Synthesis and Evaluation of Aborted and Extended CC-1065 Functional Analogues: (+)- 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

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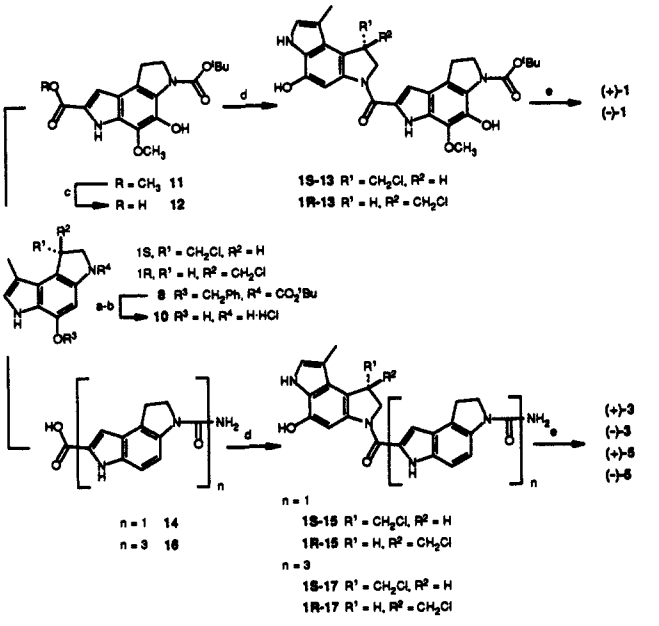
Abstract: Full details of the incorporation of (±)-(1*R**)-**8**, (−)-(1*S*)-**8**, and (+)-(1*R*)-**8** into the total syntheses of the aborted and extended CC-1065 functional analogues (+)- and (−)-CPI-PDE-I_1 [(+)- and (−)-**1**], (+)- and (−)-CPI-$CDPI_1$ [(+)- and (−)-**3**], and (±)-, (+)-, and (−)-CPI-$CDPI_3$ [(±)-, (+)-, and (−)-**5**] are described. Comparative DNA binding studies of the agents versus (+)-*N*-BOC-CPI (**6**), (+)-*N*-acetyl-CPI (**7**), (+)-CC-1065 (**2**), and (+)-/(−)-CPI-$CDPI_2$ (**4**) are presented in efforts to establish the structural and functional features of CC-1065 responsible for its sequence-selective B-DNA minor groove association and the resulting potent cytotoxic activity. The results of the comparative study suggest that the agents noncovalent binding contributes to the DNA covalent alkylation selectivity and stabilizes the DNA–agent covalent complex formation. It is suggested that it is the simple event of DNA covalent complex stabilization that potentiates the cytotoxic activity of the agents.

CC-1065 (**2**, NSC-298223), an antitumor antibiotic isolated from cultures of *Streptomyces zelensis*,[2] has been shown to possess exceptionally potent in vitro cytotoxic activity,[3] broad spectrum antimicrobial activity,[2] and potent in vivo antitumor activity.[4-6] In an extensive series of studies, (+)-CC-1065 has been shown to bind within the double-stranded B-DNA minor groove in an initial high-affinity, nonintercalative manner and subsequently forms irreversible covalent adducts.[6-9] The irreversible minor groove covalent alkylation has been shown to proceed by 3′-adenine N-3 alkylation of the electrophilic left-hand segment (CPI) of (+)-CC-1065 within two consensus sequences, 5′-d(A/GNTTA)-3′ and 5′-d(AAAAA)-3′.[10-15] The CC-1065 cytotoxic potency and antitumor activity has been correlated with its sequence-selective minor groove binding properties and has been proposed to be derived from (1) the inhibition of the normal unwinding and strand separation process required for DNA synthesis,[7,12] (2) the inhibition or alteration of replication and transcription enzyme action proximal or distal to its binding regions of DNA,[8,14] or (3) through the induction of unbalanced cell growth.[16]

Extensive efforts disclosed from the Upjohn laboratories have described the preparation and evaluation of simplified analogues of CC-1065, e.g., U-71184, bearing modified central and right-hand subunits that possess comparable in vitro cytotoxic activity and improved in vivo antitumor activity,[17,18] exhibit a comparable sequence-selective adenine N-3 alkylation of DNA,[10,17,18] lack the characteristic delayed fatal toxicity of CC-1065,[18,19] and in which the antitumor activity was determined to be restricted to the agent enantiomer bearing the natural (3b*R*,4a*S*)-CPI left-hand segment.[17-20] In sharp contrast, concurrent efforts have demonstrated that the enantiomeric pairs, (+)-CC-1065/*ent*-(−)-CC-1065 [(+)-**2** and (−)-**2**][21] and (+)-CPI-$CDPI_2$/(−)-CPI-$CDPI_2$ [(+)-**4** and (−)-**4**],[22-24] possess indistinguishable in vitro cytostatic activity and comparable in vivo antitumor activity. Consequently, our continued examination[25-30] of the CC-1065 structural features responsible for agent binding within the B-DNA minor groove has focused in part on the role and the extent to which the CC-1065 central and right-hand segments contribute to the affinity, specificity, and resulting biological consequences of the DNA–CC-1065 association.[25-27,31]

Scheme I[a]



[a] Reagents and conditions: (a) 25% aqueous HCO_2NH_4/THF (1:4), 10% Pd/C, 23 °C, 1 h, 100%; (b) 3 N HCl/EtOAc, 23 °C, 30–85 min; (c) 10 equiv of LiOH, 1.0 equiv of $Na_2S_2O_4$, THF/CH_3OH/H_2O (3:2:1), 50 °C, 1.5 h, 82%; (d) 2.7–3.1 equiv of EDCI, 0.8 equiv of **12**, 0.9 equiv of **14** or **16**, $NaHCO_3$, DMF, 23 °C, 27 h (80–90% for **13**, 90–100% for **15** and **17**); (e) Et_3N/CH_3CN/H_2O (1:1:1), 23 °C, 3 h; 79% (**1**), 83% (**3**), 50–55% (**5**).

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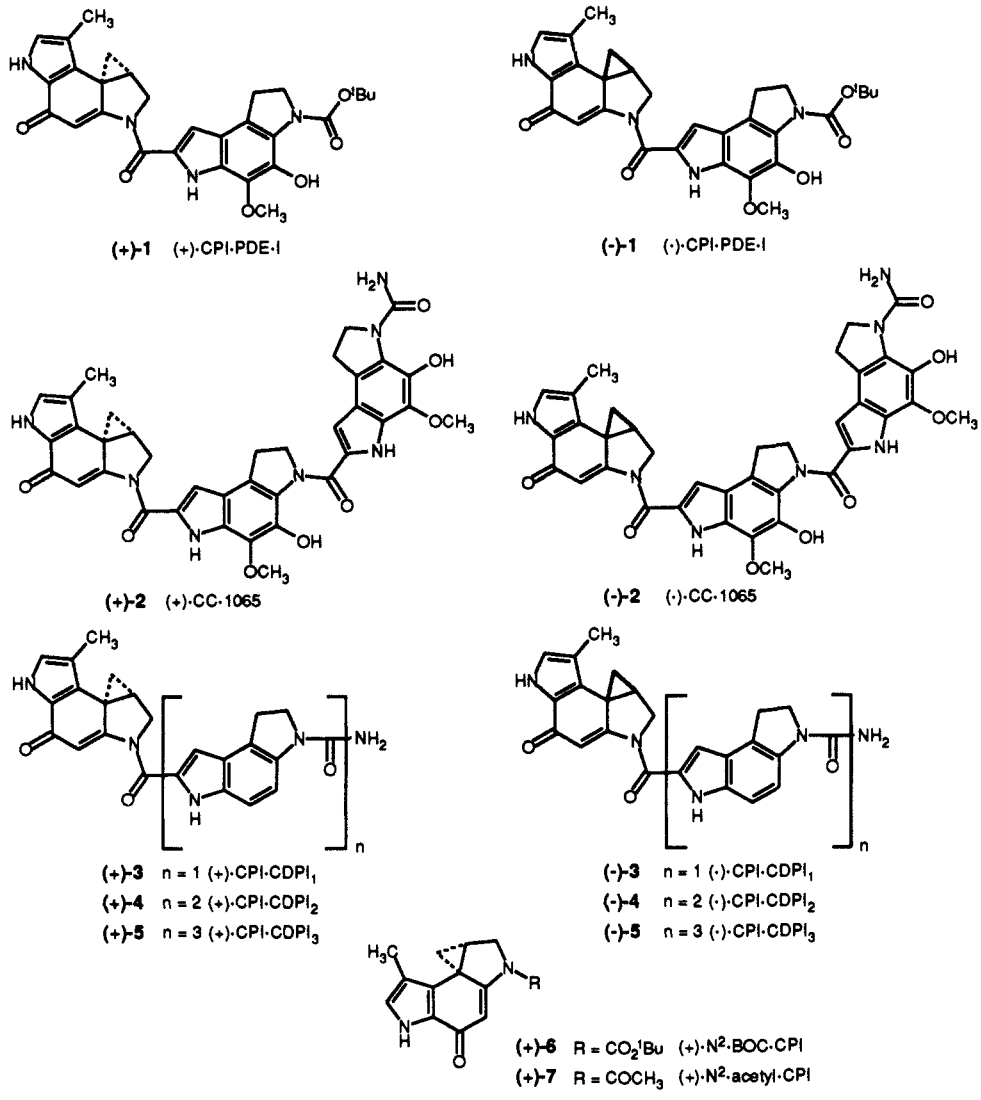

Figure 1.

