Tridentate N-Donor Palladium(II) Complexes as Efficient Coordinating Quadruplex DNA Binders


Abstract: Fifteen complexes of palladium, platinum, and copper, featuring five different N-donor tridentate (terpyridine-like) ligands, were prepared with the aim of testing their G-quadruplex–DNA binding properties. The fluorescence resonance energy transfer melting assay indicated a striking positive effect of palladium on G-quadruplex DNA stabilization compared with platinum and copper, as well as an influence of the structure of the organic ligand. Putative binding modes (non-coordinative π stacking and base coordination) of palladium and platinum complexes were investigated by ESI-MS and UV/Vis spectroscopy experiments, which all revealed a greater ability of palladium complexes to coordinate DNA bases. In contrast, platinum compounds tend to predominantly bind to quadruplex DNA in their aqua form by noncoordinative interactions. Remarkably, complexes of [Pd(tpy)] and [Pd(tMebip)] (tpy = tolylterpyridine, tMebip = 2,2’-(4-p-tolylpyridine-2,6-diyl)bis(1-methyl-1H-benzo[d]imidazole)) coordinate efficiently G-quadruplex structures at room temperature in less than 1 h, and are more efficient than their platinum counterparts for inhibiting the growth of cancer cells. Altogether, these results demonstrate that both the affinity for G-quadruplex DNA and the binding mode of metal complexes can be modulated by modifying either the metal or the organic ligand.

Keywords: DNA · FRET · G-quadruplexes · mass spectrometry · transition metals

Introduction

Guanine (G)-rich DNA and RNA sequences have the ability to fold into four-stranded helicoidal structures called G-quadruplexes (abbreviated to G4s). A large number of sequences identified by bioinformatics studies[1] may form quadruplexes (abbreviated to G4s). A large number of G-quadruplex structures, which share the G-quartet as a common monomeric motif, but differ in the loop arrangements.[2] A G-quartet is the association of four guanines by Hoogsteen hydrogen bonds in a coplanar fashion. This motif self-stacks through π–π aromatic forces and by sandwiching alkali cations (Na+, K+), which participate greatly in the stability of the quadruplex structure. It is now widely assumed that G4 DNA may interfere with various biological events related to the transfer and maintenance of genetic information (replication, transcription, telomeric functions, translation, etc.), and thereby, could be involved in the regulation of gene expression.[3] The synthesis of small molecules capable of targeting G4 DNA is a rapidly expanding field: these compounds could act pharmacologically through sequence/structure specificity and allow better understanding of the biological role(s) of quadruplexes.[4] Small molecules usually bind to G4 DNA through π-stacking interactions with external G-quartets, but some also interact with the loops[5] and grooves.[6]

Since the discovery more than 40 years ago that cisplatin derivatives could act as antitumor agents,[7] an extensive number of metal complexes aimed at binding DNA have been synthesized. Predictably, metal complexes have also emerged in the G4 DNA field and display various binding modes (π stacking, metalation of bases, cleavage).[8] The advantages of using metal complexes are numerous. Different metal cations adopt diverse geometries for a given ligand, while introducing positive charges. Moreover, the metal can withdraw electrons from the ligand, thus making it more suitable for π stacking with electron-donor bases, such as guanines. Terpyridine (tpy) derivatives with a tolyl moiety (ttpy) or an extended aromatic surface (dibenzotropolyl (BisQ); Scheme 1) have been successfully used to prepare transition-metal complexes (Pt2+, Cu2+) that have shown high binding affinity for the human telomeric G4.[8] In particular, [Pt(tppy)] selectively platinates adenine bases located in the loops of this quadruplex structure.[8] Square-planar platinum(II) complexes, with two exchangeable ligands, such as cisplatin,[11] or one, such as tpy complexes,[12] can coordi-
nate to DNA bases (with a strong preference for guanine N7) after a hydrolysis step (aquation), which is the rate-limiting step and highly dependent on the chelating ability of the ligand coordinated to the metal.[13] Palladium(II) and platinum(II) are soft Lewis acids and share the same square-planar geometry.[14] They display similar characteristics when coordinated to N-donor polydentate ligands, but interestingly, the key difference rests on the ligand-exchange kinetics, in particular, during the aquation reaction. Hydrolysis rates are much faster for palladium than for platinum complexes (10^5 times faster according to instrumental methods,[15] and 10^6 times estimated by ab initio studies [16]). This results in kinetic instability of Pd–DNA complexes relative to their Pt counterparts, which has limited the use of Pd derivatives for DNA targeting and for biomedical applications. Nevertheless, because this property can be modulated by the nature of the heterocyclic ligand surrounding the metal, interest is now gradually shifting towards palladium and other transition-metal complexes for biomedical applications.[17]

