Shape-Dependent Catalysis: Insights into the Source of Catalysis for the CC-1065 and Duocarmycin DNA Alkylation Reaction

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Introduction

CC-1065 and the duocarmycins (**1**–**3**, Figure 1) are the parent members of a potent class of antitumor antibiotics which derive their biological properties through the sequence-selective alkylation of DNA.[1,2] Although unreactive toward conventional nucleophiles at pH 7, the DNA alkylation reactions by **1**–**3** are exceptionally facile, proceeding in <1 h at 4–25 °C.

Consistent with the idea that the DNA alkylation sequence selectivity originates in the noncovalent binding selectivity of the agents (shape-selective recognition),[1,3–10] recent studies have established that the catalysis is derived from a DNA binding-induced conformational change in the agent which activates it for nucleophilic attack.[10,11] This conformational change disrupts the ability of the N² nitrogen to convey stability to the alkylation subunit through vinylogous amide conjugation. Further, we have suggested that this binding-induced activation is a general consequence of the forced adoption of a helical conformation upon AT-rich minor groove binding. Since this conformational change is dependent upon the shape of the minor groove and greatest within the narrower, deeper
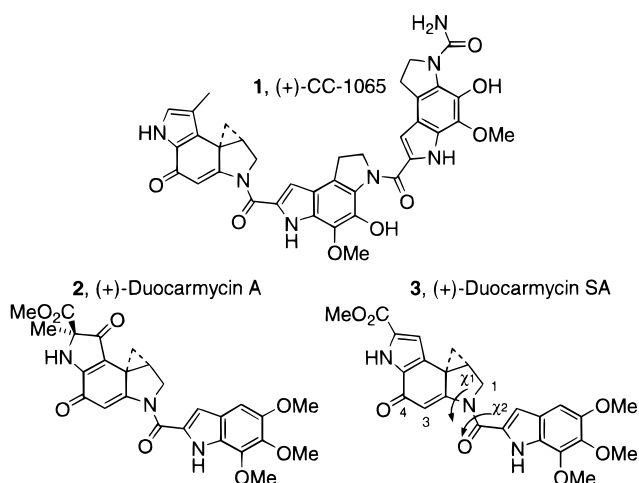
DNA Alkylation Reaction

• **Shape-dependent catalysis**: Preferential activation in AT-rich minor groove, binding-induced twist greatest in narrower, deeper AT-rich minor groove

• **Shape-selective recognition**: Preferential binding in AT-rich minor groove, noncovalent binding greatest in narrower, deeper AT-rich minor groove

• DNA bound agent adopts helical confirmation: twist ca. 45°
• DNA bound agent maintains full amide: $\chi 2$ ca. 0°
• Vinylogous amide conjugation diminished: $\chi 1$ ca. 20–40°
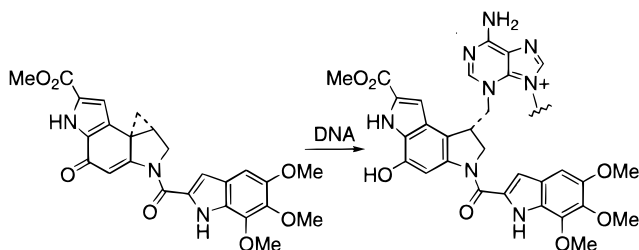• Cyclohexadienone structure destabilized

FIGURE 1. Structures of CC-1065 and the duocarmycins.

AT-rich minor groove, this leads to preferential activation within the AT-rich noncovalent binding sites. This ground-state destabilization of the agents upon binding to DNA represents a beautiful example of shape-dependent catalysis and is the key to the ability of this family of compounds to selectively alkylate DNA.

Origin of Stability: C-Ring Analogues and the Vinylogous Amide

We have examined a series of analogues of the CC-1065 and duocarmycin alkylation subunits in efforts to define the fundamental relationships between structure, chemical reactivity, and the corresponding biological properties (Figure 2). Replacing the A-ring pyrrole found in **1**–**3**[12–14] with a benzene ring allowed for a simplified synthesis of modified and effective alkylation subunit analogues. The first example of such simplified derivatives was CBI (**6**),[15] which was found to be more stable (4×), biologically more potent (4×), and synthetically more accessible than CPI

FIGURE 2. Analogue structures examined.

Scheme 1. Hydrolysis and Solvolysis of Duocarmycin Analogues

Table 1. X-ray Structures and Reactivity: Structure/Reactivity Correlations

X-ray bond lengths	N-CO$_2$Me-CBI	N-BOC-CBQ	N-CO$_2$Me-CNA
a	1.521	1.528	1.565
b	1.544	1.543	1.525
c	1.390	1.415	1.428
d	1.372	1.379	1.357
X-ray dihedral angles			
χ_1	6.9°	34.2°	86.4°
χ_2	4.5°	8.7°	3.9°
solvolysis reactivity (BOC derivatives)			
$t_{1/2}$, (pH 3)	133 h	2.1 h	0.028 h
$t_{1/2}$, (pH 7)	stable	544 h	2.1 h

X-ray bond lengths	CBI	CBQ	CNA
a	1.508	1.525	1.543
b	1.532	1.539	1.551
c	1.337	1.336	1.376
solvolysis reactivity			
$t_{1/2}$, (pH 3)	930 h	91 h	0.62 h
$t_{1/2}$, (pH 7)	stable	stable	563 h

(**5**), the alkylation subunit of CC-1065. When coupled to appropriate DNA binding subunits, CBI displayed a sequence selectivity identical to that of the natural products, a comparable or greater DNA alkylation efficiency, and a faster alkylation rate. With these properties, CBI provided an ideal template on which further studies could be conducted.

Central to the issue of catalysis was the inherent stability of the alkylation subunits. While **1**−**3** and related CBI-based analogues are exceptionally stable and exhibit no solvolysis reactivity at pH 7, they react rapidly with DNA (<1 h), it was recognized that the nitrogen in the alkylation subunits provided vinylogous amide stabilization to the spirocyclopropylcyclohexadienone, but the quantitative assessment of this stabilization had not been established. Perhaps the earliest qualitative evidence illustrating the impact of the vinylogous amide is found in the hydrolysis of the linking amide, which can be accomplished under unusually mild conditions with LiOH (25 °C, <1 h), and a representative example is provided in Scheme 1.[16] Similarly, the related *p*-quinonemethide **10**, also bearing a vinylogous amide, proved unusually stable and isolable.[17] By contrast, it was known from the studies of Baird and Winstein that simple spirocyclopropylcyclohexadienones lacking this nitrogen (e.g., **11**), were highly reactive.[18] To establish a more

quantitative assessment of the stability of the alkylation subunits, a series of analogues with modifications to the fused five-membered C-ring were prepared.
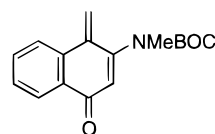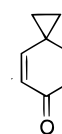
CBIn (**9**)[19] was prepared to establish directly the extent and ramifications of the vinylogous amide stabilization. The comparison of CBIn (**9**) with CBI revealed that the presence of N^2 and the vinylogous amide increases stability 3200× at pH 3 and >10^3−10^4× at pH 7. This was further documented with the synthesis and evaluation of C-ring expanded analogues, CBQ (**7**)[20] and CNA (**8**),[21] for which X-ray structures were obtained. The reactivity increases that occur in the series CNA > CBQ > CBI are the consequence of the relative extent of vinylogous amide conjugation. This feature can be observed directly in their X-ray structures with the diagnostic shortening of the C^{3a}−N^2 bond length (bond c, Table 1). *N*-Acylation (e.g., *N*-CO$_2$-Me-CBI versus CBI) reduces the vinylogous amide conjugation, lengthens bond c, and results in a substantial increase in reactivity, and this trend is observed with each of the three sets of agents (Table 1 and Figure 3).

