SYNTHESIS AND DNA BINDING PROPERTIES OF A PURINE ANALOGUE OF BISBENZIMIDE

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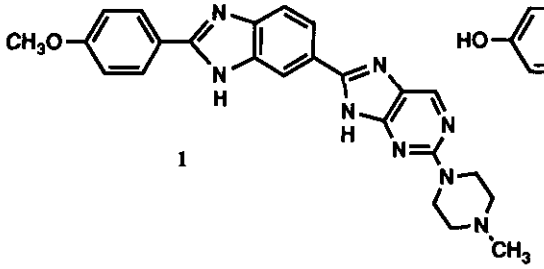
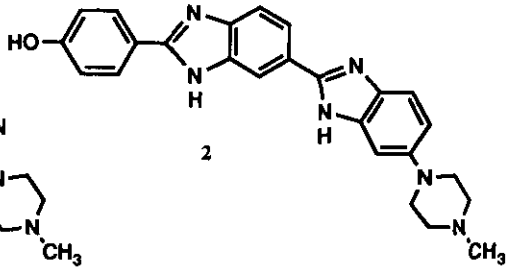
Abstract - The synthesis of a purine containing analogue (**1**) of bisbenzimide (**2**) and its DNA binding properties are described. Analogue **1** is found to have increased tolerance for binding to GC sites implying the formation of the new hydrogen bonds between guanine-2-NH_2 in the minor groove of DNA and the concave purine N3 atom of **1**.

There is increasing interest in the development of low molecular weight substances which would bind to predetermined DNA sequences.[1] Such chemical agents have potential applications as reagents for molecular biology, probes of DNA structures[2] and as chemotherapeutic agents.[3] There is particular initiative to develop ligands that can recognize DNA sequences rich in GC base pairs because these sequences are commonly found in the genomes of mammals including the 5' flank of certain oncogenes,[4] such as the sp 1 binding site of the ras proto-oncogene.[5] Consequently, there is a concerted effort in the design of low molecular weight molecules that would recognize $(GC)_n$ and mixed $(GC/AT)_n$ sequences.[1,2] Bisbenzimide, or Hoechst 33258 (**2**), readily penetrates into cells and is known to bind to B-DNA, and for these reasons has been widely used as a fluorescent cytological stain for cells.[6]



