DOI: 10.1002/ejoc.200801196

G-Quadruplex DNA Structures and Organic Chemistry: More Than One Connection

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Keywords: DNA structures / G-Quadruplex ligands / G-Quadruplexes / Telomerase inhibitors

G-Quadruplexes represent a group of unusual DNA secondary structures, based on Hoogsteen G–G paired hydrophobic planar rings consisting of four guanine units. Many studies have shown that G-quadruplex structures appear to be involved in several important biological processes, such as DNA replication, gene expression and recombination, as well as cell transformation. For these reasons, in the last few years G-quadruplexes have become the main research interest of many eminent scientists in different fields: from molecular to cellular biologists, from physicians to chemists. In this respect, organic chemists can play fundamental roles in many different areas. First of all, research into specific G-quadruplex ligands as potential drugs, in particular as telomerase inhibitors and thus potential anticancer drugs, has attracted strong attention. The rational design of and the various synthetic approaches toward these kinds of ligands represent two specific fields of interest for organic chemists. The molecules capable of stabilizing G-quadruplexes covered in this review are each characterized by an aromatic core that favours stacking interactions with the G-tetrads and, in most cases, by basic side chains that interact with DNA grooves. First generations and new classes of synthetic G-quadruplex ligands, as well as natural compounds, are presented. Moreover, because the specificities of the interactions between the studied ligands and different DNA structures (both duplex and quadruplex) are without doubt a main concern, determining the different biological effects of these compounds, several chemists are involved in developing approaches to explore this important subject. The last part of this review is devoted to a brief introduction of new fields of application of G-quadruplex structures with open synthetic challenges: quadruplex-forming modified oligonucleotides and nanostructures based on the G-quadruplex.

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1. Introduction

A few years ago we celebrated 50 years since Watson and Crick proposed the double-helical structure for duplex DNA;[1] this model represented the foundation of molecular biology, because it provided an answer to the question of the relationship between structure and function of DNA. In 1962, the first G-quartet model was proposed by Davies and co-workers,[2] but only several years later did the possible biological relevance of DNA structures based on this moiety begin to be addressed.[3]

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1.1 G-Quadruplexes

A G-quartet is composed of four guanine units, held together by a cyclic arrangement of eight Hoogsteen hydrogen bonds [Figure 1 (b)], which is quite different from the classical Watson–Crick G-C base paring for duplex DNA [Figure 1 (a)]. A number of G-quartets can overlap, giving rise to a family of nucleic acid secondary structures called G-quadruplexes.[4] The presence of a central cation (typically potassium) helps to maintain the stability of the structure, which may be very stable under physiological conditions.[5] Different G-quadruplex structures exist (Figure 2), depending on the orientations of the DNA strands and the *syn*/*anti* conformations of the guanines.[6] The level of interest in these structures has increased as a result of the hy-

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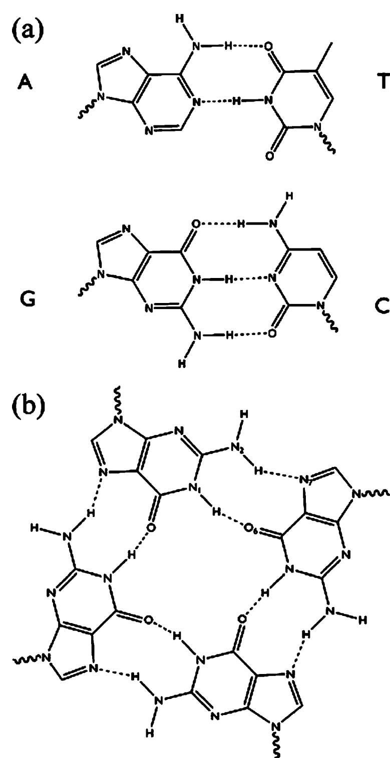

Figure 1. a) Watson–Crick base pairing. Adenine and thymine form two hydrogen bonds, whereas guanine and cytosine form three hydrogen bonds. b) Four guanines can hydrogen bond in a square arrangement to form a G-quartet, with a Hoogsteen G–G pairing pattern (J. L. Huppert, *Chem. Soc. Rev.* **2008**, *37*, 1375–1384. Reproduced by permission of The Royal Society of Chemistry, copyright 2008).

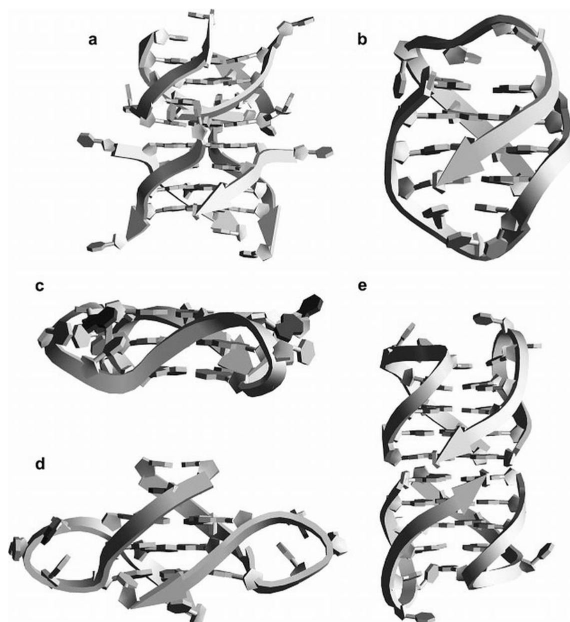

Figure 2. Views of various native quadruplex crystal structures. The backbones are represented as ribbons, and bases are drawn in cartoon form. a) d(TG_4T), b) d($G_4T_4G_4$), c) d[$AG_3(T_2AG_3)_3$], d) d(UAG_3UTAG_3T), e) lateral loop quadruplex in d($G_4UT_2G_4$) (S. Neidle, G.N. Parkinson, *Biochimie* **2008**, *90*, 1184–1196. Reproduced by permission of Elsevier, copyright 2008).

pothesis that G-quadruplex structures play roles in key biological processes.[7] The field of G-quadruplex study has greatly expanded recently as a result of the proposed in vivo existence of G-quadruplexes for oncogene promoters and telomeres through the use of specific ligands.[8]

1.2 Telomeres and Telomerase

The human telomeric G-overhang can fold into several different intramolecular quadruplex structures that differ in the positions of the adjacent loop regions.[9] In fact, human cells' telomeres represent the chromosomal ends (preventing them from fusion events), ranging in length from 3000 to 15000 bases, composed of tandem repeats of the 5′-GGTTAG-3′ sequence with a 3' overhang of the G-strand, which plays an important structural and functional role.[10] In human somatic cells, telomere length decreases with each cell division event, while reversion of this degradation by a specialized enzyme called telomerase increases cellular replicative capacity, leading to uncontrolled proliferation; in the majority of tumour cells (85–90%) this enzyme is overexpressed.[11] Optimal telomerase activity requires an unfolded single-stranded substrate, because telomerase must recognize and bind to the G-rich strand of the telomere, before addition of 5′-GGTTAG-3′ repeats to the ends of chromosomes by means of an inner RNA template. Since G-quadruplex formation directly inhibits telomerase elongation in vitro,[12] ligands that selectively bind to and stabilize G-quadruplex structures may interfere with telomere conformation and telomere elongation, acting as possible telomerase inhibitors (Figure 3), and so have attracted major attention as potential selective anticancer agents.[13]

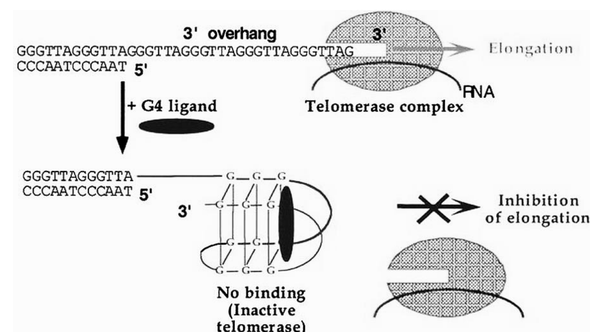

Figure 3. Possible mechanism of telomerase inhibition by G-quadruplex induction (J. F. Riou, L. Guittat, P. Mailliet, A. Laoui, E. Renou, O. Petitgenet, F. Mégnin-Chanet, C. Hélène, J. L. Mergny, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 2672–2677. Reproduced by permission of the National Academy of Sciences, USA, Copyright 2002).