FIGURE 3. Effect of *N*-acylation on structure and reactivity.

Accompanying this reduction in the vinylogous amide conjugation is an increase in length of the reacting cyclopropane bonds and a readjustment of the cyclopropane alignment to a more idealized geometry with respect to the cyclohexadienone π-system. This illustrates that both the cyclopropane conjugation and its inherent reactivity increase as the cross-conjugated vinylogous amide π-overlap is diminished.

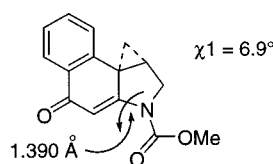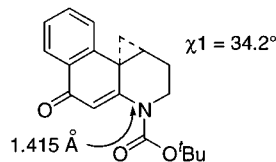More importantly, these same trends are observed within the *N*-acyl series. As one moves across the series of *N*-CO$_2$Me-CBI, *N*-BOC-CBQ, and *N*-CO$_2$Me-CNA, the length of bond c increases (1.390, 1.415, and 1.428 Å), diagnostic of the increasing loss of vinylogous amide stabilization (Table 1 and Figure 4). Correspondingly, the length, conjugation, and reactivity of the scissile cyclopropane bonds increase, tracking with the relative reactivity of the agents. Accompanying these changes and responsible for this loss of vinylogous amide conjugation is an increase in the χ_1 dihedral angle, also diagnostic of the vinylogous amide stabilization (0° = maximal orbital overlap). Throughout this series, the χ_2 dihedral angle is ca. 0°, illustrating the preferential maintenance of the carbamate versus vinylogous amide conjugation.
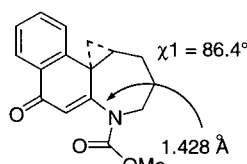
The exception to this correlation is in the NH series with CBI and CBQ and can be attributed to the perfect geometrical alignment of the CBQ cyclopropane, not accessible to CBI, which increases cyclopropane conjugation and corresponding reactivity. That is, CBQ is more reactive (ca. 10×) than CBI because of its idealized cyclopropane alignment. In contrast to CBI and CBQ, which have similar c bond lengths but different cyclopropyl alignments, CBQ and CNA have similar cyclopropane alignments but substantially different χ_1 dihedral angles and different c bond lengths of 1.336 versus 1.376 Å, respectively. This difference, diagnostic of the extent of vinylogous amide conjugation, is accompanied by an even larger increase in the CNA scissile cyclopropane bond lengths, indicative of a greater degree of conjugation, and

FIGURE 4. Effect of χ_1 dihedral angle on structure and reactivity.

accounts for over a 100-fold difference in reactivity. Thus, the degree of cyclopropane conjugation and its resulting reactivity are related not only to the extent of vinylogous amide conjugation but also to its accessible geometrical alignment.

The pH Dependence of DNA Alkylation and Model Reactions Requires a pH-Independent Alkylation Reaction

The pH dependence of the rate of DNA alkylation for duocarmycin SA over a physiologically relevant range has been examined in detail.[10,22] The rate of DNA alkylation was found to increase only slightly with decreasing pH, and the rate change was remarkably small (<2× over 2 pH units), inconsistent with a first-order dependence of acid concentration (Figure 5). Moreover, between pH 7 and 8, no rate dependence on acid concentration was observed, establishing that the DNA alkylation reaction of duocarmycin SA is not acid-catalyzed.

While **1–6** are not sufficiently reactive at pH > 3 to allow study of their pH rate dependence, the reactivity of CBQ, CNA, and CBIn approximates that required of the DNA alkylation reaction and allows more expansive pH rate profiles to be studied (Figure 5).[23] Importantly, X-ray

N-BOC Derivative Data
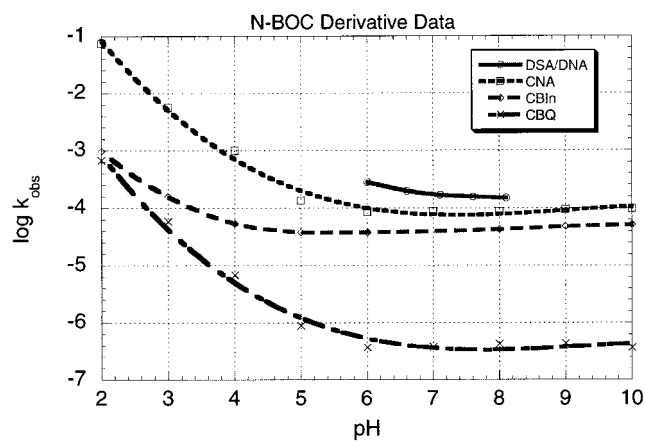
Duocarmycin SA at w794 High Affinity Site

pH	k (s^{-1})	k_{rel}
6.0	2.83 x 10^{-4}	1.89
6.6	1.99 x 10^{-4}	1.32
7.1	1.69 x 10^{-4}	1.12
7.6	1.58 x 10^{-4}	1.05
8.1	1.50 x 10^{-4}	1.00

- Acid-catalyzed at pH $\leq$ 5
- Uncatalyzed reaction dominates at pH $\geq$ 5–6
- DNA alkylation reaction is not acid catalyzed

FIGURE 5. pH rate profiles of solvolysis and the DNA alkylation reaction.

analysis of the series **6**−**8** revealed corresponding structural and reactivity changes that we suggest are analogous to those accompanying the DNA binding-induced conformational change and activation of **1**−**3**. Thus, we view the pH rate profiles of CBQ, CNA, and CBIn as being representative of those of the DNA-bound and activated structures of **1**−**3**. The solvolysis of **7**, **8**, and **9** exhibits a first-order rate dependence on hydronium ion concentration only in the region of pH 2−5, where the reaction is acid-catalyzed, and the uncatalyzed S$_N$2 reaction rate dominates at pH > 5−6. Just as surprising, the rates of the pH 7 reactions were independent of buffer concentrations, indicating not only that the reactions are not specific acid-catalyzed but also that they appear not to be general acid-catalyzed.[21,23] This established that the assumed requirement for acid catalysis of the DNA alkylation reaction is not necessary, which is consistent with the lack of an experimental pH dependence and indicates that reaction models[2,24,25] or alkylation selectivity models[2,26] based on pH 2−3 studies and a requirement for acid catalysis are unlikely to be accurate. More importantly, it illustrates that the structural and corresponding reactivity features embodied in **7**−**9**, which we suggest are analogous to those accompanying the DNA binding-induced conformational change in **1**−**3**, are sufficient to provide activation for an uncatalyzed S$_N$2 nucleophilic attack independent of pH.

