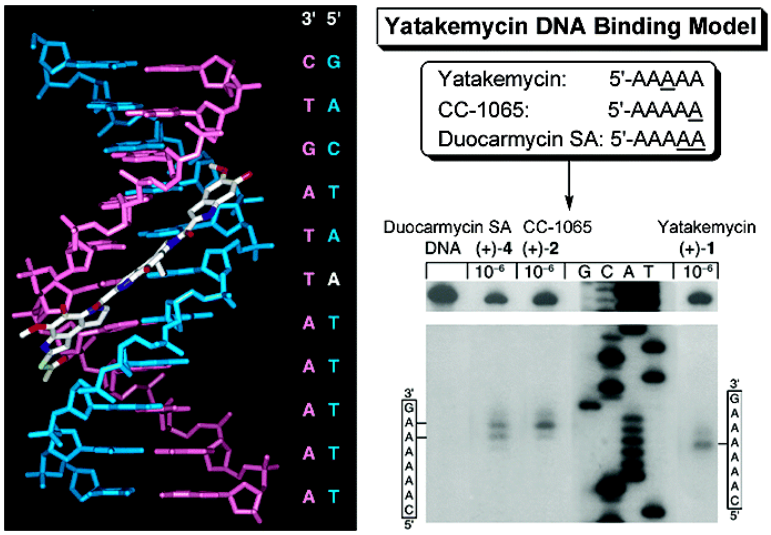
Article

DNA Alkylation Properties of Yatakemycin

Jay P. Parrish, David B. Kastrinsky, Scott E. Wolkenberg, Yasuhiro Igarashi, and Dale L. Boger

J. Am. Chem. Soc., **2003**, 125 (36), 10971-10976• DOI: 10.1021/ja035984h • Publication Date (Web): 16 August 2003



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 11 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 08/16/2003

DNA Alkylation Properties of Yatakemycin

Jay P. Parrish,[†] David B. Kastrinsky,[†] Scott E. Wolkenberg,[†] Yasuhiro Igarashi,[‡] and Dale L. Boger*,[†]

Contribution from the Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, and the Biotechnology Research Center, Toyama Perfectural University 5180, Kurokawa, Kosugi, Toyama 939-0398, Japan

Received May 6, 2003; E-mail: boger@scripps.edu

Abstract: Yatakemycin represents the newest and now most potent member of a class of naturally occurring antitumor compounds that includes CC-1065 and the duocarmycins, which derive their biological properties from a characteristic DNA alkylation reaction. Herein, the first description of the yatakemycin DNA alkylation properties is detailed, constituting the first such study of a naturally occurring "sandwiched" member of this class. Thus, the event, sequence selectivity, relative rate and efficiency, and reversibility of the DNA alkylation reaction of yatakemycin are described.

Yatakemycin (**1**)[1] was recently isolated from a culture broth of *Streptomyces* sp. TP-A0356 in the search for novel antifungals and its structure established through spectroscopic means, Figure 1. It represents the newest and now most potent (IC_{50} = 3 pM, L1210) member of a class of antitumor compounds that includes CC-1065[2] (**2**, IC_{50} = 20 pM), duocarmycin A[3] (**3**, IC_{50} = 200 pM), and duocarmycin SA[4] (**4**, IC_{50} = 10 pM), which derive their biological properties through a characteristic DNA alkylation reaction.[5−10] Yatakemycin represents a remarkable hybrid of the preceding natural products containing a central alkylation subunit identical to that of duocarmycin SA (**4**), a right-hand indole-based DNA binding subunit similar to that found in duocarmycin A and SA (**3** and **4**), and a unique left-hand binding

