G-quadruplex Interactions

DOI: 10.1002/anie.200((will be filled in by the editorial staff))

Determinants for Tight and Selective Binding of a Medicinal Dicarbene Gold(I) Complex to a Telomeric DNA G Quadruplex: a joint ESI MS and XRD Investigation

Carla Bazzicalupi, Marta Ferraroni, Francesco Papi, Lara Massai, Benoît Bertrand, Luigi Messori, Paola Gratteri,* Angela Casini.*

The dicarbene gold(I) complex [Au(9-methylcaffein-8-ylidene)$_2$]BF$_4$ (I) is an exemplary organometallic compound of outstanding interest as a prospective anticancer agent. This gold(I) complex was previously reported to be highly cytotoxic in vitro toward various cancer cell lines and behave as a tight ligand for human telomeric DNA sequences. We have analysed its interactions with various telomeric DNA models through a combined ESI MS and XRD approach. ESI MS measurements confirmed formation of stable adducts between the intact gold (I) complex and Tel 23 DNA sequence. Afterward, we solved the crystal structure of the adduct formed between [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ and Tel 23 DNA G-quadruplex. Notably, Tel 23 maintains a characteristic propeller conformation, while binding three gold(I) dicarbene moieties, at two distinct sites. Stacking interactions appear to drive noncovalent binding of this gold(I) complex. The structural bases for tight gold(I) complex /G-quadruplex recognition and for its selectivity are thus unveiled.

Gold compounds, either 3+ or 1+, form a new class of inorganic medicinal agents of great interest for cancer treatment owing to the occurrence of innovative modes of action[1]. To this regard it is worth mentioning that the established anti-arthritic gold drug auranofin has recently been repurposed as a candidate anticancer agent and is currently undergoing evaluation in three distinct US clinical trials[2]. Pairwise, other gold(I) agents and, specifically, a number of monocarbene and dicarbene gold(I) complexes, seem quite promising as they manifest relevant cytotoxic properties while being acceptably stable under physiological like conditions[3]. This behaviour might result into a higher cancer selectivity and a lower systemic toxicity compared to other cytotoxic gold drugs typically manifesting a larger and more general reactivity with biomolecules.

The modes of action of cytotoxic gold(I) compounds are still a matter of intense debate. However, there is now quite a wide consensus on the concept that they diverge profoundly from those of cisplatin and analogues, mainly grounded on a documented, poor reactivity with double helix DNA; on the other hand, there is good evidence that they often produce severe mitochondrial damage[1]. Nonetheless, alternative modes of action beyond mitochondrial insult have been proposed to explain their cytotoxicity, including proteasome inhibition and antitelomerase activity, within a probable multi-target scenario.

The latter hypothesis – *i.e.* a pronounced antitelomerase activity – distinctly emerges from previous studies on auranofin[4]. Small molecules, that are able to bind and block telomerase manifest, in general, remarkable anti-cancer properties. In turn, the G-quadruplex binding properties well correlate with antitelomerase activity[5].

G-quadruplexes (also known as G4) are nucleic acid sequences, rich in guanines, capable of forming a characteristic four-stranded fold[6]. Four guanine bases may associate through Hoogsteen hydrogen bonding to produce a square planar motif, the so called "guanine tetrad"; two or more guanine tetrads can stack on top of each other to form a G-quadruplex. The quadruplex structure is further stabilized by the presence of monovalent cations, which lie in the central channel between each pair of tetrads[7].

Human telomeric DNA consists of tandem repeats of the hexameric sequence d(TTAGGG), located at the 3'-ends of chromosomes; the quadruplexes that arise from these repeats were described in detail thanks to NMR and X-ray diffraction structural determinations[8-11]. Interestingly, the formation of quadruplexes causes a net decrease in the activity of the enzyme telomerase, responsible for maintaining the length of telomeres[12]. Therefore, molecules that template the formation or stabilize the structure of G-quadruplex DNA might pave the way to the development of new effective anticancer drugs, based on selective telomerase inhibition[13-16].

