Articles

Total Synthesis and Biological Properties of Novel Antineoplastic (Chloromethyl)furanoindolines: An Asymmetric Hydroboration Mediated Synthesis of the Alkylation Subunits†

Fariborz Mohamadi,* Michael M. Spees, Gilbert S. Staten, Philip Marder, Julia K. Kipka, and David A. Johnson

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285

Dale L. Boger and Hamideh Zarrinmayeh

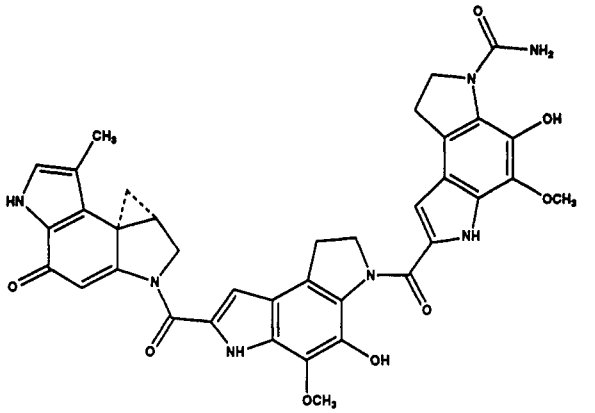
The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, California 92037

Received July 30, 1993•

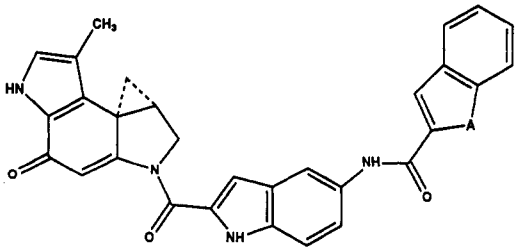
1,2-Dihydro-1-(chloromethyl)-5-hydroxy-8-methyl-3*H*-furano[3,2-*e*]indole (CFI) as a novel replacement of the cyclopropylpyrroloindoline (CPI) alkylation subunit of CC-1065, U-71184, and U-73975 (adozelesin) has been synthesized and incorporated into a series of efficacious antineoplastic agents. A partial solution to an asymmetric synthesis of the CFI alkylation subunit has been achieved by the implementation of an asymmetric hydroboration reaction of an intermediate 3-methyleneindoline (**13**). Extension to the asymmetric synthesis of the CBI and CI alkylation subunits is presented. The demonstration and comparative study of the sequence-selective DNA alkylation properties of the CFI-based agents are detailed, and the preliminary *in vitro* and *in vivo* antineoplastic propertes of these agents in the human epidermoid cell lung carcinoma (T222) are described.

CC-1065 is an antitumor antibiotic that was isolated from the fermentation cultures of *Streptomyces zelensis* (6–9 μg/mL) in 1978.[1] This molecule is an exceptionally potent cytotoxic agent that binds covalently[2] to the minor groove of double-stranded DNA and has good *in vitro* (*e.g.* IC_{50} in L1210 of 1.9 pg/mL)[3] and *in vivo* (*e.g.* ILS 111% against P388 at 30 μg/kg/d dosed days 1, 5, and 9) pharmacology.[4,5] However, administration of a single intravenous subtherapeutic dose (LD_{50} of 9 μg/kg) led to delayed deaths (50–90 days) of healthy mice as a result of the hepatoxicity of this molecule.[6] Although this preempted the clinical development of CC-1065, the synthesis of fragments and analogs of CC-1065 elucidated the structural variables responsible for this undesirable hepatotoxicity[7] and culminated in the clinical development of U-71184 and U-73975 at Upjohn.[8] Kyowa Hakko Kogyo has since isolated numerous new members of this class of natural products which possess very promising pharmacology.[9]

These agents have been shown to participate in a characteristic and sequence-selective adenine N3 alkylation of duplex DNA thought to be responsible for their therapeutic effects. Our initial studies have focused on the preparation of CC-1065 analogs bearing deep-seated changes in the agent structure with the intent of defining the fundamental structural feature contributing to DNA recognition, reactivity, and DNA alkylation selectivity and their impact on the biological properties of the agents. This study summarizes efforts on the synthesis of agents incorporating the 1,2-dihydro-1-(chloromethyl)-5-hydroxy-8-methyl-3*H*-furano[3,2-*e*]indole (CFI) alkylation subunit



(+)-CC-1065



U-71184 A = NH
U-73975 A = O

as a novel synthetic replacement for the authentic CPI alkylation subunit through an approach that provides a partial solution to the asymmetric synthesis of such agents.

One rationale for the replacement of the CPI with the CFI pharmacophore is to minimize the overlap of a fermentation with a chemical synthesis discovery program

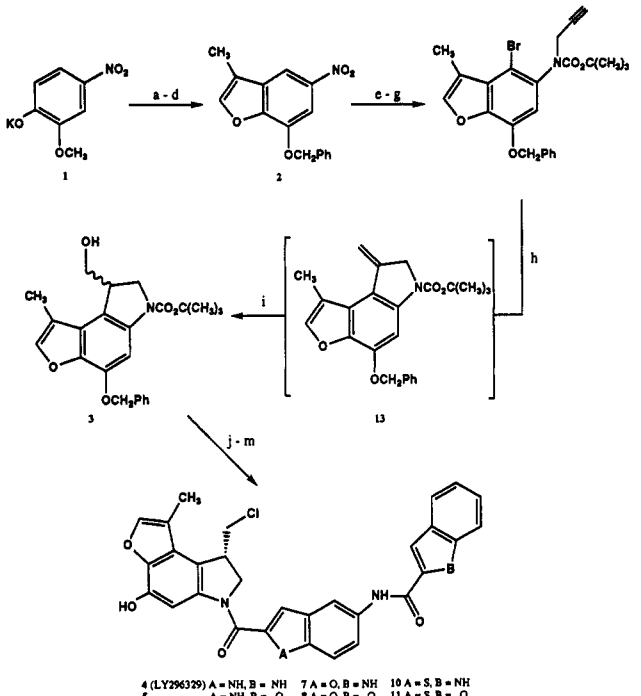
* Address correspondence to: Fariborz Mohamadi, Abbott Laboratories, 1 Abbott Park Rd., Abbott Park, IL 60064-3500.

† Presented in part at the 206th National Meeting of the American Chemical Society, Chicago, IL, August 22–27, 1993.

• Abstract published in *Advance ACS Abstracts*, December 15, 1993.

0022-2623/94/1837-0232$04.50/0

Scheme 1[a]



[a] (a) 1.3 equiv $CH_3CHCHCH_2Br$, catalytic KI, acetone, 56 °C, 24 h, 91%; (b) toluene, 180 °C, 8 h, 56%; (c) O_3, CH_2Cl_2, −78 °C; SMe_2, 24 °C, 2 h; PPA, $PhCH_3$, 110 °C, N_2, 2 h, 45%; (d) 5.9 equiv of $C_5H_5N{\cdot}HCl$, 170 °C, N_2, 2 h; 1.0 equiv of NaH, 1.0 equiv of $PhCH_2Br$, DMF, 24 °C, 5 h, 70%; (e) 1.5 atm of H_2, PtO_2, EtOAc, 30 min; 2.0 equiv of $(t\text{-}BuO_2C)_2O$, dioxane, 95 °C, 3 h, 64%; (f) 1.0 equiv of *N*-bromosuccinimide, catalytic H_2SO_4, THF, −60 °C, 5 h, 92%; (g) 1.1 equiv of NaH, 3.0 equiv of $HCCCH_2Br$, 24 °C, 3 h, 96%; (h) 2.0 equiv of *n*-Bu_3SnH, catalytic AIBN, benzene, 80 °C, 1 h; (i) 2.0 equiv of monoisopinocampheylborane, THF, 25 °C, 3 h; 2 N NaOH, 30% H_2O_2, 0–25 °C, 1 h, 45 °C, 20 min, 39% from 3-methyl-4-bromo-5-(*N*-propargyl-*N*-(*t*-butyloxycarbonyl)amino)-7-(benzyloxy)benzofuran; (j) further resolution via recrystallization of (*R*)-(−)-*O*-acetylmandelate; (k) 2.0 equiv of Ph_3P, 6 equiv of CCl_4, CH_2Cl_2, 24 °C, 24 h, quant; (l) 25% aqueous HCO_2NH_4/THF 1:5, 10% Pd/C, 0 °C, 3 h, 92%; (m) 3 N anhydrous HCl/EtOAc, 24 °C, 30 min; 3 equiv of EDCI, 1.0 equiv of arenecarboxylic acid, DMF, 24 °C, 24 h.

