III).[16] The synthetic zincophorin methyl ester was identical with a sample prepared by esterification of natural zincophorin by spectroscopic (490-MHz ^{1}H NMR, IR), optical rotation,[13] and chromatographic criteria.

Acknowledgment. This work was supported by PHS Grant HL-28548. PHS Fellowships (Grant 5 F32 GM 10369) to R.E.Z. and (Grant 3 F32 GM 10576) to H.G.S. are gratefully acknowledged. We thank Dr. J. P. Poyser of ICI Pharmaceuticals Division for a generous gift of natural zincophorin zinc salt. NMR spectra were obtained through the auspices of the Northeast Regional NSF/NMR Facility at Yale University, which was supported by NSF Chemistry Division Grant CHE 7916214.

(16) While we cannot rule out the presence of trace quantities of *Z* olefin, the *E* isomer was the only one isolated.

Alteration of the Sequence Specificity of Distamycin on DNA by Replacement of an *N*-Methylpyrrolecarboxamide with Pyridine-2-carboxamide

Warren S. Wade and Peter B. Dervan*

Arnold and Mabel Beckman Laboratories of Chemical Synthesis, California Institute of Technology Pasadena, California 91125

Received September 8, 1986

Although there has been some encouraging success with regard to building synthetic molecules that bind large sequences of pure A,T-rich double-helical DNA, there has not been corresponding success in the development of well-understood G,C recognition.[1,2] Progress in this area is an important component in an overall strategy of coupling G,C words and A,T words into sentences that uniquely recognize long sequences of right-handed DNA.[1-3]

The natural products netropsin and distamycin are DNA groove-binding molecules that bind sites of four or five successive A,T base pairs and in general avoid regions with G,C pairs[1,3,4] (Figure 1). The recent x-ray structure of a netropsin–DNA cocrystal suggests how base sequence information retrieval is accomplished.[5] The crescent-shaped netropsin sits in the middle of the minor groove of a pure A,T sequence with the aromatic hydrogens of the *N*-methylpyrrole rings set too deep in the groove to allow room for the guanine NH_2 of a G,C pair.[5] We have been making systematic substitutions on the tris(*N*-methylpyrrolecarboxamide) framework (D) of distamycin to search for altered base pair specificity.

We report that replacement of a terminal *N*-methylpyrrolecarboxamide unit of distamycin with pyridine-2-carboxamide affords a new DNA groove-binding molecule, pyridine-2-carboxamide-netropsin (2-PyN), that now accepts mixed (G,C)-(A,T) base pairs *in preference* to pure A,T stretches of DNA. The design is based on placement of the lone pair of electrons of the pyridine nitrogen proximal to the NH_2 of guanine to afford a hydrogen bond for G,C base pair recognition. Based on this model, our expectations were that 2-PyN should bind the mixed four base pair sequence 5′-(G,C)(A,T)$_3$-3′ with an orientation of the pyridinecarboxamide to the G,C side.