[*] Prof. C. Bazzicalupi, Dr. M. Ferraroni, F. Papi, Dr. L. Massai, Prof. L. Messori.
Department of Chemistry, University of Florence,
Via della Lastruccia 3, 50019 Sesto Fiorentino (FI), Italy.
Corresponding Author
Dr. Marta Ferraroni
E-mail: marta.ferraroni@unifi.it

Prof. P.Gratteri, F. Papi.
Department NEUROFARBA – Pharmaceutical and Nutraceutical section - Laboratory of Molecular Modeling Cheminformatics & QSAR, University of Firenze,
via Ugo Schiff 6, I-50019 Sesto Fiorentino (FI), Italy.
Corresponding Author

Prof. Paola Gratteri
E-mail: paola.gratteri@unifi.it

Dr. Benoît Bertrand.
Institut de Chimie Moléculaire de l'Université de Bourgogne, UMR 6302 CNRS Université de Bourgogne, Dijon, France

Prof. A. Casini
School of Chemistry, Cardiff University, Park place, Cardiff CF103AT, UK.

ŧ Tel 23-[Au(9-methylcaffein-8-ylidene)$_2$]$^+$ complex: a= 47.13 Å, b= 51.29 Å, c =58.77 Å, space group I212121, Resolution 1.9 Å, Rsym 0.069, Completeness 97.8%. Details of the crystallization, data collection and model refinement are reported in ESI. Protein coordinates have been deposited in the Protein Data Bank (PDB accession 5CCW).
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

While the majority of quadruplex DNA binders reported to date contain planar organic heteroaromatic systems, a variety of metal complexes, mostly bearing extended planar motifs, were also shown to exhibit large affinities for DNA G-quadruplexes[5,17,18]. An extensive overview of this topic is provided in two recent review articles where a number of relevant examples are illustrated[19,20]. Yet, relatively scarce structural information is available on the adducts formed between metal complexes and G4-DNA which would be of great help to develop selective G4 stabilizers: crystal structures have been reported so far only for the adducts of copper(II) and nickel(II) salphen complexes with human telomeric DNA[21]. Also, extremely scarce information on the interactions of gold compounds with G4-DNA is available[22,23]. The above arguments prompted us to explore, at a molecular level, the interactions taking place between organometallic gold(I) N-heterocyclic carbene (NHC) compounds and a representative DNA G-quadruplex – *i.e.* the one arising from Tel23 sequence – through a combined ESI MS and X-ray diffraction investigative strategy. Based on recent work by some of us[22], we selected for this study the bis-carbene cationic complex $[Au(9\text{-methylcaffein-8-ylidene})_2]^+$, complex I (Figure 1), which was earlier reported to be a selective cytotoxic agent in cancer cells. This compound is able to bind quite strongly a few representative G4-DNAs, more selectively than DNA duplexes, as well as 3- and 4-ways junctions[23]. The occurring interactions were mainly characterized by spectrofluorimetric methods but no structural data have been obtained so far for these systems.

Notably, the chemical features of the mentioned dicarbene gold(I) complex fully fulfil the basic requirements for an ideal G4 ligand: i) it is planar; ii) it is positively charged and thus prone to electrostatic interactions with negatively charged DNA; iii) it has two aromatic caffeine ligands (*i.e.* a guanine analogue) which can associate to guanines of G4-DNA through π-stacking interactions.

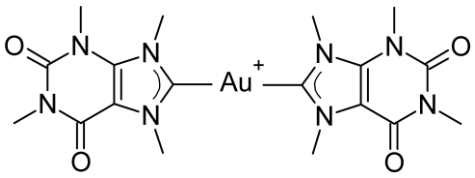


Figure 1. $[Au(9\text{-methylcaffein-8-ylidene})_2]^+$ cation (complex I) investigated in this study.

Mass spectrometry methods offer today a very powerful and straightforward tool to characterize metal binding to G-quadruplexes, at a molecular level; the suitability of ESI MS for these systems was recently highlighted[24]. The ESI MS spectrum of a solution containing both complex I and Tel 23 sequence shows three main peaks, assigned to complex I /Tel 23 adducts of various stoichiometries (1:1, 2:1 and 3:1 respectively) (Figure 2). It is inferred that complex I binds Tel 23 as the intact $[Au(9\text{-methylcaffein-8-ylidene})_2]^+$ cation, at various molar ratios (up to a maximum of 3:1). The ammonium ion, promoting the G-quadruplex assembly, is also accommodated inside the structure (see ESI-Table S1) and this confirms maintenance of the quadruplex structure.

