Synthesis and Biochemical Evaluation of the CBI-PDE-I-dimer, a Benzannelated Analog of (+)-CC-1065 That Also Produces Delayed Toxicity in Mice

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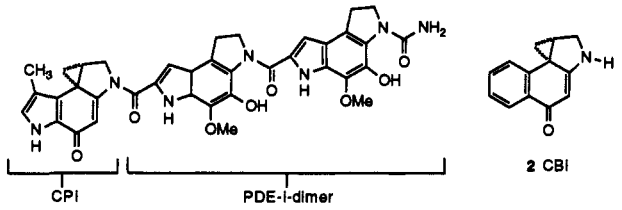
A practical synthesis of CBI (2) was developed and applied to the synthesis of benzannelated analogs of CC-1065, including CBI-PDE-I-dimer (13) and CBI-bis-indole [(+)-A'BC]. The CBI-PDE-I-dimer was shown to have similar DNA sequence selectivity and structural effects on DNA as (+)-CC-1065. Of particular importance was the observed duplex winding effect that has been associated with the pyrrolidine ring of the nonalkylated subunits of (+)-CC-1065 and possibly correlated with its delayed toxicity effects. The effect of CBI-PDE-I-dimer was also compared to (+)-CC-1065 in the inhibition of duplex unwinding by helicase II and nick sealing by T4 ligase and found to be quantitatively similar. The *in vitro* and *in vivo* potencies of the CBI compounds corresponded very closely to the corresponding CPI derivatives. Finally, CBI-PDE-I-dimer was like (+)-CC-1065 in causing delayed toxicity in mice.

Introduction

CC-1065 (1), an extremely potent antitumor antibiotic,[1] exhibits a number of interesting biological effects,[2] including the production of delayed deaths in mice at μg/kg doses.[3] Subsequent investigation of this fascinating natural product revealed that the delayed lethality of the compound resulted when the carbon skeleton of PDE-I-dimer (the right hand portion of the molecule) was attached to CPI, the left-hand alkylating segment.[4] Structurally



simplified CPI (1,2,8,8a-tetrahydro-7-methyl-cyclopropa[c]pyrrolo[3,2-e]indol-4(5H)-one) derivatives lacking the fully fledged carbon skeleton of (+)-CC-1065 and (+)-AB'C' such as (+)-ABC (Figure 1) were shown not only to be free of this detrimental toxicity but also to be much more active in mice (in terms of prolonging life or inhibiting tumor growth) than CC-1065, and one such compound, (+)-ABC'' (adozelesin), has since entered clinical testing.[5,6] To better understand the structural features of CC-1065 responsible for its biological effects, we have had an interest in preparing compounds containing an altered CPI moiety, including the benzannelated derivative CBI (2). Boger has previously prepared CBI (1,2,9,9a-tetrahydrocyclopropa[c]benz[e]indol-4-one) as well as a number of interesting analogs,[7] and Cava has reported the preparation of a protected CBI derivative.[8] In this article we describe an alternative synthesis[9] and biological evaluation of CBI and related analogs (Figure 2), including the novel derivative CBI-PDE-I-dimer (13). In addition, we describe experiments in which we compare the DNA sequence specificity and the biochemical and biological effects of CBI-PDE-I-dimer with (+)-CC-1065.

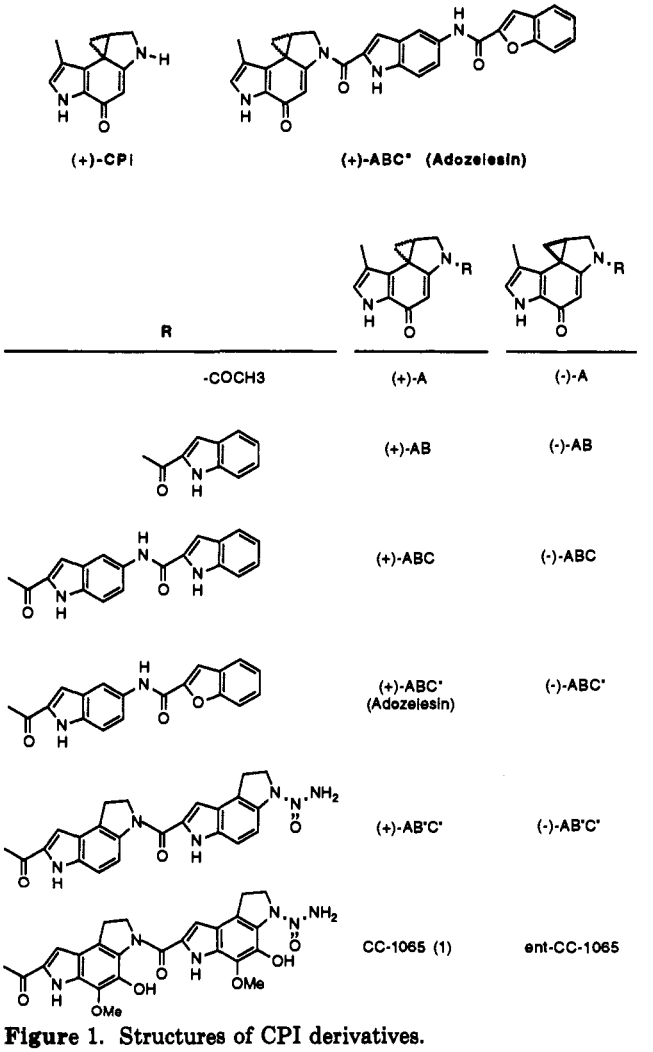


Figure 1. Structures of CPI derivatives.

Results and Discussion

Chemistry.[9] As shown in Figure 3, reaction of 1,4-naphthalenedione (3) with benzylamine cleanly gave the

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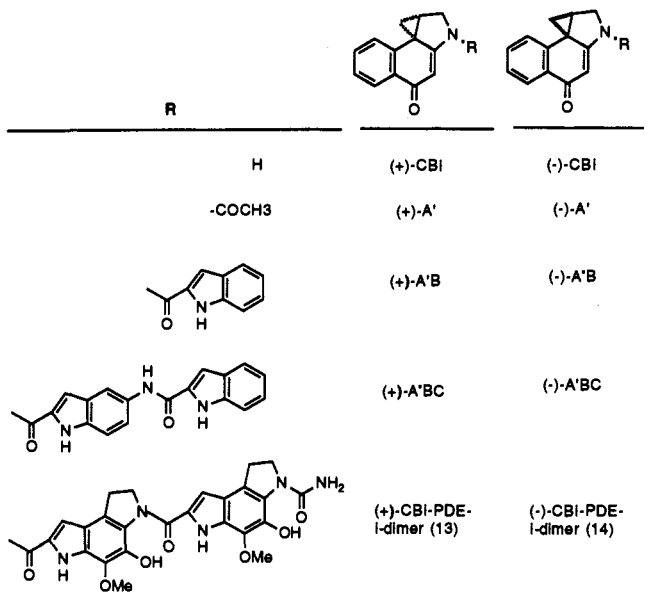

Figure 2. Structures of CBI derivatives.

