Suplementary Information

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

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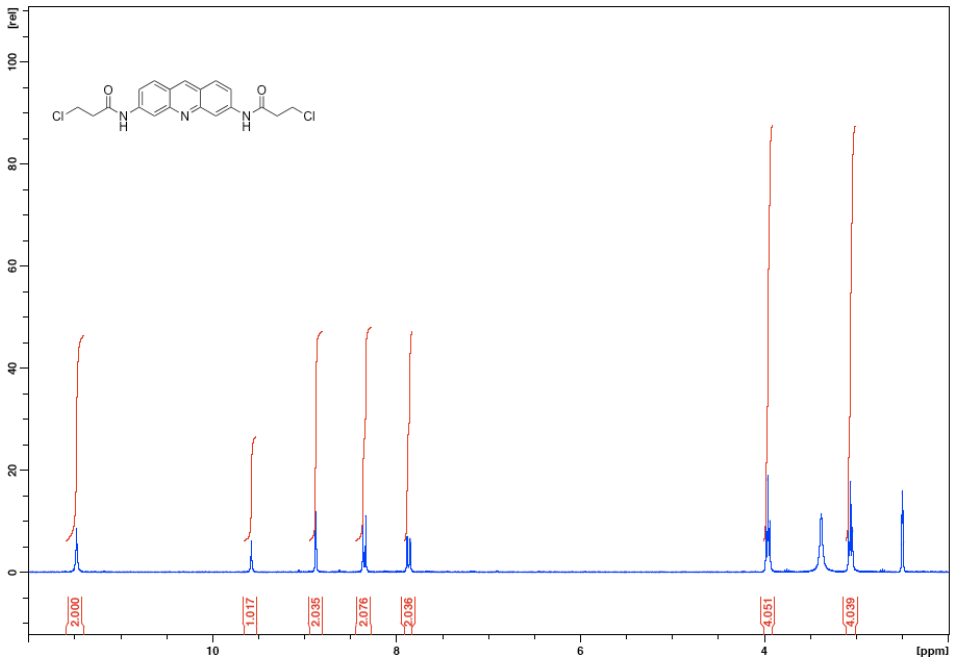
Synthesis methods

3,6-Bis(3-chloropropionamido)acridine(prepared as *J. Med. Chem,* 1999, 42, 4538 - 4546)

3,6-Diaminoacridine (1 g, 4.78 mmol) was heated under reflux in neat 3-chloropropionyl chloride (5 cm^3) for 3 h. The solution was then cooled and the product was collected by precipitation with Et_2O (20 mL). The precipitate was collected by filtration and was washed with Et_2O (2 × 10 cm^3) and dried under reduced pressure. The solid was recrystallized from EtOH:DMF (1:5) to yield 3,6-bis(3-chloropropionamido)acridine as an orange crystalline solid (1.80 g, 4.30 mmol, 90 %). **m.p.** >300 °C decomp; **^{1}H NMR** (300 MHz, d[6] – DMSO): ***δ*** 11.55 (bs, 2H; N*H*), 9.61 (s, 1 H; Ar-H), 8.91 (d, *J*=1.6 Hz, 2 H; 2 × Ar-H), 8.37 (d, *J*= 9.18 Hz, 2 H; 2 × Ar-H), 7.92 (dd, *J* = 9.12,1.6 Hz, 2 H; 2 × Ar-H), 4.00 (t, *J* = 6.15 Hz, 4 H; 2 × CH_2), 3.11 (t, *J* = 6.16 Hz, 4 H; 2 × CH_2).

