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The impact of 8-aryl- and 8-heteroaryl-2'-deoxyguanosine derivatives on G-quadruplex formation

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ABSTRACT

Guanine and G-rich oligonucleotides are known to self-assemble in the presence of a variety of cations to form higher ordered structures known as G-quadruplexes. We have synthesized a library of 8-aryl/heteroaryl-2'-deoxyguanosine derivatives (8ArGs) that are also able to self-assemble into quadruplex structures. We demonstrate that the properties of such quadruplexes can be modulated by the nature of the groups attached to the guanine base. These supramolecules are potentially useful in the development of self-assembled nanodevices.

INTRODUCTION

In the last decade supramolecular chemistry has become increasingly important as an enabling strategy for the advance of nanotechnology.[1] Nucleic acids (NAs) and related compounds have emerged as one of the most important tools in the design and construction of complex functional nanostructures.[2] However, it is increasingly clear that chemical modifications to the naturally NAs will significantly enhance the versatility and applicability of such structures.[1]

Guanosines stand out as exceptional candidates for the elaboration of a variety of supramolecular structures. The guanine base self-recognizes to form planar tetramers (G-tetrads) that that further self-assemble in the presence of a variety of different cationic templates to form higher ordered structures known as G-quadruplexes.[3] Previous work in the areas of supramolecular chemistry and nanotechnology, includes the use of lipophilic G-analogues for the construction of self-assembled ionophores, self-assembled liquid crystals, and other molecular devices.[3, 4] G-Quadruplexes are also relevant in biomedical research. For example, the G-rich DNA sequences at the ends of chromosomes (telomeres), have a propensity to form G-quadruplexes in vitro.[3] It's been suggested that molecules capable of binding to and stabilizing such quadruplexes DNA may show promising anticancer activity because of their inhibitory effect on telomerase.[5]

The significance of G-quadruplexes makes it important to elucidate structure-function relationships with guanine derivatives. In particular, the modulation might be the result of factors that are intrinsic and extrinsic to the structure of the guanine or modified guanine (8ArGs) subunits. Intrinsic factors are the disposition of the 8-aryl/heteroaryl moiety together with the nature and regiochemical arrangement of functional groups. Extrinsic factors refer to the variation of parameters such as the concentration, type of cation used as template as well as the solvent used to conduct the experiments. In here we describe the intrinsic modulation of the supramolecular properties of quadruplexes made of lipophilic of 8-aryl/heteroaryl-2'-deoxyguanosine derivatives.[6]

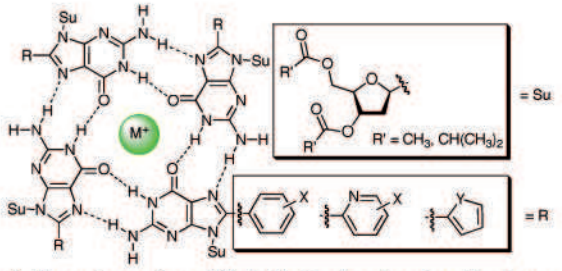
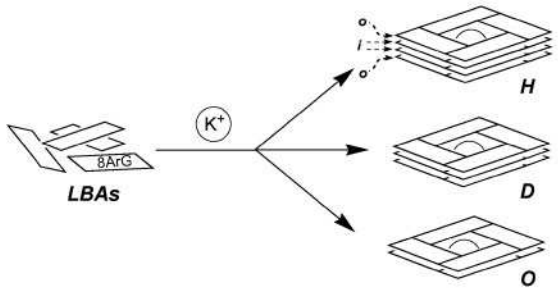


Fig.1 Top view of modified G-tetrads showing the types of substitutions possible at the C8 of the guanine base and at the 2'-deoxyribose sugar. The metal cation (K+ in this study) template can also be replaced by other cations. X = various functional groups (e.g. acetyl), Y = O, S.

RESULTS AND DISCUSSION

The 8ArGs were prepared as previously described by others and us.[6-8] In DMSO-d_6, 8ArGs show sharp and well-defined signals that are characteristic of monomeric species. On the other hand, although the peaks in $CDCl_3$ are also sharp and well resolved, the N1H peak is slightly exchange-broadened, indicating the formation of loosely bound aggregates (Scheme 1, **LBAs**). Titration of 8ArGs with KI, induces changes in the spectra, such as the sharpening of all the peaks and the downfield shifting of the N1H peaks, which is a behavior consistent with the formation of well-defined and kinetically stable quadruplexes.[6]

These titration experiments reveal the molecularity of the system by indicating the ratio of monomers to metal cation in the quadruplex and the fidelity for such quadruplexes as a function of $[K^+]$.[8] For example, the 8-(2-pyridyl) derivative **2PyGi** forms a highly symmetrical D_4 octamer (**O**) of formula $(\mathbf{2PyGi})_8 \cdot K^+$ that shows one set of peaks in the ^{1}H NMR spectrum (Fig. 2a). The 8-(2-furyl) derivative **FuGi** forms a dodecamer (**D**) of formula



Scheme 1. The 8ArGs form loosely bound aggregates (**LBAs**) in the absence of a cation template (e.g. K^+). Addition of the latter induces the formation quadruplexes different molecularities such as octamers (**O**), dodecamers (**D**), hexadecamers (**H**, **o**, **i** = outer and inner tetrads respectively). The type of quadruplex formed depends on intrinsic parameters (e.g. structure of the 8ArGs) and/or extrinsic parameters (e.g. solvent, cation).

