A click chemistry approach to C_3 symmetric, G-quadruplex stabilising ligands†

John E. Moses,*[a] Dougal J. Ritson,[a] Fengzhi Zhang,[a] Caterina Maria Lombardo,[b] Shozeb Haider,[b] Neil Oldham[a] and Stephen Neidle*[b]

Received 13th April 2010, Accepted 12th May 2010
First published as an Advance Article on the web 25th May 2010
DOI: 10.1039/c005055e

Described is the structure-based design and synthesis of a series of tris-triazole G-quadruplex binding ligands utilising the copper catalysed azide–alkyne 'click' reaction. The results of G-quadruplex stabilisation by the ligands are reported and discussed.

The maintenance of telomere integrity in the majority of human cancer cells is achieved by the telomerase enzyme complex, which acts as a reverse transcriptase and catalyses the synthesis of telomeric DNA repeats,[1] and is preferentially expressed in cancer cells.[2] This process is regulated by several other telomere-associated proteins in a cell cycle-dependent manner. Telomerase inhibition can be achieved in several ways;[3] one of particular current interest involves the sequestration of the 3′ end of telomeric DNA, to which extra telomeric DNA units would be attached by telomerase, into a higher-order quadruplex structure that is then inaccessible for attachment.[4] Quadruplex folding and stabilisation can be achieved with small-molecule ligands,[5] of which a large number have been examined.[6] Almost all share common features of a planar heteroaromatic chromophore (or equivalent) together with cationic side-chains. Selectivity for human telomeric quadruplexes over duplex DNA is a key requirement, which has been challenging to achieve for such polycyclics since their structural and electronic features are shared, at least in part, by many conventional duplex-binding agents.

The polyoxazole compound telomestatin **1**, a natural metabolite extracted from *Streptomyces annulatus*, represents a contrasting and non-polycyclic aromatic class of G-quadruplex ligand.[7] It has a high level of G-quadruplex stabilising ability and selectivity for quadruplex *versus* duplex DNA, and is a potent inhibitor of telomerase. We recently reported the synthesis of a first generation of rather simpler ligands (**2**)[8] based on the non-polycyclic aromatic concept, which showed excellent selectivity and good quadruplex-stabilising properties (Fig. 1).

Other molecules of this general non-polycyclic type that have been reported include macrocyclic hexaoxazole-, urea- and pyrimidine-based frameworks.[9] The melting temperature (ΔT_m) of the bis-triazole compound **2**[8] was comparable to the best of other previously reported synthetic G-quadruplex binding ligands, such as the acridine derivative BRACO-19[10] (ΔT_m = 28 °C) and telomestatin itself (ΔT_m = 27 °C). The bis-triazole **2** showed superior selectivity for the quadruplex structure over duplex DNA compared to BRACO-19. This trisubstituted acridine compound was designed on the basis of all three (3, 6 and 9- position) substituents being able to interact in a groove of a quadruplex structure.[11] A subsequent X-ray crystallographic study has shown that this is indeed the case.[12]

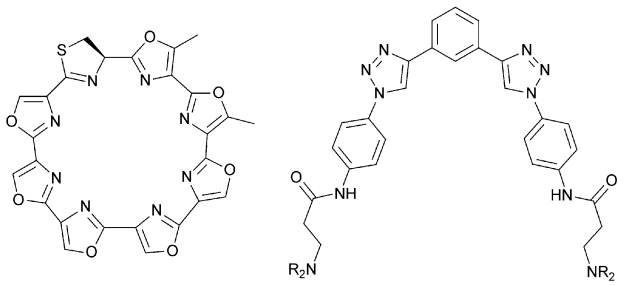


Fig. 1 G-quadruplex stabilising ligands.

Several other non-planar and non-aromatic G-quadruplex ligands have been reported, notably two steroidal molecules.[13] As yet there is no structural data, or even a model for their quadruplex interactions, but findings in other contexts,[14] that there can be attractive interactions between an aromatic system and a hydrogen atom perpendicular to it, suggests that the concept of an array of hydrogen atoms presented to a G-quartet surface might result in a stabilised complex. The present study examines this concept with a series of symmetric phenyl derivatives containing three triazole moieties linked by sp^3 carbon atoms to the benzene core (**3**, Fig. 2), *i.e.* potentially presenting a total of six hydrogen atoms to a G-quartet surface. The three symmetric substituents could perhaps maximise quadruplex groove interactions, with each one residing in a groove – analogous to the binding of the BRACO-19 substituents.