Table 2. Solvolysis Reactivity

N-BOC-**12**   *N*-PIV-**12**   *N*-Me-**12**

agent	k (s^{-1}, pH 3)	$t_{1/2}$ (h, pH 3)	regioselectivity
BOC-**12**	7.28 x 10^{-3}	95 s	>20 : 1
PIV-**12**	6.41 x 10^{-3}	108 s	nd
Me-**12**	9.00 x 10^{-3}	77 s	>20 : 1

agent	k (s^{-1}, pH 7)	$t_{1/2}$ (h, pH 7)	regioselectivity
BOC-**12**	6.79 x 10^{-4}	17 min	>20 : 1
PIV-**12**	8.88 x 10^{-4}	13 min	nd
Me-**12**	4.20 x 10^{-5}	275 min	>20 : 1
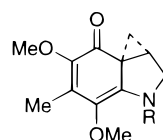
Reactivity:

R = Me > BOC > PIV (pH 3, H$^+$ catalyzed)
R = Me < BOC < PIV (pH 7, uncatalyzed)

Reversal of Inherent Reactivities of Model Alkylation Subunit Derivatives at pH 3 (Acid-Catalyzed) and pH 7 (Uncatalyzed)

Consistent with this dominant uncatalyzed reaction at pH > 5−6, the reactivity order of a series of derivatives of a reactive alkylation subunit inverted at pH 7 versus pH 3 (Table 2).[27] Thus, *N*-PIV-**12** was the most stable derivative at pH 3, followed in order by *N*-BOC-**12** and *N*-Me-**12**. For this very reactive alkylation subunit, the most easily protonated compound exhibits the greater reactivity at pH 3. At pH 7, where the uncatalyzed reaction dominates, the order is reversed, and *N*-Me-**12** is the most stable agent with the greatest degree of vinylogous amide stabilization, followed in order by *N*-BOC-**12** and *N*-PIV-**12**, in which the vinylogous amide conjugation is partially and progressively diminished by the carabamate and amide conjugation, respectively. This simple observation has significant ramifications. The reverse order of reactivity at pH 7 for the very reactive alkylation subunit is consistent only with the switch from an acid-catalyzed reaction (pH 3) to one which is uncatalyzed (pH 7). This observation, made only very recently, is expected to extend to the reactive derivatives of CNA and CBQ, and such studies have been initiated.

Critical Role of the Linking Amide

The culmination of these studies was the synthesis and evaluation of **13**, in which the linking N^2 amide was replaced with a methylene group.[28] Its examination potentially could distinguish catalysis derived from disruption of the vinylogous amide by a DNA binding-induced conformational change. This source of catalysis would be lost with **13**, rendering it ineffective, whereas acid catalysis might be enhanced by the increased basicity of the C4 carbonyl.

Table 3. Critical Impact of the Linking Amide
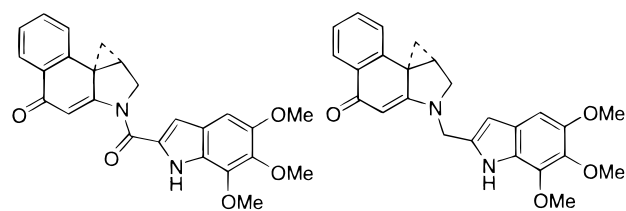
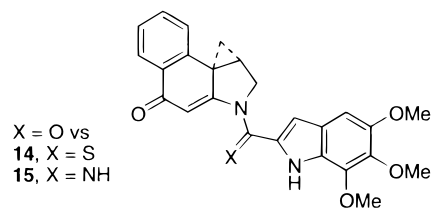|	CBI-TMI	13
$t_{1/2}$ (pH 1)	nd	80 h
$t_{1/2}$ (pH 2)	12.5 h	824 h
$t_{1/2}$ (pH 3)	133 h	30000 h
IC$_{50}$ (L1210)	30 pM	1.4 µM
DNA alkylation	$10^{-6} - 10^{-7}$	$> 10^{-1}$ M

- Demonstrates critical role of linking amide for
  - DNA alkylation rate and efficiency
  - Biological activity
  - Reactivity and catalysis

Table 4. Impact of Modifications to the Linking Amide

X = O vs
14, X = S
15, X = NH

	X = S	X = O	X = NH
IC$_{50}$ (L1210, nM)	1.0	0.02	0.75
$t_{1/2}$ (solvolysis, pH 3, h)	160	230	12 (hydrolysis)
Rotation Barrier (kcal/mol)	20.7	18.1	12.8
Hammett σ_p (-NHC(=X)CH$_3$)	0.00	0.12	-0.09 (=NH$_2^+$)

- Preferential linking amide conjugation (X = S, O)
- Preferential vinylogous amide conjugation (X = NH)
- Linking amide (X = O) represents optimal balance between competing amide (reactivity) and vinylogous amide (stability) conjugation

Compound **13** proved remarkably stable to acid-catalyzed solvolysis, consistent with an increased stabilization derived from the fully engaged vinylogous amide (Table 3). Not only was it found to be completely stable at pH 7, but it also exhibited an acid-catalyzed solvolysis half-life of ca. 30 500 h (ca. 3.5 years), even at pH 3. Removal of this linking amide also resulted in a $10^4-10^5\times$ reduction in cytotoxic potency and an inability to alkylate DNA, even under harsh conditions (37 °C, 2–14 d). Thus, the vinylogous amide stabilization against the reaction derived from the amine plays a greater role than carbonyl basicity. Rather than enhancing DNA alkylation, the removal of the linking amide abolished the capabilities for DNA alkylation, consistent with catalysis derived from a DNA binding-induced conformational change that disrupts vinylogous amide stabilization, thereby activating the agents for nucleophilic attack.

