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Supporting Information

Selective Extraction of G-quadruplex Ligands from a Rationally Designed Scaffold-Based Dynamic Combinatorial Library

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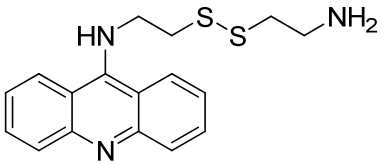
General

Commercially available chemicals and solvents were used without further purification, unless otherwise stated. Reactions were monitored by TLC (SiO_2-60, F_{254}, Merck). Purification by column chromatography was carried out using silica gel 60 (0.040-0.063 mm, Merck). NMR spectra were recorded on a Varian Gemini 2000 spectrometer in either $CDCl_3$ (Bie & Berntsen) or D_2O (Cambridge Isotope Laboratories). TMS was used as an internal standard for ^1H spectra and the residual solvent peak of $CDCl_3$ (77.16 ppm) for ^{13}C spectra. The residual solvent peak of water (4.79 ppm) was used to calibrate ^1H spectra recorded in D_2O, and 1,4-dioxane (67.19 ppm) was used as internal standard for ^{13}C spectra. MALDI-HRMS was recorded on an Ionspec 4.7 Ultima FT mass spectrometer, EI-MS on a Finnigan SSQ 710 instrument and ESI-HRMS on a Q-Star Pulsar hybrid QqTOF instrument. UV-VIS spectra were recorded on a Lambda 35 UV/VIS Spectrometer (Perkin Elmer) at 40 μM compound concentration in water. DCL building blocks were purified by HPLC (Dionex UltiMate 3000) using a Phenomenex Gemini 5u C18 150x100 mm column; 3 mL/min flow; an isocratic mixture of either 15% or 20% acetonitrile in water (0-20 min) with 0.05% TFA as modifier. Library analysis was performed using a Phenomenex Luna 3u C18 250x4.6 mm column; 0.7 mL/min flow; a gradient of 10-12% acetonitrile in water (0-240 min), then 12-16% acetonitrile in water (240-360 min) with 0.05% TFA as modifier.

Synthesis

Synthesis of scaffolds

N-(2-((2-aminoethyl)disulfanyl)ethyl)acridin-9-amine, **Aa**

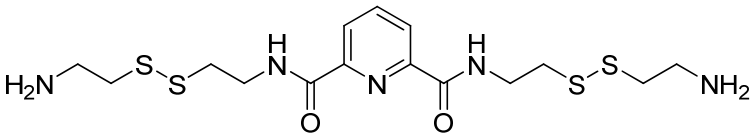


9-Chloroacridine (0.080 g, 0.37 mmol) and phenol (0.211 g, 2.25 mmol) were mixed and heated at 80 °C for 30 min. *N*-(*tert*-Butyloxycarbonyl)cystamine hydrochloride[1] (0.119 g, 0.41 mmol) and Et_3N (313 μL, 2.25 mmol) were added and the reaction mixture was stirred at 100 °C for 3 hrs, cooled to RT and the volatiles removed *in vacuo*. The residue was subjected to flash column chromatography (EtOAc/MeOH 6:1 v/v) to give the product as a yellow oil (89 mg, 55 %). ^1H NMR ($CDCl_3$, 300 MHz): δ

8.16 (d, *J* = 8.7 Hz, 2H), 8.03 (d, *J* = 9.0 Hz, 2H), 7.64 (t, *J* = 7.2 Hz, 2H), 7.36 (t, *J* = 7.2 Hz, 2H), 4.96 (br. s, 1H), 4.14 (t, *J* = 6.3 Hz, 2H), 3.41 (q, *J* = 6.0 Hz, 2H), 3.01 (t, *J* = 6.0 Hz, 2H), 2.78 (t, *J* = 6.3 Hz, 2H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 155.9, 151.7, 147.9, 130.6, 128.0, 123.6, 123.3, 117.1, 79.8, 48.5, 39.5, 38.9, 38.5, 28.5; MALDI-HRMS: calcd. for $C_{22}H_{28}N_3O_2S_2^+$ 430.1618, found 430.1608.

