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1,2,9,9a-Tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI) Analogs of CC-1065 and the Duocarmycins: Synthesis and Evaluation

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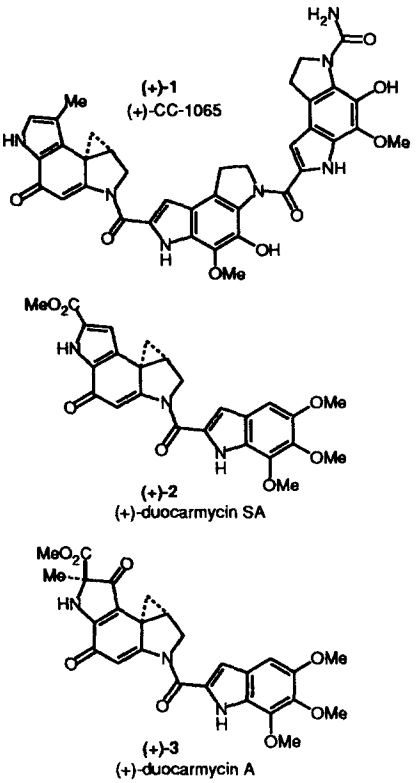
Abstract—An extensive study of analogs of the potent antitumor antibiotics CC-1065 and the duocarmycins which incorporate the 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI) alkylation subunit are detailed. In contrast to early speculation, deep-seated modifications in the CC-1065 and duocarmycin alkylation subunits are well tolerated and the CBI-based analogs proved to be potent cytotoxic agents and efficacious antitumor compounds. Full details of studies defining a direct relationship between functional stability and *in vitro* cytotoxic potency are described. As such, the readily accessible CBI-based analogs were found to be four times more stable and four times more potent than the corresponding analogs containing the authentic CPI alkylation subunit of CC-1065 and comparable in potency to agents containing the authentic alkylation subunit of duocarmycin SA. Similarly, the CBI-based agents alkylate DNA with an unaltered sequence selectivity at an enhanced rate and with a greater efficiency than the corresponding CPI analog and were comparable to the corresponding analog incorporating the duocarmycin SA alkylation subunit. Systematic and extensive modifications and simplifications in the DNA binding subunits attached to CBI were explored with the comparisons of both enantiomers of CC-1065 and the duocarmycins **2** and **3** with enantiomers of **18–24**, **25–29**, **57–61**, **62–65**, **66–68**, **72**, **73**, **78** and **79**.

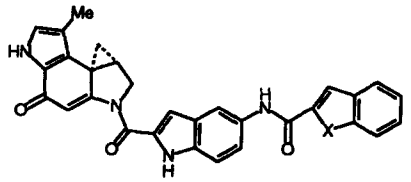
Introduction

(+)-CC-1065 (**1**),[1] and the duocarmycins **2** and **3**, disclosed in 1988 and 1990,[2–4] represent the initial members of a class of exceptionally potent antitumor antibiotics that derive their biological effects through the reversible, stereoelectronically controlled sequence selective alkylation of duplex DNA.[5–13] Subsequent to their disclosure, extensive efforts have been devoted to establish their duplex DNA alkylation selectivity and its structural origin,[5–13] to establish the link between DNA alkylation and the ensuing biological properties,[14] and to define the fundamental principles underlying the relationships between structure, chemical reactivity, and biological properties.[15–41]

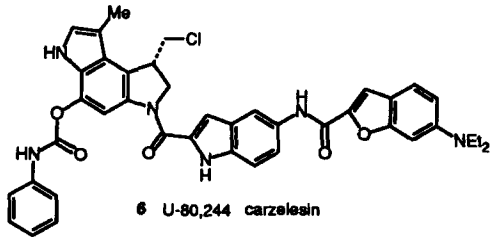
Concurrent with these studies, substantial efforts have been devoted to developing potential clinical candidates, i.e. **4–8**, based on the natural product structures.[15,42–48] In particular, efforts have focused not only on improving the *in vivo* efficacy of the agents but also on necessarily addressing the unusual delayed toxicity of natural (+)-CC-1065.[49] Importantly, this unusual property has not been observed with *ent*-(–)-CC-1065, although it is equally cytotoxic, and is not observed with the naturally-derived duocarmycins as well as simplified analogs of the natural products.

In the course of our efforts on the evaluation of CC-1065 and duocarmycin analogs bearing deep-seated structural alterations in the alkylation subunit, we described the first preparation and examination of agents containing the 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI) alkylation subunit.[24,25] Although our interest in this class of agents was derived initially from efforts to define the origin and structural features of **1–3** responsible for their sequence selective alkylation of duplex DNA and to define the fundamental relationships between structure, chemical

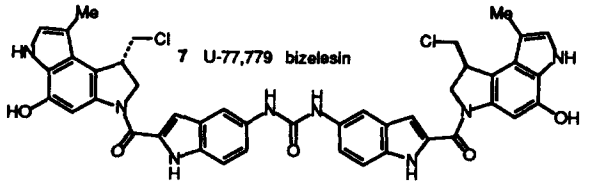




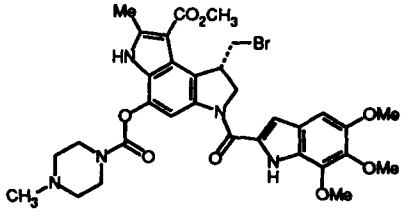
4 X = NH U-71,184
5 X = O U-73,975 adozelesin



6 U-80,244 carzelesin



7 U-77,779 bizelesin



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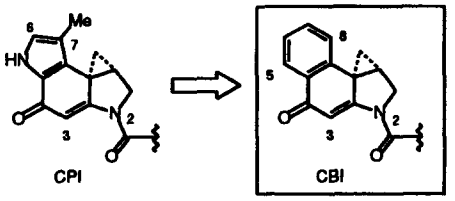
or functional reactivity, and biological properties, the properties and potential utility of the CBI-based analogs proved especially interesting. This was especially significant since preceding studies[8,12] had attributed such unique characteristics to the naturally occurring CPI alkylation subunit of CC-1065 that they left the perception that even small structural perturbations, let alone deep-seated structural changes, would have detrimental effects on the properties. Not only has this proven inaccurate, but in our studies of the natural enantiomers of the CBI-based analogs of (+)-CC-1065, we have shown that they are chemically more stable (four times), biological more potent (four times) and considerably more synthetically accessible[25-27] than the corresponding agents incorporating the natural CPI alkylation subunit of CC-1065.[30] Moreover, (+)-CBI-indole$_2$ (**27**), a selected simplified agent within our early series of CBI analogs, was prepared which not only exhibited cytotoxic potency comparable to that of the (+)-CC-1065 and greater (four times) than that of the potential clinical candidate (+)-CPI-indole$_2$ (**4**, U71,184) introduced by Upjohn, but which also exhibited potent and efficacious *in vivo* antitumor activity.[32] This constituted the first report of efficacious antitumor activity by a CC-1065 analog possessing a structurally altered and simplified DNA alkylation subunit. Moreover, the agent further lacked the delayed fatal toxicity characteristic of (+)-CC-1065.

In addition, the natural enantiomers of the CBI-based analogs have been shown to alkylate DNA with an unaltered sequence selectivity[33,34,36,37] at an enhanced rate[32-34] and with a greater efficiency than the corresponding CPI analog[32-34] indicating that the simplified CBI alkylation subunit offers distinct advantages over the natural CPI alkylation subunit of CC-1065. In recent studies, we have presented refined models of the DNA alkylation reactions of the duocarmycins[5,6] and CC-1065[9] which accomodate the reversed and offset AT-rich adenine N3 DNA alkylation selectivity of the enantiomeric agents and their structural analogs. From this work, a beautiful model has emerged which explains the unusual and apparently confusing behavior of the unnatural enantiomers. The diastereomeric adducts derived from the unnatural enantiomers suffer a significant destabilizing steric interaction between the CPI C7 center (CH_3) or the CBI C8 center with the base adjacent to the alkylated adenine which is not present with the natural enantiomer adducts. Moreover, the distinguishing features of the natural and unnatural enantiomers diminish or disappear as the inherent steric bulk surrounding this center is reduced or removed. Because of the unnatural enantiomer sensitivity to destabilizing steric interactions surrounding the CPI C7 or CBI C8 center, the unnatural enantiomers of the CBI-based analogs are particularly more effective than the corresponding CPI analog displaying an even more enhanced relative rate and efficiency of DNA alkylation. These observations have prompted us to study the CBI-based analogs of CC-1065 and the duocarmycins in detail.

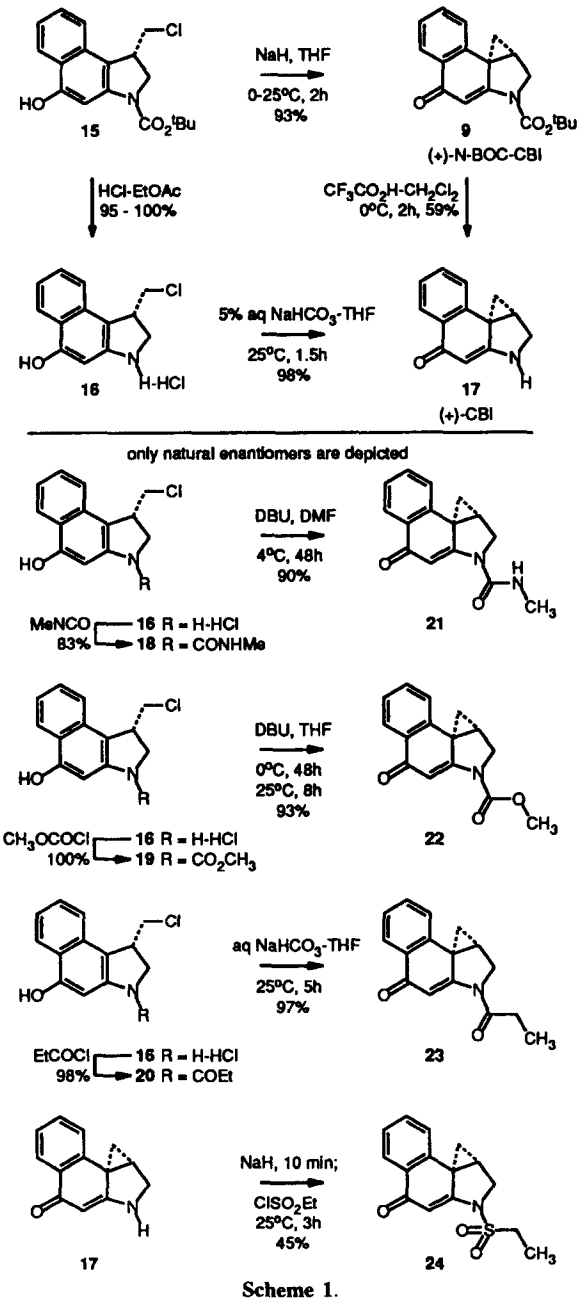


CPI CBI

Simple Derivatives of CBI. Role of the N^2 Substituent and Validation of a Direct Relationship Between Functional Stability and *In Vitro* Cytotoxic Potency

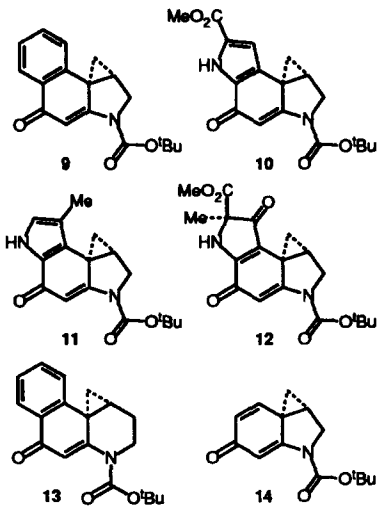
Substantial quantities of optically active natural (1*S*)- and *ent*-(1*R*)-**15** were prepared through use of two recent modifications[26,27] of our original synthesis of CBI and its precursors.[24,25] The most efficient approach[27] now proceeds in nine steps and in 38% overall yield from commercially available 1,3-dihydroxynaphthalene based on a key 5-*exo*-trig aryl radical–alkene cyclization for the direct preparation of *N*-BOC-5-benzyloxy-1-hydroxymethyl-1,2-dihydro-3*H*-benz[*e*]-indole. Moreover, the initial resolution we described based on the chromatographic separation of the diastereomeric (*R*)-*O*-acetyl madelate esters[25,30,50] of the primary alcohol precursor to **15** which has been adopted by others[29,37] has since been improved in our efforts.[36] The more advanced synthetic intermediate **15**, and in

fact the penultimate intermediate to the CBI-based analogs, may be directly and more efficiently resolved (α = 1.28) on an analytical or preparative Daicel Chiralcel OD column without recourse to diastereomeric derivatization. For our purposes, 20 mg of **15** could be separated in a single injection on a semipreparative 10 μm, 2 × 25 cm OD HPLC column (5% *i*-PrOH–hexane, 8 mL min^{-1}) with a 90–100% recovery of the total sample. Conversion of natural (1*S*)- and *ent*-(1*R*)-**15** to (+)- and *ent*-(–)-*N*-BOC-CBI (**9**), and (+)- and *ent*-(–)-CBI (**17**) have been detailed in our initial studies,[25] and provided our comparison standards for the studies detailed below (Scheme 1).



Scheme 1.

Initial studies conducted with simple derivatives of the (+)-CC-1065 alkylation subunit (CPI) led to the proposal that there exists a direct relationship between an agent's reactivity and *in vitro* cytotoxic potency (L1210, IC_{50})[15] and established the expectation that the biological potency may be enhanced as their electrophilic reactivity is increased. In our complementary series of studies conducted with agents containing deep-seated modifications in the alkylation subunit including **9–14**, the reverse relationship has been observed[16,21,25,30,36] and the agents possessing the greatest chemical solvolysis stability exhibited the most potent *in vitro* cytotoxic activity. Moreover, a direct relationship between solvolytic stability and biological potency has been observed and proved to be general with both simple and advanced analogs of the natural products.



	k (s^{-1}, pH 3)	$t_{1/2}$	IC_{50} (L1210)
10	1.1×10^{-6}	177 h	6 nM
9	1.5×10^{-6}	133 h	80 nM
11	5.3×10^{-6}	37 h	330 nM
12	1.7×10^{-5}	11 h	1000 nM
13	9.1×10^{-5}	2 h	4000 nM
14	2.0×10^{-2}	0.01 h	18000 nM

As a consequence of these studies, we became interested in the inherent role of the CC-1065 and duocarmycin N^2 substituent. Consequently, the simple derivatives **21–24** of (+)-CBI were prepared for examination and, by virtue of their structural similarities, were expected to more accurately reflect a potential relationship between functional reactivity and biological potency than the preceding studies.[15] Treatment of crude, freshly prepared **16** with methyl isocyanate (2 equiv, 3 equiv $NaHCO_3$, THF, 0–25 °C, 1 h, 83%) provided **18** and attempts to conduct this reaction in more polar solvents including DMF or in the presence of a stronger base (i.e. Et_3N) which promotes competitive closure of **16** to CBI (**17**) led to lower conversions (Scheme 1). Spirocyclization of **18** to **21** was effected by treatment with DBU (2 equiv, DMF, 4 °C, 48 h, 90%) and the use of shorter reaction periods (24 h, 55%) or less polar solvents (THF, 18 h, 35%) provided lower conversions. Treatment of the freshly generated crude indoline hydrochloride salt **16** with $ClCO_2CH_3$ (2 equiv, 3 equiv $NaHCO_3$, THF, 0–25 °C, 1.5 h) provided **19** (100%) in quantitative conversion.

Spirocyclization of **19** to provide **22** was effected by treatment with DBU (2 equiv, THF, 0 °C, 48 h and 25 °C, 10 h, 93%) and the rate of ring closure of **19** to **22** only became significant at 25 °C under these conditions. Even treatment of **19** with K_2CO_3 (1.5 equiv, THF, 25 °C, 5 d, 51%) provided **22** albeit with this latter reaction requiring a long reaction period. Similarly, treatment of crude **16** with $ClCOCH_2CH_3$ (2 equiv, 3 equiv $NaHCO_3$, THF, 0–25 °C, 5 h or 0 °C, 1h) cleanly provided **20** (94–98%). Spirocyclization of **20** to cleanly provide **23** was effected by simply dissolving **20** in a 1:1 mixture of 5% aqueous $NaHCO_3$–THF (25 °C, 5–10 h, 97%) and stirring the resulting two-phase reaction mixture at room temperature. Given the ease of hydrolysis of *N*-acyl-CBI derivatives upon exposure to aqueous base, it is of special note that this set of reaction conditions worked so well for **23**. Lower conversions to **23** were observed upon treatment of **20** with DBU (2 equiv, THF, 0–25 °C, 18 h) and, although this was not examined in detail, can be attributed to a slow cyclization under the reaction conditions resulting in significant amounts of recovered, unreacted **20**. Surprisingly, the most challenging of the derivatives to prepare was **24**. Attempts to couple freshly generated **16** with $ClSO_2CH_2CH_3$ under a wide range of reaction conditions deliberately generating or avoiding sulfene formation suffered from competitive or preferential *O*-sulfonylation or competitive closure to **17**. Although this approach could be used to generate **24**, the most productive preparation was accomplished simply by reaction of the sodium salt of CBI (**17**, 2.5 equiv NaH, THF, 0 °C, 10 min) with $ClSO_2CH_2CH_3$ (7 equiv, 3 equiv Et_3N, 25 °C, 3 h, 45%) to provide **24** directly.

The acid-catalyzed solvolysis of **21–24** conducted at pH 3 (CH_3OH–H_2O) was followed spectrophotometrically by UV with the disappearance of the characteristic long-wavelength absorption band of the CBI chromophore and with the appearance of a short-wavelength absorption band attributable to the *seco*-*N*-BOC-CBI derivative (Fig. 1). The results of these studies along with the cytotoxic activities of **21–24** are summarized in Figure 2. The cytotoxic activity of the full set of agents examined and the comparisons with the related CPI-based agents are summarized in Table 1.

The comparisons of **21–24** revealed a direct, linear relationship between the cytotoxic potency (L1210, log $1/IC_{50}$) and the solvolytic stability (–log k_{solv}, pH 3) of the agents (Fig. 2). Thus, similar to the trend observed with **9–14**, the solvolytically more stable derivatives of CBI proved to be the most potent. Similarly, a linear relationship was found between the electron-withdrawing properties of the N^2 substituents (Hammett σ_p constant) and the solvolysis reactivity (–log k_{solv}, pH 3) of the agents with the strongest electron-withdrawing substituents providing the most stable agents (Fig. 2). This latter relationship reflects the influence of the N^2 substituent on the ease of C4 carbonyl protonation required for catalysis of solvolysis and cyclopropyl ring cleavage with the stronger electron-withdrawing N^2 substituents exhibiting slower solvolysis rates. Less

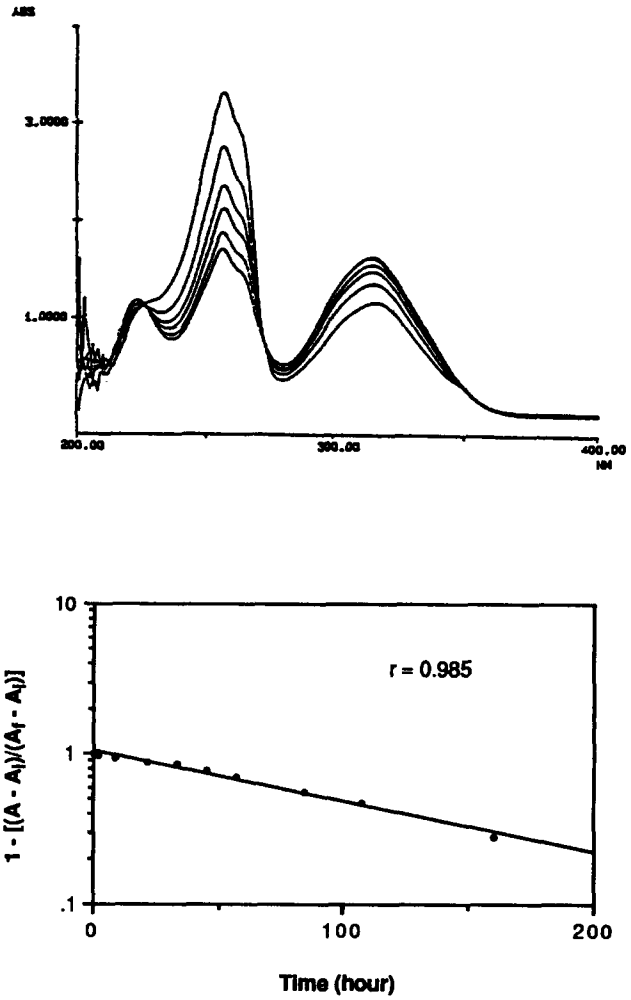
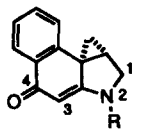


Figure 1. Solvolysis of **23**. Top: UV-visible spectra of **23** in 50% CH_3OH-aqueous buffer (pH 3) recorded at various time intervals (0, 21, 57, 84, 160, 371 h). Bottom: Plot of the disappearance of **23**, log $(1 - [(A - A_i)/(A_f - A_i)])$ versus time from which the first order solvolysis rate constant was derived.