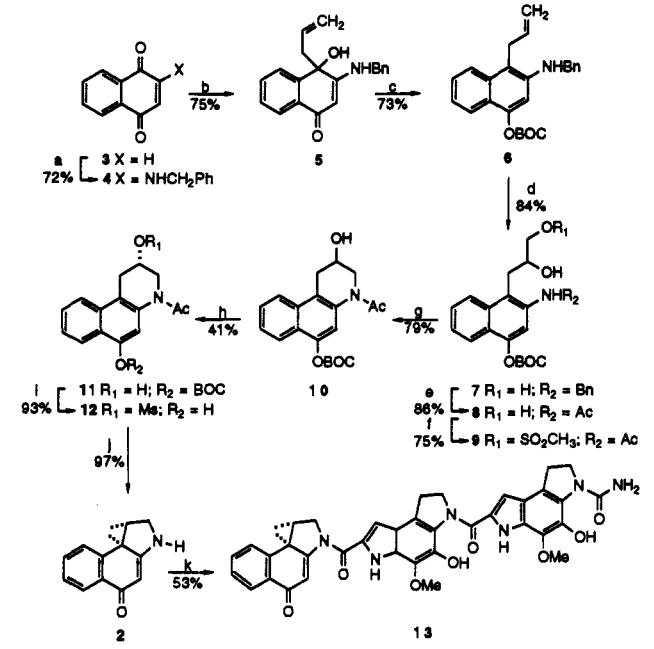

Figure 3. Synthesis of CBI (2) and (+)-CBI-PDE-I-dimer (13).[9] Key: (a) $PhCH_2NH_2$, DMF; (b) 2 equiv of $CH_2{=}CHCH_2MgBr$, THF; (c) (i) 2.5 equiv of $(t\text{-}BuO_2C)_2O$, CH_3CN; (ii) $Na_2S_2O_4$, H_2O, EtOAc; (d) 0.25 equiv of OsO_4, *N*-methylmorpholine *N*-oxide, acetone, H_2O; (e) (i) HCO_2H, Pd/C, MeOH; (ii) Ac_2O, pyridine; (iii) K_2CO_3, MeOH, H_2O; (f) 1 equiv of MsCl, pyridine, 0 °C; (g) (i) 1 equiv of Me_3SiCl, pyridine; (ii) 1.2 equiv of NaH, THF; (iii) K_2CO_3, MeOH; (h) (i) (*R*)-*O*-acetylmandelic acid, EDC, CH_2Cl_2; (ii) K_2CO_3, MeOH; (i) (i) MsCl, Et_3N, CH_2Cl_2; (ii) TFA, CH_2Cl_2; (j) 3 equiv of NaH, THF; (k) (i) HCl, EtOAc; (ii) PDE-I-dimer acid, EDC, DMF; (iii) Et_3N, CH_3CN, H_2O.

crystalline enamide **4**. Upon treatment of **4** with allylmagnesium bromide, allyl alcohol **5** was produced. Reaction of **5** initially with several equivalents of BOC anhydride followed by immediate treatment of the crude product with sodium dithionite provided the protected phenol **6**. Osmium-catalyzed dihydroxylation of **6** furnished the racemic diol **7**. Compound **7** was converted to the acetylated intermediate **8** and then treated with methanesulfonyl chloride to give the primary mesylate **9**. Protection of the secondary alcohol in **9** with trimethylsilyl chloride, treatment with sodium hydride, and hydrolytic workup furnished key alcohol **10**. Resolution of **10** utilizing mandelic acid furnished two diastereomers readily separated by column chromatography; subsequent hydrolysis afforded compound **11** in greater than 99% optical purity. Activation of the alcohol as its mesylate and BOC protecting group removal using trifluoroacetic acid furnished the cyclization precursor **12**. Compound **12** readily formed CBI (**2**)[10] upon treatment with sodium hydride.

Table I. Biological Data on CBI Derivatives

compound	*in vitro* L1210[a] IC$_{50}$ (nM)		*in vivo* L1210 OD[b] (μg/kg)	*in vivo* L1210 % ILS[c]
(+)-CBI	230	(550)	NT	NT
(−)-CBI	6600	NT[e]	NT	NT
(+)-A′	5	(2)	NT	NT
(−)-A′	70	(70)	NT	NT
(+)-A′B	0.1	(0.09)	800	50
(−)-A′B	12	(10)	NT	NT
(+)-A′BC	0.006	(0.003)	100	67
(−)-A′BC	0.3	(1)	1600[d]	(50)
13	0.02	(0.02)	200	28
14	0.01	(0.01)	200	6

[a] *In vitro* cytotoxicity test as evaluated with a 3-day drug incubation with IC$_{50}$ denoted in nanomolar. Numbers in parentheses refer to IC$_{50}$ for corresponding CPI derivative measured in same test. [b] Optimum antitumor dose (OD) in μg/kg in L1210 mouse leukemia experiment wherein L1210 cells were implanted intraperitoneally in CD2F1 mice (6 per group) on day 0 with single intravenous dose of drug 24 h later. OD for (+)-ABC in same experiment was 100 μg/kg. Except for (−)-A′BC, wherein the highest dose tested was 1600 μg/kg, OD was always 50% of the acutely lethal dose. [c] Percent increase in lifespan (ILS) at the optimum dose. (+)-ABC in this experiment gave an ILS of 83% at 100 μg/kg. [d] Highest dose tested. [e] NT = not tested.

CBI itself can be readily converted into a number of interesting analogs (Figure 2). For example, treatment of **2** with 2 equiv of sodium hydride followed by 1 equiv of the imidazolide of 5-[(1*H*-indol-2-ylcarbonyl)amino]-1*H*-indole-2-carboxylic acid[5a] afforded the CBI-bis-indole derivative (+)-A′BC (Figure 2).[7e] To prepare the more elaborated PDE-I-dimer analog **13**, it was necessary to first hydrolyze CBI to the ring-opened phenolic form[7] and then immediately couple with PDE-I-dimer acid[11] and cyclize in an analogous manner to the preparation of CC-1065.[5c] Enantiomeric CBI derivatives were synthesized from the enantiomer of compound **11**.

Biology. The CBI derivatives prepared were evaluated for their cytotoxicity against L1210 cells in side-by-side comparisons with the corresponding CPI analog. As shown in Table I, the CBI derivatives generally proved to be as potent or nearly as potent as the related CPI analog. Thus, as with the (+)-CPI analogs,[5b] elaboration of the side chain (i.e., acetyl to indole to bisindole) in the (+)-CBI series leads to increasingly more potent compounds with A′BC being more potent than (+)-CBI-PDE-I-dimer (**13**) itself. Boger has previously reported cytotoxity studies with several CBI analogs including (+)-A′BC, and his results are similar;[7] however, whereas Boger reported (+)-A′BC to be slightly more potent *in vitro* as compared to (+)-ABC, we find in our test system that the opposite is true. This may simply be due to a slightly different testing protocol, since there are only modest differences in potency between each CBI analog and the corresponding CPI derivative. In the unnatural series, as with the corresponding (+)-CPI series,[12] except for ent-CBI-PDE-I-dimer (**14**), the (−)-CBI analog is significantly less potent than the corresponding (+)-CBI analog.

The more potent CBI analogs were tested *in vivo* in an L1210 mouse leukemia model (Table I). As has been

Table II. Delayed Toxicity in Normal (Non-Tumor-Bearing) Mice Treated with a Single Intravenous Dose of Compound

compound	dose (μg/kg)	MDD[a]
(+)-A′BC	50	ND[b]
	100	ND
	200	7
(+)-ABC	50	ND
	100	ND
	200	3
13	25	48
	50	42
	100	41
	200	38
	400	5
14	100	ND
	200	ND
	400	5
CC-1065	25	42
	50	41

[a] Median day of death (six mice per dose group). [b] ND = No deaths at this dose to day 90 (termination of experiment).

previously found with the corresponding CPI analogs,[5] (+)-A′BC proved to be more potent than (+)-A′B and also more efficacious (in terms of increasing survival) than (+)-CBI-PDE-I-dimer (**13**) and its enantiomer **14**. (+)-A′BC appeared to have similar potency and survival-prolonging activity to (+)-ABC when the two compounds were compared side-by-side in this murine model.