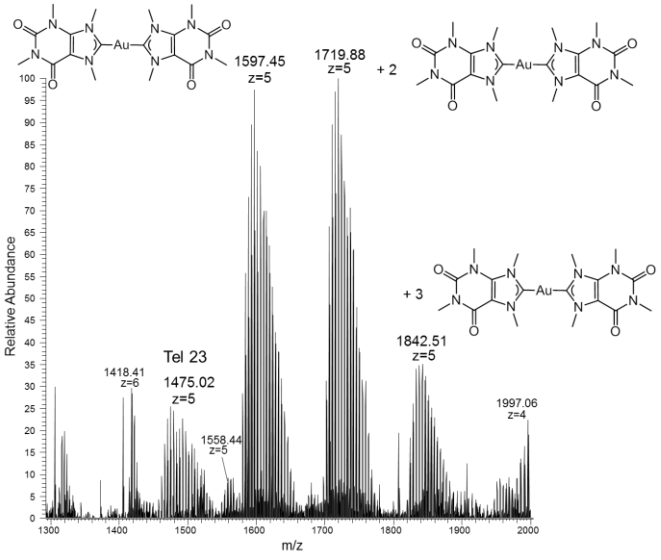


Figure 2. Section of the ESI-MS spectra of Tel23/$[Au(9\text{-methylcaffein-8-ylidene})_2]BF_4$ 25 μM in water in the presence of 60% EtOH, 3:1 metal complex:G4 molar ratio.

Afterward, crystals of the Tel23/$[Au(9\text{-methylcaffein-8-ylidene})_2]^+$ adduct were grown and analyzed by X-ray diffraction (orthorhombic system, space group I212121)[‡]. The overall organization of the adduct in the crystal (Figure 3) reveals the quadruplex in the parallel-stranded arrangement. The TTA loops are better described by using the type-9 topology (see ESI-Table S3-4), on the basis of the classification made by Collie and coworker[11]. The parallel topology results in accessible external 5' and 3' planar G-tetrad surfaces for ligand stacking. The quadruplex units, symmetry related by 2-fold rotation axes, repeat themselves in the crystal determining columns growing in the [010] direction. Along these columns, $[Au(9\text{-methylcaffein-8-ylidene})_2]^+$ ions are found at each interface between the adjacent 2-fold symmetry related quadruplexes, stacked on their external tetrads at about 3.4 Å. Noteworthy, the present structure evidences that complex I binding is possible at the 3'-3' (3'end-3'end) site, as well as at the 5'-5' (5'end-5'end) site. Interestingly, these sites differ for the relative arrangement of the two tetrads, almost perfectly coplanar in the 5'-5' site and tilted with respect to each other in the 3'-3' one, the G17 and G23 forming a dihedral angle of about 38° (Figure 3c). Moreover, the two binding sites also differ for the overall number of hosted metal complexes: either one or two $[Au(9\text{-methylcaffein-8-ylidene})_2]^+$ ions are located in the 3'-3' and 5'-5' sites, respectively (Figure 3a,c). It is worth mentioning that the number of complexes directly stacked on each single quadruplex unit is the same found in solution by the ESI mass measurements, so reinforcing the hypothesis for a maximal 3:1 gold/quadruplex binding stoichiometry. The stacked $[Au(9\text{-methylcaffein-8-ylidene})_2]^+$ ions, at each binding site, adopt a planar arrangement, with the linear geometry commonly expected for a two-coordinated Au(I) complex. Overall, the structure found for each metal complex is quite similar to that previously obtained in the $[Au(9\text{-methylcaffein-8-ylidene})_2][BF_4]$ crystal structure[22] . Interestingly, the caffeine ligands bound to the Au(I) ion are less prone to undergo ligand exchange reactions than,

for example, chlorido and N-donor ligands, therefore, preventing the Au(I) center from establishing a direct coordination bond with the G4 nucleobases.