Table 1

Agent	Configuration	IC_{50} (L1210, nM)
(+)-**9**, (+)-*N*-BOC-CBI	natural	80
(–)-**9**, (–)-*N*-BOC-CBI	unnatural	1000
(+)-**11**, (+)-*N*-BOC-CPI	natural	330
(+)-**15**	natural	80
(–)-**15**	unnatural	1000
(+)-**21**	natural	200
(+)-**22**	natural	140
(+)-**23**	natural	110
(+)-**24**	natural	25
(–)-**21**	unnatural	3000
(–)-**22**	unnatural	1500
(–)-**23**	unnatural	700
(–)-**24**	unnatural	–
18	natural	200
18	unnatural	3000
19	natural	140
19	unnatural	1500
20	natural	110
20	unnatural	700

obvious but more fundamental, the observations were found to follow a predictable linear relationship between the cytotoxic potency (L1210, log $1/IC_{50}$) and the electron-withdrawing properties of the N^2 substituent (Hammett σ_p) with the strongest electron-withdrawing substituents providing the biologically most potent agents (Fig. 2).



R	k (s^{-1}, pH 3)	$t_{1/2}$	IC_{50} (L1210)	sigma
21 CONHMe	5.4×10^{-6}	36 h	200 nM	0.36
22 CO_2Me	3.4×10^{-6}	57 h	140 nM	0.45
23 COEt	2.0×10^{-6}	96 h	110 nM	0.48
24 SO_2Et	0.5×10^{-6}	383 h	24 nM	0.72

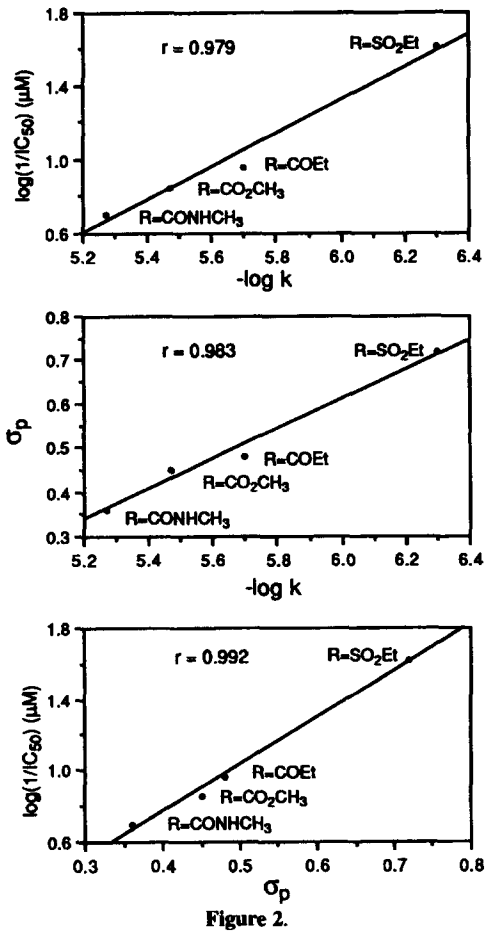


Figure 2.

These fundamental correlations between the electron-withdrawing properties of the N^2 substituent, the functional reactivity of the agents, and their biological potency should prove useful in the predictable design of new analogs.[51] In fact, it is this fundamental validation of the direct relationship between functional stability and biological potency that suggests that the CBI-based analogs, which are four times more stable than the corresponding CPI-based analogs, offer rationally based advantages that may be expected to be even further enhanced by the inherent selectivity that is intrinsic in the diminished reactivity. For agents in this class which possess sufficient reactivity to effectively alkylate duplex DNA, the chemically more stable agents may be expected to constitute the biologically more potent agents. Presumably, this may be attributed to the more effective delivery of the more stable agents to their intracellular target, and the solvolysis rates may be taken to represent a general measure of the relative functional reactivity. Notably, the consumption of the agent in route to its intracellular target need not be simply nonproductive solvolysis but competitive alkylation of nonproductive extra- and intracellular sites as well including the potential of nonproductive sites within duplex DNA. Since the chemically more stable agents provide thermodynamically less stable and more readily reversed addition products,[5,6] the observations may also represent a more effective thermodynamic partitioning of the agents to their productive intracellular target or site(s).

Consistent with prior observations, the corresponding *seco* agents **15** and **18–20** which serve as the immediate synthetic precursors to **9** and **21–23** exhibited a cytotoxic potency indistinguishable from that of the corresponding agent incorporating the preformed cyclopropane ring. Since simple C4 phenol *O*-alkyl (CH_3, CH_2Ph) and *O*-acyl derivatives of **15** exhibit substantially diminished cytotoxic potency (10–100 times),[25] this equivalency of the *seco* precursors **15** and **18–20** with **9** and **21–23** most likely may be attributed to their facile closure to the biologically relevant and more potent cyclopropane containing agents. Notably, such observations have been instrumental in the successful development of prodrug strategies for the advanced analogs of the natural products including **6–8**.

Although we have described an extensive account of the DNA alkylation properties of (+)- and *ent*-(−)-*N*-BOC-CBI (**9**)[33,36] and their comparison with those of (+)- and *ent*-(−)-*N*-BOC-CPI (**11**), the properties of **21–24** and their relationship to the biological evaluations are worth summarizing. The agents **21–24** behaved in a manner comparable to **9**. The natural and unnatural enantiomers of **21–24** were substantially less efficient (*ca* 10^4 times), less selective (selectivity = 5'-AA > 5'-TA) with 40–45% of all adenines alkylated over a 10-fold agent concentration range, and exhibited an altered DNA alkylation profile than (+)- or *ent*-(−)-**1–3**. Moreover, the natural enantiomers of **21–24**, like (+)- versus *ent*-(−)-**9**, proved to be approximately five to 10 times more efficient than the unnatural enantiomers at alkylating DNA, but were found to exhibit the same selectivity and alkylate the same sites. This alkylation selectivity of **21–24**, like that of **9**, was identical to that of (+)- or *ent*-(−)-*N*-BOC-CPI. However, both the natural enantiomers (five times) and especially the unnatural enantiomers (10–100 times) of the CBI-based agents were more effective at alkylating DNA than the corresponding CPI-based agent consistent with models that we have discussed in detail.[6,9] Importantly, the less

reactive CBI-based agents were found to alkylate DNA at a faster rate, with a greater efficiency, and with a slightly greater selectivity among the available sites than the corresponding CPI-based agent. This may be interpreted in terms of agents steric accessibility to the adenine N3 alkylation site where the C7 methyl group of the CPI alkylation subunit sterically decelerates the rate of DNA alkylation to the extent that the less reactive, but more accessible, CBI subunit alkylates DNA at a more rapid rate. Since the unnatural enantiomers are even more sensitive to destabilizing steric interactions at the CPI C7 or CBI C8 position, the unnatural enantiomers of the CBI-based agents are particularly more effective than the CPI-based agents.

Advanced Analogs of CC-1065 and the Duocarmycins: Simplification of the DNA Binding Subunits

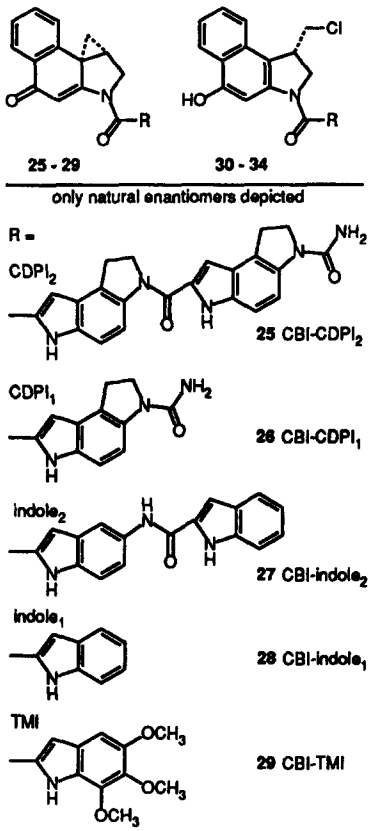
The preparation and evaluation of both enantiomers of CBI-CDPI$_2$ (**25**),[24,25,30,33,34] CBI-CDPI$_1$ (**26**),[24,25,30,33,34] CBI-indole$_2$ (**27**),[32,34] CBI-indole$_1$ (**28**),[38] and CBI-TMI (**29**)[36] and their corresponding *seco* precursors **30–34** have been disclosed in our early studies and their detailed comparisons with both enantiomers of CC-1065[9] or the duocarmycins[36,38] described. More recently, **27**, **28**, and CBI-PDE-I$_2$ have been disclosed by Aristoff and co-workers.[29,37] The comparative cytotoxic activity of these prior agents prepared in our studies is summarized in Table 2 along with that of the corresponding CPI-based analog.

In an extension of our investigations which first revealed efficacious antitumor activity for **27**,[32] we

Table 2

Agent	Configuration	IC_{50} (L1210, pM)
1, (+)-CC-1065	natural	20
1, ent-(–)-CC-1065	unnatural	20
2, (+)-duocarmycin SA	natural	10
2, ent-(–)-duocarmycin SA	unnatural	100
3, (+)-duocarmycin A	natural	500
3, ent-(–)-duocarmycin A	unnatural	≥ 22000
25, (+)-CBI-CDPI$_2$	natural	5
25, (–)-CBI-CDPI$_2$	unnatural	20
(+)-CPI-CDPI$_2$	natural	20
(–)-CPI-CDPI$_2$	unnatural	20
26, (+)-CBI-CDPI$_1$	natural	5
26, (–)-CBI-CDPI$_1$	unnatural	380
(+)-CPI-CDPI$_1$	natural	40
(–)-CPI-CDPI$_1$	unnatural	≥ 6300
27, (+)-CBI-indole$_2$	natural	10
27, (–)-CBI-indole$_2$	unnatural	≥ 3900
4, (+)-CPI-indole$_2$	natural	40
4, (–)-CPI-indole$_2$	unnatural	1000[a]
28, (+)-CBI-indole$_1$	natural	5000
(+)-CPI-indole$_1$	natural	90[a]
29, (+)-CBI-TMI	natural	30
29, (–)-CBI-TMI	unnatural	2000

[a]Taken from Ref. 37.



have expanded the studies to the preparation and evaluation of **57–61**, a larger series based on **27**. The DNA binding subunits of CC-1065 and the duocarmycins contribute in several ways to the properties of the natural products. They contribute significantly to the DNA binding affinity which serves both to increase the rate of DNA alkylation relative to **9** and to thermodynamically stabilize the inherently reversible DNA alkylation reaction.[5,6] While the former has been suggested to be the origin of the differences in the cytotoxic potency of **1** and **11** by others based principally on the comparisons of (+)-*N*-BOC-CPI (**11**), (+)-CPI-indole$_1$, and (+)-CPI-indole$_2$,[12] we have proposed that it is the latter that constitutes the biologically significant distinction. This thermodynamic versus kinetic distinction was first proposed before the reversibility of the DNA alkylation reaction was experimentally verified[5,6,8] and was based in part on the observation that the cytotoxic potency of a class of agents would plateau. For example, (+)-CC-1065, (+)-CPI-PDE-I$_1$, and (+)-CPI-CDPI$_n$ (n = 1–3) were found to be indistinguishable in our cytotoxic assays (IC_{50} = 20 pM, L1210). Although the five agents exhibit large differences in their rates of DNA alkylation,[9] all five form thermodynamically stable adducts under physiological conditions. We attribute the increase in cytotoxic potency of CPI-CDPI$_n$ (n = 1–3) versus **11** to noncovalent binding stabilization of the reversible DNA adduct formation and that it is the simple event not extent of this stabilization that results in their essentially equivalent properties. This interpretation further suggests that CPI-indole$_1$ and CBI-indole$_1$ lack the sufficient stabilization for observation of full

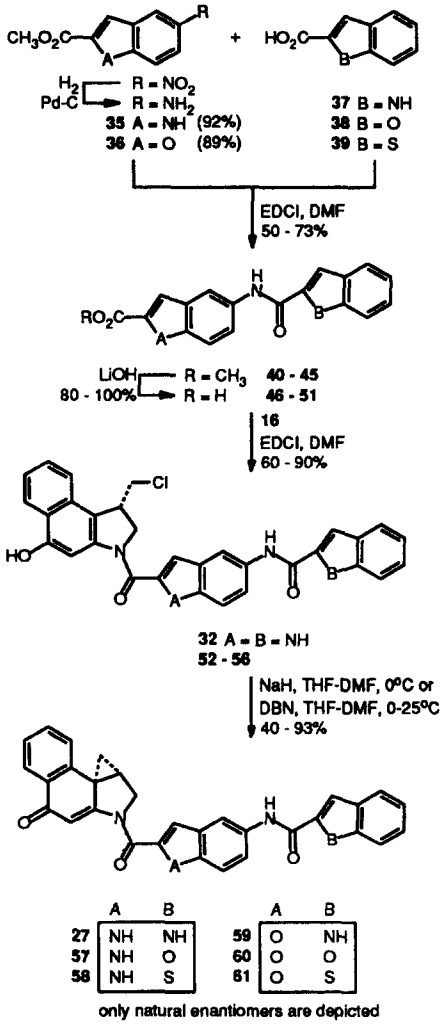
potency. Moreover, the interpretation is consistent with the observation that a maximum potency is achievable and that the level of this potency is directly related to the functional stability of the agents. Thus, the CBI-based agents examined to date exhibit a similar plateau of potency (5 pM, L1210) but at a level four times more potent than that of the corresponding CPI-based agents (20 pM, L1210).

In addition, the DNA binding subunits of CC-1065 contribute to a strong AT-rich DNA binding selectivity which we have recently shown not only contributes[13,53] to the alkylation selectivity of the agents but exerts an overriding dominate control.[52] In early studies, we were able to demonstrate that the noncovalent binding affinity was derived nearly exclusively from stabilizing van der Waals contacts and hydrophobic binding. Not only did the studies suggest that CC-1065 is best represented as a selective alkylating agent superimposed on the trimer skeleton but removal of the peripheral methoxy and hydroxy substituents (PDE-I→CDPI) had no effect on its noncovalent AT-rich binding selectivity and little effect on its binding affinity.[53] This dependence on hydrophobic binding stabilization results in preferential binding in the narrower, deeper AT-rich regions of the minor groove where the stabilizing van der Waals contacts are maximal ($\Delta G°$ = 9.5–11.5 kcal mol^{-1}). Moreover, such studies suggested seminal ways in which the DNA binding subunits could be simplified (removal of polar substituents) without altering the characteristics responsible for the essential DNA binding affinity or selectivity.

The DNA binding subunits of the agents may also have a significant impact on the physical properties and characteristics of the agents. Most apparent is the remarkable solubility properties of CC-1065 which is essentially insoluble in all solvents except DMSO or DMF including polar protic or aprotic solvents, water, or nonpolar solvents. A major impact that structural variations in the central and right hand subunits may have is in the solubility properties of the agent and hence its biodistribution and bioavailability.

Finally, we have speculated that the extent of the noncovalent binding stabilization of the inherently reversible DNA alkylation reaction may be responsible for the unusual, delayed toxicity of CC-1065.[49] That is, the extensive noncovalent binding stabilization of **1** that renders its DNA alkylation reaction irreversible while that of simpler agents including **2** and **3** are slowly reversible under physiological conditions offers a potential explanation for the apparently confusing toxicity profile among the analogs detailed to date. The only agents that have exhibited the delayed toxicity that we are aware of are (+)-CC-1065 (**1**), (+)-CPI-CDPI$_2$,[43] and (+)-CBI-PDE-I$_2$.[37] Each provide irreversible adduct formation under physiological conditions, and the unnatural enantiomers of each, which form inherently less stable and more reversible adducts, do not exhibit the delayed toxicity. Although speculative, it does suggest that simplified DNA binding subunits which provide sufficient but not extensive binding stabilization of the reversible DNA adduct might offer important advantages that relate to the inherent repair or reversal of nonproductive DNA alkylation sites. Moreover, this would also provide a further strong rationale for the use of less reactive alkylation subunits (CBI versus CPI) whose DNA adducts, while stable, are inherently less stable and more readily reversed.

The preparation of the expanded series of agents **57–61** and their corresponding *seco* derivatives **52–56** is summarized in Scheme 2. The simplified DNA binding subunits were assembled by coupling methyl 5-aminoindole-2-carboxylate (**35**) or methyl 5-aminobenzoxazole-2-carboxylate (**36**) with **37–39**. Hydrolysis of the methyl esters **40–45** (LiOH, THF–CH_3OH–H_2O, 25 °C) followed by coupling of the carboxylic acids **46–51** with freshly generated **16** (EDCI, DMF, 25 °C) deliberately conducted in the absence of added base[25] provided excellent yields of the *seco* agents **32** and **52–56**. Spirocyclization of **32** and **52–56** was effected by treatment with NaH, DBN, or P_4-*t*Bu and provided the agents **27** (CBI-indole$_2$) and **57–61**.



Scheme 2.

The results of the cytotoxic evaluations of the agents are summarized in Table 3 along with those of CBI-indole$_2$ (**27**) and CPI-indole$_2$. Several aspects of these comparative evaluations are notable. First, the natural enantiomers are substantially more potent than the unnatural enantiomers (130–1000 times). In addition, the *seco* agents **32** and **52–56** exhibited the same levels of cytotoxic activity as the cyclopropane containing agents where compared although this was not investigated in detail. Most notably and with the exception of **60**, the cytotoxic potency of natural enantiomers of the new agents were equivalent to or exceeded those of **27** and **57** and all were two to six times more potent than the corresponding CPI analog. Moreover, the potencies of **32** and **52–56** approach or are equivalent with the ceiling of potency observed with **25–36** (5 pM).

