DOI: 10.1002/chem.201002810

Recognition of G-Quadruplex DNA by Triangular Star-Shaped Compounds: With or Without Side Chains?

Hélène Bertrand,[a] Anton Granzhan,[a] David Monchaud,[a, b] Nicolas Saettel,[a] Régis Guillot,[c] Sarah Clifford,[d] Aurore Guédin,[e, f] Jean-Louis Mergny,[e, f] and Marie-Paule Teulade-Fichou*[a]

Abstract: We report the synthesis of two new series of triangular aromatic platforms, either with three aminoalkyl side chains (triazatrinaphthylene series, TrisK: six compounds), or without side chains (triazoniatrinaphthylene, TrisQ). The quadruplex–DNA binding behavior of the two series, which differ essentially by the localization of the cationic charges, was evaluated by means of FRET-melting and G4-FID assays. For the trisubstituted triazatrinaphthylenes (TrisK), the length of the substituents and the presence of terminal hydrogen-bond-donor groups (NH_2) were shown to be crucial for ensuring a high quadruplex affinity ($\Delta T_{1/2}$ values of up to 20 °C at 1 μM for the best candidate, TrisK3-NH) and selectivity versus duplex DNA. Subsequently, comparison of data collected on both the telomeric- and c-myc-quadruplex showed that the nonsubstituted TrisQ is even more efficient than TrisK3-NH, both in terms of quadruplex affinity ($\Delta T_{1/2}$ = 26 °C in K^+ buffer) and selectivity versus duplex DNA. Structural considerations conducted with the c-myc quadruplex indicate that both TrisK3-NH and TrisQ stack well onto the G-quartet but in an offset position, which might be influenced by the formation of multiple hydrogen bonds with the target in the former case. Finally, the nonsubstituted TrisQ displays a binding profile very similar to some of the best quadruplex binders, BRACO-19 and bisquinolinium 360A, used herein as references, and thereby represents a highly promising novel molecular design for quadruplex recognition.

Keywords: DNA recognition · G-quadruplexes · heterocycles · ligand design · polycycles

Introduction

It is recognized that DNA sequences containing repeats of bases are highly susceptible to aberrant replication and perturbation of other DNA-related processes such as recombination and transcription.[1] These dysfunctions may lead ultimately to modifications of the genetic material (i.e., mutations, genomic instability) and may have a role in explaining mechanisms linked to cancer development or more largely, be involved in pathogenic rearrangements genome-wide.[2] At the molecular level, the peculiar behavior of repeat sequences is attributed to the formation of secondary structures through intrastrand Watson–Crick base pairing (e.g., folded back hairpins) or inter- or intrastrand non-canonical base-assemblies (e.g., mismatched base-pairs, base-triplets or quartets). Amongst all the nucleic bases, guanine exhibits the highest propensity to self-assemble, thus forming GG pairs, G-ribbons, and four-base associations called G-quartets.[3] G-quartet motifs may coordinate to alkaline cations (K^+, Na^+) to form consecutive π stacks that initiate the formation of tetrahelical structures named quadruplexes (Figure 1).[3–4] Consequently, single-stranded DNA-domains containing G repeats are predicted to form guanine–quadruplex secondary structures.[5] Quadruplex structures exhibit a high thermodynamic stability in biological conditions, representing a fascinating example of supramolecular self-assembly. Consequently, these peculiar nucleic acid structures are

[a] Dr. H. Bertrand, Dr. A. Granzhan, Dr. D. Monchaud, Dr. N. Saettel, Dr. M.-P. Teulade-Fichou
Institut Curie, Centre de Recherche, CNRS UMR176
Centre Universitaire Paris XI, Bât. 110, 91405 Orsay (France)
Fax: (+33) 169075381
E-mail: marie-paul.teulade-fichou@curie.fr

[b] Dr. D. Monchaud
Current address: Institut de Chimie Moléculaire
CNRS UMR5260, Université de Bourgogne (ICMUB)
Faculté des Sciences Mirande, 9, Avenue Alain Savary
21000 Dijon (France)

[c] Dr. R. Guillot
Institut de Chimie Moléculaire et des Matériaux d'Orsay
CNRS UMR8182, Université Paris Sud XI
Bât. 420, 91405 Orsay (France)

[d] Dr. S. Clifford
Département de chimie minérale, analytique et appliquée
Université de Genève, quai Ernest-Ansermet 30
1211 Genève 4 (Switzerland)

[e] A. Guédin, Dr. J.-L. Mergny
Laboratoire des Régulations et Dynamique du Génome
INSERM U565, CNRS UMR5153
Muséum National d'Histoire Naturelle, 43, Rue Cuvier
75005 Paris (France)

[f] A. Guédin, Dr. J.-L. Mergny
INSERM U869, Université de Bordeaux
Institut Européen de Chimie et Biologie, 2, Rue Robert Escarpit
33607 Pessac (France)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201002810.

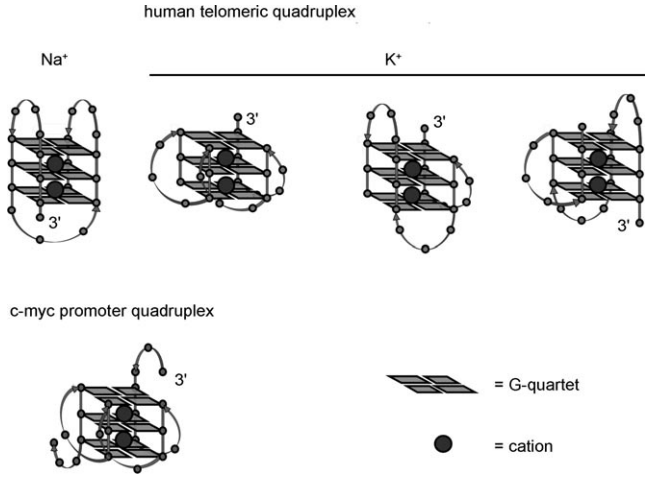
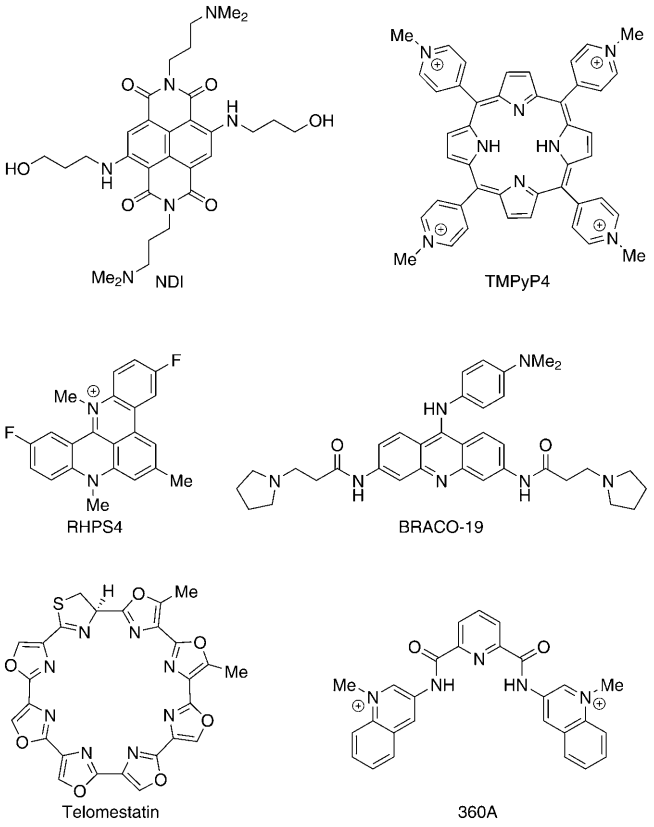


Figure 1. Schematic representation of various forms of the human telomeric quadruplex in Na^+ and K^+ conditions and of the c-myc quadruplex, identified by NMR and X-ray studies.

currently the focus of intense attention with the aim to decipher their biological roles[6] and to evaluate their potential for building DNA-based nanoobjects.[7]

Interestingly, quadruplexes may bind small molecules, which in turn stabilize these structures and may act as molecular silencers by shifting a functional DNA conformation (duplex) to a nonfunctional one (quadruplex). Over the past decade, the search for small molecules acting as quadruplex ligands has developed dramatically with essentially two major goals: finding probes to sense quadruplex formation in various biological contexts and discovering new DNA-interacting agents of potential therapeutic interest. Thus, G-quadruplex binders have great potential as drugs for anticancer research, especially in view of the fact that quadruplex-forming sequences are present in the regions involved in tumorogenesis (oncogenes)[8] or essential for the maintenance of genome stability (telomeres). Thus, these two DNA domains have been the major targets for quadruplex drug design, whereas more recently, targeting quadruplexes in messenger RNA is attracting increasing attention.[9]

