Are the Duocarmycin and CC-1065 DNA Alkylation Reactions Acid-Catalyzed? Solvolysis pH−Rate Profiles Suggest They Are Not

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Received May 7, 1999

A study of the solvolysis pH−rate profiles for two key reactive CC-1065/duocarmycin alkylation subunit analogues is detailed. Unlike the authentic alkylation subunits and *N*-BOC-CBI (**4**) which are too stable to establish complete solvolysis pH−rate profiles, the analogues *N*-BOC-CBQ (**5**) and *N*-BOC-CNA (**6**) are reactive throughout the pH range of 2−12. Moreover, they possess progressively diminished vinylogous amide conjugation resulting in a corresponding progressively increasing reactivity adopting and reflecting conformations analogous to that proposed for DNA-bound CC-1065. For both, the acid-catalyzed reaction was observed only at the lower pH of 2−5, and the uncatalyzed solvolysis reaction rate dominated at pH ≥6, indicating that the CC-1065 and duocarmycin DNA alkylation reaction observed at pH 7.4 need not be an acid-catalyzed reaction. The studies provide further strong evidence that catalysis for the DNA alkylation reaction (pH 7.4) is derived from a DNA binding-induced conformational change in the agents that disrupts the stabilizing alkylation subunit vinylogous amide conjugation activating the agents for nucleophilic attack independent of pH.

CC-1065 and the duocarmycins (**1**−**3**, Figure 1) are the parent members of an exceptionally potent class of antitumor antibiotics that derive their biological properties through the sequence-selective alkylation of duplex DNA.[1,2] Although unreactive toward conventional nucleophiles at pH 7, the DNA alkylation reactions by **1**−**3** are exceptionally facile, typically proceeding in <1 h at 4−25 °C. Two proposals for the source of this catalysis for the DNA alkylation reaction have been detailed. The first is based on the report of requisite acid or Lewis acid catalysis for solvolysis nucleophilic addition to the otherwise unusually stable alkylation subunits.[3−5] This requisite acid catalysis has been further proposed to control the DNA alkylation sequence selectivity by invoking a sequence-dependent C4 carbonyl protonation by a backbone phosphate sug-gested to be available only at the observed sites of DNA alkylation.[2,6] Although attractive, efforts to document a rate pH dependence for the DNA alkylation reaction have not been successful and the rates proved essentially independent of pH throughout the range of pH 6−8[7,8] despite inferences to the contrary.[5]

At pH 7.4, the DNA phosphate backbone is fully ionized (0.0001−0.00004% protonated), and it is unlikely that catalysis can be derived from a backbone phosphate protonation of the C4 carbonyl. Moreover, the requirement for acid catalysis at pH 7.4 is not consistent with the DNA sequence selectivity being controlled by the noncovalent binding selectivity of the agents. Extensive support for this origin of the DNA alkylation selectivity has been disclosed and includes the following: (1) the reverse and offset 3.5 or 5 base-pair AT-rich adenine N3

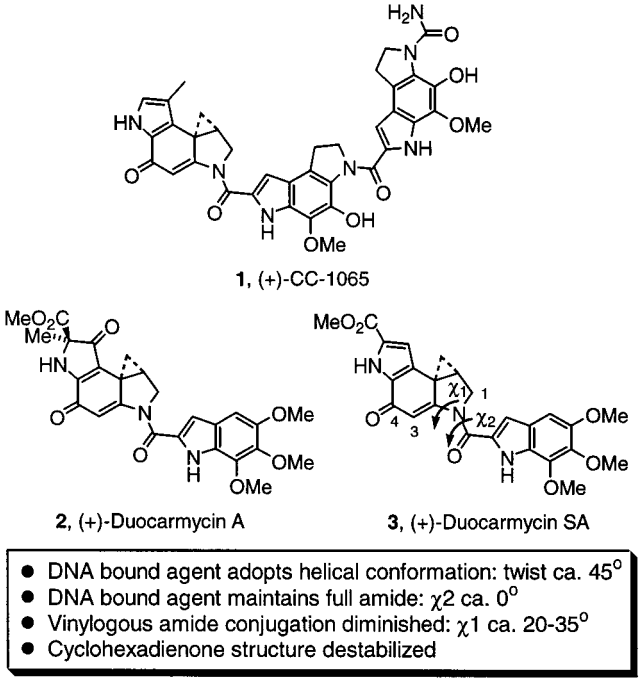

Figure 1.