Although we have described an extensive account of the DNA alkylation properties of both enantiomers of **25–27**,[34] **28**,[38] and **29**[36] elsewhere, their comparisons with the corresponding CPI-based agents and their relationship to the biological evaluations merit summarizing. In these studies, a detailed investigation leading to the definition of the 3.5–5 base pair AT-rich adenine N3 alkylation selectivity of the agents were disclosed for both the natural and unnatural enantiomers, models were disclosed which accommodate the reversed binding orientations and offset AT-rich alkylation selectivity,[5,6,9] and a beautiful explanation emerged which explains the diminished DNA capabilities of the unnatural enantiomers.[6,9,36] Moreover, a clearer picture of the origin of the DNA alkylation selectivity and the structural features of the agents responsible have emerged from these studies.[5,6,9,52] In a detailed comparative examination of the DNA alkylation properties of the CBI-based agents and the corresponding CPI-based analog or duocarmycin SA based agent, they have been found to exhibit identical DNA alkylation selectivities. This is nicely illustrated in Figures 3 and 4 with the comparisons of CBI-indole$_2$ (**27**)/CPI-indole$_2$ (**4**) and CBI-TMI (**29**)/duocarmycin SA (**2**), respectively. In addition, the CBI-based agents have been shown to alkylate DNA both at a faster rate and with a greater efficiency than the corresponding CPI-based agent. This is nicely illustrated in Figure 3 with the comparison of (+)-CBI-indole$_2$ (**27**) and (+)-CPI-indole$_2$ (**4**) where **27** is 10 times more efficient at alkylating w794 (4 °C or 37 °C, data for latter not shown). Moreover, when the relative rates of DNA alkylation were directly compared at the single high affinity site of w794 DNA, that of CBI-indole$_2$ was considerably faster, k(**27**)/k(**4**) = 14 (Fig. 5). In contrast, the natural enantiomer of CBI-based agents and corresponding duocarmycin SA based agents have been found to alkylate w794 DNA with essentially indistinguishable efficiencies (Fig. 4) and at comparable rates, k(**29**)/k(**2**) = 0.9 (Fig. 5).

In addition, because of the unnatural enantiomer sensitivity to destabilizing steric interactions surrounding the duocarmycin C7, CPI C7 or CBI C8 center, the unnatural enantiomers of the simpler CBI-based analogs are approximately four to 100 times less effective than the natural enantiomers. In comparison, the unnatural enantiomers of the CPI-based analogs are 10 to 1000 times less effective and the duocarmycin SA based analogs or agents are one to 10 times less effective in both the cytotoxic assays and in their relative DNA alkylation rate or efficiency. Moreover, this distinction in the enantiomers diminishes only with the larger agents, i.e. **25**, where the extensive noncovalent binding interactions are sufficiently large to overcome the destabilizing steric interactions of the unnatural enantiomer alkylation. Importantly, these trends follow closely the relative cytotoxic potency of the agents, the relative stabilities of the three classes of agents, and highlight the enhanced distinctions of the

Table 3

Agent	A	B	Configuration	IC_{50} (L1210, pM)
(+)-CPI-indole$_2$			natural	40
(−)-CPI-indole$_2$			unnatural	1000[a]
	A	B		
(+)-**27**	NH	NH	natural	10[b]
(+)-**57**	NH	O	natural	10[b]
(+)-**58**	NH	S	natural	5
(+)-**59**	O	NH	natural	5[b]
(+)-**60**	O	O	natural	10[b]
(+)-**61**	O	S	natural	7
(−)-**27**	NH	NH	unnatural	3900
(−)-**57**	NH	O	unnatural	30,000
(−)-**58**	NH	S	unnatural	15,000[c]
(−)-**59**	O	NH	unnatural	NT[d]
(−)-**60**	O	O	unnatural	1300
(−)-**61**	O	S	unnatural	1200[c]

[a]Taken from Ref. 37.
[b]The corresponding CPI analogs of **27**, **57**, **59**, and **60** exhibited IC_{50} values of 40, 40, 30, and 30 pM, respectively.
[c]Tested as the *seco* derivative **53** or **56**. [d]Not tested.

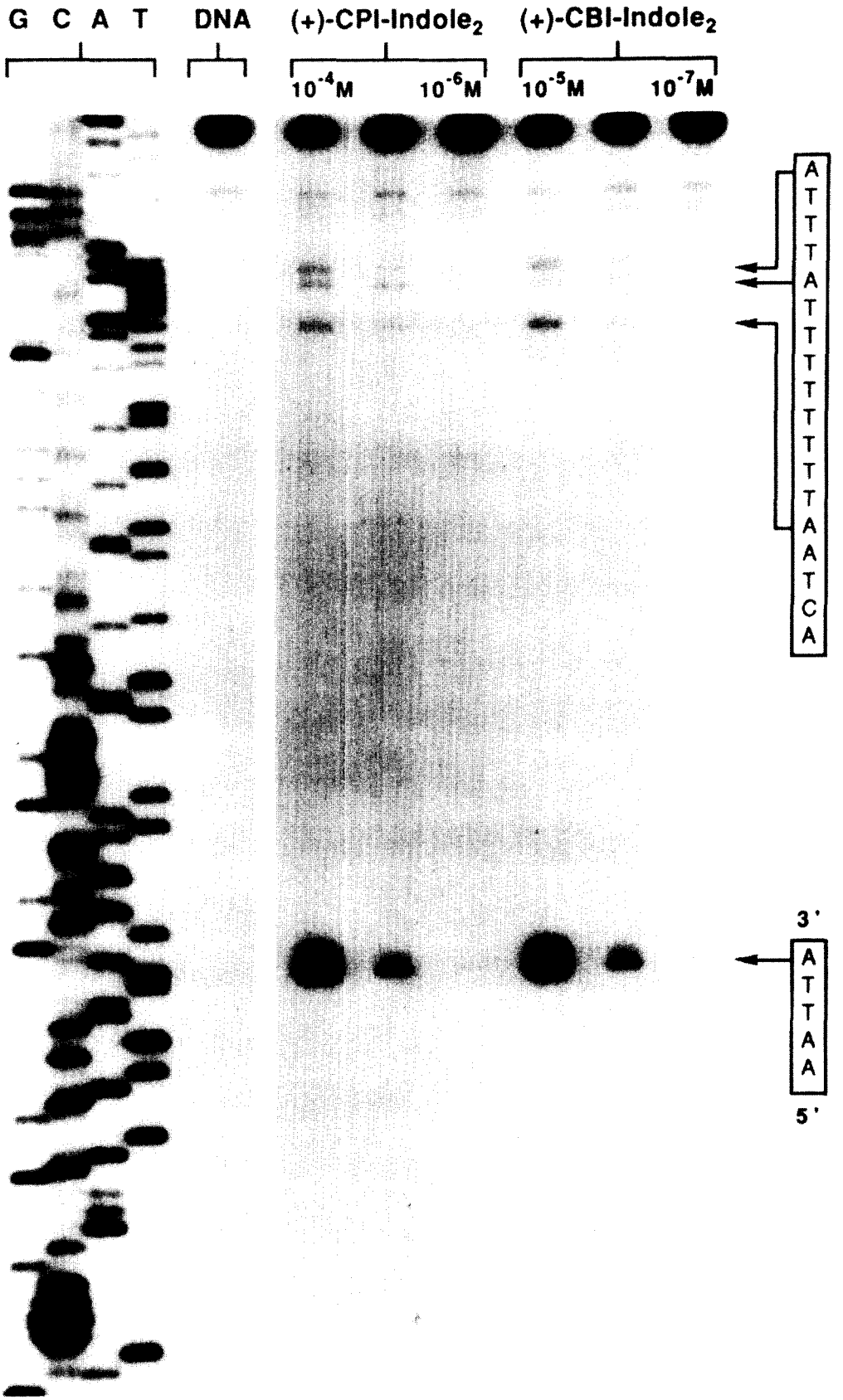


Figure 3. Thermally-induced strand cleavage of double-stranded DNA (144 bp, nucleotide no. 138–5238, clone w794) after 24 h incubation of agent–DNA at 4 °C followed by removal of unbound agent and 30 min incubation at 100 °C; denaturing 8% polyacrylamide gel and autoradiography. Lanes 1–4, Sanger G, C, A, and T sequencing reactions; lane 5, control labeled w794 DNA; lanes 6–8, (+)-CPI-indole$_2$ ((+)-**4**, 1×10^{-4}–1×10^{-6} M); lanes 9–11, (+)-CBI-indole$_2$ ((+)-**27**, 1×10^{-5}–1×10^{-7} M). The observation and origin of double bands for a single alkylation reaction with 5' end-labeled DNA have been detailed elsewhere.[10]

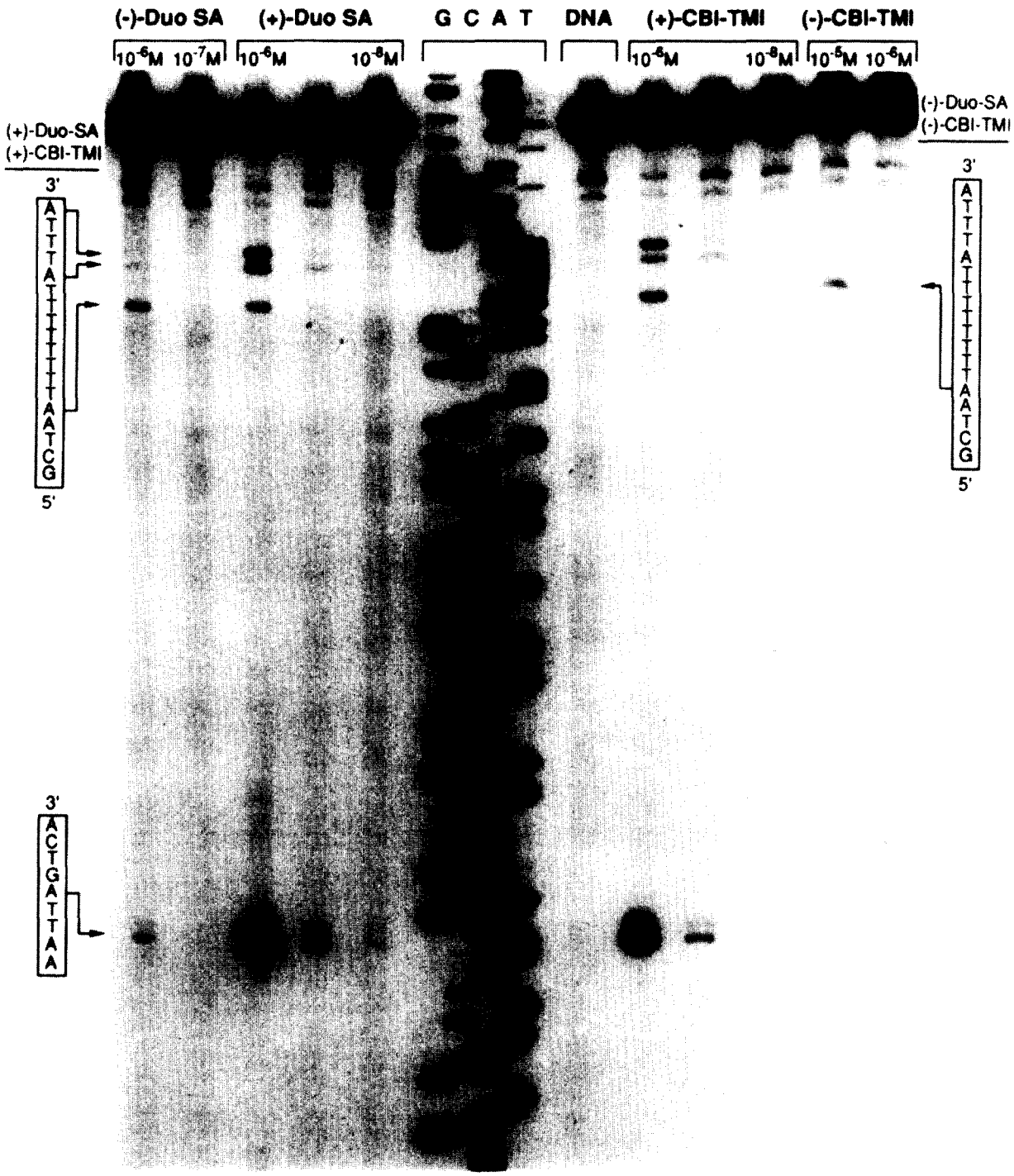


Figure 4. Thermally-induced strand cleavage of 5' end-labeled duplex DNA (clone w794, 144 bp, nucleotide no. 138–5238). Incubation of agent–DNA at 25 °C (24 h) followed by removal of unbound agent and 30 min thermolysis at 100 °C, denaturing 8% PAGE, and autoradiography. Lanes 1–2, *ent*-(–)-duocarmycin SA ((–)-**2**, 1×10^{-6} and 1×10^{-7} M); lanes 3–5, (+)-duocarmycin SA, ((+)-**2**, 1×10^{-6}–1×10^{-8} M); lanes 6–9, G, C, A and T sequencing reactions; lane 10, control labeled w794 DNA; lanes 11–13, (+)-CBI-TMI ((+)-**29**, 1×10^{-6}–1×10^{-8} M); lanes 14–15, ent-(–)-CBI-TMI ((–)-**29**, 1×10^{-5} and 1×10^{-6} M). The origin of double bands for a single cleavage site have been detailed elsewhere.[10]

CBI- versus CPI-based analogs and the comparable properties of the duocarmycin SA and CBI-based agents. Fundamental to members of this class of antitumor antibiotics, the natural enantiomers of the agents were found to follow a well-defined relationship between solvolysis (functional) stability (–log *k*, pH 3) and cytotoxic potency (1/log IC_{50}, L1210) where the chemically more stable agents within a given class exert the greatest potency (Fig. 6). Figure 6 includes data for four to six available classes of agents that bear five different DNA binding subunits which we have examined,[9,19–21,25,26,30–39,52] and although this relationship is undoubtedly a second order polynomial indicative of a parabolic relationship that will exhibit an optimal stability–reactivity/potency,[21] the agents employed in Figure 6 lie in a near linear range of such a plot. What

is unmistakable in the comparisons, is the fundamental direct correlation between functional (solvolytic) stability and cytotoxic potency.

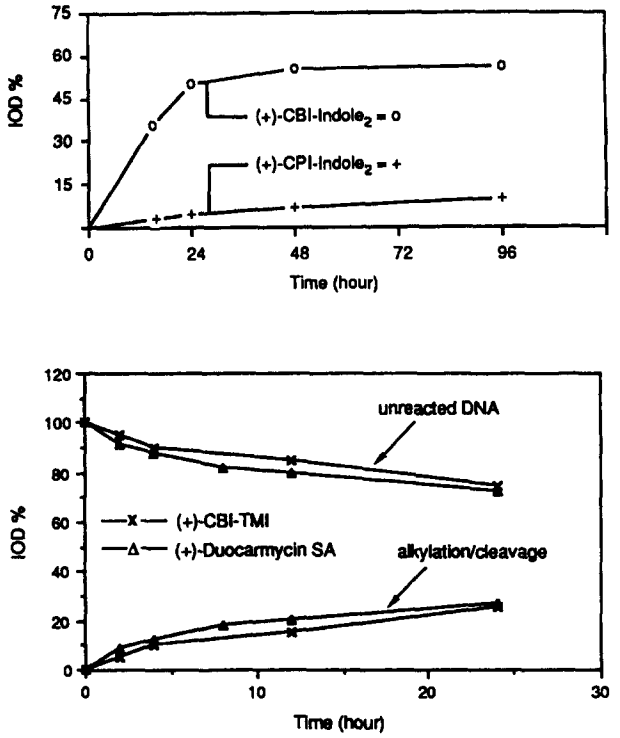


Figure 5. Top: Plot of % integrated optical density (% IOD) versus time established through autoradiography of 5' ^{32}P end-labeled DNA and used to monitor the relative rate of w794 alkylation at the 5'-AATTA high affinity site for (+)-CBI-indole$_2$ (**27**) and (+)-CPI-indole$_2$ (**4**); 37 °C, 0–5 d, 1 × 10^{-5} M agent. Bottom: Plot of % integrated optical density (% IOD) versus time established through autoradiography of 5' ^{32}P end-labeled DNA and used to monitor the relative rate of w794 alkylation at the 5'-AATTA high affinity site for (+)-duocarmycin SA (**2**) and (+)-CBI-TMI (**29**); 4 °C, 0–24 h, 1 × 10^{-6} M agent.

CBI-CDPBO$_1$ and CBI-CDPBI$_1$: Deep-Seated Structural Variations in the DNA Binding Subunits

The efforts of Lown[54] and Dervan[55] have demonstrated that the distamycin AT-rich noncovalent binding selectivity may be altered to accommodate a G–C base-pair or to exhibit progressively altered AT→GC rich binding selectivity through introduction of a nitrogen within the backbone core structure capable of serving as hydrogen bonding acceptor. Accordingly, we have investigated whether similar changes in the core structure of CC-1065 would impact on its DNA binding selectivity and resulting DNA alkylation selectivity. Key to the importance of this examination was the recognition that the more rigid structure of CC-1065, its rigid helical bound conformation, and its near exclusive dependence on stabilizing van der Waals contacts and hydrophobic binding which dictates the preference for binding and alkylation within the narrower, deeper AT-rich minor groove may not be so easily overridden by introduction of a single hydrogen bond acceptor or donor.

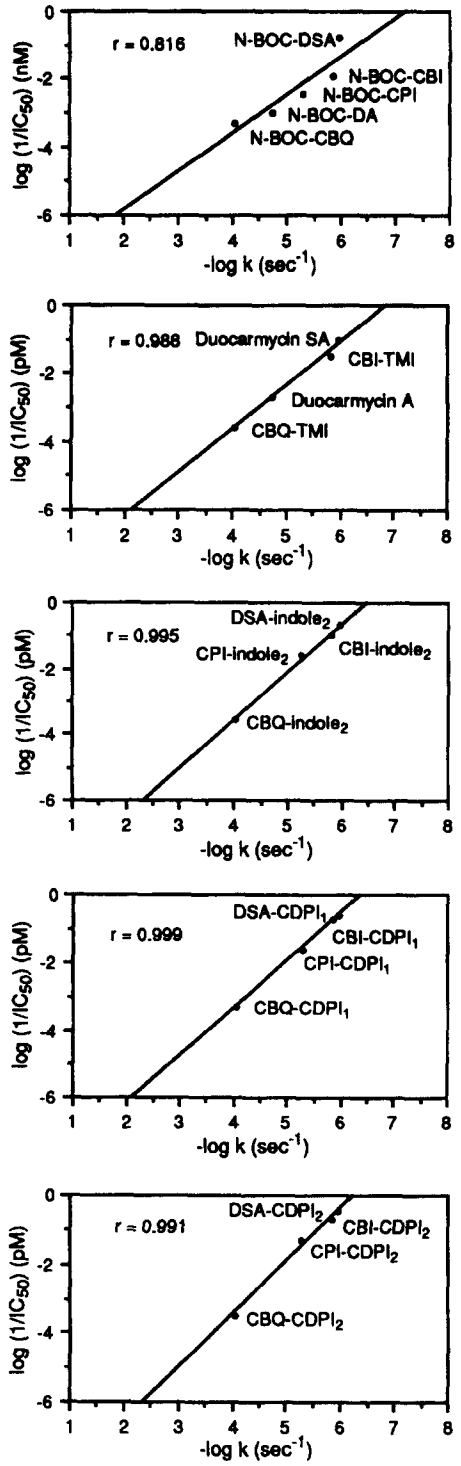
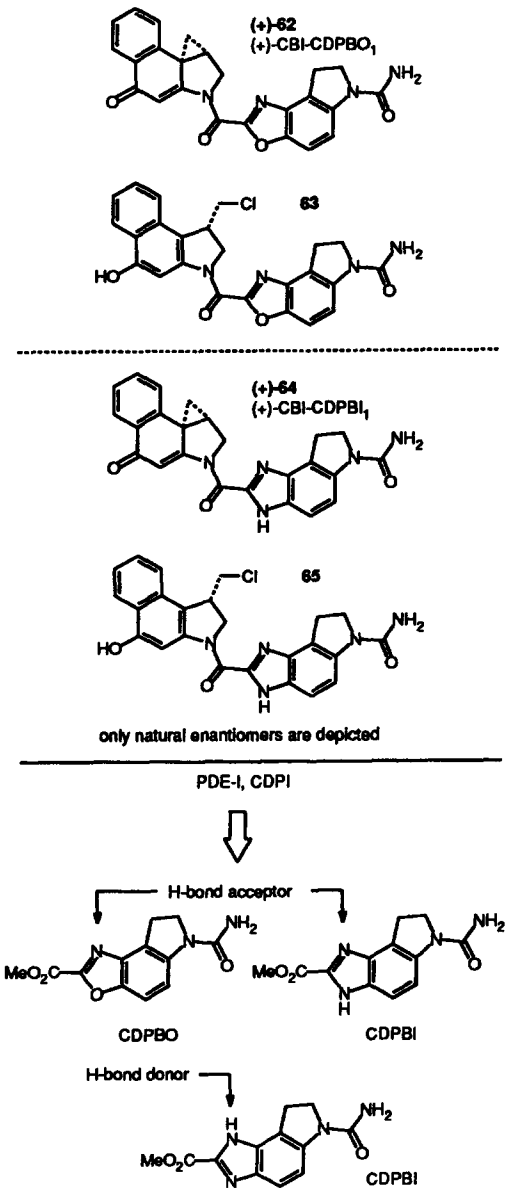


Figure 6.

