Structural Studies on Dinuclear Ruthenium(II) Complexes That Bind Diastereoselectively to an Antiparallel Folded Human Telomere Sequence

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ABSTRACT: We report DNA binding studies of the dinuclear ruthenium ligand \([(Ru(phen)_2)_2tpphz]\) in enantiomerically pure forms. As expected from previous studies of related complexes, both isomers bind with similar affinity to B-DNA and have enhanced luminescence. However, when tested against the G-quadruplex from human telomeres (which we show to form an antiparallel basket structure with a diagonal loop across one end), the \(\Lambda\Lambda\) isomer binds approximately 40 times more tightly than the \(\Delta\Delta\), with a stronger luminescence. NMR studies show that the complex binds at both ends of the quadruplex. Modeling studies, based on experimentally derived restraints obtained for the closely related \([(Ru(bipy)_2)_2tpphz]\), show that the \(\Lambda\Lambda\) isomer fits neatly under the diagonal loop, whereas the \(\Delta\Delta\) isomer is unable to bind here and binds at the lateral loop end. Molecular dynamics simulations show that the \(\Delta\Delta\) isomer is prevented from binding under the diagonal loop by the rigidity of the loop. We thus present a novel enantioselective binding substrate for antiparallel basket G-quadruplexes, with features that make it a useful tool for quadruplex studies.

INTRODUCTION

Although it has been known for five decades that guanine-rich nucleic acids can form four-stranded structures, research into quadruplex DNA has rapidly escalated in recent years. One reason for continued interest is the demonstration that telomeres can and do fold into quadruplex structures in vivo.1–3 Shortening of telomeres on chromosomal replication is considered to be a major cause of senescence, and cancer cells have been shown to generate an immortal phenotype by upregulating telomerase.4,5 The activity of telomerase is inhibited by the presence of G-quadruplexes,6 leading to the possibility of novel anticancer agents that work by binding to and stabilizing such quadruplexes.

A second reason for interest is the observation that quadruplexes are found not only in telomeres but also in other parts of the genome. Typically they are found in upstream promoters7–9 and in some cases have been shown to perform a regulatory function on downstream genes.10–13 Quadruplexes are also formed by RNA and again are likely to have regulatory roles on translation.14 For all these reasons, there is considerable interest in finding small molecules that bind to quadruplexes and stabilize them and that could act as markers for their presence.

Over the past few years, it has become abundantly clear that guanine-rich sequences can fold into quadruplexes in many different ways.15 A given sequence can also fold differently depending on solution conditions, including countercations (potassium or sodium), molecular crowding,16–18 and dehydration.19 A case in point is the human telomere sequence, HTS, \(d\AG_3(TTAG_3)_3\), which has been observed in several conformations.20–27 It appears that such behavior is common.28 This plasticity makes it all the more important to identify small molecules that bind specifically to particular conformations and stabilize them, especially if the function and dysfunction of quadruplexes in normal and abnormal cellular function are to be delineated.29

Despite this importance, there is little detailed crystallographic or NMR data on ligand–HTS quadruplex structures.28,30,31 Of relevance to this work, there is only one report on metal complexes.32 Only four X-ray structures involving an intramolecular quadruplex have been reported, all of which involve the all-parallel conformer with the ligand end-stacking on terminal G-tetrads.33–36 Although, as outlined above, telomere sequences can take up a range of topologies, virtually all the other reported structures also involve ligands bound to all-parallel conformers, comprising tetramolecular or bimolecular quadruplexes.37–41 Indeed, given this paucity of data and...
Figure 1. (A) Structures of complexes studied. (B) The two possible enantiomers of each of the metal centers in 1 and 2. (C) Schematic of the two diastereomers of 1 and 2 relevant to this study: left ΔΔ; right ΛΛ.