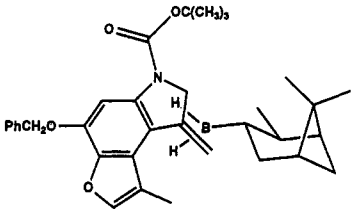
based on the biosynthesis of CC-1065. Such an approach maximizes the chemical discovery effort with regard to the number of possible permutations of accessible drug candidates. In spite of the numerous efforts in the area of the synthesis of CC-1065[10] and its subunits,[11] the Boger approach seemed the most viable for a successful asymmetric synthesis of a CFI-based agent. The demonstration of sequence specific binding of CC-1065 in an enantioselective fashion for double-stranded DNA as corroborated by nuclear Overhauser enhancement (NOE) in addition to the general decreased potency of the unnatural enantiomers prompted the effort to synthesize (+)-CFI.[12]

Synthesis of CFI and Incorporation into Analogs of CC-1065. The synthesis (Scheme 1) starts with the commercially available potassium salt of 4-nitroguaiacol (1). This phenoxide was alkylated with crotyl bromide, and the resulting crotyl ether underwent a thermolytic Claisen rearrangement to provide 6-butenyl-4-nitroguaiacol. Cleavage of the carbon–carbon double bond with ozone followed by a reductive workup provided a benzylic aldehyde that underwent dehydration to provide 3-methyl-5-nitro-7-methoxybenzofuran. As indicated by model studies, a methyl group was not a suitable protecting group for the phenolic oxygen. Pyridine hydrochloride-mediated demethylation followed by treatment of the hydroxybenzofuran with sodium hydride and benzyl bromide provided the desired 3-methyl-5-nitro-7-(benzyloxy)benzofuran (2). After the evaluation of a number of hydrogenation catalysts and hydride reducing agents, catalytic hydrogenation with platinum oxide was found to reduce the nitro group cleanly, and the crude product of this reaction treated with di-*tert*-butyl dicarbonate to provide 3-methyl-5-(*N*-(*tert*-butyloxycarbonyl)amino)-7-(benzyloxy)benzofuran. Electrophilic aromatic bromination of this molecule placed a bromine at the 4-position of this benzofuran as demonstrated by NOE studies via irradiation of the methyl group at the 3-position of the benzofuran. Treatment of the bromination product with propargyl bromide and sodium hydride provided 3-methyl-4-bromo-5-(*N*-*tert*-butyloxycarbonyl)-*N*-(2-propyn-1-yl)amino)-7-(benzyloxy)benzofuran. Treatment of this intermediate with the Boger free radical cyclization/hydroboration[13] provided in modest yield the desired racemic hydroxymethyl compound **3**. This modest chemical yield is due in part to the isomerization of the exocyclic olefin of **13** to provide the endocyclic indole.

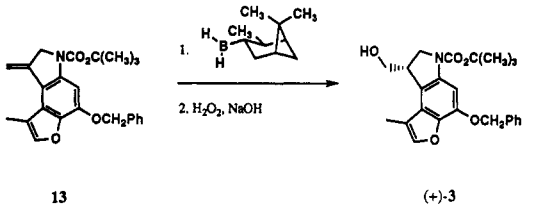
The racemic (hydroxymethyl)furanoindoline **3** was resolved via its (*R*)-(−)-*O*-acetylmandelate by preparative high-pressure liquid chromatography (HPLC).[14] The structural assignments of diastereomers was based on the NMR chemical shifts of the methine and acetyl methyl groups of the chiral auxiliary of the two diastereomeric esters and ultimately confirmed through examination of the DNA alkylation and biological properties of the agents. Treatment of the (+)-**3** with carbon tetrachloride and triphenylphosphine followed by catalytic debenzylation provided in excellent yield (+)-(1*R*)-1,2-dihydro-1-(chloromethyl)-3-(*tert*-butyloxycarbonyl)-5-hydroxy-8-methyl-3*H*-furano[3,2-*e*]indole. Deprotection of the nitrogen with hydrochloric acid followed by 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDCI)-mediated coupling with the appropriate arenecarboxylic acid provided the compounds **4**–**12**.[15]

Asymmetric Synthesis of CFI. The use of a resolution by preparative HPLC to separate diastereomeric derivatives in order to obtain chiral material is amenable to the synthesis of a large number of structural analogs of CFI but may not prove useful for a commercial-scale synthesis of a drug.[16] Consequently, an asymmetric hydroboration of the intermediate 3-methyleneindoline (**13**) generated in the 5-*exo*-*dig* aryl radical–alkyne cyclization was examined with expectations of generating the desired optically active 3-(hydroxymethyl)indoline (**3**) directly and in higher chemical conversion relative to the use of diborane. The chiral hydroboration reagents in the literature are very effective for use with 1,2-disubstituted and trisubstituted olefins but produce only low levels of asymmetric induction for 1,1-disubstituted alkenes.[17] This lack of induction is explained by the limited distance a chiral alkylborane can recognize three-dimensional space. Placement of substituents at the same carbon of the alkene removes the steric bulk of the groups on the substrate beyond the scope of the chiral environment of a hydroborating agent. However, if one examines the possible transition states for the reaction of monoisopinocampheylborane,[18] with alkene **13**, it seemed that preferential reaction through one transition state (Scheme 2) may be observed in this reaction. This deviation from the literature trend is due to the placement of an extremely large and small group on the same carbon of the olefin with a rigid geometry. This structural feature of **13** is responsible for the 50% asymmetric induction observed

Scheme 2

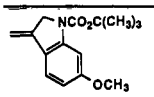
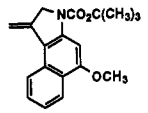
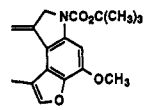


Scheme 3



13 (+)-3

Table 1

substrate	products (percent overall yield for radical cyclization and asymmetric hydroboration)	
 14	1.0:1.1 (34)	
 15	2.4:1.0 (58)	
 16	2.8:1.0 (39)	

for hydroboration with monoisopinocampheylborane (Scheme 3).[19]

In order to address the generality of this approach to the asymmetric synthesis of the existing CI, CBI, and CFI substitution of the CPI alkylation subunit, hydroboration of crude alkenes **14–16** (synthesized by tributyltin hydride-mediated cyclization of the corresponding bromoacetylene) with *R*-alpine-boramine-generated monoisopinocampheylborane (Table 1) was evaluated.[20] Notably, modest levels of asymmetric induction were observed with substrates **15** and **16**, which like **13** and unlike **14** possess large distinctions in the size of the the olefin substituents maintained on the rigid substrate structure. Subsequent derivatization of the optically-enriched sample of **3** with (*R*)-*O*-acetylmandelic acid and simple recrystallization from diethyl ether provided substantial quantities of enantiomerically pure **3**, which at the time constituted the first modestly successful approach to the asymmetric synthesis of agents related to CC-1065.[21]

Although it is conceptually possible to design reagents with extended domains of chirality such as (2,2′-bis-(methylene)-1,1′-binaphthyl)borane[22] to distinguish a large (L) and small (S) group in the course of hydroboration of a 1,1-disubstituted olefin (Figure 1), the utility of such stoichiometric chiral designer reagents is limited as noted by Masamune.[23]

DNA Alkylation Studies. Demonstration of the event

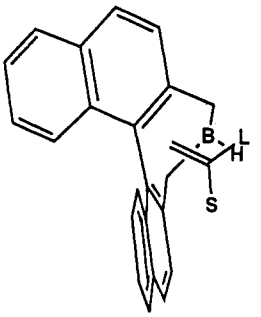
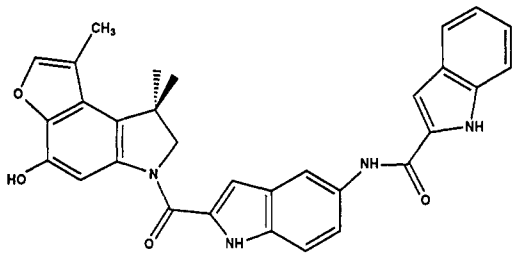
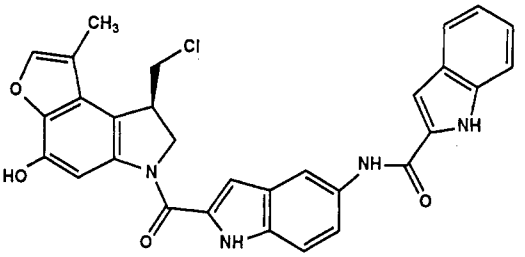


Figure 1.



17



18 (LY297950)

and relative selectivity of DNA alkylation by the agents was obtained from thermally-induced strand cleavage of singly end-labeled duplex DNA after exposure to the agents.[24] Using singly 5′-^{32}P end-labeled SV40 duplex DNA derived from clone w794 (nucleotide no. 138–5238),[25] the agents were incubated at the specified concentration with the labeled DNA at 4 °C for 24 h. The unbound agent was removed by ethanol precipitation of all DNA, and the covalently modified DNA was redissolved in aqueous buffer and warmed at 100 °C for 30 min to induce strand cleavage at the sites of alkylation. Electrophoresis of the resulting DNA under denaturing conditions adjacent to Sanger dideoxynucleotide sequencing reactions followed by autoradiography permitted the identification of the sites of DNA alkylation and subsequent cleavage (Figure 2). The alkylation reaction of (+)-seco-CBI-indole$_2$,[26] (+)-CPI-indole$_2$ (U-71184),[27] and (+)-CC-1065 were run concurrently for comparison.

The major site of alkylation with w794 duplex DNA for **4** (LY296329) and (+)-CC-1065 proved to be identical, 5′-d(AATTA)-3′-alkylation at the 3′ adenine and agent binding in the 3′ → 5′ direction. The profile of DNA alkylation selectivity for **4** proved identical to that of (+)-CBI-indole$_2$ and (+)-CPI-indole$_2$ and only subtly distinguishable from that of (+)-CC-1065 in the alkylation of the low-affinity sites. However, **18** (LY297950) exhibited a different and less selective profile of DNA alkylation. The high-affinity alkylation site for **18** with w794 duplex DNA was 5′-(AATTT)-3′, with alkylation at the 5′ adenine