Most interesting was the finding (Table II) that the CBI analogs behaved very similarly to the corresponding CPI analog in terms of delayed toxicity in mice. In particular, compound **13**, but not its enantiomer **14**, caused late deaths in mice dosed at or below the optimum dose (i.e., below the acutely toxic dose) as determined in the mouse antitumor studies. Thus compound **13** behaved very similar to CC-1065 in this regard.[3] No delayed toxicity was evidenced with (+)-A′BC, and it thus behaved much like (+)-ABC.[5a]

Besides CC-1065, only its tetradesoxy analog (+)-AB′C′ had previously been found to cause delayed toxicity in mice.[4] Since (+)-CBI-PDE-I-dimer appeared to exhibit this interesting phenomenon as well, additional investigations of **13** in comparison to CC-1065 (and selected CPI analogs) were pursued.

Biochemistry. Comparison of Sequence Selectivity of CBI-PDE-I-dimer, (+)-CC-1065, and (+)-ABC″. Using a 5′-^{32}P end-labeled DNA restriction fragment, the sequence selectivity of **13** was compared to (+)-CC-1065 and its synthetic analog (+)-ABC″ (adozelesin) at three different concentrations of drug. As shown in Figure 4, **13** showed the same sequence selectivity as (+)-CC-1065, and while (+)-ABC″ bound to many of the same sequences, there were some differences. For example, while sequences such as 5′-CGAAGA* and 5′-AGCAGA* were favored by (+)-CC-1065 and **13**, 5′-GTAATA* was preferred by (+)-ABC″. This result indicates that the pyrrolidine ring and the outside methoxy substituents of the nonalkylating subunit modulate the sequence selectivity of CPI-derived molecules, while the replacement of CPI with CBI does not affect the sequence selectivity. This confirms the results shown by Boger.[7] When an oligomer (oligomer AA in Table III) containing an A5 tract was used to determine the site of alkylation among five consecutive adenines, both (+)-CC-1065 and **13** (lanes 2 and 3) almost exclusively modified the 3′-adenine, confirming that (+)-CC-1065 and **13** have similar sequence selectivity's (see Figure 5). In contrast, (+)-ABC″ and (+)-ABC modify

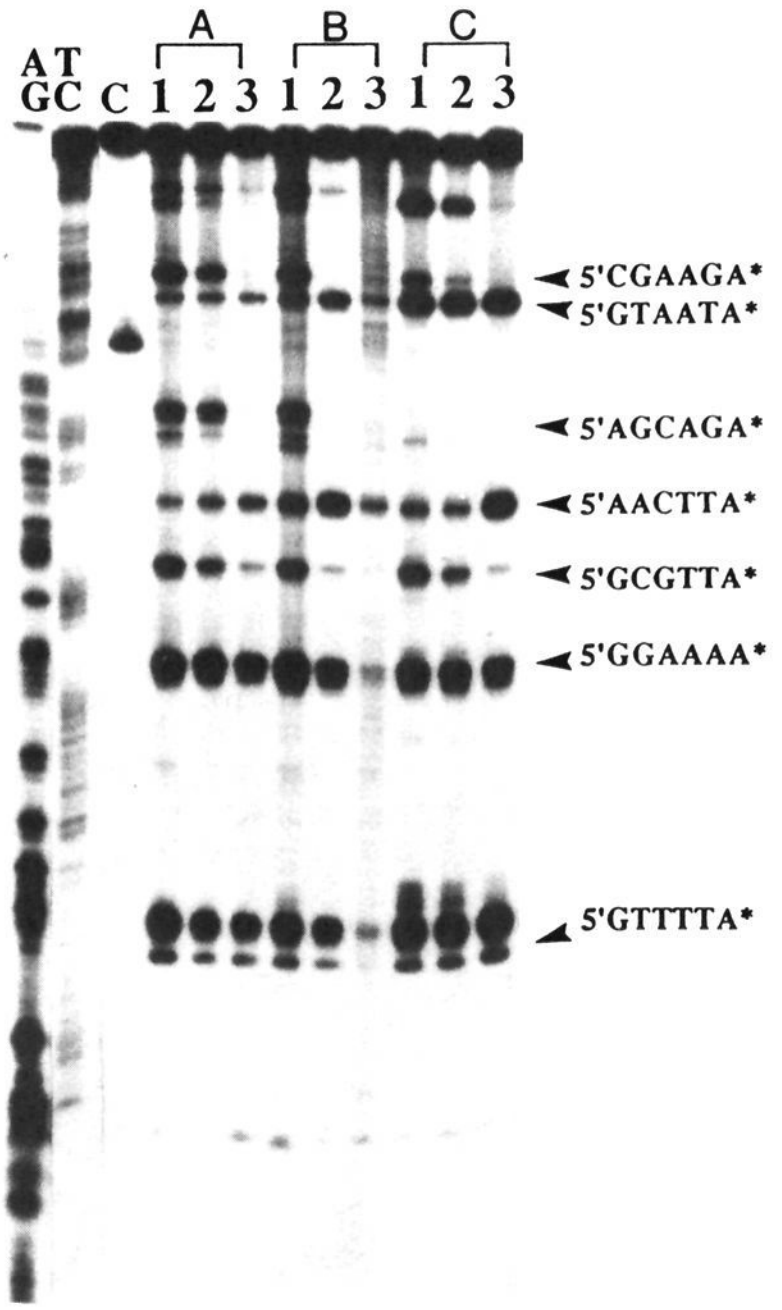

Figure 4. Comparison of sequence selectivity of **13** (B) with (+)-CC-1065 (A) and (+)-ABC″ (C). AG and TC represent purine- and pyrimidine-specific chemical cleavage reactions, respectively. Lane C is control experiment, and lanes 1–3 contain 14, 1.4, and 0.14 μM of drug molecules, respectively. An asterisk (*) indicates adenine molecules covalently modified with drug molecules.

Table III. List of Oligomer Sequence[a]

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21-D  5′-GAGGACCATAGTTACGGATTC-3′
        3′-CTGGTATCAATGCCTAAGCTC-5′

GA    5′-AATCCGTAATCGCGGTCTCAGTTCCGTAGATCACGAGATCAGCGCTAGCAGT-3′
      3′-TTAGGCATTAGCGCCAGAG-5′
                *

AA    5′-AATCCGTTTTTGCGGTCTCAGTTCCGTAGATCACGAGATCAGCGCTAGCAGT-3
      3′-TTAGGCAAAAACGCCAGAG-5′
                *

                  *                       *
40/42-A   5′-GAAAAAGGGGGGCGGGATGGCGAAAAAGGGGGGGCGGGATG-3′
        3′-GCTTTTTCCCCCCGCCCTACCGCTTTTTCCCCCCGCCCTACC-5′
                                    ↑

                  *                       *
40/42-B   5′-GAGTTAGGGGGGCGGGATGGCGAGTTAGGGGGGCGGGATG-3′
        3′-GCTCAATCCCCCCGCCCTACCGCTCAATCCCCCCGCCCTACC-5′
                                    ↑
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[a] Asterisks represent sites for covalent modification by compounds used in this study. Arrows indicate sites for T4-mediated ligation.

both 3′ adenines (lanes 4 and 5), and (+)-AB is able to modify the second 5′ adenine in addition to the two 3′ adenines (lane 6).

