Synthesis and Evaluation of a Carbocyclic Analogue of the CC-1065 and Duocarmycin Alkylation Subunits: Role of the Vinylogous Amide and Implications on DNA Alkylation Catalysis

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The synthesis and chemical properties of 1,2,9,9a-tetrahydro-1*H*-cyclopropa[*c*]benz[*e*]inden-4-one (CBIn, **10**), a carbocyclic C-ring analogue of the alkylation subunits of CC-1065 and the duocarmycins, are detailed. The core structure of CBIn was prepared with an intramolecular Heck reaction for assembly of the key tricyclic skeleton and a final Winstein Ar-3' spirocyclization to install the reactive cyclopropane. A study of the CBIn solvolysis reactivity, regioselectivity, and mechanism revealed that removal of the nitrogen and resulting vinylogous amide stabilization increased the reactivity 3200× (pH 3) and reversed the inherent regioselectivity, but did not alter the S_N2 reaction mechanism. Thus, the vinylogous amide found in the naturally occurring alkylation subunits is responsible for their unusual stability and significantly impacts the regioselectivity without altering the inherent S_N2 mechanism of nucleophilic addition. More importantly, this solvolysis reactivity proved independent of pH throughout the range of 4−12 including the physiologically relevant range of 5.0−8.0 where CBI is completely stable. Rate constants of $0.093 \pm 0.001\ M^{-1}\ s^{-1}$ and $4.2 \pm 0.4 \times 10^{-5}\ s^{-1}$ for the respective acid-catalyzed and uncatalyzed reactions were established, and the uncatalyzed reaction dominates at pH ≥ 4. These observations have important implications on the source of catalysis for the CC-1065/duocarmycin DNA alkylation reaction supporting the recent proposal that it is not derived from acid catalysis and C4 carbonyl protonation but rather a DNA binding-induced conformational change that disrupts the cross-conjugated vinylogous amide stabilization.

CC-1065 (**1**)[1] and the duocarmycins **2** and **3**[2−4] are the parent members of a novel class of exceptionally potent naturally occurring antitumor antibiotics (Figure 1). The agents derive their biological activity through a characteristic DNA alkylation reaction that has been shown to proceed by a reversible adenine N3 addition to the least substituted carbon of the activated cyclopropane at selected AT-rich sites within the minor groove.[5−13]

An important structural element of the agents is the alkylation subunit vinylogous amide which contributes to their unusual chemical stability. At least one early study[14] as well as comparisons with the exceptionally reactive Winstein cyclopropylcyclohexadienones[15,16] qualitatively established the presence but not the extent of

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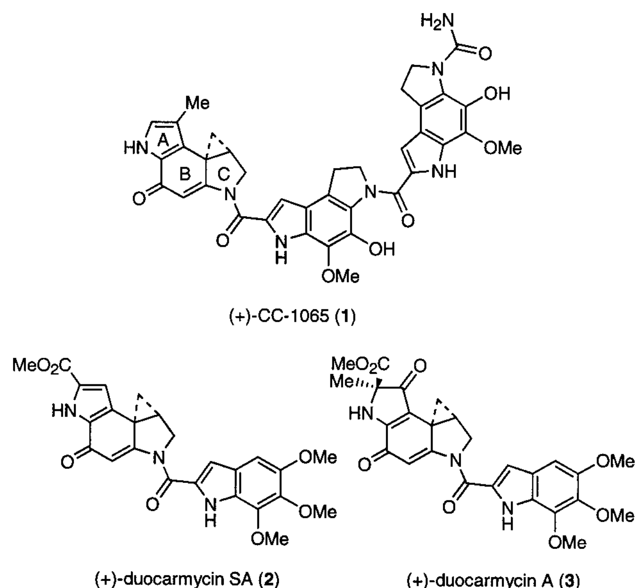

Figure 1.

this stabilization. More recently, the importance of this stabilization was revealed in studies of modified alkylation subunits including **7**–**9** in which the relative reactivities correlated with the extent of vinylogous amide conjugation established in a series of X-ray structures (Figure 2).[17–20] A remarkable range of reactivities (ca. $10^4\times$) accompanied the vinylogous amide deconjugation albeit in a series that incorporated other structural perturbations. These and a series of related studies further suggested that DNA alkylation catalysis is not derived from a long postulated[6,10] and poorly documented[21] acid-catalyzed C4 carbonyl protonation but rather a DNA binding-induced conformational change that disrupts the cross-conjugated vinylogous amide stabilization activating the agents for nucleophilic attack.[11,16] This raised the central question of what the vinylogous amide stabilization is worth and the reactivity consequences of its disruption. Herein, we report the synthesis of 1,2,9,9a-tetrahydro-1*H*-cyclopropa[*c*]benz[*e*]-inden-4-one (CBIn, **10**) and the study of its chemical properties which establishes the extent of vinylogous amide stabilization and clarifies its role.

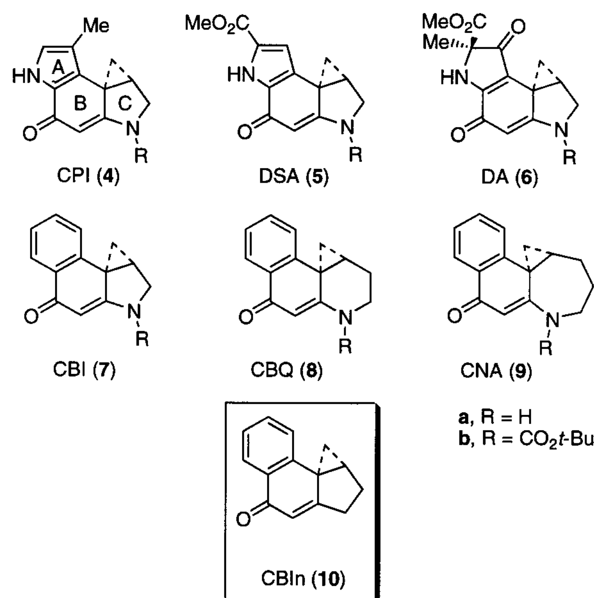

Figure 2.