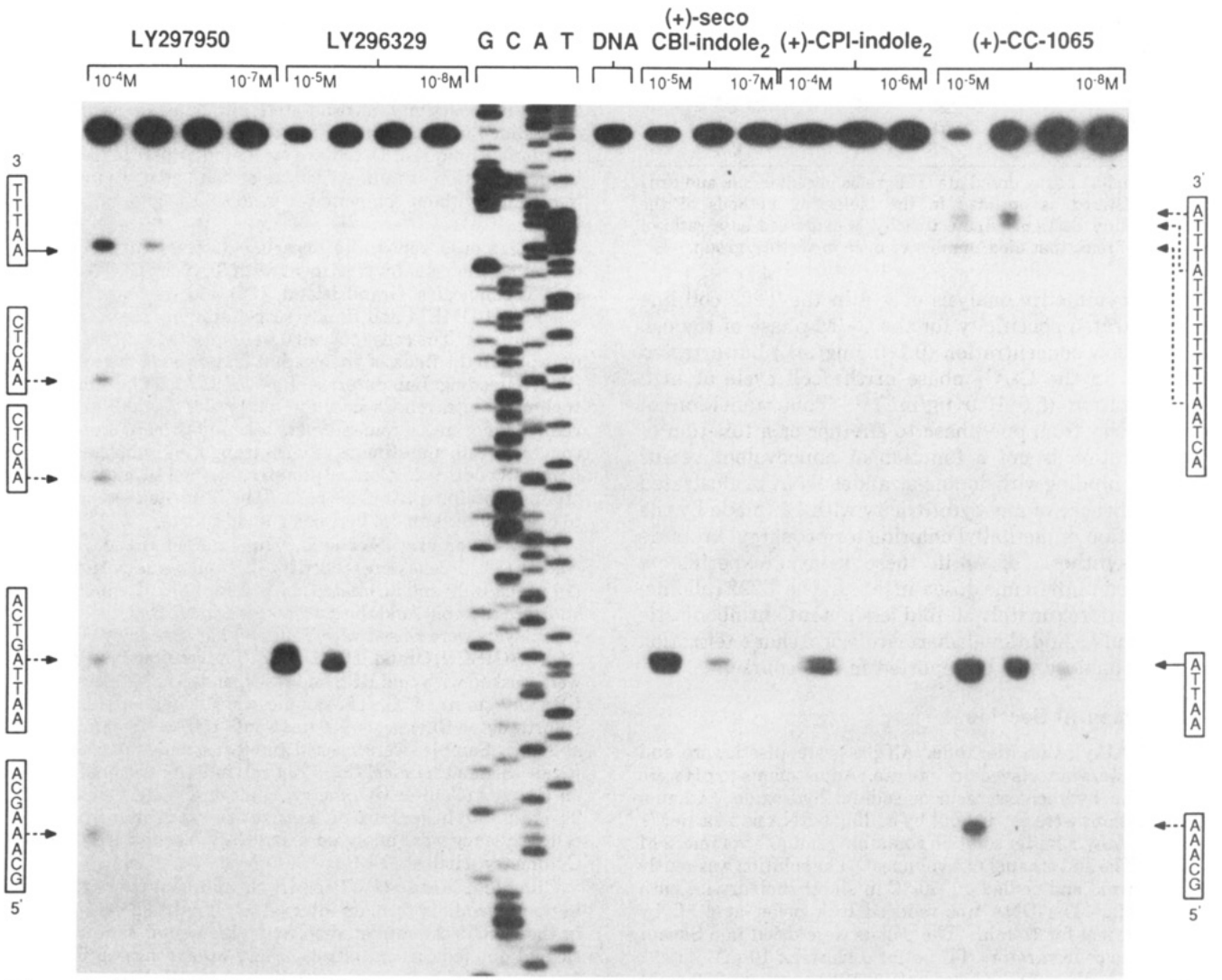


Figure 2. Thermally-induced strand cleavage of double-stranded DNA (SV40 fragment, 144 bp, nucleotide no. 138–5328, clone w794) after 24 h incubation of agent-DNA at 4 °C followed by removal of unbound agent and 30 min incubation at 100 °C; 8% denaturing PAGE and autoradiography. Lanes 1–4, **18** (LY297950) (1×10^{-4} to 1×10^{-7} M); lanes 5–8, **4** (LY296329) (1×10^{-5} to 1×10^{-8} M); lanes 9–12, Sanger G, C, A, and T reactions; lane 13, control DNA; lanes 14–16, (+)-seco-CBI-indole$_2$ (1×10^{-5} to 1×10^{-7} M); lanes 17–19, (+)-CPI-indole$_2$ (1×10^{-4} to 1×10^{-6} M); lanes 20–23, (+)-CC-1065 (1×10^{-5} to 1×10^{-8} M). The observation and origin of two close running bands for a single alkylation event have been described elsewhere.[24,25]

Table 2

agent	configuration	relative DNA alkylation efficiency[a]
4	natural	1.0–0.2
18	unnatural	0.01–0.006
(+)-CC-1065	natural	1
(−)-CC-1065	unnatural	

[a] Relative concentration of agent for which alkylation of w794 DNA is detected at incubation condition of 4 °C for 24 h.

and agent binding in the 5′ → 3′ direction. The profile of DNA alkylation exhibited by **18** proved essentially identical to that of the unnatural enantiomers (−)-CC-1065 and (−)-CPI-CDPI$_2$.[12b] A more important distinction between **4** and **18** is the relative efficiency or intensity of DNA alkylation. Consistent with the relative *in vitro* cytotoxic potency of the agents, **4** proved comparable or slightly less effective at alkylating the duplex DNA than (+)-CC-1065 (1–5 times) and much more effective than **18** (0.02–0.01 times) (Table 2). In a single, qualitative temporal dependence of the extent of DNA alkylation, **4** was found to alkylate DNA at a rate significantly faster than that of **18**. This contributes to the apparent efficiency distinctions illustrated in Figure 2.

Table 3

compound	IC_{50} (ng/mL)[a]	compound	IC_{50} (ng/mL)[a]
4	0.17	**10**	0.29
5	0.15	**11**	0.26
6	0.20	**12**	0.20
7	0.23	DAVLB hydrazide	3.0
8	0.37	**17**	NA[b]
9	0.56		

[a] Concentration for 50% growth inhibition relative to untreated controls in the T222 cell line. [b] NA = not active in this dose range.

Biological Properties. The cytotoxicity of **4–12** was determined by incubation (48 h) of drug with a human lung epidermoid carcinoma (T222)[28] and percent inhibition determined by measurement of [^{3}H]leucine uptake. Analogs **4–12** were approximately 1 order of magnitude more cytotoxic than 4-desacetylvinblastine-3-carboxhydrazide (DAVLB hydrazide) which had an IC_{50} of 3.0 ng/mL in this assay (Table 3). In a similar protocol with human lung adenocarcinoma (UCLA-P3),[29] **4** had an IC_{50} of 0.27 ng/mL. When dosed at 50–200 μg/kg intravenously on days 3, 5, and 7 in the tail vein, **4** inhibited the growth of tumors (Table 4) in mice bearing a T222 burden (injected subcutaneously at day 0).[30]

Table 4. Efficacy of 4 in Mice Bearing a T222 Tumor Burden

dose[a]	% inhibition	toxicity[b]
0.4		5/5
0.2	85	1/5
0.1	73	0/5
0.05	68	0/5

[a] The units of drug dosed are milligrams per kilogram, and drug is administered as outlined in the biological methods of the Experimental Section. [b] Acute toxicity is expressed as a ratio of number of mice that died/number of mice in the test group.

Flow cytometric analysis of 4–6 in the T222 cell line demonstrated specificity for the G_2/M phase of the cell cycle at low concentration (0.1–0.5 ng/mL) but arrested the cells in the G_0/G_1 phase of the cell cycle at high concentration (5.0–10.0 ng/mL).[31] This transition of cytotoxicity from one phase to another as a function of concentration is not a function of noncovalent versus covalent binding with double-stranded DNA as illustrated by the absence of any cytotoxicity with 17 (made by the substitution of methallyl chloride for propargyl bromide in the synthesis of 4) in these assays (experiments conducted in the same dose range). In the T222 cell line, 18 is an approximately 10-fold less potent antineoplastic agent than 4. Additional pharmacological characterization of these analogs will be reported in due course.

Experimental Section

DNA Alkylation Methods. All glassware, plasticware, and solutions were autoclaved prior to use. Adjustments to pH were made with hydrochloric acid or sodium hydroxide. Ethanol precipitations were carried out by adding t-RNA as a carrier (1 μL, 10 μg/μL), a buffer solution containing salt (0.1 volume, 2 M NaCl in TE) and ethanol (2.5 volumes). The solution was gently vortex mixed and chilled at −70 °C in an ethanol–dry ice bath for 20 min. The DNA was reduced to a pellet at 4 °C by centrifugation for 15 min. The pellets were dried in a Savant Speed Vac concentrator. TE buffer consists of 10 mM Tris, 1 mM EDTA, pH = 7.6. Kinase buffer (10×) consists of 500 mM glycine (pH = 9.5), 100 mM $MgCl_2$, and 50 mM dithiothreitol in 50% aqueous glycerol. Formamide dye contained xylene cyanol FF (0.03%), bromophenol blue (0.03%), and aqueous Na_2EDTA (8.7%, 250 mM). Electrophoresis running buffer (1 × TBE) contained Tris base (100 mM), boric acid (100 mM), and $Na_2EDTA{\cdot}2H_2O$ (0.2 mM) dissolved in water.

A solution of singly 5′-^{32}P labeled w794 double-stranded DNA (9 μL) was treated with the agent in a solution of DMSO (1 μL, at the specified concentration). The reaction mixture was mixed by gentle vortexing, brief centrifugation, and subsequent incubation at 4 °C for 24 h. The alkylated DNA was separated from unbound agent by ethanol precipitation of the DNA and resuspended in TE buffer (20 μL). The solution was warmed at 100 °C for 30 min to induce strand cleavage at the sites of alkylation. Following the addition of formamide dye (10 μL), the solution was warmed to 100 °C for 5 min, placed in an ice bath, and centrifuged, and the supernatant was loaded on a gel. Gel electrophoresis was carried out using an 8% denaturing polyacrylamide gel (19:1 acrylamide:*N,N′*-methylenebis(acrylamide); 8 M urea). The gel was prerun for 30 min to 1 h with formamide dye prior to loading the samples. Autoradiography of dried gel was carried out at −70 °C using Kodak X-Omat AR film and a Picker Spectra intensifying screen.

***Biological* Methods.** Human lung epidermoid carcinoma (T222) and human lung adenocarcinoma (UCLA-P3) cells were grown in DMEM medium supplemented with 10% fetal bovine serum and 50 μg/mL gentamicin using standard tissue culture techniques.