However, G-rich sequences with the potential to form quadruplexes are widely distributed in the genome and have also been found in mRNA, therefore the correlation of in vivo effects and in vitro data requires ligands that are capable of specific binding only to certain quadruplex motifs (telomeric vs. oncogenic, DNA vs. RNA, etc.) Currently this is the challenge, since in terms of molecular recognition the quadruplex structures have not delivered all their secrets. Indeed, although hundreds of quadruplex ligands have been reported,[10] only a handful of structures of quadruplex–ligand complexes have been resolved by X-ray or NMR analysis.[11] Most structures have been obtained with bimolecular[12] and tetramolecular[13] quadruplexes that were used as models. Only recently information has been provided from the X-ray analysis of the naphthalene diimide derivative NDI interacting with the intramolecular telomeric

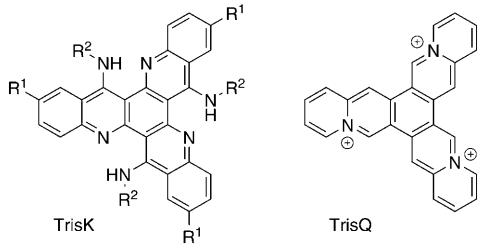


quadruplex[14] and from the NMR-derived structure of the tetracationic porphyrin TMPyP4 complexed with the intramolecular quadruplex of the c-myc oncogene.[15] On the whole, structural studies have established that large aromatic platforms stack on external G-quartets and in some cases, might be sandwiched at the junction of two quadruplex units.[13a,14] The presence of chains, (for example, aminoalkyl substituents) might be a determinant of selectivity, since in principle they allow supplementary interactions with the target DNA.[12b] The role of the side chains was initially thought to favor the anchorage in the grooves;[14,16] however, this has been reconsidered since the tetrasubstituted NDI drug was shown to interact only with loops at the interface of two quadruplex units[14] or inside a quadruplex RNA.[17] In particular, the introduction of side chains terminated with hydrogen-bond-donor groups seems to favor the hydrogen-bonding network with loops, either by direct contact or more often, mediated by water molecules, which could provide clues for the specific recognition of a given quadruplex.

Alternatively, several classes of ligands, devoid of side chains but endowed with large π surfaces that are likely to maximize π–π interactions with G-quartets, have been shown to interact strongly with quadruplexes. Amongst these ligands we will only cite RHPS4, for which an NMR-derived structure with a tetramolecular quadruplex is available,[13c] and the well-studied telomestatin.[18] Notably, in the latter case, although there are few doubts that this molecule stacks well on terminal G-quartets, no structural data is available besides a recent molecular-modeling study.[19]

In view of the structural diversity of π systems interacting with G-quartets, one question that remains to be addressed is the optimal geometry for a ligand to maximize π–π interactions with a G-quartet. At first glance, the ligands of fourfold symmetry, matching that of a G-quartet and of comparable size, represent an obvious choice. However, the porphyrin TMPyP4 that fulfills these criteria has so far not been shown to establish direct contact with G-quartets as seen from two independent studies with c-myc and the telomeric quadruplex,[12c,15] and possesses a very poor quadruplex versus duplex selectivity.[20,21] The NDI ligands that have a limited π surface (four cycles) are more potent quadruplex binders than their perylenediimide counterparts, which display a much larger π surface (seven cycles).[22] Finally, a pursuit towards an extended, fourfold-symmetric design may render the ligand nonplanar, as in the case of tetraazoniapentaphenopentaphene previously reported by Ihmels et al.,[21] and eventually disfavor its quadruplex-stacking properties. Thus, it is still difficult to define the structural features of the ligand that govern the quadruplex recognition; however, based on the structural data available, it appears that the presence of cationic charges and their localization (terminal amino groups vs. aromatic core), as well as the topology of the aromatic core, are crucial for a strong and selective interaction with the quadruplex structures.[17] Nonetheless, due to the diversity of the systems developed so far, it is difficult to dissociate contributions due to aromatic–aromatic interactions and side-chain effects. In an attempt to address this question, we report herein on the synthesis of two new series of triangular aromatic compounds, either with or without side chains, and on the comparison of their binding to two different quadruplex structures (the human telomeric quadruplex and the oncogene c-myc quadruplex) using FRET-melting, fluorescent intercalator displacement (G4-FID) assays, and molecular modeling studies.

For a systematic study aimed at establishing the structure–property relationships we synthesized two series of C_3-symmetric heterocyclic derivatives with a trinaphthylene-like polyaromatic core, which offers convenient access to structural diversity. Among these compounds, the TrisK derivatives represent an extension of the pentacyclic quinacridine series of quadruplex ligands developed by our group over the past years.[16,23] These ligands feature the neutral 5,11,17-triazatrinaphthylene core, substituted with three alkylamino substituents. In its neutral form, this motif was previously used for 2D self-assembly studies[24] and termed TrisK, with respect to the Celtic symbol Triskele meaning "three-legged". In contrast, TrisQ is devoid of any substituents and contains three quinolizinium units with quarternary nitrogen atoms in the bridgehead positions of the polyaromatic core. This compound represents a C_3-symmetric analogue of the previously reported four-branched tetraazoniapentaphenopentaphene[21] and is expected to have a permanent charge (3^+), localized at the core unit and hence a strong π-deficient character. Interestingly, in the course of our study,[25] other C_3-symmetric compounds designed for quadruplex recognition have emerged. However, these molecular systems display either moderate affinity for the quadruplex ($K_a = 10^5\,M^{-1}$, as determined by mass spectrometry)[26] or no significant quadruplex binding.[27]