As the synthesis of **2**[8] was achieved using the CuAAC reaction,[15] it was thought that this methodology could be extended to give **3** in a one pot reaction starting from 1,3,5-tris(azidomethyl)benzene **5**, itself readily available from triester **4** (Scheme 1). The complementary alkyne coupling partners were assembled *via* two routes, as the 3-(dialkyl)amino-propyl derivatives **32–36** tended to undergo elimination during the CuAAc reaction (Scheme 1). Hence, 4-ethynylaniline **7** was coupled to either chloroacetyl chloride

[a]*The School of Chemistry, University of Nottingham, University Park, Nottingham, UK NG7 2RD. E-mail: john.moses@nottingham.ac.uk; Fax: (+44) 115-951-3555; Tel: (+44) 115-951-3533*
[b]*CRUK Biomolecular Structure Group, The School of Pharmacy, University of London, 29-39 Brunswick Square, London, UK, WC1N 1AX. E-mail: stephen.neidle@pharmacy.ac.uk; Fax: (+44) 20-7753-5970; Tel: (+44) 20-7753-5969*

† Electronic supplementary information (ESI) available: Experimental procedures for the preparation of compounds **5**, **6**, **8–36**, full characterisation of all new compounds, copies of H^1 and C^{13} NMR spectra for final compounds, ESI-MS data and HPLC traces of all final compounds. See DOI: 10.1039/c005055e

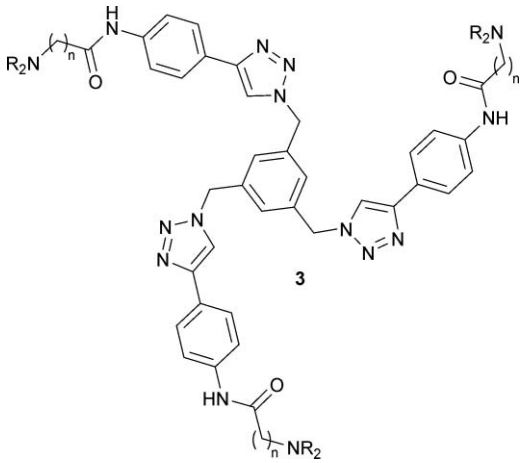


Fig. 2 C_3 Symmetric ligand design.

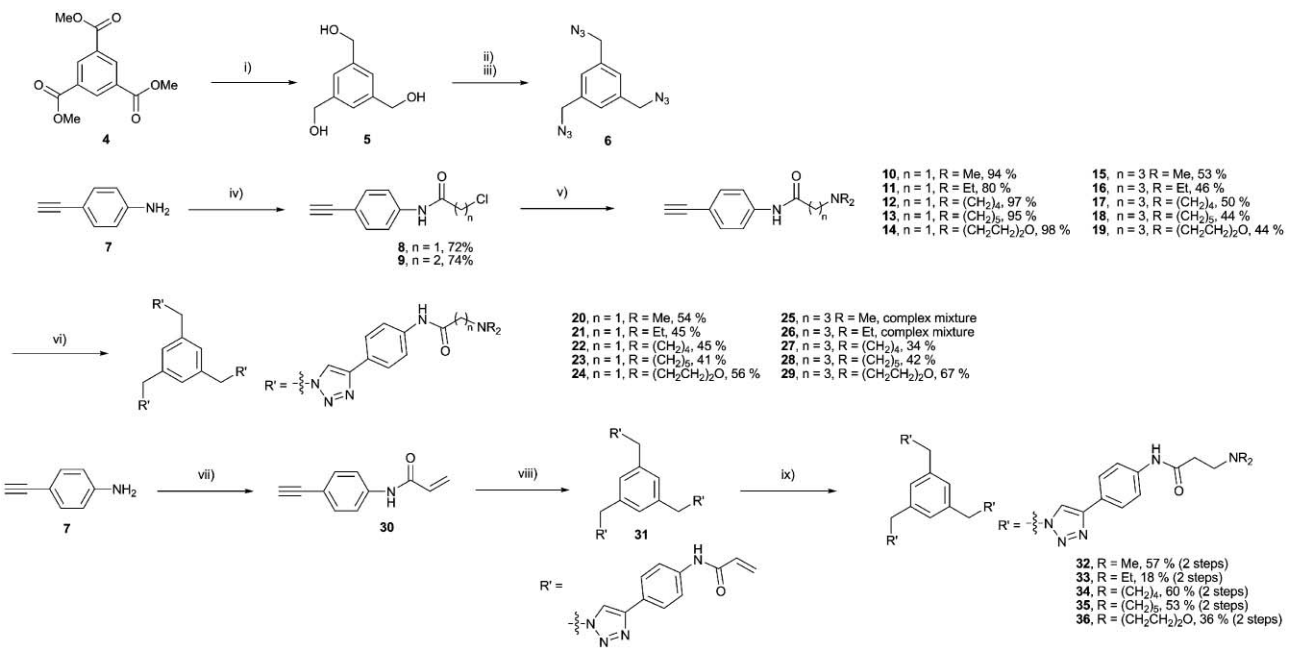
Table 1 Thermal stabilization (ΔT_m in °C) for ligands interacting with a human telomeric DNA sequence (*h*-tel) and a duplex DNA. The FRET method was used to obtain values, which are the mean of three determinations. Average esds are 0.1 °C