1



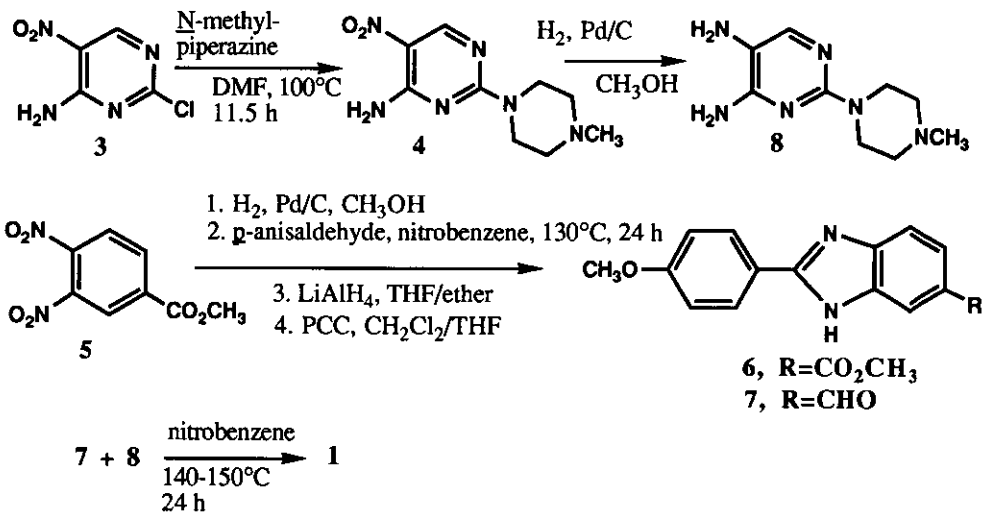
2

Several studies including absorption spectroscopy and DNA footprinting have established that bisbenzimide binds preferentially to $(AT)_4$ sequences of DNA.[7] The expected minor groove and AT specificity at such sites was recently confirmed by X-ray diffraction[8] and ^{1}H-nmr[9] studies of a 1:1 complex of **2**:d[CGCGAATTCGCG]$_2$. The sequence binding selectivity of bisbenzimide is a net result of specific van der Waals, hydrogen bonding and electrostatic interactions between the ligand and DNA.[8,9] Structural analysis of the requirements for the molecular recognition of **2** to DNA from these studies indicates that the binding preference for $(AT)_4$ is a result of unfavorable steric interactions between the concave aromatic -CH- with G-2-NH_2 which precludes the binding of **2** to the GC sites. Therefore, by analogy to the lexitropsins,[1] Lown's group[10] and we have independently proposed that replacement of the concave aromatic -CH- group of one or more of the benzimidazole unit by a sterically less demanding group -N- such that the heterocycle is capable of accepting a hydrogen bond from G-2-NH_2 should alter the preference from binding to AT sites to permit $(GC)_n$ recognition in a predictable fashion.

In this study, as shown in Scheme 1, an analogue **1** of bisbenzimide is synthesized wherein one of the benzimidazole group is replaced by a purine moiety. Reaction of 4-amino-2-chloro-5-nitropyrimidine (**3**), which was prepared from 5-nitrouracil,[11] with N-methylpiperazine gave the desired product (**4**) in 59% yield after purification by column chromatography. Ester (**5**), prepared in 92% yield by heating 3,4-dinitrobenzoic acid with methanol and sulfuric acid, was reduced by catalytic hydrogenation over 5% Pd-C to give a diaminoester intermediate which was directly condensed with p-anisaldehyde in the presence nitrobenzene at 130°C for 29 hours to afford benzimidazole (**6**) in 37% yield.[10] Reduction of **6** with refluxing lithium aluminum hydride in dry ether/THF (1:1) gave an alcohol intermediate which was then oxidized to the aldehyde (**7**) using PCC in THF/CH_2Cl_2. Compound (**7**) was immediately condensed with the diamino-intermediate (**8**), derived from catalytic hydrogenation (room temperature, atmospheric pressure, 5% Pd-C, methanol), in the presence of nitrobenzene (140-150°C, 24 hours) to give the desired analogue (**1**)[12] in an overall yield of 9% from **6** after purification by silica gel chromatography (5 to 10% methanol

in chloroform with 1% increments after every 100 ml of solvent, and then recrystallized from CH_2Cl_2).

Scheme 1



The apparent DNA binding constants of analogue (**1**) and (**2**) to calf thymus DNA, poly(dG-dC) and poly (dA-dT) were determined by measuring the decrease of ethidium bromide fluorescence as a function of the added concentrations of the ligand;[13] K_{app} of **1** for calf thymus DNA, poly(dG-dC) and poly (dA-dT) are 8.8 x 10^5, 5.2 x 10^5 and 5.1 x 10^5 M^{-1}, respectively, while K_{app} of **2** for the above DNAs are 1.6 x 10^7, 1.1 x 10^7 and 1.7 x 10^7 M^{-1}, respectively. Analogue (**1**) is assumed to be similar to **2** and binds to the minor groove of DNA. The apparent DNA binding constants of **1** are consistently lower to those of **2**; however this data show that the former agent has an improved tolerance, and hence recognition, for GC sites when compared to **1**. This increase in binding to GC sites could be a result of the acceptance of a hydrogen bond from G-2-NH_2 by the concave purine-N3 of analogue (**1**). This mode of interaction between the ligand and DNA is similar to that proposed for **2** by Lown and coworkers.[14]

Force field (MM2)[15] calculations predicted a minimum conformation of **1** wherein the aromatic system is in a common plane and the dihedral angle between the purine and

the mean plane of the piperazine ring is about 10°. Thus the piperazine terminus, also shown in the X-ray studies of the **2**:d[CGCGAATTCGCG]$_2$ complex,[8c] could bind to GC sites wherein the minor groove is wider than that for AT sites.[16] The molecule (**1**) adopts a crescent like conformation which would allow it to fit snugly into the minor groove of DNA. The distance between the two concave -NH- groups of **1** is 4.4Å which would allow them to hydrogen bond to the electron rich groups (pyrimidine-O2 and purine-N3) of contiguous base pairs which are on average about 4.8Å for B-DNA. Further investigations into the DNA sequence binding selectivity of **1** determined by footprinting studies are in the progress and the results will be reported in due course.

