Second Definitive Test of Proposed Models for the Origin of the CC-1065 and Duocarmycin DNA Alkylation Selectivity

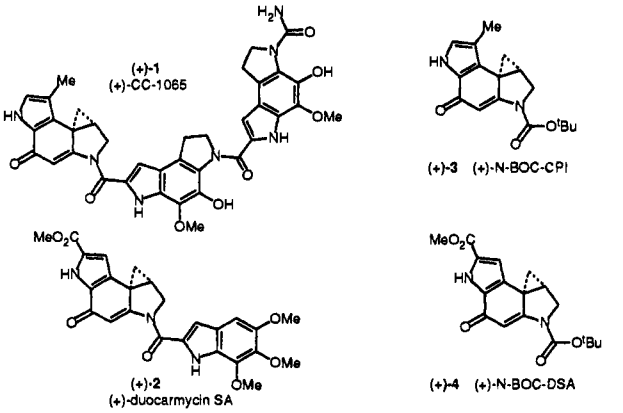
Dale L. Boger* and Douglas S. Johnson

Department of Chemistry, The Scripps Research Institute 10666 North Torrey Pines Road, La Jolla, California 92037

Received September 26, 1994

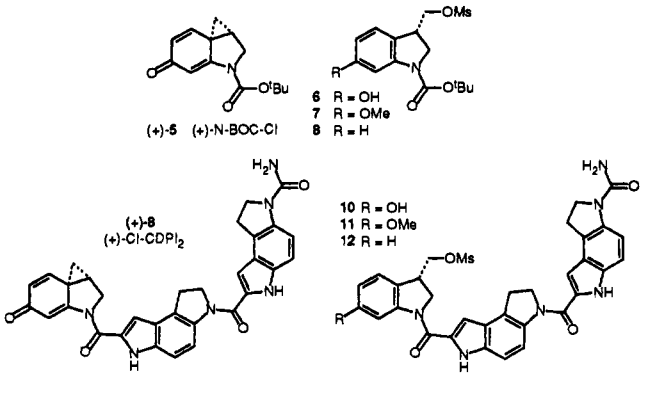
(+)-CC-1065 (**1**)[1] and the duocarmycins[2−3] are potent antitumor antibiotics that derive their biological effects through the reversible, sequence-selective alkylation of DNA.[4−10] Since their disclosure, extensive efforts have been devoted to determine their DNA alkylation selectivity and its origin,[4−10] to establish the link between DNA alkylation and the ensuing biological properties,[11] and to define the fundamental principles underlying the relationships between structure, chemical reactivity, and biological properties.[9,10]

In these studies, the alkylation selectivity of the natural enantiomers has been attributed to a sequence-dependent activation through C4 carbonyl protonation by a strategically positioned phosphate in the DNA backbone,[6,10,12] the conformational variability of DNA and alkylation at junctions of bent DNA,[12] or preferential noncovalent binding and subsequent alkylation within the narrower, deeper minor groove of AT-rich DNA.[4,5,7−9,13−18] Central to the interpretations are the perceived similarities[6,10] or distinctions[4,5,7−9,13−18] in the alkylation selectivity of simple derivatives including **3**−**4** and that of the natural products **1**−**2**. The former two proposals are based on the premise that (+)-**3** and (+)-**1** alkylate the same DNA sites and the alkylation subunit or alkylation reaction controls the selectivity irrespective of noncovalent binding. In



contrast, the latter proposal requires that the AT-rich noncovalent binding selectivity[17] of the agents and their steric accessibility to the alkylation site that accompanies deep penetration into the AT-rich minor groove control the sequence selectivity. Notably, this latter model accommodates nicely the reverse and offset 3.5- or 5-base-pair AT-rich adenine N3 alkylation selectivities of the natural and unnatural enantiomers of **2** and **1**, respectively, and requires that **3**−**4** and **1**−**2** exhibit distinct alkylation selectivities.[5,7,14]