In efforts to establish the comparative properties of a full range of agents, herein we provide details of the incorporation of (±)-(1*R**)-**8**, (-)-(1*S*)-**8**, and (+)-(1*R*)-**8** into the synthesis of the aborted and extended functional analogues of CC-1065, (+)- and

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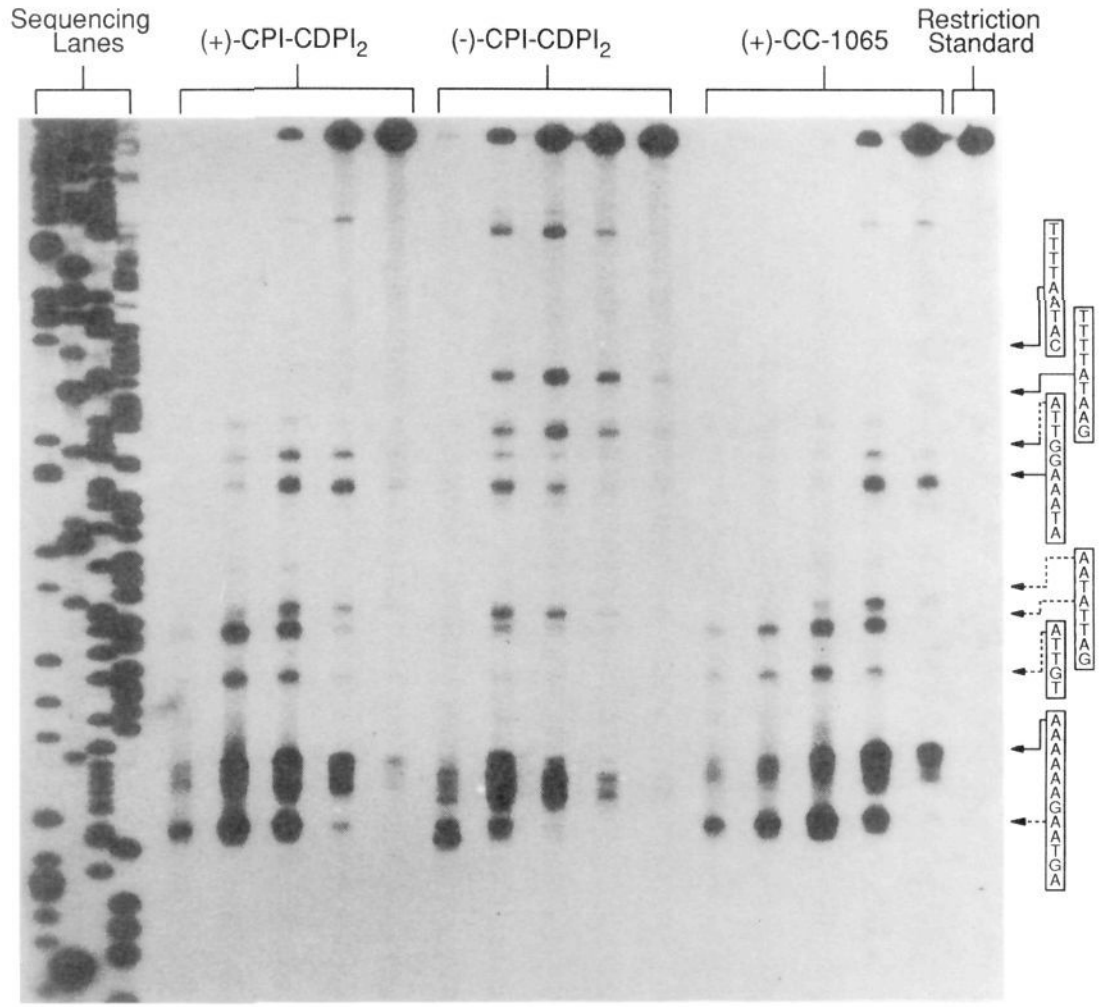

Figure 2. Thermally induced strand cleavage of a double-stranded SV40 fragment (150 bp; nucleotide no. 4210–4359, clone c988) after 24-h incubation of agent–DNA at 4 °C followed by removal of unbound agent and 30-min incubation at 100 °C; 8% denaturing poly(acrylamide) gel, 1100 V/6 h, and autoradiography. Lane 1, Sanger G reaction; lane 2, Sanger C reaction; lane 3, Sanger A reaction; lane 4, Sanger T reaction; lanes 5–9, (+)-CPI-CDPI$_2$ (5.6×10^{-4}–5.6×10^{-8} M); lanes 10–14, (−)-CPI-CDPI$_2$ (5.6×10^{-4}–5.6×10^{-8} M); lanes 15–19, (+)-CC-1065 (5.6×10^{-4}–5.6×10^{-8} M); lane 20, control DNA.

(−)-**1**, (+)- and (−)-**3**, and (+)- and (−)-**5** (Figure 1), and the definition of two additional functional roles of the CC-1065 central and right-hand subunits.

Synthesis of (+)- and (−)-CPI-PDE-I$_1$ [Aborted (+)- and (−)-CC-1065], (+)- and (−)-CPI-CDPI$_1$, and (±)-, (+)-, and (−)-CPI-CDPI$_3$. The preparation of the series of optically active CC-1065 partial structures **1**, **3**, and **5** is detailed in Scheme I. Following conditions previously described,[21a] removal of the benzyl ether of (−)-(1*S*)-**8**[22] and (+)-(1*R*)-**8**[22] by two-phase, transfer catalytic hydrogenolysis (25% aqueous HCO_2NH_4/THF 1:4, 10% Pd/C, 23 °C) provided the free phenols (1*S*)-**9** and (1*R*)-**9**. Treatment of (1*S*)-**9** and (1*R*)-**9** with anhydrous hydrochloric acid (3 N HCl in EtOAc, 23 °C, 30–85 min) provided the unstable indoline hydrochlorides (1*S*)-**10** and (1*R*)-**10**, which were coupled directly with N^3-BOC-PDE-I$_1$ (**12**),[28,32] CDPI$_1$ (**14**),[25] and CDPI$_3$ (**16**)[25] in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI, 0.8 equiv of **12** or 0.9 equiv of **14** and **16**, $NaHCO_3$, DMF, 23 °C, 27 h) to afford (1*S*)-**13**/(1*R*)-**13**, (1*S*)-**15**/(1*R*)-**15**, and (1*S*)-**17**/(1*R*)-**17**, respectively. Attempts to purify the coupled seco-CPI-CDPI$_n$ agents (**15** and **17**) by standard chromatographic techniques (SiO_2, 0–100% DMF–toluene gradient elution) served to promote the Ar-3′ intramolecular alkylation and provided CPI-CDPI$_1$ (**3**) and CPI-CDPI$_3$ (**5**). Consequently, pure samples of the coupled seco-CPI-CDPI$_n$ agents necessarily were obtained by extensive trituration. Deliberate, final Ar-3′ spirocyclization employing the conditions introduced by Kelly and co-workers (1:1:1 Et_3N/H_2O/CH_3CN, 23 °C, 3 h)[21a] provided (+)- and (−)-**1**, (+)- and (−)-CPI-CDPI$_1$ (**3**), and (±)-, (+)-, and (−)-CPI-CDPI$_3$ (**5**), respectively. As a result of the sensitivity of the seco-CPI-CDPI$_n$ and seco-CPI-PDE-I$_n$ to chromatographic purification, the final coupling and closure were found to be most effectively conducted without the intermediate purification of the seco agents.[33,34]

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(31) The magnitude and relative importance of this binding selectivity goes beyond the "mass action" role initially assigned to the CC-1065 central and right-hand subunits; see refs 21c and 10c.
(32) Conducting the lithium hydroxide promoted methyl ester hydrolysis of **11** in the absence of sodium dithionite afforded discolored product of unsuitable purity for subsequent use.

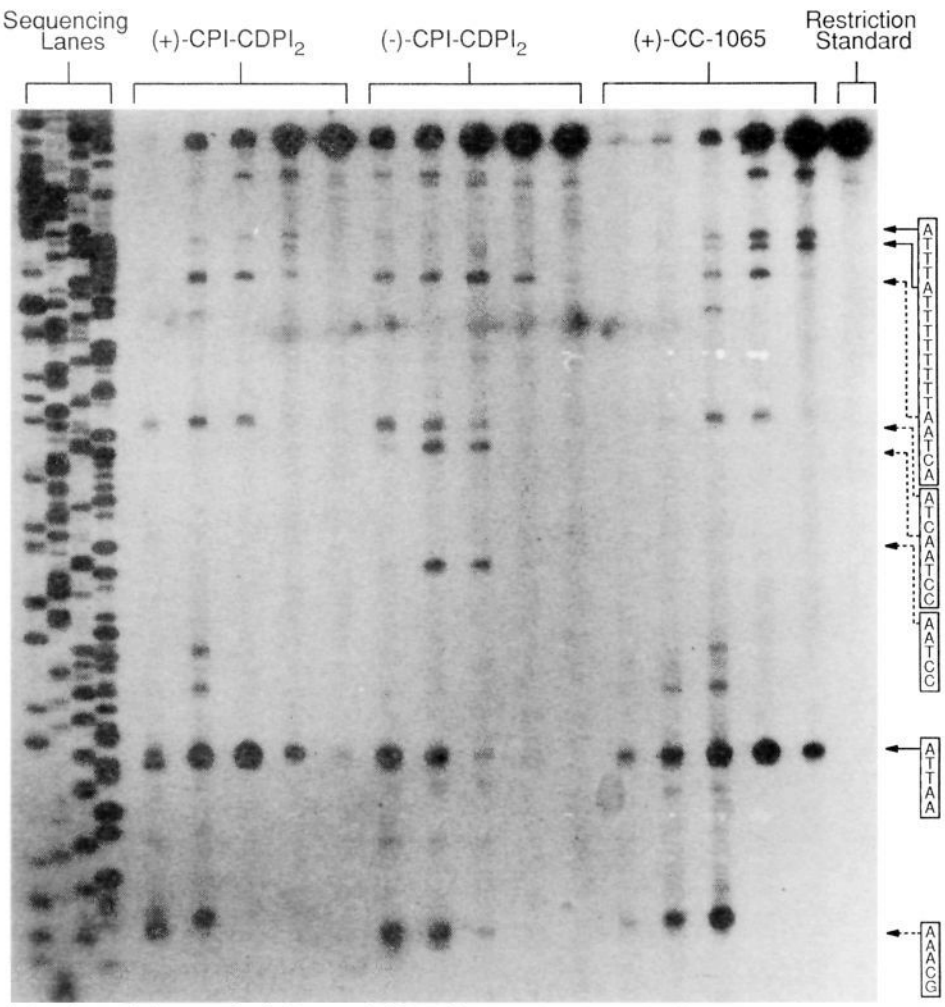

Figure 3. Thermally induced strand cleavage of a double-stranded SV40 fragment (144 bp; nucleotide no. 138–5238, clone w794) after 24-h incubation of agent–DNA at 4 °C followed by removal of unbound agent and 30-min incubation at 100 °C; 8% denaturing poly(acrylamide) gel, 1100 V/6 h, and autoradiography. Lane 1, Sanger G reaction; lane 2, Sanger C reaction; lane 3, Sanger A reaction; lane 4, Sanger T reaction; lanes 5–9, (+)-CPI-$CDPI_2$ (5.6×10^{-4}–5.6×10^{-8} M); lanes 10–14, (−)-CPI-$CDPI_2$ (5.6×10^{-4}–5.6×10^{-8} M); lanes 15–19, (+)-CC-1065 (5.6×10^{-4}–5.6×10^{-8} M); lane 20, control DNA.

