ORGANIC LETTERS

2004
Vol. 6, No. 20
3505−3507

Delineating Noncovalent Interactions between the Azinomycins and Double-Stranded DNA: Importance of the Naphthalene Substitution Pattern on Interstrand Cross-Linking Efficiency

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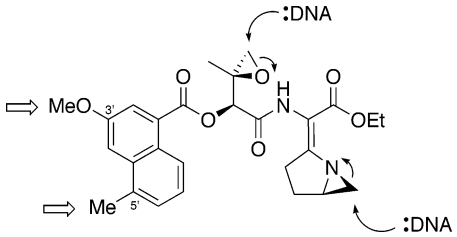
Received July 14, 2004

ABSTRACT



Using a series of synthetic azinomycin analogues, it is shown that the efficiency of in vitro DNA interstrand cross-linking is markedly reduced when either the C-5′ methyl group or both the C-5′ methyl and C-3′ methoxy groups are deleted from the naphthalene ring.

The antitumor antibiotics azinomycin A (**1**) and B (**2**) are structurally unique natural products containing the strained 1-azabicyclo[3.1.0]hexane ring system (Figure 1).[1] These compounds possess potent in vitro cytotoxic activity and significant in vivo antitumor activity and appear to act by disruption of cellular DNA replication by interstrand cross-link (ISC) formation.[1] In 2001, the first total synthesis of azinomycin A was achieved by Coleman.[2] The epoxide and aziridine are known to be responsible for the cross-linking process which occurs via N-7 of purine bases two residues apart on the complementary DNA strands.[3] At present, there is essentially no data available concerning the role of other functionalities contained within the azinomycin skeleton on the selectivity of DNA recognition and efficiency of interstrand cross-linking. Such information would provide important insights into the mode of action of the azinomycins and assist in the design of new DNA targeting agents.

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10.1021/ol048653y CCC: $27.50
Published on Web 09/03/2004

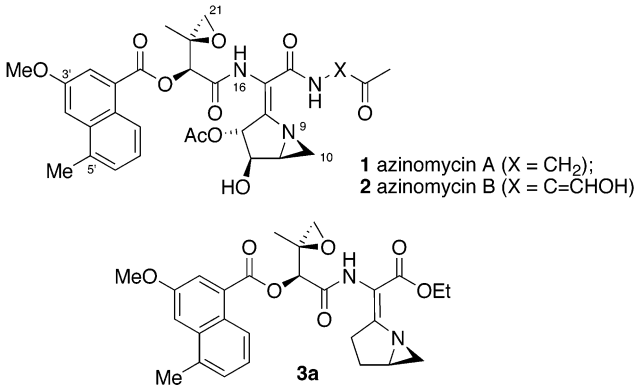


Figure 1. Structures of the naturally occurring DNA interstrand cross-linking azinomycins and functional synthetic analogue **3a**.

In systems based on just the "left-hand" epoxide domain, the naphthoate group plays a critical role in the efficiency of DNA alkylation,[4,5] DNA sequence selectivity,[4,5] and cytotoxicity.[5,6] By extrapolation, one can postulate that the naphthalene ring and its 5-methyl and 3-methoxy substituents play pivotal roles in noncovalent association between the azinomycins and double-stranded DNA. However, testing this hypothesis is difficult because modification of the aromatic ring within the natural products is precluded because of their inherent chemical instability. Recently, we described the synthesis of azinomycin analogue **3a**, which acts on double-stranded DNA in a manner similar to that of the natural products (Figure 1).[3d] We reasoned that by studying a series of derivatives related to **3a** in which the naphthoate fragment was modified, we would be able to probe if the substituents on the naphthalene ring play a critical role in DNA binding and ISC efficiency or indeed if they are simply unnecessary artifacts of the biosynthesis.[7] Herein, we report the preparation of modified azinomycin analogues **3b**−**d** and demonstrate that the nature of the naphthalene substitution pattern does indeed influence in vitro DNA ISC efficiency.

The synthesis of epoxy aziridines **3b**−**d** parallels our earlier synthesis of **3a**. Thus, esterification of (2*S*,3*S*)-epoxy alcohol **4**[8] with the appropriate acid chlorides[9] furnished esters **5b**−**d** (Scheme 1). Removal of the benzyl ester by catalytic hydrogenation yielded **6b**−**d**, which were immediately coupled with dehydroamino ester **8**, formed by removal of the *N*-Boc group from **7**,[3d] to give **9b**−**d** in good yields. Finally, ring closure to 1-azabicyclo[3.1.0]hexanes **3b**−**d** was accomplished according to methodology originally devised by Terashima via the corresponding mesylate.[10] These ring closures were best performed using TBAF. The low yields for the ring closure step largely reflect problems associated with obtaining **3b**−**d** in an acceptable state of purity.[11]