	G4 *h*-tel DNA		ds DNA
Compound	1 μM	2 μM	1 μM
20	1	1	0
21	0	1	0
22	0	0	0
23	2	2	0
24	0	0	0
27	1	7	0
28	4	11	0
29	0	0	0
32	2	7	0
33	1	2	0
34	2	5	0
35	1	1	0
36	0	0	0

or 4-chlorobutyryl chloride to give the acylated products **8** and **9**, which were then reacted with the appropriate dialkylamine to afford trialkylamines **10–19**.

The alkynes were then 'clicked' together with **6** to arrive at the C_3 symmetric ligands **20–29**. For reasons as yet unknown, the dimethyl and diethyl derivatives **25** and **26** gave complex mixtures after the CuAAc reaction and could not be obtained in appreciable amounts. To arrive at the propyl analogues, **7** was added to acryloyl chloride, and then subjected to the CuAAc reaction with **6**. Michael addition of the desired dialkyl amines to **31** in DMSO led to the corresponding ligands **32–36** in varying yields.

The ΔT_m values for the compounds **20–24** and **27–36**, determined by a FRET method,[16] are given in Table 1. The data show that these second generation tris-triazole ligands do not match those of the first generation for their ability to stabilise G-quadruplexes.[16] The ligands with acetylamide appendages are uniformly poor quadruplex stabilisers, and in all cases the morpholino analogues (**24**, **29**, **36**) show no significant G-quadruplex stabilisation, which is likely to result from reduced electrostatic interactions in the backbone loops and grooves by the uncharged morpholine moiety. The butanamide analogues **27** and **28** show the greatest quadruplex stabilisation. The analogous propanamides **34** and **35** show far less stabilisation, which suggests that the longer side-chains of **27** and **28** enable more effective positioning of the protonated amines in the phosphate backbone loops, or simply provide more surface area for the ligand to bind to the quadruplex. A general trend is that longer side-chains of the tris-triazole



Scheme 1 Synthesis of the azido coupling partner **6** and click reactions to form the tris-triazole ligands. *Reagents and conditions*: (i) $LiAlH_4$, THF, 85 °C, 45%; (ii) PBr_3, Et_2O, rt; (iii) NaN_3, DMF, 80 °C, 92% (2 steps); (iv) chloroacetyl chloride or 4-chlorobutyryl chloride, Et_3N, THF, 0 °C to rt; (v) HNR_2, MeOH; (vi) **6**, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF–H_2O, 120 °C, μWave; (vii) acryloyl chloride, Et_3N, CH_2Cl_2, 0 °C to rt, 85%; (viii) **6**, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF–H_2O, 120 °C, μWave; (ix) HNR_2, DMSO, 40 to 70 °C.