Based on the reasons outlined above, we speculated that the introduction of palladium to our metal tpy G4 binders could modulate both noncoordinative binding and the coordination capability of quadruplex structures. Therefore, we launched a program aimed at modulating both the ligand surface and the coordinating metallic cation. It is worth noting that palladium complexes devoted to quadruplex DNA recognition have already been synthesized by Vilar et al. but the series investigated did not display strong binding affinities for the targeted DNA.[18]

Bis(N-methylbenzimidazolyl)pyridine (Mebip) is a tridentate nitrogen ligand often used in inorganic chemistry as an analogue of tpy, but compared with the latter, it is a moderate σ donor and also a π acceptor.[19] The larger aromatic surface, relative to tpy, seems to be more suited to π overlap with a G-quartet. [46] For instance, the benzimidazole motif is a purine mimic that may stack efficiently on DNA bases[20] and has already been used to construct quadruplex-DNA ligands.[21] In addition, the bis(benzimidazole)pyridine core was recently shown to lead to efficient quadruplex ligands.[21a] including luminescent platinum complexes.[22] In the case of the tpy complexes, a tolyl group in the para position of the central pyridine ring dramatically increased the quadruplex binding affinity.

We therefore envisaged the preparation of the tolyl derivative of the Mebip ligand (tMebip; 2,2’-(4′-tolylypyridine-2,6-diyl)bis(1-methyl-1H-benzimidazole)), which combined the structural features of tpy and benzimidazole scaffolds, to prepare a metal complex with improved G4-recognition properties. Similarly, the tolyl moiety was introduced into the dibenzo[pyrylidine (BisO) scaffold used in previous studies[10] thereby affording a second ligand (tBisQ) featuring a tolylpyridine core and an extended aromatic surface. The five ligands derived from the tpy model (Scheme 1) were combined with the three metallic cations (Pd^{2+}, Cu^{2+}, Pt^{2+}). Binding affinities, binding modes, and binding kinetics of the five metal complex families for G4 DNA were investigated by fluorescence resonance energy transfer (FRET) melting,[23] electrospray mass spectrometry experiments,[24] and time-dependent UV/Vis absorbance measurements.

Results and Discussion

Synthesis of complexes: tMebip was prepared in three steps from chelidamic acid, with an overall yield of 45% (Scheme 2). Formation of benzimidazoles was achieved by thermal cyclization of chelidamic acid and N-methyl-1,2-phenylenediamine in the presence of polyphosphoric acid, which was used as a solvent.[25] Position 4 of the pyridine was then brominated by phosphorus oxybromide,[26] allowing

Scheme 1. Structures of tridentate N-donor ligand described herein.

Scheme 2. Synthesis of tMebip complexes. Reagents and conditions: a) HOOC\N\COOH, N-methylbenzene-1,2-diamine, polyphosphoric acid (PPA), 215°C, 17 h; b) POBr3, 140°C, 17 h; c) p-tolylboronic acid, [Pd(AcAc)2] (cod = 1,5-cyclooctadiene), DMF, 50°C, 48 h, 42% yield; d) K2PtCl4, nitromethane, reflux, 24 h, 45% yield; e) [Pd(Mebip)] d) M = Pt, L = Cl, n = 1 [Pd(Mebip)] e) M = Pd, L = Cl, n = 1 [Cu(Mebip)] f) M = Cu, L = NO3, n = 2

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the introduction of the tolyl group through a Suzuki coupling.[27] The final product was easily obtained because it precipitated at room temperature in the THF/water mixture used as the solvent. This synthesis can be performed on the gram scale and requires few purification steps.

BisQ was prepared by following procedure described in the literature[10] based on a double Friedländer condensation between two equivalents of 2-aminobenzaldehyde and 2,6-acetylpyridine (Scheme 3). 2-Aminobenzaldehyde is not stable,[28] and thus, it was generated in situ by reduction of 2-nitrobenzaldehyde.[29] An overall yield of 75% was obtained for the two-step, one-pot process.

The synthesis of tBisQ was less straightforward and was achieved in 4 steps (Scheme 4) with an overall yield of 27%. The key intermediate, 2-acetylquinoline (5), was obtained by esterification of quinaldic acid, followed by Claisen condensation, and then a saponification/decarboxylation step. The ligand was finally obtained in 54% yield by a double Kröhnke reaction, using a double condensation with aqueous ammonia on 2-acetylquinoline, ammonium acetate, and 4-methylbenzaldehyde under harsh conditions in a sealed tube at 100°C for 24 h.[30]

Palladium, platinum, and copper complexes of BisQ, tBisQ, and tMebip were prepared by adapting procedures described in the literature.[9,31] These complexes were obtained in lower yields than those with tpy and ttpy, presumably because of their larger aromatic surface, which delocalizes the nitrogen lone-pair electrons.