$(\mathbf{FuGi})_{12}{\cdot}2K^+$ that shows three sets of peaks in the ^{1}H NMR spectrum corresponding to the three different tetrads that make up the quadruplex (Fig. 2b). The 8-(*meta*-acetylphenyl) derivative **mAGi** forms a hexadecamer (**H**) of formula $(\mathbf{mAGi})_{16}{\cdot}3K^+$ that shows two sets of peaks in the ^{1}H NMR spectrum corresponding to the four tetrads that make up the quadruplex (Fig. 2c). The high D_4 symmetry of $(\mathbf{mAGi})_{16}{\cdot}3K^+$ results in a relatively simple spectrum because the outer (**o**) and inner (**i**) tetrads are equivalent between them (Scheme 1, **H**).

Most other titration experiments with other 8ArGs indicate that the octamer is the favoured quadruplex formed. Then, what drives the formation of a dodecamer or even a hexadecamer? In the absence of X-Ray diffraction data molecular modelling and two-dimensional NOESY experiments offer important clues. Firstly, one side of the tetrad made by 8ArGs is less crowded than the other because the monomers stay in the *syn* conformation around the glycosidic bond. That means that one of the tetrads forming a dodecamer will have a more sterically crowded interphase, therefore the smaller five-membered furan in **FuGi** can accommodate such sterically crowded tetrad interphase more easily than the bigger 2-pyridyl moiety of **2PyGi**. But if a smaller ring allows a dodecamer to form how can we explain the formation $(\mathbf{mAGi})_{16}{\cdot}3K^+$ hexadecamer? Similar studies indicate that the inner tetrads in such hexadecamer are held together by attractive π-stacking and CH-π interactions between the acetylphenyl moieties of the neighbouring inner tetrads that are disposed almost perpendicular to the plane of the tetrads. Such interactions are not possible in **2PyGi**, which seems to prefer a more co-planar conformation of the 2-pyridyl moiety in order to maximize favourable secondary H-bond interactions between the 2-pyridyl nitrogen and the $N2H_2$ of the adjacent monomer. The outer tetrads in $(\mathbf{mAGi})_{16}{\cdot}3K^+$ appear to also adopt a more co-planar conformation that allows the formation of up to four additional hydrogen bonds per tetrad that further stabilizes such quadruplex.

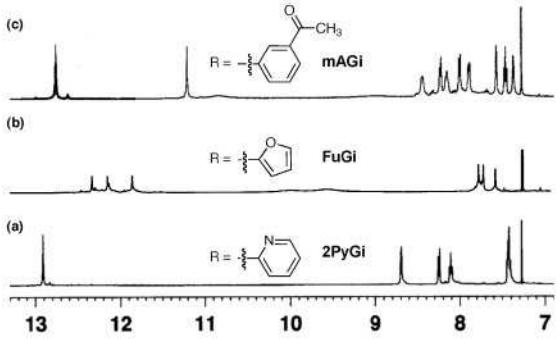


Fig. 2. Partial ^{1}H NMR spectra (500 MHz, 295 K, ~50 mM $CDCl_3$, saturated in KI) showing the aromatic region (7.3 - 9.0 ppm) and the N1H region (11.0 - 13.0 ppm) for (a) the $(\mathbf{2PyGi})_8{\cdot}K^+$ octamer (**O**), (b) the $(\mathbf{FuGi})_{12}{\cdot}2K^+$ dodecamer (**D**) and (c) the $(\mathbf{mAGi})_{16}{\cdot}3K^+$ hexadecamer (**H**). In all the derivatives shown the R' = $CH(CH_3)_2$, see fig. 1 for further details.

CONCLUSION

The 8ArdGs described in this article offer an attractive strategy for obtaining easily prepared recognition motifs that can be used to modulate supramolecular properties such as molecularity, fidelity and stability of G-quadruplexes. Combining the variation of such intrinsic parameters with extrinsic parameters such as the nature of the cation template and the solvent it is also possible to obtain a wide variety of quadruplexes with customizable supramolecular properties. These supramolecules are in turn potentially useful in the development of self-assembled functional nanodevices.

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9. The molecularity of the quadruplexes is corroborated by other techniques such as VPO, ESI-MS, etc.

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