), 5.11 (m, 1H, H^3), 4.57-4.39 (m, 2H, H^5), 4.38 (m, 1H, H^4), 2.59-2.49 (m, 2H, H^2), 1.55 (s, 3H, H^8) ppm.



Compound **7**

The performed competitive cleavage of compound **4** and **7** shows, that the new developed 3'-*O*-anthracenyl thymidine is faster cleaved than the previous reported 5'-O-anthracenyl thymidine (Figure **S11**).

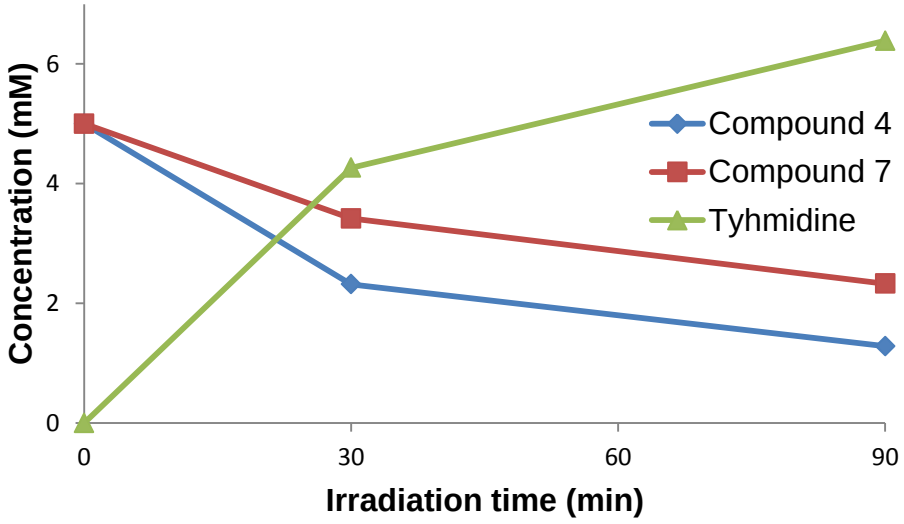


Figure **S11**. Changes of concentrations of **4** and **7** as a function of the irradiation time as determined by ^{1}H NMR spectroscopy. In this experiment a mixture of **4** (5 mM), **7** (5 mM) and **P-OH** (0.1 eq) in $CDCl_3$ containing additionally 1% DMSO-d6 (v/v) was irradiated with red light (650 nm, 0.29 W).

For the examination of the cleavage of compound **3** by HR-ESI mass spectrometry a solution of compound **3** (5 mM) and **P-OH** (0.1 eq) in $CDCl_3$ was prepared with 1% DMSO-*d6* (v/v) and with/without 1% trifluoroacetic acid (v/v). To generate $^1O_2$ the resulting mixture was exposed to red light (λ = 650 nm, 0.29 W) for 30 minutes. The

resulting mixture was diluted 100-fold in THF/Acetonitrile (1/1, v/v). In the presence of 1% trifluoroacetic acid (v/v) a peak corresponding to thymidine could be detected in the mass spectrum: MS (HR-ESI$^+$) calculated for $C_{10}H_{14}O_5N_2$ m/z 265.07949 ([M+Na]$^+$); found m/z 265.0794.

Synthesis of chemically modified DNAs

Oligoucleotides were synthesized using an H-8 DNA/RNA synthesizer on a 1 µmol-scale by the standard (3′→5’), respectively the 3’ anthracenyl modified DNA was synthesized by reverse (5’→3’) solid phase synthesis in accordance to the recommendations of the manufacturers. Commercially available DNA phosphoramidites (dA-bz, dC-bz, dG-dmf, dT; Aldrich, Germany) as well as reverse DNA phopshoramidites (dT-5’-CE, dA-5’-CE, dG-dmf-5’-CE, dC-5’-CE; Link Technologies, Scotland), 5’-Amino-modifier C6 phosphoramidite (Link Technologies, Scotland) and solid supports (3′-Amino-Modifier C7 CPG and dC-5’-SynBase CPG; Link Technologies, Scotland) were used in the synthesis. Unmodified Primers were purchased from Sigma-Aldrich (Germany) in a 100 µM scale. Synthesized oligonucleotides were cleaved from the solid support and deprotected by treatment with a 26% ammonium hydroxide solution at 55 °C for 2 h. After purging of the samples with nitrogen to remove the remaining ammonia from the aqueous solution, the DNA samples were washed out from the support with a 150 mM (NH_4)OAc aqueous solution buffered at pH 7.4 and purified by HPLC by using the following gradient of solution B (CH_3CN) in solution A (0.1 M aqueous (NEt_3H)(OAc)):