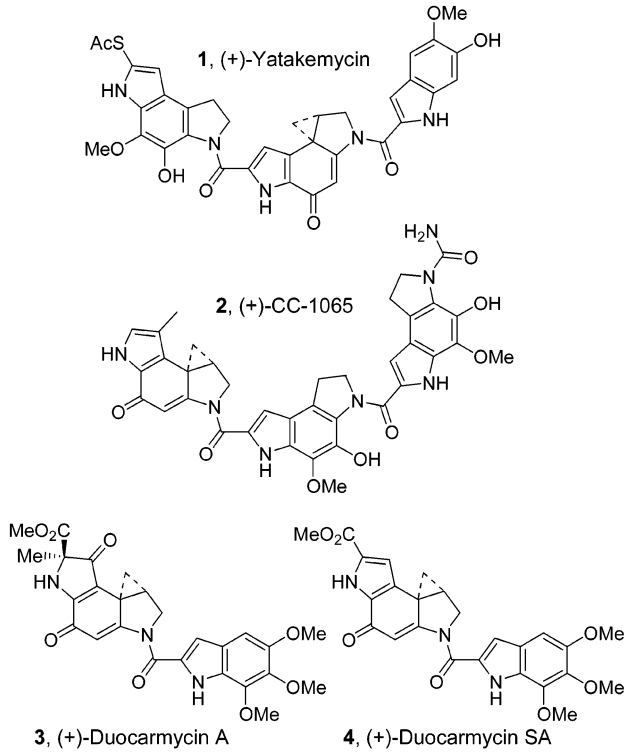
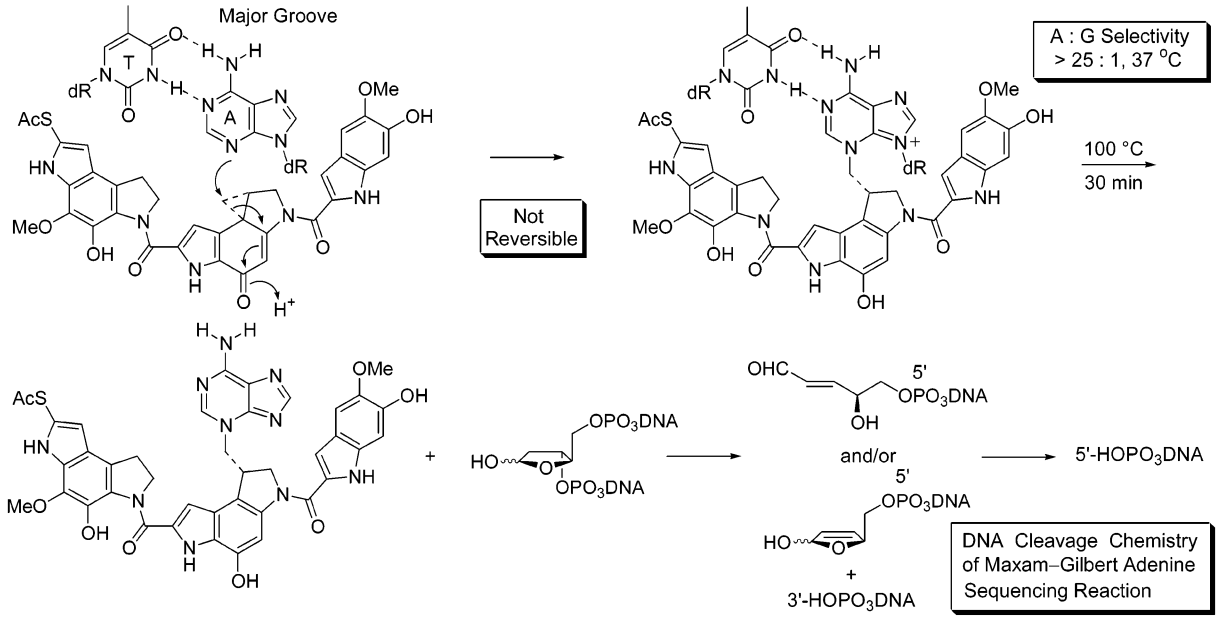


Figure 1.

[†] The Scripps Research Institute.
[‡] Toyama Perfectural University.

