Parallel Synthesis and Evaluation of 132 (+)-1,2,9,9a-Tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI) Analogues of CC-1065 and the Duocarmycins Defining the Contribution of the DNA-Binding Domain

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The solution-phase, parallel synthesis and evaluation of a library of 132 (+)-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI) analogues of CC-1065 and the duocarmycins containing dimeric monocyclic, bicyclic, and tricyclic heteroaromatic replacements for the DNA-binding domain are described. This systematic study revealed clear trends in the structural requirements for observation of potent cytotoxic activity and DNA alkylation efficiency, the range of which spans a magnitude of ≥10 000-fold. Combined with related studies, these results highlight that the role of the DNA-binding domain goes beyond simply providing DNA-binding selectivity and affinity (10−100-fold enhancement in properties), consistent with the proposal that it contributes significantly to catalysis of the DNA alkylation reaction accounting for as much as an additional 1000-fold enhancement in properties.

Introduction

CC-1065 (**1**)[1] and the duocarmycins (**2** and **3**)[2] are among the most potent antitumor antibiotics discovered to date, and they have been shown to derive their biological activity through the sequence selective alkylation of duplex DNA (Figure 1).[2−4] An extensive series of studies has defined the nature of the alkylation reaction, which proceeds by adenine N3 addition to the least-substituted cyclopropane carbon of the left-hand alkylation subunit, and the alkylation sequence selectivity.[5−8] For the natural enantiomers, this entails 3′ adenine N3 alkylation with binding across a 3.5−4 (duocarmycins) or 5 (CC-1065) base-pair AT-rich site (e.g., 5′-AAAAA), whereas the unnatural enantiomers bind in the reverse 5′→3′ direction (e.g., 5′-AAAAA) across analogous 3.5−5 base-pair AT-rich sites.[4] An alternative

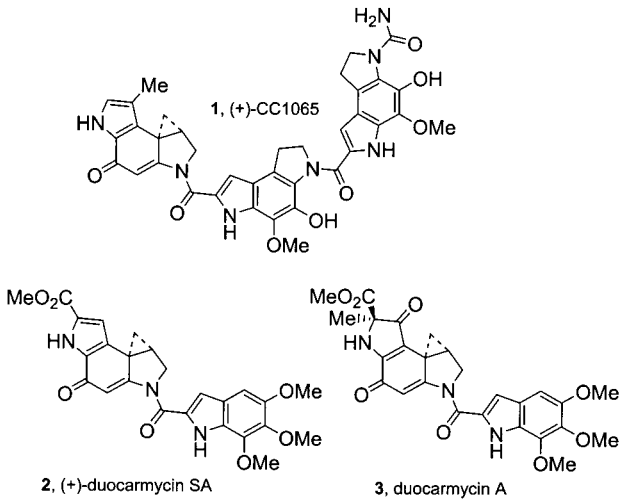

Figure 1.

way of visualizing this behavior of the two enantiomers is that, from a common bound orientation and within a common binding site, they alkylate adenine on complementary strands of duplex DNA at sites offset by one base pair (e.g., $^{5'-\text{AATTA(natural)}}_{3'-\text{TTAAT(unnatural)}}$).[9] Early studies demonstrated that the right-hand segment(s) of the natural products effectively delivers the alkylation subunit to AT-rich sequences of duplex DNA increasing the selectivity and efficiency of DNA alkylation.[10] Because the preferential AT-rich noncovalent binding affinity, like that of distamycin and netropsin,[11] is related to the deeper and narrower shape of the AT-rich minor groove, it is often

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referred to as shape-selective recognition. However, it is only in more recent studies that it has become apparent that the DNA-binding domain also plays an important role in catalysis of the DNA alkylation reaction.[12] Because this is also related to the shape characteristics of the minor groove and results in preferential activation in the narrower, deeper AT-rich minor groove, we have come to refer to this as shape-dependent catalysis.[12] We have suggested that this catalysis is derived from a DNA-binding-induced conformational change in the agents, which adopt a helical DNA-bound conformation requiring a twist in the amide linking of the alkylation subunit and the first DNA-binding subunit. This conformational change serves to partially deconjugate the stabilizing vinylogous amide, activating the cyclopropane for nucleophilic attack. For activation, this requires a rigid, extended heteroaromatic N2-amide substituent,[12−14] and the shape, length, and strategically positioned substituents on the first DNA-binding subunit can have a pronounced effect on the DNA alkylation rate and efficiency and the resulting biological properties of the agents.

The combination of the effects is substantial. The DNA alkylation rate and efficiency increase approximately 10 000-fold, and the resulting biological potency also increases proportionally 10 000-fold when simple *N*-acetyl or *N*-Boc derivatives of the alkylation subunits, which lack the DNA-binding domain, are compared with **1**−**3**. In three independent studies, the DNA-binding subunit contribution to the DNA alkylation rate could be partitioned into that derived from an increased binding selectivity/affinity and that derived from a contribution to catalysis of the DNA alkylation reaction. The former was found to increase the rate approximately 10−100-fold, whereas the latter increases the rate approximately 1000-fold indicating a primary importance.[13,15,16]

Throughout these investigations, the complementary roles of the DNA-binding subunits have been examined with relatively limited numbers of compounds, and no systematic study has been disclosed. Moreover, there is some confusion in the disclosures as to the relative effectiveness of the distamycin/lexitropsin substitutions for the DNA-binding subunits, with regard to both DNA alkylation selectivity and alkylation efficiency.[14,17−20] Consequently, we have examined and describe herein the effects of the DNA-binding subunits with a complete series of dimeric monocyclic, bicyclic, and tricyclic heteroaromatics. The results of these studies underscore the observation that the contribution of the DNA-binding domain goes beyond simply providing AT-rich noncovalent binding affinity supporting an additional primary role in catalysis.

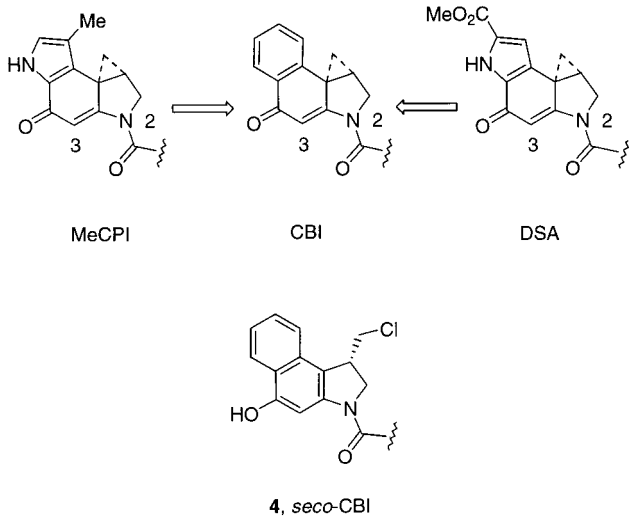

Figure 2.

Because of its synthetic accessibility, its potency and efficacy, which matches or exceeds that of the CC-1065 MeCPI alkylation subunit, and the extensive documentation of the biological properties of its derivatives, the library was assembled using the seco precursor **4** to the (+)-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI) alkylation subunit (Figure 2).[21−24] To date, no distinctions between the *seco*-CBI and CBI derivatives have been detected in a range of in vitro and in vivo assays in accordance with past studies of all such alkylation subunits,[25] indicating that in situ spirocyclization is not rate-determining or property-limiting.

