# CAN ACETYL INTERCALATION BE INCORPORATED INTO A DNA DOUBLE HELIX?

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## ABSTRACT

From molecular model-building studies it is suggested that an intercalating acetyl group can replace a pyrimidine base in a DNA double helical structure. The studies are based on the conformation of an acetyl group found in a single crystal study of the nucleoside 2′, 3′, 5′-tri-O-acetylguanosine.

## INTRODUCTION

MUCH interest has been focused in recent years on the flexibility of the DNA double helix. The suspicion that double helical DNA could exist solely as a right-handed structure has been disproven, with the discovery that left-handed DNA (Z-DNA) can exist[1]. Further, it has been found that in oligonucleotide-drug structures[2], intercalation of a planar drug can lead to a local increase in base-base separation in the oligonucleotide from 3.4 to 6.8Å. The postulated activity mechanism of such drugs relies strongly on the fact that the usual base-stacking interactions can be interrupted and modified. The mechanisms involve backbone conformational changes to accommodate the increased base-base separation. Such models show that base-base stacking is not a prerequisite for stable oligonucleotide structure, despite its wide occurrence in studies of nucleosides and nucleotides.

## SINGLE CRYSTAL RESULTS

We have found in a recent crystal structure, of 2′, 3′, 5′-tri-O-acetylguanosine[TAG][3], an interesting conformation of the 2′-acetyl group. As can be seen in figures 1 and 2, this acetyl adopts a "scorpion-tail" position such that the O atom of the polar C=O group is sandwiched in an intercalating position between two guanine bases. The acetyl oxygen, 02′1, is at 3.15(2)Å from the plane of the base in the residue to which it is attached, and at 3.02(2)Å from the base plane of the residue translated by one cell repeat along the a direction. In previous acetylated nucleoside structures studied in this laboratory[4–7] such a contact of an acetyl carbonyl oxygen above a base has been observed to play an important part in the stacking of the molecules Such contacts are stabilised by dipolar and London dispersion forces. Previously these contacts have involved 3′ or 5′-acetyl carbonyl O atoms stacking above a solitary base, but in this latter case the participation of the 2′ acetyl group, and its positioning between two parallel bases, are both novel and interesting.

In the structure of unsubstituted guanosine dihydrate [GD][8], the base rings stack in a position of almost maximum overlap, this stacking obviously playing an important role in the structure, as evidenced by the fact that inosine hydrate[8,9] adopts the same crystal structure in spite of having one fewer hydrogen bond to stabilise the structure. Since this base-base interaction is obviously so important, it is of special interest that, in TAG, it is superseded by the base-acetyl-base stacking observed. This interaction therefore seems likely to represent a low energy, stable form of