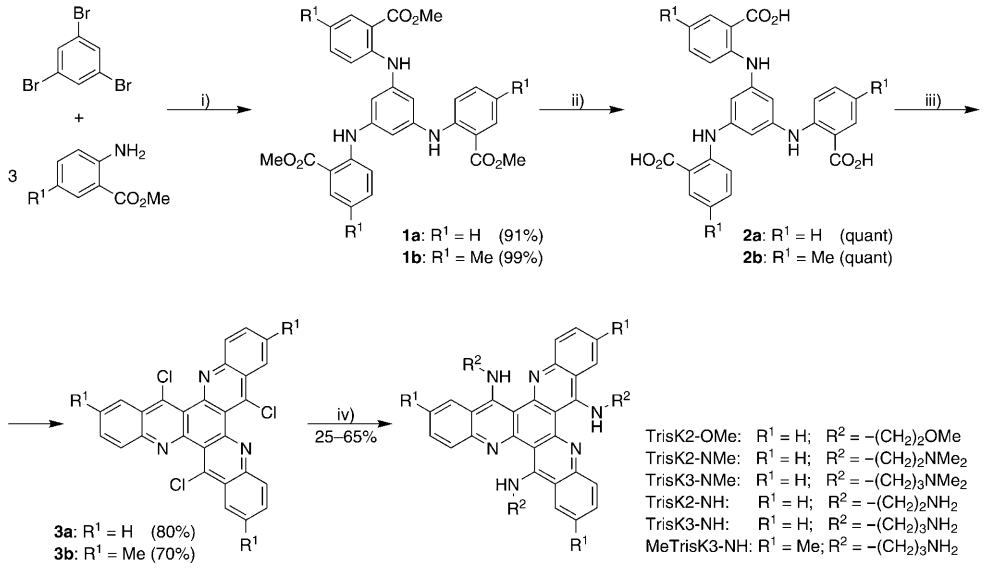


Results

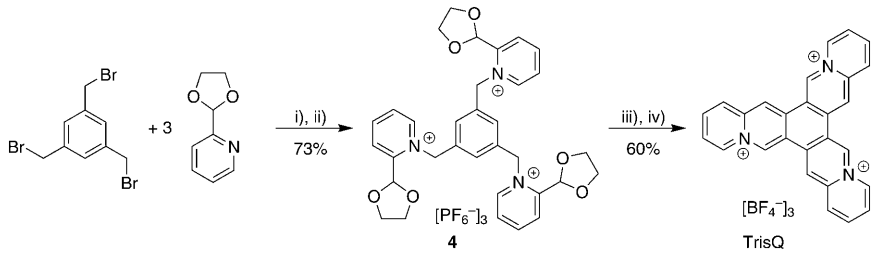
Synthesis of the ligands

TrisK series: The synthetic strategy for the 5,11,17-triazatrinaphthylene derivatives was based on our previously published synthesis of the parent trimethyl-TrisK[24] and quinacridines.[28] This pathway enabled structural diversity by the introduction of substituent chains in the final step through reaction of the common intermediates, 6,12,18-trichloro-5,11,7-triazatrinaphthylene derivatives **3a–3b** with primary amines (Scheme 1). The synthesis of **3a–3b** was readily performed through a high-yielding three-step procedure from commercially available materials, 1,3,5-tribromobenzene and methyl esters of anthranilic or 5-methylanthranilic acids. After the highly efficient palladium-catalyzed triple amination of 1,3,5-tribromobenzene by methyl anthranilates, basic hydrolysis of the resulting triester derivatives **1a–1b** enabled the quantitative formation of the triacids **2a–2b**, which after cyclization at reflux with $POCl_3$, afforded the trichloro derivatives **3a–3b** in high yield. In the final step, the alkylamino side chains were introduced by nucleophilic substitution of chlorine with primary amines upon heating in solvent-free conditions. The chains introduced were namely 1-amino-2-methoxyethane, to give TrisK2-OMe ("2" for the number of carbon atoms in the chain and OMe for the methoxy terminal group), *N,N*-dimethyl-1,2-diaminoethane to give TrisK2-NMe (NMe for the *N,N*-dimethylamino terminal group), *N,N*-dimethyl-1,3-diaminopropane to give TrisK3-NMe; 1,2-diaminoethane to give TrisK2-NH (NH for the NH_2 terminal function), and 1,3-diaminopropane to give TrisK3-NH. The trimethyl analogue of the latter, MeTrisK3-NH, was also synthesized. After purification by column chromatography, the alkylamino-substituted derivatives TrisK were obtained as free bases and characterized by ^1H and ^{13}C NMR spectroscopy and HRMS analysis.

TrisQ: The synthesis of 4a,10a,16a-triazoniatrinaphthylene (TrisQ) was performed through the cyclodehydration pathway previously established for the other polycyclic quinolizinium derivatives (Scheme 2).[21,29] The triacetal precursor



Scheme 1. Synthesis of tris(aminoakyl)-TrisK compounds. Reagents and conditions: i) $Pd(OAc)_2$, $P(tBu)_3$, Cs_2CO_3, toluene, 40 h; ii) 5% NaOH (aq), acetone, 16 h, room temp.; iii) $POCl_3$, 16 h, reflux; iv) R^2NH_2, 3 d, reflux.



Scheme 2. Synthesis of TrisQ$[BF_4^-]_3$. Reagents and conditions: i) *N*-methylpyrrolidone (NMP), 7 d, RT; ii) $NaPF_6$ (aq); iii) $MeSO_3H$, 72 h, 210 °C; iv) $NaBF_4$ (aq).

4$[PF_6^-]_3$, obtained upon reaction of 1,3,5-tris(bromomethyl)-benzene with 2-(1,3-dioxolan-2-yl)pyridine, was subjected to cyclodehydration upon heating in methanesulfonic acid to give TrisQ$[PF_6^-]_3$, which was converted by ion metathesis into the more stable tetrafluoroborate salt TrisQ$[BF_4^-]_3$ and characterized by ^1H and ^{13}C NMR spectroscopy, mass spectrometry, and elemental analysis. The structure of TrisQ$[BF_4^-]_3$ was supported by single-crystal X-ray diffraction analysis data (see the Supporting Information, Figure S1). The compound crystallized from aqueous solution in the $P2_12_12_1$ space group with the inclusion of one molecule of water; notably, no π–π stacking between the aromatic cations in the solid state was observed.

Potentiometric titrations and evaluation of the charge state of the ligands at pH 7.2: In the TrisK structure, up to three types of nitrogen atom can be identified (see the Supporting Information, Figure S2); namely intracyclic (N1), aromatic amino (N2, located in the *para* position to N1), and aliphatic amino nitrogen atoms located at the end of the side chains (N3). Compounds TrisK can consequently exhibit variable cationic charge distribution depending on the pH. Thus, to enable a reliable comparison between the two series of ligands, it was necessary to determine the protonation state of the TrisK compounds under the experimental conditions. To this end, potentiometric titrations of TrisK2-NH were performed in MeOH/H_2O (50:50 v/v). The results allowed us to determine the pK_a values of 10.87, 10.03, 8.57, 5.88, 5.09, 3.78, and 3.66 corresponding to seven of its nine nitrogen atoms (see the Supporting Information, Figure S3, A); two pK_a values could not be determined, most likely due to low pK_a values (<1). The three highest pK_a values were assigned to the primary amino groups of the aminoalkyl side chains (N3), with the remaining assigned to the aromatic secondary amino groups (N2) and the heterocyclic core (N1). This assignment was made by analogy with related systems, that is, quinacridines and phenanthrolines, which possess ring nitrogens with low pK_a values.[16,30] The analysis of the charged-species distribution curve (see the Supporting Information, Figure S3, B) reveals that at pH 7.2 (conditions used for the DNA-binding studies), TrisK2-NH exists predominantly as a tricationic species with the charges localized on the side chains, whereas its heteroaromatic core is not protonated. This fully validates the direct comparison between the two series, as TrisQ is tricationic independently of the pH except at pH ≥ 9, at which decomposition of the quinolizinium moieties may occur.[31] Finally, it may be assumed that most derivatives of the TrisK series (TrisK3-NH, MeTrisK3-NH, TrisK2-NMe, and TrisK3-NMe) have a protonation profile similar to that of TrisK2-NH and thereby exist as tricationic species at pH 7.2, except for TrisK2-OMe,

which is assumed to be neutral at physiological pH given the lack of protonable groups on its side chains.

Interaction with human telomeric quadruplex

Evaluation of quadruplex-DNA affinity and selectivity by FRET-melting: The quadruplex–DNA affinity of the six TrisK compounds and of TrisQ was evaluated by using the FRET-melting assay.[32] This assay is based on the thermal denaturation of the quadruplex-forming oligonucleotide F21T mimicking the human telomeric repeat (F21T: FAM-$G_3[T_2AG_3]_3$-TAMRA, in which FAM is 6-carboxyfluorescein and TAMRA is 6-carboxytetramethylrhodamine), monitored by a fluorescence resonance energy transfer (FRET) effect. A semi-quantitative evaluation of the ligand binding affinity is obtained by measuring the increase in melting temperature ($\Delta T_{1/2}$) induced by the ligand ($\Delta T_{1/2} = T_{1/2}(+\text{ligand}) - T_{1/2}(\text{no ligand})$, in which $T_{1/2}$ is the temperature at which 50% of the oligonucleotide is unfolded). In view of the polymorphism of the human telomeric quadruplex as a function of the cation present in solution,[33] FRET-melting experiments have been carried out both in Na^+- and K^+-rich buffers. A great advantage of the FRET-melting assay is the direct insight into the quadruplex- versus duplex-DNA selectivity, which is a critical issue for the design of specific ligands. To this end, FRET-melting experiments are performed in the presence of various amounts of competitive duplex-DNA ds26 (self-complementary sequence $d(CA_2TCG_2ATCGA_2T_2CGATC_2GAT_2G)$). If the stabilization of the quadruplex imparted by the ligand is affected by the excess of duplex-DNA, a drop in the $\Delta T_{1/2}$ value is observed. The extent of this drop is inversely proportional to the selectivity and can be expressed by the ratio $S_{FRET} = \Delta T_{1/2}(+\text{ds26})/\Delta T_{1/2}(-\text{ds26})$, which reflects the preferential binding of a compound to the quadruplex-DNA ($S \rightarrow 1$) or a complete lack of selectivity ($S \rightarrow 0$).[34]

As depicted in Figure 2 and Table S1 (Supporting Information), low ($\Delta T_{1/2} = 0.2\,°C$ for TrisK2-OMe) to very high ($\Delta T_{1/2} = 19.8\,°C$ for TrisK3-NH) levels of stabilization were obtained with TrisK derivatives. The comparison of the performances of the various compounds in Na^+ and K^+ conditions indicates that globally the series is rather insensitive to the cationic nature of the buffer because the results obtained were similar in either buffer (average difference: 3.6 °C). For this reason, only values obtained in K^+ conditions will be used hereafter for comparison purposes. In contrast, the various side chains have a dramatic effect and the following trends can be inferred: 1) the neutral TrisK2-OMe does not stabilize the G-quadruplex, which implies that terminal amino groups are required for F21T stabilization as shown by the $\Delta T_{1/2}$ values measured for all the amino terminated derivatives; 2) the length of the side chains has a significant influence because 5-membered chains lead to a better F21T stabilization compared with 4-membered ones (typically 5–7 °C) both for NMe_2- or NH_2-terminated arms; 3) stabilization from compounds with terminal NMe_2 groups are modest (TrisK2-NMe and TrisK3-NMe, with $\Delta T_{1/2}$ values measured to be between 6.2 and 13.9 °C) compared with compounds that have terminal NH_2 groups (TrisK2-NH and TrisK3-NH, with $\Delta T_{1/2}$ values measured to be between 13.0 and 19.8 °C); 4) the additive effects of these two features are seen from comparing TrisK2-NMe and TrisK3-NH; and 5) the presence of three methyl groups on the TrisK core is clearly deleterious to the interaction, since a drop of almost 11 °C is observed for MeTrisK3-NH compared with TrisK3-NH ($\Delta T_{1/2} = 9.3$ vs. 19.9 °C, respectively).