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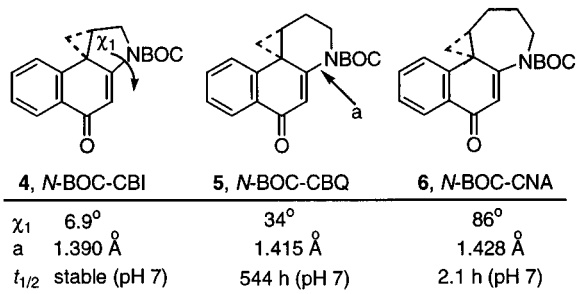
10.1021/jo990762g CCC: $18.00
Published on Web 07/02/1999

alkylation selectivity of the natural and unnatural enantiomers of **1**−**3** corresponding nicely to the length of the agent and the required size of the binding region surrounding the alkylation site;[9,10] (2) the distinct and smaller alkylation selectivity of simple derivatives such as **4**−**6** versus **1**−**3**, which proved independent of the absolute stereochemistry;[9,10] (3) the AT-rich noncovalent binding selectivity of **1**−**3**,[11] which is coincidental with the sites of DNA alkylation;[12] (4) the demonstration that the characteristic DNA alkylation selectivity may be observed with other electrophiles incorporated into the structures and does not require the C4 carbonyl or the activated cyclopropane;[13,14] and (5) the remarkable switch in the enantiomeric alkylation selectivity of reversed analogues.[7,15] Consistent with this origin of the DNA alkylation selectivity, we have proposed that the catalysis is derived from a DNA binding-induced conformational change in the agent, which twists the linking amide in a manner that diminishes the alkylation subunit stabilizing vinylogous amide conjugation and activates the agent for nucleophilic attack.[7,16] A growing number of indirect observations support this as the source of catalysis including the demonstration that the rates of DNA alkylation between closely related alkylation subunit analogues do not follow their acid-catalyzed reactivity,[17−19] that the linking amide is essential for observation of DNA alkylation,[20] that disruption or removal of the alkylation subunit vinylogous amide provides agents of sufficient reactivity to account for DNA alkylation catalysis,[21−23] and that the rates and efficiencies of DNA alkylation correlate with the degree of DNA binding-induced intersubunit twist.[24]



	4, *N*-BOC-CBI	**5**, *N*-BOC-CBQ	**6**, *N*-BOC-CNA
χ_1	6.9°	34°	86°
a	1.390 Å	1.415 Å	1.428 Å
$t_{1/2}$	stable (pH 7)	544 h (pH 7)	2.1 h (pH 7)

Figure 2.

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 AT-rich versus wider, shallower GC-rich minor groove, this leads to preferential activation within the preferred AT-rich noncovalent binding sites. As such, DNA binding cocks the pistol, but does not pull the trigger for further reaction. What was yet uncertain was whether nucleophilic addition to such a bound, activated agent requires further acid catalysis or whether it participates in a direct uncatalyzed nucleophilic addition. Consistent with the lack of pH dependence on the rate of DNA alkylation,[7,8] herein we report a study of the solvolysis pH−rate profiles of two reactive alkylation subunit analogues for which the uncatalyzed reaction rate dominates at pH ≥6, indicating that the DNA alkylation reaction need not be an acid-catalyzed reaction.

Although the authentic alkylation subunits of **1**−**3**[4,25−27] and the analogue *N*-BOC-CBI (**4**)[28] are exceptionally stable and exhibit no detectable solvolysis reactivity at pH 7 (pH >5), the analogues **5** and **6** proved sufficiently reactive to observe solvolysis at pH 7 (Figure 2). Moreover, X-ray structural analysis of the series **4**−**6**[22] revealed corresponding structural and reactivity changes that we suggest are analogous to those accompanying the DNA binding-induced conformational change and activation of **1**−**4**. Thus, although **1**−**4** are too stable to establish a meaningful solvolysis pH−rate profile, we believe those measurable for **5** and **6** are analogous to those of the DNA bound and activated structures of **1**−**4**.