(1) Igarashi, Y.; Futamata, K.; Fujita, T.; Sekine, A.; Senda, H.; Naoki, H.; Furumai, T. *J. Antibiot.* **2003**, *56*, 107.
(2) Martin, D. G.; Biles, C.; Gerpheide, S. A.; Hanka, L. J.; Krueger, W. C.; McGovren, J. P.; Mizsak, S. A.; Neil, G. L.; Stewart, J. C.; Visser, J. *J. Antibiot.* **1981**, *34*, 1119. Gilvusmycin: Tokoro, Y.; Isoe, T.; Shindo, K. *J. Antibiot.* **1999**, *52*, 263.
(3) Takahashi, I.; Takahashi, K.; Ichimura, M.; Morimoto, M.; Asano, K.; Kawamoto, I.; Tomita, F.; Nakano, H. *J. Antibiot.* **1988**, *41*, 1915. Yasuzawa, T.; Muroi, K.; Ichimura, M.; Takahashi, I.; Ogawa, T.; Takahashi, K.; Sano, H.; Saitoh, Y. *Chem. Pharm. Bull.* **1995**, *43*, 378. Pyrindamycin A and B: Ohba, K.; Watabe, H.; Sasaki, T.; Takeuchi, Y.; Kodama, Y.; Nakazawa, T.; Yamamoto, H.; Shomura, T.; Sezaki, M.; Kondo, S. *J. Antibiot.* **1988**, *41*, 1515. Ishii, S.; Nagasawa, M.; Kariya, Y.; Yamamoto, H.; Inouye, S.; Kondo, S. *J. Antibiot.* **1984**, *42*, 1713.
(4) Ichimura, M.; Ogawa, T.; Takahashi, K.; Kobayashi, E.; Kawamoto, I.; Yasuzawa, T.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1990**, *43*, 1037. Ichimura, M.; Ogawa, T.; Katsumata, S.; Takahashi, K.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1991**, *44*, 1045.
(5) Reviews: Boger, D. L.; Johnson, D. S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1439. Boger, D. L.; Johnson, D. S. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 3642. Boger, D. L. *Acc. Chem. Res.* **1995**, *28*, 20. Boger, D. L. *Chemtracts: Org. Chem.* **1991**, *4*, 329. Warpehoski, M. A.; Hurley, L. H. *Chem. Res. Toxicol.* **1988**, *1*, 315. Hurley, L. H.; Needham-Van Devanter, D. R. *Acc. Chem. Res.* **1986,** *19*, 230.
(6) CC-1065: Hurley, L. H.; Reynolds, Y. L.; Swenson, D. H.; Petzold, G. L.; Scahill, T. A. *Science* **1984**, *226*, 843. Hurley, L. H.; Lee, C.-S.; McGovren, J. P.; Warpehoski, M. A.; Mitchell, M. A.; Kelly, R. C.; Aristoff, P. A. *Biochemistry* **1988**, *27*, 3886. Hurley, L. H.; Warpehoski, M. A.; Lee, C.-S.; McGovren, J. P.; Scahill, T. A.; Kelly, R. C.; Mitchell, M. A.; Wicnienski, N. A.; Gebhard, I.; Johnson, P. D.; Bradford, V. S. *J. Am. Chem. Soc.* **1990**, *112*, 4633. Boger, D. L.; Johnson D. S.; Yun, W.; Tarby, C. M. *Bioorg. Med. Chem.* **1994**, *2*, 115. Boger, D. L.; Coleman, R. S.; Invergo, B. J.; Sakya, S. M.; Ishizaki, T.; Munk, S. A.; Zarrinmayeh, H.; Kitos, P. A.; Thompson, S. C. *J. Am. Chem. Soc.* **1990**, *112*, 4623.
(7) Duocarmycin A: Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Kitos, P. A.; Suntornwat, O. *J. Org. Chem.* **1990**, *55*, 4499. Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *J. Am. Chem. Soc.* **1990**, *112*, 8961. Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H. *J. Am. Chem. Soc.* **1991**, *113*, 6645. Sugiyama, H.; Hosoda, M.; Saito, I.; Asai, A.; Saito, H. *Tetrahedron Lett.* **1990**, *31*, 7197.
(8) Duocarmycin SA: Boger, D. L.; Johnson, D. S.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 1635.
(9) Boger, D. L.; Garbaccio, R. M. *Acc. Chem. Res.* **1999**, *32*, 1043.

Scheme 1. Yatakemycin DNA Alkylation and Cleavage Reaction



subunit similar in structure to the central and right-hand subunits of CC-1065 (**2**). Distinct from the preceding natural products, it represents the first naturally occurring member of this class that contains DNA binding subunits flanking each side of the alkylation subunit. Prior to the disclosure of yatakemycin, we had prepared and characterized the first series of such "sandwiched" analogues and established their remarkable properties including their potent cytotoxic activity, enhanced DNA alkylation rate, and uniquely altered DNA alkylation selectivity.[11] Herein, we report the first study of yatakemycin assessing its DNA alkylation properties.

DNA Alkylation Selectivity. The DNA alkylation properties of yatakemycin were examined within five 150 base-pair segments of DNA for which comparative results are available for **2**−**4**. Five clones of phage M13mp10 were examined and contain SV40 nucleosomal DNA inserts w794 (nucleotide no. 5238−138) and its complement w836 (nucleotide no. 5189−91), c988 (nucleotide no. 4359−4210) and its complement c820 (nucleotide no. 4196−4345), and c1346 (nucleotide no. 1632−1782).[12] The alkylation site identification and the assessment of the selectivity among the available sites were obtained by thermally induced strand cleavage of the singly 5′ end-labeled duplex DNA after exposure to the agents (Scheme 1).[6,12] Following treatment of the end-labeled duplex DNA with a range of compound concentrations (24−96 h, 4−37 °C), the unbound compound was removed by EtOH precipitation of the DNA. Redissolution of the DNA in aqueous buffer, thermolysis (100 °C, 30 min) to induce strand cleavage at the sites of DNA alkylation, denaturing high-resolution polyacrylamide gel electrophoresis (PAGE) adjacent to Sanger dideoxynucleotide sequencing standards,[13] and autoradiography led to identification of the DNA cleavage and alkylation sites.

The statistical treatment of the alkylation sites provided herein proved more revealing than a conventional analysis that considers only the observed alkylation sites. That is, an evaluation that includes the consideration of sites *not* alkylated helped distinguish the composite consensus sequence and highlighted subtle features not apparent from a simple examination of the alkylated sites.

