SUPPLEMENTARY INFORMATION:

Pulsed EPR spectroscopy distance measurements of DNA internally labelled with Gd^{3+}-DOTA

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Experimental procedures

General

All reactions were performed in oven-dried glassware under nitrogen. Chemicals and solvents were purchased from Sigma. Solvents were of laboratory grade. Thin-layer chromatography (TLC) was performed on aluminium-backed plates pre-coated with silica gel 60 (0.2 mm, $UV_{254,}$ Merck, Germany). Flash column chromatography (FCC) was performed using silica gel 60 (Merck, Germany). ESI mass spectra were recorded on a Bruker Daltonics esquire 3000plus. NMR spectra were recorded using Avance 400 (400 MHz, Bruker, Germany). ^{31}P NMR spectra was recorded at 161.8 MHz using 85% H_3PO_4 as an external standard. All spectra were referenced to the signals of the corresponding solvent. Chemical shifts are given in ppm (δ scale) and coupling constants (*J*) in Hz. High resolution electrospray ionization (ESI) mass spectra were recorded on a micrOTOF II (Bruker Daltonics).

5-[6-hydroxy-hexynyl]-5′-O-(4,4′-dimethoxytrityl)-2′-deoxyuridine (1),

To a 50 mL round-bottom flask, is added 5′-DMT-5-iodo-2′-deoxyuridine (1.3 g, 1.98 mmol) and 15 mL of THF under a nitrogen atmosphere. NEt_3 (2.0 mL, 14.5 mmol) followed by 5-hexyn-1-ol (0.6 mL, 5.44 mmol) are added and the solution is cooled below 0°C with a dry-ice/acetone bath. The solution is deoxygenated three times using freeze-pump-thaw cycling. To the solution is added $Pd(PPh_3)_2Cl_2$ (200 mg, 0.285 mmol) and CuI (80 mg, 0.421 mmol) and the suspension is deoxygenated once using freeze-pump-thaw cycling. The reaction is removed from the dry-ice/acetone bath and stirred for 16 hours at room temperature. The reaction is diluted with CH_2Cl_2 (200 mL) and extracted with an aqueous solution of EDTA (5%, 50 mL)/$NaHCO_3$ (5%, 50 mL), $NaHCO_3$ (5%, 100 ml), water (100 mL) and brine (100 mL) dried over anhydrous Na_2SO_4, concentrated *in vacuo* and purified by FCC, eluting with stepwise ethyl acetate/hexane gradient (6:4, 8:2, 1:0 + ~0.1% NEt_3). The compound was dissolved in CH_2Cl_2 (2 mL) and precipitated by adding diethyl ether (5 mL) and hexane (25 mL), the solvents were decanted and the residue was dried in vacuo to give (0.89 g, 72%) of an off-white foam. ^{1}H NMR (400MHz , $CDCl_3$): δ 8.02 (s, 1 H), 7.27 - 7.50 (m, 10 H), 6.77 - 6.93 (m, 4 H), 6.34 (m, 1 H), 4.52 - 4.53 (m, 1 H), 4.10 (m, 1 H), 3.79 (s, 6 H), 3.51 (t, *J* = 6.3 Hz, 2 H), 3.43 (dd, *J* = 3.0, 10.5 Hz, 1 H), 3.33 (dd, *J* = 3.5, 10.8 Hz, 1 H), 2.52 (ddd, *J* = 2.5, 5.7, 13.6 Hz, 1 H), 2.23 - 2.33 (m, 1 H), 2.14 (t, *J* = 6.9 Hz, 2 H), 1.46 - 1.56 (m, 2 H), 1.33 - 1.41 (m, 2 H). ^{13}C NMR (100 MHz , $CDCl_3$): δ 19.1, 24.2, 31.7, 41.4, 55.2, 61.9, 63.6, 71.2, 72.2, 76.7, 77.0, 77.2, 77.3, 85.7, 86.6, 86.9, 95.2, 101.0, 113.3, 126.8, 128.0, 130.0, 135.6, 141.4, 144.5, 149.5, 158.5, 162.5. HRMS (ESI) *m*/*z* calcd for $C_{36}H_{38}N_2O_8Na$ [MNa^+] 649.2526, found 649.2520.