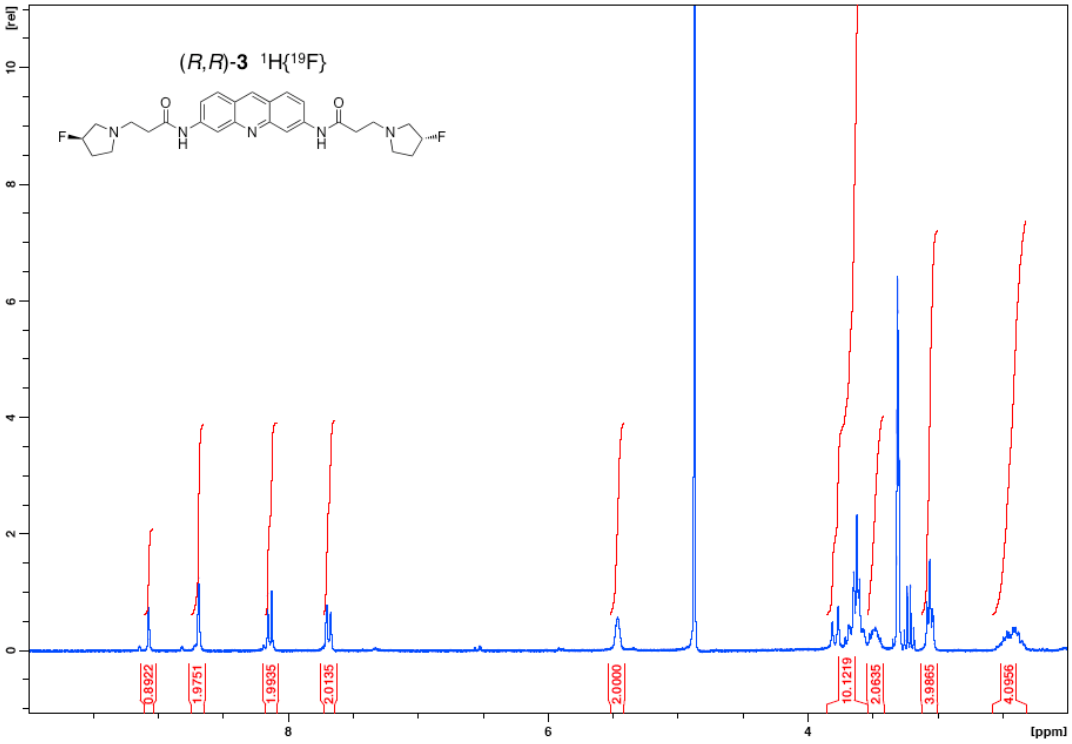


^{1}H-NMR (DMSO, 300MHz) -3,6-bis(3-chloropropionamido)acridine

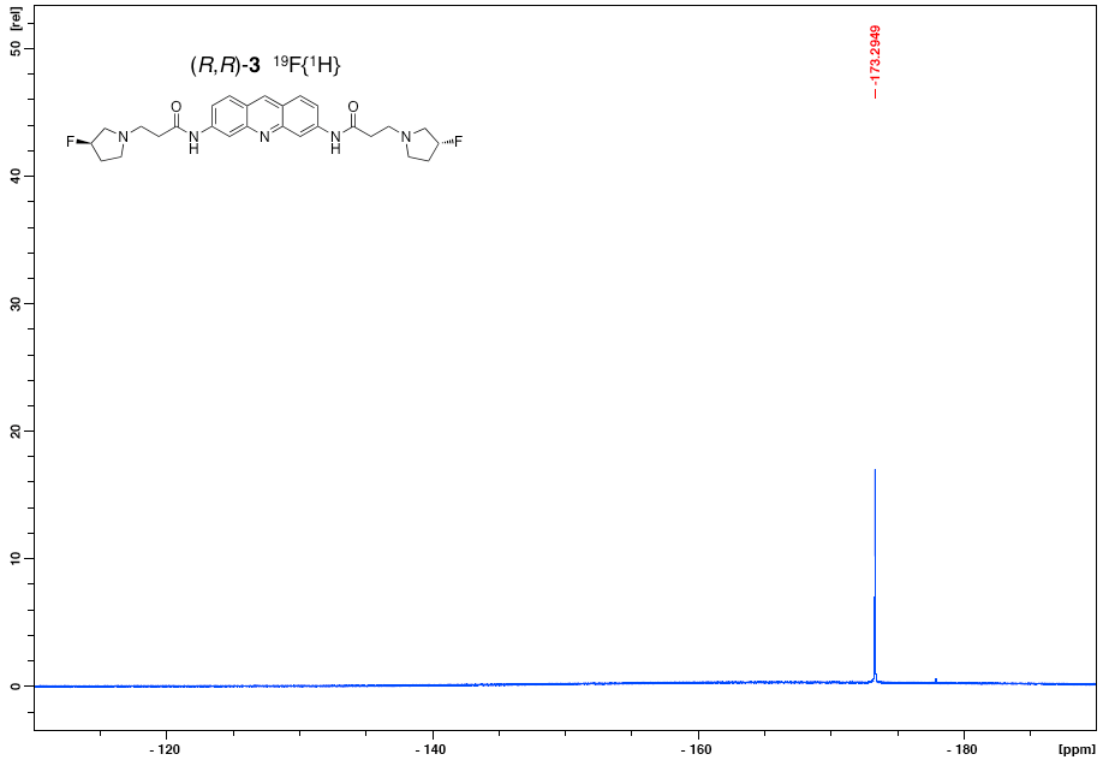
3,6-Bis(3-(3'-(*R*)fluoropyrrolinido)propionamido)acridine (*R, R*) -3