DNA Binding Properties.[35] The initial confirmed binding of the aborted and extended functional analogues of CC-1065 with DNA was demonstrated through stabilization of the thermally induced helix to coil melting transition of poly[dA]poly[dT] (Table I).[36] Evidence for DNA covalent alkylation was obtained from the thermally induced strand cleavage of double-stranded DNA by the agents (autofootprinting).[10,35] The autofootprinting was carried out by a protocol to be described elsewhere employing two ^{32}P singly 5′-end-labeled fragments of SV40 DNA (w794, 144 bp, nucleotides no. 5238–438), and c988, 150 bp, nucleotides no. 4359–4210 cloned into the *Sma*1 site of the M13mp10 polylinker region. The agents were incubated with the singly ^{32}P 5′-end-labeled double-stranded DNA at 4 °C for 24 h, unbound agent

Table I. In Vitro Cytotoxic Activity (L1210) and Preliminary DNA Binding Studies

	agent	IC_{50}, 10^{-5} μg/mL (nM, relative IC_{50})[a]	ΔT_m,[b] °C	autofootprinting[c]
(+)-**2**	(+)-CC-1065	1.1 (0.02, 1)	40	+
(−)-**2**	(−)-CC-1065	1.3 (0.02, 1)	27	+
(+)-**4**	(+)-CPI-$CDPI_2$	1.2 (0.02, 1)	25	+
(−)-**4**	(−)-CPI-$CDPI_2$	1.3 (0.02, 1)	20	+
(+)-**1**	(+)-CPI-PDE-I_1	0.8 (0.02, 1)	nt	+
(−)-**1**	(−)-CPI-PDE-I_1	≥125 (≥2.4, ≤0.01)	nt	±
(+)-**3**	(+)-CPI-$CDPI_1$	1.7 (0.04, 0.5)	37	+
(−)-**3**	(−)-CPI-$CDPI_1$	≥270 (≥6.3, ≤0.003)	17	±
(+)-**5**	(+)-CPI-$CDPI_3$	1.6 (0.02, 1)	15	+
(−)-**5**	(−)-CPI-$CDPI_3$	10 (0.13, 0.15)	13	+
(+)-**6**	(+)-N^2-BOC-CPI	10000 (330, 0.00006)	0	*c*

[a] IC_{50}, inhibitory concentration for 50% cell growth relative to untreated controls. The L1210 cell culture cytotoxicity assays were performed as described: Boger, D. L.; Yasuda, M.; Mitscher, L. A.; Drake, S. D.; Kitos, P. A.; Thompson, S. C. *J. Med. Chem.* **1987**, *30*, 1918. [b] ΔT_m relative to poly[dA]·poly[dT]. The DNA hyperchromicity at 260 nm was monitored as a function of temperature; 1:10 agent–base pair, incubated 24 °C, 3 days (CPI-$CDPI_1$ and CPI-$CDPI_2$) or 6 days (CPI-$CDPI_3$) in 10 mMP_i (pH = 7.2) with 0.3% DMSO. [c] See text.

(33) The enantiomeric purity of the natural (3b*R*,4a*S*)-CPI and unnatural (3b*S*,4a*R*)-CPI agents was determined to be ≥99%.

(34) The seco agents were found to close upon attempted chromatographic purification. Purity of the crude agents were established by HPLC (1 mL/min, 7.5% DMF/THF): seco-CPI-$CDPI_1$ (80% **15**, 14% **3**; R_T = 6.6 min); seco-CPI-$CDPI_3$ (92% **17**, 6% **5**; R_T = 6.4 min). CPI-PDE-I, CPI-C-DPI_1, and CPI-$CDPI_3$ were purified to homogeneity by chromatography (**1**, ≥98%; **3** ≥ 98%; **5**, ≥97%; ^1H NMR and HPLC).

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(36) The ΔT_m data correlates with total DNA binding (noncovalent binding *and* covalent alkylation) and does not correlate directly with the relative extent of DNA covalent alkylation. Since the potent cytotoxic activity of the agents may be correlated with the relative extent or sites of DNA covalent alkylation, the use of ΔT_m values does not provide a useful predictive correlation, cf. (+)- and (−)-CPI-$CDPI_1$.

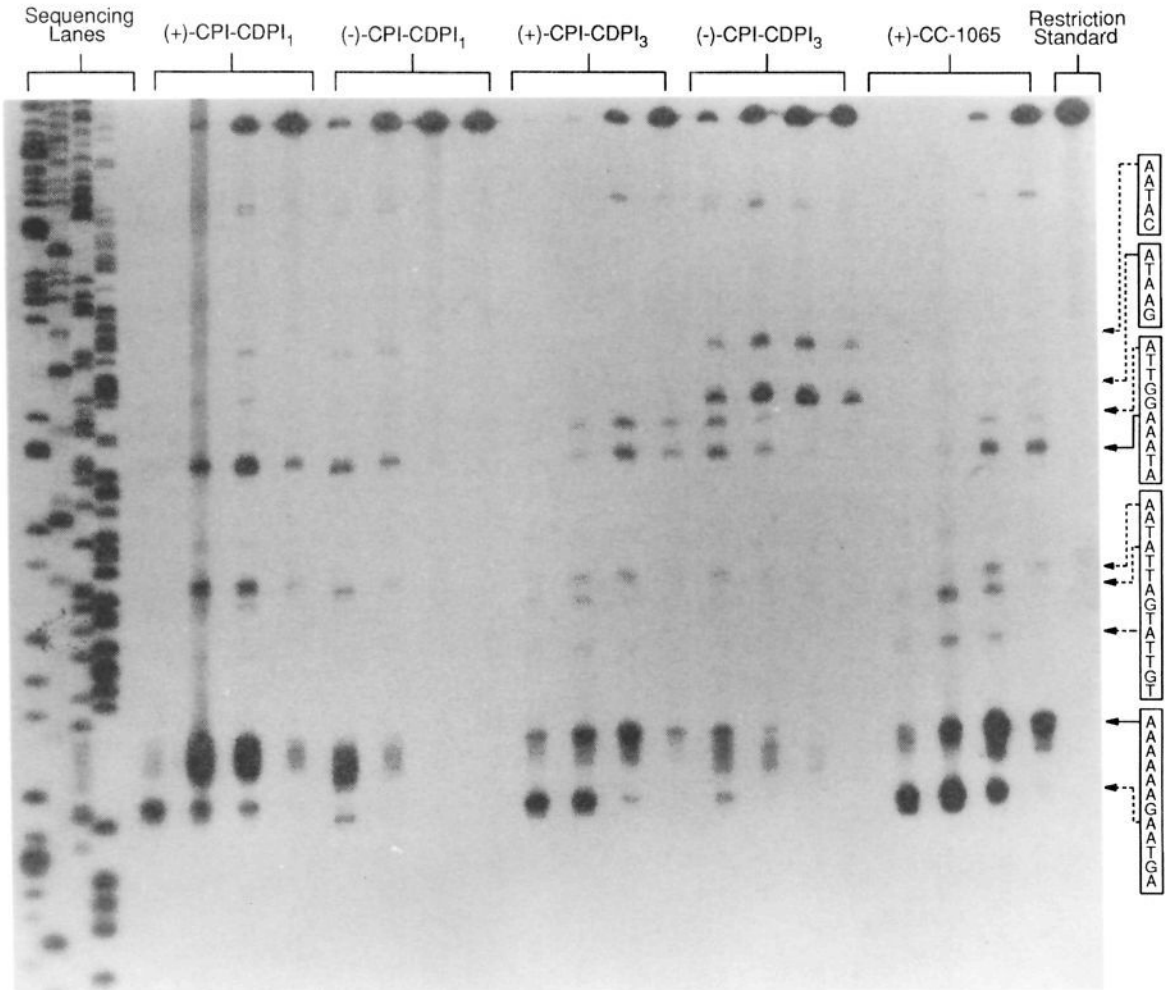


Figure 4. Thermally induced strand cleavage of a double-stranded SV40 fragment (150 bp; nucleotide no. 4210–4359, clone c988) after 24-h incubation of agent–DNA at 4 °C followed by removal of unbound agent and 30-min incubation at 100 °C; 8% denaturing poly(acrylamide) gel, 1100 V/6 h, and autoradiography. Lane 1, Sanger G reaction; lane 2, Sanger C reaction; lane 3, Sanger A reaction; lane 4, Sanger T reaction; lanes 5–8, (+)-CPI-CDPI$_1$ (5.6×10^{-5}–5.6×10^{-8} M); lanes 9–12, (−)-CPI-CDPI$_1$ (5.6×10^{-5}–5.6×10^{-8} M); lanes 13–16, (+)-CPI-CDPI$_3$ (5.6×10^{-5}–5.6×10^{-8} M); lanes 17–20, (−)-CPI-CDPI$_3$ (5.6×10^{-5}–5.6×10^{-8} M); lanes 21–24, (+)-CC-1065 (5.6×10^{-5}–5.6×10^{-8} M); lane 25, control DNA.

was removed by ethanol precipitation, and the DNA–agent covalent complex was warmed at 100 °C for 30 min to induce strand cleavage.[10] The natural enantiomer of CPI-CDPI$_1$ and both enantiomers of CPI-CDPI$_2$[10c] and CPI-CDPI$_3$ were shown to cleave DNA at a site of covalent alkylation under these conditions (Figures 2–5). (+)-CPI-CDPI$_2$ (**4**) and (+)-CC-1065 (**2**) proved virtually indistinguishable in their profile (sites and their relative intensity) of DNA covalent alkylation although a slightly higher concentration of (+)-CPI-CDPI$_2$ was required to induce the same intensity (profile) of covalent alkylation under the assay conditions (Figures 2 and 3). Thus, while the sequence selectivity of the covalent alkylation for the two agents has proven *indistinguishable*, the intensity of the DNA modification at 4 °C (24 h) is slightly different.[37] The enantiomer, (−)-CPI-CDPI$_2$, maintained the ability to alkylate most of the prominent (+)-CC-1065 alkylation sites at high concentrations and exhibited a notably different profile of covalent alkylation at low concentrations.[10,21,35] Thus, while (−)-CPI-CDPI$_2$ maintains the ability to alkylate within the c988 5′-d(AAAAAA)-3′ site preferentially at the 5′- versus 3′-end as well as the 5′-d(AGTAA)-3′, 5′-d(GATTA)-3′, 5′-d(TATAA)-3′, and 5′-d(ATAAA)-3′ sites, additional (+)-CC-1065 minor sites are not alkylated [5′-d(TGTTA)-3′], and additional prominent sites unique to (−)-CPI-CDPI$_2$ are observed [5′-d(GAA-T*A*TTTT)-3′ and 5′-d(CATA*A*TTTT)-3′] for which a 5′ alkylation site and 5′ → 3′ binding directionality may be envisioned.[33,38] Similar observations may be drawn from w794 binding studies (Figure 2) in which the major sites of (+)-CC-1065 are alkylated by (−)-CPI-CDPI$_2$ at high concentrations, some minor alkylation sites of (+)-CC-1065 are not alkylated, and major alkylation sites unique to (−)-CPI-CDPI$_2$ are observed at the pertinent low concentrations.