(+)-Yatakemycin alkylated DNA with a selectivity distinct from either (+)-CC-1065[6] or (+)-duocarmycin SA/A (Figure 2).[7,8] The consensus alkylation sequence for (+)-**1** is summarized in Table 1, a summary of the sequence preferences is provided in Table 2, and a statistical treatment of the available alkylation sites is provided in the supporting information. Without exception, all alkylation sites were adenine and no minor guanine alkylation was detected, and essentially all the

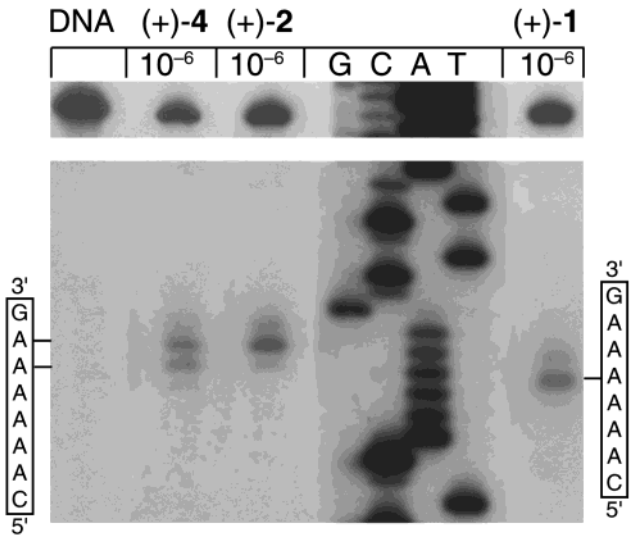


Figure 2. Thermally induced strand cleavage of w836 DNA (146 bp, nucleotide 5189−91) after DNA−agent incubation at 25 °C (24 h), removal of unbound agent by EtOH precipitation and 30 min thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography. Lane 1, control DNA; lane 2, (+)-duocarmycin SA (1×10^{-6} M); lane 3, (+)-CC-1065 (1×10^{-6} M); lanes 4−7, Sanger G, C, A, and T sequencing standards; lane 8, (+)-yatakemycin (1×10^{-6} M).

(10) Wolkenberg, S. E.; Boger, D. L. *Chem. Rev.* **2002**, *102*, 2477.
(11) Boger, D. L.; Bollinger, B.; Hertzog, D. L.; Johnson, D. S.; Cai, H.; Mesini, P.; Garbaccio, R. M.; Jin, Q.; Kitos, P. A. *J. Am. Chem. Soc.* **1997**, *119*, 4987. Boger, D. L.; Hertzog, D. L.; Bollinger, B.; Johnson, D. S.; Cai, H.; Goldberg, J.; Turnbull, P. *J. Am. Chem. Soc.* **1997**, *119*, 4977.
(12) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661.
(13) Sanger, F.; Nicklen, S.; Coulsen, A. R. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *74*, 5463.

Table 1. Yatakemycin Consensus DNA Alkylation Sequence

base[a]	–3	–2	–1	0	1	2	3	3′
A (30)[b]	56	61	78	100	83	50	36	
T (26)	19	23	19		14	22	23	
G (21)	17	8	0		3	22	19	
C (23)	8	8	3		0	6	22	
A/T (56)	75	84	97	100	97	72	59	
composite	A/T > G/C	A/T > G/C	A/T	A	A/T	A/T > G/C	N	

[a] Percentage of the indicated base located at the designated position relative to the adenine-N3 alkylation site. [b] Percentage composition within the DNA examined.

Table 2. Yatakemycin Sequence Preferences

sequence	no. AS[a]	no. TS[b]	%[c]
5′-(NAAAN)-3′	25	39	64
5′-(NTAAN)-3′	5	18	28
5′-(NAATN)-3′	3	14	21
5′-(NTATN)-3′	2	15	13

[a] Number of such alkylated sites in the DNA examined. [b] Total number of such sites in the DNA examined. [c] No. AS/No. TS × 100, % of such sites alkylated in the DNA examined.

adenine N3 alkylation sites were flanked by a 5′ and 3′ A or T base. The preference for this three base sequence follows the order: 5′-AAA > 5′-AAT ≥ 5′-TAA > 5′-TAT[14] (Table 2). In addition, there was a strong preference, but not absolute requirement, for both the second 5′ and 3′ bases to be A or T. Exceptions typically involved one but not both of these locations, and the preference was strongest on the 5′ side of the alkylation site (e.g., 5′-AAAAG > 5′-CAAAA). In addition, this preference for a five base A/T site was observed most prominently with the higher versus lower affinity alkylation sites. Thus, alkylation was observed at adenines central to a five base pair A/T sequence (e.g., 5′-AAAAA). This is summarized in Table 1 and illustrated nicely in Figure 2 with w836 DNA where (+)-**1** alkylated adenine central to the stretch of six adenines rather than the 3′ terminal adenines characteristic of (+)-CC-1065 or (+)-duocarmycin SA. The comparisons highlighted in Figure 2 are actually misleading in that the distinctions between **1** and CC-1065 or duocarmycin SA are more pronounced than this might suggest. Very few of the alkylation sites for **1** overlap with those of either enantiomer of **2**−**4**, and those that do are typically found in a long stretch of A's containing multiple alkylation sites for the compounds.

Thus, yatakemycin was established to alkylate adenine central to a five base pair A/T site differing significantly from (+)-CC-1065 or (+)-duocarmycin SA/A which alkylate the 3′ terminal adenine of a 5 base-pair (CC-1065) or 3−4 base-pair (duocarmycins) A/T site (5′-AAA > 5′-TTA > 5′-TAA > 5′-ATA),[5−8,14] see Figure 2.

