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 Alkylation Subunit: Hammett Quantitation of the Magnitude of Electronic Effects on Functional Reactivity

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The synthesis of 7-cyano-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CCBI), a substituted CBI derivative bearing a C7 cyano group, is described in efforts that establish the magnitude of potential electronic effects on the functional reactivity of the agents. The CCBI alkylation subunit was prepared by a modified Stobbe condensation/Friedel−Crafts acylation for generation of the appropriately functionalized naphthalene precursors followed by 5-*exo-trig* aryl radical−alkene cyclization for synthesis of the 1,2-dihydro-3*H*-benz[*e*]indole skeleton and final Ar-3′ alkylation for introduction of the activated cyclopropane. The most concise approach provided the CCBI subunit and its immediate precursor in 14−15 steps in superb overall conversions (15−20%). Resolution of an immediate CCBI precursor and its incorporation into both enantiomers of **34**−**39**, analogs of CC-1065 and the duocarmycins, are detailed. A study of the solvolysis reactivity and regioselectivity of *N*-BOC-CCBI (**25**) revealed that introduction of the C7 nitrile slowed the rate of solvolysis but only to a surprisingly small extent. Classical Hammett quantitation of the effect provided a remarkably small ρ (−0.3), indicating an exceptionally small C7 substituent electronic effect on functional reactivity. Additional kinetic studies of acid-catalyzed nucleophilic addition proved inconsistent with C4 carbonyl protonation as the slow and rate-determining step but consistent with a mechanism in which protonation is rapid and reversible followed by slow and rate-determining nucleophilic addition to the cyclopropane requiring both the presence and assistance of a nucleophile (S_N2 mechanism). No doubt this contributes to the DNA alkylation selectivity of this class of agents and suggests that the positioning of an accessible nucleophile (adenine N3) and not C4 carbonyl protonation is the rate-determining step controlling the sequence selectivity of the DNA alkylation reaction. This small electronic effect on the solvolysis rate had no impact on the solvolysis regioselectivity, and stereoelectronically-controlled nucleophilic addition to the least substituted carbon of the activated cyclopropane was observed exclusively. Consistent with past studies, a direct relationship between solvolysis stability and cytotoxic potency was observed with the CCBI-derived agents providing the most potent analogs in the CBI series, and these observations were related to the predictable Hammett substituent effects. For the natural enantiomers, this unusually small electronic effect on functional reactivity had no perceptible effect on their DNA alkylation selectivity. Similar effects of the C7 cyano substituent on the unnatural enantiomers were observed, and they proved to be 4−10× more effective than the corresponding CBI-based unnatural enantiomers and 4−70× less potent than the CCBI natural enantiomers.

(+)-CC-1065 (**1**)[1] and the duocarmycins **2**−**3**[2−4] constitute the parent members of a class of potent antitumor antibiotics that derive their biological effects through the reversible, sequence-selective alkylation of DNA.[5−13] Since their disclosure, extensive efforts have been devoted to define and exploit their properties. In these studies, the use of synthetic samples of the natural and unnatural enantiomers of the natural products[14−16] and

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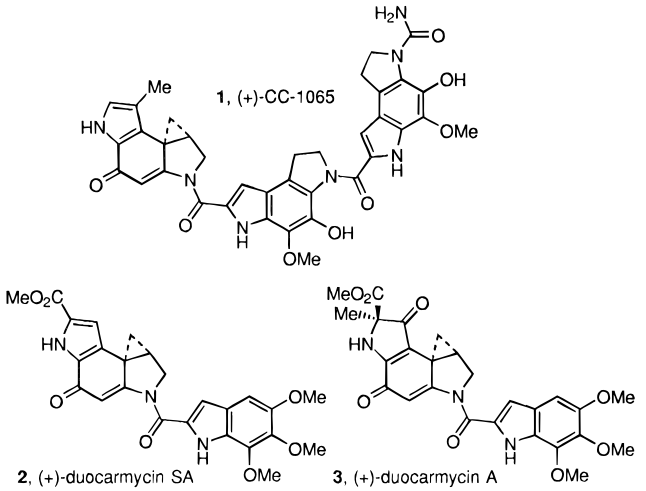
synthetic analogs bearing deep-seated structural changes[17−40] have proven especially valuable in the definition and examination of such properties.[41]



In a preceding paper we described the preparation and preliminary evaluation of MCBI, a substituted 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one bearing a methoxy group para to the C4 carbonyl.[42] Herein, we report the complementary preparation and preliminary evaluation of 7-cyano-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]-indol-4-one (CCBI) bearing a cyano group para to the C4 carbonyl. The comparative evaluation of this key set of agents incorporating the CBI, MCBI, and CCBI alkyla-

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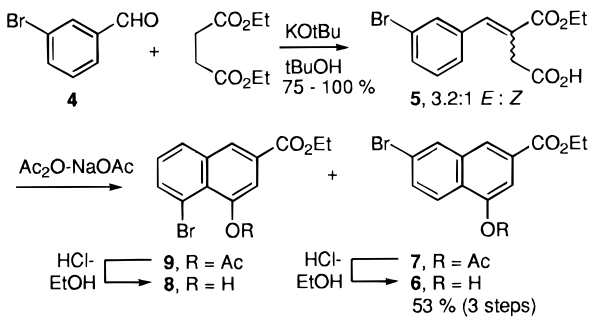
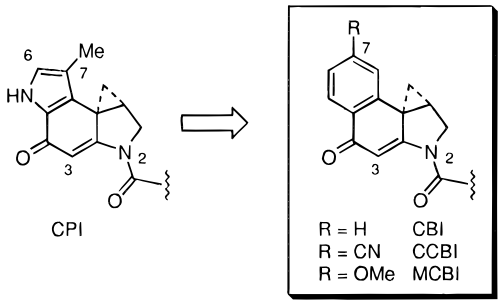
Scheme 1

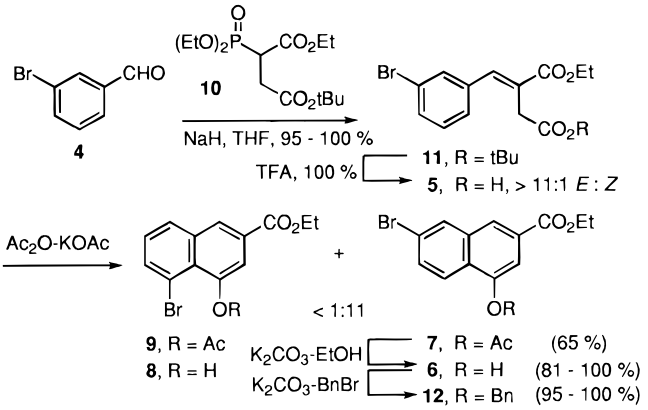


tion subunits was designed to permit an accurate assessment of the electronic influence of the C7 substituent on the chemical and functional reactivity of CBI and the fundamental role this may play in establishing the biological properties of the agents.



Synthesis of CCBI (26) and *N*-BOC-CCBI (25). The approach implemented for the synthesis of **25** and **26** and their advanced analogs of CC-1065 and the duocarmycins was developed concurrent with that of MCBI and consequently relies on the strategy outlined in our preceding work.[42] However, recognizing that the incorporation of a strong electron-withdrawing substituent into the fused benzene ring would decelerate the Friedel−Crafts acylation reaction required of its construction, this was conducted with the substrate **5** in which a halogen occupies this para position. The expectation was that conversion of the bromide to the nitrile could be accomplished at a later stage. Stobbe condensation[43] of 3-bromobenzaldehyde (**4**) with diethyl succinate (1.5 equiv) affected by treatment with *t*-BuOK[44] (1.1 equiv, *t*-BuOH, reflux, 2 h, 75−100%) provided a 3.2:1 mixture of the half esters **5** in excellent conversion (Scheme 1). Subjection of this mixture of **5** to Friedel−Crafts acylation (1−1.1 equiv of NaOAc, Ac_2O, reflux, 6 h)[45] provided a mixture of **6**, its *O*-acylation product **7**, and significant amounts of the isomeric products **8** and **9**. The best conversions were observed when the Friedel−Crafts acylation was conducted under moderately dilute reaction conditions (0.1 M versus 0.5 M). Subsequent ethanolysis (3 M HCl−EtOH) of the resulting mixture served to hydrolyze the *O*-acetates **7** and **9**, providing a 7:1 mixture of **6** and its isomer **8**. Both **6** and **8** were readily separated by chromatography or more conveniently by simple recrystallization of the mixture. Although this procedure has provided **6** in conversions as high as 53% overall yield for the three steps, it generally provided the material in lower conversions of 20−30%. A number of alternatives on this direct approach were investigated[46−49] and failed to improve on the conversions or the isomer ratio of **6** to **8**. In part, this diminished conversion may be attributed to the mixture of (*E*)- and (*Z*)-**5** taken into the Friedel−Crafts acylation reaction and the vigorous reaction conditions and time required to affect the *Z* to *E* isomerization for productive cyclization.

Scheme 2



This was improved significantly by conducting the Stobbe condensation in a more controlled manner. Condensation of **4** with the Wadsworth−Horner−Emmons reagent **10**[50] (1.03 equiv, 1.1 equiv of NaH, THF, 0−25 °C, 12 h, 95−100%) provided **11** in which the required *E*-isomer predominated (>11:1 *E*:*Z*), Scheme 2. Acid-catalyzed deprotection of **11** (100%) followed by Friedel−Crafts acylation of **5** affected by treatment with Ac_2O−KOAc[50] (reflux, 1 h, 30 min) provided **7** in 65% recrystallized yield free of minor amounts of **9**. In part, the improved conversions to provide **7** may be attributed to the facile closure of the correct *E* isomer of **5** and the milder reaction conditions and shorter reaction times employed since *Z* to *E* isomerization under the reaction conditions was no longer required. Hydrolysis of the *O*-acetate **7** by treatment with K_2CO_3−EtOH (reflux, 0.5−1 h, 81−100%) cleanly provided **6**. This approach dependably provided **6** in overall conversions of 45−50% for the four steps and offered the additional advantages of the clean generation of the required (*E*)-**5** and the ability to purify and characterize intermediates in route to **6**. Protection of the free phenol **6** as its benzyl ether provided **12** (100%).

At this stage, the C6 nitrile was introduced in a remarkably clean and effective reaction by simply treating **12** with CuCN in refluxing DMF[51] to provide **13** (96%), Scheme 3. Hydrolysis of the ethyl ester (99−100%) followed by Curtius rearrangement of **14** effected by treatment of the carboxylic acid with the Shioiri−

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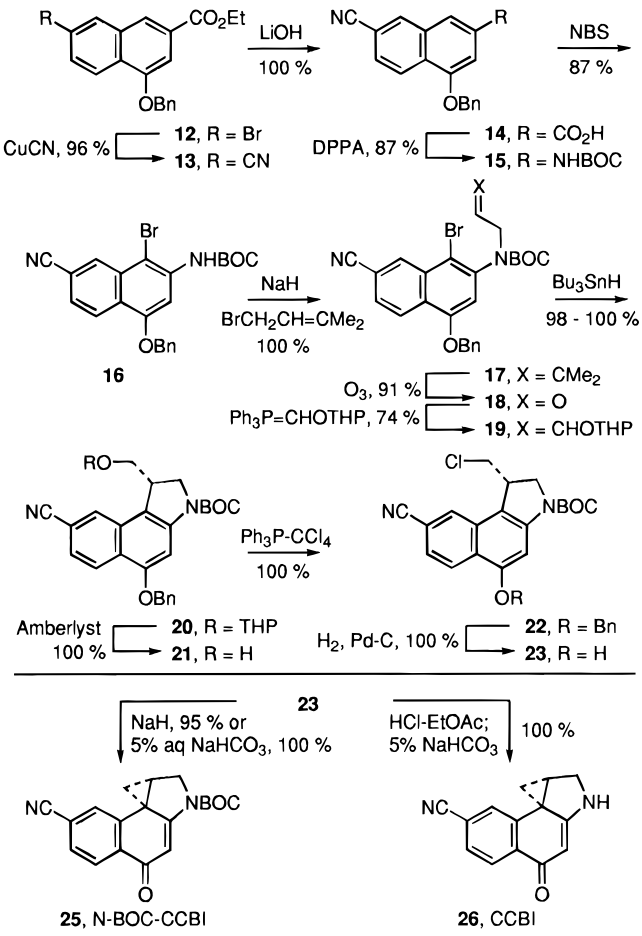
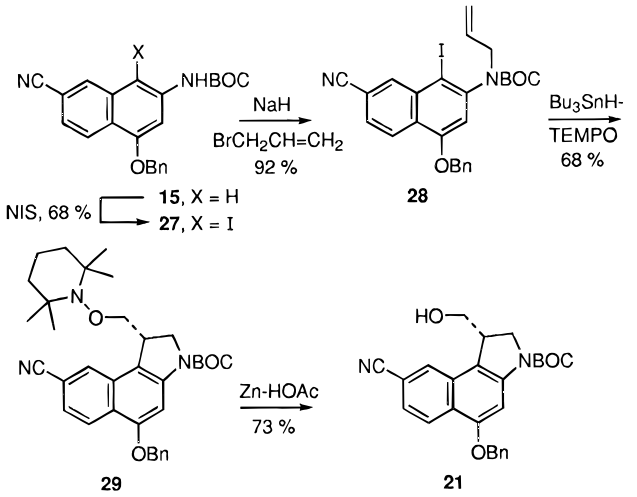
Scheme 3



Yamada reagent[52] (1.2 equiv of DPPA, 1.2 equiv of Et_3N, *t*-BuOH, reflux, 14 h, 87%) cleanly provided **15**. In the optimization of this latter reaction, it was determined that use of rigorously dried *t*-BuOH, the use of dilute (0.005 M) or moderately dilute reaction conditions (0.025 M), and the maintenance of anhydrous reaction conditions through addition of 4 Å molecular sieves served to significantly reduce the amount of competitive symmetrical urea generation.[53] Preliminary efforts to reverse the order of the steps in the conversion of **12** to **15** by first converting the ethyl ester to the corresponding *tert*-butyl carbamate followed by CuCN replacement of the C6 bromide failed to provide **15** cleanly. Low-temperature, acid-catalyzed C4 bromination of **15** (1.2 equiv of NBS, catalytic H_2SO_4, THF, −60 °C, 4 h, 87%) cleanly provided **16**, and alkylation of the sodium salt of **16** (1.3 equiv of NaH, DMF, 25 °C, 30 min) with 1-bromo-3-methyl-2-butene (3 equiv, DMF, 0−25 °C, 12 h, 99−100%) afforded **17**. Low-temperature ozonolysis of **17** under carefully controlled reaction conditions followed by immediate reductive workup (Me_2S) of the crude ozonide provided the aldehyde **18** (91%). The use of extended reaction times or the failure to immediately quench the excess O_3 led to the rapid generation of a further oxidation product. Introduction of the vinyl ether **19** (74%) proved most effective with low-temperature generation of $Ph_3P{=}CHOTHP$[54] in THF followed by reaction with **18** in THF−HMPA[55] over a sustained reaction period. Treatment of **19** with Bu_3SnH (2 equiv, 0.2 equiv of AIBN, C_6H_6, 80 °C, 2 h, 98−100%) provided the product of clean 5-*exo*-*trig* aryl radical−alkene cyclization **20** in excellent yield. Subsequent THP deprotection[56] (100%), conversion[57] of the primary alcohol **21** to the chloride **22** (100%), and phenol deprotection[58] (100%) provided **23**. Spirocyclization of **23** to provide *N*-BOC-CCBI (**25**) was effected by treatment with NaH (3 equiv, 0 °C, 30 min, 95%), and acid-catalyzed deprotection of **23** (4 M HCl−EtOAc, 25 °C, 30 min) followed by treatment of the crude indoline hydrochloride salt **24** with 5% aqueous $NaHCO_3$−THF (1:1, 25 °C, 1.5 h, 100%) cleanly provided CCBI (**26**). Notably and because of the enhanced acidity of the phenol, simple treatment of **23** with 5% aqueous $NaHCO_3$−THF (1:1, 25 °C, 9 h, 100%) led to clean spirocyclization to provide **25** without evidence of subsequent hydrolysis of the labile BOC.

Scheme 4



This approach to **25** and **26** was further shortened and improved with implementation of the Tempo trap[26] of the 5-*exo*-*trig* aryl radical−alkene cyclization of the substrate **28** containing an unactivated and unfunctionalized acceptor alkene. Selective, acid-catalyzed C4 iodination of **15** effected by low-temperature treatment with NIS followed by alkylation of the sodium salt of **27** (1.5 equiv of NaH, DMF, 25 °C, 2 h) with allyl bromide (5 equiv, DMF, 25 °C, 2 h, 92%) provided **28**. Treatment of **28** with Bu_3SnH (5 equiv, 60 °C, 1.5−2 h) in benzene in the presence of Tempo (5 equiv) cleanly provided **29** (Scheme 4). Reductive cleavage of **29** to provide **21** was effected by treatment with Zn (80 equiv, 3:1:1 THF−HOAc−H_2O, 70 °C, 7 h, 73%).

Resolution. The resolution of a late stage synthetic intermediate was accomplished by the chromatographic separation of the enantiomers of **22** on a Chiralcel-OD semipreparative HPLC column (2 × 25 cm) using a 7% *i*-PrOH−hexane eluent (7 mL/min), $\alpha = 1.38$.[35,42] This routinely provided the two enantiomers of **22** in greater than 99.9% ee and with a 97% recovery. The intermediate **22** proved to be the only late-stage intermediate that was effectively resolved on a Chiracel-OD column, and similar efforts to separate **21** or **23** as well as *N*-BOC-

(53) For the symmetrical urea byproduct: ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.21 (s, 2H), 8.44 (s, 2H), 8.20 (d, 2H, J = 8.3 Hz), 7.82 (s, 2H), 7.57 (m, 6H), 7.45 (m, 8H), 5.34 (s, 4H); IR (KBr) ν_{max} 3400, 2240, 1650, 1590 cm^{-1}; FABMS (NBA) m/z 575 (M + H$^+$).

(54) Schlude, H. *Tetrahedron* **1975**, *31*, 89.

(55) Corey, E. J.; Arai, Y.; Mioskowski, C. *J. Am. Chem. Soc.* **1979**, *101*, 6748.

(56) Bongini, A.; Cardillo, G.; Orena, M.; Sandri, S. *Synthesis* **1979**, 618.