The profile of covalent alkylation by (+)-CPI-CDPI$_1$ and (+)-CPI-CDPI$_3$ proved nearly identical with that of (+)-CC-1065/(+)-CPI-CDPI$_2$, with (+)-CPI-CDPI$_3$ exhibiting a nearly indistinguishable profile and (+)-CPI-CDPI$_1$ exhibiting a perceptibly different profile (Figures 4 and 5). For example, within w794 (Figure 5) (+)-CPI-CDPI$_1$ fails to alkylate the two minor (+)-CC-1065/(+)-CPI-CDPI$_2$ sites just above 5′-d(AATTA)-3′ and alkylates the minor (+)-CC-1065/(+)-CPI-CDPI$_2$ site of 5′-d(TAATA)-3′ with greater intensity and as a major alkylation site. It is this indistinguishable DNA binding profile of (+)-CC-1065/(+)-CPI-CDPI$_2$ versus that of (+)-CPI-CDPI$_1$ *and* the behavior of the unnatural enantiomers [(−)-CC-1065 ≡ (−)-CPI-CDPI$_2$ ≠ (−)-CPI-CDPI$_1$] that suggests that CPI-CDPI$_2$ versus CPI-CDPI$_1$ embodies the precise structural and functional features

(37) The initial assessment of the properties of CPI-CDPI$_2$ has led to the initial conclusion "that the *o*-catechol substituents of CC-1065 are important in stabilizing its noncovalent binding to DNA".[24] This assessment has been qualified upon further investigation[21c] and since the publication of related efforts.[23]

(38) Although it is possible that contaminate (+)-CPI-CDPI$_2$ (HPLC, <0.5%) in (−)-CPI-CDPI$_2$ is responsible for the (+)-CC-1065 comparable alkylation sites, the direct comparison of the alkylation profile of (+)- and (−)-CPI-CDPI$_2$ requires that it be present in >10% quantity.

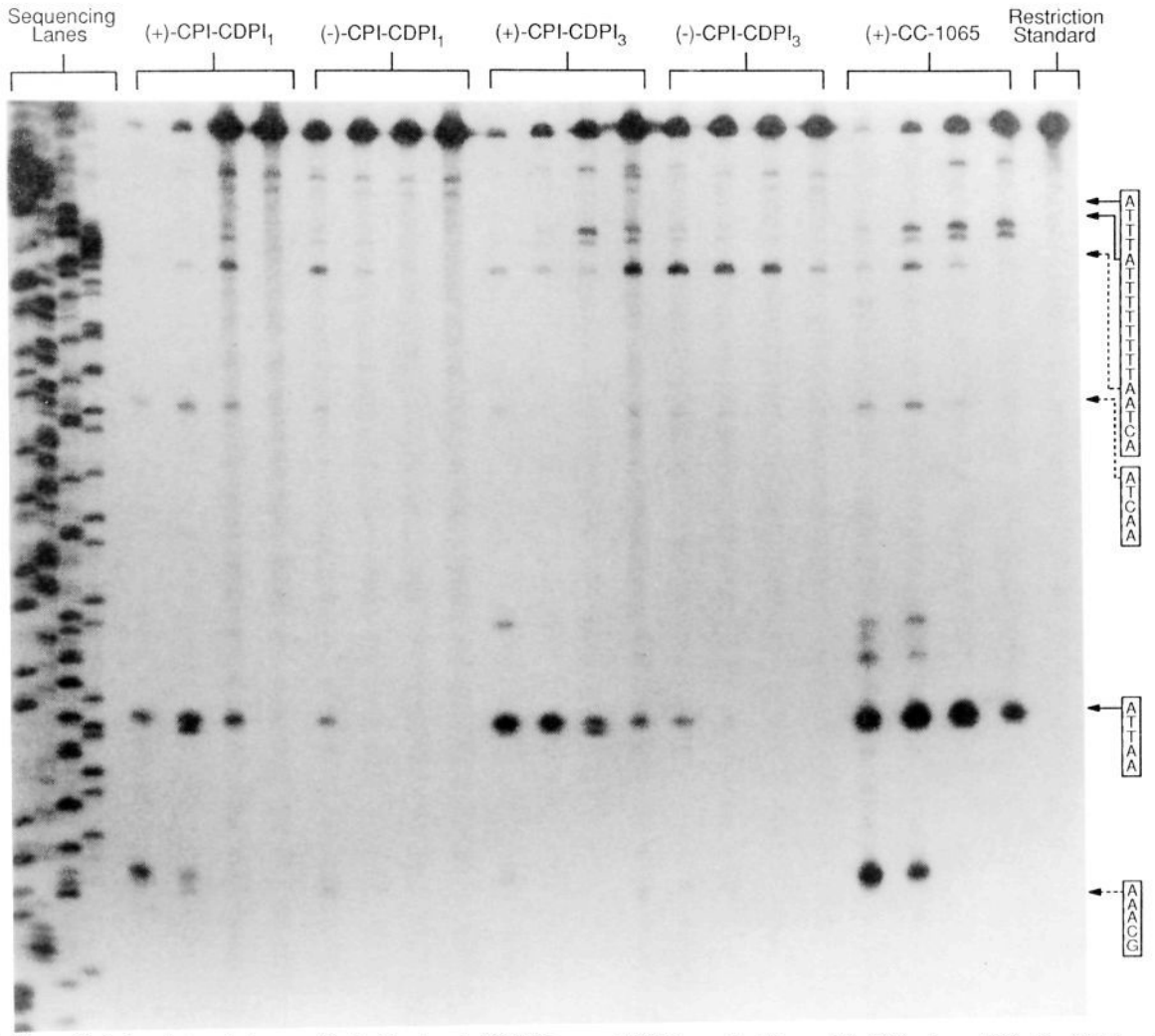


Figure 5. Thermally induced strand cleavage of a double-stranded SV40 fragment (144 bp; nucleotide no. 138–5238, clone w794) after 24-h incubation of agent–DNA at 4 °C followed by removal of unbound agent and 30-min incubation at 100 °C; 8% denaturing poly(acrylamide) gel, 1100 V/6 h, and autoradiography. Lane 1, Sanger G reaction; lane 2, Sanger C reaction; lane 3, Sanger A reaction; lane 4, Sanger T reaction; lanes 5–8, (+)-CPI-$CDPI_1$ (5.6×10^{-5}–5.6×10^{-8} M); lanes 9–12, (−)-CPI-$CDPI_1$ (5.6×10^{-5}–$5.6\ 10^{-8}$ M); lanes 13–16, (+)-CPI-$CDPI_3$ (5.6×10^{-5}–5.6×10^{-8} M); lanes 17–20, (−)-CPI-$CDPI_3$ (5.6×10^{-5}–5.6×10^{-8} M); lanes 21–24, (+)-CC-1065 (5.6×10^{-5}–5.6×10^{-8} M); lane 25, control DNA.

of CC-1065 responsible for its sequence-selective DNA binding properties.

The differences in the DNA covalent alkylation profile between (+)- and (−)-CPI-$CDPI_3$ at low concentration are quite marked. (−)-CPI-$CDPI_3$ appears to be more selective than (+)-CPI-$CDPI_3$ in that fewer and unique sites are alkylated by the unnatural enantiomer. Even at higher concentrations (−)-CPI-$CDPI_3$ maintains significant sequence selectivity and (−)-CPI-$CDPI_3$ proved distinguishable from (−)-CPI-$CDPI_2$ in this respect. The major (−)-CPI-$CDPI_2$ and (−)-CPI-$CDPI_3$ sites of covalent alkylation observed at low concentration proved identical and unique from the (+)-CC-1065/(+)-CPI-$CDPI_2$ high-affinity sites. However, the (−)-CPI-$CDPI_3$ covalent alkylation proved somewhat more selective than that observed with (−)-CPI-$CDPI_2$, especially at the higher concentrations. Most notable was the lack of prominent DNA modification by (−)-CPI-$CDPI_1$ (Figures 4 and 5). (−)-CPI-$CDPI_1$ required 100–1000× the concentration of (+)-CPI-$CDPI_1$ to induce a comparable covalent alkylation footprint. While we cannot unambiguously establish the origin of this footprint, its indistinguishable profile from that of (+)-CPI-$CDPI_1$ suggests that this (−)-CPI-$CDPI_1$ DNA modification most likely may be attributed to the contaminant natural enantiomer [<1% (+)-CPI-$CDPI_1$].[33]

The comparative DNA binding properties of the parent agents, (+)-*N*-BOC-CPI (**6**) and (+)-*N*-acetyl-CPI (**7**), with those of (+)-CC-1065 were particularly revealing (Figure 6). Both agents were shown to alkylate DNA when incubated under the standard conditions (4 °C, 24 h) but required concentrations of (0.25–25) × 10^{-2} M [10^5–10^7× the concentration of (+)-CC-1065] for observtaion, and the alkylation profile of (+)-**7** proved to be approximately 100× more intense than that of (+)-**6** (not shown). When (+)-**6** and (+)-**7** were incubated with DNA at 37 °C (24 h), the intensity of the DNA strand scission increased without significantly altering the relative intensity of the various cleavage sites (selectivity). Even at 37 °C the induced DNA strand scission was weak, requiring 10^4–10^7× the concentration of (+)-CC-1065. Significantly, (+)-**6** and (+)-**7** behaved in a comparable fashion in that their profile of DNA covalent alkylation proved nearly identical although (+)-**6** requires approximately 10–100× the concentration of (+)-**7** to exhibit the comparable intensity of DNA covalent binding.[39] More significant is the recognition that the DNA covalent alkylation sequence selectivity for (+)-**6** and (+)-**7** is quite distinct from that of (+)-CC-1065 (4 or 37 °C). The two agents alkylate the minor (+)-CC-1065 site of 5′-d(AC-

(39) Because of the reduced stability of some agents, e.g., *N*-acetyl-CI versus *N*-BOC-CI,[29] and in efforts to maintain a direct comparison of DNA binding properties of the agents, we have elected to make such comparisons with the BOC derivatives of the modified or natural left-hand subunits of the agents. As detailed herein, this use of (+)-*N*-BOC-CPI versus (+)-*N*-acetyl-CPI affects the intensity (concentration) at which an autofootprint is observed, but does not affect the selectivity (relative intensity of alkylation sites).