Synthesis of CBIn (10). The key to the synthesis of the CBIn core was implementation of the intramolecular Heck reaction (Scheme 1).[22] Starting material for the approach was prepared by condensation of benzaldehyde with the Wadsworth–Horner–Emmons reagent **11**[23] (1 equiv, 1.1 equiv NaH, THF, 0–25 °C, 2 h) to provide **12** in which the preferred *E*-isomer predominated (10:1). Selective acid-catalyzed deprotection of the *tert*-butyl ester followed by intramolecular Friedel–Crafts acylation (Ac_2O–NaOAc, 2 h) provided **14** in 73% overall yield from benzaldehyde. Subsequent hydrolysis of the *O*-acetate gave **15** in excellent yield (90%). Bromination in a polar aprotic solvent (DMF) with NBS[24] was followed by direct benzylation (BnBr, K_2CO_3) to afford **16** in 74% overall yield.[25] Although not investigated in detail, the reversal of the order of benzylation and bromination resulted in no product formation upon NBS treatment under a

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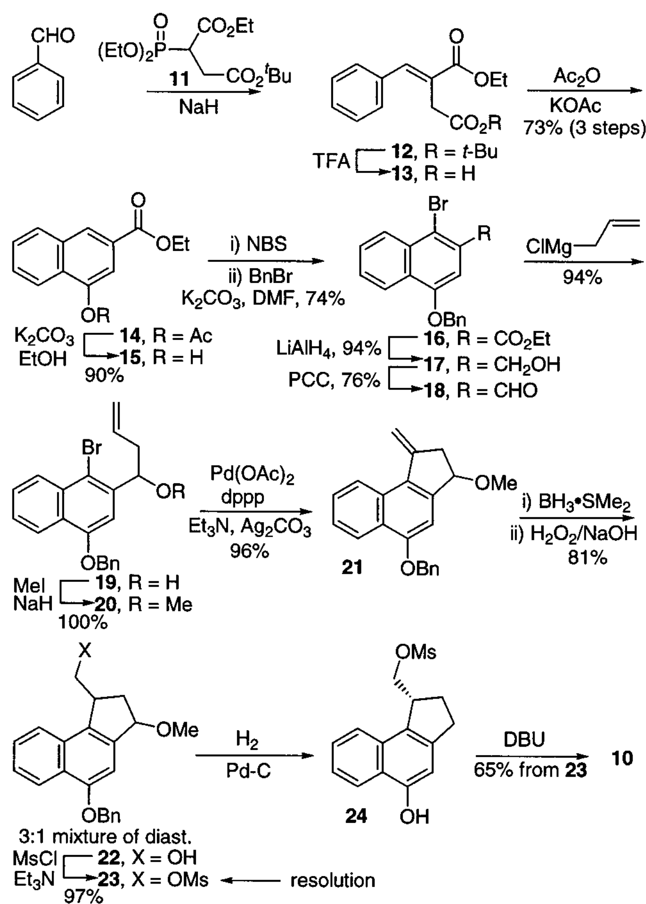
Scheme 1



variety of conditions. Reduction of ethyl ester **16** afforded alcohol **17** (94%) which was oxidized (PCC) to provide aldehyde **18** (76%). Treatment of **18** with allylmagnesium chloride gave the alcohol **19** and set the stage for an intramolecular Heck reaction. Use of $Pd(PPh_3)_4$ as catalyst and Et_3N as base[20] gave a maximum yield of 30% for the desired five-membered ring cyclization product[25] with predominant amounts of recovered starting material under a range of conditions, and protection of the hydroxy group did little to increase the reaction yield. However, the use of **20**, $Pd(OAc)_2$, bis(diphenylphosphino)propane, and Ag_2CO_3[22b] provided a reaction that proceeded in a reproducible 96% yield with no evidence of the endocyclic methylene product resulting from readdition of the intermediate palladium hydride species.

Securing **21** allowed completion of the synthesis following protocols introduced with our synthesis of CC-1065.[17,26] Hydroboration followed by oxidative workup converted **21** to the desired alcohol **22** (81%).[17,20,26] Treatment of **22** with methanesulfonyl chloride in the presence of Et_3N furnished **23** in excellent yield (97%).

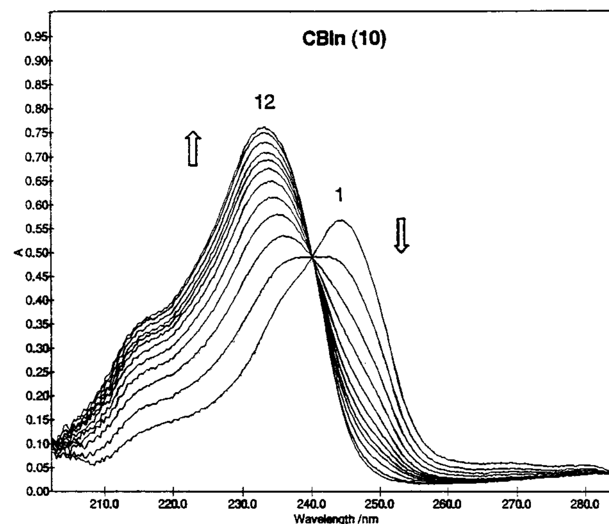


Figure 3. Solvolysis study (UV spectra) of CBIn (**10**) in 50% CH_3OH–aqueous buffer (pH 4.0, 0.02 M boric acid, 0.005 M citric acid, 0.02 M NaH_2PO_4). The spectra were recorded at regular intervals, and only a few are shown for clarity: 1, 0 min; 2, 100 min; 3, 200 min; 4, 300 min; 5, 400 min; 6, 500 min; 7, 600 min; 8, 700 min; 9, 800 min; 10, 1000 min; 11, 1400 min; 12, 2000 min.

Simultaneous removal of the benzyl protecting group and hydrogenolysis of the C3 methoxy group provided the unstable seco-derivative **24** which had a propensity to undergo spirocyclization during purification. Consequently, the final agent was obtained by treating crude **24** directly with DBU to furnish CBIn (**10**) in 65% overall yield for the two steps. The major diastereomer of **23** was chromatographically resolved (>99.9% ee) on a Chiracel OD semipreparative HPLC column (2 × 25 cm, 20% *i*-PrOH/hexane, 7 mL/min, α = 1.26) providing optically active **23** and **10**.

Solvolysis Reactivity. The rate of solvolysis and the regioselectivity of cyclopropyl ring opening have proven important in understanding the structural features underlying the chemical and biological properties of the duocarmycins and CC-1065.[5] In the case of CBIn, the direct comparison with CBI (**7a**) and *N*-BOC–CBI (**7b**) was expected to establish the extent of vinylogous amide stabilization within the authentic alkylation subunits and help define its role. In a preliminary evaluation, the solvolysis reactivity of CBIn (**10**) was followed spectrophotometrically by UV at both 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) and pH 7 (50% CH_3OH–H_2O) [see Figure 3 for a representative run]. CBIn displayed half-lives of 1.3 and 4.0 h at pH 3 and 7, respectively. The direct comparison of **10** with CBI (**7a**) which contains N^2 and the vinylogous amide characteristic of the natural products revealed that **10** is 3200× more reactive at pH 3 but $>10^3$–10^4× more reactive at pH 7 where CBI is completely stable and exhibits no solvolytic reactivity. Thus, removal of the vinylogous amide stabilization from **7** results in a $\geq 10^3$ increase in reactivity at pH 3 and an even larger increase at pH 7. In addition, the exceptionally small 3.2× difference in the rate of solvolysis for **10** over a pH range of 4 units was surprising. A first-order dependence on hydronium ion concentration on the rate of solvolysis would require a difference of 10^4. However, two prior agents with reactivities allowing measurable solvolysis at pH 7, *N*-BOC–CBQ (**8b**) and *N*-BOC–CNA (**9b**), also