2. G-Quadruplex Interactive Compounds as Telomerase Inhibitors

A range of G-quadruplex ligands have been shown to bind quadruplexes in vitro.[14] Although some very interesting examples of compounds capable of stabilizing G-quad-

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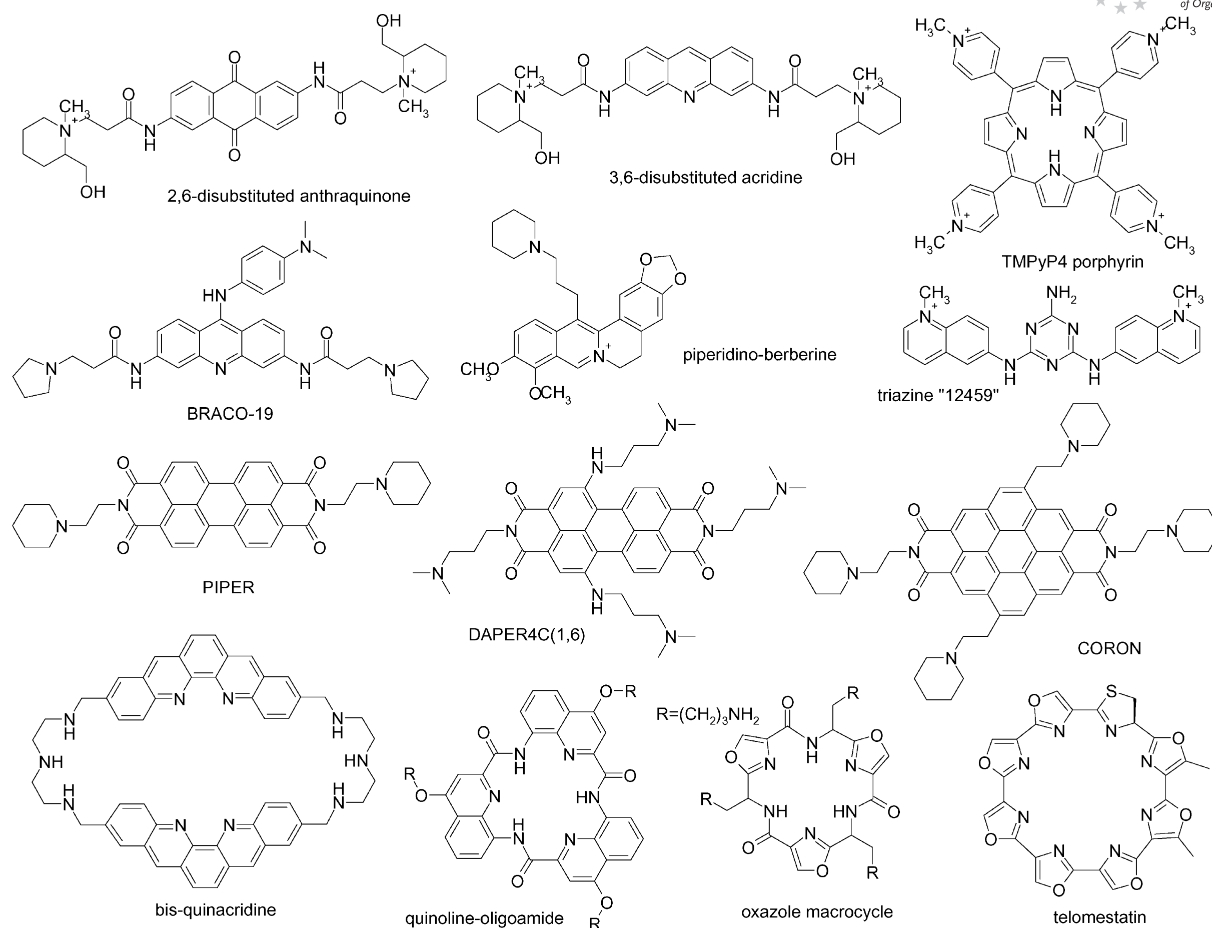

Figure 4. Structures of known G-quadruplex ligands.

ruplexes showing a binding mode based on loop and groove interactions have been reported,[15] the types of molecules presented in this review are each characterized by an aromatic core, which favours stacking interactions with the G-tetrads, and, in most cases, by basic side chains (positively charged under physiological conditions), which interact with the quadruple helix grooves (Figure 4).[16] These molecules recognize quadruplex DNA, adopting a terminal stacking mode (Figure 5), and are active in the telomere repeat amplification protocol (TRAP) assay.[17] Some of these molecules have also been shown to induce telomere shortening and/or telomere instability, triggering apoptosis and/or senescence programs in various cell lines.[18] It is worth noting that, as a result of their direct interaction with telomeres, G-quadruplex ligands have shown more rapid and specific effects than those that would be expected for simple telomerase inhibitors, for which a lag phase was predicted,[19] probably by perturbing the equilibrium of capped/uncapped telomeres.[20]

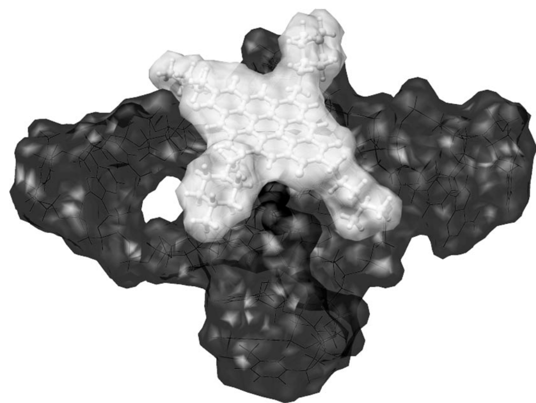

Figure 5. Representation of a complex between a coronene derivative (CORON3)[48] as a ball-and-stick model with a grey surface and a monomeric G-quadruplex (black surface), obtained by simulated annealing.[48]

2.1 The First Generations: Anthraquinones, Porphyrins and Acridines

Many molecules with the characteristics described above have been synthesized as potential telomerase inhibitors: anthraquinones[21] and 3,6-disubstituted acridines[22] are examples of the first generation of these inhibitors. Subsequently, trisubstituted acridines,[23] porphyrins,[24] and triazines[25] were synthesised to improve efficiency in telomerase inhibition (Figure 4).