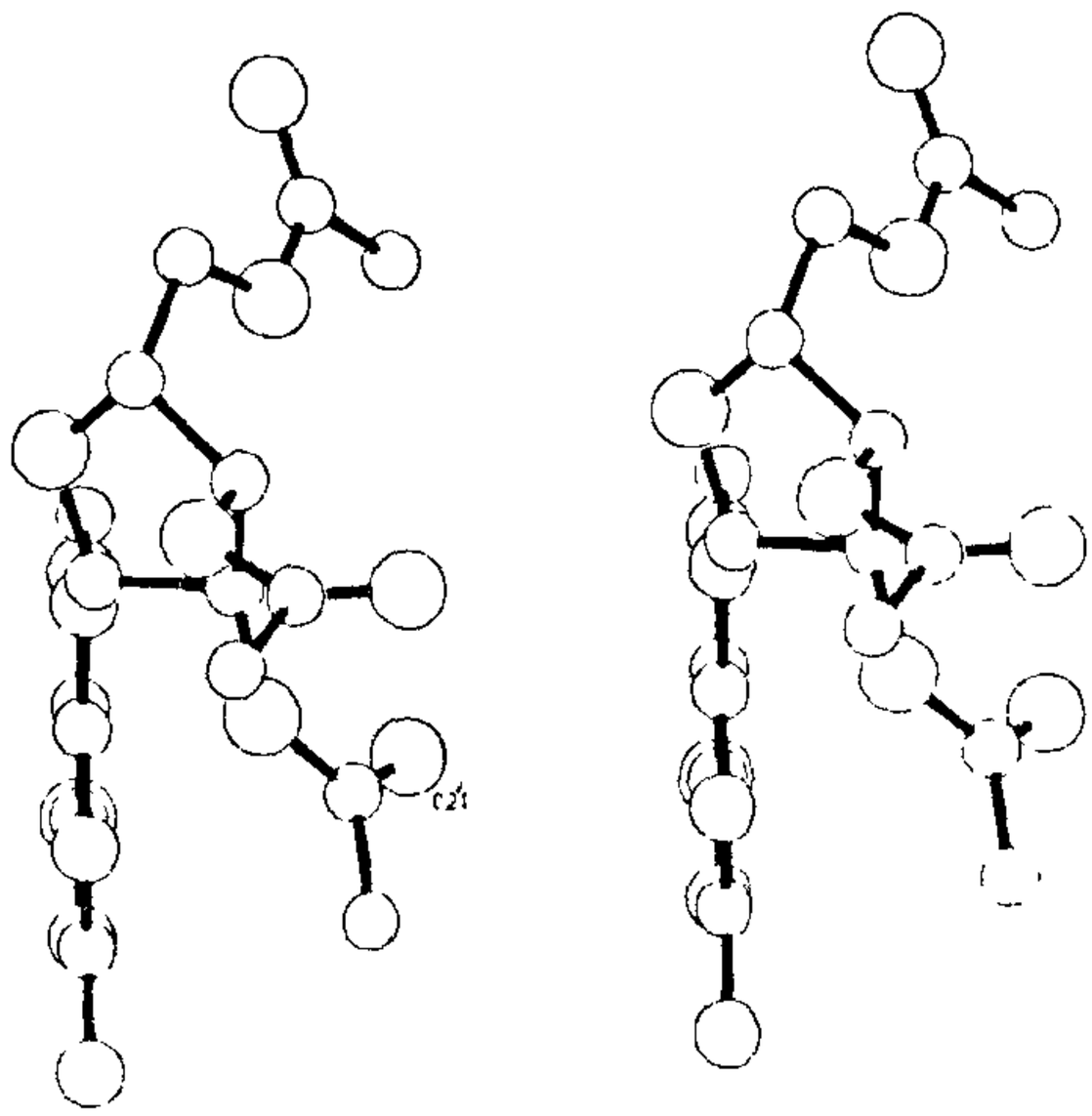


**Figure 1.** Intercalation geometry of 02′1 viewed in the plane of the bases.

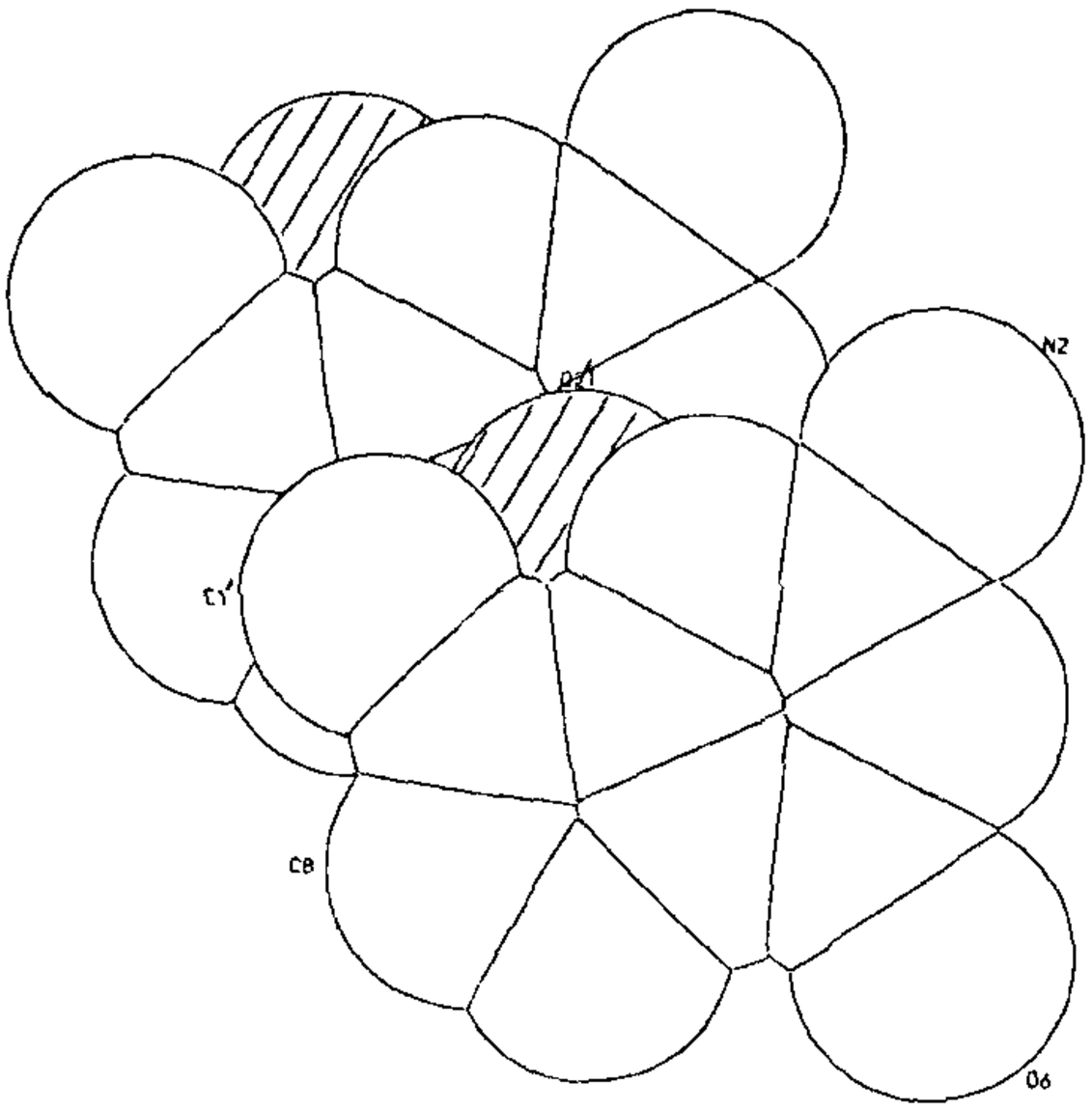


**Figure 2.** Space-filling diagram viewed perpendicular to the bases, showing 02'1 (shaded) position.

stacking. In addition, it was noted that, in the structure of TAG, the guanosine bases were base-paired, with one of the bases mimicking the role of cytosine in a "Hoogsteen-like"[10] arrangement. Thus the situation in TAG is of parallel bases, paired in Hoogsteen fashion, with an acetyl carbonyl group O atom intercalated.

Since base-stacking is considered to play an important role in stabilising nucleic acid structures[11] and because in this case base stacking is excluded by a new base-dipole-base stacking pattern, it is tempting to try to incorporate such a conformation into a double helical structure. It has been previously suggested by Parthasarathy *et al*[12] that a water molecule could intercalate and replace a pyrimidine base in DNA, but the geometric constraints in the present case are more stringent with the acetyl group bound to one nucleoside.

## INTERCALATION MODELS

Model-building studies were carried out of possible double helical arrangements, using as a starting point the actual conformation of the TAG molecule. As the contact of interest involves the 2'-acetyl group, the 3' and 5' positions were assumed to be non-acetylated in the model-building, and able to participate in the sugar-phosphate backbone. Two models which showed particular promise, although others can certainly be envisaged, are detailed below.

If the 2'-acetylguanosine moiety (anti, $X_{cn} = -160.6(9)°$, sugar pucker = C2'-endo) is assumed to hydrogen bond in Watson-Crick fashion to a cytosine base, a right-handed B-DNA helix can be constructed in which the acetyl carbonyl oxygen replaces a pyrimidine base and is stabilised by one hydrogen bond, as illustrated in figure 3, to a purine on the opposite strand of the double helix. In this way one full nucleoside is missed out, and the back-bone at the site of disruption is more extended than usual, to compensate. The base conformation at the glycosyl bond of all the nucleosides in this model is anti, and sugar puckers are C2'-endo, as found in TAG, which is quite normal.