Comparison of the DNA Bending and Winding Effects of CBI-PDE-I-dimer and (+)-CC-1065. In previous studies, by comparing the migration of ligated multimers in a gel electrophoresis system, it has been shown that as the consequence of drug modification, (+)-CC-1065 and its analogs induce DNA bending.[13] In order to compare the bending effects of **13** and (+)-CC-1065, site-

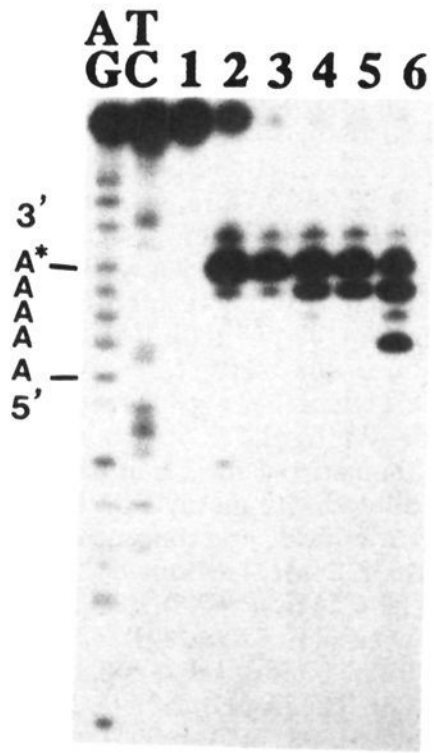

Figure 5. Autoradiogram of the thermally induced strand-breakage assay of the partial duplex oligomer (AA in Table III) containing A5 tracts after covalent modification with drug molecules. AG and TC are purine and pyrimidine-specific chemical cleavage reactions, respectively. Lane 1 represents control DNA without drug modification, and lanes 2–6 represent DNA modified with (+)-CC-1065, **13**, (+)-ABC″, (+)-ABC, and (+)-AB, respectively. Drug concentrations used in this experiment were 14 μM in lanes 2–5 and 140 μM in lane 6.

directed drug–oligomer adducts (21D in Table III) were prepared. The autoradiogram of ligation products of unmodified (lane 1), (+)-CC-1065-modified (lane 2), and **13**-modified 21D oligomers (lane 3) is shown in Figure 6A, and a plot of the R_L values (apparent vs actual length) vs size of ligated multimers is shown in Figure 6B. Both the autoradiogram of the gel and R_L plot show that the multimers of both drug-modified oligomers show comparable retardation in electrophoretic mobility compared to control ligation products, indicating that **13** induces the same magnitude of DNA bending upon covalent modification. The winding effect of **13** was also investigated using a method in which a comparison of electrophoretic mobility of ligated multimers of drug-modified 20-mer and 21-mer containing the sequence 5′ AGTTA was made.[13] The results show that compound **13** produced a winding effect similar in magnitude and direction to (+)-CC-1065, since the ligation product of drug-modified 20-mer showed more retardation in electrophoretic mobility than those of drug-modified 21-mer for both drugs (data not shown).

Effect of CBI-PDE-I-dimer on the Helicase II-Mediated Unwinding of a Tailed Oligomer and the Nick Sealing Activity of T4 DNA Ligase. Previous studies have shown that (+)-CC-1065 and its analog, (+)-AB′C′, have an unusual winding effect on DNA molecules,[13] and this winding effect is primarily responsible for the production of a pronounced inhibition of unwinding of 3′-tailed oligomer duplexes by helicase II when the displaced strand is covalently modified.[14,15] Likewise, this DNA winding effect of (+)-CC-1065 and (+)-AB′C′ can also cause the proximal inhibition of the ligation of the noncovalently modified strand.[16] Therefore, both helicase II and T4 ligase were used in this study to determine whether **13** is able to produce a comparable effect on the unwinding of tailed duplex mediated by helicase II and the nick sealing activity mediated by T4 DNA ligase.

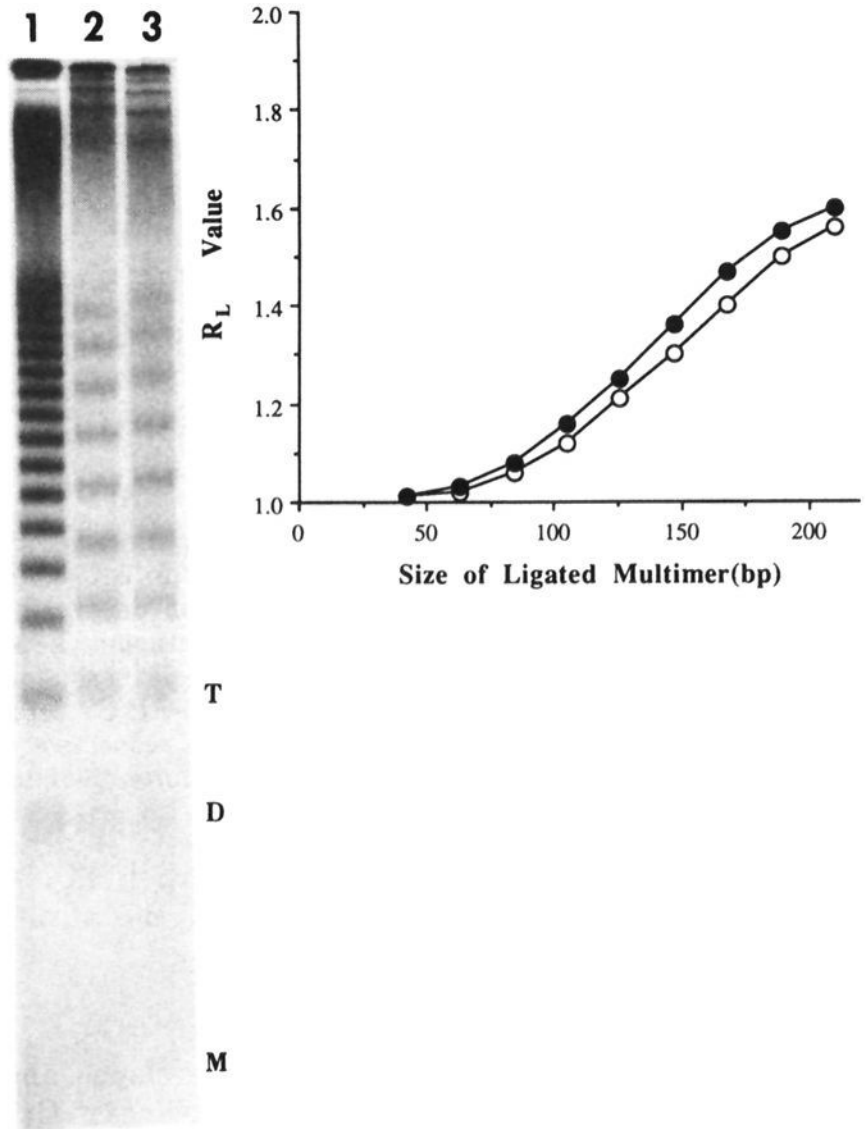

Figure 6. (A, left) Autoradiogram of the ligation products of oligomer 21D (Table III) (lane 1), oligomer 21D modified with (+)-CC-1065 (lane 2), and oligomer 21D modified with **13** (lane 3). M and D indicate monomer and dimer of unit oligomer, respectively. (B, right) Plot of R_L values vs the multimer size for the ligation products of oligomer 21D modified with (+)-CC-1065 (E) and compound **13** (J).

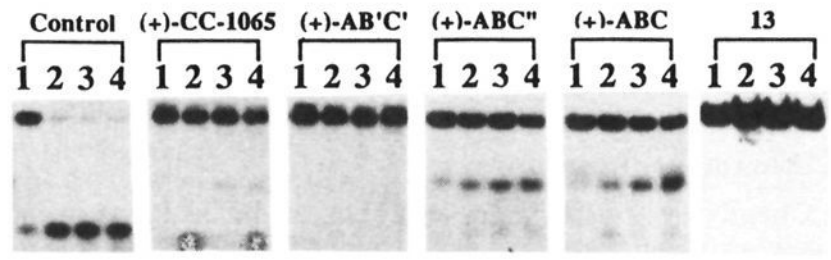

Figure 7. Nondenaturing polyacrylamide gel electrophoresis of the helicase II-catalyzed unwinding of drug-modified and unmodified oligomers (GA in Table III). Lane headings refer to the drug molecules used in modification of DNA. Lanes 1–4 represent reactions containing 0, 10, 20, and 80 ng of helicase II, respectively.