Balance between Stability and Reactivity: Modifications to the Linking Amide

The loss in properties with **13** led to a further probe of the role of the N^2 amide with the synthesis and evaluation of **14** and **15**, in which the linking amide was replaced with an amidine and thioamide, respectively (Table 4).[29] The thioamide proved to be a more reactive agent, consistent with the greater thioamide conjugation reducing the vinylogous amide stabilization of the alkylation subunit. In contrast, the amidine was far more susceptible to hydrolysis rather than solvolysis even at neutral pH, indicating preferential vinylogous amide conjugation and stabilization at the expense of the stability of the linking amidine. These observations are consistent with the predicted relative conjugation derived from estimates of barriers to rotation [thioamide > amide > vinylogous amide (ca. 12.2–14.5 kcal/mol) > amidine].[29] The enhanced properties of the amide versus amidine or thioamide establish the N^2 amide as the optimum linking unit

Table 5. Key Substituent Effects on Reactivity

ρ = −0.3

ρ = −3.0

X (R = BOC)		$t_{1/2}$ (pH = 3)	σ_p
OMe	(**16**)	110 h	−0.28
H	(**6**)	133 h	0.00
CN	(**17**)	213 h	0.70
R (X = H)		$t_{1/2}$ (pH = 3)	σ_p
CONHCH$_3$		36 h	0.72
CO$_2$CH$_3$		59 h	0.48
COEt		96 h	0.45
SO$_2$Et		383 h	0.36

- C7 substituent effect is exceptionally small
- N2 substituent effect is exceptionally large
- Large change in reactivity accompanies a small perturbation in the vinylogous amide

and reveal that it provides a beautiful balance between competing amide (reactivity) and vinylogous amide (stability) conjugation.

Substituent Effects on Reactivity: N2- versus C7-Substituted Analogues

We have conducted two studies which employ a classical Hammett series to establish the magnitude of substituent effects on reactivity (Table 5).[30,31] The C7 substituent effects were established with a series of CBI derivatives and were found to be exceptionally small: $\rho = -0.30$. Although the introduction of a strong electron-withdrawing group slowed acid-catalyzed solvolysis and the introduction of a strong electron-donating substituent accelerated solvolysis, the effect was very small, and only a 2-fold solvolysis rate difference between *N*-BOC-CCBI (**17**, X = CN)[30] and *N*-BOC-MCBI (**16**, X = OMe)[32] was observed. In contrast, the nature of the N^2 substituent had

Table 6. Key Substituent Effects on DNA Alkylation Rate

	X =	CN	H	OMe
rel k (solv., pH 3)		0.7	1.0	1.2
rel k (DNA alkyl.)		2.9	1.0	1.9

- Impact of C7 substituent on DNA alkylation rate is related to its presence rather than electronic nature
- Extended length of alkylation subunit increases inherent twist of linking amide upon DNA binding

an exceptionally large effect: $\rho = -3.0$. Thus, consistent with previous observations, a large change in reactivity accompanies even a small perturbation in the nature of the vinylogous amide.

Noncorrelation between pH-Dependent Reactivity and DNA Alkylation Rates

Another important observation made with the CBI analogues was that the relative rates of DNA alkylation did not follow the relative rates of acid-catalyzed solvolysis, consistent with the pH-independent catalysis derived from a DNA binding-induced conformational change (Table 6).[30] Because of the structural similarity of **6**, **16**, and **17**, it is unlikely that the subtle structural differences could contribute to an alteration of the expected reactivity order. Rather, the unexpected order of DNA alkylation rates was influenced by an effect intimately linked to catalysis. In this series, the impact of the C7 substituent (R = CN > OCH$_3$ > H) is related to its simple presence rather than its electronic nature (R = OCH$_3$ > H > CN). This effect may be attributed to the resulting extended length of the alkylation subunit and the corresponding increase in the inherent twist of the linking N^2 amide that would accompany minor groove binding.

Right-Hand Subunit Rigid Length Requirements for Catalysis: Simple, Extended, Reversed, and Sandwiched Analogues

The rates of DNA alkylation by simple derivatives of the alkylation subunits (**4**−**6**) are much slower than those for **1**−**3**. Their DNA alkylation reaction requires much higher agent concentration (10$^4\times$), more vigorous reaction conditions (37 °C), and much longer reaction times (24−72 h).[2−4,10] Several explanations have been advanced to account for this, including the rate enhancement derived from the noncovalent minor groove binding of the full agents, proximity effects imposed on the DNA-bound
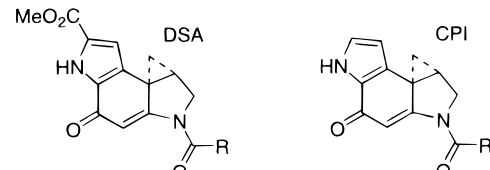
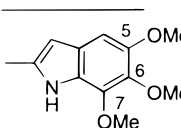**Table 7. Structural Features Required for DNA Alkylation**

18, (+)-DSA-CDPI$_2$

19, (+)-CDPI-DSA-CDPI

20, (+)-CDPI$_2$-DSA
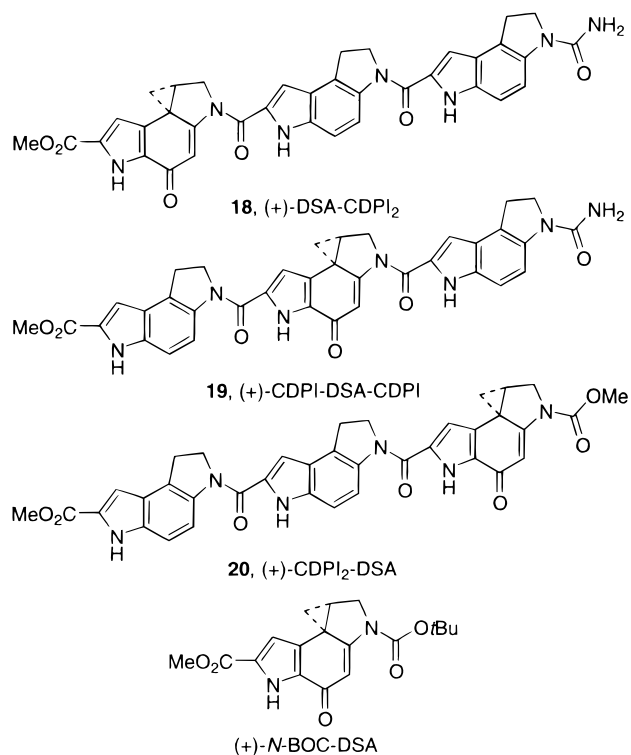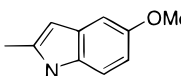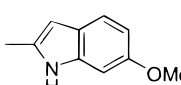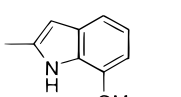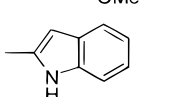
(+)-*N*-BOC-DSA

selectivity		rel. DNA alkyl. rate	
(+)-**18** = (−)-**20**	(+)-**18**	13000	
(−)-**18** = (+)-**20**	(+)-**19**	16500	
(+)-**19** = (−)-**19**	(+)-**20**	1	
	(+)-*N*-BOC DSA	0.1	

- Rapid reaction requires rigid, extended N^2 amide substituent
- DNA alkylation rate of reversed agent **20** is similar to *N*-BOC-DSA (no catalysis)

agents, or the relative degree of reversibility of the adenine N3 alkylation. All invoke a benefit derived from the DNA binding or stabilization provided by the noncovalent contacts of the attached right-hand subunit. We additionally now know that, in the absence of the extended and rigid right-hand subunit, minor groove binding no longer requires an induced twist in the linking amide, depriving the agent of the activation toward DNA alkylation.