As part of a program to develop luminescent metal complexes as sequence and structure specific DNA binding substrates, we have studied the quadruplex binding properties of dinuclear ruthenium(II) complexes containing the tetrapyrido[3,2-′:2′,3′-c:3′,2′-h:2″,3″-j]phenazine (tpphz) ligand. Although the central tpphz ligand in such complexes is planar, the octahedral coordination geometry about the ruthenium centers gives rise to a “dumbbell” structure with bulky, and chiral, “stopper” groups. These studies have revealed that both [Ru(bipy)2]tpphz]+, 1, and [Ru(phen)2]tpphz]+, 2, (where bipy = 2,2′-bipyridine, phen = 1,10-phenanthroline), bind to quadruplex DNA with high affinities (>10^7 M^-1).47

Both these complexes display a “DNA light-switch” effect, being essentially nonemissive in aqueous solution until DNA binding induces a several orders of magnitude increase in their Ru → tpphz MLCT-based luminescence. Uniquely, the emission and binding affinities of 1 and 2 are sensitive to DNA structure. While groove binding to all duplexes produces a relatively weak emission at >675 nm, binding to quadruplexes produces more complex emission changes. Intense blue-shifted luminescence (∼630 nm) and high affinity binding is observed only when the complex binds to antiparallel quadruplex structures containing external diagonal loops at least three bases in length. The presence of shorter lateral loops limits binding affinities by several orders of magnitude and results in negligible emission.48 This difference in luminescence output means that, despite the only modest selectivity in binding affinities, quadruplex structures can be detected in the presence of duplex DNA. Indeed, this concept has been illustrated by recently reported cell studies with these complexes.49

Fascinatingly, in cellulo studies reveal that, while 1 is only taken up by fixed cells, 2 is actively transported into live cells.49,50 Confocal microscopy studies confirm that 2 is a selective luminescent stain for heterochromatin. Furthermore, 2 displays distinctive noncolocalized multiple emission peaks, whose wavelengths are consistent with those obtained through in vitro studies, indicating that the complex is an in cellulo probe of DNA structure.49

Until now, these studies have used racemic mixtures of complexes 1 and 2. However, recently the Qu group have shown that the ΛΛ-enantiomer of a nonemissive dinuclear nickel(II) trislicicate complex displays a strong binding preference for specific quadruplex structures over duplex DNA,51,52 while Sugiyama and co-workers have demonstrated that a chiral helicene macrocycle can enantioselectively recognize quadruplex DNA.53 Furthermore, recent spectroscopic,54,55 crystallographic,56–58 and NMR59 studies on mononuclear Ru(III) (dpdz = dipyrido[3,2-′:2′,3′-c]-phenazine) systems with duplex DNA have also illustrated the importance of chirality in such interactions. In light of these studies, we discuss the DNA binding preferences of enantiopure samples of 2 and also report NMR-based studies designed to delineate the structural details of quadruplex binding by 1 and 2, followed by further rationalization of the results based on molecular dynamics (MD) simulations in water.

RESULTS AND DISCUSSION

Spectroscopic Binding Studies. In previous studies, we have found that the luminescent binding response of rac-1 and rac-2 to duplex and quadruplex DNA is effectively identical. In both cases, binding to duplex DNA produces a 60-fold increase in emission, while binding to quadruplex produces a >150 times increase in blue-shifted emission. Furthermore comparisons of K0 values for rac-1 and rac-2 revealed they are almost identical within experimental error.47 In the study reported herein, the interaction of enantiomerically pure complexes ΔΔ-2 and ΛΛ-2 with duplex and quadruplex DNA was investigated through luminescence titrations using calf thymus DNA (CT-DNA) (Supporting Information Figure S01) and the human telomere sequence (HTS) d[AG3(TTAG3)3] (Figure 2).
To aid comparisons, data for rac-2 in these conditions are also included. The CD spectrum of the HTS sequence in crowded K⁺ solutions confirmed that it adopts an antiparallel structure after DNA binding. Furthermore, the markedly greater increase in luminescence for bound ΔΔ-2 implies significantly better shielding from solvent for this isomer. Further evidence for this hypothesis was obtained by NMR studies.

**NMR Binding Studies.** To provide structural insights into the effects observed in our optical studies, the binding of 1 and 2 to HTS was further investigated using a combination of NMR spectroscopy and simulated annealing coupled with restrained molecular dynamics simulations for structure determination. Assignments of the folded quadruplex before addition of any complex are given in the Supporting Information (Table S1).