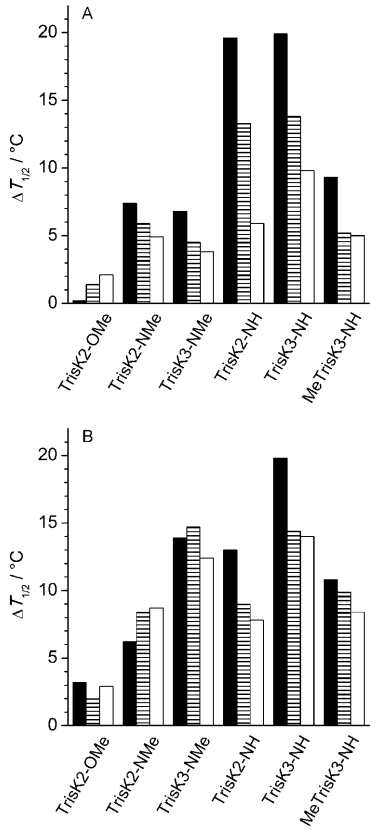


Figure 2. FRET-melting results of TrisK derivatives; experiments were carried out with F21T (0.2 μM), ligand (1 μM), without (black bars) or with ds26 (3 μM: horizontally striped bars; 10 μM: white bars); concentration expressed in strand in lithium cacodylate buffer (10 mM), pH 7.2, with A) NaCl (100 mM) or B) LiCl (90 mM) + KCl (10 mM).

This data implies firstly that the three positive charges, due to the three terminal amines, promote the ligand–quadruplex interaction on the basis of electrostatic attraction; however, it is not yet known if this is mediated by direct contact with the DNA phosphates. In this regard the chain length could play a role for the interaction, because a five-membered chain would enable better flexibility and DNA accessibility. Secondly, the results indicate that terminal primary amines have a crucial influence that could be a result of their hydrogen-bond-donating ability, thereby suggesting that hydrogen bonding may be involved between the ligand and the quadruplex grooves or loops. Finally, tight π-stacking interactions are certainly involved between the planar TrisK core and the external G-quartets since the stabiliza-

tion is highly sensitive to the enhancement of the steric hindrance around the ligand aromatic core.

If we examine the effect of the TrisQ compound (Figure 3 and Table S1 in the Supporting Information), we note a very high stabilization in K^+ conditions ($\Delta T_{1/2}=26.2\,°C$), making this compound a more potent binder than TrisK3-NH, which is the best candidate from the TrisK series. However, their comparison is not straightforward since, in contrast to the TrisK series, TrisQ shows a remarkable dependence on the cationic nature of the buffer, displaying strikingly modest results in the Na^+ buffer ($\Delta T_{1/2}=10.2\,°C$). Interestingly, this situation is reminiscent of that of BRACO-19, (Figure 3), in which $\Delta T_{1/2}=16.5$ and 27.3 °C in Na^+ and K^+ buffers, respectively (i.e., $\Delta\Delta T_{1/2}=10.8\,°C$) but with an even higher amplitude ($\Delta\Delta T_{1/2}=16.4\,°C$ for TrisQ). This preference for K^+ over Na^+ has been reported for several classes of quadruplex binders[32] without being well understood. Nonetheless, this trend may reflect the greater accessibility of the terminal quartets inside the K^+-promoted conformation(s), which is in agreement with the structures determined in the solid state and in solution. However, the possibility for external binding out of the quartets observed for certain ligands[12c,14] and the coexistence of several differently folded quadruplex conformations[33,36] make this trend difficult to be fully rationalized.

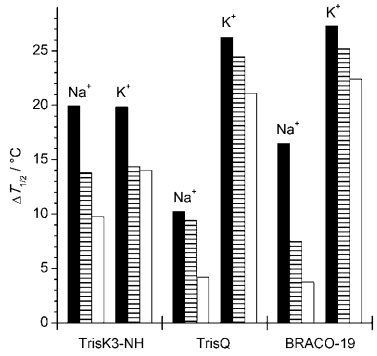


Figure 3. Comparison of FRET-melting results in Na^+ and K^+ buffers for TrisK3-NH, TrisQ and BRACO-19. The same experimental conditions were used as in Figure 2.[35]

In terms of selectivity, the excess of ds26 affects the quadruplex stabilization to various extents, depending on the ligand and the cationic conditions, but generally the resistance to the competition is better in K^+ than in Na^+ conditions (Figure 3 and Figure 4). In the TrisK series, compound TrisK3-NH clearly emerges as the most selective ligand because it exhibits a moderate to high selectivity both in Na^+ and in K^+ buffers ($S_{FRET}=0.49$ and 0.70, Figure 4). In the case of TrisQ, an even higher selectivity is observed in K^+ buffer ($S_{FRET}=0.80$), which makes this compound one of the strongest and most selective quadruplex binders reported so far.[37] On the other hand, it elicits a rather low selectivity in Na^+ buffer, in line with its low stabilizing effect ($S_{FRET}=0.41$). Again, this situation is reminiscent of BRACO-19,

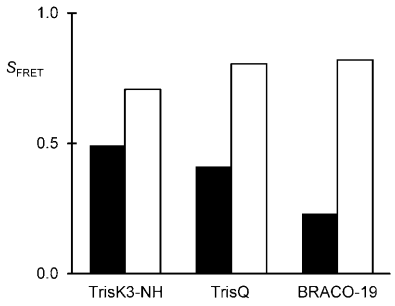


Figure 4. Quadruplex- versus duplex-DNA selectivity estimated by FRET-melting ($S_{FRET}=\Delta T_{1/2(+10\,\mu M)}/\Delta T_{1/2}$) for TrisK3-NH, TrisQ, and BRACO-19 in Na^+ (black) and in K^+ (white) conditions.

which features a very similar profile but with an even lower selectivity in Na^+ buffer ($S_{FRET}=0.23$ in Na^+ vs. 0.82 in K^+ buffer). In conclusion, although TrisK3-NH is worth considering due to its excellent recognition properties irrespective of the cation used, TrisQ may be a more promising candidate because of its high efficiency in K^+, rivaling the FRET-melting performances of BRACO-19, one of the most widely studied G-quadruplex ligands.[38]

Evaluation of quadruplex-DNA affinity and selectivity by the G4-FID assay: To further evaluate the quadruplex-binding properties of the two best candidates TrisK3-NH and TrisQ, fluorescent indicator displacement titrations (G4-FID assay) were performed. This assay enables evaluation of ligand affinity for DNA structures on the basis of its ability to displace the fluorescent probe thiazole orange (TO) from both quadruplex- and duplex-DNA matrices. The displacement efficiency that reflects the affinity is quantified by $^{G4}DC_{50}$ and $^{ds}DC_{50}$ representing the concentrations required to displace 50% of TO from the given DNA matrix.[20b,39] Herein, we used two DNA matrices previously used to calibrate this assay:[39] the telomeric quadruplex 22AG ($AG_3[T_2AG_3]_3$), which differs from F21T used in FRET-melting only by one supplementary adenine at the 5′-end, and the duplex-DNA ds26. Finally, since the FRET-melting results highlight the strong impact of the cation on quadruplex recognition, the experiments performed with 22AG have been carried out both in Na^+- and K^+-rich conditions.

As depicted in Figure 5A and Table 1, in Na^+ conditions TrisK3-NH displaces TO from 22AG with a much better efficiency ($^{G4}DC_{50}=0.58\,\mu M$) than from ds26 ($^{ds}DC_{50}>2.5\,\mu M$). Unexpectedly, the displacement of TO from 22AG is significantly less efficient in K^+ conditions ($^{G4}DC_{50}=2.05\,\mu M$), in contrast to FRET-melting results. Currently, we do not have any satisfactory explanation for the discrepancy between the two methods, however, the existence of allosteric or external binding sites in the K^+ form(s), which would induce stabilization but not displacement of the TO probe, might be reasoned. This situation has already been observed with the side-arm substituted quinacridines.[20b] In contrast, the G4-FID curves (Figure 5B) show that TrisQ is a much better TO displacer from 22AG in K^+ than in Na^+ conditions ($^{G4}DC_{50}=0.3$ and 2.3 μM respectively); the ratio of the two

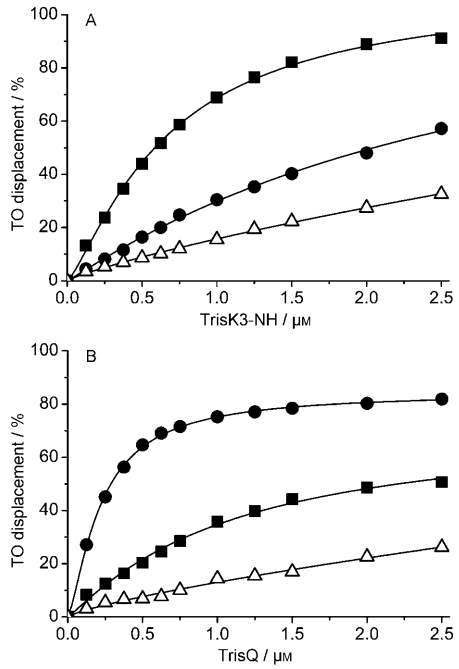


Figure 5. G4-FID titration curves obtained with A) TrisK3-NH and B) TrisQ, for experiments carried out with 22AG (Na^+ = filled square; K^+ = filled circle) and ds26 (K^+ = open triangle). Buffer: lithium cacodylate (pH 7.2, 10 mM) and NaCl or KCl (100 mM).

