Supporting Information

Triggering RNA interference by photoreduction under red light irradiation

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1 Synthesis

1.1. Chemicals

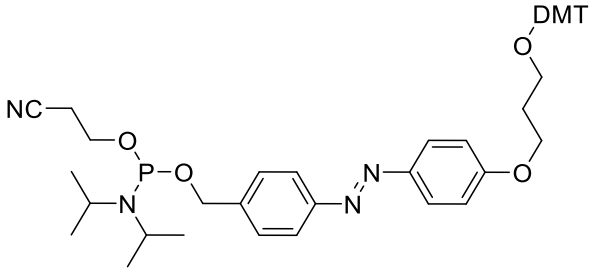
If not stated otherwise, all chemicals were acquired commercially from Sigma-Aldrich (Germany) in at least 98 percent purity. The reagents were used without further purification. Anhydrous solvents and NMR solvents were stored under nitrogen or argon atmosphere and, if possible, with use of molecular sieve. As solid phase for DNA synthesis dC(bz) CPG (1000 Å, 28 µmol/g, Sigma Aldrich) and for RNA synthesis dT CPG (1000 Å, 25 – 35 µmol/g, Sigma Aldrich) were used. The strands were synthesized with DMT-dT and DMT-2´-O-TC (rA(bz), rC(ac), rU, rG(ib)) phosphoramidites purchased from Sigma Aldrich. Gibco™ Opti-MEM™ Reduced Serum Media (Opti-MEM), Lipofectamine® RNAiMAX, random hexamer primer and dNTPs, the reverse transcriptase and its 5x buffer were all obtained from Thermo Fisher Scientific, Germany. The Zymo-Spin IC Columns were purchased from Zymo Research Corporation, US.

LightCycler® 480 SYBR® Green Master from Roche Diagnostics GmbH (Germany) was used for the qPCR analysis. For the microscopy 35 mm µ-imaging dishes (ibidi GmbH, Germany) were used.

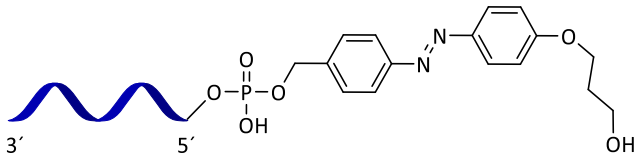
1.2. Instruments

Automated oligonucleotide synthesis was performed on a H-8 synthesizer (K&A Laborgeräte GbR, Germany). Purification of oligonucleotide conjugates was done on a Prominence UFPLC (Shimadzu) equipped with a EC 250/4.6 Nucleosil 100-10 C18 column (Macherey-Nagel, Germany). The chromatographic separations were performed at 22 °C. HPLC fractions were analyzed on a UltrafleXtreame MALDI-TOF mass spectrometer (Bruker). Samples were prepared by dried droplet method on a MTP 384 polished steel plate (Bruker). Concentrations of modified and non-modified RNAs and DNAs were determined with a NanoDrop™ UV-visible spectrophotometer (Thermo Fisher Scientific, Germany). For irradiation a red LED lamp compiled of eighteen 2-watt super bright red LEDs (λ = 660 nm, Flux = 1086 lm, efficacy: 56.6 lm/w; half width at half maximum = 26.4 nm) with 30 cm distance to the sample was used. The reverse transcription was performed with a Dual Block Gradient PCR Thermal Cycler GE4852T™ (Biogen, US). For the qPCR experiments the LightCycler® 480 from Roche Molecular Systems, Inc. (US) was used.

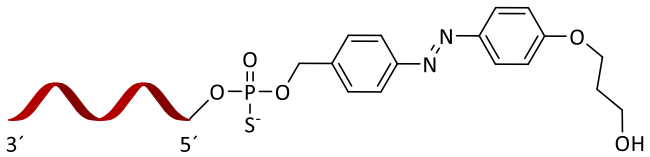
1.3. Synthesis of phosphoramidite compound and modified oligonucleotides



Scheme **S1**: Structure of DMT-RF-carrying phosphoramidite used for DNA/RNA synthesis.



Scheme **S2:** Structure of DNA **1**



Scheme **S3:** Structure of RNA **2**.