The yellow oil (0.067 g, 0.16 mmol) was dissolved in dry DCM (2.5 mL) and TFA (0.5 mL) was added. The reaction was stirred under argon for 40 min and concentrated to give the TFA salt of **Aa** in quantitative yield as a bright yellow thick oil, which was purified by HPLC (15% MeCN, Rt 12.5 min). ^{1}H NMR (D_2O, 300 MHz): δ 7.94 (d, *J* = 8.4 Hz, 2H), 7.77 (t, *J* = 7.2 Hz, 2H), 7.33-7.40 (m, 4H), 4.14 (t, *J* = 6.3 Hz, 2H), 3.40 (t, *J* = 6.3 Hz, 1H), 3.30 (t, *J* = 6.3 Hz, 2H), 3.07 (t, *J* = 6.3 Hz, 2H), 3.01 (t, *J* = 6.3 Hz, 1H), 2.90 (t, *J* = 6.3 Hz, 2H); ^{13}C NMR (D_2O, 75 MHz): δ 158.0, 139.5, 136.0, 125.1, 124.7, 118.9, 112.4, 47.7, 38.1, 35.9, 33.9; MALDI-HRMS: calcd. for $C_{17}H_{20}N_3S_2^+$ 330.1093, found 330.1081.

N^2,N^6-Bis(2-((2-aminoethyl)disulfanyl)ethyl)pyridine-2,6-dicarboxamide, **Ba$_2$**

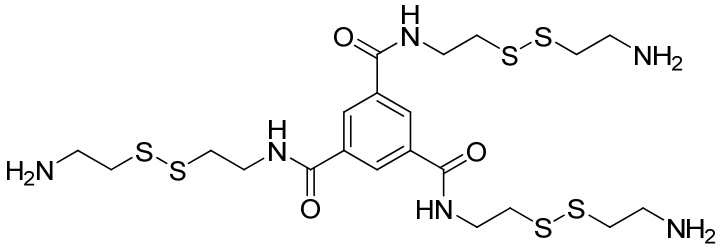


EDC hydrochloride (0.518g, 2.70 mmol), HOBt hydrate (0.365g, 2.70 mmol) and Et_3N (0.37 mL, 2.7 mmol) were added a solution of pyridine-2,6-dicarboxylic acid (0.205 g, 1.23 mmol) in dry DMF (15 mL) under argon. *N*-(tert-Butyloxycarbonyl)cystamine[1] (0.620g, 2.26 mmol) was dissolved in dry DMF (10 mL) and added the reaction mixture on an ice bath. After stirring at RT for 23 hrs the DMF was concentrated and the residue was dissolved in EtOAc (100 mL). The organic phase was sequentially washed with water (2x 50 mL), 1 M hydrochloric acid (3x 50 mL), saturated $NaHCO_3$ solution (3x 50 mL) and brine (1x 50 mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo*. Purification by dry column chromatography (5-50% EtOAc in petroleum ether) gave the desired product as white foam (0.483g, 62 %). ^{1}H NMR ($CDCl_3$): δ 8.61 (br. s, 2H), 8.35 (d, *J* = 7.8 Hz, 2H), 8.03 (t, *J* = 7.8 Hz, 1H), 5.16 (br s, 2H), 3.81 (q, *J* = 6.0 Hz, 4H), 3.48 (q, *J* = 6.0 Hz, 4H), 2.98 (t, *J* = 6.0 Hz, 4H), 2.85 (t, *J* = 6.0 Hz, 4H), 1.43(s, 18H); ^{13}C NMR ($CDCl_3$): δ 164.0, 156.3, 148.8, 139.1, 125.1, 79.9, 39.5, 38.8, 38.6, 38.3, 28.5; MALDI-HRMS: calcd. for $C_{25}H_{41}N_5NaO_6S_4^+$ 658.1832, found 658.1810.

The Boc-protected target compound (0.256 g, 0.40 mmol) was deprotected as described for **Aa** to give the TFA salt of **Ba$_3$** in quantitative yield as white foam, which was purified by HPLC (20% MeCN, Rt 6.6 min). ^{1}H NMR (D_2O): δ 8.20-8.10 (m, 3H), 3.79 (t, *J* = 6.3 Hz, 4H), 3.36 (t, *J* = 6.3 Hz, 4H), 2.98-

3.05 (2x t, J = 6.3 Hz, 8H); ^{13}C NMR (D_2O): δ 166.3, 148.4, 140.5, 125.6, 38.9, 38.4, 37.0, 34.3; MALDI-HRMS: calcd. for $C_{15}H_{25}N_5NaO_2S_4^+$: 458.0783, found 458.0780.