Synthesis of the 132-Membered Library

In a recent study,[26] we detailed the parallel synthesis of a 132-membered library of heteroaromatic dimers

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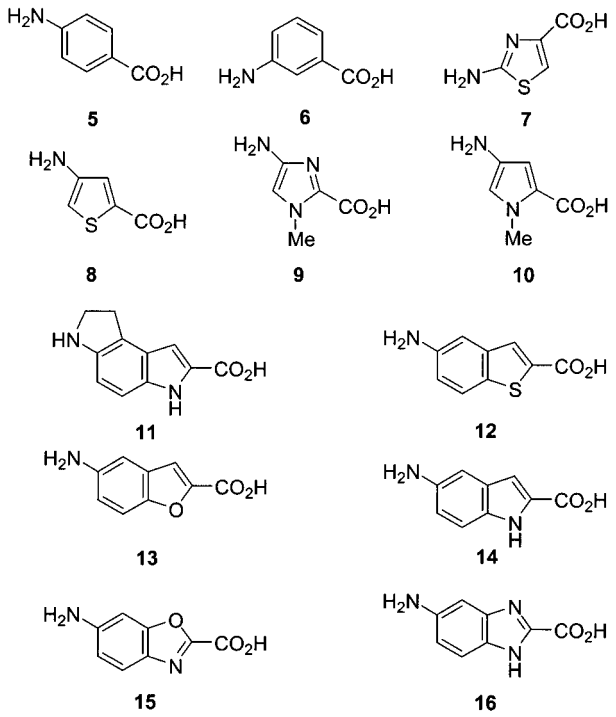
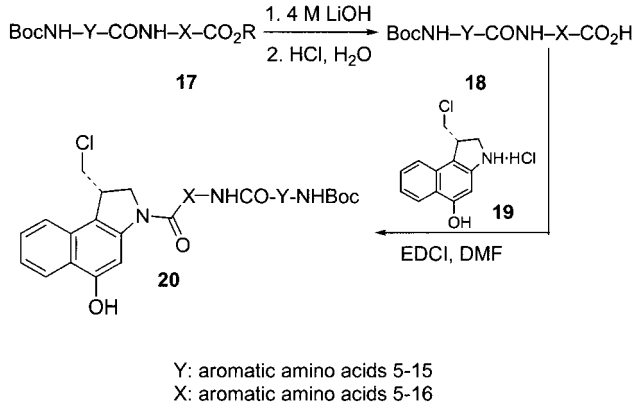


Figure 3.

Scheme 1



Y: aromatic amino acids 5-15
X: aromatic amino acids 5-16

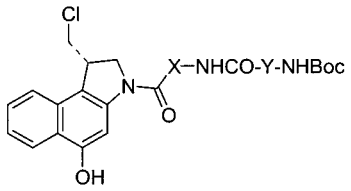
related to the structures of distamycin and CC-1065. This included the monocyclic, bicyclic, and tricyclic heteroaromatic amino acids **5**−**16** (Figure 3), which have been explored in the examination of these two natural products. The 132 dimers composed of these subunits were assembled by parallel synthesis through formation of the linking amide enlisting a simple acid−base liquid−liquid extraction protocol for isolation and purification. Each of the 132 dimers was fully characterized[26] and used for the formation of the library of CBI analogues. Each dimer was saponified by treatment with LiOH (4 M aqueous solution in dioxane−water 4:1 for 12 h at 25 °C) to afford the lithium salts of the carboxylic acids (Scheme 1). Hydrolysis of the compounds that possessed the 4-amino-1-methylpyrrole-2-carboxylate (**10**) or 5-aminoindole-2-carboxylate (**14**) subunits at the C-terminus was slower, and the reactions were conducted at 40 °C. Acidifying the aqueous Li-salt solutions gave the free carboxylic acids **18** that were used for the subsequent couplings without further purification. Notably, the dimers with the 6-aminobenzoxazole-2-carboxylate (**15**) and 5-aminobenzimidazole-2-carboxylate (**16**) subunits at the C-terminus, which are prone to decarboxylation,[26] were sufficiently stable for use in the next conversion. After deprotection of **4** (4 M HCl−EtOAc, 25 °C, 45 min), the resulting hydrochloride **19** was coupled with the dimer carboxylic acids using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) to provide **20**. Simple acid−base extraction and purification with aqueous 3 N HCl and saturated aqueous Na_2CO_3 yielded each analogue sufficiently pure for direct assay.

Cytotoxic Activity

Evaluation of the CBI-based analogues in a cellular functional assay for L1210 cytotoxic activity revealed a clear relationship between the potency of the agents and the structure of the DNA-binding domain (Figure 4). For comparison, the L1210 IC_{50} for (+)-*N*-Boc-CBI, which lacks an attached DNA-binding domain, is 80 nM (80 000 pM). With a few exceptions, all group 1 compounds containing two monocyclic subunits (**5**−**10** in positions X and Y) exhibited IC_{50} values between 1 and 10 nM or higher indicating an increase in potency of approximately 10-fold relative to *N*-Boc-CBI. The exception is the thiophene subunit **8**, which, when incorporated as the X subunit adjacent to the DNA alkylation subunit, exhibited slightly greater potency. The best in this series were X**8**−Y**8** (290 pM, 275-fold enhancement) and X**8**−Y**10** (310 pM, 260-fold enhancement). Notably, the distamycin/netropsin dipyrrole was also effective with X**10**−Y**10** (440 pM) exhibiting a 180-fold enhancement. Nonetheless, even the best in this series exhibited a modest ca. 100-fold enhancement over (+)-*N*-Boc-CBI, and typically the series exhibited a much more modest 10−100-fold enhancement. Within the group 1 dimers, it is also interesting that the 4-aminobenzoic acid subunit (**5**, X group) compares favorably with the distamycin *N*-methyl-4-aminopyrrole-2-carboxylic acid subunit (**10**) providing IC_{50}'s that are within 2−3-fold of one another, whereas the 3-aminobenzoic acid subunit (**6**) and the imidazole (**9**) are not effective.

An analogous level of potency (10−100-fold enhancement) was observed with the group 2 monocyclic heteroaromatics (X group) when they were coupled to a terminal bicyclic heteroaromatic subunit (**12**−**15**), and a slightly greater enhancement was observed when the Y subunit was tricyclic (**11**). Notably, none of the compounds in this group 1 or 2 series exhibit IC_{50}'s lower than 100 pM or approach the potency of the natural products.

In contrast to these analogues, the group 3 dimers with the bicyclic and tricyclic subunits **11**−**14** bound directly to the DNA alkylation subunit constitute an array of substances with much greater cytotoxic potency. The potency enhancement observed with the analogues containing a bicyclic or tricyclic X subunit linked directly to the alkylation subunit (group 3, X**11**−**14** subunits) typically ranges from 27 000−1 000 (IC_{50} = 3−80 pM) relative to *N*-Boc-CBI. This is also roughly a 100−1000-fold enhancement over the monocyclic X subunits. All compounds in the library with IC_{50}'s below 10 pM can be found in this collection, and two-thirds of them contain the tricyclic CDPI subunit (**11**) in this key position, i.e., X**11**−Y**7** (5 pM), X**11**−Y**8** (3 pM), X**11**−Y**9** (3 pM), X**11**−Y**10** (5 pM), X**11**−Y**11** (5 pM), and X**11**−Y**14** (7 pM). In this regard, it seems advantageous to have a five-membered heterocycle in the Y position with CDPI (**11**) in the X position.

