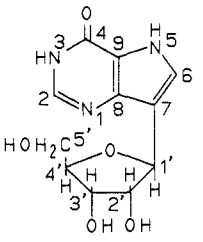

1

(200 μg/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.

(15) Marr, J. J.; Berens, R. L.; Cohn, N. K.; Nelson, D. J.; Klein, R. S. *Antimicrob. Agents Chemother.* **1984**, *25*, 292-295.

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

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Among the emerging class of diynene antitumor antibiotics, calicheamicin γ_1^I (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

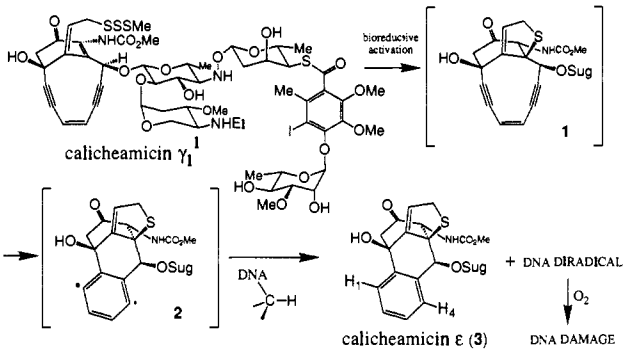
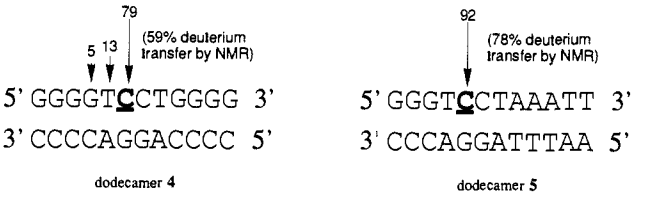


Chart I



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 precedented between tritiated thymidine residues in λ DNA and the neocarzinostatin chromophore (NCS-chrom)[6] and for reaction of both the NCS-chrom[7] and CLM[8] in deuteriated 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^I 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

(1) Zein, N.; Sinha, A. M.; McGahren, W. J.; Ellestad, G. A. *Science* **1988**, *240*, 1198-1201. Compare the sequence selectivities of the neocarzinostatin chromophore,[2] esperamicin A,[3] and dynemicin A.[4]

(2) Goldberg, I. H. In *Mechanisms of DNA Damage and Repair*; Simic, M. G., Grossman, L., Upton, A. C., Eds.; Plenum: New York, 1987; pp 231-244.

(3) Long, B. H.; Golik, S.; Forenza, S.; Ward, B.; Rehfuss, R.; Dabrowiak, J. C.; Catino, J. J.; Musial, S. T.; Brookshire, K. W.; Doyle, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2-6. Sugiura, Y.; Uesawa, Y.; Takahashi, Y.; Kuwahara, J.; Golik, J.; Doyle, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7672-7676.

(4) Sugiura, Y.; Shiraki, T.; Konishi, M.; Oki, T. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3831-3835.

(5) Lee, M. D.; Dunne, T. S.; Siegel, M. M.; Chang, C. C.; Morton, G. O.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3464-3466. Lee, M. D.; Dunne, T. S.; Chang, C. C.; Ellestad, G. A.; Siegel, M. M.; Morton, G. O.; McGahren, W. J.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3466-3468. De Voss, J. J.; Hangeland, J. J.; Townsend, C. A. *J. Am. Chem. Soc.* **1990**, *112*, 4554-4556.

(6) Kappen, L. S.; Goldberg, I. H. *Nucleic Acids Res.* **1985**, *13*, 1637-1648.

(7) Chin, D.-H.; Zeng, C.-h; Costello, C. E.; Goldberg, I. H. *Biochemistry* **1988**, *27*, 8106-8114.

(8) Zein, N.; McGahren, W. J.; Morton, G. O.; Ashcroft, J.; Ellestad, G. A. *J. Am. Chem. Soc.* **1989**, *111*, 6888-6890.

(9) Zein, N.; Poncin, M.; Nilakantan, R.; Ellestad, G. A. *Science* **1989**, *244*, 697-699.

(10) The syntheses of [5'-$^2H_2$]DMT-d(cytidine)(Bz)cyanoethyl phosphoramidite will be described elsewhere. The synthesis and purification of dodecamers **4** and **5** were carried out by conventional methods and will be reported in a full paper.