Solvolysis pH−Rate Profiles. With the reactivity of both **5** and **6**, it was possible to measure the rate of

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Table 1. Solvolysis Rates for *N*-BOC-CBQ (5) under Universal Buffer Conditions[a]

pH	k_{obs} (s^{-1})	$t_{1/2}$ (h)	pH	k_{obs} (s^{-1})	$t_{1/2}$ (h)
2	6.7×10^{-4}	0.28	7	3.8×10^{-7}	494
3	5.8×10^{-5}	3.3	8	4.3×10^{-7}	448
4	6.8×10^{-6}	28.3	9	4.4×10^{-7}	428
5	8.9×10^{-7}	214	10	3.7×10^{-7}	509
6	3.7×10^{-7}	515			

[a] Buffer consists of 0.2 M $B(OH)_3$, 0.05 M citric acid, and 0.1 M Na_3PO_4.

Table 2. Solvolysis Rates for *N*-BOC-CNA (6) under Universal Buffer Conditions[a]

pH	k_{obs} (s^{-1})	$t_{1/2}$ (h)	pH	k_{obs} (s^{-1})	$t_{1/2}$ (h)
2	7.5×10^{-2}	0.003	7	8.8×10^{-5}	2.19
3	5.6×10^{-3}	0.03	8	8.8×10^{-5}	2.18
4	9.9×10^{-4}	0.19	9	9.5×10^{-5}	2.01
5	1.4×10^{-4}	1.40	10	9.7×10^{-5}	1.97
6	8.6×10^{-5}	2.23			

[a] Buffer consists of 0.2 M $B(OH)_3$, 0.05 M citric acid, and 0.1 M Na_3PO_4.

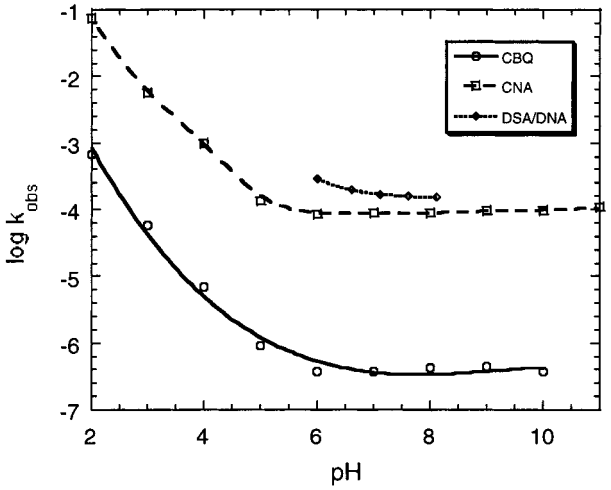

Figure 3. Plot of log k_{obs} versus pH for solvolysis of **5** and **6** using a universal buffer (0.11−0.24 M, pH 2−11, 0.2 M $B(OH)_3$, 0.05 M citric acid, 0.1 M Na_3PO_4) and a corresponding pH−rate profile (pH 6−8) for the duocarmycin SA DNA alkylation reaction at a high-affinity alkylation site taken from refs 7 and 8.

solvolysis over the full pH range under universal buffer conditions (boric acid/citric acid/Na_3PO_4),[29] Tables 1 and 2. The solvolysis of **5** and **6** was followed spectrophotometrically by UV as previously detailed.[21,22] No solvolysis rate dependence on the buffer concentration, ionic strength, or pH was observed at pH ≥6. This is apparent even in the simple comparison of the pH 7 rates measured under the universal buffer conditions (Tables 1 and 2) with those previously obtained in 50% H_2O-CH_3OH (Figure 2). The solvolysis of both **5** and **6** exhibits a first-order rate dependence on hydronium ion concentration in the region of pH 2−5 where the reaction is acid-catalyzed (Figure 3). However, the uncatalyzed reaction rate dominates at pH ≥6 and represents that of a direct rate-determining S_N2 nucleophilic attack. From a regression analysis best fit of k_{obs} versus $[H^+]$, the rate constants obtained are 0.057 ± 0.0003 M^{-1} s^{-1} and $4.6 \pm 1.0 \times 10^{-7}$ s^{-1} for the acid-catalyzed and uncatalyzed reactions of **5** and 5.6 ± 0.13 M^{-1} s^{-1} and $1.3 \pm 0.4 \times 10^{-4}$ s^{-1} for the acid-catalyzed and uncatalyzed reactions of **6**. The lack of a significant base-catalyzed solvolysis reaction with either **5** or **6** at the higher pH is interesting and analogous to observations made with a closely related carbocycle analogue of **4**[23] but is unlike those made by Winstein with simple spirocyclic cyclopropylcyclohexadienones.[30] In fact, hydrolysis of the linking amide rather than additional solvolysis catalysis was observed at the higher pH of 11 (for **5**) and 12 (for **6**). The solvolysis nucleophilic addition to optically active **1**−**6** has been shown to occur by an S_N2 mechanism producing a single enantiomer of the ring expansion solvolysis products.[21,22,25−27] Thus, the switch from an acid-catalyzed to uncatalyzed reaction at pH ≥6 is not accompanied by a change in S_N1 to S_N2 mechanism of addition.