FRET melting assay: The binding performances of the free ligands and their corresponding complexes were first evaluated by FRET melting assays. This well-known assay is based on monitoring the stability induced by binding of a ligand to a fluorescently labeled quadruplex structure, such as the human telomeric sequence F21T (FAM-G3A C H T U N G T R E N N U N G (T2AG3)3-Tamra)] or other G4-forming sequences (F21RT, F25CebT, F21CTAT, FmycT, Fkit1T, Fkit2T; see the Experimental Section and the Supporting Information).[23] The stabilization, which was measured by a FRET effect, was expressed as the increase in melting temperature of the labeled oligonucleotide ($\Delta T_{1/2}$) induced by the ligand. Addition of an unlabeled DNA competitor (for example, duplex DNA) enables one to evaluate the selectivity for the targeted quadruplex structure.

The FRET melting data, summarized in Figure 1, indicate little or no stabilizing effect of the free organic ligands (BisQ, tBisQ, and tMebip), thereby confirming the crucial effect of the metallic cation, as previously observed.[9] Remarkably, compared with the corresponding Pt$^{2+}$ and Cu$^{2+}$ complexes, Pd$^{2+}$ derivatives are clearly the most efficient at

**Scheme 3.** Synthesis of BisQ complexes. Reagents and conditions: a) iron powder, 0.1 x HCl (aq), 95°C, 30 min; b) 2,6-diacyetylpyridine, potassium hydroxide, 95°C, 1 h; 75% yield over two steps in a one-pot procedure; c) K$_2$PtCl$_4$, nitromethane, reflux, 24 h, 21% yield; d) [Pd(cod)Cl$_2$], DMF, 50°C, 24 h, 84% yield; e) Cu(NO$_3$)$_2$, CH$_3$Cl, acetonitrile, 4°C, 48 h, 52% yield. 

**Scheme 4.** Synthesis of tBisQ complexes. Reagents and conditions: a) sulfuric acid, MeOH, reflux, 16 h, 89% yield; b) BuOK, ethyl acetate, RT, 15 min, 79% yield; c) HCl, dioxane, 100°C, 16 h, 70% yield; d) 4-methylbenzaldehyde, KOH, aqueous ammonia, EtOH, 100°C, sealed tube, 24 h, 54% yield; e) K$_2$PtCl$_4$, nitromethane, reflux, 24 h, 94% yield; f) [Pd(cod)Cl$_2$], DMF, 50°C, 24 h, 40% yield; g) Cu(NO$_3$)$_2$, CH$_3$Cl, acetonitrile, 65%.

![Figure 1. FRET melting stabilization ($\Delta T_{1/2}$ in °C) of the human telomeric sequence F21T (0.2 μM) in lithium cacodylate buffer (10 mM), KCl (10 mM), and LiCl (90 mM) in the presence of metal complexes or the corresponding free ligand (5 equiv). Ligand: white, Cu$^{2+}$: diagonal hatching, Pt$^{2+}$: horizontal hatching, Pd$^{2+}$: black.](/C23/2011/Wiley-VCH-Verlag-GmbH-Co-KGaA-Weinheim/Chem-Eur-J-2011-17-13274-13283)
stabilizing human telomeric G4. The tpy series represents a striking example of the pronounced effect of Pd$^{2+}$. [Pd(tpy)] induces strong stabilization of the quadruplex structure ($\Delta T_{1/2} = +17.3^\circ$C), whereas the two other tpy complexes exhibit a negligible effect ($\Delta T_{1/2} < +2^\circ$C). Similarly, stabilization in the tMebip series is particularly impressive: $\Delta T_{1/2}$ for the Pd$^{2+}$ complex almost reaches the limit of the test with a $\Delta T_{1/2}$ value of around $+35^\circ$C, whereas $\Delta T_{1/2}$ values of around $10-12^\circ$C are recorded for the Cu$^{2+}$ and Pt$^{2+}$ counterparts. The same trend was observed in the tpy series, but to a lesser extent ($\Delta T_{1/2} = +20.3$, $+12.9$, $+6.3^\circ$C for Pd, Pt, and Cu derivatives, respectively). Conversely, the effect of palladium was much less striking in the BisQ and tBisQ series, for which the three complexes showed similar performances ($7-10^\circ$C $< \Delta T_{1/2} < 14^\circ$C). The hindrance of the aromatic surface of these ligands is likely to counterbalance the effect of the metallic cations, suggesting that in those cases the interaction is dominated by the aromatic ligand. On the other hand, the interaction is clearly governed by the nature of the metallic cation in the three other series (tpy, tpy, and tMebip). The beneficial effects of cation and ligand are clearly additive in the case of [Pd(tMebip)], which appears to be the best candidate of the fifteen complexes studied.