The helicase II substrate (oligomer GA in Table III) was prepared containing a single drug-modified site on the short strand. The results in Figure 7 show that, like (+)-CC-1065 and (+)-AB′C′, **13** also produces the pronounced inhibition of duplex unwinding by helicase II. As shown previously, (+)-ABC″ and (+)-ABC are much less potent in this regard (Figure 7). To evaluate the effect of **13** vs (+)-CC-1065, (+)-AB′C′, (+)-ABC″, and (+)-ABC on T4-mediated ligation of oligomers, two different oligomers (40/42 A and 40/42 B in Table III) were prepared as site-directed DNA adducts and incubated with T4 ligase. The results in Figure 8 show that while (+)-ABC″ and (+)-ABC (lanes 4 and 5) show relatively little inhibition of ligation of the single-stranded break, **13**, (+)-CC-1065, and (+)-AB′C′ (lanes 6, 2, and 3) show a much more pronounced inhibition. Overall, these results support our previous proposal that DNA helical changes such as this

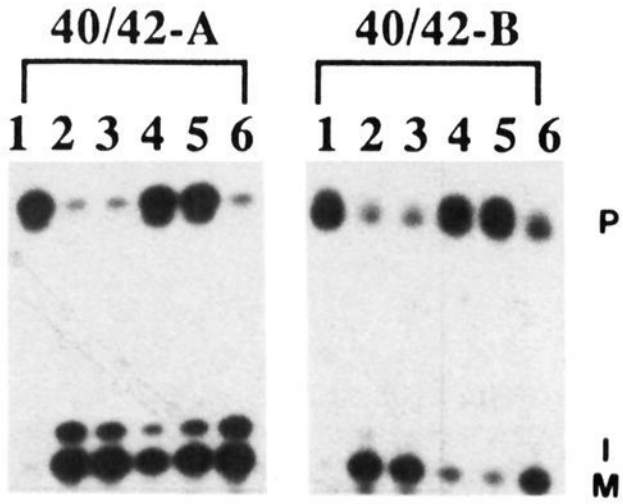

Figure 8. The effect of drug modification on nick-sealing activity of T4-DNA ligase. The ligated products of nicked duplexes (40/42A and 40/42B in Table III) were analyzed on denaturing polyacrylamide gel electrophoresis after heat treatment. Lane headings refer to the nicked duplex used in this experiment. Lanes 1–6 represent control, (+)-CC-1065-, (+)-AB′C′-, (+)-ABC″-, (+)-ABC-, and **13**-modified nicked duplex, respectively. P, I, and M refer to ligated product, adenylated intermediate, and nonligated product, respectively.

winding effect can produce a significant inhibition of DNA metabolizing enzymes and, consequently, the strong cytotoxic potency of these drugs.

Conclusions

A practical synthesis of CBI (**2**) was developed and applied to the synthesis of benzannelated analogs of CC-1065, in particular, (+)-CBI-PDE-I-dimer (**13**) and (+)-A′BC. Changing the (+)-CPI alkylating moiety of (+)-CC-1065 to a (+)-CBI moiety does not affect sequence specificity or the structural, biochemical, or biological effects of the natural product. As anticipated, the fully fledged carbon skeleton in **13**, just like the same structural feature in (+)-CC-1065 and (+)-AB′C′, produces the same helix winding mediated inhibition of helicase II and T4 ligase activities as well as producing delayed toxicity in mice.

Experimental Section

Chemistry. Mass spectra were obtained by the Physical and Analytical Chemistry Unit of The Upjohn Company. UV spectra were recorded on a Beckman DU7500. HPLC was conducted on a Perkin-Elmer Series 4 chromatograph or a Waters 600E chromatograph equipped with an ISCO V4 detector. NMR spectra were recorded on a Bruker Aspect 3000 300-MHz spectrometer, and chemical shifts are reported in ppm (parts per million) on the δ scale relative to internal tetramethylsilane. Coupling constants are reported in hertz. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. All reactions were carried out under a nitrogen atmosphere in glassware that was oven-dried. Flash chromatography refers to the method of Still and utilized silica gel 60 (Merck, particle size 0.04–0.063). Solvents were reagent grade distilled from glass (Burdick and Jackson or Mallinkrodt). Dry tetrahydrofuran (THF) refers to THF distilled from benzophenone ketyl. Reagents were used as purchased. Combustion analyses of the final cytotoxic products were not obtained in order to minimize the exposure of personnel to these extremely potent cytotoxic agents. However all compounds exhibited acceptable high-resolution mass spectra and were >96% pure by HPLC.

(−)-(2*S*)-4-Acetyl-2-[(methylsulfonyl)oxy]-6-hydroxy-1,2,3,4-tetrahydrobenzo[*f*]quinoline (12). A solution of **11**[9] (150 mg, 0.42 mmol) in methylene chloride (5 mL) at 5 °C was treated with triethylamine (100 μL, 0.72 mmol) and methanesulfonyl chloride (40 μL, 0.51 mmol) and then stirred at ambient temperature for 1 h. The reaction solution was washed with water, dried (sodium sulfate), and flash chromatographed, eluting with 30% ethyl acetate in methylene chloride to give the mesylate (170 mg, 93%) as a white foam [^{1}H NMR (acetone-d_6, 300 MHz, ppm) 7.99 (d, 1H, J = 7.8), 7.93 (d, 1H, J = 7.8), 7.5–7.7 (m, 3H), 5.47 (m, 1H), 4.4 (m, 1H), 3.92 (dd, 1H, J = 2.7 and 13.5), 3.66 (dd, 1H, J = 6.0 and 18.0), 3.42 (dd, 1H, J = 3.6 and 18.3), 3.24 (s, 3H), 2.32 (s, 3H), 1.57 (s, 9H); ^{13}C NMR (acetone-d_6, 300 MHz, ppm) 23.29, 27.75, 31.36, 38.33, 75.15, 84.09, 117.47, 122.25, 124.06, 125.65, 126.80, 128.21, 133.41, 136.73, 145.81, 152.57, 170.96; IR (mull) ν_{max} 1759, 1669, 1465, 1406, 1371, 1335, 1277, 1270, 1251, 1175, 1142 cm^{-1}; EIMS m/e (relative intensity) 335 (32), 293 (18), 239 (34), 197 (69), 196 (100), 57 (80); FABHRMS m/e 436.1411 ($C_{21}H_{25}NO_7S$ requires 436.1430); $[\alpha]^{23}_D$ = −87.0° (c = 0.47, methanol)]. The mesylate (150 mg, 0.34 mmol) was dissolved in methylene chloride (7 mL) at 5 °C, treated with trifluoroacetic acid (2 mL), and then stirred for 2 h at ambient temperature. The solution was diluted with methylene chloride, washed with water, dried (sodium sulfate), and concentrated *in vacuo* to give **12** (115 mg, 100%): ^{1}H NMR (acetone-d_6, 300 MHz, ppm) 8.25 (d, 1H, J = 7.8), 7.89 (d, 1H, J = 7.8), 7.59 (t, 1H, J = 7.1), 7.47 (t, 1H, J = 7.1), 7.1 (bs, 1H), 5.4 (m, 1H), 4.35 (m, 1H), 3.92 (dd, 1H, J = 3.1 and 11.1), 3.59 (dd, 1H, J = 6.3 and 12.3), 3.30 (dd, 1H, J = 4.2 and 12.3), 3.24 (s, 3H), 2.93 (s, 1H), 2.28 (s, 3H); IR (mull) ν_{max} 3141, 1622, 1596, 1454, 1450, 1422, 1398, 1377, 1362, 1353, 1347, 1332, 1174, 964, 901, 758 cm^{-1}; EIMS m/e (relative intensity) 335 (M^+, 4), 239 (50), 197 (80), 196 (100), 168 (27), 44 (68); FABHRMS m/e 335.0817 ($C_{16}H_{17}NO_5S$ requires 335.0827); $[\alpha]^{23}_D$ = −98.8° (c = 0.400, methanol).

ent-**12** was synthesized in a similar manner (94%) and had identical spectral characteristics. FABHRMS m/e 336.0921 M + H ($C_{16}H_{17}NO_5S$ + H requires 336.0906); $[\alpha]^{23}_D$ = +99.8° (c = 0.475, methanol).