The examination of the reversed (**18**) versus extended (**19**) and sandwiched (**20**) analogues of duocarmycin SA established that the presence of the extended heteroaryl N^2 amide substituent conveys a special DNA alkylation reactivity that is independent of the alkylation sites (Table 7).[9,10] It was shown that reversal of the orientation of the DNA binding subunits results in the complete reversal of the inherent enantiomeric DNA alkylation selectivity: *ent*-(−)-CDPI$_2$-DSA = (+)-DSA-CDPI$_2$ and (+)-CDPI$_2$-DSA = *ent*-(−)-DSA-CDPI$_2$. In addition, both enantiomers of the sandwiched agents (CDPI-DSA-CDPI) alkylated the same sites and did so with a selectivity distinct from those of the extended or reversed agents. These studies established that the preferential AT-rich noncovalent binding selectiv-

Table 8. Substituent Effects on DNA Alkylation Efficiency, Rate, and Biological Potency

R	DSA rel. alkylation efficiency and rate		IC$_{50}$ L1210	CPI rel. alkylation efficiency and rate	
	efficiency	k_{rel}		efficiency	k_{rel}
(5-OMe, 6-OMe, 7-OMe)	1.0	1.0	10 pM	0.1	0.08
(5-OMe)	1.0	1.0–0.5	12 pM	0.1–0.05	nd
(6-OMe)	0.2	nd	25 pM	nd	nd
(7-OMe)	0.1	nd	60 pM	nd	nd
(none)	0.05	0.05	65 pM	0.01	0.004

Minor groove imbedded substituents that extend rigid subunits length

DNA Binding Subunits
- Stabilize reversible DNA adduct
- Accelerate rate of DNA alkylation
- Extend AT-rich binding selectivity
- DNA alkylation catalysis: extended length increases conformational change upon DNA binding

ity of the agents controls the DNA alkylation sequence selectivity and that the natural and unnatural enantiomers are subject to the same polynucleotide recognition features. In addition, there was a substantial change in the rate of DNA alkylation with the reversed agents. While the DNA alkylation rate of the extended and sandwiched agents is exceptionally fast, that of the reversed agents is exceptionally slow, proceeding at rates similar to those of the simple derivatives lacking a DNA binding subunit, e.g., *N*-BOC-DSA. The distinguishing feature between the extended or sandwiched analogues and the reversed analogues is the presence of the right-hand heteroaryl N^2 amide substituent. Thus, a rigid extended N^2 amide substituent is required for rapid and effective alkylation of DNA. With the sandwiched analogues, this effect is independent of the sites of DNA alkylation and the enantiomeric configuration of the alkylation subunit. In contrast, the N^2 amide of the reversed agents is not altered upon binding; thus, the agents are not activated for nucleophilic addition. Consequently, they undergo DNA alkylation at rates comparable to those of the simple derivatives themselves, which also lack a rigid, extended N^2 amide substituent.

Substituent Effects on the Rate and Efficiency of DNA Alkylation

Additional and related subtle factors contribute to the properties of duocarmycin SA. Both the left-hand subunit C6 methyl ester and the right-hand subunit C5′ methoxy group independently increase biological potency (ca. 5−10×) and DNA alkylation efficiencies and rates (ca. 5−20×) (Table 8).[10,33] Both of these substituents are deeply embedded in the minor groove with the DNA-bound agent, and both can provide stabilizing, noncovalent binding contacts that may account, in part, for their importance. However, it is the simple presence of these substituents which increase the rigid length of each subunit, and increase the inherent twist in the DNA-bound conformation, that serves to enhance the properties. This more effectively disrupts the vinylogous amide stabilization in the alkylation subunit and further increases the inherent reactivity of the DNA-bound agent.

Notably, similar observations with the substituted CBI analogues **16** and **17** (Table 6) have been made and illustrate that the effect is unrelated to the electronic nature of the substituent and related simply to the presence of such a substituent. Consistent with this, (+)-duocarmycin SA alkylates DNA 12−13× faster and with a 10-fold greater efficiency than the unsubstituated CPI-TMI (Table 8), despite being intrinsically 6× less reactive.[33]

NMR Studies: Bound Conformation

In collaboration with Chazin and co-workers,[34] the assessment of the binding-induced conformational change in duocarmycin and related analogues by NMR analysis of the covalent adducts with d(GACTAATTGTC)·d(GAC-AATTAGTC) has been studied. The high-resolution structures of three closely related adducts have been determined, and the most pronounced feature of the structures is the relative intersubunit twist in the agents induced by binding in the minor groove. Moreover, there is a good correlation between binding-induced twist in the χ_1 dihedral angle and the relative rate and efficiency of alkylation, accounting for not only the behavior of simplified analogues but also that of the unnatural enantiomers (Table 9). In the three structures established to date, little perturbation in the DNA structure is observed, and it appears that it is the DNA that alters the conformation of the bound agent and not the bound agent which alters the DNA conformation.

Table 9. Structures of DNA-Bound Agents and the Importance of χ_1

(+)-**3**
(+)-duocarmycin SA

(+)-**21**
(+)-DSA-Indole
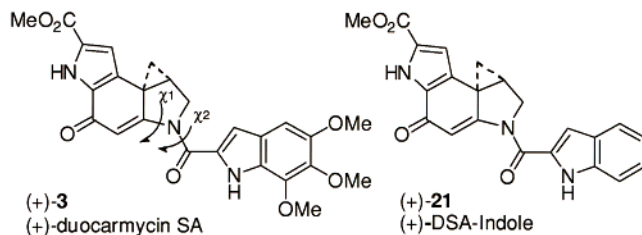
χ_1	22.4°	14.2°
χ_2	11.0°	13.4°
Intersubunit Twist	44.8°	37.3°
Rel. DNA Alkylation Efficiency	1.0	0.05
Rel. DNA Alkylation Rate	1.0	0.04
Rel. IC$_{50}$ (L1210)	1.0 (5 pM)	0.08 (65 pM)

- Terminal C5 methoxy group increases inherent twist in DNA bound conformation (χ_1)
- Accounts for distinctions in DNA alkylation rate and biological potency