Therefore, the tMebip series was similarly assayed for a wider spread of quadruplex-forming sequences of biological interest. The results shown in Figure 2 confirm the stronger stabilization of almost all G4s by [Pd(tMebip)], compared with copper and platinum derivatives. Importantly, very low binding was observed when using the doubly labeled duplex (FdxT, 2.9$^\circ$C), revealing the strong preference of the compound for G4 structures. Notably, high quadruplex selectivity was also observed for the Cu$^{2+}$ and Pt$^{2+}$ complexes. The selectivity of [Pd(tMebip)], with regards to duplex structure, was also evaluated by competitive FRET melting using F21T and a 26 bp duplex (ds26) as a competitor. A high decrease was observed when adding the first dose (3 $\mu$m) of the duplex competitor, whereas the second dose (10 $\mu$m) did not cause a further decrease in stabilization (Figure 3). The same trend was observed for all Pd$^{2+}$ complexes, irrespective of the ligand (see Figure S1 in the Supporting Information), but not for the Pt$^{2+}$ and Cu$^{2+}$ complexes. Nevertheless, [Pd(tMebip)] retains a significant effect (+17$^\circ$C) under these conditions of harsh competition (50 equiv of duplex), and thus, remains the best candidate. The same experiment conducted at a lower concentration of [Pd(tMebip)] indicated that the decrease was abolished under these conditions of lower compound/DNA ratio, thereby suggesting that the excess compound may participate “nonspecifically” in the stabilization observed (Figure 3, left part).

This unusual behavior raises questions about the nature of the interaction and how to rationalize the effect of the palladium relative to the other cations, in particular, platinum. It could be hypothesized that a portion of the complex was bound externally, thus being easily redistributed on the competitor. In addition, metal coordination to DNA bases may occur during the experiment favored by the temperature increase. The $\Delta T_{1/2}$ value measured could thus result both from noncoordinative binding and from in situ coordination. To investigate further the nature of the interaction, in the case of Pd$^{2+}$ and Pt$^{2+}$ complexes, complementary experiments were conducted for the tpy and tMebip families by using isothermal methods, namely, ESI-MS and UV/Vis spectroscopy.

**ESI-MS:** Mass spectrometry was used to characterize the amount of ligand bound to duplexes and G4 structures.[24] Furthermore, analysis of the masses of the complexes formed between [Pt(tppy or tMebip)]/[Pd(tppy or tMebip)] and DNA revealed a particular binding mode. Figure 4 shows a magnification of the complexes detected with the tetramolecular G4 Q1, [TGGGGT]$_4$. Not shown is the signal of the free Q1 at charge state 5$,^+$ with an average $m/z$ of 1499.67. In the region corresponding to the complexes, three species can be distinguished: the association between DNA and the metal complex [Pt(tppy or tMebip)]/[Pd(tppy or tMebip)], the association between DNA and the aqua...
form of the metal complex [PtOH(tpy or tMebip)]/[PdOH(tpy or tMebip)] and with its chlorido form [PtCl(tpy or tMebip)]/[PdCl(tpy or tMebip)].

In all cases, the free complex is in the chlorido form, as shown by high-resolution mass spectrometry (HRMS) analysis. For the two platinum complexes (Figure 4A and B), the major form bound to the DNA is, however, the aqua form (Cl⁻ is replaced by OH⁻ bound to the metallic cation). For palladium derivatives (C and D), direct coordination to the DNA is the major form, the aqua form is minor, and the chloride form is undetected.

Figure 4. Nature of the 1:1 (DNA/ligand) complex of various metal derivatives ([Pt(tpy)] (A), [Pt(tMebip)] (B), [Pd(tpy)] (C), [Pd(tMebip)] (D)) with [d(TGGGGT)]₄ obtained 1 h after mixing. For platinum complexes (A and B) the binding of the aqua form (coordination of the Pt with OH) to Q₁ is predominant. Minor species are complexed with the chloride form and direct coordination with DNA (neither OH nor Cl bound to the metallic cation). For palladium derivatives (C and D), direct coordination to the DNA is the major form, the aqua form is minor, and the chloride form is undetected.

Scheme 5. Binding modes of [M(tMebip)] (M=Pt, Pd) observed by ESI-MS and time-dependent UV/Vis absorbance measurements. Complexes (A) can quickly π stack on a G-quartet (chlorido (B) and hydrolyzed (C) forms) and/or coordinate to DNA bases (D).

Figure 5. Amounts of metal complexes ([Pt(tpy)] (A), [Pt(tMebip)] (B), [Pd(tpy)] (C), [Pd(tMebip)] (D)) bound to different DNA G4s (Q₁ = [d(TGGGGT)]₄, Q₂ = human telomeric sequence d(GGGTTA)₃GGG) and duplexes (D₁ = d(CGCGGGGGCGCG), D₂ = d(CGCGAATTCGCG), D₃ = d(CGTAATTTACG)). The concentration was 4.8 μM of DNA and 8 μM of compound. The black bars correspond to the fraction of ligand directly coordinated to DNA and the grey bars correspond to the fraction of noncoordinately bound complexes (aqua and chlorido forms) 1 h after mixing.