Table 1. G4-FID results: DC_{50} and selectivity values for TrisK3-NH and TrisQ.

Ligand	$^{G4}DC_{50}$[a] [μM]		$^{ds}DC_{50}$ [μM]	S_{G4-FID}[b]	
	22AG (K^+)	22AG (Na^+)	ds26	22AG (K^+)	22AG (Na^+)
TrisK3-NH	2.0	0.6	>2.5	2	7
TrisQ	0.3	2.3	>2.5	17	5

[a] Experimental errors are estimated at ±5%. [b] Estimated selectivity: $S_{G4-FID} = {}^{ds}DC_{50}/{}^{G4}DC_{50}$; however, the $^{ds}DC_{50}$ value cannot be determined when the interaction with duplex-DNA is very weak. In this case, the selectivity is estimated as follows: the TO displacement obtained with 2.5 μM of ligand on duplex DNA is determined and the concentration C of ligand required to induce the same fluorescence decrease on quadruplex-DNA is determined. The estimated selectivity value corresponds to the ratio of these two concentrations: $S_{G4-FID} = 2.5/C$.

$^{G4}DC_{50}$ (Na^+/K^+) indicates an eightfold preference. In addition, TrisQ hardly displaces TO from the duplex matrix ds26 ($^{ds}DC_{50} > 2.5$ μM). In this case, the preference for binding the telomeric quadruplex in K^+ conditions and the high quadruplex versus duplex selectivity ($S_{G4-FID} = 17$) are fully consistent with the FRET-melting results. Of note, the displacement of TO is incomplete and levels off at 80%, which again may indicate the existence of allosteric binding sites both for the ligand and the fluorescent probe resulting in indirect competition.

Interaction with the c-myc quadruplex

It can be assumed that in case of TrisK3-NH, the association is mainly driven by π–π stacking interactions between the broad aromatic surface of the ligand and the most accessible G-quartet of the quadruplex, and furthermore stabilized by the side chains through additional electrostatic interactions and hydrogen bonding. In contrast, TrisQ cannot interact with loops or any other surrounding structural features, for example, phosphates or walls of the grooves. It was thus of interest to determine if the presence and the nature of the loops that surround the quartets could influence significantly the binding behavior of the two compounds. We decided to study the interaction of the two ligands with another well-defined G-quadruplex structure, the quadruplex formed in the sequence of the c-myc promoter, which has been extensively studied since dysregulation of this element is involved in many cancers and formation of quadruplexes has been shown to promote repression of its transcription.[40] In addition, a number of ligands have been reported recently for their interaction with c-myc.[41] The interaction was evaluated by the two methods used previously. Firstly, the competitive FRET-melting assay was performed in the presence of F21T and unlabeled c-myc ($T_2GAG_3TG_3TAG_3TG_3TA_2$) as competitor. The results shown in Figure 6A indicate a very

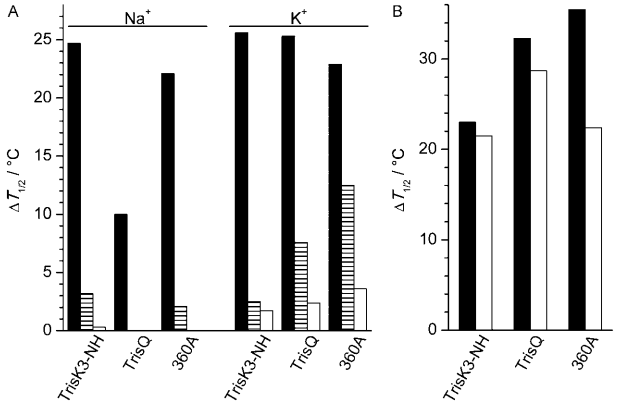


Figure 6. A) Competitive FRET-melting with F21T and c-myc as competitor and various compounds. Experiments were carried out with F21T (0.2 μM), ligand (1 μM) without (black bars) or with c-myc (3 μM: horizontally striped bars; 10 μM: white bars), with the concentration expressed in strand. Buffer: lithium cacodylate (pH 7.2, 10 mM), with NaCl (100 mM) or LiCl (99 mM) + KCl (1 mM); B) FRET-melting stabilization obtained with FmycT in the same buffer conditions (black: Na^+; white: K^+).

efficient competition since, for both ligands, the stabilization of F21T is dramatically decreased at the lowest concentration in c-myc competitor (3 μM) and totally removed at the highest concentration (10 μM). This phenomenon, which indicates redistribution of ligands from the telomeric to the c-myc quadruplex, seems to be rather independent of the cation present in the medium although the resistance is slightly stronger in K^+ for TrisQ. The effects of the two ligands are very similar to that of the bisquinolinium 360A, one of the best quadruplex ligands,[42] although the latter shows a slightly higher resistance to the competition in K^+ conditions.

These first results indicate that the two ligands have a strong affinity for c-myc and hence a poor ability to discrim-

inate between the two quadruplexes. This was confirmed by FRET-melting using the doubly labeled c-myc sequence FmycT (FAM-$T_2GAG_3TG_3TAG_3TG_3TA_2$-TAMRA). In Na^+ and K^+ conditions, remarkably strong stabilization is induced, with $\Delta T_{1/2}$ values (Na^+/K^+) ranging from 23/21.5 to 32.3/28.7 °C (Figure 6B). Again, the TrisQ compound appears slightly more efficient than the three-armed TrisK3-NH, but altogether the effect of the two ligands compares favorably with that of the reference compound 360A ($\Delta T_{1/2}(Na^+/K^+) = 35.5/22.4$ °C), thereby confirming that the two compounds are strong c-myc binders.[40,41c–d,43] Of note, due to the high stability of c-myc, the FRET-melting was performed at lower KCl concentration than that used for F21T (1 vs. 10 mM KCl) and the values cannot be directly compared with those of Table 1 (see the Supporting Information, Tables S2 A and B).

The G4-FID assay was also performed with TrisK3-NH, TrisQ, and the c-myc quadruplex and the resulting titrations curves are shown in Figure 7. A significant difference is observed between the two ligands, indicating that TrisQ displaces the fluorescent probe with a much higher efficiency than TrisK3-NH ($^{G4}DC_{50} < 0.1$ μM for TrisQ vs. 1.3 μM for TrisK3-NH). Although the higher binding affinity of TrisQ was suggested by the FRET-melting measurements, this large difference was somewhat unexpected. Again, this may indicate an indirect competition with the TO probe due to external binding in case of TrisK3-NH, thereby suggesting a clear difference in the binding mode of the two compounds in the concentration range examined. Finally, the data demonstrates that a combination of the two assays is necessary to gain detailed information on ligand–quadruplex interactions.

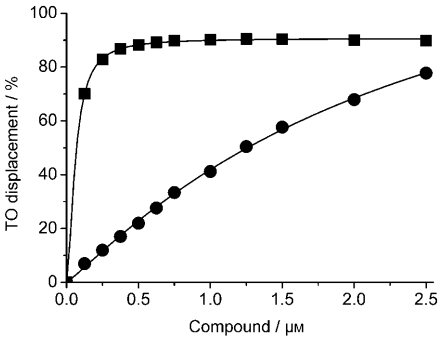


Figure 7. G4-FID titrations curves obtained with TrisK3-NH (circles) and TrisQ (squares) for experiments carried out with c-myc quadruplex in lithium cacodylate buffer (pH 7.2, 10 mM), in the presence of KCl (100 mM) at 20 °C.