In the conduct of these studies, we reported the preparation of (+)- and *ent*-(–)-CBI-CDPBO$_1$ (**62**), (+)- and *ent*-(–)-CBI-CDPBI$_1$ (**64**) and their corresponding *seco* precursors **63** and **65** bearing deep-seated modifications in the DNA binding subunit including the incorporation of a nitrogen atom capable of functioning as a hydrogen bond acceptor (CDPBO, CDPBI) or hydrogen bond donor (CDPBI) on their inside convex face which is projected to be in intimate contact with the minor groove floor.[39]

The initial comparisons were made with agents containing a single DNA binding subunit where the single deep-seated structural modification in the DNA binding subunit might be expected to exert a more pronounced effect. In these studies, the DNA alkylation selectivities and efficiencies of the natural enantiomers of **62** and **64** were found to be essentially identical. Moreover, both were approximately 100 times less efficient at alkylating DNA than (+)-CBI-CDPI$_1$ (**26**). Thus, the simple incorporation of a single nitrogen into **64** versus **26** has a pronounced and detrimental effect on the relative efficiency of DNA alkylation. Identical to trends detailed in our prior work on the CBI-derived agents,[34,36] the unnatural enantiomers of **62** and **64** proved to be 10–100 times times less efficient at alkylating DNA than the corresponding natural enantiomers.

More interesting was the observed DNA alkylation selectivities of **62** and **64**. The DNA alkylation selectivities of (+)-**62** and (+)-**64** were essentially identical and both were comparable to the selectivity observed with (+)-**26**. Although the DNA alkylation selectivity of (+)-**62** and (+)-**64** potentially could have been significantly altered or have become increasingly more tolerant of a GC base-pair in the alkylation sequence, the selectivity proved more revealing than this simple expectation. Not only did (+)-**62** and (+)-**64** alkylate DNA with the near identical selectivity of (+)-**26**, but the unnatural enantiomer selectivity for **62** and **64** proved essentially identical to that of *ent*-(−)-**26**. Thus, in a manner essentially identical to (+)- and *ent*-(−)-**26** which exhibit distinct alkylation selectivities (5'-A/TA/TA/TA versus 5'-A/TAA/TA/T, respectively)[9] characteristic of the reverse binding orientations and offset 3.5 base-pair AT-rich binding sites surrounding the alkylation site,[9] the two entantiomers of **62** and **64** alkylated essentially the same sites as the corresponding enantiomers of **26** within duplex DNA. Moreover, this was observed to occur not with the increasing tolerance for incorporation of GC base-pairs in the alkylation sequence, but rather with a diminished DNA alkylation efficiency (100 times) relative to that of (+)- and *ent*-(−)-CBI-CDPI$_1$ (**26**). The potential origin of these effects have been discussed elsewhere.[39]



The cytotoxic properties of **62–65** and that of the closely related CBI agents are summarized in Table 4. Consistent with their relative efficiencies of DNA alkylation, the natural enantiomers of **62** and **64** were essentially indistinguishable (500–1000 pM, L1210) and 100–200 times less potent than (+)-CBI-CDPI$_1$ (**26**). Thus, the introduction of the single nitrogen atom in the DNA binding subunit of **64** reduced the biological potency 100–200-fold. Consistent with prior observations, the natural enantiomers of **62** and **64** were 10–100 times more potent than the corresponding unnatural enantiomers.

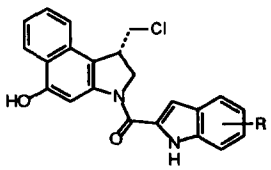
Table 4

Agent	Configuration	IC_{50} (L1210, pM)
26, (+)-CBI-CDPI$_1$	natural	5
26, (−)-CBI-CDPI$_1$	unnatural	380
62, (+)-CBI-CDPBO$_1$	natural	500
62, (−)-CBI-CDPBO$_1$	unnatural	20,000
64, (+)-CBI-CDPBI$_1$	natural	1000
64, (−)-CBI-CDPBI$_1$	unnatural	60,000
63	natural	500
63	unnatural	20,000
65	natural	1000
65	unnatural	60,000
1, (+)-CC-1065	natural	20
1, (−)-CC-1065	unnatural	20
2, (+)-duocarmycin SA	natural	10
2, (−)-duocarmycin SA	unnatural	100

CBI-Indole-NMe_3^+: Electropositive Substituents Capable of Enhancing DNA Alkylation Efficiency Through Stabilizing Electrostatic Interactions

In recent studies, we have studied the impact that electronegative and electropositive substituents placed

on peripheral face of the agents have on the noncovalent DNA binding affinity and selectivity. In these studies, we defined a destabilizing contribution to the DNA binding affinity that results from the introduction of a strong electronegative substituent and described a substantial enhancement of noncovalent binding affinity that results from introduction of an electropositive substituent.[56] This was attributed to a spatially well-defined destabilizing or stabilizing electrostatic interaction with the negatively charged DNA phosphate backbone, respectively, and was found to have little impact on the intrinsic AT-rich binding selectivity of the parent agents. These studies were recently extended to the preparation of **66–68**, close analogs of **28/33**, containing a peripheral quaternary ammonium salt capable of providing a strong, stabilizing electrostatic interaction with the DNA phosphate backbone.[38] Consistent with expectations, the agents **66–68** alkylated DNA with the same relative efficiency as **1** and **2** and were approximately 100 times more effective than **28** or **33** which lack the ammonium salt substituent. Because of the smaller size of the agents, they exhibited a DNA alkylation selectivity that was subtly altered from that of (+)-CC-1065, but comparable to that of (+)-duocarmycin SA. In addition, the agents were water soluble and offer potential advantages over the existing agents.

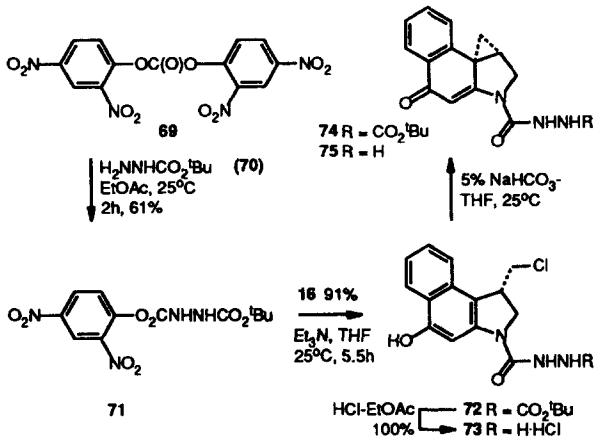


33 R = H
66 R = 5-NMe_3^+
67 R = 6-NMe_3^+
68 R = 7-NMe_3^+

Consequently, we were interested in the relative cytotoxic properties of **66–68** and the results of their evaluations are summarized in Table 5. Although **66–68** were essentially identical in their cytotoxic potencies (10 nM), they proved to be slightly less potent than (+)-CBI-indole$_1$ (**28**) and approximately 1000 times less potent than (+)-**1** and (+)-**2**. This is in contrast to expectations based on their relative efficiencies of DNA alkylation. Although this was not investigated, we attribute this diminished cytotoxic potency to ineffective cellular penetration required for the agents to reach their intracellular target.

Additional Analogs

In the course of our investigations, several additional agents have been examined including **73** and **75**, simple derivatives of the CBI alkylation subunit which possess enhanced DNA alkylation capabilities and *in vitro* cytotoxic potency by virtue of stabilizing electrostatic DNA binding. That is, in place of the DNA binding affinity derived from hydrophobic binding and stabilizing van der Waals contacts provided by the



Scheme 3.

Table 5

Agent	IC_{50} (L1210, nM)	Rel DNA Alkylation[a]
(+)-**1**, (+)-CC-1065	0.02	1
(+)-**2**, (+)-duocarmycin SA	0.01	1
(+)-**28**, (+)-CBI-indole$_1$	5	0.01
(+)-**33**	5	0.01
(+)-**66**	10	1
(+)-**67**	10	1
(+)-**68**	10	1

[a]Relative efficiency for alkylation of w794 DNA (4 °C, 24 h), Ref. 38.

Table 6

Agent	IC_{50} (L1210, nM)	Rel DNA alkylation[a]
9, (+)-*N*-BOC-CBI	80	0.00001
72	100	0.00001
73	0.8	0.01
78	0.005	1
79	0.005	1
25, (+)-CBI-CDPI$_2$	0.005	1

[a]Relative efficiency of alkylation of w794 DNA; 37 °C, 24 h, Ref. 31.

central and right-hand subunits of **1–3**, the simple electrostatic binding affinity provided by the protonated amine of **73** and **75** with the negatively charged phosphate backbone of DNA proved sufficient to substantially enhance the DNA alkylation intensity and *in vitro* cytotoxic activity.

The semicarbazide of CBI and its *seco* chloride precursor were prepared as detailed in Scheme 3. Treatment of bis(2,4-dinitrophenyl)carbonate (**69**)[57] with *tert*-butylcarbazate[58] (**70**, 1 equiv, 24 °C, 2 h, EtOAc) provided **71** (61%) and a convenient acylating agent for introduction of the *tert*-butyloxycarbonyl protected hydrazide. N-deprotection of **15** (3 N HCl–EtOAc, 24 °C, 20 min, 100%) followed by immediate treatment of the unstable amine hydrochloride salt **16** with **71** (1.3 equiv, 1 equiv Et_3N, 24 °C, 5.5 h, THF,

91%) provided **72** in excellent yield. Acid-catalyzed N-BOC deprotection of **72** provided **73** and exposure of **72** or **73** to 5% aqueous $NaHCO_3$–THF (24 °C) provided **74** or **75**, respectively.

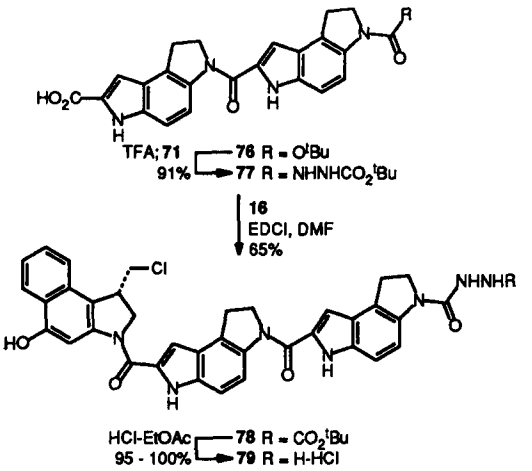
The results of the *in vitro* cytotoxic evaluation of the *N*-semicarbazide of CBI conducted on its more stable *seco* precursor **73** are detailed in Table 6 along with the comparative results from the evaluation of *N*-BOC-CBI (**15**) and **72**. Notably, **73** which possesses the free amine exhibited more potent *in vitro* cytotoxic activity than its precursor possessing the *tert*-butylcarbazate (**72**, *ca* 100 times) or *N*-BOC-CBI (**9**) itself, and proved to be only 100 times less potent than (+)-CC-1065.

Consistent with the trends observed in the relative cytotoxic potency of the agents, the intensity of DNA alkylation similarly increased with the introduction of the free semicarbazide and the results of these studies have been detailed elsewhere.[31] Thus, the introduction of a positively charged functionality (protonated amine) onto the simple CBI alkylation subunit served to enhance the DNA alkylation intensity of the agent presumably by providing noncovalent electrostatic DNA binding affinity to the agents. Consistent with the enhancement in the DNA alkylation intensity (100 times), the *in vitro* cytotoxic activity of the agents increased correspondingly (100 times).

The introduction of a terminal semicarbazide onto CBI-$CDPI_2$ was carried out for comparison purposes (Scheme 4). Acid-catalyzed deprotection of *N*-BOC-$CDPI_2$[59] (**76**, CF_3CO_2H, 25 °C, 1 h) followed by coupling of crude amine salt with **71** (1.5 equiv, 1 equiv Et_3N, 25 °C, 19 h, 91% overall) provided **77**[60] in excellent conversion. Direct coupling of **77** with freshly generated **16** (3 equiv EDCI, DMF, 25 °C, 10 h) provided **78** (65%) in good conversions. Acid-catalyzed deprotection (3 M HCl–EtOAc, 25 °C, 30 min) cleanly provided **79** (95–100%).

The examination of **78** and **79** revealed that this alteration in the C-terminus of CBI-$CDPI_2$ (**25**) did not impact on the inherent properties of the agent (Table 6). Thus, in contrast to **73** where the introduction of a stabilizing electrostatic interaction enhances the DNA alkylation efficiency and cytotoxic potency of the agent, it had no impact on the properties of **79** versus **78/25**. Presumably, this may be attributed to the fact that the noncovalent hydrophobic binding affinity of **25** is already sufficient to provide full stabilization of the reversible DNA adduct and the maximal cytotoxic potency and that the additional electrostatic stabilization provided in **79** is unnecessary.

Notably, the terminal acyl hydrazides of **73**, **75** and **79** may serve as useful functionality for subsequent reversible or irreversible conjugation with tumor selective delivery systems and such studies are underway.



Scheme 4.

Further Characterization of the Cytotoxic Activity

The cytotoxic activity of the more potent agents was further examined in drug resistant tumor cell lines. For this purpose, the natural enantiomers of **25**, **27**, **29** and **57–61** were examined alongside (+)-CPI-$indole_2$ (**4**, U-71,184) and (+)-duocarmycin SA (**2**) in a human colon carcinoma cell line (HCT116) and two resistant variants, HCT116/VM46 and HCT116/VP35. The HCT116/VM46 resistant cell line overexpresses a gp 170 glycoprotein and embodies the MDR phenotype while the HCTV116/VP35 cell line is resistant to topoisomerase II inhibitors by virtue of its lower expression. The results which are an average of three separate experiments are summarized in Table 7. Importantly, none of the agents exhibited reduced activity in the resistant cell lines and most were in fact substantially more potent against the resistant cell lines. In addition, all the CBI-based agents were substantially more potent than (+)-CPI-$indole_2$ (**4**, > 60 times) in all three cell lines. With the exception of **25**, the CBI-based agents typically were 2–3 times more potent against the multidrug resistant (MDR) cell line HCT116/VM46 and, with the exception of **59–60**, they were typically 5–20 times more potent against HCT116/VP35 which is resistant to topoisomerase II inhibitors. As such, the results suggest the agents may prove especially effective against resistant variants of tumor cell lines and particularly useful in initial combination therapy or upon relapse chemotherapy. Notably, this hypersensitivity of the resistant cell lines was more pronounced with the CBI-based agents than with (+)-CPI-$indole_2$ (**4**) which proved essentially equipotent in all three cell lines.

In Vivo Antitumor Activity

In preliminary studies, the *in vivo* antitumor activity of a set of representative agents was examined. The murine P388 leukemia was passaged ip as ascites once weekly in syngeneic DBA/2 mice. The murine M109 lung carcinoma and B16 melanoma were passaged sc

Table 7. *In Vitro* cytotoxicity in HCT116 sensitive and resistant cell lines (pM)[a]

Agent	HCT116	HCT116/VM46	HCT116/VP35
(+)-**2**, (+)-duocarmycin SA	30	10	2
(+)-**29**, (+)-CBI-TMI	7	2	1
(+)-**4**, (+)-CPI-indole$_2$	12000	8000	8000
(+)-**25**, (+)-CBI-CDPI$_2$	90	70	10
(+)-**27**, (+)-CBI-indole$_2$	200	60	10
(+)-**57**	100	30	20
(+)-**58**	100	30	10
(+)-**59**	150	40	100
(+)-**60**	100	40	150
(+)-**61**	200	90	40

[a]XTT assay after 72 h drug exposure.

biweekly in syngeneic Balb/c and C57BL/6 mice, respectively. The human A2780 ovarian carcinoma was passaged sc biweekly in athymic mice. For the *in vivo* testing, P388 cells were implanted iv into CDF_1 mice, the M109 tumor was implanted sc into CDF_1 mice, the B16 tumor was implanted sc into BDF_1 mice, and the A2780 tumor was implanted sc into athymic mice. Therapeutic results from the experiments are presented either in terms of increases in lifespan reflected by the medium survival times of treated (T) versus control (C) groups of mice (% T/C) and any long-term survivors (> 60 days) or by primary tumor growth inhibition determined by calculating the times for T versus C mice to grow tumors of 1 g expressed as T-C values (in days). The activity criterion for increased lifespan was T/C ≥ 125% and the activity criterion for tumor inhibition was a delay in primary tumor consistent with ≥ 1 gross log 10 cell kill (LCK). The absolute T-C value needed to attain this level of efficacy varied from experiment and depended upon the tumor volume doubling time (TVDT) of the control mice in each study (i.e., LCK = T-C/TVDT × 3.32). Statistical evaluations of the data were performed using Gehan's generalized Wilcoxon test. Group sizes consisted of 6 mice in the P388 examination and 8 mice in all other tests.

(+)-CBI-indole$_2$ (**27**) was evaluated in detail to characterize the efficacy of the class of agents and to characterize the response profile of several tumor models. A summary of the results are provided in Table 8. Importantly, (+)-CBI-indole$_2$ was found to be active in disseminated and distal site tumor models. When injected iv on an intermittent schedule versus iv implanted P388 leukemia (disseminated tumor), the mice receiving the optimal dose of 10 μg kg^{-1} inj^{-1} produced a T/C of 306%. Impressively in a distal site tumor model, (+)-CBI-indole$_2$ (**27**) proved highly effective against a human ovarian tumor, A2780. In this model, an optimal dose of 8 μg kg^{-1} inj^{-1}, administered iv to mice bearing 50–100 mg sc tumors, achieved a 50% cure rate when the mice were inspected visually for tumor residue 63 days post-tumor implant. At an optimal dose of 10 μg kg^{-1} inj^{-1}, **27** produced an 8.5 day delay in the median time for mice to grow 1 gm sc M109 tumors. This tumor growth inhibition translated to 0.6 LCK, accompanied by a T/C of 114%; neither outcome was considered an active result. An attempt was made in a subsequent sc M109 experiment (Expt B) to improve the therapeutic outcome by modifying the treatment regimen. Despite the application of two new treatment schedules and a repeat of the intermittent schedule used in the previous study, LCK of only between 0.5 and 0.7 were obtained accompanied by minimal increases in lifespan reflected by T/C values of 122–129. The sc B16 melanoma model was evaluated for its response to (+)-CBI-indole$_2$ (**27**). In B16 Expt A, intermittent iv injections of 10 μg kg^{-1} inj^{-1} resulted in a nearly 10 day delay in tumor growth, which for this fast growing tumor translated into 3.6 LCK. The greatest increase in lifespan found in this experiment, a T/C of 165%, occurred using 20 μg kg^{-1} inj^{-1} of **27**. Confirmation of the activity in the sc B16 tumor model was realized in two subsequent experiments.