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Quantification experiments with different quadruplexes (Q2 is the 21-nt human telomeric sequence and D1–D3 are 12 bp duplexes with decreasing GC content). Globally, the amount of compound bound to DNA was higher for ttpy complexes (Figure 5A and C) than for the tMebip counterparts (Figure 5B and D). The platinum derivative of
Palladium(II) Complexes Coordinating Quadruplex DNA

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Time-dependent UV/Vis absorbance spectra: In an attempt to gain further insight into the coordination capability of the two series, the kinetics of coordination to DNA were followed by UV/Vis spectroscopy measurements, as previously described for [Pt(tpy)] complexes.[35] The absorption spectrum of [Pd(tMeibp)] has a broad band with two local maxima at 330 and 400 nm, corresponding to the ligand-centered band (Figure 6). In the tail of this band, the weak contribution lying in the visible region is assigned to the metal-to-ligand charge-transfer band (MLCT).[36] Indeed this contribution cannot be the result of a metal centered d-d transition because it appears at a wavelength higher than 400 nm and is characterized by a low intensity.[37] The addition of tetceteric quadruplex DNA [22AG, 5'-AG₃(T₃AG₃)₃-3'] (1 equiv) resulted in moderate hypochromism, suggesting π stacking of the complex, presumably on a G4. After this initial step, the spectrum further evolved over time with prominent hyperchromism of the ligand band, displaying a maximum at 330 nm. Concomitantly, the MLCT band increased in intensity and was slightly redshifted, while one isosbestic point common to all curves remained at 425 nm. This indicates the existence of interconversion between two species and agrees with coordination of the complex to DNA, as evidenced by the ESI-MS data. Indeed, the substitution of an electron-withdrawing atom (chlorine) with a neutral donor (nitrogen lone pair from a DNA base) can increase the electron density of the metal center, and therefore, decrease the MLCT energy.[37–38] Finally, two other isosbestic points appeared successively (395 nm, plain lines (Figure 6, short dashed lines)), suggesting the existence of two reactions with dissimilar rate constants, which may correspond to two differently coordinated species.[39] In the case of [Pd(tpy)], the addition of G4 DNA also resulted in a rapid change of the absorption spectrum of the complex (Figure S3 in the Supporting Information). A significant hypochromism of the ligand band also suggested direct stacking of the complex on G4. The time evolution of the system

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was comparable to that of [Pd(tMebip)], that is, an increase in the absorbance of the ligand together with significant bathochromism of the MLCT. Overall, the UV data for [Pd(tMebip)] and [Pd(tpy)] agree with the mass spectral data and suggest an interaction with quadruplex DNA through first stacking over a G4 and subsequently by fast coordination (on a minute timescale).

In contrast, no detectable immediate change occurred after the addition of 22AG to [Pt(tMebip)] (Figure S4 in the Supporting Information). Then the spectrum evolved in a similar fashion to that of [Pd(tMebip)], but to a much lower extent, with almost no change monitored in the MLCT band. Conversely, addition of 22AG to [Pt(tpy)] led to rapid hypochromism followed by an increase in absorbance over time, along with the appearance of two simultaneous isosbestic points (350 and 415 nm; Figure S5 in the Supporting Information). Similar to [Pt(tMebip)], no significant modification of the MLCT was detected.

In vitro cytotoxicity: Finally, we evaluated the effect of tMebip and tpy complexes on the growth of several cancer cell lines. For both families, Pd complexes were highly efficient (Table S1 in the Supporting Information). In particular, [Pd(tpy)] strongly inhibited the proliferation of the three tested cell lines (KB, A549, and MCF7) with IC50 values in the nanomolar range (65–115 nM; Table S2 in the Supporting Information). This palladium complex was, in this regard, more efficient than [Pt(tpy)], which has IC50 values in the micromolar range. Whether the higher cytotoxicity of palladium derivatives is related to differences in the DNA interaction observed in vitro is not known and requires further investigations. Nevertheless, these results indicate that the novel compounds penetrate the cell membrane and exhibit a promising drug-like potential.

