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A Comparative Study Of The Solvolysis Reactivity, Regioselectivity, And Stereochemistry Of The Duocarmycin A And SA Alkylation Subunits

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Abstract. The comparative solvolysis reactivity, regioselectivity, and stereochemistry of *N*-BOC-DSA (**4**) and *N*-BOC-DA (**5**), simple derivatives of the DNA alkylation subunits of duocarmycin SA and A, are detailed. Most important of the observations is the substantially greater reactivity of **5** versus **4** (16×), the modest regioselectivity of both **4** (6.5–4:1) and **5** (1.5:1), and the establishment that the abnormal ring expansion solvolysis proceeds by S_N2 addition to the activated cyclopropane with clean inversion of the reacting center stereochemistry.
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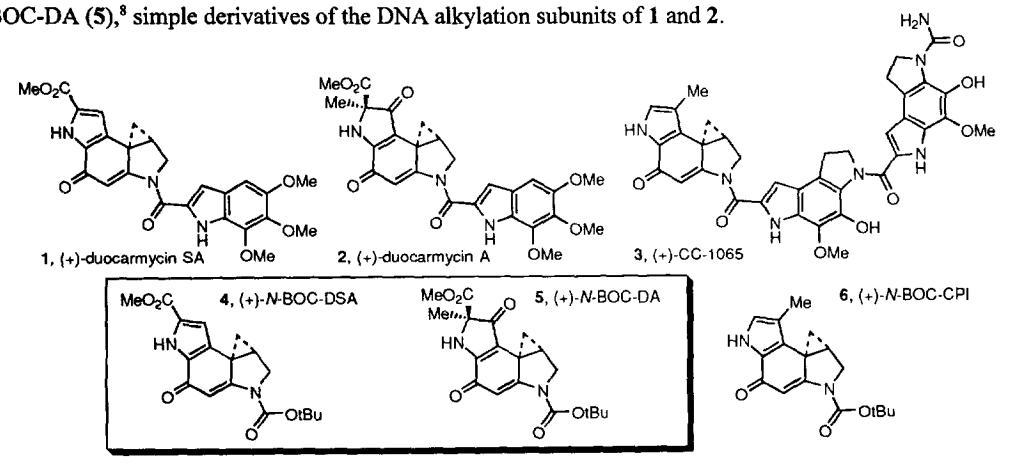
Duocarmycin SA (**1**)[1] and duocarmycin A (**2**)[2] constitute the parent members of a class of potent antitumor antibiotics[3] related to CC-1065 (**3**)[4] that derive their biological properties through a sequence selective alkylation of duplex DNA.[5,6] Substantial efforts have been devoted to defining the characteristics of their DNA alkylation reactions, to determining the origin of their DNA alkylation selectivity, to establishing the link between DNA alkylation and the ensuing biological properties, and to defining fundamental principles underlying the relationships between structure, functional reactivity, and biological properties.[5,6] Two fundamental characteristics of the alkylation subunits have proven important in these studies. The first is the regioselectivity of the acid-catalyzed ring opening of the cyclopropane which occurs with preferential addition of a nucleophile to the least substituted cyclopropane carbon. The second is the relative rate of acid-catalyzed solvolysis which has been found to reflect their functional reactivity and follow a direct relationship between stability and cytotoxic potency. Herein, we detail a study of the acid-catalyzed solvolysis reactivity, regioselectivity, and stereochemistry of *N*-BOC-DSA (**4**)[7] and *N*-BOC-DA (**5**),[8] simple derivatives of the DNA alkylation subunits of **1** and **2**.



Reactivity. The solvolysis reactivity of **5** was established spectrophotometrically by UV at pH 3 (50% CH_3OH–buffer, buffer = 4:1:20 v/v/v 0.1 M citric acid, 0.2 M Na_2HPO_4, H_2O) measuring the disappearance of the long-wavelength absorption band at 336 nm following a procedure previously detailed for **4**.[7] As expected, *N*-

BOC-DA (**5**, $t_{1/2}$ = 11 h, $k = 1.75 \times 10^{-5}$ s^{-1}) proved to be more reactive than the alkylation subunit of CC-1065, *N*-BOC-CPI (**6**, $t_{1/2}$ = 37 h), or duocarmycin SA ($t_{1/2}$ = 177 h),[7] Table 1. Thus, *N*-BOC-DA proved to be 3.4× more reactive than *N*-BOC-CPI and 16× more reactive than *N*-BOC-DSA. The magnitude of this latter difference is substantial and significant and can be attributed to a combination of the diminished gain in delocalization energy upon aromatization as well as the electronic deactivation solvolysis by the conjugated C6 methyl ester of **4**.