Based on the antitumor response profile of **27**, the distal sc B16 tumor model was chosen for an additional comparative efficacy testing. The agent **58** was evaluated for its relative antitumor efficacy and the results were comparable and are also summarized in Table 8.

Conclusions

In contrast to early speculation, deep-seated modifications in the CC-1065 and duocarmycin alkylation subunit are well tolerated and the CBI-based analogs proved to be potent cytotoxic agents and efficacious antitumor compounds. A direct relationship between functional stability and cytotoxic potency was defined and validated. As such, the readily accessible CBI-based analogs were found to be four times more stable and four times more potent than the corresponding analogs containing the CPI alkylation subunit of CC-1065 and comparable in potency to the agents containing the duocarmycin SA alkylation subunit. Similarly, the CBI-based agents alkylate DNA with an unaltered sequence selectivity at an enhanced rate and with a greater efficiency than the corresponding CPI

Table 8. Summary of *in vivo* antitumor test results

Tumor, site	Expt. No.	Treatment Od[a] ($\mu g\ kg^{-1}\ inj^{-1}$)	Schedule, route	Maximum %T/C	T-C (days)	LCK(C/T)[b]
(+)-CBI-indole$_2$ (**27**)						
P388, iv	A	10	d 1, 4 & 7, iv	306	-	-
A2780, sc	A	8	d 11, 15 & 19, iv	-	> 46	> 3.5 (4/8)
M109, sc	A	10	d 1, 5 & 9, iv	114	8.5	0.6
	B	9	d 1, 5 & 9, iv	129	7.8	0.7
		4	d 1, 3, 5, 7 & 9, iv	129	5.0	0.5
		3	qd 1-5, iv	122	4.8	0.5
B16, sc	A	20/10	d 1, 5 & 9, iv	165	9.8	3.6
	B	100	d 1, 5 & 9, iv	176	8.3	1.4
	C	100	d 1, 5 & 9, iv	210	12.0	2.0
(+)-**58**						
B16, sc	A	25	d 1, 5 & 9, iv	154	7.3	1.2

analogs and were comparable to the corresponding DSA analog. Systematic modification and simplification of the attached DNA binding subunits have provided a series of synthetic and potent cytotoxic agents including **25–29** and **57–61** whose biological profile are under further study. In preliminary studies, a number of the agents detailed herein exhibit potent and efficacious antitumor activity.

Experimental

1-(Chloromethyl)-5-hydroxy-3-[(methylaminocarbonyl]-1,2-dihydro-3H-benz[e]indole (18). Phenol **15** (6.9 mg, 21 μmol) was treated with anhydrous 3 M HCl–EtOAc at 24 °C for 30 min under Ar. The solvent was removed *in vacuo* to afford crude, unstable **16** (quantitative). A solution of **16** and $NaHCO_3$ (5.2 mg, 62 μmol, 3 equiv) in THF (0.3 mL) was cooled to 0 °C and treated with CH_3NCO (2.4 μL, 41 μmol, 2 equiv). The reaction mixture was kept at 0 °C for 1 h under Ar before the solvent was removed under a stream of N_2. Flash chromatography (SiO_2, 0.5 × 3 cm, 50–80% EtOAc–hexane gradient elution) afforded **18** (5.0 mg, 6.0 mg theoretical, 83%) as a pale greenish solid: ^1H NMR (CD_3OD, 400 MHz): δ 8.11 (*d*, 1H, *J* = 8.4 Hz, C6-H), 7.68 (*s*, 1H, C4-H), 7.65 (*d*, 1H, *J* = 8.4 Hz, C9-H), 7.44 (*t*, 1H, *J* = 8.2 Hz, C8-H), 7.24 (*t*, 1H, *J* = 8.3 Hz, C7-H), 4.06–4.14 (*m*, 3H, C2-H_2, C1-H), 3.92 (*d*, 1H, *J* = 11.4 Hz, CHHCl), 3.51–3.53 (*m*, 1H, CHHCl), 2.83 (*s*, 3H, CH_3); IR (film) ν_{max} 3816, 1624, 1585, 1522, 1384, 1339, 1250, 1121 cm^{-1}; FABHRMS (NBA) m/z 290.0818 (M^+ + H, $C_{15}H_{15}ClN_2O_2$ requires 290.0822).

Natural (1*S*)-**18**: $[\alpha]_D^{25}$ –4.5 (*c* 0.36, CH_3OH).
ent-(1*R*)-**18**: $[\alpha]_D^{25}$ +5.7 (*c* 0.11, CH_3OH).

N^2-[(Methylamino)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[c]benz[e]indol-4-one (21). A solution of **18** (3.0 mg, 10.3 μmol) in DMF (0.9 mL) was cooled to 0 °C and treated with DBU (3.2 μL, 21 μmol, 2 equiv) and the mixture was stirred at 4 °C for 2 d. The solvent was removed *in vacuo* and flash chromatography (SiO_2, 0.5 × 3 cm, 0–10% CH_3OH–EtOAc gradient elution) afforded **21** (2.3 mg, 2.6 mg theoretical, 90%) as a pale yellow solid: ^1H NMR (CD_3OD, 400 MHz): δ 8.08 (*d*, 1H, *J* = 8.0 Hz, C5-H), 7.55 (*t*, 1H, *J* = 7.7 Hz, C7-H), 7.40 (*t*, 1H, *J* = 8.0 Hz, C6-H), 7.08 (*d*, 1H, *J* = 7.7 Hz, C8-H), 6.93 (*s*, 1H, C3-H), 4.05 (*dd*, 1H, *J* = 10.0, 5.1 Hz, C1-H), 3.95 (*d*, 1H, *J* = 10.0 Hz, C1-H), 3.08 (*m*, 1H, C9a-H), 2.79 (*s*, 3H, CH_3), 1.73 (*dd*, 1H, *J* = 7.8, 4.2 Hz, C9-H), 1.48 (*t*, 1H, *J* = 4.6 Hz, C9-H); IR (neat) ν_{max} 3358, 2920, 1680, 1622, 1594, 1539, 1466, 1458, 1410, 1281 cm^{-1}; UV (CH_3OH) ν_{max} 311 (ε 15,000), 257 (7300), 217 (17,000), 200 (17,000) nm; UV (THF) λ_{max} 304 (ε 11,000), 248 (7200), 216 (17,000), 208 (15,000) nm; FABHRMS (NBA) m/z 255.1140 (M^+ + H, $C_{15}H_{14}N_2O_4$ requires 255.1134).

Natural (+)-**21**: $[\alpha]_D^{23}$ +183 (*c* 0.08, CH_3OH).
ent-(–)-**21**: $[\alpha]_D^{25}$ –184 (*c* 0.13, CH_3OH).

1-(Chloromethyl)-5-hydroxy-3-(methoxycarbonyl)-1,2-dihydro-3H-benz[e]indole (19). A solution of freshly prepared, crude **16** (52 μmol) and $NaHCO_3$ (13.2 mg, 157 μmol, 3 equiv) in THF (0.5 mL) was cooled to 0 °C and treated with $ClCO_2CH_3$ (8.1 μL, 104 μmol, 2 equiv). The reaction mixture was warmed to 25 °C and stirred for 1.5 h before it was concentrated *in vacuo*. Flash chromatography (SiO_2, 1 × 10 cm, 20–40% EtOAc–hexane gradient elution) afforded **19** (15 mg, 15 mg theoretical, 100%) as a white solid: ^1H NMR ($CDCl_3$, 400 MHz): δ 8.59 (*br s*, 1H, OH), 8.25 (*d*, 1H,

J = 8.1 Hz, C6-H), 7.94 (*br s*, 1H, C4-H), 7.62 (*d*, 1H, J = 8.3 Hz, C9-H), 7.50 (*t*, 1H, J = 8.1 Hz, C8-H), 7.35 (*t*, 1H, J = 8.2 Hz, C7-H), 4.31 (*d*, 1H, J = 11.4 Hz, C2-H), 4.12 (apparent *t*, 1H, J = 9.3 Hz, C2-H), 3.90–3.99 (*m*, 5H, C1-H, C<u>H</u>HCl, CO_2CH_3), 3.40 (*t*, 1H, J = 10.5 Hz, CH<u>H</u>Cl); IR (film) ν_{max} 3275, 2918, 1678, 1442, 1388, 1335 cm^{-1}; FABHRMS (NBA) m/z 291.0664 ($[M]^+$, $C_{15}H_{14}ClNO_4$ requires 291.0662).

Natural-(1*S*)-**19**: $[\alpha]_D^{23}$ –30.3 (*c* 0.11, CH_3OH).
ent-(1*R*)-**19**: $[\alpha]_D^{23}$ +31.8 (*c* 0.24, CH_3OH).

N^2-(Methoxycarbonyl)-1,2,9,9a-tetrahydrocyclopropa[c]benz[e]indol-4-one (22). A solution of **19** (10.0 mg, 34 μmol) in THF (3 mL) was cooled to 0 °C and treated with DBU (10.5 μL, 68 μmol, 2 equiv). The reaction mixture was stirred at 4 °C for 41 h and then warmed to 24 °C and stirred for 10 h.[61] The reaction mixture was treated with saturated aqueous NH_4Cl (3 mL) and extracted with CH_2Cl_2 (3 × 2 mL). The combined organic layer was dried (Na_2SO_4) and concentrated. Flash chromatography (SiO_2, 1 × 10 cm, 10–50% EtOAc–hexane gradient elution) afforded **22** (8.1 mg, 8.7 mg theoretical, 87%) as a yellow solid: ^1H NMR ($CDCl_3$, 400 MHz): δ 8.21 (*d*, 1H, J = 7.8 Hz, C5-H), 7.48 (*t*, 1H, J = 7.6 Hz, C7-H), 7.39 (*t*, 1H, J = 7.8 Hz, C6-H), 6.87 (*s*, 1H, C3-H), 6.86 (*d*, 1H, J = 7.6 Hz, C8-H), 3.86–4.08 (*m*, 2H, CH_2N), 3.86 (*s*, 3H, CH_3), 2.79 (*m*, 1H, C9a-H), 1.56–1.59 (*m*, 1H, C9-H), 1.39 (*t*, 1H, J = 4.8 Hz, C9-H); IR (neat) ν_{max} 3283, 2984, 1728, 1626, 1559, 1436, 1405, 1380, 1328, 1277, 1246, 1195, 1118, 1077, 1021, 764 cm^{-1}; UV (CH_3OH) λ_{max} 307 (ε 32,000), 255 (24,000), 216 (32,000), 200 (33,000) nm; UV (THF) λ_{max} 296 (ε 33,000), 253 (23,000), 217 (38,000), 203 (44,000) nm; FABHRMS (NBA) m/z 256.0986 (M^+ + H, $C_{15}H_{13}NO_3$ requires 256.0974).

Natural (+)-**22**: $[\alpha]_D^{23}$ +198 (*c* 0.48, CH_3OH).
ent-(–)-**22**: $[\alpha]_D^{25}$ –196 (*c* 0.14, CH_3OH).

1-(Chloromethyl)-5-hydroxy-3-propionyl-1,2-dihydro-3H-benz[e]indole (20). A solution of freshly prepared, crude **16** (45 μmol) and $NaHCO_3$ (11.3 mg, 135 μmol, 3 equiv) in THF (0.4 mL) was cooled to 0 °C and treated with ClCOEt (8 μL, 90 μmol, 2 equiv). The reaction mixture was warmed to 24 °C and stirred for 5 h under N_2. The solvent was removed under a stream of N_2. Flash chromatography (SiO_2, 1 × 10 cm, 10–40% EtOAc–hexane gradient elution) afforded **20** (12.8 mg, 13 mg theoretical, 98%) as a white solid: ^1H NMR ($CDCl_3$, 400 MHz): δ 9.70 (*br s*, 1H, OH), 8.39 (*s*, 1H, C4-H), 8.32 (*d*, 1H, J = 8.0 Hz, C6-H), 7.66 (*d*, 1H, J = 8.3 Hz, C9-H), 7.52 (*t*, 1H, J = 8.3 Hz, C8-H), 7.39 (*t*, 1H, J = 8.3 Hz, C7-H), 4.32 (dd, 1H, J = 2.0, 10.9 Hz, C2-H), 4.23 (*d*, 1H, J = 10.8 Hz, C2-H), 4.04 (*m*, 1H, C1-H), 3.97 (*dd*, 1H, J = 2.9, 11.3 Hz, C<u>H</u>HCl), 3.41 (*t*, 1H, J = 10.8 Hz, CH<u>H</u>Cl), 2.59–2.72 (*m*, 2H, C<u>H</u>$_2$CH$_3$), 1.39 (*t*, 3H, J = 7.4 Hz, $CH_2C\underline{H}_3$); IR (film) ν_{max} 3170, 2918, 1628, 1582, 1427, 1389 cm^{-1}; FABHRMS (NBA) m/z 290.0953 (M^+ + H, $C_{16}H_{16}ClNO_2$ requires 290.0953).

Natural (1*S*)-**20**: $[\alpha]_D^{25}$ –54 (*c* 0.08, THF).
ent-(1*R*)-**20**: $[\alpha]_D^{25}$ +59 (*c* 0.13, THF).

N^2-(Propionyl)-1,2,9,9a-tetrahydrocyclopropa[c]benz[e]indol-4-one (23). A solution of **20** (5.0 mg, 17 μmol) in THF (0.9 mL) was treated with 0.9 mL of 5% aqueous $NaHCO_3$ and the two-phase mixture was stirred at 24 °C for 5 h under N_2. The reaction mixture was extracted with EtOAc (3 × 3 mL). The organic layer was dried (Na_2SO_4) and concentrated. Flash chromatography (Florisil, 1 × 5 cm, 60% EtOAc–hexane) afforded **23** (4.2 mg, 4.3 mg theoretical, 97%) as a pale yellow solid: ^1H NMR ($CDCl_3$, 400 MHz): δ 8.22 (*d*, 1H, J = 7.8 Hz, C5-H), 7.51 (*t*, 1H, J = 7.5 Hz, C7-H), 7.40 (*t*, 1H, J = 7.9 Hz, C6-H), 6.89 (*br s*, 1H, C3-H), 6.88 (*d*, 1H, J = 7.8 Hz, C8-H), 4.13–4.16 (*m*, 1H, C1-H), 4.03 (*dd*, 1H, J = 10.6, 4.9 Hz, C1-H), 2.76–2.81 (*m*, 1H, C9a-H), 2.54–2.56 (*m*, 2H, C<u>H</u>$_2$CH$_3$), 1.67 (*dd*, 1H, J = 7.6 Hz, 4.5 Hz, C9-H), 1.43 (*t*, 1H, J = 4.8 Hz, C9-H), 1.22 (*t*, 3H, J = 7.3 Hz, CH_3); IR (neat) ν_{max} 2924, 1698, 1626, 1599, 1562, 1461, 1406, 1241 cm^{-1}; UV (CH_3OH) λ_{max} 311 (ε 16,000), 258 (9100), 218 (14,000), 201 (19,000) nm; UV (THF) λ_{max} 301 (ε 15,000), 253 (9400), 219 (15,000), 204 (15,000) nm; FABHRMS (NBA) m/z 254.1173 (M^+ + H, $C_{16}H_{15}NO_2$ requires 254.1181).

Natural (+)-**23**: $[\alpha]_D^{23}$ +193 (*c* 0.03, CH_3OH).
ent-(–)-**23**: $[\alpha]_D^{25}$ –197 (*c* 0.12, CH_3OH).

N^2-(Ethylsulfonyl)-1,2,9,9a-tetrahydrocyclopropa[c]benz[e]indol-4-one (24). NaH (1.5 mg, 60% oil dispersion, 38 μmol, 2.5 equiv) in a flame-dried flask was treated with (+)-CBI (**17**, 3.0 mg, 15.2 μmol) in THF (0.8 mL) and the mixture was stirred for 10 min at 24 °C under N_2. A premixed solution of Et_3N (7 μL, 50 μmol, 3.3 equiv) and $ClSO_2Et$ (10 μL, 106 μmol, 7 equiv) in THF (0.8 mL) was added and the reaction mixture was stirred at 24 °C for 3 h before being concentrated. Flash chromatography (SiO_2, 0.5 × 3 cm, 40–60% EtOAc–hexane gradient elution) afforded **24** (2.0 mg, 4.4 mg theoretical, 45%) as a pale yellow solid: ^1H NMR ($CDCl_3$, 400 MHz): δ 8.19 (*d*, 1H, J = 7.8 Hz, C5-H), 7.49 (*t*, 1H, J = 8.3 Hz, C7-H), 7.39 (*t*, 1H, J = 7.8 Hz, C6-H), 6.85 (*d*, 1H, J = 7.8 Hz, C8-H), 6.46 (*s*, 1H, C3-H), 4.09 (*m*, 2H, CH_2N), 3.21–3.28 (*m*, 2H, C<u>H</u>$_2$CH$_3$), 2.83 (*m*, 1H, C9a-H), 1.69 (*dd*, 1H, J = 7.8, 4.6 Hz, C9-H), 1.54 (*t*, 1H, partially obscured by H_2O, C9-H), 1.43 (*t*, 3H, J = 7.4 Hz, CH_3); IR (neat) ν_{max} 2923, 1618, 1559, 1354, 1149 cm^{-1}; UV (CH_3OH) λ_{max} 301 (ε 12,000), 248 (11,000), 214 (15,000) nm; UV (THF) λ_{max} 293 (ε 13,000), 248 (14,000), 216 (16,000), 208 (14,000) nm; FABHRMS (NBA-NaI) m/z 290.0850 (M^+ + H, $C_{15}H_{15}NO_3S$ requires 290.0851).

Natural (+)-**24**: $[\alpha]_D^{23}$ +73 (*c* 0.10, $CHCl_3$).
ent-(–)-**24**: $[\alpha]_D^{25}$ –70 (*c* 0.12, $CHCl_3$).

Solvolytic reactivity of compounds 21-24. Compounds **21–24** were dissolved in CH_3OH (1.5 mL). The CH_3OH solution was mixed with aqueous buffer (pH 3, 1.5 mL).

The buffer contained 4:1:20 (v:v:v) 0.1 M citric acid, 0.2 M Na_2HPO_4, and H_2O, respectively. After mixing, the solvolysis solutions were stoppered and kept at 25 °C in the dark. The UV spectrum of the solutions was measured 3–4 times in the first two days and twice a day for 2–4 weeks for **21–23** and 3 months for **24**. The UV monitoring was continued until no further change was detectable. The long-wavelength absorption at 316 nm (**21–23**) or 306 nm (**24**) and short-wavelength absorption at 256 nm (**21–23**) or 248 nm (**23**) were monitored. The solvolysis rate constant and half-life were calculated from the data recorded at the short wavelength (256 nm for **21–23** and 248 nm for **24**) from the least square treatment (r = 0.995, **21**; r = 0.997, **22**; r = 0.985, **23**; r = 0.994, **24**) of the slopes of plots of time versus log $(1 - [(A - A_{initial})/A_{final} - A_{initial})])$ (Fig. 1). The results are summarized in Figure 2.