N^1,N^3,N^5-Tris(2-((2-aminoethyl)disulfanyl)ethyl)benzene-1,3,5-tricarboxamide, **Ca$_3$**

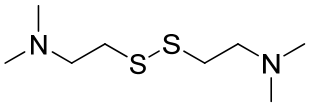


EDC hydrochloride (0.810g, 4.23 mmol), HOBt hydrate (0.571g, 4.23 mmol) and Et_3N (0.59 mL, 4.23 mmol) were added a solution of 1,3,5-benzenetricarboxylic acid (0.269 g, 1.28 mmol) in dry DMF (20 mL) under argon. *N*-(tert-Butyloxycarbonyl)cystamine[1] (0.975g, 3.84 mmol) was dissolved in dry DMF (10 mL) and added the reaction mixture on an ice bath. After stirring at RT for 24 hrs the DMF was evaporated and the residue was dissolved in EtOAc (100 mL). The organic phase was sequentially washed with water (2x 50 mL), 1 M hydrochloric acid (3x 50 mL), saturated $NaHCO_3$ solution (3x 50 mL) and brine (1x 50 mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo*. Purification by dry column chromatography (5-50% EtOAc in petroleum ether) gave the desired product as white foam (0.537g, 46 %). ^{1}H NMR ($CDCl_3$): δ 8.30 (br. s, 3H), 7.55 (br. s, 3H), 5.21 (br. s, 3H), 3.77 (q, J = 6.0 Hz, 6H), 3.46 (q, J = 6.0 Hz, 6H), 2.96 (t, J = 6.2 Hz, 6H), 2.84 (t, J = 6.5 Hz, 6H), 1.39 (s, 27H); ^{13}C NMR ($CDCl_3$): δ 166.6, 156.2, 135.3, 128.7, 79.8, 39.7, 39.4, 38.3, 38.1, 28.5; MALDI-HRMS: calcd. for $C_{36}H_{60}N_6NaO_9S_6^+$: 935.2638, found 935.2603.

The Boc-protected target compound (0.311g, 0.34 mmol) was deprotected as described for **Aa** to give the TFA salt of **Ca$_3$** in quantitative yield as white foam, which was purified by HPLC (20% MeCN, Rt 4.6 min). ^{1}H NMR (D_2O): δ 8.27 (s, 3H), 3.79 (t, J = 6.3 Hz, 6H), 3.37 (t, J = 6.3 Hz, 6H), 3.03 (t, J = 6.6 Hz, 12H); ^{13}C NMR (D_2O): δ 169.4, 135.5, 129.6, 39.4, 38.4, 36.8, 34.3; MALDI-HRMS: calcd. for $C_{21}H_{36}N_6NaO_3S_6^+$: 635.1065, found 635.1065.

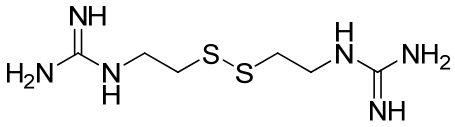
Synthesis of side chains

N,*N*,*N*´,*N*´-Tetramethylcystamine, **b_2**



1,2-Dimethylamino-ethanethiol hydrochloride (5.000 g, 35.3 mmol) was dissolved in MeOH (100 mL) and titrated with 0.5 M iodine in MeOH until a yellow color persisted. The reaction was quenched with 10 % $NaHSO_3$ solution (30 mL) to a colorless solution. MeOH was removed in vacuo and the remaining brownish water solution was added additional water (50 mL) and 2 M NaOH (aq.) until pH > 10. The solution was continuously extracted from the water phase overnight with Et_2O. The extract was concentrated to pink oil (3.33 g, 16 mmol), which was afterwards dissolved in dry Et_2O (20 mL) and added 2.0 M HCl solution in Et_2O (16 mL, ~32 mmol). This resulted in a white precipitate which was filtered and recrystallised from abs. EtOH to afford the disulphide as white crystals (3.942g, 80 %). ^{1}H NMR (D_2O, 300 MHz): δ 3.58 (t, *J* = 6.9 Hz, 4H), 3.14 (t, *J* = 6.9 Hz, 4H), 2.97 (s, 12 H); ^{13}C NMR (D_2O, 75 MHz): δ 56.2, 43.5, 31.2; EI MS *m/z*: 208 (100 %, M^+).

1,1'-(2,2'-Disulfanediylbis(ethane-2,1-diyl))diguanidine, **c_2**



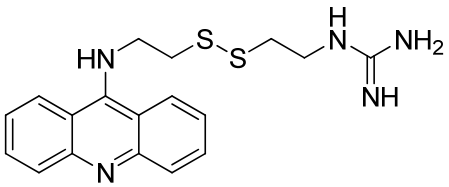
Cystamine dihydrochloride (0.040 g, 0.18 mmol) and *N*,*N*'-di-Boc-*N*''-trifluoromethanesulfonyl-guanidine[2] (0.140 g, 0.36 mmol) were suspended in dry DCM (3 mL) under argon. Et_3N (154 μL, 1.11 mmol) was added and the mixture was stirred for 10 hrs at RT, during which the white solids dissolved. DCM (15 mL) was added and the organic phase was washed with 2 M $NaHSO_4$ solution (10 mL), saturated $NaHCO_3$ solution (10 mL), brine (15 mL) and dried ($MgSO_4$). After concentration *in vacuo* the crude product was purified by flash column chromatography (Initially DCM, then EtOAc/PE 1:1 v/v) giving the desired tetra-Boc-bisguanidine compound as white solid (0.103 g, 91 %). ^{1}H NMR ($CDCl_3$, 300 MHz): 11.47 (s, 2H), 8.62 (t, *J* = 5.4 Hz, 2H), 3.77 (q, *J* = 6.3 Hz, 4H), 2.88 (t, *J* = 6.3 Hz, 4H), 1.50 (s, 36H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 163.7, 156.3, 153.3, 83.4, 79.5, 39.3, 37.2, 28.4, 28.2.