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REFERENCES AND NOTES

1. (a) D. E. Thurston and A. S. Thompson, Chem. in Britain, Aug. 1990, 767; (b) K. Krowicki, M. Lee, J. A. Hartley, B. Ward, K, Kissinger, J. C. Dabrowiak, and J. W. Lown, 'Structure and Expression,' Vol. 2, eds. by R. H. Sarma and M. H. Sarma, Adenine Press, Albany, New York, 1988, p. 251; (c) L. H. Hurley, J. Med. Chem., 1989, **32**, 2027; (d) L. H. Hurley and F. L. Boyd, Annual Rep. Med. Chem., 1987, **22**, 259.
2. (a) P. B. Dervan, Science, 1986, **232**, 464; (b) P. B. Dervan, 'Molecular Mechanism of Carcinogenic and Antitumor Activity,' eds. by C. Chagas and B. Pullman, Adenine Press, Albany, New York, 1986, p. 25.
3. (a) J. W. Lown, Anti-Cancer Drug Design, 1988, **3**, 25 and references given therein.
4. (a) W. B. Mattes, J. A. Hartley, K. W. Kohn, and D. W. Matheson, Carcinogenesis, 1988, **9**, 2065; (b) J. A. Hartley, J. W. Lown, W. B. Mattes, and K. W. Kohn, Acta Oncologica, 1988, **27**, 503.

5. S. Ischii, J. T. Kadonaga, R. Tjian, J. N. Brady, G. T. Merlino, and I. Patsan, Science, 1986, **232**, 1410.

6. H. D. Preisler, Cancer Treat. Rep., 1978, **62**, 1393.

7. K. Harshman and P. B. Dervan, Nucl. Acids Res., 1985, **13**, 4825.

8. (a) M. -K. Teng, N. Ulsman, C. A. Frederick, and A. H. -J. Wang, Nucl. Acids Res., 1988, **16**, 2671; (b) M. A. A. F. de C. T. Carrondo, M. Coll, J. Aymami, A. H. -J. Wang, G. A. van der Marel, J. H. vand Boom, and A. Rich, Biochem., 1989, **28**, 7849; (c) P. E. Pjura, K. Grzeskowiak,and R. E. Dickerson, J. Mol. Biol., 1987, **197**, 257.

9. J. A. Parkinson, J. Barber, K. T. Douglas, J. Rosamond, and D. Sharpless, Biochem., 1990, **29**, 10181.

10. (a) B. Yadagiri and J. W. Lown, Syn. Commun., 1990, **20**, 955; (b) B. Yadagiri and J. W. Lown, ibid., 1990, **20**, 175.

11. D. J. Brown, J. Appl. Chem.,1952, **2**, 239.

12 The products are characterized by ir, ^{1}H-nmr (300 MHz), ms (FAB) and accurate mass measurements.

13. A. R. Morgan, J. S. Lee, D. E. Pulleyblank, N. L. Murray, and D. H. Evans, Nucl. Acids Res., 1979, **7**, 545.

14. (a) S. Kumar, B. Yadagiri, J. Zimmerman, and J. W. Lown, J. Biomol. Struct. Dyn., 1990, **8**, 331; (b) B. Yadagiri, K. E. Rao, R. G. Shea, and J. W. Lown, Chem. Res. Toxicol., 1990, **3**, 268.

15. The MM2 energy minimization iterations were performed using the Chem 3Dplus program (Cambridge Scientific, MA) on a Mac IIci platform.

16. A. V. Fratini, M. I. Kopka, H. R. Drew, and R. E. Dickerson, J. Biol.Chem., 1983, **257**, 14686.

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