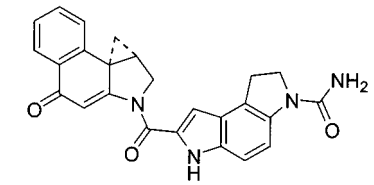


	Group3		Group 1				Group 2				Group 4
	Y5	**Y6**	**Y7**	**Y8**	**Y9**	**Y10**	**Y11**	**Y12**	**Y13**	**Y14**	**Y15**
X5	2400	10000	2600	1200	4800	830	100	1300	270	300	4300
X6	>10000	>10000	>10000	>10000	>10000	>10000	140	6000	650	770	>10000
X7	10000	10000	3300	6300	9400	2100	100	4200	880	330	3500
X8	460	6100	840	290	1600	310	250	670	540	310	600
X9	>10000	>10000	10000	>10000	>10000	7500	3700	4300	3200	1000	1000
X10	1200	5500	3900	3400	10000	440	240	920	330	340	10000
X11	48	150	5	3	3	5	5 (5)	100	56	7	1300
X12	38	270	160	53	130	6	49	13	26	56	65
X13	43	47	45	6	120	38	5	20 (7)	7 (10)	22 (5)	240
X14	67	2400	66	120	100	31	64	22 (5)	46 (10)	19 (10)	570
X15	1900	130	5000	2500	56	330	6800	5000	10000	160	2800
X16	230	>10000	410	310	4000	150	680	3500	2700	210	10000

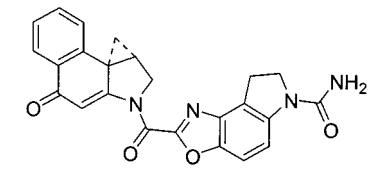
Figure 4. Cytotoxic activity (IC_{50} values ± 10%, average of two or more assays run in triplicate) of the CBI analogues, L1210 IC_{50} (pM). The values in parentheses represent those previously reported (see ref. 22).

The proposal of binding-induced catalysis for DNA alkylation by CC-1065 (**1**) and related compounds in which the shape and size of the substituent directly bound to the vinylogous amide make a major contribution to the properties is supported by the trends within the library. Compounds having the extended subunits **11**−**14** in the X position and smaller subunits **7**−**10** in the Y position show higher potency (typically 10−100-fold) than the corresponding compounds with inverted sequences. Because the bound agent is forced to follow the inherent helical twist of the minor groove, the helical rise induced in the molecule can only be adjusted by twisting the linking amide that connects the noncovalent binding subunit with the vinylogous amide of the alkylation subunit. The more extended the subunit is, the greater the twist is in the linking amide resulting in an increased activation of the agent. The lower cytotoxicity exhibited by analogues made from dimers consisting of the five-membered heterocycles **7**−**10** is also consistent with this explanation. Although these subunits are well-known as minor groove-binding constituents of distamycin, netropsin, and lexitropsins, they lack the rigid length that the fused aromatic heterocycles possess.

Compared to the analogues possessing benzothiophene **(12)**, benzofuran (**13**), or indole (**14**) at the X position of the dimer, agents containing benzoxazole (**15**) or benzimidazole (**16**) in this position (group 4) exhibit a considerable decrease in potency, up to 130-fold for X**15**−Y**13**. Similar observations have been made in a previous study concerning deep-seated modifications of the DNA-binding subunit of CC-1065 (Figure 5).[22,23] The introduction of an additional heteroatom in the carboxylate bearing aromatic ring of (+)-CBI−CDPI (**21**) led to a 40-fold decrease in cytotoxic activity and an analogous decrease in the DNA alkylation efficiency observed with (+)-CBI−CDPBO (**22**) and (+)-CBI−CDPBI (**23**) but no alteration in the alkylation selectivity compared to that of the parent compound. This was attributed to the



21, (+)-CBI-CDPI



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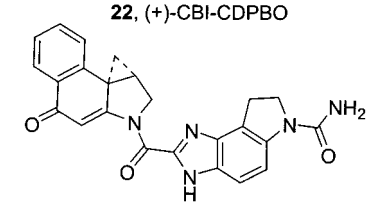

23, (+)-CBI-CDPBI

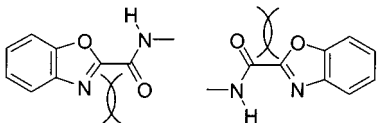

Figure 5.

destabilizing electrostatic interactions between the amide carbonyl lone pair and the heteroatom lone pairs present when the amide carbonyl adopts either of the in-plane conjugated conformations (Figure 5). This interaction results in a twist of the C-terminal bicyclic aromatic ring out of the plane defined by the carboxamide precluding

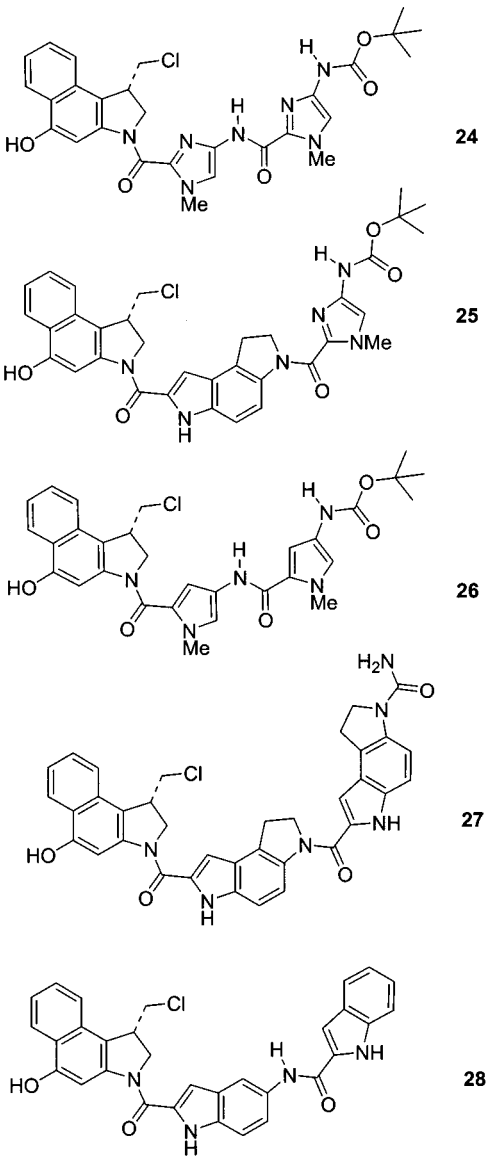

Figure 6.

preferential adoption of a near-planar conformation that facilitates minor groove binding.

