Supporting Information

Scaffold stabilization of a G-triplex and study of its interactions with G-quadruplex targeting ligands

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Abbreviations

CD: Circular Dichroism; CuAAC: Copper Catalyzed Alkyne-Azide Cycloaddition; DCM: dichloromethane; DIEA: diisopropylamine; DMF: dimethylformamide; DTT: dithiothreitol; EDTA: Ethylenediaminetetraacetic acid; ESI-MS: Electrospray Ionisation Mass Spectrometry; RP-HPLC: Reverse-Phase High Performance Liquid Chromatography; Rt: Retention time; SEC: Size Exclusion Chromatography; BLI: Biolayer Interferometry; TDS: Thermal Difference Spectrum; TFA: trifluoroacetic acid; THPTA: Tris-(hydroxypropyltriazolylmethyl)amine; TIS: triisopropylsilane; TRIS: 2-amino-2-hydroxymethyl-propane-1,3-diol; UPLC: Ultra Performance Liquid Chromatography; UV: Ultraviolet.

General details

ESI mass spectra were performed on an Esquire 3000 spectrometer from Bruker or on an Acquity UPLC/MS system from Waters equipped with a SQ Detector 2. Peptides were analyzed in positive mode and oligonucleotides and conjugates in negative mode. The mass spectra display either the relative abundance of ion signals (total ion counts) against the m/z ratios or the total ion counts against the m/z ratios. All solvents and reagents used were of highest purity commercially available.

Peptide synthesis

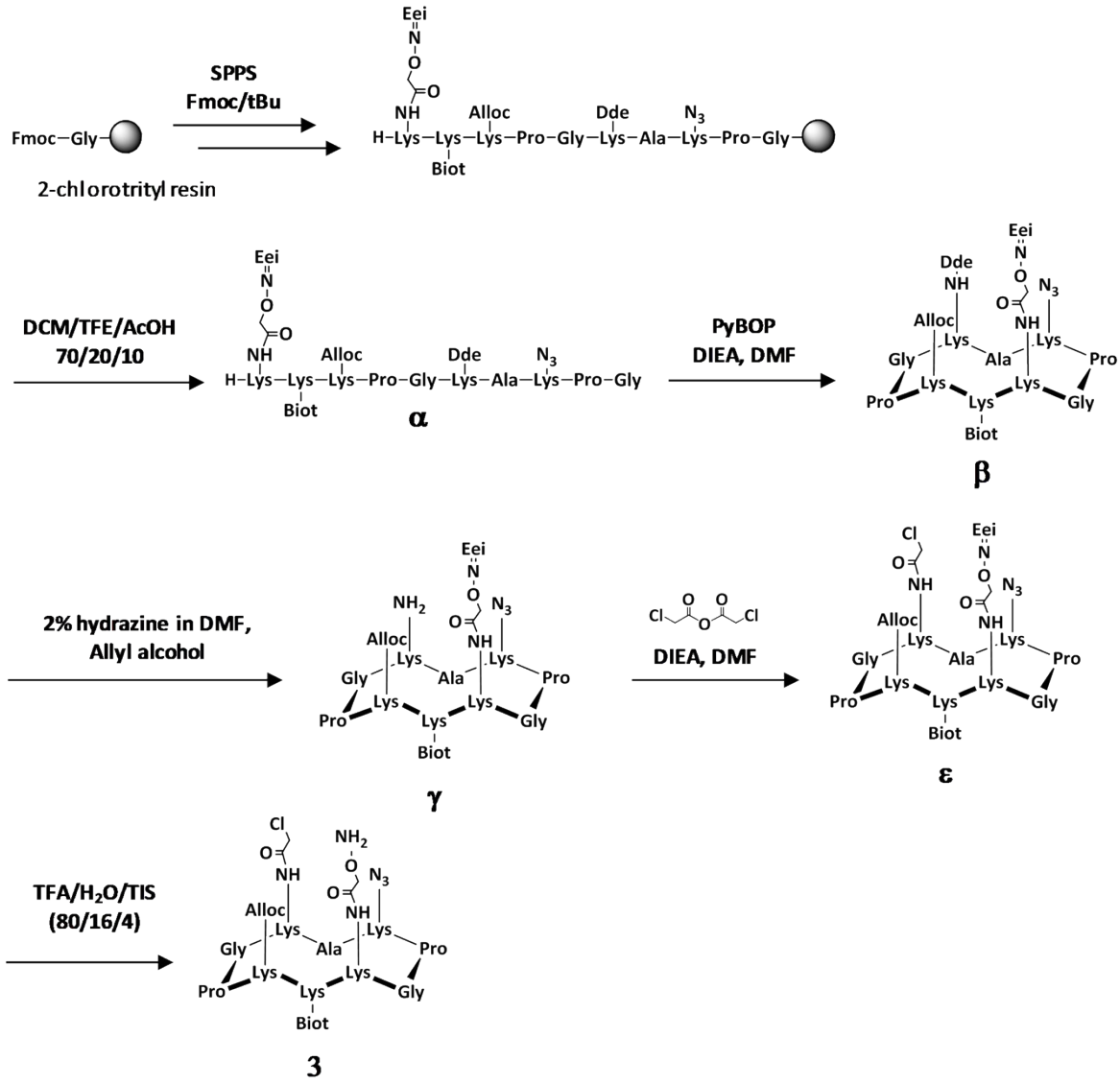
General details

The synthesis was performed on a Syro II synthesizer using Fmoc/tBu strategy on a 2-Chlorotrityl resin.

The course of reactions were monitored by using UPLC system Waters, it includes reverse phase chromatography using Nucleosil C18 column (130 Å, 2.1 x 50 mm, 1.7 µm) and detection by UV at 214 nm and 250 nm. A 1mL/min flow linear gradient from 95% solvent A (0.1% formic acid in water) and 5% solvent B (0.1% formic acid in acetonitrile/water: 9/1) to 100% B for 3 minutes was applied. The purified products were analyzed by the same UPLC system and the chromatograms display the UV absorbance at 214 nm against time.

RP-HPLC purifications were performed on a Gilson system with Nucleosil C18 column (100 Å, 250 x 21 mm, 7 µm) with UV monitoring at 214 nm and 250 nm. A 20 mL/min flow linear gradient was applied from 95% solvent A (0.1% trifluoroacetic acid in water) and 5% solvent B (0.1% trifluoroacetic acid in acetonitrile/water: 9/1) to 100% B for 20 minutes.

Scheme S1. Synthesis of peptide 3



a) Linear peptide α

Compound α was synthesis using commercially available 2-Chlorotrityl resin (loading of 0.83 mmol/g). Fmoc-Gly-OH (3 eq) was coupled on the resin in anhydrous DCM in a glass reaction vessel fitted with a sintered glass. pH 8 was adjusted using DIEA. The mixture was stirred for 1h at room temperature. A DCM/MeOH/DIEA solution (17:2:1, v:v:v) was then added. Fmoc protecting group was removed using three washes with 20% piperidine in DMF (40 mL). The resin loading was monitored by quantification of free dibenzofulvene using UV absorbance at 299 nm (loading of 0.40 mmol/g, yield: 48%). The elongation was performed on a Syro II using Fmoc/tBu strategy on the above-prepared resin, Fmoc-Ala-OH, Fmoc-Gly-OH, Fmoc-Lys(Alloc)-OH, Fmoc-Lys(Dde)-OH, Fmoc-Pro-OH and Fmoc-Lys(biotin)-OH were commercially available. Fmoc-azidononorleucine and Fmoc-Lys(-CO-CH_2-O-N=Eei)-OH were obtained using reported protocols.[1] The linear peptide was cleaved from the resin using a DCM/TFE/AcOH solution (70/20/10, v/v/v) (5 x 20 mL). The solution was evaporated under vacuum and the peptide was precipitated in ether as a white powder. The crude product was used without any further purification.

b) Cyclic peptide β

Peptide α (365 mg) was dissolved in DMF to reach a 10^{-3} M concentration and 1.2 eq of PyBOP (0.250 mmol, 130 mg) was added. The pH was adjusted to 8-9 using DIEA and the solution was stirred at room temperature until the complete peptide cyclisation (UPLC monitoring). The solvent was evaporated under vacuum then the crude peptide was precipitated in ether. The crude product was purified on RP-HPLC and freeze-dried to obtain a white powder (0.15 mmol, 238 mg, yield: 66% for two steps). Rt = 1.86 min.

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[1] E. D. Goddard-Borger, R.V. Stick, *Org. Lett.* 2007, **9**, 3797-3800 ; S. Foillard *et al*, *J. Org. Chem*. 2008, **73**, 983-9991

ESI-MS (+): *m/z* calcd for $C_{77}H_{123}N_{20}O_{19}S$: 1663.8, *m/z* found: 1664.6 $[M+H]^+$.

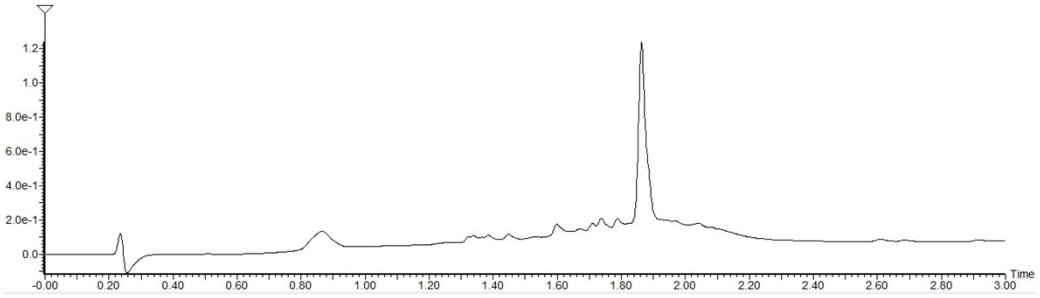


Fig. S1. UPLC chromatogram of cyclic peptide β

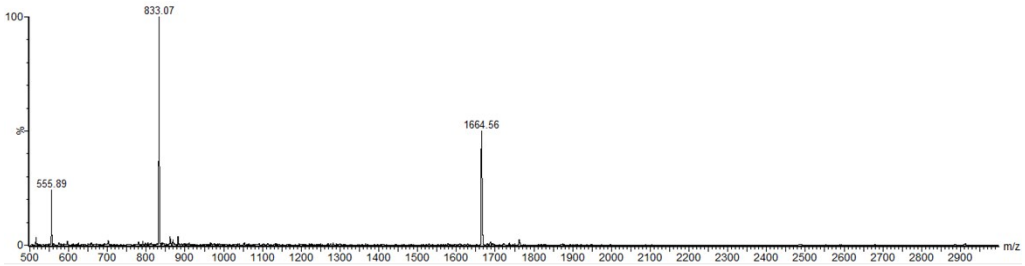


Fig. S2. ESI mass spectrum of cyclic peptide β

c) Peptide γ

Peptide **β** (0.15 mmol, 250 mg) was dissolved in a 2% hydrazine solution in DMF in presence of 20 eq. of allylic alcohol. The solution was stirred at room temperature until the complete

deprotection (UPLC monitoring). The solvent was evaporated under vacuum then the crude peptide was precipitated in ether as a white powder. The crude product was used without any further purification.

d) Peptide ε

Peptide **γ** was dissolved in DMF to reach a 3.10^{-3} M concentration. The pH was adjusted to 8-9 using DIEA and chloroacetic anhydride (2 eq, 0.3 mmol) was added. The reaction was stirred for 1h30 at room temperature. The solvent was evaporated under vacuum then the crude peptide was precipitated in ether. The crude product was purified on RP-HPLC and freeze-dried to obtain a white powder (0.09 mmol, 142 mg, yield: 60% for two steps). Rt = 1.74 min.