Table 1. Solvolysis Reactivity

	k (s^{-1}, pH 3)	$t_{1/2}$ (pH 3)	IC_{50} (L1210)
4	1.08×10^{-6}	177 h	6 nM
5	1.75×10^{-5}	11 h	2000 nM
6	5.26×10^{-6}	37 h	330 nM

This relative reactivity closely follows the trends established for the biological potency[7,8] and DNA alkylation efficiency[9–11] of the agents where the more stable agents exhibit the more effective properties. In addition, the observation of a minor guanine N3 alkylation with duocarmycin A[12] that is not detected even under forcing conditions with duocarmycin SA[10] may be attributed to this greater reactivity of **2**. Similarly, the greater ease of reversibility of the duocarmycin SA DNA alkylation reaction[10,11] may be attributed to the lower degree of adduct stability resulting from its lower reactivity. Thus, consistent with its relative reactivity, the DNA alkylation reaction of **2** is less efficient, less selective among the available sites, and less reversible than that of **1**.

Solvolysis Regioselectivity, Stereochemistry, and Mechanism of Acid-catalyzed Nucleophilic Addition. A study of the acid-catalyzed nucleophilic additions to **4** and **5** established that solvolysis preferentially occurs with cleavage of the C7b–C8 bond with addition of a nucleophile to the least substituted C8 cyclopropane carbon versus cleavage of the C7b–C8a bond with ring expansion and addition to C8a. The latter cleavage would place a developing partial positive charge on a preferred secondary versus primary center and, with preceding agents, this preference was overridden by the inherent stereoelectronic control of the reaction regioselectivity.

Preparative acid-catalyzed addition of CH_3OH to *N*-BOC-DSA (0.12 equiv CF_3SO_3H, 0.01 M in CH_3OH, 0 or 25 °C, 1–3 h, 88–93%) cleanly provided two products **7** and **8** in a 6.5–4:1 ratio with the greater selectivity observed at 0 versus 25 °C (equation 1). Similarly, solvolysis of **4** (0.24 equiv CF_3SO_3H, 0.01 M in 20% H_2O–THF, 25 °C, 48 h, 95–96%) provided a 6:1 ratio of **9** to **10**. The mechanistic course of the reaction was established by subjecting both racemic and natural (+)-**4** to the acid-catalyzed methanolysis or solvolysis to provide mixtures of **7** and **8** or **9** and **10**, respectively. Resolution on a Diacel ChiralCel AD HPLC column separated both enantiomers of the two reaction products and those derived from optically active (+)-**4** were found to consist of a single enantiomer. This is illustrated in Figure 1 for the addition of CH_3OH. Although the generation of a single enantiomer of **7** would be consistent with either a S_N1 or S_N2 ring opening reaction, the generation of a single enantiomer of **8** unambiguously establishes that the abnormal ring expansion cleavage of the internal cyclopropane bond proceeds with clean inversion of the reaction center stereochemistry in a S_N2 ring opening reaction.

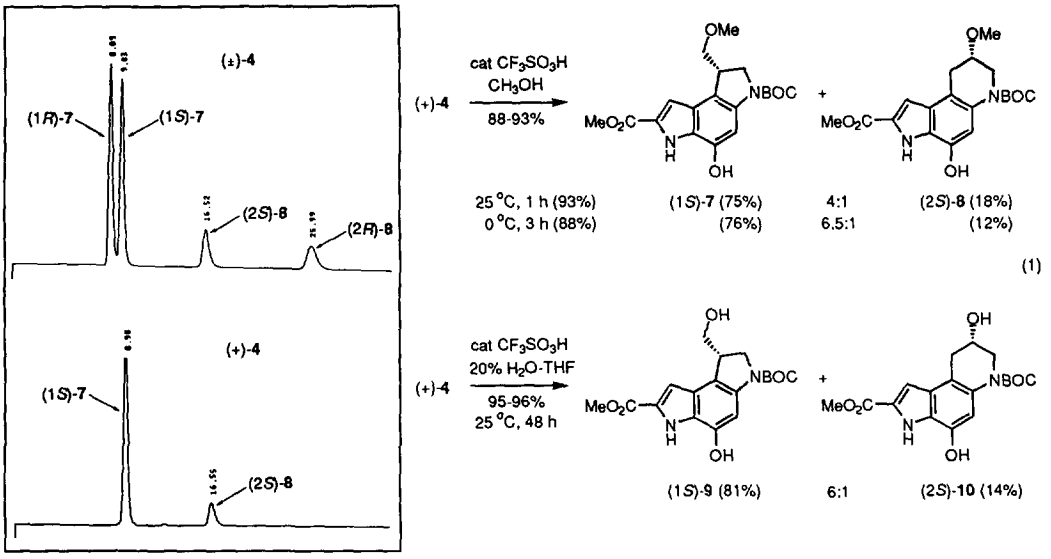
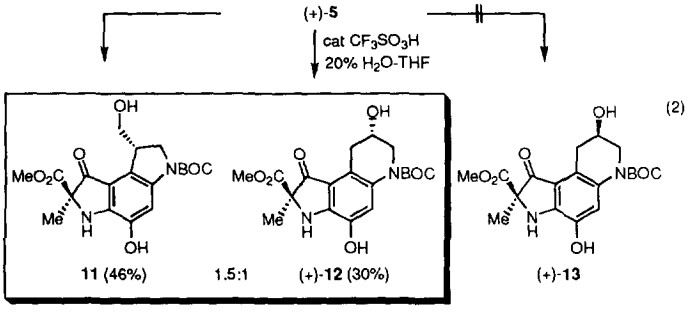