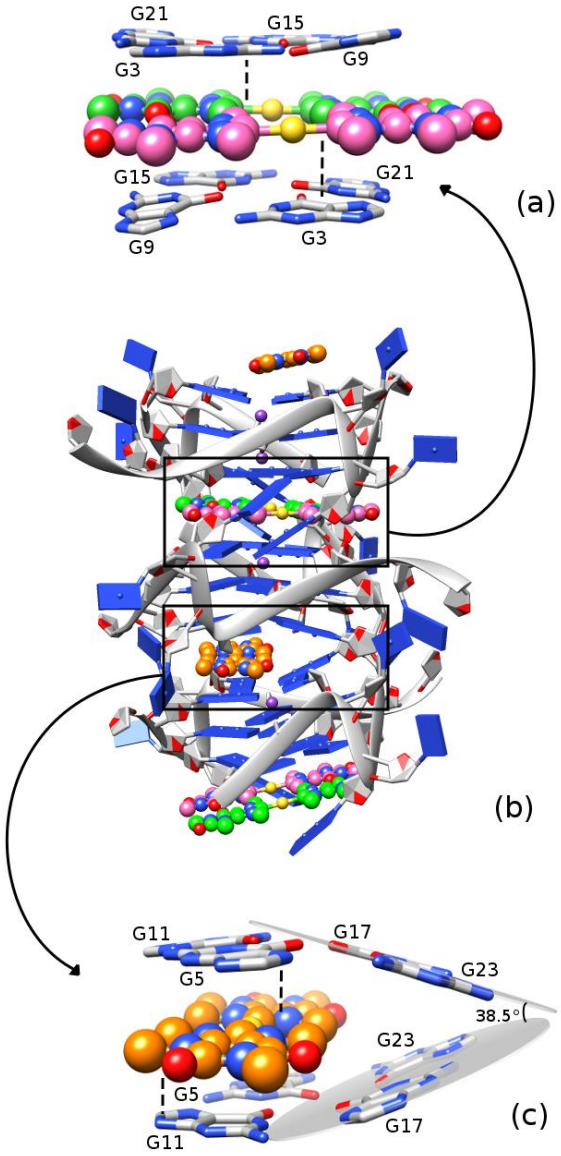


Figure 3. (a) 5'-5' end binding site of the Tel 23:[Au(9-methylcaffein-8-ylidene)2]+ adducts; (b) columnar disposition of the propeller Tel 23:[Au(9-methylcaffein-8-ylidene)2]+ adducts featuring the overall crystal packing; (c) 3'-3' end binding site of the Tel 23:[Au(9-methylcaffein-8-ylidene)2]+ adducts, with indication of the dihedral angle formed by symmetry related G17 and G23 residues. All interplanar distances (dashed lines) are ca. 3.4 Å. Symmetry independent complex molecules are indicated by using different colors.

The 2-fold symmetry, featuring the binding sites, also applies to **complex I**: in the 3'-3' end site the metal ion perfectly lies on the crystallographic axis while the caffeine units are disordered and shared between the 2-fold symmetry related positions (Figure ESI-S2). In the 5'-5' site both stacked [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ ions place their metal center near, but not above, the 2-fold axis (Figure 4). This kind of 2-fold pseudo-symmetric disorder is rather common, having been found in several of the crystal structures reported so far for human telomeric quadruplex/ligand adducts[25-28], and it is irrespective of the nature of the interacting ligand. This observation supports the view that binding processes are mainly driven by adirectional forces, such as electrostatic, Van der Waals and π-π stacking interactions, which, require broad contact surfaces.

Nevertheless, as clearly shown in Figure 4 and Figure ESI-S2, only one caffeine unit, out of two potentially available per metal complex, interacts with the guanine residues, the other one protruding outside and giving little or almost no overlap with the tetrad. Actually, the [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ ion is longer than the tetrad side, but it could give a good dimensional matching with the tetrad diagonal (about 16 Å for both the metal complex and the tetrad diagonal vs. 11 Å for the tetrad side). In the 3'-3' end site, the 38.5° dihedral angle formed by the symmetry related G17 and G23 residues (Figure 3c) prevents the [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ ion to be diagonally located (Figure 3c). However, in the 5'-5' end site, the tetrad is completely available, and one can only suppose that the partial coordination of two metal complexes provides a greater extent of stabilization than the stacking of two caffeines belonging to only one metal compound in diagonal position with respect to the guanine tetrad. In this respect, it should be noted that all the gold(I) centers in the Tel23:[Au(9-methylcaffein-8-ylidene)$_2$]$^+$ adduct are far from the central channel defined by the guanine carbonyl oxygens. Instead, they are always sandwiched between two pyrimidine rings, one from each symmetric guanine tetrad, at about 3.5 Å from a nitrogen atom (Figure 4) or from a carbon atom (Figure ESI-S2). This feature highlights a significant difference from the salphen metal complexes previously reported by Campbell et al.[21], where the metal centers are placed almost in line with the central ion channels, without specific contact with the quadruplex unit.