The tetra-Boc-bisguanidine compound (0.072 g, 0.11 mmol) was dissolved in dry DCM (1.5 mL) under argon and TFA (300 μL) was added. The mixture was stirred for 1 h at RT and concentrated to give

the TFA salt of **c_2** as white solid in quantitative yield. ^{1}H NMR (D_2O, 300 MHz): δ 3.54 (t, *J* = 6.2 Hz, 4H), 2.91 (t, *J* = 6.3 Hz, 4H); ^{13}C NMR (D_2O, 75 MHz): δ 163.9, 41.0, 37.2; MALDI-HRMS: calcd. for $C_6H_{16}N_6NaS_2^+$: 259.0770, found 259.0769.

Synthesis of the extracted compound

1-(2-((2-(Acridin-9-ylamino)ethyl)disulfanyl)ethyl)guanidine, **Ac**



The TFA salt of **Aa** (0.061 g, 0.11 mmol) and *N*,*N*'-Di-Boc-*N*''-trifluoromethanesulfonyl-guanidine (0.041 g, 0.11 mmol) were dissolved in dry DCM (1 mL) under argon and Et_3N (30 μL, 0.22 mmol) was added. The mixture was stirred for 2 hrs at RT and then washed with 2 M $NaHSO_4$ solution (2x 2 mL), saturated $NaHCO_3$ solution (2x 2 mL) and brine (2 mL). The organic phase was concentrated and purified by flash column chromatography (EtOAc/MeOH 8:1 v/v) to give yellow oil (0.037 g, 65%). ^{1}H NMR ($CDCl_3$, 300 MHz): 11.48 (s, 1H), 8.63 (s, 1H), 8.15 (m, 2H), 8.05 (m, 2H), 7.67 (m, 2H), 7.39 (m, 2H), 7.25 (br s, 1H), 4.13 (m, 2H), 3.77 (m, 2H), 2.99 (m, 2H), 2.90 (m, 2H), 1.48, 1.47 (2x s, 18H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 163.6, 156.4, 153.4, 151.2, 130.3, 129.0, 123.7, 123.1, 117.7, 83.5, 79.6, 48.5, 39.4, 39.1, 37.5, 28.4, 28.2.

The yellow oil (0.0127 g, 0.002 mmol) was dissolved in dry DCM (0.3 mL) under argon and TFA (0.3 mL) was added. The mixture was stirred for 2 hrs at RT and concentrated to give the TFA salt of **Ac** as a yellow semi-solid in quantitative yield. The crude product was purified by HPLC (20% MeCN, Rt 11.0 min). ^{1}H NMR (D_2O, 300 MHz): 8.01 (d, *J* = 8.7 Hz, 2H), 7.80 (t, *J* = 6.9 Hz, 2H), 7.43-7.38 (m, 4H), 4.19 (t, *J* = 6.3 Hz, 2H), 3.36 (t, *J* = 6.3 Hz, 2H), 3.09 (t, *J* = 6.3 Hz, 2H), 2.71 (t, *J* = 6.3 Hz, 2H); ^{13}C NMR (D_2O, 75 MHz): δ 163.7, 157.9, 139.5, 136.0, 125.1, 124.7, 118.9, 115.0, 47.8, 40.3, 36.7, 36.4; MALDI-HRMS: calcd. for $C_{18}H_{22}N_5S_2^+$: 372.1311, found 372.1306.

Procedures for DCC experiments

Oligonucleotides

Oligonucleotides were synthesized by Sigma-Genosys (0.2 μmol scale) and received HPLC purified. All concentrations are expressed in strand molarity, using the nearest-neighbor approximation for the absorption coefficients of the unfolded species at 260 nm.[3] Two 5′-biotinylated oligonucleotides were used in the studies described: the human telomeric 5′-(biotin-d[$(GT_2AG_2)_5$])-3′, and a random coil DNA control strand 5′-(biotin-d[$(AGT_2AG)_5$])-3′. The oligonucleotides were annealed in DCL buffer (150 mM KCl, 300 mM Tris-HCl, pH 7.4) by heating to 95 °C for 5 min and then slowly cooling to room temperature. After overnight incubation at 4 °C, the stock solutions were stored at -20 °C.