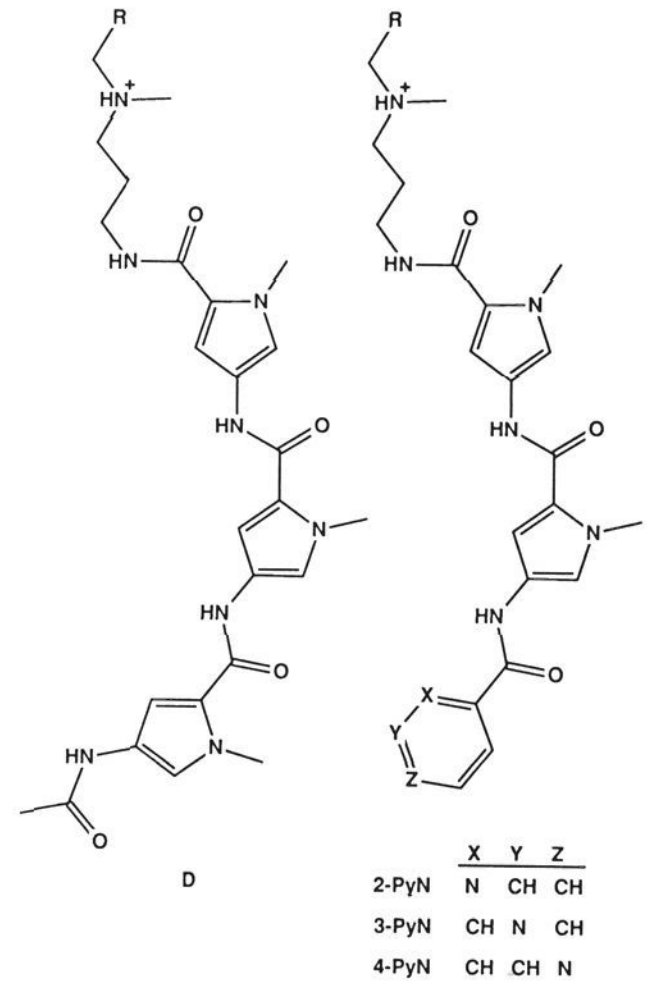

Figure 1. R = H for D and 2-, 3-, and 4-PyN. R = $(CH_2)_2NHCOCH_2N(CH_2CO_2H)CH_2CH_2N(CH_2CO_2H)_2$ for ED and 2-, 3-, and 4-PyNE.

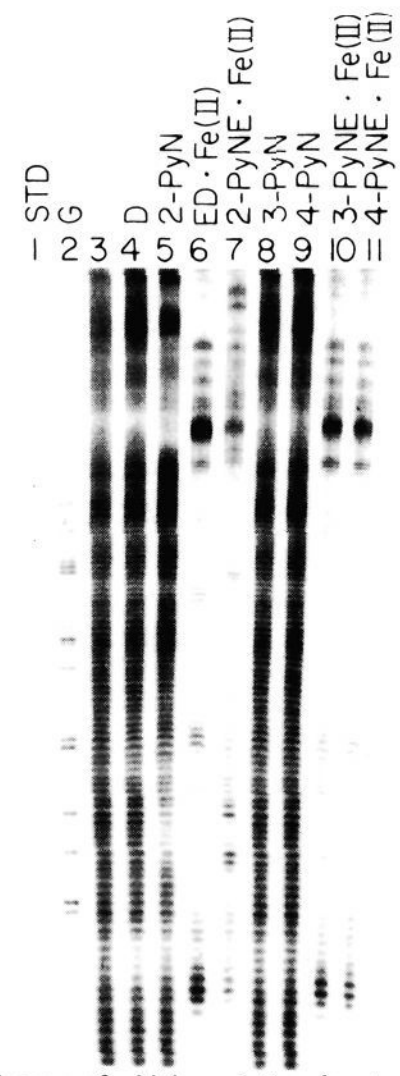

Figure 2. Autoradiogram of a high-resolution denaturing polyacrylamide gel, ^{32}P 5′ end-labeled DNA. Lane 1, intact DNA; lane 2, Maxam–Gilbert chemical sequencing G reactions; lanes 3–5, 8, and 9, footprinting lanes with MPE·Fe(II) at 5 μM; lane 3, MPE·Fe(II) control; lane 4, D at 1 μM concentration; lane 5, 2-PyN at 10 μM; lane 6, ED·Fe(II) at 2.5 μM; lane 7, 2-PyNE·Fe(II) at 50 μM; lane 8, 3-PyN at 4 μM; lane 9, 4-PyN at 4 μM; lane 10, 3-PyNE-Fe(II) at 10 μM; lane 11, 4-PyNE·Fe(II) at 7 μM.

(1) For a review: Dervan, P. B. *Science* (*Washington, D.C*) **1986**, *232*, 464–471.

(2) For an example of a synthetic hybrid intercalator-groove binders that mix G,C/A,T specificity, see: (a) Kristrova, M. A.; Moroshkina, E. B.; Gliben, E. N.; Frisman, E. V. *Mol. Biol.* **1984**, *18*, 950. (b) Dervan, P. B.; Sluka, J. *Proceedings of the 3rd international Kyoto Conference on New Aspects of Organic Chemistry* in *New Synthetic Methodology and Functionally Interesting Compounds* Kodamska LTD: Tokyo, 1986, p 307.

(3) (a) Goodsell, D.; Dickerson, R. E. *J. Med. Chem.* **1986**, *29*, 727. Lown, J. W.; Krowicki, K.; Bhat, U. G.; Skorobogaty, A.; Ward, B.; Dabrowiak, J. C. *Biochemistry* **1986**, *25*, 7408.

(4) For a review: Zimmer, C.; Wöhnert, U. *Prog. Biophys. Mol. Biol.* **1986**, *47*, 31–112.