A solution of (3*R*)-fluoropyrrolidine hydrochloride (479.0 mg, 3.84 mmol) in EtOH (1 cm^3) was added dropwise to a stirred solution of 3,6-bis(3-chloropropionamido)acridine(150 mg, 0.384 mmol) and NaI(57.6 mg, 0.384 mmol) in EtOH (5 cm^3). The reaction was stirred under reflux for 5 h before being cooled to 0 °C, filtered and washed with Et_2O (10 cm^3). The product was purified by column chromatography (MeOH/DCM, 1:9 with 5% Et_3N) to yield 3,6-bis(3-(3'-(*R*)fluoropyrrolindino)propionamido)acridine**3** as an orange powder (120.6 mg, 0.243 mmol, 63 %).

R_f0.1 (MeOH/Et_3N/DCM, 5:1:94); m.p. >300 °C decomp;**[α]$_D$**- 8.0 ° (*c*. 2, CH_3OH); ^1H {^{19}F} (300 MHz, CD_3OD): δ 9.05 (1 H, s, Ar), 8.67 (2 H, s, Ar), 8.12 (2 H, d, *J*= 9.1, Ar), 7.67 (2 H, dd, *J* = 9.1, 1.4 Hz, Ar), 5.44-5.49 (2 H, m, 2 × C*H*F), 3.55-3.79 (6 H, m, CH_2), 3.60 (4 H, t, *J* = 6.6 Hz, CH_2), 3.42-3.50 (2 H, m, 2 × CH_aH$_b$), 3.04 (4 H, t, *J* = 6.6 Hz, CH_2), 2.33-2.52 (2 H, m, 2 × CH_2); ^{19}F {^1H} (280 MHz, CD_3OD): δ 173.3 (2F, s, CHF).



