Chemical and Structural Comparison of *N*-BOC-CBQ and *N*-BOC-CBI: Identification and Structural Origin of an Unappreciated but Productive Stability of the CC-1065 and Duocarmycin SA Alkylation Subunits

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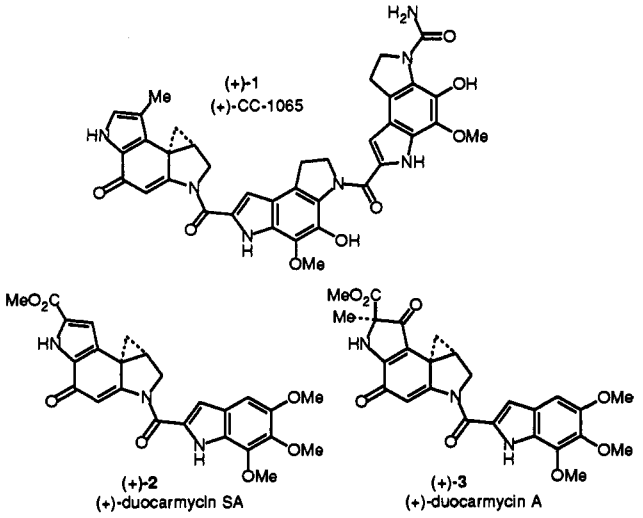
(+)-CC-1065 (**1**),[1] duocarmycin SA (**2**),[2] and duocarmycin A (**3**)[3] constitute the parent agents of a class of potent antitumor antibiotics that derive their biological properties through a sequence selective alkylation of DNA.[4-7] The now characteristic

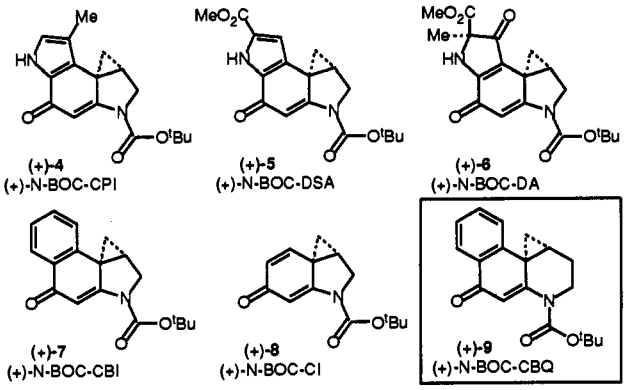


alkylation reaction has been shown to proceed by a stereoelectronically-controlled adenine N3 addition to the least substituted carbon of the activated cyclopropane within selected AT-rich sites of the minor groove.[8-12] Although the intracellular target for the agents has been shown to be DNA, the mechanism by which DNA alkylation translates into productive antitumor activity has remained elusive until the recent disclosure that apoptotic cell death is initiated by DNA alkylation in sensitive cell lines.[13] Two fundamental characteristics of the alkylation subunits **4-8**[11,14,15] have proven important in studies to date. The first is the stereoelectronically-controlled ring opening of the cyclopropane which dictates addition of a nucleophile to the least substituted carbon. The second is the relative rate of acid-catalyzed solvolysis representative of the functional reactivity of the agents and the demonstration of a fundamental linear relationship between chemical stability and cytotoxic potency.[11,15,16]

Table 1[a]

agent	k (s^{-1}, pH 3)	$t_{1/2}$ (h, pH 3)	IC_{50} (μM, L1210)
5	1.08×10^{-6}	177	0.006
7	1.45×10^{-6}	133	0.08
4	5.26×10^{-6}	37	0.3
6	1.75×10^{-5}	11	1
9	9.07×10^{-5}	2.1	4
8	1.98×10^{-2}	0.01	18

[a] Solvolysis rates (k) and half-lives ($t_{1/2}$) were determined spectrophotometrically (UV) at pH 3 in 1:1 CH_3OH–buffer, buffer = 4:1:20 (v:v:v) 0.1 M citric acid, 0.2 M Na_2HPO_4, and H_2O, as detailed in ref 15.