Structural considerations: The nature of the binding interaction is difficult to rationalize if the appropriate geometry of interaction is not available. To gain further insights into the ligand–quadruplex interaction, manual docking was carried out with minimized structures of TrisK3-NH and TrisQ and the c-myc quadruplex (ChemAxon Calculator Plugins)[44]. This quadruplex structure provides a clear advantage given that an NMR-derived structure of its complex with TMPyP4 has been recently reported (PDB entry: 2A5R).[15] This structure, obtained with the 24-nucleotide modified sequence Pu24I ($TGAG_3TG_2IGAG_3TG_4A_2G_3$, with the guanine in position 10 being substituted by an inosine), is one of the few examples of a solution structure of a complex with a small molecule bound (within a more or less defined pocket), to an intramolecular quadruplex of biological relevance. Manual docking within Maestro[45] was then performed (minimizing steric clashes and maximizing the π–π overlap and hydrogen bonding when applicable), resulting in a few selected positions shown in Figure 8.

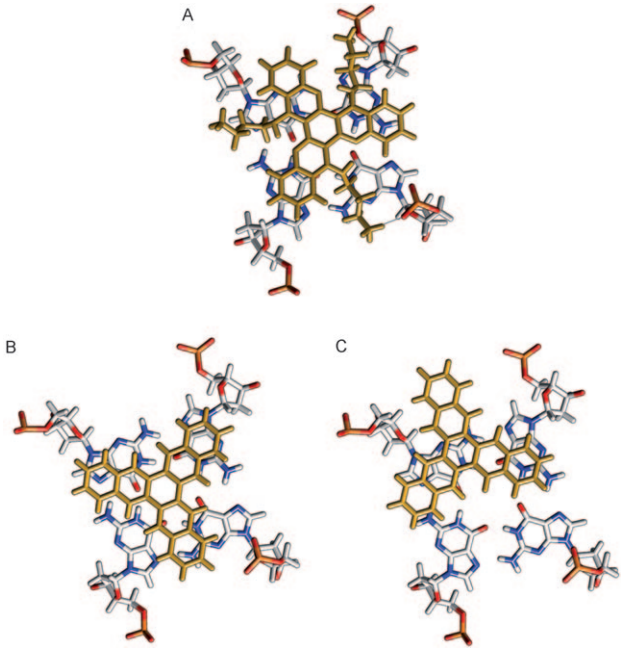


Figure 8. Superposition onto G-tetrad of A) TrisK3-NH; B) and C) TrisQ (two possible orientations). Molecular graphics images were produced using the UCSF Chimera package.[46]

In the case of TrisK3-NH (Figure 8, A), at least one hydrogen bond between one amino terminated chain and a phosphate group and/or the deoxyribose can always be present, maintaining an optimal overlap with the quartet and indicating the ability of the molecule to establish supplementary interactions with the residues surrounding the quartet. This model is in agreement with recent observations that the interaction of drugs with cationic side-arms with structural features surrounding the quartets can be mediated by hydrogen bonding[12b,14,17] and suggests that a combination of the ligand's side-chain–DNA interactions and π–π accessibility governs the interaction. In the case of TrisQ, π stacking between the electron-rich G-tetrad and the electron-poor TrisQ core is achieved through at least two guanine bases and two quinolizinium branches of TrisQ (Figure 8, B and C). However, due to its smaller steric hindrance and the lack of potential hydrogen bonding, this π system could adopt several positions, resulting in various geometries of

the interaction. The third quinolizinium branch could either position itself on the center of the G-quartet (Figure 8B) or adopt a clearly offset position pointing outside the G-quartet, towards the entrance of the groove (Figure 8C).

Notably, in both cases, the cofacial arrangement does not imply a maximum overlap between the aromatic three-branched core and the quartet, as would have been the case if van der Waals and solvophobic effects were dominating. More likely, the relative orientation of the ligand and the quartet is guided by minimizing the electrostatic repulsions between the two π systems and external constraints (e.g., steric hindrance of the loop residues in proximity).

Discussion

Although several molecular guidelines have been established and used to enhance the binding selectivity of small molecules to quadruplexes, the question of ligand structural predictability still remains with respect to the large structural diversity of quadruplex binders, the conformational heterogeneity of quadruplexes, and the small amount of structural data available for drug–quadruplex complexes. Herein, we have chosen to explore the potential of an aromatic three-branched scaffold of C_3-symmetry to compare compounds possessing similar geometrical features but differing by the presence or the absence of side chains in an attempt to evaluate the various contributions due to, on one hand, aromatic–aromatic interactions and on the other hand, side-chain–DNA interactions. To this end, two synthetic schemes have been devised; the first scheme gave access to the TrisK series (six analogues) possessing a neutral core endowed with three ammonium-terminated side chains and the second involved the preparation of a unique compound TrisQ, an electron-poor system featuring three permanently charged bridgehead nitrogen atoms.

Evaluation by FRET-melting and G4-FID assays led to the identification of two compounds (TrisK3-NH and TrisQ) that display binding efficiencies close to that of the best binders reported so far, such as 360A and BRACO-19, thereby fully validating the choice of the molecular design. Clearly, the three-branched scaffold is a structural advantage that provides not only a large seven-ring π-surface favorable to a strong interaction with G-quartets, but most importantly, creates a certain steric hindrance that prevents duplex binding. This is evidenced in the case of TrisQ, which has an excellent binding selectivity for quadruplex- versus duplex-DNA in spite of the absence of side chains. More mitigated results were obtained with TrisK3-NH, which exhibits a more modest quadruplex- versus duplex-DNA selectivity, although it remains at a satisfactory level relative to many reported quadruplex binders.

Another remarkable point is the large difference in the binding performances of TrisQ with respect to the cation present in the solution. Indeed, TrisQ binds the human telomeric quadruplex with a very poor efficiency in the presence of Na^+, which is in stark contrast to its high stabilizing ability in K^+. Consistent with this, the quadruplex- versus duplex-DNA selectivity is better in K^+ conditions. These observations tend to demonstrate that the interaction is stronger in K^+ and suggest that the Na^+ form is not well-recognized by the trisquinolizinium scaffold, thus revealing steric hindrance and/or a reduced accessibility of the external quartets. This trend has been observed for a number of ligands including BRACO-19, but there is currently no satisfactory rational explanation, although understanding this difference would be of importance for the development of ligands able to discriminate between different quadruplexes. Conversely, TrisK3-NH shows a relatively similar level of stabilization in Na^+ and K^+ conditions, whereas the G4-FID data indicate a significant preference for the Na^+ form.

Using the same methods, the interaction of the two ligands with the c-myc quadruplex was evaluated. Both TrisQ and TrisK3-NH show exceptional stabilization effects ($\Delta T_{1/2}$ values of up to 32 °C), which are close to that of the reference compound 360A. Again, the G4-FID measurements helped to confirm the superior binding ability of the nonsubstituted TrisQ scaffold, which seems to have tighter binding likely to be attributable to a unique binding mode.

Globally, the data obtained for TrisQ shows good accordance between the two methods used (FRET-melting and G4-FID). In contrast, for TrisK3-NH, divergences are found; G4-FID data showed differences that were not revealed by FRET-melting or at least less clearly. This suggests that the trisubstituted compound has a more complex binding behavior; the side chains may be responsible for multiple binding modes (stacking on G-quartets, external binding to loops, and external binding to duplex-DNA), which makes the data more difficult to evaluate because it represents several contributions. These observations confirm the importance of using at least two or ultimately several methods of evaluation based on different principles, with the aim to better understand the contributions that come into play. Our study also shows the necessity of comparing the performance of new ligands with well-studied reference compounds to help place newly proposed molecular designs in the very dense context of quadruplex ligands.

Molecular modeling indicates that TrisK3-NH has a good capacity for quartet overlapping that might explain its high quadruplex-stabilization activity and in this respect, this ligand seems more efficient than its two-branched counterpart, that is, the trisubstituted quinacridine developed previously.[16] In addition, the presence of free amino groups may promote hydrogen bonding with the target either directly or through water molecules.[44] This would be consistent with experimental measurements, which have shown the importance of chain length in optimizing the properties in the TrisK series (at least a four-membered chain is required). The potential of TrisK3-NH for establishing hydrogen bonding with residues surrounding the quartets could also explain the preference for the Na^+ form of the telomeric quadruplex, which exhibits accessible diagonal and edgewise loops. However, whereas the side chains might enable supplementary interactions with the quadruplex, they appear not to be

a strong determinant of selectivity in the present case. In the case of TrisQ, the relative position of the aromatic platform on the quartet may vary significantly thus providing more possibilities in the geometry of the arrangement than for the trisubstituted compound. The partial overlap of the aromatic surface area in contact is in line with the basic principle of aromatic–aromatic interactions[47] and suggests that the notion of shape complementarity and optimal aromatic overlap between a ligand and a G-quartet should be taken with care and is not necessarily a successful guide for optimizing interaction.[48] It is also likely that a simple π-stacking interaction mode is also dependent on the external constraints and susceptible to influences of the surrounding structural features and of their local dynamics.