On addition of ΔΔ-2 to HTS, severe line broadening of DNA signals was observed by NMR, mainly from the lateral loop end (red dots, bottom end of Figure 3). This implies that the ligand is binding at this end and causing structural perturbations concomitant with binding, which occur on a time scale in the millisecond range. Unfortunately, this is a common observation in studies of DNA/ligand interactions and it makes structure determination of the complexes difficult by severely reducing the information content of spectra. By contrast, addition of the more tightly binding ΔΔ-2 produced less severe relaxation-induced broadening although enough to abolish intermolecular NOEs. Chemical shift changes occurred at both ends of the quadruplex, and intramolecular NOEs were broadened and lost at both ends. It therefore appears that whereas ΔΔ-2 binds mainly at the lateral loop end, ΔΔ-2 binds at both ends. Taken together with the luminescence data, the implication is that binding of ΔΔ-2 at the diagonal loop end (top end of Figure 3) is accompanied by a high degree of shielding of the ligand from solvent. This in turn implies that the ligand is stacked onto the tetrad underneath the diagonal loop.

All attempts to alter the solution conditions so as to bring back intermolecular NOEs were unsuccessful. We therefore carried out titrations with 1. The absorption and emission response of this complex to HTS binding is very similar to that of 2: in particular, the enhancement and blue-shifting of its...
luminescence are very similar, indicating analogous modes of binding. These studies were carried out on a diastereomeric mixture as this had the advantage that both putative binding sites could be potentially investigated in a single experiment.

Addition of 1 to HTS gave rise to chemical shift changes at both ends of the quadruplex. There was broadening of signals throughout, which led to a general reduction in the intensities of intramolecular NOEs, although the largest effects were seen at the lateral loop end (Table 2). However, gratifyingly, a large number of new intermolecular NOEs could be seen to the ligand (Figure 4).

In free solution, NMR resonances from the four symmetry-related positions of 1 have identical chemical shifts. However, in the presence of HTS, most signals were split into four, in some cases with fairly large chemical shift changes. The increased complexity made it impossible to assign the ligand signals in the complex to individual positions and meant that, although we were able to observe 20 intermolecular NOEs in the complex, we were only able to assign the nucleotide signals (Table 2).

Analysis of the NOEs reveals that they are not compatible with a single structure for the complex, because 13 derive from contacts at the diagonal loop end and seven from contacts at the lateral loop end. On the basis of the discussion above, this is not surprising. In particular, by analogy with 2, we expect ΔΔ-I to bind mainly at the lateral loop end and ΔΛ-I (and probably also ΔΔ-I) mainly at the diagonal loop end. NOE intensities are compatible with this expectation. As anticipated, the NOEs at the diagonal loop end are consistent with 1 binding under the diagonal loop. NMR-Based Structures of Complexes. The experimentally observed NOEs from the lateral loop end were used to calculate structures for both ΔΛ-I and ΔΔ-I bound at the lateral loop end using restrained simulated annealing. The structure generated for ΔΔ-I is shown in Figure 5 and has no violations of the NOE constraints greater than 0.5 Å.

Table 2. Intermolecular NOE Crosspeaks and the Corresponding DNA Residues Identified for the Interaction of 1 with HTS

| complex signal | DNA signal | assigned DNA atom |
|----------------|------------|------------------|
| 7.79           | 4.38       | G10 H5'          |
| 7.79           | 2.89       | G14 H2'          |
| 7.79           | 2.74       | G14 H2'          |
| 7.80           | 5.65       | T11 H1'          |
| 7.80           | 6.30       | G10 H1'          |
| 7.76           | 6.31       | G10 H1'          |
| 7.74           | 3.31       | G22 H2'          |
| 7.55           | 2.40       | G22 H2'          |
| 6.95           | 5.58       | T12 H1'          |
| 6.95           | 1.14       | T12 H2'          |
| 6.29           | 5.46       | G2 H1'           |
| 8.36           | 2.61       | G9 H2'           |
| 8.36           | 2.94       | G9 H2'           |
| 8.36           | 2.51       | G8 H2'           |
| 8.36           | 3.08       | G8 H2'           |
| 8.11           | 1.91       | T5 CH3           |
| 6.93           | 1.30       | T18 H2'          |
| 6.93           | 2.07       | T17 CH3          |
| 6.93           | 4.04       | T6 H5'           |
| 8.22           | 2.83       | A19 H2'          |