Herein, we detail a comparison of the chemical and structural properties of *N*-BOC-CBQ (**9**)[17] and *N*-BOC-CBI (**7**)[15] which confirm the stereoelectronic control of the solvolysis and alkylation reactions of **4–8**, reveal an unappreciated but important and productive functional stability of the CC-1065 and duocarmycin SA alkylation subunits and its structural origin, and verify the direct relationship between chemical stability and cytotoxic potency.

The solvolysis reactivity and cytotoxic activity of **9** and **4–8** are summarized in Table 1. In addition to the increased reactivity (63×) and diminished cytotoxic activity (50×) of **9** versus the closely related *N*-BOC-CBI (**7**), the solvolysis of **9** occurs with nucleophilic addition to both C10 and C10a while that of **4–8**[11,14]

(1) Chidester, C. G.; Krueger, W. C.; Mizsak, S. A.; Duchamp, D. J.; Martin, D. G. *J. Am. Chem. Soc.* **1981**, *103*, 7629.
(2) Ichimura, M.; Ogawa, T.; Takahashi, K.; Kobayashi, E.; Kawamoto, I.; Yasuzawa, T.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1990**, *43*, 1037.
(3) Takahashi, I.; Takahashi, K.; Ichimura, M.; Morimoto, M.; Asano, K.; Kawamoto, I.; Tomita, F.; Nakano, H. *J. Antibiot.* **1988**, *41*, 1915.
(4) Warpehoski, M. A.; Hurley, L. H. *Chem. Res. Toxicol.* **1988**, *1*, 315.
(5) Hurley, L. H.; Needham-VanDevanter, D. R. *Acc. Chem. Res.* **1986**, *19*, 230.
(6) Coleman, R. S.; Boger, D. L. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1989; Vol. 3, p 301. Boger, D. L. In *Advances in Heterocyclic Natural Products Synthesis*; Pearson, W. H., Ed.; JAI Press: Greenwich, CT, 1992; Vol. 2, p 1.
(7) Boger, D. L. *Chemtracts: Org. Chem.* **1991**, *4*, 329. Boger, D. L. in *Proc. Robert A. Welch Found. Conf. Chem. Res.: Chem. Front. Med.* **1991**, *35*, 137. Boger, D. L. *Pure Appl. Chem.* **1994**, *66*, 837.
(8) Hurley, L. H.; Reynolds, V. L.; Swenson, D. H.; Petzold, G. L.; Scahill, T. A. *Science* **1984**, *226*, 843. 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.
(9) 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.
(10) 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. Boger, D. L.; Yun, W. *J. Am. Chem. Soc.* **1993**, *115*, 9872.
(11) Boger, D. L.; Johnson, D. S.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 1635. Synthesis: Boger, D. L.; Machiya, K.; Hertzog, D. L.; Kitos, P. A.; Holmes, D. *J. Am. Chem. Soc.* **1993**, *115*, 9025.
(12) Boger, D. L.; Yun, W.; Terashima, S.; Fukuda, Y.; Nakatani, K.; Kitos, P. A.; Jin, Q. *BioMed. Chem. Lett.* **1992**, *2*, 759.
(13) Boger, D. L.; Johnson, D. S; Wrasidlo, W. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 631.
(14) Boger, D. L.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1431. Boger, D. L.; Munk, S. A.; Zarrinmayeh, H. *J. Am. Chem. Soc.* **1991**, *113*, 3980. Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661. Boger, D. L.; Wysocki, R. J., Jr.; Ishizaki, T. *J. Am. Chem. Soc.* **1990**, *112*, 5230.
(15) Boger, D. L.; Munk, S. A. *J. Am. Chem. Soc.* **1992**, *114*, 5487. Boger, D. L.; Ishizaki, T.; Wysocki, R. J., Jr.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *J. Am. Chem. Soc.* **1989**, *111*, 6461. Boger D. L.; Ishizaki, T.; Kitos, P. A.; Suntornwat, O. *J. Org. Chem.* **1990**, *55*, 5823. Boger, D. L.; Yun, W.; Teegarden, B. R. *J. Org. Chem.* **1992**, *57*, 2873. Boger, D. L.; Ishizaki, T. *Tetrahedron Lett.* **1990**, *31*, 793. Boger, D. L.; Munk, S. A.; Ishizaki, T. *J. Am. Chem. Soc.* **1991**, *113*, 2779.
(16) Boger, D. L.; Yun, W. *J. Am. Chem. Soc.*, in press.
(17) Boger, D. L.; Mesini, P. Manuscript in preparation.