(57) Hooz, J.; Gilani, S. S. H. *Can. J. Chem.* **1968**, *46*, 86.

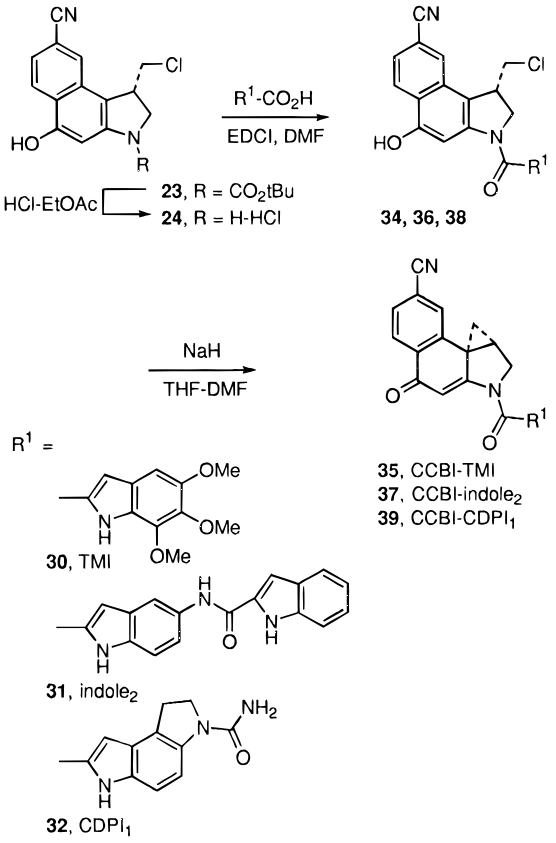
(58) Beig, T.; Szeja, W. *Synthesis* **1985**, 76.

Table 1. Chromatographic Resolution[a]

agent	solvent	α
21	3−7% *i*-PrOH/hexane gradient	1.09
22	5% *i*-PrOH/hexane	1.33
22	7% *i*-PrOH/hexane	1.38
23	2 or 3% *i*-PrOH/hexane	1.00
25	15, 25 or 30% *i*-PrOH/hexane	1.00

[a] Analytical 4.6 × 250 mm, 10 μm Chiralcel-OD column, 1 mL/min.

Scheme 5



CCBI (**25**) itself were not as successful, Table 1. Subjection of both enantiomers of **22** to the conditions of catalytic hydrogenation for removal of the benzyl ether provided the enantiomers of **23** which were incorporated into the optically active agents **25**, **26**, and **34**−**39**. The assignment of the absolute configuration was tentatively based on the optical rotations of the final agents **25**, **26**, **33**, **37**, and **39** for which the strong positive rotation was assigned the natural configuration in analogy to all closely related studies and unambiguously established in the subsequent biological studies. Most notably, the distinct DNA alkylation selectivities characteristic of the natural and unnatural enantiomers for which prior stereochemical assignments have been unambiguously established were found to be in agreement with the initial assignments.

CCBI−TMI (35), CCBI−Indole$_2$ (37), and CCBI−CDPI$_1$ (39). The CCBI alkylation subunit was incorporated into the CC-1065 and duocarmycin analogs **34**−**39** as detailed in Scheme 5. Acid-catalyzed deprotection of **23** (4 M HCl−EtOAc, 25 °C, 30 min, quantitative) followed by coupling of the unstable indoline hydrochloride salt **24** with 5,6,7-trimethoxyindole-2-carboxylic acid (**30**,[14] 3 equiv of EDCI, DMF, 25 °C, 14 h, 85%), **31**[39] (3 equiv of EDCI, DMF, 25 °C, 16 h, 87%), and CDPI$_1$[59] (**32**, 3 equiv of EDCI, DMF, 25 °C, 16 h, 81%) conducted in the absence of added base provided the agents **34**, **36**, and **38**, respectively. Interestingly, the agent **24** was found to couple less effectively than prior agents, and the slower coupling reactions in the series (*e.g.*, CDPI$_2$) were less successful. These slower coupling reactions, which can be attributed principally to the insolubility of the carboxylic acids even in DMF, suffered competitive ring closure of **24** to CCBI (**26**). In part, this distinction of **24** may be attributed to the increased phenol acidity leading to a more facile deprotonation and spirocyclization competitive with coupling. Subsequent treatment of the coupled agents with NaH (3 equiv, 20% DMF−THF, 0 °C, 30 min) provided CCBI−TMI (**35**, 99%) and CCBI−indole$_2$ (**37**, 66%) in good conversion. More remarkable, simple exposure of **36** to 3% aqueous NaHCO$_3$−THF (1:1, 25 °C, 3 h, 68%) or **38** to KHCO$_3$ in DMF−H$_2$O (5:2, 25 °C, 9−10 h, 69%) provided spirocyclization to provide **37** and **39** without evidence of significant subsequent hydrolysis of the labile amide.

Table 2. Aqueous Solvolysis of *N*-BOC-CCBI and Related Agents

	N-BOC-MCBI (**40**)	*N*-BOC-CBI (**41**)	*N*-BOC-CCBI (**25**)
k, s^{-1} (pH 3)	1.75×10^{-6}	1.45×10^{-6}	0.99×10^{-6}
$t_{1/2}$, h (pH 3)	110	133	213
k, s^{-1} (pH 2)	1.62×10^{-5}	1.53×10^{-5}	0.79×10^{-5}
$t_{1/2}$, h (pH 2)	11.8	12.5	24.2

Chemical Solvolysis: Reactivity. Hammett Quantitation of the Magnitude of the C7 Substituent Electronic Effect on Functional Reactivity. The first important characteristic of the CCBI agents that was examined was its relative reactivity toward solvolysis which may be considered to reflect the relative functional reactivity of the agents for acid or Lewis acid-catalyzed DNA alkylation. Expectations were that the introduction of the C7 cyano group would slow acid-catalyzed solvolysis, which requires C4 carbonyl protonation, but the magnitude of this electronic effect of the C7 substituent was unknown. In preceding studies with the comparisons of the MCBI and CBI-based agents in which the effect of a C7 electron-donating substituent (OCH$_3$) was examined, the magnitude of this effect was found to be exceptionally small.[42]

Similar observations were made with the comparisons now extended to *N*-BOC-CCBI (**25**) and CCBI (**26**). Consistent with expectations, solvolysis at both pH 3 and pH 2 revealed that *N*-BOC-CCBI (**25**) was the most stable agent in the series while *N*-BOC-MCBI (**40**) was the most reactive. However, the difference in reactivity was remarkably modest and *N*-BOC-CCBI (**25**) was only approximately two times more stable than *N*-BOC-MCBI (**40**), which in turn was only slightly more reactive than *N*-BOC-CBI (**41**) itself at both pH 2 and pH 3 (Table 2). All three agents, including *N*-BOC-CCBI, were stable at pH 7 (1:1 CH$_3$OH−H$_2$O), and no solvolysis was detected over a 2 week monitoring period. Nearly identical observations were made in the examination of CCBI (**26**) in which it was found to be the most stable agent in the series, but the differences in solvolysis rates at either pH 2 or 3 were surprisingly small: CCBI, $k = 1.5 \times 10^{-6}$ s^{-1}, $t_{1/2} = 127$ h (pH 2) and $k = 1.65 \times 10^{-7}$ s^{-1}, $t_{1/2} = 1182$ h (ph 3); CBI, $k = 2.07 \times 10^{-7}$ s^{-1}, $t_{1/2}$, 930 h (pH 3); MCBI, $k = 5.76 \times 10^{-7}$ s^{-1}, $t_{1/2} = 334$ h (pH 3). Like the observations disclosed in prior studies, CCBI was sub-

(59) Boger, D. L.; Coleman, R. S.; Invergo, B. J. *J. Org. Chem.* **1987**, *52*, 1521. Boger, D. L.; Coleman, R. S. *J. Org. Chem.* **1984**, *49*, 2240.

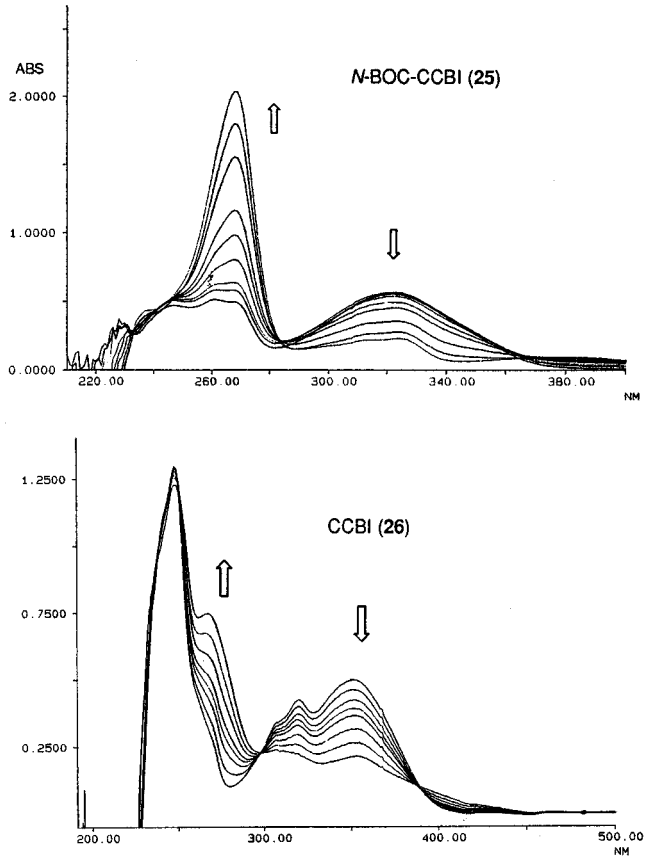


Figure 1. Top: UV spectra of solvolysis study of *N*-BOC-CCBI at pH 2 conducted in 50% CH_3OH buffer (4:1:20 1.0 M citric acid, 0.2 M Na_2HPO_4, and H_2O). The spectra shown were recorded at 0, 1, 2, 6, 11, 17, 37, and 63 h. Bottom: UV spectra of solvolysis study of CCBI at pH 2 conducted in 50% CH_3OH buffer. The spectra shown were recorded at 0, 20, 45, 70, 94, 142, 214, and 361 h.

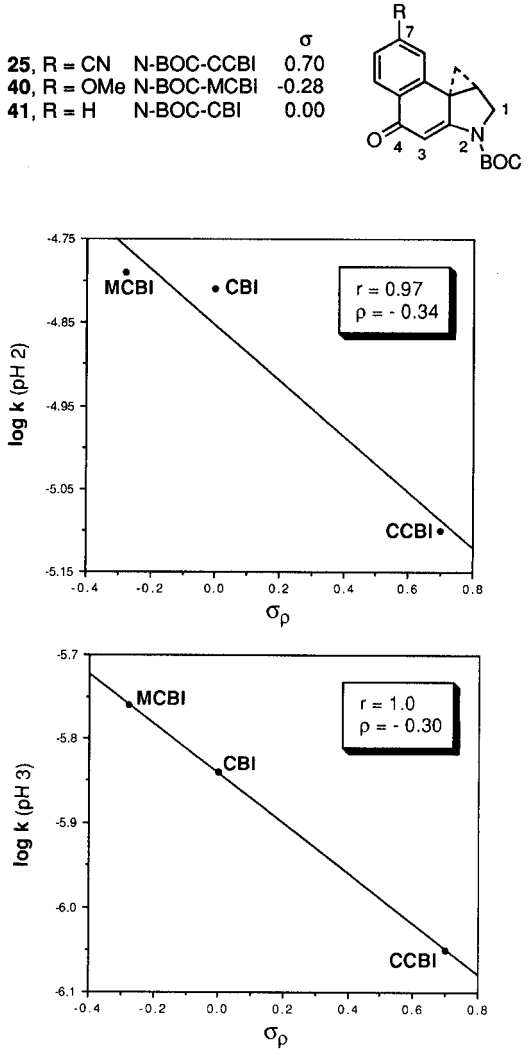


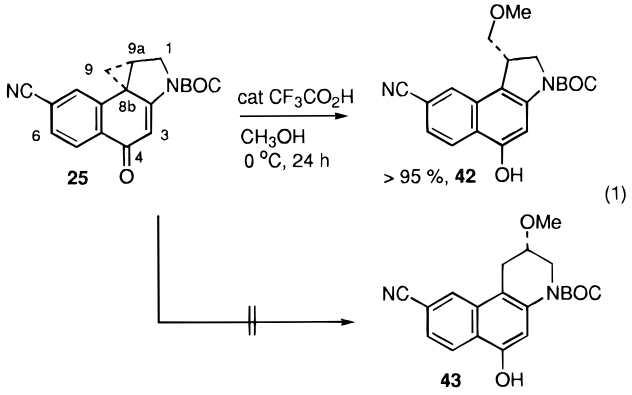
Figure 2.

stantially more stable than *N*-BOC-CCBI presumably because of preferential N versus O protonation.

The solvolysis was conducted in 1:1 CH_3OH−buffer (pH 3.1; buffer = 4:1:20 v:v:v 0.1 M citric acid, 0.2 M Na_2HPO_4, H_2O; pH 2.05: buffer = 4:1:20 v:v:v 1.0 M citric acid, 0.2 M Na_2HPO_4, H_2O) and was followed spectrophotometrically by UV with the disappearance of the long-wavelength absorption of the CCBI chromophore and with the appearance of a short-wavelength absorption attributable to the solvolysis products (Figure 1). Although this was not examined in the present case, past studies[24] with *N*-BOC-CBI have demonstrated the production of two solvolysis products derived from addition of H_2O and CH_3OH to the least-substituted carbon of the cyclopropane. Consistent with these observations, solvolysis in CH_3OH produced a single product as detailed below.

Hammett plots revealed unusually small ρ values of −0.30 (pH 3) or −0.34 (pH 2) for the acid-catalyzed solvolysis (Figure 2). This exceptionally small ρ value of −0.3 indicates little differential positive charge buildup in the reaction transition state consistent with a strict S_N2 versus S_N1 mechanism requiring the presence and assistance of the nucleophile for ring opening of the cyclopropane. Moreover, the observation of the remarkably small 2× solvolysis rate difference in going from a strong C7 electron-donating substituent to a strong electron-withdrawing substituent (OMe versus CN) is consistent with nucleophilic addition and not C4 carbonyl protonation as the rate-determining step.

Chemical Solvolysis: Regioselectivity. Treatment of *N*-BOC-CCBI (**25**) with 0.1 equiv of CF_3CO_2H in CH_3OH (0 °C, 24 h) resulted in the clean solvolysis to provide a single product **42** (95%), eq 1. No BOC deprotection



or olefin was observed, and the methanolysis proceeded without alteration of the stereoelectronically-controlled regioselectivity. Clean cleavage of the C8b−C9 bond with addition of CH_3OH to the least substituted C9 cyclopropane carbon was observed to provide **42**, and no cleavage of the C8b−C9a bond with ring expansion and addition

Table 3. CH_3OH−THF Solvolysis of 25[a]

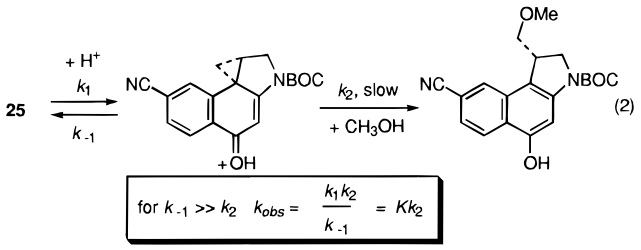
equiv of H^+	equiv of CH_3OH	k (s^{-1})	$t_{1/2}$ (h)
0.25	100	7.4×10^{-4}	0.26
0.1	100	2.1×10^{-4}	0.92
0.1	500	5.6×10^{-4}	0.34
0.1	20	0.2×10^{-4}	9.2

[a] Catalytic CF_3SO_3H, 0.3 mM in THF, 25 °C.

of CH_3OH to C9a to provide **43** was detected (>20:1). This is in sharp contrast to solvolysis studies of the more reactive alkylation subunits of CC-1065,[60] duocarmycin A,[7] and CBQ[20] where significant or substantial amounts of the abnormal ring expansion solvolysis products have been detected. Nonetheless, the observations are consistent with prior studies with *N*-BOC-CBI (**41**) and *N*-BOC-MCBI (**40**) where no (>20:1) ring expansion solvolysis product was detected.[24,42] Thus, within the reactivity range represented by the agents **25**, **40**, and **41** and their C7 substituents, no effect of the substituents on the regioselectivity of acid-catalyzed nucleophilic addition was observed. Given the potential range represented by the C7 substituents examined, it is unlikely that any substituent will effect a perceptible change in this inherent regioselectivity with the CBI-based agents.

To date, the abnormal ring expansion solvolysis products have only been detected with the chemically more reactive agents and are only especially prevalent in the CBQ system where the stereoelectronic alignment of both cyclopropane bonds are equivalent.[20] However, this stereoelectronically-controlled acid-catalyzed nucleophilic addition to the CBI-based agents including the CCBI-based agents that proceed with >20:1 regioselectivity provides an additional advantage of the agents over the CPI-based analogs of CC-1065 that have been found to exhibit a more modest 4:1 selectivity.[60]

Chemical Solvolysis: Mechanism. As illustrated by the relative aqueous solvolysis rates measured at pH 3 versus pH 2, the 10-fold rate increase at pH 2 for all three agents is indicative of a solvolysis first-order dependence on the acid concentration. In additional studies conducted with *N*-BOC-CCBI (**25**), the solvolysis not only exhibited this first-order rate dependence on acid concentration but also exhibited a first-order rate dependence on the concentration of the nucleophile when the acid-catalyzed addition of CH_3OH was conducted in THF (0.1−0.25 equiv of CF_3SO_3H, 20−500 equiv of CH_3OH, 25 °C), Table 3.[61] Together, these studies illustrate that the slow step for acid-catalyzed nucleophilic addition to the activated cyclopropane under the conditions examined is not C4 carbonyl protonation, but rather nucleophilic addition to the activated cyclopropane following rapid and reversible C4 carbonyl protonation (eq 2).



Although these latter studies were conducted under conditions different than that employed for DNA alkylation, the results have significant implications on the origin of the DNA alkylation selectivity of the agents and suggests that positioning of an accessible nucleophile[5,6,9,11] (adenine N3) and not C4 carbonyl protonation[8,12,60] is the rate-determining step controlling the DNA alkylation selectivity.