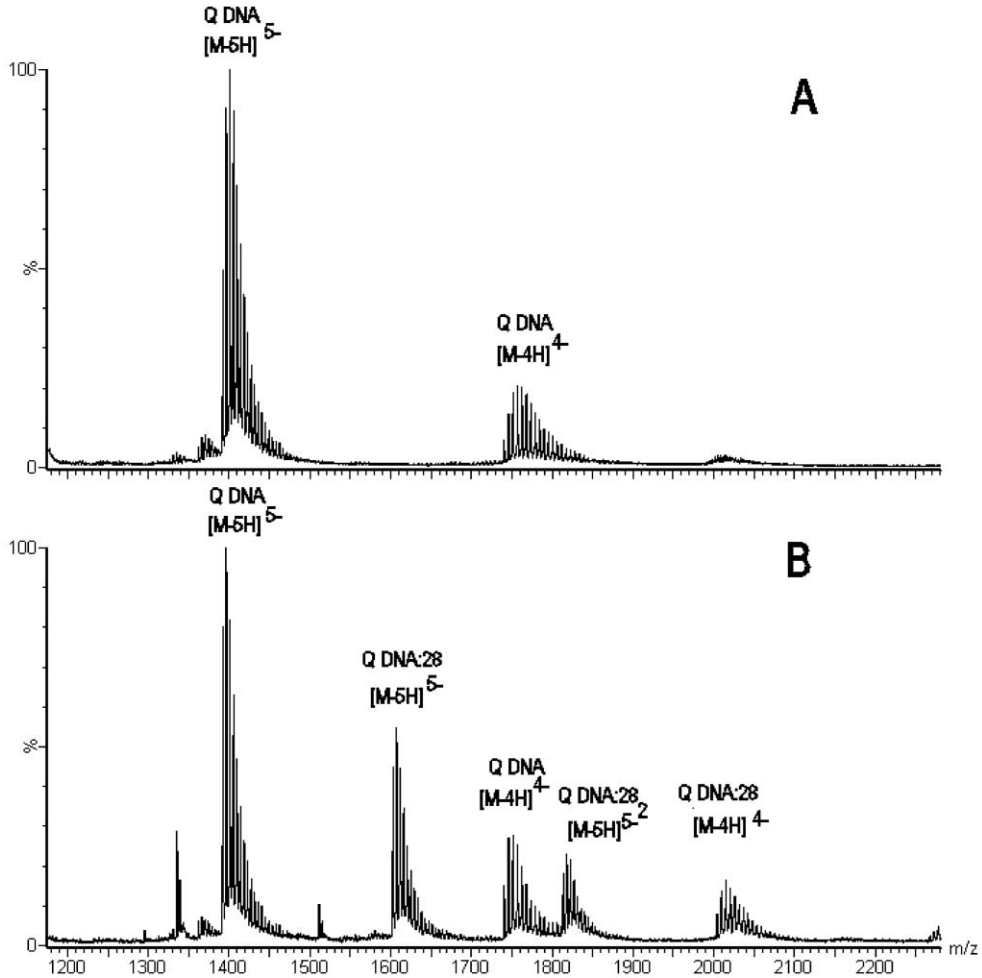


Fig. 3 ESI-MS of *h*-tel (10 μM) alone (A) and in the presence of **28** (25 μM) (B) showing the presence of one and two binding events.

compound lead to improved quadruplex stabilisation, in accord with the original findings.[8] It should be noted that for all ligands there is no increase in duplex (ds) DNA ΔT_m at concentrations of 1 μM. At higher concentrations the ΔT_m values generally increase, although the selectivity is not compromised *e.g.* at a concentration of 3 μM of **27** the quadruplex ΔT_m is 17 °C, and the ds DNA ΔT_m is 2 °C.

Further analysis of the quadruplex binding of **28** by electrospray ionisation-mass spectrometry (ESI-MS) was then conducted (Fig. 3), which revealed the presence of one and two binding events (as previously seen for the bis-triazole ligands).[17] An estimated apparent K_D of 23 μM was obtained for the first binding event and a small degree of positive cooperativity was evident (see SI). However, considering that the first generation of bis-triazole ligands gave ΔT_m of up to 19 °C for quadruplex DNA with complete selectivity over ds DNA at a concentration of 1 μM, further investigation of this new series of ligands is not being pursued.

The key difference between the bis-triazole and tris-triazole series is the presence of the sp^3 methylene groups in the latter. Molecular modelling has been used[18] to better understand the ligand–quadruplex interactions of these two series. Compound **28** was selected for this study as it has the greatest G-quadruplex stabilising ability, together with the parallel telomeric G-quadruplex[19] as the quadruplex structure. Other quadruplex folds such as (3 + 1) hybrids are likely to be present, at least in dilute solution,[20] but the results found here are not dependent on the fold itself, and so interactions with these other folded structures have not been explored further.