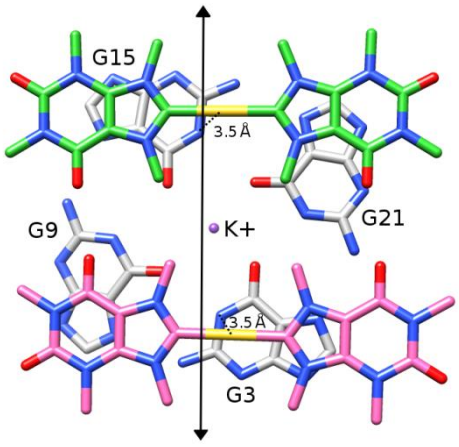


Figure 4. Structural details for the gold(I) complex in the 5'-5' binding site

It should be mentioned that the symmetric binding sites found in the Tel 23:[Au(9-methylcaffein-8-ylidene)$_2$]$^+$ adduct could not be formed by adjacent quadruplex units along the same telomeric polymer. Nevertheless, the actual structure for human telomeric DNA *in vivo* is still unknown, and different kinds of higher-order arrangements, afforded by non-consecutive G-quadruplexes belonging to long telomeric sequences, should be taken into account, which may still produce the observed adducts.

In conclusion, we have described here, through a joint ESI-MS and XRD investigation, the adduct formed between a human telomeric DNA G-quadruplex and a cationic gold(I) dicarbene complex. In agreement with ESI-MS results, XRD data evidence that three gold(I) complexes binds the G quadruplex at two distinct sites. Details of the contacts between the metal complex and DNA G-quadruplex were carefully analyzed to dissect the molecular basis of such tight interaction. Interestingly, the greater selectivity of [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ for DNA G-quadruplex structures over double helix DNA can be tentatively explained as follows. Actually, the double helix base pair, dimensionally similar to a G4 tetrad side, is too short in comparison to the elongated size of our gold(I) complex. In ds-DNA the phosphate backbones limit the space available to a parallel intercalating ligand. Instead in Tel 23:[Au(9-methylcaffein-8-ylidene)2]+ adduct, only one caffeine unit per ligand molecule interacts with the guanine residues, while the other one protrudes outside the tetrad surface, which in the all parallel propeller folding is not hindered by lateral or diagonal loops. Overall, the reported results shed further light into the possible mechanisms of anticancer activity of complex I, for which G4-DNA seems to be a relevant pharmacological target and inhibition of the enzyme telomerase a realistic mode of action.

Acknowledgment

We gratefully acknowledge ECRF-Ente Cassa di Risparmio di Firenze for a grant to FP. Beneficentia Stiftung (Vaduz, Liechtenstein),COST Action CM1105 and AIRC16049 are also acknowledged for generous financial support.

Received: ((will be filled in by the editorial staff))
Published online on ((will be filled in by the editorial staff))

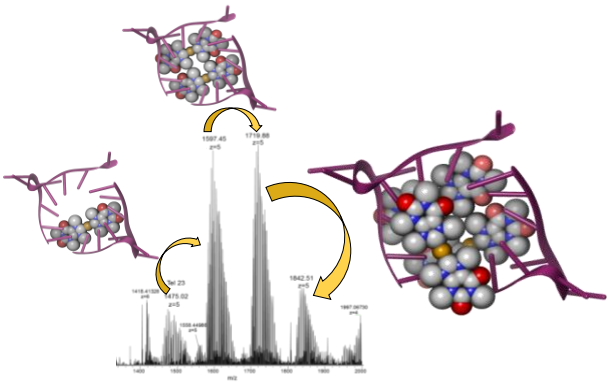
Keywords: G-quadruplex · gold compounds · cancer ·ESI-Mass Spectrometry· X-ray diffraction