Conclusion

Using straightforward synthetic pathways, we prepared fifteen metal complexes, featuring three different metals, namely, copper(II), platinum(II), and palladium(II). Five structurally related tridentate N-donor ligands were used, including well known tpy and tpy, and the already studied dibenzo[24]crown-6 ether. To enhance the capability of metal complexes binding G4 DNA, two larger ligands (tBisQ and tMebip) were prepared. Unexpectedly, FRET melting experiments revealed, for the first time, a significant positive effect of palladium on the stabilization properties of the complexes for G4 DNA. We hypothesize that this striking difference between platinum and palladium complexes is due to a difference in the nature of the DNA binding interaction despite the fact that they share the same square-planar geometry. Consequently, ESI-MS and UV/Vis spectroscopy experiments were carried out and showed that, under the conditions examined, platinum complexes tended to predominantly bind G4 DNA in their aqua form by noncoordinative interactions, whereas palladium complexes coordinated DNA bases to a large extent in a short time. Notably, mass spectrometry revealed that, after incubation for 1 h at room temperature, a major fraction of the complexes involved palladated DNA. In addition, the palladium complexes may coordinate to thymines in the vicinity of the G4 core when no purine base is accessible. Overall, we demonstrated that tpy-like metal complexes could be finely tuned, by varying the nature of both metal and ligand, to target G4 by a multiple interaction based on noncoordinative π stacking and coordination to residues surrounding terminal G4s.

This particular interaction may open up perspective for specifically targeting G4 structures exhibiting proper loop conformations and/or dynamics.

Experimental Section

General: 1H and 13C NMR spectra were recorded at 25°C on a Bruker Avance 300 spectrometer using tetramethylsilane (TMS) as an internal standard. Deuterated CDCl3, and [D6]DMSO were purchased from SDS. The following abbreviations are used: singlet (s), doublet (d), triplet (t) and multiplet (m). Low-resolution ESI mass spectra were recorded on a micromass ZQ 2000 (High-resolution ESI mass spectra and elemental analyses were provided by the Institut de Chimie des Substances Naturelles (I.C.S.N., Gif-sur-Yvette, France). TLC analysis was carried out on silica gel (Merck 60F-254) plates with visualization at 254 and 366 nm. Preparative flash chromatography was carried out with Merck silica gel (Si 60, 40–63 lm). Reagents and chemicals were purchased from Sigma-Aldrich, Acros, or Alfa-Aesar unless otherwise stated. Solvents were purchased from SDS. Melting points were recorded on a Kofer melting point apparatus and are uncorrected. UV/Vis spectra were recorded on a Seccom Uvikon XL spectrophotometer and fluorescence melting curves were recorded on a Stratagene Mx 3005P real-time PCR machine. Oligonucleotides purified by reversed-phase HPLC were purchased from Eurogentec (Belgium).

Preparation of palladium complexes: A solution of dichloro(1,5-cyclooctadiene)palladium (1.2 equiv) in a minimum amount of CH2Cl2 was added to a solution of ligand (1 equiv) in a minimum amount of DMF. The resulting yellow solution was stirred for 2–3 d at room temperature under argon and protected from light. The resulting suspension was filtered. The solid was washed with DMF, CH2Cl2, and then diethyl ether. The powder was then dried under vacuum.

Preparation of platinum complexes: Platinum complexes with tpy and tpy were prepared as previously described. Other complexes were prepared as follows: Potassium platinum(IV) chloride (1 equiv) and sodium tetrafluoroborate (2 equiv) were added to a solution of ligand in dry nitromethane. The mixture was heated at reflux for 48 h under argon and protected from light. The NaCl precipitate was removed by hot filtration. The product was precipitated by addition of diethyl ether (when necessary), filtered through a membrane (Schleicher & Schuell, 1 lm), washed with CH2Cl2 and diethyl ether, then dried under vacuum.

Preparation of copper complexes: A solution of copper nitrate (1.1 equiv) in a minimum amount of anhydrous acetonitrile was carefully added dropwise to a solution of the ligand (1 equiv) in a minimum amount of CH2Cl2 to form two immiscible layers. The biphasic solution was kept at 4°C until complete coloration of the upper phase (typically 1–3 days). Green needles were filtered off; washed carefully with acetonitrile, CH2Cl2, and diethyl ether; then dried under vacuum.

Synthesis of 1: The procedure for the synthesis of 1 was adapted from literature. Chelidanic acid (3 g, 15 mmol) and N-methylphenylenediamine (3.4 mL, 30 mmol) in polyphosphoric acid (55 g) were added to a 250 mL round-bottomed flask to afford a red viscous solution that was stirred under argon at 250°C. After 48 h, the solution was poured (hot) into cold water (300 mL) under vigorous stirring. The blue solid was col-
lected by filtration, taken up into aqueous Na2CO3 (10%) at 110°C for 15 min, then filtered off. The solid was suspended in water (300 mL) and the pH was adjusted to four. The foamy solid was collected again by filtration. It was then recrystallized from hot DMSO by adding water until the solution became cloudy. After the solution cooled, compound I was recovered as white needles (4.4 g, 12.38 mmol, 83%). M.p. 135°C (decomp); 1H NMR (300 MHz, D2DMSO): δ = 11.0 (brs 1H), 7.84–7.58 (m, 2H), 7.47–7.12 (m, 4H), 4.25 ppm (s, 6H); 13C NMR (75 MHz, D2DMSO): δ = 165.5, 150.2, 149.5, 140.7, 136.7, 123.7, 123.0, 119.0, 113.1, 111.2, 32.7 ppm.