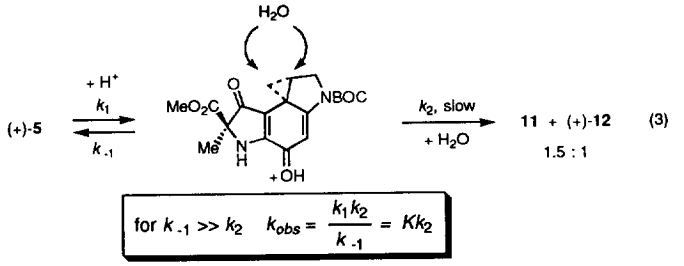
Figure 1. Acid-catalyzed CH_3OH addition to (±)-4 (top) and (+)-4 (bottom). ChiralCel AD HPLC (0.46 x 25 cm), 0.75 mL/min, 15% 2-PrOH/hexane.

Preparative solvolysis of (+)-**5** (20% H_2O–THF, 0.1 equiv CF_3SO_3H, 25 °C, 24 h) cleanly provided both the normal solvolysis product **11** (46%) and the abnormal ring expansion solvolysis product (+)-**12**[13] (30%) in a 1.5:1 ratio (equation 2). Moreover, no trace of the diastereomer (+)-**13**[13] was detected indicating that under these conditions, the ring expansion solvolysis occurs with clean inversion of the reacting center stereochemistry indicative of S_N2, not S_N1, addition to the activated cyclopropane.



These results are in agreement with the similarly unambiguous observations made in our studies of the CBQ-based agents[14] but contrasts the conclusions reached in a study of the CPI solvolysis where a free carbocation has been invoked to explain the observation of minor ring expansion products.[15] The results herein and those of our related study,[14] suggest that this later work should be reexamined using a more definitive experimental basis for assessing the stereochemical course of the reaction. This is fully consistent with kinetic studies of the acid-catalyzed nucleophilic addition conducted on related systems[15,16,17] where the rate of reaction exhibits a first order dependence on both the acid concentration (pH) as well as the nucleophile indicative of a mechanism involving rapid and

reversible C4 carbonyl protonation followed by a slow, rate determining S_N2 nucleophilic attack on the activated cyclopropane. This is illustrated in equation 3 for duocarmycin A.



X-ray Structure of *N*-BOC-DSA: Correlation with Solvolysis Reactivity and Regioselectivity. The single-crystal X-ray structure determination of *N*-BOC-DSA (**4**) was conducted on crystals grown from hexane/ /THF/EtOH/CH_3OH (2:1:1:1)[18] in expectations that its comparison with prior structures would provide insights into the origin of the solvolysis regioselectivity (Figure 2). To date, agents incorporating the CBI nucleus have exhibited the greatest regioselectivity (≥20:1)[16,17,19] for the cyclopropane ring opening reaction including the exceptionally reactive F_2CBI (≥9:1)[20] and more modest selectivity has been observed with CPI derivatives including CC-1065 (*ca.* 4:1),[15] duocarmycin A (1.5–1:1),[12] duocarmycin SA (6–4:1), or CBQ derivatives (3:2).[14] The distinguishing features controlling the regioselectivity of addition appear to be the relative stereoelectronic alignment of the two cyclopropane bonds available for cleavage and the relative reactivity of the agent. Within a class of agents whose cyclopropane alignment with the π-system would be expected to be similar due to structural constraints, the regioselectivity