ESI-MS (+): *m/z* calcd for $C_{69}H_{112}ClN_{20}O_{18}S$: 1575.8, *m/z* found: 1576.5 $[M+H]^+$.

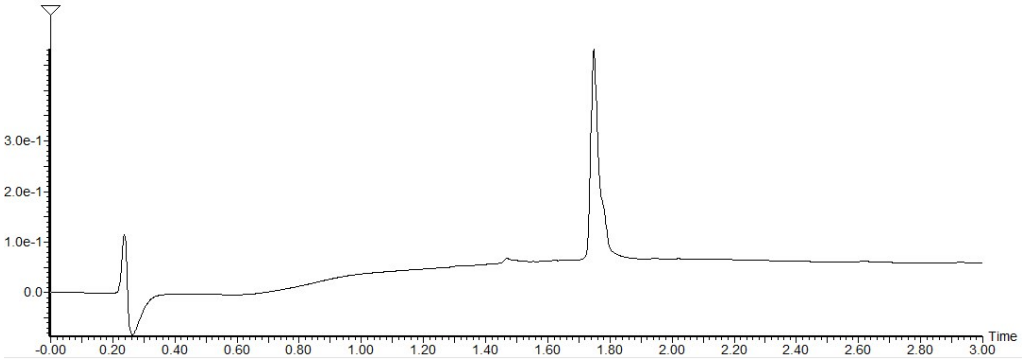


Fig. S3. UPLC chromatogram of peptide **ε**

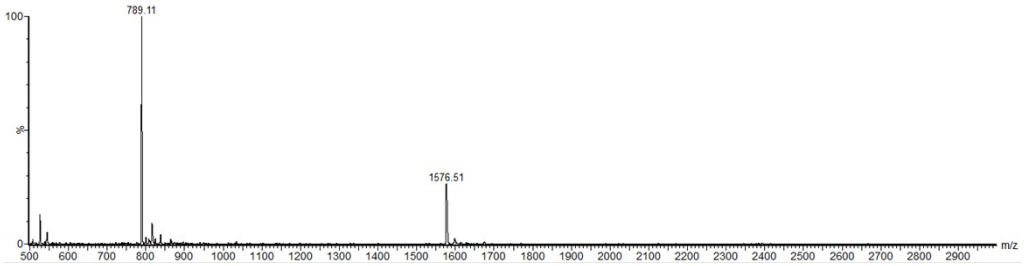


Fig. S4. ESI mass spectrum of peptide **ε**

e) Peptide 3

Peptide **ε** (3 mg) was dissolved in a TFA/H_2O/TIS (80/16/4, v/v/v) solution (4 mL) and the reaction was stirred 2h at room temperature. The solvent was evaporated under vacuum then the crude peptide was precipitated in ether as a white powder. The crude product was used without any further purification. The yield was considered as quantitative. Rt = 1.42 min.

ESI-MS (+): *m/z* calcd for $C_{65}H_{106}ClN_{20}O_{17}S$: 1507.1, *m/z* found: 1506.5 $[M+H]^+$.

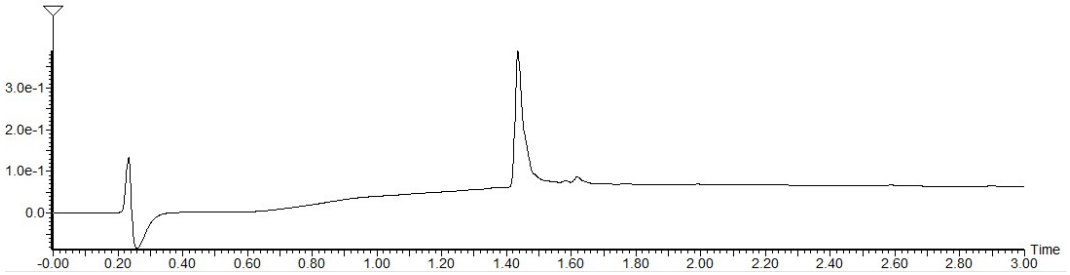


Fig. S5. UPLC chromatogram of precipitated peptide **3**

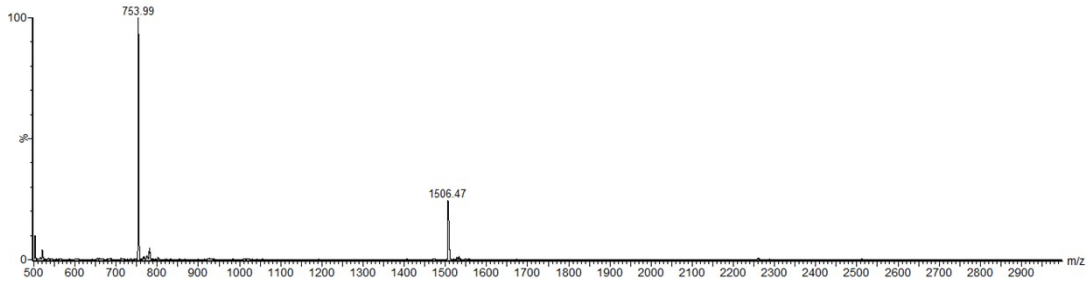


Fig. S6. ESI mass spectrum of precipitated peptide **3**

Oligonucleotides and conjugates synthesis

General details

Oligonucleotides were prepared using β-cyanoethylphosphoramidite chemistry on a 3400 DNA synthesizer at 1 μmol scale.

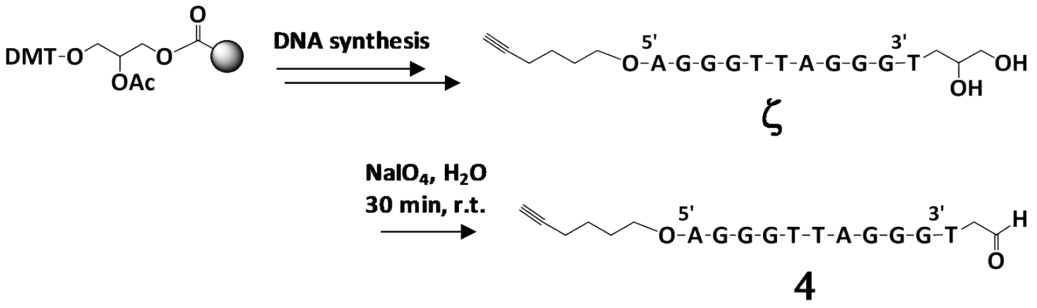
RP-HPLC analyses were performed on a Waters HPLC system using C18 Nucleosil column (Macherey-Nagel, 100 Å, 250 x 4.6 mm, 5 μm) with UV-monitoring at 260 nm and 280 nm. A 1 mL/min flow linear gradient was applied. Solvent A (50 mM triethylammonium acetate buffer with 5% acetonitrile) and solvent B (acetonitrile with 5% water) were used. A stepwise gradient of 0-30% B in 20 min then from 30 to 100% B in 10 min was applied for the gradient. The chromatograms of the purified products display the UV absorbance at 260 nm against time. The RP-HPLC purifications of oligonucleotides were performed on a Gilson system with Nucleosil C-18 colunm (Macherey-Nagel, 100 Å, 250 x 10 mm, 7 μm) with UV-monitoring at 260 nm and 280 nm using 4 mL/min flow linear gradient. A stepwise gradient of 0-30% B in 20 min then from 30 to 100% B in 10 min was applied.

Desalting of oligonucleotides was performed by SEC on NAP 25 cartridge using manufacturer's protocol.

Quantification of oligonucleotides was performed at 260 nm using Nanodrop apparatus (molar extinction Ɛ260nm was estimated according to the nearest neighbor model).

Oligonucleotides synthesis

Scheme S2. Synthesis of oligonucleotide 4



a) Oligonucleotide ζ

Oligonucleotide ζ was obtained from automated synthesis on a 3'-glyceryl CPG resin at 1 µmol scale using a 3400 DNA synthesizer from Applied Biosystems. The last coupling was carried using commercially available 5' hexynyl (β-cyanoethyl) phosphoramidite (GlenReseach). After synthesis, cyanoethyl protecting groups were removed using 20% piperidine in acetonitrile.

Cleavage from the resin and deprotection was performed in 28% NH_4OH for 16h at 55°C. The product was purified on RP-HPLC and readily oxidized toward oligonucleotide **4**.

b) Oligonucleotide 4

Sodium metaperiodate (20 eq; 8 µmol; 1.7 mg) was added to a solution of oligonucleotide **ζ** (1 eq; 400 nmol) in water (400 µL). The reaction was stirred for 1h at room temperature in dark conditions. The product was then desalted on NAP 25 and the fractions were collected to obtain the crude product (UV-monitored at 260 nm). The oxidation was considered quantitative and the crude containing oligonucleotide **4** was used in the next step without further purification. The purity of the crude material was assessed by ESI-MS and RP-HPLC.

Rt = 14.8 min, ESI-MS (-) *m/z* calcd for $C_{118}H_{147}N_{46}O_{72}P_{12}$: 3733.4, *m/z* found: 3733.3 $[M-H]^-$.

