Supplementary Material (ESI) for Chemical Communications

Supporting Information

Redesigned Tetrads with altered Hydrogen Bonding Patterns enable Programming of Quadruplex Topologies

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Experimental Details

Phosphoramidite Synthesis. Using deoxyguanosine monohydrate dG x H_2O as starting material the two sugar hydroxyls were protected by acetic acid anhydride leading to deoxyguanosine diacetate (AcdG).[1] The 2-amino group of AcdG was tritylated with DMT-Cl followed by introduction of the NPE group at the O^6 position via the Mitsunobu reaction using NPE-OH, PPH_3 and DEAD.[2, 3] The DMT group was easily removed in the next step with benzenesulfonic acid. Deamination with nitrous acid yielded to the O^6 NPE protected diacetylxanthine.[3] Double NPE protection at position O^2 was archived again via the Mitsunobu reaction.[4] After elimination of the acetyl protecting groups the 5'-hydroxyl was protected with DMT-Cl in dry pyridine. Subsequent treatment with 2-cyanoethyl diisopropylphosphoramidochloridite yielded into the double NPE protected xanthosine phosphoramidite.[5]

Oligonucleotide Synthesis. Oligonucleotides were synthesized on a ABI 392 DNA/RNA synthesizer (reagents purchased from J. T. Baker and ABI, 200-nmol LV columns from ABI) applying solid phase phosphoramidite chemistry. The synthesis was performed with standard conditions, except for a longer coupling time for phosphoramidites (2 minutes total in several pushes) and an activator solution of 5-Ethylthio-1H-Tetrazole in MeCN (0.25 M).

Oligonucleotide Deprotection. Cleavage of the NPE protecting groups was performed with 1 M DBU delivered in several pushes and following waiting steps (total wait time: 15 h). The solid support bearing "trityl off" oligonucleotides was incubated with 25 % Ammonium hydroxide solution at 37 °C for 16 hours.

PAGE Purification and Analysis of Oligonucleotides. Oligonucleotides were purified on a 10 % polyacrylamide gel and precipitated with EtOH and Sodium Acetate. Concentrations of the Oligonucleotides were determined by measurement of absorption at 260 nm. For the determination of the extinction coefficient of modified DNAs, ε of 7800 M^{-1} cm^{-1} for xanthosine and 5900 M^{-1} cm^{-1} for 8-oxo-G was used. The purified oligonucleotides were analysed by ESI-MS (HT-c: calc. 7574.9 g/mol, found 7574.1 g/mol; HT-ap: calc. 7608.9 g/mol, found 7607.3 g/mol ; HT-p: calc. 7608.9 g/mol, found 7608.2 g/mol).

Electrophoretic mobility shift assay. 20 pmol of each oligonucleotide strand were radioactively labelled by adding 2 U T4 PNK (Fermentas), 1 μCi ^{32}P-γ-ATP and reaction buffer (50 mM Tris-HCl (pH 7.6 at 25°C), 10 mM $MgCl_2$, 5 mM DTT, 0.1 mM spermidine and 0.1 mM EDTA.). Reactions were incubated at 37 °C for 30 minutes, heat inactivated at 65 °C for 10 minutes and purified using a G25 column (GE Healthcare).

Denaturing PAGE: Probes were denatured by addition of 1 volume page loading buffer (80 % [v/v] formamide, 50 mM EDTA pH = 8.0, 0.025 % [w/v] bromphenolblue and 0.025 % [w/v] xylene cyanole) and heating to 95 °C for 2 minutes. Subsequently the oligonucleotides were separated on a 10 % denaturing PAGE. Visualization was performed using phosphorimaging.

Native PAGE: The ^{32}P labelled oligonucleotides were mixed with 1 volume of native PAGE loading buffer (1x TBE, 100 mM KCl, 40 % [v/v] Glycerol) and were heat denatured at 95 °C for 2 minutes. After cooling slowly over night, the samples were analyzed on a 10 % native PAGE at 20 °C containing 100 mM KCl in the buffer and gel, followed by visualization by phosphorimaging.

CD Spectra. Oligonucleotides were prepared at 1.2 μM concentration in water with 10 mM Tris-HCl (pH 7.5) and 25 mM KCl. Annealing was performed by heating to 95 °C followed by slow cooling to 20 °C over 16 hours. CD spectra were recorded on a Jasco 715 spectrometer in cuvettes with a 1 cm path length, resolution of 0.5 nm, band width of 1.0 nm and speed of 50 nm/min at 25 °C. Each spectrum was accumulated 5 times and averaged. For thermal denaturation studies, the sample was heated from 20 °C to 90 °C with a heating rate of 1 °C min^{-1}. The CD signal at 265 nm and 290 nm, respectively, was recorded every 1 °C and the melting temperature was obtained by determining the temperature at the half-maximum decrease of the signal.