Gradient A for 2 min 0% solution B, in 5 min from 0 to 6% solution B, for 13 min 6% solution B, in 25 min from 6 to 60% solution B, in 1 min from 60 to 90% solution B, for 9 min 90% solution B, in 1 min from 90 to 0% solution B.

Gradient B for 2 min 0% solution B, in 40 min from 0 to 60% solution B, in 1 min from 60 to 90% solution B, for 7 min 90% solution B, in 1 min from 00 to 0% solution B.

The new conjugates were identified by MALDI-TOF mass spectrometry and their purity was confirmed by analytical HPLC. Purified DNAs were dissolved in

ammonium acetate buffer (150 mM $(NH_4)OAc$ in RNase-free water, pH 7.4) and stored at -25 °C.

Synthesis of 3' protected and 5' InPPa modified DNA: 3′-Amino-Modifier C7 CPG solid support (30 mg) containing a DMT-protected alcohol group and an Fmoc-protected amino group (1 µmol of the Fmoc-group on the solid support) was used for the standard (3′→5') DNA synthesis until the last nucleotide by using DNA/RNA synthesizer, including the 5'-end DMT deprotection. 1*H*-Imidazole-4,5-dicarbonitrile solution in CH_3CN (0.25 M, 0.1 mL, Sigma-Aldrich) was added to a solution of 5' MMT-Amino modified C6 phosphoramidite (0.1 M) dissolved in anhydrous acetonitrile (0.1 mL) just before coupling to the synthesized DNA on solid support. After 20 min the solution was removed from the solid support, washed with anhydrous CH_3CN (2x0.2 mL), oxidized with iodine in THF (0.02 M, 0.3 mL), washed with CH_3CN (2 x 0.2 mL) and dried for 2 h. Deprotection of the MMT-group was performed by treatment of the solid support with 2% trifluoroacetic acid in dichloromethane (v/v) (0.5 ml). Then the solid support was washed with anhydrous DMF (3×1 mL), anhydrous CH_3CN (2×1 mL) and lyophilized overnight. The photosensitizer containing a carboxylic acid group (**PS**, 16.5 µmol), *N,N,N′,N′*-tetramethyl-*O*-(*1H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU; 1.8 eq, 11.4 mg, 30 µmol), and 1-hydroxy-*1H*-benzotriazole (HOBT; 2.0 eq, 4.5 mg, 33 µmol) were dissolved in anhydrous DMF (0.6 mL) and *N,N*-diisopropylethylamine (DIPEA; 4.5 eq, 12.9 µL, 74.3 µmol) was added. This solution was immediately added to the solid support and shaken for 4 hours. The obtained green slurry was washed with DMF (3×1 mL), CH3CN (2×1 mL) and lyophilized overnight. Deprotection, cleavage from the support and purification was performed as previously described.

Synthesis of 3' anthracenyl modified "caged" Primer: Synthesis of the DNA was done by the reverse (5′→3') DNA synthesis until the second last nucleotide by using DNA/RNA synthesizer, including the 3'-end DMT deprotection. Phosphoramidite **4** (12.4 mg, 20.0 µmol) was dried for 24 h in vacuum (0.01 mbar), then dissolved in anhydrous acetonitrile (0.2 mL) and separated evenly in two portions. Just before coupling of each portion to the synthesized DNA on solid support *1H*-Imidazole-4,5-

dicarbonitrile solution (0.25 M, 0.1 mL, Sigma-Aldrich) was added to the solution of **4** and the resulting mixture was added to the solid support. After 3 min the solution was removed from the solid support, washed with anhydrous CH_3CN (2x0.2 mL) and the next portion activated with *1H*-Imidazole-4,5-dicarbonitrile was added. After two such couplings were completed, the solid support was oxidized with iodine in THF (0.02 M, 0.3 mL), washed with CH_3CN (2 x 0.2 mL) and dried overnight. Deprotection, cleavage from the support and purification was performed as previously described.