In an earlier study,[18] it was shown that **5**−**8** exhibited identical alkylation selectivities (5′-A<u>A</u> > 5′-T<u>A</u>) independent of their absolute configuration and identical to those of both enantiomers of **3** and **4**. In addition, the natural enantiomers of **9**−**12**



exhibited identical DNA alkylation selectivities (5′-A/TA/TAA<u>A</u> or 5′-A/TA/TTT<u>A</u>) with alkylation of the same sites as (+)-**1** and were more selective and more efficient than **5**−**8**. The observation that **7**−**8** and **11**−**12**, which lack the C4 carbonyl, alkylate the same sites as **5/3**−**4** or **9/1**, respectively, established that a sequence-dependent phosphate protonation and activation cannot be the event that determines the alkylation selectivity. In addition, the more selective DNA alkylation by **9**−**12/1**−**2** versus **5**−**8/3**−**4** independent of the nature of the electrophile was fully consistent with the noncovalent binding model and inconsistent with the proposal that alkylation is observed preferentially at junctions of bent DNA irrespective of binding selectivity. Herein we report a second test of the proposed models which provides further support for the noncovalent binding model and definitively excludes alternative models.

(1) Chidester, C. G.; Krueger. W. C.; Mizsak, S. A.; Duchamp, D. J.; Martin, D. G. *J. Am. Chem. Soc.* **1981**, *103*, 7629.
(2) Takahashi, I.; Takahashi, K.; Ichimura, M.; Morimoto, M.; Asano, K.; Kawamoto, I.; Tomita, F.; Nakano, H. *J. Antibiot.* **1988**, *41*, 1915.
(3) Ichimura, M.; Ogawa, T.; Takahashi, K.; Kobayashi, E.; Kawamoto, I.; Yasuzawa, T.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1990**, *43*, 1037.
(4) 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. Boger, D. L.; Yun, W. *J. Am. Chem. Soc.* **1993**, *115*, 9872.
(5) Boger, D. L.; Johnson, D. S.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 1635.
(6) 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.
(7) 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.
(8) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661.
(9) Boger, D. L. *Acc. Chem. Res.*, in press. Boger, D. L. *Chemtracts: Org. Chem.* **1991**, *4*, 329. Boger, D. L. In *Proc. R. A. Welch Found. Conf. Chem. Res., XXXV. Chem. Front. Med.* **1991**, *35*, 137. Boger, D. L. In *Advances in Heterocyclic Natural Products Synthesis;* Pearson, W. H., Ed.; JAI Press: Greenwich, CT, 1992; Vol. 2, pp 1−188. Coleman, R. S.; Boger, D. L. *Stud. Nat. Prod. Chem.* **1989**, *3*, 301.
(10) Warpehoski, M. A. In *Advances in DNA Sequence Specific Agents*; Hurley, L. H., Ed.; JAI Press: Greenwich, CT, 1992; Vol. 1, p 217. Warpehoski, M. A. *Drugs Fut.* **1991**, *16*, 131. Warpehoski, M. A.; McGovren, J. P.; Mitchell, M. A. Hurley, L. H. In *Molecular Basis of Specificity in Nucleic Acid-Drug Interactions*; Pullman, B., Jortner, J., Eds.; Kluwer: Netherlands, 1990; p 531. Warpehoski, M. A.; Hurley, L. H. *Chem. Res. Toxicol.* **1988**, *1*, 315.
(11) Boger, D. L.; Johnson, D. S.; Wrasidlo, W. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 631.
(12) Sun, D.; Lin, C. H.; Hurley, L. H. *Biochemistry* **1993**, *32*, 4487. Lin, C. H.; Hill, G. C.; Hurley, L. H. *Chem. Res. Toxicol.* **1992**, *5*, 167. Lin, C. H.; Beale, J. M.; Hurley, L. H. *Biochemistry* **1991**, *30*, 3597.
(13) Boger, D. L.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1431.
(14) Boger, D. L.; Munk, S. A. *J. Am. Chem. Soc.* **1992**, *114*, 5487.
(15) Boger, D. L.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 7996.
(16) Boger, D. L.; Munk, S. A.; Ishizaki, T. *J. Am. Chem. Soc.* **1991**, *113*, 2779.
(17) Boger, D. L.; Invergo, B. J.; Coleman, R. S.; Zarrinmayeh, H.; Kitos, P. A.; Thompson, S. C.; Leong, T.; McLaughlin, L. W. *Chem.-Biol. Interact.* **1990**, *73*, 29. Boger, D. L.; Sakya, S. M. *J. Org. Chem.* **1992**, *57*, 1277.
(18) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H. *J. Am. Chem. Soc.* **1991**, *113*, 3980.

