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NEW APPROACH TO CONFORMATIONAL ANALYSIS OF HETEROBIARYLS IN SOLUTION

Lucjan Strekowski,* Farial A. Tanious, Subramanian Chandrasekaran,
Rebecca A. Watson and W. David Wilson*

Department of Chemistry, Georgia State University, Atlanta, GA 30303

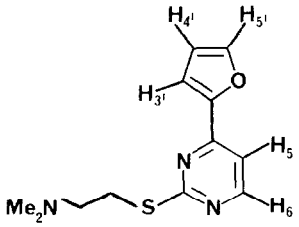
Summary: Native DNA is used in conformational studies of heterobiaryls. Evidence is presented that the 4-(2'-furyl)pyrimidine and 4-(2'-thienyl)-pyrimidine systems exist in solution in an essentially planar s-trans and s-cis conformation, respectively. The 5-methyl-4-(2'-thienyl)pyrimidine system is also s-cis.

The conformational study of heterobiaryls in solution is a difficult task. The methods that have been used to estimate the torsional angle in these compounds are mainly analyses of the infinite-dilution Kerr constants, Cotton-Mouton constants, and dipole moments, in addition to spectral methods and theoretical calculations.[1] In this communication we present a new approach for studying conformations of heterobiaryls using native, double-helix DNA as a stereochemical template for molecular conformations with low torsional angles.

Compounds to be studied must (i) intercalate with DNA and (ii) have protons or substituents with protons at the ortho and ortho' positions with respect to the torsional bond. After the unfused heterobicyclic system slides (intercalates) between the sandwiches of DNA base pairs, it is planar or only slightly deviated from co-planarity (<8°) in the resultant DNA intercalation complex.[2] The stereochemistry of the molecule (s-cis or s-trans) in the complex is studied by the NOE effect for the ortho and ortho' protons. The equilibrium conformation of heterobiaryl free in solution is then evaluated through comparison of its NOE difference spectrum with that for the molecule in the DNA intercalation complex.

An important structural feature in compounds to be studied is a basic side chain (or cationic, see **2**) attached to the heterobiaromatic system. The basic site becomes cationic in D_2O, the solvent used for NMR work with DNA, and as such interacts electrostatically with the outer, anionic DNA backbone providing additional stabilization for the intercalator-DNA complex. This often helps to obtain an intercalation complex for aromatics which without the cationic group fail to intercalate with DNA.[3] In addition, the cationic side chain increases solubility of an organic compound in D_2O.

The method has been successfully applied to estimate the planar s-trans conformation for furylpyrimidine **1** and the planar s-cis conformation for three thienylpyrimidines[4] **2-4**, and to show low torsional angle in sterically hindered thienylpyrimidine **5**. All these compounds bind strongly to DNA through intercalation.[5] Irradiation of H5 of the pyrimidine ring in **1** bound to DNA resulted only in a strong NOE at H6 of the same ring. In the same way irradiation of H3' of the furan ring gave a strong NOE to H4' only, indicating the s-trans form of **1** in



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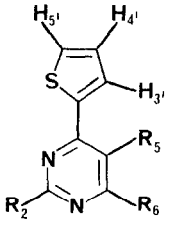

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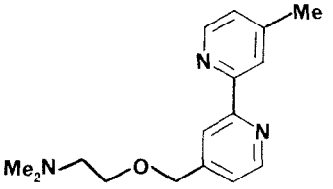

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2. $R_2 = I^{\ominus}\,Me_3^{\oplus}N\!\sim\!S$, $R_5 = R_6 = H$

3. $R_2 = Me_2N\!\sim\!S$, $R_5 = H$, $R_6 = Me$

4. $R_2 = MeS$, $R_5 = H$, $R_6 = NH\!\sim\!NMe_2$

5. $R_2 = Me_2N\!\sim\!S$, $R_5 = Me$, $R_6 = H$

the intercalation complex. The same results were obtained for **1** free in solution (D_2O and $CDCl_3$). These results together with the UV spectrum of **1** ($\lambda_{max}^{H_2O}$ 321 nm, ε 17960 $M^{-1}cm^{-1}$) and a substantial NMR deshielding effect for H5 in **1** in comparison to the NMR absorption of H5 in the respective pyrimidine without a furyl substituent ($\Delta\delta$ 0.34 ppm) indicate that the 4-(2'-furyl)pyrimidine system must be in the essentially planar, s-trans conformation in solution.