In Vitro Cytotoxic Activity. The results of a study of the comparative cytotoxic properties of the CCBI-based agents alongside the corresponding CBI and MCBI agents are detailed in Table 4. In preliminary studies, the natural enantiomers of the CCBI-based agents have been found to exhibit the most potent cytotoxic activity in CBI series (Table 4).

Consistent with past observations, the agents were found to follow a direct relationship between chemical stability (−log k) and in vitro cytotoxic potency (L1210, log $1/IC_{50}$) over the narrow range of reactivity examined by the series. This is illustrated in Figure 3 with the *N*-BOC derivatives. Presumably, this may be attributed to the more effective delivery of the more stable agents to their intracellular target, and the solvolysis rates may be taken to accurately represent the relative functional reactivity/stability of the agents.

Less obvious, but more fundamental, the observations were found to follow a predictable direct relationship between in vitro cytotoxic activity and the Hammett σ_ρ constant of the C7 substituent with CCBI providing the most potent agent (Figure 4). This fundamental relationship should prove useful in the design of new analogs possessing further enhanced properties.

While the latter two correlations are limited to the comparisons made on the three presently available agents, they also follow trends established in the examination of **44**−**49** (Figure 5) and their related full structure analogs (Figure 6), further supporting their potential generality. Notably, **25** is the most stable and one of the most synthetically accessible alkylation subunits disclosed to date.

Analogous to prior observations, the corresponding seco precursors **23**, **34**, **36**, and **38** exhibited cytotoxic activity indistinguishable from the cyclopropane-containing agents.

DNA Alkylation Selectivity and Efficiency. The DNA alkylation properties of the agents were examined within w794 duplex DNA,[10] a 144 base-pair segment of duplex DNA for which comparative results are available for related agents. The alkylation site identification and the assessment of the relative selectivity among the available sites were obtained by thermally-induced strand cleavage of the singly 5′ end-labeled duplex DNA after exposure to the agents. Following treatment of the end-labeled duplex DNA with a range of agent concentrations, the unbound agent was removed by EtOH precipitation of the DNA. Redissolution of the DNA in aqueous buffer, thermolysis (100 °C, 30 min) to induce strand cleavage at the sites of DNA alkylation, denaturing high resolution polyacrylamide gel electrophoresis (PAGE) adjacent to Sanger dideoxynucleotide sequencing standards, and autoradiography led to identification of the DNA cleavage and alkylation sites. The full details of this procedure have been disclosed and discussed elsewhere.[10] The DNA alkylation reaction selectivities observed under the incubation conditions for the agents detailed herein have proven identical to the alkylation selectivities observed with shorter or extended reaction periods or when the

(60) Warpehoski, M. A.; Harper, D. E. *J. Am. Chem. Soc.* **1994**, *116*, 7573. Warpehoski, M. A.; Harper, D. E. *J. Am. Chem. Soc.* **1995**, *117*, 2951.

(61) Boger, D. L.; McKie, J. A.; Han, N.; Tarby, C. M.; Riggs, H. W.; Kitos, P. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 659.

Table 4. Cytotoxic Activity

agent	IC_{50} (L1210)	agent	IC_{50}(L1210)	agent	IC_{50}(L1210)
		Natural Enantiomers			
25, (+)-CCBI	2 mM	(+)-CBI	nd	(+)-MCBI	5 mM
26, (+)-*N*-BOC-CCBI	20 nM	(+)-*N*-BOC-CBI	80 nM	(+)-*N*-BOC-MCBI	90 nM
35, (+)-CCBI−TMI	7 pM	(+)-CBI−TMI	30 nM	(+)-*N*-MCBI-TMI	8 pM
37, (+)-CCBI−indole$_2$	7 pM	(+)-CBI−indole$_2$	10 pM	(+)-MCBI−indole$_2$	10 pM
39, (+)-CCBI−$CDPI_1$	6 pM	(+)-CBI−$CDPI_1$	5 pM	(+)-MCBI−$CDPI_1$	6 pM
(+)-CCBI−$CDPI_2$	nd	(+)-CBI−$CDPI_2$	5 pM	(+)-MCBI−$CDPI_2$	6 pM
		Unnatural Enantiomers			
25, (−)-CCBI	3 mM	(−)-CBI	11 mM	(−)-MCBI	30 mM
26, (−)-*N*-BOC-CCBI	80 nM	(−)-*N*-BOC-CBI	900 nM	(−)-N-BOC-MCBI	200 nM
35, (−)-CCBI−TMI	450 pM	(−)-CBI−TMI	2000 pM	(−)-MCBI-TMI	400 pM
37, (−)-CCBI−indole$_2$	400 pM	(−)-CBI−indole$_2$	4000 pM	(−)-MCBI-indole$_2$	30 pM
39, (−)-CCBI−$CDPI_1$	80 pM	(−)-CBI−$CDPI_1$	380 pM	(−)-MCBI-$CDPI_1$	10 pM
(−)-CCBI−$CDPI_2$	nd	(−)-CBI−$CDPI_2$	40 pM	(−)-MCBI-$CDPI_2$	10 pM

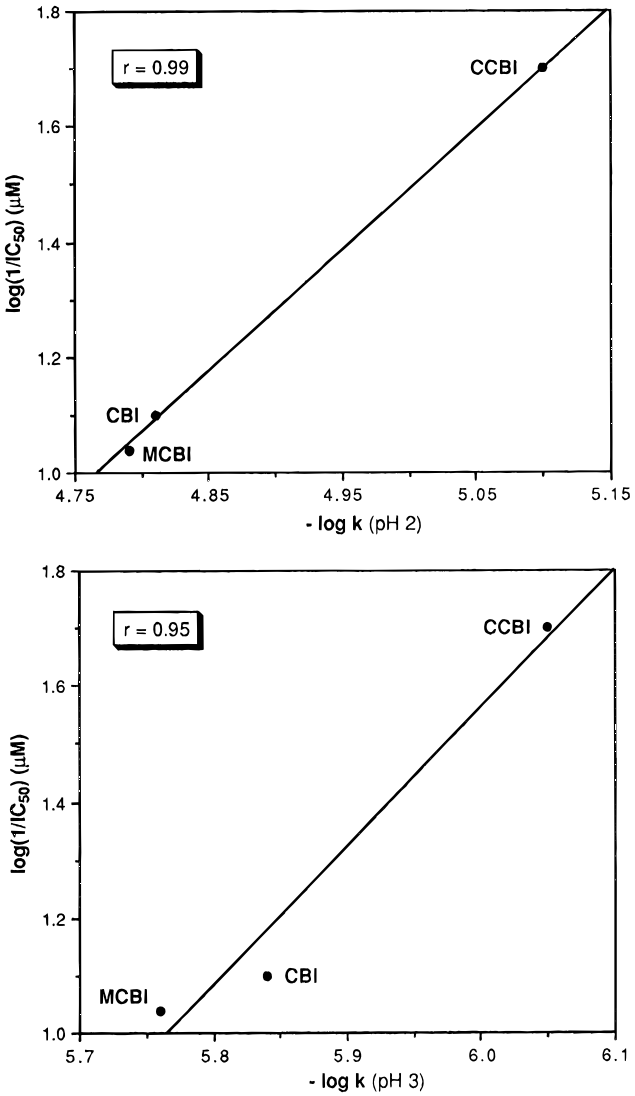


Figure 3.

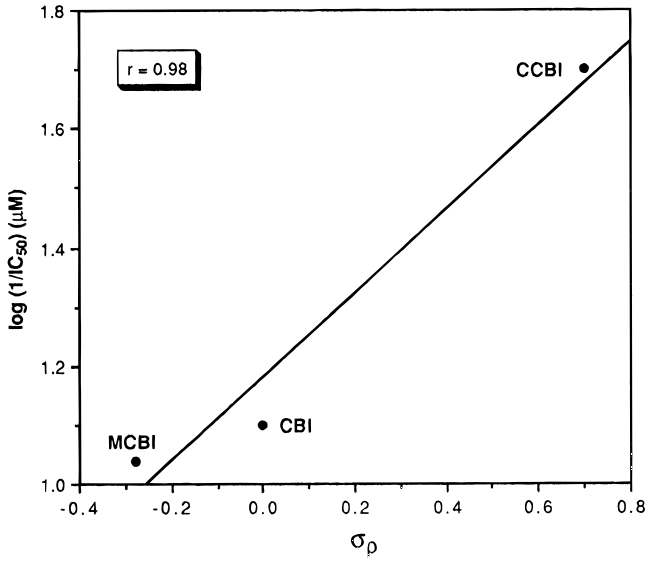
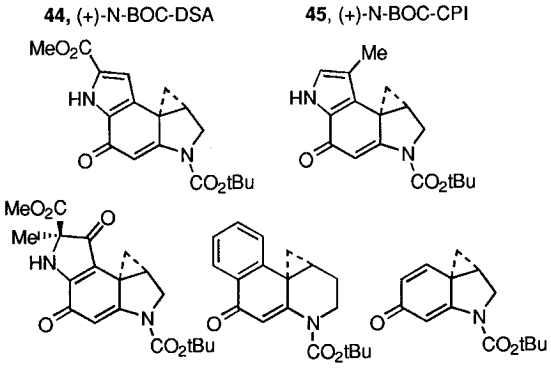


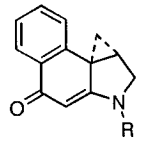
Figure 4.

reactions were conducted at different temperatures (37 or 4 °C, 0.5−7 d). As discussed below, the rates and efficiencies but not final relative efficiencies of DNA alkylation were altered by changing the reaction temperatures.

DNA Alkylation Properties of (+)- and *ent*-(−)-*N*-BOC-CCBI. A representative comparison of the DNA alkylation properties of both enantiomers of *N*-BOC-CCBI (**25**) alongside both enantiomers of *N*-BOC-MCBI (**40**) within w794 DNA is illustrated in Figure 7. No substantial distinctions between *N*-BOC-CCBI (**25**) and *N*-BOC-MCBI (**40**) were detected. Both natural enantiomers exhibited comparable efficiences of DNA alkylation detectable at 10^{-3} M (37 °C, 72 h) and prominent at 10^{-2} M, and both were only slightly more efficient than the corresponding unnatural enantiomers (1−2×). This efficiency of DNA alkylation proved analogous to that of (+)-*N*-BOC-CBI (**41**) but distinct from the unnatural enantiomer of *N*-BOC-CBI: (+)-*N*-BOC-CBI/*ent*-(−)-*N*-BOC-CBI (5−10×)[33] versus (+)-*N*-BOC-CCBI/*ent*-(−)-*N*-BOC-CCBI and (+)-*N*-BOC-MCBI/*ent*-(−)-*N*-BOC-MCBI (1−2×). This distinction between the enantiomers of **41** which was not observed with **40** or **25** was also accurately reflected in the relative cytotoxic potencies of the agents where the unnatural enantiomers of both *N*-BOC-CCBI (4×) and *N*-BOC-MCBI (2×) more closely approach that of the corresponding natural enantiomers than that of *N*-BOC-CBI (11×). Like the preceding BOC derivatives examined,[18,20,33,42] the two enantiomers of **25** alkylated DNA much less efficiently than **34**−**39** (10^4×), providing detectable alkylation at 10^{-2}−10^{-3} M (37 °C, 24−72 h) much less selectively than **34**−**39** exhibiting a two base-pair AT-rich alkylation selectivity (5′-A<u>A</u> > 5′-T<u>A</u>), and did so with alkylation of the same sites. This unusual behavior of the two enantiomers alkylating the same sites is analogous to past observations.[11,12] It is a natural consequence of the reversed binding orientations of the two enantiomers and the diastereomeric relationship of the two adducts that result in the two enantiomers covering the exact same binding site surrounding the alkylated adenine. This has been discussed in detail and



	k (s^{-1}, pH 3)	$t_{1/2}$ (pH 3)	IC_{50} (L1210)
44	1.08×10^{-6}	177 h	6 nM
41	1.45×10^{-6}	133 h	80 nM
45	5.26×10^{-6}	37 h	330 nM
46	1.75×10^{-5}	11 h	1000 nM
47	9.07×10^{-5}	2.1 h	2000 nM
48	1.98×10^{-2}	0.01 h	18000 nM



pH = 3

R	k (s^{-1})	$t_{1/2}$	IC_{50} (L1210)	σ
SO_2Et	0.5×10^{-6}	383 h	24 nM	0.72
COEt	2.0×10^{-6}	96 h	110 nM	0.48
CO_2Me	3.4×10^{-6}	57 h	140 nM	0.45
CONHMe	5.3×10^{-6}	36 h	200 nM	0.36

Figure 5.

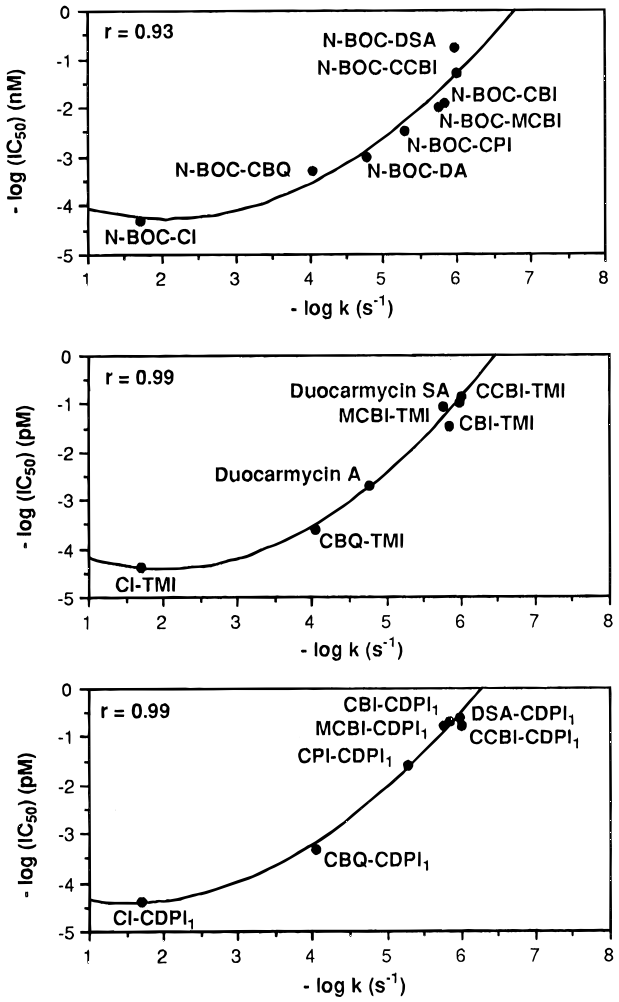


Figure 6.

illustrated elsewhere,[6,9,11,35] and *N*-BOC-CCBI (**25**) conforms nicely to these past observations and models.

DNA Alkylation Properties of the Natural Enantiomers of CCBI-TMI (35), CCBI−Indole$_2$ (37), and CCBI−CDPI$_1$ (39). A comparison of the DNA alkylation by the natural enantiomers of **35**, **37**, and **39** alongside (+)-duocarmycin SA (**2**) and (+)-CC-1065 (**1**) within w794 DNA is illustrated in Figure 8 and is representative of comparisons that have been made with the agents. (+)-Duocarmycin SA (**2**) and (+)-CCBI−TMI (**35**) were indistinguishable, and the two agents exhibited the same selectivity and efficiency of DNA alkylation. This is illustrated nicely in Figure 8 where the two agents detectably alkylate the same high affinity site of 5′-AATTA at 10^{-6}−10^{-7} M (25 °C, 24 h). This is analogous to the observations made in our prior comparisons of duocarmycin SA (**2**) and CBI−TMI[35] or MCBI−TMI.[42]

The alkylation selectivities have been documented and described in detail elsewhere[5,6,9,34,36] and summarized in reviews,[11] and each alkylation site detected was adenine followed by two 5′ A or T bases in a three base-pair site that follows the following preference: 5′-AAA > 5′-TTA > 5′-TAA > 5′-ATA. For the shorter agents CCBI−TMI and CCBI−CDPI$_1$, there was also a strong preference but not absolute requirement for the fourth 5′ base to be A or T versus G or C, and this preference distinguished many of the high versus low affinity sites (*e.g.*, 5′-AAAA). For the longer agent, CCBI−indole$_2$, not only was there a stronger preference for the fourth base to be A or T but that preference extended to include a fifth 5′ A or T base (*e.g.*, 5′-AAAAA). Thus, like the preceding agents, the CCBI-based agents exhibited AT-rich adenine N3 alkylation selectivities that start at the 3′ adenine N3 alkylation site with agent binding in the minor groove in the 3′ → 5′ direction covering 3.5 or 5 base pairs.

DNA Alkylation Properties of the Unnatural Enantiomers of CCBI−TMI (35), CCBI−Indole$_2$ (37), and CCBI−CDPI$_1$ (39). A representative comparison of the DNA alkylation by the unnatural enantiomers of the CCBI-based agents alongside the unnatural enantiomer of duocarmycin SA (**1**) and the natural enantiomer of CC-1065 (**1**) in w794 of DNA is illustrated in Figure 9. Several important findings analogous to those made in our prior studies with the CBI-based agents are also observed with the CCBI-based agents. First, the unnatural enantiomer DNA alkylation is considerably slower, and the results shown in Figure 9 for the unnatural enantiomers were obtained only with incubation at 25 °C (72 h) versus incubation at 25 °C (24 h, Figure 8) for the natural enantiomers. Even with the more vigorous reaction conditions (37 °C) or the more extended reaction periods, the extent of alkylation by the unnatural enantiomers is lower, requiring higher agent concentrations to detect. This distinguishing difference in the rate and efficiency of DNA alkylation was most prominent with the smaller agents CCBI−TMI (**35**) and duocarmycin SA (**2**) and readily perceptible but less prominent with the intermediate-sized agents CCBI−CDPI$_1$ (**39**) and CCBI−indole$_2$ (**37**). This trend is similar to that observed in the relative cytotoxic potency of the enantiomeric pairs.