Methyl 5-nitrobenzofuran-2-carboxylate. 5-Nitrobenzofuran-2-carboxylic acid[62] (500 mg, 2.4 mmol) in 20 mL of CH_3OH was treated with 5 drops of H_2SO_4. The reaction mixture was stirred at 24 °C for 24 h and warmed at 50 °C for 2 h. The mixture was cooled to 24 °C, diluted with H_2O (20 mL) and saturated aq. $NaHCO_3$ (20 mL), and extracted with EtOAc (3 × 30 mL). The combined organic phase was dried (Na_2SO_4) and concentrated *in vacuo*. Flash chromatography (SiO_2, 2 × 20 cm, 40–60% EtOAc–hexane) afforded the methyl ester (469 mg, 534 mg theoretical, 88%) as a white solid: mp > 230 °C (dec); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.64 (*d*, 1H, *J* = 2.3 Hz, C4-H), 8.37 (*dd*, 1H, *J* = 2.3, 9.2 Hz, C6-H), 7.69 (*d*, 1H, *J* = 9.1 Hz, C7-H), 7.64 (*s*, 1H, C3-H), 4.02 (*s*, 3H, CH_3); ^{13}C NMR ($CDCl_3$–CD_3OD, 100 MHz): δ 157.8 (C), 122.7 (CH), 120.9 (C), 119.4 (CH), 118.5 (C), 114.1 (CH), 112.7 (CH), 112.0 (C), 109.2 (C), 52.4 (CH_3); IR (film) ν_{max} 3383, 3108, 1731, 1620, 1571, 1521, 1441, 1349, 1270, 1177, 827, 750 cm^{-1}; FABHRMS (NBA) m/z 222.0405 (M^+ + H, $C_{10}H_7NO_5$ requires 222.0402).

Methyl 5-aminobenzofuran-2-carboxylate (36). A solution of methyl 5-nitrobenzofuran-2-carboxylate (469 mg, 2.12 mmol) in 50 mL of EtOAc was treated with 10% Pd-C (235 mg, 0.5 wt equiv), placed under 1 atm of H_2, and stirred at 25 °C (12 h). The catalyst was removed by filtration through Celite, and the solvent was removed *in vacuo*. Flash chromatography (SiO_2, 2 × 20 cm, 40–60% EtOAc–hexane) afforded **36** (360 mg, 404 mg theoretical, 89%) as a pale yellow solid: mp 109–111 °C (CH_2Cl_2, pale yellow fine needles); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.36 (*s*, 1H, C3-H), 7.36 (*d*, 1H, *J* = 8.1 Hz, C7-H), 6.89 (*d*, 1H, *J* = 2.4 Hz, C4-H), 6.83 (*dd*, 1H, *J* = 2.4, 8.9 Hz, C6-H), 3.94 (*s*, 3H, CH_3), 3.45 (*br s*, 2H, NH_2); IR (film) ν_{max} 3359, 1725, 1562, 1488, 1434, 1331, 1301, 1222, 1158 cm^{-1}; FABHRMS (NBA) m/z 192.0663 (M^+ + H, $C_{10}H_9NO_3$ requires 192.0661).

General procedure for the preparation of compounds 40–45. Methyl 5-aminoindole-2-carboxylate (**35**)[38] or methyl 5-aminobenzofuran-2-carboxylate (**36**), EDCI (3 equiv) and indole-2-carboxylic acid (**37**), benzofuran-2-carboxylic acid (**38**) or benzo[*b*]thiophene-2-carboxylic acid (**39**) (1 equiv) were stirred in DMF (0.04–0.06 M) at 24 °C under Ar for 12 h. The solvent was removed *in vacuo*, and the dry residue was mixed with H_2O and stirred for 30 min. The precipitate was collected by centrifugation and washed with 1 N aq. HCl, saturated aq. $NaHCO_3$, and H_2O. Drying the solid *in vacuo* afforded the desired aqueous methyl esters **40–45** in typical yields of 50–73%.

Methyl 5-[((1H-indol-2'-yl)carbonyl)amino]-1H-indole-2-carboxylate (40). 5 h, 61%; mp > 270 °C (dec); ^1H NMR (DMSO-d_6, 400 MHz): δ 11.92 (*s*, 1H, NH), 11.69 (*s*, 1H, NH), 10.16 (*s*, 1H, NH), 8.16 (*d*, 1H, *J* = 1.6 Hz, C4-H), 7.67 (*d*, 1H, *J* = 8.0 Hz, C4'-H), 7.60 (*dd*, 1H, *J* = 2.0, 8.9 Hz, C6-H), 7.47 (*d*, 1H, *J* = 8.3 Hz, C7'-H), 7.45 (*d*, 1H, *J* = 9.0 Hz, C7-H), 7.41 (*s*, 1H, C3-H), 7.21 (*t*, 1H, *J* = 8.2 Hz, C6'-H), 7.18 (*s*, 1H, C3'-H), 7.07 (*t*, 1H, *J* = 7.1 Hz, C5'-H), 3.88 (*s*, 3H, CH_3); IR (neat) ν_{max} 3277, 1700, 1652, 1553, 1535, 1310, 1247, 1225, 1022, 999, 742 cm^{-1}; FABHRMS (NBA-NaI) m/z 356.1004 (M^+ + Na, $C_{19}H_{15}N_3O_3$ requires 356.1011).

Methyl 5-[((benzofuro-2'-yl)carbonyl)amino]-1H-indole-2-carboxylate (41). 5 h, 73%; mp > 230 °C; ^1H NMR (DMSO-d_6, 400 MHz): δ 11.91 (*br s*, 1H, NH), 10.48 (*br s*, 1H, NH), 8.18 (*d*, 1H, *J* = 1.8 Hz, C4-H), 7.83 (*d*, 1H, *J* = 7.8 Hz, C4'-H), 7.76 (*s*, 1H, C3'-H), 7.72 (*d*, 1H, *J* = 8.4 Hz, C7'-H), 7.61 (*dd*, 1H, *J* = 1.9, 8.9 Hz, C6-H), 7.50 (*t*, 1H, *J* = 8.4 Hz, C6'-H), 7.44 (*d*, 1H, *J* = 8.8 Hz, C7-H), 7.37 (*t*, 1H, *J* = 7.6 Hz, C5'-H), 7.18 (*s*, 1H, C3-H), 3.88 (*s*, 3H, CH_3); IR (film) ν_{max} 3333, 1695, 1658, 1591, 1535, 1442, 1303, 1255, 746 cm^{-1}; FABHRMS (NBA) m/z 335.1036 (M^+ + H, $C_{19}H_{14}N_2O_4$ requires 335.1032).

Methyl 5-[((benzo[b]thieno-2'-yl)carbonyl)amino]-1H-indole-2-carboxylate (42). 7 h, 62%; mp > 230 °C; ^1H NMR (DMSO-d_6, 400 MHz): δ 11.93 (*br s*, 1H, NH), 10.47 (*br s*, 1H, NH), 8.35 (*s*, 1H, C3'-H), 8.13 (*d*, 1H, *J* = 1.9 Hz, C4-H), 8.05 (*d*, 1H, *J* = 7.0 Hz, C7'-H), 7.99 (*d*, 1H, *J* = 6.7 Hz, C4'-H), 7.57 (*dd*, 1H, *J* = 2.0, 8.9 Hz, C6-H), 7.44–7.50 (*m*, 2H, C6'-H, C5'-H), 7.44 (*d*, 1H, *J* = 9.0 Hz, C7-H), 7.17 (*s*, 1H, C3-H), 3.87 (*s*, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 161.7, 160.1, 140.5, 140.4, 139.2, 134.6, 131.6, 127.7, 126.6, 126.4, 125.3 (two CH), 125.0, 122.8, 119.9, 113.2, 112.6, 107.9, 51.8; IR (film) ν_{max} 3336, 1694, 1633, 1532, 1455, 1336, 1309, 1257, 1232 cm^{-1}; FABHRMS (NBA) m/z 351.0810 (M^+ + H, $C_{19}H_{14}N_2O_3S$ requires 351.0803).

Methyl 5-[((1H-indol-2'-yl)carbonyl)amino]benzofuran-2-carboxylate (43). 8 h, 52%; mp > 230 °C; ^1H NMR (DMSO-d_6, 400 MHz): δ 11.75 (*s*, 1H, NH), 10.36 (*s*, 1H, NH), 8.34 (*d*, 1H, *J* = 2.1 Hz, C4-H), 7.84 (*dd*, 1H, *J* = 2.1, 9.0 Hz, C6-H), 7.83 (*s*, 1H, C3-H), 7.73 (*d*, 1H, *J* = 9.0 Hz, C7-H), 7.68 (*d*, 1H, *J* = 8.0 Hz, C4'-H), 7.47 (*d*, 1H, *J* = 8.3 Hz, C7'-H), 7.43 (*s*, 1H, C3'-H), 7.22 (*t*, 1H, *J* = 7.1 Hz, C6'-H), 7.07 (*t*, 1H, *J* = 7.1 Hz, C5'-H), 3.89 (*s*, 3H, CH_3); IR (film) ν_{max} 3346, 1712, 1643, 1577, 1543, 1308, 1289, 1234, 745 cm^{-1}; FABHRMS (NBA) m/z 335.1040 (M^+ + H, $C_{19}H_{14}N_2O_4$ requires 335.1032).

Methyl 5-[((benzofuro-2'-yl)carbonyl)amino]benzofuran-2-carboxylate (44). 12 h, 62%; mp > 230 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.44 (*br s*, 1H, NH), 8.28 (apparent *t*, 1H, *J* = 1.3 Hz, C4-H), 7.69 (*d*, 1H, *J* = 7.7 Hz, C4'-H), 7.60 (*d*, 1H, *J* = 0.8 Hz, C3-H or C3'-H), 7.54–7.56 (apparent *d*, 3H, *J* = 8.4 Hz), 7.51 (*s*, 1H, C3-H or C3'-H), 7.45 (*t*, 1H, *J* = 7.2 Hz, C6'-H), 7.31 (*t*, 1H, *J* = 7.9 Hz, C5'-H), 3.97 (*s*, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.8 (C), 156.7 (C), 154.8 (C), 152.8 (C), 148.3 (C), 146.3 (C), 133.3 (C), 127.6 (C), 127.4 (C), 127.3 (CH), 124.0 (CH), 122.9 (CH), 121.0 (CH), 114.1 (CH), 114.0 (CH), 112.7 (CH), 111.8 (CH), 111.7 (CH), 52.5 (CH_3); IR (film) ν_{max} 3382, 1729, 1663, 1562, 1541, 1475, 1431, 1291, 1204, 1151, 1103 cm^{-1}; FABHRMS (NBA) m/z 336.0878 (M^+ + H, $C_{19}H_{13}NO_5$ requires 336.0872).

Methyl 5-[((benzo[b]thieno-2'-yl)carbonyl)amino]benzofuran-2-carboxylate (45). 8 h, 50%; mp > 230 °C ; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.67 (*s*, 1H, NH), 8.38 (*s*, 1H, C3'-H), 8.31 (*d*, 1H, *J* = 2.0 Hz, C4-H), 8.06 (*dd*, 1H, *J* = 1.7, 6.9 Hz, C7'-H), 8.00 (*dd*, 1H, *J* = 1.8, 6.9 Hz, C4'-H), 7.83 (*s*, 1H, C3-H), 7.80 (*dd*, 1H, *J* = 2.1, 9.0 Hz, C6-H), 7.74 (*d*, 1H, *J* = 9.0 Hz, C7-H), 7.50 (*dt*, 1H, *J* = 1.7, 7.1 Hz, C6'-H), 7.47 (*dt*, *J* = 2.0, 7.1 Hz, C5'-H), 3.89 (*s*, 3H, CH_3); IR (film) ν_{max} 3287, 1728, 1657, 1546, 1473, 1436, 1296, 1216, 1154 cm^{-1}; FABHRMS (NBA) m/z 352.0650 (M^+ + H, $C_{19}H_{13}NO_4S$, requires 352.0644).

General procedure for the preparation of compounds 46–51. A solution of the methyl esters **40–45** prepared above in THF:CH_3OH:H_2O (3:1:1) was treated with 4 equiv of LiOH·H_2O. The reaction mixture was stirred at 24 °C for 4–6 h. The solvent was removed and the dry residue was mixed with H_2O, acidified with 1 N aq. HCl to pH 1. The precipitate was collected by centrifugation and washed with H_2O (two times). Drying the solid *in vacuo* afforded the desired acid **46–51** with yields 80–100%.

5-[((1H-Indol-2'-yl)carbonyl)amino]-1H-indole-2-carboxylic acid (46). 3 h, 89%; mp > 270 °C (dec); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.82 (*s*, 1H, NH), 11.23 (*br s*, 1H, NH), 10.14 (*s*, 1H, NH), 7.99 (*s*, 1H, C4-H), 7.66 (*d*, 1H, *J* = 7.6 Hz, C4'-H), 7.48 (*d*, 1H, *J* = 8.0 Hz, C6-H), 7.41 (*s*, 1H, C3-H), 7.39–7.41 (*m*, 2H, C7'-H and C7-H), 7.20 (*t*, 1H, *J* = 7.6 Hz, C6'-H), 7.06 (*t*, 1H, *J* = 7.2 Hz, C5'-H), 6.69 (*br s*, 1H, C3'-H); IR (film) ν_{max} 3413, 3354, 3315, 1665, 1596, 1532, 1463, 1444, 1409, 1306, 1222, 1159, 1080 cm^{-1}; FABHRMS (NBA) m/z 320.1041 (M^+ + H, $C_{18}H_{13}N_3O_3$ requires 320.1035).

5-[((Benzofuro-2'-yl)carbonyl)amino]-1H-indole-2-carboxylic acid (47). 5 h, 77%; mp > 230 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.62 (*br s*, 1H, NH), 10.43 (*s*, 1H, NH), 8.12 (*s*, 1H, C4-H), 7.83 (*d*, 1H, *J* = 7.6 Hz, C4'-H), 7.75 (*s*, 1H, C3'-H), 7.73 (*d*, 1H, *J* = 8.4 Hz, C7'-H), 7.55 (*d*, 1H, *J* = 9.2 Hz, C6-H), 7.50 (*t*, 1H, *J* = 8.4 Hz, C6'-H), 7.40 (*d*, 1H, *J* = 8.8 Hz, C7-H), 7.37 (*t*, 1H, *J* = 7.2 Hz, C5'-H), 7.00 (*br s*, 1H, C3-H); IR (film) ν_{max} 3297, 1661, 1594, 1537, 1299, 1258, 1229, 743 cm^{-1}; FABHRMS (NBA) m/z 321.0880 (M^+ + H, $C_{18}H_{12}N_2O_4$ requires 321.0875).

5-[((Benzo[b]thieno-2'-yl)carbonyl)amino]-1H-indole-2-carboxylic acid (48). 3 h; 80%; mp > 230 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.63 (*s*, 1H, NH), 10.46 (*s*, 1H, NH), 8.36 (*s*, 1H, C3'-H), 8.08 (*s*, 1H, C4-H), 8.04 (*d*, 1H, *J* = 6.8 Hz, C7'-H), 7.99 (*d*, 1H, *J* = 6.6 Hz, C4'-H), 7.52 (*d*, 1H, *J* = 8.9 Hz, C6-H), 7.44–7.48 (*m*, 2H, C6'-H, C5'-H), 7.41 (*d*, 1H, *J* = 8.8 Hz, C7-H), 7.00 (*s*, 1H, C3-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.1, 160.0, 140.6, 140.4, 139.3, 134.2, 131.1, 126.9, 126.3, 125.3, 125.3 (CH and C), 125.0, 122.9, 118.9, 113.1, 112.4, 106.4; IR (film) ν_{max} 3429, 3375, 1648, 1542, 1431, 1305, 1249, 739 cm^{-1}; FABHRMS (NBA) m/z 337.0654 (M^+ + H, $C_{18}H_{12}N_2O_3S$ requires 337.0647).

5-[((1H-Indol-2'-yl)carbonyl)amino]benzofuran-2-carboxylic acid (49). 4 h, 80%; mp > 230 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 13.54 (*br s*, 1H, CO_2H), 11.74 (*s*, 1H, NH), 10.34 (*s*, 1H, NH), 8.30 (*d*, 1H, *J* = 1.8 Hz, C4-H), 7.81 (*dd*, 1H, *J* = 1.9, 9.0 Hz, C6-H), 7.72 (*s*, 1H, C3-H), 7.70 (*d*, 1H, *J* = 7.6 Hz, C7-H), 7.68 (*d*, 1H, *J* = 8.6 Hz, C4'-H), 7.47 (*d*, 1H, *J* = 8.3 Hz, C7'-H), 7.43 (*s*, 1H, C3'-H), 7.22 (*t*, 1H, *J* = 8.0 Hz, C6'-H), 7.06 (*t*, 1H, *J* = 7.6 Hz, C5'-H); IR (film) ν_{max} 3285, 1703, 1649, 1547, 1475, 1420, 1312, 1231, 1195, 1159, 744 cm^{-1}; FABHRMS (NBA) m/z 321.0870 (M^+ + H, $C_{18}H_{12}N_2O_4$ requires 321.0875).

5-[((Benzofuro-2'-yl)carbonyl)amino]benzofuran-2-carboxylic acid (50). 12 h, 100%; mp > 230 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 13.50 (*br s*, 1H, CO_2H), 10.61 (*s*, 1H, NH), 8.22 (*d*, 1H, *J* = 1.7 Hz, C4-H), 7.83 (*d*, 1H, *J* = 7.7 Hz, C4'-H), 7.78 (*d*, 1H, *J* = 0.8 Hz, C3'-H), 7.73 (*dd*, 1H, *J* = 0.7, 8.4 Hz, C7'-H), 7.72 (*dd*, 1H, *J* = 2.1, 9.0 Hz, C6-H), 7.61 (*d*, 1H, *J* = 8.9 Hz, C7-H), 7.50 (*t*, 1H, *J* = 8.4 Hz, C6'-H), 7.37 (*t*, 1H, *J* = 7.9 Hz, C5'-H), 7.35 (*br s*, 1H, C3-H); IR (film) ν_{max} 3362, 1709, 1659, 1564, 1473, 1438, 1292, 1226, 1152, 790 cm^{-1}; FABHRMS (NBA) m/z 322.0720 (M^+ + H, $C_{18}H_{11}NO_5$ requires 322.0715).

5-[((Benzo[b]thieno-2'-yl)carbonyl)amino]benzofuran-2-carboxylic acid (51). 12 h, 82%; mp > 230 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 13.61 (*br s*, 1H, CO_2H), 10.67 (*s*, 1H, NH), 8.40 (*s*, 1H, C3'-H), 8.30 (*d*, 1H, *J* = 1.9 Hz, C4-H), 8.09 (*dd*, 1H, *J* = 1.7, 6.8 Hz, C7'-H), 8.05 (*dd*, 1H, *J* = 1.8, 6.2 Hz, C4'-H), 7.79 (*dd*, 1H, *J* = 2.0, 9.1 Hz, C6-H), 7.73 (*d*, 1H, *J* = 9.0 Hz, C7-H), 7.70 (*br s*, 1H, C3-H), 7.53 (*dt*, 1H, *J* = 1.6, 7.1 Hz, C6'-H), 7.50 (*dt*, 1H, *J* = 1.5, 7.1 Hz, C5'-H); IR (film) ν_{max} 3395, 1697, 1653, 1551, 1479, 1296, 1273, 1231, 1155, 1024, 991, 762 cm^{-1}; FABHRMS (NBA) m/z 338.0480 (M^+ + H, $C_{18}H_{11}NO_4S$ requires 338.0487).

General procedure for the coupling of compound 16 with 46–51. Phenol **15**[26,36] was treated with anhydrous 3 M HCl–EtOAc at 24 °C for 30 min. The solvent was removed *in vacuo* to afford crude unstable **16** (quantitative). A solution of **16**, the carboxylic acids **46–51** (1 equiv), and EDCI (2–3 equiv) in DMF (0.04–

0.06 M) was stirred at 24 °C under N_2 for 8–12 h. The reaction mixture was concentrated *in vacuo*, suspended in H_2O and the precipitate was collected by centrifugation, and washed with H_2O (two times). Flash chromatography (SiO_2, 40–60% THF–hexane) afforded **32** and **52–56** in yields of 60–90%.