(11) Earlier experiments to investigate the sequence selectivity of DNA cleavage by CLM had shown TCCT sites to be particularly favored for cutting.[1]

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putative 1,4-diyl intermediate **2**. Presuming further a nonrandom orientation of the activated **1** at the TCCT cleavage sites of these oligonucleotides, deuterium transfer to the calicheamicin ϵ (**3**) product should be site specific at C-1 or C-4 and assayable by ^{1}H NMR to reveal the general organization of the drug/DNA complex.

A control experiment was carried out first with unlabeled dodecamer **4** in a deuteriated medium as described previously.[9] No deuterium was observed to be incorporated, and hence, **2** abstracted hydrogens solely from nonexchangeable positions of the DNA dodecamer, consistent with analogous experiments carried out earlier with sonicated calf thymus DNA.[8] Next, the specifically labeled dodecamers **4** and **5** were incubated in the presence of CLM in a nondeuteriated medium.[8,9] Integration of the aromatic proton signals in the NMR spectrum of calicheamicin ϵ (**3**) from both experiments indicated that deuterium was transferred *only* to the C-4 position of the newly formed aromatic ring (see Scheme I).

However, inspection of the ^{1}H NMR spectra revealed that the deuterium incorporations from the isotope-transfer experiments were not complete (62 ± 5% from dodecamer **4**, 82 ± 5% from dodecamer **5**[12]). Scanning densitometry of high-resolution sequencing gels provided the relative proportions of the cleavage products from dodecamers **4** and **5**, as shown in Chart I. Cleavage at the expected cytidine in dodecamer **4** amounted to 79 ± 3% of the observed products and 92 ± 3% in dodecamer **5**. To test the possibility that a kinetic isotope effect in the 5′-deuterium abstraction step might result in isotope-induced branching[13] or solvent abstraction, two further experiments were conducted. With respect to the latter possibility, dodecamer **5** was incubated as above but in a deuteriated medium. The extent of deuterium transfer was unchanged within experimental error (77 ± 5%[12]). With regard to the former, deuterium-labeled and unlabeled dodecamer **4** was 5′-^{32}P-end labeled and treated with CLM in parallel incubations. The fragmentation products were analyzed on sequencing gels where, remarkably, essentially *no* difference was observed between the cleavage patterns exhibited by the labeled and unlabeled oligonucleotide, indicating little or no isotope-induced branching in the distribution of the DNA cleavage products.[14] The origin of the remaining 5–20% hydrogen content in the spent drug presumably either owes to experimental error in NMR integration and densitometry or involves alternate modes of hydrogen abstraction from the 5′-deuterium-labeled deoxycytidine residue itself for it to fail to be readily distinguished on a sequencing gel.[15] Resolution of this issue will have to await additional experiments.

In conclusion, however, the major DNA cleavage process by far (>80%) within the TCCT sequence is initiated by discrete deuterium transfer from the C-5′-labeled deoxycytidine (C) to the proposed activated form of calicheamicin, the diradical **2**. Unlike the NCS-chrom, however, this process unexpectedly is accompanied by no apparent isotope-induced branching, indicating that alternate reaction pathways are not kinetically accessible or that no kinetic isotope effect exists. The deuterium from dodecamers **4** and **5** is transferred to the acetylene nearer the glycosidic linkage in CLM and thus emerges at C-4 in the spent drug leaving an imprint of the minor groove on the aglycon portion of calicheamicin. Given the absolute configuration of calicheamicin γ_1[1], the aryl-linked carbohydrate segment will, therefore, be directed to the 3′-side of the TCCT cleavage site in these oligomers.[16] While we have no direct evidence for the location of this side chain, it presumably extends along the minor groove to establish hydrophobic, electrostatic, and hydrogen-bonding interactions[9,17] that would account, at least in part, for the specificity of the drug for its cognate DNA receptor,[18] as discussed previously.[17]

The atom-transfer method described herein is a discriminating and direct means to overcome the pseudo-C_2 symmetry of the DNA helix and the inherent geometric ambiguity of drug/DNA interactions. For the growing class of diynene antibiotics, application of this method in principle can not only reveal the orientation of the drug at any cleavage site, but also identify the hydrogen(s) that are abstracted to initiate DNA cleavage events, whether or not isotope-induced branching is observed.[14]

Acknowledgment. We are pleased to thank R. D. Rulli and S. Mah for providing the scanning densitometry measurements, V. Dean for the CD determinations, and V. Axelrod for carrying out oligonucleotide syntheses. Fellowship support from the Royal Commission for The Exhibition of 1851 and the Dimitri V. d'Arbeloff Fund (J.J.De V.) and the S.E.R.C. for a NATO Award (A.B.T.) is gratefully acknowledged. This research was supported, in part, by the NIGMS (GM 40883 to S.L.S.).