(25) Trace amounts of the 2,4-dibromide were isolated (5–10%) in the bromination of **15**: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.27–8.25 (m, 1H), 8.12–8.10 (m, 1H), 7.67–7.56 (m, 4H), 7.47–7.38 (m, 3H), 5.11 (s, 2H), 4.56 (q, J = 7.2 Hz, 2H), 1.49 (t, J = 7.2 Hz, 3H); IR (film) ν_{max} 3062, 3034, 2978, 2922, 1738, 1575 cm^{-1}. For 5-(benzyloxy)-3-hydroxy-1-methylidene-2,3-dihydro-1*H*-benz[*e*]indene: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.20–8.17 (m, 1H), 7.86–7.83 (m, 1H), 7.74 (s, 1H), 7.58–7.56 (m, 2H), 7.48–7.42 (m, 5H), 6.08–6.07 (m, 1H), 5.40–5.36 (m, 2H), 5.10 (d, J = 11.1 Hz, 1H), 5.02 (d, J = 11.1 Hz, 1H), 3.26 (dddd, J = 16.3, 7.2, 2.0, 2.0 Hz, 1H), 2.78 (dddd, J = 16.2, 4.4, 2.3, 2.3 Hz, 1H); IR (film) ν_{max} 3327, 3064, 3035, 2898, 2839, 1639 cm^{-1}; FABHRMS (NBA–NaI) *m*/*z* 302.1317 (M^+, $C_{21}H_{18}O_2$ requires 302.1307).

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Table 1. Solvolysis Rates under Phosphate Buffer Conditions[a]

pH	k_{obs} (s^{-1})	$t_{1/2}$ (h)
5.0	3.31×10^{-5}	5.81
6.0	3.89×10^{-5}	4.95
7.0	4.27×10^{-5}	4.51
8.0	5.37×10^{-5}	3.59

[a] Buffer consists of 0.1 M NaH_2PO_4-NaH_2PO_4.

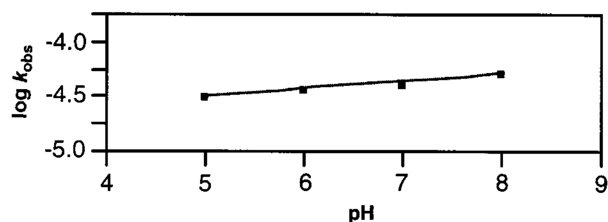

Figure 4. Plot of pH versus log k_{obs} for solvolysis using potassium phosphate buffer (0.1 M, pH 5−8).

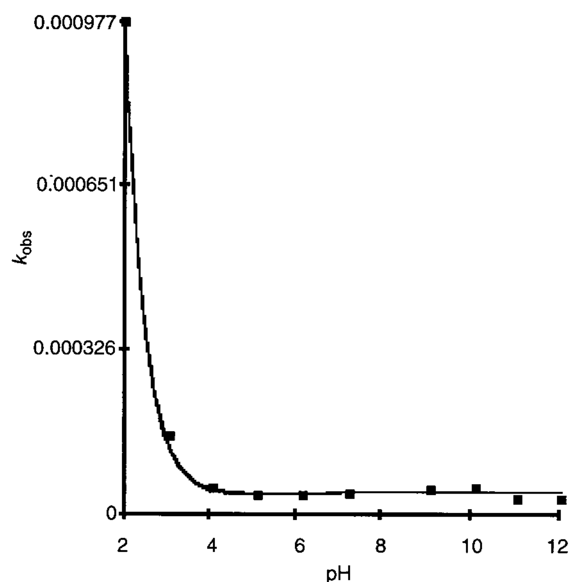

Figure 5. Plot of pH versus k_{obs} for solvolysis using a universal buffer (0.11−0.24 M, pH 2−12, 0.2 M $B(OH)_3$, 0.05 M citric acid, 0.1 M Na_3PO_4).

displayed incongruent differences of 259× and 75×, respectively, in this pH range.[19,20] Consequently, a closer examination of the solvolysis pH dependence was conducted.

Solvolysis pH Dependence. The first such study addressed the rate of cyclopropane ring cleavage in a physiologically relevant pH range. A sodium phosphate buffer system (0.2 M NaH_2PO_4/Na_2HPO_4) amenable to a pH range of 5−8 was used. Table 1 summarizes the results, and Figure 4 illustrates a plot of the log k_{obs} versus pH where once again little difference in k_{obs} was seen over a pH range of 3 units. In fact, a slight inverse correlation of hydronium ion concentration to rate was observed in which the $t_{1/2}$ at pH 5 was 5.9 h while that at pH 8 was 3.8 h.

With an agent that has a relatively short $t_{1/2}$ throughout the full pH spectrum, it was possible to measure the rate of solvolysis over a wide range under universal buffer conditions (0.2 M boric acid, 0.05 M citric acid, and 0.1 M Na_3PO_4, pH 2−12, 0.11−0.24 M), Figure 5. A slight linear dependence on buffer concentration was observed at pH 4, and no dependence was observed at pH 8 (Table

Table 2. Solvolysis Rates under Universal Buffer Conditions[a]

pH	k_{obs} (s^{-1})	$t_{1/2}$ (h)
2.0	9.77×10^{-4}	0.20
3.0	1.58×10^{-4}	1.21
4.0	5.37×10^{-5}	3.59
5.0	3.80×10^{-5}	5.06
6.0	3.80×10^{-5}	5.06
7.1	4.37×10^{-5}	4.41
8.0	4.25×10^{-5}	4.51[b]
8.0	4.42×10^{-5}	4.35[c]
8.0	3.72×10^{-5}	5.17[d]
9.0	4.79×10^{-5}	4.02
10.0	5.13×10^{-5}	3.75
11.0	2.88×10^{-5}	6.68
12.0	2.34×10^{-5}	8.21

[a] Buffer consists of 0.1 M $B(OH)_3$, 0.05 M citric acid, and 0.1 M Na_3PO_4. [b] 0.16 M buffer, 0.173 M ionic strength adjusted with $NaClO_4$. [c] 0.08 M buffer, 0.173 M ionic strength adjusted with $NaClO_4$. [d] 0.04 M buffer, 0.173 M ionic strength adjusted with $NaClO_4$.