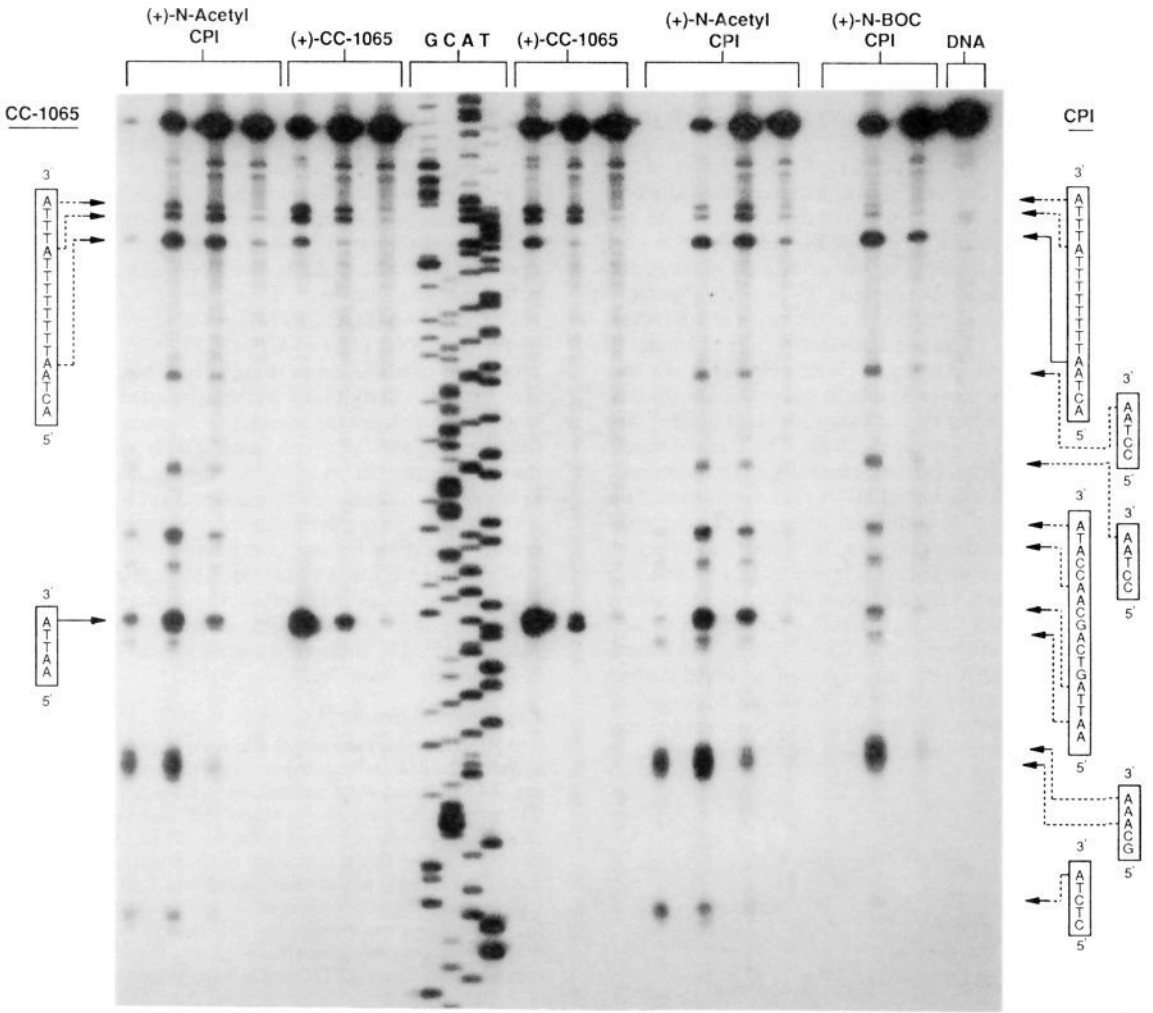


Figure 6. Thermally induced strand cleavage of a double-stranded SV40 fragment (144 bp; nucleotide no. 138–5238, clone w794) after 24-h incubation of agent–DNA at 4 or 37 °C followed by removal of unbound agent and 30-min incubation at 100 °C; 8% denaturing poly(acrylamide) gel and autoradiography. Lanes 1–4, (+)-*N*-acetyl-CPI (4 °C, 2.5×10^{-1}–2.5×10^{-4} M); lanes 5–7, (+)-CC-1065 (4 °C, 1×10^{-6}–1×10^{-8} M); lanes 8–11, Sanger G, C, A, and T reactions; lanes 12–14, (+)-CC-1065 (37 °C, 1×10^{-6}–1×10^{-8} M); lanes 15–18, (+)-*N*-acetyl-CPI (37 °C, 2.5×10^{-2}–2.5×10^{-5} M); lanes 19–21, (+)-*N*-BOC-CPI (37 °C, 2.5×10^{-1}–2.5×10^{-3} M); lane 22, control DNA.

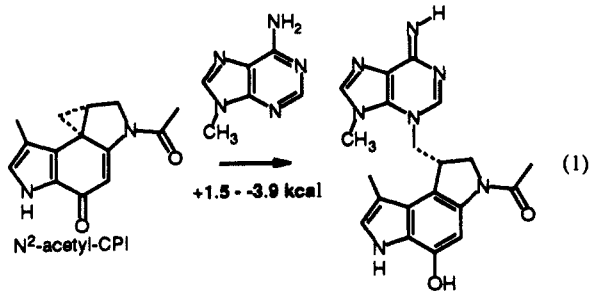
TAA)-3′ as their major alkylation site, a number of additional sites not alkylated by (+)-CC-1065 are alkylated, *and* the *three* (+)-CC-1065 high-affinity sites in w794 [5′-d(AATTA)-3′ > 5′-d(ATTTA)-3, 5′-d(TTTTA)-3] constitute less prominent or minor alkylation sites for the parent agents. As such, the profile of the DNA alkylation by (+)-**6** and (+)-**7** is substantially different and less selective than that of (+)-CC-1065. Consequently, contrary to the conclusions drawn by others[10,21] we interpret such behavior to indicate that the sequence selectivity of the (+)-CC-1065 covalent alkylation is derived only in part from the inherent selectivity of the CPI covalent alkylation. The complementary A–T-rich DNA noncovalent binding selectivity of the agents[25] additionally restricts the number of accessible CPI adenine alkylation sites and productively contributes to the agents DNA covalent alkylation selectivity.

Thus, in summary: (1) (+)-CPI-$CDPI_2$ and (+)-CC-1065 proved *indistinguishable* in their profile of covalent alkylation of DNA supporting the anticipated[27,28] *precise* properties of the two agents, (2) (+)-CPI-$CDPI_1$ and (+)-CPI-$CDPI_3$ exhibit DNA binding properties comparable [(+)-CPI-$CDPI_1$] or nearly indistinguishable [(+)-CPI-$CDPI_3$] from the alkylation profile of (+)-CC-1065/(+)-CPI-$CDPI_2$, (3) (−)-CPI-$CDPI_2$ and (−)-CPI-$CDPI_3$ exhibit a comparable DNA covalent alkylation profile that at high concentrations includes the major sites of (+)-CC-1065 covalent alkylation, excludes some minor (+)-CC-1065 alkylation sites, and at pertinent low concentrations includes major sites unique to the unnatural enantiomers, (4) (−)-CPI-$CDPI_1$ exhibits a substantially weaker or imperceptible covalent modification of DNA, (5) (+)-*N*-BOC-CPI and (+)-*N*-acetyl-CPI exhibit a comparable profile of covalent alkylation of DNA under the standard conditions of assay (4 °C, 24 h) albeit at 10^5–$10^7\times$ the concentration required of (+)-CC-1065, (6) (+)-*N*-BOC-CPI and (+)-*N*-acetyl-CPI exhibit a more pronounced covalent alkylation of DNA under more vigorous conditions (37 °C, 24 h) albeit still requiring approximately 10^4–$10^7\times$ the concentration of (+)-CC-1065 for observable alkylation, and which proceeds with a profile easily distinguished from and less selective than that of (+)-CC-1065 (4 or 37 °C), and (7) the differences in the DNA alkylation profile of the agents can be interpreted on the basis of a more prominent role for the noncovalent binding selectivity of the agents.[25,27]

In Vitro Cytotoxic Activity. The results of the in vitro evaluation of the cytotoxic activity of the CC-1065 partial structures (**1**, **3**, and **5**) are summarized in Table I along with the comparative results for (+)- and (−)-CC-1065 (**2**),[22] (+)- and (−)-CPI-$CDPI_2$ (**4**),[22,23] and (+)-N^2-BOC-CPI (**6**). Within the limits of experimental error, the aborted and extended agents possessing the natural (3b*R*,4a*S*)-CPI left-hand segment proved equally potent to (+)-CC-1065. Like the simplified analogues of (+)-CC-1065,[17–19] the aborted agents **1** and **3** bearing the natural (3b*R*,4a*S*)-CPI left-hand segment proved substantially more potent that the unnatural, enantiomeric (3b*S*,4a*R*)-CPI aborted agents.[33] This is in sharp contrast to the observation that the enantiomeric pairs of the precise [(+)-CPI-$CDPI_2$/(−)-CPI-