DNA Alkylation Efficiency and Selectivity

The DNA alkylation properties of the compounds including those of CBI-X**9**-Y**9** (**24**), CBI-X**11**-Y**9** (**25**), and CBI-X**10**-Y**10** (**26**) (Figure 6) were examined within a 150 base-pair segment of duplex DNA and compared with (+)-duocarmycin SA (**2**), (+)-CBI−$CDPI_2$ (**27**), and (+)-CBI−$indole_2$ (**28**). One clone of phage M13mp10 that contained the SV40 nucleosomal DNA insert w794 (nucleotide no. 5238-138) was selected for the study.[27] The alkylation site identification and the assessment of the relative selectivity among the available sites were obtained by thermally induced strand cleavage of the singly 5′-end-labeled duplex DNA after exposure to the agents. After treatment of the end-labeled duplex DNA with a range of agent concentrations, the unbound agent was removed by EtOH precipitation of the DNA. Redissolution of the DNA in aqueous buffer, thermolysis (100 °C, 30 min) to induce strand cleavage at the sites of DNA alkylation, denaturing high-resolution polyacrylamide gel electrophoresis (PAGE) adjacent to Sanger dideoxynucleotide sequencing standards, and autoradiography led to identification of the DNA cleavage and alkylation sites.[28]

Representative of the comparisons made and the trends observed, the analogues **25** and **26** were found to detectably alkylate DNA at 10^{-5}−10^{-6} and 10^{-3} M, respectively, whereas alkylation by **24** (not shown) could not be observed even at 10^{-3} M (Figure 7). Throughout the comparisons, the relative DNA alkylation efficiencies were found to parallel the cytotoxic potencies of the compounds. Thus, the 100-fold lower cytotoxicity of **26** compared to **25** is also reflected in the 100−1000-fold lower alkylation efficiency of **26**. This behavior is dramatic with **26** being only 10−100-fold more effective than *N*-Boc-CBI which alkylates DNA at 10^{-1}−10^{-2} M under comparable reaction conditions albeit with a reduced selectivity. Thus, while the dipyrrole binding subunit does enhance the DNA alkylation efficiency and selectivity relative to *N*-Boc-CBI, it is also substantially less effective (100−1000-fold) than the compounds containing bicyclic or tricyclic X groups. The significance of those observations should not be underestimated, and the observations suggest that hybrid agents composed of the CC-1065/duocarmycin-related alkylation subunits and distamycin/netropsin DNA-binding subunits are intrinsically poor DNA alkylating agents.

Notably, no alterations in the DNA alkylation selectivities were observed despite the changes in the DNA-binding domain except for the minor differences noted before. Thus, although the efficiency of DNA alkylations was altered greatly, the selectivity was not. Within the w794 DNA, a major alkylation site (5′-AATTA-3′) and two minor sites (5′-ACTAA-3′, 5′-GCAAA-3′) are observed with the natural enantiomers. The relative extent to which alkylation at the minor sites is observed is dependent on the overall size (length) of the agent and the extent of DNA alkylation. For example, neither **27** nor **28** alkylates the minor 5′-ACTAA-3′ site to a significant extent, while the shorter agent **25**, like **21**,[24] does. In addition, the minor 5′-GCAAA-3′ site only appears on the gel after nearly complete consumption of the end-labeled DNA indicative of extensive, multiple DNA alkylations resulting in cleavage to shorter fragments of DNA. Other than these minor distinctions in the DNA alkylation selectivity, which have been noted in prior studies of CBI derivatives,[24] no significant changes were observed with variations in the DNA-binding subunits. Thus, while it may appear reasonable to suggest that the alkylation of the 5′-ACTAA-3′ site by **25** is a result of imidazole H-bonding to the intervening GC base pair, the identical behavior of (+)-CBI−CDPI (**21**), which lacks this subunit, suggests it is simply a natural consequence of a shorter agent binding and alkylating DNA within a shorter AT-rich sequence.[24,29]

Conclusions

The parallel synthesis of 132 CBI analogues of CC-1065 and the duocarmycins was described utilizing the solution-phase technology of acid−base liquid−liquid extraction for their isolation and purification. The 132 ana-

(27) Ambrose, C.; Rajadhyaksha, A.; Lowman, H.; Bina, M. *J. Mol. Biol.* **1989**, *210*, 255.

(28) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661.

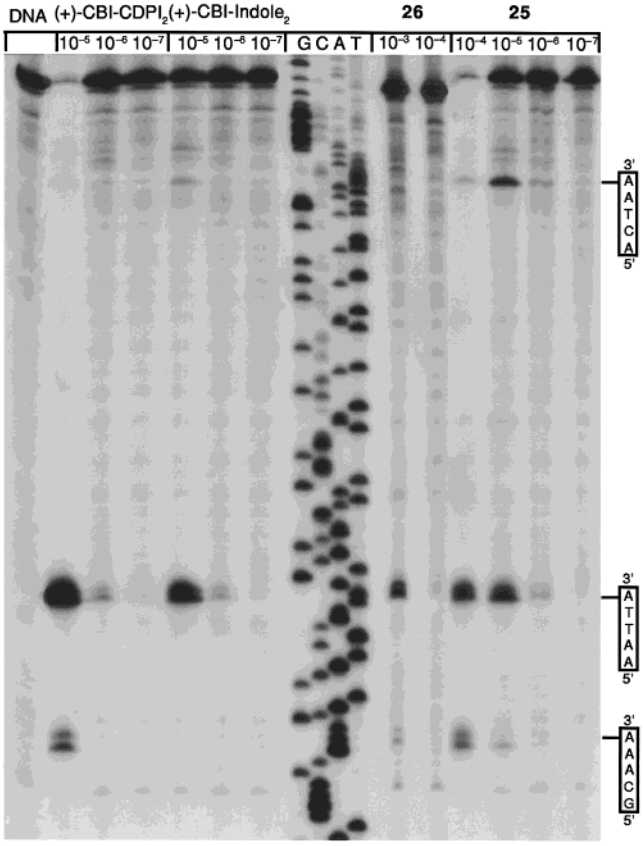

Figure 7. Thermally induced strand cleavage of double-stranded DNA (SV40 DNA fragment, 144 bp, nucleotide no. 5238-138, clone w794) after 24 h of incubation of agent−DNA at 25 °C followed by removal of unbound agent and 30 min incubation at 100 °C, denaturing PAGE, and autoradiography: lane 1, control DNA; lanes 2−4, (+)-CBI−$CDPI_2$ (**27**, 1×10^{-5} to 1×10^{-7} M); lanes 5−7, (+)-CBI−$indole_2$ (**28**, 1×10^{-5} to 1×10^{-7} M); lanes 8−11, Sanger G, C, A, and T reactions; lanes 12 and 13, CBI-X**10**-Y**10** (**26**, 1×10^{-3} and 1×10^{-4} M); lanes 14−17, CBI-X**11**-Y**9** (**25**, 1×10^{-4} to 1×10^{-7} M).

logues constitute a systematic study of the DNA-binding domain with the incorporation of dimers composed of monocyclic, bicyclic, and tricyclic heteroaromatic subunits. From examination of these analogues, clear trends in cytotoxic potency and DNA alkylation efficiency emerge highlighting the principle importance of the first attached DNA-binding subunit (X subunit): tricyclic > bicyclic > monocyclic heteroaromatic subunits. Notably, the trends observed in the cytotoxic potencies parallel those observed in the relative efficiencies of DNA alkylation. Our interpretation of these results is that the trends represent the partitioning of the role of the DNA-binding subunit(s) into two distinct contributions. The first is derived from an increase in DNA-binding selectivity and affinity which leads to property enhancements of 10−100-fold and is embodied in the monocyclic group 1 series. The second, which we suggest is additionally and effectively embodied in the bicyclic and tricyclic heteroaromatic subunits, is a contribution to catalysis of the DNA alkylation reaction that provides additional enhancements of 100−1000-fold for a total that exceeds a 25 000-fold enhancement. Aside from the significance of these observations in the design of future CC-1065/duocarmycin analogues, their significance to the design of hybrid structures containing the CC-1065/duocarmycin alkylation subunit should not be underestimated. Those that lack an attached bicyclic or tricyclic X subunit, i.e., duocarmycin/distamycin or CC-1065/distamycin hybrids, can be expected to be intrinsically poor or slow DNA alkylating agents.