2). Similarly, no dependency on the ionic strength was observed. This was apparent even by comparing the solvolysis rate constants (pH 7) measured under the three conditions of CH_3OH-H_2O, phosphate buffer, and universal buffer where the rates were 4.81, 4.27, and 4.37 $\times 10^{-5}$ s^{-1}, respectively. At very low pH (2−3), the solvolysis of CBIn appears to exhibit a near first-order rate dependence on pH which is in agreement with observations made in previous studies with **4**−**9** and related agents.[21,27] When the pH is increased above 3.5, the dependence on acid concentration disappears. In fact, there is no difference in the solvolysis rate at pH 4 and pH 8 and essentially no difference over the full pH 4−12 range (2×). This along with the lack of a buffer concentration dependence indicates that, above pH 4, the mechanism of solvolysis changes from acid-catalyzed to one which is uncatalyzed and represents a direct rate-determining S_N2 nucleophilic attack of H_2O onto **10** conducted under pseudo-first-order reaction conditions. From a regression analysis best fit of the k_{obs} versus pH data, rate constants of 0.093 ± 0.001 M^{-1} s^{-1} and $4.2 \pm 0.4 \times 10^{-5}$ s^{-1} for the acid-catalyzed and uncatalyzed reactions, respectively, were established. The rate of the uncatalyzed reaction is substantial and dominates at pH ≥ 4. The lack of a significant base-catalyzed reaction at the higher pH is interesting and was unexpected.[15]

Solvolysis Regioselectivity and Mechanism. Treatment of CBIn (**10**) with 0.36 equiv of CF_3SO_3H in a mixture of H_2O and THF resulted in clean solvolysis (>88%) to provide a 15:1 mixture of products (Scheme 2). Comparison of this mixture to **26**, which would result from attack of H_2O at the less substituted cyclopropane carbon, confirmed that ring expansion had occurred predominantly to afford **25**. In contrast, treatment of **10** with HCl (1.5 equiv, −78 °C, 5 min) provided a 1:2 mixture of **27** and **28** with the non ring expanded product **28** predominating. The preference for the site of nucleophilic attack by H_2O has been shown to be dependent on the relative stereoelectronic alignment of the sissile cyclopropyl bonds with the cyclohexadienone.[19,28,29] Nu-

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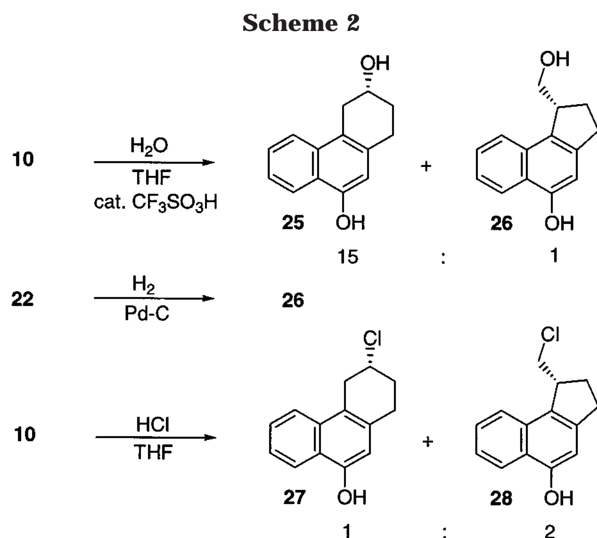
(28) Boger, D. L.; Goldberg, J.; McKie, J. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1955.

(29) Boger, D. L.; McKie, J. A.; Nishi, T.; Ogiku, T. *J. Am. Chem. Soc.* **1997**, *119*, 311.

Scheme 2



cleophilic additions of H_2O affording ring expansion products have been shown to possess a better orbital overlap of the bond extending to the more substituted cyclopropane carbon with the π-system of the cyclohexadienone.[20]

Conducting the acid-catalyzed solvolysis reaction (0.36 equiv CF_3SO_3H, THF−H_2O, 25 °C, 2 h) with optically active **10** produced a single enantiomer of **25** indicating S_N2, not S_N1, solvolysis (Figure 6) and identical observations were made with the pH 3 and 7 solvolysis product **25**.[30] Thus, the switch in selectivity from near exclusive ring expansion addition of H_2O to predominate non ring expansion addition of chloride may be attributed to characteristics of an S_N2 reaction where the intrinsic stereoelectronic preference for ring expansion is overridden in part by the steric preference for addition to the least substituted position by the larger nucleophiles. Although we were not successful at securing suitable crystals of **10** for an X-ray that would define the cyclopropane structural disposition needed to confirm these conclusions, analogous prior observation of the enhanced regioselectivity of chloride versus H_2O or CH_3OH addition to the least substituted cyclopropane carbon has been made in a number of our[11,19] and related studies.[21] Thus, the removal of N^2 reverses the intrinsic stereoelectronic preference for acid-catalyzed addition of H_2O from >20:1 for CBI[17] to 1:15 for CBIn. Like the CBI derivatives, the nucleophilic additions to **10** occur exclusively by a S_N2, not S_N1, addition at pH ≥ 3 where this has been examined.

Discussion. The remarkable chemical stability of **1**−**3** and the requirement for acid catalysis for addition of typical nucleophiles have led to the assumption that the DNA alkylation reaction must similarly be an acid- or Lewis acid-catalyzed reaction. Although efforts have gone into supporting the role of such acid catalysis,[21] it remains undocumented for the DNA alkylation reaction. At pH 7.4, one can estimate that the DNA phosphate backbone (pK_a 1−1.5) is fully ionized with less than 1 in 2500000−800000 phosphates bearing a proton (0.0001−0.00004% protonated). It is unlikely that catalysis for DNA alkylation originates from a backbone phosphate delivery of a proton to the C4 carbonyl. Although increases in the local hydronium ion concentration surrounding "acidic domains" of DNA have been invoked to explain DNA-mediated acid catalysis[31] and nucleotide reactivity[6] and extrapolated to alkylation site catalysis for **1**−**3**,[21] the intrinsic acid stability of **1**−**3** even at pH 5 suggests such proposals cannot account for catalysis.