(12) These deuterium incorporations are corrected for the actual deuterium contents of **4** and **5** (95 ± 1%) accurately determined by mass spectrometry of stable intermediates in the respective chemical syntheses. Drug/DNA ratios of 1:2–1:1 gave identical extents of deuterium transfer. The latter ratio was used, therefore, in the experiments described herein.

(13) Arigoni, D. *Pure Appl. Chem.* **1975**, *41*, 219–245. Samuelson, A. G.; Carpenter, B. K. *J. Chem. Soc., Chem. Commun.* **1981**, 354–356. Korzekwa, T.; Trager, W. F.; Gillette, J. R. *Biochemistry* **1989**, *28*, 9012–9018.

(14) This finding is in notable contrast to recent experiments with NCS-chrom: Kappen, L. S.; Chen, C.-q.; Goldberg, I. H. *Biochemistry* **1988**, *27*, 4331–4340. Kappen, L. S.; Goldberg, I. H.; Wu, S. H.; Stubbe, J.; Worth, L., Jr., Kozarich, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 2797–2798. However, recently Kozarich and Stubbe have made an analogous observation in the reaction of esperamicin A_1 with a restriction fragment of pBR322 in which the thymidine residues had been labeled at C-4′ with deuterium: Kozarich, J. W.; Worth, L., Jr., Frank, B. L.; Christner, D. F.; Vanderwall, D. E.; Stubbe, J. *Science* **1989**, *245*, 1396–1399.

(15) For possibly related chemistry, see: Saito, I.; Kawabata, H.; Fujiwara, T.; Sugiyama, H.; Matsuura, T. *J. Am. Chem. Soc.* **1989**, *111*, 8302–8303.

(16) We note that the aglycon can rotate to place the carbamate near the base pairs and the enediyne along the minor groove, in analogy to neocarzinostatin,[17] without violating the distance constraint of the atom-transfer experiment.

(17) Hawley, R. C.; Kiessling, L. L.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1105–1109.

(18) CD measurements of dodecamers **4** and **5** showed spectral characteristics of B-form DNA: Aboul-ela, F.; Varani, G.; Walker, G. T.; Tinoco, I. *Nucleic Acids Res.* **1988**, *16*, 3559–3572.

A New Approach to Cyclitols Based on Rabbit Muscle Aldolase (RAMA)[1]

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The synthesis of cyclitols having well-defined stereochemistry starting with nonchiral precursors is a current challenge in organic synthesis. Most synthetic strategies either start from chiral precursors (e.g., carbohydrates),[3-5] or resolve the racemic adduct formed in a Diels–Alder reaction.[6,7] Here we report the application of rabbit muscle aldolase (RAMA; EC 4.1.2.13) to the preparation of cyclitols and *C*-glycosides (Scheme I).

RAMA catalyzes the aldol condensation of dihydroxyacetone phosphate (DHAP) and aldehydes and forms products with the

(1) This work was supported by NIH, Grant GM 30367.

(2) "Erwin Schrödinger" Postdoctoral Fellow of the "Fonds zur Förderung der wissenschaftlichen Forschung in Österreich" (Austrian Science Foundation).

(3) Paulsen, H.; Mielke, B. *Liebigs Ann. Chem.* **1990**, 169–180.

(4) Köhn, A.; Schmidt, R. R. *Liebigs Ann. Chem.* **1987**, 1045–1054.

(5) Tadano, K.; Maeda, H.; Hoshino, M.; Iimura, Y.; Suami, T. *J. Org. Chem.* **1987**, *52*, 1946–1956.

(6) (a) Ogawa, S.; Nose, T.; Ogawa, T.; Toyokuni, T.; Iwasawa, Y.; Suami, T. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2369–2374. (b) Ogawa, S.; Iwasawa, Y.; Nose, T.; Suami, T.; Ohba, S.; Ito, M.; Saito, Y. *J. Chem. Soc., Perkin Trans. 1* **1985**, 903–906.

(7) Micobial oxidation of benzene with *Pseudomonas putida* followed by appropriate transformations of the products also yields cyclitols: (a) Ley, S.; Sternfeld, F. *Tetrahedron* **1989**, *45*, 3463–3476. (b) Ley, S.; Sternfeld, F. *Tetrahedron Lett.* **1988**, *29*, 5305–5308. (c) Ley, S.; Sternfeld, F.; Tylor, S. *Tetrahedron Lett.* **1987**, *28*, 225–226.

0002-7863/90/1512-9670$02.50/0