DCL studies

All solutions were prepared from degassed double distilled water. Stock solutions containing 1 mM of each scaffold component (**Aa**, $\mathbf{Ba_2}$ or $\mathbf{Ca_3}$), 100 mM of each side chain component ($\mathbf{a_2}$, $\mathbf{b_2}$ or $\mathbf{c_2}$) and of initiator **a**, and 50 mM of the alternative initiator dithiothreitol (DTT) were prepared in DCL buffer (150 mM KCl, 300 mM Tris-HCl, pH 7.4).

Scaffold stock solutions of **Aa**, (2.5 μL, 1 mM, 2.5 nmol), $\mathbf{Ba_2}$ (5.0 μL, 1 mM, 5.0 nmol) and $\mathbf{Ca_3}$ (8.3 μL, 1 mM, 8.3 nmol), side chain stock solutions of $\mathbf{a_2}$ (3.85 μL, 100 mM, 385 nmol), $\mathbf{b_2}$ (5 μL, 100 mM, 500 nmol) and $\mathbf{c_2}$ (5 μL, 100 mM, 500 nmol) and DCL buffer (18.35 μL) were mixed in an HPLC vial under a blanket of argon, and disulfide scrambling was initiated by addition of **a** (2 μL, 100 mM, 200 nmol) or DTT (2 μL, 50 mM, 100 nmol) to give a total library volume of 50 μL. In templated DCL studies, a part of the DCL buffer solution was substituted with biotinylated G-quadruplex folded or random coil DNA stock solution to a final DNA concentration of 50 μM (2.5 nmol). The mixture was shaken gently in the capped HPLC vial. The DCL mixture was incubated for typically 3 hours, at which point the disulfide exchange was stopped by the addition of 10 μL of 1.7% aqueous TFA. The library solutions were analyzed by HPLC, and the individual peaks were analyzed by ESI-HRMS.

The solution was added to 2 mg of magnetic streptavidin beads (Aureon biosystems, Streptavidin A-beads™) and incubated at room temperature for 20 min. The beads were separated from solution with a magnet and washed with DCL buffer (3x 100 μL, pH 2). The washings were combined with the remaining library solution and analyzed by HPLC. The DNA was subsequently denatured by incubating

the beads with 0.05% TFA in water at 85 °C for 10 minutes (3x 200 µL), and the combined solutions from DNA denaturing were separately analyzed by HPLC.

Confirming conservation of G-quadruplex folding in presence of the DCL

Libraries containing biotinylated DNA were diluted with buffer (65 µL, pH 2) to give a 20 µM DNA solution, and corresponding DNA solutions with identical salt concentrations but without the DCL were prepared. CD spectra were recorded in a 1 mm quartz cuvette (J-815 CD-Spectrometer, JASCO, Figure S1).

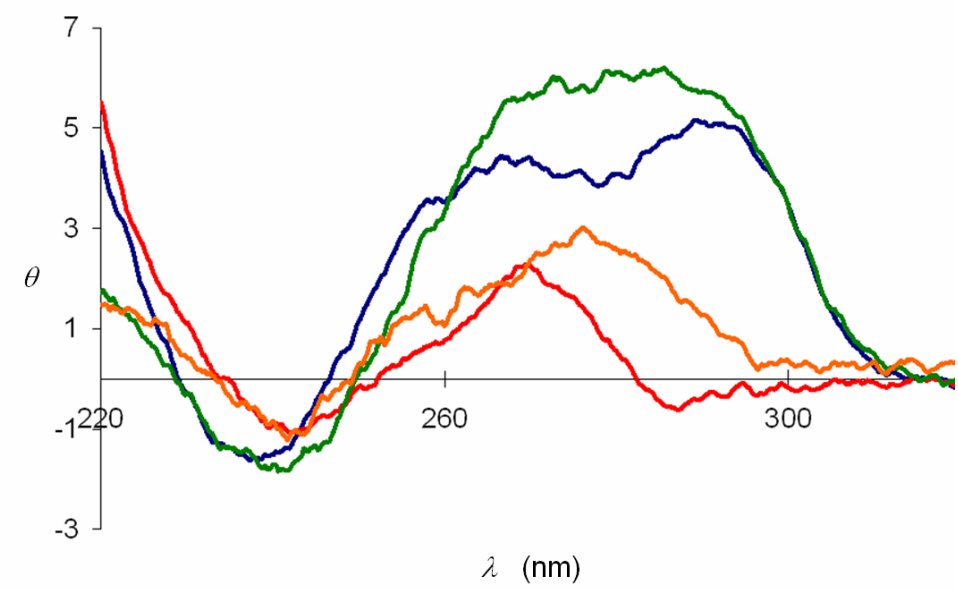


Figure S1. CD spectra of: 20 µM 5´-biotin-d[(GTTAGG)$_5$] (blue: G-quadruplex; green: G-quadruplex + DCL mixture) and 20 µM 5´-biotin-d[(AGTTAG)$_5$] (red: ssDNA; orange: ssDNA + DCL mixture) in DCL buffer.