Forward Primer: 5' TCA CCC ACA CTG TGC CCA TCT ACG A 3'
Purchased from Sigma-Aldrich.

*Reverse Primer (**ON1**): 5' CAG CGG AAC CGC TCA TTG CCA AT 3'*
HPLC gradient A (70°C), R_t = 15.06 min; HPLC gradient B (65°C), R_t = 11.45 min; MALDI-TOF MS, negative mode, calculated for $C_{222}H_{281}N_{87}O_{134}P_{22}$ ($[M-H]^-$): 6992.53 *m/z*, found 6994.01 *m/z*.

*3' caged reverse Primer (**ON1-AN**): 5' CAG CGG AAC CGC TCA TTG CCA A [Anthracene-dT] 3'*
HPLC gradient A (70°C), R_t = 30.06 min; HPLC gradient B (65°C), R_t = 18.12 min; MALDI-TOF MS, negative mode, calculated for $C_{236}H_{289}N_{87}O_{134}P_{22}$ ($[M-H]^-$): 7168.75 *m/z*, found 7168.71 *m/z*.

*Template (**Target**): 5' GCC TGA CGG CCA GGT CAT CAC CAT TGG CAA TGA GCG GTT CCG CTG CCC TG 3'*
Purchased from Sigma-Aldrich
HPLC gradient A (70°C), R_t = 25.64 min.

*3' C7-Amino linker protected and 5' InPPa modified template DNA (**PS-ON2a**): InPPa-C6-Linker - 5' TGACCTGGCCGT 3' - C7-Amino linker*
HPLC gradient A (70°C), R_t = 41.76 min; MALDI-TOF MS, negative mode, calculated for $C_{162}H_{208}N_{49}O_{81}P_{13}InCl$ ($[M-Cl]^-$): 4655.15 *m/z*, found 4654.46 *m/z*.

*3' C7-Amino linker protected protected and 5' InPPa modified complementary DNA to reverse Primer (**PS-ON2b**): InPPa-C6-Linker - 5' ATT GGC AAT 3' - C7-Amino linker*
HPLC gradient B (65°C), R_t = 29.56 min; MALDI-TOF MS, negative mode, calculated for $C_{135}H_{172}N_{40}O_{61}P_{10}InCl$ ($[M-Cl]^-$): 3755.64 *m/z*, found 3752.71 *m/z*.