2 Tests of conjugates

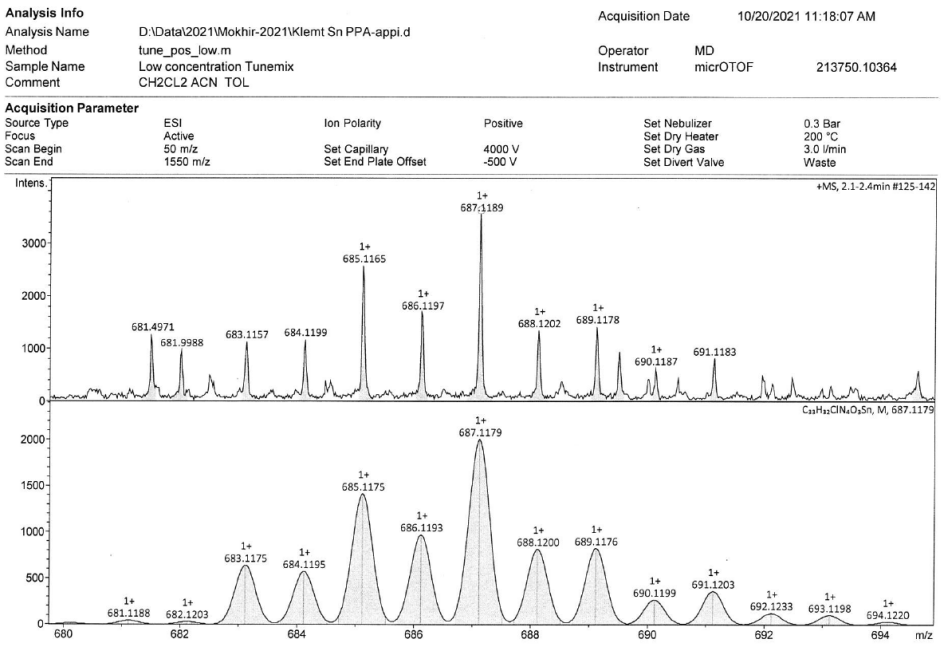


Figure **S1**: High resolution mass spectrum of [Sn(P~OH)Cl2]*EtOH.

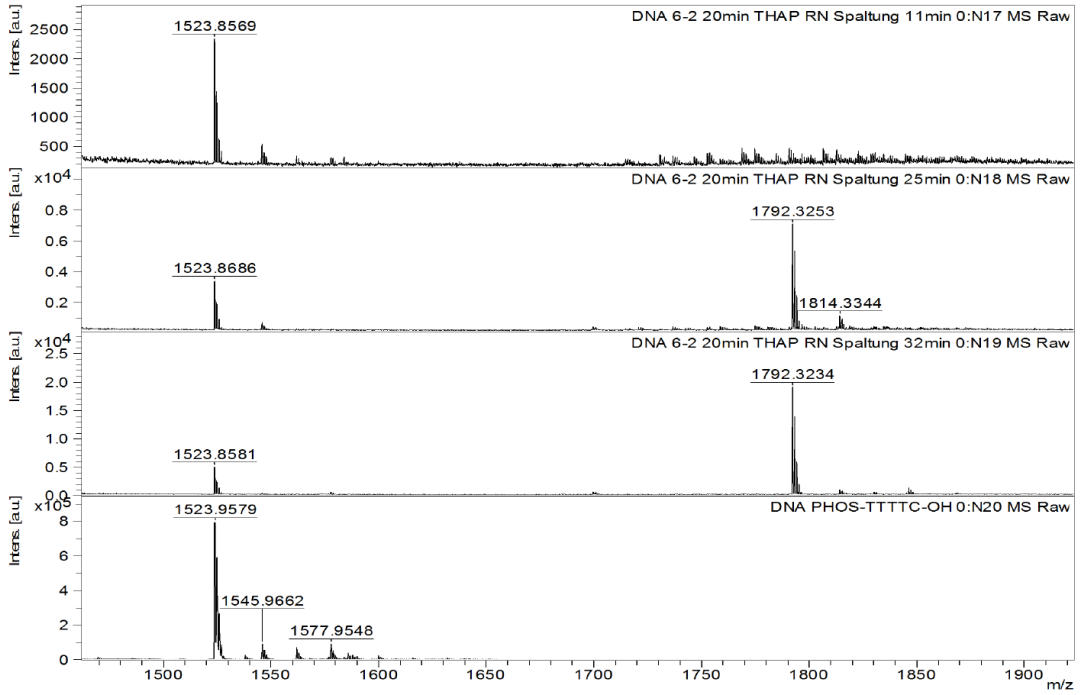
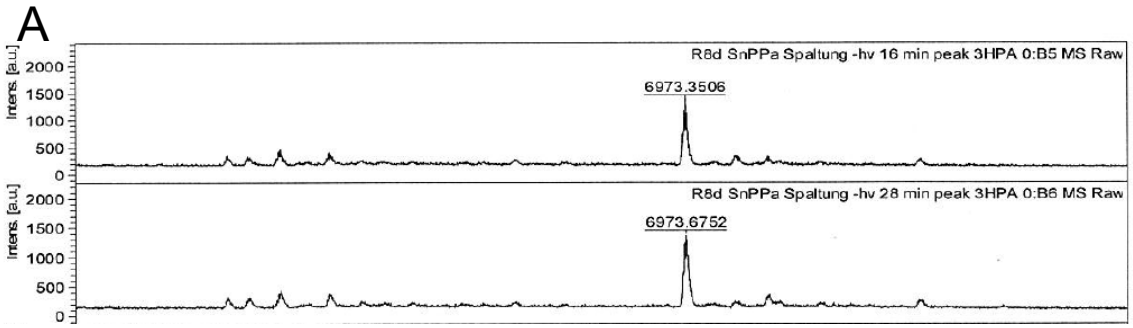