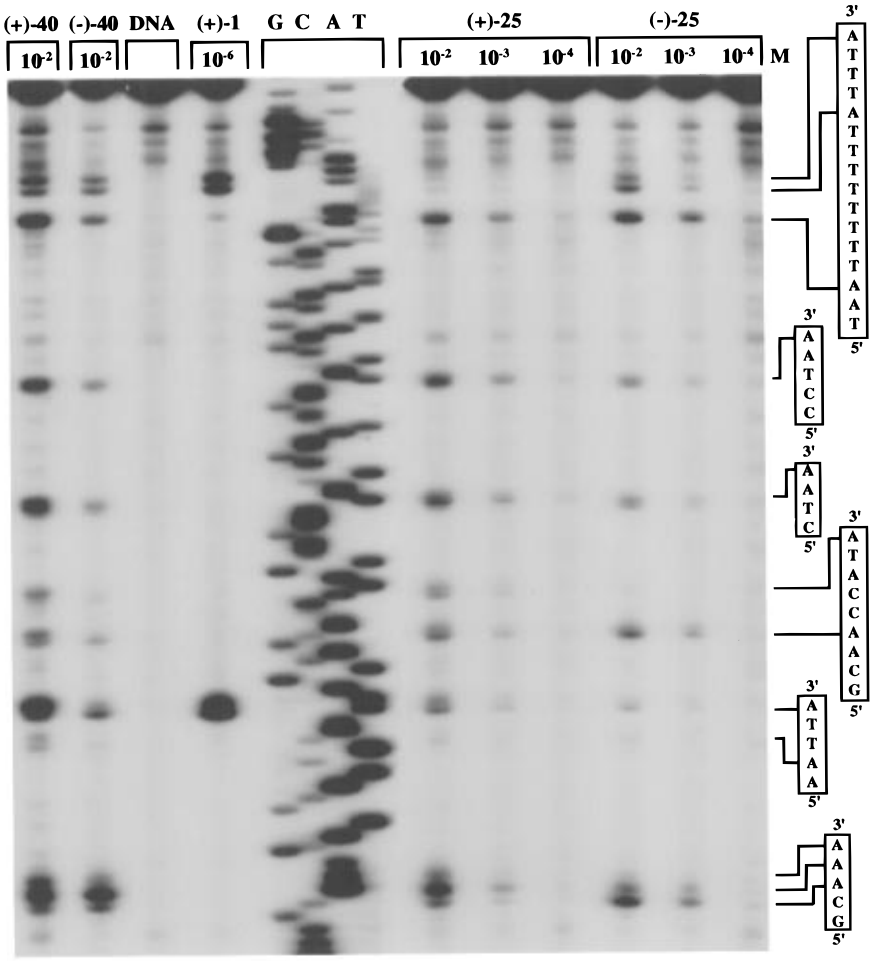


Figure 7. Thermally-induced strand cleavage of double-stranded DNA (SV40 DNA fragment, 144 bp, nucleotide no. 5238−138, clone w794) after 72 h incubation of agent with DNA at 37 °C followed by removal of unbound agent, and 30 min incubation at 100 °C, 8% denaturing PAGE, and autoradiography: lane 1, (+)-*N*-BOC-MCBI (1×10^{-2} M); lane 2, *ent*-(−)-*N*-BOC-MCBI (1×10^{-2} M); lane 3, control DNA; lane 4, (+)-CC-1065 (1×10^{-6} M); lanes 5−8, Sanger G, C, A, and T reactions; lanes 9−11 (+)-*N*-BOC-CCBI (1×10^{-2} to 1×10^{-4} M); lanes 12−14, *ent*-(−)-*N*-BOC-CCBI (1×10^{-2} to 1×10^{-4} M).

The DNA alkylation selectivity and efficiency observed with *ent*-(−)-CCBI−TMI (**35**) and *ent*-(−)-duocarmycin SA (**2**) were nearly indistinguishable with the latter agent being slightly more effective. This observation is analogous to that made in our prior comparisons with (−)-MCBI−TMI but different from that made with (−)-CBI−TMI where the distinction was even larger (10×).[35] The larger agents were more effective at alkylating DNA, (−)-CCBI−indole$_2$ > (−)-CCBI−CDPI$_1$ > (−)-CCBI−TMI, and even with incubation at 25 °C for 72 h the more effective agents did not achieve the efficiency observed with the natural enantiomers. This is illustrated nicely in Figure 9 with the comparison of the unreacted DNA observed at 10^{-6} M for (+)-CC-1065 (**1**) versus the full set of CCBI unnatural enantiomers. Again, no distinctions in the DNA alkylation selectivity of the unnatural enantiomers of the CCBI-based agents and the agents described previously were perceptible. Each of the alkylation sites proved to be adenine, which was flanked on both sides nearly always by an A or T base and the preference for this three-base AT-rich site was 5′-A$\underline{A}$A > 5′-T$\underline{A}$A > 5′-A$\underline{A}$T > 5′-T$\underline{A}$T. For the shorter agents, there was a strong preference for the second 3′ base to be A or T (*e.g.*, 5′-A$\underline{A}$AA), which for the larger agents extended to the third 3′ base as well (*e.g.*, 5′-A$\underline{A}$AAA). Thus, each alkylation site for the unnatural enantiomers proved consistent with adenine N3 alkylation with agent binding in the minor groove in the reverse 5′ → 3′ direction across a 3.5 or 5 base-pair AT-rich site surrounding the alkylation site. This is analogous to the natural enantiomer alkylation selectivity except that it extends in the reverse 5′ → 3′ direction in the minor groove and, because of the diastereomeric nature of the adducts, is offset by one base pair relative to the natural enantiomers.

Enantiomer Distinctions. Prior studies have suggested an attractive explanation for the confusing behavior of enantiomeric pairs of agents that we have proposed may be attributed to a single structural feature—the degree of steric bulk surrounding the CPI/DSA C7 or CBI/MCBI C8 center in the alkylation subunit for which the unnatural enantiomers are especially sensitive.[6,35] The enantiomer differences have proven distinguishable with simple derivatives of the alkylation subunits themselves (*i.e.*, *N*-BOC−CCBI), are most prominent with the dimer-based agents (*i.e.*, CCBI−TMI), and are less prominent or not readily distinguishable with the larger trimer- or tetramer-based agents (*i.e.*, CCBI−CDPI$_2$). In general, less distinction in the biological potency and relative DNA alkylation efficiency was observed with the CI and duocarmycin SA enantiomeric pairs, both of which lack substituents or steric bulk at this position. Moreover, the distinctions among the enantiomeric CI-based agents that lack the pyrrole ring

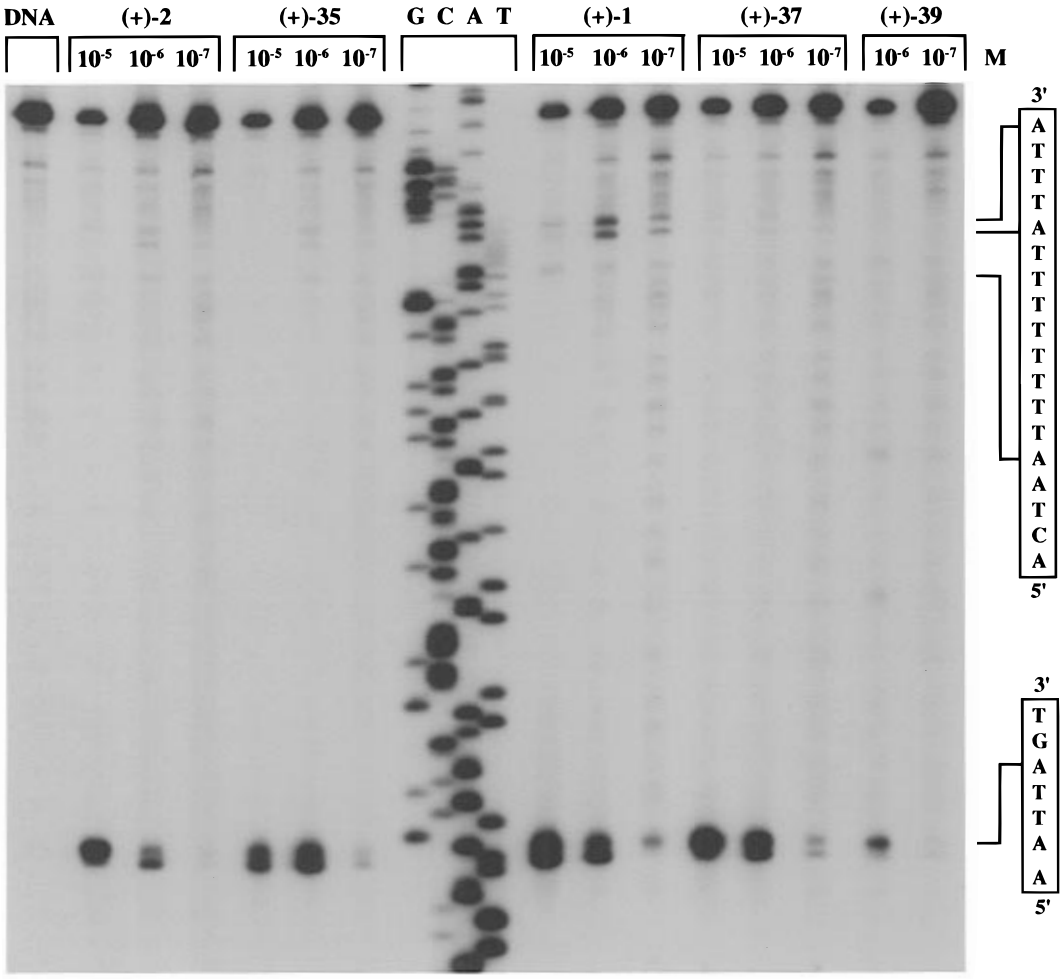


Figure 8. Thermally-induced strand cleavage of double-stranded DNA (SV40 DNA fragment, 144 bp, nucleotide no. 5238−138, clone w794) after 24 h incubation of agent−DNA at 25 °C followed by removal of unbound agent, 30 min incubation at 100 °C, 8% denaturing PAGE, and autoradiography: lane 1, control DNA; lanes 2−4, (+)-duocarmycin SA (**2**, 1×10^{-5} to 1×10^{-7} M); lanes 5−7, (+)-CCBI−TMI (1×10^{-5} to 1×10^{-7} M); lanes 8−11, Sanger G, C, A, and T reactions; lanes 12−14, (+)-CC-1065 (**1**, 1×10^{-5} to 1×10^{-7} M); lanes 15−17, (+)-CCBI−indole$_2$ (1×10^{-5} to 1×10^{-7} M); lanes 18−19, (+)-CCBI−CDPI$_1$ (1×10^{-6} and 1×10^{-7} M).

altogether are small and less pronounced than those observed with the DSA-based agents (DSA > CI). In contrast, the CPI-, CBI-, and DA-based agents exhibit more pronounced distinctions (CPI > DA > CBI), following an order that reflects the relative steric differences. Consistent with these observations, the CCBI−TMI enantiomers were found to exhibit analogous distinctions (Table 5). As detailed and illustrated elsewhere,[6,35] this distinguishing behavior of the unnatural enantiomers is derived from a pronounced steric interaction of the CPI/DSA C7 or CBI/CCBI C8 center with the 5′ base adjacent to the adenine N3 alkylation site present in the unnatural enantiomer 5′ → 3′ binding model.

Rate of DNA Alkylation. Analogous to two prior studies,[35,42] the relative rates of DNA alkylation for the natural enantiomers of CCBI−TMI (**35**) and CCBI−indole$_2$ (**37**) versus those of the corresponding CBI and MCBI agents were measured at the single high affinity site in w794, 5′-AATTA. In these prior studies, the relative rates of DNA alkylation for MCBI−TMI, CBI−TMI, and duocarmycin SA were determined to be quite similar at 4 °C, 1.8:1.0:0.9, respectively. The same relative rates were observed in the present study now extended to include CCBI−TMI (**35**), and this latter agent proved to alkylate DNA with the fastest relative rate (10^{-6} M, 25 °C): CCBI−TMI (2.5×) > MCBI−TMI (1.9×) > CBI−TMI (1.0) > duocarmycin SA (0.9×) (Figure 10). The relative rates of DNA alkylation by CCBI−indole$_2$ (2.5×), MCBI−indole$_2$ (1.4×), and CBI−indole$_2$ (1.0) at 25 °C were determined to exhibit nearly identical trends. Although the studies are limited to a very narrow reactivity range difficult to distinguish, the rate of DNA alkylation did not correlate with the relative reactivity of the agents toward acid-catalyzed solvolysis suggesting that other or additional factors may contribute to the rate or are responsible for the catalysis of the DNA alkylation reaction.[20] In prior studies, we have highlighted similar observations with agents that span a much larger range of relative reactivities.[32]

More interestingly, the final relative efficiency of DNA alkylation observed under these reaction conditions did more closely follow the trends of the relative reactivities of the agents. The chemically more stable CCBI-based agents **35** and **37** alkylated DNA 1.5−2× more efficiently than the MCBI- or CBI-based agents which in turn were essentially indistinguishable. Nearly identical trends were observed in the relative stability of the agents with CCBI being 1.6−2.1× more stable than CBI or MCBI which in turn were very close in stability (1.06−1.2×). Such observations are consistent with our prior studies that suggest it is not the rate[12,60] of DNA alkylation that may be related to the cytotoxic potency of the agents, but

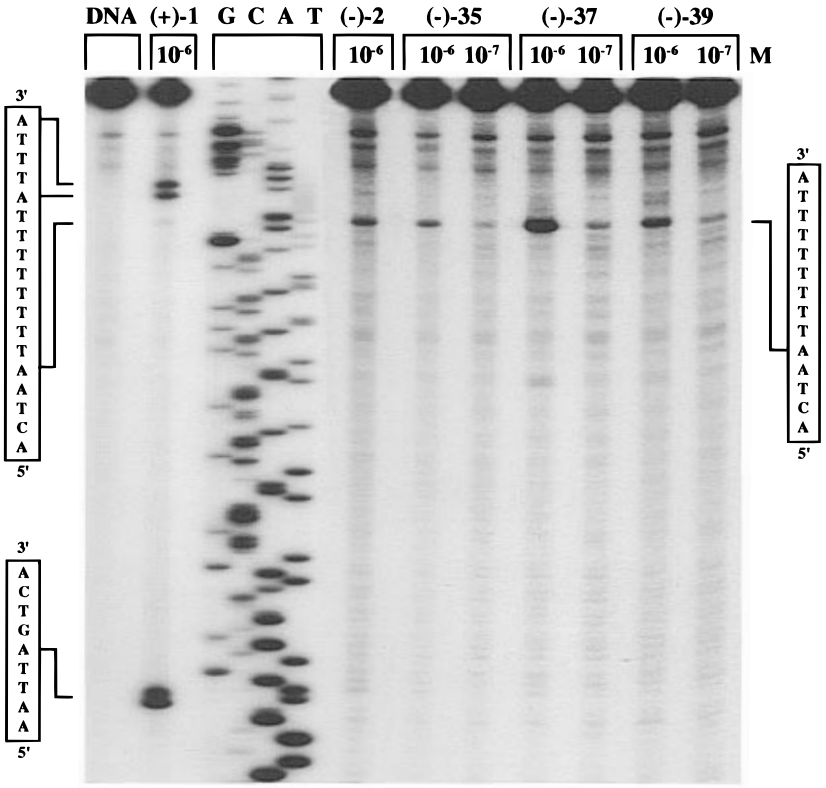


Figure 9. Thermally-induced strand cleavage of duplex DNA (SV40 DNA segment, 144 bp, nucleotide no. 5238−138, clone w794) after 72 h incubation at 25 °C followed by removal of unbound agent, 30 min incubation at 100 °C, 8% denaturing PAGE, and autoradiography: lane 1, control DNA; lane 2, (+)-CC-1065 (1×10^{-6} M); lanes 3−6, Sanger G, C, A, and T sequencing reactions; lane 7, *ent*-(−)-duocarmycin SA (1×10^{-6} M); lanes 8−9, *ent*-(−)-CCBI-TMI (1×10^{-6} and 10^{-7} M); lanes 10−11, *ent*-(−)-CCBI−indole$_2$ (1×10^{-6} and 10^{-7} M); lanes 12−13, *ent*-(−)-CCBI−CDPI$_1$ (1×10^{-6} and 10^{-7} M).

Table 5. Enantiomer Distinctions

agent	rel IC_{50} (L1210)[a]	rel DNA alkylation intensity[b]
CI−TMI	1	0.5−2.0
duocarmycin SA	10	10
MCBI−TMI	50	50
CCBI−TMI	65	50−100
CBI−TMI	70	100
duocarmycin A	>100	>100

[a] IC_{50} (unnatural)/(natural) enantiomer. [b] Concentrations of unnatural/natural enantiomer required to detect DNA alkylation.

rather the ultimate efficiency of DNA alkylation that may be more relevant to the expression of their biological properties.[6,9,11,13,29,31−35,42,61] Just as importantly, they also suggest that other factors are contributing to the DNA alkylation reaction and that other or additional features[20] beyond C4 carbonyl protonation[60] or Lewis acid complexation may be responsible for catalysis. Most prevalent among these possibilities is activation by ground-state destabilization derived through a DNA binding induced conformational change that activates the agent for nucleophilic addition.[20] Studies which address such issues are in progress and will be reported in due course.