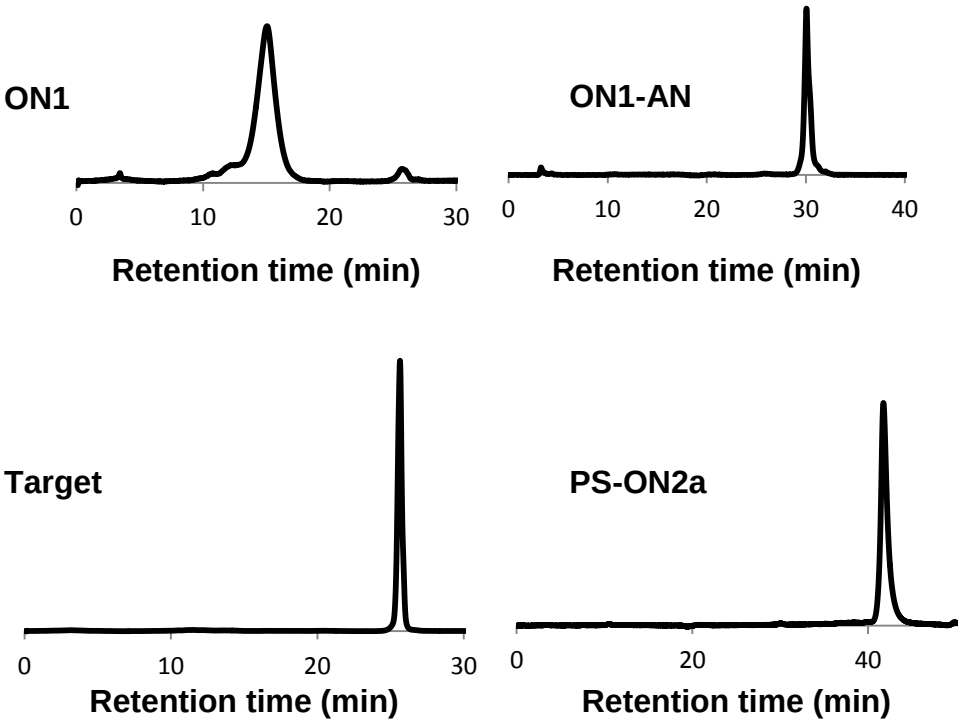


Figure **S12**. HPLC profiles (gradient A, 70 °C) of **ON1**, **ON1-AN**, **Target** and **PS-ON2a**. Absorbance at 260 nm was monitored. Other conditions are described above.

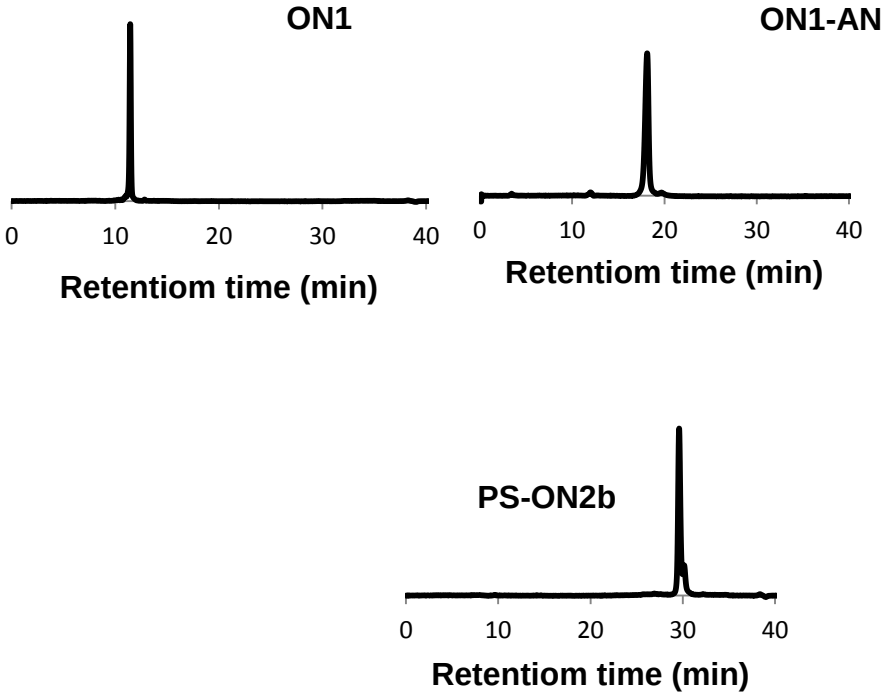


Figure **S13**. HPLC profiles (gradient B, 65 °C) of pure **ON1**, **ON1-AN** and **PS-ON2b**. Absorbance at 260 nm was monitored. Other conditions are described above.

Annealing of DNA

Annealing of the primer to the template or the conjugated 5' InPPa strand (**PS-ON2b**) of desired concentrations were prepared in RNase-free water with 20 volume percent of 5X annealing buffer (100 mM KCl, 2 mM $MgCl_2$, 25 mM KOH, 30 mM HEPES in RNase-free water, pH 7.4; all bio grade, Sigma). The mixtures were heated up to 90 °C and then slowly (5 °C / min) cooled down to 22 ° C.