Relative Efficiency of DNA Alkylation. The final efficiency of DNA alkylation conducted under our experimental conditions (4−25 °C, 24−96 h) for the sequencing studies did not differ significantly for yatakemycin (**1**) versus duocarmycin SA (**4**) or CC-1065 (**2**) and the lowest concentration at which the alkylation was detectable was 10^{-6}−10^{-7} M in our assay. However, all three were significantly more effective (ca. 10-fold) than duocarmycin A (**3**) at 25 °C which, because of its limited stability, undergoes competitive reactions including solvolysis.[8] However, as detailed below, significant differences in the rates of DNA alkylation were observed.

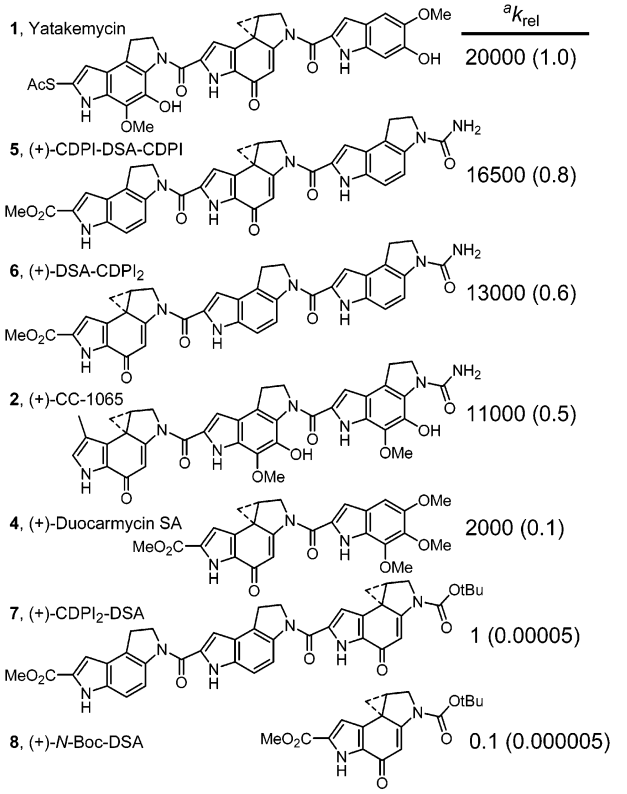


Figure 3. Relative rates of DNA alkylation.

Relative Rate of DNA Alkylation. A more significant distinction in the natural products and related compounds was observed in a comparison of their rates, versus efficiencies, of DNA alkylation. These comparisons were made by establishing relative rate constants (k_{rel}, 1 × 10^{-5} M, 25 °C) for alkylation of the w836 alkylation sites within the 6 base A site (5′-AAAAAA) that both enantiomers of all classes of compounds alkylate effectively.[11] The results are summarized in Figure 3. Impressively, yatakemycin alkylated DNA faster than any of the preceding natural products. Moreover, it was found to be slightly faster than the preceding sandwiched analogue, (+)-CDPI-DSA-CDPI (**5**), and both were notably faster than the typical compounds in the class including (+)-CC-1065 (**2**) and (+)-DSA-CDPI$_2$ (**6**). Most interestingly, yatakemycin was found to alkylate DNA much faster (10-fold) than duocarmycin SA even though the final efficiencies of DNA alkylation were not distinguishable. Compared to *N*-Boc-DSA (**8**), yatakemycin alkylates DNA roughly 200 000 times faster, which is a consequence of both its enhanced noncovalent binding (ca. 10- to 100-fold enhancement)[11,15] and catalysis derived from a binding-induced conformational change that disrupts the alkylation subunit stabilizing vinylogous amide (ca. 10 000-

(14) Notably and unlike 5′-AAA, the mixed sequences can contain competitive alkylation sites on the complementary unlabeled strand that diminishes the apparent alkylation efficiency on the labeled strand. It is likely that a majority of the apparent three base A/T selectivity is simply a statistical preference exaggerated by competitive unlabeled strand alkylation rather than unique characteristics embodied in the individual sequences.

(15) Boger, D. L.; Schmitt, H.; Fink, B. E.; Hedrick, M. P. *J. Org. Chem.* **2001**, *66*, 6654. Ellis, D. A.; Wolkenberg, S. E.; Boger, D. L. *J. Am. Chem. Soc.* **2001**, *123*, 9299.

fold).[9−11,15] The latter catalysis effect is only observed with compounds bearing a rigid and extended right-hand heteroaromatic chromophore and is absent with **7**.[11] The extent of the DNA binding induced conformational change, and hence the extent of the catalysis, benefits from the presence of rigid left-hand subunits (or substituents),[9,16] which effectively extend the rigid length of the alkylation subunit. Consequently, it is not surprising that yatakemycin exhibits exceptional rates of DNA alkylation that exceeds those of the prior natural products.