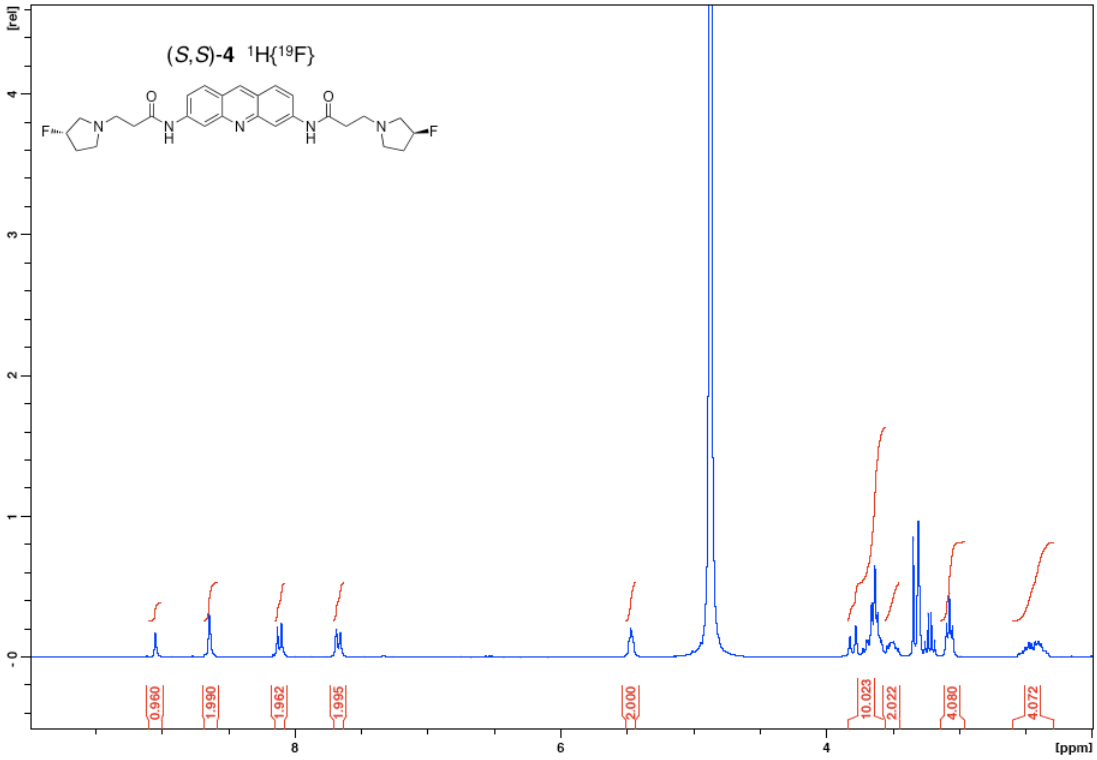
^1H\{^{19}F\}-NMR (MeOD, 300MHz) -(***R, R*)-3**



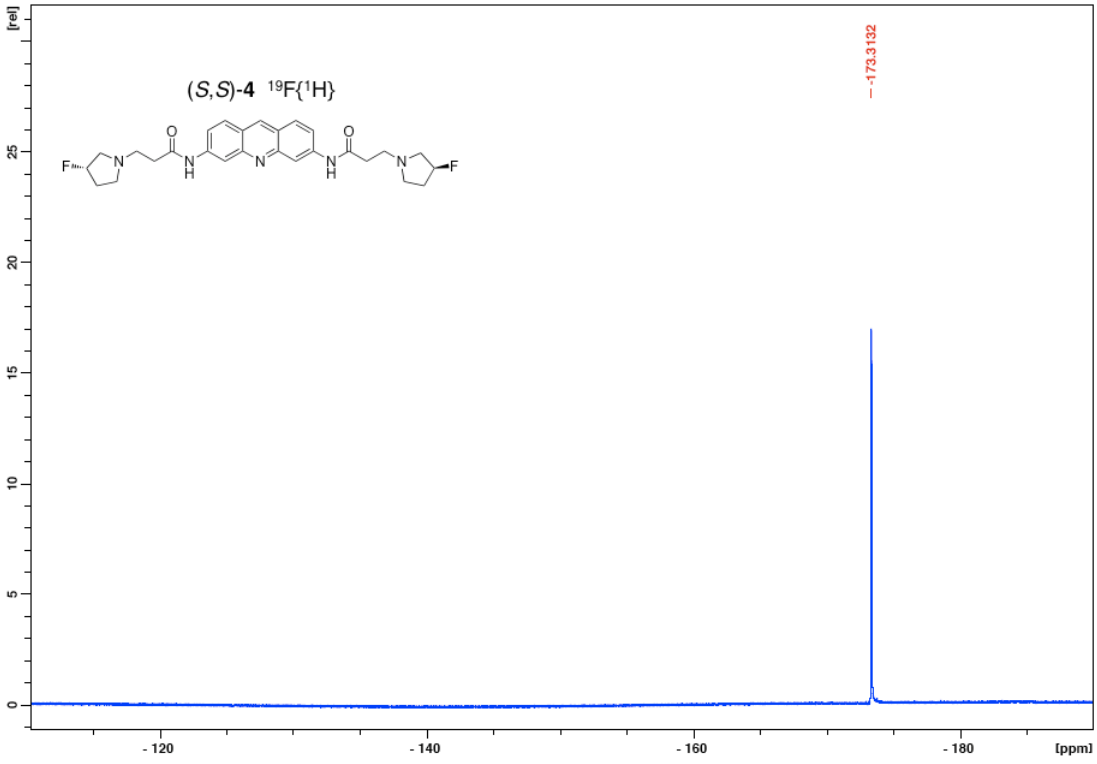
$^{19}F\{^{1}H\}$-NMR (MeOD, 280MHz) -(***R, R***)-**3**