3-[(5'-(((1H-Indol-2"-yl)carbonyl)amino)-1H-indol-2'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3H-benz[e]indole (32). 8.5 h, 73%; mp > 255 °C (dec); ^{1}H NMR (DMF-d_7, 400 MHz): δ 11.76 (*s*, 1H, NH), 11.69 (*s*, 1H, NH), 10.58 (*s*, 1H, NH), 10.28 (*s*, 1H, OH), 8.40 (*d*, 1H, *J* = 1.7 Hz, C4'-H), 8.25 (*d*, 1H, *J* = 8.4 Hz, C6-H), 8.10 (*br s*, 1H, C4-H), 7.96 (*d*, 1H, *J* = 8.3 Hz, C4"-H), 7.73 (*d*, 1H, *J* = 2.0, 8.9 Hz, C6'-H), 7.70 (*d*, 1H, *J* = 8.0 Hz, C9-H), 7.61 (*d*, 1H, *J* = 8.2 Hz, C7"-H), 7.59 (*d*, 1H, *J* = 8.8 Hz, C7'-H), 7.57 (*t*, 1H, *J* = 8.1 Hz, C8-H), 7.53 (*s*, 1H, C3'-H), 7.41 (*t*, 1H, *J* = 8.0 Hz, C7-H), 7.30 (*s*, 1H, C3"-H), 7.26 (*t*, 1H, *J* = 8.0 Hz, C6"-H), 7.10 (*t*, 1H, *J* = 7.9 Hz, C5"-H), 4.90 (apparent *t*, 1H, *J* = 10.6 Hz, C2-H), 4.76 (*dd*, 1H, *J* = 1.8, 10.9 Hz, C2-H), 4.30–4.34 (*m*, 1H, C1-H), 4.13 (*dd*, 1H, *J* = 3.1, 11.0 Hz, CHHCl), 3.96 (*dd*, 1H, *J* = 7.8, 11.0 Hz, CHHCl); IR (film) ν_{max} 3258, 2923, 1659, 1624, 1578, 1512, 1411, 1395, 1233, 745 cm^{-1}; FABHRMS (NBA) m/z 535.1526 (M^+ + H, $C_{31}H_{23}ClN_4O_3$ requires 535.1537).

Natural (1*S*)-**32**: $[\alpha]_D^{25}$ +70 (*c* 0.17, DMF).
ent-(1*R*)-**32**: $[\alpha]_D^{25}$ –70 (*c* 0.17, DMF).

3-[(5'-(((Benzofuro-2"-yl)carbonyl)amino)-1H-indol-2'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3H-benz[e]indole (52). 14 h, 60%; mp > 230 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.73 (*s*, 1H, NH), 10.59 (*s*, 1H, NH), 10.56 (*s*, 1H, OH), 8.44 (*d*, 1H, *J* = 1.0 Hz, C4'-H), 8.24 (*d*, 1H, *J* = 8.4 Hz, C6-H), 8.10 (*br s*, 1H, C4-H), 7.94 (*d*, 1H, *J* = 8.3 Hz, C4"-H), 7.85 (*d*, 1H, *J* = 7.8 Hz, C7"-H), 7.80 (*dd*, 1H, *J* = 2.0, 8.8 Hz, C6'-H), 7.78 (*s*, 1H, C3"-H), 7.68 (*d*, 1H, *J* = 8.4 Hz, C9-H), 7.61 (*d*, 1H, *J* = 8.6 Hz, C7'-H), 7.56 (*t*, 1H, *J* = 7.0 Hz, C6"-H), 7.52 (*t*, 1H, *J* = 8.4 Hz, C8-H), 7.40 (*t*, 1H, *J* = 7.8 Hz, C5"-H or C7-H), 7.39 (*t*, 1H, *J* = 7.7 Hz, C5"-H or C7-H), 7.32 (*s*, 1H, C3'-H), 4.90 (apparent *t*, 1H, *J* = 10.8 Hz, C2-H), 4.75 (*dd*, 1H, *J* = 2.0, 10.8 Hz, C2-H), 4.32–4.34 (*m*, 1H, C1-H), 4.13 (*dd*, 1H, *J* = 3.2, 11.2 Hz, CHHCl), 3.96 (*dd*, 1H, *J* = 7.6, 11.2 Hz, CHHCl); ^{13}C NMR (THF-d_8, 100 MHz): δ 161.1 (C), 156.9 (C), 155.9 (C), 155.7 (C), 151.2 (C), 143.6 (C), 134.8 (C), 132.8 (C), 132.6 (C), 131.3 (C), 129.0 (two C), 128.0 (CH), 127.4 (CH), 124.5 (CH), 124.4 (CH), 123.7 (C), 123.6 (CH), 123.4 (CH), 123.1 (CH), 119.9 (CH), 116.0 (C), 114.1 (CH), 112.5 (CH), 112.4 (CH), 110.9 (CH), 106.7 (CH), 101.4 (CH), 56.1 (CH), 47.2 (CH_2), 43.9 (CH_2); IR (film) ν_{max} 3272, 2954, 1610, 1585, 1513, 1408, 1253, 1135, 741 cm^{-1}; FABHRMS (NBA) m/z 536.1390 (M^+ + H, $C_{31}H_{22}ClN_3O_4$ requires 536.1377).

Natural (1*S*)-**52**: $[\alpha]_D^{23}$ +56 (*c* 0.23, THF).

3-[(5'-(((Benzo[b]thieno-2"-yl)carbonyl)amino)-1H-indol-2'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3H-benz[e]indole (53). 11 h, 68%; mp > 230 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.80 (*s*, 1H, NH), 10.52 (*s*, 1H, NH), 10.48 (*s*, 1H, OH), 8.40 (*s*, 1H, C3"-H), 8.20 (*d*, 1H, *J* = 1.6 Hz, C4'-H), 8.15 (*d*, 1H, *J* = 8.2 Hz, C6-H), 8.08 (*d*, 1H, *J* = 6.9 Hz, C7"-H), 8.03 (*d*, 1H, *J* = 6.7 Hz, C4"-H), 8.01 (*br s*, 1H, C4-H), 7.88 (*d*, 1H, *J* = 8.4 Hz, C9-H), 7.60 (*dd*, 1H, *J* = 1.9, 8.9 Hz, C6'-H), 7.48–7.57 (*m*, 4H, C6"-H, C5"-H, C8-H, C7'-H), 7.39 (*t*, 1H, *J* = 8.1 Hz, C7-H), 7.25 (*s*, 1H, C3'-H), 4.85 (apparent *t*, 1H, *J* = 10.8 Hz, C2-H), 4.60 (*dd*, 1H, *J* = 1.8, 11.0 Hz, C2-H), 4.24–4.28 (*m*, 1H, C1-H), 4.06 (*dd*, 1H, *J* = 3.1, 11.1 Hz, CHHCl), 3.91 (*dd*, 1H, *J* = 7.3, 11.1 Hz, CHHCl); IR (film) ν_{max} 3286, 1655, 1628, 1587, 1518, 1409, 1262, 1239 cm^{-1}; FABHRMS (NBA) m/z 552.1152 (M^+ + H, $C_{31}H_{22}ClN_3O_3S$ requires 552.1149).

Natural (1*S*)-**53**: $[\alpha]_D^{23}$ +111 (*c* 0.15, DMF).

3-[(5'-(((1H-Indol-2"-yl)carbonyl)amino)benzofuro-2'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3H-benz[e]indole (54). 13 h, 80%; mp > 230 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.78 (*s*, 1H, NH), 10.51 (*s*, 1H, NH), 10.38 (*s*, 1H, OH), 8.35 (*d*, 1H, *J* = 2.0 Hz, C4'-H), 8.12 (*d*, 1H, *J* = 8.3 Hz, C6-H), 7.92 (*br s*, 1H, C4-H), 7.86 (*d*, 1H, *J* = 8.8 Hz, C9-H), 7.84 (*dd*, 1H, *J* = 2.1, 9.0 Hz, C6'-H), 7.80 (*s*, 1H, C3'-H), 7.76 (*d*, 1H, *J* = 9.0 Hz, C7'-H), 7.69 (*d*, 1H, *J* = 8.0 Hz, C4"-H), 7.53 (*t*, 1H, *J* = 8.2 Hz, C8-H), 7.48 (*d*, 1H, *J* = 8.4 Hz, C7"-H), 7.45 (*s*, 1H, C3"-H), 7.38 (*t*, 1H, *J* = 8.0 Hz, C7-H), 7.23 (*t*, 1H, *J* = 8.0 Hz, C6"-H), 7.07 (*t*, 1H, *J* = 7.7 Hz, C5"-H), 4.79 (apparent *t*, 1H, *J* = 9.8 Hz, C2-H), 4.58 (*d*, 1H, *J* = 9.9 Hz, C2-H), 4.24 (*m*, 1H, C1-H), 4.01 (*dd*, 1H, *J* = 3.1, 11.1 Hz, CHHCl), 3.89 (*dd*, 1H, *J* = 7.4, 11.1 Hz, CHHCl); IR (film) ν_{max} 3274, 2928, 1655, 1621, 1579, 1546, 1414, 1390, 1329, 1240 cm^{-1}; FABHRMS (NBA) m/z 536.1360 (M^+ + H, $C_{31}H_{22}ClN_3O_4$ requires 536.1377).

Natural (1*S*)-**54**: $[\alpha]_D^{23}$ +26 (*c* 0.36, DMF).

3-[(5'-(((Benzofuro-2"-yl)carbonyl)amino)benzofuro-2'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3H-benz[e]indole (55). 11 h, 88%; mp > 230 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.72 (*s*, 1H, NH), 10.48 (*s*, 1H, OH), 8.38 (*d*, 1H, *J* = 2.0 Hz, C4'-H), 8.14 (*d*, 1H, *J* = 8.3 Hz, C6-H), 7.71–7.95 (*m*, 7H), 7.54 (*t*, 1H, *J* = 7.2 Hz, C8-H), 7.52 (*t*, 1H, *J* = 7.4 Hz, C6"-H), 7.39 (*t*, 2H, *J* = 7.9 Hz, C7-H and C5"-H), 4.79 (apparent *t*, 1H, *J* = 10.6 Hz, C2-H), 4.59 (*d*, 1H, *J* = 9.8 Hz, C2-H), 4.25 (*m*, 1H, C1-H), 4.02 (*dd*, 1H, *J* = 3.0, 11.1 Hz, CHHCl), 3.90 (*dd*, *J* = 7.4, 11.1 Hz, CHHCl); IR (film) ν_{max} 3267, 2923, 1664, 1581, 1554, 1410, 1390, 1328, 1256 cm^{-1}; FABHRMS (NBA) m/z 537.1210 (M^+ + H, $C_{31}H_{21}ClN_2O_5$ requires 537.1217).

Natural (1*S*)-**55**: $[\alpha]_D^{23}$ +26 (*c* 0.28, DMF).

3-[(5'-(((Benzo[b]thieno-2"-yl)carbonyl)amino)benzofuro-2'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3H-benz[e]indole (56). 18 h, 68%; mp > 230 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.69 (*s*, 1H, NH), 10.52 (*s*, 1H, OH), 8.40 (*s*, 1H, C3"-H), 8.32 (*d*, 1H, *J* = 1.9

Hz, C4'-H), 8.13 (*d*, 1H, *J* = 8.3 Hz, C6-H), 8.07 (*d*, 1H, *J* = 8.7 Hz, C7"-H), 8.03 (*d*, 1H, *J* = 6.2 Hz, C4"-H), 7.94 (*br s*, 1H, C4-H), 7.86 (*d*, 1H, *J* = 8.3 Hz, C9-H), 7.82 (*dd*, 1H, *J* = 2.0, 9.0 Hz, C6'-H), 7.80 (*s*, 1H, C3'-H), 7.77 (*d*, 1H, *J* = 9.0 Hz, C7'-H), 7.45-7.55 (*m*, 3H, C8-H, C6"-H, C5"-H), 7.38 (*t*, 1H, *J* = 7.9 Hz, C7-H), 4.79 (apparent *t*, *J* = 10.0 Hz, C2-H), 4.59 (*d*, 1H, *J* = 10.0 Hz, C2-H), 4.22–4.26 (*m*, 1H, C1-H), 4.02 (*dd*, 1H, *J* = 3.0, 11.1 Hz, CHHCl), 3.88 (*dd*, 1H, *J* = 7.4, 11.0 Hz, CHHCl); IR (film) ν_{max} 3259, 2923, 1659, 1630, 1583, 1549, 1413, 1392, 1336, 1244, 1211 cm^{-1}; FABHRMS (NBA) m/z 553.0985 (M^+ + H, $C_{31}H_{21}ClN_2O_4S$ requires 553.0989).

Natural (1*S*)-**56**: $[\alpha]_D^{23}$ +30 (*c* 0.33, DMF).

General procedures for the spirocyclization and preparation of compounds 27 and 57–61. Method A: a suspension of NaH (60% oil dispersion, 2 equiv) in THF at 0 °C under Ar was treated with a solution of **32** and **52–55** prepared above in THF:DMF (1:1, *ca* 0.015 M reaction concentration). The reaction mixture was stirred at 0 °C for 30 min–1 h. The solvent was removed *in vacuo* and the solid residue was washed with H_2O and dried *in vacuo*. Flash chromatography (SiO_2, 50–70% THF–hexane) afforded **27** and **57–60** in 50–93% yield.

Method B: a solution of compounds **32** and **52–56** in THF:DMF (2:1, *ca* 0.015 M) was cooled to 0 °C and treated with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN, 2 equiv). The reaction mixture then was allowed to warm to 24 °C and stirred for 2–4 h. The solvent was removed *in vacuo*, and flash chromatography (SiO_2, 50–70% THF–hexane) afforded **27** and **57–61** with 40–75% yield.

Method C: a sample of **32** (1.6 mg, 0.0030 mmol) in THF (0.20 mL) was treated with the phosphazene base P_4-*t*-Bu (3.3 μL, 1 M solution in hexane, 1.1 equiv) at –78 °C. The mixture was stirred under Ar at –78 °C for 40 min, at 0 °C for 6 h, and at 25 °C for 2 h. The crude mixture was purified directly by chromatography (SiO_2, 60% THF–hexane) to provide **27** (1.4 mg, 1.5 mg theoretical, 93%) as a yellow solid.

N^2-*[5'-(((1H-Indol-2"-yl)carbonyl)amino)-1H-indol-2'-yl)-carbonyl]-1,2,9,9a-tetrahydrocyclopropa[c]benz[e]-indol-4-one (27, CBI-indole$_2$).*[32] Method A, 93%; method B, 75%; method C, 93%; mp > 240 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): 11.86 (*br s*, 1H, NH), 11.73 (*br s*, 1H, NH), 10.19 (*s*, 1H, NH), 8.24 (*d*, 1H, *J* = 2.6 Hz, C4'-H), 8.02 (*d*, 1H, *J* = 8.0 Hz, C5-H), 7.67 (*d*, 1H, *J* = 7.8 Hz, C4"-H), 7.63 (*m*, 2H, C6-H and C7-H), 7.47 (*m*, 4H), 7.29 (*s*, 1H, C3'-H or C3"-H), 7.25 (*m*, 2H, C8-H and C6"-H), 7.07 (*t*, 1H, *J* = 7.3 Hz, C5"-H), 6.98 (*s*, 1H, C3-H), 4.65 (*dd*, 1H, *J* = 4.9, 10.2 Hz, C1-H), 4.53 (apparent *d*, 1H, *J* = 10.2 Hz, C1-H), 3.20 (*m*, 1H, obscured by H_2O, C9a-H), 1.77 (*dd*, 1H, *J* = 4.2, 7.4 Hz, C9-H), 1.73 (*t*, 1H, *J* = 4.2 Hz, C9-H); IR (KBr) ν_{max} 3432, 1648, 1522, 1384, 1266, 1126, 744 cm^{-1}; UV (DMF) λ_{max} 316 (ε = 45,000), 274 nm (25,000); FABHRMS (NBA) m/z 499.1792 (M^+ + H, $C_{31}H_{22}N_4O_3$ requires 499.1770).

Natural (+)-**27**: $[\alpha]_D^{23}$ +114 (*c* 0.03, DMF), $[\alpha]_D^{25}$ +81 (*c* 0.12, THF).
ent-(–)-**27**: $[\alpha]_D^{25}$ –120 (*c* 0.1, DMF), $[\alpha]_D^{25}$ –81 (*c* 0.12, THF).

N^2-*[(5'-(((Benzofuro-2"-yl)carbonyl)amino)-1H-indol-2'-yl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[c]benz[e]-indol-4-one (57).* Method A, 60%; method B, 49%; mp > 230 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.89 (*s*, 1H, NH), 10.51 (*s*, 1H, NH), 8.25 (*d*, 1H, *J* = 1.4 Hz, C4'-H), 8.04 (*d*, 1H, *J* = 7.8 Hz, C5-H), 7.85 (*d*, 1H, *J* = 7.7 Hz, C4"-H), 7.79 (*s*, 1H, C3"-H), 7.75 (*d*, 1H, *J* = 8.3 Hz, C7"-H), 7.66 (*dd*, 1H, *J* = 1.8, 8.9 Hz, C6'-H), 7.63 (*t*, 1H, *J* = 7.4 Hz, C7-H), 7.53 (*t*, 1H, *J* = 8.3 Hz, C6"-H), 7.50 (*d*, 1H, *J* = 8.7 Hz, C7'-H), 7.46 (*t*, 1H, *J* = 7.2 Hz, C6-H), 7.39 (*t*, 1H, *J* = 7.4 Hz, C5"-H), 7.31 (*s*, 1H, C3'-H), 7.28 (*d*, 1H, *J* = 7.8 Hz, C8-H), 7.00 (*s*, 1H, C3-H), 4.66 (*dd*, 1H, *J* = 5.0, 10.3 Hz, C1-H), 4.53 (*d*, 1H, *J* = 10.3 Hz, C1-H), 3.28–3.32 (*m*, 1H, C9a-H), 1.79 (*dd*, 1H, *J* = 4.1, 7.6 Hz, C9-H), 1.73 (apparent *t*, 1H, *J* = 4.4 Hz, C9-H); ^{13}C NMR (THF-d_8, 100 MHz): δ 185.2 (C), 162.3 (C), 160.7 (C), 157.0 (C), 155.9 (C), 151.1 (C), 141.4 (C), 135.2 (C), 134.1 (C), 132.8 (C), 132.2 (CH), 131.6 (C), 128.7 (C), 127.5 (CH), 127.1 (CH), 126.9 (CH), 124.5 (CH), 123.4 (CH), 122.5 (CH), 120.5 (CH), 114.1 (CH), 112.7 (CH), 112.4 (two CH), 110.9 (CH), 108.4 (C), 107.9 (CH), 55.3 (CH_2), 33.2 (C), 29.9 (CH) 28.5 (CH_2): IR (film) ν_{max} 3299, 1654, 1595, 1517, 1388, 1262, 1127, 744 cm^{-1}; FABHRMS (NBA) m/z 500.1610 (M^+ + H, $C_{31}H_{21}N_3O_4$ requires 500.1610).

Natural (+)-**57**: $[\alpha]_D^{23}$ +91 (*c* 0.13, THF).