One of the most promising compounds of this series is represented by BRACO19, a 3,6,9-trisubstituted acridine

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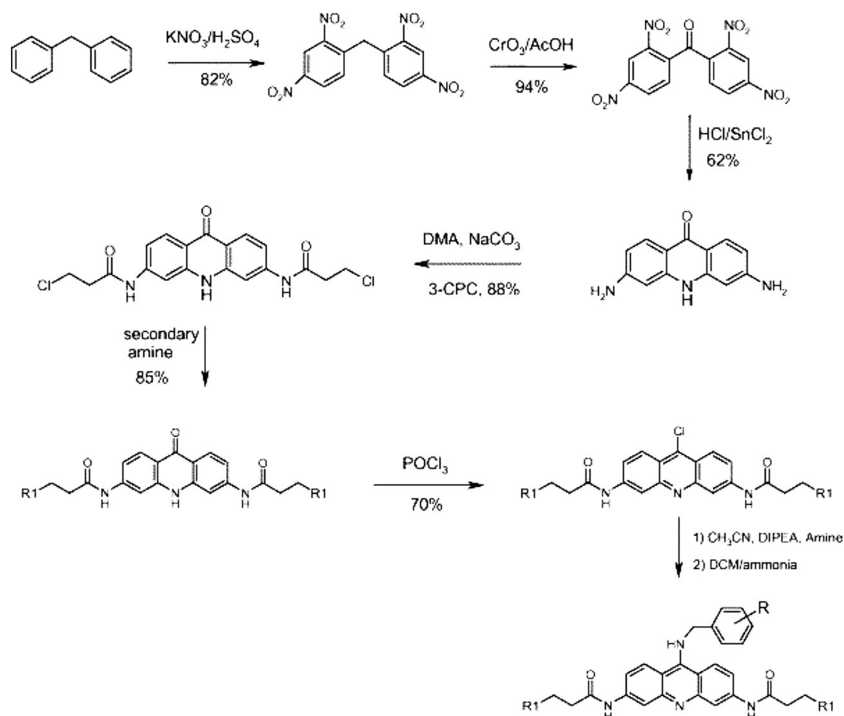


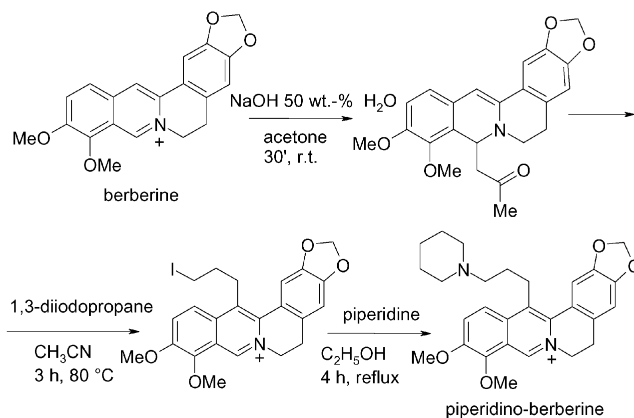
Scheme 1. General synthetic scheme for 3,6,9-trisubstituted acridine derivatives. (DCM = dichloromethane, DIPEA = *N*,*N*-diisopropylethylamine) (C. Martins, M. Gunaratnam, J. Stuart, V. Makwana, O. Greciano, A.P. Reszka, L.R. Kelland, S. Neidle, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2293–2298. Reproduced by permission of Elsevier, copyright 2007).

synthesized and studied by Neidle's group.[26] In fact, 9-[4-(dimethylamino)phenylamino]-3,6-bis(3-pyrrolidinopropionamido)acridine has been shown to inhibit tumour growth in vitro and in vivo at concentrations under 1 μM.[27] The synthesis of this class of molecules proceeds through acridone intermediates, by treatment of the acridone precursor with excess $POCl_3$ and substitution of the resulting 9-chloroacridine, as shown in Scheme 1.[28] Very recently, a series of trisubstituted acridine–peptide conjugates has been reported to show 10-fold discrimination between different G-quadruplex structures.[29]

2.2 Natural Compounds: Berberine and Telomestatin

Natural compounds such as berberine[30] and telomestatin[31] (Figure 4) have also been shown to inhibit human telomerase, the latter with high efficiency. Telomestatin was isolated from *Streptomyces anulatus* 3533-SV4, after a wide range of specific screening for telomerase inhibitors.[32] Indeed its name is due to its potent inhibitory activity against human telomerase at 5 nM concentration. After its structural characterization, showing the macrocyclic linkage of two methyloxazoles, five oxazoles and one thiazoline ring, the total synthesis of telomestatin was reported, and its absolute configuration was determined to be (*R*).[33] Telomestatin has been shown to be able to reduce cell growth both in vitro and in vivo in a number of human tumours, such as multiple myeloma,[34] neuroblastomas[35] and leukaemia.[36]

Berberine is an antibiotic alkaloid originating from Chinese herbal medicine, and its antibacterial activity against many species has been demonstrated.[37] The drug was subsequently screened for anticancer activity after evidence of antineoplastic properties.[38] More recently, berberine's anticancer activity was associated with down-regulation of telomerase activity[39] and, ultimately, with its ability to bind to G-quadruplex DNA.[40] As a result of this, several semisynthetic derivatives were prepared with the goal of improving quadruplex binding; in particular, 13-[41] and 9-substituted[42] berberine derivatives attracted strong attention. As an example, the synthetic pathway for 13-[3-(1-piperidino)propyl]berberine is shown in Scheme 2. The synthetic



Scheme 2. Synthetic scheme for 13-[3-(1-piperidino)propyl]berberine (piperidino-berberine).[41]

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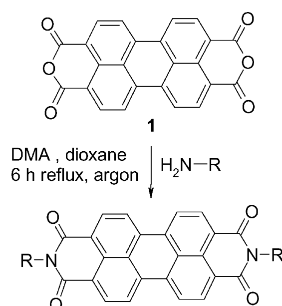
strategy uses acetonyl-berberine, obtained by condensation of berberine with acetone, as a key intermediate. It was next subjected to displacement of the acetonyl group with 1,3-diiodopropane, and subsequent substitution of the iodine atom with piperidine gave the berberine derivative with the desired side chain. Piperidino-berberine showed a better ability to stabilize G-quadruplex structures and a better antitelomerase activity, as determined by TRAP assay, than berberine, but a much higher activity was obtained with coralyne, a synthetic analogue of berberine.[41]

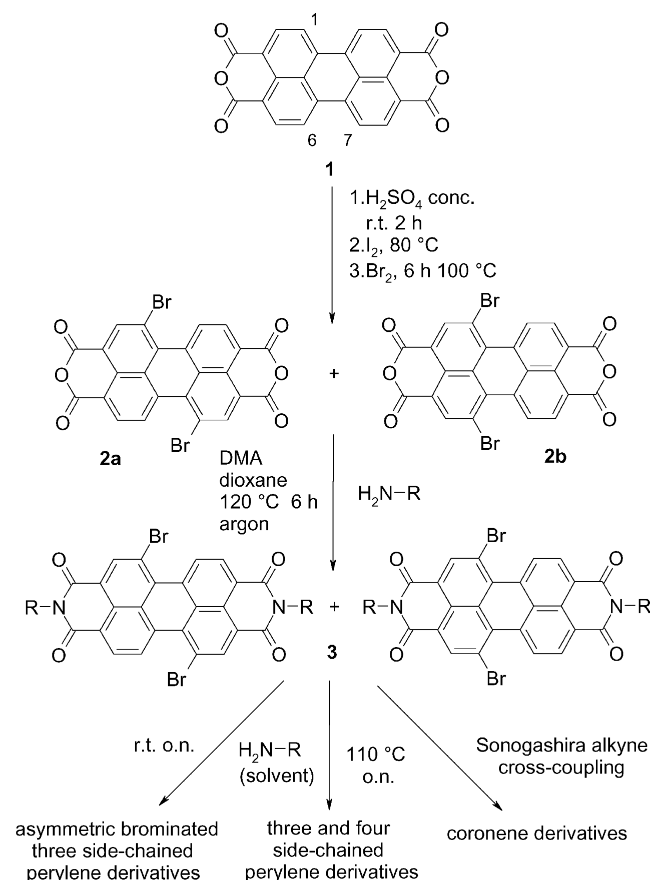
2.3 Perylene and Coronene Derivatives

Perylenedicarboximides present optimal features for interaction with G-quadruplexes[43] and show good abilities to induce different G-quadruplex structures and to inhibit telomerase,[44] depending on the side chain basicity and length.[45] Simple perylenedicarboximides with two basic side chains are easily prepared as shown in Scheme 3,[46] the first reported and most intensely studied being PIPER (Figure 4). Our research group has recently reported a series of new highly hydrosoluble perylene [such as DAPER4C(1,6)][47] and coronene (CORON)[48] derivatives with three or four side chains bearing various basic substituents (Figure 4). All of them exhibit enhanced ability to interact with G-quadruplex structures and to inhibit human telomerase, with respect to the previously reported perylene derivatives. Our study was carried out through investigation of the abilities of the synthesized compounds to induce inter- and intramolecular G-quadruplex structures as gauged by polyacrylamide gel electrophoresis (PAGE) and to inhibit telomerase in a modified TRAP assay. The two properties appear to be satisfactorily correlated.