(29) It is important to recognize that the X subunit C5 substituent contributes significantly to the rate and efficiency of DNA alkylation and cytotoxic activity presumably by extending the rigid length of the X subunit. In studies of analogues which lack a third Y subunit, the presence of a C5 substituent on the bicyclic X subunit substantially (≥10−1000-fold) enhances the properties providing analogues comparable in cytotoxic potency and DNA alkylation efficiency to the best analogues detailed herein. See the following: (a) ref 13. (b) Boger, D. L.; Stauffer, F.; Hedrick, M. P. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2021.

Experimental Section

General Procedure for Preparation of the CBI Analogues. A solution of the dimer ester **17**[26] (20 μmol) in dioxane−water (4:1, 250−300 μL) was treated with aqueous LiOH (4 M, 20 μL), and the mixture was stirred for 12 h at 20−25 °C. After lyophilization, the crude material was dissolved in water (500 μL) and treated with aqueous HCl (3 M, 100 μL), and the precipitate was collected by centrifugation. Decantation and lyophilization of the residue from water (500 μL) yielded material (**18**) that was sufficiently pure for the subsequent coupling. A sample of **4**[21] (1 mg, 3 μmol) was treated for 45 min with HCl−EtOAc (4 M, 300 μL). After evaporation of the solvent under a steady stream of N_2, the residue was dried in vacuo. The crude material was dissolved in DMF (40 μL) together with EDCI (9 μmol, 1.7 mg) and **18** (4.5 μmol) and allowed to stand at 20−25 °C. The reaction was quenched after 12 h by adding saturated aqueous NaCl (400 μL). Isolation of the product was performed by extraction with EtOAc (4 × 600 μL) and subsequent washing of the organic layer with aqueous 3 M HCl (4 × 400 μL), saturated aqueous Na_2CO_3 (4 × 400 μL), and saturated aqueous NaCl (1 × 400 μL). The combined organic layers were dried (Na_2SO_4) and concentrated to afford the CBI analogue in yields between 30 and 97%.

The diagonal elements of the library and additional selected members were characterized by ^{1}H NMR and high-resolution matrix-assisted laser desorption ionization (HRMALDI)-FTMS.

1-(Chloromethyl)-5-hydroxy-3-{4-[4-(*tert*-butoxycarbonylamino)benzoylamino]benzoyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X5-Y5): 0.99 mg, 58%; ^{1}H NMR (acetone-d_6, 600 MHz) δ 9.67 (s, 1H), 9.27 (s, 1H), 8.75 (s, 1H), 8.21 (d, J = 8.3 Hz, 1H), 8.03−7.94 (m, 4H), 7.84 (d, J = 8.3 Hz, 1H), 7.70 (d, J = 8.8 Hz, 2H), 7.67 (d, J = 8.3 Hz, 2H), 7.53 (dd, J = 7.7, 7.9 Hz, 1H), 7.36 (dd, J = 7.4, 7.5 Hz, 1H), 4.44 (dd, J = 9.6, 9.9 Hz, 1H), 4.30−4.24 (m, 1H), 4.10−4.05 (m, 1H), 3.99 (dd, J = 3.1, 10.9 Hz, 1H), 3.82−3.75 (m, 1H), 1.50 (s, 9H); HRMALDI-FTMS (DHB) m/z 572.1943 ($C_{32}H_{30}ClN_3O_5$ + H^+ requires 572.1952).

1-(Chloromethyl)-5-hydroxy-3-{3-[3-(*tert*-butoxycarbonylamino)benzoylamino]benzoyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X6-Y6): 0.95 mg, 55%; ^{1}H NMR (acetone-d_6, 600 MHz) δ 9.73 (s, 1H), 9.29 (s, 1H), 8.61 (s, 1H), 8.25−8.20 (m, 1H), 8.17 (s, 1H), 8.12 (br s, 1H), 8.05 (br s, 1H), 7.84 (d, J = 8.5 Hz, 1H), 7.73 (d, J = 7.1 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.55−7.48 (m, 2H), 7.43−7.35 (m, 3H), 4.47−4.40 (m, 1H), 4.22 (br s, 1H), 4.14−4.08 (m, 1H), 3.99 (d, J = 9.5 Hz, 1H), 3.84−3.76 (m, 1H), 1.48 (s, 9H); HRMALDI-FTMS (DHB) m/z 558.1995 ($C_{32}H_{30}ClN_3O_5$ − HCl + Na^+ requires 558.2005).

1-(Chloromethyl)-5-hydroxy-3-{[2-[[2-(*tert*-butoxycarbonylamino)-1,3-thiazol-4-yl]carbonylamino]-1,3-thiazol-4-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X7-Y7): 1.12 mg, 64%; ^{1}H NMR (acetone-d_6, 300 MHz) δ 10.64 (br s, 1H), 9.29 (s, 1H), 8.23 (d, J = 8.8 Hz, 1H), 8.09 (s, 2H), 7.94 (br s, 1H), 7.86 (d, J = 8.3 Hz, 1H), 7.57−7.50 (m, 1H), 7.43−7.35 (m, 1H), 4.74−4.67 (m, 2H), 4.20−4.11 (m, 1H), 4.04−3.94 (m, 1H), 3.78−3.68 (m, 1H), 1.54 (s, 9H); HRMALDI-FTMS (DHB) m/z 608.0814 ($C_{26}H_{24}ClN_5O_5S_2$ + Na^+ requires 608.0805).

1-(Chloromethyl)-5-hydroxy-3-{[2-[[4-(*tert*-butoxycarbonylamino)-1-methylimidazol-2-yl]carbonylamino]-1,3-thiazol-4-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X7-Y9): 1.10 mg, 63%; ^{1}H NMR (acetone-d_6, 600 MHz) δ 10.73 (br s, 1H), 9.27 (s, 1H), 8.69 (br s, 1H), 8.23 (d, J = 8.7 Hz, 1H), 8.09 (br s, 1H), 7.93 (br s, 1H), 7.85 (d, J = 8.2 Hz, 1H), 7.53 (dd, J = 7.4, 7.6 Hz, 1H), 7.42−7.35 (m, 2H), 4.76−4.65 (m, 2H), 4.19−4.13 (m, 1H), 4.12 (s, 3H), 3.99 (dd, J = 3.1, 11.2 Hz, 1H), 3.73 (dd, J = 8.7, 11.3 Hz, 1H), 1.48 (s, 9H); HRMALDI-FTMS (DHB) m/z 583.1519 ($C_{27}H_{27}ClN_6O_5S$ + H^+ requires 583.1525).