Assessing thermodynamic equilibrium

Thermodynamic equilibrium is a requirement of regeneration of target-bound compounds in the mixture, and although it is not a requirement for the extraction procedure described here, it is still expected to have a beneficial influence on the outcome and to be important for the reproducibility of the library.[4]

With the concentration of free thiol used here (4 mM), the mixture consistently reached a perfectly reproducible distribution of library members within 3 hours. Comparing a representative cysteamine initiated library with a DTT initiated library revealed that all peak integrals matched within 0.5%. The presence of free thiols in the mixture at equilibrium was ensured by Ellman's test.[5]

Library analysis

Table S1. UV-VIS absorbances for initial compounds relative to 245 nm.

	245 nm	254 nm	285 nm	420 nm
Aa	100	217	37	58
Ba_2	100	81	43	--
Ca_3	100	65	7	--

Table S2. Analysis of library mixture incubated without DNA.

Peak	Peak integrals [a]				Peak absorption ratios [b]					% library composition [c]				*m/z*		Formula	Cmpd.
#	245 nm	254 nm	285 nm	420 nm	245 nm	254 nm	285 nm	420 nm	Scaffold	245 nm	254 nm	285 nm	Average	Calcd.	Found		
1	4,1750	2,6680	0,3308		100	64	8	--	**C**	4,4	4,5	4,4	**4,4**	635,1065	635,1135	$C_{21}H_{36}N_6NaO_3S_6^+$	**Ca_3**
2	3,3648	2,7202	1,5457		100	81	46	--	**B**	4,2	4,3	4,1	**4,2**	458,0783	458,0783	$C_{15}H_{25}N_5NaO_2S_4^+$	**Ba_2**
3	4,6287	2,9969	0,3760		100	65	8	--	**C**	4,9	5,0	5,0	**5,0**	641,1559	641,1565	$C_{23}H_{41}N_6O_3S_6^+$	**Ca_2b**
4	7,2687	5,8577	3,2720		100	81	45	--	**B**	9,1	9,3	8,6	**9,0**	464,1277	464,1295	$C_{17}H_{30}N_5O_2S_4^+$	**Bab**
5	6,8821	15,1457	2,6192	4,2438	100	220	38	62	**A**	6,6	6,9	6,2	**6,6**	330,1093	330,1069	$C_{17}H_{20}N_3S_2^+$	**Aa**
6	3,8260	2,3954	0,3060		100	63	8	--	**C**	4,0	4,0	4,1	**4,1**	669,1872	669,1843	$C_{25}H_{45}N_6O_3S_6^+$	**Cab_2**
7	1,5515	1,0045	0,1153		100	65	7	--	**C**	1,6	1,7	1,5	**1,6**	655,1464	655,1458	$C_{22}H_{39}N_8O_3S_6^+$	**Ca_2c**
8	11,3723	19,3309	4,5514	4,3851	100	170	40	39	**A+B**	10,9	8,9	10,7	**10,2**				
									A					492,1590	492,1593	$C_{19}H_{34}N_5O_2S_4^+$	**Ab**
									B					358,1406	358,1395	$C_{19}H_{24}N_3S_2^+$	**Bb_2**
9	8,3955	5,3715	0,6881		100	64	8	--	**C**	8,9	9,0	9,2	**9,0**	683,1776	683,1765	$C_{24}H_{43}N_8O_3S_6^+$	**Cabc**
10	7,0662	5,5733	3,1541		100	79	45	--	**B**	8,8	8,9	8,3	**8,7**	478,1181	478,1179	$C_{16}H_{28}N_7O_2S_4^+$	**Bac**
11	4,4474	2,8187	0,3954		100	63	9	--	**C**	4,7	4,7	5,3	**4,9**	697,2185	697,2195	$C_{27}H_{49}N_6O_3S_6^+$	**Cb_2**
12	8,6172	18,8118	3,4217	5,3004	100	218	40	62	**A**	8,3	8,6	8,0	**8,3**	372,1311	372,1333	$C_{18}H_{22}N_5S_2^+$	**Ac**
13	7,5496	5,9556	3,5043		100	79	46	--	**B**	9,4	9,5	9,2	**9,4**	506,1495	506,1493	$C_{18}H_{32}N_7O_2S_4^+$	**Bbc**
14	3,6530	2,3579	0,2790		100	65	8	--	**C**	3,9	4,0	3,7	**3,9**	697,1682	697,1675	$C_{23}H_{41}N_{10}O_3S_6^+$	**Cac_2**
15	4,2295	2,9060	0,3814		100	69	9	--	**C**	4,5	4,9	5,1	**4,8**	725,1995	725,1976	$C_{25}H_{45}N_{10}O_3S_6^+$	**Cbc_2**
16	3,6024	2,8949	1,6866		100	80	47	--	**B**	4,5	4,6	4,4	**4,5**	543,1292	543,1315	$C_{17}H_{30}N_9O_2S_4^+$	**Bc_3**
17	1,1849	0,7798	0,1411		100	66	12	--	**C**	1,3	1,3	1,9	**1,5**	370,0986	370,0941	$C_{24}H_{44}N_{12}O_3S_2^{2+}$	**Cc_3**