Scheme 3. General synthetic scheme for the preparation of simple perylene diimides, where R does not contain any primary or secondary amino group or alcoholic function. (DMA = *N*,*N*-dimethylacetamide).

We have proposed a general synthetic protocol for these new derivatives, in which dibromo bay-substituted perylene diimides serve as key intermediates (Scheme 4).[49] The first step in the synthetic strategy is the bromination of the bay area of 3,4:9,10-perylenetetracarboxylic acid dianhydride (**1**) to provide two functionalized positions capable of reacting in the subsequent steps. Four positions (1, 6, 7 and 12) in the perylene moiety are reactive, but electronic and steric factors allow only two dibromo derivatives to be obtained; their ratio is determined by the reaction conditions, but the major isomer has always been 1,7-dibromoperylene-3,4:9,10-tetracarboxylic acid dianhydride (**2a**) and the minor one the corresponding 1,6-derivative (**2b**). Temperature and reaction time have been shown to be key parameters in determining the ratio between the two isomers. In particular, the highest proportion of the major isomer, in an approximately 6:1 ratio with respect to the minor 1,6-isomer, was obtained when the temperature was kept at 100 °C for 4–6 hours. The second step consists of the addition of the side chains on the major axis of the perylene core, through reactions between the anhydride groups and primary amines containing suitably functionalized chains, analogously to what has been reported for simple perylene diimides (Scheme 3).[46] In this way we obtained the desired dibromo bay-substituted perylene diimides **3**. It is important to note that the two isomers could not be separated at this stage, so the isomeric mixtures were used in the following steps.



Scheme 4. Synthetic scheme for the preparation of dibromo bay-substituted perylene diimides and their subsequent use to afford multiply substituted perylene and coronene derivatives (o.n. = overnight).

Treatment of **3** with a large excess of the same amine as used in the preceding step, at 110 °C under argon, led to the displacement of bromine by the nitrogen of the primary amine (Scheme 5).[50] This reaction produced a mixture of different products, which were successfully separated by col-

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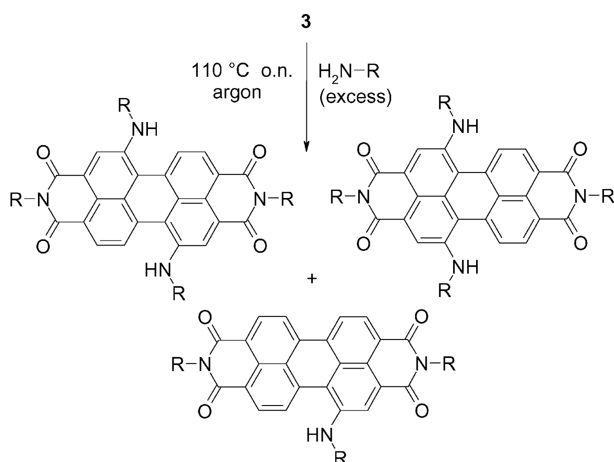
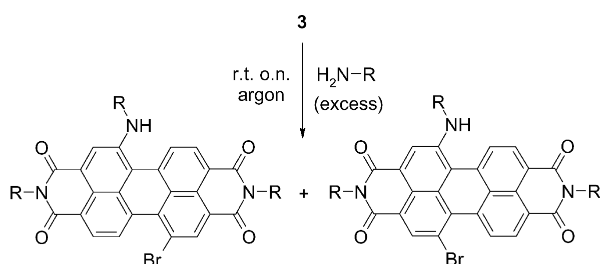
umn chromatography: two isomers of the tetrasubstituted perylene derivatives, with the additional chains in the 1,7 and 1,6 positions, together with one trisubstituted derivative resulting from partial dehalogenation of the dibromo diimides, a side-reaction also described by Wasielewski and co-workers.[51] An attempt to obtain the tetrasubstituted molecules in a one-pot procedure, by direct addition of excess primary amine to the dibromoperylene anhydride **2**, was not successful. It is worth noting that, in principle, the step shown in Scheme 5 could be performed with a primary amine different from the one used in the previous step.



Scheme 5. Synthetic route from **3** to tri- and tetrasubstituted perylene derivatives.

In addition, we also performed the substitution of bromine on the dibromoperylene diimides **3** at room temperature (Scheme 6)[50] and found that only one bromine atom was readily substituted by the amine nitrogen while the other still remained on the aromatic core even after a prolonged reaction time (12–24 hours). The obtained asymmetric compounds present one polar side chain and one bromine atom on the perylene bay area, allowing further substitution with a different polar side chain.



Scheme 6. Synthetic route from **3** to asymmetric monobrominated three-side-chained perylene derivatives.

Three- and four-chained perylene derivatives have been shown to be very soluble in aqueous media, and their tendencies to self-aggregate are significantly weaker than those of the previously reported two-chained perylene diimides.[50] The self-aggregation of the synthesized compounds in aqueous solution must be carefully considered in models of the interaction between the different ligands and G-quadruplex DNA. In fact, self-association of perylene derivatives is reported to favour the specific recognition of the G-quadruplex with respect to duplex DNA.[52] Nevertheless, it has recently been shown by Palumbo and co-workers[44] that strong drug self-aggregation is related to lower telomerase inhibition and weaker interactions with G-quadruplexes, suggesting that the higher selectivity for G-quadruplex arrangements upon aggregation is due to reduced binding efficiency to duplex and single-stranded DNA rather than to a higher affinity for G-quartets.

According to the terminal stacking mode of interaction, widely demonstrated for G-quadruplex ligands (Figure 5),[16] it is reasonable to suppose that the presence of a wider aromatic core and four positively charged side chains should improve the interactions between these ligands and G-quadruplexes, leading to higher binding constants and consequently to increased telomerase inhibition and higher selectivity with respect to duplex DNA. For these reasons we designed the synthesis of new hydrosoluble coronene derivatives. In order to proceed towards the formation of the coronene aromatic core, a strategy similar to that described by Mullen and co-workers for lipophilic derivatives was used.[53] Suitably functionalized alkynes were prepared and subsequently used in Sonogashira cross-couplings[54] with **3** (Scheme 7); these reactions are catalysed by Pd^0 complexes in the presence of CuI and a suitable base, leading to new C–C bonds. If a secondary amine is used as a base, alternative substitution of the bromine atoms can occur:[49] for this reason only tertiary amines can be used, both as solvent and in the substituents of all the side chains. During the Sonogashira coupling, the cyclization described in the next step also partially occurs. Separation was not useful at this stage, so the mixtures of each intermediate compound and the related partially closed products were used in the following step. In order to complete the coronene aromatic cores, base-catalysed cyclization was performed on the intermediate compounds with DBU. The synthesis of four hydrosoluble coronene ligands as potential G-quadruplex interactive telomerase inhibitors by this synthetic pathway has been reported (Scheme 7).[48]

