Major Groove Binding and 'DNA-Induced' Fit in the Intercalation of a Derivative of the Mixed Topoisomerase I/II Poison *N*-(2-(Dimethylamino)ethyl)acridine-4-carboxamide (DACA) into DNA: X-ray Structure Complexed to d(CG(5-BrU)ACG)$_2$ at 1.3-Å Resolution

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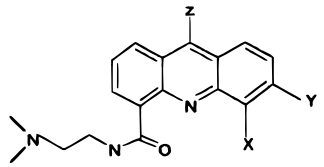
Received August 17, 1998

The design of improved mixed poisons of topoisomerases (topo) I and II is an important aim of cancer chemotherapy.[1,2] Structural information on both enzymes is now available,[3−5] and at least in yeast topo II, a major groove recognition domain has been identified.[3] However, although the DNA major groove therefore seems a preferred target for ligands (particularly topoisomerase poisons) incorporating sequence-dependent recognition elements, the vast majority of such agents in fact bind in the minor groove.[6] We now show that one acridine-4-carboxamide topoisomerase poison intercalates into duplex DNA with a specific hydrogen-bonded interaction to the N7 of guanine in the major groove. The only other specific major groove hydrogen-bonded interactions in intercalators which have been crystallographically characterized are those to N7 and O6 of guanine in nogalamycin[7,8] and to N7 in the bis-intercalators ditercalinium[9] and 'flexi-di'.[10]

The acridine-4-carboxamides have been intensively studied, and one example (**1**, DACA, *N*-(2-(dimethylamino)ethyl)acridine-4-carboxamide) (Figure 1) is in clinical trial as an antitumor agent and is known to be a mixed topo I/topo II poison.[11]

Extensive previous studies in both this series of compounds and the related 9-amino series have shown consistent structure−activity relationships for cytotoxicity[12,13] and inhibition of both topoisomerases I and II.[14,15] We have also recently shown a detailed pattern of dependence of cytotoxicity on steric and electronic effects,[16] but until now without an underlying structural rationale. The minimum requirements for therapeutic activity in the larger class of tricyclic carboxamides have been defined as a carboxamide side chain *peri* to an electron-withdrawing atom in the central ring.[17] Here we describe the near-atomic resolution (1.3-Å) determination of 9-amino-6-bromo-DACA (**2**) complexed to the sequence d(CG(5-BrU)ACG). It is part of a larger study which is revealing a pattern of specific major groove interactions combined with a consistent core binding which includes the novel feature of a 'DNA-induced' side-chain orientation generated by the combination of the negative charge at the intercalation site and a conserved bound water molecule.



1 = DACA (X = Y = Z = H)
2 X=H; Y = Br; Z = NH_2
3 X = Br; Y = H; Z = NH_2

Figure 1. Structural formula for DACA.

The structure was solved using coordinates of an isomorphous structure[18] which was solved ab initio by a MAD methodology. It was refined using SHELX97[21] (with distance and angle but no torsional (or 1,4 distance) restraints) to give the symmetric duplex shown in Figure 2.

The refined model has 2-fold symmetry with two molecules of **2** intercalated between the d(CG)$_2$ base pairs. The intercalated drug is clearly shown in an 'omit map' in Figure 3a with the final refined density for comparison.

The first feature of interest is the single hydrogen bond formed by the $-NMe_2H^+$ group of the *N*−(2-(dimethylamino)ethyl)-4-carboxamide side chain to N7 of one of the two guanines at the intercalation site (Figure 4), which was an unexpected side-chain orientation. The calculated position for this hydrogen gives a good hydrogen bond (ND2−N7, 2.85 Å; ND2−H···N7, 137°), and the side-chain torsion angles show a *gauche* conformation relating the two side-chain nitrogen atoms.

Second, the carboxamide nitrogen NI1 and oxygen OI1 are 0.72 and 0.42 Å from the acridine ring plane on the same side as the side chain. This finding suggests that the delocalized positive charge on N10 is further extended over the carboxamide group, generating an unexpected DNA-induced conformation which can presumably only exist in such a stabilizing local environment. N10 is here a hydrogen bond donor to the carbonyl group of the carboxamide. All prior models[22−25] assumed that the carboxamide carbon CI1 would be planar, but probably noncoplanar with the acridine in the dicationic form of the drug. The negatively charged environment of the major groove intercalation site therefore indirectly generates a favorable side-chain orientation for hydrogen bond formation to the guanine N7.

