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Design, synthesis and evaluation of 4,7-diamino-1,10-phenanthroline G-quadruplex ligands

Mads Corvinius Nielsen [a], Jonas Borch [b], Trond Ulven [a,*]

[a] Department of Physics and Chemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark
[b] Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark

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ABSTRACT

A series of 4,7-diamino-1,10-phenanthroline derivatives carrying positively charged side chains has been synthesized, and their G-quadruplex interaction evaluated by circular dichroism (CD) and surface plasmon resonance (SPR). In absence of side chains, 4,7-diamino-1,10-phenanthroline exhibits a weak but significant G-quadruplex stabilizing effect, compared to no stabilization by 1,10-phenanthroline. We hypothesize that this effect is due to increased basicity of the phenanthroline nitrogens and protonation or ion chelation to form a central positive charge which stack on the G-tetrad above the central ionic column. Introduction of positively charged side chains results in compounds with appreciable G-quadruplex stabilizing properties and high aqueous solubility, with the longer side chains giving more potent compounds. Ligands carrying guanidine side chains in general show higher quadruplex stabilizing activity and distinctly slower kinetic properties than their amino and dimethylamino analogues, possibly due to specific hydrogen bond interactions with the G-quadruplex loops.

1. Introduction

Nucleic acid sequences containing multiple close segments of three or more consecutive guanosine residues have the propensity to form secondary structures known as G-quadruplexes and consisting of stacked layers of guanine tetrads.[1,2] Such motifs are found throughout the human genome and are currently receiving attention as potential anticancer targets.[3–5] The telomeric regions at the chromosome terminals play a critical role in the regulation of cellular proliferation, and are among the most studied quadruplex-forming sequences.[6] Stabilization of G-quadruplex structures in the 5′-d[TTAGGG]$_n$-3′ repeat of the telomeric single-stranded 3′-overhang inhibits the activity of telomerase, an enzyme not found in most normal somatic cells, but present in 85–90% of cancer cells and contributing to their immortality. Ligands capable of G-quadruplex stabilization have indeed exhibited anticancer activity, although some observations, for example, activity against ALT cancer cell lines devoid of telomerase, indicate that telomerase inhibition may not be the only mechanism of this activity.[7] G-quadruplex prone motifs are also enriched in gene promoter regions,[8,9] including promoter regions of proto-oncogenes such as *c-Myc*, *c-kit*, *Bcl-2*, *VEGF*, and *RET*.[10,11] Stabilization of such G-quadruplex structures can potentially inhibit the expression of oncogenes and disrupt development of cancer.

The recent interest in G-quadruplex structures as potential anticancer drug targets has resulted in the identification of a large number of quadruplex stabilizing compounds.[3,4] These compounds frequently consist of a central aromatic structure substituted with positively charged side chains, generally presumed to interact by G-tetrad stacking and with backbone phosphate groups, respectively. The four guanines of the G-tetrad interact by Hoogsteen base pairing, and the dipolar moments directed towards the center of the tetrad by the guanines are neutralized by central potassium ions or other cations localized between the stacked G-tetrads, with each ion coordinated to eight carbonyl groups.[1,2] Some crystal structures, like those with a stabilizing diagonal loop, also show ions in the loop regions above and below the outer tetrads, whereas this ion is not visible in other structures.[12–14] In fact, several potent quadruplex ligands, like RHPS4,[15,16] berberine derivatives,[17–20] ethidium derivatives,[21] and papaverine derivatives,[22–24] are based on aromatic scaffolds with a permanent positive central charge which can serve the same function as the central metal ions. Other ligands, for example, the potent quadruplex ligand BRACO19, have a central basic site which is protonated at physiological pH. The crystal structure of BRACO19 in complex with a telomeric G-quadruplex shows that a hydronium ion hydrogen bound to the acridine-N indeed is placed directly above the central column of potassium ions.[25] Thus, central positive charges on the outside of the G-tetrad stack appear to be favored but not required, and ligands carrying such a charge may have an advantage over ligands based on a neutral aromatic scaffold.