Melting Experiments

UV/Vis melting experiments were performed on a Jasco V-550 UV/Vis spectrophotometer measuring absorbance at 260 nm in 1 cm semi-microcuvettes with sample volume of 1.0 mL, 1 µM strand concentration in annealing buffer (pH 7.4, 200 µL 5X annealing buffer in 800 µL RNase-free water, Sigma). Cooling and heating rates were 0.5 °C/min. Melting points were averages of the extrema of the first derivative of at least 4 melting curves.

ON1/Target: T_m = 73.9 ± 0.6 °C.

PS-ON2a/Target: T_m = 62.3 ± 1.1 °C.

ON1/PS-ON2b: T_m = 28.0 ± 1.5 °C.

Uncaging of 3'caged Primer

The activation of the 3'caged Primer was examined by MALDI-TOF mass spectrometry and analytical HPLC. Solutions of the desired Primer (25 µM in case of templated activation, 20 µM in case of the complementary 5' PS-containing conjugate) were prepared in annealing solution and annealed. The mixtures were exposed to red light (λ = 650 nm, 0.29 W) for different time periods. Probes (10 µL) were analyzed by HPLC at 65 °C or 70 °C (temperature in the column). The remaining 5 µL were used to acquire MALDI-TOF mass spectra.

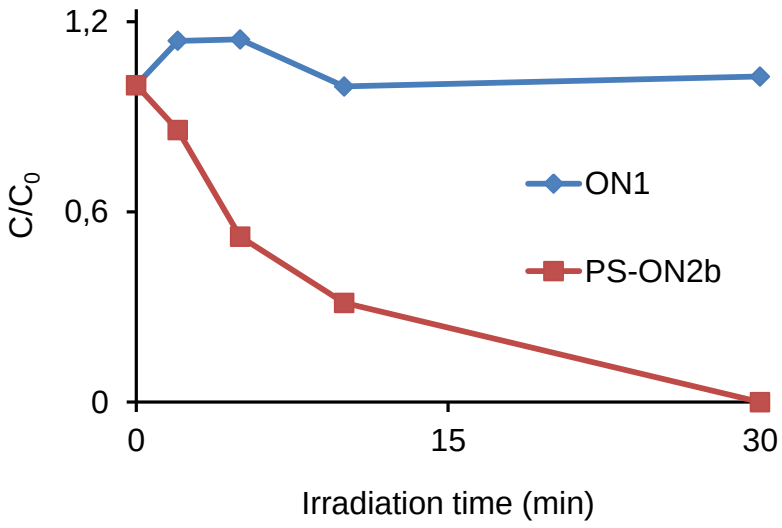


Figure S14. Change of relative concentrations of conjugates ON1 (20 μM) and PS-ON2a (1 eq) (C/C_0, where C is the concentration of a corresponding conjugate at the time point t and C_0 – before the irradiation), which were mixed together in the buffer (pH 7.4, HEPES 6 mM, KOH 5 mM, KCl 20 mM, $MgCl_2$ 0.4 mM) and irradiated with red light (LED array, 650 nm, 0.29 W) for the time shown on the OX axis.

Cells and cell culture

The human cervical cancer cells line (HeLa TK) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin/streptomycin. 24 h before transfection the cells were detached from the surface by removing the medium, washing the cells with DPBS (2x10 mL) and adding trypsin (0.05%)/ethylenediamine tetracetic acid (EDTA, 0.02%) solution (1.5 mL) to the cells. The cells were incubated for 2 min at 22 °C, the trypsin/EDTA solution was removed and the dry cells were incubated for 5 min at 37 °C, 95% air humidity and 5% CO_2. Then, the cells were resuspended in the fresh medium and diluted with the medium up to the concentration of 80 cells/μL. Next, 2.5 mL aliquots were placed into wells of 6-well plates, and the cells were allowed to get attached to the surface for 24 h in the incubator at 37 °C, 95% air humidity and 5% CO_2 content.

Isolation of cell RNA

TRI Reagent® (Sigma-Aldrich, Germany) was used for the isolation of cell RNA.