Synthesis of 2: Compound 2 was prepared from 1 as described in the literature.[8]

Synthesis of tMebip: Compound 2 (100 mg, 0.24 mmol), 4-tolylboronic acid (32.6 mg, 0.24 mmol), potassium acetate (99 mg, 0.72 mmol), and tetrazole (triphenylphosphine)palladium (17.3 mg, 0.015 mmol) were added to a degassed solution of THF (80 mL) and water (35 mL) to give a brown solution that was heated at reflux under argon for 24 h. The reaction mixture was allowed to cool slowly to room temperature. Light brown needles were filtered off. Recrystallization from THF/water afforded pure tMebip (66.8 mg, 65% yield). M.p. 215°C (decump); 1H NMR (300 MHz, D2DMSO): δ = 11.0 (brs 1H), 7.84–7.58 (m, 2H, 7.47–7.12 (m, 4H), 4.25 ppm (s, 6H); 13C NMR (75 MHz, D2DMSO): δ = 165.5, 150.2, 149.5, 140.7, 136.7, 123.7, 123.0, 119.0, 113.1, 111.2, 32.7 ppm.

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Synthesis of 3: In a 250 mL flask, quinaldic acid (4.12 g, 23.79 mmol) was dissolved in MeOH (40 mL). Concentrated H2SO4 (1 mL) was added and the mixture was heated at reflux for 16 h. After cooling to RT, the mixture was neutralized with a saturated aqueous solution of NaHCO3 and extracted with CH2Cl2 (3 × 100 mL). The organic phases were combined, dried with MgSO4, and evaporated to afford 3 as a white solid (3.97 g, 71.21 mmol, 89%). M.p. 83°C (81–83°C lit. [40]); 1H NMR (300 MHz, CDCl3): δ = 8.32 (d, J = 8.3 Hz, 2H), 8.21 (d, J = 8.5 Hz, 1H), 7.89 (d, J = 8.2 Hz, 1H), 7.80 (t, J = 7.7 Hz, 1H), 7.66 (t, J = 7.5 Hz, 1H), 4.09 ppm (s, 3H); 13C NMR (75 MHz, CDCl3): δ = 166.0, 147.9, 147.5, 137.33, 130.7, 130.3, 129.4, 128.6, 127.6, 121.0, 52.2 ppm; LRMS (m/z): 188.2 (M+H+).

Synthesis of 4:[9] Solid dibuOT (1.539 g, 13.39 mmol) was slowly added to a solution of 3 (2 g, 10.66 mmol) in ethyl acetate (35 mL). The mixture was stirred for 15 min at RT and then quenched with H2O (60 mL). The organic layer was separated and the aqueous phase was extracted with EtOAc (3 × 50 mL). The organic phases were combined, dried with MgSO4, and evaporated. The residue was purified by flash chromatography (EtOAc/cyclohexane, 1:4) to afford 4 as an off-white paste (2.06 g, 8.47 mmol, 79%). 1H NMR (300 MHz, CDCl3): δ = 8.27 (d, J = 8.3 Hz, 1H), 8.16 (d, J = 7.2 Hz, 2H), 7.78 ppm (d, J = 8.1 Hz, 1H), 7.64 (t, J = 7.5 Hz, 1H), 4.36 (s, 2H), 4.22 (q, J = 7.2 Hz, 2H), 1.26 ppm (t, J = 7.2 Hz, 3H).

Synthesis of 5:[10] In a 250 mL flask, compound 4 (2.06 g, 7.95 mmol) was dissolved in dioxane (20 mL). HCl (1M, 20 mL) was added and the solution was stirred at 100°C for 16 h, then it was concentrated under reduced pressure. The residual aqueous phase was extracted with EtOAc (3 × 50 mL). The organic phases were combined, washed with a saturated aqueous solution of NaHCO3 and dried with MgSO4, and evaporated. The residue was purified by chromatography on a small silica gel column to afford 5 as a white solid (954 mg, 5.57 mmol, 70%). M.p. 49°C; 1H NMR (300 MHz, CDCl3): δ = 8.27 (d, J = 8.5 Hz, 1H), 8.20 (d, J = 8.5 Hz, 1H), 8.13 (d, J = 8.5 Hz, 1H), 7.87 (d, J = 8.1 Hz, 1H), 7.79 (t, J = 7.7 Hz, 1H), 7.65 (t, J = 7.5 Hz, 1H), 2.38 (s, 3H) ppm; 13C NMR (75 MHz, CDCl3); δ = 200.7, 153.2, 147.2, 136.9, 130.6, 129.6, 126.6, 127.7, 118.0, 25.6 ppm; LRMS: m/z: 194.2 [M+N]+.

