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(54) **ANALOGUES DE CBI DE CC-1065 ET DES DUOCARMYCINES**

(54) **CBI ANALOGS OF CC-1065 AND THE DUOCARMYCINS**

(57) L'invention concerne des analogues d'antibiotiques antitumoraux CC-1065 et de duocarmycines comportant la sous-unité d'alcoylation 1,2,9,9a-tétrahydrocyclopropa[c]benz[e]indol-4-one (CBI). Ces analogues à base de CBI, qui sont doués d'une forte action cytotoxique, montrent leur efficacité en tant que composés antitumoraux. L'invention a également trait à une relation directe existant entre une stabilité fonctionnelle et une capacité cytotoxique in vitro. Les analogues à base de CBI que l'on synthétise facilement sont quatre fois plus stables et plus puissants que les analogues correspondants contenant la véritable sous-unité d'alcoylation de CC-1065 (CPI) et ont une capacité comparable à celle d'agents contenant la véritable sous-unité d'alcoylation de duocarmycine SA. De la même manière, Les agents à base de CBI, qui alcoylent l'ADN avec une sélectivité séquentielle inchangée, à une cadence renforcée ainsi qu'avec un rendement plus élevé que l'analogue de CPI correspondant, se sont avérés comparables à l'analogue correspondant dans lequel figure la sous-unité d'alcoylation de duocarmycine SA. L'invention porte, en outre, sur des modifications et des simplification, systématiques et à grande échelle, au sein des sous-unités de liaison d'ADN attachées au CBI.

(57) Analogs of the antitumor antibiotics CC-1065 and the duocarmycins incorporate the 1,2,9,9a-tetrahydrocyclopropa[c]benz[e]indol-4-one (CBI) alkylation subunit. The CBI-based analogs have potent cytotoxic activity and are useful as efficacious antitumor compounds. A direct relationship between functional stability and in vitro cytotoxic potency is disclosed. The CBI-based analogs are easily synthesized and are 4x more stable and 4x 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 are also described.



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(54) Title: CBI ANALOGS OF CC-1065 AND THE DUOCARMYCINS		
(57) Abstract		
<p> Analogs of the antitumor antibiotics CC-1065 and the duocarmycins incorporate the 1,2,9,9a-tetrahydrocyclopropa[c]benz[e]indol-4-one (CBI) alkylation subunit. The CBI-based analogs have potent cytotoxic activity and are useful as efficacious antitumor compounds. A direct relationship between functional stability and in vitro cytotoxic potency is disclosed. The CBI-based analogs are easily synthesized and are 4x more stable and 4x 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 are also described. </p>		

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CBI ANALOGS OF CC-1065 AND THE DUOCARMYCINS

Description

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Field of Invention:

The invention relates to antitumor antibiotics. More particularly, the invention relates to analogs of CC-1065 and the duocarmycins having antitumor antibiotic activity.

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Background:

(+)-CC-1065 (1) and the duocarmycins 2-3 represent the initial members of a class of exceptionally potent antitumor antibiotics. Members of this class of antitumor antibiotic derive their biological effects through the reversible, stereoelectronically-controlled sequence selective alkylation of duplex DNA. (H. Sugiyama, et al., *Tetrahedron Lett.* 1990, 31, 7197; C.H. Lin, et al., *J. Am. Chem. Soc.* 1992, 114, 10658; H. Sugiyama, et al., *Tetrahedron Lett.* 1993, 34, 2179; K. Yamamoto, et al., *Biochemistry* 1993, 32, 1059; A. Asai, et al., *J. Am. Chem. Soc.* 1994, 116, 4171; and D.L. Boger, et al., *Tetrahedron* 1991, 47, 2661.) (+)-CC-1065 (1) was first disclosed in 1981 by L.J. Hanka, et al. (*J. Am. Chem. Soc.* 1981, 103, 7629.) The duocarmycins 2-3 were first disclosed in 1988 and 1990. (Takahashi, et al., *J. Antibiot.* 1988, 41, 1915; T. Yasuzawa, et al., *Chem. Pharm. Bull.* 1988, 36, 3728; M. Ichimura, et al., *J. Antibiot.* 1988, 41, 1285; M. Ichimura, et al., *J. Antibiot.* 1990, 43, 1037; M.H. Ichimura, et al., *J. Antibiot.* 1991, 44, 1045; K. Ohba, et al., *J. Antibiot.* 1988, 41, 1515; and S. Ishii, *J. Antibiot.* 1989, 42, 1713.)

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Subsequent to their disclosure, extensive efforts have been devoted to establish their duplex DNA alkylation selectivity and its structural origin. (D.L. Boger, *Acc. Chem. Res.* 1995, 28, 20; D.L. Boger, *Proc. Natl. Sci. U.S.A.* in