* Corresponding author. Tel.: +45 6550 2568; fax: +45 6615 8780.
E-mail address: ulven@ifk.sdu.dk (T. Ulven).

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Several structures containing 1,10-phenanthrolines have been investigated as G-quadruplex ligands, including the highly potent bisquinolinium amides,[26] a neomycin-capped structure,[27,28] and complexes with platinum,[29,30] ruthenium and other transition metals.[31–33] A common feature observed for most of the compounds is the 2,9-substitutional pattern around the central phenanthroline scaffold. The only 4,7-disubstituted 1,10-phenanthroline evaluated as G-quadruplex ligand is MOP1,[27,28] which showed very limited G-quadruplex stabilizing abilities in a FRET quadruplex melting assay despite four positive charges.[27,28]

The extended aromatic structure of a 1,10-phenanthroline scaffold provides a basis for π–π stacking interactions with terminal G-tetrads. Phenanthroline is known as a powerful metal ion chelator, and evidence indicates that it is also capable of interaction with potassium ions despite their large ionic radii.[34–37] We hypothesized that introduction of 4,7-diamine substituents would significantly increase the basicity of the phenanthroline and thereby increase the quadruplex stabilizing properties either by protonation or by favoring chelation with a central ion. Herein we demonstrate that 4,7-diaminophenanthroline carrying positively charged side chains is indeed sufficient for significant stabilization of telomeric G-quadruplex structures.

2. Results and discussion

The unsubstituted 4,7-diamino-1,10-phenanthroline (**2**) and the side chain substituted analogues **3a**–**c** and **4a**–**c** were synthesized from 4,7-dichlorophenanthroline (**1**)[38] by microwave assisted nucleophilic aromatic substitution with excess primary amines or aqueous ammonia (Scheme 1). The guanidine analogues **5a**–**c** were synthesized from the corresponding primary amines **3a**–**c** in quantitative yields by reactions with 1*H*-pyrazole-1-carboxamidine.

Circular dichroism (CD) is a well-established method for determining the presence and to some degree the folding of G-quadruplex structures, with a positive band near 270 nm together with a negative around 240 nm indicating a parallel quadruplex form, and a positive band near 295 nm together with a negative around 265 nm indicating an anti-parallel quadruplex form.[39,40] The compounds were evaluated in a buffer containing 10 mM potassium chloride, in which the quadruplex adopts a mixed parallel/anti-parallel conformation in the absence of ligands. CD spectra of the telomeric G-quadruplex together with ligands showed that all compounds induce the same equilibrium structure (Fig. 1A, data only shown for phenanthroline, **2** and **5c**). The positive bands near 295 and 268 nm are enhanced and a negative band appears at 255 nm together with a small shoulder around 245 nm. Similar spectra were also observed with higher concentrations of phenanthroline, 4,7-diamino-1,10-phenanthroline (**2**) and 4,7-dimethyl-1,10-phenanthroline, despite the very low activity of these compounds. Since phenanthroline absorbs highly in this region of the spectrum, the new bands are more likely a consequence from induced CD in the phenanthroline scaffold by the chiral environment than from changes in quadruplex structure. Preliminary investigation indicate that the compounds also stabilize duplex DNA, as the representative compounds **3c** and **5c** at 20 μM concentrations stabilized the melting temperature of the duplex sequence 5′-d[$(AT)_{10}$]-3′ from 41 °C to 57 and 63 °C, respectively, as measured by CD at 265 nm. Thus, further optimization is required to produce selective G-quadruplex stabilizers.