Irradiation of H5 of the pyrimidine ring in **2** in the DNA intercalation complex resulted in a strong NOE at H6 of the same ring and a strong NOE at H3' of the thiophene. Moreover, irradiation of H3' of the thiophene ring gave a strong NOE to H4' of the same ring and an even stronger NOE to H5 of the pyrimidine, which shows the s-cis stereochemistry for **2** in the DNA intercalation complex. Since the same NOE difference spectra, with the same relative enhancement intensities,[6] were obtained for **2** free in solution (D_2O), these results show that the 4-(2'-thienyl)pyrimidine system exists in solution in the planar or only slightly skewed s-cis conformation. In a similar way the s-cis conformation has been obtained for compounds **3** and **4**, which indicates that the buttressing effect of the methyl group and the alkylamino group, respectively, on H5 in the pyrimidine ring has no effect on the stereochemistry of the 4-(2'-thienyl)pyrimidine system. To our astonishment, however, the 5-methyl substituted thienylpyrimidine **5** gave identical NOE difference spectra, for the DNA intercalation complex and free in solution (D_2O and $CDCl_3$). The spectra were in full agreement with the s-cis stereochemistry with a small value of the torsional angle. This result was further supported by the following comparative studies of structurally related compounds **3** and **5**. Thus **3** and sterically hindered **5** gave the same upfield shift for H3' of the thiophene ($\Delta\delta$ -0.61 ppm) upon intercalation with DNA, under the same binding conditions. This indicates that the ring current effect of the pyrimidine ring on H3' is similar for **3** and **5**, in agreement with similar conformations for these compounds. Secondly, the UV spectra of **3** ($\lambda_{max}^{H_2O}$ 322 nm, ε 15,300 $M^{-1}cm^{-1}$) and **5** ($\lambda_{max}^{H_2O}$ 327 nm, ε 13,150 $M^{-1}cm^{-1}$) show strong conjugation between the two heteroaromatic subunits in both compounds.

Two major factors responsible for the equilibrium conformation of heterobiaryls in solution are the π-electron delocalization energy which reaches a maximum for co-planar rings, and the interactions between groups ortho to the central bond. The conjugation factor is especially important for compounds **1-5**, which are combinations of π-electron deficient and π-electron excessive heterocycles. These biaromatic systems are strongly polarized with the electron density transfer to the electron-deficient pyrimidine.[7] The s-trans conformation of **1** can be understood in terms of favorable stereoelectronic interactions between electropositive H3' of the furan and electronegative N3 of the pyrimidine. In addition, the preferred oxygen environment is also in the plane of the aromatic ring.[8] The suggested planarity of system **1** is further supported by comparison of the calculated[9] distances H3'···N3 and H5···O with those allowed using the respective van der Waals radii.[10] Similar analysis of the 4-(2'-thienyl)pyrimidine system reveals a favorable N3···S interaction which offsets the repulsive steric ortho-ortho' interaction between H5···H3' in compounds **2-4** and between CH_3···H3' in compound **5**. Nonbonded contacts of a nucleophile with sulfur are known to greatly reduce the normally accepted van der Waals radius of sulfur, and are the basis of some of the strongest intermolecular forces in crystals.[11] The calculated values of interatomic distances N3···S and H5···H3' in the planar s-cis conformation of **2-4** are 3.13 Å and 2.17 Å, respectively. Since both values are not lower than the allowed minimum contact distances between the respective atoms,[8,11] we believe that compounds **2-4** are planar in solution. Sterically hindered compound **5** is apparently non-planar but, using the minimum contact distances, its conformational model with the torsional angle lower than 8° has been computed. It is quite remarkable that **5** has the highest DNA binding constant of all the compounds tested in this work.[5]

The NOE experiments with bipyridine **6** in $CDCl_3$ revealed the s-trans orientation in agreement with the generally accepted view.[1] This compound failed to intercalate with DNA and, apparently, has the torsional angle higher than 8°. Similarly, biphenyls do not intercalate with DNA and are known to exist in a twisted form (30°) in solution.

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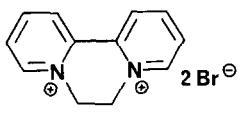
2. A polyaromatic compound must be essentially planar or be able to acquire such a conformation in order to interact with DNA through intercalation. This has been shown recently by one of our group (W.D.W.) using bipyridinium compound **7** and two phenanthrolinium derivatives **8** and **9**. Compounds **7** and **8** have two positive charges in the molecule, and on this basis alone they can be expected to be strong intercalators.