press; D.L. Boger, *Chemtracts: Org. Chem.* 1991, 4, 329; D.L. Boger, In *Proceed. R. A. Welch Found. Conf. on Chem. Res., XXXV. Chem. at the Frontiers of Medicine* 1991, 35, 137; D.L. Boger, In *Advances in Heterocyclic Natural Products Synthesis, Vol. 2*, Pearson, W. H. Ed.; JAI Press: Greenwich, CT, 1992, 1-188; D.L. Boger, *Pure Appl. Chem.* 1993, 65, 1123; D.L. Boger, *Pure Appl. Chem.* 1994, 66, 837; R.S. Coleman, In *Studies in Nat. Prod. Chem.*, Vol 3, Rahman, A.-u.-, Ed.; Elsevier: Amsterdam, 1989, 301; and D.L. Boger, In *Heterocycles in Bioorganic Chemistry*; J. Bergman, H.C. van der Plas, and M. Simonyi, Eds; Royal Society of Chemistry: Cambridge, 1991, 103.) Progress has also been made with respect to characterizing the link between DNA alkylation and the ensuing biological properties. (D.L. Boger, et al., *Bioorg. Med. Chem. Lett.* 1994, 4, 631.) Extensive efforts have also been devoted to define the fundamental principles underlying the relationships between structure, chemical reactivity, and biological properties. (W. Wierenga, et al., *Adv. Enzyme Regul.* 1986, 25, 141; M.A. Warpehoski, et al., *J. Med. Chem.* 1988, 31, 590; D.L. Boger, et al., *J. Am. Chem. Soc.* 1993, 115, 9025; D.L. Boger, et al., *J. Am. Chem. Soc.* 1992, 114, 10056; H. Muratake, et al., *Tetrahedron Lett.* 1994, 35, 2573; Y. Fukuda, et al., *Tetrahedron* 1994, 50, 2793; Y. Fukuda, et al., *Tetrahedron* 1994, 50, 2809; Y. Fukuda, et al., *Bioorg. Med. Chem. Lett.* 1992, 2, 755; Y. Fukuda, et al., *Tetrahedron Lett.* 1990, 31, 6699; W. Wierenga, *J. Am. Chem. Soc.* 1981, 103, 5621; P. Magnus, et al., *J. Am. Chem. Soc.* 1987, 109, 2706; G.A. Kraus, et al., *J. Org. Chem.* 1985, 50, 283; D.L. Boger, et al., *J. Am. Chem. Soc.* 1988, 110, 1321, 4796; R.E. Bolton, et al., *J. Chem. Soc., Perkin Trans. 1* 1988, 2491; R.J. Sundberg, et al., *J. Org. Chem.* 1988, 53, 5097; R.J. Sundberg, et al., *J. Org. Chem.* 1991, 56, 3048; V.P. Martin, *Helv. Chim. Acta* 1989, 72, 1554; M. Toyota, et al., *J. Chem. Soc., Perkin Trans. 1* 1992, 547; and L.F. Tietze, et al., *J. Org. Chem.* 1994, 59, 192.) The relationships between structure, chemical reactivity, and biological properties of CI-based analogs have also been characterized. (D.L. Boger, et al., *Proc. Natl. Acad. Sci. U.S.A.* 1991, 88, 1431; D.L. Boger, et al., *J. Am. Chem. Soc.* 1991, 113, 3980; D.L. Boger, et al., *J. Org. Chem.* 1989, 54,

1238; D.L. Boger, et al., *J. Am. Chem. Soc.* 1990, 112, 5230; K.J. Drost, et al., *J. Org. Chem.* 1989, 54, 5985; J.H. Tidwell, et al., *J. Org. Chem.* 1992, 57, 6380; J. Sundberg, et al., *Tetrahedron Lett.* 1986, 27, 2687; Y. Wang, et al., *Heterocycles* 1993, 36, 1399; Y. Wang, et al., *J. Med. Chem.* 1993, 36, 4172; L.F. Tietze, et al., *Chem. Ber.* 1993, 126, 2733; and T. Sakamoto, et al., *J. Chem. Soc., Perkin Trans. I* 1993, 1941.) The relationships between structure, chemical reactivity, and biological properties of C₂BI-based analogs have also been characterized. (D.L. Boger, et al., *J. Am. Chem. Soc.* 1992, 114, 9318; and D.L. Boger, et al., *Bioorg. Med. Chem.* 1993, 1, 27.) The relationships between structure, chemical reactivity, and biological properties of CBQ-based analogs have also been characterized. (D.L. Boger, et al., *J. Am. Chem. Soc.* 1994, 116, 6461; and D.L. Boger, et al., *J. Am. Chem. Soc.* 1994, 116, 11335.) F. Mohamadi et al. have characterized the relationships between structure, chemical reactivity, and biological properties of CFI-based analogs (*J. Med. Chem.* 1994, 37, 232.) A *p*-quinonemethide analog was characterized by D.L. Boger, et al. (*J. Org. Chem.* 1994, 59, 4943.)

Concurrent with the above structure/function studies, substantial efforts have been devoted to developing potential clinical candidates based on the natural product structures having enhanced *in vivo* efficacy. Compounds 4-8 are analogs of the natural product structures having enhanced *in vivo* efficacy with clinical potential. (D.L. Boger, et al., *J. Org. Chem.* 1984, 49, 2240; M.A. Warephoski, M. A. *Tetrahedron Lett.* 1986, 27, 4103; Li, L. H.; *Invest. New Drugs* 1991, 9, 137; B.K. Bhuyan, et al., *Cancer Res.* 1992, 52, 5687; B.K. Bhuyan, et al., *Cancer Res.* 1993, 53, 1354; L.H. Li, et al., *Cancer Res.* 1992, 52, 4904; M.A. Mitchell, et al., *J. Am. Chem. Soc.* 1991, 113, 8994. Lee, C.-S.; Gibson, N. W. *Cancer Res.* 1991, 51, 6586. Lee, C.-S.; Gibson, N. W. *Biochemistry* 1993, 32, 9108; Wierenga, W. *Drugs Fut.* 1991, 16, 741; K. Gomi, et al., *Jpn. J. Cancer Res.* 1992, 83, 113. Okamoto, A.; Okabe, M.; Gomi, K. *Jpn. J. Cancer Res.* 1993, 84, 93; E. Kobayashi, et al., *Cancer Res.* 1994, 54, 2404; and H. Ogasawara, *Jpn. J. Cancer Res.* 1994, 85, 418.) A Phase I clinical trial one one drug candidate in this

class is described by G. F. Fleming, et al., (*J. Natl. Cancer Inst.* 1994, 86, 368.) Efforts have also focused on the development of analogs having decreased delayed toxicity as compared to the natural form of (+)-CC-1065. (J.P. McGovren, et al., *Cancer Res.* 1993, 53, 5690.) Importantly, this unusual property has not been
5 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.