5-[6-iodo-hexynyl]-5′-O-(4,4′-dimethoxytrityl)-2′-deoxyuridine-3′-O-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (2),

To a 25 mL round-bottom flask is added **1** (0.338 g, 0.54 mmol) and anhydrous DMF (2.5 mL) under a nitrogen atmosphere, followed by *N,N*-diisopropylethylamine (0.280 mL, 1.62 mmol). The solution is cooled to 0°C with an ice bath and the flask is charged with $(PhO)_3PCH_3I$ (0.294 g, 0.65 mmol). The reaction is removed from the ice bath and stirred for at room temperature for 6 hours. The reaction is diluted with CH_2Cl_2 (100 mL) and extracted with saturated aqueous $NaHCO_3$ (50 mL), water (50 mL) and brine (100 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue is passed through a silica plug (50 g) eluting with ethyl acetate/hexane (8:2 + ~0.1% NEt_3). The compound containing fractions are concentrated in vacuo. The crude compound is dissolved in anhydrous CH_2Cl_2 (10 mL), cooled to 0 ˚C, and *N,N*-diisopropylethylamine (0.28 mL, 1.62 mmol) is added followed by 2-cyanoethyl diisopropylethylamine (130 µL, 0.59 mmol). The reaction is removed from the ice-bath and stirred for 2.0 h at rt, diluted with CH_2Cl_2 (50 mL) and the organic phase is washed with saturated aqueous $NaHCO_3$ (25 mL), brine (25 mL), dried over anhydrous Na_2SO_4, concentrated in vacuo and purified by FCC, eluting with hexane/ethyl acetate (8:2 to 1:1 + ~0.1% NEt_3). The resulting oil was dissolved in a minimal amount of CH_2Cl_2 and precipitated by the addition of hexane (20 mL), after 2h at room temperature the hexane was decanted and the residue was dried under reduced pressure to give a white foam (263 mg, 52%, 2 steps). ^{1}H NMR (400MHz , $CDCl_3$); δ 8.12 (s, 0.5 H), 8.07 (s, 0.5 H), 7.40 - 7.49 (m, 2 H), 7.28 - 7.40 (m, 7 H), 7.20 - 7.25 (m, 1 H), 6.80 - 6.91 (m, 4 H), 6.26 - 6.37 (m, 1 H), 4.58 (m, 1 H), 4.12 - 4.25 (m, 1 H), 3.81(s, 3H), 3.80 (s, 3 H), 3.42 - 3.70 (m, 4 H), 3.22 - 3.31 (m, 1 H), 2.90 (m, 2 H), 2.62 (t, *J* = 6.3 Hz, 1 H), 2.43 (t, *J* = 6.3 Hz, 1 H), 2.23 - 2.36 (m, 1 H), 1.96 - 2.14 (m, 2 H), 1.51 - 1.70 (m, 4 H), 1.21 - 1.37 (m, 4 H), 1.17 (m, 9 H), 1.06 (d, *J* = 6.8 Hz, 3 H). ^{13}C NMR (100 MHz , $CDCl_3$): δ 6.3, 6.3, 18.3, 20.1, 20.2, 20.3, 20.4, 24.4, 24.5, 24.6, 24.6, 28.7, 28.7, 32.2, 40.6, 43.1, 43.2,43.3, 43.3, 55.3, 55.3, 58.1, 58.2, 58.2, 58.4, 63.0, 63.2, 71.2, 73.3, 73.4, 73.6, 73.8, 77.2, 85.4, 85.5, 86.9, 94.2, 100.9, 100.9, 113.3, 126.9, 127.9, 128.0, 130.0, 130.0, 135.4, 135.5, 135.5, 135.6, 141.6, 144.4, 144.5, 149.0, 149.1, 158.6, 161.5. ^{31}P NMR (161.8 MHz ,$CDCl_3$): δ 149.1, 148.6. HRMS (ESI) *m*/*z* calcd for $C_{45}H_{54}IN_4O_8PNa$ [MNa^+] 959.2622, found 959.2616.

DNA synthesis

DNA synthesis was performed on a 1.0 µmole scale on a Applied-Biosystem 392 DNA/RNA synthesizer. Chemicals and the solid support was purchased from AzcoBiotech (USA), J.T. Baker (Netherlands) and Link-Technologies (Scotland, UK). 5-(ethylthio)-1H-tetrazole (0.25 M in acetonitrile) was used as the activator and coupling times of 3 min were used for the modified building block **2**.