Finally, if we consider that ligands may interact at the junction of two quadruplex units through π-stacking and eventually dimerization,[12b,14] it is likely that TrisK3-NH and TrisQ will not display the same ability to interact with a quadruplex junction, since the former is able to dimerize by analogy with the behavior of NDI, whereas TrisQ should not be prone to dimerization due to strong electrostatic repulsion as shown by the X-ray analysis of its structure (Figure S1 in the Supporting Information). This might explain the differences in their binding behavior but a full evaluation requires further investigation.

Conclusion

Although TrisK3-NH and TrisQ share common features (shape, size, and triscationic charge under the experimental conditions) and can stack on G-quartets, they clearly interact with quadruplexes with different strengths and differ by their capacity to discriminate one quadruplex from another and quadruplex- from duplex-DNA. TrisK3-NH interacts certainly in a dual mode involving quartet and loops, whereas the interaction of TrisQ appears most exclusively driven by efficient π-stacking forces with the quartet surface. However, a comparison of data collected on both telomeric and c-myc quadruplexes suggests that the naked, nonsubstituted platform is more efficient both in terms of quadruplex-binding strength and in terms of quadruplex- over duplex-DNA selectivity (at least in K^+, the physiologically relevant conditions). It is likely that the presence of flexible amino-terminated side chains represents a double-edged sword which, on one hand, holds a great potential for establishing hydrogen bonding interactions with the residues surrounding the quartets and on the other hand, favors nonspecific external electrostatic interactions with both quadruplex and duplex architectures. The trisusbstituted TrisK scaffold remains nonetheless an attractive design, which retains a high affinity and selectivity for the telomeric form in Na^+ and may be optimized by chemical engineering of the pendant side chains.

Acknowledgements

The authors would like to thank the Centre National de la Recherche Scientifique (CNRS) and the Commissariat à l'Energie Atomique (CEA) for funding H.B., the Institut National de la Santé et de la Recherche Médicale (INSERM), the Région Aquitaine, Fondation pour la Recherche Médicale (FRM) (J.L.M.), and a grant ANR-09-BLAN-0355 from the "G4Toolbox" (M.P.T.F. and J.L.M.). Anne De Cian (MNHN) is gratefully acknowledged for preliminary FRET-melting experiments and Eric Largy (Institut Curie) for help with the G4-FID experiments. Prof. Heiko Ihmels (Universität Siegen, Germany) is greatly acknowledged for fruitful discussions.

[1] a) R. D. Wells, R. Dere, M. L. Hebert, M. Napierala, L. S. Son, *Nucleic Acids Res.* **2005**, *33*, 3785–3798; b) J. Eddy, N. Maizels, *Mol. Carcinog.* **2009**, *48*, 319–325.
[2] F. Bena, S. Gimelli, E. Migliavacca, N. Brun-Druc, K. Buiting, S. E. Antonarakis, A. J. Sharp, *Hum. Mol. Genet.* **2010**, *19*, 1967–1973.
[3] J. T. Davis, *Angew. Chem.* **2004**, *116*, 684–716; *Angew. Chem. Int. Ed.* **2004**, *43*, 668–698.
[4] a) D. J. Patel, A. T. Phan, V. Kuryavyi, *Nucleic Acids Res.* **2007**, *35*, 7429–7455; b) S. Burge, G. N. Parkinson, P. Hazel, A. K. Todd, S. Neidle, *Nucleic Acids Res.* **2006**, *34*, 5402–5415; c) A. N. Lane, J. B. Chaires, R. D. Gray, J. O. Trent, *Nucleic Acids Res.* **2008**, *36*, 5482–5515.
[5] a) A. Piazza, J. B. Boule, J. Lopes, K. Mingo, E. Largy, M. P. Teulade-Fichou, A. Nicolas, *Nucleic Acids Res.* **2010**, *38*, 4337–4348; b) A. Mendez-Bermudez, M. Hills, H. A. Pickett, A. T. Phan, J. L. Mergny, J. F. Riou, N. J. Royle, *Nucleic Acids Res.* **2009**, *37*, 6225–6238.
[6] H. J. Lipps, D. Rhodes, *Trends Cell Biol.* **2009**, *19*, 414–422.
[7] a) N. Sreenivasachary, J. M. Lehn, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 5938–5943; b) T. Giorgi, S. Lena, P. Mariani, M. A. Cremonini, S. Masiero, S. Pieraccini, J. P. Rabe, P. Samori, G. P. Spada, G. Gottarelli, *J. Am. Chem. Soc.* **2003**, *125*, 14741–14749; c) D. Gonzalez-Rodriguez, P. G. A. Janssen, R. Martin-Rapun, I. De Cat, S. De Feyter, A. Schenning, E. W. Meijer, *J. Am. Chem. Soc.* **2010**, *132*, 4710–4719; d) M. Nikan, J. C. Sherman, *Angew. Chem.* **2008**, *120*, 4978–4980; *Angew. Chem. Int. Ed.* **2008**, *47*, 4900–4902.
[8] a) S. Neidle, G. Parkinson, *Nat. Rev. Drug Discovery* **2002**, *1*, 383–393; b) L. H. Hurley, *Nat. Rev. Cancer* **2002**, *2*, 188–200.
[9] a) M. Wieland, J. S. Hartig, *Chem. Biol.* **2007**, *14*, 757–763; b) S. Kumari, A. Bugaut, J. L. Huppert, S. Balasubramanian, *Nat. Chem. Biol.* **2007**, *3*, 218–221; c) D. Gomez, A. Guedin, J. L. Mergny, B. Salles, J. F. Riou, M. P. Teulade-Fichou, P. Calsou, *Nucleic Acids Res.* **2010**, *38*, 7187–7198.
[10] a) D. Monchaud, M. P. Teulade-Fichou, *Org. Biomol. Chem.* **2008**, *6*, 627–636; b) M. Franceschin, *Eur. J. Org. Chem.* **2009**, 2225–2238; c) D. Monchaud, A. Granzhan, N. Saettel, A. Guédin, J.-L. Mergny, M.-P. Teulade-Fichou, *J. Nucleic Acids* **2010**, DOI: 10.4061/2010/525862; d) S. N. Georgiades, N. H. Abd Karim, K. Suntharalingam, R. Vilar, *Angew. Chem.* **2010**, *122*, 4114–4128; *Angew. Chem. Int. Ed.* **2010**, *49*, 4020–4034.
[11] a) S. Neidle, *Curr. Opin. Struct. Biol.* **2009**, *19*, 239–250; b) A. T. Phan, *FEBS J.* **2010**, *277*, 1107–1117.
[12] a) S. M. Haider, G. N. Parkinson, S. Neidle, *J. Mol. Biol.* **2003**, *326*, 117–125; b) N. H. Campbell, G. N. Parkinson, A. P. Reszka, S. Neidle, *J. Am. Chem. Soc.* **2008**, *130*, 6722–6724; c) G. N. Parkinson, R. Ghosh, S. Neidle, *Biochemistry* **2007**, *46*, 2390–2397.
[13] a) G. R. Clark, P. D. Pytel, C. J. Squire, S. Neidle, *J Am Chem. Soc* **2003**, *125*, 4066–4067; b) E. Gavathiotis, R. A. Heald, M. F. G. Stevens, M. S. Searle, *Angew. Chem.* **2001**, *113*, 4885–4887; *Angew. Chem. Int. Ed.* **2001**, *40*, 4749–4751; c) E. Gavathiotis, R. A. Heald, M. F. G. Stevens, M. S. Searle, *J. Mol. Biol.* **2003**, *334*, 25–36; d) S. Cosconati, L. Marinelli, R. Trotta, A. Virno, S. De Tito, R. Romagnoli, B. Pagano, V. Limongelli, C. Giancola, P. G. Baraldi, L. Mayol, E. Novellino, A. Randazzo, *J. Am. Chem. Soc.* **2010**, *132*, 6425–