3,6-Bis(3-(3'-(*S*)fluoropyrrolindino)propionamido)acridine(*S, S*) -4

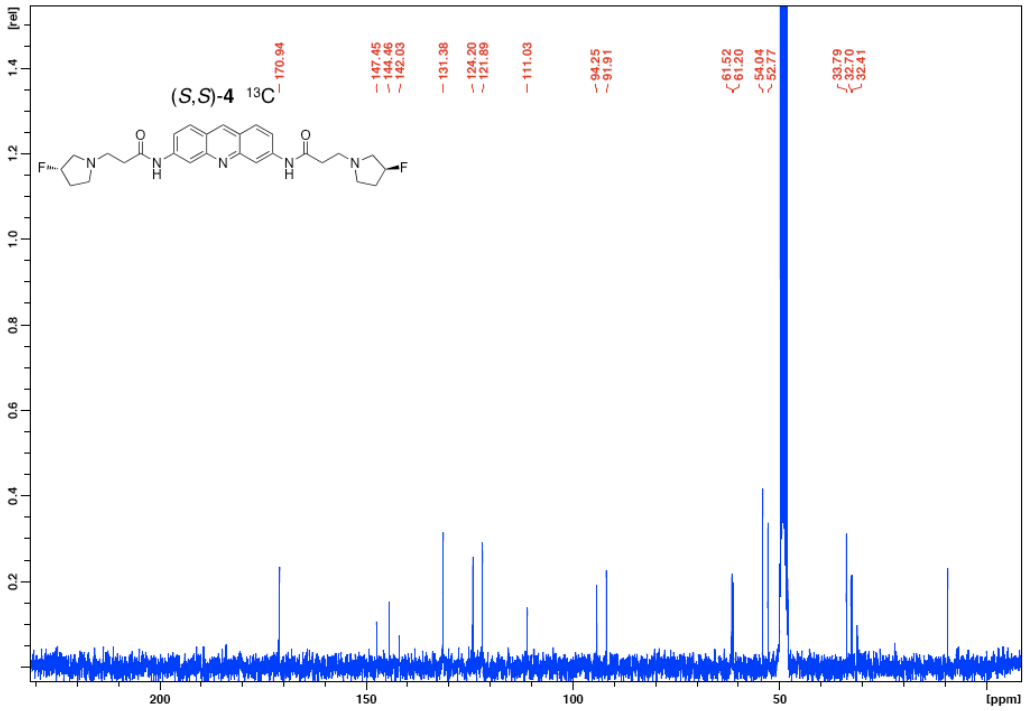
3,6-Bis(3-(3'-(*S*)fluoropyrrolindino)propionamido)acridinewas synthesized in an analogous manner to compound **3** but starting from (3*S*)-fluoropyrrolidine hydrochloride (479.0 mg, 3.84 mmol) to yield the title compound **4** as an orange powder (122.8 mg, 0.248 mmol, 65 %).R_f0.1 (MeOH/Et_3N/DCM, 5:1:94); m.p. >300 °C decomp; $[\alpha]_D^{20}$ + 8.0 ° (*c*. 2, CH_3OH); ^{1}H {^{19}F} (300 MHz, CD_3OD): δ 9.05 (1 H, s, Ar), 8.65 (2 H, s, Ar), 8.12 (2 H, d, *J*= 9.1, Ar), 7.68 (2 H, dd, *J* = 9.1, 1.7 Hz, Ar), 5.44-5.49 (2 H, m, 2 × C*H*F), 3.58-3.83 (6 H, m, CH_2), 3.64 (4 H, t, *J* = 6.8 Hz, CH_2), 3.46-3.54 (2 H, m, 2 × C$H_a$$H_b$), 3.08 (4 H, t, *J* = 6.8 Hz, CH_2), 2.35-2.56 (2 H, m, 2 × CH_2); ^{19}F {^{1}H} (280 MHz, CD_3OD): δ 173.3 (2F, s, CHF); ^{13}C NMR (75 MHz, CD_3OD): δ 170.9 (2 C), 147.4 (2 C), 144.4 (2 C), 142.0 (2 C), 131.4 (2 C), 124.2 (2 C), 121.9 (2 C), 111.0 (1 C), 93.0 (2 C, d, *J* = 176.9 Hz, CHF), 61.4 (2 C, d, *J* = 24.0 Hz, CH_2), 54.0 (2 C), 52.8 (2 C), 33.8 (2 C), 32.55 (2 C, d, *J* = 22.5 Hz, CH_2); *m/z* (%): 496.22 (100); HRMS (ESI^+) m/z calc for $C_{27}H_{32}N_5O_2F_2$: 496.2524 $[M+H]^+$, found: 496.2540.



