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Fluorine in medicinal chemistry: β-fluorination of peripheral pyrrolidines attached to acridine ligands affects their interactions with G-quadruplex DNA†‡

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Comparative X-ray structure studies reveal that C–F bond incorporation into the peripheral pyrrolidine moieties of the G-quadruplex DNA binding ligand BSU6039 leads to a distinct pyrrolidine ring conformation, relative to the non-fluorinated analogue, and with a different binding mode involving reversal of the pyrrolidinium N^+–H orientation.

G-quadruplexes (G4s) are four-stranded higher-order structures which can be formed in guanine-rich sequences of genomic DNA and RNA in which the core structure comprises layers of four-guanine base motifs (G-quartets) which become proximate in the folded structures.[1] The G-quartets are stabilised by co-ordination to alkali metal ions, preferably K^+ or Na^+ and by Hoogsteen hydrogen bonding. A range of G4 topologies have been characterised depending on backbone folding patterns and conformations/lengths of the loop sequences connecting successive G-quartets.[2]

G4 motifs in particular genomic environments[3] such as oncogene promoters,[4] 5′-untranslated regions[5] and telomere ends[6] can play a role in regulating selective gene transcription, translation or DNA damage. G4s can be induced in the single-stranded 3′ end sequences of telomeric DNA at the termini of eukaryotic chromosomes.[7] Telomeric DNA length maintenance in cancer cells is performed by the telomerase enzyme complex, which plays a major role in the immortalization of these cells. Low molecular-weight ligands which stabilize these G4 structures, are being developed as novel therapies in a range of disease states, notably in cancer.[8] One such compound is the 3,6,9-trisubstituted planar acridine BRACO-19 (**2**).[9a] The acridine core stacks onto a terminal G-quartet in the crystal structure of a human telomeric G4 complex, with the N^+ ring atom positioned above the central channel of potassium ions.[9b] The 3- and 6-substituents are flexible side-chains that terminate in protonated pyrrolidine residues which interact with quadruplex loops and grooves. The simpler 3,6-disubstituted acridine **1** (BSU6039)[10a] similarly binds to human telomeric G4 DNAs. It also forms a complex with the bimolecular G4 formed by the *Oxytricha nova* telomeric sequence $dG_4T_4G_4$.[10b] Crystal structures of several derivatives of **1** with this G4 have been reported,[10c] and are useful model systems for more complex G4-ligand structures.

In this study we have explored modification of BSU6039 side-chains by fluorine substitution at C-3 of the pyrrolidine rings to generate the *bis*-3-fluoropyrrolidine enantiomers (*R*,*R*)-**3** and (*S*,*S*)-**4**. It is known that when a C–F bond is positioned vicinal to a quarternary amine (positively charged) then this sets up a charge-dipole interaction between the C–F bond and the ammonium N^+–H, an interaction estimated up to 5.0 kcal $mole^{-1}$ *i.e.* approximately similar in strength to a hydrogen bond.[11] We hypothesized that this would have an effect on the hydrogen-bonding properties of the side-chains.

This interaction has been described for 3-fluoropiperidinium **5**,[12] 3-fluoroazetidinium **6**[13] and the 3-fluoro-1,5-diazacyclooctane **7**[13] (Fig. 1). Although not formally described for pyrrolidines, the interaction is anticipated to result in a particular ring pucker for the pyrrolidinium rings of **3** and **4** when fluorine is placed at the 3-position. It was envisaged that enantiomers of 3-fluoropyrrolidinium at the periphery of the acridine could induce subtle and distinct binding modes to G4 DNA as well as generating diastereoisomeric complexes. The co-crystal structure of **1** with *Oxytricha nova* G4 DNA shows that both of the pyrrolidine rings are positioned each in a groove, with one projecting outwards (Figures 2a and b) and hydrogen bonding to a water molecule which in turn interacts with a guanosine residue, through a second hydrogen bond.[10b] The protonated nitrogen atoms of the other pyrrolidinium ring however forms a hydrogen bond contact to the ether oxygen of the deoxyribose ring of a guanosine residue located below the plane of the acridine. This is a rather long hydrogen bond (2.67 Å) suggesting a relatively weak contact as illustrated in Fig. 2b. For comparison the fluorinated *bis*-pyrrolidine enantiomers (*R*,*R*)-**3** and (*S*,*S*)-**4** were synthesised as shown in Scheme 1. The key step involved pyrrolidine ring substitutions on the *bis*-3-chloropropionamide diaminoacridine **5**, with the appropriate 3-fluoropyrrolidine enantiomer, to generate (*R*,*R*)-**3** and (*S*,*S*)-**4**.