6433; e) M. J. Cocco, L. A. Hanakahi, M. D. Huber, N. Maizels, *Nucleic Acids Res.* **2003**, *31*, 2944–2951.
[14] G. N. Parkinson, F. Cuenca, S. Neidle, *J. Mol. Biol.* **2008**, *381*, 1145–1156.
[15] A. T. Phan, V. Kuryavyi, H. Y. Gaw, D. J. Patel, *Nat. Chem. Biol.* **2005**, *1*, 167–173.
[16] C. Hounsou, L. Guittat, D. Monchaud, M. Jourdan, N. Saettel, J. L. Mergny, M. P. Teulade-Fichou, *ChemMedChem* **2007**, *2*, 655–666.
[17] G. W. Collie, S. M. Haider, S. Neidle, G. N. Parkinson, *Nucl. Acids Res.* **2010**, *38*, 5569–5580.
[18] a) K. Shin-ya, K. Wierzba, K. Matsuo, T. Ohtani, Y. Yamada, K. Furihata, Y. Hayakawa, H. Seto, *J. Am. Chem. Soc.* **2001**, *123*, 1262–1263; b) T. Tauchi, K. Shin-ya, G. Sashida, M. Sumi, A. Nakajima, T. Shimamoto, J. H. Ohyashiki, K. Ohyashiki, *Oncogene* **2003**, *22*, 5338–5347; c) N. Temime-Smaali, L. Guittat, A. Sidibe, K. Shin-ya, C. Trentesaux, J.-F. Riou, *PLoS ONE* **2009**, *4*, e6919.
[19] S. Agrawal, R. P. Ojha, S. Maiti, *J. Phys. Chem. B* **2008**, *112*, 6828–6836.
[20] a) A. De Cian, L. Guittat, K. Shin-ya, J.-F. Riou, J.-L. Mergny, *Nucleic Acids Symposium Series* **2005**, *49*, 235–236; b) D. Monchaud, C. Allain, M. P. Teulade-Fichou, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4842–4845.
[21] A. Granzhan, H. Ihmels, K. Jäger, *Chem. Commun.* **2009**, 1249–1251.
[22] a) J. T. Kern, P. W. Thomas, S. M. Kerwin, *Biochemistry* **2002**, *41*, 11379–11389; b) V. Casagrande, A. Alvino, A. Bianco, G. Ortaggi, M. Franceschin, *J. Mass Spectrom.* **2009**, *44*, 530–540.
[23] a) J. L. Mergny, L. Lacroix, M. P. Teulade-Fichou, C. Hounsou, L. Guittat, M. Hoarau, P. B. Arimondo, J. P. Vigneron, J. M. Lehn, J. F. Riou, T. Garestier, C. Helene, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 3062–3067; b) M. P. Teulade-Fichou, C. Carrasco, L. Guittat, C. Bailly, P. Alberti, J. L. Mergny, A. David, J. M. Lehn, W. D. Wilson, *J. Am. Chem. Soc.* **2003**, *125*, 4732–4740.
[24] N. Saettel, N. Katsonis, A. Marchenko, M. P. Teulade-Fichou, D. Fichou, *J. Mater. Chem.* **2005**, *15*, 3175–3180.
[25] H. Bertrand, PhD Thesis, Université Pierre et Marie Curie, Paris VI (France), **2008**.
[26] L. Ginnari-Satriani, V. Casagrande, A. Bianco, G. Ortaggi, M. Franceschin, *Org. Biomol. Chem.* **2009**, *7*, 2513–2516.
[27] J. E. Moses, D. J. Ritson, F. Zhang, C. M. Lombardo, S. Haider, N. Oldham, S. Neidle, *Org. Biomol. Chem.* **2010**, *8*, 2926–2930.
[28] R. Lartia, H. Bertrand, M. P. Teulade-Fichou, *Synlett* **2006**, 610–614.
[29] a) S. Arai, M. Hida in *Advances in Heterocyclic Chemistry, Vol. 55* (Ed.: A. R. Katritzky), Academic Press, **1992**, pp. 261–358; b) H. Ihmels in *Science of Synthesis, Vol. 15: Six-Membered Hetarenes with One Nitrogen or Phosphorus Atom* (Ed.: D. Black), Georg Thieme Verlag, Stuttgart, **2004**, pp. 907–946.
[30] O. Baudoin, M. P. Teulade-Fichou, J. P. Vigneron, J. M. Lehn, *J. Org. Chem.* **1997**, *62*, 5458–5470.
[31] C. K. Bradsher, J. P. Sherer, *J. Org. Chem.* **1967**, *32*, 733–737.
[32] A. De Cian, L. Guittat, M. Kaiser, B. Sacca, S. Amrane, A. Bourdoncle, P. Alberti, M. P. Teulade-Fichou, L. Lacroix, J. L. Mergny, *Methods* **2007**, *42*, 183–195.
[33] R. D. Gray, L. Petraccone, J. O. Trent, J. B. Chaires, *Biochemistry* **2010**, *49*, 179–194.
[34] A. De Cian, P. Grellier, E. Mouray, D. Depoix, H. Bertrand, D. Monchaud, M. P. Teulade-Fichou, J. L. Mergny, P. Alberti, *ChemBioChem* **2008**, *9*, 2730–2739.
[35] A. De Cian, PhD Thesis, Université Pierre et Marie Curie, Paris VI (France) **2007**.
[36] a) K. W. Lim, S. Amrane, S. Bouaziz, W. X. Xu, Y. G. Mu, D. J. Patel, K. N. Luu, A. T. Phan, *J. Am. Chem. Soc.* **2009**, *131*, 4301–4309; b) G. N. Parkinson, M. P. H. Lee, S. Neidle, *Nature* **2002**, *417*, 876–880; c) A. T. Phan, D. J. Patel, *J. Am. Chem. Soc.* **2003**, *125*, 15021–15027; d) A. Ambrus, D. Chen, J. X. Dai, T. Bialis, R. A. Jones, D. Z. Yang, *Nucleic Acids Res.* **2006**, *34*, 2723–2735.
[37] A. Arola-Arnal, J. Benet-Buchholz, S. Neidle, R. Vilar, *Inorg. Chem.* **2008**, *47*, 11910–11919.
[38] a) M. Read, R. J. Harrison, B. Romagnoli, F. A. Tanious, S. H. Gowan, A. P. Reszka, W. D. Wilson, L. R. Kelland, S. Neidle, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 4844–4849; b) S. Neidle, *FEBS J.* **2010**, *277*, 1118–1125.
[39] D. Monchaud, C. Allain, H. Bertrand, N. Smargiasso, F. Rosu, V. Gabelica, A. De Cian, J. L. Mergny, M. R. Teulade-Fichou, *Biochimie* **2008**, *90*, 1207–1223.
[40] V. Gonzalez, L. H. Hurley, *Annu. Rev. Pharmacol. Toxicol.* **2010**, *50*, 111–129.
[41] a) P. Wu, D. L. Ma, C. H. Leung, S. C. Yan, N. Y. Zhu, R. Abagyan, C. M. Che, *Chem. Eur. J.* **2009**, *15*, 13008–13021; b) E. A. Owen, M. A. Keniry, *Aust. J. Chem.* **2009**, *62*, 1544–1549; c) J. Dash, P. S. Shirude, S. Balasubramanian, *Chem. Commun.* **2008**, 3055–3057; d) T. Lemarteleur, D. Gomez, R. Paterski, E. Mandine, P. Mailliet, J. F. Riou, *Biochem. Biophys. Res. Commun.* **2004**, *323*, 802–808.
[42] a) A. De Cian, L. Lacroix, C. Douarre, N. Temime-Smaali, C. Trentesaux, J. F. Riou, J. L. Mergny, *Biochimie* **2008**, *90*, 131–155; b) C. Granotier, G. Pennarun, L. Riou, F. Hoffschir, L. R. Gauthier, A. De Cian, D. Gomez, E. Mandine, J. F. Riou, J. L. Mergny, P. Mailliet, B. Dutrillaux, F. D. Boussin, *Nucleic Acids Res.* **2005**, *33*, 4182–4190.
[43] a) P. Wang, C.-H. Leung, D.-L. Ma, S.-C. Yan, C.-M. Che, *Chem. Eur. J.* **2010**, *16*, 6900–6911; b) J. E. Reed, A. J. P. White, S. Neidle, R. Vilar, *Dalton Trans.* **2009**, 2558–2568; c) J. Alzeer, B. R. Vummidi, P. J. C. Roth, N. W. Luedtke, *Angew. Chem.* **2009**, *121*, 9526–9529; *Angew. Chem. Int. Ed.* **2009**, *48*, 9362–9365; d) T. Agarwal, S. Roy, T. K. Chakraborty, S. Maiti, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4346–4349.
[44] Marvin, version 5.2, ChemAxon, **2009**.
[45] Maestro, version 9.0.211, Schrödinger, LLC, New York, NY, **2009**.
[46] E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, T. E. Ferrin, *J. Comput. Chem.* **2004**, *25*, 1605–1612.
[47] C. A. Hunter, K. R. Lawson, J. Perkins, C. J. Urch, *J. Chem. Soc. Perkin Trans. 2* **2001**, 651–669.
[48] J. Debray, W. Zeghida, M. Jourdan, D. Monchaud, M.-L. Dheu-Andries, P. Dumy, M.-P. Teulade-Fichou, M. Demeunynck, *Org. Biomol. Chem.* **2009**, *7*, 5219–5228.

Received: September 30, 2010
Revised: January 3, 2011
Published online: March 17, 2011