The stereochemical assignment about the tetrasubstituted double bond of **3a** was originally based on NOE measurements and correlations with the literature.[3d] In undertaking this study, we were fortunate that alcohol **9c** devoid of the methoxy group at C-3′ was highly crystalline, and using a single crystal grown from EtOAc/*n*-hexane, we were able to solve its structure unambiguously using X-ray crystallography (Figure 2).[12] As this is the first solid-state structure

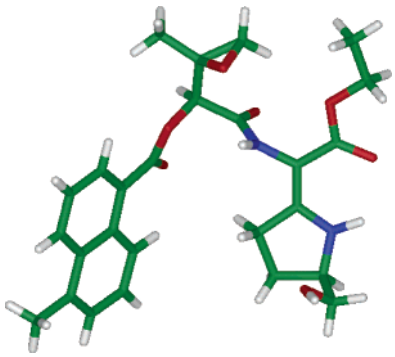
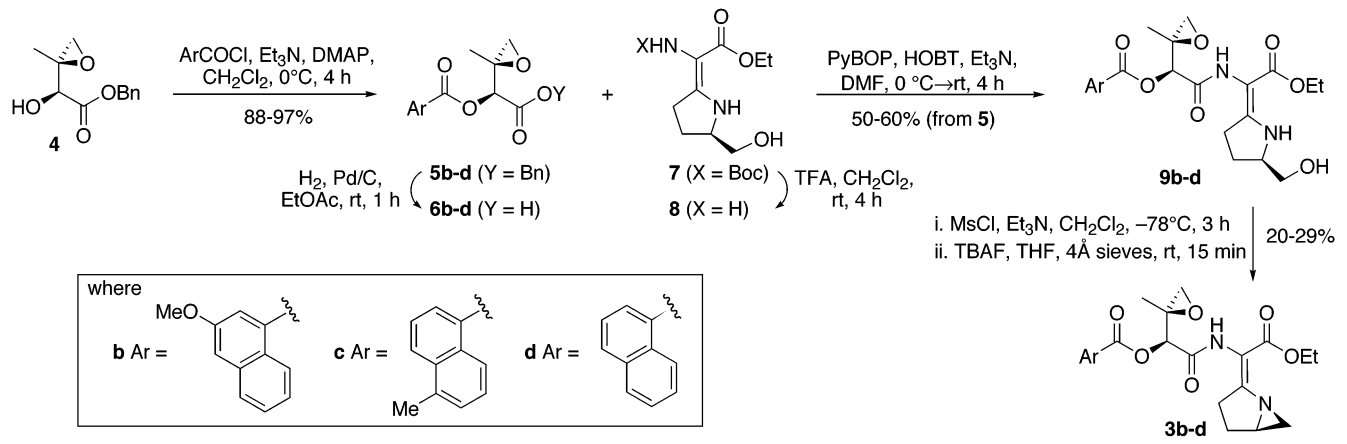


Figure 2. X-ray crystal structure of **9c**.[12]

of an "azinomycin-like" material, several features merit comment. First, the distance between the cross-linking carbon atoms (C-10 and C-21) is determined to be 8.70 Å in **9c**,

Scheme 1



which is close to the estimated linear distance of 8.5 Å between the N-7's of the two reacting guanines in B-form DNA.[13] Second, the lone pairs of N-9 and N-16 are nearly orthogonal (CO−N−C═C torsional angle = 84°), presumably to minimize electron repulsion. Finally, it is apparent that there are differences between the solid-state conformation of **9c** and that of azinomycin B (**2**) derived by mechanism filtered molecular modeling.[13] These potentially important conformational differences warrant scrutiny in the future.

The DNA interstrand cross-linking activities of epoxy aziridines **3b**−**d** were compared with resynthesized **3a** using an agarose gel assay using the pUC18 linearized plasmid.[14] The ^{32}P 5′-end-labeled duplex was incubated with **3a**−**d** (1.0−200 μM) for 1 h at 37 °C prior to denaturing with alkali and subsequent gel electrophoresis. The extent of cross-linking was determined by quantifying the relative amounts of double-stranded and single-stranded DNA by storage phosphorimage analysis. The experiment was performed four times, and a representative gel is presented (Figure 3).

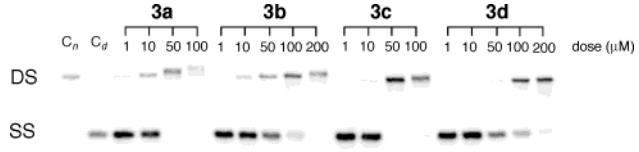


Figure 3. Agarose cross-linking gel for epoxy aziridines **3a**−**d**. Plasmid DNA was treated with the agents at the concentrations shown for 1 h prior to alkali denaturation and gel electrophoresis. C_n and C_d are control nondenaturated and denatured samples, respectively. DS and SS indicate the positions of double and single stranded DNA, respectively. At ≫100% cross-linking, the intensity of the DS band reduces as other DNA products begin to appear (not shown).