The modelling clearly shows that the three methylene bridges perturb the planarity of the aromatic region in **28** to such an extent, that effective π–π stacking of the ligand onto the terminal G-quartet is no longer possible (Fig. 4). This lack of planarity and loss of ability to π-stack would also prevent intercalation in duplex DNA, hence the observed selectivity of the tris-triazole ligands for quadruplex DNA over duplex DNA. The observed (though low) affinity of some members of the present family of ligands for the quadruplex structure, thus arises in large part from interactions between the side-chains and the grooves/loops of the quadruplex. The details of these interactions will depend on the nature of these binding sites *i.e.* on the particular fold adopted by the quadruplex. These results also suggest that much of the observed high degree of stabilisation found for compound **2** and its analogues is a consequence of π–π stacking interactions and that groove/loop non-bonded interactions are relatively minor contributors to the overall stabilisation.

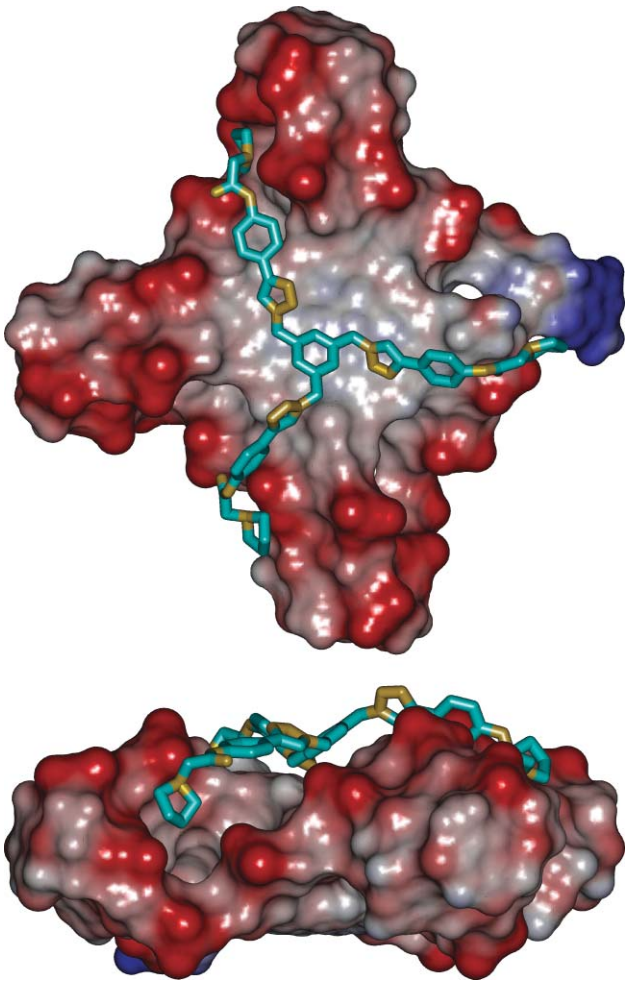


Fig. 4 Two views of compound **28** bound to the parallel human telomeric quadruplex structure (shown as a solvent-accessible surface colored by charge). Note the non-planar nature of the ligand, colored cyan.

The overwhelming majority of quadruplex-binding ligands have planar groupings, which stack onto terminal G-quartets. The steroid molecules[13] that are exceptions to this rule are highly non-planar and their axial hydrogen atoms are oriented in an analogous manner to the hydrogen atoms attached to the sp^3 carbon bridge atoms in the present compounds. The high level of quadruplex stabilisation and affinity shown by these steroids suggests that they are unlikely to be bound on the G-quartet surface, but rather in the grooves. The present results highlight the need to have a coplanar ligand core in order for effective interactions with a terminal G-quartet and suggest that the lack of such a core cannot be fully compensated by side-chain electrostatic interactions. These issues need to be taken into account in the future design of novel quadruplex-binding molecules.

Conclusions

In summary, we have synthesised a second generation of tris-triazole compounds designed on previously reported G-quadruplex ligands[8] and using a rationale that has been proven to be effective. The best of the ligands synthesised, the piperidino compound **28**, showed a modest G-quadruplex stabilising ability at a concentration of 1 μM and good stabilisation at a concentration of 2 μM whilst displaying excellent selectivity for quadruplex DNA over duplex DNA.

