

ductive polymers. We are currently developing a mechanistic model for this enhanced order. We are also exploring the extent to which other types of polymers can be ordered via the template method. Template synthesis may prove to be a general procedure for obtaining highly ordered nanoscopic polymeric fibrils.

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Structure of Pyrrolosine: A Novel Inhibitor of RNA Synthesis, from the Actinomycete *Streptomyces albus*

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During the last 20 years, our knowledge of the biosynthesis of RNA has expanded greatly, due largely to the discovery and use of various inhibitors of RNA synthesis. Several inhibitors of RNA synthesis have been used as anticancer, antiviral, and antibacterial agents.¹ However, there are very few, if any, specific inhibitors of RNA synthesis without undesirable side effects on cellular functions. We have searched for microbial products capable of arresting the development of starfish embryos specifically at the early blastula stage, when RNA synthesis becomes active, without interfering with cell division at the morula stage or with blastula formation, which are independent of RNA synthesis.² We encountered a culture broth conditioned by the actinomycete *Streptomyces albus* A282, which showed marked activity in the starfish assay and which led us to isolate the active component. Now we report the structure elucidation of the active constituent, pyrrolosine.

The culture broth filtrate (17 L) was passed through a column of Diaion HP-20.³ The absorbed material was eluted with MeOH. The eluate was subjected to low-pressure column chromatography on silanized silica gel (H₂O/5% MeOH), then alumina (70% MeOH), and finally Robar RP-8⁴ (10% MeOH), to give pyrrolosine (1) (246 mg) as colorless plates, $[\theta]_{240}^{2300} +2300^{\circ}$ (c 0.0014, MeOH). Pyrrolosine (10 μ g/mL) inhibited RNA synthesis of starfish (*Asterina pectinifera*) embryos at blastulation and halted embryonic development just after completion of blastulation. Furthermore, pyrrolosine inhibited cell growth of transformed human fibroblast KMST-6 cells and mouse mammary carcinoma FM3A cells at IC₅₀ 13 and 27 ng/mL, respectively. The molecular formula of C₁₁H₁₃N₃O₅ was established by an MH⁺ ion peak at m/z 268 in the FAB mass spectrum and by elemental analysis. Pyrrolosine showed UV absorption maxima [$\lambda_{\max}^{\text{H}_2\text{O}}$ (pH 7.0) 220 (ϵ 10000), 242 (4400), 272 nm (4200); (pH 1.5) 225 (5800), 268 (7500 sh), 276 (7800), 285 nm (5200 sh)]. ¹³C NMR spectra⁵ revealed 11 carbon signals, five of which are assignable to a ribosyl unit. Detailed 270- and 400-MHz NMR analyses⁶ including H-H

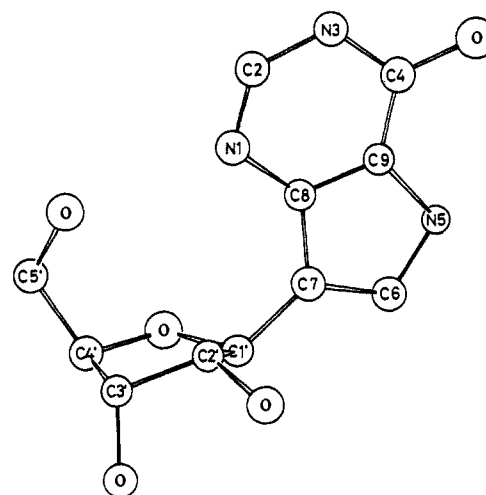


Figure 1. Computer-generated perspective drawing of pyrrolosine. Hydrogens are omitted for clarity, and no absolute configuration is implied.

COSY⁷ and C-H COSY experiments⁸ (¹J and long range) suggested strongly that the structure of pyrrolosine is 7-(β -ribofuranosyl)-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidine. This C-nucleoside analogue of inosine was reported as having been synthesized and was designated 9-deazainosine by Lim et al.⁹ However, HPLC analysis (Radialpak NVC18,¹⁰ 0.8 \times 10 cm; 20% MeOH) revealed that the retention volume of 9-deazainosine¹¹ was 2.9 mL whereas that of pyrrolosine was 3.8 mL. Furthermore, the UV spectrum¹² of 9-deazainosine was different from that of pyrrolosine. Therefore, the structure of pyrrolosine was deduced by single-crystal X-ray diffraction. Repeated crystallization from a mixture of MeOH and ethyl acetate afforded colorless hexagonal plates (methanol monosolvate; mp 105–110 $^{\circ}$ C) belonging to the orthorhombic space group $P2_12_12_1$ with $a = 9.994$ (1) \AA , $b = 19.441$ (1) \AA , $c = 7.026$ (1) \AA , and $Z = 4$. Intensities were measured in the ω -1.33 θ scan mode on an Enraf-Nonius CAD4 diffractometer, using graphite-monochromated Cu K α radiation ($\lambda = 1.5418$ \AA). Correction was made for Lorentz and polarization factors but not for absorption. Of 1655 independent reflections measured in a range of $2^{\circ} < 2\theta < 149^{\circ}$, 31 reflections with $|F_o| < 1.0\sigma(|F_o|)$ were considered unobserved.

The structure was determined by direct methods coupled with the MULTAN 11/82 program.¹³ Its parameters were refined by the full-matrix least-squares method using anisotropic temperature factors for non-hydrogen atoms. The final R value for 1624 reflections used in the refinement process was 0.065. A computer-generated perspective drawing of the final X-ray model of pyrrolosine (1) is given in Figure 1. The X-ray experiment did not define the absolute configuration, so the enantiomer shown is arbitrary. The structure of pyrrolosine is the same as that proposed for synthetic 9-deazainosine.