While the tpphz ligand stacks on top of the tetrads, this interaction is reinforced by electrostatics: the positively charged ruthenium centers are located at the edge of the tetrads close to the anionic phosphate backbone. There is little perturbation to the quadruplex structure, with slight movement of the lateral loops to accommodate the ligand. Bases in the loops partially shield the tpphz rings, as expected from the luminescence. There is little direct contact between the bipy ligands and the quadruplex, and therefore both the ΔΛ and ΔΔ complexes bind in a similar way, with similar energies.

The observed NOEs were also used to calculate a structure for the complex bound at the diagonal loop end. The bound ΛΛ-I structure is shown in Figure 6. The bipy rings fit neatly against the phosphate backbone making close van der Waals contact. The diagonal loop holds the tpphz in place and shields it from solvent, as expected from the luminescence data.

By contrast, attempts to use the same NOEs to calculate a structure with ΔΔ-I bound at the same site result in a very high-energy state (roughly 10 times higher). In this structure, one end of the ligand is able to fit straightforwardly, by displacing the terminal nucleotide of the quadruplex DNA chain (Figure 7). However, in this calculation, the other end of the ligand does not displace the DNA backbone. Instead, the calculation generates a number of physically impossible solutions, of which the lowest energy is shown in Figure 7: the phosphate backbone passes through the middle of one of the aromatic bipy rings. The other solution is to displace one bipy ligand completely away from the tpphz plane. Structures have been calculated for the ΛΛ and ΔΔ isomers of 2 in the same way and show similar features (Supporting Information Figures S04 and S05). These calculations imply that the experimental NOE data are compatible with the ΛΛ isomer binding at the diagonal loop end but not with the ΔΔ isomer binding at the same site. This result is thus in agreement with the conclusion reached above, that both isomers bind at the...
lateral loop end, but only the ΛΛ isomer (and probably the ΛΔ isomer also) binds at the diagonal loop end. To investigate this issue further, unconstrained molecular dynamics simulations were carried out in a TIP3P water model.

Unconstrained MD Simulations. To evaluate the impact of ΛΛ-1 and ΔΔ-1 complexes on the quadruplex structure when binding under the diagonal loop, molecular dynamics simulations were performed on free HTS and its association with ΛΛ-1 and ΔΔ-1. For free HTS, three independent simulations were undertaken using the structures presented in Figures 5–7 (henceforth denoted A, B, and C) as starting conformations. These results were then compared with simulations of the associations formed between the quadruplex and both diastereomers ΛΛ-1 and ΔΔ-1, labeled ΛΛ-1-HTS and ΔΔ-1-HTS, respectively. For the ΛΛ-1-HTS simulation, the NMR-based structure shown in Figure 6 was used as starting point, while for simulation of ΔΔ-1-HTS, the starting structure was generated from ΛΛ-1-HTS by replacing ΛΛ-1 with ΔΔ-1, superimposing the two ruthenium atoms and the lateral loop end.

Figure 4. Selected sections of spectra showing the new crosspeaks identified upon addition of a diastereomeric mixture of 1 to the HTS sequence.

Figure 5. Molecular dynamics-based structure of ΔΔ-1 bound to the lateral loop end of HTS, generated using experimentally observed NOEs for the interaction.

Figure 6. Molecular dynamics-based structure of ΛΛ-1 bound to the diagonal loop end of HTS, generated using experimentally observed NOEs for the interaction. Left: Illustrating the bipy ring at front left parallel to the DNA backbone. Right: Showing how the 5’ end of the DNA backbone (front center) moves to avoid the ligand.

Figure 7. Molecular dynamics-based structure of ΔΔ-1 bound to the diagonal loop end of HTS, generated using experimentally observed NOEs for the interaction.
tpphz bridging ligand. Thus, only the spatial arrangement of the two Ru(bipy)$_2$ moieties was changed, in agreement with the respective diastereomers in the octahedral metal coordination sphere. Further molecular dynamics simulation details, together with an extended discussion, are provided in Supporting Information.