Third, the pK_a of this DACA derivative is close to the pH of crystallization, 6.5, and both the acid and conjugate base forms of the drug can actually be seen in this crystal structure. A third spacer drug is found between the duplexes. It is striking that this third drug molecule is present as the monocationic (conjugate base) form, with a neutral acridine ring. The role of N10 here is as

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10.1021/jm980479u CCC: $18.00
Published on Web 02/25/1999

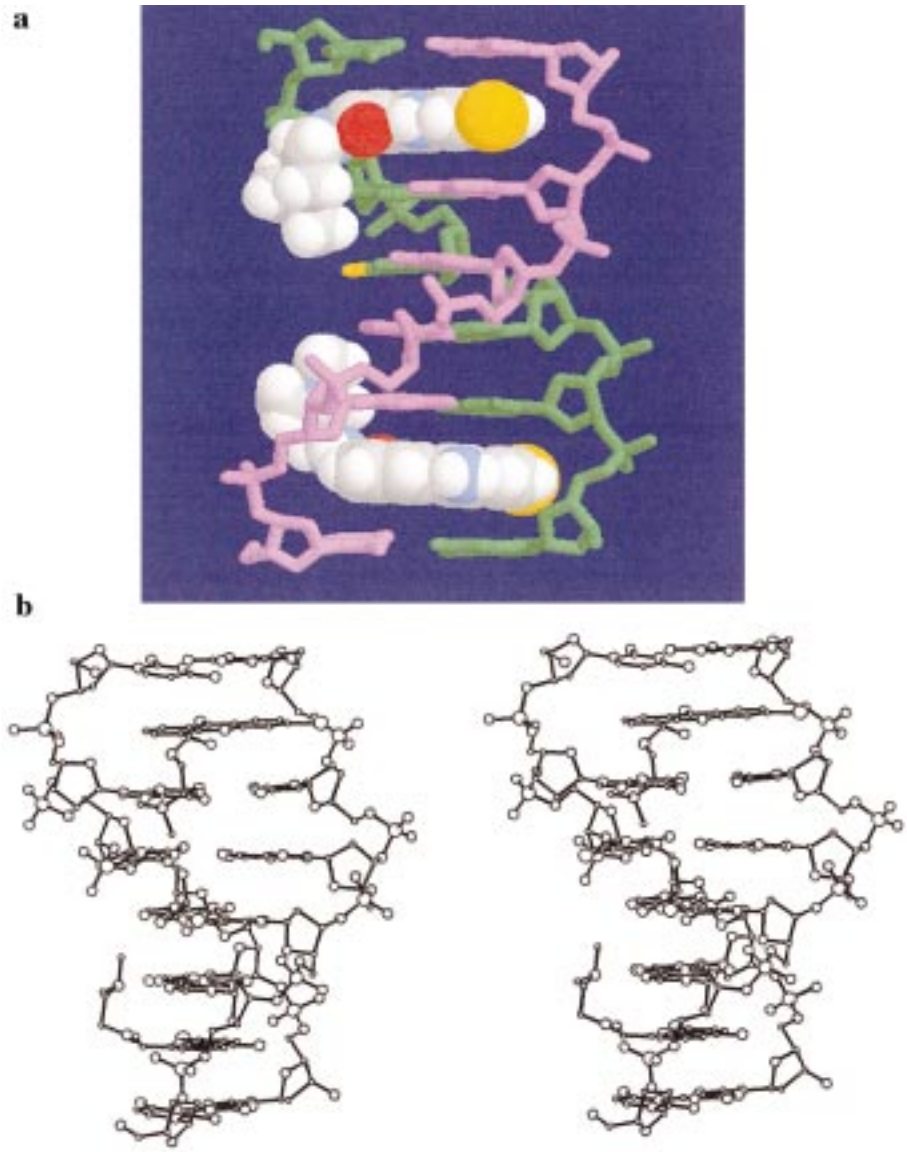

Figure 2. (a) RasMol plot representing the intercalated drug as a space-filling model and the DNA backbone as wireframe. (b) Stereoview of the d(CG(5-BrU)ACG)$_2$ duplex with two intercalated molecules of the drug **2**. The spacer drug has been omitted for clarity.

an acceptor of the amide hydrogen bond, as in the original crystal structure of the conjugate base and in the original modeling work.[20] This is clearly shown by the carboxamide orientation adopted, with a hydrogen bond between N10 and ND1 rather than the carbonyl but retaining the terminal NMe_2H^+ group (ND2) hydrogen bond to N7, albeit a longer and less optimally oriented one. In this form of the drug, the carboxamide group is in the plane of the acridine ring (dihedral angle between the planes, 0.7°) and is itself planar, and the conformation about the C−C single bond of the side chain is *trans* rather than *gauche* as it is in the dication form. The present work thus clearly demonstrates both the importance of local electrostatic potential (presence or absence of phosphate) in determining the state of protonation of this drug and the change in carboxamide orientation and geometry produced by this change in protonation.