HeLa cells were lysed directly on the 6-well culture dish. TRI Reagent® (600 µL/well) was added to the surface area. After addition of the reagent, the cell lysate was passed several times through a pipette to form a homogenous lysate.

To ensure complete dissociation of nucleoprotein complexes, the samples were allowed to stand for 5 minutes at room temperature. 1-Bromo-3-chloropropane (60 µL/well) was added to the lysate. The samples were closed tightly, shaked vigorously for 15 seconds, and were allowed to stand for 15 minutes at room temperature. The resulting mixture of each well was transferred to an Eppendorf tube and these were centrifuged at 12,000 *x* g for 15 minutes at 2 °C. The Centrifugation separated the mixture into 3 phases: a red organic phase (containing protein), an interphase (containing DNA), and a colorless upper aqueous phase (containing RNA).

The aqueous phase of each tube was transferred to a fresh Eppendorf tube and 2-propanol (300 µL/tube) was added and mixed. The samples were allowed to stand for 10 minutes at room temperature. Then, each tube was centrifuged at 12,000 *x* g for 10 minutes at 2 °C. The RNA precipitate formed a pellet on the side and bottom of the tube.

The supernatant was removed and the RNA pellet washed by adding 1 ml of 75% ethanol. The samples were vortexed and then centrifuged at 7,500 *x* g for 5 minutes at 2 °C. The washing step was repeated two times to ensure, that all phenol impurities were removed.

The RNA pellets were briefly dried for 5 minutes under a vacuum. It was ensured, that the pellet did not dry completely, as this would greatly decrease its solubility. RNase-free water (20 µL/tube) was added to each tube. To facilitate dissolution, the RNA pellets were mixed by repeated pipetting with a micropipette at 55 °C for 10 minutes. Then, the dissolved RNA pellets were combined in one Eppendorf tube and the concentration/purity of RNA was determined by UV-visible absorbance (Figure **S15**).

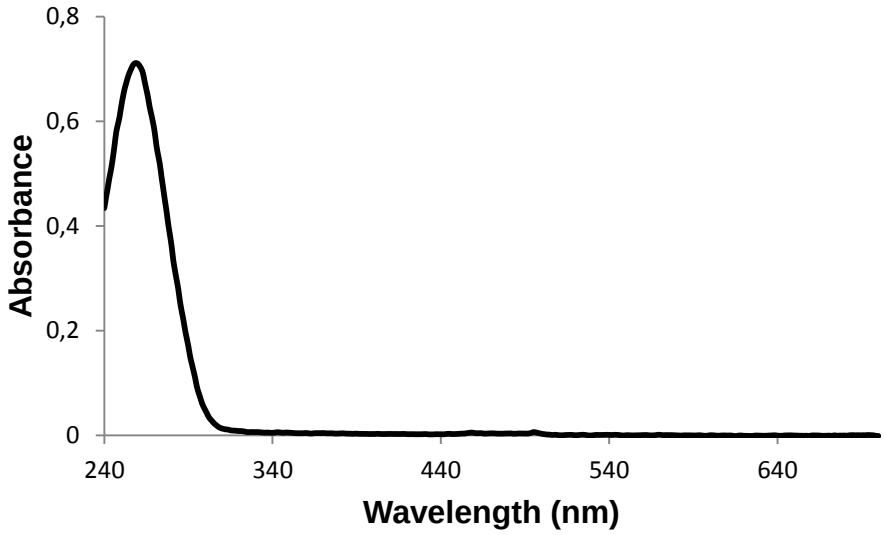


Figure **S15**: UV-visible absorbance of isolated RNA.

Polymerase chain reaction

To amplify the isolated RNA a carousel-based LightCycler® 2.0 system (Roche, Germany) was used for qRT-PCR. The LightCycler® RNA Master SYBR Green I kit for one-step qRT-PCR was obtained by Roche (Germany). The isolated RNA was used as template RNA.