Reversibility of DNA Alkylation Reaction. Although the nature of the (+)-duocarmycin SA and (+)-duocarmycin A DNA alkylations has proven similar to that of (+)-CC-1065, one important feature of the reactions distinguish the two classes of natural products. Unlike (+)-CC-1065, which essentially irreversibly alkylates duplex DNA,[17] (+)-duocarmycin SA and (+)-duocarmycin A, like simplified analogues of CC-1065,[17] were found to reversibly alkylate DNA.[8,18] The relative rate or ease of reversibility proved dependent upon the relative reactivity of the compound and hence the stability of the adducts as well as the extent of the noncovalent binding interactions.

The reversible nature of the yatakemycin DNA alkylation reaction was monitored through measurement of its transfer from unlabeled duplex DNA (w836) covalently modified with **1** to unmodified radiolabeled w836 duplex DNA. Detection of the compound transfer reaction and the identification of the sites of labeled DNA alkylation were established following the procedure in which thermolysis (100 °C, 30 min) of the covalently modified DNA induces strand cleavage at the adenine N3 alkylation sites. Under all conditions examined, no transfer of yatakemycin from the unlabeled to labeled DNA was observed, indicating that the DNA alkylation is not reversible under the conditions explored. This included the previously disclosed conditions[8] and DNA where both duocarmycin A and SA exhibit extensive reversal (range examined: pH 6−8.4, 25−50 °C, 1−8 days).

Like CC-1065,[17] the yatakemycin DNA alkylation is not reversible under the conditions that we and others have explored. This distinguishes both **1** and **2** from the duocarmycins, which exhibit readily reversible DNA alkylation reactions (SA > A). Clear from these compound comparisons are two distinct structural features that effect the reversibility of the reaction. The first is embodied in the comparison of duocarmycin A and SA at common sites where the relative reactivity of the compounds (A > SA, and hence the stability of the duocarmycin A vs SA adenine adducts) correlates with a slower, less effective retroalkylation.[8] The second is embodied in the comparison of yatakemycin with duocarmycin SA, which contain identical alkylation subunits (comparable intrinsic reactivities) but of considerably different sizes, which impacts the extent of the noncovalent binding stabilization. Analogous to observations made with CC-1065 (irreversible) versus its simpler analogues (reversible),[17] yatakemycin alkylates DNA essentially irreversibly, whereas duocarmycin SA is readily reversible.[8]

Cytotoxic Activity. The in vitro cytotoxic activity of yatakemycin was established against a cell line (L1210) for which we have extensive comparisons, Table 3. Paralleling observations made in the DNA alkylation studies, yatakemycin was found to be the most potent member of this class of natural products. In part, the enhanced potency of yatakemycin and duocarmycin SA relative to CC-1065 and duocarmycin A can be attributed to the significantly enhanced stability of their alkylation subunit (DSA > MeCPI (5-fold) > DA (16-fold)).[19] In turn, the enhanced potency of yatakemycin over duocarmycin SA correlates well with its enhanced rate of DNA alkylation.

Table 3. Cytotoxic Activity, L1210

compound	IC_{50} (pM)
yatakemycin (**1**)	3
duocarmycin SA (**4**)	10
CC-1065 (**2**)	20
duocarmycin A (**3**)	200

Yatakemycin Absolute Stereochemistry. Unique to the sandwiched analogues CDPI-DSA-CDPI (**5**) was the observation that both enantiomers exhibited a nearly identical alkylation selectivity that was distinct from either the natural or unnatural enantiomers of CC-1065 and related compounds. In past studies, the distinct alkylation profile of the natural and unnatural enantiomers could be used to confidently assign stereochemistry. In the case of yatakemycin, this is not possible since the natural and unnatural enantiomers, like those of CDPI-DSA-CDPI,[11] would be expected to exhibit indistinguishable selectivities. Similarly, the rates and efficiencies of DNA alkylation typically differ substantially for the enantiomers of CC-1065 and related compounds, easily distinguishing the natural and unnatural enantiomers. In contrast, those of CDPI-DSA-CDPI were much more difficult to distinguish (ca. 2-fold differences).[11] Nonetheless, the exceptional rate of DNA alkylation observed with yatakemycin that exceeds that of the natural enantiomer of CDPI-DSA-CDPI, or any prior natural enantiomer, suggests it is unlikely yatakemycin possesses an inverted, "unnatural" stereochemistry. Additionally, yatakemycin exhibited the most potent cytotoxic activity of any member of this class of natural products. Typically, the natural enantiomers are significantly more potent, although the prior enantiomeric sandwiched analogues **5** exhibited little or no distinction (1- to 2-fold).[11] Thus, although the comparison could not be considered definitive, the exceptional potency of yatakemycin makes it unlikely that the stereochemistry differs from that of the prior natural products. Consistent with this "natural" stereochemistry assignment, yatakemycin exhibits a strong dextrarotatory $[\alpha]_D$ that is characteristic of all such natural enantiomers, including not only **1**−**4** but also CDPI-DSA-CDPI (**5**) and related sandwiched analogues.[11] Thus, although there is no comparison that allows an unambiguous assignment of the yatakemycin "natural" stereochemistry and several observations (e.g., $[\alpha]_D$) which implicate it, there are no observations that would suggest an unusual inversion of stereochemistry to that of an "unnatural" enantiomer would be warranted.