$^{1}H\{^{19}F\}$-NMR (MeOD, 300MHz) -(***S, S***)-**4**



$^{19}F\{^{1}H\}$-NMR (MeOD, 280MHz) -(***S, S***)-**4**



$^{13}C\{^{1}H\}$-NMR (MeOD, 75MHz) -(***S, S***)- **4**

Crystallisation of G-quadruplexes

Oligonucleotide synthesis, purification and annealing

The RP(reverse phase)-cartridge-purified Oxytricha*nova*telomericdeoxyribo-oligonucleotide sequence d[GGGGTTTTGGGG] was purchased from Eurogentec Ltd and used without further purification. The oligonucleotide was annealed at 3 mM (quadruplex concentration where one quadruplex is formed of two strands of DNA) before use by incubation in a heating block at 80°C for 15 minutes in 20 mM potassium cacodylate buffer at pH 6.5 and left overnight to cool gradually to room temperature.

Ligand preparation

Stock solutions were prepared for each of the disubstituted fluorinated acridine ligands (compounds 3 and 4) for crystallisation experiments by dissolving in 100% DMSO (dimethyl sulfoxide) at a concentration of 20 mM. The stock solutions were kept at -20°C. The relevant stock solution was thawed before setting up the crystallisation drops and used immediately.

Complex preparation

The complex was prepared by adding the ligand stock solution to the annealed quadruplex stock solution to make a working solution of the complex at 2mM quadruplex and 2mM ligand (1:1). The crystallisation cocktail was then added to the solution of the prepared DNA-ligand complex to produce the initial crystallisation conditions mentioned in the next section.

Crystallisation conditions

The hanging-drop vapour-diffusion method was used. The 24-well VDX plates and 22 mm circular siliconised glass cover slips (Hampton Research Corporation) were used in the setup.

Crystals of the Quadruplex-Ligand (R,R) complex formed in the following crystallisation conditions: a 5 µl drop containing 1.2 mMquadruplex DNA, 1.2 mM ligand, 4% MPD, 4 mM potassium chloride, 4 mM magnesium chloride, 4 mM sodium chloride, 4 mM lithium sulphate, 3.2 mM spermine.4HCl and 16 mM potassiumcacodylate buffer at pH 6.5. This was equilibrated against a reservoir well solution of 500 µl of 45% MPD.