Two resin-bound modified oligodeoxynucleotides were synthesized ODN 1 5′-GCGAGTGACTGGTATGA**X**GATGCT-3′ and ODN 2 5′-AGCATCATCATACCAG**X**CACTCGC-3′ containing the modified building block **X**. The resin-bound DNA was placed in a 1.5 mL microcentrifuge tube with a screw cap. The resin-bound DNA was placed in a 1.5 mL microcentrifuge tube with a screw cap, anhydrous DMF (1.0 mL) was added followed by sodium azide (45 mg, 0.69 mmol). The vial was placed in an eppendorf thermomixer, and shaken for 24 hours at 55°C. The microcentrifuge tube was cooled to room temperature and spun down in an eppendorf centrifuge MiniSpin. The DMF was removed and the resin was washed with acetonitrile/water (1:1, 3 x 1 mL), then acetonitrile (3 x 1 mL).

To the resin-bound azide containing DNA was added DMF (0.5 mL), water (0.5 mL), tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) (1 mg, 1.88 µmol), $CuSO_4$ (1.5mg, 9.4 µmol), and sodium ascorbate (10 mg, 51 µmol) and 12 mg (20 µmol) of alkyne-(Gd^{3+}-DOTA) was added. The vial was placed in an eppendorf thermomixer, and shaken for 24 hours at 50°C. The microcentrifuge tube was cooled to room temperature and spun down in an eppendorf centrifuge MiniSpin. The resin was washed with DMF (3 x 1 mL)), then acetonitrile (3 x 1 mL).

Cleavage from the solid support and final deprotection was achieved with 30 % NH_4OH solution (50°C, 12 h). HPLC purification was performed using a Prominence HPLC (Shimadzu, Japan) equipped with a Phenomenex Jupiter semi-preparative RP-HPLC column (5µm, C18, 300Å) using A = 0.1 M triethylammonium acetate in H_2O, pH 7.0; B = 0.1 M triethylammonium acetate in CH_3CN/H_2O 4:1, pH 7.0, detection wavelength at 260 nm. The oligonucleotides were desalted using Sephadex-G10.

The modified oligonucleotides were confirmed by ESI-MS; ODN 1 5′-GCGAGTGACTGGTATGA**X**GATGCT-3′ $C_{261}H_{328}GdN_{101}O_{151}P_{23}$ Exact Mass: 8162.4 found 8161.3 and ODN 2 5′-AGCATCATCATACCAG**X**CACTCGC-3′ $C_{255}H_{326}GdN_{95}O_{147}P_{23}$ Exact Mass: 7940.4 found 7939.4.

Thermal denaturation studies

Thermal denaturation studies were recorded on a Varian Cary 100-Bio UV/VIS spectrophotometer (Varian Inc.), equipped with a Peltier element at 260 nm with a heating/cooling rate of 0.5°C/min. A heating cycle in the temperature range 20–90°C was applied. All measurements were carried out in a buffer consisting of 50 mM NaCl and 10 mM Tris (pH = 7.4) at 1.0 µM strand concentration.

CD spectra

CD spectra were measured on a JASCO J-715 spectropolarimeter using quartz cuvettes with a path length of 1 cm. All measurements were carried out in a buffer consisting of All measurements were carried out in a buffer consisting of 50 mM NaCl and 10 mM Tris (pH = 7.4) at 1.0 µM strand concentration.

Microinjection of DNA samples

Sample preparation of intracellular EPR samples were done as described by Qi *et al.*[9] Briefly, the *Xenopus laevis* oocytes on stage V/VI (purchased from EcoCyte Bioscience, Castrop-Rauxel, Germany) were kept in MBS at 18°C. Three oocytes were prepared on a poly (tetrafluoroethylene) holder and microinjected with 50 nL of a 3.7 mM solution of the Gd(III)-labelled duplex DNA sample solution and washed with MBS buffer. The oocytes were collected in a Q-band tube (quartz glass, 1 mm inner diameter, Bruker Biospin) via slightly applied negative pressure and were visually inspected directly afterwards and after 15 min of incubation time using a Stemi 2000-C binocular microscope mounted with an AxiaCam ERc 5s camera (ZEISS). After removal of supernatant MBS buffer the oocytes were shock-frozen in liquid nitrogen and stored at -80°C until measuring them without de-freezing them.

Investigating cytotoxic effects of injected samples

Gd(III) is highly toxic. The toxicity can significantly be reduced by complexation.[1-4] Consequently, Gd(III) chelates are routinely used as MRI contrast agents in medicine.

However, even for injection of 50 nL 8 mM uncomplexed $GdCl_3$ per *Xenopus laevis* oocyte, we observe morphological stability for several hours, see Figure S1.

In contrast, upon microinjection 50 nL 3.7 mM Gd(III)-DOTA labelled duplex DNA we found an increased cytotoxic effect, resulting in apoptosis after 1.5 h. This suggests that the duplex DNA contributes to the toxic effect.