More recently we have synthesized perylene diimides containing polyamine side chains, with the aim of conjugating the efficiency of perylene derivatives in stabilizing G-quadruplex structures with the polyamines' biological activity arising from specific interactions with different DNA domains.[55] Although in the case of the natural occurring spermine the conjugation with the perylene moiety resulted in poor water solubility, probably due to the formation of micellar aggregates, some very interesting results, in terms both of binding selectivity and of efficient telomerase inhibition, were obtained when triethylenetetramine was used as a side chain; these were probably related to the possible multiple hydrogen bonds between this side chain and the G-quadruplex. As shown in Scheme 8, the synthetic strategy for the preparation of perylene diimides with polyamine side chains is based on the use of the polyamine itself as a solvent in order to avoid polymerization processes, although in the cases of spermine and other solid amines DMA and dioxane were added.[55]

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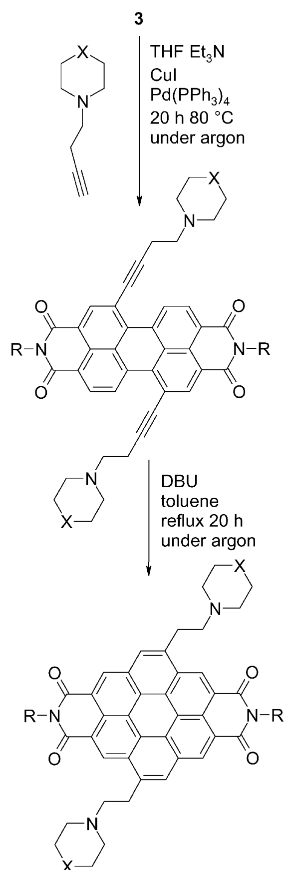
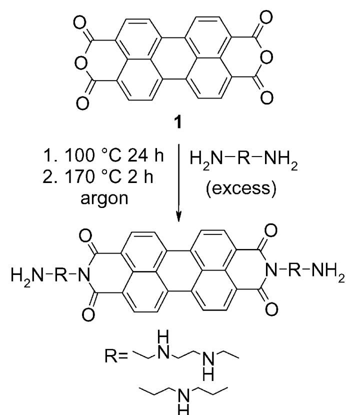


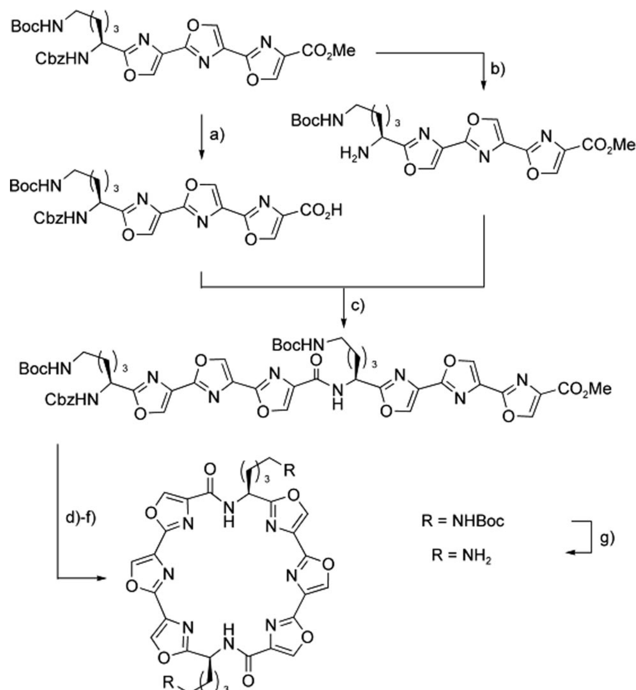
Scheme 7. Synthetic pathway for the preparation of coronene derivatives by Sonogashira cross-coupling. (THF = tetrahydrofuran, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, X = CH_2, NCH_3).



Scheme 8. Synthetic scheme for the preparation of perylene diimides bearing polyamine side chains.

2.4 New Classes of G-Quadruplex Ligands: Macrocycles and Metal Complexes

Following the promising results obtained with the naturally occurring telomestatin,[31] several synthetic macrocycles, based on quinacridine,[56] oxazole[57] and quinoline[58] moieties (Figure 4), were proposed and studied as G-quadruplex ligands. The last two classes of compounds are trimers characterized by amide bonds and obtained by classical peptide chemistry with protecting groups such as BOC.[57–58] More recently, an analogous strategy has been used to synthesize macrocyclic hexaoxazoles (Scheme 9), which bear a closer structural resemblance to telomestatin than the previously reported macrocycles.[59] These compounds combine a planar macrocyclic hexaoxazole pharmacophore with basic side chains: they showed good specificities for G-quadruplexes and potent telomerase-inhibitory activities in both cell-free and cell-based assay systems.[59]



Scheme 9. Synthesis of macrocyclic hexaoxazoles. a) LiOH, THF/H_2O; b) $Pd(OH)_2$/C, H_2, THF/MeOH; c) DMT-MM, *N*-methylmorpholine, THF/H_2O/MeOH; d) LiOH, THF/H_2O; e) $Pd(OH)_2$/C, H_2, THF/MeOH; f) Et*i*Pr_2N, DMAP, BOPCl, DMF/CH_2Cl_2; g) TFA, CH_2Cl_2 99%. [Boc = *tert*-butoxycarbonyl, Cbz = benzyloxycarbonyl, DMT-MM = 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride, DMAP = 4-(dimethylamino)pyridine, BOPCl = bis(2-oxooxazolidin-3-yl)phosphinic chloride, TFA = trifluoroacetic acid] (M. Tera, H. Ishizuka, M. Takagi, M. Suganuma, K. Shin-ya, K. Nagasawa, *Angew. Chem. Int. Ed.* **2008**, *47*, 5557–60. Reproduced by permission of Wiley-VCH, copyright 2008).

Finally, it is worth citing several metal complexes that have been shown to bind to G-quadruplexes through terminal stacking, with high affinities and selectivities,[60] in some cases depending on their metal-mediated conformations.[61] These interesting compounds, developed in the field of bioinorganic chemistry, have recently been reviewed.[62]

3. Study of Ligand Interactions with Quadruplex and Duplex DNA

Detailed studies on drug–quadruplex complexes are essential for understanding of quadruplex recognition and for addressing drug design.[63] Several techniques can be used

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to this end, including absorption spectroscopy,[64] circular dichroism,[65] emission spectroscopy,[66] calorimetry,[67] nuclear magnetic resonance (NMR),[68] surface plasmon resonance (SPR),[69] mass spectrometry,[70] X-ray diffraction[71] and competition dialysis.[72] In particular, fluorescence-based melting assays for study of quadruplex ligands have been developed; specifically, Fluorescence Resonance Energy Transfer (FRET) assays have been widely used to study the thermal stabilization of preformed G-quadruplex structures upon binding of different ligands.[73] If two probes forming a donor–acceptor system are linked at the end of a G-quadruplex-forming sequence, a FRET assay represents a powerful tool for the study of G-quadruplex ligands, due to its potential to allow large numbers of simultaneous measurements to be performed under different conditions (including ionic strength and various drug concentrations).