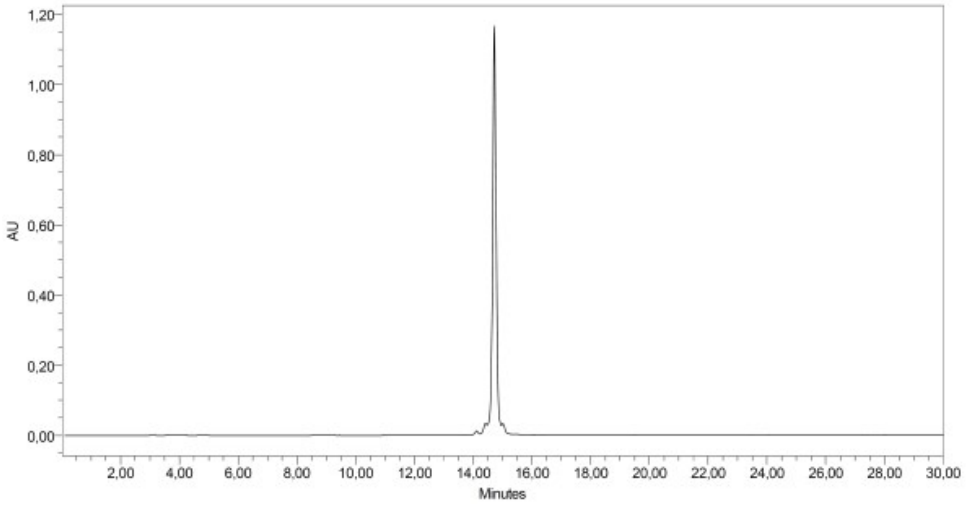


Fig. S7. RP-HPLC chromatogram of oligonucleotide **4**

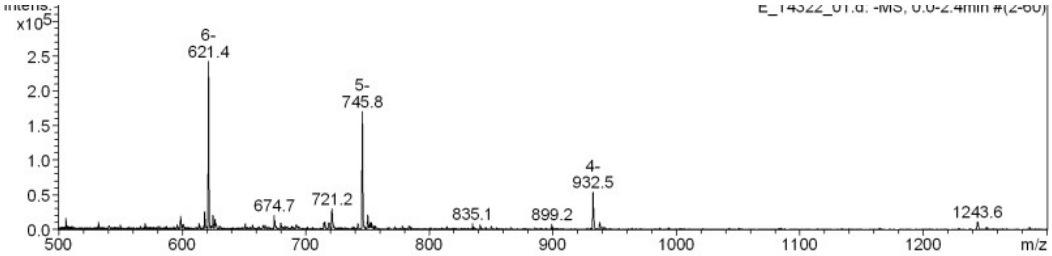
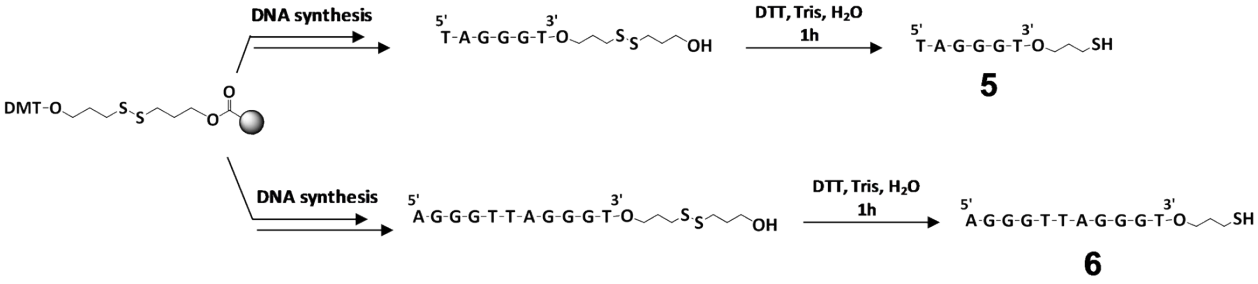


Fig. S8. ESI mass spectrum of oligonucleotide **4**

Scheme S3. Synthesis of oligonucleotides 5 and 6



c) Oligonucleotide 5

Oligonucleotide **5** was obtained from automated synthesis on a 3'-thiol-modifier C3 S-S CPG resin at 1 μmol scale using a 3400 DNA synthesizer from Applied Biosystems. After synthesis, cyanoethyl protecting groups were removed using 20% piperidine in acetonitrile. Cleavage from the resin and deprotection was performed in 28% NH4OH for 16h at 55°C. The product was

purified on RP-HPLC and the DMT protecting groups was removed using in 80% aqueous acetic acid solution for 20 min at room temperature. After concentration of the acetic acid solution, dithiothreitol (100 eq) was added to a solution of the 3'SSOH oligonucleotide (500 nmoles) in Tris.HCl buffer 1 M, pH 8.5 (500 uL). The mixture was stirred for 1h at room temperature. The product was purified on RP-HPLC then desalted and freeze-dried.

Rt = 15.9 min, ESI-MS (-) *m/z* calcd for $C_{63}H_{81}N_{24}O_{38}P_6S$: 2000.4, *m/z* found: 1999.9 $[M-H]^-$.

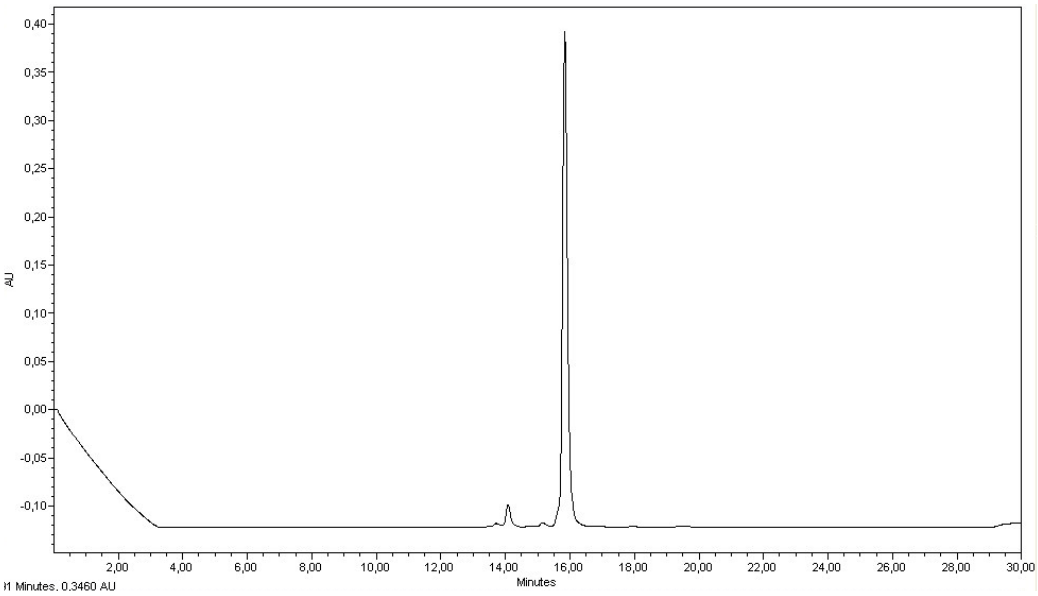


Fig. S9. RP-HPLC chromatogram of oligonucleotide **5**

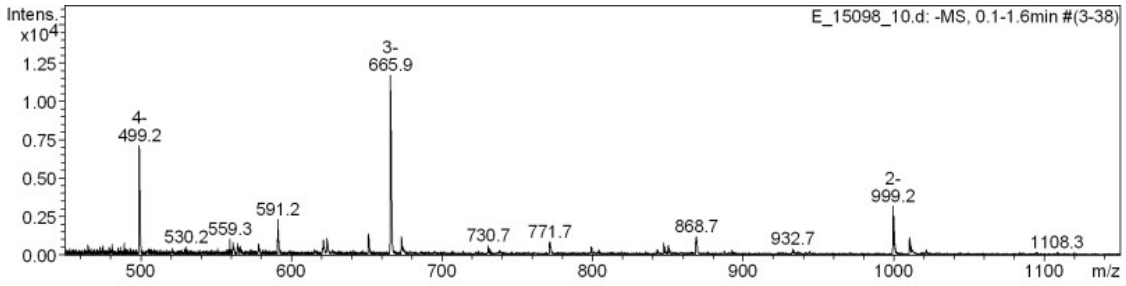


Fig. S10. ESI mass spectrum of oligonucleotide **5**

c) Oligonucleotide 6

Oligonucleotide **6** was obtained from automated synthesis on a 3'-thiol-modifier C3 S-S CPG resin at 1 µmol scale using a 3400 DNA synthesizer from Applied Biosystems. After synthesis, cyanoethyl protecting groups were removed using 20% piperidine in acetonitrile. Cleavage from the resin and deprotection was performed in 28% NH4OH for 16h at 55°C. The product was purified on RP-HPLC and the DMT protecting groups was removed using in 80% aqueous acetic acid solution for 20 min at room temperature. After concentration of the acetic acid solution, dithiothreitol (100 eq) was added to a solution of the 3'SSOH oligonucleotide (400 nmoles) in Tris.HCl buffer 1 M, pH 8.5 (400 uL). The mixture was stirred for 1h at room temperature. The product was purified on RP-HPLC then desalted and freeze-dried.

Rt = 15.9 min, ESI-MS (-) *m/z* calcd for $C_{113}H_{142}N_{46}O_{68}P_{11}S$: 3605.4, *m/z* found: 3605.4 $[M-H]^-$.