The first preparation and examination of agents containing the 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI) alkylation subunit were described
10 in connection with efforts to evaluate CC-1065 and duocarmycin analogs bearing deep-seated structural alterations in the alkylation subunit. (D.L. Boger, et al., *J. Am. Chem. Soc.* 1989, 111, 6461; and D.L. Boger, et al., *J. Org. Chem.* 1990, 55, 5823.) These agents were employed as tools to identify the structural features of
15 compounds 1-3 associated with their sequence selective alkylation of duplex DNA and to define the fundamental relationships between structure, chemical or functional reactivity and biological properties.

Prior to the present invention, it had been assumed that the unique
20 alkylating activity of the naturally occurring CPI subunit of CC-1065 would be degraded if this portion of the molecule were structurally altered. (L.H. Hurley, et al., *Science* 1984, 226, 843; V.L. Reynolds, et al., *Biochemistry* 1985, 24, 6228. L.H. Hurley, et al., *Biochemistry* 1988, 27, 3886; L.H. Hurley, et al., *J. Am. Chem. Soc.* 1990, 112, 4633; M.A. Warpehoski, et al., *J. Biochemistry* 1992, 31, 2502; D.L. Boger, et al., *Bioorg. Med. Chem.* 1994, 2, 115; D.L. Boger, et al., *J. Am. Chem. Soc.* 1990, 112, 4623; M.A. Warpehoski, et al., In *Advances in DNA Sequence Specific Agents*; Hurley, L. H., Ed.; JAI Press: Greenwich, CT, 1992, Vol 1, 217; M.A. Warpehoski, *Drugs Fut.* 1991, 16, 131; M.A. Warpehoski, et al., in *Molecular Basis of Specificity in Nucleic Acid-Drug Interactions*;
25 B. Pullman and J. Jortner, Eds.; Kluwer: Netherlands; 1990, 531; M.A.

Warpehoski, et al., *Chem. Res. Toxicol.* 1988, 1, 315; Hurley, L. H.; In *Molecular Aspects of Anticancer Drug-DNA Interactions*; Neidle, S., Waring, M., Eds.; CRC Press: Ann Arbor, MI 1993, Vol 1, 89; and L.H. Hurley, et al., *Acc. Chem. Res.* 1986, 19, 230.) The above assumption is disclosed herein to be

5 inaccurate. Furthermore, the natural enantiomers of the CBI-based analogs of (+)-CC-1065, have been shown to be approximately four times more stable chemically and approximately four times more potent biologically as compared to the corresponding agents incorporating the natural CPI alkylation subunit of CC-1065. (D.L. Boger, et al., *Tetrahedron Lett.* 1990, 31, 793; D.L. Boger, et al., *J. Org.*

10 *Chem.* 1992, 57, 2873; and D.L. Boger, et al., *J. Org. Chem.* 1995, 60, 0000.) The CBI analogs are also considerably more synthetically accessible as compared to the naturally occurring CPI compounds. (+)-CBI-indole₂ (27) exhibits cytotoxic potency comparable to that of the (+)-CC-1065 and greater (4x) than that of the potential clinical candidate (+)-CPI-indole₂ (4, U71,184) introduced by Upjohn.

15 (+)-CBI-indole₂ (27) also exhibits potent and efficacious *in vivo* antitumor activity. (D.L. Boger, et al., *Bioorg. Med. Chem. Lett.* 1991, 1, 115.) (+)-CBI-indole₂ (27) was the first 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.

20 The natural enantiomers of the CBI-based analogs have been shown to alkylate DNA with an unaltered sequence selectivity as compared to the corresponding CPI analog. (D.L. Boger, et al., *J. Am. Chem. Soc.* 1994, 116, 7996; and P.A. Aristoff, et al., *J. Med. Chem.* 1993, 36, 1956.) Furthermore, the

25 DNA alkylation of CBI-based analogs occurs at an enhanced rate as compared to the corresponding CPI analogs (D.L. Boger, et al., *J. Am. Chem. Soc.* 1991, 113, 2779) and with a greater efficiency than the corresponding CPI analog. (D.L. Boger, et al., *J. Am. Chem. Soc.* 1992, 114, 5487)

30 Refined models of the DNA alkylation reactions of the duocarmycins

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have been developed which accomodate the reversed and offset AT-rich adenine N3 DNA alkylation selectivity of the enantiomeric agents and their structural analogs.

(D. L. Boger, et al., *J. Org. Chem.* 1990, 55, 4499; D.L. Boger, et al., *J. Am.*

Chem. Soc. 1990, 112, 8961; D.L. Boger, et al., *J. Am. Chem. Soc.* 1991, 113,

5 6645; D.L. Boger, et al., *J. Am. Chem. Soc.* 1993, 115, 9872; D.L. Boger, et al.,

Bioorg. Med. Chem. Lett. 1992, 2, 759; and D.L. Boger, et al., *J. Am. Chem. Soc.*

1994, 116, 1635.) A similar refined model of the DNA alkylation reactions of CC-

1065 have been developed which also accomodate the reversed and offset AT-rich

adenine N3 DNA alkylation selectivity of the enantiomeric agents and their

10 structural analogs. (D.L. Boger, et al., *Bioorg. Med. Chem.* 1994, 2, 115; and D.L.

Boger, et al., *J. Am. Chem. Soc.* 1990, 112, 4623.) These models teach that the

diastereomeric adducts derived from the unnatural enantiomers suffer a significant

destabilizing steric interaction between the CPI C7 center (CH₃) or the CBI C8

center with the base adjacent to the alkylated adenine which is not present with the

15 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

20 more effective than the corresponding CPI analog displaying an even more

enhanced relative rate and efficiency of DNA alkylation.