$CDPI_2$][22,23] and extended [(+)-CPI-$CDPI_3$/(−)-CPI-$CDPI_3$] functional analogues of CC-1065 possess potent cytotoxic properties. The precursor (1*S*)- and (1*R*)-(chloromethyl)-seco-CPI agents **13**, **15**, and **17** possess cytotoxic activity at levels comparable to that of the CPI agents and presumably suffer Ar-3′ alkylative ring closure in vitro.[34]

Covalent Alkylation. Computational studies have indicated that the adenine N-3 alkylation of CPI is a thermodynamically poor alkylation that may be attributed to the destabilizing effect of the adenine N-3 alkylation (+27.5 kcal, AM1; +28.8 kcal, MNDO).[40-42] Errors inherent in the calculated heats of formation[42] suggest that accurate estimates of the heats of reaction for the CPI adenine N-3 alkylation lie in the range of 1.5 (AM1) to −3.9 (MNDO) kcal. This modest electrophilic character of CPI appears to be well reflected in its chemical behavior in which CPI-based agents have proven stable to aqueous base (N-deacylation versus cyclopropane ring opening), stable at pH 5–7, and modestly stable at pH 3 ($t_{1/2}$ = ca. 35–39 h). This thermal neutral nature of the adenine-CPI alkylation, coupled with the nonbonded destabilization of double-stranded DNA that accompanies the covalent alkylation,[27] and an additional, albeit small, conformational destabilization of the agent and DNA required to permit binding[27] places the simple CPI alkylation of DNA close to a destabilizing event. This is well reflected in the inability to observe a detectable interaction of CPI with double-stranded DNA under conventional assay conditions (ΔT_m, CD)[18] and its weak capabilities for observable DNA covalent alkylation as judged through its thermally induced cleavage of DNA. To us, this has suggested that it is the dominant noncovalent binding of the agents **1–5** that drives (stabilizes) a thermodynamically poor covalent alkylation (binding-driven bonding).



(1)

Thus, the substantially reduced DNA covalent alkylation efficiency and cytotoxic properties of CPI (≥10000× CC-1065) versus the comparable properties of the natural enantiomers of **1–5** suggest that an additional functional role of the CC-1065 central and right-hand subunits is simply to stabilize the DNA–CC-1065 covalent complex formation. Thus, a potential relationship between the (3b*R*,4a*S*)-CPI-based agents' cytotoxic potency and DNA binding properties lies not with the relative *rate*[21c] of the agents' DNA covalent alkylation or the *extent* of the agents' noncovalent stabilization of the covalent complex (**2** > **4** ≥ **5** > **1** > **3**)[43] but with the *simple event of noncovalent binding stabilization of this thermal neutral covalent alkylation* (**6** ≪ **1–5**; IC_{50} 330 nM ≪ 0.02, 0.02, 0.04, 0.02, 0.02 nM).[44]

Conclusions. The potent and comparable cytotoxic properties of (+)-**1–5** relative to CPI, e.g. **6** and **7**, may be derived from the simple event of noncovalent stabilization of the thermal neutral CPI-adenine covalent alkylation. Thus, a potentially important functional role of the central subunit of the natural enantiomers of **1–5** is noncovalent binding stabilization of a thermal neutral and potentially reversible covalent alkylation. In contrast, the unnatural enantiomers (−)-**1–5** require the full trimer size of the agent for observation of effective DNA alkylation properties and potent cytotoxic activity.[44] The distinct DNA covalent alkylation profiles of (+)-*N*-BOC-CPI (**6**)/(+)-*N*-acetyl-CPI (**7**) versus that of (+)-CC-1065 (**2**)/(+)-CPI-$CDPI_n$ (**3–5**) further suggest a prominent contribution to the agents' DNA covalent alkylation sequence selectivity that may be attributed to the noncovalent binding selectivity of the agents.[25,45] Consequently, an additional functional role of the agents' central and/or right-hand subunits may be the restriction of the number of accessible adenine alkylation sites through selective noncovalent binding preferentially within the narrower, sterically more accessible A–T-rich minor groove (accessible hydrophobic binding).[25,27] As such, the strict A–T preference for the first three base pairs of the agents' alkylation sequences may reflect the combination of the initial 3′-adenine alkylation site with the required noncovalent stabilization provided by the agents' central subunit and its two base-pair A–T binding selectivity.[46]

Experimental Section[47]

3-[(*tert*-Butyloxy)carbonyl]-4-hydroxy-5-methoxy-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylic Acid (12). A suspension of **11**[28b] (3.4 mg, 9.4 μmol) and sodium dithionite[32] (1.7 mg, 9.8 μmol, 1.0 equiv) in 0.12 mL of tetrahydrofuran/methanol (3:2) under argon was treated with an aqueous solution of lithium hydroxide (23 μL of a 4.0 M solution, 92 μmol, 9.8 equiv) and the reaction mixture was warmed at 50 °C (90 min). The reaction mixture was diluted with 1 mL of saturated aqueous sodium chloride and 5–6 drops of 1% aqueous hydrochloric acid (pH = 3). The mixture was extracted with EtOAc (5 × 2 mL), the combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo to afford pure **12** (2.7 mg, 3.3 mg theoretical, 82%): ^1H NMR

(40) AM1: Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.

(41) MNDO: Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899.

(42) This estimation was derived through comparison of the experimental versus calculated heat of reaction ($\Delta H°$) derived from heats of formation (ΔH_f MOPAC, AM1 and MNDO) for the addition of ammonia to cyclopropane as taken from ref 40 (ΔH_f values in kilocalories).

	c-C_3H_6	+ NH_3	→	$CH_3CH_2CH_2NH_2$
exptl	12.7	−11.0		−16.8
AM1	17.8	−7.3		−22.1
error	+5.1	+3.7	(−14.1)	−5.3
MNDO	11.2	−6.4		−18.2
error	−1.5	+4.6	(−4.5)	−1.4

(43) This expected mode of binding of **5** relative to **4** is based on the experimental comparison of $CDPI_4$ versus $CDPI_3$ methyl ester noncovalent DNA minor groove binding in which partial bound forms of $CDPI_4$ constitute the predominant, kinetic mode of binding; see ref 25a.

(44) This assessment also suggests that the unnatural enantiomer binding requires the full trimer structure of (−)-CC-1065, i.e., (−)-CPI-$CDPI_2$, to provide sufficient noncovalent stabilization of a covalent complex and this appears to be verified through the lack of observation of (−)-CPI-$CDPI_1$ covalent alkylation.

(45) The covalent alkylation profile for (+)-**6**/(+)-**7** and (+)-CC-1065 within selected sequences of double-stranded DNA, e.g., c988, has proven comparable and in our experience reflects the use of double-stranded DNA that lacks a pronounced selectivity for (+)-CC-1065. Under the conditions detailed herein, (−)-**6** failed to provide a detectable DNA covalent alkylation through autofootprinting.

(46) The loss of selectivity for the terminal two base pairs of the five base-pair consensus sequences may reflect the opportunity for DNA full structure bound agents, e.g., CPI-$CDPI_2$-bound CPI-$CDPI_2$, *or* stable DNA partial-bound agents, e.g., CPI-$CDPI_1$-bound CPI-$CDPI_2$. Thus, in instances where full structure binding is precluded or less accessible, partial-bound agents may constitute productive and potentially relevant binding modes for minor groove association.[25a]

(47) Melting points (mp) were determined with a Thomas-Hoover capillary melting point apparatus or Fisher micromelting apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Hewlett-Packard Model 8451 spectrophotometer. HPLC analysis were performed on a Gilson 320 chromatography system equipped with an Isco V[4] variable-wavelength absorbance detector on an Alltech 600 Si column. Flash chromatography[48] was performed on 230–400-mesh silica gel. Tetrahydrofuran (THF) and benzene were distilled from sodium benzophenone ketyl under nitrogen immediately prior to use. Methanol was distilled immediately before use from magnesium methoxide. Methylene chloride (CH_2Cl_2) was distilled from phosphorus pentoxide (P_2O_5) immediately before use. *N*,*N*-Dimethylformamide (DMF), toluene, and triethylamine (Et_3N) were distilled from calcium hydride and stored under nitrogen or argon. Pyridine was distilled from barium oxide, and acetonitrile was distilled from P_2O_5. All chromatography and extraction solvents [hexane, methylene chloride (CH_2Cl_2), ethyl acetate (EtOAc), and ether (Et_2O)] were distilled before use. 1-[3-(Dimethylamino)-3-propyl]-3-ethylcarbodiimide hydrochloride (EDCI) was purchased from Aldrich Chemical Co. The potency and potential fatal toxicity of the agents required that the work detailed herein be conducted at a scale permitting the preparation of 1–3 mg of the agents.

(48) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

($CDCl_3$, 300 MHz) δ 11.64 (s, 1 H, NH), 8.86 (s, H, ArOH), 7.13 (d, 1 H, J = 1.9 Hz, C8-H), 4.12 (t, 2 H, J = 8.6 Hz, NCH_2CH_2), 4.03 (s, 3 H, OCH_3), 3.20 (t, 2 H, J = 8.6 Hz, NCH_2CH_2), 1.57 (s, 9 H, $C(CH_3)_3$); IR (solid film) ν_{max} 3855, 3839, 3822, 3752, 3736, 3691, 3677, 3650, 3630, 2931, 1654, 1541, 1431, 1407, 1368, 1328, 1290, 1257, 1154, 1099, 1056, 1018, 961, 883, 751 cm^{-1}; FABMS (dithiothreitol/dithioerythritol) m/e 371 (M^+ + Na), 349 (M^+ + H).