The crystal packing is novel, and the crystallographic repeat is generated by stacking of the terminal residues of the duplex onto the spacer drug molecule. (The incorporation of an extra stacked acridine into the lattice had already been found in the 9-aminoacridine/(iodoCG)$_2$ and related structures.[26]) The helix axis is parallel to the crystallographic direction 100 and is intersected by a 2-fold axis (Figure 5), with the spacer drugs forming hydrogen bonds to adjacent duplexes and generating a pseudopolymeric infinite stack.

The major groove of the duplex is aligned orthogonal to the 001 crystallographic direction. This arrangement results in a highly diffracting, commensurate lattice by generating an overall 180° turn of the duplex, although the total twist of the intercalated hexamer is only 155°.

Table 1. Backbone Torsion Angles (deg) for **2**

	α	β	γ	δ	ϵ	ζ	χ	sugar pucker
C			59	90	−161	−75	−136	C3′-*endo*
G	175	−176	−166	154	−177	−106	−103	C2′-*endo*
5-BrU	−68	−177	51	137	−153	−166	−99	C2′-*endo*
A	−33	143	41	136	177	−92	−94	C2′-*endo*
C	−69	175	53	131	−155	−138	−93	C1′-*exo*
G	63	172	−64	160			−69	C2′-*endo*
B-DNA[27]	−65	167	51	129	−157	−120	−103	

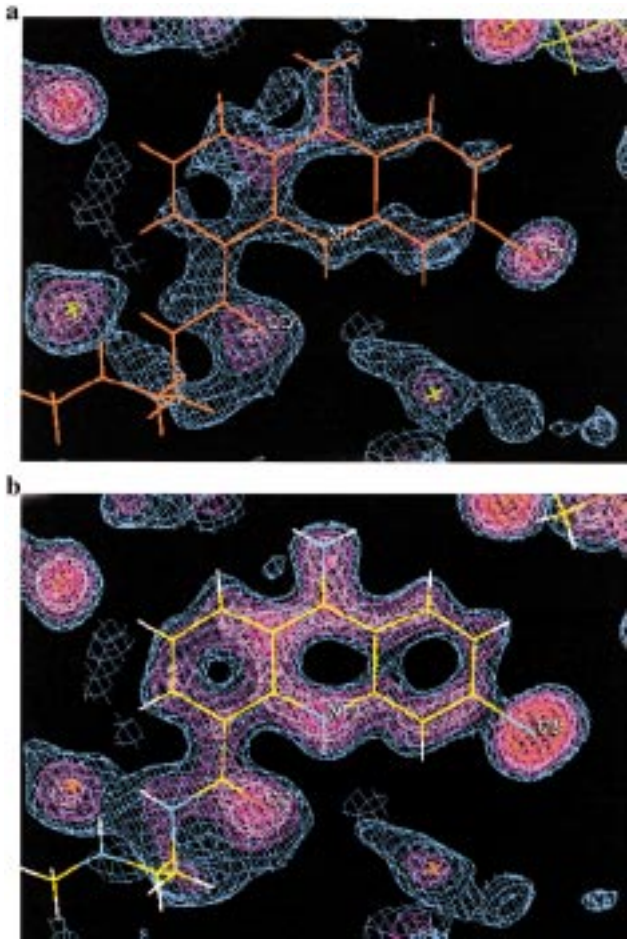

Figure 3. (a) Omit map showing the intercalated drug density for **2**. (A $2F_o - F_c$ map omitting the intercalated drug residue from the F_c calculation generated from within XtalView; the actual side-chain location was determined from a series of difference maps generated by SHELX97 refinements.) (b) Final σ-A map for the same residue.

It is only obtained in the presence of the 9-NH_2 substituent of the chromophore due to an interduplex hydrogen bond, but this observation is probably of no pharmacological significance, as a similar structural model seems to be valid for DACA itself, currently undergoing refinement in a different, less well diffracting, lattice.

The hydrogen bonds of the base pairs remain essentially undistorted by intercalation. The overall ligand orientation places the long axis of the acridine chromophore aligned with the long axes of the base pairs. The stacking interaction on the ring containing the side chain maximizes the overlap with the six-membered rings of the cytosine and guanine on either side, in a remarkably similar pattern to that in the original 9-aminoacridine/(iodoCG)$_2$ complex[23] (Figure 6).