Sequences

HT-s: 5’- GTGAGTGTGAGTGTGAGTGTGAGT -3’

HT-c: 5’- TAGGGTTAGGGTTAGGGTTAGGGT -3’

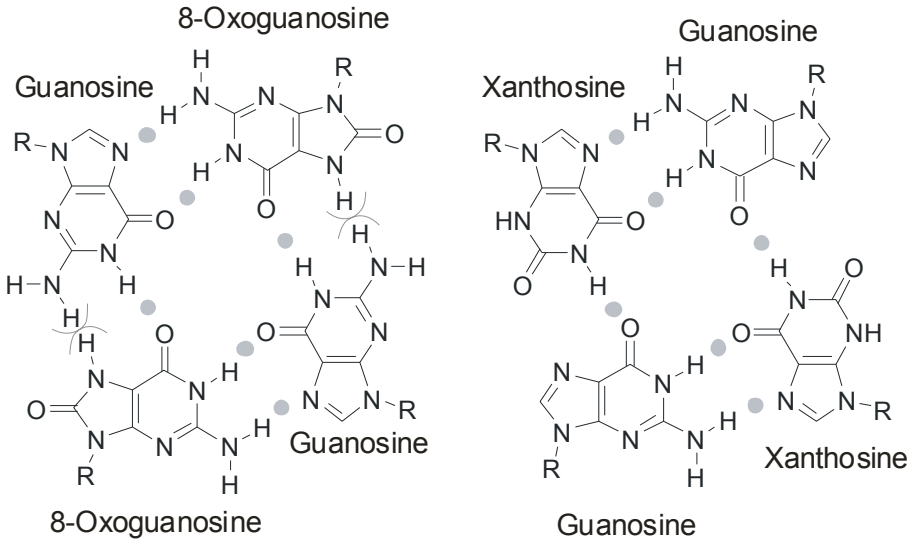
HT-ap: 5’- TA**O**GGTTAGG**X**TTA**X**GGTTAGG**O**T -3’

HT-p: 5’- TA**O**GGTTA**X**GGTTA**O**GGTTA**X**GGT -3’

HT-ap (inv): 5’- TA**X**GGTTAGG**O**TTA**O**GGTTAGG**X**T -3’

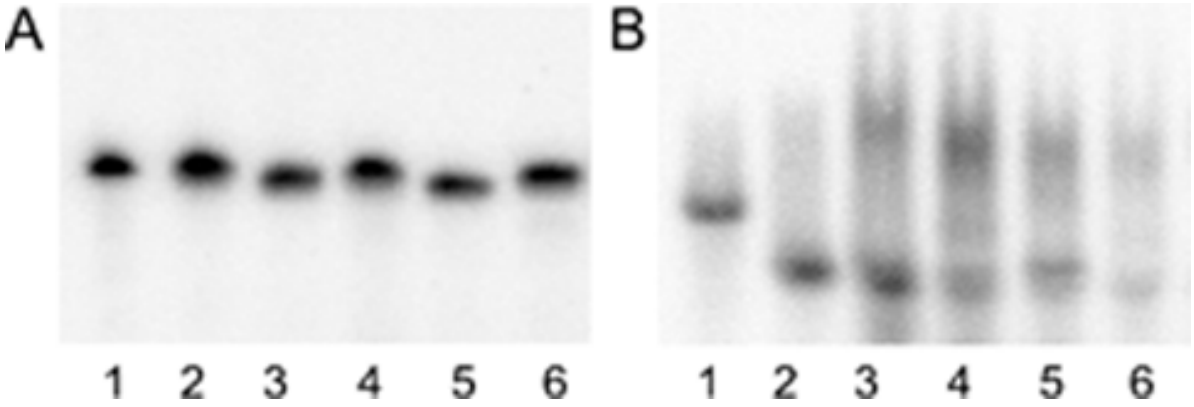
HT-p (inv): 5’- TA**X**GGTTA**O**GGTTA**X**GGTTA**O**GGT -3’

Quadruplex tetrads



Parallel folding of the anti-parallel programmed quadruplexes are lacking four hydrogen-bonds. In the tetrad composed of two 8-oxoguanines and two guanines the H7 of 8-oxoguanine and the amino proton of guanine are interfering with each other. The tetrad composed of two xanthines and two guanines lacks two hydrogen bonds between the 2-carbonyl group of xanthine and the N7 of guanine. According to this the parallel topology of an anti-parallel programmed quadruplex should be disfavoured and vice versa.

Electrophoretic mobility shift assay of quadruplex-forming sequences



Electrophoretic mobility shift assay (EMSA) of quadruplex-forming sequences. A: Denaturing PAGE, B: Native PAGE. 1: HT-s, scrambled control sequence unable to fold a quadruplex; 2: HT-c; 3: HT-ap; 4: HT-p; 5: HT-ap (inv); 6: HT-p (inv). The quadruplex-forming sequences 2–6 fold into monomolecular species as indicated by the higher mobility in the native EMSA together with a fraction of higher order aggregates.