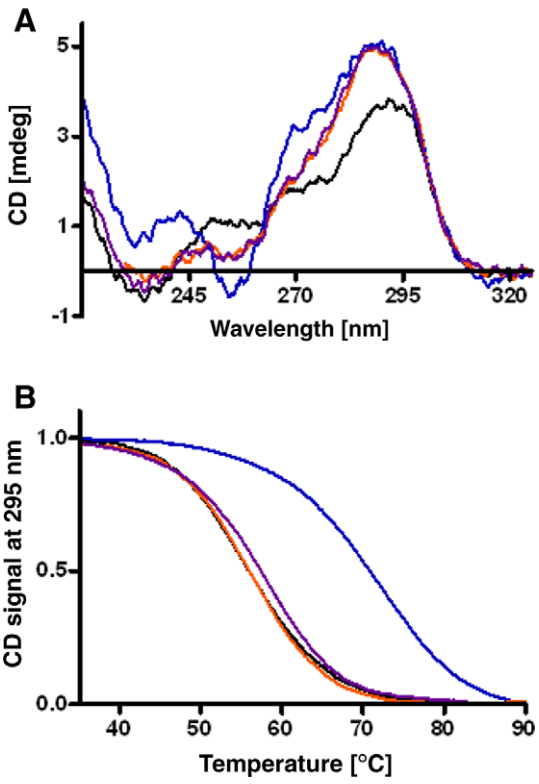
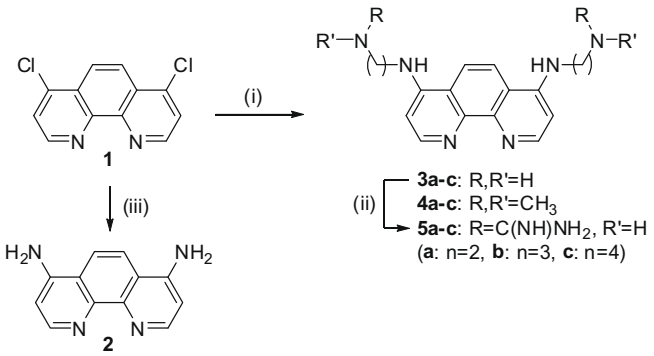


Figure 1. CD spectra (A) and thermal denaturation curves from a CD signal at 295 nm (B), with 2 μM 5′-d[$(G_3T_2A)_3G_3$]-3′ quadruplex concentration without ligand (—) and in presence of 20 μM 1,10-phenanthroline (—), 20 μM **2** (—), or 10 μM **5c** (—).

Thermal denaturation studies were carried out monitoring the CD signal at 295 nm (Table 1 and Fig. 1B). 4,7-Diamino-1,10-phenanthroline (**2**) displays a weak stabilizing effect at 20 μM concentration, whereas unsubstituted 1,10-phenanthroline (Phe) and 4,7-dimethyl-1,10-phenanthroline (4,7-diMePhe) show no stabilizing effect. This supports the hypothesis that the 4,7-diamino substituents alone have a favorable influence on the quadruplex stabilizing effect of the scaffold. Introduction of positively charged side chains on this scaffold results in compounds with significant quadruplex stabilizing effects and high aqueous solubility. Increasing side chain length from two to four methylene groups is paralleled by an increasing stabilizing effect. The guanidine ligands (**5a**–**c**) stabilize the G-quadruplex more effectively than their amino (**3a**–**c**) and dimethylamino (**4a**–**c**) analogues, a tendency that is most pronounced at the lowest ligand concentrations. The small increase in thermal stabilization between **5b** and **5c** indicate that not much can be gained by extending the guanidine side chains further.

The kinetics of the ligand—quadruplex interactions were studied by surface plasmon resonance (SPR) using biotinylated quadruplex-folded DNA attached to a streptavidin-coated sensor chip. In agreement with the CD denaturation studies, a tendency towards



Scheme 1. Reagents and conditions: (i) Amine, EtOH, 165 °C (μν), 16 h (54–87%); (ii) 1*H*-pyrazole-1-carboxamidine hydrochloride, DIPEA, DMF, 60 °C, overnight (100%); (iii) NH_3 (concd), 120 °C, 1 h (98%).