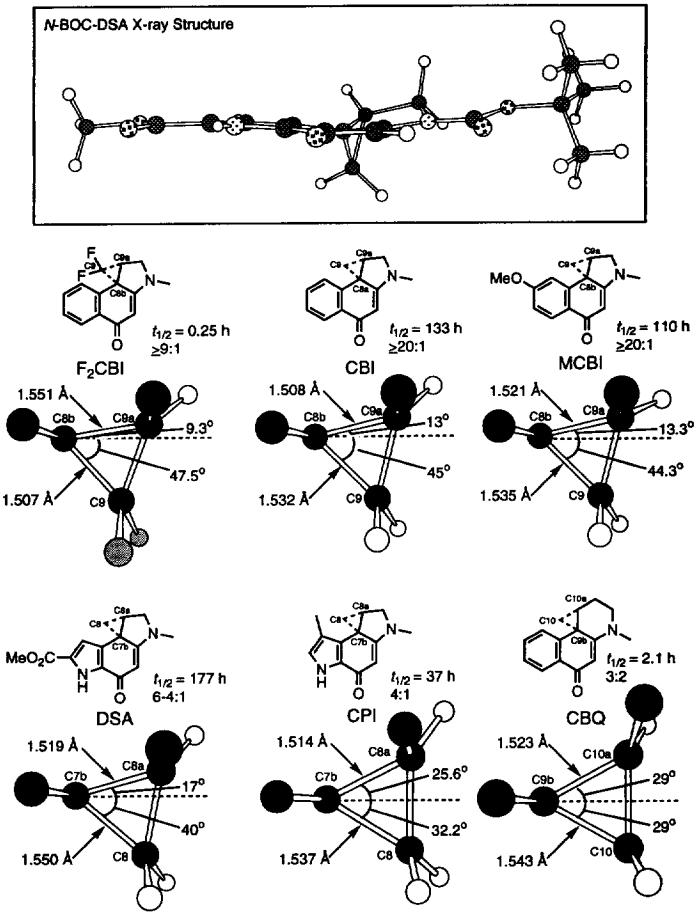


Figure 2. Stick models of the side view of the activated cyclopropanes of F_2CBI, CBI, MCBI, DSA, CPI and CBQ illustrating data taken from the X-ray crystal structures. Solvolysis half-life (pH 3) and regioselectivity of the *N*-BOC derivatives are given.

nicely follows the relative reactivity with the more stable agents providing the more selective reaction: *eg.* *N*-BOC-DSA (6–4:1) > CPI (4:1)[15] > *N*-BOC-DA (3:2). However, this fails to hold true when comparing between classes of agents: *eg.* *N*-BOC-CBI (≥20:1) versus *N*-BOC-DSA (6–4:1). Thus, additional important factors contribute to this reaction regioselectivity. In the comparisons that can be made from the available structural studies of CPI,[21] CBI,[19] MCBI,[17] CBQ,[14] and F_2CBI,[20] the degree of selectivity also reflects the relative degree of stereoelectronic alignment of the two available cyclopropane bonds and this alone could account for the reaction regioselectivity (Figure 2). Similar structural studies on *N*-BOC-DA are in progress and should prove revealing.

DNA Alkylation Regioselectivity. The observation of exclusive adenine N3 addition to the C8 cyclopropane carbon in the DNA alkylation studies of **1**[10] and **2**[9] is not consistent with expectations that the inherent acid-catalyzed nucleophilic addition regioselectivity solely controls the DNA alkylation regioselectivity.[14] This exclusive DNA alkylation regioselectivity was not only observed in our studies with **1** or **2** and their enantiomers but is general with all agents examined to date that undergo solvolysis with a mixed regioselectivity including the CPI-based agents and CC-1065 (4:1 regioselectivity)[22,23] and the CBQ-based agents (3:2 regioselectivity). Examination of each of these classes of agents has led only to detection of adducts derived from adenine N3 addition to the least substituted cyclopropane carbon. Moreover, each of these studies also quantitated the adduct formation and, in the case of duocarmycin A (86–92%),[9] duocarmycin SA (95–100%),[10] CC-1065 (> 85%),[22] and the CBQ-based agents (>75%),[14] established that the regioselectivity of the DNA alkylation reaction is greater than that of solvolysis. Although several explanations may be advanced for these observations,[14] the two most prominent are preferential adoption of binding orientations that favor normal adenine N3 addition (proximity effects) and the significant destabilizing torsional strain and steric interactions that accompany the abnormal addition especially when the reactants are restricted to the relative orientations found when bound in the minor groove. Figures illustrating these effects have been disclosed in our work and we would suggest that this latter subtle effect is most substantial.[8,14] Consequently, the clean regioselectivity of the characteristic adenine N3 alkylation reaction benefits not only from stereoelectronic control but additional important subtle effects that complement the normally observed regioselectivity as well.

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