0002-7863/95/1517-1443$09.00/0

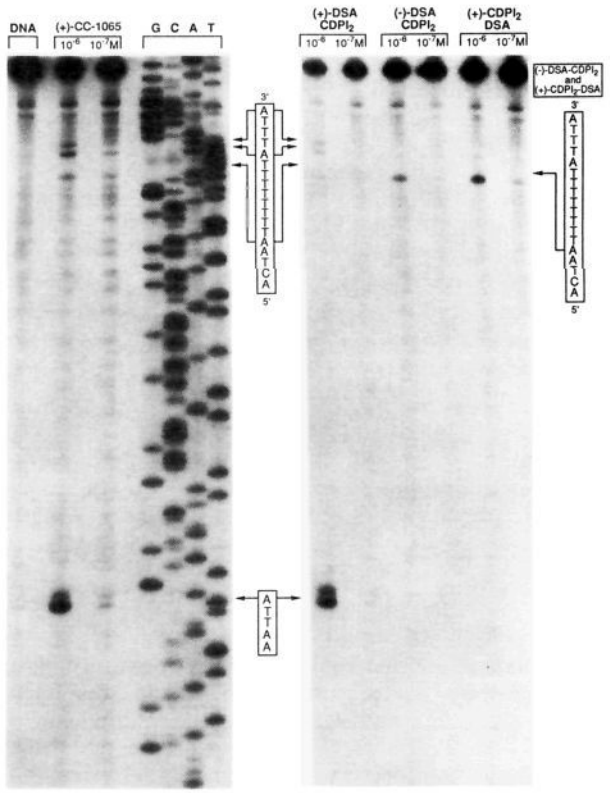
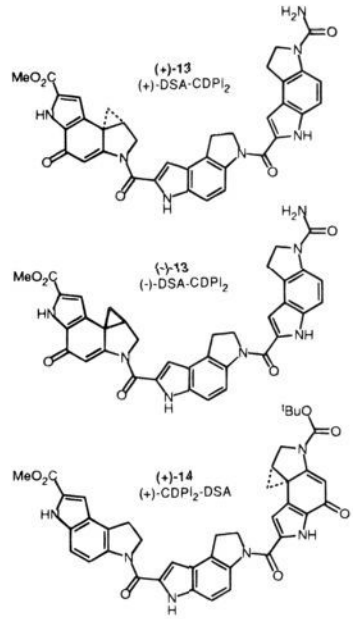


Figure 1. Thermally-induced strand cleavage of w794 DNA (144 bp, nucleotide no. 138−5238) after DNA−agent incubation with **13** and **14**, removal of unbound agent by EtOH precipitation and 30 min thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography. Lane 1, control DNA; lanes 2 and 3, (+)-CC-1065 ((+)-**1**, 1×10^{-6} and 1×10^{-7} M); lanes 4−7, Sanger G, C, A, and T sequencing standards; lanes 8 and 9, (+)-DSA-CDPI$_2$ ((+)-**13**, 1×10^{-6} and 1×10^{-7} M); lanes 10 and 11, *ent*-(−)-DSA-CDPI$_2$ ((−)-**13**, 1×10^{-6} and 1×10^{-7} M); lanes 12 and 13, (+)-CDPI$_2$-DSA ((+)-**14**, 1×10^{-6} and 1×10^{-7} M).