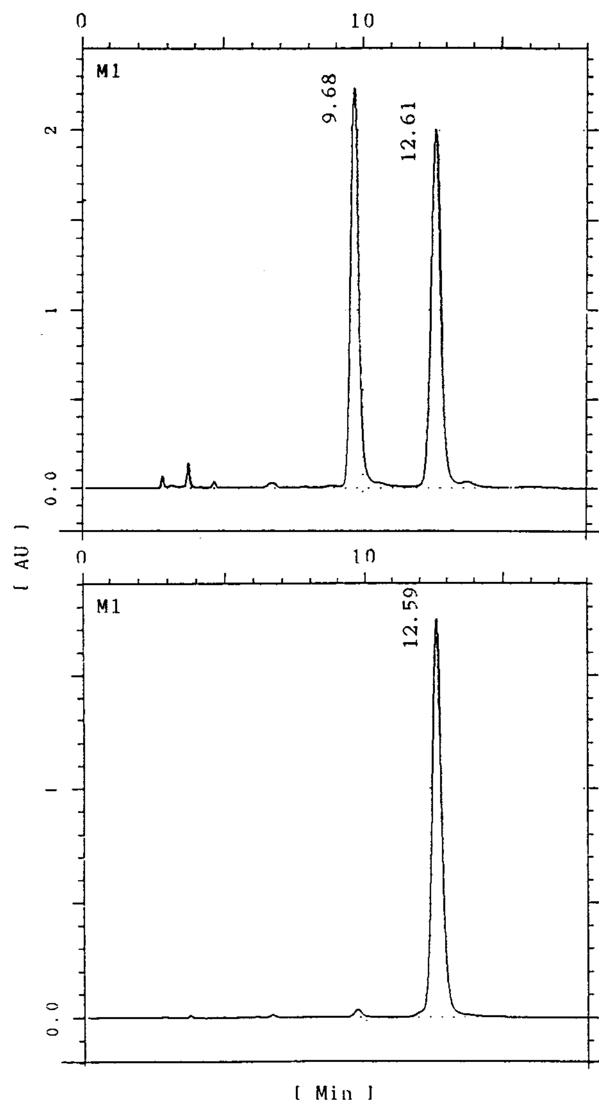


Figure 6. Chiral phase HPLC separation of the product **25** of acid-promoted addition of H_2O to racemic **10** (top) and (−)-**10** (bottom). Chiracel AD HPLC column (10 μm, 0.46 × 25 cm), 15% *i*-PrOH/hexane, 1 mL/min.

Recent studies have shown that pH has essentially no effect on the rate of DNA alkylation,[11,12] and the rate change upon lowering the pH from 8 to 6 was less than 2−3× inconsistent with a first-order dependence. A number of unrelated observations also support these observations and indirectly do not support an acid-catalyzed DNA alkylation reaction. These include the lack of correlation between acid-catalyzed reactivity and DNA alkylation rate[5,7,11,12,16,17,32] even with closely related structural analogues bearing minor structural perturbations,[17c] the structural requirement for an extended rigid N^2 amide substituent for observation of DNA alkyla-

(30) Figures illustrating this may be found in the Supporting Information.

(31) Jayaram, B.; Sharp, K. A.; Honig, B. *Biopolymers* **1989**, *28*, 975. Lamm, G.; Pack, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9033.

(32) Boger, D. L.; Munk, S. A.; Ishizaki, T. *J. Am. Chem. Soc.* **1991**, *113*, 2779.

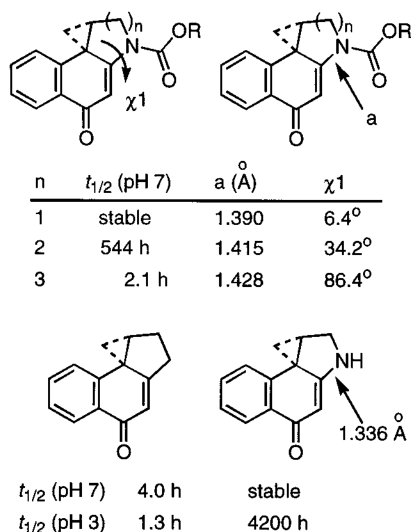


Figure 7.

tion catalysis,[11] and the fact that relocation of the C4 carbonyl from the periphery of the complex to the interior of the minor groove inaccessible to the phosphate backbone or solvent has no impact on the intrinsic selectivity or rate of DNA alkylation.[33] More fundamental to the source of the sequence selectivity, studies have also shown that alternative electrophiles incorporated into the structures exhibit identical selectivities even though they are not subject to acid catalysis, thus removing its requirement for or role in the sequence-determining step of DNA alkylation.[7]

In a recent series of studies involving the X-ray comparisons of **7**−**9**, a direct correlation between the extent of vinylogous amide conjugation and their relative reactivities was established (Figure 7). Tracking with a decrease in cross-conjugated vinylogous amide stabilization (increases in the "a" bond length) and the increase in reactivity was an increase in the χ_1 dihedral angle and a concomitant increase in the bond lengths and conjugation of the cyclopropane.[20] These studies established a direct relationship between diminished vinylogous amide conjugation and reactivity and demonstrated that sufficient reactivity changes accompany even partial disruption of the vinylogous amide conjugation to account for DNA alkylation catalysis (i.e., **8**).[34] These and related studies led us to propose that the rate enhancement for DNA alkylation is not due to acid catalysis, but rather a DNA binding-induced conformational change in the agent that disrupts the cross-conjugated vinylogous amide stabilization of the alkylation subunit and activates the agent for nucleophilic addition.[11,16,20] These observations were consistent with a prior disclosure of ours where the Hammett ρ value for the N^2 substituent was established to be exceptionally large, −3.0, indicating that even small perturbations to the vinylogous amide have a large impact on reactivity.[35]

The comparison of CBIn (**10**), which lacks N^2 and the vinylogous amide, with prior agents including the structural homologue CBI (**7**), permits the direct assessment of the vinylogous amide stabilization. Its presence in CBI (**7a**) increases the stability by 3200× at pH 3 and >10^3–10^4× at pH 7 relative to **10**. More interestingly, **10** exhibited a solvolysis first-order dependence on hydronium ion concentration only at pH 2−3, and above pH 4 there was no apparent dependence on acid concentration. Therefore, the extrapolation of the pH 2−3 acid-catalyzed reactivity of typical agents, including **1**−**9** which are not reactive at pH 5−8, to a pH of 7 is not a viable means of assessing reactivity in a biologically relevant pH range.[21] Throughout the range examined (pH ≥ 3) nucleophilic addition to **10** exhibited S_N2, not S_N1, characteristics with a rate that was independent of pH. Above pH 4, this would be consistent with a reaction that is uncatalyzed and entails direct rate-determining S_N2 nucleophilic attack. In addition, CBIn (**10**) addition of H_2O occurred nearly exclusively at the more substituted cyclopropane carbon with ring expansion while the larger nucleophile chloride underwent predominate addition at the least substituted center. Thus, removal of N^2 altered the intrinsic preference for non ring expansion addition observed with **1**−**7** and, extrapolating from prior structural studies, may be attributed to a potential conformational role in defining the structural disposition of the cyclopropane.

Importantly, CBIn exhibits a reactivity at pH 7 that is similar to that seen for the natural agents in the presence of DNA ($t_{1/2}$ for alkylation ca. 1 h) suggesting that a DNA binding-induced conformational change that disrupts the vinylogous amide stabilization would provide sufficient activation (reactivity) for the reaction and would do so independent of pH. This reactivity, the lack of a DNA alkylation rate pH dependence,[11,12] the structural characterization of DNA-bound helical (twisted) conformations of the agents consistent with this source of activation,[36] the demonstration of the requirement for a rigid extended N^2 amide substituent for catalysis,[11] and the documentation of the absolute critical role of the N^2 amide for activation[37] provide compelling support for this proposal.