This kits is designed for LightCycler® capillaries with a final reaction volume of 20 μL. Before the qRT-PCR experiments started the following solutions were prepared: **RT-PCR Primer mix** (5' InPPa containing DNA, forward and reverse primer) of desired primers in RNase-free water with a concentration of 5.0 μM (10.0 μM in the case of 5' InPPa containing DNA) of each primer. **Primer $p(dT)_{15}$** for cDNA synthesis (reverse transcription) with a concentration of 8.0 μM.

The **RT-PCR mix** for one 20 μL reaction was prepared by adding the following components in the order mentioned: H_2O (7.2 μL, PCR grade), $Mn(OAc)_2$ (1.3 μL, 50 mM, final concentration 3.25 mM), RT-PCR Primer Mix (2.0 μL, 5.0 μM, final concentration 0.5 μM), $p(dT)_{15}$ primer (1.0 μL, 8.0 μM, final concentration 0.4 μM), template RNA (1.0 μL of desired amount between 1.5 and 150.0 ng) and LightCycler® RNA Master SYBR Green I. The reaction mixture was mixed gently by pipetting up

and down. Then, each capillary was sealed with a stopper, placed in the centrifuge adapters and centrifuged at 3000 rpm for 5 s. The samples were transferred into the LightCycler® sample carousel and then into the LightCycler® instrument to start the reverse transcription and the following amplification program.

The following parameters were used for qRT-PCR.

1. *Reverse transcription* (61 °C, 20 minutes hold time, 20 °C/s ramp rate)
2. *Initial denaturation* (95 °C, 30 seconds hold time, 20 °C/s)
3. *Amplification* (fluorescence acquisition after every cycle)

   (i) template mediated activation of the primer with **PS-ON2a** (45 cycles)
   Annealing (50 °C, 15 seconds hold time, 5 °C/s ramp rate)
   Amplification (69 °C, 30 seconds hold time, 20 °C/s ramp rate)
   Denaturation (95 °C, 5 seconds hold time, 20 °C/s ramp rate)

   (ii) activation of the primer with **PS-ON2b** (60 cycles)
   Annealing (55 °C, 15 seconds hold time, 5 °C/s ramp rate)
   Amplification (69 °C, 30 seconds hold time, 20 °C/s ramp rate)
   Denaturation (95 °C, 5 seconds hold time, 20 °C/s ramp rate)

4. *Melting curve*
   Denaturation (95 °C, 5 seconds hold time, 20 °C/s ramp rate)
   Annealing (65 °C, 60 seconds hold time, 20 °C/s ramp rate)
   Melting curve (95 °C, 0.1 °C/s ramp rate, continuous fluorescence acquisition)

Exposure of the probes to red light (λ = 650 nm, 0.29 W) for 5 or 10 minutes was conducted during different steps of RT-PCR: (i) Irradiation of the annealed PCR Primer Mix at 22 °C in Eppendorf tubes before addition to the final 20 µL reaction mixture; (ii) Irradiation of the RT-PCR mix at 22 °C in 20 µL capillaries, after annealing of the PCR Primer mix and addition of this to the final 20 µL reaction mixture; (iii) Irradiation of the RT-PCR mix after reverse transcription at 22 °C in 20 µL capillaries or Eppendorf tubes.