For the in vitro cytotoxicity assays, 10^4 target cells were distributed in each well of 96-well tissue culture plates and incubated in leucine-deficient medium (leucine free DMEM, 13 μg/mL L-leucine, 29.2 μg/mL L-glutamine, 50 μg/mL gentamicin, and 10% dialyzed fetal calf serum) for 16 h at 37 °C in 5% carbon dioxide/air atmosphere. The medium was then removed aseptically, and compound dilutions were added in 200 μL of leucine-deficient medium. Following an additional 48-h incubation, 4 μCi[^{3}H]leucine (New England Nuclear, Boston, MA) were added to each well, and the plates were returned to the incubator for 24 h. Radioactivity incorporated into macromolecules was determined using an automated cell harvester and liquid scintillation techniques. Data were evaluated as percent reduction in incorporation of radioactivity relative to controls incubated in medium without compound to yield a 50% cytotoxic concentration (IC_{50}).

Nude mouse xenografts experiments were carried out by collecting the cells by treatment with Trypsin/EDTA (Gibco, Life Technologies, Grand Island, NY) and washing with supplemented DMEM and finally suspending in Hanks balanced salt solution. The cells (10^7 cells) were injected subcutaneously (day 0) into the flank of young adult female nude mice (Charles River Breeding Laboratories, Boston, MA). The mice were treated by intravenous injection on days 3, 5, and 7 in the tail vein. Tumor measurements were taken in two dimensions and converted into an estimate of mass using the formula weight = (length × width^2)/2. Control groups contained 10 mice, with test groups containing five mice each. The Student *t* test was used to evaluate differences between tumor masses.

Flow Cytometry Methods. Human epidermoid lung carcinoma (T222) cells were seeded into 24-well tissue culture plates (10^5 cells/well) and incubated at 37 °C for 24 h. Compound was added to the wells, and the cultures were incubated for 20 h. The monolayers were rinsed with PBS and harvested with trypsin–EDTA (GIBCO, Grand Island, NY). The resultant suspensions were washed with cold PBS and resuspended in 50% methanol/PBS for 10 min at 4 °C. The samples were incubated with RNAse (Worthington Chemicals, 150 units/mL, DNAse-free) for 30 min at 37 °C. Samples were washed and resuspended in propidium iodide (Sigma Chemical Co., 50 μg/mL in PBS) and analyzed on an Epics C (Coulter Cytometry, Hialeah, FL) flow cytometer. The resultant histograms of linear fluorescence intensity versus cell frequency were analyzed using EASY-2 software (Coulter Cytometry, Hialeah, FL).

Chemical Methods. ^{1}H NMR chemical shifts are reported in ppm downfield from an internal tetramethylsilane standard in the specified deuterated solvent. Elemental analyses were carried out for carbon, nitrogen, and oxygen by the Eli Lilly Microanalysis Laboratory. Tetrahydrofuran (THF) was distilled from sodium/benzophenone, and benzene was distilled from sodium metal under nitrogen prior to use. Anhydrous *N,N′*-dimethylformamide was purchased from the Aldrich Chemical Co.

1-(1-(1-Oxa-2-butenyl))-2-methoxy-4-nitrobenzene. A mixture of 4-nitroguaiacol, potassium salt hydrate (200 g, 0.97 mol), and crotyl bromide (175 g, 1.16 mol) was refluxed in 2 L of acetone overnight. The solvent was removed under vacuum and the residue filtered through silica gel (2 L) with 15% EtOAc/hexanes. The filtrate was collected in 4-L fractions, and the appropriate fractions were concentrated under vacuum to provide 195 g of solid (91% yield): ^{1}H NMR (CD_3SOCD_3) δ 7.88 (dd, *J* = 3, 9 Hz, 1 H), 7.73 (d, *J* = 3 Hz, 1 H), 7.16 (d, *J* = 9 Hz, 1 H), 5.96–5.84 (m, 1 H), 5.76–5.66 (m, 1 H), 4.64 (d, *J* = 7 Hz, 2 H), 3.89 (s, 3 H), 1.72 (d, *J* = 7 Hz, 3 H); MS (FD) *m/z* 223 (M^+).

2-(3-(1-Butenyl))-4-nitro-6-methoxyphenol. A solution of 1-(1-(1-oxa-2-butenyl))-2-methoxy-4-nitrobenzene (194 g, 0.87 mol) dissolved in 1.8 L of toluene was heated in a stainless steel bomb at 180 °C overnight. The reaction mixture was cooled to room temperature and concentrated under vacuum. The residue was dissolved in 2 L of diethyl ether and extracted with 1.5 L of 1 N NaOH. The aqueous layer was separated, acidified (pH 2) with 5 N HCl and extracted with methylene chloride. The organic layer was filtered through silica gel (2 L) with 15% EtOAc/hexanes, and the appropriate fractions were combined and concentrated under vacuum. The residue was triturated with hexanes to provide 108 g of solid (56% yield): ^{1}H NMR (CD_3SOCD_3) δ 10.30 (bs, 1 H), 7.68 (s, 2 H), 6.05 (ddd, *J* = 6, 9, 16 Hz, 1 H), 5.13–5.11 (m, 1 H), 5.08 (bs, 1 H), 3.96 (s, 1 H), 3.90 (dt, *J* = 3, 6 Hz, 1 H), 1.30 (d, *J* = 6 Hz, 3 H); MS (FAB) *m/z* 223 (M^+). Anal. ($C_{11}H_{13}NO_4$) C, H, N.

3-Methyl-5-nitro-7-methoxybenzofuran. A stream of 3% ozone/oxygen was bubbled through a solution of 2-(3-(1-butenyl))-4-nitro-6-methoxyphenol (63.3 g, 0.284 mol) dissolved in 2 L of

methylene chloride at −78 °C until the solution turned light blue (~2 h). The solution was purged with a stream of nitrogen at −78 °C, dimethyl sulfide (62 mL) was added, and the reaction mixture was stirred at ambient temperature for 30 min. The reaction mixture was placed in a separatory funnel, and the organic layer was washed with water (2 × 1 L). The methylene chloride layer was dried with anhydrous magnesium sulfate, filtered, and concentrated under vacuum. The residue was added to a solution of polyphosphoric acid (60 g) dissolved in 1 L of benzene and stirred at reflux for 6 h. The reaction mixture was extracted with water (2 × 500 mL), and the organic layer was dried with anhydrous magnesium sulfate, filtered, and concentrated under vacuum. The residue was purified by a preparative high-pressure liquid chromatogram (Waters Prep 500 A) with elution of 7.5% EtOAc/hexanes on two silica gel cartridges to provide 26.5 g of solid (45% yield): ^{1}H NMR (CD_3SOCD_3) δ 8.21 (d, J = 3 Hz, 1 H), 8.00 (d, J = 2 Hz, 1 H), 7.76 (d, J = 3 Hz, 1 H), 4.06 (s, 3 H), 2.27 (d, J = 2 Hz, 3 H); MS (FAB) m/z 208 (M^+ + 1). Anal. ($C_{10}H_9NO_4$) H, N; C: calcd, 57.97; found, 58.81.

3-Methyl-5-nitro-7-(benzyloxy)benzofuran. A mixture of 3-methyl-5-nitro-7-methoxybenzofuran (39.1 g, 189 mmol) and pyridinium hydrochloride (130 g, 1.12 mol) was melted in a 170 °C oil bath under nitrogen for 2 h. The reaction mixture was cooled to room temperature, dissolved in 500 mL of methylene chloride, and extracted with water (2 × 300 mL) and 1 N sodium hydroxide (3 × 150 mL). The methylene chloride layer was dried with anhydrous sodium sulfate, filtered, and concentrated under vacuum to provide 15.6 g of starting material (this material is recycled). The sodium hydroxide layer was acidified with 1 N HCl (585 mL) and extracted with methylene chloride (3 × 300 mL). The combined organic layer was dried with anhydrous sodium sulfate, filtered, and concentrated under vacuum to provide 24.3 g of solid. To this precipitate and benzyl bromide (15.5 mL, 130 mmol) dissolved in 250 mL of anhydrous N,N-dimethylformamide (DMF) was added 55% sodium hydride/oil (5.7 g, 130 mmol). This mixture was stirred under a drying tube for 5 h. The reaction mixture was concentrated under vacuum and dissolved in 1 L of methylene chloride. The organic layer was washed with water (3 × 300 mL), dried with anhydrous sodium sulfate, filtered, and concentrated under vacuum to provide 25.5 g of solid. Subjecting the 15.6 g of isolated starting material to the above procedure provided an additional 12 g of product (70% combined yield). Although this material was used crude in the next procedure, purification of an analytic sample by flash chromatography with 15% EtOAc/hexanes provided a solid: ^{1}H NMR (CD_3SOCD_3) δ 8.21 (d, J = 2 Hz, 1 H), 8.00 (d, J = 1 Hz, 1 H), 7.88 (d, J = 2 Hz, 1 H), 7.56–7.36 (m, 5 H), 5.43 (s, 2 H), 2.27 (d, J = 1 H, 3 H); MS (FAB) m/z 284 (M^+ + 1). Anal. ($C_{16}H_{13}NO_4$) H, N; C: calcd, 67.84; found, 69.89.

3-Methyl-5-(*N*-(*tert*-butyloxycarbonyl)amino)-7-(benzyloxy)benzofuran. A solution of 3-methyl-5-nitro-7-(benzyloxy)-benzofuran (10 g, 35 mmol) and platinum oxide (3 g) in 200 mL of ethyl acetate was hydrogenated at 20 psi of hydrogen for 1.5 h. The reaction was filtered through a plug of Celite and concentrated under vacuum. This residue and di-*tert*-butyl dicarbonate (15.4 g, 70.7 mmol) were dissolved in 130 mL of 1,4-dioxane, and the mixture was refluxed under nitrogen for 3 h. The reaction mixture was concentrated under vacuum and purified by flash chromatography with 15% EtOAc/hexanes to provide 8.0 g of solid (64% yield): ^{1}H NMR (CD_3SOCD_3) δ 9.27 (bs, 1 H), 7.68 (bs, 1 H), 7.51 (dd, J = 2, 9, Hz, 2 H), 7.46–7.34 (m, 4 H), 7.12 (bs, 1 H), 5.12 (s, 2 H), 2.16 (s, 3 H), 1.51 (s, 9 H); MS (FD) m/z 353 (M^+). Anal. ($C_{21}H_{23}NO_4$) C, H, N.