Simulations A, B, and C of the free HTS show that the G-tetrads yield low root mean-square deviations (RMSD), calculated relative to the starting structures, consistent with a minor conformational rearrangement experienced by these subunits throughout the 50 ns of simulation time, which is to be expected because they are held in place by hydrogen bonds. In contrast, the diagonal loops are more mobile (see Supporting Information Figure S06) showing higher RMSD values. Overall, the three independent simulations sampled significant HTS conformational space as suggested by the representative conformations of simulations A, B, and C, which have slightly different structures with cross RMSD values collected in Supporting Information Table S03 between 2.71 and 3.88 Å. We then went on to look at the interaction of HTS with the metal complex using the same method.

Figure 8 shows the RMSD values over the simulation time (using the unrelaxed starting structures as reference) for both the ΛΛ-1-HTS and ΔΔ-1-HTS associations. In both cases, after an initial jump due to the geometry relaxation using the ideal force field parameters, the values tend to stabilize. Focusing on the RMSD values for ΛΛ-1-HTS, the G-tetrad and the HTS structure typically stabilize after the first five ns while the values for the diagonal loop oscillate. This loop mobility is assigned to the presence of ΛΛ-1, as it was not observed in simulations A, B, and C of free HTS. Apart from this increased loop mobility, the antiparallel basket is as stable as free HTS was in simulations A, B, and C, so unsurprisingly the RMSDs between the representative conformations of ΛΛ-1-HTS and simulations A, B, and C are within the variability found for the calculated cross RMSDs obtained in these three simulations (see Supporting Information Table S03).

By contrast, for ΔΔ-1-HTS, all the RMSD values converge very quickly, including those associated with the diagonal loop. This indicates that complex ΔΔ-1 is able to fit under the loop without causing major G-DNA conformational changes. Indeed, the representative conformation is comparable to the representative conformation of the ΛΛ-1-HTS simulation (with a RMSD value of only 1.55 Å) and the representative frames of the free HTS simulations A, B, and C, with cross RMSD values ranging from 1.54 to 2.81 Å. This indicates that the observed experimental recognition of only ΛΛ-1 under the diagonal loop is not caused by the intrinsic loop cavity shape or size because both isomers are able to fit into the cavity.

Analyzing the representative conformations of ΛΛ-1-HTS and ΔΔ-1-HTS represented in Figure 9, both ruthenium complexes bind under the diagonal loop with the tpphz ligand stacking over the top G-tetrad. However, some differences are evident. Complex ΛΛ-1 adopts a diagonal arrangement over

Figure 8. Variation of the RMSD values throughout the course of the MD simulations for ΛΛ-1-HTS (left) and ΔΔ-1-HTS (right). The vertical black line marks the separation between the equilibration and collection simulation stages.

Figure 9. Representative snapshots of simulations ΛΛ-1-HTS (top, magenta) and ΔΔ-1-HTS (bottom, aquamarine). Side and top views are presented on the left and center, respectively. Right pictures represent the complexes stacked over the top G-quartet.
the guanine bases and under the loop whereas in $\Delta\Delta^{-1}$, the stacking of the tpphz ligand is more localized over two guanines of only one-half of the G-tetrad.

To clarify this point, a surface representation was constructed for the position occupied by the tpphz ligand (excluding the hydrogen atoms) over a 50 ns collection period for the HTS association with each of the diastereomers (Figure 10). These surfaces clearly show a striking difference: in $\Lambda\Lambda^{-1}$-HTS, the tpphz ligand is able to "float" over the G-tetrad interacting with the four guanines. In contrast, in $\Delta\Delta^{-1}$-HTS, the tpphz ligand remains "locked" over two guanines of the G-tetrad throughout the entire simulation time. Because tpphz is present in both dinuclear complexes, the difference in dynamic behavior must be caused by the stereochemistry of the bipy ligands in the octahedral Ru(II) coordination spheres. This is consistent with the observation that in $\Lambda\Lambda^{-1}$-HTS the diagonal loop appears to be more flexible, oscillating over the middle of both Ru(bipy)$_2$ moieties. On the other hand, in $\Delta\Delta^{-1}$-HTS, the loop is more rigid and locks the $\Delta\Delta^{-1}$ complex movement over the G-tetrad.