Model of the DNA Alkylation Reaction. Illustrated in Figure 4 are models of the tentatively assigned natural and unnatural enantiomers of yatakemycin at the w794 high-affinity alkylation site. The assigned natural enantiomer alkylates adenine in the minor groove with the alkylation subunit extending in the 3′ to 5′ direction with the attached binding subunits covering two

(16) Boger, D. L.; Santillan, A., Jr.; Searcey, M.; Brunette, S. R.; Wolkenberg, S. E.; Hedrick, M. P.; Jin, Q. *J. Org. Chem.* **2000**, *65*, 4101.
(17) Warpehoski, M. A.; Harper, D. E.; Mitchell, M. A.; Monroe, T. J. *Biochemistry* **1992**, *31*, 2502. For related studies, see: Lee, C.-S.; Gibson, N. W. *Biochemistry* **1993**, *32*, 9108.
(18) Boger, D. L.; Yun, W. J. *J. Am. Chem. Soc.* **1993**, *115*, 9872.
(19) Boger, D. L.; McKie, J. A.; Nishi, T.; Ogiku, T. *J. Am. Chem. Soc.* **1997**, *119*, 311.

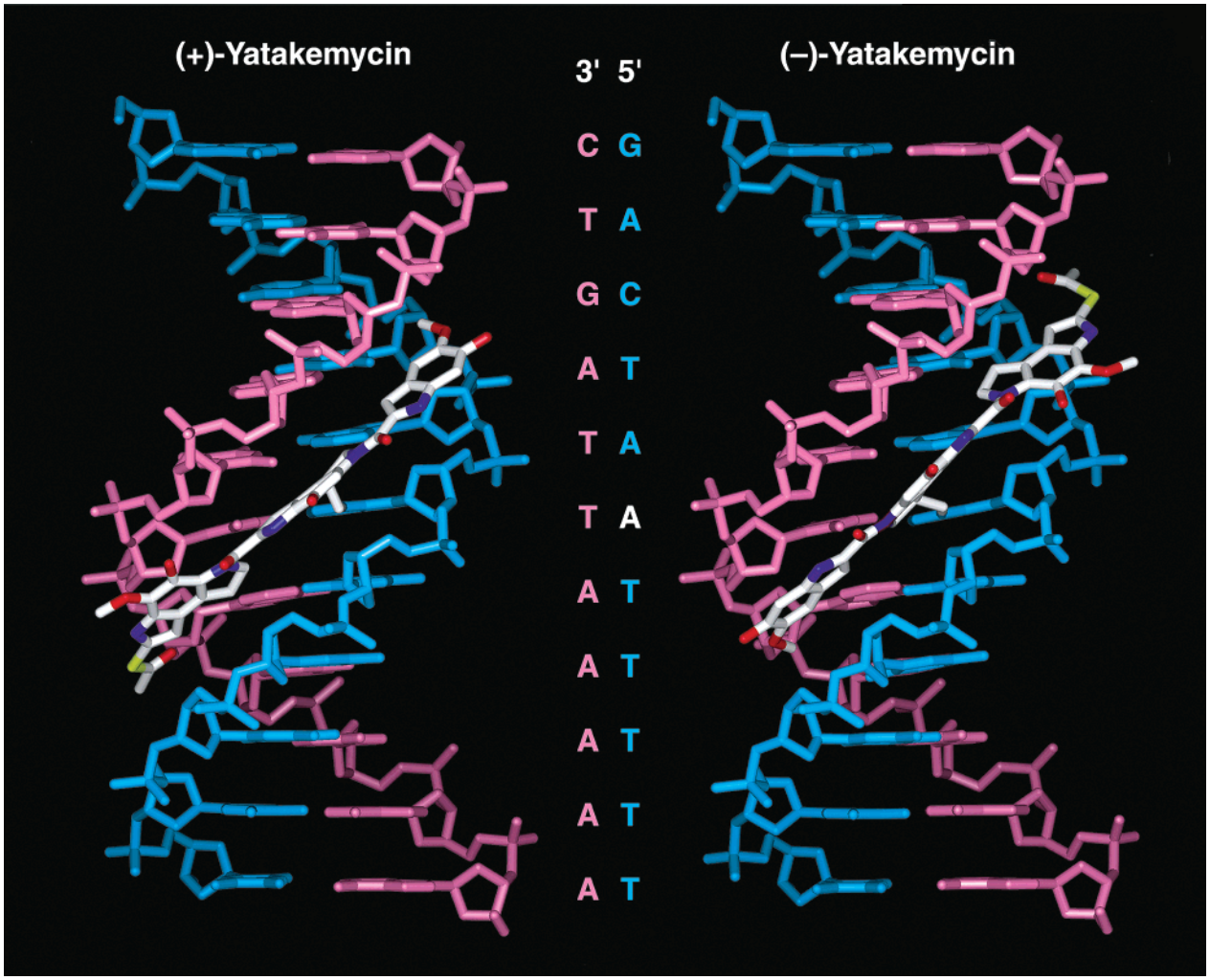


Figure 4. Models of the assigned natural (left) and unnatural (right) enantiomer alkylation and binding at the high affinity site in w794 DNA. Both enantiomers alkylate and bind (cover) the same 5′-TAATT site, but with reversed binding orientations.