Table 1
CD melting temperatures and dissociation constants (K_D) on-rates (k_a) and off-rates (k_d) determined by SPR

Compound	ΔT_m [°C][a]			SPR data		
	5 μM	10 μM	20 μM	K_D (μM)	k_a [10^5 M^{-1} s^{-1}]	k_d (s^{-1})
Phe[b]	—	—	0.0	—	—	—
4,7-diMePhe[c]	—	—	0.1	—	—	—
2	—	—	1.3	—	—	—
3a	2.0	7.6	9.9	0.72 ± 0.21	3.30 ± 0.51	0.23 ± 0.03
3b	2.4	10.6	13.1	0.45 ± 0.01	4.08 ± 0.32	0.19 ± 0.02
3c	2.5	14.6	15.3	0.23 ± 0.02	4.28 ± 0.66	0.10 ± 0.01
4a	1.8	8.8	10.5	0.82 ± 0.06	3.44 ± 0.32	0.28 ± 0.05
4b	1.9	10.6	13.3	0.30 ± 0.05	5.10 ± 0.53	0.15 ± 0.01
4c	2.3	13.0	14.4	0.33 ± 0.12	5.15 ± 0.76	0.21 ± 0.03
5a	3.0	9.9	13.7	0.40 ± 0.03	1.18 ± 0.42	0.04 ± 0.01
5b	6.0	14.2	16.6	0.41 ± 0.04	0.94 ± 0.11	0.04 ± 0.01
5c	6.1	14.7	17.3	0.33 ± 0.03	1.14 ± 0.16	0.04 ± 0.01

[a] ΔT_m values determined from the CD signal at 295 nm for different ligand concentrations in presence of 2 μM 5′-d[$(G_3T_2A)_3G_3$]-3′ quadruplex. T_m = 56 °C for the quadruplex in absence of any ligand. Values are averaged from three measurements within ±0.5 °C.
[b] 1,10-Phenanthroline.
[c] 4,7-Dimethyl-1,10-phenanthroline.

lower equilibrium dissociation constants (K_D) was observed in the SPR studies of the interaction between the telomeric G-quadruplex and amino (**3a**–**c**) and dimethylamino (**4a**–**c**) ligands with longer side chains (Table 1). The introduction of guanidines in ligands **5a**–**c** results in equilibrium dissociation constants comparable to the long amine and dimethylamine side chains.

The guanidine ligands **5a**–**c** display distinctly different kinetic properties with significantly lower association rate constants (k_a) and dissociation rate constants (k_d) than those of **3a**–**c** and **4a**–**c** (Fig. 2). This remarkable difference may be a consequence of the ability of the guanidinium group to form hydrogen bond interactions. Specific interactions with phosphate groups or bases of the G-quadruplex loops and potentially cation–π interactions may thus account for higher binding energies and significantly lower off-rates, which are also reflected in the higher melting temperatures exhibited by the guanidinium ligand complexes.[41,42] The lower on-rates may on the other hand be explained by the larger solvation shell and resulting slower dehydration of the guanidinium groups. The low on-rates of the guanidinium ligands even out their low off-rates and give equilibrium dissociation constants that are comparable with the other ligands. The clear trend towards higher melting temperatures for the guanidinium ligands thus indicates that the off-rates of the ligands have higher influence on thermal stability than the on-rates. A preferred role of the guanidinium group in quadruplex ligands is supported by two independent dynamic combinatorial libraries targeting quadruplex DNA, which both identified the guanidinium-containing compounds as the favored library members,[43,44] and by the higher efficiency of the guanidinium-containing steroid FG exhibited compared to the analogous trimethylammonium steroid maoquetine.[45]