Figure **S2**: Incubation of DNA **1** with sodium ascorbate in the presence of SnPPA (Figure 4 B). Upper spectrum of cleavage product at 11 min. Both middle spectra correspond the peaks of residual DNA **1** at 25 min and 32 min which was not cleaved (calculated for $C_{65}H_{82}N_{13}O_{35}P_5$ [MH^-]: m/z=1791.36 Da; found: m/z=1791.3 Da) (Figure 4 B) and lowest spectrum of the reference (phosphorylated strand, Phos-TTTTC, Figure 4 C). Expected mass: calculated for $C_{49}H_{66}N_{11}O_{35}P_5$ (MH-): m/z=1523.242 Da.



B

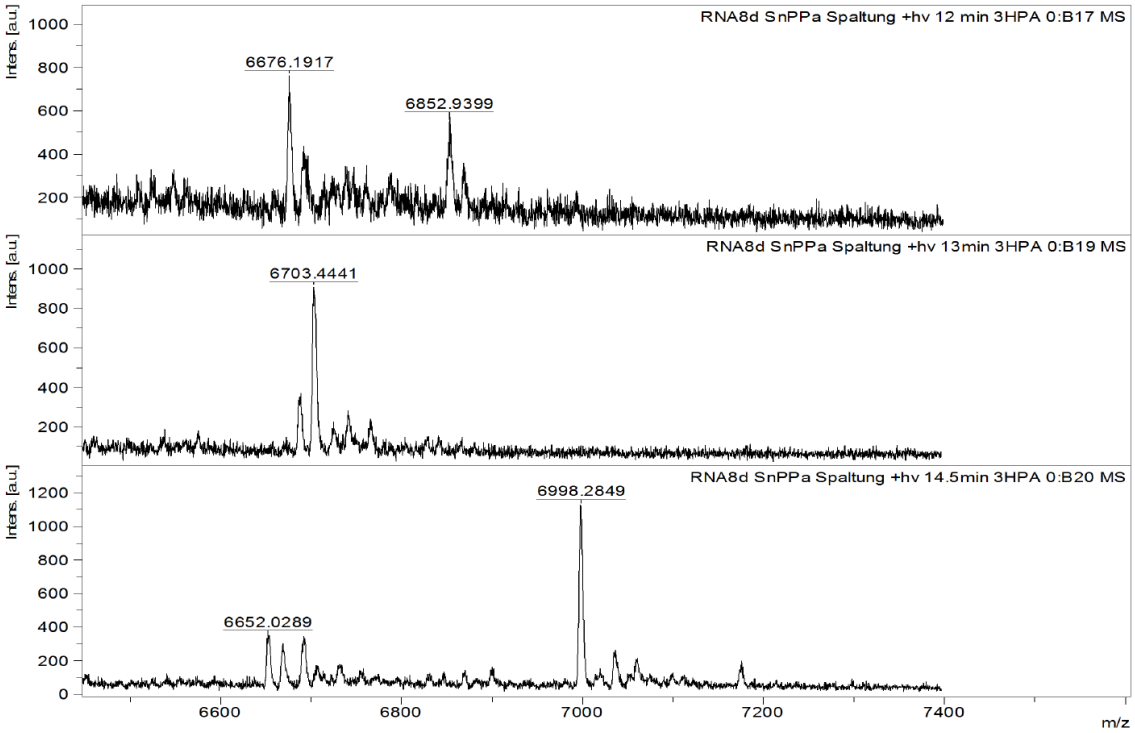


Figure **S3**: MALDI-TOF masses for cleavage of RNA **2** with SnPPa (HPLC traces and details see Figure 6). A: Sample without irradiation (-hv) for both isomeric peaks (elution after 16 min and 28 min, respectively) of RNA **2** (calculated for $C_{216}H_{266}N_{71}O_{150}P_{21}$ [M^-]: m/z=6970.0 Da). B: Sample with irradiation (+hv): 12 min peak (5´-phos-antisense strand, calculated for $C_{200}H_{250}N_{69}O_{151}P_{21}$ [MH^-]: m/z=6687 Da), 13 min peak (5´-thiophos-antisense strand, calculated for $C_{200}H_{250}N_{69}O_{150}P_{21}S$ [MH^-]: m/z=6701 Da)), 14.5 min peak (product unknown).

3. Cells and cell culture

Human ovarian cancer cell line A2780 was purchased from Sigma-Aldrich and cultivated in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with foetal bovine serum (FBS, 10 %), penicillin/streptomycin (1 %) and L-Glutamine (1 %). Cells were cultivated to around 80 % confluence at 37 °C with 5 % CO_2.