3-Methyl-4-bromo-5-(*N*-(*tert*-butyloxycarbonyl)amino)-7-(benzyloxy)benzofuran. To a solution of 3-methyl-5-(*N*-(*tert*-butyloxycarbonyl)amino)-7-(benzyloxy)benzofuran (8.0 g, 22.0 mmol) dissolved in 120 mL of anhydrous tetrahydrofuran (THF) cooled to −61 °C ($CHCl_3/CO_2$) was added one drop of concentrated sulfuric acid and N-bromosuccinimide (4.0 g, 22.0 mmol). This mixture was stirred at −61 °C for 5 h and then warmed to room temperature. The reaction mixture was concentrated under vacuum and purified by flash chromatography with 10% EtOAc/hexanes to obtain 8.7 g of solid (92% yield): ^{1}H NMR (CD_3SOCD_3) δ 8.57 (s, 1 H), 7.83 (s, 1 H), 7.50 (dd, J = 2, 9 Hz, 2 H), 7.44–7.34 (m, 3 H), 7.14 (s, 1 H), 5.15 (s, 2 H), 2.17 (s, 3 H), 1.47 (s, 9 H); MS (FD) m/z 431 (M^+) and 433 (M^+ + 2). Anal. ($C_{21}H_{22}BrNO_4$) C, H, N.

3-Methyl-4-bromo-5-(*N*-(*tert*-butyloxycarbonyl)-*N*-(2-propyn-1-yl)amino)-7-(benzyloxy)benzofuran. To a solution of 3-methyl-4-bromo-5-(*N*-(*tert*-butyloxycarbonyl)amino)-7-(benzyloxy)benzofuran (8.7 g, 20 mmol) and an 80% toluene solution of propargyl bromide (8.9 g, 60 mmol) dissolved in 130 mL of anhydrous DMF was added 60% sodium hydride in oil (0.84 g, 21 mmol). This mixture was stirred at room temperature for 3 h. The reaction was concentrated under vacuum, dissolved in 1 L of toluene, and extracted with water (3 × 300 mL). The organic layer was dried with anhydrous sodium sulfate, filtered, and concentrated under vacuum. The residue was purified by flash chromatography with 12.5% EtOAc/hexanes to obtain 9.0 g of oil (96% yield): ^{1}H NMR ($CDCl_3$) δ 7.50–7.30 (m, 6 H), 6.85 (s, 1 H), 5.25 (s, 2 H), 4.83 (d, J = 15 Hz, 1 H), 3.88 (d, J = 15 Hz, 1 H), 2.42 (s, 3 H), 2.05 (s, 1 H), 1.55 (s, 9 H); MS (FD) m/z (M^+) and 471 (M^+ + 2).

(+)-(1*R*)-1,2-Dihydro-1-(hydroxymethyl)-3-(*tert*-butyloxycarbonyl)-5-(benzyloxy)-8-methyl-3*H*-furano[3,2-*e*]indole. A solution of 3-methyl-4-bromo-5-(*N*-(*tert*-butyloxycarbonyl)-*N*-(2-propyn-1-yl)amino)-7-(benzyloxy)benzofuran (8.7 g, 18.5 mmol), tri-*n*-butyltin hydride (9.9 mL, 37 mmol), and 2,2′-azobis(2-methylpropionitrile) (0.62 g) in 320 mL of anhydrous benzene was refluxed for 35 min. The reaction mixture was cooled to room temperature and concentrated under vacuum. To this residue was added a ~0.7 M THF solution of monoisopinocampheylborane (38.2 mmol, freshly prepared from *S*-alpine boramine[18]) and the reaction mixture stirred at room temperature under nitrogen for 2 h. The reaction mixture was cooled to 0 °C, and to it were added sequentially dropwise water (20 mL), 2 N NaOH (20 mL), and 30% hydrogen peroxide (13 mL). This mixture was stirred at room temperature for 1 h and at 50 °C for 20 min. The reaction mixture was extracted with diethyl ether (3 × 100 mL), and the ether layer was dried with anhydrous magnesium sulfate, filtered, and concentrated under vacuum. The aliphatic tin byproducts were removed by dissolving this residue in acetonitrile and extracting with hexanes. The acetonitrile layer was concentrated under vacuum and the resulting foam purified by flash chromatography with 45% EtOAc/hexanes to provide 2.95 g of foam. This material had an enantiomeric excess of 50% of the (+)-(1*R*) isomer. Although further purification could be achieved by preparative high-pressure liquid chromatography of the (*R*)-(−)-*O*-acetylmandelate derivative of this foam,[14] recrystallization of the enhanced diastereomeric mixture from ether followed by lithium hydroxide mediated removal of the chiral auxiliary[25] provided 1.32 g of solid (17.5% overall yield): ^{1}H NMR ($CDCl_3$) δ 7.80 (bs, 1 H), 7.53–7.47 (d, J = 8 Hz, 2 H), 7.40–7.33 (m, 5 H), 5.25 (s, 2 H), 4.23–4.13 (m, 1 H), 4.05–3.95 (m, 1 H), 4.85–4.75 (m, 1 H), 4.61–4.60 (m, 2 H), 2.32 (s, 3 H), 1.57 (s, 9 H); MS (FD) m/z 409 (M^+); $[\alpha]_{589}$ +19.8° (c = 0.35 mg/dL, CH_2Cl_2). Anal. ($C_{24}H_{27}NO_5$) C, H, N.

(+)-(1*R*)-1,2-Dihydro-1-(chloromethyl)-3-(*tert*-butyloxycarbonyl)-5-(benzyloxy)-8-methyl-3*H*-furano[3,2-*e*]indole. To a solution of (+)-(1*R*)-1,2-dihydro-1-(hydroxymethyl)-3-(*tert*-butyloxycarbonyl)-5-(benzyloxy)-8-methyl-3*H*-furano[3,2-*e*]indole (0.47 g, 1.15 mmol) dissolved in 5 mL of anhydrous methylene chloride were added triphenylphosphine (0.6 g, 2.3 mmol) and carbon tetrachloride (0.67 mL, 6.9 mmol). This mixture was stirred under nitrogen at room temperature overnight. The reaction mixture was concentrated under vacuum and purified by flash chromatography with 7% EtOAc/hexanes to obtain 493 mg of solid (quantitative yield): ^{1}H NMR ($CDCl_3$) δ 7.75 (bs, 1 H), 7.49 (d, J = 7 Hz, 2 H), 7.39–7.29 (m, 5 H), 5.25 (s, 2 H), 4.24 (d, J = 11 Hz, 1 H), 4.02 (dd, J = 9, 11 Hz, 1 H), 3.82–3.69 (m, 2 H), 3.39 (dd, J = 11, 11 Hz, 1 H), 2.31 (s, 3 H), 1.62 (s, 9 H); MS (FD) m/z 429 (M^+ + 1); $[\alpha]_{589}$ −5.5° (c = 0.36 mg/dL, CH_2Cl_2). Anal. ($C_{24}H_{26}ClNO_4$) C, H, N.

(+)-(1*R*)-1,2-Dihydro-1-(chloromethyl)-3-(*tert*-butyloxycarbonyl)-5-hydroxy-8-methyl-3*H*-furano[3,2-*e*]indole. To a solution of (+)-(1*R*)-1,2-dihydro-1-(chloromethyl)-3-(*tert*-butyloxycarbonyl)-5-(benzyloxy)-8-methyl-3*H*-furano[3,2-*e*]indole (0.49 g, 1.2 mmol) dissolved in 15 mL of tetrahydrofuran (THF) under nitrogen at 0 °C were added 10% Pd/C (0.2 g) and a 25% aqueous solution of ammonium formate (2.0 mL). This suspension was stirred at 0 °C under nitrogen for 2.5 h. The reaction mixture was diluted with 250 mL of diethyl ether, filtered through a plug of Celite, and concentrated under vacuum to provide 359 mg of solid (92% yield): ^{1}H NMR ($CDCl_3$) δ 7.62 (bs,

1 H), 7.37 (d, J = 1 Hz, 1 H), 6.45 (bs, 1 H), 4.22 (d, J = 11 Hz, 1 H), 4.00 (dd, J = 9, 11 Hz, 1 H), 3.79–3.67 (m, 2 H), 3.38 (dd, J = 11, 11 Hz, 1 H), 2.29 (d, J = 1 Hz, 3 H), 1.58 (s, 9 H); MS (FD) m/z 337 (M^+); $[\alpha]_{589}$ −28.6° (c = 0.35 mg/dL, CH_2Cl_2). Anal. ($C_{17}H_{20}ClNO_4$) C, H, N.

General Method for Condensation of Arenecarboxylic Acid with Alkylation Subunit. (+)-(1*R*)-1,2-Dihydro-1-(chloromethyl)-3-(*tert*-butyloxycarbonyl)-5-hydroxy-8-methyl-3*H*-furano[3,2-*e*]indole (1 mmol) was stirred in 23 mL of freshly prepared 3 N HCl in ethyl acetate for 30 min. The reaction mixture was concentrated under vacuum. A solution of the residue, arenecarboxylic acid (1 mmol), and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (3 mmol) in 20 mL of anhydrous DMF was stirred at room temperature overnight. The reaction mixture was concentrated under vacuum and suspended in water and the precipitate collected by filtration. The residue was purified by flash chromatography with 20–40% THF/toluene.