Intercalation produces characteristic modifications of torsion angles in the DNA duplex, as shown in Table 1.

One obvious feature is the *antiperiplanar* conformation about the C4′−C5′ bond of the G2 sugar (γ torsion angle). This conformational change is not found for intercalation by the anthracycline antibiotics, even nogalamycin, which causes considerable buckling of the base pair in the corresponding position, but was observed in the ethidium/(iodoCG)$_2$ structure.[27] The reasons for this difference are not yet understood, since that

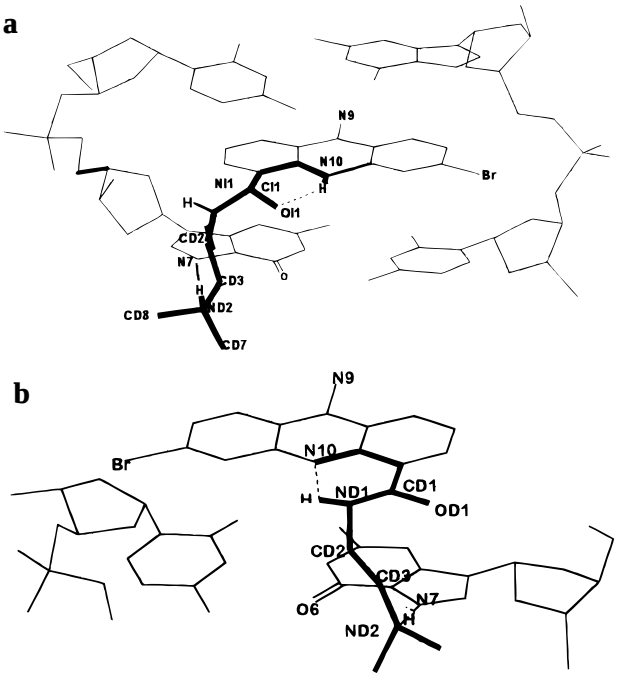

Figure 4. (a) d(CG)$_2$ pair containing the intercalated drug **2**, showing the specific hydrogen bonds. The NI2−N7 distance is 2.85 Å, and the N10−OI1 distance is 2.58 Å. The torsion angle CI1−NI1−CD2−CD3 is −123(3)°, NI1−CD2−CD3−ND2 is −63(6)°, CD1−CD2−ND2−CD7 is −176(3)°, and CD1−CD2−ND2−CD8 is −57(7)°. The unusual *antiperiplanar* conformation at C4′−C5′, torsion angle O5′−C5′−C4′−C3′ −169°, on G2 is highlighted. (b) Alternative conformation adopted by the spacer drug **2**, in the monocationic form, with the NH of the carboxamide hydrogen bonded to the N10 of the neutral acridine.

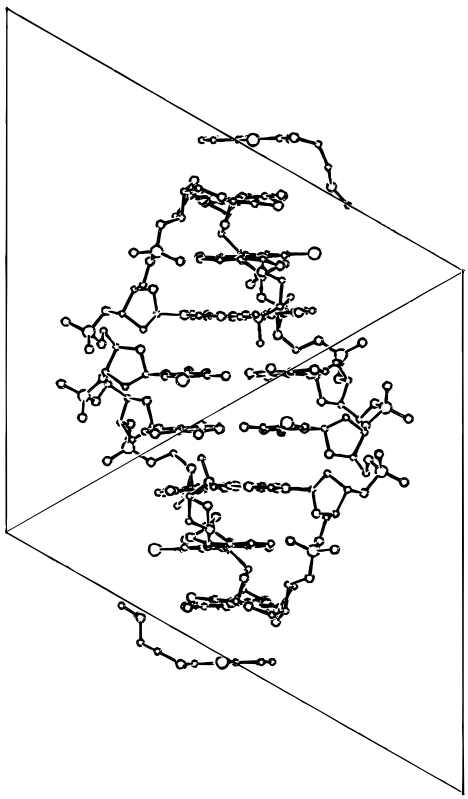

Figure 5. View down the 001 direction of the $P6_4$ cell, looking directly into the major groove.