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Summary:

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 4x more stable and 4x 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 1-3 with both enantiomers of 18-24, 25-29, 57-61, 62-65, 66-68, 72-73 and 78-79.

CPI and CBI Structures

Simple Derivatives of CBI. Role of the N² 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 our original synthesis of CBI and its precursors, as referenced above with two recent modifications. (D.L. Boger, et al., *J. Org. Chem.* 1992, 57, 2873; and D.L. Boger, et al., *J. Org. Chem.* 1995, 60, 0000.) The most efficient approach now proceeds in 9 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 of the primary alcohol precursor to 15 which has been adopted by others has been since improved in our efforts. The more
5 advanced synthetic intermediate 15, and in fact the penultimate intermediate to the CBI-based analogs, may be directly and more efficiently resolved ($\alpha = 1.28$) 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 μ m, 2 x 25 cm OD HPLC column (5% *i*-
10 PrOH-hexane, 8 mL/min) 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, and provided our comparison standards for the studies detailed below (Figure 1).

15 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, IC₅₀) and established the expectation that the biological potency may be enhanced as their electrophilic reactivity is increased. In our complementary series of studies
20 conducted with agents containing deep-seated modifications in the alkylation subunit including 9-14, the reverse relationship has been observed 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
25 and advanced analogs of the natural products.

As a consequence of these studies, we became interested in the inherent role of the CC-1065 and duocarmycin N² substituent. Consequently, the simple derivatives 21-24 of (+)-CBI were prepared for examination and, by virtue of their
30 structural similarities, were expected to more accurately reflect a potential

relationship between functional reactivity and biological potency than the preceding studies. Treatment of crude, freshly prepared 16 with methyl isocyanate (2 equiv, 3 equiv NaHCO₃, 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. Et₃N) which promotes competitive closure of 16 to CBI (17) led to lower conversions. 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 ClCO₂CH₃ (2 equiv, 3 equiv NaHCO₃, 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 K₂CO₃ (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 ClCOCH₂CH₃ (2 equiv, 3 equiv NaHCO₃, 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 NaHCO₃-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 ClSO₂CH₂CH₃ 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

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simply by reaction of the sodium salt of CBI (**17**, 2.5 equiv NaH, THF, 0 °C, 10 min) with ClSO₂CH₂CH₃ (7 equiv, 3 equiv Et₃N, 25 °C, 3 h, 45%) to provide **24** directly.

5 The acid-catalyzed solvolysis of **21-24** conducted at pH 3 (CH₃OH-H₂O) were 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, Figures 19A-19B. The results of these studies along with the
10 cytotoxic activities of **21-24** are summarized in Figures 20A-20D. The cytotoxic activity of the full set of agents examined and the comparisons with the related CPI-based agents are summarized in Figure 7.

 The comparisons of **21-24** revealed a direct, linear relationship between
15 the cytotoxic potency (L1210, log 1/IC₅₀) and the solvolytic stability (-log *k*_{solv}, pH 3) of the agents (Figures 20A-20D). 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 N² substituents (Hammett σ_p constant) and the solvolysis
20 reactivity (-log *k*_{solv}, pH 3) of the agents with the strongest electron-withdrawing substituents providing the most stable agents (Figures 20A-20D). This latter relationship reflects the influence of the N² substituent on the ease of C4 carbonyl protonation required for catalysis of solvolysis and cyclopropyl ring cleavage with the stronger electron-withdrawing N² substituents exhibiting slower solvolysis
25 rates. Less obvious but more fundamental, the observations were found to follow a predictable linear relationship between the cytotoxic potency (L1210, log 1/IC₅₀) and the electron-withdrawing properties of the N² substituent (Hammett σ_p) with the strongest electron-withdrawing substituents providing the biologically most potent agents (Figures 20A-20D).

30 These fundamental correlations between the electron-withdrawing

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properties of the N² substituent, the functional reactivity of the agents, and their biological potency should prove useful in the predictable design of new analogs. 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
5 4x 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.

10 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
15 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, the observations may also represent a more effective thermodynamic partitioning of the agents to their productive intracellular target or site(s).

20 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
25 (CH₃, CH₂Ph) and *O*-acyl derivatives of 15 exhibit substantially diminished cytotoxic potency (10-100x), 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
30 strategies for the advanced analogs of the natural products including 6-8.

- 12 -

Although we have described an extensive account of the DNA alkylation properties of (+)- and *ent*-(-)-*N*-BOC-CBI (9) 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 x), 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 (+)- vs *ent*-(-)-9, proved to be approximately 5-10x 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 (5x) and especially the unnatural enantiomers (10-100x) 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. 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-CDPI₂ (25), CBI-CDPI₁ (26), CBI-indole₂ (27), CBI-indole₁ (28), and CBI-TMI (29) and their corresponding seco precursors 30-34

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have been disclosed in our early studies and their detailed comparisons with both enantiomers of CC-1065 or the duocarmycins described. More recently, 27, 28, and CBI-PDE-I₂ have been disclosed by Aristoff and co-workers. The comparative cytotoxic activity of these prior agents prepared in our studies is summarized in
5 Figure 9 along with that of the corresponding CPI-based analog.