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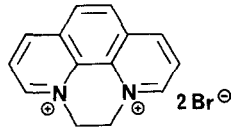

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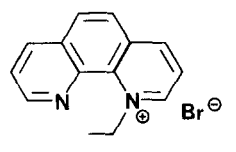

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These compounds, however, do not intercalate with DNA, which is due to the torsional angles of 20° (**7**) and 8° (**8**) between two pyridinium moieties in the molecules. Compound **9** has the torsional angle 2° and intercalates with DNA (V. Vaishnav, M. Williamson, and W.D. Wilson, unpublished results). Other studies have shown that actinomycin has the ring system twisted less than 8° and intercalates with DNA [H.M. Sobell, Prog. Mol. Biol., **13**, 153 (1973)]. Based on these data we believe that a biaryl molecule has a torsional angle <8° in the DNA intercalation complex. Evidence for the intercalation (for a review see: G.L. Cantoni and D.R. Davies, "Procedures in Nucleic Acid Research," Harper and Row, New York, 1971) is based on (i) increases in DNA viscosity (DNA becomes longer), (ii) downfield shifts in DNA ^{31}P-NMR spectra, (iii) upfield shifts for the signals of the DNA imino base pair protons (anisotropic effect of the intercalator's aromatic system), and (iv) upfield shifts for the signals of the aromatic protons of the intercalator molecule (anisotropic effect of the aromatic systems of the DNA bases).

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4. In the preparations of **1-5** the addition reactions (Et_2O, -30°C) of 2-furyllithium or 2-thienyllithium with the respective pyrimidines were followed by aromatization with DDQ of the resultant intermediate dihydropyrimidines. The trimethylammonium derivative **2** was prepared through quaternization of the dimethylamino compound (**2**, R=Me_2N S) with MeI and crystallized from EtOH; m.p. 244-246°C. All new compounds gave satisfactory elemental analyses (C, ±0.2 and H, ±0.1%). DNA for the NMR experiments was sonicated to the range of approximately 100-150 base pairs to reduce the viscosity of the polymer solution [W.D. Wilson and R.L. Jones, Nucl. Acids Res., **10**, 1399 (1982)]. Proton (270 MHz) NMR spectra were obtained on a JEOL GX 270 spectrometer under the following conditions: typically 2000 scans; 2.15-s pulse repetition rate; 0.1-Hz line broadening; 16K data points; TSP reference; 4000-Hz spectral width; 100% D_2O/phosphate buffer containing 15 mM NaH_2PO_4, 0.1 mM EDTA, 0.1 M NaCl (pD 7.0); 5 mM of the intercalator (**1-5**); 0-33 mM DNA (concentration of base pairs DNA); 0.8-mL sample volume in a 5-mm NMR tube. All NMR experiments were conducted at 50°C, below the denaturation temperature of DNA (approximately 80°C as determined by NMR and UV). Assignments of the chemical shifts of the protons of **1-5** were obtained using coupling patterns and 2D COSY experiments; compound (D_2O, no DNA), δ for H5 (or 5-Me), H6 (or 6-Me), H3', H4', H5': **1**, 7.45, 8.45, 7.76, 6.70, 7.36; **2**, 7.59, 8.52, 7.94, 7.29, 7.78; **3**, 7.41, 2.44, 7.87, 7.27, 7.74; **4**, 6.63, 2.58, 7.76, 7.23, 7.66; **5**, 2.47, 8.39, 7.83, 7.32, 7.78. Proton NOE difference spectra were obtained using 10-s saturation time, 21-μs pulse width (corresponding to 90° pulse), 4-Hz line broadening, and 1:3 molar ratio of intercalator/base pairs DNA. It should be noted that the saturation time required for NOE observation varies with the proton relaxation time and must be optimized for each system studied. For closely related compounds such as **2-5** the relaxation and saturation times are very similar under similar conditions.
5. Rigorous proof for the intercalation was obtained using the methods discussed in note 2. The shift difference in the range of 0.6-0.8 ppm for all aromatic protons of **1-5** was obtained between the compounds free in solution and with excess of DNA (ratio 0.15). This indicates that both aromatic subunits of **1-5** are fully intercalated. The following DNA binding constants were obtained for the solution conditions of note 4: **1**, 3500; **2**, >20000; **3**, 24800; **4**, >5000; **5**, 47300 M^{-1}.
6. Because sonicated DNA and high temperatures were used (see note 4), the NOE signals for **2** (and other intercalators) in the DNA intercalation complex were only slightly broader in comparison to the NOE spectrum of the compound free in solution.
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8. R.O. Gould, A.M. Gray, P. Taylor, and M.D. Walkinshaw, J. Am. Chem. Soc., **107**, 5921 (1985).
9. For the computations, the nearest-neighbor interatomic distances and bond angles were taken from the X-ray crystallographic data of structurally related compounds.
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