base pairs on both the 5′ and 3′ sides of the alkylation subunit and alkylated adenine. The binding extends slightly farther to the 5′ side than the 3′ side and this may account for the stronger and slightly extended AT preference on the 5′ side of the alkylation site.

The unnatural enantiomer would be expected to exhibit identical characteristics, except that the alkylation subunit binds in the minor groove extending in the reverse 5′ to 3′ direction relative to the alkylated strand covering the same five base A/T site surrounding the central alkylated adenine. This unusual feature of the two enantiomers alkylating the same five base pair site has been observed with the prior sandwiched analogue CDPI-DSA-CDPI.[11] It is also analogous to the behavior of simple derivatives of the alkylation subunit, e.g., *N*-Boc-DSA,[8] except that these latter smaller agents only cover two base pairs. An array of NMR studies of CC-1065,[20] duocarmycin SA,[21] or duocarmycin A[22] and related agents bound to deoxyoligonucleotides have established the accuracy of analogous models and the minor groove binding orientations relative to the alkylated strands.

Conclusions

The (+)-yatakemycin DNA alkylation properties were established and are presumed to proceed by adenine N3 addition to the least substituted carbon of the cyclopropane found in the central subunit analogous to the reactions established for all prior members in this class, including the closely related duocarmycin SA. Each alkylation site detected proved to be adenine flanked essentially exclusively by a 5′ and 3′ A or T base with a preference that follows the order 5′-A<u>A</u>A > 5′-A<u>A</u>T ≥ 5′-T<u>A</u>A > 5′-T<u>A</u>T,[14] with a strong preference for an additional 5′ and 3′ A or T base flanking these sites. Thus, yatakemycin preferentially alkylates the central adenine of a five-base AT site (e.g., 5′-AA<u>A</u>AA) and requires the central three-base A/T site.

Assessments of the relative efficiencies of DNA alkylation revealed that yatakemycin (**1**), CC-1065 (**2**), and duocarmycin SA (**4**) were not distinguishable under our assay conditions and that all three were 10-fold more effective than duocarmycin A (**3**), which suffers competitive solvolysis. In contrast, significant differences in the rates of DNA alkylation were observed with yatakemycin exhibiting the fastest rate among the natural products or related compounds and being complete within

(20) Scahill, T. A.; Jensen, R. M.; Swenson, D. H.; Hatzonbuhler, N. T.; Petzold, G.; Wierenga, W.; Brahme, N. D. *Biochemistry* **1990**, *29*, 2852.
(21) Eis, P. S.; Smith, J. A.; Rydzewski, J. M.; Case, D. A.; Boger, D. L.; Chazin, W. J. *J. Mol. Biol.* **1997**, *272*, 237. Smith, J. A.; Bifulco, G.; Case, D. A.; Boger, D. L.; Gomez-Paloma, L.; Chazin, W. J. *J. Mol. Biol.* **2000**, *300*, 1195. Schnell, J. R.; Ketchem, R. R.; Boger, D. L.; Chazin, W. J. *J. Am. Chem. Soc.* **1999**, *121*, 5645.
(22) Lin, C. H.; Patel, D. J. *J. Am. Chem. Soc.* **1992**, *114*, 10 658. Lin, C. H.; Patel, D. J. *J. Mol. Biol.* **1995**, *248*, 162.

minutes at 25 °C under our conditions of assay. Yatakemycin alkylated DNA 1.2-fold faster than a prior synthetic sandwiched analogue CDPI-DSA-CDPI (**5**), roughly 2-fold faster than typical extended compounds including CC-1065 (**2**), 10-fold faster than duocarmycin SA (**4**), 20 000-fold faster than a representative reversed analogue $CDPI_2$-DSA (**7**), and 200 000-fold faster than the simple derivative *N*-Boc-DSA (**8**).

Additionally distinguishing yatakemycin from the duocarmycins, its DNA alkylation reaction was not reversible under the range of conditions that were examined.

Acknowledgment. We gratefully acknowledge the financial support of the National Institutes of Health (CA41936, 42056) and the Skaggs Institute for Chemical Biology. We especially thank Dr. Inkyu Hwang for establishing the cytotoxic activity of yatakemycin (L1210). David B. Kastrinsky and Scott E. Wolkenberg are Skaggs Fellows.

Supporting Information Available: Experimental details for the DNA alkylation studies and a figure illustrating the DNA alkylation sites (Figure S1) are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

JA035984H