Conclusions. A short and efficient synthesis of CCBI and its immediate precursors is detailed. Its evaluation permitted an accurate assessment of the electronic effect of substituents on the chemical and functional reactivity of the agents and the impact this may have on their biological properties. A study of the solvolysis reactivity of *N*-BOC-CCBI and its comparison with related agents revealed that the introduction of a strong electron-withdrawing C7 cyano group slowed the rate of solvolysis but the effect was very small. Classical Hammett quantitation of the effect provided a remarkably small ρ (−0.3), indicating an exceptionally small C7 substituent electronic effect on functional reactivity. Additional kinetic studies demonstrated that protonation of the C4 carbonyl is not the rate-determining step of solvolysis or acid-catalyzed nucleophilic addition, but rather that it is rapid and reversible followed by slow and rate-determining nucleophilic addition to the cyclopropane requiring the presence and assistance of a nucleophile (S_N2 mechanism). No doubt this contributes to the DNA alkylation selectivity and suggests that the positioning of an accessible nucleophile (adenine N3) and not C4 carbonyl protonation or Lewis acid complexation is the rate-determining step controlling the sequence selectivity of DNA alkylation. This exceptionally small electronic effect on the solvolysis rate had no impact on the solvolysis regioselectivity, and stereoelectronically-controlled nucleophilic addition to the least substituted carbon of the activated cyclopropane was observed exclusively. Consistent with past studies, a direct relationship between solvolysis stability and cytotoxic potency was demonstrated and related to the predictable Hammett substituent effects. For the natural enantiomers, this very small electronic effect on functional reactivity had no perceptible effect on their DNA alkylation selectivity. Although the range of reactivity spanned by the agents is quite small and the effects are difficult to distinguish, the efficiencies but not the rates of DNA alkylation were found to correlate with the relative reactivities of the agents with the most stable agent in the series providing the most efficient DNA alkylation as well as most potent cytotoxic activity. Similar effects

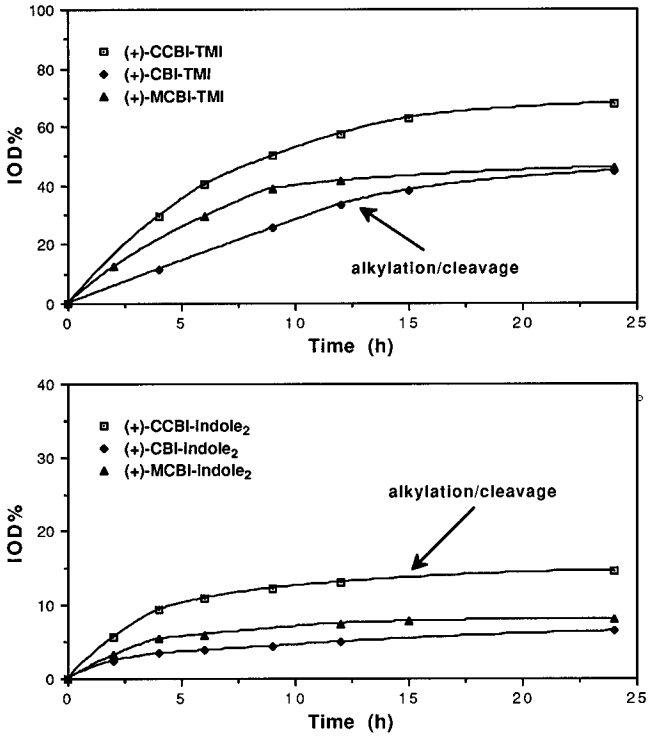


Figure 10. Plot of percent integrated optical density (IOD%) versus time established through autoradiography of 5′-^{32}P end-labeled DNA and used to monitor the relative rate of w794 alkylation at the 5′-AATTA high affinity site for **35**, **37**, (+)-CBI−TMI, (+)-MCBI−TMI, (+)-CBI−indole$_2$, and (+)-MCBI−indole$_2$.

of the C7 cyano group on the unnatural enantiomers were detected and they proved to be 4−10× more effective than the corresponding CBI-based unnatural enantiomer and 4−70× less potent than the corresponding CCBI natural enantiomer.

Experimental Section

Ethyl 7-Bromo-4-hydroxy-2-naphthalenecarboxylate (6). Method A. A solution of *t*-BuOK (7.19 g, 64.1 mmol) in *t*-BuOH (100 mL) at 45 °C was treated with a mixture of *m*-bromobenzaldehyde (**4**, 10.78 g, 58.3 mmol) and diethyl succinate (15.23 g, 87.4 mmol) dropwise. The reaction mixture was warmed at reflux for 2 h. After cooling to 25 °C, the mixture was neutralized with the addition of 10% aqueous HCl (pH = 1), and the *t*-BuOH was removed from the organic layer under reduced pressure. The residue was extracted with EtOAc (3 × 30 mL). The half ester was extracted from the organic layer with 5% aqueous $NaHCO_3$ (5 × 30 mL). The combined basic aqueous layers were reacidified with aqueous 3 M HCl and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with saturated aqueous NaCl and dried ($MgSO_4$). Solvent removal yielded a mixture of the two isomeric half-esters **5** (13.7 g, 18.2 g theoretical, 75%) as an amber oil.

The half-esters **5** (13.66 g) were dissolved in 300 mL of Ac_2O. Anhydrous NaOAc (3.93 g, 48.0 mmol) was added, and the reaction mixture was warmed at reflux for 6 h. The Ac_2O was removed under reduced pressure, and the residue was suspended in 10% aqueous Na_2CO_3 (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was dissolved in 150 mL of 3 M HCl−EtOH at 0 °C, and the solution was allowed to warm to 25 °C and stir for 16 h. The solvent was removed under reduced pressure. Chromatography (SiO_2, 5 × 20 cm, 20% EtOAc−hexane) and subsequent recrystallization from toluene afforded pure **6** as a white solid free of contaminant **8**.

Method B. NaH (2.99 g, 74.6 mmol, 1.05 equiv, 60% dispersion in mineral oil) was washed with Et_2O (3 × 20 mL) and suspended in anhydrous THF (100 mL) under Ar. The suspension was cooled to 0 °C, **10**[50] (24.77 g, 72.3 mmol, 1.03 equiv) was added dropwise under Ar, and the reaction mixture was stirred at 0 °C for 2 h. The solution was then transferred by cannula to a solution of *m*-bromobenzaldehyde (**4**, 13.15 g, 71.1 mmol, 1 equiv) in 80 mL of THF at 0 °C. The resulting reaction mixture was stirred at 0 °C for 1 h before being allowed to warm to 25 °C, and the mixture was stirred overnight. The solvent was removed under vacuum, and the residue was partitioned between H_2O and CH_2Cl_2. The aqueous phase was extracted with CH_2Cl_2 (3 × 200 mL), and the combined organic layers were dried ($MgSO_4$) and concentrated. The diester was purified by passage through a plug of SiO_2 (10% EtOAc−hexane) to provide **11** (26.3 g, 26.3 g theoretical, 100%) as a clear oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.73 (s, 1H), 7.48 (s, 1H), 7.45 (m, 1H), 7.26 (d, 1H, J = 5.8 Hz), 7.25 (d, 1H, J = 6.8 Hz), 4.25 (q, 2H, J = 7.1 Hz), 3.38 (s, 2H), 1.44 (s, 9H), 1.30 (t, 3H, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 169.6, 166.7, 139.2, 137.0, 131.4, 131.3, 129.8, 127.9, 127.3, 122.3, 80.9, 60.9, 34.5, 27.7, 14.0; IR (film) ν_{max} 2978, 2930, 1728, 1641, 1560, 1474, 1368, 1329, 1279, 1198, 1154, 1097, 786, 682 cm^{-1}; FABHRMS (NBA−CsI) m/z 500.9664 (M^+ + Cs, $C_{17}H_{21}BrO_4$ requires 500.9678).

A solution of **11** (16.6 g, 46.5 mmol) in 90% aqueous CF_3CO_2H (75 mL) was stirred for 30 min at 25 °C. The solvent was removed under reduced pressure, and the residue was azeotroped two times with benzene. The half-ester **5** was purified by dissolution in saturated aqueous $NaHCO_3$ solution (50 mL). Acidification of the aqueous phase with 10% aqueous HCl (pH = 1) and extraction with EtOAc (3 × 50 mL) followed by drying the combined organic phase (Na_2SO_4) and concentration afforded pure **5** (14.6 g, 14.6 g theoretical, 100%) as a pale yellow oil that crystallized under vacuum: mp 78−80 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.83 (s, 1H), 7.50 (m, 2H), 7.29 (m, 2H), 4.30 (q, 2H, J = 7.1 Hz), 3.52 (s, 2H), 1.33 (t, 3H, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.6, 167.1, 140.4, 136.8, 131.9, 131.8, 130.3, 127.3, 126.8, 122.8, 61.5, 33.5, 13.6; IR (neat) ν_{max} 2984, 1709, 1639, 1556, 1472, 1370, 1278, 1194, 1092, 1018, 782 cm^{-1}; FABHRMS (NBA−NaI) m/z 334.9883 (M^+ + Na, $C_{13}H_{13}BrO_4$ requires 334.9895).

The half-ester **5** (14.56 g, 46.5 mmol) was dissolved in Ac_2O (310 mL, 0.15 M) under Ar. KOAc (5.93 g, 60.5 mmol, 1.3 equiv) was added, and the reaction mixture was warmed at reflux for 30 min. The hot solution was poured into H_2O (400 mL), and the resulting mixture was stirred and allowed to cool to 25 °C. The product was collected by filtration and recrystallized from CH_3OH to yield pure **7** (10.06 g, 15.68 g theoretical, 65%) free of contaminant **9**. For **7**: mp 113−114 °C (CH_3OH); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.34 (s, 1H, C1-H), 8.08 (d, 1H, J = 1.6 Hz, C8-H), 7.77−7.59 (m, 3H), 4.38 (q, 2H, J = 7.1 Hz), 2.45 (s, 3H), 1.37 (t, 3H, J = 7.1 Hz); IR (neat) ν_{max} 2986, 1770, 1717, 1591, 1369, 1273, 1238, 1190, 1155, 1099, 1068, 1057, 1020, 907, 815 cm^{-1}; FABHRMS (NBA) m/z 358.9882 (M^+, $C_{15}H_{13}BrO_4$ requires 358.9895).

The acetate **7** (15.7 g, 46.6 mmol) was dissolved in EtOH (200 mL), and K_2CO_3 (32.2 g, 233 mmol, 5 equiv) was added. The reaction mixture was warmed at reflux for 1 h, cooled to 25 °C, and poured into H_2O (200 mL). The mixture was acidified with the addition of 10% aqueous HCl (pH = 1), and the desired product was extracted into EtOAc (3 × 250 mL). The combined organic layers were washed with saturated aqueous NaCl and dried ($MgSO_4$), and the solvent was removed under reduced pressure. The resulting solid was recrystallized from toluene to yield **6** (14.62 g, 14.62 g theoretical, 100%): mp 180 °C (needles, toluene); ^{1}H NMR (acetone-d_6, 250 MHz) δ 9.53 (br s), 8.23 (d, 1H, J = 1.8 Hz), 8.18 (d, 1H, J = 9.0 Hz), 8.09 (s, 1H), 7.68 (dd, 1H, J = 2.0, 8.9 Hz), 7.50 (d, 1H, J = 1.4 Hz), 4.37 (q, 2H, J = 7.1 Hz), 1.38 (t, 3H, J = 7.1 Hz); ^{13}C NMR (acetone-d_6, 100 MHz) δ 166.5, 154.3, 136.0, 131.6, 130.9, 130.5, 126.3, 125.2, 121.7, 121.6, 108.4, 61.7, 14.5; IR (KBr) ν_{max} 3379, 2989, 1701, 1588, 1476, 1420, 1399, 1386, 1360, 1284, 1252, 1080, 1026, 966, 893, 816, 768 cm^{-1}; FABHRMS (NBA) m/z 293.9888 (M^+, $C_{13}H_{11}BrO_3$ requires 293.9892).

Anal. Calcd for $C_{13}H_{11}BrO_3$: C, 52.91; H, 3.76. Found: C, 53.17; H, 3.46.

Ethyl 4-(Benzyloxy)-7-bromo-2-naphthalenecarboxylate (12). A solution of **6** (8.04 g, 2.2 mmol) in anhydrous DMF (150 mL) under Ar was treated with K_2CO_3 (5.65 g, 40.9 mmol), benzyl bromide (5.59 g, 32.7 mmol, 1.2 equiv), and Bu_4NI (402 mg, 1.1 mmol, 0.04 equiv). After being stirred for 11 h at 25 °C, the reaction mixture was poured into H_2O (200 mL) and extracted with EtOAc (3 × 200 mL). The combined organic layers were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated. The resulting solid was recrystallized from 5% EtOAc−hexane to afford **12** (8.36 g, 10.49 g theoretical, 80%) as white needles. An additional 2.13 g (20%) of **12** was obtained by chromatography (SiO_2, 4 × 20 cm, 10% EtOAc−hexane) of the crystallization mother liquors: mp 105 °C (needles, hexane); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.19 (d, 1H, J = 8.8 Hz), 8.10 (s, 1H), 8.05 (d, 1H, J = 1.8 Hz), 7.61 (dd, 1H, J = 1.9, 8.9 Hz), 7.50 (m, 6H), 5.28 (s, 2H), 4.45 (q, 2H, J = 7.1 Hz), 1.46 (t, 3H, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.5, 154.7, 136.4, 134.7, 130.8, 130.7, 129.1, 128.7, 128.3, 127.6, 126.3, 124.2, 122.5, 121.5, 104.6, 70.4, 61.4, 14.4; IR (KBr) ν_{max} 2986, 1716, 1589, 1578, 1406, 1369, 1331, 1279, 1238, 1098, 1025, 962, 892, 816, 767, 725, 691 cm^{-1}; FABHRMS (NBA) m/z 384.0370 (M^+, $C_{20}H_{17}BrO_3$ requires 384.0361).

Anal. Calcd for $C_{20}H_{17}BrO_3$: C, 62.35; H, 4.45. Found: C, 62.23; H, 4.61.

Ethyl 4-(Benzyloxy)-7-cyano-2-naphthalenecarboxylate (13). A solution of **12** (9.46 g, 27.2 mmol) in anhydrous DMF (12.6 mL) under Ar was treated with CuCN (2.92 g, 32.6 mmol, 1.2 equiv), and the mixture was warmed at reflux for 20 h. The reaction mixture was cooled to 25 °C and poured into 250 mL of H_2O to which $FeCl_3$ (5.29 g, 32.6 mmol, 1.2 equiv) was added with swirling. The solution was extracted with EtOAc (3 × 200 mL), and the combined organic layers were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated. Recrystallization from 20% EtOAc−hexane provided **13** (6.70 g, 9.00 g theoretical, 74%) as a white solid. Chromatography (SiO_2, 4 × 20 cm, 10% EtOAc−hexane) of the crystallization mother liquors afforded additional **13** (2.00 g, 22%, 96% combined) as white solid: mp 125 °C (needles, EtOH); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.43 (d, 1H, J = 8.7 Hz), 8.29 (s, 1H), 8.25 (s, 1H), 7.69 (dd, 1H, J = 1.6, 8.7 Hz), 7.66 (s, 1H), 7.56−7.39 (m, 5H), 5.32 (s, 2H), 4.46 (q, 2H, J = 7.2 Hz), 1.46 (t, 3H, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 165.9, 154.3, 135.9, 134.6, 132.3, 129.8, 128.6, 128.3, 128.2, 127.8, 127.5, 123.8, 123.2, 118.6, 110.7, 106.9, 70.5, 61.5, 14.3; IR (KBr) ν_{max} 2994, 2226, 1711, 1577, 1502, 1284, 1252, 1093, 1029, 916, 827 cm^{-1}; FABHRMS (NBA) m/z 332.1276 (M^+ + H, $C_{21}H_{17}NO_3$ requires 332.1287).

Anal. Calcd for $C_{21}H_{17}NO_3$: C, 76.11; H, 5.17; N, 4.23. Found: C, 75.96; H, 5.42; N, 4.31.

4-(Benzyloxy)-7-cyano-2-naphthalenecarboxylic Acid (14). A solution of **13** (8.67 g, 26.2 mmol) in 260 mL of THF−CH_3OH−H_2O (3:1:1) was treated with LiOH−H_2O (5.49 g, 131 mmol, 5 equiv), and the mixture was stirred at 25 °C for 25 h. The solution was acidified with the addition of 10% aqueous HCl (pH < 1) and the product partially precipitated. The product was collected by filtration, and the remaining aqueous phase was extracted with EtOAc (3 × 200 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo to afford a combined **14** (7.94 g, 7.94 g theoretical, 100%): mp 235 °C (white powder, EtOH); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.73 (d, 1H, J = 1.1 Hz), 8.34 (d, 1H, J = 8.9 Hz), 8.32 (s, 1H), 7.88 (dd, 1H, J = 1.5, 8.9 Hz), 7.63 (s, 1H), 7.57−7.34 (m, 5H), 5.38 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.3, 153.8, 136.6, 135.5, 132.3, 130.3, 128.6, 128.3, 128.1, 127.6, 126.4, 123.4, 121.9, 118.8, 110.0, 107.5, 70.0; IR (KBr) ν_{max} 3000 (br), 2227, 1687, 1577, 1499, 1419, 1343, 1283, 1250, 1105, 996, 915, 836, 740 cm^{-1}; FABHRMS (NBA) m/z 304.0964 (M^+ + H, $C_{19}H_{13}NO_3$ requires 304.0974).

***N*-(*tert*-Butyloxycarbonyl)-4-(benzyloxy)-7-cyano-2-naphthylamine (15).** A solution of **14** (500 mg, 1.65 mmol) in freshly distilled *t*-BuOH (165 mL) was treated with Et_3N (0.276 mL, 1.98 mmol, 1.2 equiv) and 5 g of activated 4 Å molecular sieves. Diphenyl phosphorazidate (0.426 mL, 1.98 mmol, 1.2 equiv) was added, and the reaction mixture was warmed at reflux for 14 h. The mixture was cooled to 25 °C, and the solvent was removed under vacuum. The residue was dissolved in EtOAc, and the organic phase was washed with 10% aqueous HCl, dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 3 × 20 cm, 20% EtOAc−hexane) afforded **15** (534 mg, 618 mg theoretical, 87%) as a white crystalline solid: mp 145 °C (white needles, 10% EtOAc−hexane); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.26 (d, 1H, J = 8.7 Hz), 8.01 (s, 1H), 7.45 (m, 7H), 7.23 (s, 1H), 6.84 (s, 1H), 5.21 (s, 2H), 1.57 (s, 9H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 154.9, 152.5, 138.1, 136.1, 133.6, 132.6, 128.6, 128.2, 127.4, 124.1, 123.6, 123.5, 119.3, 110.5, 106.5, 101.7, 81.1, 70.4, 28.3; IR (KBr) ν_{max} 3327, 2980, 2223, 1701, 1586, 1545, 1420, 1369, 1341, 1251, 1160, 1107, 1064, 1002, 910, 820, 744, 694 cm^{-1}; FABHRMS (NBA) m/z 374.1641 (M^+, $C_{23}H_{22}N_2O_3$ requires 374.1630).

Anal. Calcd for $C_{23}H_{22}N_2O_3$: C, 73.78; 5.92; N, 7.48. Found: C, 73.40; H, 5.84; N, 7.23.