Crystals of the Quadruplex-Ligand (S,S) complex formed in the following crystallisation conditions: a 4 µl drop containing 0.75 mMquadruplex DNA, 0.75 mM ligand, 10 % MPD, 12.5 mM magnesium chloride, 12.5 mM spermine.4HCl and 10 mM potassiumcacodylate buffer at pH 6.5. This was equilibrated against a reservoir well solution of 600 µl of 30% MPD.

In both cases, crystals grew as yellowrhombohedrons after 10 days at 12°C (285.15 K) and the dimensions of the crystals used for data collection were approximately 0.2 x 0.2 x 0.05 mm.

Mounting the crystal

The crystals were harvested using a synthetic fibre loop and immersed in liquid nitrogen prior to transport for data collection.

Data collection and processing

Data were collected at the Diamond Light Source synchrotron (Beamline IO4) at wavelength 0.9702Å. Indexing and data processing were carried out using the MOSFLM software interfaced by IMOSFLM (Leslie, 1992). The space group was determined as $P2_12_12$. The crystallographic data isgiven in Table 1.

Preparation of library file for ligand

The ligand was constructed using the SYBYL 7.3 suite of programmes (Tripos, Inc., St. Louis, USA), with charges and atom types applied using the MMFF94 method (Halgren, 1996; Halgren, 1996). Stepwise minimisations using the MMFF94s force field were subsequently carried out to a convergence of 0.02 kcal.mol^{-1}/Å over 5000 steps using the Powell method (Powell, 1977) with a cutoff of 11 Å.

The final coordinates of the ligand were saved in PDB format and used as input for eLBOW (electronic Ligand Builder and Optimisation Workbench) (Moriarty et al., 2009) and the output geometry restraints (in CIF format (Brown & McMahon, 2002)) were checked using the programme module REEL (Restraints Editor Especially Ligands). ELBOW and REEL are part of the PHENIX suite of computational crystallographic software (Adams et al., 2010).

Structure solution and verification

The program Phaser (McCoy et al., 2007) from the CCP4 package (Collaborative Computational Project, 1994) was used with default settings to solve the structure by molecular replacement (MR). The crystal structure of the complex formed of the bimolecular Oxytricha nova telomeric quadruplex and the ligand BSU6039 determined to 1.75Å (PDB ID 1L1H) (Haider et al., 2003)was used as a search model. Prior to use, the starting model was stripped of ligand, ions and waters.

The unit cell and space group ($P2_12_12$) were identical in the complexes to the previously reported complex (PDB ID 1L1H). The asymmetric unit contained one ligand bound in one of the diagonal loops in the quadruplex.

Upon visual inspection of the electron density maps using Coot version 0.6.1 (Emsley and Cowtan, 2004), a volume of electron density identical to the corresponding disubstitutedacridine ligand in shape and size was observed in one of the diagonal loops.

Crystallographic data are shown in Table 1.

Deposition in the Protein Data Bank Database

The structures solved in this work were deposited in the Protein Data Bank Database on 28th February 2008 and can be accessed using PDB IDs 3NYP and 3NZ7

Visualisation and Image production

Coot version 0.6.1 (Emsley&Cowtan, 2004), Chimera version 1.4.1 (Pettersen et al., 2004) and PyMOL version 0.99rc6 (DeLano, 2008) were used in model visualisation and image production in this work.

Table 1: Crystallographic data.

PDB ID	3NYP *	3NZ7 **
Data collection		
Total number of reflections collected	85 447	248 167
Number of unique reflections	23 982	25 994
Space Group	$P2_12_12$	$P2_12_12$
Cell dimensions: a, b and c (Å)	57.80, 44.46, 28.14	55.57, 42.70, 27.00
Angle (degrees)		
♋ ♌ ♑	90.00, 90.00, 90.00	90.00, 90.00, 90.00
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
Refinement		
Resolution range used in refinement (Å)	21.99 - 1.18	7.80 - 1.10
Number 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
Number of asymmetric units per unit cell	4	4
Number of atoms		
DNA	506	506
Ligand	36	36
Potassium ions	4	4
Water	177	188

**One crystal was used. ** Two crystals were used.*

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