On the other hand, if the 2'-acetylguanosine were to be incorporated into a (left-handed) Z-DNA-like but Hoogsteen-paired G-C double helix, the acetyl O would be in a position to make two hydrogen bonds to a purine, as in figure 4. In this model the conformations of all the pyrimidine nucleosides are anti at the glycosidic bond, and those of the purines all syn, apart from the 2'-acetylguanosine, which is anti, and the purine to which the acetyl O hydrogen bonds, which is also forced into an anti conformation. Thus both helical chains are kinked in this model, and there is a concomitant unwinding of the helix locally. Sugar puckers are again normal. A left-handed Hoogsteen linked structure has been suggested for double helical polynucleotides[13], and its compactness (C1'-C1' separation within the base-pair = 8.5Å) would favour the present suggestion, by bringing the intercalating O atom sufficiently close to the purine base to hydrogen bond. Whilst G-C Hoogsteen pairs are very rare due to

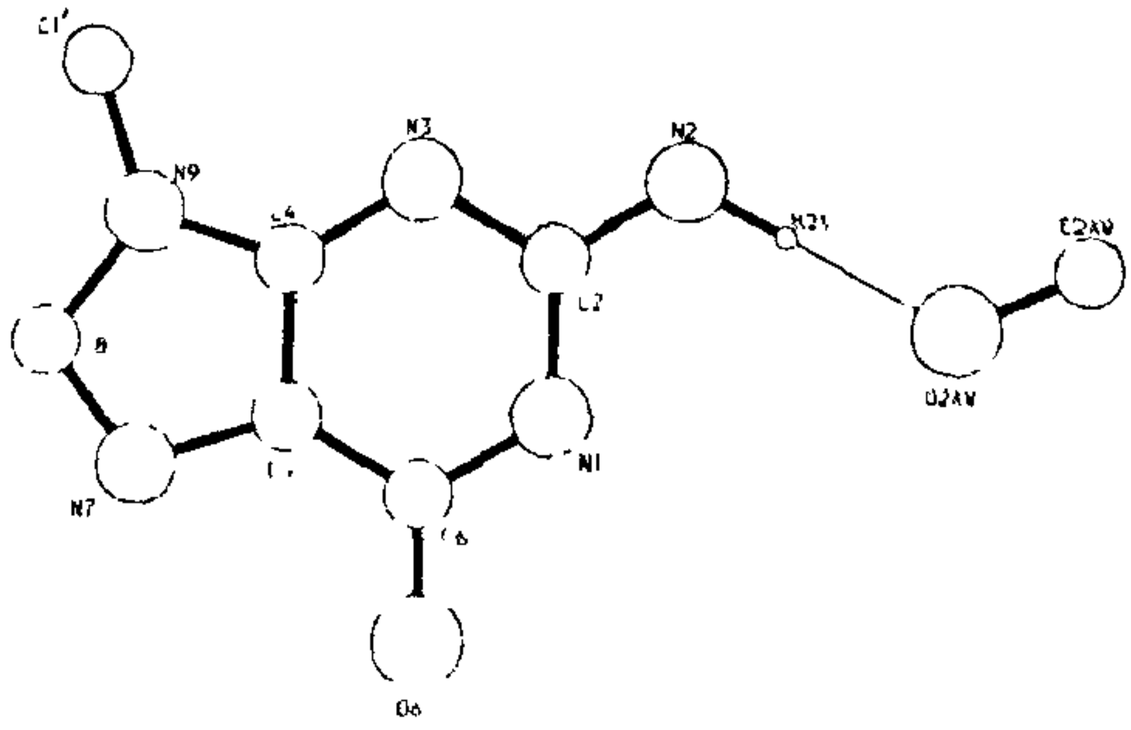


**Figure 3.** Hydrogen bonding of the acetyl oxygen (02AW) in the right-handed Watson-Crick model

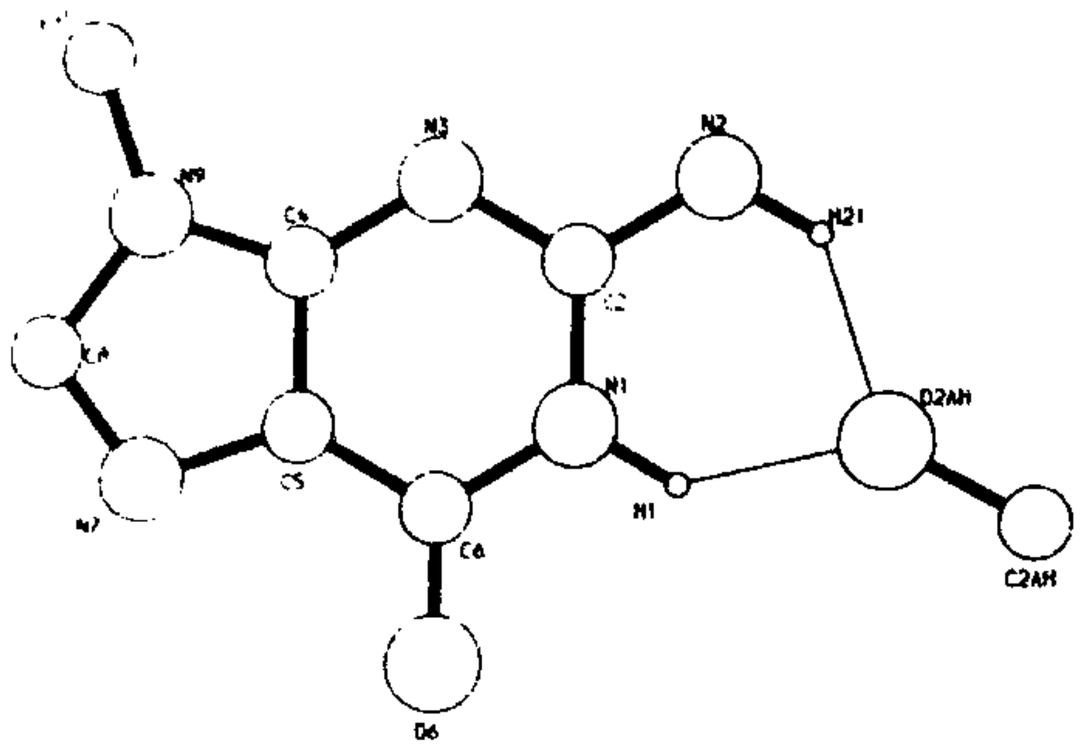


**Figure 4.** Hydrogen bonding of the acetyl oxygen (02AH) in the left-handed Hoogsteen model.

the greater stability of the Watson-Crick G-C pair, it is feasible that such a modification could occur under favourable pH conditions.