The above results suggest that, although both $\Lambda\Lambda^{-1}$ and $\Delta\Delta^{-1}$ complexes can fit under the loop, sufficient HTS loop conformational freedom to permit the complex entrance into the prefolded G-DNA structure is only present for $\Lambda\Lambda^{-1}$. In contrast, the stereochemistry of $\Delta\Delta^{-1}$ appears to induce increased rigidity on the loop, preventing complex entrance into the loop arch. In other words, $\Delta\Delta^{-1}$ does not bind under the diagonal loop because the loop arch does not fulfill the complex’s "stereochemical requirements".

As mentioned above, the simulated annealing structure proposed for the binding of $\Delta\Delta^{-1}$ to the diagonal loop end of HTS corresponds to an impossible solution (Figure 7), which could also be rationalized by our unconstrained MD simulations in water. If one calculates the expected NOEs (not shown) from the representative conformations of simulations $\Lambda\Lambda^{-1}$-HTS and $\Delta\Delta^{-1}$-HTS (or from the relaxed structures obtained by Molecular Mechanics minimization), the protons that contact the ligand are quite different, meaning that the interaction of $\Lambda\Lambda^{-1}$ and $\Delta\Delta^{-1}$ under the loop results in clearly distinct NOEs. Therefore, the experimental interactions (presumably generated only by the "$\Lambda\Lambda^{-1}$-HTS" interaction) are not suitable as restraints to generate a hypothetical "$\Delta\Delta^{-1}$-HTS" association with this binding mode.

**CONCLUSIONS**

We have shown that $\Lambda\Lambda^{-2}$ binds mainly at the diagonal loop end of HTS, stabilized by a good steric fit under the loop. By contrast, $\Delta\Delta^{-2}$ binds >40-fold more weakly and almost entirely at the other end. Both isomers also bind to B-DNA, but the affinity of $\Lambda\Lambda^{-2}$ is greater for HTS than for B-DNA and, importantly, when bound to HTS the luminescence is more intense and is blue-shifted from ~675 to ~630 nm. Studies with other ligands that bind to quadruplex DNA have shown that compounds that bind strongly to one conformation also stabilize that conformation, as expected from thermodynamic arguments.6-52,60-62 We have previously shown49,50 that 2 is actively taken up by cells, and confocal images show that it generates punctate images centered mainly in the heterochromatin, as might be expected for a probe that highlights G-quadruplexes. It also has a wavelength and intensity of emission that is different for different quadruplex structures.48 $\Lambda\Lambda^{-2}$ is therefore a useful tool for specifically stabilizing and imaging antiparallel basket structures with a diagonal loop. This study also confirms that the antiparallel structure of HTS can be selectively targeted. In comparison with the use of antibodies to detect quadruplexes,63-65 small molecules like complex 2 and its analogues are much simpler to rationally design and potentially have wider applicability as they can be used directly on living cells. Furthermore, and as this study indicates, because such systems can be made to target specific features of a quadruplex, they can be made specific to individual quadruplex structures. Consequently, with the structural information obtained by this study, we are exploring this potential for enhanced specificity through informed design of derivatives of 2. Although this work is intended to image antiparallel basket quadruplexes in living cells, it could subsequently be extended into molecular tools for selectively stabilizing quadruplexes.

**EXPERIMENTAL SECTION**

Ligands were synthesized using published methods. Ru(phen)$_2$Cl$_2$·2H$_2$O and Ru(bipy)$_2$Cl$_2$·2H$_2$O were prepared following a standard literature method.66 The racemic mixtures of the tpphz complexes were prepared by methods we have previously described.47 Enantiomers of 2 were prepared by the route first described by MacDonnell and Bodige.68 Characterization data for these complexes were identical to the original reports.