3' 5'
T A
A T
A T
T A
C G

5' G A C T A A T T G A C 3'
3' C T G **A** T T A A C T G 5'
↑
(+)-Duocarmycin SA
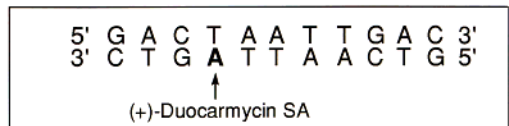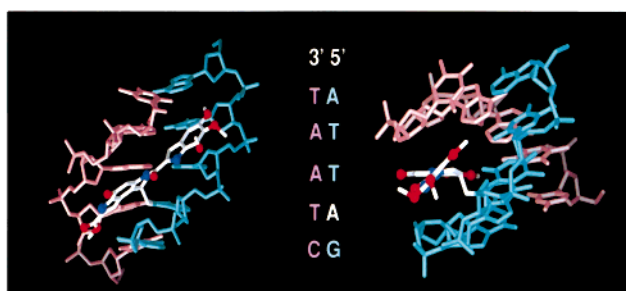
Reversibility: The DNA-Bound Conformation Disfavors Retroalkylation and Stabilizes the DNA Adduct

In contrast to intuitive expectations, the cyclopropane ring is very easily introduced through an Ar-3′ spirocyclization (e.g., NaHCO₃). In early studies, this suggested that the DNA adenine N3 adduct should be formed in a readily reversible manner.[1,4] Ultimately, this proved to be accurate, although the degree of reversibility was lower and the rate of retroalkylation was slower than chemical precedent would suggest.[35,36] The unusual stability of the DNA adducts and the slow rates of retroalkylation have been attributed to the noncovalent binding stabilization provided by the right-hand subunits. We now suggest that the adoption of the DNA-alkylated conformation no longer facilitates Ar-3′ spirocyclization with reversal of the DNA alkylation reaction and that this contributes to the unusual stability of the DNA adducts. Thus, not only does this conformational change that results in ground-state destabilization resulting from diminished vinylogous amide

conjugation account for the rate acceleration for formation of the adduct by lowering the apparent activation energy, but it also contributes to a shift in the reaction equilibrium to favor adduct formation since the DNA adduct is not similarly destabilized by adopting a helical conformation.

Conclusions

The examination of a series of CC-1065 and duocarmycin analogues containing well-devised, deep-seated structural changes resulted in the discovery and establishment of an unusual and initially unappreciated source of catalysis for the DNA alkylation reaction. This catalysis is derived from a DNA binding-induced conformational change that disrupts the alkylation subunit vinylogous amide conjugation and stabilization, activating the agent for nucleophilic attack. This activation, which is greatest within the narrower, deeper AT-rich minor groove (shape-dependent catalysis), represents a beautiful complement to the preferential AT-rich minor groove binding of the agents (shape-selective recognition), where the binding-induced activation simply cocks the pistol but does not pull the trigger, for reaction. Hence, only upon binding to its target DNA site does the agent acquire sufficient reactivity to undergo nucleophilic attack.

References

(1) Boger, D. L.; Johnson, D. S. CC-1065 and the Duocarmycins: Understanding Their Biological Function through Mechanistic Studies. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1439. Boger, D. L.; Boyce, C. W.; Garbaccio, R. M.; Goldberg, J. CC-1065 and the Duocarmycins: Synthetic Studies. *Chem. Rev.* **1997**, *97*, 787. Boger, D. L.; Johnson, D. S. CC-1065 and the Duocarmycins: Unraveling the Keys to a New Class of Naturally Derived DNA Alkylating Agents. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 3642. Boger, D. L. The Duocarmycins: Synthetic and Mechanistic Studies. *Acc. Chem. Res.* **1995**, *28*, 20. Boger, D. L. Duocarmycins: A New Class of Sequence Selective DNA Minor Groove Alkylating Agents. *Chemtracts: Org. Chem.* **1991**, *4*, 329.

(2) Warpehoski, M. A.; Hurley, L. H. Sequence Selectivity of DNA Covalent Modification. *Chem. Res. Toxicol.* **1988**, *1*, 315. Hurley, L. H.; Needham-VanDevanter, D. R. Covalent Binding of Antitumor Antibiotics in the Minor Groove of DNA. Mechanism of Action of CC-1065 and the Pyrrolo[1,4]-benzodiazepines. *Acc. Chem. Res.* **1986**, *19*, 230. Warpehoski, M. A. The DNA Sequence Selectivity of CC-1065. In *Advances in DNA Sequence Specific Agents*; Hurley, L. H., Ed.; JAI: Greenwich, CT, 1992; Vol. 1, p 217. Aristoff, P. A. In *Advances in Medicinal Chemistry*; Maryanoff, B. E., Maryanoff, C. E., Eds; JAI: Greenwich, CT, 1993; Vol. 2, p 67. Warpehoski, M. A.; McGovren, P.; Mitchell, M. A., Contrasting Mechanisms for the Sequence Recognition of DNA by (+)- and (−)-CC-1065. In *Molecular Basis of Specificity in Nucleic Acid-Drug Interactions*; Pullman, B., Jortner, J., Eds.; Kluwer: Dordrecht, The Netherlands, 1990; p 531.

(3) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Kitos, P. A.; Suntornwat, O. Synthesis and Preliminary Evaluation of Agents Incorporating the Pharmacophore of the Duocarmycin/Pyrindamycin Alkylation Subunit: Identification of the CC-1065/Duocarmycin Common Pharmacophore. *J. Org. Chem.* **1990**, *55*, 4499. Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. Duocarmycin-Pyrindamycin DNA Alkylation Properties and Identification, Synthesis and Evaluation of Agents Incorporating the Pharmacophore of the Duocarmycin-Pyrindamycin Alkylation Subunit: Identification of the CC-1065/Duocarmycin Common Pharmacophore. *J. Am. Chem. Soc.* **1990**, *112*, 8961. Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. An Alternative and Convenient Strategy for Generation of Substantial Quantities of Singly 5′-^{32}P-End-labeled Double-stranded DNA for Binding Studies: Development of a Protocol for the Examination of the Functional Features of (+)-CC-1065 and the Duocarmycins That Contribute to Their Sequence-selective DNA Binding Properties. *Tetrahedron* **1991**, *47*, 2661. Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H. Isolation and Characterization of the Duocarmycin-adenine DNA Adduct. *J. Am. Chem. Soc.* **1991**, *113*, 6645. Boger, D. L.; Yun, W.; Terashima, S.; Fukuda, Y.; Nakatani, K.; Kitos, P. A.; Jin, Q. DNA Alkylation Properties of the Duocarmycins: (+)-Duocarmycin A, *epi*-(+)-Duocarmycin A, *ent*-(−)-Duocarmycin A, and *epi,ent*-(−)-Duocarmycin A. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 759. Boger, D. L.; Yun, W. Reversibility of the Duocarmycin A and SA DNA Alkylation Reaction. *J. Am. Chem. Soc.* **1993**, *115*, 9872. Boger, D. L.; Johnson, D. S.; Yun, W. (+)- and *ent*-(−)-Duocarmycin SA and (+)- and *ent*-(−)-*N*-BOC-DSA DNA Alkylation Properties. Alkylation Site Models That Accommodate the Offset AT-rich Adenine N3 Alkylation Selectivities of the Enantiomeric Agents. *J. Am. Chem. Soc.* **1994**, *116*, 1635.