***N*-(*tert*-Butyloxycarbonyl)-4-(benzyloxy)-1-bromo-7-cyano-2-naphthylamine (16).** A solution of **15** (137 mg, 0.366 mmol) in freshly distilled THF (7.3 mL) and cooled to −78 °C under Ar was treated with 10 μL of a 1 μL/mL solution of H_2SO_4 in THF, and the solution was stirred for 20 min before the addition of NBS (78 mg, 439 mmol, 1.2 equiv). The reaction mixture was allowed to warm to −60 °C and was stirred for 4 h at which time the reaction was complete by TLC. Et_2O (7.3 mL) was added, and the resulting organic phase was washed with 5% aqueous $NaHCO_3$ (1 × 10 mL) and saturated aqueous NaCl (1 × 10 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography (SiO_2, 2 × 20 cm, 10% EtOAc−hexane) afforded **16** (145 mg, 166 mg theoretical, 87%) as a white crystalline solid: mp 179 °C dec (white needles, 10% EtOAc−hexane); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.40 (s, 1H), 8.26 (d, 1H, J = 8.5 Hz), 8.23 (s, 1H), 7.5−7.3 (m, 6H), 5.23 (s, 2H), 1.58 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.4, 152.4, 137.0, 136.0, 132.2, 132.0, 128.7, 128.6, 128.4, 128.0, 124.9, 124.8, 124.2, 119.1, 111.9, 102.0, 81.9, 70.8, 28.3; IR (KBr) ν_{max} 3416, 2978, 2230, 1738, 1623, 1603, 1570, 1498, 1405, 1364, 1335, 1229, 1158, 990, 970, 878, 850, 823, 758, 696 cm^{-1}; FABHRMS (NBA−CsI) m/z 584.9793 (M^+ + Cs, $C_{23}H_{21}BrN_2O_3$ requires 584.9790).

Anal. Calcd for $C_{23}H_{21}BrN_2O_3$: C, 60.94; H, 4.67; N, 6.18. Found: C, 61.12; H, 4.75; N, 6.04.

***N*-(*tert*-Butyloxycarbonyl)-*N*-(3-methyl-2-buten-1-yl)-4-(benzyloxy)-1-bromo-7-cyano-2-naphthylamine (17).** A solution of **16** (1.77 g, 3.90 mmol) in anhydrous DMF (20 mL) under Ar was treated with NaH (206 mg, 5.1 mmol, 1.3 equiv, 60% oil dispersion), and the reaction mixture was stirred for 30 min. The mixture was cooled to 0 °C, and 4-bromo-2-methyl-2-butene (1.35 mL, 11.7 mmol, 3 equiv) was added dropwise by cannula. The solution was stirred at 0 °C for 1 h before being allowed to warm to 25 °C and stirred overnight. Water (20 mL) was added, and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic phases were washed with saturated aqueous NaCl (1 × 30 mL) and dried (Na_2SO_4), and the solvent was removed under vacuum. Chromatography (SiO_2, 4 × 20 cm, 10% EtOAc−hexane) afforded **17** (2.05 g, 2.03 g theoretical, >99%) as an amber oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.66 (d, 1H, J = 0.5 Hz), 8.40 (d, 1H, J = 8.6 Hz), 7.63 (d, 1H, J = 8.6 Hz), 7.51−7.34 (m, 5H), 6.85 (s, 1H), 5.24 (d, 1H, J = 9.5 Hz), 5.20 (d, 1H, J = 11.7 Hz), 5.17 (d, 1H, J = 11.7 Hz), 4.40 (dd, 1H, J = 6.1, 14.5 Hz), 4.01 (dd, 1H, J = 7.7, 14.9 Hz), 1.61 (s, 3H), 1.57 (s, 3H), 1.37 and 1.30 (two s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.7, 140.9, 136.3, 135.8, 133.5, 128.8, 128.5, 127.3, 126.7, 124.1, 119.8, 119.5, 118.8, 111.7, 110.9, 81.0, 80.5, 70.8, 47.7, 46.3, 28.5, 28.2, 25.7; IR (film) ν_{max} 3467 (br), 2928, 2229, 1701, 1676, 1596, 1503, 1438, 1387, 1335, 1255, 1164, 1090, 863, 738 cm^{-1}.

Anal. Calcd for $C_{28}H_{29}BrN_2O_3$: C, 64.60; H, 5.62; N, 5.38. Found: C, 64.51; H, 5.74; N, 5.18.

***N*-(*tert*-Butyloxycarbonyl)-*N*-(formylmethyl)-4-(benzyloxy)-1-bromo-7-cyano-2-naphthylamine (18).** A solution of **17** (180 mg, 0.345 mmol) in 22 mL (0.016 M) of 20% CH_3OH−CH_2Cl_2 was cooled to −78 °C. A stream of 3% O_3/O_2

(160 L/min) was bubbled through the solution for 72 s. The reaction was immediately quenched with the addition of 0.81 mL of dimethyl sulfide, and the mixture was allowed to stir at −78 °C for 5 min before being allowed to warm to 25 °C and stirred for 5 h. The solvent was removed in vacuo. Chromatography (SiO_2, 2 × 20 cm, 30% EtOAc−hexane) afforded **18** (155 mg, 171 mg theoretical, 91%) as a white foamy solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.76 and 9.73 (two s), 8.63 and 8.61 (two s, 1H), 8.41 and 8.36 (two d, 1H, J = 8.7 Hz), 7.64 and 7.60 (two dd, 1H, J = 1.4, 8.7 Hz), 7.50−7.34 (m, 5H), 7.17 and 7.14 (two s, 1H), 5.26, 5.29 and 5.17 (one s and two d, 2H, J = 11.4 Hz), 4.76 and 4.65 (two d, 1H, J = 18.8 Hz), 4.01 and 3.95 (two d, 1H, J = 18.8 Hz), 1.31 and 1.52 (two s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz, major rotamer) δ 197.3, 154.1, 140.8, 135.6, 133.4, 132.0, 128.8, 128.7, 128.5, 127.9, 127.6, 127.1, 124.2, 118.7, 113.8, 112.0, 110.5, 81.8, 70.9, 58.9, 28.1; IR (film) ν_{max} 2976, 2229, 1706, 1596, 1415, 1369, 1335, 1259, 1225, 1152, 1096, 848 cm^{-1}; FABHRMS (NBA) m/z 495.0922 (M^+ + H, $C_{25}H_{23}BrN_2O_4$ requires 495.0919).

Anal. Calcd for $C_{25}H_{23}BrN_2O_4$: C, 60.62; H, 4.68; N, 5.65. Found: C, 60.39; H, 4.61; N, 5.69.

2-[*N*-(*tert*-Butyloxycarbonyl)-*N*-[3-(tetrahydropyranyloxy)-2-propen-1-yl]amino]-4-(benzyloxy)-1-bromo-7-cyanonaphthalene (19). A suspension of triphenyl[(2-tetrahydropyranyloxy)methyl]phosphonium chloride[54] (635 mg, 1.51 mmol, 3 equiv) in THF (5 mL) at −78 °C was treated dropwise with *n*-BuLi (1.44 mmol, 0.58 mL, 2.5 M in hexane, 2.86 equiv). The reaction mixture was stirred at −78 °C for 10 min and allowed to warm to 0 °C over 20 min. The mixture was recooled to −78 °C, and HMPA (2.11 mL, 12.1 mmol, 24 equiv) was added followed immediately by the addition of **18** (250 mg, 0.505 mmol) in 2.5 mL of THF. The reaction was stirred at −78 °C for 1.5 h and 24 h at 25 °C before being quenched with the addition of 20 mL of phosphate buffer (pH 7.0). The mixture was extracted with EtOAc (3 × 50 mL), and the combined organic phase was dried (Na_2SO_4) and concentrated in vacuo. Chromatography (SiO_2, 2 × 30 cm, 10% EtOAc−hexane with 2% Et_3N) afforded **19** (221 mg, 300 mg theoretical, 74%) as an oil and as a mixture of *E*- and *Z*-isomers: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.65 (s, 1H), 8.38 (m, 1H), 7.62 (d, 1H, J = 8.1 Hz), 7.49−7.36 (m, 6H), 6.96−6.79 (m, 1H), 6.22−6.07 (m, 1H), 5.28−5.15 (m, 2H), 4.83−4.29 (m, 3H), 3.85−2.77 (m, 2H), 1.98−1.22 (m, 15H); IR (film) ν_{max} 2940, 2229, 1704, 1596, 1415, 1367, 1332, 1258, 1225, 1163, 1021, 965, 902, 849, 739, 697 cm^{-1}.

5-(Benzyloxy)-3-(*tert*-butyloxycarbonyl)-8-cyano-1-[(tetrahydropyranyloxy)methyl]-1,2-dihydro-3*H*-benz[*e*]indole (20). A solution of **19** (41 mg, 69 μmol) in freshly distilled benzene (3.5 mL) under Ar was treated with AIBN (2 mg, 0.2 equiv) followed by Bu_3SnH (40 mg, 0.014 mmol, 2 equiv). The reaction mixture was warmed at reflux for 2 h and cooled to 25 °C, and the solvent was removed under a stream of N_2. The residue was azeotroped with THF (1 × 2 mL). Chromatography (SiO_2, 1 × 20 cm, 10% EtOAc−hexane) afforded **20** (35 mg, 35.5 mg theoretical, 99%) as a clear oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.29 (d, 1H, J = 8.7 Hz), 8.24 and 8.19 (s and d, 1H, J = 1.3 Hz), 8.01 (br s, 1H), 7.50 (br d, 1H, J = 7.1 Hz), 7.44−7.34 (m, 5H), 5.24 (s, 2H), 4.58 and 4.55 (two br m, 1H), 4.12 (br s, 1H), 4.10 (br s, 1H), 3.96 (dd, 1H, J = 5.8, 9.6 Hz), 3.87 (m, 1H), 3.78 (dd, 1H, J = 8.9, 9.6 Hz), 3.64 and 3.58 (ddd and dd, 1H, J = 3.1, 8.1, 11.3 Hz and 6.2, 9.4 Hz), 3.50−3.38 (m, 1H), 1.73−1.55 (m, 15H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.3, 153.0, 136.3, 129.8, 129.5, 128.6, 128.2, 127.7, 124.5, 123.4, 123.1, 119.5, 110.3, 99.7, 99.1, 80.5, 70.5, 70.1, 62.6, 62.4, 52.8, 38.9, 30.5, 28.4, 28.2, 25.3, 19.5; IR (film) ν_{max} 2943, 2226, 1703, 1622, 1592, 1454, 1367, 1328, 1258, 1141, 1033, 967, 854, 736, 697 cm^{-1}; FABHRMS (NBA−CsI) m/z 647.1549 (M^+ + Cs, $C_{31}H_{34}N_2O_5$ requires 647.1552).

5-(Benzyloxy)-3-(*tert*-butyloxycarbonyl)-8-cyano-1-(hydroxymethyl)-1,2-dihydro-3*H*-benz[*e*]indole (21). From 20. A solution of **20** (35 mg, 69 μmol) in freshly distilled CH_3OH (1 mL) was treated with 0.5 mg of Amberlyst-15 ion exchange resin, and the reaction mixture was stirred at 45 °C for 5 h. The resin was removed by filtration and washed with CH_3OH (1 × 2 mL). The filtrates were combined, and the solvent was removed under a stream of N_2. Chromatography (SiO_2, 1 × 20 cm, 20% EtOAc−hexane) afforded **21** (29.7 mg, 29.7 mg theoretical, 100%) as an off-white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.32 (d, 1H, J = 8.7 Hz, C6-H), 8.09 (s, 1H, C9-H), 8.02 (br s, 1H, C4-H), 7.51 (d, 1H, J = 8.7 Hz, C7-H), 7.43 (m, 5H), 5.25 (s, 2H, CH_2Ph), 4.21 (dd, 1H, J = 11.5, 2.3 Hz, C2-H), 4.14 (dd, 1H, J = 11.5, 8.9 Hz, C2-H), 3.92 (dd, 1H, J = 4.0, 10.3 Hz, C*H*HOH), 3.80 (m, 1H, C1-H), 3.75 (dd, 1H, J = 7.2, 10.3 Hz, CH*H*OH), 1.59 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.5, 153.0, 136.2, 129.7, 128.7, 128.5, 128.2, 127.7, 124.9, 123.5, 123.2, 119.4, 115.0, 110.7, 99.1, 80.5, 70.6, 64.8, 52.5, 47.0, 41.1, 28.4; IR (KBr) ν_{max} 3450 (br), 2926, 2226, 1702, 1592, 1458, 1410, 1368, 1328, 1143, 1032, 735, 696 cm^{-1}; FABHRMS (NBA) m/z 430.1896 (M^+, $C_{26}H_{23}N_2O_4$ requires 430.1893).

From 29. A solution of **29** (341 mg, 0.599 mmol) in THF−HOAc−H_2O (3:1:1, 20 mL) was treated with Zn powder (3.13 g, 80 equiv), and the mixture was warmed at 70 °C for 6 h. The Zn powder was removed by filtration through Celite, and the mixture was concentrated under vacuum. Chromatography (SiO_2, 1.3 × 13 cm, 0−25% EtOAc−hexane) afforded **21** (188 mg, 258 mg theoretical, 73%) as an off-white solid identical in all respects to that described above.

5-(Benzyloxy)-3-(*tert*-butyloxycarbonyl)-1-(chloromethyl)-8-cyano-1,2-dihydro-3*H*-benz[*e*]indole (22). A solution of **21** (47 mg, 0.10 mmol) in freshly distilled anhydrous CH_2Cl_2 (0.35 mL) under Ar was treated sequentially with Ph_3P (82 mg, 0.31 mmol, 3 equiv) and CCl_4 (91 μL, 0.94 mmol, 9 equiv). The reaction mixture was stirred at 25 °C for 2 h. The solvent was evaporated under a stream of N_2. Chromatography (SiO_2, 0.8 × 10 cm, 10% EtOAc−hexane) afforded **22** (49 mg, 49 mg theoretical, 100%) as a white solid: mp 212−214 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.33 (d, 1H, J = 8.7 Hz, C6-H), 7.98 (s, 1H, C9-H), 7.70 (br s, 1H, C4-H), 7.50−7.24 (m, 6H), 5.24 (s, 2H), 4.23 (d, 1H, J = 11.0 Hz, C2-H), 4.14 (dd, 1H, J = 11.0, 8.9 Hz, C2-H), 3.95 (dddd, 1H, J = 2.6, 9.2, 11.1, 12.1 Hz, C1-H), 3.83 (dd, 1H, J = 3.1, 11.1 Hz, CH*H*Cl), 3.47 (dd, 1H, J = 9.9, 11.0 Hz, C*H*HCl), 1.54 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.9, 144.3, 136.1, 134.3, 133.8, 133.6, 130.4, 128.7, 128.5, 128.4, 128.3, 127.7, 125.1, 123.5, 119.2, 111.1, 99.0, 70.6, 53.1, 46.3, 41.3, 28.3; IR (KBr) ν_{max} 2978, 2231, 1692, 1594, 1478, 1409, 1374, 1337, 1166, 1146, 1084, 973, 960, 859, 756, 697 cm^{-1}; FABHRMS (NBA) m/z 448.1570 (M^+, $C_{26}H_{25}ClN_2O_3$ requires 448.1554).

Resolution of 22. Samples of racemic **22** were resolved by preparative HPLC chromatography on a Diacel Chiralcel-OD column (10 μm, 2 × 25 cm) using 7% *i*-PrOH−hexane eluant (7 mL/min). The enantiomers eluted with retention times of 20.70 min (unnatural enantiomer) and 28.54 min (natural enantiomer), α = 1.38.

(1*S*)-22: $[\alpha]^{25}_D$ −9.5 (*c* 0.5, $CHCl_3$).

***ent*-(1*R*)-22:** $[\alpha]^{25}_D$ +9.5 (*c* 0.3, $CHCl_3$).

3-(*tert*-Butyloxycarbonyl)-1-(chloromethyl)-8-cyano-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (23). A solution of **22** (71.5 mg, 0.159 mmol) and 10% Pd−C (40 mg) in anhydrous EtOAc (5 mL) was degassed with a stream of N_2 for 30 s. The resulting mixture was placed under an atmosphere of H_2 and stirred at 25 °C for 2.5 h. The mixture was diluted with THF (1 mL) and filtered through Celite (EtOAc wash). The solvent was removed in vacuo. Chromatography (SiO_2, 1.5 × 6 cm, 20% EtOAc−hexane) afforded **23** (57.1 mg, 57.1 mg theoretical, 100%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.25 (d, 1H, J = 8.7 Hz, C6-H), 7.97 (s, 1H, C9-H), 7.87 (br s, 1H, C4-H), 7.42 (dd, 1H, J = 1.5, 8.7 Hz, C7-H), 6.67 (br s, 1H, OH), 4.23 (d, 1H, J = 11.4 Hz, C2-H), 4.14 (dd, 1H, J = 8.8, 11.8 Hz, C2-H), 3.95 (m, 1H, C1-H), 3.82 (dd, 1H, J = 3.2, 11.3 Hz, C*H*HCl), 3.46 (dd, 1H, J = 9.8, 11.2 Hz, CH*H*Cl), 1.59 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.2, 153.1, 142.9, 129.4, 127.8, 125.2, 123.2, 122.8, 119.3, 114.7, 111.0, 101.7, 82.5, 53.3, 46.3, 41.3, 28.4; IR (film) ν_{max} 3315 (br), 2964, 2923, 2227, 1676, 1585, 1421, 1369, 1331, 1235, 1141, 729 cm^{-1}; FABHRMS (NBA) m/z 358.1076 (M^+ + H, $C_{19}H_{19}ClN_2O_3$ requires 358.1084).

(1*S*)-23: $[\alpha]^{23}_D$ −15 (*c* 0.08, $CHCl_3$).