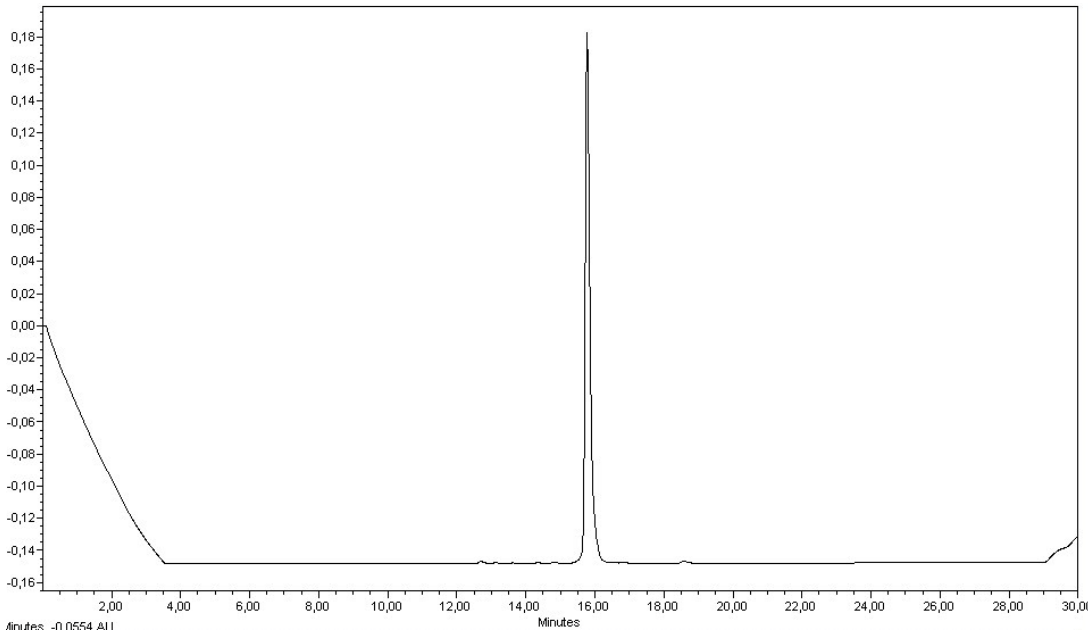


Fig. S11. RP-HPLC chromatogram of oligonucleotide **6**

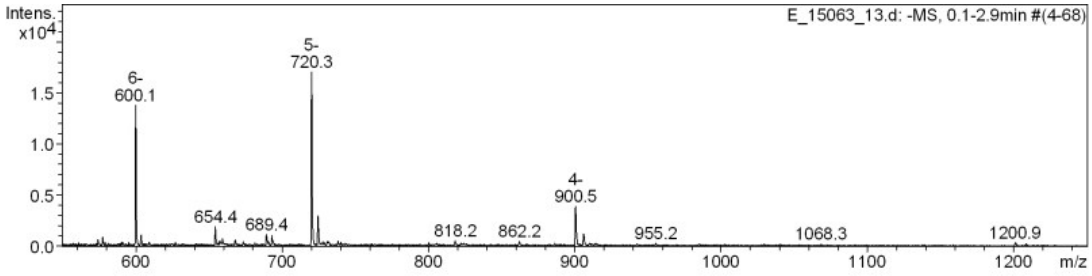
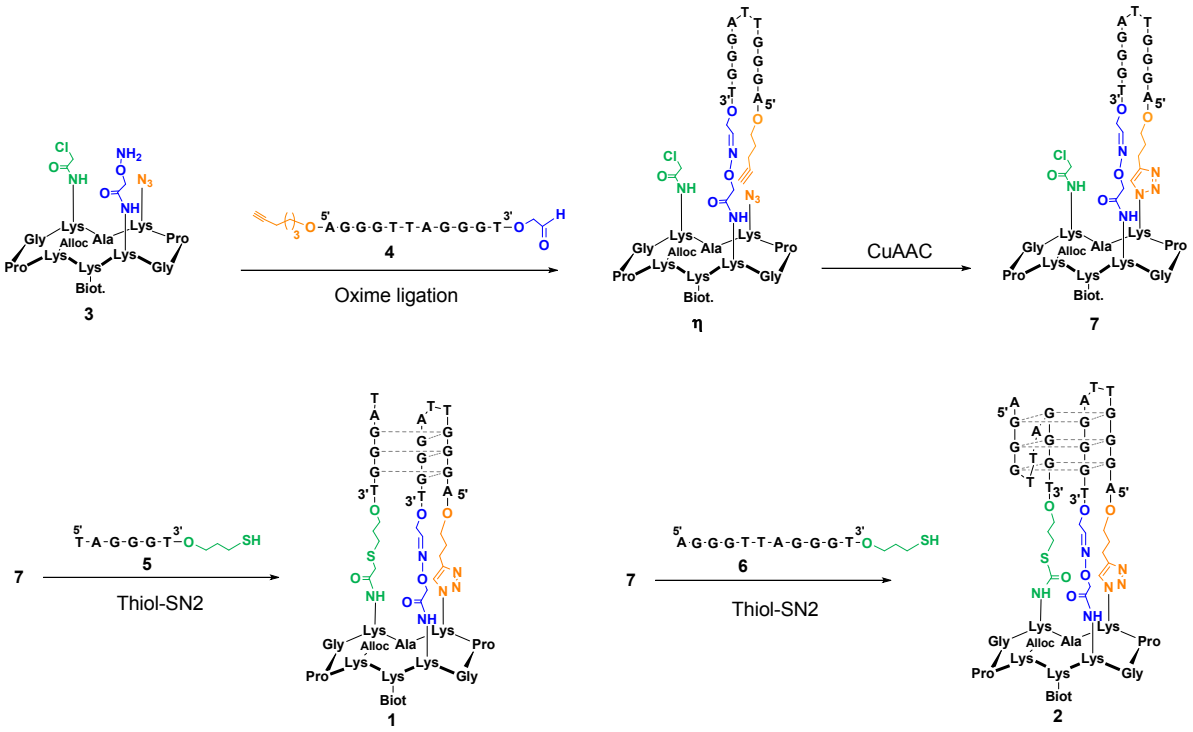


Fig. S12. ESI mass spectrum of oligonucleotide **6**

Conjugates synthesis

Scheme S4. Synthesis of conjugates 1 and 2



Synthesis of conjugate η

3' aldehyde containing oligonucleotide **4** (1 eq, 490 nmoles) was dissolved in 0.4 M ammonium acetate buffer (pH 4.5, concentration 10^{-3} M) and aminooxy peptide **3** (1.2 eq, 588 nmoles) was added. The solution was stirred at 55°C for 45 min. The crude was purified using RP-HPLC conjugate **η** was desalted by SEC and freeze dried. Quantification was performed by UV-spectrometry (294 nmoles, yield 60%, ε_{260nm} = 114800 $M^{-1}.cm^{-1}$). Rt = 22.2 min

ESI-MS (-) m/z calcd for $C_{183}H_{250}N_{66}O_{88}P_{12}SCl$: 5221.5, m/z found: 5221.4 $[M-H]^-$.

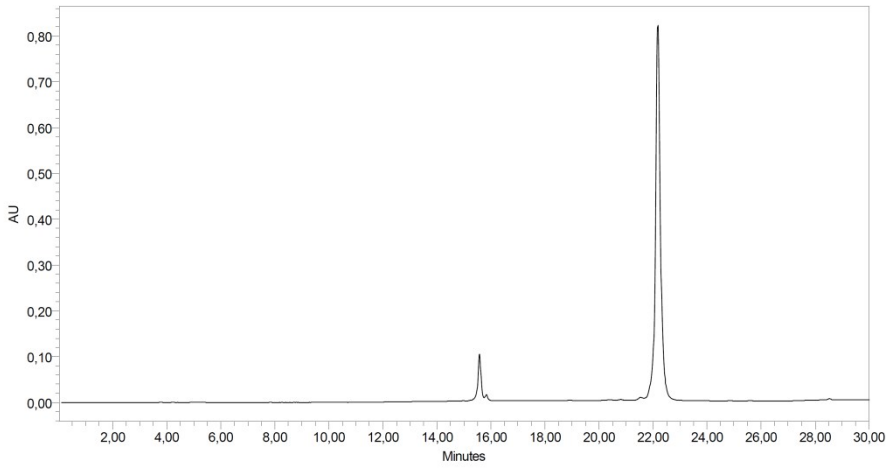


Fig. S13. RP-HPLC chromatogram of conjugate **η**

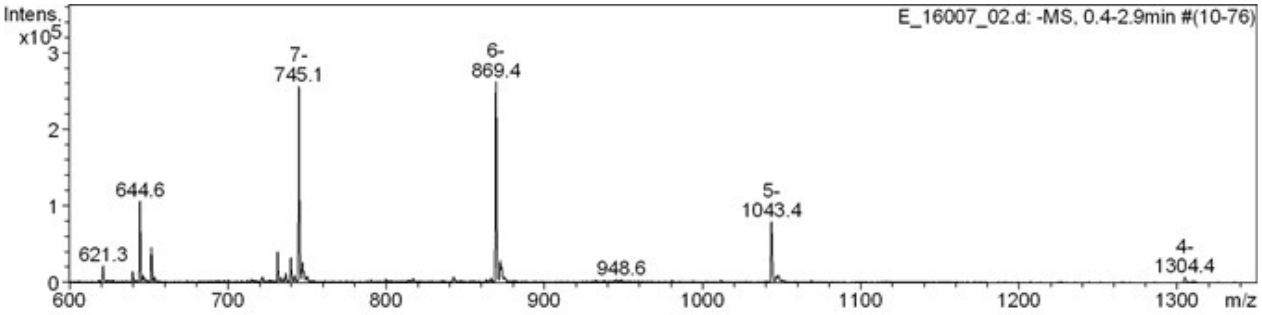


Fig. S14. ESI mass spectrum of conjugate **η**

Synthesis of conjugate 7

Conjugate **η** (237 nmoles) was dissolved in 100 mM HEPES buffer (pH 7.4, concentration 10^{-4} M) in presence of 10% DMF (v,v), CuSO4 (6 eq by azido function), THPTA (30 eq by azido function) and sodium ascorbate (30 eq by azido function) were added. The reaction was stirred at 37°C for 2h and quenched with 0.5 M EDTA solution (50 eq by azido function). The resulting mixture was desalting by SEC. The crude was purified using RP-HPLC then conjugate **7** was desalted by SEC

and freeze dried. Quantification was performed by UV-spectrometry (156 nmoles, yield 66%, ε_{260nm} = 114800 $M^{-1}.cm^{-1}$). Rt = 19.5 min

ESI-MS (-) m/z calcd for $C_{183}H_{250}N_{66}O_{88}P_{12}SCl$: 5221.5, m/z found: 5221.4 $[M-H]^-$.