(4) Boger, D. L.; Johnson, D. S.; Yun, W.; Tarby, C. M. Molecular Basis for Sequence Selective DNA Alkylation by (+)- and *ent*-(−)-CC-1065 and Related Agents: Alkylation Site Models That Accommodate the Offset AT-rich Adenine N3 Alkylation Selectivity. *Bioorg. Med. Chem.* **1994**, *2*, 115. Boger, D. L.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. Demonstration of a Pronounced Effect of Noncovalent Binding Selectivity on the (+)-CC-1065 DNA Alkylation and Identification of the Pharmacophore of the Alkylation Subunit. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1431. 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. Synthesis and Evaluation of Aborted and Extended CC-1065 Functional Analogs: (+)- and (−)-CPI-PDE$_1$; (+)- and (−)-CPI-CDPI$_1$; and (±)-, (+)-, and (−)-CPI-CDPI$_3$. Preparation of Key Partial Structures and Definition of an Additional Functional Role of the CC-1065 Central and Right-hand Subunits. *J. Am. Chem. Soc.* **1990**, *112*, 4623.

(5) Boger, D. L.; Coleman, R. S.; Invergo, B. J.; Zarrinmayeh, H.; Kitos, P. A.; Thompson, S. C.; Leong, T.; McLaughlin, L. W. A Demonstration of the Intrinsic Importance of Stabilizing Hydrophobic Binding and Noncovalent Van Der Waals Contacts Dominant in the Noncovalent CC-1065:DNA Binding. *Chem. Biol. Interact.* **1990**, *73*, 29.

(6) Boger, D. L.; Zhou, J.; Cai, H. Demonstration and Definition of the Noncovalent Binding Selectivity of Agents Related to CC-1065 by an Affinity Cleavage Agent: Noncovalent Binding Coincidental with Alkylation. *Bioorg. Med. Chem.* **1996**, *4*, 859. Boger, D. L.; Zhou, J. CDPI$_3$-enediyne and CDPI$_3$-EDTA Conjugates: A New Class of DNA Cleaving Agents. *J. Org. Chem.* **1993**, *58*, 3018.

(7) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H. The (+)-CC-1065 DNA Alkylation: Key Studies Demonstrating a Noncovalent Binding Selectivity Contribution to the Alkylation Selectivity. *J. Am. Chem. Soc.* **1991**, *113*, 3980.

(8) Boger, D. L.; Garbaccio, R. M.; Jin. Q. Synthesis and Evaluation of CC-1065 and Duocarmycin Analogs Incorporating the Iso-CI and Iso-CBI Alkylation Subunits: Impact of Relocation of the C4 Carbonyl. *J. Org. Chem.* **1997**, *62*, 8875.

(9) Boger, D. L.; Johnson, D. S. Second Definitive Test of Proposed Models for the Origin of the CC-1065 and Duocarmycin DNA Alkylation Selectivity. *J. Am. Chem. Soc.* **1995**, *117*, 1443

(10) Boger, D. L.; Hertzog, D. L.; Bollinger, B.; Johnson, D. S.; Cai, H.; Goldberg, J.; Turnbull, P. Duocarmycin SA Shortened, Simplified, and Extended Agents: A Systematic Examination of the Role of the DNA Binding Subunit. *J. Am. Chem. Soc.* **1997**, *119*, 4977. Boger, D. L.; Bollinger, B.; Hertzog, D. L.; Johnson, D. S.; Cai, H.; Mesini, P.; Garbaccio, R. M.; Jin, Q.; Kitos, P. A. Reversed and Sandwiched Analogs of Duocarmycin SA: Establishment of the Origin of the Sequence-Selective Alkylation of DNA and New Insights into the Source of Catalysis. *J. Am. Chem. Soc.* **1997**, *119*, 4987.

(11) Boger, D. L.; Garbaccio, R. M. Catalysis of the CC-1065 and Duocarmycin DNA Alkylation Reaction: DNA Binding Induced Conformational Change in the Agent Results in Activation. *Bioorg. Med. Chem.* **1997**, *5*, 263.

(12) Boger, D. L.; Machiya, K.; Hertzog, D. L.; Kitos, P. A.; Holmes, D. Total Synthesis and Preliminary Evaluation of (+)- and *ent*-(−)-Duocarmycin SA. *J. Am. Chem. Soc.* **1993**, *115*, 9025

(13) Boger, D. L.; McKie, J. A.; Nishi, T.; Ogiku, T. Total Synthesis of (+)-Duocarmycin A, *epi*-(+)-Duocarmycin A and Their Unnatural Enantiomers: Assessment of Chemical and Biological Properties. *J. Am. Chem. Soc.* **1997**, *119*, 311.

(14) Boger, D. L.; Goldberg, J. A.; McKie, J. A. A Comparative Study of the Solvolysis Reactivity, Regioselectivity, and Stereochemistry of the Duocarmycin A and SA Alkylation Subunits. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1955.

(15) Boger, D. L.; Ishizaki, T.; Kitos, P. A.; Suntornwat, O. Synthesis of *N*-(*tert*-Butyloxycarbonyl)-CBI, CBI, CBI-CDPI$_1$, and CBI-CDPI$_2$: Enhanced Functional Analogs Incorporating the 1,2,9, 9a-Tetrahydrocyclo prop[1,2-*c*]benz[1,2-*e*]indol-4-one (CBI) Lefthand Subunit. *J. Org. Chem.* **1990**, *55*, 5823. Boger, D. L.; Munk, S. A. DNA Alkylation Properties of Enhanced Functional Analogs of CC-1065 Incorporating the 1,2,9,9a-Tetrahydrocycloprop[1,2-*c*]benz-[1,2-*e*]indol-4-one (CBI) Alkylation Subunit. *J. Am. Chem. Soc.* **1992**, *114*, 5487.

(16) Boger, D. L.; Jenkins, T. J. X-ray Structure, and Properties of Fluorocyclopropane Analogs of the Duocarmycins Incorporating the 9,9-Difluoro-1,2,9,-9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (F$_2$-CBI) Alkylation Subunit. *J. Am. Chem. Soc.* **1996**, *118*, 8860.

(17) Boger, D. L.; Nishi, T.; Teegarden, B. R. A *p*-Quinonemethide Analog of the CC-1065 and Duocarmycin Alkylation Subunits. *J. Org. Chem.* **1994**, *59*, 4943.

(18) Baird, R.; Winstein, S. Neighboring Carbon and Hydrogen. LI. Dienones from Ar₁ σ3 Participation. Isolation and Behavior of Spiro(2,5)octa-1,4-diene-3-one. *J. Am. Chem. Soc.* **1963**, *85*, 567.

(19) Boger, D. L.; Turnbull, P. Synthesis and Evaluation of a Carbocyclic Analog of the CC-1065 and Duocarmycin Alkylation Subunits: Role of the Vinylogous Amide and Implications on DNA Alkylation Catalysis. *J. Org. Chem.* **1998**, *63*, 8004.