(+)-(1*R*)-1,2-Dihydro-1-(chloromethyl)-3-((5-((1*H*-indol-2-ylcarbonyl)amino)-1*H*-indol-2-yl)carbonyl)-5-hydroxy-8-methyl-3*H*-furano[3,2-*e*]indole: ^{1}H NMR (CD_3SOCD_3) δ 10.13 (s, 1 H), 8.18 (s, 1 H), 7.79 (s, 1 H), 7.71 (s, 1 H), 7.63 (d, J = 8 Hz, 1 H), 7.53 (d, J = 8 Hz, 1 H), 7.45 (s, 1 H), 7.42 (s, 1 H), 7.38 (s, 1 H), 7.17 (dd, J = 8, 8 Hz, 1 H), 7.13 (s, 1 H), 7.02 (dd, J = 8, 8 Hz, 1 H), 4.67 (dd, J = 9, 9 Hz, 1 H), 4.52 (d, J = 9 Hz, 1 H), 4.03 (m, 1 H), 3.85 (dd, J = 2, 9 Hz, 1 H), 3.64 (dt, J = 2, 9 Hz, 1 H), 2.32 (s, 3 H); HRMS m/z 539.1482 ($C_{30}H_{24}ClN_4O_4$ requires 539.1486); $[\alpha]_{589}$ +85.8° (c = 0.5 mg/dL, DMF). Anal. ($C_{30}H_{23}ClN_4O_4$) H, N; C: calcd, 66.85; found, 66.16.

(+)-(1*R*)-1,2-Dihydro-1-(chloromethyl)-3-((5-((1*H*-indol-2-ylcarbonyl)amino)benzofuran-2-yl)carbonyl)-5-hydroxy-8-methyl-3*H*-furano[3,2-*e*]indole: ^{1}H NMR (CD_3SOCD_3) δ 10.42 (s, 1 H), 10.12 (s, 1 H), 8.18 (s, 1 H), 7.80 (s, 1 H), 7.76 (d, J = 6 Hz, 1 H), 7.70 (m, 3 H), 7.56 (d, J = 7 Hz, 1 H), 7.44 (m, 2 H), 7.34 (d, J = 7 Hz, 1 H), 7.14 (s, 1 H), 4.70 (t, J = 6 Hz, 1 H), 4.54 (d, J = 6 Hz, 1 H), 4.04 (t, J = 6 Hz, 1 H), 3.90 (d, J = 6 Hz, 1 H), 3.66 (t, J = 6 Hz, 1 H), 2.32 (s, 3 H); HRMS m/z 540.1326 ($C_{30}H_{23}ClN_3O_5$ requires 540.1326); $[\alpha]_{589}$ + 76.9° (c = 0.51 mg/dL, DMF). Anal. ($C_{30}H_{22}ClN_3O_5$) C, H, N.

(+)-(1*R*)-1,2-Dihydro-1-(chloromethyl)-3-((5-((1*H*-indol-2-ylcarbonyl)amino)benzo[*b*]thiophene-2-yl)carbonyl)-5-hydroxy-8-methyl-3*H*-furano[3,2-*e*]indole: ^{1}H NMR (CD_3SOCD_3) δ 10.24 (s, 1 H), 10.10 (s, 1 H), 8.32 (s, 1 H), 8.14 (s, 1 H), 8.02 (d, J = 7 Hz, 1 H), 7.98 (d, J = 7 Hz, 1 H), 7.80 (s, 1 H), 7.72 (s, 1 H), 7.56–7.40 (m, 5 H), 7.14 (s, 1 H), 4.66 (t, J = 6 Hz, 1 H), 4.52 (d, J = 6 Hz, 1 H), 4.02 (t, J = 6 Hz, 1 H), 3.90 (s, J = 6 Hz, 1 H), 3.64 (t, J = 6 Hz, 1 H), 2.30 (s, 3 H); HRMS m/z 556.1096 ($C_{30}H_{23}ClN_3O_4S$ requires 556.1098); $[\alpha]_{589}$ +73.7° (c = 0.5 mg/dL, DMF). Anal. ($C_{30}H_{22}ClN_3O_4S$) C, H; N: calcd, 7.56; fond, 6.62.

(+)-(1*R*)-1,2-Dihydro-1-(chloromethyl)-3-((5-((benzofuran-2-ylcarbonyl)amino)-1*H*-indol-2yl)carbonyl)-5-hydroxy-8-methyl-3*H*-furano[3,2-*e*]indole: ^{1}H NMR (CD_3SOCD_3) δ 10.32 (s, 1 H), 10.16 (s, 1 H), 8.30 (s, 1 H), 7.81–7.64 (m, 7 H), 7.46–7.42 (m, 2 H), 7.19 (dd, J = 8, 8 Hz, 1 H), 7.04 (dd, J = 8, 8 Hz, 1 H), 4.63 (t, J = 8 Hz, 1 H), 4.52 (d, J = 11 Hz, 1 H), 4.03 (t, J = 8 Hz, 1 H), 3.88 (d, J = 11 Hz, 1 H), 3.67 (t, J = 8 Hz, 1 H), 2.27 (s, 3 H); HRMS m/z 540.1322 ($C_{30}H_{23}ClN_3O_5$ requires 540.1326); $[\alpha]_{589}$ +68.6° (c = 0.35 mg/dL, DMF). Anal. ($C_{30}H_{22}ClN_3O_5 \cdot H_2O$) C, H, N.

(+)-(1*R*)-1,2-Dihydro-1-(chloromethyl)-3-((5-((benzofuran-2-ylcarbonyl)amino)benzofuran-2-yl)carbonyl)-5-hydroxy-8-methyl-3*H*-furano[3,2-*e*]indole: ^{1}H NMR (CD_3SOCD_3) δ 10.16 (s, 1 H), 8.30 (s, 1 H), 7.81–7.69 (m, 9 H), 7.47 (dd, J = 8, 8 Hz, 1 H), 7.34 (dd, J = 8, 8 Hz, 1 H), 4.62 (t, J = 9 Hz, 1 H), 4.52 (d, J = 10 Hz, 1 H), 4.03 (t, J = 9 Hz, 1 H), 3.87 (d, J = 10 Hz, 1 H), 3.67 (t, J = 9 Hz, 1 H), 2.26 (s, 3 H); HRMS m/z 541.1166 ($C_{30}H_{22}ClN_2O_6$ requires 541.1166); $[\alpha]_{589}$ +57.1° (c = 0.35 mg/dL, DMF). Anal. ($C_{30}H_{21}ClN_2O_6$) C: calcd, 66.61; found, 65.44; H: calcd, 3.91; found 4.68; N: calcd, 5.18; found, 4.16.

(+)-(1*R*)-1,2-Dihydro-1-(chloromethyl)-3-((5-((benzofuran-2-ylcarbonyl)amino)benzo[*b*]thiophene-2-yl)carbonyl)-5-hydroxy-8-methyl-3*H*-furano[3,2-*e*]indole: ^{1}H NMR (CD_3SOCD_3) δ 10.16 (s, 1 H), 8.35 (s, 1 H), 8.26 (s, 1 H), 8.04–7.97 (m, 3 H), 7.78–7.70 (m, 4 H), 7.49–7.43 (m, 3 H), 4.62 (t, J = 9 Hz, 1 H), 4.52 (d, J = 10 Hz, 1 H), 4.03 (t, J = 9 Hz, 1 H), 3.86 (d, J = 10 Hz, 1 H), 3.66 (t, J = 9 Hz, 1 H), 2.26 (s, 3 H); HRMS m/z 557.0919 ($C_{30}H_{22}ClN_2O_5S$ requires 557.0938); $[\alpha]_{589}$ + 57.1° (c = 0.35 mg/dL, DMF). Anal. ($C_{30}H_{21}ClN_2O_5S$) C, H, N.

(+)-(1*R*)-1,2-Dihydro-1-(chloromethyl)-3-((5-((benzo[*b*]thiophene-2-ylcarbonyl)amino)-1*H*-indol-2-yl)carbonyl)-5-hydroxy-8-methyl-3*H*-furano[3,2-*e*]indole: ^{1}H NMR (CD_3SOCD_3) δ 10.35 (s, 1 H), 10.14 (s, 1 H), 8.54 (s, 1 H), 8.08 (s, 1 H), 8.00 (d, J = 8 Hz, 1 H), 7.78 (d, J = 9 Hz, 1 H), 7.71 (s, 1 H), 7.65 (d, J = 8 Hz, 1 H), 7.45 (d, J = 9 Hz, 1 H), 7.43 (s, 1 H), 7.18 (dd, J = 8, 8 Hz, 1 H), 7.04 (dd, J = 8, 8 Hz, 1 H), 4.64 (t, J = 9 Hz, 1 H), 4.41 (d, J = 9 Hz, 1 H), 4.03 (m, 1 H), 3.92 (d, J = 8 Hz, 1 H), 3.68 (t, J = 9 Hz, 1 H), 2.26 (s, 3 H); HRMS m/z 556.1110 ($C_{30}H_{23}ClN_3O_4S$ requires 556.1098); $[\alpha]_{589}$ +117.1° (c = 0.35 mg/dL, DMF).

(+)-(1*R*)-1,2-Dihydro-1-(chloromethyl)-3-((5-((benzo[*b*]thiophene-2-ylcarbonyl)amino)benzofuran-2-yl)carbonyl)-5-hydroxy-8-methyl-3*H*-furano[3,2-*e*]indole: ^{1}H NMR (CD_3SOCD_3) δ 10.14 (s, 1 H), 8.57 (s, 1 H), 8.09 (s, 1 H), 8.01 (d, J = 9 Hz, 1 H), 7.83–7.80 (m, 3 H), 7.78–7.72 (m, 2 H), 7.48 (dd, J = 8, 8 Hz, 1 H), 7.34 (dd, J = 8, 8 Hz, 1 H), 7.20–7.12 (m, 2 H), 4.67 (t, J = 8 Hz, 1 H), 4.41 (d, J = 9 Hz, 1 H), 4.02 (t, J = 8 Hz, 1 H), 3.89 (d, J = 9 Hz, 1 H), 3.68 (t, J = 8 Hz, 1 H), 2.26 (s, 3 H); HRMS m/z 557.0945 ($C_{30}H_{22}ClN_2O_5S$ requires 557.0938); $[\alpha]_{589}$ +108.6° (c = 0.35 mg/dL, DMF). Anal. ($C_{30}H_{21}ClN_2O_5S$) C, H, N.