1-(Chloromethyl)-5-hydroxy-3-{[2-[[5-(*tert*-butoxycarbonylamino)benzofuran-2-yl]carbonylamino]-1,3-thiazol-4-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X7-Y13): 1.00 mg, 54%; ^{1}H NMR (acetone-d_6, 600 MHz) δ 9.27 (br s, 1H), 8.58 (br s, 1H), 8.23 (d, J = 8.7 Hz, 1H), 8.13 (br s, 2H), 7.96 (br s, 1H), 7.87−7.83 (m, 2H), 7.63−7.58 (m, 1H), 7.57−7.51 (m, 2H), 7.38 (dd, J = 7.4, 7.7 Hz, 1H), 4.70 (br s, 2H), 4.18−4.13 (m, 1H), 4.01−3.96 (m, 1H), 3.75−3.69 (m, 1H), 1.50 (s, 9H); HRMALDI-FTMS (DHB) m/z 641.1215 ($C_{31}H_{27}ClN_4O_6S$ + Na^+ requires 641.1232).

1-(Chloromethyl)-5-hydroxy-3-{[4-[[4-(*tert*-butoxycarbonylamino)thiophen-2-yl]carbonylamino]thiophen-2-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X8-Y8): 1.51 mg, 86%; ^{1}H NMR (acetone-d_6, 500 MHz) δ 10.30 (br s, 1H), 9.26 (s, 1H), 8.82 (s, 1H), 8.23 (d, J = 8.3 Hz, 1H), 8.00 (d, J = 1.2 Hz, 1H), 7.97 (br s, 1H), 7.92 (s, 1H), 7.87 (d, J = 8.3 Hz, 2H), 7.54 (t, J = 8.3 Hz, 1H), 7.41−7.36 (m, 2H), 4.68 (t, J = 10.3 Hz, 1H), 4.61 (dd, J = 1.9, 10.7 Hz, 1H), 4.25−4.20 (m, 1H),4.05 (dd, J = 3.1, 11.5 Hz, 1H), 3.81 (dd, J = 8.5, 11.1 Hz, 1H), 1.48 (s, 9H); HRMALDI-FTMS (DHB) m/z 570.1118 ($C_{28}H_{26}ClN_3O_5S_2$ − HCl + Na^+ requires 570.1133).

1-(Chloromethyl)-5-hydroxy-3-{[4-[[4-(*tert*-butoxycarbonylamino)-1-methylimidazol-2-yl]carbonylamino]-1-methylimidazol-2-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]-indole (*seco*-CBI-X9-Y9): 1.48 mg, 85%; ^{1}H NMR (acetone-d_6, 600 MHz) δ 9.26 (br s, 1H), 9.21 (s, 1H), 8.56 (br s, 1H), 8.24 (d, J = 8.4 Hz, 1H), 8.10 (br s, 1H), 7.87 (d, J = 8.2 Hz, 1H), 7.57−7.52 (m, 3H), 7.39 (t, J = 7.2 Hz, 1H), 7.24 (br s, 1H), 4.95−4.88 (m, 1H), 4.80−4.71 (m, 1H), 4.19−4.13 (m, 1H), 4.08 (s, 3H), 4.05−3.98 (m, 4H), 3.75−3.69 (m, 1H), 1.48 (s, 9H); HRMALDI-FTMS (DHB) m/z 580.2060 ($C_{28}H_{30}ClN_7O_5$ + H^+ requires 580.2075).

1-(Chloromethyl)-5-hydroxy-3-{[4-[[4-(*tert*-butoxycarbonylamino)-1-methylpyrrol-2-yl]carbonylamino]-1-methylpyrrol-2-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X10-Y10): 1.18 mg, 68%; ^{1}H NMR (acetone-d_6, 600 MHz) δ 9.30 (br s, 1H), 9.21 (s, 1H), 8.21 (d, J = 8.7 Hz, 1H), 8.14 (br s, 1H), 7.82 (d, J = 8.2 Hz, 1H), 7.66 (br s, 1H), 7.52 (t, J = 6.7 Hz, 1H), 7.46 (d, J = 1.5 Hz, 1H), 7.36 (t, J = 8.2 Hz, 1H), 6.93 (br s, 1H), 6.81 (br s, 1H), 6.72 (br s, 1H), 4.50 (d, J = 5.1 Hz, 2H), 4.10−4.04 (m, 1H), 3.99 (dd, J = 3.6, 11.0 Hz, 1H), 3.92 (s, 3H), 3.83 (s, 3H), 3.74−3.69 (m, 1H), 1.44 (s, 9H); HRMALDI-FTMS (DHB) m/z 564.2233 ($C_{30}H_{32}ClN_5O_5$ − HCl + Na^+ requires 564.2223).

1-(Chloromethyl)-5-hydroxy-3-{[3-[[2-(*tert*-butoxycarbonylamino)-1,3-thiazol-4-yl]carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indol-7-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]-indole (*seco*-CBI-X11-Y7): 1.23 mg, 64%;^{1}H NMR (acetone-d_6, 600 MHz) δ 10.92 (br s, 1H), 10.42 (br s, 1H), 9.28 (s, 1H), 8.40 (br s, 1H), 8.24 (d, J = 8.2 Hz, 1H), 8.08 (br s, 1H), 7.88 (d, J = 8.7 Hz, 1H), 7.73 (br s, 1H), 7.54 (dd, J = 7.1, 7.2 Hz, 1H), 7.47−7.42 (m, 1H), 7.39 (dd, J = 7.4, 7.7 Hz, 1H), 7.20 (s, 1H), 4.88−4.82 (m, 1H), 4.82−4.77 (m, 1H), 4.62−4.52 (m, 2H), 4.31−4.25 (m, 1H), 4.06 (dd, J = 3.6, 11.8 Hz, 1H), 3.86−3.80 (m, 1H), 3.43−3.35 (m, 1H), 1.54 (s, 9H); HRMALDI-FTMS (DHB) m/z 544.1195 ($C_{33}H_{30}ClN_5O_5S$ − Boc + H^+ requires 544.1205).

1-(Chloromethyl)-5-hydroxy-3-{[3-[[4-(*tert*-butoxycarbonylamino)-1-methylpyrrol-2-yl]carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indol-7-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X11-Y10): 1.19 mg, 62%; ^{1}H NMR (acetone-d_6, 600 MHz) δ 10.90 (br s, 1H), 9.27 (br s, 1H), 8.24 (d, J = 8.2 Hz, 1H), 8.13 (br s, 1H), 8.09−8.02 (m, 2H), 7.88 (d, J = 8.7 Hz, 1H), 7.54 (dd, J = 7.7, 8.2 Hz, 1H), 7.45−7.36 (m, 2H), 7.20 (s, 1H), 7.00 (s, 1H), 6.56 (s, 1H), 4.88−4.83 (m, 1H), 4.82−4.78 (m, 1H), 4.41 (dd, J = 8.2, 8.2 Hz, 2H), 4.31−4.26 (m, 1H), 4.06 (dd, J = 3.1, 11.2 Hz, 1H), 3.83 (dd, J = 8.7, 11.3 Hz, 1H), 3.78 (s, 3H), 3.42−3.31 (m, 2H), 1.46 (s, 9H); HRMALDI-FTMS (DHB) m/z 626.2377 ($C_{35}H_{34}ClN_5O_5$ − HCl + Na^+ requires 626.2374).