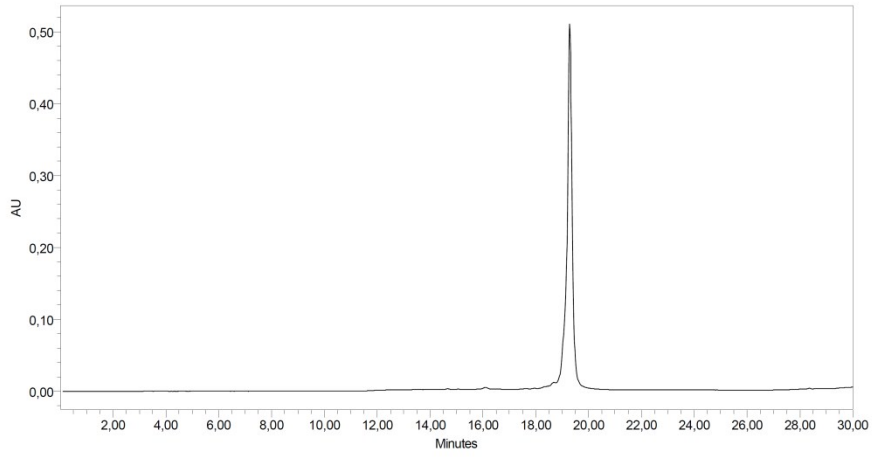


Fig. S15. RP-HPLC chromatogram of conjugate **7**

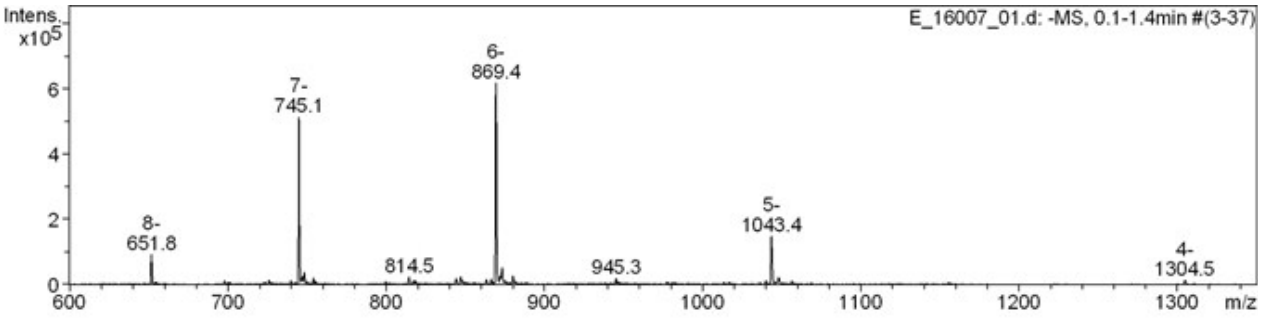


Fig. S16. ESI mass spectrum of oligonucleotide **7**

Synthesis of conjugate 1

Conjugate **7** (1 eq, 50 nmol) and oligonucleotide **5** (2 eq, 100 nmol) were dissolved in H_2O/CH_3CN solution (9/1, v/v, concentration 5.10^{-4} M) and TCEP (2 eq by thiol function), 500 mM KCl, DIEA (45 eq by chloroacetamide function to obtain pH 8.5), KI (60 eq by chloroacetamide function) were added. The reaction mixture was stirred at room temperature for 5h. The crude was purified using RP-HPLC then conjugate **1** was desalted by SEC and freeze dried. Quantification was performed by UV-spectrometry (25 nmol, yield 50%, ε_{260nm} = 176500 $M^{-1}.cm^{-1}$). Rt = 17.8 min

ESI-MS (-) m/z calcd for $C_{246}H_{329}N_{90}O_{126}P_{18}S_2$: 7184.4, m/z found: 7186.7 $[M-H]^-$.

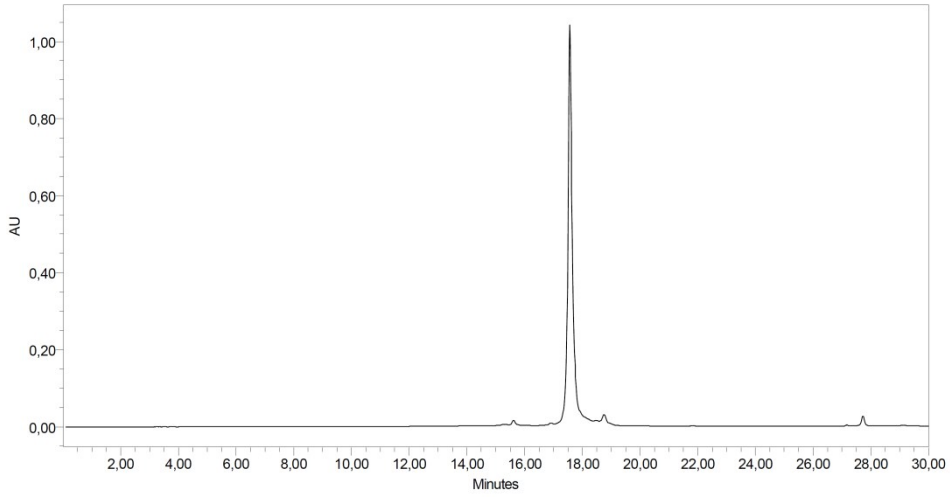


Fig. S17. RP-HPLC chromatogram of conjugate **1**

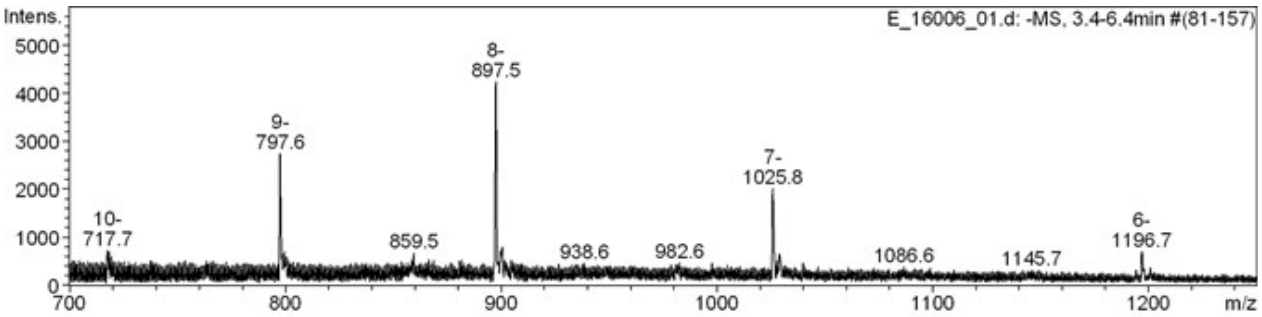


Fig. S18. ESI mass spectrum of conjugate **1**

Synthesis of conjugate 2

Conjugate **7** (1 eq, 50 nmol) and oligonucleotide **6** (2 eq, 100 nmol) were dissolved in H_2O/CH_3CN solution (9/1, v/v, concentration 5.10^{-4} M) and TCEP (2 eq by thiol function), 500 mM KCl, DIEA (45 eq by chloroacetamide function to obtain pH 8.5), KI (60 eq by chloroacetamide function) were added. The reaction mixture was stirred at room temperature for 5h. The crude was purified using RP-HPLC then conjugate **2** was desalted by SEC and freeze dried. Quantification was performed by UV-spectrometry (30 nmol, yield 60%, ε_{260nm} = 229600 $M^{-1}.cm^{-1}$). Rt = 17.1 min

ESI-MS (-) m/z calcd for $C_{296}H_{390}N_{112}O_{156}P_{23}S_2$: 8789.4, m/z found: 8792.1 $[M-H]^-$.

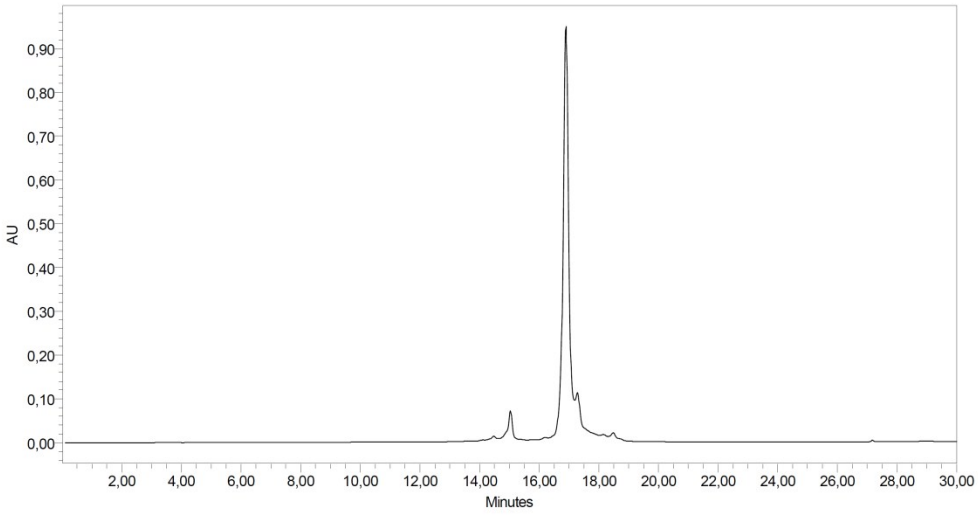


Fig. S19. RP-HPLC chromatogram of conjugate **2**

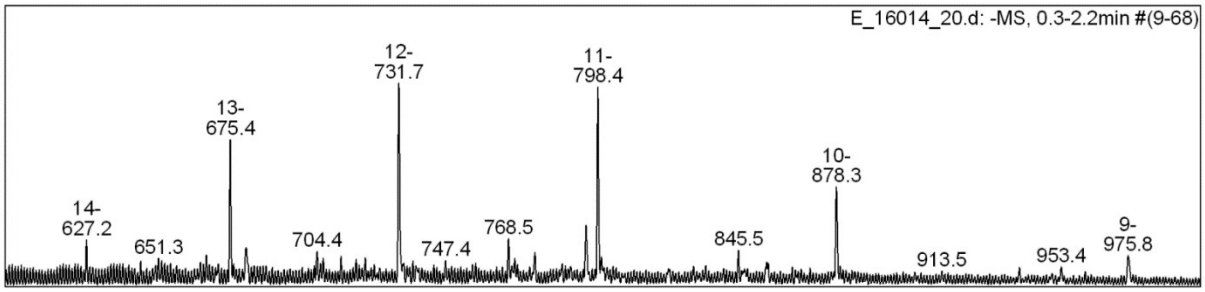


Fig. S20. ESI mass spectrum of conjugate **2**

Circular Dichroism studies

Thermal denaturation studies

Circular dichroism studies were performed after thoroughly desalting the products by SEC on NAP 25 cartridge. An annealing step was applied by heating the sample at 90°C for 5 min in buffer (Tris 10 mM pH 7.4 with 100 mM KCl) and cooling it over 2h to room temperature. Analyses were recorded on a Jasco J-810 spectropolarimeter using 1 cm length quartz cuvette. Spectra were recorded at 20°C or every 5°C in a range from 5°C to 90°C with wavelengths range from 220 to 330 nm. For each temperature, the spectrum was an average of three scans with a 0.5 s response time, a 1 nm data pitch, a 4 nm bandwidth and a 200 $nm.min^{-1}$ scanning speed. Melting temperatures were obtained using Boltzmann fit on Origin software.