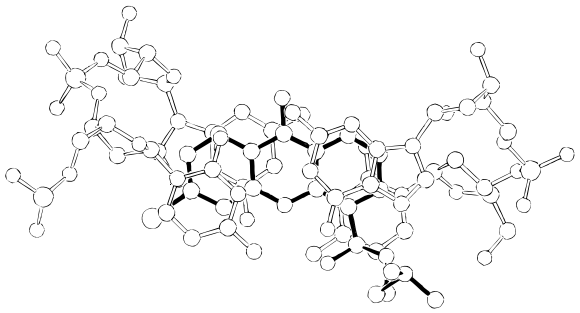

Figure 6. Stacking interaction, showing the optimization of overlap between the guanine (G2) six-membered ring and the phenyl ring of the drug **2** bearing the carboxamide substituent.

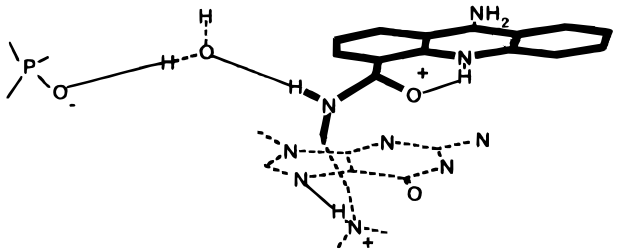

Figure 7. Conserved core of the acridine-4-carboxamide interaction.

part of the intercalation cavity contains solvent. The local twist angle at the intercalation site is 25° and is 28° at the adjacent d(GT/AC) step, rising to 46° at the $d(TA/TA)_2$ step, so that there is clear evidence of unwinding at the intercalation and adjacent sites. The unwinding angle for DACA, using closed circular DNA, is 12°,[28] in excellent agreement with an overall unwinding caused by two drugs from 180° to the observed 155°.

The change in electrostatic potential in the major groove, which results from neutralization of the guanine partial negative charge by the NMe_2H^+ group, combined with the steric blocking of the groove by the side chain are likely to be important contributors to topoisomerase inhibition. In the acridine series, the presence of a water molecule in the intercalation cavity, conserved in all datasets so far examined, also suggests a role for a hydrogen bond to the carboxamide NH in anchoring the DNA-induced side-chain orientation. This water molecule seems also to have a conserved interaction with a phosphate oxygen of the intercalation cavity. Figure 7 summarizes the major interactions. Several related systems are currently under examination with a view to confirming the generality of these observations.

Acknowledgment. Financial support from the BBSRC (to A.K.T.), AICR (to A.A. and L.P.G.W.), and University of Reading (to J.H.T.) is gratefully acknowledged. We thank Professor S. Neidle (CRC Laboratories) for helpful discussions and for reading a draft of the manuscript. We are also grateful to Professor D. J. Cardin, Dr. H. R. Powell, Mrs. E. Dodson, and Dr. P. R. Evans for many helpful discussions during the course of this work and to Dr. A. Savoia and his colleagues at Sincrotrone Elettra, Trieste, for their capable technical support during the MAD data collections.

Supporting Information Available: ORTEP plot, line diagram of numbering scheme for drug, and crystal data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) **2** with $d(CG(5\text{-}BrU)ACG)_2$: hexagonal, space group $P6_4$ (no. 172); $a = b = 30.087$ Å, $c = 39.316$ Å, $Z = 3$; solvent content 54%; $\lambda = 1.0000$ Å. Data were collected on the X-ray diffraction beamline Sincrotrone Elettra, Trieste, using a Mar 345 image plate detector running in large Mar mode at 100 K and using other procedures already described in detail.[19] The structure of the isomorphous 5-Br analogue of the drug (**3**) with the same sequence DNA was solved by MAD. The Br atom positions were determined from the anomalous differences at the white line maximum. Phasing was carried out with the CCP4 versions of MLPHARE and DM, and the model was built ab initio into the MAD map using O. Since the 6- and 5-Br structures were isomorphous, the structure of the 6-Br analogue was refined using the model for the 5-Br structure, by omitting the Br atoms and locating their new positions from the $F_o - F_c$ map. Refinement of both structures was carried out using SHELX97, restrained anisotropic temperature factors, and all data in the range 8−1.3 Å for the 6-bromo compound (**2**) (31 514 reflections measured, 9 306 unique observations, $R_{merg} = 6.75\%$ overall, 37% in range 1.4−1.3 Å) to a conventional R factor of 14.68%. The final model includes 29 full occupancy and 1 half-occupancy

water molecules. The coordinates and structure factors for the work reported here have been deposited with the Nucleic Acid Databank, accession number DDF073. The 5-bromo data is being refined at 1.2 Å,[20] and the current conventional *R* factor for that structure is 14.2%.

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JM980479U