1-(Chloromethyl)-5-hydroxy-3-{[3-[[3-(*tert*-butoxycarbonyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indol-7-yl]carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indol-7-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X11-Y11): 1.06 mg, 50%; ^{1}H NMR (acetone-d_6, 600 MHz) δ 10.93 (br s, 1H), 10.75 (br s, 1H), 9.26 (s, 1H), 8.47−8.38 (m, 1H), 8.25 (d, J = 8.2 Hz, 1H), 8.09 (s, 1H), 7.88 (d, J = 8.2 Hz, 1H), 7.55 (dd J = 7.1, 7.2 Hz, 1H), 7.47 (d, J = 8.7 Hz, 1H), 7.43−7.37 (m, 2H), 7.24 (s, 1H), 7.06 (s, 1H), 4.89−4.85 (m, 1H), 4.82 (dd, J = 1.5, 10.7 Hz, 1H), 4.79−4.73 (m, 2H), 4.32−4.27 (m, 1H), 4.11−4.04 (m, 3H), 3.84 (dd, J = 8.7, 11.3 Hz, 1H), 3.63−3.52 (m, 2H), 3.32 (t, J = 9.2 Hz, 2H), 1.56 (s, 9H); HRMALDI-FTMS (DHB) m/z 702.2478 ($C_{40}H_{36}ClN_5O_5$ + H^+ requires 702.2478).

1-(Chloromethyl)-5-hydroxy-3-{[3-[[5-(*tert*-butoxycarbonylamino)indole-2-yl]carbonyl]-1,2-dihydro-3*H*-pyrrolo-[3,2-*e*]indol-7-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X11-Y14): 0.91 mg, 45%; ^{1}H NMR (acetone-d_6, 600 MHz) δ 10.94 (br s, 1H), 10.70 (br s, 1H), 9.27 (s, 1H), 8.44−8.40 (m, 1H), 8.29−8.21 (m, 2H), 8.09 (br s, 1H), 7.93−8.01 (m, 2H), 7.89 (d, J = 8.2 Hz, 1H), 7.55 (dd, J = 7.7, 7.9 Hz, 1H), 7.48 (dd, J = 9.0, 12.2 Hz, 2H), 7.41−7.37 (m, 2H), 7.25 (s, 1H), 7.12 (s, 1H), 4.90−4.86 (m, 1H), 4.80−4.83 (m, 1H), 4.77−4.73 (m, 1H), 4.32−4.27 (m, 1H), 4.07 (dd, J = 3.1, 11.3 Hz, 1H), 3.84 (dd, J = 8.4, 10.7 Hz, 1H), 3.62−3.52 (m, 2H), 1.50 (s, 9H); HRMALDI-FTMS (DHB) m/z 676.2309 ($C_{38}H_{34}ClN_5O_5$ + H^+ requires 676.2321).

1-(Chloromethyl)-5-hydroxy-3-{[5-[[4-(*tert*-butoxycarbonylamino)-1-methylpyrrol-2-yl]carbonylamino]benzothiophen-2-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X12-Y10): 1.05 mg, 57%; ^{1}H NMR (acetone-d_6, 600 MHz) δ 9.39 (br s, 1H), 9.31 (br s, 1H), 8.61 (br s, 1H), 8.25 (d, J = 8.2 Hz, 1H), 8.18 (br s, 1H), 8.06 (s, 1H), 7.93 (d, J = 8.7 Hz, 1H), 7.89 (d, J = 8.7 Hz, 1H), 7.77 (d, J = 8.2 Hz, 1H), 7.55 (dd, J = 7.2, 7.4 Hz, 1H), 7.40 (dd, J = 7.4, 7.7 Hz, 1H), 6.99 (br s, 1H), 6.94 (br s, 1H), 4.84−4.79 (m, 1H), 4.70−4.67 (m, 1H), 4.26−4.21 (m, 1H), 4.05 (dd, J = 3.1, 11.3 Hz, 1H), 3.93 (s, 3H), 3.83 (dd, J = 8.4, 10.7 Hz, 1H), 1.46 (s, 9H); HRMALDI-FTMS (DHB) m/z 495.1504 ($C_{33}H_{31}ClN_4O_5S$ − Boc − HCl + H^+ requires 495.1491).

1-(Chloromethyl)-5-hydroxy-3-{[5-[[5-(*tert*-butoxycarbonylamino)benzothiophene-2-yl]carbonylamino]benzothiophene-2-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X12-Y12): 1.81 mg, 88%; ^{1}H NMR (acetone-d_6, 600 MHz) δ 9.91 (br s, 1H), 9.33 (br s, 1H), 8.61−8.59 (m, 2H), 8.28−8.23 (m, 2H), 8.18 (s, 1H), 8.10 (s, 1H), 8.01, (d, J = 8.7 Hz, 1H), 7.95 (br s, 1H), 7.94−7.88 (m, 2H), 7.85−7.80 (m, 1H), 7.62 (dd, J = 1.8, 8.4 Hz, 1H), 7.55 (t, J = 7.0 Hz, 1H), 7.40 (t, J = 8.0 Hz, 1H), 4.87−4.81 (m, 1H), 4.72−4.67 (m, 1H), 4.28−4.23 (m, 1H), 4.05 (dd, J = 3.1, 11.3 Hz, 1H), 3.85 (dd, J = 8.2, 11.2 Hz, 1H), 1.51 (s, 9H); HRMALDI-FTMS (DHB) m/z 684.1366 ($C_{36}H_{29}ClN_3O_5S_2$ + H^+ requires 684.1388).

1-(Chloromethyl)-5-hydroxy-3-{[5-[[4-(*tert*-butoxycarbonylamino)benzoylamino]benzofuran-2-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X13-Y5): 1.78 mg, 97%; ^{1}H NMR (acetone-d_6, 600 MHz) δ 9.58 (br s, 1H), 9.34 (br s, 1H), 8.73 (br s, 1H), 8.41 (d, J = 2.0 Hz, 1H), 8.25 (d, J = 8.7 Hz, 1H), 8.03−7.98 (m, 2H), 7.89 (d, J = 8.2 Hz, 1H), 7.84 (dd, J = 2.0, 8.7 Hz, 1H), 7.74−7.63 (m, 5H), 7.56 (dd, J = 7.4, 7.7 Hz, 1H), 7.41 (dd, J = 7.7, 8.2 Hz, 1H), 4.86−4.80 (m, 1H), 4.31−4.24 (m, 1H), 4.05 (dd, J = 3.1, 10.7 Hz, 1H), 3.83 (dd, J = 10.7, 11.3 Hz, 1H), 3.60−3.56 (m, 1H), 1.50 (s, 9H); HRMALDI-FTMS (DHB) m/z 598.1946 ($C_{34}H_{30}ClN_3O_6$ − HCl + Na^+ requires 598.1949).

1-(Chloromethyl)-5-hydroxy-3-{[5-[[4-(*tert*-butoxycarbonylamino)thiophen-2-yl]carbonylamino]benzofuran-2-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X13-Y8): 0.91 mg, 48%; ^{1}H NMR (acetone-d_6, 600 MHz) δ 9.88 (br s, 1H), 9.33 (br s, 1H), 8.85 (br s, 1H), 8.36−8.33 (m, 1H), 8.24 (d, J = 8.2 Hz, 1H), 8.18 (br s, 1H), 8.00 (br s, 1H), 7.89

(d, J = 8.7 Hz, 1H), 7.82−7.88 (m, 1H), 7.67−7.63 (m, 2H), 7.55 (dd, J = 7.1, 7.2 Hz, 1H), 7.42−7.38 (m, 2H), 4.82−4.79 (m, 2H), 4.28−4.24 (m, 1H), 4.04 (dd, J = 3.1, 11.3 Hz, 1H), 3.84 (dd, J = 8.7, 11.3 Hz, 1H), 1.49 (s, 9H); HRMALDI-FTMS (DHB) m/z 517.0855 ($C_{32}H_{28}ClN_3O_6S^+$ − Boc requires 517.0863).