(±)-(1*R)-, (1*S*)-, and (1*R*)-3-[(*tert*-Butyloxy)carbonyl]-1-(chloromethyl)-5-hydroxy-8-methyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole [(±)-(1*R**)-9, (1*S*)-9,[33] and (1*R*)-9[33]].** In a typical procedure, a solution of **8**[22] (2.5 mg, 5.9 μmol) in 0.10 mL of tetrahydrofuran under nitrogen at 23 °C was treated sequentially with 25% aqueous ammonium formate[21] (25 μL) and 10% palladium/carbon (ca. 2 mg), and the reaction mixture was stirred vigorously for 65 min (23 °C). The catalyst was removed by filtration through Celite (0.5 × 1 cm, EtOAc wash) and the filtrate was evaporated in vacuo. The residue was purified by flash chromatography (0.5 × 1 cm SiO_2, 0–25% EtOAc–CH_2Cl_2 gradient elution) to afford **9** (2.0 mg, 2.0 mg theoretical, 100%) as an unstable, white crystalline solid. An analytical sample was secured by chromatography (SiO_2, 0.5 × 6 cm, 40% EtOAc–hexane): mp 157 °C (sharp); ^1H NMR ($CDCl_3$, 200 MHz) δ 8.20 (br s, 1 H, OH), 7.48 (br s, 1 H, C4-H), 6.96 (s, 1 H, C7-H), 6.74 (br s, 1 H, NH), 4.32–3.76 (m, 3 H, C1-H and C2-H), 3.38 (apparent t, 2 H, J = 11 Hz, CH_2Cl), 2.38 (s, 3 H, $ArCH_3$), 1.58 (s, 9 H, $CO_2C(CH_3)_3$); IR (KBr) ν_{max} 3377, 2976, 2927, 1662, 1590, 1499, 1409, 1368, 1333, 1256, 1165, 1141, 1043, 896, 834, 759, 737, 705 cm^{-1}; EIMS, m/e (relative intensity) 336 (M^+, 1), 302 (1), 280 (1), 246 (3), 231 (9), 199 (4), 187 (4), 56 (base); CIMS (2-methylpropane), m/e (relative intensity) 303 (M^+ + 2H − Cl, 31), 302 (M^+ + H − Cl, 17), 301 (M^+ + H − HCl, 26), 281 (9), 278 (12), 247 (base), 203 (13), 201 (14); EIHRMS, m/e 336.1236 ($C_{17}H_{21}N_2O_3Cl$ requires 336.1240).

(+)- and (−)-*N*-BOC-CPI-PDE-I_1 [(+)-(3b*R*,4a*S*)-1[33] and (−)-(3b*S*,4a*R*)-1[33]]. In a typical procedure, **9** (1.6 mg, 4.8 μmol) was treated with anhydrous 3 N hydrochloric acid in ethyl acetate (0.5 mL) under argon at 23 °C (60–85 min). The solvent was removed in vacuo to afford crude, unstable **10**. A mixture of crude **10**, the carboxylic acid **12**[28] (*N*-BOC-PDE-I_1; 1.3 mg, 3.7 μmol), sodium bicarbonate (1.9 mg, 23 μmol, 4.8 equiv), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (2.5 mg, 13 μmol, 2.7 equiv) was dissolved in *N*,*N*-dimethylformamide (0.1 mL) at 23 °C under nitrogen and the mixture was stirred for 27 h (23 °C). The solvent was removed in vacuo and the residue was slurried in 0.5 mL of water containing 2 drops of 5% aqueous hydrochloric acid (pH = 3). The suspension was stirred vigorously for 60 min (23 °C) and the solids were collected by centrifugation. The solids were washed with dilute aqueous hydrochloric acid (0.5 mL of water containing 1 drop of 5% aqueous hydrochloric acid, 4×) and with water (0.5 mL). Drying the solids in vacuo afforded **13** (1.7 mg, 2.1 mg theoretical, 81%) as a grey powder.

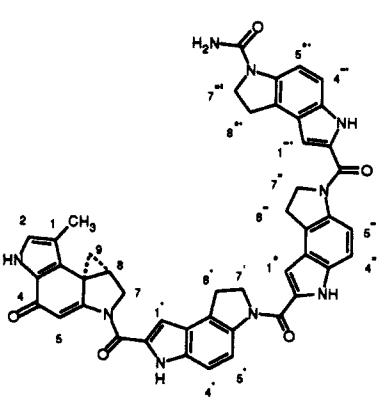
In a typical procedure, **13** (0.7 mg, 1.2 μmol) was treated with 1:1:1 $Et_3N/H_2O/CH_3CN$[21] (70 μL) and the reaction mixture was stirred vigorously for 3 h (23 °C) under nitrogen. The solvent was removed under a stream of nitrogen and the residue was suspended in 0.5 mL of water containing 1 drop of saturated aqueous ammonium chloride. The solid was collected by centrifugation and was washed with water (0.5 mL). Drying the solid in vacuo and flash chromatography (0.5 × 2.7 cm SiO_2, 0–100% EtOAc–CH_2Cl_2 gradient elution) afforded **1** (0.5 mg, 0.64 mg theoretical, 79%) as a grey powder:[34] mp >280 °C; ^1H NMR ($CDCl_3$, 300 MHz)[49] δ 11.60 (s, 1 H, OH), 9.13 (br s, 1 H, NH), 8.93 (br s, 1 H, NH), 6.91 (s, 1 H, C2-H), 6.82 (d, 1 H, J = 1.9 Hz, C5-H or C1′-H), 6.81 (d, 1 H, J = 1.2 Hz, C5-H or C1′-H), 4.39 (d, 2 H, J = 3.4 Hz, C7-H), 4.11 (t, 2 H, J = 8.6 Hz, C7′-H), 4.02 (s, 3 H, OCH_3), 3.16 (apparent dd, 3 H, J = 10, 8 Hz, C8-H and C8′-H), 2.06 (s, 3 H, $ArCH_3$), 2.04 (m, 1 H, C9-H), 1.57 (s, 9 H, $C(CH_3)_3$), 1.45 (apparent t, 1 H, J = 5 Hz, C9-H); IR (solid film) ν_{max} 3903, 3871, 3854, 3839, 3822, 3802, 3745, 3735, 3712, 3690, 3676, 3650, 3630, 3620, 3588, 3568, 3228, 2923, 2851, 2361, 1773, 1734, 1718, 1700, 1684, 1654, 1636, 1577, 1559, 1541, 1522, 1507, 1474, 1457, 1437, 1396, 1379, 1303, 1267, 1146, 1059, 858 cm^{-1}; FABMS (dithiothreitol/dithioerythritol) m/e 531 (M^+ + H); HRFABMS (dithiothreitol/dithioerythritol) m/e 531.2280 ($C_{29}H_{30}N_4O_6$ + H requires 531.2244).

(1*S*)- and (1*R*)-seco-CPI-CDPI$_1$ [(1*S*)-15[33] and (1*R*)-15[33]]. In a typical procedure, **9** (1.7 mg, 5.0 μmol) was treated with anhydrous 3 N hydrochloric acid in ethyl acetate (ca. 0.4 mL) at 23 °C for 30 min. The solvent was removed in vacuo to afford crude, unstable **10**. A mixture of crude **10**, sodium bicarbonate (3.3 mg, 40 μmol, 8.0 equiv), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI; 2.9 mg, 15 μmol, 3.0 equiv), and **14**[25b] (CDPI$_1$; 1.1 mg, 4.6 μmol, 0.9 equiv) was slurried in dry *N*,*N*-dimethylformamide (0.2 mL) at room temperature under argon and the reaction mixture was stirred vigorously for 21 h at 23 °C. The solvent was removed in vacuo, the residue was slurried in 0.5 mL of water containing 2 drops of 5% aqueous hydrochloric acid, and the mixture was stirred vigorously for 60 min (23 °C). The solids were collected by centrifugation and were washed with dilute aqueous hydrochloric acid (0.5 mL of water containing 1 drop of 5% aqueous HCl, 4×), and water (0.5 mL, 1×). Drying the solid in vacuo afforded **15** (2.30 mg, 2.30 mg theoretical, 100%) as a grey powder:[34] mp >280 °C; ^1H NMR (DMF-d_7, 300 MHz)[49] δ 11.48 (br s, 1 H, NH), 10.79 (br s, 1 H, NH), 9.84 (s, 1 H, O*H*), 8.14 (d, 1 H, J = 9.0 Hz, C4′-H), 7.75 (s, 1 H, C5-H), 7.36 (d, 1 H, J = 9.0 Hz, C5′-H), 7.16 (s, 1 H, C1′-H), 7.07 (s, 1 H, C2-H), 6.11 (br s, 2 H, $CONH_2$), 4.71 (apparent t, 2 H, C7-H_2), 4.15 (t, 2 H, J = 8.6 Hz, C7′-H_2), 3.95 (apparent t, 1 H, J = 10 Hz, C9-H), 3.66 (t, 1 H, J = 10 Hz, C9-H), 3.39 (t, 2 H, J = 8.6 Hz, C8′-H_2), 2.43 (s, 3 H, $ArCH_3$); ^1H NMR (DMSO-d_6, 300 MHz) δ 11.55 (br s, 1 H, NH), 10.72 (br s, 1 H, NH), 9.79 (s, 1 H, OH), 7.99 (d, 1 H, J = 9.0 Hz, C4′-H), 7.61 (br s, 1 H, C5-H), 7.21 (d, 1 H, J = 9.0 Hz, C5′-H), 7.05 (s, 1 H, C1′-H), 6.90 (s, 1 H, C2-H), 6.12 (br s, 2 H, $CONH_2$), 4.66 (apparent t, 1 H, J = 8.6 Hz, C7-H), 4.51 (apparent d, 1 H, J = 10 Hz, C7-H), 4.00 (t, 2 H, J = 8.6 Hz, C7′-H_2), 3.90 (dd, 1 H, J = 10, 1 Hz, C9-H), 3.59 (t, 1 H, J = 8.6 Hz, C9-H), 3.16 (t, 2 H, J = 8.6 Hz, C8′-H_2), 2.98 (m, 1 H, C8-H), 2.37 (s, 3 H, $ArCH_3$); IR (solid film) ν_{max} 3242, 2924, 2854, 1701, 1664, 1604, 1503, 1434, 1389, 1258, 1098, 808, 758 cm^{-1}; FABMS (dithiothreitol/dithioerythritol) m/e 464 (M^+ + H); HRFABMS (dithiothreitol/dithioerythritol) m/e 464.1488 ($C_{24}H_{22}ClN_5O_3$ + H^+ requires 464.1489).