In an extension of our investigations which first revealed efficacious antitumor activity for 27, we have expanded the studies to the preparation and evaluation of 57-61, a larger series based on 27. The DNA binding subunits of CC-
10 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. While the former has been suggested to be the origin of the differences in the
15 cytotoxic potency of 1 and 11 by others based principally on the comparisons of (+)-*N*-BOC-CPI (11), (+)-CPI-indole₁, and (+)-CPI-indole₂, 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 and was based in part
20 on the observation that the cytotoxic potency of a class of agents would plateau. For example, (+)-CC-1065, (+)-CPI-PDE-I₁, and (+)-CPI-CDPI_n (n = 1-3) were found to be indistinguishable in our cytotoxic assays (IC₅₀ = 20 pM, L1210). Although the five agents exhibit large differences in their rates of DNA alkylation, all five form thermodynamically stable adducts under physiological conditions. We
25 attribute the increase in cytotoxic potency of CPI-CDPI_n (n = 1-3) vs 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-indole₁ and CBI-indole₁ lack the sufficient stabilization for observation of full potency.
30 Moreover, the interpretation is consistent with the observation that a maximum

- 14 -

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 4x more potent than that of the corresponding CPI-based agents (20 pM, L1210).

5

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 to the alkylation selectivity of the agents but exerts an overriding dominate control. 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. 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 ($\Delta G^\circ = 9.5-11.5$ kcal/mol). 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.

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15
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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.

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Finally, we have speculated that the extent of the noncovalent binding stabilization of the inherently reversible DNA alkylation reaction may be responsible

- 15 -

for the unusual, delayed toxicity of CC-1065. That is, the extensive noncovalent binding stabilization of 1 that renders its DNA alkylation reaction irreversible while that of simpler agents including 2-3 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-CDPI₂, and (+)-CBI-PDE-I₂. 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 Figure 10. 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-CH₃OH-H₂O, 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 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 P₄-tBu and provided the agents 27 (CBI-indole₂) and 57-61.

The results of the cytotoxic evaluations of the agents are summarized in Figure 11 along with those of CBI-indole₂ (27) and CPI-indole₂. Several aspects of these comparative evaluations are notable. First, the natural enantiomers are

- 16 -

substantially more potent than the unnatural enantiomers (130-1000x). 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 2-6x 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, 28, and 29 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, and a beautiful explanation emerged which explains the diminished DNA capabilities of the unnatural enantiomers. 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. 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-indole₂ (27)/CPI-indole₂ (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 21 with the comparison of (+)-CBI-indole₂ (27) and (+)-CPI-indole₂ (4) where 27 is 10x 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

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single high affinity site of w794 DNA, that of CBI-indole₂ was considerably faster, $k(27)/k(4) = 14$, Figures 23A-23B. 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 (Figure 22) and at comparable rates, $k(29)/k(2) = 0.9$, Figures 23A-23B.

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 4-100x less effective than the natural enantiomers. In comparison, the unnatural enantiomers of the CPI-based analogs are 10-1000x less effective and the duocarmycin SA based analogs or agents are 1-10x 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, *ie.* **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 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, Figures 24A-24E. Figures 24A-24E include data for 4-6 available classes of agents that bear five different DNA binding subunits which we have examined, and although this relationship is undoubtedly a second order polynomial indicative of a parabolic relationship that will exhibit an optimal stability-reactivity/potency, the agents employed in Figures 24A-24E 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.

CBI-CDPBO₁ and CBI-CDPBI₁: Deep-Seated Structural Variations in the DNA Binding Subunits.

The efforts of Lown and Dervan 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.

In the conduct of these studies, we reported the preparation of (+)- and *ent*-(-)-CBI-CDPBO₁ (62), (+)- and *ent*-(-)-CBI-CDPBI₁ (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.

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 100x less efficient at alkylating DNA than (+)-CBI-CDPI₁ (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, the unnatural enantiomers of 62 and 64 proved to be 10-100x less efficient at alkylating DNA than the corresponding natural enantiomers.

5 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) characteristic of the reverse binding orientations and offset 3.5 base-pair AT-rich binding sites surrounding the alkylation site, the two enantiomers 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 (100x) relative to that of (+)- and *ent*-(-)-CBI-CDPI₁ (26). The potential origin of these effects have been discussed elsewhere.

25 The cytotoxic properties of 62-65 and that of the closely related CBI agents are summarized in Figure 13. Consistent with their relative efficiencies of DNA alkylation, the natural enantiomers of 62 and 64 were essentially indistinguishable (500-1000 pM, L1210) and 100-200x less potent than (+)-CBI-CDPI₁ (26). Thus, the introduction of the single nitrogen atom in the DNA binding subunit of 64 reduced the biological potency 100 to 200-fold. Consistent with prior observations, the natural enantiomers of 62 and 64 were 10-100x more potent than

- 20 -

the corresponding unnatural enantiomers.

CBI-Indole-NMe₃⁺: Electropositive Substituents Capable of Enhancing DNA Alkylation Efficiency Through Stabilizing Electrostatic Interactions. In recent

5 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
10 substituent. 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. Consistent with expectations, the agents 66-68
15 alkylated DNA with the same relative efficiency as 1-2 and were approximately 100x 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.

25 Consequently, we were interested in the relative cytotoxic properties of 66-68 and the results of their evaluations are summarized in Figure 15. Although 66-68 were essentially identical in their cytotoxic potencies (10 nM), they proved to be slightly less potent than (+)-CBI-indole₁ (28) and approximately 1000x less potent than (+)-1 and (+)-2. This is in contrast to expectations based on their
30 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 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 Figure 16. Treatment of bis(2,4-dinitrophenyl)carbonate (69) with *tert*-butylcarbazate (70, 1 equiv, 24 °C, 2 h, EtOAc) provided 71 (61%) and a convenient acylating agent for introduction of the *tert*-butoxycarbonyl 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 Et₃N, 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 NaHCO₃-THF (24 °C) provided 74 or 75, respectively.

The results of the in vitro cytotoxic evaluation of the *N*-seminecarbazide of CBI conducted on its more stable seco precursor 73 are detailed in Figure 18 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.* 100x) or *N*-BOC-CBI (9) itself, and proved to be only 100x 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. 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 (100x), the in vitro cytotoxic activity of the agents increased correspondingly (100x).