Acknowledgements

JEM thanks EPSRC, the Association for International Cancer Research (AICR), and The University of Nottingham for financial support. SN is grateful to CRUK for support. Work at the School of Pharmacy was supported by Cancer Research UK (programme grant to S. N.) and a studentship to support C. L. (grant to J.E.M and S.N.).

References

1 M. J. McEachern, A. Krauskopf and E. H. Blackburn, *Annu. Rev. Genet.*, 2000, **34**, 331; E. H. Blackburn, *Cell*, 2001, **106**, 661; C. Autexier and N. F. Lue, *Annu. Rev. Biochem.*, 2006, **75**, 493.
2 N. W. Kim, M. A. Piatyszek, K. R. Prowse, C. B. Harley, M. D. West, P. L. C. Ho, G. M. Coviello, W. E. Wright, S. L. Weinrich and J. W. Shay, *Science*, 1994, **266**, 2011; C. M. Counter, H. W. Hirte, S. Bacchetti and C. B. Harley, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 2900; J. W. Shay and S. Bacchetti, *Eur. J. Cancer*, 1997, **33**, 787; J. Feng, W. D. Funk, S. S. Wang, S. L. Weinrich, A. A. Avilion, C.-P. Chiu, R. R. Adams, E. Chang, R. C. Allsopp, J. Yu, S. Le, M. D. West, C. B. Harley, W. H. Andrews, C. W. Greider and B. Villeponteau, *Science*, 1995, **269**, 1236.
3 See for example: W. C. Hahn, S. A. Stewart, M. W. Brooks, S. G. York, E. Eaton, A. Kurachi, R. L. Beijersbergen, J. H. M. Knoll, M. Meyerson and R. A. Weinberg, *Nat. Med.*, 1999, **5**, 1164; S. E. Hamilton, A. E. Pitts, R. R. Katipally, X. Jia, J. P. Rutter, B. A. Davies, J. W. Shay, W. E. Wright and D. R. Corey, *Biochemistry*, 1997, **36**, 11873; A. E. Pitts and D. R. Corey, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 11549; K. Damn, U. Hemmann, P. Garin-Chesa, N. Hauel, I. Kauffmann, H. Priepke, C. Niestroj, C. Daiber, B. Enenkel, B. Guilliard, I. Lauritsch, E. Műller, E. Pascolo, G. Sauter, M. Pantic, U. M. Martens, C. Wenz, J. Lingner, N. Kraut, W. J. Rettig and A. Schapp, *EMBO J.*, 2001, **20**, 6958; J. W. Shay and W. E. Wright, *Nat. Rev. Drug Discovery*, 2006, **5**, 577.
4 A. M. Zahler, J. R. Williamson, T. R. Cech and D. M. Prescott, *Nature*, 1991, **350**, 718.
5 D. Sun, B. Thompson, B. E. Cathers, M. Salazar, S. M. Kerwin, J. O. Trent, T. C. Jenkins, S. Neidle and L. H. Hurley, *J. Med. Chem.*, 1997, **40**, 2113.
6 See for example: M.-K. Cheng, C. Modi, J. C. Cookson, I. Hutchinson, R. A. Heald, A. J. McCarroll, S. Missailidis, F. Tanious, W. D. Wilson, J.-L. Mergny, C. A. Laughton and M. F. G. Stevens, *J. Med. Chem.*, 2008, **51**, 963; T. P. Garner, H. E. L. Williams, K. I. Gluszyk, S. Roe, N. J. Oldham, M. F. G. Stevens, J. E. Moses and M. S. Searle, *Org. Biomol. Chem.*, 2009, **7**, 4194For recent reviews see: A. De Cian, L. Lacroix, C. Douarre, N. Temime-Smaali, C. Trentesaux, J. F. Riou and J. L. Mergny, *Biochimie*, 2008, **90**, 131; D. Monchaud and M.-P. Teulade-Fichou, *Org. Biomol. Chem.*, 2008, **6**, 627; T. M. Ou, Y. J. Lu, J. H. Tan, Z. S. Huang, K. Y. Wong and L. Q. Gu, *ChemMedChem*, 2008, **3**, 690.
7 M. Y. Kim, H. Vankayalapati, K. Shin-Ya, K. Wierzba and L. H. Hurley, *J. Am. Chem. Soc.*, 2002, **124**, 2098; D. Gomez, T. Wenner, B. Brassart, C. Douarre, M. F. O'Donohue, V. El Khoury, K. Shin-Ya, H. Morjani, C. Trentesaux and J.-F. Riou, *J. Biol. Chem.*, 2006, **281**, 38721.
8 A. D. Moorhouse, A. M. Santos, M. Gunaratnam, M. Moore, S. Neidle and J. E. Moses, *J. Am. Chem. Soc.*, 2006, **128**, 15972; A. D. Moorhouse, S. Haider, M. Gunaratnam, D. Munnur, S. Neidle and J. E. Moses, *Mol. BioSyst.*, 2008, **4**, 629.
9 W. C. Drewe and S. Neidle, *Chem. Commun.*, 2008, 5295; J. Dash, P. S. Shirude, S. T. D. Hsu and S. Balasubramanian, *J. Am. Chem. Soc.*, 2008, **130**, 15950; M. Satyanarayana, S. G. Rzuczek, E. J. Lavoie, D. S. Pilch, A. Liu, L. F. Liu and J. E. Rice, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 3802; R. Rodriguez, S. Müller, J. A. Yeoman, C. Trentesaux, J.-F. Riou and S. Balasubramanian, *J. Am. Chem. Soc.*, 2008, **130**, 15758.
10 A. M. Burger, F. Dai, C. M. Schultes, A. P. Reszka, M. J. Moore, J. A. Double and S. Neidle, *Cancer Res.*, 2005, **65**, 1489; M. Gunaratnam,