(20) Boger, D. L.; Mesini, P. Design, Synthesis and Evaluation of CC-1065 and Duocarmycin Analogs Incorporating the 2,3,10,10a-Tetrahydro-1*H*-cyclopropa[*d*]benzo[*f*]quinol-5-one (CBQ) Alkylation Subunit: Identification and Structural Origin of Subtle Stereoelectronic Features That Govern Reactivity and Regioselectivity. *J. Am. Chem. Soc.* **1994**, *116*, 11335. Boger, D. L.; Mesini, P. DNA Alkylation Properties of CC-1065 and Duocarmycin Analogs Incorporating the 2,3,10,10a-Tetrahydrocyclopropa[*d*]benzo[*f*]quinol-5-one (CBQ) Alkylation Subunit: Identification of Subtle Structural Features That Contribute to the Regioselectivity of the Adenine N3 Alkylation Reaction. *J. Am. Chem. Soc.* **1995**, *117*, 11647.

(21) Boger, D. L.; Turnbull, P., Synthesis and Evaluation of CC-1065 and Duocarmycin Analogs Incorporating the 1,2,3,4,11,11a-Hexahydrocyclopropa[*c*]naphtho[2,1-*b*]azepin-6-one (CNA) Alkylation Subunit: Structural Features That Govern Reactivity and Reaction Regioselectivity. *J. Org. Chem.* **1997**, *62*, 5849.

(22) Boger, D. L.; Boyce, C. W.; Johnson, D. S. pH Dependence of the Rate of DNA Alkylation for (+)-Duocarmycin SA and (+)-CCBI-TMI. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 233.

(23) Boger, D. L.; M. Garbaccio, R. M. Are the Duocarmycin and CC-1065 DNA Alkylation Reactions Acid-catalyzed? Solvolysis pH Rate Profiles Suggest They Are Not. *J. Org. Chem.* **1999**, *64*, 5666.

(24) Warpehoski, M. A.; Harper, D. E. Acid-dependent Electrophilicity of Cyclopropylpyrroloindoles. Nature's Masking Strategy for a Potent DNA Alkylator. *J. Am. Chem. Soc.* **1994**, *116*, 7573.

(25) Warpehoski, M. A.; Harper, D. E. Enzyme-like Rate Acceleration in the DNA Minor Groove. Cyclopropylpyrroloindoles as Mechanism-based Inactivators of DNA. *J. Am. Chem. Soc.* **1995**, *117*, 2951.

(26) Reynolds, V. L.; Molineux, I. J.; Kaplan, D. J.; Swenson, D. H.; Hurley, L. H. Reaction of the Antitumor Antibiotic CC-1065 with DNA. Location of the Site of Thermally Induced Strand Breakage and Analysis of DNA Sequence Specificity. *Biochemistry* **1985**, *24*, 6228. Hurley, L. H.; Lee, C.-S.; McGovren, J. P.; Warpehoski, M. A.; Mitchell, M. A.; Kelly, R. C.; Aristoff, P. A. Molecular Basis for Sequence Specific DNA Alkylation by CC-1065. *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. Sequence Specificity of DNA Alkylation by the Unnatural Enantiomer of CC-1065 and its Synthetic Analogues. *J. Am. Chem. Soc.* **1990**, *112*, 4633.

(27) Boger, D. L.; Garbaccio, R. M. A Novel Class of CC-1065 and Duocarmycin Analogues Subject to Mitomycin Related Reductive Activation. *J. Org. Chem.* **1999**, *64*, 8350.

(28) Boger, D. L.; Santillán, A., Jr.; Searcey, M.; Jin, Q. The Critical Role of the Linking Amide in CC-1065 and the Duocarmycins: Implications on the Source of DNA Alkylation Catalysis. *J. Am. Chem. Soc.* **1998**, *120*, 11554.

(29) Boger, D. L.; Santillán, A., Jr.; Searcey, M.; Jin, Q. Synthesis and Evaluation of Duocarmycin and CC-1065 Analogues Containing Modifications in the Subunit Linking Amide. *J. Org. Chem.* **1999**, *64*, 5241.

(30) Boger, D. L.; Han, N.; Tarby, C. M.; Boyce, C. W.; Cai, H.; Jing, Q.; Kitos, P. A. Synthesis, Chemical Properties, and Preliminary Evaluation of Substituted CBI Analogs of CC-1065 and the Duocarmycins Incorporating the 7-Cyano-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CCBI) Alkylation Subunit: Hammett Quantitation of the Magnitude of Electronic Effects on Functional Reactivity. *J. Org. Chem.* **1996**, *61*, 4894. Boger, D. L.; McKie, J. A.; Han, N.; Tarby, C. M.; Riggs, H. W.; Kitos, P. A. A Hammett Correlation for CC-1065 and Duocarmycin Analogs: Magnitude of Substituent Electronic Effects on Functional Reactivity. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 659.

(31) Boger, D. L.; Yun, W. Role of the CC-1065 and Duocarmycin N² Substituent: Validation of a Direct Relationship Between Solvolysis Chemical Stability and in Vitro Biological Potency. *J. Am. Chem. Soc.* **1994**, *116*, 5523.

(32) Boger, D. L.; McKie, J. A.; Cai, H.; Cacciari, B.; Baraldi, P. G. Synthesis and Properties of Substituted CBI Analogs of CC-1065 and the Duocarmycins Incorporating the 7-Methoxy-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (MCBI) Alkylation Subunit: Magnitude of Electronic Effects on the Functional Reactivity. *J. Org. Chem.* **1996**, *61*, 1710.

(33) Boger, D. L.; Santillán, A., Jr.; Searcey, M.; Brunette, S. R.; Wolkenberg, S. E.; Hedrick, M.; Jin, Q. Submitted.

(34) Schnell, J. R.; Ketchem, R. R.; Boger, D. L.; Chazin, W. J. DNA Binding Induced Alkylation: Insights from the Structure of a DNA Duplex Alkylated by the Indole Derivative of Duocarmycin SA. *J. Am. Chem. Soc.* **1999**, *121*, 5645. Eis, P. S.; Smith, J. A.; Rydzewski, J. M.; Case, D. A.; Boger, D. L.; Chazin, W. J. High-Resolution Solution Structure of a DNA Duplex Alkylated by the Antitumor Agent Duocarmycin SA. *J. Mol. Biol.* **1997**, *272*, 237. Smith, J. A.; Schnell, J. R.; Chazin, W. J. Unpublished studies.

(35) Boger, D. L.; Yun, W. Reversibility of the Duocarmycin A and SA DNA Alkylation Reaction. *J. Am. Chem. Soc.* **1993**, *115*, 9872.

(36) Warpehoski, M. A.; Harper, D. H.; Mitchell, M. A.; Monroe, J. J. Reversibility of the Covalent Reaction of CC-1065 and Analogs with DNA. *Biochemistry* **1992**, *31*, 2502.