(+)- and (−)-CPI-CDPI$_1$ [(+)-(3b*R*,4a*S*)-3[33] and (−)-(3b*S*,4a*R*)-3[33]]. In a typical procedure, **15** (3.56 mg, 7.7 μmol) was slurried in 1:1:1 $Et_3N/H_2O/CH_3CN$ (300 μL)[21] under argon and the reaction mixture was stirred vigorously for 3 h (23 °C). The solvents were removed in vacuo. The residue was dissolved in *N*,*N*-dimethylformamide (ca. 0.4 mL) and 60 mg of flash chromatography silica gel was added. The solvent was removed in vacuo and the dry silica gel containing the crude product was placed on top of a flash chromatography column (0.5 cm × 6 cm). Flash chromatography (0–50% *N*,*N*-dimethylformamide–toluene gradient elution) afforded **3** (2.74 mg, 3.28 mg theoretical, 83%) as a tan solid:[34] mp >275 °C; ^1H NMR (DMF-d_7, 300 MHz)[49] δ 11.64 (br s, 1 H, NH), 11.53 (br s, 1 H, NH), 8.17 (d, 1 H, J = 9 Hz, C4′-H), 7.38 (d, 1 H, J = 9 Hz, C5′-H), 7.14 (s, 1 H, C1′-H), 7.00 (s, 1 H, C2-H), 6.77 (s, 1 H, C5-H), 6.15 (br s, 2 H, $CONH_2$), 4.56 (apparent t, 2 H, J = 8 Hz, C7-H_2), 4.15 (t, 2 H, J = 8.6 Hz, C7′-H_2), 3.39 (t, 2 H, J = 8.6 Hz, C8′-H_2), 3.14 (m, 1 H, C8-H), 2.08 (s, 3 H, $ArCH_3$), 2.04 (m, 1 H, C9-H), 1.49 (t, 1 H, J = 4.7 Hz, C9-H); ^1H NMR (DMSO-d_6, 300 MHz) δ 11.68 (br s, 1 H, NH), 11.54 (br s, 1 H, NH), 8.01 (d, 1 H, J = 9.0 Hz, C4′-H), 7.20 (d, 1 H, J = 9.0 Hz, C5′-H), 6.99 (s, 1 H, C2-H), 6.88 (s, 1 H, C1′-H), 6.66 (s, 1 H, C5-H), 6.13 (br s, 1 H, $CONH_2$), 4.50 (m, 2 H, C7-H_2), 3.98 (t, 2 H, J = 8.9 Hz, C7′-H_2), 3.28 (t, 2 H, J = 8.9 Hz, C8-H_2), 3.18 (m, 1 H, C8-H), 2.00 (s, 3 H, $ArCH_3$), 1.96 (m, 1 H, C9-H), 1.39 (apparent t, 1 H, J = 3.5 Hz, C9-H); IR (solid film) ν_{max} 2925, 2857, 1728, 1659, 1604, 1507, 1454, 1388, 1262, 1123, 1099, 805 cm^{-1}; UV (DMF) λ_{max} 355 nm (ϵ = 20 300), 318 (22 200), 270 (20 800); FABMS (dithiothreitol/dithioerythritol) m/e 428 (M^+ + H); HRFABMS (dithiothreitol/dithioerythritol) m/e 428.1726 ($C_{24}H_{21}N_5O_3$ + H^+ requires 428.1723). (+)-CPI-CDPI$_1$: $[\alpha]^{25}_{578}$ = +95.0° (c = 0.13, DMF). (−)-CPI-CDPI$_1$: $[\alpha]^{25}_{578}$ = −94.3° (c = 0.091, DMF).

(1*R)-, (1*S*)-, and (1*R*)-seco-CPI-CDPI$_3$ [(1*R**)-17, (1*S*)-17,[33] and (1*R*)-17[33]].** In a typical procedure, **9** (2.0 mg, 5.9 μmol) was treated with anhydrous 3 N hydrochloric acid in ethyl acetate (0.6 mL) at 23 °C for 40 min. The solvent was removed in vacuo to afford crude, unstable **10**. A mixture of crude **10**, sodium bicarbonate (4.1 mg, 49 μmol, 8 equiv), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI;

(49) ^1H NMR assignments are reported based on the indicated numbering system.



3.5 mg, 18 μmol, 3.1 equiv), and **16**[25b] ($CDPI_3$; 3.3 mg, 5.4 μmol, 0.9 equiv) was slurried in dry *N,N*-dimethylformamide (0.15 mL) at 23 °C under nitrogen and the reaction mixture was stirred vigorously for 32 h (23 °C). The solvent was removed in vacuo and the residue was slurried in 0.6 mL of water. Two drops of 5% aqueous hydrochloric acid was added and the mixture was stirred vigorously for 60 min (23 °C). The solid was collected by centrifugation and was washed with dilute aqueous hydrochloric acid (0.5 mL of water containing 1 drop of 5% HCl, 4×) and water (0.5 mL). Drying the solid in vacuo afforded **17** (4.5 mg, 4.5 mg theoretical, 100%) as a grey powder:[34] mp >280 °C; ^{1}H NMR (DMSO-d_6, 300 MHz)[49] δ 11.77 (br s, 2 H, NH), 11.57 (s, 1 H, NH), 10.73 (s, 1 H, NH), 9.78 (s, 1 H, OH), 8.29 (m, 2 H, C4′-H and C4″-H), 7.98 (d, 1 H, J = 8.8 Hz, C4‴-H), 7.61 (br s, 1 H, C2-H or C5-H), 7.38 (br d, 2 H, J = 7.3 Hz, C5′-H and C2-H or C5-H), 7.23 (d, 1 H, J = 8.8 Hz, C5‴-H), 7.14 (s, 1 H, C5″-H), 7.09 (s, 1 H, C1‴-H), 7.06 (s, 1 H, C1″-H), 6.98 (s, 1 H, C1′-H), 6.12 (s, 2 H, $CONH_2$), 4.69–4.53 (m, 6 H, C7-H, C7′-H, and C7″-H), 3.99 (t, 2 H, J = 8 Hz, C7‴-H), 3.61 (apparent t, 2 H, J = 9.9 Hz, CH_2Cl), 3.48–3.14 (m, 7 H, partially obscured by H_2O, C8-H, C8′-H, C8″-H, and C8‴-H), 2.36 (s, 3 H, $ArCH_3$); IR (KBr) ν_{max} 3406, 2925, 1607, 1581, 1506, 1425, 1364, 1343, 1286, 1147, 805, 755, 693 cm^{-1}; FABMS (dithiothreitol/dithioerythritol) m/e 832 (M^+ + H).

(±)-, (+)-, and (−)-CPI-CDPI$_3$ [(±)-(3b*R,4a*S**)-5, (+)-(3b*R*,4a*S*)-5,[33] and (−)-(3b*R*,4a*S*)-5[33]].** In a typical procedure, **17** (2.6 mg, 3.1 μmol) was slurried in 1:1:1 $Et_3N/H_2O/CH_3CN$[21] (0.26 mL) and the reaction mixture was stirred vigorously at 23 °C for 3 h under nitrogen. The solvents were removed in vacuo, the residue was dissolved in 0.6 mL of *N,N*-dimethylformamide, and 50 mg of flash chromatography silica gel was added. The solvent was removed in vacuo and the dry silica gel was placed at the top of a flash chromatography column (0.5 × 6 cm). Flash chromatography (0–90% *N,N*-dimethylformamide–toluene gradient elution) afforded **5** (1.3 mg, 2.5 mg theoretical, 53%) as a grey powder:[34] mp >280 °C; ^{1}H NMR (DMSO-d_6, 300 MHz)[49] δ 11.88 (s, 1 H, NH), 11.77 (s, 1 H, NH), 11.56 (d, 1 H, J = 1.3 Hz, NH), 11.55 (s, 1 H, NH), 8.28 (br m, 2 H, C4′-H and C4″-H), 7.98 (d, 1 H, J = 9 Hz, C4‴-H), 7.38 (d, 1 H, J = 8.9 Hz, C5′-H), 7.23 (d, 1 H, J = 8.8 Hz, C5‴-H), 7.15 (d, 1 H, J = 9.1 Hz, C5″-H), 7.15 (br s, 1 H, C1′-H), 6.98 (s, 2 H, C1″-H and C1‴-H), 6.89 (d, 1 H, J = 2.2 Hz, C2-H), 6.68 (s, 1 H, C5-H), 6.11 (s, 2 H, $CONH_2$), 4.72–4.45 (m, 6 H, C7-H, C7′-H, and C7″-H), 3.99 (t, 2 H, J = 8.6 Hz, C7‴-H), 3.50–3.16 (m, 7 H, partially obscured by H_2O, C8-H, C8′-H, C8″-H, C8‴-H), 2.02 (s, 3 H, $ArCH_3$), 1.97 (m, 1 H, C9-H), 1.42 (t, 1 H, J = 4.7 Hz, C9-H); IR (solid film) ν_{max} 3855, 3822, 3817, 3808, 3802, 3753, 3745, 3713, 3676, 3584, 3283, 2921, 1719, 1664, 1612, 1580, 1503, 1421, 1391, 1365, 1342, 1285, 1018 cm^{-1}; UV (DMF) λ_{max} 330 nm (ϵ = 52 300), 270 (26 200); FABMS (dithiothreitol/dithioerythritol) m/e 796 (M^+ + H); HRFABMS (dithiothreitol/dithioerythritol) m/e 796.2835 ($C_{46}H_{37}N_9O_5$ requires 796.2995). (+)-CPI-CDPI$_3$: $[\alpha]^{25}_{578}$ = +45.5° (c = 0.044 DMF). (−)-CPI-CDPI$_3$: $[\alpha]^{25}_{578}$ = −44.3° (c = 0.070, DMF).

DNA Binding Studies. The DNA binding studies were carried out as detailed elsewhere.[35] Singly ^{32}P 5′-end-labeled segments of SV40 double-stranded DNA were treated with agent at 4 °C (24 h). After ethanol precipitation of the agent–DNA complex to remove unbound agent, the complex was warmed at 100 °C for 30 min to induce strand cleavage at the sites of covalent alkylation. Electrophoresis of the resulting DNA was carried out using an 8% denaturing poly(acrylamide) gel and autoradiography of the dried gel was carried out at −78 °C with the aid of an intensifying screen.

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Supplementary Material Available: A summary of computational studies, a full summary (L1210, B16, P388, KB) of the in vitro cytotoxic evaluation and preliminary DNA binding properties of the agents, a summary of the autofootprinting study, and a figure illustrating the full structure bound and partial-bound structures of (+)-CC-1065 and (+)-CPI-$CDPI_2$ within 5′-d-(GC*GCTTA*AGCGC)$_2$-3′ (14 pages). Ordering information is given on any current masthead page.