Synthesis of BisQ: Compound 5 (100 mg, 0.584 mmol) was added in a solution of 4-methylbenzaldehyde (35.1 mg, 0.292 mmol), potassium hydroxide (EtOAc/cyclohexane, 1:4) to afford 5 as an off-white paste (2.06 g, 8.47 mmol, 79%). 1H NMR (300 MHz, CDCl3): δ = 8.27 (d, J = 8.3 Hz, 1H), 8.16 (d, J = 7.2 Hz, 2H), 7.78 ppm (d, J = 8.1 Hz, 1H), 7.64 (t, J = 7.5 Hz, 1H), 4.36 (s, 2H), 4.22 (q, J = 7.2 Hz, 2H), 1.26 ppm (t, J = 7.2 Hz, 3H).

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Palladium(II) Complexes Coordinating Quadruplex DNA


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2.34 ppm (s, 3H); LRMS (in presence of acetonitrile): m/z: 654.1 [M+H]+; HRMS: m/z calcd for C60H42N7CI6Pt: 654.1073; found: 654.1124.

[C(5,11)G]: Green needles (65%); LRMS: m/z (%): 581.15 (15) [M+H]+, 486.1 (100) [M–2NO3]+; HRMS: m/z calcd for C59H37N7CI4Cu: 486.1031; found: 486.1038.

**Pd(tpp)**: Yellow powder (99%); m.p. > 260°C (decomp): [1] H1NMR (300 MHz, D2-DMSO): δ: 8.95 (d, J = 5.4 Hz, 2H), 8.86 (d, J = 7.8 Hz, 2H), 8.71 (d, J = 5.4 Hz, 2H), 8.50 (d, J = 8.0 Hz, 2H), 8.13 (d, J = 8.1 Hz, 2H), 7.89 (J = 6.8 Hz, 2H), 7.49 (δ = 8.2 Hz, 2H), 7.24 (s, 3H) ppm; LRMS (in presence of acetonitrile): m/z: 376 [M+H]+; 365.0 [M–C4H4+]; HRMS: m/z calcd for C58H34N6CI5Pd: 375.0870; found: 375.0968.

**Pd(tpp)**: Orange powder (78%); m.p. > 260°C (decomp): [1] H1NMR (300 MHz, D2-DMSO): δ: 8.95 (d, J = 5.4 Hz, 2H), 8.86 (d, J = 7.8 Hz, 2H), 8.71 (d, J = 5.4 Hz, 2H), 8.50 (d, J = 8.0 Hz, 2H), 8.13 (d, J = 8.1 Hz, 2H), 7.89 (J = 6.8 Hz, 2H), 7.49 (δ = 8.2 Hz, 2H), 7.24 (s, 3H) ppm; LRMS (in presence of acetonitrile): m/z: 466.0 [M+H]+; 455.1 [M–C4H4+]; HRMS: m/z calcd for C58H34N6CI5Pd: 464.0146; found: 464.0168.

**FRET melting**: FRET melting assays were performed with oligonucleotides that mimic the human telomeric sequence, as well as other quadruplex-forming oligonucleotides (25Ceb, 21CTA, c-Myc, c-Kit1, c-Kit2), and the control duplex d26, equipped with FRET partners at each extremity (see sequences given in the Supporting Information). Measurements were made with excitation at 492 nm and detection at 516 nm in a 10 mM lithium cacodylate pH 7.2 buffer supplemented with 10 mM KCl and 90 mM LiCl.

**ESI mass spectrometry**: ESI-MS experiments were performed on a Solar-ix 9.4 FTICR mass spectrometer (Bruker Daltonics, Bremen, DE). The electrospray source was used in negative ion mode with a capillary voltage of –3.1 kV. The source parameters were tuned so as to minimize collisional activation (source pressure 3.1 mbar, skimmer at –20 V). The instrument was externally calibrated with sodium iodide (1 ppm accuracy). High-resolution mass spectra and comparison with theoretical isomeric patterns were performed to unambiguously assign the nature of the observed complexes. Oligodeoxynucleotides d-CGTAATAATTACG (3644.45 Da), d-CCGCCATTCCGGC (3646.44 Da), d-CCGGCGCCGGCG (3678.40 Da), and d-CCGGTGTACGGG (3655.35 Da) were purchased from Eurogentec (Angleur, Belgium) and used without further purification. Duplex and quadruplex solutions were prepared in ammonium acetate (150 mM) according to previous reports.[38] The quantification of free DNA and complexes was performed on the most abundant charge state (5−).

**Molecular modeling**: Optimized structures of OH-Pt[Pd(Mebip)] and Thymine-Pt[Pd(Mebip)] were obtained by using DFT with the hybrid functional B3LYP and the 6–1G(d) basis set. The LANL2DZ effective core potential was used to model the ligand. [Pd(ttpy)] appeared to be a much more potent ligand and IC50 values were determined.

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