The introduction of a terminal semicarbazide onto CBI-CDPI₂ was carried for comparison purposes (Figure 17). Acid-catalyzed deprotection of *N*-BOC-CDPI₂ (76, CF₃CO₂H, 25 °C, 1 h) followed by coupling of crude amine salt with 71 (1.5 equiv, 1 equiv Et₃N, 25 °C, 19 h, 91% overall) provided 77 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 (3M 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-CDPI₂ (25) did not impact on the inherent properties of the agent, Figure 18. 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.

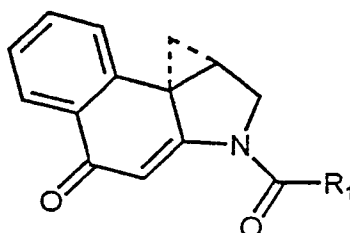
Notably, the terminal acyl hydrazides of 73, 75 and 79 may serve as

useful functionality for subsequent reversible or irreversible conjugation with tumor selective delivery systems and such studies are underway.

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 4x more stable and 4x 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 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. A number of the agents detailed herein exhibit potent and efficacious antitumor activity.

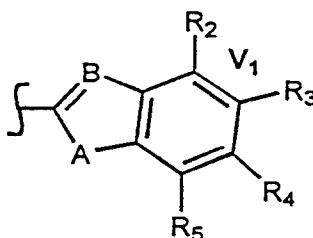
- 24 -

One aspect of the invention is directed to a compound represented by the following structure:



5 wherein R_1 is selected from the group consisting of -
 CH_2CH_3 (alkyl), $-\text{NHCH}_3$ (-N-alkyl), $-\text{OCH}_3$ (O-alkyl), $-\text{NH}_2$,
 $-\text{NHNH}_2$, $-\text{NHNHCO}_2^t\text{Bu}$, and a radical. The radical is
 represented by the following structure:

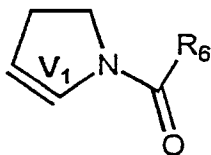
10



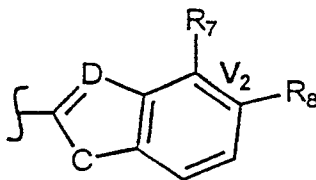
wherein **A** is selected from the group consisting of **NH**
 and **O**; **B** is selected from the group consisting of **C** and
N; R_2 is selected from the group consisting of hydrogen,
 hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, and a first
 15 N-substituted pyrrolidine ring; R_3 is selected from the
 group consisting of hydrogen, hydroxyl, O-alkyl (C1-
 C6), N-alkyl (C1-C6)₃, the first N-substituted
 pyrrolidine ring; R_4 is selected from the
 group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6),
 and N-alkyl (C1-C6)₃; R_5 is selected from the group
 20 consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), and

- 25 -

N-alkyl (C1-C6)₃; and V₁ represents a first vinylene group between R₂ and R₃. The following provisos apply: if R₂ participates in the first N-substituted pyrrolidine ring, then R₃ also particlates in the first N-substituted pyrrolidine ring; if R₃ participates in the first N-substituted pyrrolidine ring, then R₂ also particlates in the first N-substituted pyrrolidine ring; if R₂ and R₃ participate in the first N-substituted pyrrolidine ring, then R₄ and R₅ are hydrogen; and if R₂ is hydrogen, then R₄ and R₅ are hydrogen and R₃ is N-alkyl (C1-C6)₃. The first N-substituted pyrrolidine ring is fused to the first vinylene group between R₂ and R₃ and is represented by the following structure:



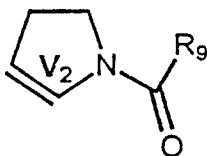
wherein V₁ represents the first vinylene group between R₂ and R₃; R₆ is selected from the group consisting of -CH₂CH₃ (alkyl), -NHCH₃ (-N-alkyl), -OCH₃ (O-alkyl), -NH₂, -NHNH₂, -NHNHCO₂^tBu, and a radical. The radical is represented by the following structure:



wherein C is selected from the group consisting of NH and O; D is selected from the group consisting of C and N; R₇ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, and a

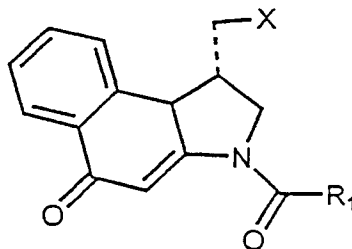
- 26 -

second N-substituted pyrrolidine ring; R_8 is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6), the second N-substituted pyrrolidine ring; and V_2 represents the second vinylene group between R_7 and R_8 . The following provisos apply: if R_7 participates in the N-substituted pyrrolidine ring, then R_8 also participates in the N-substituted pyrrolidine ring; and if R_8 participates in the N-substituted pyrrolidine ring only if R_7 also participates in the N-substituted pyrrolidine ring. The second N-substituted pyrrolidine ring is fused to the second vinylene group between R_7 and R_8 and is represented by the following structure:



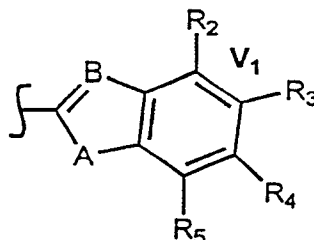
wherein V_2 represents the second vinylene group between R_7 and R_8 ; and R_9 is selected from the group consisting of $-CH_2CH_3$ (alkyl), $-NHCH_3$ (N-alkyl), $-OCH_3$ (O-alkyl), $-NH_2$, $-NHNH_2$, and $-NHNHCO_2^tBu$.