(5) (a) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *Proc. Natl. Acad. Sci., U.S.A.* **1985**, *82*, 1376–1380. (b) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *J. Mol. Biol.* **1985**, *183*, 553–563.

0002-7863/87/1509-1574$01.50/0

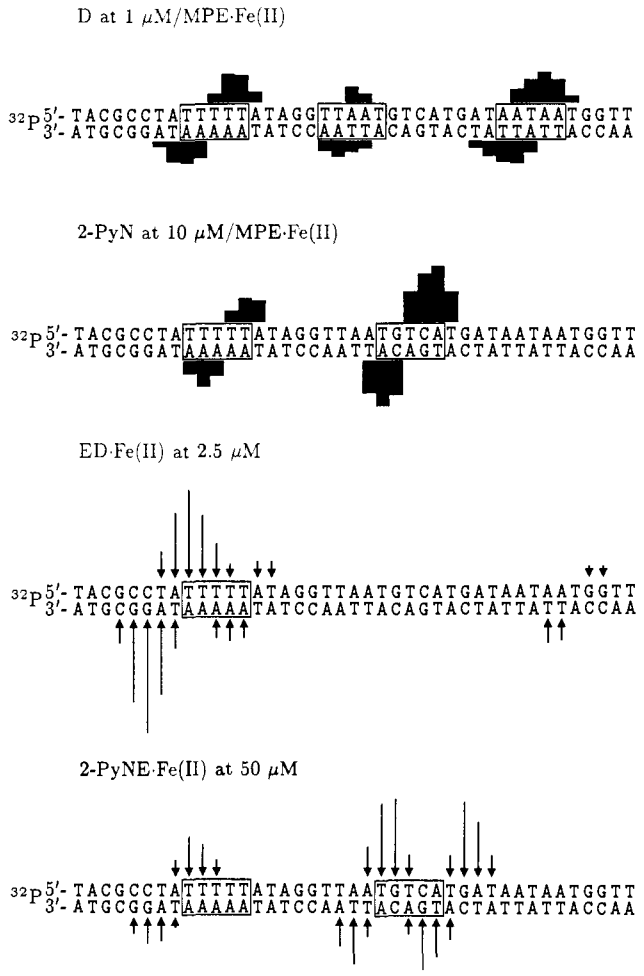

Figure 3. Base pairs 4337 to 4295 of plasmid pBR322. Top strand (5′-3′) data from Figure 2. Bottom strand (3-5) data not shown. (Top half) MPE·Fe(II) footprints of D and 2-PyN. Bar heights of histogram represent protection from cleavage by MPE·Fe(II). (Bottom half) Affinity cleavage patterns of ED·Fe(II) and 2-PyNE. Length of arrows represents intensity of cleavage. Boxes are binding sites assigned on the basis of models presented in ref 1.

Comparison of equilibrium binding sites of the natural product analogue D and the synthetic 2-PyN was determined to nucleotide resolution by MPE·Fe(II) footprinting[1,6] and by the affinity cleaving method[1,7] using EDTA–distamycin (ED) and pyridine-2-carboxamide–netropsin–EDTA (2-PyNE). Sequencing analysis was carried out on a 517 *EcoRI/Rsal* fragment of plasmid pBR322[7c] (Figures 2 and 3). MPE·Fe(II) footprinting reveals that the tripeptide D binds three sites (5′–3′)TTTTT, TTAAT, and AATAA. 2-PyN binds only the TTTTT site and a new site 5′-TGTCA-3′ (Figure 3). Affinity cleaving reveals that ED·Fe(II) binds one strong site TTTTT. 2-PyNE·Fe(II) binds the same TTTTT site weakly and one new strong site 5′-TGTCA-3′ (Figure 3). Further up on the 517 bp restriction fragment, densitometric analysis reveals another strong site, 5′-TGTCT-3′, and two weak sites, 5′-TGTAT-3′ for 2-PyNE. These weak sites show no strong orientation preference. With regard to relative binding affinities, DE·Fe(II) and 2-PyNE·Fe(II) cleave their respective strong sites with comparable efficiency at 2.5 and 50 μM, respectively. D and 2-PyN afford comparable footprints at 0.4 and 10 μM, respectively.