0002-7863/94/1516-6461$04.50/0

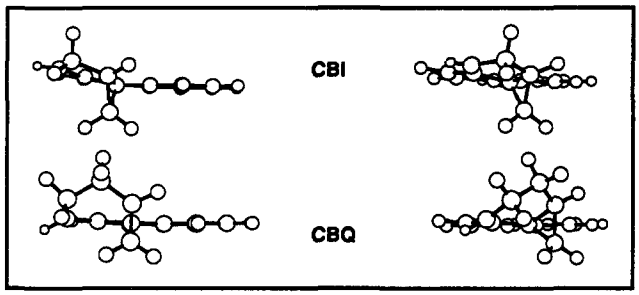


Figure 1. End-on (left) and 30–45° rotation views (right) of the X-ray structures of CBI[15] and *N*-BOC-CBQ.

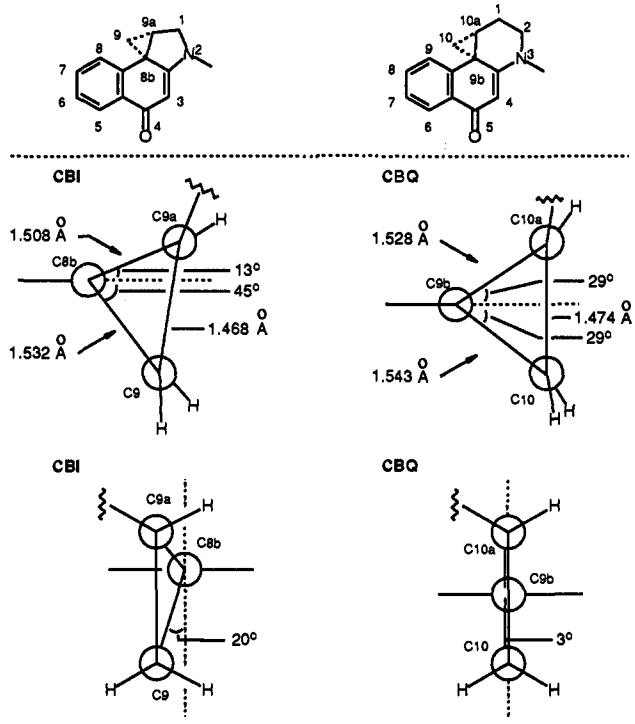


Figure 2. Stick models of the side and 90° rotation view of the cyclopropane of CBI and CBQ highlighting the idealized overlap and alignment of the CBQ cyclopropane with the cyclohexadienone π-system.

including **7**[15] occurs exclusively or near exclusively at the least substituted cyclopropane carbon (exclusively at C9 for **7**).[17]

The X-ray structure of *N*-BOC-CBQ (**9**) provided key structural insights into the CBQ solvolysis reactivity and regioselectivity. It is clear from the X-ray structures of the CPI subunit taken from CC-1065[1] and of the closely related CBI subunit itself[15] that the bent orbital[18] of the cyclopropane bond extending to the least substituted carbon is nearly perpendicular to the plane of the cyclohexadienone and consequently overlaps[19] nicely with the developing π-system of the solvolysis product phenol (Figures 1 and 2). In contrast, the cyclopropane bond extending to the tertiary carbon is nearly in the plane of the cyclohexadienone, and its orbital is nearly orthogonal to the π-system of the product phenol. Thus, opening of the cyclopropane occurs under stereoelectronic control with addition of a nucleophile to the least substituted carbon, and the stereoelectronic control overrides the intrinsic electronic preference for ring expansion.