3.1 Mass Spectrometry in the Study of G-Quadruplex Ligands and their Selectivities

Mass spectrometry is a powerful tool for the study of biomolecular structures and non-covalent interactions; it can provide data on the functional properties of biomacromolecules complementary to that obtained from the traditional techniques discussed above.[74] Specifically, electrospray ionization-mass spectrometry (ESI-MS, a well known technique for the characterization of organic compounds widely used by organic chemists) is very useful in the analysis of non-covalent complexes between nucleic acids and small molecules,[75] because under certain conditions it allows the transfer of non-covalently bound complexes into the gas phase without the disruption of the complex and therefore the mass spectrometric determination of their modes and energies of interaction.[76] This technique has been successfully applied to the study of the binding of G-quadruplex ligands to their target sequences.[77] In this respect, we have recently reported an extensive ESI-MS study of the non-covalent interactions between different inter- and intramolecular G-quadruplex structures and several perylene and coronene ligands.[78] If suitable experimental conditions are used, the structures of the complexes formed in the solution sample are not altered during the electrospray process, providing a method for the determination of stoichiometries and relative binding affinities of such complexes.[79] The formation of stable complexes between the studied ligands and G-quadruplex-forming oligonucleotides is clearly demonstrated by the presence in the mass spectra of intense peaks corresponding to 1:1 and 2:1 drug/quadruplex complexes (Figure 6). Quantitative analysis of binding affinities with quadruplex DNA structures is possible, because the association constants can be calculated directly from the relative intensities of the corresponding peaks found in the mass spectra, under the assumption that the relative intensities in the spectrum are proportional to the relative concentrations in the injected solution, as has been shown previously.[79]

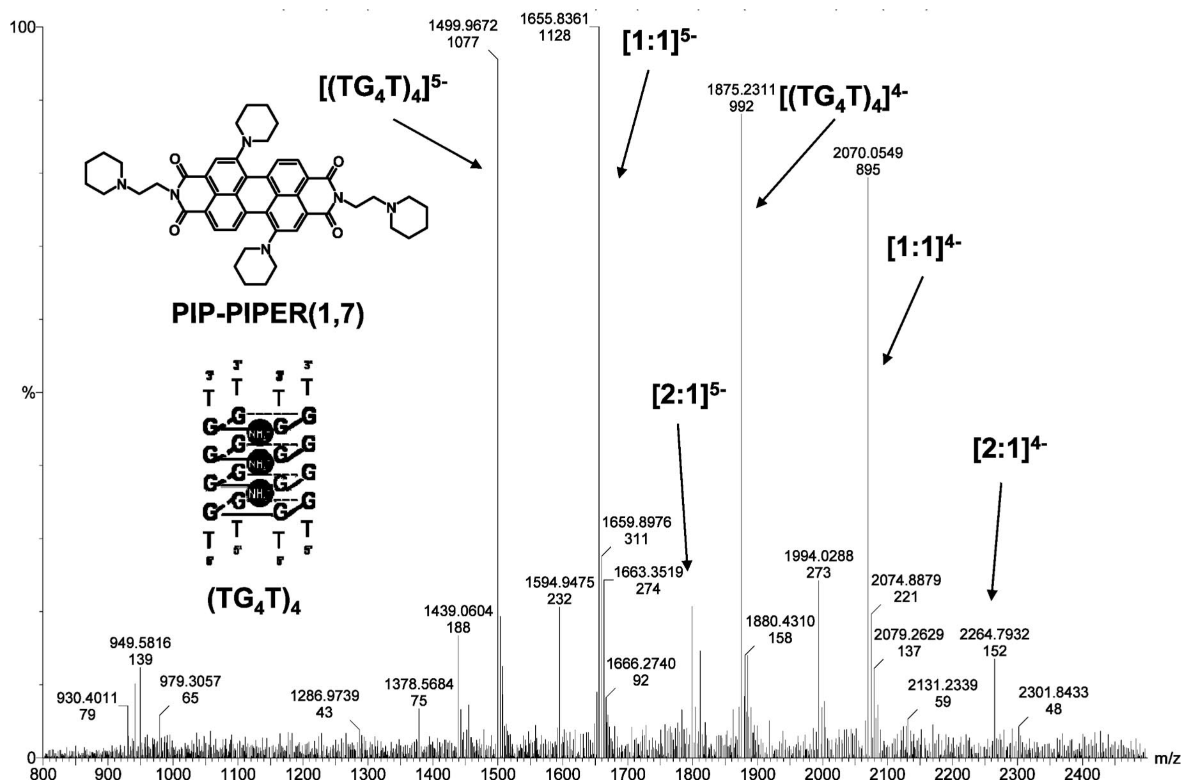

Figure 6. ESI mass spectrum of complexes formed between $[d(TG_4T)]_4$ and a perylene derivative [PIP-PIPER(1,7)].[49] Labels and arrows indicate the main ions identified, corresponding to the oligomer alone folded into an intermolecular G-quadruplex and the 1:1 and 2:1 ligand/DNA complexes, in different charge states.

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Differential selectivity between quadruplex and duplex DNA is certainly a highly relevant topic and could be related to the specificities of the biological activities of these compounds:[80] the drug must be able to recognize the telomeric DNA in the cellular nucleus in the presence of a large amount of duplex DNA. Several studies directed towards exploration of this very important topic in detail have been reported, and have made use of many of the techniques discussed above, ranging from FRET to SPR.[56] In order to evaluate selectivity between quadruplex and duplex DNA it is necessary to choose an appropriate model for duplex DNA: Dickerson-like dodecamers are among the simplest models and have been widely used in the literature.[81] The possibility of performing experiments in the simultaneous presence of G-quadruplex structures and a double-stranded genomic DNA is of particular interest for the biological relevance of this system, so after exploring this topic by a classical approach based on the very simple duplex model of an autocomplementary dodecamer, we extended our analysis, reporting for the first time a competition ESI-MS experiment in the presence of genomic DNA fragments.[78] Whereas those ligands showing high levels of selectivity between quadruplex and duplex oligonucleotides in terms of binding constants confirmed their selectivities in the competition experiment, the contrary was not always true: some ligands showing poor selectivity with respect to the autocomplementary dodecamer were selective in the presence of genomic DNA fragments. This result suggests that physiologically nonrelevant interactions are possible with short duplex oligonucleotides. This is the case with the coronene derivative CORON, which would have been said not to be selective according to the data obtained with the short duplex oligomer, whereas its binding to quadruplex DNA is poorly affected by the presence of calf thymus DNA.[78]

3.2 Molecular Modelling Simulations

Molecular modelling is widely used to explore possible ligands for a biological target or to study the structural features of known ligands that determine their pharmacological behaviour. For G-quadruplex ligands, this kind of study is particularly challenging for two main reasons. First of all, the G-quadruplex can exist in many different conformations with specific loop geometries (Figure 2):[6] very recently Neidle and co-workers have addressed the importance of TTA loops in defining the modes of interactions between ligands and G-quadruplexes, as well as the relative binding sites.[82] This means that different results can arise from different G-quadruplex structures analysed as ligand targets in such simulations. The second factor that makes the complete study of G-quadruplex ligands by molecular modelling very hard – with respect to enzyme inhibitors, for instance – is that the site of interaction between the ligand and the macromolecule is not in a hydrophobic environment, as typically occurs inside the active site of an enzyme, but in a region at the interface with water. Despite these difficulties, there have been several approaches to the design of G-quadruplex ligands and interpretation of their interactions with the target, ranging from molecular docking to simulated annealing and more complex dynamics simulations.[83] The G-quadruplex structure used in many of these simulations is the X-ray-derived monomeric structure of the 22-mer human telomeric DNA sequence AGGG-$(TTAGGG)_3$ folded at a K^+ concentration that approximates its intracellular concentration (PDB code 1KF1):[84] in this structure all four DNA strands are parallel, with the three linking trinucleotide loops positioned on the exterior of the quadruplex core. As an example, this structure was used to obtain the model of a complex with a coronene derivative shown in Figure 5.[48] As can be seen, in this model all the obtained data agree with the terminal stacking mode of interaction between the G-quadruplex ligands and their target, in which the aromatic core of the ligand stacks on terminal G-tetrad and the basic side chains interact with DNA grooves.[16] In some cases these simulations have led to relative binding energy evaluations.[28,48] It is worth citing two cases in which molecular modelling simulations were used to explain the role of an additional side chain of the ligand. The first case involves acridine derivatives in which the third side chain conferred enhanced G-quadruplex interaction because it was able to fit into the G-quadruplex third groove: the choice of the substituents of this additional side chain was subjected to molecular modelling simulations.[85] The second case involves three- and four-chained perylene derivatives: the fourth side chain of a perylene derivative such as DAPER4C(1,6) (Figure 4) cannot reach the fourth groove when the ligand is anchored with the perylene area superimposed on approximately half the G-tetrad area, so its presence is not useful for improving the binding to the G-quadruplex relative to three-chained perylene derivatives.[47]