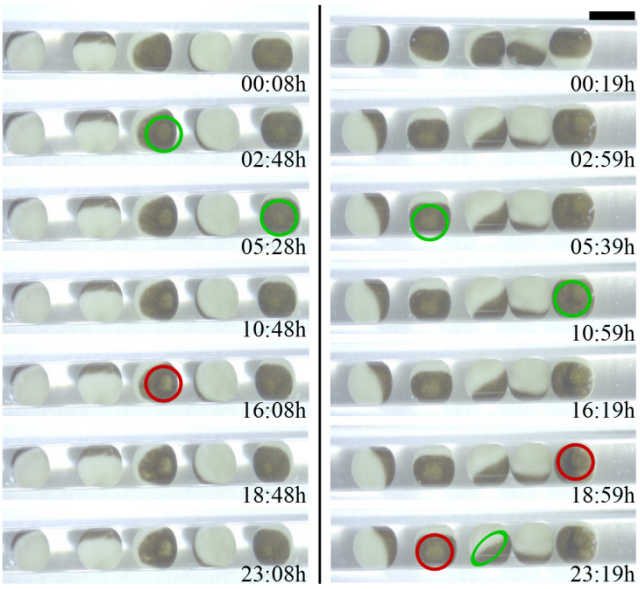

Figure S1: Micrographs of *Xenopus laevis* oocytes after microinjection of 50 nL 8 mM $GdCl_3$ (left) and untreated oocytes (right) after the indicated incubation times at 18°C. Signs for apoptosis tagged by green circles and progressed apoptosis tagged by red circles. Scale bar = 1 mm.

EPR measurements

Q-band EPR experiments were performed at 34 GHz microwave frequency using an ELEXSYS E500 spectrometer equipped with an EN 5107D2 Q-Band EPR probehead (both Bruker Biospin) and a 10 W microwave power solid state amplifier (HBH Microwave). A CF935 helium gas flow system (Oxford Instruments) was used for temperature control.

Two pulse echo detected field sweeps were obtained at T = 10 K using a Hahn-echo sequence with τ = 400 ns, $\pi/2$ pulse = 8 ns,

π pulse = 16 ns and a sweep width of 4000 G with B_0 = ~12200 G.

The four-pulse, dead-time free DEER was obtained using the following pulse sequence:

$$(\pi/2)_{obs} \cdots \tau_1 \cdots (\pi)_{obs} \cdots \mathrm{t} \cdots (\pi)_{pump} \cdots (\tau_1 + \tau_2 - t) \cdots (\pi)_{obs} \cdots \tau_2 \cdots echo.$$

The pump pulse (length 16 ns for *in vitro*, resp. 18 ns for *in cellulo* experiments) was set to the maximum of the Gd(III) spectrum and the observer pulses (lengths 14/14/28 ns) were shifted Δυ = 125 MHz to lower frequencies. The time separation τ_1 was 400. Shot repetition time was set to 2 µs.

The transverse relaxation time T_2 was determined by incremental elongation of the time separation τ_1 between π/2 pulse and π pulse shown in Figure S2 *in vitro* and *in cellula*.

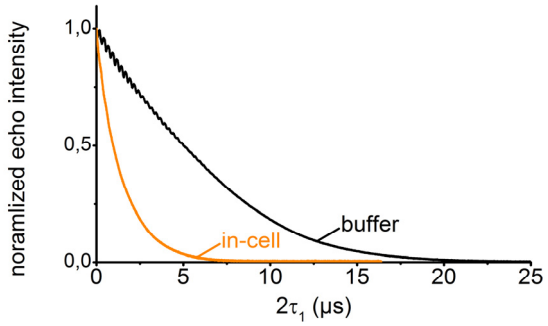

Fig. S2 T_2 relaxation time experiment of doubly Gd(III)-DOTA labelled duplex DNA at 10 K in deuterated Tris-HCl buffer (pH = 7.4, DNA concentration = 100 µM) (black) and inside *Xenopus laevis* oocytes after microinjection of 50 nL 3.7 mM labelled duplex DNA in Tris-HCl buffer (pH = 7.4) (15 min incubation time, absolute spin concentration = 180 µM) (orange); initial pulse separation time = 400 ns, mw-frequency = 34 GHz, pulse lengths: 14/28 ns. The observed ESEEM in the *in vitro* experiment (black) is due to Gd(III) interaction with the deuterated solvent.

References of Supporting Information:

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