Both fluorinated stereoisomers (*R*,*R*)-**3** and (*S*,*S*)-**4** formed high-quality co-crystals with the *Oxytricha nova* telomeric G4

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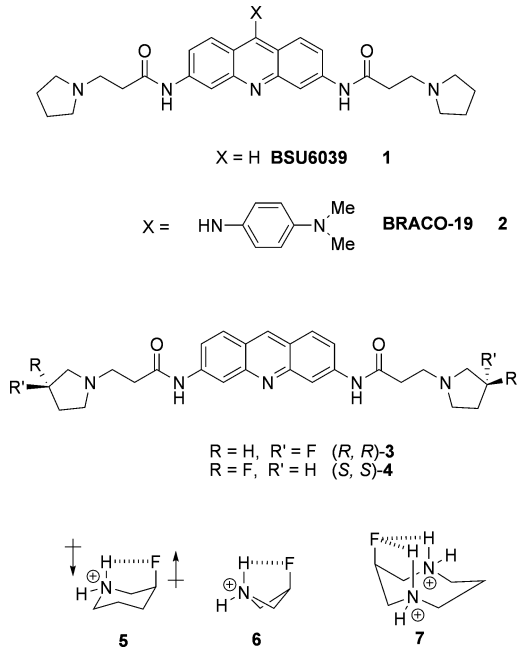
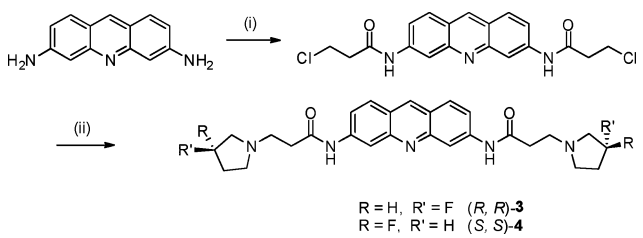


Fig. 1 β-Fluoroammonium interactions lead to favoured low energy conformations of nitrogen heterocycle rings where the C–F bond occupies an axial/pseudoaxial orientation. Such conformational preferences have been attributed to attractive charge–dipole interactions in **5–7** (or dipole–dipole relaxation) between the $C^{\delta+}$–$F^{\delta-}$ and $N^{\delta-}$–$H^{\delta+}$ bonds.



Scheme 1 *Reagents and conditions*: (i) 3-Chloropropionyl chloride, reflux, 3 h, 90–93%; (ii) 3-fluoropyrrolidine, EtOH, NaI (2.2 eq), 5 h, 59–65%.

sequence $(GGGGTTTTGGGG)_2$, which diffracted to near-atomic resolution at a synchrotron source compared to the co-crystal of **1** (1.75 Å for **1** compared to 1.05 Å for (*R*,*R*)-**3** and 1.18 Å for (*S*,*S*)-**4**).[14] Both crystal structures are isomorphous with the original **1** complex and were solved by molecular replacement methods (Table 1). The acridine moiety is in an identical position in all three structures. The high resolution of the fluorinated complexes suggests a more ordered arrangement for (*R*,*R*)-**3** and (*S*,*S*)-**4** possibly due to reduced conformational flexibility of the fluorinated pyrrolidinium motifs. The crystal structures of the co-crystals of (*R*,*R*)-**3** and (*S*,*S*)-**4** are shown in Fig. 2c, d and 3a, b respectively. Although hydrogen atoms are not observed in these crystal structures, their positions may be unambiguously calculated. Strikingly the pyrrolidinium N^+–H in each case is oriented in an opposite direction to that in the non-fluorinated **1** structure (Fig.2a and b). This reversal of orientation is observed for both of the peripheral pyrrolidinium rings in each co-crystal structure of (*R*,*R*)-**3** and (*S*,*S*)-**4**, showing that the hydrogen bonding interactions are very different after introduction of the C–F bond. Comparison of the complexes of the two enantiomers (*R*,*R*)-**3** and (*S*,*S*)-**4** reveals that the pyrrolidinium N^+–H is located