4. Two Other Relevant Synthetic Aspects Relating to G-Quadruplexes: Quadruplex-Forming Modified Oligonucleotides and Nanostructures Based on G-Quadruplexes

Although they would surely deserve an entire review, it is worth briefly introducing two emerging fields of application of G-quadruplex structures presenting synthetic open challenges: quadruplex-forming oligonucleotides and nanostructures based on G-quadruplexes. In fact, artificial G-quadruplex structures, whether in the form of modified oligonucleotides or not, have been pointed out as tools with many interesting applications.[5]

G-rich synthetic oligonucleotides have shown promising biological and possibly pharmacological properties ranging from anticancer to anti-HIV activities; G-quadruplex formation has been shown to be essential in determining these biological effects in all cases.[86] When a specific target molecule is defined for the biochemical action of these oligonucleotides, the oligonucleotide is referred to as an “aptamer”. The most widely studied G-quadruplex-forming

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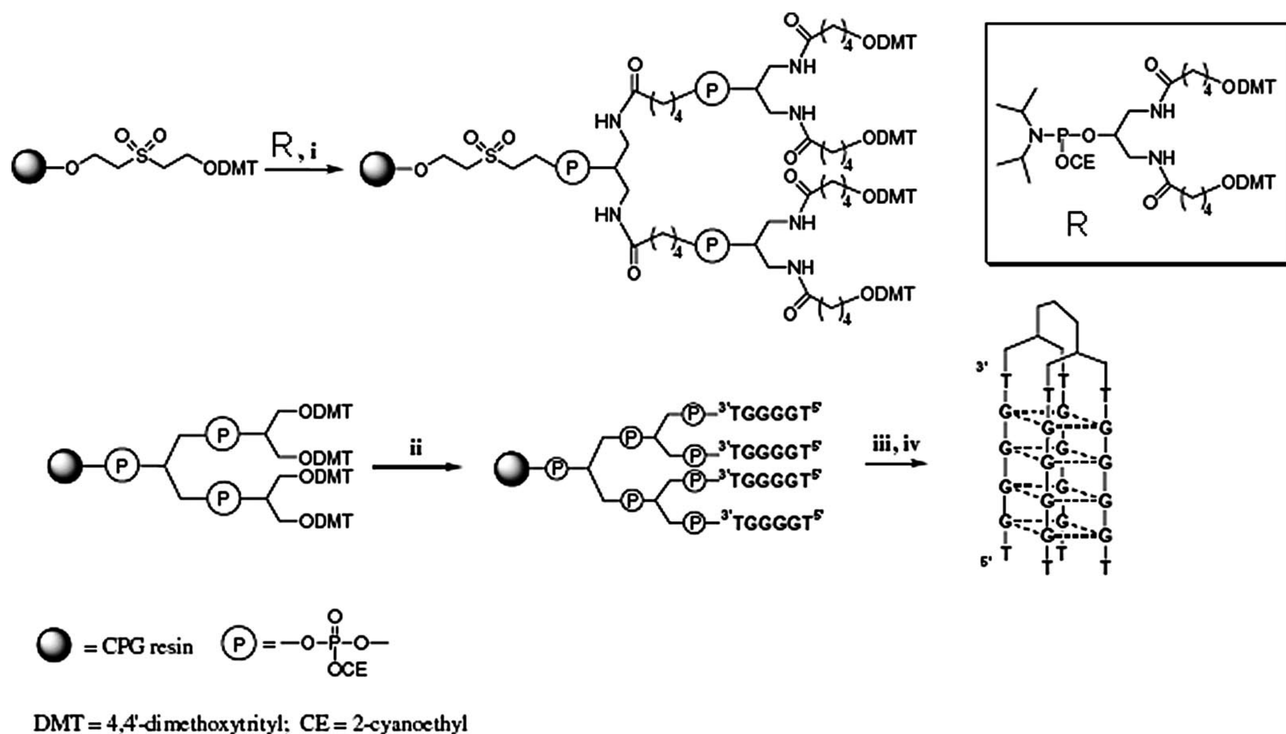
aptamer class is that of the thrombin-binding aptamers (TBAs), which bind to α-thrombin, a fundamental protein involved in blood coagulation, preventing thrombosis.[87] G-quadruplex conformation and stability have been determined to be key parameters in defining the biophysical and biological properties of these aptamers.[88] Modified TBAs, including oligonucleotides containing an acyclic nucleotide,[89] have been reported to enhance G-quadruplex stability and anticoagulant activity. Another bioactive quadruplex-forming oligonucleotide, a 26-mer nucleotide named "AS1411" (Antisoma, London, UK), has been shown specifically to inhibit cancer cell proliferation: the activity of this aptamer has been related to its binding to certain nucleolin-containing complexes.[90] AS1411 has completed phase I clinical trials, and phase II clinical trials have recently begun.[91] Another family of guanine-rich oligonucleotides has been developed as potential anti-HIV therapeutic drugs.[92] These compounds have demonstrated strong interaction with HIV-1 integrase in vitro and the ability to inhibit the integration of viral DNA into host DNA.[93] Among several different G-quartet oligonucleotides proposed and studied for their ability to inhibit HIV, the most efficient was the 17-mer oligonucleotide 5′-GTGGTGGGTGGGTGGGT, referred to as Zintevir (AR177 or T30177), as well as two 16-mer oligonucleotides (T30695 and 93del) and the shorter sequence TTGGGGTT (ISIS 5320).[94] Because of the importance of the structural stabilities of the G-quadruplexes formed by these aptamers for their activities,[95] several synthetic approaches to improving the stabilities of the G-quadruplexes formed by short G-rich sequences have been proposed, either through the introduction of large aromatic substituents at their 5′-ends[96] or by tethering them to a suitable linker to afford constrained quadruplexes (Scheme 10).[97]

G-quartets show molecular self-assembly features typical of supramolecular chemistry: these properties have awakened interest in the use of such structures in nanotechnology.[5] Such self-organization of noncovalent assemblies of G-quartets, for instance, leads to liquid crystals in water: even without any covalent bridge, G-quartets stack to give columnar structures with hydrophilic surfaces and lipophilic cores. Depending on the concentration and other physico-chemical parameters, these aggregates generate arrangements in the cholesteric phase and in the hexagonal liquid-crystalline phase (Figure 7).[98] In another case, a lipophilic G-quadruplex is reported to act as a Na^+ ion transporter when inserted in a phospholipid membrane.[99] In this case olefin metathesis was used to cross-link all 16 appropriately substituted guanosine subunits after self-assembly into a G-quadruplex structure in the presence of K^+ ions (Figure 8). Because G-quadruplex-forming sequences combined with two fluorescent probes forming donor–acceptor systems have proven to be efficient FRET systems for study of G-quadruplex ligands,[73] the same principle has been applied to obtain some nanodevices. This is the case with quadruplex molecular beacons, fluorescent nucleic acid probes with hairpin-shaped structures used for the diagnosis of single-stranded DNA or RNA with high mismatch discrimination,[100] and of quadruplex probes for the fluorimetric detection of K^+ ions (Figure 9).[101]



Scheme 10. Synthesis of G-quadruplex-forming bunch-oligonucleotides. i) Two coupling procedures with R, ii) ODN synthesis, iii) detachment and deprotection with NH_4OH conc. 32% (7 h, 55 °C), and iv) HPLC purification and annealing (G. Oliviero, N. Borbone, A. Galeone, M. Varra, G. Piccialli, L. Mayol, *Tetrahedron Lett.* **2004**, *45*, 4869–4872. Reproduced by permission of Elsevier, copyright 2004).