The HTS oligonucleotide d[AG$_3$(TTAG)$_3$]$_3$ was purchased from Eurogentec Biotechnology (Southampton, UK), purified by HPLC, and used without further annealing. Samples for UV−vis and luminescence titrations were in 10 mM KH$_2$PO$_4$/K$_2$HPO$_4$ and 1 mM KOAc/K$_2$PO$_4$ and 1 mM K$_2$EDTA in 50–200 mM KCl (pH 7.0, 298 K), in which DNA was added to 10 μM ligand, while samples for NMR were prepared in 50 mM NaCl, pH 7, using 300 mM DNA at 298 K. Titrations were conducted with DNA and ligand of the same order of concentration as 1/K$_D$ shown to be the optimum values for obtaining accurate binding constants.67 The optimum temperature for observing NOEs to 1 was 283 K. CD spectra were recorded on a Jasco J-810 spectrophotometer using a 1 cm path length. UV−vis titrations were performed using a Cary 3 Bio. Binding affinities were obtained by fitting using Origin 7.0 software to a standard one-set-of-binding-sites model.

NMR experiments were carried out on Bruker Avance 800, 600, and 500 spectrometers. Assignments were made using COSY, TOCSY, and NOESY spectra (mixing times 60 or 90 ms for TOCSY, 100 ms for NOESY), supported by $^1$H−$^31$P HSQC (Supporting Information Table S01). Spectra were analyzed using FELIX (Felix NMR, Inc., San Diego, CA).

Structure calculations were carried out using Xplor with the parallhdg.dna parameters. Calculations used simulated annealing over 8000 steps from 2000 to 100 K, using standard square-well potentials.

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**Figure 10.** Surface (isovalue = 1) representing the histogram of positions occupied by the tpphz atoms (excluding hydrogen) over the 50 ns collection period in $\Lambda\Lambda^{-1}$-HTS (left) and $\Delta\Delta^{-1}$-HTS (right).
for NOEs. All calculations imposed planarity and hydrogen bonding in the tetrazas plus planarity in tpphz and bipy polypryridyl ligands and octahedral geometry around the ruthenium. The force field included van der Waals and electrostatic terms. Threading of the ligand into the complex was accomplished by starting with almost zero van der Waals radii and increasing the radius in a geometric progression during the simulated annealing. For each complex, only the intramolecular NOEs relevant to that end were included, all specified as ambiguous NOEs to any ligand proton, with an upper limit of 5 Å. Convergence was improved by including restraints to position the tpphz ring close to the relevant tetrad plane. NOEs for the ΔΔ-1-HTS and ΔΔ-1-HTS complexes were calculated by numerical integration of the Solomon equations.68

Unconstrained MD simulations were carried out using the AMBER ff99bsc0 set of parameters and charges for DNA69 combined with General Amber Force Field (GAFF) parameters70 with extra terms71,72 and RESP charges73 for both ΔΔ-1 and ΔΔ-1 complexes. These simulations were undertaken with the pmemd.cuda AMBER executable, able to accelerate explicit solvent Particle Mesh Ewald (PME)74,75 calculations through the use of GPUs with the new Single Precision Fixed Point (SPFP) model.76

■ ASSOCIATED CONTENT

2 Supporting Information
Details of MD; luminescent titrations with CT-DNA; circular dichroism spectrum of HTS quadruplex; titration with ΔΔ-2; NMR assignments of the HTS quadruplex; models of ΛΔ-2 and ΔΔ-2 bound to HTS. This material is available free of charge via the Internet at the http://pubs.acs.org.

Accession Codes
Coordinates and chemical shift data have been deposited at the PDB with the entry codes 2mcc (ΔΔ-1 complex) and 2mco (ΔΔ-1 complex) and also at BMRB with the entry codes 19435 and 19448, respectively.

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Notes
The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

HTS, human telomere sequence; tpphz, tetrapyrido[3,2-a:2′,3′- c:3′,2-h:2′,3′,2′-j]phenazine; bipy, 2,2′-bipyridine; phen, 1,10-phenanthroline; dppz, dipyrido[3,2-a:2′,3′-c]phenazine; MD, molecular dynamics; CD, circular dichroism; CT-DNA, calf thymus DNA; NOE, nuclear Overhauser effect

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