In contrast, the *N*-BOC-CBQ X-ray structure exhibits unique characteristics. Most notably, the cyclopropane is ideally conjugated[19] with the cyclohexadienone π-system, and the plane defined by the cyclohexadienone ideally bisects the cyclopropane with the bonds extending to the secondary and tertiary carbons equally aligned with the π-system. Thus, the two available cyclopropane bonds of **9** are equally aligned for cleavage, and addition to both is experimentally observed.[17]

More surprising was the unusually rapid solvolysis of *N*-BOC-CBQ (**9**) and important insights into this enhanced reactivity are clear from the X-ray structures. Although they reside in a cyclopropane fused to an inherently less strained six- versus five-membered ring, the bond lengths of the CBQ C9b–C10a (1.528 Å) and C9b–C10 (1.543 Å) bonds are longer than those found in CBI (1.508 and 1.532 Å, respectively) and nicely reflect the enhanced cleavage reactivity,[20,21] Figure 2. This lengthening of the cyclopropane bonds may be attributed to the idealized conjugation or π-delocalization of both the C9b–C10a and C9b–C10 cyclopropane bonds with the cyclohexadienone π-system.[19,20] Contributing to this enhanced conjugation is the ideal geometrical alignment of C10 and C10a with C9b, C5, and the carbonyl oxygen. For CPI and CBI, the constraints of the fused five-membered ring place its C9 and C9a at a 20–25° angle offset from this plane and prevent ideal alignment and overlap of either the C8b–C9a or C8b–C9 bond with the cyclohexadienone π-system. This idealized CBQ cyclopropane conjugation[19] with the cyclohexadienone π-system results in the observed longer bond lengths,[20] weaker bond strengths, and higher solvolysis reactivity.[21,22]

Thus, the geometrical constraints of the fused five-membered ring found in the alkylation subunits of CC-1065 and the duocarmycins impose the stereoelectronic control on the nucleophilic cleavage of the cyclopropane requiring addition to the least substituted carbon. In addition, the nonideal alignment and overlap of the cyclopropane with the cyclohexadienone π-system found in CPI, CBI, or DSA result in productively diminished electrophilic reactivity. The fundamental insight derived from these comparisons is not the surprising solvolysis reactivity of *N*-BOC-CBQ, but rather the surprising stability of the CBI, CPI, and DSA alkylation subunits. In spite of the structural features that intuitively suggest high reactivity, the latter agents are uncharacteristically stable. This unusual but productive stability is imposed by fusion of the activated cyclopropane to the five-membered ring, which constrains it to a nonideal alignment and overlap with the cyclohexadienone π-system.

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Supplementary Material Available: Details of the X-ray structure determination of **9** (17 pages); observed and calculated structure factors for **9** (4 pages). 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.

(18) Walsh, A. D. *Nature (London)* **1947**, *159*, 712. Sugden, T. M. *Nature (London)* **1947**, *160*, 367. Coulson, C. A.; Moffitt, W. E. *J. Chem. Phys.* **1947**, *15*, 151. Coulson, C. A.; Moffitt, W. E. *Philos. Mag.* **1949**, *40*, 1.
(19) Hoffmann, R.; Davidson, R. B. *J. Am. Chem. Soc.* **1971**, *93*, 5699.
(20) Clark, T.; Spitznagel, G. W.; Klose, R.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1984**, *106*, 4412.
(21) Baird, R.; Winstein, S. *J. Am. Chem. Soc.* **1963**, *85*, 567.
(22) Danishefsky, S. *Acc. Chem. Res.* **1979**, *12*, 66.