(+)-(8b*R*,9a*S*)-1,2,9,9a-Tetrahydrocyclopropa[*c*]benz[*e*]-indol-4-one [2, (+)-CBI]. A solution of **12** (35 mg, 0.10 mmol) in THF (4 mL) was added dropwise to sodium hydride (50% in mineral oil, 15 mg, 0.3 mmol) in tetrahydrofuran (2 mL) at 5 °C and then stirred at ambient temperature for 6 h. The solution was then diluted with water and extracted with ethyl acetate (3 × 20 mL), and the combined organics were dried (sodium sulfate), concentrated *in vacuo*, and flash chromatographed, eluting with 60% acetone in hexane to give **2** (20 mg, 97%) as a pale yellow solid: $[\alpha]^{23}_D$ = +335° (c = 0.200, MeOH) [lit.[6c] $[\alpha]^{23}_D$ = +332° (c = 0.052, MeOH)]. Compound **2** had spectroscopic characteristics that were identical to the published data:[7c] ^{1}H NMR ($CDCl_3$, 300 MHz, ppm) 8.21 (d, 1H, J = 7.8), 7.3–7.4 (m, 2H), 6.82 (d, 1H, J = 7.5), 5.75 (s, 1H), 5.40 (bs, 1H), 3.84 (ddd, 1H, J = 1, 4.5, and 10.5), 3.65 (d, 1H, J = 10.5), 2.84 (m, 1H), 1.58 (dd, 1H, J = 3.9 and 7.8), 1.41 (t, 1H, J = 4.5); UV (THF): (316) nm (ε 11 200); EIMS m/e (relative intensity) 197 (M^+, 100), 180 (32), 168 (44), 154 (14), 139 (21), 83 (20); EIHRMS m/e 197.0841 ($C_{13}H_{11}NO$ requires 197.0841); chiral HPLC[17] t_R = 18.1 min, >99% ee (<1% ent-**2**).

(−)-CBI was synthesized in a similar manner and had identical spectral characteristics to (+)-CBI and previously published data:[7c] chiral HPLC[17] t_R = 21.3 min, >98% ee; $[\alpha]^{23}_D$ = −336° (c = 0.125, MeOH).

(+)-(8b*R*,9a*S*)-5-Acetyl-1,2,9,9a-tetrahydrocyclopropa[c]-benz[*e*]indol-4-one [(+)-A′]. A solution of (+)-CBI, **2** (4 mg, 0.02 mmol), in dry THF (1 mL) was added dropwise to a suspension of sodium hydride (50% in oil, 2 mg, 0.04 mmol) in dry THF at −20 °C under nitrogen. The resulting solution was stirred for 30 min, cooled to −40 °C, and treated with acetyl chloride (3 μL, 0.04 mmol). The solution was stirred at −40 °C for 4 h, diluted with water, extracted with methylene chloride, dried (sodium sulfate), concentrated in vacuo, and flash chromatographed, eluting with 40% acetone in hexane to give (+)-A′ (4 mg, 82%) as a white solid: ^{1}H NMR (acetone-d_6, 300 MHz, ppm) 8.07 (d, 1H, J = 8.7), 7.54 (t, 1H, J = 8.3), 7.40 (t, 1H, J = 8.3), 7.12 (d, 1H, J = 9.0), 7.10 (bs, 1H), 4.23 (s, 2H), 3.08 (m, 1H), 2.25 (s, 3H), 1.68 (dd, 1H, J = 4.7 and 8.7), 1.55 (t, 1H, J = 5.0); UV (MeOH) 220 (ε 14 500), 261 (ε 7800), 311 nm (ε 15 900); EIMS m/e (relative intensity) 239 (M^+, 58), 197 (92), 196 (100), 180 (23), 168 (32), 139 (19), 115 (14), 43 (52); EIHRMS m/e 239.0955 ($C_{15}H_{13}NO_2$ requires 239.0946); $[\alpha]^{23}_D$ = +243° (c = 0.200, methanol); HPLC[18] t_R = 5.5 min, >99%.

(−)-A′ was synthesized in a similar manner from (−)-CBI and had identical spectral characteristics to (+)-A′: $[\alpha]^{23}_D$ = −245°

(c = 0.175, methanol). EIHRMS m/e 239.0955 ($C_{15}H_{13}NO_2$ requires 239.0946), HPLC:[18] t_R = 5.5 min, >99%.

(+)-(8b*R*,9a*S*)-2-[(1*H*-Indol-2-yl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one [(+)-CBI-indole]. A solution of indole-2-carboxylic acid (20 mg, 0.12 mmol) in DMF (1 mL) was treated with 1,1′-carbonyldiimidazole (24 mg, 0.15 mmol), stirred for 18 h at room temperature and then at 40 °C for 1 h, and diluted with water (20 mL), and the resulting yellow solid was collected by filtration. The filter cake was washed with water and dried *in vacuo* to give the imidazolide (24 mg, 92%): ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) 12.32 (s, 1H), 8.57 (s, 1H), 7.92 (s, 1H), 7.75 (d, 1H, J = 9.0), 7.5 (m, 2H), 7.37 (t, 1H, J = 8.0), 7.22 (s, 1H), 7.15 (t, 1H, J = 7.8). A suspension of sodium hydride (50% in mineral oil washed with hexane, 2 mg, 0.04 mmol) in DMF (1 mL) at 5 °C was treated with a solution of **2** (4 mg, 0.02 mmol) in DMF (0.5 mL), stirred 30 min, treated with the imidazolide (vide supra, 5 mg, 0.025 mmol) in DMF (1 mL), stirred 4 h at 5 °C, diluted with ethyl acetate (50 mL), washed with water, dried (sodium sulfate), and concentrated *in vacuo*, adsorbing the crude material on Celite (1 g). The Celite mixture was placed on top of a column of silica gel and flash chromatographed eluting with 10% DMF in toluene to give (+)-A′B (6 mg, 87%): ^{1}H NMR (DMF-d_8, 300 MHz, ppm) 11.37 (s, 1H), 8.0 (d, 1H, J = 7.5), 7.7–7.5 (m, 3H), 7.36 (t, 1H, J = 7.5), 7.2 (m, 3H), 7.02 (t, 1H, J = 7.8), 6.94 (s, 1H), 4.62 (dd, 1H, J = 4.8 and 10.2), 4.48 (d, 1H, J = 10.2), 3.2 (m, 1H), 1.7 (m, 2H); UV (1% DMF in MeOH) 338 nm (ϵ 34 800); FABMS m/e (relative intensity) 341 (M + H, 5), 167 (45), 153 (46), 135 (57), 121 (54), 103 (100); FABHRMS (M + H) m/e 341.1288 ($C_{22}H_{16}N_2O_2$ + H requires 341.1290); $[\alpha]^{23}_D$ = +87.7° (c = 0.180, DMF); HPLC[18] t_R = 11.2 min, >99%.

(−)-A′B was synthesized in a similar manner (58% yield) and had identical spectral characteristics to (+)-A′B: FABMS m/e (relative intensity) 341 (M^+ + H, 100), 167 (45), 198 (67), 144 (73), 118 (84), 103 (40); FABHRMS (M + H) m/e 341.1281 ($C_{22}H_{16}N_2O_2$ + H requires 341.1290); HPLC[18] t_R = 11.2 min, >99%.

