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SYNTHESIS AND DNA BINDING PROPERTIES OF AN AMIDINE-LINKED AND PHENYL-CONTAINING ANALOGUE OF DISTAMYCIN A

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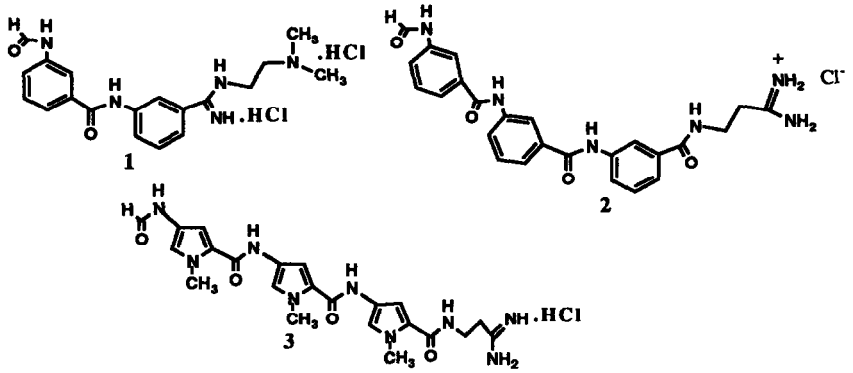
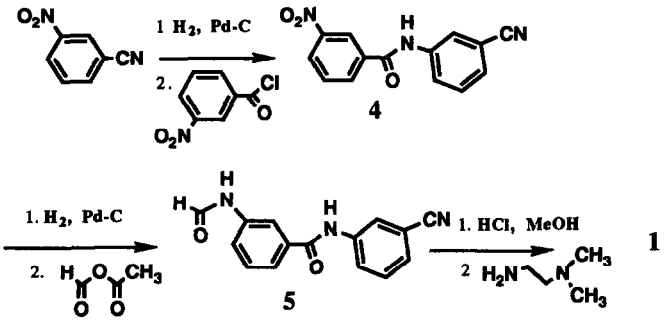
Abstract: The synthesis of a novel amidine-linked analogue **1** of the phenyl-containing congener **2** of distamycin A and its DNA binding properties are described. The amidine group in **1** improves its water solubility while retaining the minor groove and AT sequence binding selectivety.

Interest in the development of sequence-specific DNA binding agents for chemotherapy,[1] for use as structural probes of DNA[1] and as artificial restriction enzymes[2] has blossomed in recent years. A group of naturally occurring oligopeptides including distamycin A **3**[3] exhibit specific binding to the minor groove and to $(AT)_5$ sequences as determined from x-ray[4], footprinting analysis of restriction DNA fragments, [2a,5] and ^{1}H-NMR studies.[6] The firm and site-specific binding of these sequence reading oligopeptides to DNA is a net result of specific van der Waals, hydrogen bonding[7], and electrostatic interactions.[8] If the human genome is the target of sequence selective agents, the distinguishable sequence must have a binding site size of 15 to 16 base pairs.[2] Dervan[2,9] and others[10] have shown that by increasing the number of methylpyrrolecarboxamido moieties (i.e. increasing the number of amide NHs) the DNA binding site size increases by N+1 contiguous base pairs. There are, however, a number of problems associated with the development of polypyrrolecarboxamide analogues wherein N is more than 3, such as the 'phasing problem'[1a,11] and low water solubility. Consequently, there is a concerted effort in the development of distamycin analogues that have improved water solubility.[12] Our approach to overcome the solubility problem involves the incorporation of readily accessible ionic groups into the analogues. A phenyl-containing analogue **2** of



distamycin recognizes AT rich sequences of DNA through the minor groove,[13] and it serves as a model for the current study. To our knowledge this communication presents the first synthesis and DNA binding properties of an analogue, **1**, of distamycin wherein one of the amide groups is replaced with its isosteric, amidine moiety.[14] The C-terminus in **1** contains a dimethylaminoethyl moiety which, like the propioamidine group of **2** and **3**, recognizes an AT base pair.[5,9] At pH 7.4, analogue **1** would exist as a dication which should have good water solubility, and since the amidine moiety has similar size and shape to an amide, the crescent shape and thus the groove and DNA sequence selectivity of **1** should not be affected.

Scheme 1



The synthesis of analogue **1** as shown in scheme 1 starts with the catalytic hydrogenation of 3-nitrobenzonitrile followed by coupling with 3-nitrobenzoyl chloride to give amide **4** in 90 percent yield. Reduction of **4** (H_2, Pd/C methanol) gave an unstable amine intermediate which was coupled directly with a freshly prepared solution of acetic formic anhydride in the presence of a catalytic amount of

dimethylaminopyridine to afford formamide **5** in 46 percent yield. Finally, conversion of the nitrile moiety into the desired amidine group in **1** was achieved by the Pinner reaction wherein compound **5** was first treated with HCl(g)/dry methanol to give an imidate ester intermediate which was then reacted with N,N-dimethylethylenediamine to give **1**[15] in 17 percent after purification by silica gel column chromatography.

The apparent DNA binding constant, Kapp, to DNA was determined by the ethidium displacement assay,[16] and the values of Kapp for **1** for calf thymus DNA and T_4 DNA are 6.5 x 10^4 and 1.2 x 10^5 M^{-1}, respectively. The binding constant of **3** to calf thymus DNA is 7.7 x 10^5 M^{-1} under identical conditions. The major groove of T_4 DNA is occluded by α-glycosylation of the 5-hydroxymethyl group of cytosine residues, and so binding of **1** to this DNA indicates that the interaction must occur in the minor groove. The DNA sequence binding selectivity of **1** is confirmed by MPE footprinting[2] on the Hind III-EcoR I fragment of pBR322 which showed weak footprints at similar long AT rich sequences as **3**.[17] The lower affinity for DNA of **1** compared to **3** (and hence to **2**)[18] could be explained by the reduced number of amide groups and/or by a decrease in the number of aryl moieties, thus less van der Waals contacts in the former compound. Furthermore the lower DNA binding affinity could also be due to an increase in the curvature of the former molecule resulting from the replacement of the pyrrole moieties with benzene groups.[13]

The binding of **1** to AT rich sequences suggests that the concave amide and amidine NH can form hydrogen bonds to adenine-N3 or thymine-O2 on the floor of the minor groove. Thus, the concave aromatic hydrogen atoms of **1** would be in close proximity to adenine-2-H, and thereby preventing it from binding to GC sites due to steric interactions with the guanine-2-NH_2 groups.[1a,b] The positively charged dimethylammonium and amidinium moieties in **1** provide favorable electrostatic attraction to the negative electrostatic potential of the DNA.

In conclusion, amidines are found to be good isosteres of amide moieties in the class of DNA minor groove binders studied, and they can improve the water solubility while not affecting the DNA sequence binding selectivity of these compounds.

Synthesis of pyrrole-containing and amidine-linked analogues of **3** are in progress and will be reported in due course.

Acknowledgements

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17. MPE footprinting was performed on a 5'-end labeled Hind III-EcoR 1 fragment pBR322. The strongest footprint was observed in the sequence 5'-A(89)AATCTAACAAT(100) which was also the strongest footprint site for **2** in this fragment.
18. Based on the molar ellipticities of the induced CD bands of the ligand-calf thymus DNA complexes, the binding affinity of **2** is only slightly lower than that of **3**.