Experimental Section

5-(Benzyloxy)-3-methoxy-1-methylidene-2,3-dihydro-1*H*-benz[*e*]indene (21). A solution of **20**[38] (0.926 g, 2.34 mmol, 1 equiv) in CH_3CN (degassed, 44 mL) was treated sequentially with Et_3N (0.651 mL, 4.68 mmol, 2 equiv), Ag_2CO_3 (0.645 g, 2.34 mmol, 1 equiv), 1,3-bis(diphenylphosphino)propane (0.232 g, 0.56 mmol, 0.24 equiv), and $Pd(OAc)_2$ (0.063 g, 0.286 mmol, 0.12 equiv). The resulting mixture was stirred in a sealed tube at 25 °C for 10 min and at 80 °C for 2 h. Radial chromatography (SiO_2, 4 mm plate, 10% EtOAc−hexane) provided **21** as a yellow oil (0.710 g, 96%): ^1H NMR (400 MHz, $CDCl_3$) δ 8.20−8.17 (m, 1H), 7.87−7.83 (m, 1H), 7.73 (s, 1H), 7.59−7.58 (m, 2H), 7.49−7.36 (m, 5H), 6.08 (d, J = 1.0 Hz, 1H), 5.41 (d, J = 1.1 Hz, 1H), 5.12 (d, J = 11.1 Hz, 1H), 5.03 (d, J = 11.1 Hz, 1H), 4.98 (ddd, J = 6.9, 3.9, 0.7 Hz, 1H), 3.48 (s, 3H), 3.15 (dddd, J = 16.1, 7.0, 2.0, 2.0 Hz, 1H), 2.86 (dddd, J = 16.1, 3.8, 2.2, 2.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.0, 144.2, 144.1, 137.3, 135.3, 128.9, 128.6 (2C), 128.3, 128.1, 127.7 (2C), 126.3, 126.0, 122.4, 120.7, 110.7, 87.4, 80.8, 74.0, 56.2, 40.4; IR (film) ν_{max} 3056, 3026, 2923, 2821

(33) Boger, D. L.; Garbaccio, R. M., Jin, Q. *J. Org. Chem.* **1997**, *62*, 8875.

(34) This is especially true if one considers the additional entropic contribution to the DNA alkylation rate provided by the high affinity noncovalent minor groove binding (ca. 10^2×).

(35) Boger, D. L.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 5523.

(36) Eis, P. S.; Smith, J. A.; Rydzewski, J. M.; Case, D. A.; Boger, D. L.; Chazin, W. J. *J. Mol. Biol.* **1997**, *272*, 237.

(37) Boger, D. L.; Santillán, A.; Searcey, M.; Jin. Q. *J. Am. Chem. Soc.* **1998**, *120*, in press.

(38) Experimental details for the preparation of **15**−**20** are provided in Supporting Information.

1641, 1569 cm^{-1}; FABHRMS (NBA−NaI) *m/z* 316.1473 (M^+, $C_{22}H_{20}O_2$ requires 316.1463).

5-(Benzyloxy)-1-(hydroxymethyl)-3-methoxy-2,3-dihydro-1*H*-benz[*e*]indene (22). A solution of **21** (0.751 g, 2.38 mmol, 1 equiv) in THF (24 mL) was cooled to 0 °C prior to dropwise addition of $BH_3 \cdot SMe_2$ (0.713 mL, 10 M, 3(9) equiv). The cooling bath was removed after 5 min, and the mixture was stirred at 25 °C for 3 h. The excess borane was quenched with slow addition of H_2O (11.0 mL). Oxidative workup was accomplished by addition of 2.5 M aqueous NaOH (5.0 mL) followed by 30% aqueous H_2O_2 (3.20 mL, 12 equiv), and the resulting heterogeneous solution was stirred vigorously at 25 °C (1 h) and 45 °C (2 h). The cooled reaction mixture was treated with saturated aqueous NaCl (5.0 mL), and the layers were separated. The aqueous portion was extracted with EtOAc (2 × 30 mL), and the combined organic portions were dried ($MgSO_4$), filtered, and concentrated. Radial chromatography (SiO_2, 4 mm plate, 30% EtOAc−hexane) provided **22** as a colorless oil (0.645 g, 81%): mixture of diastereomers (major diastereomer); ^1H NMR (400 MHz, $CDCl_3$) δ 8.15−8.09 (m, 1H), 7.88−7.85 (m, 1H), 7.71 (s, 1H), 7.51−7.35 (m, 7H), 5.17−5.06 (m, 2H), 4.78 (dd *J* = 6.2, 1.8 Hz, 1H), 3.99 (dd, *J* = 10.7, 4.7 Hz, 1H), 5.12 (dd, *J* = 10.6, 5.3 Hz, 1H), 3.54−3.48 (m, 1H), 3.46 (s, 3H), 2.51 (ddd, *J* = 14.0, 8.7, 6.3 Hz, 1H), 2.25 (t, *J* = 6.4 Hz, 1H), 2.03 (ddd, *J* = 14.0, 2.7, 2.7 Hz, 1H); IR (film) ν_{max} 3403, 3055, 2923, 2882, 2820, 1603, 1573 cm^{-1}; FABHRMS (NBA−NaI) *m/z* 335.1659 ($M + H^+$, $C_{22}H_{22}O_3$ requires 335.1647).

5-(Benzyloxy)-1-[((methanesulfonyl)oxy)methyl]-3-methoxy-2,3-dihydro-1*H*-benz[*e*]indene (23). A solution of **22** (0.596 g, 1.78 mmol, 1 equiv) in CH_2Cl_2 (18.0 mL) was cooled to 0 °C prior to sequential addition of Et_3N (1.24 mL, 8.92 mmol, 5 equiv) and CH_3SO_2Cl (0.276 mL, 3.57 mmol, 2 equiv). The cooling bath was removed after 20 min, and the mixture was stirred at 25 °C for 1 h. The reaction was quenched with the addition of saturated aqueous $NaHCO_3$ (0.25 mL), and the layers were separated. The aqueous portion was extracted with EtOAc (2 × 30 mL), and the combined organic portions were dried ($MgSO_4$), filtered, and concentrated. Radial chromatography (SiO_2, 4 mm plate, 50% EtOAc−hexane) furnished **23** as a white solid (0.709 g, 97%): mixture of diastereomers (major diastereomer); mp 101−103 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.15−8.11 (m, 1H), 7.89−7.86 (m, 1H), 7.70 (s, 1H), 7.52−7.36 (m, 7H), 5.12 (s, 2H), 4.81−4.77 (m, 2H), 4.25 (t, *J* = 9.8 Hz, 1H), 3.61−3.51 (m, 1H), 3.44 (s, 3H), 2.91 (s, 3H), 2.46 (m, 1H), 2.25 (ddd, *J* = 14.0, 3.2, 3.2 Hz, 1H); IR (film) ν_{max} 3058, 3025, 2926, 2816, 1600, 1573 cm^{-1}; FABHRMS (NBA−CsI) *m/z* 545.0414 ($M + Cs^+$, $C_{23}H_{24}O_5S$ requires 545.0399).