(+)-(1*R*)-1,2-Dihydro-1-(chloromethyl)-3-((5-((benzo[*b*]thiophene-2-ylcarbonyl)amino)benzo[*b*]thiophene-2-yl)carbonyl)-5-hydroxy-8-methyl-3*H*-furano[3,2-*e*]indole: ^{1}H NMR (CD_3SOCD_3) δ 10.14 (s, 1 H), 8.51 (s, 1 H), 8.37 (s, 1 H), 8.08 (s, 1 H), 8.04–7.98 (m, 3 H), 7.76–7.68 (m, 3 H), 7.50–7.41 (m, 3 H), 4.66 (t, J = 8 Hz, 1 H), 4.40 (d, J = 9 Hz, 1 H), 3.99 (t, J = 8 Hz, 1 H), 3.87 (d, J = 9 Hz, 1 H), 3.67 (t, J = 8 Hz, 1 H), 2.26 (s, 3 H); HRMS m/z 573.0710 ($C_{30}H_{22}ClN_2O_4S_2$ requires 573.0673); $[\alpha]_{589}$ + 113.5° (c = 0.52 mg/dL, DMF). Anal. ($C_{30}H_{21}ClN_2O_4S_2$) H, N; C: calcd, 62.88; found, 63.76.

Acknowledgment. The authors would like to thank Paul Pranc for providing us with 45 g of 3-methyl-5-nitro-7-methoxybenzofuran, the Lilly Cancer Strategy Group, and the National Institutes of Health for financial support (D.L.B., CA41986).

References

(1) Martin, D. G.; Chidester, C.G.; Duchamp, D. J.; Mizsak, S. A. Structure of CC-1065 (NSC-298223), a New Antitumor Antibiotic. *J. Antibiot.* **1980**, *33*, 902–903. Hanka, L. J.; Deitz, A.; Gerpheide, S. A.; Kuentzel, S. L.; Martin, D. G. CC-1065 (NSC-298223), a New Antitumor Antibiotic. Production, In Vitro Biological Activity, Microbiological Assays and Taxonomy of the Producing Microorganism. *J. Antiobiot.* **1978**, *31*, 1211–1217.

(2) Hurley, L. H.; Reynolds, V. L.; Swenson, D. H.; Petzold, G. L.; Scahill, T. A. Reaction of the Antitumor Antibiotic CC-1065 with DNA: Structure of a DNA Adduct with DNA Sequence Specificity. *Science* **1984**, *226*, 843–844.

(3) Li, L. H.; Swenson, D. H.; Schpok, S.; Kuentzel, S. L.; Dayton, B. D.; Krueger, W. C. CC-1065 (NSC 298223), a Novel Antitumor Agent that Interacts Strongly with Double-Stranded DNA. *Cancer Res.* **1982**, *42*, 999–1004. Bhuyan, B. K.; Newell, K. A.; Crampton, S. L.; Von Hoff, D. D. CC-1065 (NSC 298223), a Most Potent Antitumor Agent: Kinetics of Inhibition of Growth, DNA Synthesis, and Cell Survival. *Cancer Res.* **1982**, *42*, 3532–3537.

(4) Martin, D. G.; Biles, C.; Gerpheide, S. A.; Hanka, L. J.; Krueger, W. C.; McGovren, J. P.; Mizsak, S. A.; Neil, G. L.; Stewart, J. C.; Visser, J. CC-1065 (NSC 298223), a Potent New Antitumor Agent. Improved Production and Isolation, Characterization and Antitumor Activity. *J. Antibiot.* **1981**, *34*, 1119–1125.

(5) Reynolds, V. L.; McGovren, J. P.; Hurley, L. H. The Chemistry, Mechanism of Action and Biological Properties of CC-1065, a Potent Antitumor Antibiotic. *J. Antibiot.* **1986**, *39*, 319–334. Hurley, L. H.; Needham-VanDevanter, D. R. Covalent Binding of Antitumor Antibiotics in the Minor Groove of DNA. Mechanism of Action of CC-1065 and the Pyrrolo(1,4)benzodiazepines. *Acc. Chem. Res.* **1986**, *19*, 230–237.

(6) McGovren, J. P.; Clarke, G. L.; Pratt, E. A.; DeKoning, T. F. Preliminary Toxicity Studies with the DNA-Binding Antibiotic, CC-1065. *J. Antibiot.* **1984**, *37*, 63–70.

(7) Warpehoski, M. A.; Bradford, V. S. Bis-dehydroxy, Bis-demethoxy CC-1065. Synthesis, DNA Binding, and Biological Activity. *Tetrahedron Lett.* **1988**, *29*, 131–134.

(8) Wierenga, W. Sequence-selective DNA-interactive Antitumor Agents. *Drugs Future* **1991**, *16*, 741–750. Li, L. H.; Kelly, R. C.; Warpehoski, M. A.; McGovren, J. P.; Gebhard, I.; DeKoning, T. F. Adozelesin. A Selected Lead Among Cyclopropylpyrroloindole

Analogs of the DNA-binding Antibiotic CC-1065. *Invest. New. Drugs.* **1991**, *9*, 137–148. Warpehoski, M. A. Dissecting the Complex Structure of CC-1065. *Drugs Future* **1991**, *16*, 131–141. Li, L. H.; Wallace, T. L.; DeKoning, T. F.; Warpehoski, M. A.; Kelly, R. C.; Prairie, M. D.; Krueger, W. C. Structure and Activity Relationship of Several Novel CC-1065 Analogs. *Invest. New Drugs* **1987**, *5*, 329–338. Warpehoski, M. A.; Gebhard, I.; Kelly, R. C.; Krueger, W. C.; Li, L. H.; McGovren, J. P.; Prairie, M. D.; Wicnienski, N.; Wierenga, W. Stereoelectronic Factors Influencing the Biological Activity and DNA Interaction of Synthetic Antitumor Agents Modeled on CC-1065. *J. Med. Chem.* **1988**, *31*, 590–603. Wierenga, W.; Bhuyan, B. K.; Kelly, R. C.; Krueger, W. C.; Li, L. H.; McGovren, J. P.; Swenson, D. H.; Warpehoski, M. A. Antitumor Activity and Biochemistry of Novel Analogs of the Antibiotic CC-1065. *Adv. Enzyme Regul.* **1986**, *25*, 141–156. Warpehoski, M. A. Total Synthesis of U-71184. A Potent New Antitumor Agent Modeled on CC-1065. *Tetrahedron Lett.* **1986**, *27*, 4103–4106.

(9) Ichimura, M.; Ogawa, T.; Takahashi, K.-I.; Kobayashi, E.; Kawamoto, I.; Yasuzawa, T.; Takahashi, I.; Nakano, H. Duocarmycin SA. A New Antitumor Antibiotic from Streptomyces sp. *J. Antibiot.* **1990**, *43*, 1037–1038. Ishii, S.; Nagasawa, M.; Kariya, Y.; Yamamoto, H.; Inouye, S.; Kondo, S.; Antitumor Activity of Pyrindamycins A and B. *J. Antibiot.* **1989**, *42*, 1713–1717. Ogawa, T.; Ichimura, M.; Katsumata, S.; Morimoto, M.; Takahashi, K. New Antitumor Antibiotics, Duocarmycins B_1 and B_2. *J. Antibiot.* **1989**, *42*, 1299–1301. Ohba, K.; Watabe, H-O.; Sasaki, T.; Takeuchi, Y.; Kodama, Y.; Nakazama, T.; Yamamoto, H.; Shomura, T.; Sezaki, M.; Kondo, S. Pyrindamycins A and B, New Antitumor Antibiotics. *J. Antibiot.* **1988**, *41*, 1515–1519. Takahashi, I.; Takahashi, K.-I.; Ichimura, M.; Morimoto, M.; Asano, K.; Kawamoto, I.; Tomita, F.; Nakano, H. Duocarmycin A, A New Antitumor Antibiotic from Streptomyces. *J. Antibiot.* **1988**, *41*, 1915–1917. Fukuda, Y.; Nakatani, K.; Ito, Y.; Terashima, S. First Total Synthesis of dl-Duocarmycin A. *Tetrahedron Lett.* **1990**, *31*, 6699–6702. Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. Duocarmycin-Pyrindamycin DNA Alkylation Properties and Identification, Synthesis, and Evaluation of Agents Incorporating the Pharmacophore of the Duocarmycin-Pyrindamycin Alkylation Subunit. Identification of the CC-1065 Duocarmycin Common Pharmacophore. *J. Am. Chem. Soc.* **1990**, *112*, 8961–8971. Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H. *J. Am. Chem. Soc.* **1991**, *113*, 6645. Sugiyama, H.; Hosoda, M.; Saito, I. Covalent Alkylation of DNA with Duocarmycin A. Identification of Abasic Site Structure. *Tetrahedron Lett.* **1990**, *31*, 7197–7200.

(10) Boger, D. L.; Coleman, R. S. Total Synthesis of (+)- and (−)-CPI-CDPI$_2$: (+)-(3*βR*,4*αS*)- and (−)-(3*βS*,4*αR*)-deoxy-CC-1065. *J. Am. Chem. Soc.* **1988**, *110*, 4796–4807. Boger, D. L.; Coleman, R. S. Total Synthesis of (+)-CC-1065 and ent-(−)-CC-1065. *J. Am. Chem. Soc.* **1988**, *110*, 1321–1323. Kelly, R. C.; Gebhard, I.; Wicnienski, N.; Aristoff, P. A.; Johnson, P. D.; Martin, D. G. Coupling of Cyclopropapyrroloindole (CPI) Derivatives. The Preparation of CC-1065, ent-CC-1065, and Analogs. *J. Am. Chem. Soc.* **1987**, *109*, 6837–6838.