***ent*-(1*R*)-23**: $[\alpha]^{23}_D$ +16 (*c* 0.09, $CHCl_3$).

***N*-(*tert*-Butyloxycarbonyl)-7-cyano-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (25, *N*-BOC-CCBI). Method A.** A solution of **23** (1.4 mg, 3.91 μmol) in 2:1 DMF−

THF (112 μL) at 0 °C was treated with NaH (1.6 mg, 39 μmol, 60% oil dispersion), and the mixture was stirred for 30 min. The solvent was removed under a stream of N_2 and vacuum. PTLC (SiO_2, 0.25 mm × 10 × 15 cm, 30% EtOAc−hexane) afforded **25** (1.20 mg, 1.26 mg theoretical, 95%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.29 (d, 1H, J = 8.1 Hz, C5-H), 7.64 (dd, 1H, J = 1.5, 8.1 Hz, C6-H), 7.14 (d, 1H, J = 1.1 Hz, C8-H), 6.87 (br s, 1H, C3-H), 4.03 (m, 2H, C1-H_2), 2.81 (dt, 1H, J = 4.9, 7.5 Hz, C9a-H), 1.65 (dd, 1H, J = 4.7, 7.9 Hz, C9-H), 1.55 (s, 10H, C9-H and C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.4, 159.8, 151.4, 140.9, 135.8, 129.6, 127.7, 125.3, 118.2, 115.0, 108.8, 83.9, 52.9, 33.2, 29.4, 28.1, 23.8; IR (film) ν_{max} 2977, 2230, 1727, 1634, 1608, 1396, 1369, 1295, 1277, 1250, 1159, 1131, 843 cm^{-1}; UV (THF) λ_{max} 259 (ε = 22 200), 267 (ε = 24 000), 300 nm (ε = 12 000); UV (CH_3OH) λ_{max} 260 (ε = 24 700), 266 (ε = 25 200), 317 nm (ε = 13 400); FABHRMS (NBA) m/z 323.1383 (M^+ + H, $C_{19}H_{18}N_2O_3$ requires 323.1396).

(+)-*N*-BOC-CCBI (25): [α]$^{23}_D$ +124 (*c* 0.04, THF).

***ent*-(−)-*N*-BOC-CCBI (25)**: [α]$^{23}_D$ −121 (*c* 0.03, THF).

Method B. A solution of **23** (8.6 mg, 24.0 μmol) was dissolved in 1:1 THF−5% aqueous $NaHCO_3$ (2 mL), and the mixture was stirred at 25 °C for 9 h. The THF was removed by evaporation, and the product was extracted with EtOAc (4 × 1 mL). Chromatography (SiO_2, 0.8 × 5 cm, 0−25% EtOAc−hexane gradient) afforded **25** (7.7 mg, 7.7 mg theoretical, 100%) as a white solid identical to that described above.

7-Cyano-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (26, CCBI). A solution of **23** (2.5 mg, 7.0 μmol) in 4 M HCl−EtOAc (400 μL) was stirred at 25 °C under Ar for 30 min. The solvent was removed under a stream of N_2. After being dried in vacuo, the residue **30**[14] was dissolved in THF (200 μL) and treated with 200 μL of 5% aqueous $NaHCO_3$. The reaction mixture was stirred at 25 °C for 5 h before the solvent was removed in vacuo. PTLC (SiO_2, 0.25 mm × 20 × 20 cm, 70% THF−hexane) afforded **26** (1.6 mg, 1.6 mg theoretical, 100%) as a cream colored solid: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.30 (d, 1H, J = 8.1 Hz, C5-H), 7.61 (dd, 1H, J = 1.5, 8.1 Hz, C6-H), 7.11 (d, 1H, J = 1.5 Hz, C8-H), 5.77 (s, 1H, C3-H), 4.88 (br s, 1H, NH) 3.87 (dd, 1H, J = 5.2, 10.3 Hz, C1−H), 3.69 (d, 1H, J = 10.3 Hz, C1-H), 2.92 (dt, J = 3.6, 6.7 Hz, 1H, C9a-H), 1.63 (dd, 1H, J = 4.3, 7.9 Hz, C9-H), 1.49 (t, 1H, J = 4.7 Hz, C9-H); IR (film) ν_{max} 3097, 2851, 2227, 1622, 1583, 1519, 1328, 1241, 1092, 809 cm^{-1}; UV (THF) λ_{max} 337 (ε = 4400), 312 (ε = 4000), 266 (ε = 6300), 246 (ε = 13 500), 222 nm (ε = 15 000); UV (CH_3OH) λ_{max} 348 (ε = 5500), 317 (ε = 4300), 264 (ε = 6500), 248 (ε = 16 300), 221 nm (ε = 12 800); FABHRMS (NBA) m/z 223.0865 (M^+ + H, $C_{14}H_{10}N_2O$ requires 223.0871).

(+)-CCBI (26): [α]$^{23}_D$ +64 (*c* 0.05, THF).

***ent*-(−)-CCBI (26)**: [α]$^{23}_D$ −67 (*c* 0.05, THF).

***N*-(*tert*-Butyloxycarbonyl)-4-(benzyloxy)-7-cyano-1-iodo-2-naphthylamine (27).** A solution of **23** (250 mg, 0.67 mmol) in 10 mL of THF−CH_3OH (1:1) at −40 °C was treated with a catalytic amount of TsOH−H_2O (20 mg) and NIS (180 mg, 0.80 mmol, 1.2 equiv) in 2 mL of THF. The reaction mixture was stirred under Ar at −40 °C for 1 h and then warmed to 0 °C. Additional TsOH−H_2O (10 mg) and NIS (75 mg, 0.5 equiv) were added. After the reaction mixture was stirred for 1 h at 25 °C, it was quenched with the addition of 5 mL of saturated aqueous $NaHCO_3$ and extracted with Et_2O (4 × 15 mL). The combined organic layer was washed with saturated aqueous NaCl and dried (Na_2SO_4). PCTLC (2 mm SiO_2, 0−10% EtOAc−hexane gradient) afforded **27** (230 mg, 338 mg theoretical, 68%) as a solid: mp 177 °C dec (needles, EtOAc-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.38 (d, 1H, J = 1.5 Hz), 8.26 (d, 1H, J = 8.6 Hz), 8.21 (s, 1H), 7.52 (dd, 2H, J = 1.0, 7.8 Hz), 7.47 (dd, 1H, J = 1.5, 8.6 Hz), 7.45−7.35 (m, 3H), 7.33 (br s, 1H), 5.26 (s, 2H), 1.59 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.4, 152.5, 140.2, 137.0, 135.9, 134.2, 128.7, 128.4, 127.9, 125.1, 125.0, 124.3, 119.0, 112.2, 102.2, 81.8, 78.8, 70.7, 28.3; IR (film) ν_{max} 3383, 2978, 2228, 1732, 1600, 1563, 1495, 1397, 1366, 1331, 1230, 1154, 987, 879, 735 cm^{-1}; FABHRMS (NBA-CsI) m/z 632.9674 (M^+ + Cs, $C_{23}H_{21}IN_2O_3$ requires 632.9651).

Anal. Calcd for $C_{23}H_{21}IN_2O_3$: C, 55.21; H, 4.23; N, 5.60. Found: C, 55.28; H, 4.03; N, 5.40.

***N*-(*tert*-Butyloxycarbonyl)-*N*-(2-propenyl)-4-(benzyloxy)-7-cyano-1-iodo-2-naphthylamine (28).** A solution of **27** (160 mg, 0.31 mmol) in anhydrous DMF (5 mL) under Ar was treated with NaH (19 mg, 0.47 mmol, 1.5 equiv, 60% oil dispersion), and the reaction mixture was stirred for 30 min at 0 °C. Allyl bromide (194 mg, 139 μL, 1.55 mmol, 5 equiv) was added dropwise over 5 min, and the solution was allowed to warm to 25 °C and stirred for 2 h. Saturated aqueous $NaHCO_3$ (10 mL) was added, and the aqueous phase was extracted with EtOAc (4 × 10 mL), dried (Na_2SO_4), and concentrated. Chromatography (SiO_2, 2 × 15 cm, 10% EtOAc−hexane) afforded **28** (155 mg, 168 mg theoretical, 92%) as a clear oil that crystallized under vacuum: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.60 (s, 1H), 8.38 (d, 1H, J = 8.6 Hz), 7.63 (d, 1H, J = 8.4 Hz), 7.47−7.34 (m, 5H), 6.90 and 6.79 (two s, 1H), 5.94−5.84 (m, 1H), 5.27 and 5.22 (two d, 2H, J = 12.3 Hz), 5.00 (m, 2H), 4.50 (dd, 1H, J = 5.3, 14.4 Hz), 3.81 (dd, J = 7.0, 14.4 Hz), 1.57 and 1.29 (two s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.8, 144.9, 138.7, 135.7, 134.8, 133.2, 128.8, 128.3, 127.3, 126.9, 126.8, 124.2, 118.8, 118.4, 117.2, 112.2, 110.7, 80.8, 70.7, 53.3, 52.0, 28.4; IR (film) ν_{max} 2977, 2228, 1703, 1593, 1503, 1410, 1368, 1324, 1250, 1223, 1150, 1108, 931, 830, 735, 696 cm^{-1}; FABHRMS (NBA-CsI) m/z 672.9948 (M^+ + Cs, $C_{26}H_{25}IN_2O_3$, requires 672.9964).

Anal. Calcd for $C_{26}H_{25}IN_2O_3$: C, 57.99; H, 4.66; N, 5.18. Found: C, 57.60; H, 4.41; N, 5.27.

5-(Benzyloxy)-3-(*tert*-butyloxycarbonyl)-8-cyano-1-[(2′,2′,6′,6′- tetramethylpiperidino)oxy]methyl-1,2-dihydro-3*H*-benz[*e*]indole (29). A solution of **28** (200 mg, 0.37 mmol) in freshly distilled benzene (12 mL) was treated sequentially with TEMPO (3.0 equiv) and Bu_3SnH (1.0 equiv). The reaction mixture was warmed to 60 °C. After 20 min, an additional 1 equiv of Bu_3SnH was added. After 30 min, additional TEMPO (2 equiv) and Bu_3SnH (1.0 equiv) were added. After 20 min, 2 equiv of TEMPO and Bu_3SnH in two separate portions at 15 min intervals were added. After 45 min at 60 °C, the solvent was removed by evaporation. PCTLC (4 mm SiO_2, 0−25% EtOAc−hexane gradient) afforded **29** (144 mg, 212 mg theoretical, 68%) as a semisolid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.31 (d, 1H, J = 8.7 Hz), 8.19 (s, 1H), 8.01 (s, 1H), 7.53 (d, 2H, J = 7.0 Hz), 7.44 (t, 2H, J = 7.0 Hz), 7.40 (m, 2H), 5.26 (s, 2H), 4.13 (m, 2H), 3.96 (m, 1H), 3.88 (t, 1H, J = 7.0 Hz), 3.80 (m, 1H), 1.59 (s, 9H), 1.39 (m, 4H), 1.10 (s, 3H), 1.03 (s, 6H), 0.96 (s, 3H), 0.92 (t, 2H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.2, 152.6, 143.0, 136.4, 129.8, 129.6, 128.7, 128.2, 127.7, 124.5, 123.3, 123.1, 119.5, 117.1, 110.3, 99.1, 81.1, 78.7, 70.5, 59.9, 52.8, 39.6, 39.5, 38.1, 33.0, 28.5, 26.6, 20.4, 20.2, 17.1, 13.6; IR (film) ν_{max} 2968, 2926, 1701, 1623, 1586, 1450, 1407, 1352, 1326, 1143, 1041, 855 cm^{-1}; FABHRMS (NBA) m/z 570.3330 (M^+ + H, $C_{35}H_{43}N_3O_4$ requires 570.3332).

***seco*-CCBI−TMI (34).** A solution of **23** (1.5 mg, 4.2 μmol) in 150 μL of 4 M HCl−EtOAc was stirred at 25 °C for 20 min. The solvent was removed under a stream of N_2. After being dried in vacuo, the residue, **30**[14] (1.0 mg, 4.2 μmol, 1 equiv), and EDCI (2.9 mg, 12.6 μmol, 3 equiv) were dissolved in anhydrous DMF, and the reaction mixture was stirred at 25 °C for 16 h. The solvent was removed under vacuum, and the residue was dissolved in THF and loaded directly onto a silica gel column. Chromatography (SiO_2, 0.5 × 6 cm, 50% EtOAc−hexane) afforded **34** (1.7 mg, 2.0 mg theoretical, 85%) as a mustard-colored solid: ^{1}H NMR (DMSO-d_6, 250 MHz) δ11.50 (br s, 1H, NH), 10.86 (s, 1H, OH), 8.53 (s, 1H, C9-H), 8.22 (d, 1H, J = 8.7 Hz, C6-H), 8.04 (s, 1H, C4-H), 7.59 (dd, 1H, J = 1.5, 8.7 Hz, C7-H), 7.07 (d, 1H, J = 1.7 Hz, C4′-H), 6.95 (s, 1H, C3′-H), 4.75 (t, 1H, J = 10.0 Hz, C2-H), 4.46 (d, 1H, J = 10.5 Hz, C2-H), 4.26 (m, C1-H), 4.04 (dd, 1H, J = 2.8, 11.3 Hz, CH*H*Cl), 3.92 (s, 3H), 3.88 (dd, 1H, J = 3.9, 11.3, Hz), 3.81 (s, 3H), 3.79 (s, 3H); IR (film) ν_{max} 3422, 3122, 2938, 2225, 1587, 1525, 1493, 1454, 1389, 1312, 1235, 1110, 1050, 997, 824, 794 cm^{-1}; FABHRMS (NBA) m/z 492.1347 (M^+ + H, $C_{26}H_{22}ClN_3O_5$ requires 492.1326).

(1*S*)-34: [α]$^{25}_D$ −19 (*c* 0.12, $CHCl_3$).

***ent*-(1*R*)-34:** [α]$^{25}_D$ +19 (*c* 0.12, $CHCl_3$).

CCBI−TMI (35). A solution of **34** (2.2 mg, 4.4 μmol) in 20% DMF-THF (0.25 mL, 0.018 M) under Ar was cooled to 0

°C, and NaH (0.5 mg, 3 equiv) was added. The reaction mixture was stirred at 0 °C for 30 min before the solvent was removed under a stream of N_2 with care to maintain the 0 °C temperature. PTLC (SiO_2, 0.25 mm × 10 cm × 15 cm, 50% EtOAc−hexane) afforded **35** (2.1 mg, 2.1 mg theoretical, 99%) as a pale yellow solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.66 (br s, 1H, NH), 8.10 (d, 1H, J = 8.3 Hz, C5-H), 7.87 (s, 1H, C8-H), 7.85 (dd, 1H, J = 1.5, 8.3 Hz, C6-H), 7.12 (d, 1H, J = 2.2 Hz, C4′-H), 6.92 (s, 1H, C3′-H), 6.74 (s, 1H, C3-H), 4.54 (dd, 1H, J = 5.6, 10.5 Hz, C1-H), 4.37 (d, 1H, J = 10.5 Hz, C1-H), 3.89 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H), 3.4 (1H, C9a-H masked by H_2O), 1.93 (dd, 1H, J = 4.0, 7.6 Hz, C9-H), 1.80 (t, 1H, J = 5.0 Hz, C9-H); IR (film) ν_{max} 3440, 2920, 2850, 2226, 1734, 1653, 1457, 1389, 1307, 1233, 1108 cm^{-1}; FABHRMS (NBA) m/z 456.1546 (M^+ + H, $C_{26}H_{21}N_3O_5$ requires 456.1559).

(+)-CCBI−TMI (35): $[\alpha]^{23}_D$ +144 (*c* 0.05, acetone).

***ent*-(−)-CCBI−TMI (35):** $[\alpha]^{23}_D$ −135 (*c* 0.04, acetone).

***seco*-CCBI−Indole$_2$ (36).** A solution of **23** (2.8 mg, 7.8 μmol) in 250 μL of 4 M HCl−EtOAc under Ar was stirred at 25 °C for 20 min. The solvent was removed under a stream of N_2, and the crude hydrochloride salt was dried under vacuum. A solution of **24, 31**[39] (2.5 mg, 7.8 μmol, 1.0 equiv), and EDCI (4.5 mg, 23.5 μmol, 3 equiv) was added, and the mixture was slurried in 142 μL (0.55 M) of anhydrous DMF. The reaction mixture was stirred at 25 °C for 16 h before the solvent was removed under vacuum. PTLC (SiO_2, 0.25 mm × 15 × 20 cm, 30% DMF−toluene) afforded **36** (3.8 mg, 87%) as a tan solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.76 (s, 1H, NH), 11.71 (s, 1H, NH), 10.85 (s, 1H, OH), 10.16 (s, 1H, NH), 8.55 (s, 1H, C9-H), 8.23 (d, 1H, J = 8.6 Hz, C6-H), 8.22 (s, 1H, C4′-H), 8.14 (s, 1H, C4-H), 7.67 (d, 1H, J = 7.8 Hz, C4″-H), 7.59 (m, 2H), 7.48 (apparent t, 2H, J = 8.8 Hz, C7′- and C7″-H), 7.41 (s, 1H, C3″-H), 7.25 (s, 1H, C3′-H), 7.22 (t, 1H, J = 8.1 Hz, C6″-H), 7.06 (t, 1H, J = 7.3 Hz, C5″-H), 4.85 (t, 1H, J = 10.0 Hz, C2-H), 4.60 (d, 1H, J = 11.2 Hz, C2-H), 4.34 (m, 1H, C1-H), 4.06 (d, 1H, J = 10.9 Hz, C*H*HCl), 3.92 (dd, 1H, J = 6.9, 11.4 Hz, CH*H*Cl); IR (film) ν_{max} 3284, 2921, 2225, 1651, 1589, 1557, 1516, 1411, 1391, 1316, 1230, 1137, 1059, 805, 743 cm^{-1}; FABHRMS (NBA) m/z 560.1470 (M^+ + H, $C_{32}H_{22}ClN_5O_3$ requires 560.1489).

(1*S*)-36: $[\alpha]^{25}_D$ +49 (*c* 0.17, DMF).