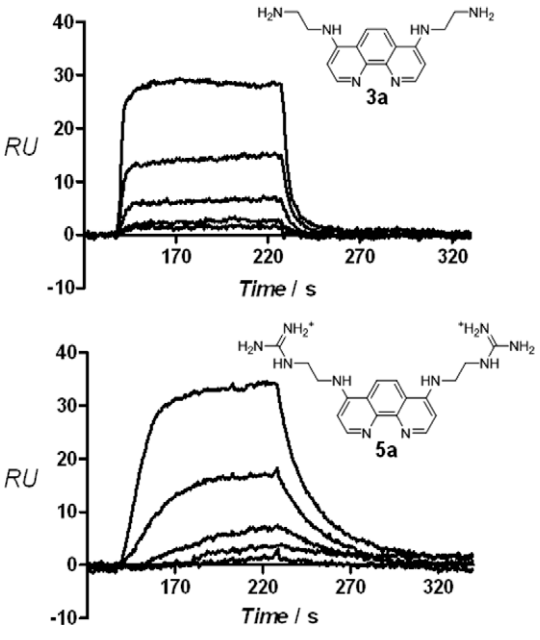


Figure 2. Representative SPR sensograms shown for ligands **3a** and **5a**.

3. Conclusions

In summary, we have synthesized and evaluated a series of 4,7-diamino-1,10-phenanthroline derivatives as G-quadruplex stabilizers. The 4,7-diamino substituents were shown to have beneficial effect on the quadruplex stabilizing effect of phenanthroline, although positively charged side chains are necessary to obtain useful effects. Activity on duplex DNA was also observed. We suggest that the effect of the 4,7-diamino substituents is due to increased basicity of the phenanthroline, resulting in a central positive charge in the structure, either by protonation or by metal chelation. Longer side chains on the 1,10-phenanthroline scaffold result in ligands with appreciable potency, and side chains with guanidinium groups were superior to their amino and dimethylamino analogues. The guanidinium compounds further exhibit distinctly slower kinetics than the other compounds. These results indicate that the 4,7-diamino-1,10-phenanthroline is an appropriate central scaffold for G-quadruplex stabilizers, and furthermore point at the guanidine as a preferred loop-interacting group for such compounds.

4. Experimental

4.1. General

Commercially available chemicals and solvents were used without further purification, unless otherwise stated. Microwave reactions were performed in an Emrys Creator microwave reactor. NMR was recorded on a Varian Gemini 2000 or a Bruker Avance III 400 spectrometer. 1,4-Dioxane (δ 67.19 ppm) and the residual solvent peak of water (δ 4.79 ppm) were used as internal standards for ^{13}C and ^{1}H NMR spectra recorded in D_2O, whereas TMS (δ 0 ppm) and the residual solvent peak of $CDCl_3$ (δ 77.16 ppm) were used as an internal standards for spectra recorded in $CDCl_3$. MALDI-HRMS was recorded on an Ionspec 4.7 Ultima FT mass spectrometer and ESI-HRMS on a Q-Star Pulsar hybrid QqTOF instrument. The products were purified by HPLC (Dionex UltiMate

3000) using a Waters Deltapack 15 μm C18 19 × 300 mm column; 7 mL/min flow rate; an isocratic mixture of acetonitrile in water with 0.05% TFA as modifier.

4.2. 1,10-Phenanthroline-4,7-diamine (2)

4,7-Dichloro-1,10-phenanthroline (**1**, 30 mg, 0.12 mmol) and concentrated aqueous ammonia (4 mL) was heated in a sealed tube in a microwave reactor at 120 °C for 1 h. The mixture was filtered, washed with a 5% aqueous ammonia solution (10 mL) and dried over P_2O_5 to give **2** as a light yellow solid (24.9 mg, 98%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.08 (d, J = 4.8 Hz, 2H), 8.36 (s, 2H), 7.76 (d, J = 4.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 150.5, 147.2, 143.1, 127.1, 124.0, 123.4; ESI-HRMS: calcd for $C_{12}H_{11}N_4$ (M+H) 211.0979, found: 211.0988. The compound was converted to the corresponding HCl salt before CD testing.