[1] Ott, I. *Coordin. Chem. Rev*. **2009**, 253, 1670.
[2] Roder, C.; Thomson, *M.J. Drugs R. D*. **2015**, 15, 13.
[3] Bertrand, B.; Casini, A. *Dalton Trans*. **2014**, 43, 4209.
[4] Kim, N.H.; Park, H.J.; Oh M.K.; Kim, I.S. *BMB Rep*. **2013**, 46, 59.
[5] Maji, B.; Bhattacharya, S. *Chem. Commun*. **2014**, 50, 6422.
[6] Murat, P.; Balasubramanian, S. *Curr. Opin. Genet. Dev*. **2014**, 25, 22.
[7] Burge, S.; Parkinson, G.N.; Hazel, P.; Todd, A.K.; Neidle, S. *Nucleic Acids Res*. **2006**, 34(19), 5402.
[8] Adrian, M.; Heddi, B.; Phan, A.T. *Methods* **2012**, 57, 11.
[9] Salgado, G.F.; Cazenave, C.; Kerkour, A.; Mergny, J.L. *Chem. Sci*. **2015**, 6, 3314.
[10] Parkinson, G.N.; Lee, M.P.H.; Neidle, S. *Nature* **2002**, 417, 876.
[11] Collie, G.W.; Campbell, N.H.; Neidle, S. *Nucleic Acids Res*. **2015**, 43(10), 4785.
[12] Tan, Z.; Tang, J.; Kan, Z.Y.; Hao, Y.H. *Curr. Top. Med. Chem*. **2015**, 15(19), 1940
[13] Gonçalves, D.P.N.; Rodriguez, R.; Balasubramanian, S.; Sanders, J.K.M. *Chem. Commun.* **2006**, 4685.
[14] Chen, Z.F.; Qin, Q.P.; Qin, J.L.; Liu, Y.C.; Huang, K.B.; Li, Y.L.; Meng, T.; Zhang, G.H.; Peng, Y.; Luo, X.J.; Liang, H. *J. Med. Chem.* **2015**, 58, 2159.
[15] Chen, Z.F.; Qin, Q.P.; Qin, J.L.; Zhou, J.; Li, Y.L.; Li, N.; Liu, Y.C.; Liang, H. *J. Med. Chem.* **2015**, 58, 4771.
[16] Düchler, M. J. *Drug Targeting* **2012**, 20, 389.
[17] Arola, A.; Vilar, R. Curr. Top. Med. Chem. 2008, 8, 1405.
[18] Suntharalingam, K.; White, A.J.P.; Vilar, R. *Inorg. Chem*. **2010**, 49, 8371.
[19] Georgiades, S.N.; Abd Karim, N.H.; Suntharalingam, K.; Vilar, R. *Angew. Chem. Int. Ed.* **2010**, 49, 4020.
[20] Ralph, S.F. *Curr. Top. Med. Chem.* **2011**, 11, 572.
[21]Campbell, N.H.; Abd Karim, N.H.; Parkinson, G.N.; Gunaratnam, M.; Petrucci, V.; Todd, A.K.; Vilar, R.; Neidle, S. *J. Med. Chem* **2012**, 55, 209.
[22] Bertrand, B.; Stefan, L.; Pirrotta, M.; Monchaud, D.; Bodio, E.; Richard, P.; Le Gendre, P.; Warmerdam, E.; de Jager, M.H.; Groothuis, G.M.M.; Picquet, M.; Casini, A. *Inorg. Chem*. **2014**, 53, 2296.
[23] Stefan, L.; Bertrand, B.; Richard, P.; Le Gendre, P.; Denat, F.; Picquet, M.; Monchaud, D. *Chem. Bio. Chem*. **2012**, 13, 1905.
[24] Gratteri, P.; Massai, L.; Michelucci, E.; Rigo, R.; Messori, L.; Cinellu, M.A.; Musetti, C.; Sissi, C.; Bazzicalupi, C. *Dalton Trans.* **2015**, 44, 3633.
[25] Parkinson, G.N.; Cuenca, F.; Neidle, S. *J. Mol. Biol*. **2008**, 381, 1145.
[26] Micco, M.; Collie, G.W.; Dale, A.G.; Ohnmacht, S.A.; Pazitna, I.; Gunaratnam, M.; Reszka, A.P.; Neidle, S. *J. Med. Chem.* **2013**, 56, 2959
[27] Bazzicalupi, C.; Ferraroni, M.; Bilia, A.R.; Scheggi, F.; Gratteri, P. *Nucleic Acids Res*. **2013**, 41, 632.
[28] Parkinson, G.N.; Ghosh, R.; Neidle, S. *Biochemistry* **2007**, 46, 2390.

Entry for the Table of Contents

Carla Bazzicalupi, Marta Ferraroni, Francesco Papi, Lara Massai, Benoît Bertrand, Luigi Messori, Paola Gratteri,* Angela Casini.*

Determinants for Tight and Selective Binding of a Medicinal Dicarbene Gold(I) Complex to a Telomeric DNA G Quadruplex: a joint ESI MS and XRD Investigation



Crystal structure of the adduct formed between $[Au(9\text{-methylcaffein-8-ylidene})_2]^+$ and Tel 23 DNA G quadruplex. Tel 23 maintains, in the adduct, a characteristic propeller conformation, while binding three gold(I) dicarbene moieties, at two distinct sites.