***ent*-(1*R*)-36**: $[\alpha]^{25}_D$ −44 (*c* 0.21, DMF).

CCBI−Indole$_2$ (37). Method A. A solution of **36** (3.4 mg, 6.10 μmol) in 20% DMF−THF (0.34 mL) under Ar was cooled to 0 °C and treated with NaH (0.8 mg, 18.3 μmol, 60% in oil, 3 equiv). The reaction mixture was stirred for 30 min at 0 °C before the solvent was removed under a stream of N_2 with care to maintain the 0 °C temperature. PTLC (SiO_2, 0.25 mm × 15 × 20 cm, 15% DMF−toluene) afforded **37** (2.1 mg, 3.2 mg theoretical, 66%) as a tan solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.87 (s, 1H, NH), 11.72 (s, 1H, NH), 10.18 (s, 1H, NH), 8.23 (s, 1H, C4′-H), 8.12 (d, 1H, J = 8.2 Hz, C5-H), 7.88 (s, 1H, C8-H), 7.86 (d, 1H, J = 8.2 Hz, C6-H), 7.66 (d, 1H, J = 7.9 Hz, C4″-H), 7.61 (dd, 1H, J = 8.9, 2.0, Hz, C6′-H), 7.49 (d, 1H, J = 8.2, Hz, C7″-H), 7.50 (d, 1H, J = 8.9 Hz, C7′-H), 7.41 (s, 1H, C3″-H), 7.31 (s, 1H, C3′-H), 7.20 (t, 1H, J = 7.2 Hz, C6″-H), 7.06 (t, 1H, J = 7.2 Hz, C5″-H), 7.05 (s, 1H, C3-H), 4.66 (dd, J = 4.8, 10.2 Hz, C1-H), 4.53 (d, 1H, J = 10.2 Hz, C1-H), 3.37 (m, 1H, C9a-H), 1.91 (dd, 1H, J = 4.4, 8.4 Hz, C9-H), 1.78 (t, 1H, J = 4.8 Hz, C9-H); IR (neat) ν_{max} 3274, 1644, 1601, 1549, 1516, 1454, 1388, 1308, 1265, 1237, 1133, 806, 745 cm^{-1}; FABHRMS (NBA) m/z 524.1737 (M^+ + H, $C_{32}H_{21}N_5O_3$ requires 524.1723).

(+)-CCBI−Indole$_2$ (37): $[\alpha]^{25}_D$ +80 (*c* 0.04, THF).

***ent*-(−)-CCBI−Indole$_2$ (37):** $[\alpha]^{25}_D$ −81 (*c* 0.09, THF).

Method B. A solution of **36** (2.7 mg, 4.8 μmol) in 1:1 THF−3% aqueous $NaHCO_3$ (700 μL) was stirred at 25 °C for 10 h. The solvent was removed under a stream of N_2. PTLC (SiO_2, 0.25 mm × 20 × 20 cm, 10% DMF−toluene) afforded **37** (1.7 mg, 2.5 mg theoretical, 68%) as a tan solid.

***seco*-CCBI−CDPI$_1$ (38).** A solution of **23** (4.0 mg, 11.1 μmol) in 500 μL of 4 M HCl−EtOAc was stirred at 0 °C for 30 min. The solvent was removed under a stream of N_2, and the crude hydrochloride salt was dried under vacuum. The salt and **32**[59] (3.0 mg, 12.3 μmol, 1.1 equiv) were dissolved in 300 μL of anhydrous DMF and treated with EDCI (6.4 mg, 33.3 μmol, 3 equiv). The reaction mixture was stirred at 25 °C for 14 h. The crude reaction mixture was concentrated and loaded directly onto a preparative TLC plate. Chromatography (SiO_2, 0.25 mm × 20 × 15 cm, 20% DMF−toluene) afforded **38** (4.4 mg, 5.4 mg theoretical, 81%) as a pale yellow solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ11.68 (s, 1H, NH), 10.88 (br s, 1H, OH), 8.55 (s, 1H, C9-H), 8.22 (d, 1H, J = 8.7 Hz, C6-H), 8.13 (s, 1H, C4-H), 8.00 (d, 1H, J = 8.9 Hz, C4′-H), 7.59 (d, 1H, J = 8.7 Hz, C7−H), 7.23 (d, 1H, J = 8.9 Hz, C5′-H), 7.04 (s, 1H, C8′-H), 6.10 (s, 2H, NH_2), 4.82 (t, 1H, J = 10.1 Hz, C2-H), 4.55 (dd, 1H, J = 2.0, 11.0 Hz, C2-H), 4.33 (m, 1H, C1-H), 4.05 (dd, 1H, J = 3.1, 11.1 Hz, C*H*HCl), 3.99 (m, 3H, CH*H*Cl and C2′-H_2), 3.26 (m, 2H, C1′-H_2 obscured by H_2O); IR (film) ν_{max} 3350, 2921, 2260, 1723, 1658, 1620, 1590, 1503, 1452, 1414, 1342, 1283, 1252, 1024, 799 cm^{-1}; FABHRMS (NBA) m/z 486.1340 (M^+ + H, $C_{26}H_{20}ClN_5O_3$ requires 486.1333).

(1*S*)-38: $[\alpha]^{25}_D$ +37 (*c* 0.15, DMF).

***ent*-(1*R*)-38:** $[\alpha]^{25}_D$ −36 (*c* 0.22, DMF).

CCBI−CDPI$_1$ (39). A solution of **38** (3.6 mg, 7.4 μmol) in DMF−H_2O (5:2, 600 μL + 240 μL) was treated with $KHCO_3$ (20 equiv). The reaction mixture was stirred at 25 °C for 9 h. After removal of solvent, PTLC (SiO_2, 0.25 mm × 20 × 20 cm, 20% DMF−toluene) afforded **39** (2.3 mg, 3.3 mg theoretical, 69%) as a tan solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.74 (s, 1H, NH), 8.12 (d, 1H, J = 8.1 Hz, C5-H), 8.02 (d, 1H, J = 8.9 Hz, C4′-H), 7.87 (s, 1H, C8-H), 7.85 (d, 1H, J = 8.1 Hz, C6-H), 7.21 (d, 1H, J = 8.9 Hz, C5′-H), 7.10 (s, 1H, C3-H), 7.01 (s, 1H, C8′-H), 6.13 (s, 2H, NH_2), 4.63 (dd, 1H, J = 5.1, 10.3 Hz, C1-H), 4.51 (d, 1H, J = 10.3 Hz, C1-H), 3.97 (t, 2H, J = 8.8 Hz, C2′-H), 3.42 (m, 1H, C9a-H), 3.28 (t, 2H, J = 8.8 Hz, C1′-H), 1.91 (dd, 1H, J = 4.1, 7.7 Hz, C9-H), 1.76 (t, 1H, J = 4.6 Hz, C9-H); IR (neat) ν_{max} 3364, 2911, 1652, 1598, 1499, 1455, 1386, 1263, 1130 cm^{-1}; FABHRMS (NBA) m/z 450.1578 (M + H^+, $C_{26}H_{19}N_5O_3$ requires 450.1566).

(+)-CCBI−CDPI$_1$ (39): $[\alpha]^{23}_D$ +124 (*c* 0.10, DMF).

***ent*-(−)-CCBI−CDPI$_1$ (39):** $[\alpha]^{23}_D$ −117 (*c* 0.07, DMF).

Aqueous Solvolysis Reactivity. pH 3. *N*-BOC-CCBI (**25**, 100 μg) and CCBI (**26**, 50 μg) were dissolved in CH_3OH (1.5 mL) and mixed with pH 3 aqueous buffer (1.5 mL). The buffer contained 4:1:20 (v:v:v) 0.1 M citric acid, 0.2 M Na_2HPO_4, and H_2O, respectively. The solvolysis solution was sealed and kept at 25 °C protected from light. The UV spectrum was measured at regular intervals every 2 h during the first day, every 12 h for another week, and every 24 h for an additional week. For **26**, the measurements continued every 5 days for 6 months. For **25**, the decrease in the long-wavelength absorption at 322 nm and the increase in the short-wavelength absorption at 268 nm were monitored, Figure 1. The solvolysis rate constant (k = 9.90 × 10^{-7} s^{-1}) and half-life ($t_{1/2}$ = 213 h) were calculated from data recorded at the short wavelength from the least-squares treatment (r = 0.999) of the slope of the plot of time versus 1n[($A_f - A_i$)/($A_f - A$)], Figure 11. For **26**, the decrease in the long-wavelength absorption at 353 nm was monitored providing k = 1.65 × 10^{-7} s^{-1} ($t_{1/2}$ = 1182 h, r = 0.99) by the same treatment.

pH 2. Samples of **25** (100 μg) and **26** (50 μg) were dissolved in CH_3OH (1.5 mL), and the solutions were mixed with aqueous buffer (pH 2.05, 1.5 mL). The buffer contained 4:1:20 (v:v:v) 1.0 M citric acid, 0.2 M Na_2HPO_4, and H_2O, respectively. Immediately after mixing, the UV spectra of the solutions were measured against a reference solution containing CH_3OH (1.5 mL) and the aqueous buffer (1.5 mL), and these readings were used for the initial absorbance values. The solutions were stoppered, protected from the light, and allowed to stand at 25 °C. UV spectra were recorded at regular intervals until constant values were obtained for the long and short wavelength absorbances. The solvolysis rate constants were determined from the slope of the lines obtained from linear least-squares treatment of plots of 1n[($A_f - A_i$)/($A_f - A$)] versus time using the short wavelength measurements for **25** and long wavelength measurements for **26** (Figure 11). The first-order rate constant determined under these conditions was 7.94 × 10^{-6} s^{-1} ($t_{1/2}$ = 24.2 h, r = 0.999) for *N*-BOC-CCBI (**25**) and 1.5 × 10^{-6} s^{-1} ($t_{1/2}$ = 127 h, r = 0.99) for CCBI (**26**).

Solvolysis of *N*-BOC-CCBI in THF−CH_3OH. The solvolysis of *N*-BOC-CCBI (**25**) was carried out in THF with 20−

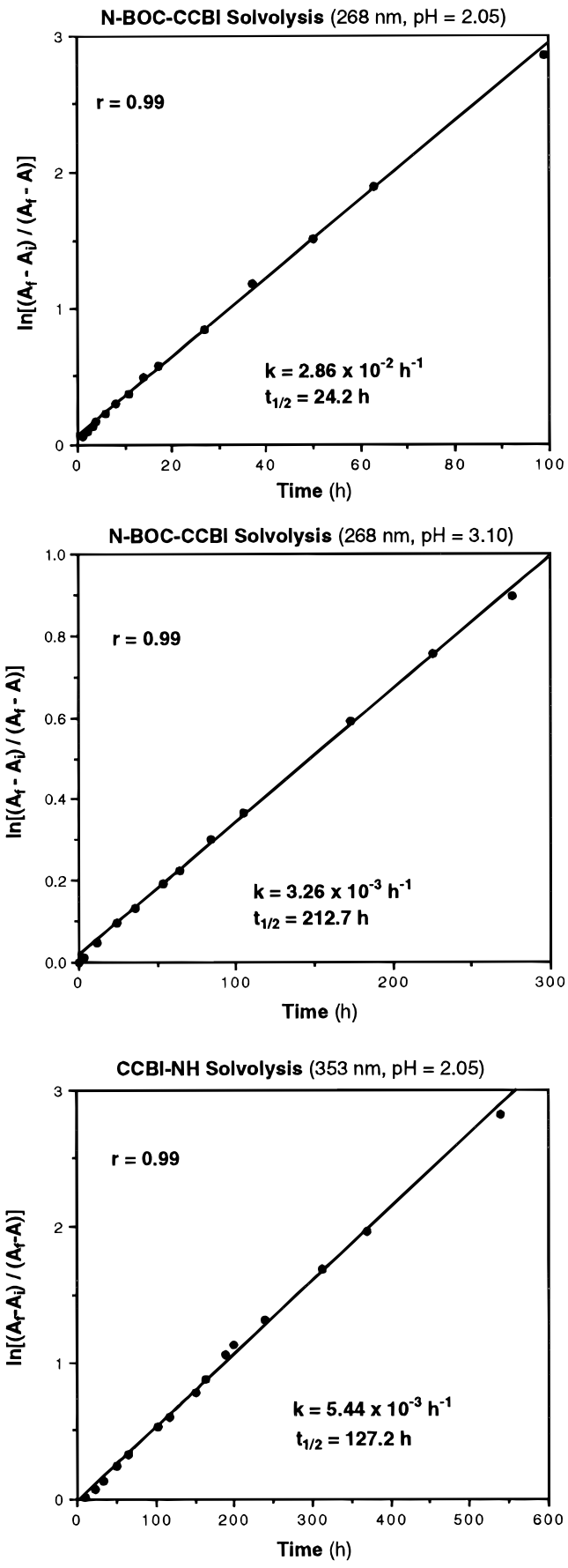


Figure 11.

500 equiv of CH_3OH in the presence of 0.1−0.25 equiv of CF_3SO_3H. The following is the procedure for the solvolysis of **25** in THF with 20 equiv of CH_3OH in the presence of CF_3SO_3H (0.1 equiv). A stock solution was prepared by addition of CF_3SO_3H (5.6 μL) and anhydrous CH_3OH (502 μL) to anhydrous THF (99.49 mL). A sample of **25** (100 μg) in a UV cell was dissolved in THF (1950 μL), and the THF stock solution (50 μL) that contained CF_3SO_3H (0.1 equiv) and CH_3OH (20 equiv) was added. The solvolysis solution was sealed, and the UV spectrum was measured with an automated cycle program at regular intervals (10 min/cycle). The decrease in the long wavelength absorption at 313 nm was monitored, and the solvolysis was complete after 80 h. The solvolysis rate ($k = 0.2 \times 10^{-4}$ s^{-1}) and half-life ($t_{1/2} = 9.2$ h) were calculated from data recorded at 313 nm from the least-squares treatment ($r = 0.984$) of the slope of the plot of time versus $\ln[(A_f - A_i)/(A_f - A)]$.

Solvolysis Regioselectivity: 3-(*tert*-Butyloxycarbonyl)-8-cyano-5-hydroxy-1-(methoxymethyl)-1,2-dihydro-3*H*-benz[*e*]indole (42). A solution of **25** (2.5 mg, 7.8 μmol) in CH_3OH (1 mL) containing 0.12 equiv of CF_3CO_2H was stirred at 0 °C for 24 h. $NaHCO_3$ (2.5 mg) was added, and the reaction mixture was stirred at 0 °C, warmed to 25 °C, filtered through a plug of Celite, and concentrated. PTLC (SiO_2, 0.25 mm × 20 × 20 cm, 20% EtOAc−hexane) provided **42** as a semisolid (2.3 mg, 2.7 mg theoretical, 85%, 95% based on conversion) and recovered **25** (0.3 mg, 12%). For **42**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.23 (d, 1H, $J = 8.7$ Hz), 8.08 (s, 1H), 7.88 (br s, 1H), 7.40 (dd, 1H, $J = 1.4$, 8.7 Hz), 6.79 (br s, 1H), 4.10 (apparent d, 2H, $J = 7.9$ Hz), 3.85 (m, 1H), 3.65 (dd, 1H, $J = 4.6$, 9.4 Hz), 3.39 (s, 3H), 3.35 (t, 1H, $J = 9.2$ Hz), 1.59 (s, 9H); IR (film) ν_{max} 3339, 2974, 2925, 1704, 1674, 1620, 1586, 1452, 1418, 1369, 1329, 1250, 1220, 1137 cm^{-1}; FABHRMS (NBA−NaI) *m/z* 377.1488 (M + Na$^+$, $C_{20}H_{22}N_2O_4$ requires 377.1477).

DNA Alkylation Studies: Selectivity and Efficiency. General procedures, the preparation of singly ^{32}P 5′ end-labeled double-stranded DNA, the agent binding studies, gel electrophoresis, and autoradiography were conducted according to procedures described in full detail elsewhere.[10] Eppendorf tubes containing the 5′ end-labeled DNA (9 μL) in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) were treated with the agent in DMSO (1 μL at the specified concentration). The solution was mixed by vortexing and brief centrifugation and subsequently incubated at 4 or 25 °C for 24 h (natural enantiomers) and 25 or 37 °C (unnatural enantiomers) for 72 h. The covalently modified DNA was separated from unbound agent by EtOH precipitation and resuspended in TE buffer (10 μL). The solution of DNA in an Eppendorf tube sealed with parafilm was warmed at 100 °C for 30 min to induce cleavage at the alkylation sites, allowed to cool to 25 °C, and centrifuged. Formamide dye (0.03% xylene cyanol FF, 0.03% bromophenol blue, 8.7% Na_2EDTA 250 mM) was added (5 μL) to the supernatant. Prior to electrophoresis, the sample was denatured by warming at 100 °C for 5 min, placed in an ice bath, and centrifuged, and the supernatant (3 μL) was loaded directly onto the gel. Sanger dideoxynucleotide sequencing reactions were run as standards adjacent to the reaction samples. Polyacrylamide gel electrophoresis (PAGE) was run on an 8% sequencing gel under denaturing conditions (8 M urea) in TBE buffer (100 mM Tris, 100 mM boric acid, 0.2 mM Na_2EDTA) followed by autoradiography.

DNA Alkylation Relative Rate of (+)-CCBI−TMI (35), (+)-CBI−TMI, and (+)-MCBI−TMI. Following the procedure detailed above, Eppendorf tubes containing 5′ end-labeled w794 DNA (9μL) in TE buffer (pH 7.5) were treated with (+)-CCBI−TMI (**35**), (+)-CBI−TMI, or (+)-MCBI−TMI (1 μL, 10^{-6} M in DMSO). The solutions were mixed and incubated at 25 °C for 2, 4, 6, 9, 12, 15, and 24 h, respectively. Subsequent isolation of the alkylated DNA by EtOH precipitation, resuspension in TE buffer (10 μL, pH 7.5), thermolysis (30 min, 100 °C), PAGE, and autoradiography were conducted as detailed above. Relative rates for alkylation at the w794 high-affinity 5′-AATTA site were derived from the slopes of the plots of percent integrated optical density (IOD) of the high-affinity alkylation cleavage bands versus time.

DNA Alkylation Relative Rate of (+)-CCBI−Indole$_2$ (37), (+)-CBI−Indole$_2$, and (+)-MCBI−Indole$_2$. Following

the procedure detailed above, Eppendorf tubes containing 5′ end-labeled w794 DNA (9 μL) in TE buffer (pH 7.5) were treated with (+)-CCBI−indole$_2$ (**37**), (+)-CBI−indole$_2$, and (+)-MCBI−indole$_2$ (1 μL, 10^{-6} M in DMSO). The solutions were mixed and incubated at 25 °C for 2, 4, 6, 9, 12, 15, and 24 h, respectively. Subsequent isolation of the alkylated DNA by EtOH precipitation, resuspension in TE buffer (10 μL, pH 7.5), thermolysis (30 min, 100 °C), PAGE, and autoradiography were conducted as detailed above. Relative rates for alkylation at the w794 high-affinity 5′-AATTA site were derived from the slopes of the plots of percent integrated optical density (IOD) of the high-affinity alkylation cleavage bands versus time.

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Supporting Information Available: Summary tables of the in vitro cytotoxic activity (L1210) employed for Figure 6 and ^{1}H NMR spectra of **5**, **7**, **11**, **14**, **19**−**23**, **25**, **26**, **29**, **34**−**39**, and **42** (22 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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