(11) For excellent reviews, see: Coleman, R. S.; Boger, D. L. Natural and Synthetic Antitumor Agents: Synthetic Studies on CC-1065 and Functionally Related Agents. *Studies in Natural Products Chemistry*; Elsevier: Amsterdam, 1989; Vol. 3, pp 301–383. Remers, W. A. CC-1065. *The Chemistry of Antitumor Antibiotics*; John Wiley and Sons: New York, 1988; Vol. 2, pp 146–185. For the first synthesis of the alkylating portion of CC-1065, see: Wierenga, W. Synthesis of the Left-Hand Segment of the Antitumor Agent CC-1065. *J. Am. Chem. Soc.* **1981**, *103*, 5621–5623.

(12) (a) Hurley, L. H.; Warpehoski, M. A.; Lee, C.-S.; McGovren, J. P.; Scahill, T. A.; Kelly, R. C.; Mitchell, M. A.; Wicniehski, N. A.; Gebhard, I.; Johnson, P. D.; Bradford, V. S. Sequence Specificity of DNA Alkylation by the Unnatural Enantiomer of CC-1065 and its Synthetic Analogs. *J. Am. Chem. Soc.* **1990**, *112*, 4633–4649. (b) Boger, D. L.; Coleman, R. S.; Invergo, B. J.; Sakya, S. M.; Ishizaki, T.; Munk, S. A.; Zarrinmayeh, H.; Kitos, P. A. Synthesis and Evaluation of Aborted and Extended CC-1065 Functional Analogs: (+)- and (−)-CPI-PDE-I_1, (+)- and (−)-CPI-CDPI$_1$, and (.+-.)-, (+)-, and (−)-CPI-CDPI$_3$. Preparation of Key Partial Structures and Definition of an Additional Functional Role of the CC-1065 Central and Right-Hand Subunits. *J. Am. Chem. Soc.* **1990**, *112*, 4623–4632. (c) Scahill, T. A.; Jensen, R. M.; Swenson, D. H.; Hatzenbuhler, N. T.; Petzold, G.; Wierenga, W.; Brahme, N. D. An NMR Study of the Covalent and Noncovalent Interactions of CC-1065 and DNA. *Biochemistry* **1990**, *29*, 2852–2860. Hurley, L. H.; Needham-VanDevanter, D. R.; Lee, C.-S. Demonstration of the Asymmetric Effect of CC-1065 on Local DNA Structure Using a Site-Directed Adduct in a 117-Base-Pair Fragment from M13mp1. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 6412–6417.

(13) Boger, D. L.; Ishizaki, T.; Kitos, P. A.; Suntornwat, O. *J. Org. Chem.* **1990**, *55*, 5823. Boger, D. L.; Ishizaki, T.; Wysocki, R. J.; Munk, S. A. Total Synthesis and Evaluation of (.+-.)-N-(*tert*-butoxycarbonyl)-CBI, (.+-.)-CBI-CDPI$_1$, and (.+-.)-CBI-CDPI$_2$: CC-1065 Functional Agents Incorporating the Equivalent 1,2,9,9*a*-Tetrahydrocyclopropa[1,2-*c*]benz[1,2-*e*]indol-4-one (CBI) Left-Hand Subunit. *J. Am. Chem. Soc.* **1989**, *111*, 6461–6463.

(14) Boger, D. L.; Ishizaki, T. Resolution of a CBI Precursor and Incorporation into the Synthesis of (+)-CBI, (+)-CBI-CDPI$_1$, (+)-CBI-CDPI$_2$: Enhanced Functional Analogs of (+)-CC-1065. A Critical Appraisal of a Proposed Relationship Between Electrophile Reactivity, DNA Binding Properties, and Cytotoxic Potency. *Tetrahedron Lett.* **1990**, *31*, 793–796.

(15) The arenecarboxylic acid was synthesized by the coupling of indole-2-carboxylic acid, benzofuran-2-carboxylic acid or benzothiophene-2-carboxylic acid with ethyl 5-aminoarene-2-carboxylate in DMF with EDCI, and saponification of the ester. Ethyl 5-aminoindole-2-carboxylate was formed by catalytic hydrogenation of ethyl 5-nitroindole-2-carboxylate (Parmerter, S. M.; Cook, A. G.; Dixon, W. B. *J. Am. Chem. Soc.* **1958**, *80*, 4621). Ethyl 5-aminobenzothiophene-2-carboxylate was synthesized as described in the following: Zambias, R. A.; Hammond, M. L. The Synthesis of 5-Hydroxy-2,3-dihydrobenzo(b)thiophene via an Efficient One Step Preparation of 5-Nitrobenzo(b)thiophene-2-carboxylate. *Synth. Commun.* **1991**, *21*, 959–964. Ethyl 5-aminobenzofuran-2-carboxylate was prepared from the commercially available 5-nitrobenzofuran-2-carboxylic acid.

(16) The Upjohn synthesis uses a recrystallization of a *N*-(*tert*-butyloxycarbonyl)trypthophan ester of an intermediate in their synthesis to obtain chiral material.

(17) Brown, H. C.; Jadhav, P. K.; Mandal, A. K. Asymmetric Syntheses via Chiral Organoborane Reagents. *Tetrahedron* **1981**, *37*, 3547–3587.

(18) *R*-Alpine-boramine is available from Aldrich Chemical Company, but *S*-alpine-boramine was synthesized from (−)-pinene and used to prepare monoisopinocampheylborane as described in: Mandal, A. K.; Jadhav, P. K.; Brown, H. C. Monoisopinocampheylborane: An Excellent Chiral Hydroborating Agent for Phenyl-Substituted Tertiary Olefins. Synthesis of Alcohols Approaching 100% Enantiomeric Excess. *J. Org. Chem.* **1980**, *45*, 3543–3544.

(19) Although useful with regard to the formation of larger quantities of the desired enantiomer, purification via the (*R*)-(−)-*O*-acetylmandelate is necessary.

(20) The chemical yield is calculated from the amount of bromoacetylene used and is not optimized. The extent of asymmetric induction is determined by analytical HPLC and ^{1}H NMR analysis of the (*R*)-(−)-*O*-acetylmandelate.

(21) For a chiral osmylation approach to the synthesis of the CBI alkylation subunit, see: Aristoff, P. A.; Johnson, P. D. Synthesis of CBI-PDE-I-Dimer, the Benzannelated Analogue of CC-1065. *J. Org. Chem.* **1992**, *57*, 6234–6239.

(22) This reagent can in principle be prepared from (*S*)-2,2′-bis-(bromomethyl)-1,1′-binaphthyl (Maigrot, N.; Mazaleyrat, J.-P. New and Improved Synthesis of Optically Pure (*R*)- and (*S*)-2,2′-Dimethyl-1,1′-binaphthyl and Related Compounds. *Synthesis* **1985**, 317–320) in analogy to the method described in ref 22 for the synthesis of 2,5-dimethylborolane.

(23) Masamune, S.; Kim, B. M.; Petersen, J. S.; Sato, T.; Veenstra, S. J.; Imai, T. Organoboron Compounds in Organic Synthesis. 1. Asymmetric Hydroboration. *J. Am. Chem. Soc.* **1985**, *107*, 4549–4551.

(24) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. An Alternative and Convenient Strategy for Generation of Substantial Quantities of Singly 5′-^{32}P-End-Labeled Double-Stranded DNA for Binding Studies: Development of a Protocol for Examination of Functional Features of (+)-CC-1065 and the Duocarmycins that Contribute to their Sequence-Selective DNA Alkylation Properties. *Tetrahedron* **1991**, *47*, 2661–2682.

(25) Ambrose, C.; Rajadhyaksha, A.; Lowman, H.; Bina, M. Locations of Nucleosomes on the Regulatory Region of Simian Virus 40 Chromatin. *J. Mol. Biol.* **1989**, *210*, 255–263.

(26) Boger, D. L.; Munk, S. A. DNA Alkylation Properties of Enhanced Functional Analogs of CC-1065 Incorporating the 1,2,9,9*a*-Tetrahydrocyclopropa[1,2-*c*]benz[1,2-*e*]indol-4-one (CBI) Alkylation Subunit. *J. Am. Chem. Soc.* **1992**, *114*, 5487–5496.

(27) Boger, D. L.; Ishizaki, T.; Sakya, S. M.; Munk, S. A.; Kitos, P. A.; Jin, Q.; Besterman, J. Synthesis and Preliminary Evaluation of (+)-CBI-indole$_2$: An Enhanced Functional Analog of (+)-CC-1065. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 115–120.

(28) Masui, H.; Kawamoto, T.; Sato, J. D.; Wolf, B.; Sato, G.; Mendelsohn, J. Growth Inhibition of Human Tumor Cells in Athymic Mice by Anti-Epidermal Growth Factor Receptor Monoclonal Antibodies. *Cancer Res.* **1984**, *44*, 1002–1007.

(29) Varki, N. M.; Reisfield, R. A.; Walker, L. E. Antigens Associated with a Human Lung Adenocarcinoma Defined by Monoclonal Antibodies. *Cancer Res.* **1984**, *44*, 681–687.

(30) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems. *Cancer Chemotherap. Rep. Part 3*; Natl. Cancer Inst., Natl. Inst. Health: Bethesda, MD, 1972; 103 pp.

(31) Marder, P.; Apelgren, L. D.; Bumol, T. F. Comparative Analysis of Monoclonal Antibody-Drug Conjugate Binding by Flow Cytometry. *J. Immunol. Methods* **1987**, *96*, 165–170.