Anal. Calcd for $C_{23}H_{24}O_5S$: C, 66.97; H, 5.87; S, 7.76. Found: C, 66.80; H, 5.60; S, 7.88.

A solution of **23** (0.100 g) in 50% *i*-PrOH/hexane was resolved on a semipreparative Diacel Chiracel OD column (10 μm, 2 × 25 cm) using 20% *i*-PrOH/hexane as eluent (7 mL/min). The effluent was monitored at 254 nm, and the enantiomers of the major diastereomer were eluted with retention times of 23.9 and 30.1 min, respectively (α = 1.26). The fractions were collected and concentrated to afford (+)-**23** (t_R = 23.9 min/0.031 g) and (−)-**23** (t_R = 30.1 min/0.032 g) with an 84% recovery based on the starting diastereomeric ratio (>99.9% ee).

(+)-**23**: $[\alpha]^{25}_D$ +10 (*c* 0.016, THF).

(−)-**23**: $[\alpha]^{25}_D$ −11 (*c* 0.016, THF).

2,3,9,9a-Tetrahydro-1*H*-cyclopropa[*c*]benz[*e*]inden-4-one (CBIn, 10). A solution of **23** (0.256 g, 0.62 mmol, 1 equiv) in EtOAc (13 mL) was treated with 10% Pd−C (0.126 g, 0.18 mmol, 0.2 equiv) and the reaction vessel was equipped with an H_2-filled balloon. After 2 h of stirring at 25 °C, the crude mixture was filtered through a plug of Celite. Concentration gave **24** as a pale yellow oil that was dissolved in CH_3CN (15 mL), treated with DBU (0.143 mL, 0.68 mmol, 1.1 equiv), and stirred at 25 °C for 5 min. Radial chromatography of the crude reaction solution (SiO_2, 1 mm plate, 20% EtOAc−hexane) furnished **10** as a bright yellow solid (0.079 g, 65%): mp 53−54 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.07−8.05 (m, 1H), 7.55 (ddd, *J* = 8.9, 7.5, 1.4 Hz, 1H), 7.33−7.26 (m, 2H), 6.54 (s, 1H), 2.87 (ddd, *J* = 7.2, 5.9, 5.9 Hz, 1H), 2.51 (dd, *J* = 16.3, 8.6 Hz, 1H), 2.48−2.38 (m, 1H), 2.20−2.13 (m, 1H), 2.10 (dd, *J* = 8.1, 3.1 Hz, 1H), 2.03 (dd, *J* = 11.8, 7.0 Hz, 1H), 1.76 (dd, *J* = 6.0, 3.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.2, 149.9, 140.4, 133.8, 128.6, 127.2, 126.4, 125.9, 116.7, 43.5, 38.5, 31.6, 27.3, 27.2; IR (film) ν_{max} 3056, 2923, 2851, 1662, 1641, 1595 cm^{-1}; UV (THF) λ_{max} 241 (ε 35 260) nm; FABHRMS (NBA−NaI) *m/z* 197.0958 ($M + H^+$, $C_{14}H_{12}O$ requires 197.0966).

(+)-**10**: $[\alpha]^{25}_D$ +12 (*c* 0.0032, EtOAc) from (+)-**23**.

(−)-**10**: $[\alpha]^{25}_D$ −11 (*c* 0.0017, EtOAc) from (−)-**23**.

5-Hydroxy-1-(hydroxymethyl)-2,3-dihydro-1*H*-benz[*e*]indene (26). A solution of **22** (0.006 g, 0.018 mmol, 1 equiv) in EtOAc (0.4 mL) was treated with 10% Pd−C (0.002 g, 0.002 mmol, 0.1 equiv), and the reaction vessel was equipped with an H_2-filled balloon. After 1 h of stirring at 25 °C, the crude mixture was filtered through a plug of Celite. Radial chromatography (SiO_2, 1 mm plate, 20% EtOAc−hexane) furnished **26** as a pale yellow oil (0.0028 g, 74%): ^1H NMR (400 MHz, acetone-d_6) δ 10.32 (s, 1H), 8.19−8.17 (m, 1H), 7.69−7.67 (m, 1H), 7.36−7.31 (m, 2H), 7.17 (s, 1H), 6.07−6.03 (m, 1H), 4.12−4.07 (m, 1H), 3.70−3.61 (m, 1H), 3.61−3.52 (m, 1H), 3.07−2.88 (m, 2H), 2.30−2.21 (m, 1H), 1.68−1.57 (m, 1H); ^1H NMR (400 MHz, $CDCl_3$) δ 9.35 (s, 1H), 8.25−8.23 (m, 1H), 7.69−7.66 (m, 1H), 7.41−7.35 (m, 2H), 7.21 (s, 1H), 4.15 (dd, *J* = 9.1, 3.3 Hz, 1H), 3.83−3.78 (m, 1H), 3.68−3.60 (m, 1H), 3.09−2.98 (m, 2H), 2.61 (bs, 1H), 2.29 (dddd, *J* = 13.0, 8.1, 8.1, 5.0 Hz, 1H), 1.66 (ddd, *J* = 16.6, 12.7, 8.2 Hz, 1H); IR (film) ν_{max} 3190, 3038, 2918, 2853, 1634, 1569 cm^{-1}; FABHRMS (NBA−NaI) *m/z* 214.0988 (M^+, $C_{14}H_{14}O_2$ requires 214.0994).