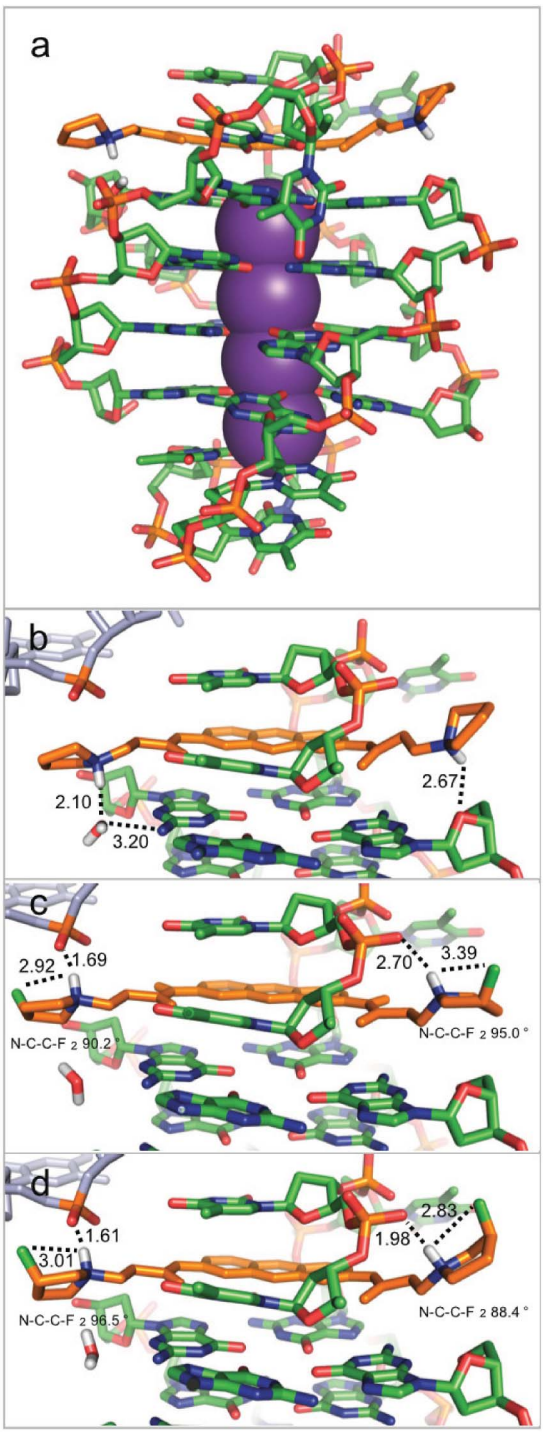


Fig. 2 *Oxytricha nova* G4 complex X-ray structures; (a) with **1** showing the entire complex including the four potassium ions (purple) and (b) a close up of the complex with **1** (potassium ions omitted). The phosphate group (top left) is from a second molecule in the unit cell. (c) Is a close up of the complex with (*R*,*R*)-**3** and (d) is a close up of the complex with (*S*,*S*)-**4**. Both (c) and (d) show an additional interaction to a phosphate group (top left) from a neighbouring molecule of the unit cell. Distances are in Å.

similarly in each structure (Fig. 2b and c), and that the fluorine adopts a pseudo axial orientation in all four pyrrolidinium ring moieties. To a first approximation the structures of (*R*,*R*)-**3** and

Table 1 Crystallographic data

PDB ID	3NYP[a]	3NZ7[b]
Total no of reflections collected	85 447	248 167
No of unique reflections	23 982	25 994
Space Group	$P2_12_12$	$P2_12_12$
Cell dimensions: (Å)	57.80, 44.46, 28.14	55.57, 42.70, 27.00
Maximum resolution (Å)	1.18	1.10
R_{merge}	0.051	0.073
I/σ	15.5	25.4
I/σ (highest resolution shell)	5.7	6.8
Completeness (%)	97.7	97.1
Redundancy	3.6	5.0
Resolution range used in refinement (Å)	21.99–1.18	7.80–1.10
No of unique reflections used in refinement	23 275	25 901
Completeness (%)	94.6	97.0
R_{factor}(%)	16.6	13.8
R_{free}(%)	18.8	15.9
Number of G-quadruplexes per asymmetric unit	1	1
Number of ligands per asymmetric unit	1	1
No of DNA atoms	506	506
No of ligand atoms	36	36
No of potassium ions	4	4
No of water molecules	177	188

[a] One crystal was used. [b] Two crystals were used.

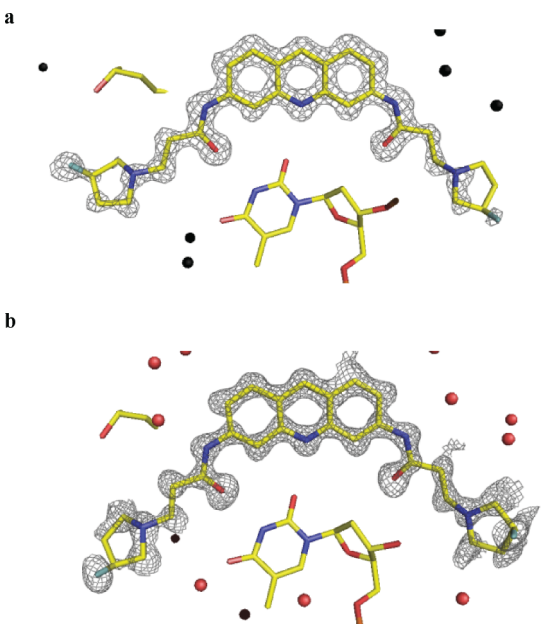


Fig. 3 Electron density in the plane of the acridine ring for (a) (*R*,*R*)-**3** and (b) (*S*,*S*)-**4**. Contours are at the 1.0 σ level.