[a] Crude integrals from HPLC chromatogram.
[b] Absorption ratios relative to the 245 nm absorption.
[c] Calculated from peak integral corrected for relative absorption (see table inserted in Figure 2a).

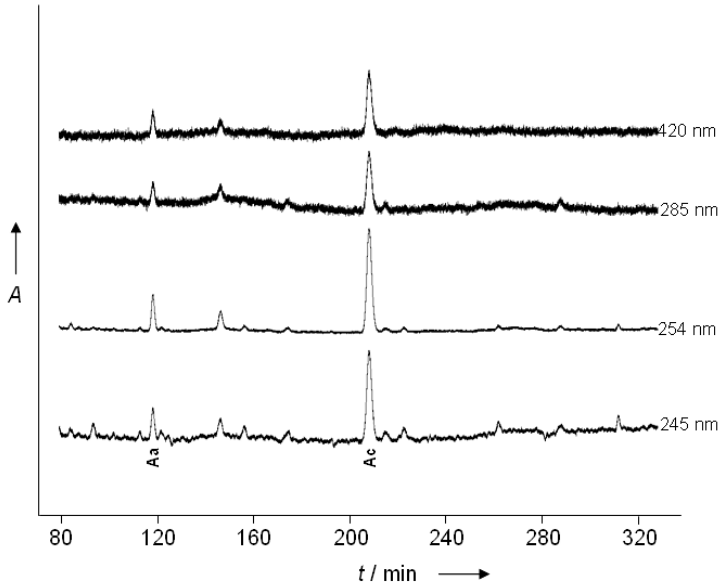


Figure S2. All monitored wavelengths of the HPLC chromatogram from the supernatant after denaturation of G-quadruplex 5′-(biotin-d[$(GT_2AG_2)_5$])-3′.

Evaluation of compounds

Circular Dichroism

Samples were prepared to provide a Tris-HCl buffer (10 mM KCl, 10 mM Tris-HCl, pH 7.4) and a 2 μM final concentration of oligonucleotide 5′-d[$(G_3(T_2A_3G_3)_3]$-3′ in double distilled water. Test compounds were added from aqueous stock solutions to give a final concentration of 10 μM. All samples were annealed by heating to 95 °C for 10 min and slowly cooled to room temperature. CD spectra were recorded on a J-815 CD-Spectrometer (JASCO) using a quartz cell of 5 mm optical path length and 100 nm/min scanning speed with a response time of 1 second in the wavelength ranges 220-320 nm with a data pitch of 0.1 nm. The final CD spectra are results of five averaged scans at 25 °C. Data were corrected for signal contributions due to the buffer. T_m experiments were performed with a heating rate of 0.2 °C/min at a 295 nm wavelength with a data pitch of 0.1 °C in the temperature interval 25-95 °C. T_m curves were afterwards smoothened using a moving average method and normalized for comparison of data.

Surface Plasmon Resonance

Surface plasmon resonance (SPR) measurements were performed with a four-channel BIAcore 3000 optical biosensor system (Biacore Inc.) equipped with a streptavidin-coated sensor chip. The oligomer 5'-biotin-d[$T_7(T_2AG_3)_4$]-3' was heated at 95 °C for 5 min and annealed by slow cooling to form quadruplex in filtered and degassed buffer 20 mM Tris-HCl, 200 mM KCl, pH 7.4. The quadruplex was then immobilized (~400 RU) on flow cell 3. Similarly, 5'-biotin-d[$(AGT_2AG)_5$]-3' was immobilized (~400 RU) on flow cell 2, leaving flow cell 1 and 4 as blank. Data from the blank flow cells were subtracted from the sample flow cells (2 and 3) to remove bulk responses caused by different refractive indexes of sample and running buffer. The baselines were adjusted to zero on the y (SPR response) axis and aligned to the injection time on the x (time) axis.