O. Greciano, C. Martins, A. P. Reszka, C. M. Schultes, H. Morjani, J. F. Riou and S. Neidle, *Biochem. Pharmacol.*, 2007, **74**, 679.
11 M. Read, R. J. Harrison, B. Romagnoli, F. A. Tanious, S. H. Gowan, A. P. Reszka, W. D. Wilson, L. R. Kelland and S. Neidle, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 4844.
12 N. H. Campbell, G. N. Parkinson, A. P. Reszka and S. Neidle, *J. Am. Chem. Soc.*, 2008, **130**, 6722.
13 B. Brassart, D. Gomez, A. De Cian, R. Paterski, A. Montagnac, K. H. Qui, N. Temime-Smaali, C. Trentesaux, J.-L. Mergny, F. Gueritte and J.-F. Riou, *Mol. Pharmacol.*, 2007, **72**, 631.
14 S. Scheiner, T. Kar and J. Pattanayak, *J. Am. Chem. Soc.*, 2002, **124**, 13257.
15 C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057; V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596; H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004; J. E. Moses and A. D. Moorhouse, *Chem. Soc. Rev.*, 2007, **36**, 1249.
16 The FRET DNA melting assay was performed as described previously (B. Guyen, C. M. Schultes, P. Hazel, J. Mann and S. Neidle, *Org. Biomol. Chem.*, 2004, **2**, 981). The tagged DNA sequences used were: 5′-*FAM*-d(GGG[TTAGGG]$_3$)-*TAMRA*-3′ for the G4 and 5′-*FAM*-dTATAGCTATA-*HEG*-TATAGCTATA-*TAMRA*-3′ (*HEG* linker: [(-CH_2-CH_2–O-)$_6$]) for the duplex experiment.
17 T. P. Garner, H. E. L. Williams, K. I. Gluszyk, S. Roe, N. J. Oldham, M. F. G. Stevens, J. E. Moses and M. S. Searle, *Org. Biomol. Chem.*, 2009, **7**, 4194.
18 See supporting information for details.
19 G. N. Parkinson, M. P. Lee and S. Neidle, *Nature*, 2002, **417**, 876.
20 A. T. Phan, V. Kuryavyi, K. N. Luu and D. J. Patel, *Nucleic Acids Res.*, 2007, **35**, 6517; A. Ambrus, D. Chen, J. Dai, T. Bialis, R. A. Jones and D. Yang, *Nucleic Acids Res.*, 2006, **34**, 2723; K. W. Lim, S. Amrane, S. Bouaziz, W. Xu, Y. Mu, D. J. Patel, K. N. Luu and A. T. Phan, *J. Am. Chem. Soc.*, 2009, **131**, 4301; D. Renciuk, I. Kejnovská, P. Skoláková, K. Bednárová, J. Motlová and M. Vorlícková M, *Nucleic Acids Res.*, 2009, **37**, 6625.