Key to the study was the disclosure that both the natural and unnatural enantiomers of duocarmycin SA alkylate DNA efficiently.[5,15] One unique feature of **2** is the C6 methyl ester on the left-hand side of the alkylation subunit that complements the right-hand side linking amide. This feature provides the ability to introduce DNA binding subunits on either side of the duocarmycin SA alkylation subunit. In our studies, both the natural and unnatural enantiomers of the extended and reversed duocarmycin SA analogs **13** and **14** have been prepared.[19] It was anticipated that (+)- and *ent*-(−)-DSA-CDPI$_2$ (**13**) would exhibit a more extended 5-base-pair AT-rich alkylation selectivity than **2** and behave identically to (+)-CC-1065 (*i.e.*, 5′-AAAAA) and *ent*-(−)-CC-1065 (*i.e.*, 5′-AAAAA), respectively.[7] From the noncovalent binding model, the reversed agents, (+)- and *ent*-(−)-CDPI$_2$-DSA (**14**), were projected to exhibit a similar 5-base-pair AT-rich alkylation selectivity but one that extends in the atypical reverse direction from an alkylation site. Moreover, the predicted alkylation sites for the natural enantiomer of the reversed agent (+)-CDPI$_2$-DSA (**14**) coincide with those of *ent*-(−)-CC-1065 and *ent*-(−)-DSA-CDPI$_2$ (**13**). Similarly, the predicted alkylation sites for the unnatural enantiomer of the reversed agent **14** coincide with those of natural (+)-CC-1065 or (+)-DSA-CDPI$_2$ (**13**). In contrast, the models in which the natural enantiomer alkylation selectivity is independent of binding selectivity would require that the two natural enantiomers (+)-DSA-CDPI$_2$ (**13**) and (+)-CDPI$_2$-DSA (**14**) alkylate the same sites. Thus, the examination of the DNA alkylation selectivities of both enantiomers of **13** and **14** provides an unambiguous test of the two models with a definitive resolution.

(19) Boger, D. L.; Hertzog, D. L. Unpublished studies.



The results of the comparative examination of **13**−**14** are illustrated with the alkylation of w794 DNA[8] which contains a single high-affinity alkylation site for both (+)-CC-1065 (5′-AATTA) and *ent*-(−)-CC-1065 (5′-AATTT), Figure 1. Both (+)-DSA-CDPI$_2$ and *ent*-(−)-DSA-CDPI$_2$ (**13**) alkylate the single high-affinity alkylation sites of (+)- and *ent*-(−)-CC-1065 (**1**), respectively. The natural enantiomer of the reversed agent (+)-CDPI$_2$-DSA (**14**) was found to alkylate the high-affinity alkylation site observed with *ent*-(−)-DSA-CDPI$_2$ and *ent*-(−)-CC-1065.[20] This incorporation and conversion of a natural enantiomer of the duocarmycin SA alkylation subunit into an agent that exhibits the DNA alkylation selectivity of a typical unnatural enantiomer by simple reversal of the orientation of the DNA binding subunits of the agent is only consistent with a model where the AT-rich noncovalent binding selectivity and depth of minor groove penetration surrounding the alkylation site are controlling the sites of alkylation. The observations are inconsistent with alternative models based on the premise that the natural enantiomer alkylation subunit controls the alkylation selectivity.

Similarly, the unnatural enantiomer of the reversed agent *ent*-(−)-CDPI$_2$-DSA (**14**) was found to alkylate the same sites as (+)-DSA-CDPI$_2$ (**13**) and (+)-CC-1065, typical natural enantiomers (supplementary material, Figure 2). Thus, this reversal of the enantiomeric alkylation selectivity that accompanies the simple reversal of agent orientation is general and confirms that the same fundamental recognition features are operative for both the natural and unnatural enantiomers.

Acknowledgment. This work was assisted financially through the support of the National Institutes of Health (CA41986 and 55276) and the award of an ACS Organic Division Graduate Fellowship (D.S.J., 1993−1994) sponsored by Zeneca Pharmaceuticals.

Supplementary Material Available: PAGE figure of (+)- and *ent*-(−)-**13** and (+)- and *ent*-(−)-**14** illustrating w836 DNA alkylation (1 page). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JA9431768

(20) This identical DNA alkylation sequence selectivity for (+)-**14** and *ent*-(−)-**1**/**13** is general and is observed in all segments of DNA examined to date. Similarly, *ent*-(−)-CDPI$_2$-DSA (**14**) exhibited the same DNA alkylation sequence selectivity as (+)-CC-1065 (**1**) and (+)-DSA-CDPI$_2$ (**13**). Both of these features are illustrated with supplementary material Figure 2 (w836 DNA).