Just as significant, the duocarmycin SA DNA alkylation reaction exhibits the nearly identical subtle pH dependence in the pH range of 6−8, where it can be examined (Figure 3). Only a small pH dependence is observed at pH 6, indicative of a dominant uncatalyzed reaction rate, and no pH dependence was observed at the most pertinent pH 7−8. Thus, the pH−rate profiles for solvolysis of **5** and **6** are identical to that of the DNA alkylation reaction by **1**−**3**, and both are consistent with an uncatalyzed nucleophilic addition reaction independent of pH and C4 carbonyl protonation.

Discussion and Conclusions. The establishment of the solvolysis pH−rate profiles for **5** and **6** and the determination that the uncatalyzed S_N2 reaction dominates at pH ≥6 has important ramifications on the source of catalysis for the DNA alkylation reactions of **1**−**3**. First, it established that the assumed requirement for acid catalysis is not necessary[2] consistent with recent experimental observations[7,8] and indicates that reaction models[3] or alkylation selectivity models[2,6] based on pH 2−3 studies[4,5] and a requirement for acid catalysis are unlikely to be accurate. More importantly, it illustrates that the structural and corresponding reactivity features embodied in **5** and **6**, 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_N2 nucleophilic attack independent of pH. This activation results from adoption of a helical-bound conformation leading to disruption of the alkylation subunit cross-conjugated vinylogous amide, and it is a beautiful complement to proposals that the DNA alkylation selectivity of **1**−**3** is derived from their preferential noncovalent AT-rich binding selectivity where the minor groove binding cocks the pistol (activates), but does not pull the trigger for reaction.

Experimental Section

Solvolysis of *N*-BOC-CBQ (5) and *N*-BOC-CNA (6). Samples of **5** (70 μg) and **6** (150 μg) were dissolved in CH_3OH

compd	pH	scan taken every	duration
5	2	3 min	1 h
		20 min	6 h
	3	30 min	30 h
	4	1 h	1 d
		1 d	12 d
	5	12 h	7 d
		1 d	2 mo
	6−12	3 d	3 mo

compd	pH	scan taken every	duration
6	2	0.5 s	3 min
	3	30 s	30 min
	4	2 min	2 h
	5	10 min	12 h
	6−12	20 min	24 h

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(1.5 mL), and the resulting solutions were mixed with a universal aqueous buffer (pH 2−12; 1.5 mL, 0.2 M boric acid, 0.05 M citric acid, 0.1 M Na_3PO_4, and deionized H_2O respectively). The blanks and the solvolysis reaction solutions were stoppered, protected from the light, allowed to stand at 25 °C, and periodically monitored by UV. The total reaction times shown below reflect those required to observe no further change in absorbance, and the UV spectra were taken at regular intervals corresponding with the reactivity of the molecule at the particular pH (see below). The decrease in absorbance at 318 nm for **5** and 319 nm for **6** were monitored. The solvolysis rates were calculated from the least-squares treatment of the slope of plots of time versus ln $[(A_f - A_i)/(A - A_f)]$.

Acknowledgment. We gratefully acknowledge the financial support of the National Institutes of Health (CA41986), The Skaggs Institute for Chemical Biology, and the award of an ACS Organic Division graduate fellowship sponsored by Zeneca Pharmaceuticals Group and an Eli Lilly graduate fellowship (R.M.G.).

JO990762G