4.3. General procedure A: nucleophilic aromatic substitution

A mixture of 4,7-dichloro-1,10-phenanthroline (**1**), the corresponding amine (1 mL) and EtOH (2 mL) was heated in a sealed tube in a microwave reactor at 165 °C for 16 h. The resulting solution was filtered, concentrated, and purified by HPLC. The products were isolated as the corresponding TFA salts.

4.4. N^1,$N^{1\prime}$-(1,10-Phenanthroline-4,7-diyl)diethane-1,2-diamine (3a)

Following general procedure A using **1** (50 mg, 0.20 mmol) and ethylenediamine (1 mL, 15 mmol), and purification by HPLC (isocratic, 6% MeCN in water, t_R 18.1 min) gave **3a** as a film (64 mg, 61%). ^{1}H NMR (D_2O, 300 MHz): δ 8.68 (d, J = 7.2 Hz, 2H), 8.37 (s, 2H), 7.22 (d, J = 7.2 Hz, 2H), 4.05 (t, J = 6.3 Hz, 4H), 3.48 (t, J = 6.3 Hz, 4H); ^{13}C NMR (D_2O, 75 MHz): δ 156.7, 143.9, 131.8, 120.3, 119.5, 102.3, 41.2, 37.9; MALDI-HRMS: calcd for $C_{16}H_{21}N_6$ (M+H) 297.1822, found: 297.1815.

4.5. N^1,$N^{1\prime}$-(1,10-Phenanthroline-4,7-diyl)dipropane-1,3-diamine (3b)

Following general procedure A using **1** (50 mg, 0.20 mmol) and 1,3-propylenediamine (1 mL, 12 mmol), and purification by HPLC (isocratic, 6% MeCN, t_R 21.3 min) gave **3b** as a film (96 mg, 87%). ^{1}H NMR (D_2O, 300 MHz): δ 8.58 (d, J = 7.2 Hz, 2H), 8.31 (s, 2H), 7.14 (d, J = 7.2 Hz, 2H), 3.78 (t, J = 6.9 Hz, 4H), 3.18 (t, J = 7.5 Hz, 4H), 2.20 (quin., J = 7.5 Hz 4H); ^{13}C NMR (D_2O, 75 MHz): δ 156.8, 142.9, 130.6, 120.1, 119.4, 102.0, 41.3, 37.7, 26.0; MALDI-HRMS: calcd for $C_{18}H_{25}N_6$ (M+H) 325.2135, found: 325.2149.

4.6. N^1,$N^{1\prime}$-(1,10-Phenanthroline-4,7-diyl)dibutane-1,4-diamine (3c)

Following general procedure A using **1** (50 mg, 0.20 mmol) and butane-1,4-diamine (1 mL, 10 mmol), and purification by HPLC (isocratic, 8% MeCN, t_R 20.8 min), gave **3c** as a film (93 mg, 80%). ^{1}H NMR (D_2O, 300 MHz): δ 8.54 (d, J = 7.2 Hz, 2H), 8.30 (s, 2H), 7.12 (d, J = 7.2 Hz, 2H), 3.72 (t, J = 6.9 Hz, 4H,), 3.08 (t, J = 6.9 Hz, 4H), 1.88–1.84 (m, 8H); ^{13}C NMR (D_2O, 75 MHz): δ 156.7, 142.6, 130.6, 120.0, 119.3, 101.9, 43.7, 49.7, 25.0, 24.9; MALDI-HRMS: calcd for $C_{20}H_{29}N_6$ (M+H) 353.2448, found: 353.2437.

4.7. N^1,$N^{1\prime}$-(1,10-Phenanthroline-4,7-diyl)bis(N^2,N^2-dimethylethane-1,2-diamine) (4a)

Following general procedure A using **1** (50 mg, 0.20 mmol) and N^1,N^1-dimethyl-1,2-ethanediamine (1 mL, 9 mmol), and purification by HPLC (isocratic, 9% MeCN, t_R 9.5 min) gave **4a** as a film (63 mg, 54%;). ^{1}H NMR (D_2O, 300 MHz): δ 8.69 (d, J = 7.2 Hz, 2H), 8.28 (s, 2H), 7.17 (d, J = 7.2 Hz, 2H), 4.10 (t, J = 6.9 Hz, 4H), 3.65 (t, J = 6.9 Hz, 4H), 3.04 (s, 12H); ^{13}C NMR (D_2O, 75 MHz): δ 155.7, 145.0, 134.0, 119.8, 119.2, 102.5, 55.3, 43.7, 38.8; MALDI-HRMS: calcd for $C_{20}H_{29}N_6$ (M+H) 353.2448, found: 353.2441.