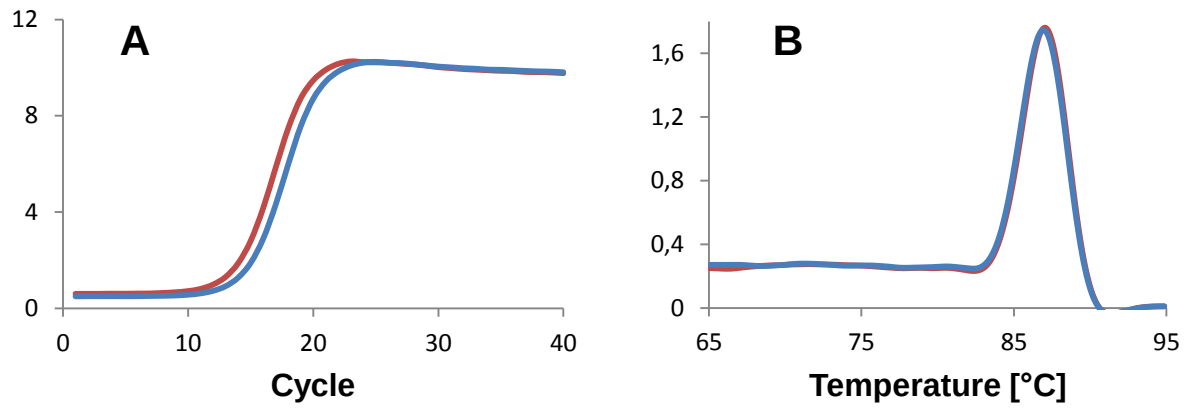


Figure **S16**: Fluorescence histogramm received during qRT-PCR (**A**) and measured melting curves after amplification (**B**) with **ON1**, **ON3** and 150 ng total RNA (red); **ON1**, **ON3**, **PS-ON2a** and 150 ng total RNA (blue).

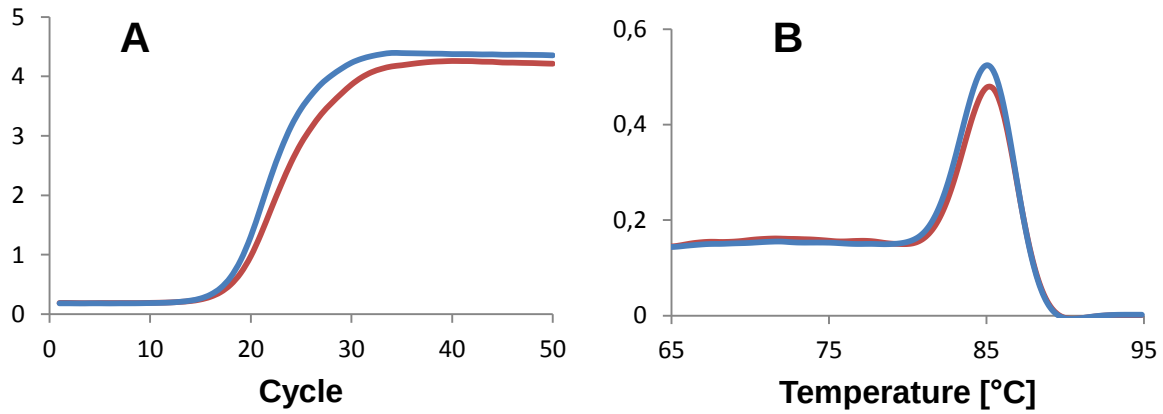


Figure **S17**: Fluorescence histogramm received during qRT-PCR (**A**) and measured melting curves after amplification (**B**) with **ON1**, **ON3** and 150 ng total RNA (red); **ON1**, **ON3**, **PS-ON2b** and 150 ng total RNA (blue).

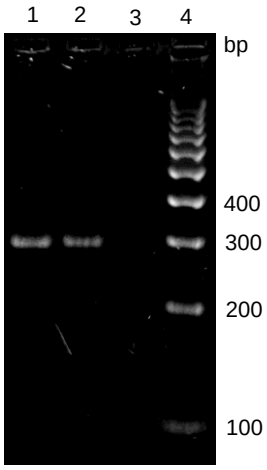


Figure **S18**. Agarose gel electrophoresis (3 % gel) of PCR products obtained in the result of amplification of β-actin cDNA (obtained by reverse transcription of total RNA (150 ng) isolated from HeLa cells as described above, predicted product size – 295 bp) using caged and control primers (Figure 2C, main part of the paper) - lane 1: control mixture containing unmodified primers ON1 and ON3; lane 2: a mixture containing “caged” primer ON1-AN/PS-ON2b, which was activated by irradiation with red light for 10 min; lane 3: the same as 2, but not irradiated (no product is observed); lane 4: DNA ladder (molecular weight marker), the number of basepairs (bp) in DNAs from calibration bands is indicated on the right.