Acid-Catalyzed Addition of H_2O to 10: 2,6-Dihydroxy-1,2,3,4-tetrahydrophenanthrene (25). A solution of **10** (0.0029 g, 0.015 mmol, 1 equiv) in THF (0.3 mL) was treated with H_2O (0.10 mL) followed by CF_3SO_3H (0.053 mL, 0.1 M in H_2O, 0.36 equiv) at 25 °C, and the mixture was stirred for 2 h. The reaction mixture was treated with $NaHCO_3$ (0.01 g) followed by H_2O (0.5 mL). The aqueous portion was extracted with EtOAc (2 × 1 mL), and the combined organic portions were dried ($MgSO_4$), filtered, and concentrated to a colorless oil. ^1H NMR analysis of the crude mixture and comparison with **26** indicated the presence of the ring expansion solvolysis product in great excess (>15:1). Radial chromatography (SiO_2, 1 mm plate, 50−75% EtOAc−hexane) provided **25** as a colorless oil (0.0028 g, 88%): ^1H NMR (400 MHz, acetone-d_6) δ 8.17−8.14 (m, 1H), 7.93 (s, 1H), 7.70−7.67 (m, 1H), 7.37−7.30 (m, 2H), 7.17 (s, 1H), 4.17−4.10 (m, 1H), 3.95 (d, *J* = 4.2 Hz, 1H), 3.25 (dd, *J* = 16.7, 5.2 Hz, 1H), 3.10 (ddd, *J* = 16.6, 5.8, 5.8 Hz, 1H), 2.96−2.87 (m, 1H), 2.72 (dd, *J* = 16.7, 7.8 Hz, 1H), 2.05−2.03 (m obscured by solvent, 1H), 1.84−1.71 (m, 1H); ^1H NMR (400 MHz, $CDCl_3$) δ 8.03−8.00 (m, 1H), 7.70−7.67 (m, 1H), 7.42−7.37 (m, 2H), 7.23 (s, 1H), 5.16 (bs, 1H), 4.31−4.25 (m, 1H), 3.24−3.12 (m, 2H), 3.03−2.95 (m, 1H), 2.74 (dd, *J* = 15.9, 7.8 Hz, 1H), 2.14−2.09 (m, 1H), 1.92−1.85 (m, 1H); IR (film) ν_{max} 3333, 3046, 2923, 1651, 1595, 1569 cm^{-1}; UV (THF) λ_{max} 238 (ε 26 680), 216 (ε 12 920) nm; FABHRMS (NBA−NaI) *m/z* 214.0986 (M^+, $C_{14}H_{14}O_2$ requires 214.0994).

A solution of (−)-**10** (0.0020 g, 0.010 mmol, 1 equiv) in THF (0.3 mL) was treated with H_2O (0.1 mL) followed by CF_3SO_3H (0.37 mL, 0.1 M in H_2O, 0.36 equiv) at 25 °C, and the mixture was stirred for 2 h. Workup as described for racemic **10** and radial chromatography (SiO_2, 1 mm plate, 50−75% EtOAc−hexane) provided **25** as a colorless oil (0.0015 g, 69%). This material was identical with racemic **25** except the solvolysis provided a single enantiomer (Figure 6) established by chiral phase HPLC separation on a Chiralcel AD column (10 μm, 0.46 × 25 cm, 15% *i*-PrOH/hexane, 2 mL/min).

(+)-**25**: $[\alpha]^{25}_D$ +26 (*c* 0.00075, EtOAc).

A solution of (+)-**10** (0.0021 g, 0.011 mmol, 1 equiv) in THF (0.3 mL) was treated with H_2O (0.1 mL) followed by CF_3SO_3H (0.039 mL, 0.1 M in H_2O, 0.36 equiv at 25 °C, and the mixture was stirred for 2 h. Workup as described for racemic **25**, and radial chromatography (SiO_2, 1 mm plate, 50−75% EtOAc−hexane) provided **25** as a colorless oil (0.0018 g, 79%). This material was identical with racemic **25** except the solvolysis of (+)-**10** provided a single enantiomer established by chiral

phase HPLC separation on a Chiracel AD column (10 μm, 0.46 × 25 cm, 15% *i*-PrOH/hexane, 1 mL/min).

(−)-**25**: $[\alpha]^{25}_{D}$ −23 (*c* 0.0009, EtOAc).

Addition of HCl to 10: 2-Chloro-6-hydroxy-1,2,3,4-tetrahydrophenanthrene (28). A solution of **10** (0.0030 g, 0.015 mmol, 1 equiv) in THF (0.3 mL) was treated with 3.0 M HCl−THF (0.0080 mL, 1.5 equiv) at −78 °C for 5 min. Evaporation of the volatiles left a yellow oil. ^{1}H NMR analysis of the crude mixture indicated the presence of the normal and the ring expansion addition product (2:1). Radial chromatography (SiO_2, 1 mm plate, 5% EtOAc−hexane) provided **28** as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.10−8.07 (m, 1H), 7.74−7.71 (m, 1H), 7.46−7.39 (m, 2H), 7.30 (s, 1H), 3.85−3.78 (m, 3H), 3.15 (dddd, *J* = 16.2, 9.7,7.9, 1.5 Hz, 1H), 2.99 (dddd, *J* = 15.7, 8.3, 2.9, 1.0 Hz, 1H), 2.41−2.34 (m, 1H), 2.16−2.11 (m, 1H); IR (film) ν_{max} 3507, 3056, 2946, 2916, 2844, 1637, 1601 cm^{-1}; FABHRMS (NBA−CsI) *m/z* 232.0650 (M^+, $C_{14}H_{13}$-ClO requires 232.0655). The ring expansion product **27** coeluted with **10** and the closure of **27** and **28** back to **10** upon chromatography precluded isolation of a pure sample. For **27** observed in the crude ^{1}H NMR of the reaction mixture: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.98−7.95 (m, 1H), 7.72−7.68 (m, 1H), 7.44−7.40 (m, 2H), 7.30 (s, 1H), 5.13 (s, 3H), 4.57−4.49 (m, 1H), 3.43 (dd, *J* = 17.0, 6.0 Hz, 1H), 3.28−3.22 (m, 1H), 3.19−3.11 (m, 1H), 3.36−3.32 (m, 1H), 2.36−2.33 (m, 1H), 2.17−2.11 (m, 1H).

Aqueous Solvolysis of CBIn (10). Samples of **10** (10 μg) were dissolved in CH_3OH (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 UV spectra of the solutions were measured immediately after mixing with the appropriate aqueous solution. The blanks and the solvolysis reaction solutions were stoppered, protected from light, and allowed to stand at 25 °C. The total reaction times reflect those required to observe no further change in absorbance. The UV spectrum was taken every 20 min. The increase of the absorbance at 235 nm was monitored except in the high pH ranges where the decrease in absorbance at 245 nm was monitored. The solvolysis rate was calculated from the least squares treatment of the slope of a plot of time versus $\ln[(A_f - A_i)/(A - A_f)$ or $\ln[(A_f - A_i)/(A_f - A)]$. The effect of buffer concentration on the rate of solvoysis was determined by plotting the k_{obs} versus concentration. The order with respect to acid concentration was determined from a plot of pH versus log k_{obs}.

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Supporting Information Available: Experimental procedures for the preparation of **15**−**20**, chiral phase HPLC traces of the optically active pH 3 and 7 solvolysis product **26**, and ^{1}H NMR spectra of **10**, **16**−**23**, and **25**−**28** (16 pages). This material is contained in 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.

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