DNA binding experiments were carried out in running buffer (filtered and degassed 20 mM KH_2PO_4, 150 mM KCl, 0.005 % surfactant P20, 0.5 mM EDTA, pH 7.4) at a flow rate of 20 μl/min. Ligand solutions at different concentrations (4, 12, 37, 111, 333 and 1000 nM) were prepared in degassed running buffer by serial dilutions from stock solution. These solutions were injected (80 μL/min for 120 s) in random series to avoid any systematic error. Chip regeneration was performed using 1 M KCl. Data was analyzed using BIAevaluation 4.0.1. The curves were fitted to a 1:1 model (Figure S3).

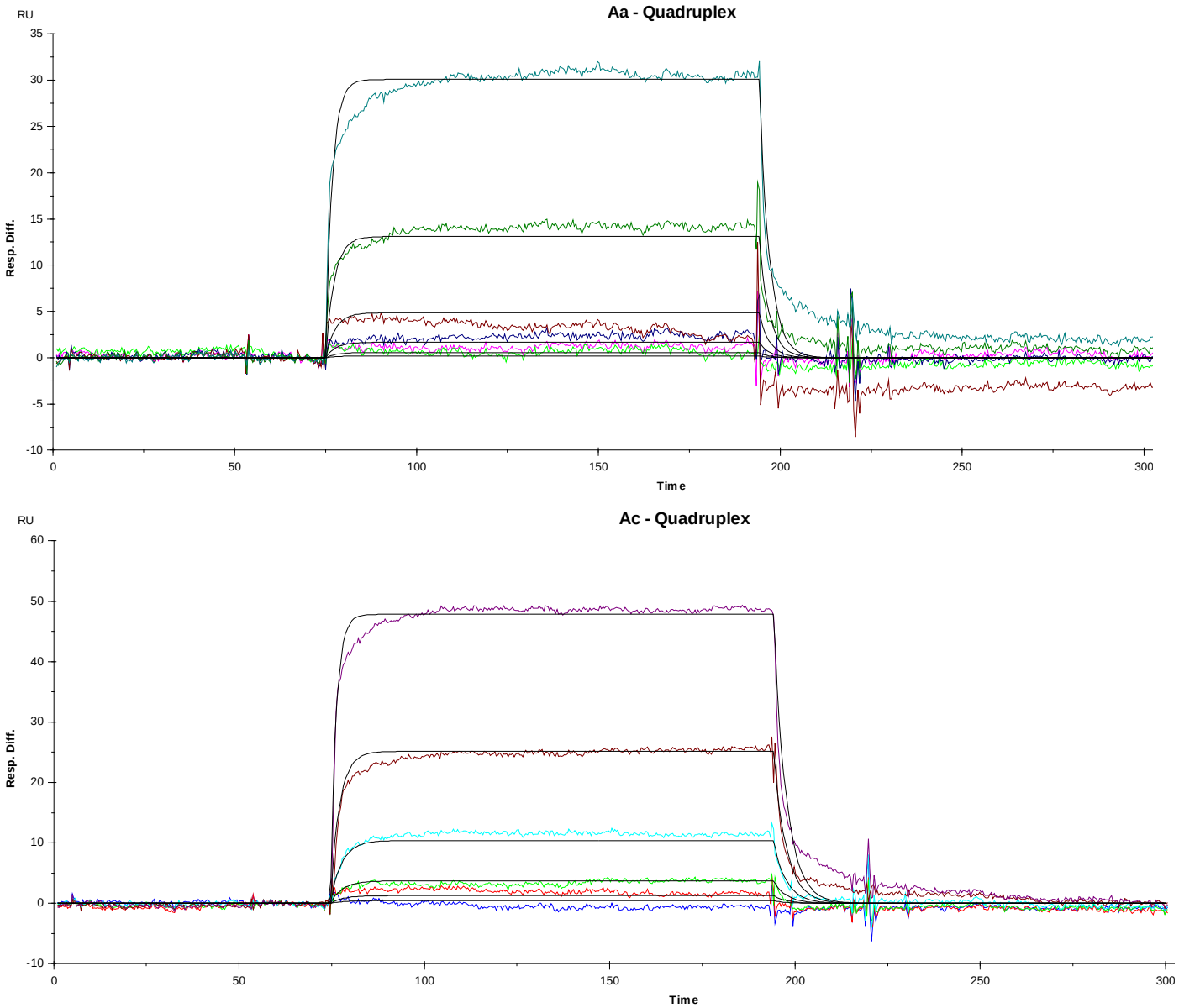


Figure S3. The x-axis represents time (s) and the y-axis the observed response (RU). Sensorgrams are shown for the binding of ligands **Aa** (top) and **Ac** (bottom) to 5'-biotin-d[$T_7(T_2AG_3)_4$]-3'.

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