4.8. N^1,$N^{1\prime}$-(1,10-Phenanthroline-4,7-diyl)bis(N^3,N^3-dimethylpropane-1,3-diamine) (4b)

Following general procedure A using **1** (50 mg, 0.20 mmol) and N^1,N^1-dimethyl-1,2-propanediamine (1 mL, 8 mmol), and purification by HPLC (isocratic, 9% MeCN, t_R 13.4 min) gave **4b** as a film (73 mg, 60%). ^{1}H NMR (D_2O, 300 MHz): δ 8.60 (d, J = 7.2 Hz, 2H), 8.32 (s, 2H), 7.14 (d, J = 7.2 Hz, 2H), 3.79 (t, J = 6.9 Hz, 4H), 3.34 (t, J = 7.5 Hz, 4H), 2.93 (s, 12H), 2.27 (quin., J = 7.5 Hz, 4H); ^{13}C NMR (D_2O, 75 MHz): δ 156.7, 143.1, 130.9, 120.1, 119.3, 102.0, 55.7, 43.4, 41.1, 23.5; MALDI-HRMS: calcd for $C_{22}H_{33}N_6$ (M+H) 381.2761, found: 381.2751.

4.9. N^1,$N^{1\prime}$-(1,10-Phenanthroline-4,7-diyl)bis(N^4,N^4-dimethylbutane-1,4-diamine) (4c)

Following general procedure A using **1** (50 mg, 0.20 mmol) and N^1,N^1-dimethyl-1,2-butanediamine (1 mL, 7 mmol), and purification by HPLC (isocratic, 8% MeCN, t_R 29.7 min) gave **4c** as a film (83 mg, 65%). ^{1}H NMR (D_2O, 300 MHz): δ 8.55 (d, J = 7.2 Hz, 2H), 8.30 (s, 2H), 7.11 (d, J = 7.2 Hz, 2H), 3.72 (t, J = 6.9 Hz, 4H), 3.21 (t, J = 7.2 Hz, 4H), 2.89 (s, 12H), 1.89–1.84 (m, 8H); ^{13}C NMR (D_2O, 75 MHz): δ 156.7, 142.6, 130.5, 120.0, 119.3, 101.9, 57.8, 43.6, 43.2, 24.9, 22.2; ESI-HRMS: calcd for $C_{24}H_{37}N_6$ (M+H) 409.3074, found: 409.3074.

4.10. General procedure B: guanylation of primary amines

The corresponding primary amine (**3a**, **3b** or **3c**) was dissolved in DMF. After addition of DIPEA (3 equiv) and 1*H*-pyrazole-1-carboxamidine hydrochloride (2 equiv) the mixture was heated at 60 °C overnight, cooled to room temperature and purified by HPLC. The products were isolated as TFA salts.

4.11. 1,1′-(2,2′-(1,10-Phenanthroline-4,7-diyl)bis(azanediyl)bis(ethane-2,1-diyl))diguanidine (5a)

Following general procedure B using **3a** (20 mg, 38 μmol) in DMF (600 μL), DIPEA (20 μL, 114 μmol) and 1*H*-pyrazole-1-carboxamidine hydrochloride (11 mg, 76 μmol), and purification by HPLC (isocratic, 8% MeCN, t_R 22.5 min) gave **5a** as a film (23 mg, 100%). ^{1}H NMR (D_2O, 300 MHz): δ 8.62 (d, J = 7.2 Hz, 2H), 8.33 (s, 2H), 7.19 (d, J = 7.2 Hz, 2H), 3.93 (t, J = 6.9 Hz, 4H), 3.67 (t, J = 7.2 Hz, 4H); ^{13}C NMR (D_2O, 75 MHz): δ 163.7, 157.2, 143.1, 130.9, 120.2, 119.4, 101.9, 42.9, 40.3; ESI-HRMS: calcd for $C_{18}H_{25}N_{10}$ (M+H) 381.2258, found: 381.2243.