(+)-(8b*R*,9a*S*)-1,2,9,9a-2-[[5-[(1*H*-Indol-2-ylcarbonyl)amino]-1*H*-indol-2-yl]carbonyl]tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one [(+)-CBI-bis-indole, 17]. A solution of 5-[(1*H*-indol-2-ylcarbonyl)amino]-1*H*-indole-2-carboxylic acid (100 mg, 0.29 mmol) in DMF (2 mL) was treated with 1,1′-carbonyldiimidazole (55 mg, 0.34 mmol), stirred 18 h at room temperature and then at 40 °C for 1 h, and diluted with water (20 mL), and the resulting yellow solid was collected by filtration. The filter cake was washed with water and dried *in vacuo* to give the imidazolide (102 mg, 95%): ^{1}H NMR (DMSO-d_8, 300 MHz, ppm) 12.32 (s, 1H), 11.75 (s, 1H), 10.26 (s, 1H), 8.57 (s, 1H), 8.31 (s, 1H), 7.94 (s, 1H), 7.5–7.8 (m, 6H), 7.2 (m, 2H), 7.07 (t, 1H, 7.8); ^{13}C NMR (DMSO-d_6, 300 MHz, ppm) 103.45, 111.79, 112.27, 112.68, 113.17, 118.30, 119.77, 121.48, 121.56, 123.53, 126.64, 126.99, 127.60, 130.20, 131.61, 132.35, 135.40, 136.65, 137.86, 158.26, 159.53; FABMS m/e (relative intensity) 369 (M^+, 96), 302 (28), 226 (57), 158 (100), 144 (74); FABHRMS m/e 369.1236 ($C_{21}H_{15}N_5O_2$ requires 369.1236). A suspension of sodium hydride (50% in mineral oil washed with hexane, 2 mg, 0.04 mmol) in DMF (1 mL) at 5 °C was treated with a solution of **2** (4 mg, 0.02 mmol) in DMF (0.5 mL), stirred for 30 min, treated with the imidazolide (*vide supra*, 9 mg, 0.025 mmol) in DMF (1 mL), stirred for 4 h at 5 °C, diluted with ethyl acetate (50 mL), washed with water, dried (sodium sulfate), and concentrated *in vacuo*, adsorbing the crude material on Celite (1 g). The Celite mixture was placed on top of a column of silica gel and flash chromatographed, eluting with 10% DMF in toluene to give (+)-A′BC (6 mg, 59%): ^{1}H NMR (DMF-d_7, 300 MHz, ppm) 11.82 (s, 1H), 11.75 (s, 1H), 10.30 (s, 1H), 8.39 (bs, 1H), 8.04 (d, 1H, J = 9.0), 7.4–7.7 (m, 7H), 7.3 (m, 3H), 7.10 (d, 1H, J = 7.2), 7.05 (s, 1H), 4.74 (dd, 1H, J = 4.8 and 10.2), 4.60 (d, 1H, J = 10.2), 3.3 (m, 1H), 1.81 (t, 1H, J = 4.0), 1.78 (apparent dd, 1H, J = 4.0 and 5.5); UV (1% DMF in methanol) 314 nm (ϵ 45 200), 338 nm (ϵ 46 100); MS m/e (relative intensity), 499 (M + H, 12), 376 (40), 320 (67), 275 (42), 198 (41), 172 (38); FABHRMS m/e 499.1773 (M^+ + H), ($C_{31}H_{22}N_4O_3$ + H requires 499.1770); $[\alpha]^{23}_D$ = +87.7° (c = 0.180, DMF); HPLC[18] t_R = 14.3 min, >96%).

(−)-A′BC was synthesized in similar manner (50% yield) and had identical spectral characteristics to (+)-A′BC: FABMS m/e (relative intensity) 499 (M^+ + H, 12), 376 (40), 320 (67), 275 (42), 198 (41), 172 (38); FABHRMS m/e 499.1791 (M + H), ($C_{31}H_{22}N_4O_3$ + H requires 499.1770); $[\alpha]^{23}_D$ = −86.0° (c = 0.180, DMF); HPLC[18] t_R = 14.3 min, >98%).

(+)-(8b*R*,9a*S*)-2-(PDE-I-dimer)-1,2,9,9a-Tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (13). Anhydrous hydrochloric acid was introduced via a Teflon tube extending below the surface of a solution of **2** (10 mg, 0.051 mmol) in ethyl acetate (2 mL) for 30 min at room temperature. The resulting yellow solution of **18** was concentrated *in vacuo*, dissolved in dimethyl formamide (1 mL), treated with PDE-I-dimer (26 mg, 0.049 mmol) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (12 mg, 0.06 mmol), stirred at room temperature for 4 h, quenched with water (0.5 mL), and diluted with 20% dimethylformamide in toluene (15 mL). The aqueous layer was separated, Celite (1 g) was added to the organic layer, and the mixture was concentrated *in vacuo*. The resulting Celite mixture was placed on top of a column of silica gel and flash chromatographed, eluting with 10% DMF in toluene to give a yellow powder (21 mg, 56%): ^{1}H NMR (DMF-d_7, 300 MHz, ppm) 13.05 (s, 1H), 11.40 (s, 1H), 11.34 (s, 1H), 11.10 (s, 1H), 8.34 (s, 1H), 8.23 (d, 1H, J = 8.1), 7.92 (d, 1H, J = 8.4), 7.69 (s, 1H), 7.40 (t, 1H, J = 8.1), 7.24 (s, 1H), 7.20 (s, 1H), 6.98 (bs, 2H), 4.82 (t, 2H, J = 8.7), 4.70 (d, 1H, J = 8.7), 3.8–4.3 (m, partially obscured by residual water, including: 4.22, t, J = 8.7; 4.1, dd, J = 1.8 and 8.7; 3.99, s, 3H; 3.93, s, 3H), 3.46 (t, 1H, J = 9.3), 3.36 (t, 1H, J = 9.3); UV (1% DMF in methanol) 358 nm (ϵ 53 400); FABMS m/e (relative intensity) 737 (M + H_2, 0.5), 736 (M + H, 0.4), 279 (15), 202 (50), 177 (17), 167 (13), 135 (17), 118 (21), 103 (22), 91 (100); FABHRMS, the spectra was too weak for a peak match; $[\alpha]^{23}_D$ = +54.9° (c = 0.133, DMF). A portion of this yellow powder (14 mg, 0.019 mmol) was dissolved in acetonitrile/water/triethylamine (3:1:1, 10 mL), stirred at room temperature for 1 h, diluted with ethyl acetate (50 mL), washed with water (3 × 20 mL), dried (sodium sulfate), concentrated *in vacuo* adsorbing the crude material on Celite (1 g), and flash chromatographed, eluting with 20% DMF in toluene to give **13** (12 mg, 94%) as a yellowish brown solid: ^{1}H NMR (DMF-d_7, 300 MHz, ppm) 13.08 (s, 1H), 11.64 (s, 1H), 11.37 (s, 1H), 11.22 (s, 1H), 8.10 (d, 1H, J = 7.8), 7.62 (t, 1H, J = 8.7), 7.47 (t, 1H, J = 8.7), 7.29 (s, 1H), 7.28 (d, 1H, J = 7.8), 7.20 (s, 1H), 7.00 (s, 2H), 6.93 (s, 1H), 4.80 (t, 2H, J = 10.2), 4.68 (dd, 1H, J = 6.0 and 10.2), 4.54 (d, 1H, J = 10.2), 4.22 (t, 2H, J = 10.2), 3.97 (s, 3H), 3.93 (s, 3H), 3.3–3.5 (m, 5H), 1.82 (d, 2H, J = 6.2); UV (1% DMF in methanol) 367 nm (ϵ 32 100); FABMS m/e (relative intensity) 701 (M + H, 4), 504 (4), 436 (4) 411 (6), 274 (6), 198 (17), 73 (100); FABHRMS m/e 701.2399 (M + H) ($C_{38}H_{32}N_8O_8$ + H requires 701.2360); $[\alpha]^{23}_D$ = +37.3° (c = 0.166, DMF); HPLC[18] t_R = 4.8 min, >99%.