(*S*,*S*)-**4** are similar with all fluorine atoms orientated in the same direction though the C–F bond is positioned on different ring carbons for each enantiomer.

In the co-crystal for the (*R*,*R*)-**3** enantiomer (Fig. 2c) the right hand pyrrolidinium N^+–H is rotated by 180° relative to **1** (Fig. 2b) and makes a contact (2.7 Å) to the upper phosphate backbone of the quadruplex. The pyrrolidinium ring is puckered (N–C–C–F torsion angle = 95°) with an axially orientated fluorine atom. The N^+–H atom of the left hand pyrrolidinium ring of (*R*,*R*)-**3** (Fig. 2c) extends clear of the G4 complex and makes a short contact (1.69 Å) to a phosphate oxygen of a neighbouring G4 complex in the unit cell. In this case the ring pucker is tighter (N–C–C–F torsion angle = 90.2°) and the CF···H–$^+$N distance shorter (2.92 Å *versus* 3.39 Å) indicating stronger overall electrostatic interactions. For the (*S*, *S*)-**4** complex (Fig. 2d) the situation is similar. The right hand pyrrolidinium ring is significantly puckered (N–C–C–F torsion angle = 88.4°) with the fluorine axial and there is a contact (1.98 Å) between the pyrrolidinium hydrogen and the upper phosphate. The left hand ring also extends clear of the complex and contacts a phosphate of another molecule within the unit cell. Overall, the pyrrolidinium rings of (*R*,*R*)-**3** and (*S*,*S*)-**4** are both significantly more puckered than those found in the G4 complex with **1**.

It is striking that for all four of the fluoro-substituted pyrrolinidium rings in **3** and **4** the N^+–H bonds rotate by around 180° relative to **1** and all interact with phosphate ions, whereas for the non-fluorinated pyrrolidiniums in **1**, they form more classical hydrogen bonds to either a ribose ring or a water molecule. It should be noted that for each pyrrolidine ring of **1** there is a phosphate positioned above it, no doubt offering additional electrostatic stability through the axial ring hydrogens. Taken together there are therefore contacts to the top and bottom of the ligand which will have a role in overall quadruplex stability. For (*R*,*R*)-**3** and (*S*,*S*)-**4**, steric factors may play a role with the observed change in ring conformation; however the pK_a of the protonated pyrrolidines will reduce from about 10.7 to 9.0 due to *β*-fluorination,[15] thus the N^+–H hydrogens should tend towards stronger hydrogen bonding interactions. This appears to result in matching with the better available hydrogen bond acceptor (charged phosphate oxygen) coupled with a strong electrostatic interaction.

Binding affinities for these compounds to the *Oxytricha* quadruplex are not available but it is clear from ΔT_m data (Table 2) that the fluorinated ligands **3** and **4** are actually less able to stabilise the G-quadruplex structure than unsubstituted **1**. In general replacement of C–H for C–F is anticipated to be almost sterically neutral and not prone to additional hydrogen bonding interactions, unlike for example an OH replacement. The introduction of the fluorines which puckers and changes the orientation of the pyrrolidine rings, does not appear to offer much in the way of additional stabilisation, in this case, despite the perhaps stronger electrostatic interactions with the phosphate backbone. This may be due to the fact that hydrogen bonding is now to the top loop of the G-quadruplex, and the ligand is no longer acting as an anchor to secure the top and bottom layers of the structure, hence weakening the overall integrity of the complex. However we demonstrate that this C–H for C–F change retains ligand binding, and induces a substantial change on the pyrrolidine ring conformations and the mode of ligand binding. This structural study exemplifies the

Table 2 Thermal stabilization, as ΔT_m values, measured from a Fluorescence Resonance Energy Transfer (FRET) study. Values are ± 0.1 °C, and are the mean of three or more determinations with a 1 μM ligand concentration

Ligand	ΔT_mdeg C
BSU6039:**1**	13.3
BRACO-19:**2**	28.0
3	4.5
4	6.1

potential of the fluorine···ammonium charge dipole interaction as a strategy to influence the molecular conformation of ligands (pharmaceutical products) binding to macromolecules. The BSU6093,[10b] **1**, and BRACO-19,[9b] **2**, ligands bind quite differently to human telomeric quadruplex sequences relative to that for *Oxytricha nova*. Our ligands are currently being explored in that context, where hydrogen-bonding interactions to the phosphate groups are no longer possible within the propeller loops.[9c]

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