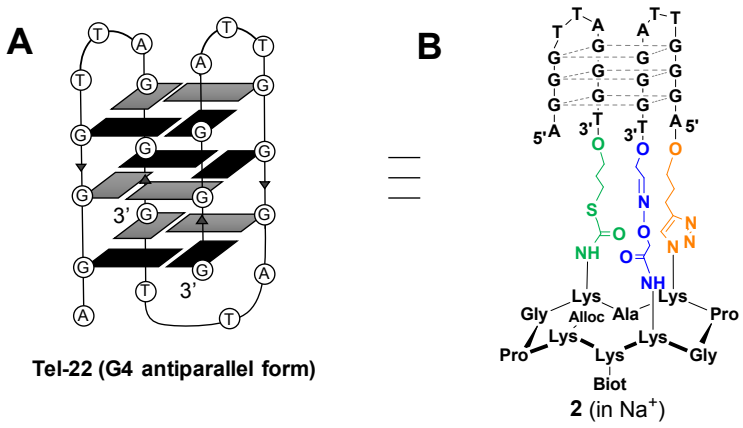


Fig. S21. A/ Schematic drawing of the G4 structure formed by the human telomeric DNA sequence (Tel-22) in presence of Na^+ and corresponding G-tetrad arrangements (black box =(anti) guanine, grey box = (syn) guanine B/ Schematic drawing of G4 forming DNA conjugate **2** in presence of Na^+

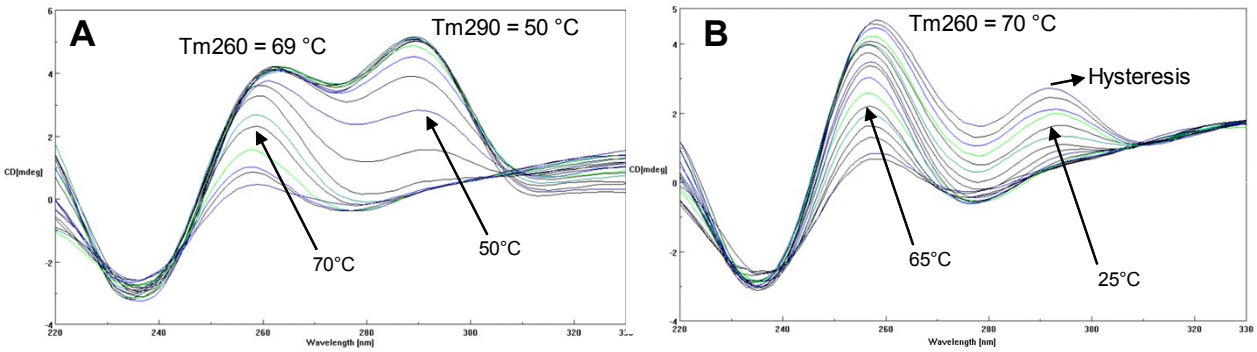


Fig. S22. Raw CD traces for **1** (3.2 µM in 10 mM Tris-HCl pH 7.4 with 100 mM KCl) during melting (**A**, 5°C to 90°C) and annealing (**B**, 90°C to 5°C).

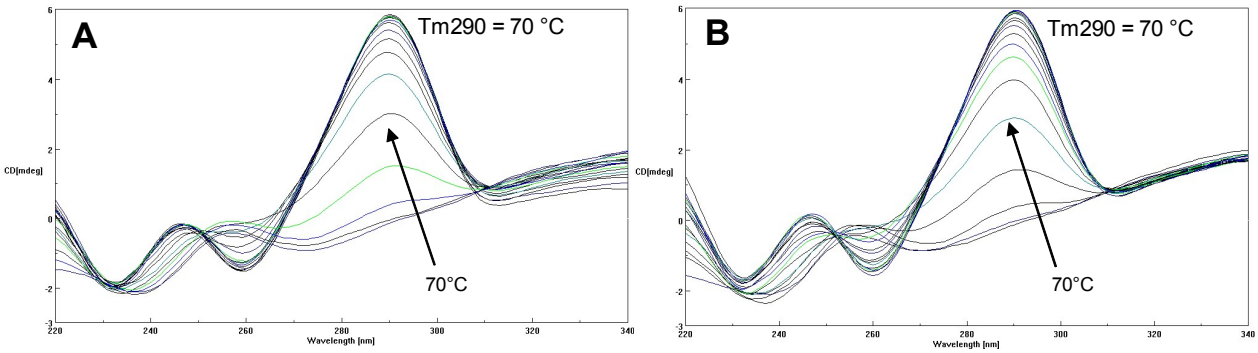


Fig. S23. Raw CD traces for **2** (3.2 μM in 10 mM Tris-HCl pH 7.4 with 100 mM KCl) during melting (**A**, 5°C to 90°C) and annealing (**B**, 90°C to 5°C).

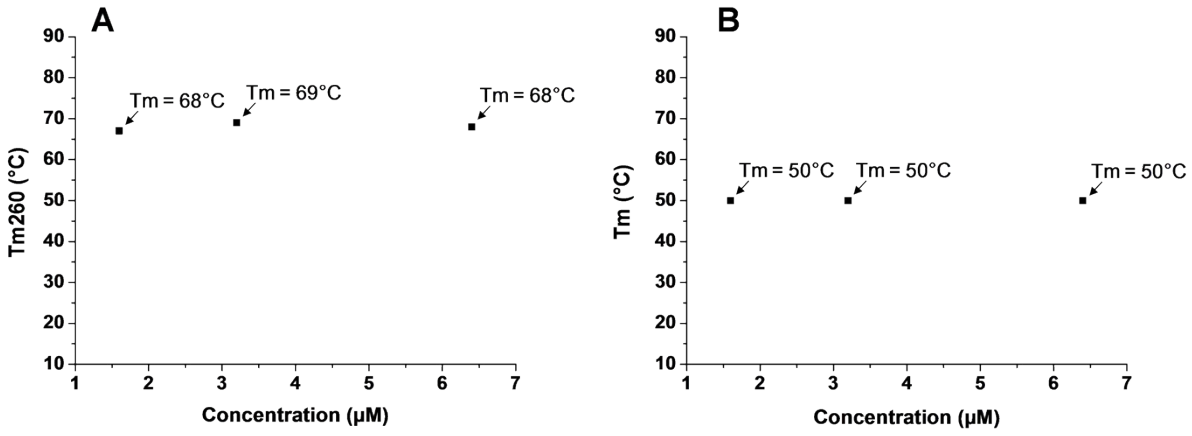


Fig. S24. Influence of the concentration on the melting temperature of **1** (in 10 mM Tris-HCl pH 7.4 with 100 mM KCl). Tm = f(C) at 260 nm (**A**) and 290 nm (**B**)

Thermal difference spectrum (TDS)

Thermal difference spectrum was obtained from the subtraction of UV spectrum performed at 5°C from UV spectrum performed at 90°C. UV spectra were recorded on a Varian Cary 400 UV-visible spectrophotometer using 1 cm length quartz cuvette. Spectra were recorded at 5°C and 90°C with wavelengths range from 230 to 320 nm. The difference spectra were normalized by dividing the raw data by its maximum value, so that the highest positive peak gets a Y-value of +1 as recommended in reference 21 of the manuscript.

BLI experiments

Bio-layer interferometry experiments were performed using sensors coated with streptavidin (SA sensors) purchased from Forte Bio (PALL). Prior functionalization, they were washed for 10 minutes by incubation in buffer (HEPES buffer 10 mM, KCl 150 mM, pH 7.5 and 0.5% v/v surfactant P20) to dissolve the sucrose layer. Then, the sensors were dipped for 15 minutes in solutions containing the conjugates **1**,**2** or **8** (Figure S28) at 100 nM and then washed in buffer solution (HEPES buffer 10 mM, KCl 150 mM, pH 7.5 and 0.5% v/v surfactant P20) for 10 minutes. The functionalized sensors were then dipped in G4-ligand (TMPyP4, Braco-19, PDS and PhenDC3) containing solution at different concentrations (Table S1) interspersed by a rinsing step in the buffer solution. The concentrations and association and dissociation time were optimized for

each ligand (Table S1). For TMPyP4 and Braco-19 the same sensors were used for the 6 concentrations whereas for PDS and PhenDC3, one sensor was used for each concentration as we couldn't achieve full dissociation of those ligands. A reference sensor without DNA was used to subtract the non-specific adsorption on the SA layer. The sensorgrams were fitted using a 1:1 interaction model. The reported values are the means of representative independent experiments, and the errors provided are standard deviations from the mean. Each experiment was repeated at least two times

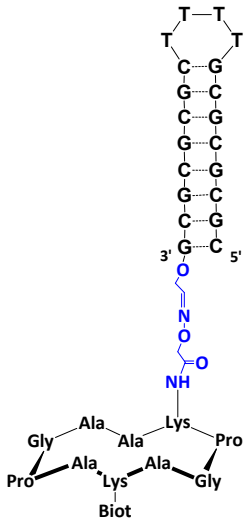


Fig. S25. Hairpin mimicking conjugate **8**

G4-ligands	Association time (s)	Dissociation time (s)	Concentration (nM)
TMPyP4	120	240	50, 100, 300, 500, 1000, 1500
Braco-19	120	240	75, 200, 400, 800, 1500, 3000
PDS	600	600	50, 100, 150, 200, 350
PhenDC3	600	600	50, 75, 100, 150, 250

Table S1. Experimental conditions for the recognition of G4 ligand on molecule **1**, **2** and **8**