Such an intrastrand intercalation, introducing openings in the backbone, and leading to "vacancies" in certain base-occupied positions, could be a precursive step to drug action, for example. The scenario envisaged is either of incorporation of one or more 2′-O-acetylguanosine moieties into a DNA double helix, or incorporation into a DNA : RNA double helical hybrid, although models of the latter case have not as yet been investigated.

The acetyl group is situated in the minor groove of the right-handed model above, but in the broad shallow major groove in the left-handed one. The acetylated guanosine nucleoside is situated within the double helix as shown in figure 5.

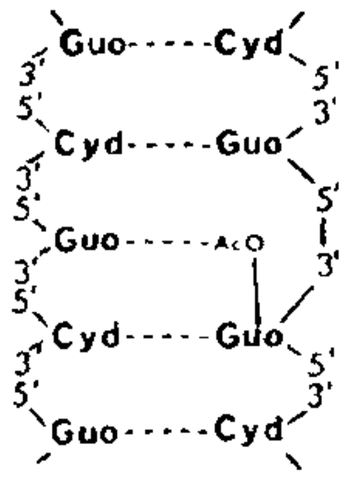


**Figure 5.** Schematic representation of the double helix with intercalated acetyl group.

Whilst G-C Hoogsteen pairing is unlikely, the closely related inosine-cytosine is less so[13]. The possibility of a similar interaction in crystals of 2′,3′,5′-tri-O-acetylinosine is under investigation in this laboratory, and attempts are being made to form the 2′-monoacetylated nucleoside used in the model building, to see if the interaction is present in the crystal structure of this compound.

## ACKNOWLEDGEMENTS

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