N^2-*[(5'-(((Benzo[b]thieno-2"-yl)carbonyl)amino)-1H-indol-2'-yl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[c]benz-[e]indol-4-one (58).* Method A, 50%; method B, 46%; mp > 230 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.88 (*s*, 1H, NH), 10.50 (*s*, 1H, NH), 8.36 (*s*, 1H, C3"-H), 8.18 (*s*, 1H, C4'-H), 8.06 (*d*, 1H, *J* = 6.7 Hz, C7"-H), 8.01 (*d*, 2H, *J* = 7.2 Hz, C4"-H, C5-H), 7.61 (*t*, 1H, *J* = 8.2 Hz, C7-H), 7.59 (*d*, 1H, *J* = 8.9 Hz, C6'-H), 7.42–7.51 (*m*, 4H, C6-H, C6"-H, C5"-H, C7'-H), 7.28 (*s*, 1H, C3'-H), 7.26 (*d*, 1H, *J* = 7.8 Hz, C8-H), 6.98 (*s*, 1H, C3-H), 4.65 (*dd*, 1H, *J* = 5.0, 10.3 Hz, C1-H), 4.51 (*d*, 1H, *J* = 10.2 Hz, C1-H), 3.28 (*m*, 1H, partially obscured by H_2O, C9a-H), 1.76 (*dd*, 1H, *J* = 4.2, 7.6 Hz, C9-H), 1.71 (apparent *t*, 1H, *J* = 4.8 Hz, C9-H); IR (film) ν_{max} 3321, 1652, 1593, 1554, 1516, 1386, 1256, 1121 cm^{-1}; FABHRMS (NBA) m/z 516.1391 (M^+ + H, $C_{31}H_{21}N_3O_3S$ requires 516.1382).

Natural (+)-**58**: +73 (*c* 0.05, DMF).

N^2-*[(5'-(((1H-Indol-2"-yl)carbonyl)amino)benzofuran-2'-yl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[c]benz[e]-indol-4-one (59).* Method A, 63%; method B, 45%; mp > 230 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.77 (*s*, 1H, NH), 10.38 (*s*, 1H, NH), 8.35 (*d*, 1H, *J* = 2.0 Hz, C4'-H), 8.01 (*d*, 1H, *J* = 7.8 Hz, C5-H), 7.87 (*s*, 1H,

C3'-H), 7.85 (*dd*, 1H, *J* = 2.1, 9.0 Hz, C6'-H), 7.75 (*d*, 1H, *J* = 9.0 Hz, C7'-H), 7.68 (*d*, 1H, *J* = 8.1 Hz, C4"-H), 7.61 (*t*, 1H, *J* = 7.8 Hz, C7-H), 7.47 (*d*, 1H, *J* = 8.8 Hz, C7"-H), 7.44 (*s*, 1H, C3"-H), 7.44 (*t*, 1H, *J* = 8.0 Hz, C6-H), 7.25 (*d*, 1H, *J* = 7.0 Hz, C8-H), 7.22 (*t*, 1H, *J* = 8.2 Hz, C6"-H), 7.07 (*t*, 1H, *J* = 7.0 Hz, C5"-H), 6.91 (*s*, 1H, C3-H), 4.53–4.59 (*m*, 2H, C1-H_2), 3.27–3.28 (*m*, 1H, partially obscured by H_2O, C9a-H), 1.72–1.78 (*m*, 2H, C9-H_2); IR (film) ν_{max} 3330, 1660, 1548, 1382, 1300, 1242, 1035 cm^{-1}; FABHRMS (NBA) m/z 500.1600 (M^+ + H, $C_{31}H_{21}N_3O_4$ requires 500.1610).

Natural (+)-**59**: +176 (*c* 0.09, DMF).

N^2-*[(5'-(((Benzofuro-2"-yl)carbonyl)amino)benzofuro-2'-yl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[c]benz[e]-indol-4-one (60).* Method A, 93%; method B, 49%; mp > 230 °C; ^1H NMR (DMSO-d_6, 400 MHz): δ 10.74 (*s*, 1H, NH), 8.39 (*d*, 1H, *J* = 2.0 Hz, C4'-H), 8.04 (*d*, 1H, *J* = 7.9 Hz, C5-H), 7.90 (*s*, 1H, C3'-H), 7.89 (*dd*, 1H, *J* = 2.2, 9.0 Hz, C6'-H), 7.87 (*d*, 1H, *J* = 7.8 Hz, C4"-H), 7.82 (*s*, 1H, C3"-H), 7.78 (*d*, 1H, *J* = 9.0 Hz, C7'-H), 7.77 (*d*, 1H, *J* = 8.4 Hz, C7"-H), 7.64 (*t*, 1H, *J* = 7.5 Hz, C7-H), 7.54 (*t*, 1H, *J* = 8.3 Hz, C6"-H), 7.47 (*t*, 1H, *J* = 7.6 Hz, C6-H), 7.41 (*t*, 1H, *J* = 8.0 Hz, C5"-H), 7.27 (*d*, 1H, *J* = 7.6 Hz, C8-H), 6.94 (*s*, 1H, C3-H), 4.62 (*dd*, 1H, *J* = 4.7, 10.5 Hz, C1-H), 4.57 (*d*, 1H, *J* = 10.4 Hz, C1-H), 3.30 (*m*, 1H, partially obscured by H_2O, C9a-H), 1.80 (*dd*, 1H, *J* = 4.1, 7.7 Hz, C9-H), 1.76 (*t*, 1H, *J* = 4.6 Hz, C9-H); IR (film) ν_{max} 3369, 2921, 1660, 1600, 1549, 1378, 1295, 1244, 1050 cm^{-1}; FABHRMS (NBA) m/z 501.1470 (M^+ + H, $C_{31}H_{20}N_2O_5$ requires 501.1450).

Natural (+)-**60**: $[\alpha]_D^{23}$ +90 (*c* 0.10, DMF).

N^2-*[(5'-(((Benzo[b]thieno-2"-yl)carbonyl)amino)benzofuro-2'-yl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[c]-benz[e]indol-4-one (61).* Method B, 50%; mp > 230 °C; ^1H NMR (DMSO-d_6, 400 MHz): δ 10.72 (*s*, 1H, NH), 8.41 (*s*, 1H, C3"-H), 8.34 (*d*, 1H, *J* = 2.2 Hz, C4'- H), 8.09 (*d*, 1H, *J* = 7.0 Hz, C5-H), 8.05 (*d*, 1H, *J* = 7.0 Hz, C7"-H), 8.04 (*d*, 1H, *J* = 6.2 Hz, C4"-H), 7.90 (*s*, 1H, C3'-H), 7.85 (*dd*, 1H, *J* = 2.0, 8.9 Hz, C6'-H), 7.78 (*d*, 1H, *J* = 9.0 Hz, C7'-H), 7.64 (*t*, 1H, *J* = 6.2 Hz, C7-H), 7.45–7.55 (*m*, 3H, C6-H, C6"-H, C5"-H), 7.27 (*d*, 1H, *J* = 8.0 Hz, C8-H), 6.94 (*s*, 1H, C3-H), 4.58–4.62 (*m*, 2H, C1-H_2), 3.27–3.28 (*m*, 1H, obscured by H_2O, C9a-H), 1.76–1.80 (*m*, 2H, C9-H_2); IR (film) ν_{max} 2920, 2851, 1661, 1599, 1555, 1466, 1381, 1297, 1243 cm^{-1}; FABHRMS (NBA) m/z 517.1233 (M^+ + H, $C_{31}H_{20}N_2O_4S$ requires 517.1222).

Natural (+)-**61**: $[\alpha]_D^{23}$ +69 (*c* 0.04, DMF).

2,4-Dinitrophenyl N^1-*[N^2-(tert-butyloxycarbonyl)hydrazino]carboxylate (71).* A suspension of bis(2,4-dinitrophenyl)carbonate[57] (**69**, 394 mg, 1.0 mmol) in 1.5 mL of EtOAc at 24 °C under N_2 was treated with a solution of *tert*-butylcarbazate[58] (70, 132 mg, 1.0 mmol) in EtOAc (6 mL), and the reaction mixture was stirred for 2 h (24 °C). The reaction mixture was filtered through a glass filter. The filtrate was concentrated to 2 mL below 24 °C *in vacuo* and mixed with hexane (10 mL). The resulting precipitate was collected by filtration to afford **71** (271 mg, 72% pure as a mixture with 2,4-dinitrophenol) and a second crop of crystals was obtained from the mother liquor to afford pure **71** (12 mg) as colorless flakes: mp 105–107 °C (hexane, colorless flakes); ^1H NMR ($CDCl_3$ 200 MHz): δ 8.93 (*d*, 1H, *J* = 2.7 Hz, C3-H), 8.51 (*dd*, 1H, *J* = 2.7, 9.0 Hz, C5-H), 7.62 (*d*, 1H, *J* = 9.0 Hz, C6-H), 7.05 (*br s*, 1H, NH), 6.43 (*br s*, 1H, NH), 1.48 (*s*, 9H, $C(CH_3)_3$); IR (KBr) ν_{max} 3414, 3268, 3112, 2978, 1754, 1738, 1612, 1538, 1484, 1394, 1346, 1240, 1166, 1070, 1024, 918, 858, 834, 752, 728, 642 cm^{-1}.

*3-[N^1-[N^2-(tert-Butyloxycarbonyl)hydrazino]carbonyl]-1-chloromethyl-5-hydroxy-1,2-dihydro-3*H*-benz[e]indole (72).* Phenol **15** (6.0 mg, 18 μmol) was treated with anhydrous 3 N HCl–EtOAc (0.5 mL) at 24 °C for 20 min. the solvent was removed *in vacuo* to afford crude, unstable **16** quantitatively. A solution of **16** in THF (0.4 mL) at 24 °C under Ar was treated sequentially with **71** (11 mg, 72% pure, 23.4 μmol, 1.3 equiv) and Et_3N (2.5 μL, 18 μmol, 1 equiv), and the reaction mixture was stirred for 5.5 h (24 °C). Flash chromatography (1.5 × 15 cm SiO_2, 66% EtOAc–hexane) afforded **72** (6.4 mg, 7.0 mg theoretical, 91%) as a white solid: mp 221 °C; ^1H NMR ($CDCl_3$–DMF-d_7, 200 MHz): δ 9.82 (*s*, 1H, OH), 8.25 (*d*, 1H, *J* = 8 Hz, C6-H), 7.82 (*s*, 1H, C4-H), 7.64 (*d*, 1H, *J* = 2 Hz, NH), 7.58 (*d*, 1H, *J* = 8 Hz, C9-H), 7.46 (*ddd*, 1H, *J* = 1.4, 7, 8 Hz, C8-H), 7.30 (*ddd*, 1H, *J* = 1.4, 7, 8 Hz, C7-H), 6.86 (*br s*, 1H, NH), 4.24 (*dd*, 1H, *J* = 3, 10 Hz, C2-H), 4.17 (*t*, 1H, *J* = 10 Hz, C2-H), 3.98 (*m*, 1H, C1-H), 3.92 (*dd*, 1H, *J* = 3, 11 Hz, C<u>H</u>HCl), 3.37 (*t*, 1H, *J* = 11 Hz, CH<u>H</u>Cl), 1.50 (*s*, 9H, $C(CH_3)_3$); IR (KBr) ν_{max} 3408, 2926, 1718, 1654, 1584, 1522, 1476, 1394, 1246, 1160, 862, 758 cm^{-1}; UV (THF) λ_{max} 318 (ε = 9500), 308 (8200), 260 (31,000), 254 nm (32,000); FABHRMS (DTT-DTE) m/z 392.1364 (M^+ + H, $C_{19}H_{22}ClN_3O_4$ requires 392.1377).

*1-(Chloromethyl)-3-(hydrazino)carbonyl-5-hydroxy-1,2-dihydro-3*H*-benz[e]indole hydrochloride (73).* A sample of **72** (1.0 mg, 2.6 μmol) was treated with anhydrous 3 N HCl–EtOAc (1 mL) at 24 °C for 30 min. The solvent was removed *in vacuo* to afford **73** (0.9 mg, 0.87 mg theoretical, 100%) as a white solid: mp 225 °C (dec); ^1H NMR ($CDCl_3$–DMSO-d_6, 300 MHz): δ 10.00 (*br s*, N^+H_3), 9.96 (*s*, 1H, OH), 9.77 (*s*, 1H, CONH), 8.18 (*d*, 1H, *J* = 8.3 Hz, C6-H), 7.78 (*s*, 1H, C4-H), 7.63 (*d*, 1H, *J* = 8.3 Hz, C9-H), 7.48 (*t*, 1H, *J* = 7.4 Hz, C8-H), 7.30 (*t*, 1H, *J* = 7.5 Hz, C7-H), 4.26 (*m*, 2H, C2-H), 4.07 (*m*, 1H, C1-H), 3.93 (*dd*, 1H, *J* = 2, 11 Hz, C<u>H</u>HCl), 3.51 (*t*, 1H, *J* = 10.2 Hz, C<u>H</u>HCl); IR (KBr) ν_{max} 3400 (br), 3200 (br), 2926, 1670, 1632, 1584, 1520, 1478, 1420, 1394, 1352, 1242, 1154, 1126, 1074, 1024, 756 cm^{-1}; UV (DMF) λ_{max} 322 (ε = 9300), 310 (sh, 7900), 270 nm (23,000); FABHRMS (DTT-DTE) m/z 292.0867 (M^+ + H, $C_{14}H_{14}ClN_3O_2$ requires 292.0853).

N^1-*[N^2-(tert-Butyloxycarbonyl)hydrazino]carbonyl-CDPI$_2$ (77).* *N*-BOC-CDPI$_2$[59] (**76**, 6.2 mg, 12.8 μmol) was treated with CF_3CO_2H (0.5 mL) at 24 °C for 1 h.

The CF_3CO_2H was removed by a stream of N_2 and the residue was dried *in vacuo*. A solution of the crude salt in DMF (0.2 mL) at 24 °C under Ar was treated sequentially with **71** (72% pure in 2,4-dinitrophenol, 9.1 mg, 19.3 μmol, 1.5 equiv) and Et_3N (1.8 μL, 12.8 μmol, 1 equiv) and the reaction mixture was stirred for 19 h (24 °C). The solvent was removed *in vacuo* and the residue was washed with saturated aqueous $NaHCO_3$ (1 mL), H_2O (0.5 mL), 10% aqueous citric acid (1 mL), and H_2O (4 × 1 mL). Drying the solid *in vacuo* afforded **77** (6.3 mg, 6.9 mg theoretical, 91%) as a pale yellow solid: mp 257 °C (dec); ^1H NMR (DMSO-d_6, 300 MHz): δ 11.84 (*s*, 1H, NH), 11.61 (*s*, 1H, NH), 8.62 (*s*, 1H, CONH), 8.42 (*s*, 1H, CONH), 8.28 (*br d*, 1H, *J* = 9 Hz, C4-H), 7.94 (*d*, 1H, *J* = 8.9 Hz, C4'-H), 7.32 (*d*, 1H, *J* = 9 Hz, C5-H), 7.26 (*d*, 1H, *J* = 8.9 Hz, C5'-H), 7.06 (*s*, 1H, C8'-H), 6.98 (*s*, 1H, C8-H), 4.64 (*t*, 2H, *J* = 8.3 Hz, C2-H_2), 4.02 (*t*, 2H, *J* = 8.3 Hz, C2'-H_2), 3.2–3.6 (*m*, 4H, partly obscured by H_2O, C1-H_2, C1'-H_2), 1.43 (*s*, 9H, $C(CH_3)_3$); IR (KBr) ν_{max} 3424, 1686, 1508, 1438, 1372, 1160, 800, 684 cm^{-1}.

N^1-N^2-*(tert-Butyloxycarbonyl)hydrazino]carbonyl-seco-CBI-CDPI$_2$ (78).* A slurry of crude **16** freshly prepared from **15** (3.7 mg, 11.1 μmol), EDCI (6.4 mg, 33 μmol, 3 equiv), and **77** (6.0 mg, 11.1 μmol, 1 equiv) in DMF (0.2 mL) at 24 °C under Ar was vigorously stirred for 10 h. The solvent was removed *in vacuo* and the residue washed with H_2O (2 × 2 mL) and dried *in vacuo*. Flash chromatography (0.5 × 5 cm SiO_2, 0–66% DMF–toluene gradient elution) afforded **78** (5.5 mg, 8.4 mg theoretical, 65%) as a pale yellow solid: mp 250 °C (dec); ^1H NMR (DMSO-d_6, 300 MHz): δ 11.83 (*s*, 1H, NH), 11.63 (*s*, 1H, NH), 10.45 (*s*, 1H, OH), 8.63 (*s*, 1H, CONH), 8.43 (*s*, 1H, CONH), 8.29 (*br d*, 1H, *J* = 9 Hz, C4'-H), 8.13 (*d*, 1H, *J* = 8.5 Hz, C6-H), 7.99 (*s*, 1H, C4-H), 7.95 (*d*, 1H, *J* = 8.9 Hz, C4"-H), 7.87 (*d*, 1H, *J* = 8.3 Hz, C9-H), 7.54 (*t*, 1H, *J* = 7.6 Hz, C8-H), 7.40 (*d*, 1H, *J* = 9.3 Hz, C5'-H), 7.38 (*t*, 1H, *J* = 7.7 Hz, C7-H), 7.27 (*d*, 1H, *J* = 8.9 Hz, C5"-H), 7.19 (*s*, 1H, C8'-H), 7.01 (*s*, 1H, C8"-H), 4.85 (*t*, 1H, *J* = 10 Hz, C2-H), 4.68 (*t*, 2H, *J* = 8 Hz, C2'-H_2), 4.59 (*d*, 1H, *J* = 10 Hz, C2-H), 4.26 (*m*, 1H, C<u>H</u>HCl), 4.03 (*t*, 2H, *J* = 8 Hz, C2"-H_2), 3.9–4.0 (*m*, 2H, C1-H), CH<u>H</u>Cl), 3.2–3.6 (*m*, 4H, partly obscured by H_2O, C1'-H_2, C1"-H_2), 1.44 (*s*, 9H, $C(CH_3)_3$); IR (KBr) ν_{max} 3410, 3315, 2962, 2927, 1664, 1610, 1582, 1508, 1416, 1370, 1340, 1262, 1158, 1098, 802, 762, 528 cm^{-1}; UV (DMF) λ_{max} 340 (ε = 43,000), 310 (44,000), 270 nm (26,000); FABHRMS (DTT-DTE) m/z 760.2635 (M^+ + H, $C_{41}H_{38}ClN_7O_6$ requires 760.2650).

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51. We anticipate that this full relationship will be parabolic in nature displaying an optimal reactivity– stability/activity[21] but that the studies detailed herein which span a 10-fold reactivity range lie in range where the relationship is linear.

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60. The corresponding methyl ester was prepared as well by treating *N*-BOC-CDPI$_2$ methyl ester with CF_3CO_2H (25 °C, 1 h) followed by coupling with **71** (1.2 equiv, 1 equiv Et_3N, DMF, 25 °C, 16 h, 98%). Although this was not examined in detail, a single attempted hydrolysis of the methyl ester (5 equiv LiOH, THF:CH_3OH:H_2O 3:2:1, 25 °C, 10 h) did not provide **77**. For the methyl ester of **77**: pale yellow solid; mp 245 °C (dec); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.01 (*s*, 1H, NH), 11.62 (*s*, 1H, NH), 8.62 (*s*, 1H, CONH), 8.42 (*s*, 1H, CONH), 8.29 (*br d*, 1H, *J* = 8.9 Hz, C4-H), 7.95 (*d*, 1H, *J* = 8.9 Hz, C4'-H), 7.34 (*d*, 1H, *J* = 9.0 Hz, C5-H), 7.26 (*d*, 1H, *J* = 8.9 Hz, C5'-H), 7.14 (*s*, 1H, C8'-H), 6.98 (*s*, 1H, C8-H), 4.64 (*t*, 2H, *J* = 8.2 Hz, C2-H_2), 4.03 (*t*, 2H, *J* = 8.4 Hz, C2'-H_2), 3.88 (*s*, 3H, CO_2CH_3), 3.2–3.5 (*m*, 4H, partly obscured by H_2O, C1-H_2, C1'-H_2); IR (KBr) ν_{max} 3330, 2978, 1706, 1610, 1578, 1508, 1436, 1372, 1334, 1288, 1254, 1210, 1162, 1048, 1026, 1004, 806, 764 cm^{-1}.

61. After 2 days at 4 °C, most **19** remained and after 8 h at 25 °C the reaction was complete.

62. Commercially available from Transworld Chemicals.

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