4.12. 1,1′-(3,3′-(1,10-Phenanthroline-4,7-diyl)bis(azanediyl)bis(propane-3,1-diyl))diguanidine (5b)

Following general procedure B using **3b** (17 mg, 31 μmol) in DMF (460 μL), DIPEA (17 μL, 92 μmol) and 1*H*-pyrazole-1-carboxamidine hydrochloride (9.1 mg, 62 μmol), and purification by HPLC (isocratic, 10% MeCN, t_R 21.1 min) gave **5b** as a film (19 mg, 100%). ^{1}H NMR (D_2O, 300 MHz): δ 8.56 (d, J = 7.2 Hz, 2H,), 8.29 (s, 2H), 7.11 (d, J = 7.2 Hz, 2H,), 3.76 (t, 4H), 3.39 (t, J = 6.9 Hz, 4H,), 2.14 (quin., J = 6.6 Hz, 4H,); ^{13}C NMR (D_2O, 75 MHz): δ 163.7, 156.6, 142.8, 130.9, 119.7, 119.2, 101.9, 41.5, 39.2, 27.0; ESI-HRMS: calcd for $C_{20}H_{29}N_{10}$ (M+H) 409.2572, found: 409.2539.

4.13. 1,1′-(4,4′-(1,10-Phenanthroline-4,7-diyl)bis(azanediyl)bis-(butane-4,1-diyl))diguanidine (5c)

Following general procedure B using **3c** (25 mg, 50 μmol) in DMF (800 μL), DIPEA (27 μL, 160 μmol) and 1*H*-pyrazole-1-carboxamidine hydrochloride (15.1 mg, 100 μmol), and purification by HPLC (isocratic, 12% MeCN, t_R 21.9 min) gave **5c** as a film (28 mg, 100%). ^{1}H NMR (D_2O, 300 MHz): δ 8.53 (d, J = 7.2 Hz, 2H), 8.29 (s, 2H), 7.11 (d, J = 7.2 Hz, 2H), 3.70 (t, J = 6.9 Hz, 4H), 3.26 (t, J = 6.9 Hz, 4H), 1.88–1.76 (m, 8H); ^{13}C NMR (D_2O, 75 MHz): δ 163.3, 156.6, 142.5, 130.5, 119.9, 119.2, 101.9, 43.9, 41.4, 26.0, 25.1; ESI-HRMS: calcd for $C_{22}H_{33}N_{10}$ (M+H) 437.2884, found: 437.2834.

4.14. 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 oligonucleotides 5′-d[$(G_3(T_2A_3G_3)_3]$-3′ or 5′-d[$(AT)_{10}$]-3′ in double distilled water. After annealing, by heating to 95 °C for 10 min followed by slow cooling to room temperature, test compounds were added from aqueous stock solutions in appropriate amounts to give the desired final concentrations. For samples containing 1,10-phenanthroline or 4,7-dimethyl-1,10-phenanthroline DMSO was added to a final concentration of 0.1%. 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 s 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 25–100 °C. T_m curves were afterwards smoothened using a moving average method and normalized for comparison of data.

4.15. 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 cells 1 and 4 as blank.

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. 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. To obtain the kinetic association and dissociation rate constants, k_a and k_d, the curves were fitted by the least sum of squares residual to a first order Langmuir interaction model. The equilibrium dissociation constants were derived from the ratio of the rate constants: $K_D = k_d/k_a$.[46]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.09.055.

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