Synthetic "9-deazainosine" was shown to be toxic in vitro to protozoan cells but not to mammalian cells.¹⁴ Starfish embryos developed normally in the presence of the reputed "9-deazainosine"

(6) ¹H NMR (D₂O, 270 MHz): δ 3.84 (2 H, br AB q, $J = 14.0$ Hz, H-5'), 4.22 (1 H, br m, H-4'), 4.32 (1 H, br d, $J = 7.2$ Hz, H-3'), 4.52 (1 H, dd, $J = 7.4$ and 7.2 Hz, H-2'), 5.04 (1 H, d, $J = 7.4$ Hz, H-1'), 8.02 (1 H, s, H-6), 8.15 (1 H, s, H-2).

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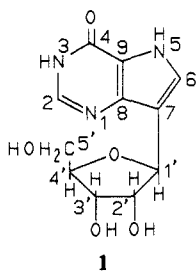
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(5) ¹³C NMR (D₂O, 67.8 MHz): δ 62.9 (t, C-5'), 72.7 (d, C-3'), 75.6 (d, C-2'), 76.4 (d, C-1'), 86.2 (d, C-4'), 119.3 (s), 135.3 (s), 147.2 (s), 149.4 (d, C-6), 150.6 (s, C-4), 153.1 (d, C-2).



(200 $\mu\text{g}/\text{mL}$). Synthetic "9-deazainosine" was active in vivo against several pathogenic hemoflagellate species including one affecting AIDS patients.¹⁵ The remarkable specificity in the pharmacological action of the reported "9-deazainosine"⁹ requires prompt reinvestigation of its chemical structure.

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Supplementary Material Available: Tables of final atomic coordinates, thermal parameters, bond distances, and bond angles for **1** (4 pages); table of observed and calculated structure factors for **1** (7 pages). Ordering information is given on any current masthead page.

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Site-Specific Atom Transfer from DNA to a Bound Ligand Defines the Geometry of a DNA-Calicheamicin γ_1^1 Complex

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Among the emerging class of diynene antitumor antibiotics, calicheamicin γ_1^1 (CLM) shows the greatest sequence selectivity in its cleavages of double-stranded DNA.¹ We describe in this paper atom-transfer studies that both establish the identity of one of the two principal DNA-bound hydrogens abstracted by the proposed thiol-activated form of the drug (**1** in Scheme I)⁵ and

Scheme I

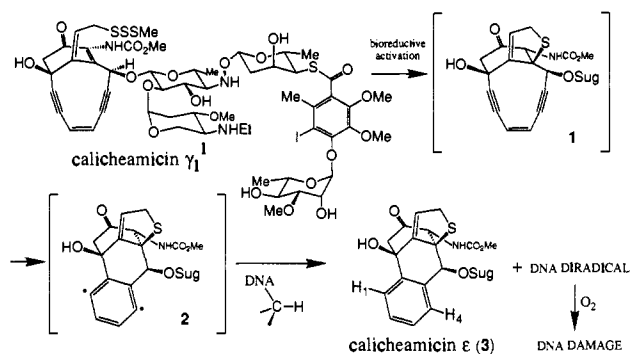
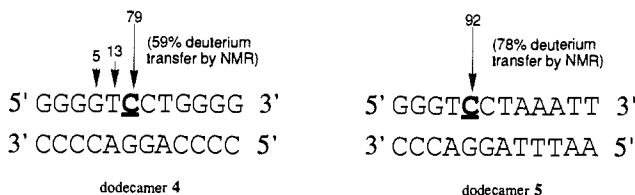


Chart I



quantify the efficiency of the overall process. As the transfer is specific to only one of the acetylenes in CLM, it is possible to define the major orientation of the drug in the minor groove at a particular cleavage site. These preliminary findings illustrate the utility of the site-specific atom-transfer method to derive detailed structural information, and they point to further experimental steps to be taken toward a deeper understanding of the underlying issues of molecular recognition and mechanism posed by the reactions of CLM with DNA.

Atom-transfer experiments are preceded between tritiated thymidine residues in λ DNA and the neocarzinostatin chromophore (NCS-chrom)⁶ and for reaction of both the NCS-chrom⁷ and CLM⁸ in deuterated media in the presence and absence of calf thymus DNA. However, in none of these experiments was it possible to correlate the precise location of the abstracted hydrogens in the reduced drug to specific loci on the DNA strand from which they were transferred. While for NCS-chrom the identities of the DNA hydrogens removed in major and minor reaction processes are well studied, for CLM it has only been possible to infer these sites on the basis of electrophoretic mobilities of cleavage fragments.^{1,9} To secure experimentally the suspected transfer of a deoxyribose 5'-hydrogen from DNA to CLM and to establish unambiguously the orientation of the calicheamicin γ_1^1 aglycon in the minor groove at a specific cleavage site, two site specifically labeled duplex oligonucleotides **4** and **5** (Chart I) were synthesized and purified by HPLC.¹⁰ Each dodecamer contained an internal 5'-TCCT/AGGA in which the 5'-cytidine (C) carbon bore two deuteriums (C^2H_2).¹¹ Reaction, therefore, of CLM with these oligonucleotides in the presence of a thiol would be anticipated to result in transfer of a single deuterium to the

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