1-(Chloromethyl)-5-hydroxy-3-{[5-[[5-(*tert*-butoxycarbonylamino)benzofuran-2-yl]carbonylamino]benzofuran-2-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X13-Y13): 1.32 mg, 67%; ^{1}H NMR (acetone-d_6, 600 MHz) δ 9.90 (br s, 1H), 9.34 (s, 1H), 8.55 (br s, 1H), 8.47−8.44 (m, 1H), 8.25 (d, J = 8.7 Hz, 1H), 8.08 (br s, 1H), 7.93−7.88 (m, 2H), 7.71−7.68 (m, 2H), 7.64 (s, 1H), 7.60−7.54 (m, 2H), 7.51 (d, J = 8.7 Hz, 1H), 7.41 (ddd, J = 1.0, 7.7, 7.7 Hz, 1H), 4.84−4.80 (m, 2H), 4.30−4.25 (m, 1H), 4.05 (dd, J = 3.1, 11.3 Hz, 1H), 3.83 (dd, J = 8.7, 11.3 Hz, 1H), 1.50 (s, 9H); HRMALDI-FTMS (DHB) m/z 638.1883 ($C_{36}H_{30}ClN_3O_7$ − HCl + Na^+ requires 638.1903).

1-(Chloromethyl)-5-hydroxy-3-{[5-[[5-(*tert*-butoxycarbonylamino)indole-2-yl]carbonylamino]indole-2-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X14-Y14): 1.39 mg, 71%; ^{1}H NMR (acetone-d_6, 600 MHz) δ 10.86 (br s, 1H), 10.83 (d, J = 1.0 Hz, 1H), 9.59 (br s, 1H), 9.23 (s, 1H), 8.38−8.34 (m, 1H), 8.24 (d, J = 8.2 Hz, 1H), 8.08 (br s, 1H), 7.96 (br s, 1H), 7.89 (d, J = 8.2 Hz, 1H), 7.63 (dd, J = 2.0, 8.9 Hz, 1H), 7.59−7.52 (m, 2H), 7.49 (d, J = 8.7 Hz, 1H), 7.41−7.37 (m, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.26 (d, J = 5.6 Hz, 1H), 4.89−4.84 (m, 1H), 4.83−4.77 (m, 1H), 4.32−4.27 (m, 1H), 3.84 (dd, J = 8.2, 11.2 Hz, 1H), 1.49 (s, 9H); HRMALDI-FTMS (DHB) m/z 650.2149 ($C_{36}H_{32}ClN_5O_5$ + H^+ requires 650.2165).

1-(Chloromethyl)-5-hydroxy-3-{[6-[[6-(*tert*-butoxycarbonylamino)benzoxazole-2-yl]carbonylamino]benzoxazole-2-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X15-Y15): 1.06 mg, 50%; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 11.51 (s, 1H), 10.57 (s, 1H), 9.83 (s, 1H), 8.51 (s, 1H), 8.14 (d, J = 8.2 Hz, 1H), 8.15−8.00 (m, 4H), 7.88 (d, J = 8.7 Hz, 1H), 7.84 (d, J = 8.4 Hz, 1H), 7.60−7.51 (m, 2H), 7.45−7.39 (m, 1H), 4.98 (d, J = 12.3 Hz, 1H), 4.86−4.79 (m, 1H), 4.29−4.24 (m, 1H), 3.91−3.86 (m, 1H), 1.51 (s, 9H); HRMALDI-FTMS (DHB) m/z 653.1692 ($C_{34}H_{28}ClN_5O_7^+$ requires 653.1671).

1-(Chloromethyl)-5-hydroxy-3-{[5-[[4-(*tert*-butoxycarbonylamino)thiophene-2-yl]carbonylamino]benzimidazole-2-yl]carbonyl}-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI-X16-Y8): 1.40 mg, 75%; ^{1}H NMR (acetone-d_6, 500 MHz) δ 9.89 (br s, 1H), 9.82 (br s, 1H), 9.30 (s, 1H), 8.84 (br s, 1H), 8.48−8.45 (m, 1H), 8.26 (d, J = 8.5 Hz, 1H), 8.23 (s, 1H), 8.01−7.98 (m, 1H), 7.95−7.90 (m, 1H), 7.78 (d, J = 8.9 Hz, 1H), 7.60−7.54 (m, 2H), 7.44−7.37 (m, 2H), 5.50 (dd, J = 10.9, 11.1 Hz, 1H), 4.98−4.88 (m, 1H), 4.36−4.27 (m, 1H), 4.12−4.04 (m, 1H), 3.88−3.80 (m, 1H), 1.49 (s, 9H); HRMALDI-FTMS (DHB) m/z 618.1584 ($C_{31}H_{28}ClN_5O_5S$ + H^+ requires 618.1572).

DNA Alkylation Studies: Selectivity and Efficiency. The preparation of singly ^{32}P-5′-end-labeled double-stranded DNA, agent binding studies, gel electrophoresis, and autoradiography were conducted according to procedures described in full detail elsewhere.[28] Eppendorf tubes containing the 5′-end-labeled DNA (9 μL) in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) were treated with the agent in DMSO (1 μL at the specified concentration). The solution was mixed by vortexing and brief centrifugation and subsequently incubated at 25 °C for 24 h. The covalently modified DNA was separated from the unbound agent by EtOH precipitation and resuspended in TE buffer (10 μL). The solution of DNA in an Eppendorf tube sealed with Parafilm was warmed at 100 °C for 30 min to introduce cleavage at the alkylation sites, allowed to cool to 25 °C, and centrifuged. Formamide dye (0.03% xylene cyanol FF, 0.03% bromophenol blue, 8.7% Na_2EDTA 250 mM) was added (5 μL) to the supernatant. Prior to electrophoresis, the sample was denatured by warming at 100 °C for 5 min, placed in an ice bath, and centrifuged, and the supernatant (3 μL) was loaded directly onto the gel. Sanger dideoxynucleotide sequencing reactions were run as standards adjacent to the reaction samples. Polyacrylamide gel electrophoresis (PAGE) was run on an 8% sequencing gel under denaturing conditions (8 M urea) in TBE buffer (100 mM Tris, 100 mM boric acid, 0.2 mM Na_2EDTA) followed by autoradiography.

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Supporting Information Available: ^{1}H NMR spectra of the diagonal elements of the library, *seco*-CBI-X**5**-Y**5**−*seco*-CBI-X**15**-Y**15**, *seco*-CBI-X**7**-Y**9**, *seco*-CBI-X**7**-Y**13**, *seco*-CBI-X**11**-Y**7**, *seco*-CBI-X**11**-Y**10**, *seco*-CBI-X**11**-Y**14**, *seco*-CBI-X**12**-Y**10**, *seco*-CBI-X**13**-Y**5**, *seco*-CBI-X**13**-Y**8**, and *seco*-CBI-X**16**-Y**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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