Although all of the epoxy aziridines produce DNA cross-links at micromolar concentrations of drug, the efficiency of cross-linking varied with the naphthalene substitution pattern. At 50 μM, **3a** produced 95.0 ± 1.9% cross-links (mean ± SD); **3b**, 27.1 ± 3.8% cross-links; **3c**, 95.1 ± 3.9% cross-links; and **3d**, 5.1 ± 1.6% cross-links. Thus, the order of ISC efficiency follows the trend **3a** ≈ **3c** > **3b** > **3d** with ca. 18-fold difference in efficiency across the group. At 10 μM, **3a** induces more cross-links than **3c**, indicating that it is a little more potent. Because **3c** and **3a** possess the 5′-methyl group and **3b** and **3d** do not, we conclude that this substituent plays an important role in noncovalent association between **3** and the DNA duplex. Interestingly, the 3′-methoxy group, which is proposed to be introduced relatively late in the biosynthesis,[7] has less influence upon the efficiency of ISC formation (**3a** cf. **3c**), although the deletion of both substituents does have an appreciable effect (**3a** cf. **3d**).

How the 5′-methyl promotes binding remains unclear, although we believe that it is most probably enhancing hydrophobic interactions between the DNA duplex and the epoxy aziridines. Currently, we do not know whether this arises from specific contacts between the 5′-methyl and the DNA backbone or a more general enhancement of the hydrophobicity of the drug. Mechanisms based on more active involvement of the naphthalene ring such as intercalation are most likely not operating, as extensive efforts by Coleman to detect intercalative binding using azinomycin B have not yet been successful.[3e]

To conclude, we have established that the 5′-methyl group is an important element in noncovalent association between DNA and azinomycin-like compounds. Future efforts will be focused on using this strategy to identify other important noncovalent binding interactions in this class of agent.

Acknowledgment. We are indebted to BBSRC (B15997) for financial support of this work and to Dr. Kevin Moffat of the University of Warwick for his help and assistance.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **3b**−**d** and crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

OL048653Y

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(9) 5-Methylnaphthoyl chloride was made from ethyl 3-hydroxy-5-methylnaphthoate[8] in 4 steps [(i) Tf_2O, Et_3N, CH_2Cl_2; (ii) cat. Pd(dppf)Cl_2, Et_3SiH, DMF, 60 °C; (iii) LiOH, MeOH, H_2O; (iv) $(COCl)_2$, cat. DMF, CH_2Cl_2, 4 h, 54% over 4 steps]. 3-Methoxynaphthoic acid was made according to published methods. See: Horii, Z.; Matsumoto, Y.; Momose, T. *Chem. Pharm. Bull.* **1971**, *19*, 1245. Newman, M. S.; Sankaran, V.; Olsen, D. R. *J. Am. Chem. Soc.* **1976**, *98*, 3237 and references therein. Ester **5d** has been made previously (see ref 6).
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(11) Epoxy aziridines **3a**−**d** are unstable and cannot be readily purified by chromatography. After extensive experimentation, we have devised a purification protocol that involves, after aqueous workup, selective precipitation of the impurities by addition of *n*-hexane to a chloroform solution of the crude product. After removal of the precipitate using a centrifuge, the mother liquor provides **3a**−**d** in a good state of purity (ca. 80−90%) as judged by ^{1}H and ^{13}C NMR spectroscopy (see Supporting Information).
(12) **Crystallographic data for 9c**: X-ray diffraction studies on a colorless crystal grown from EtOAc/*n*-hexane were performed at 125 K using a Bruker SMART diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The structure was solved by direct methods. $C_{26}H_{30}N_2O_7$, M = 482.52, monoclinic, space group $P2_1$, a = 9.010(1), b = 12.978(2), c = 10.722(1) Å, V = 1181.1(3) Å^3, Z = 2, D_c = 1.357 M gm^{-3}, μ = 0.099 mm^{-1}, $F(000)$ = 512, crystal size = 0.18 × 0.1 × 0.1 mm^3. Flack parameter 0.0(9). Of 5101 measured data, 3105 were unique (R_{int} = 0.0155) and 2810 observed ($I > 2\sigma(I)$) to give R_1= 0.035 and wR_2 = 0.0821. All non-hydrogen atoms were refined with anisotropic displacement parameters; the NH and OH protons were located from ΔF maps and allowed to refine isotropically subject to a distance constraint (O−H/N−H = 0.98 Å). All remaining hydrogen atoms bound to carbon were idealized. Structural refinements were by the full-matrix least-squares method on F^2.
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