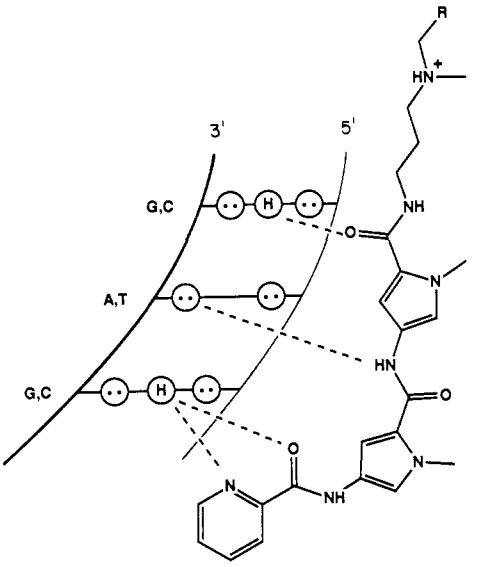

Figure 4. Hypothetical model for 2-PyN binding to 5′-GTC-3′. Circles with two dots represent lone pairs of electrons on N3 of adenine and guanine and on O2 of cytidine and thymine. Circles containing an H represent the N2 hydrogen of guanine. Dashed lines are proposed hydrogen bonds.

As controls, two isomers of 2-PyN were synthesized. From footprinting (Figure 2, lanes 8, 9) and affinity cleaving (Figure 2, lanes 10, 11) we find that pyridine-3-carboxamide–netropsin (3-PyN and 3-PyNE) and pyridine-4-carboxamide–netropsin (4-PyN and 4-PyNE) showed binding specificity similar to the distamycin analogue D and *not* 2-PyN. This suggests that the position of the nitrogen in pyridine-2-carboxamide is a key feature for the recognition of G,C base pairs.

Although the apparent strong site 5′-TGTCA-3′ for 2-PyN from footprinting and affinity cleaving data might be explained by two four base pair sites overlapping with opposite orientation preference, 5′-TGTC-3′ and 5′-TGAC-3′, some observations remain unexplained. The initial working hypothesis predicts 5′-TGTAT-3′ should be a strong binding site with the picolinamide oriented to the G,C side. Yet we observe that 5′-TGTAT-3′ is a weak binding site with no orientation preference. Moreover, the strong binding sites for 2-PyN contain a central 5′-GTC-3 sequence. This specificity of 2-PyN for G,C base pairs in the first *and third* base pair positions demands a revision of the original model. It is known that picolinamide chelates divalent metals with both the nitrogen and carbonyl functional groups.[8] Therefore, one possible explanation for G,C recognition in the first base pair position is the formation of a three-center hydrogen bond between the NH_2 of guanine and the lone pairs of electrons on the nitrogen of pyridine and the carbonyl of the picolinamide. Rotation of the pyridine carbonyl toward the floor of the helix would shift the position of the bis(*N*-methylpyrroles) of 2-PyN in the minor groove of DNA such that one could imagine that the carbonyl on the *terminal* pyrrolecarboxamide of 2-PyN also rotates inward to hydrogen bond to the NH_2 of a guanine located in the *third* base pair position (Figure 4). If this is true, this suggests that the carbonyl of pyrrolecarboxamides as well as pyridine-2-carboxamide could be used as a G,C recognition feature if properly aligned in the minor groove of DNA.[9]

Acknowledgment. We are grateful to the National Institutes of Health for research support (GM-27681) and to the National Science Foundation for a predoctoral fellowship to W.S.W.

Registry No. 2-PyN, 106500-21-4.

(6) (a) Van Dyke, M. W.; Hertzberg, R. P.; Dervan, P. B. *Proc. Natl. Acad. Sci., U.S.A.* **1982**, *79*, 5470. (b) Van Dyke, N. W.; Dervan, P. B. *Nucl. Acids Res.* **1983**, *11*, 5555. (c) Harshman, K.; Dervan, P. B. *Nucl. Acids Res.* **1985**, *13*, 4825.

(7) (a) Taylor, J. S.; Schultz, P. G.; Dervan, P. B. *Tetrahedron* **1984**, *40*, 457. (b) Schultz, P. G.; Dervan, P. B. *J. Biomol. Struct. Dynam.* **1984**, *1*, 1133. (c) Youngquist, R. S.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2565.

(8) (a) Brown, D. H.; MacSween, D. R.; Mercer, M.; Sharp, D. W. A. *J. Chem. Soc., A* **1971**, 1574. (b) Sekizaki, M.; Yamasaki, K. *Spectrochim. Acta, Part A* **1969**, *25A*, 475–485.

(9) We cannot rule out the possibility that the local DNA conformation of the bound GTC site may match more favorably the structure of 2-PyN.