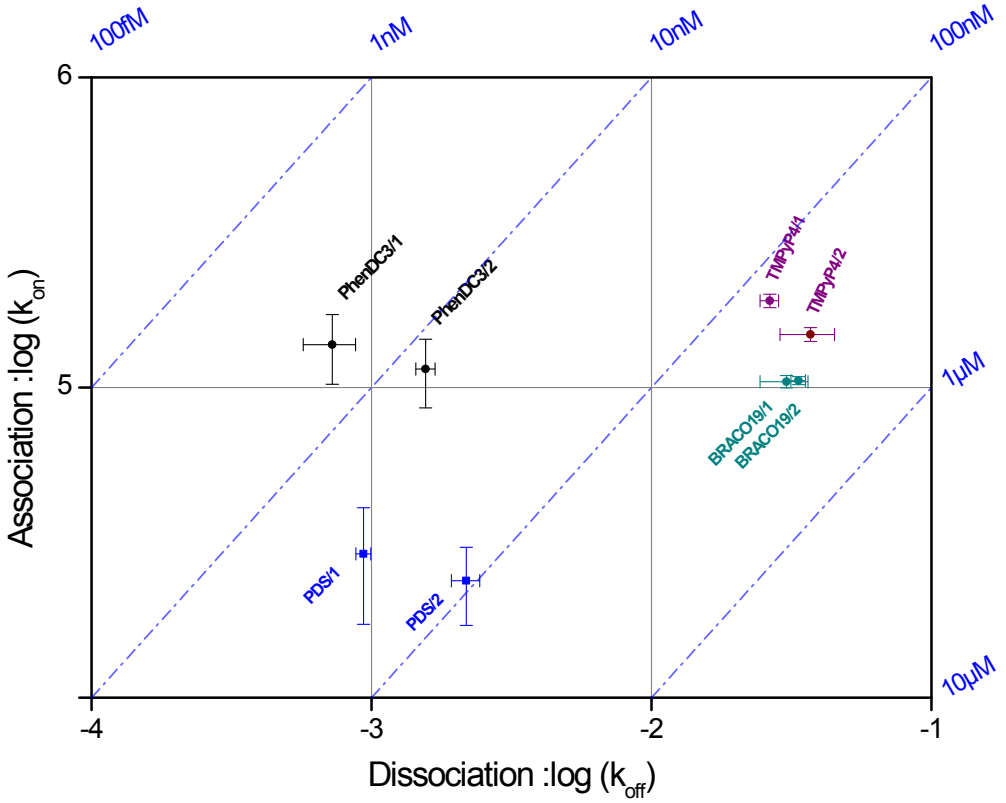


Fig. S26. Isoaffinity plot and kinetic characterization as established by BLI. K_D (parallel diagonal lines) k_{on} (association kinetic constant, y axis), k_{off} (dissociation kinetic constant, x axis).

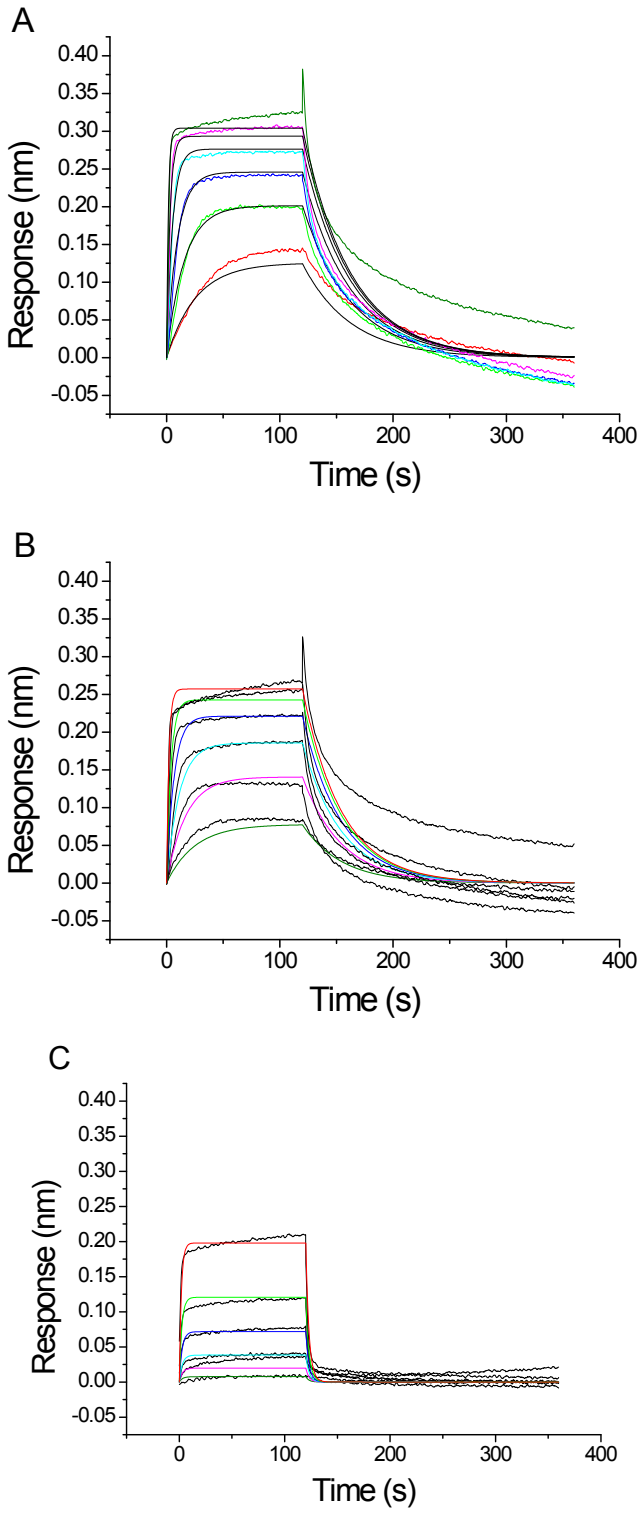
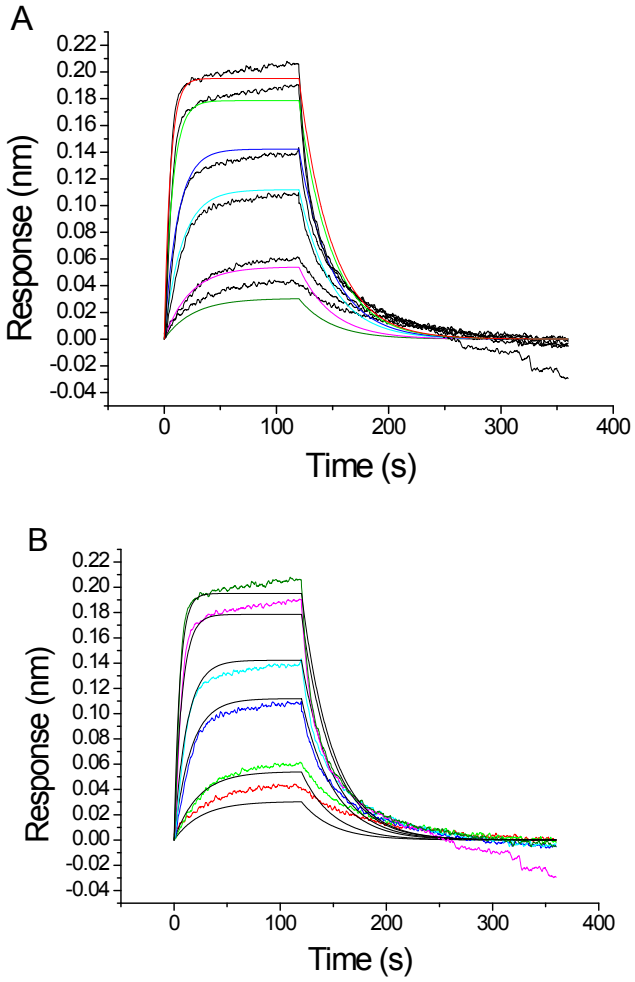

A
Response (nm)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
0
100
200
300
400
Time (s)
B
Response (nm)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
0
100
200
300
400
Time (s)
C
Response (nm)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
0
100
200
300
400
Time (s)

Fig. S27. BLI sensorgrams for the interaction of TMPyP4 with molecule **1** (A), **2** (B) and **8** (C). The analyte concentrations were 75, 200, 400, 800, 1500 and 3000 nM. The color curves are the raw data and the black curves are the fitting result.



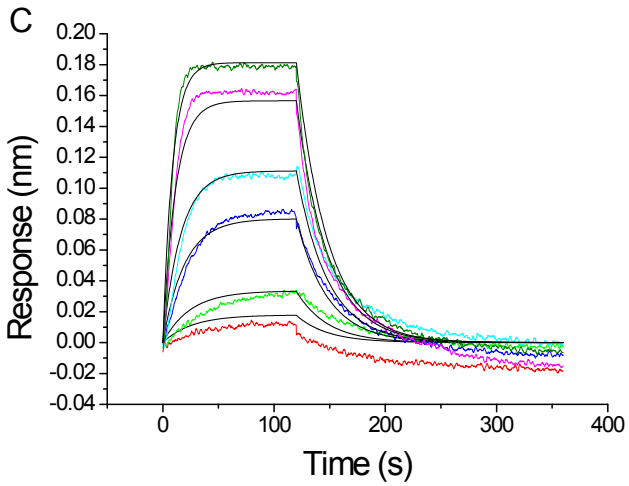
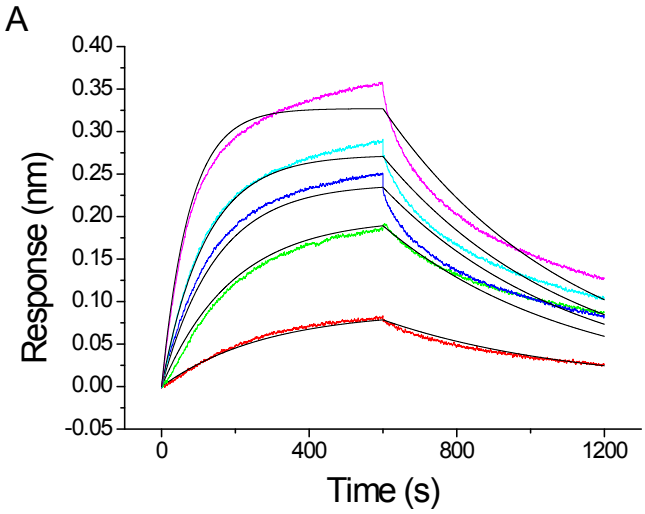


Fig. S28. BLI sensorgrams for the interaction of BRACO-19 with molecule **1** (A), **2** (B) and **8** (C). The analyte concentrations were 50, 100, 300, 500, 1000 and 1500 nM. The color curves are the raw data and the black curves are the fitting result.