(−)-(8b*S*,9a*R*)-2-(PDE-I-dimer)-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one, 14, was synthesized in a similar manner (56% yield) and had spectral properties identical to (+)-CBI-PDE-I-dimer, **13**: FABMS m/e (relative intensity) 701 (M + H, 4), 504 (4), 436 (4) 411 (6), 274 (6), 198 (17), 73 (100); FABHRMS m/e 701.2399 (M + H) ($C_{38}H_{33}N_8O_8$ requires 701.2360); $[\alpha]^{23}_D$ = −37.3° (c = 0.166, DMF); HPLC[18] t_R = 4.8 min, >99%.

L1210 Cell Growth in Culture. The basal medium used for growing mouse leukemia L1210 cells was RPMI medium 1634. Fetal calf serum (5%), sodium bicarbonate (0.075%), and a mixture of penicillin (0.1 mg/mL of medium) and streptomycin (50 mg/mL of medium) were added as supplements. Aliquots of drug (0.25 mL) were pipetted into each culture tube. The experiment was then initiated by the addition of 4.75 mL of cells (approximately 5 × 10^3 cells/mL), as described elsewhere,[19] and the tubes were incubated at 37 °C for 3 days. Cell number was then determined with a Coulter counter (Coulter Electronics, Hialeah, FL).[5b,6]

Antitumor Activity and Delayed Toxicity. L1210 leukemia (10^5 cells/mouse) was maintained by passage in syngeneic DBA/2 female mice. Tumor cells (10^6 cells/mouse) were inoculated intraperitoneally in male B_6D_2F1 (C57 BL/6 female × DMF/2 male) mice on day 0. The drug was given intravenously on day 1. Compounds were prepared in a vehicle containing 2% DMA, 10% Emulphor EL-620P (GAF Corporation, New York, NY), and 88% sterile water (McGaw). A dose response was determined in all experiments, with the optimal dose defined as that dose

producing the greatest efficacy and causing an acceptable level (≤15%) of drug-related deaths. The antitumor efficacy of the compound was expressed as the percent increase in life span (ILS), which was calculated from the median survival time of 6 mice per group (inclusive of long-term survivors) according to the following equation:

$$\% \text{ ILS} = \left(\frac{\text{median survival time of drug-treated group}}{\text{median survival of vehicle-treated control group}} - 1\right) \times 100$$

Based on criteria set by the U.S. National Cancer Institute (NCI), an ILS value of ≥25% for L1210 leukemia (iv) is considered active. A confirmed ILS value of ≥50% is considered highly active (DN2 level of activity). The potential of these compounds to cause delayed lethality in mice was determined by observing the survival of normal (non-tumor-bearing) mice for 90 days after administration of a single intravenous injection of the compound. "Delayed death" is defined as deaths occurring at times greater than 28 days after single-dose iv administration. Acute toxicity of the analogs was estimated in the chemotherapy experiments from the early death (prior to vehicle-treated controls).

Chemicals and Enzymes. Electrophoretic reagents [acrylamide, TEMED, ammonium persulfate, and bis(acrylamide)] were purchased from Bio-Rad. Other chemicals for chemical DNA sequencing were from Aldrich Chemical Co.; [γ-^{32}P]ATP was from ICN; and X-ray film, intensifying screens, and developing chemicals were from Kodak. Helicase II was generously provided by Professor Thomas Kodadek at the University of Texas, Austin, TX. The T4-polynucleotide kinase and T4-DNA ligase were from United States Biochemical.

Oligonucleotide Synthesis. The oligonucleotides were synthesized on an automated DNA synthesizer (Applied Bio System 381A) by the phosphoramidite method. The oligomers were then deprotected separately by heating at 55 °C overnight with saturated ammonium hydroxide, dried under vacuum, and redissolved in double-distilled water (DDW).

5′-^{32}P End Labeling of DNA Restriction Fragment. Plasmid pUC9 was digested with EcoRI, dephosphorylated with bacterial alkaline phosphatase, and digested with HaeII. DNA restriction fragments were 5′-^{32}P end labeled by T4-polynucleotide kinase (10 units) in 25 μL of solution containing 70 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 5 mM dithiothreitol, and 30 μCi of [γ-^{32}P]ATP for 1 h at 37 °C. The reaction mixture was applied to 8% polyacrylamide gel electrophoresis, and the labeled restriction fragment (162 bp) was located by autoradiography. The resulting band was cut from the gel, minced with a blade, and extracted with DDW. After lyophilization of the extracted solution containing DNA, the pellet was resuspended in DDW and further purified by phenol/chloroform extraction and ethanol precipitation.

Construction of Drug-Modified Template for Helicase II-Mediated Unwinding Assay and T4-DNA Ligase-Mediated Nick-Sealing Assay. To construct the substrates for the helicase II experiments, the short oligomer strand of the partial duplex (GA), as shown in Table III, were 5′-end labeled with [γ-^{32}P]ATP and annealed to the complementary long strand to produce a partial duplex, and resulting duplexes were purified by using the 8% polyacrylamide gel electrophoresis, as described previously.[15,16] The DNA substrates for T4-DNA ligase assay were constructed by annealing 5′-^{32}P end-labeled 21-mer oligomers to the complementary 40-mers. The purification of resulting nicked duplexes was achieved using a previously published method.[18]

Drug Modification and Strand-Breakage Assay. For drug modification of restriction fragment, 50-μL aliquots of DNA (50 ng) were incubated with the same volume of 28, 2.8, or 0.28 μM of (+)-CC-1065 (13) and (+)-ABC″ at room temperature for 1 day, followed by phenol/chloroform extraction and ethanol precipitation to remove unbound drug molecules.

For the drug modification of oligomers, 100 mL of DNA solution (~1 mg DNA) were incubated with the same volume of 28 μM of (+)-CC-1065 (13), (+)-ABC″, (+)-ABC, and 280 μM of (+)-AB for 3 days. Unbound drug molecules were removed as described above. For strand-breakage assay, drug-modified duplexes were resuspended in 10 μL of DDW and heated at 95 °C for 30 min to produce strand breakage at the covalent modification site.

Bending Experiments and Ligation. Drug-modified and unmodified oligomer 21D labeled at both complementary strands with [^{32}P] were self-ligated to produce multimers in 20 μL of ligation buffer with 1 unit of T4 DNA ligase at room temperature overnight. The ligation buffer contains 25 mM Tris-HCl (pH 7.6), 5 mM $MgCl_2$, 25% (w/v) poly(ethylene glycol) 8000, 0.5 mM ATP, and 0.5 mM dithiothreitol. Ligated multimers were electrophoresed on an 8% nondenaturing polyacrylamide gel and visualized by autoradiography, as described previously.[13] Ligation of nicked duplexes was carried out in 20 μL of ligation buffer containing 20 ng of DNA and 1 unit of T4-DNA ligase at room temperature for 12 h.

Helicase II-Catalyzed Unwinding of Drug-Modified or Unmodified Tailed Duplex. Each reaction was carried out in 10 μL of helicase II unwinding buffer containing 20 ng of DNA substrate and the indicated amount of helicase II. After preincubation for 5 min, an 8 M excess of unlabeled ss DNA (the same strand as the labeled strand) was added to the reaction as a trap to prevent the released labeled strand from reannealing. Each reaction was incubated for 30 min at 30 °C and then quenched with helicase stop buffer. The reaction buffer and helicase stop buffer were described elsewhere.[14,15]

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