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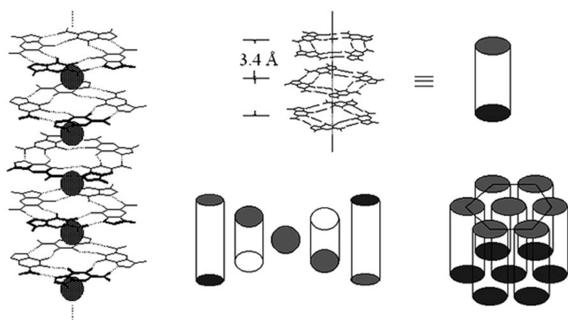

Figure 7. Formation of liquid crystals from self-assembled guanosines (J.T. Davis, G.P. Spada, *Chem. Soc. Rev.* **2007**, *36*, 296–313. Reproduced by permission of The Royal Society of Chemistry, copyright 2007).

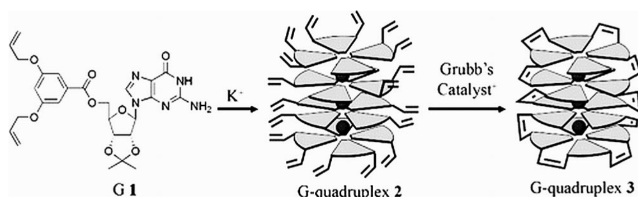

Figure 8. Two-step synthesis of a lipophilic G-quadruplex by olefin metathesis (M.S. Kaucher, W.A. Harrell Jr., J.T. Davis, *J. Am. Chem. Soc.* **2006**, *128*, 38–39. Reproduced by permission of the American Chemical Society, copyright 2006).

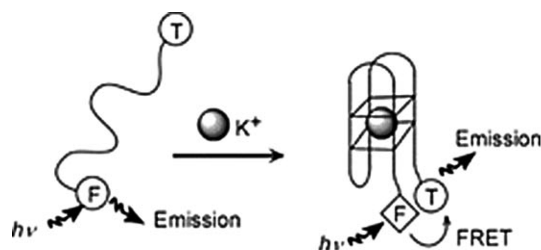

Figure 9. Effect of K^+ ions on the structures of fluorescence-labelled G-rich oligonucleotides and consequent FRET detection (S. Nagatoishi, T. Nojima, E. Galezowska, A. Gluszynska, B. Juskowiak, S. Takenaka, *Anal. Chim. Acta* **2007**, *581*, 125–131. Reproduced by permission of Elsevier, copyright 2007).

Another intriguing potential application of quadruplex-based nanodevices is represented by the so called G-wires. The term "G-wires" was coined by Marsh and Henderson to describe the continuous parallel-stranded DNA superstructures formed by $G_4T_2G_4$ self-assembly as a consequence of the slippage of chains, visualized by AFM imaging.[102] Guanine nanowires incorporating 2,2′-bipyridine units have been reported to be controllable and switchable by external signals (Figure 10).[103] Such G-wire switches present important properties that make them useful for the development of DNA-based functional nanomaterials.[104] Being controllable by chemical input signals – namely, divalent metal ions – they are important for the development of molecular electronic technologies: together with previously reported DNA logic gates based on the effect of monovalent ions on telomeric sequences[105] they can constitute the basis of "liquid computing". Finally, it is worth reporting that G-wire assembly has very recently been proposed to have a role in prebiotic nucleic acid organization.[106]

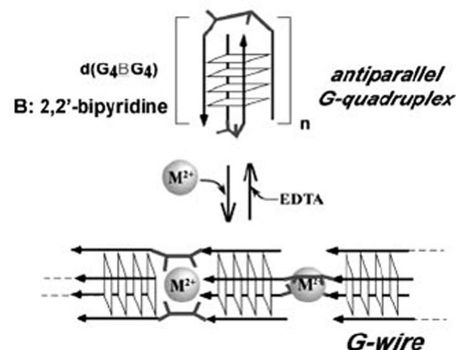

Figure 10. G-wire formation controlled by divalent cations (H. Karimata, D. Miyoshi, T. Fujimoto, K. Koumoto, Z.M. Wang, N. Sugimoto, *Nucleic Acids Symp. Ser.* **2007**, *51*, 251–252. Reproduced by permission of the author and Oxford University Press, copyright 2007).

5. Conclusions

Since their discovery, G-quadruplex structures have attracted the attention of chemists and biologists for a number of different reasons. The interest in these DNA structures is demonstrated by the impressive amount of papers on their structural and biological studies by scientists throughout the world. Many of these studies have increased the interest of these structures for organic chemists and given rise to several synthetic challenges. In particular, compounds that interact with G-quadruplexes definitively represent a very interesting class of bioactive molecules, not only as possible telomerase inhibitors, but as specific agents acting directly on telomere structures as well as on other G-rich regions of the genome, especially the promoters of several oncogenes. Many aspects still need to be fully clarified, above all the structural features that can improve selectivity for different G-quadruplex structures and for quadruplexes with respect to duplex DNA, but the search for G-quadruplex ligands as potential anticancer drugs continues to attract the interest of many organic chemists. As a matter of fact, selective G-quadruplex ligands such as BRACO19 have shown short-term effects on cancer cells (senescence from 7 days post-exposure to non-acute cytotoxic concentrations) and are entering Phase I clinical trials.[107]

As explained at the end of this review, other emerging fields of interest for organic chemists are represented by quadruplex-forming modified oligonucleotides and nanostructures based on G-quadruplexes. Also in these cases, several examples suggest promising perspectives, such as aptamers currently in Phase II/III clinical trials as antican-

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cer[91] and antiviral agents,[108] thus arousing the search for new synthetic methods to improve the stabilities of the designed G-quadruplex structures.

It is worth underlining the fact that, in these interdisciplinary fields, the collaboration between research groups and scientists with different areas of expertise is particularly significant and stimulating.

Acknowledgments

Financial support for the work at the Department of Chemistry, including the author's postdoctoral fellowship, came from the Ministero dell'Università e della Ricerca (MIUR) and Sapienza Università di Roma. The author would like to thank all the people working on this project, in particular Professors A. Bianco, M. Savino and G. Ortaggi, and PhD students Antonello Alvino, Valentina Casagrande and Luca Ginnari, for their fundamental support, and Professors S. Neidle (London, UK) and E. De Pauw (Liège, Belgium) for fruitful collaborations. Thanks are due to Christoph Schultes for help in the final revision of the manuscript and to Lorenzo Velluti and Giorgio Franceschin for graphical aid.

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Received: December 1, 2008
Published Online: February 27, 2009

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