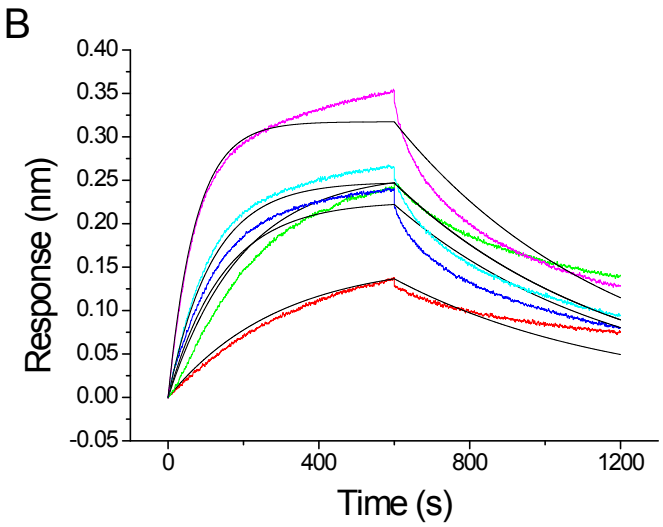
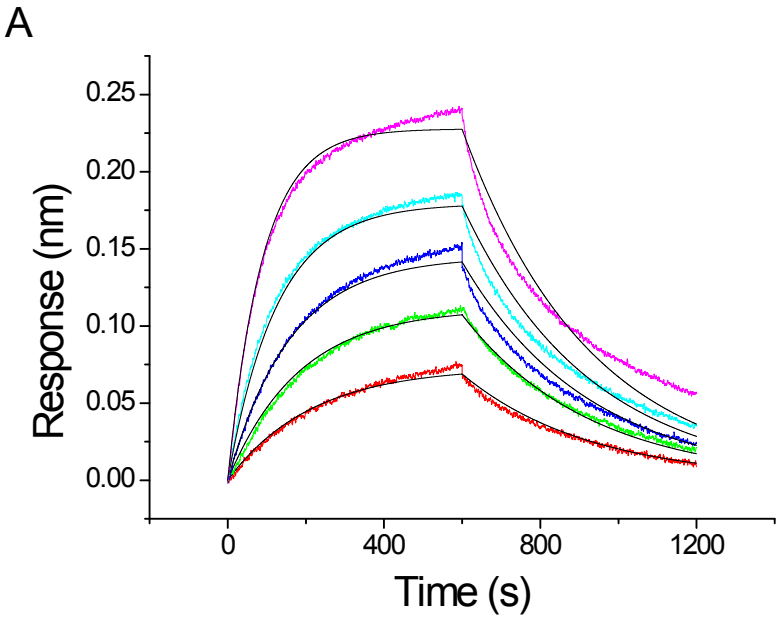


Fig. S29. BLI sensorgrams for the interaction of PDS with molecule **1** (A) and **2** (B). The analyte concentrations were 50, 100, 150, 200 and 350 nM. The color curves are the raw data and the black curves are the fitting result.



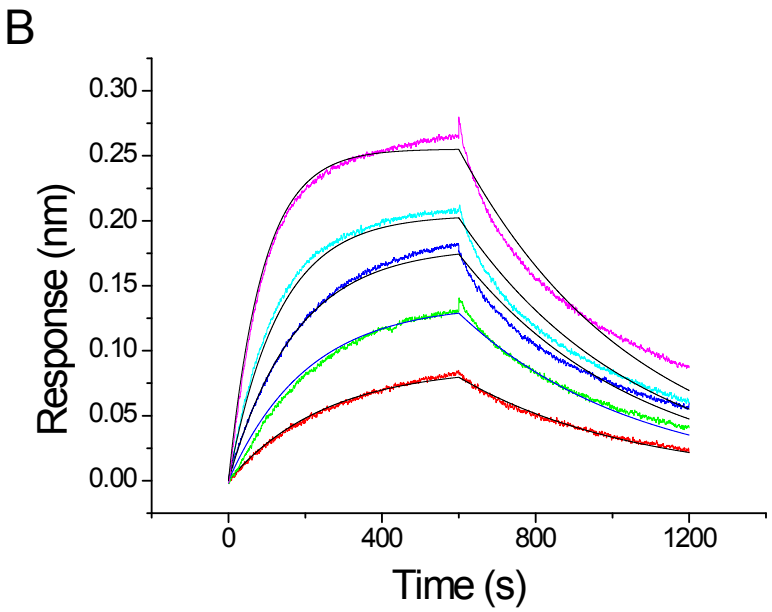


Fig. S30. BLI sensorgrams for the interaction of PhenDC3 with molecule **1** (A) and **2** (B). The analyte concentrations were 50, 75, 100, 150 and 250 nM. The color curves are the raw data and the black curves are the fitting result.

SPR experiments

HS-$(CH_2)_{11}$-EG_6-Biotin was procured from Prochimia. All other chemical products were purchased from Sigma-Aldrich. Cleaning procedure of the gold sensor chips included UV-ozone treatment during 10 min followed by rinsing with MilliQ water and ethanol. The cleaned gold surfaces were then functionalized according to the following procedure. Firstly, mixed self-assembled monolayers (SAMs) were formed at room temperature by dipping overnight gold sensors in the thiol mixture: 80% HS-$(CH_2)_{11}$-EG_4-OH and 20% HS-$(CH_2)_{11}$-EG_6-Biotin (1 mM total thiol concentration in EtOH). After overnight adsorption, gold sensors were rinsed with ethanol and dried under nitrogen. The surface is then inserted in the BIAcore T200 device. All measurements

were performed at 25°C, using a running buffer (R.B.) composed of HEPES buffered saline: (HEPES buffer 10 mM, KCl 150 mM, pH 7.5 and 0.5% v/v surfactant P20. Streptavidin (100 ng/mL) was injected (10 µL/min) on the biotinylated SAM until saturation of the surface (around 2400 R.U.). The different systems **1**, **2** and **8** were injected at 2 µL/min on streptavidin-coated SAM surfaces until surface saturation.

Binding experiments were conducted by injection at 80 $\mu L.min^{-1}$ of PDS and Phend-DC3 dissolved in R.B at five different concentrations using a single cycle kinetic method (SCK). A streptavidin surface, prepared as described below, was used as reference. Curves obtained on the reference surface were deduced from the curves recorded on the recognition one, allowing elimination of refractive index changes due to buffer effects. The binding rate constants of DNA/ligands interactions were calculated by a non-linear analysis of the association and dissociation curves using the SPR kinetic evaluation software BIAcore T200 evaluation Software. The data were fitted using a heterogeneous ligand model taking into account the possible heterogeneity of the ligand presentation. The association rate constants, k_{on1} and k_{on2}, and the dissociation rate constants, k_{off1} and k_{off2} as well as the theoretical maximal response R_{max1} and R_{max2} of the two interactions were calculated. Finally, the equilibrium dissociation constants were obtained from the binding rate constants as $K_{D1} = k_{off1}/k_{on1}$ and $K_{D2} = k_{off2} / k_{on2}$ We reported the thermodynamic dissociation constants that were consistent between independent experiments and for which the theoretical maximum response (R_{max}) is consistent with 1:1 interaction. We chose to not report the parameters for the second interaction that might involve non-stoichiometric binding and/or non-specific interactions.

Analytes	Association time (s)	Dissociation time (s)	Concentration (nM)
PDS	240	300	10, 25, 50, 100, 250
PhenDC3	180	300	2, 10, 25, 50, 150

Table S2. Experimental conditions for the recognition of G4 ligand on molecule **1**, **2** and **8**

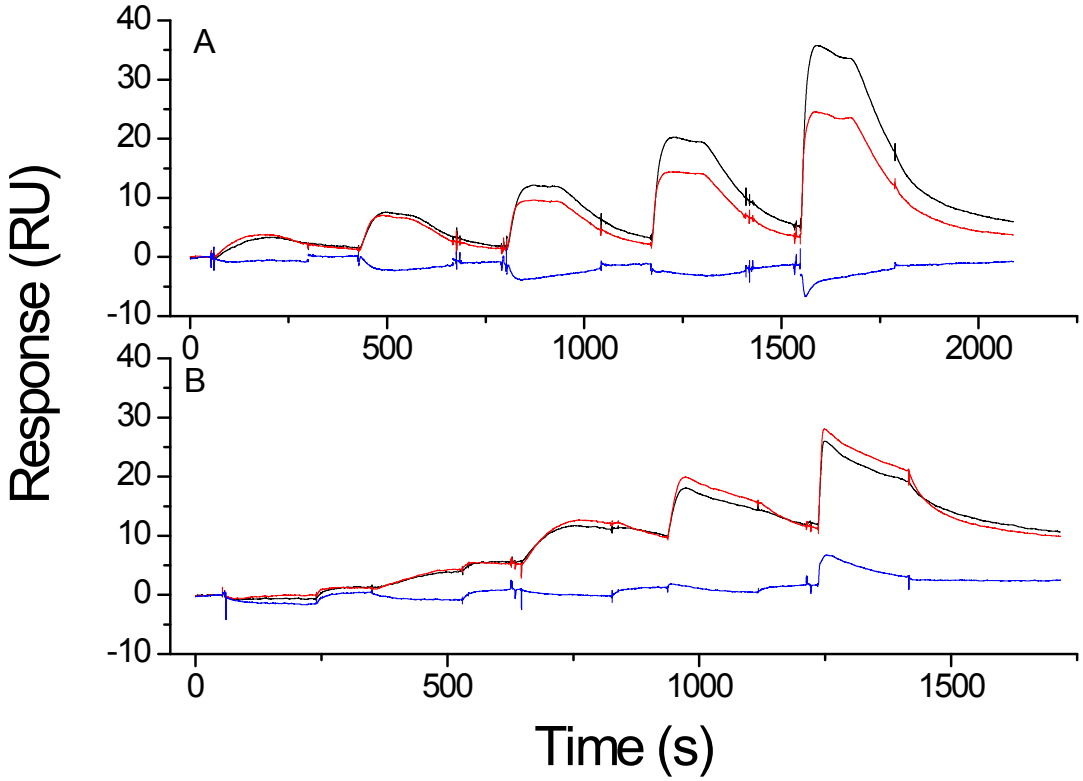


Fig. S31. SPR sensorgrams for the interaction of PDS (**A**) and PhenDC3 (**B**) with molecule **1** (black), **2** (red) and **hairpin 8** (blue).

	G3 structure **1**	G4 structure **2**	Hairpin structure **8**
PhenDC3	4.5 ± 0.5	4.4 ± 0.9	nd
PDS	21.0 ± 13.4	17.0 ± 15.3	nd

Table S3. Thermodynamic dissociation constants (K_D) for the interaction of PDS and PhenDC3 with G3 structure **1**, G4 structure **2** and hairpin structure **8**. nd: not determinable as no signals were observed in the range of concentrations.