Another aspect of the invention is directed to a compound represented by the following structure:



- 27 -

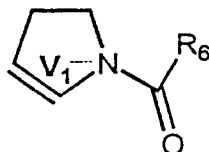
wherein X is selected from the group consisting of chlorine, bromine, iodine, and OTOS; and R₁ is selected from the group consisting of -CH₂CH₃ (alkyl), -NHCH₃ (-N-alkyl), -OCH₃ (O-alkyl), -NH₂, -NHNH₂, -NHNHCO₂^tBu, and a radical. The radical is represented by the following structure:



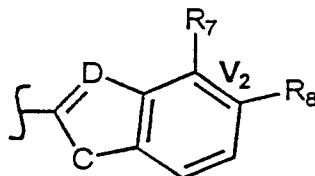
wherein A is selected from the group consisting of NH and O; B is selected from the group consisting of C and N; R₂ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, and a first N-substituted pyrrolidine ring; R₃ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, the first N-substituted pyrrolidine ring; R₄ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), and N-alkyl (C1-C6)₃; R₅ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), and N-alkyl (C1-C6)₃; and V₁ represents a first vinylene group between R₂ and R₃. The following provisos apply: if R₂ participates in the first N-substituted pyrrolidine ring, then R₃ also participates in the first N-substituted pyrrolidine ring; if R₃ participates in the first N-substituted pyrrolidine ring, then R₂ also participates in the first N-substituted pyrrolidine ring; if R₂ and R₃ participate in the first N-substituted pyrrolidine ring, then R₄ and R₅ are

- 28 -

hydrogen; and if R_2 is hydrogen, then R_4 and R_5 are hydrogen and R_3 is N-alkyl (C1-C6)₃. The first N-substituted pyrrolidine ring is fused to the first vinylene group between R_2 and R_3 and is represented by
 5 the following structure:



wherein V_1 represents the first vinylene group between R_2 and R_3 ; R_6 is selected from the group consisting of -
 10 CH_2CH_3 (alkyl), $-NHCH_3$ (-N-alkyl), $-OCH_3$ (O-alkyl), $-NH_2$, $-NHNH_2$, $-NHNHCO_2^tBu$, and a radical. The radical is represented by the following structure:

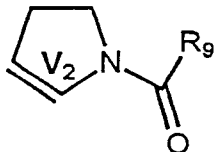


wherein C is selected from the group consisting of NH
 15 and O; D is selected from the group consisting of C and N; R_7 is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, and a second N-substituted pyrrolidine ring; R_8 is selected from the group consisting of hydrogen, hydroxyl, O-
 20 alkyl (C1-C6), N-alkyl (C1-C6)₃, the second N-substituted pyrrolidine ring; and V_2 represents the second vinylene group between R_7 and R_8 . The following provisos apply: if R_7 participates in the N-substituted pyrrolidine ring, then R_8 also particlates in the N-
 25 substituted pyrrolidine ring; and if R_8 participates in the N-substituted pyrrolidine ring only if R_7 also

- 29 -

particulates in the N-substituted pyrrolidine ring. The second N-substituted pyrrolidine ring is fused to the second vinylene group between R_7 and R_8 and is represented by the following structure:

5



wherein V_2 represents the second vinylene group between R_7 and R_8 ; and R_9 is selected from the group consisting of $-\text{CH}_2\text{CH}_3$ (alkyl), $-\text{NHCH}_3$ ($-\text{N}$ -alkyl), $-\text{OCH}_3$ (O -alkyl), $-\text{NH}_2$, $-\text{NHNH}_2$, and $-\text{NHNHCO}_2^t\text{Bu}$.

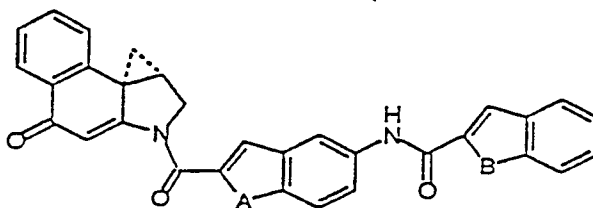
10

Another aspect of the invention is directed to a compound represented by the following structure:

15

20

57-61



wherein A is selected from the group consisting of NH and O and B is selected from the group consisting of NH , O , and S .

25

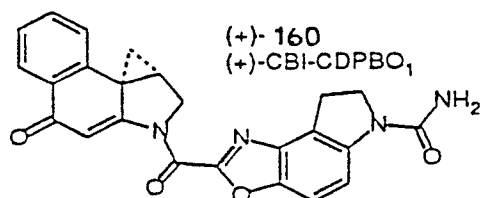
Another aspect of the invention is directed to a compound compound represented by the following structure:

30

- 30 -

62

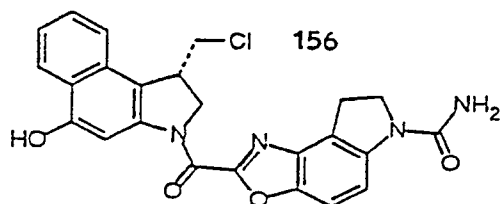
5



Another aspect of the invention is directed to a compound compound represented by the following structures:

15

63

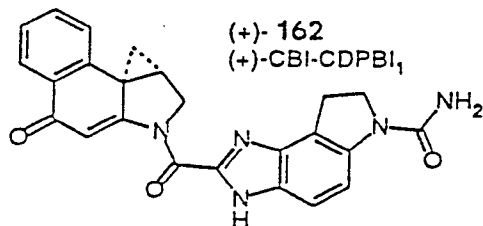


20

Another aspect of the invention is directed to a compound compound represented by the following structure:

25

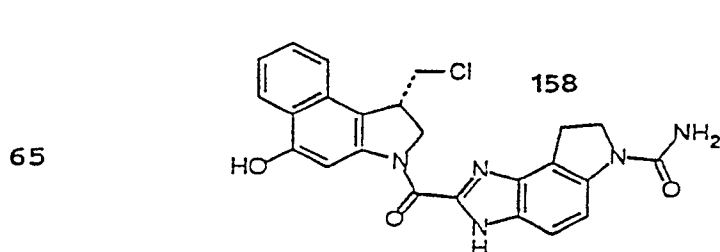
64



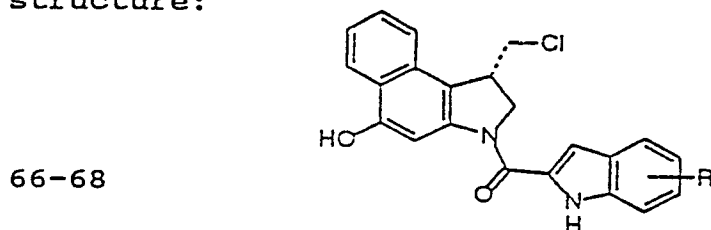
30

- 31 -

Another aspect of the invention is directed to a compound represented by the following structure:

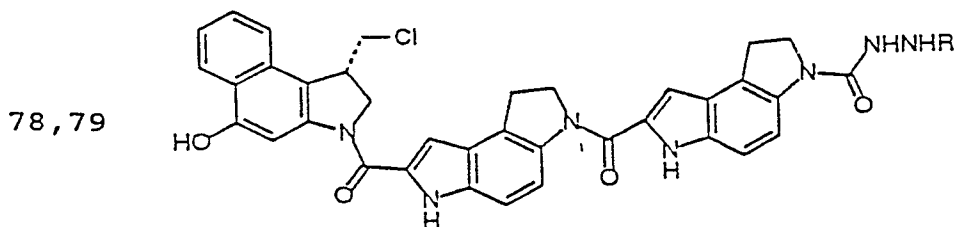


Another aspect of the invention is directed to a compound represented by the following structure:



where R is selected from the group comprising of: H, 5-NMe₃⁺, 6-NMe₃⁺, 7-NMe₃⁺.

Another aspect of the invention is directed to a compound represented by the following structure:



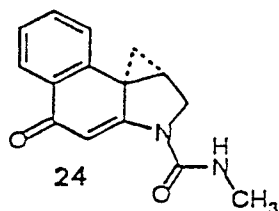
- 32 -

where R is selected from the group comprising of:
 CO_2^tBu , H-HCl .

5 Another aspect of the invention is directed to a
compound compound represented by the following
structure:

10

21



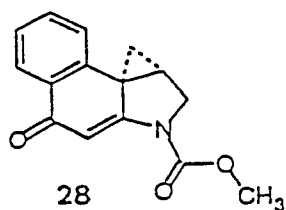
15

Another aspect of the invention is directed to a
compound compound represented by the following
structure:

20

22

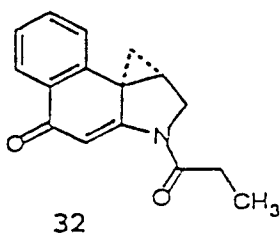
25



30 Another aspect of the invention is directed to, a
compound compound represented by the following
structure:

35

23



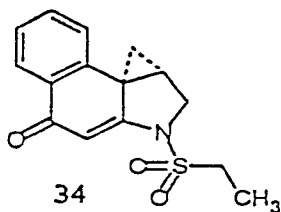
WO 97/12862

PCT/US96/16481

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Another aspect of the invention is directed to a
compound compound represented by the following
5 structure:

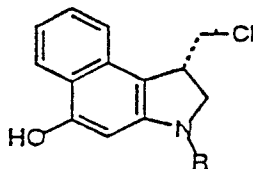
10 24



15 Another aspect of the invention is directed to a
compound compound represented by the following
structure:

20

16, 18-20



25

where R is selected from the group comprising of: H-
HCl, CONHMe, CO₂CH₃, COEt.

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Another aspect of the invention is directed to a compound represented by the following structure:

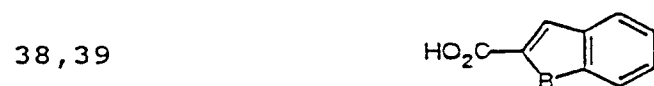


Another aspect of the invention is directed to a compound represented by the following structure:



wherein A is selected from the group consisting of O and R is selected from the group consisting of NO₂ and NH₂.

Another aspect of the invention is directed to a compound represented by the following structure:



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wherein B is selected from the group consisting of O
and S.

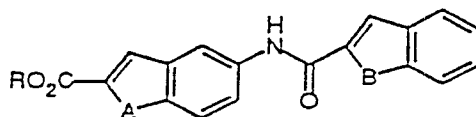
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- 36 -

Another aspect of the invention is directed to a compound represented by the following structure:

5

41-51



10

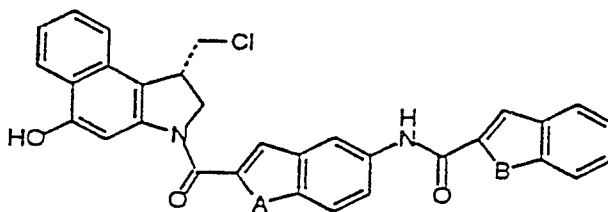
wherein A is selected from the group consisting of NH and O and B is selected from the group consisting of NH, O, and S and R is selected from the group consisting of H and CH₃.

15

Another aspect of the invention is directed to a compound represented by the following structure:

20

52-56



25

wherein A is selected from the group consisting of NH and O and B is selected from the group consisting of NH, O, and S.

30

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Description of Figures:

Figure 1 illustrates the structures of (+)-CC-1065 (+)-
1b, (+)-duocarmycin SA (+)-2, (+)-duocarmycin A and
5 (+)-3.

Figure 2 illustrates the structures of adozelesin
(derivatives 4 and 5), carzelesin (6) and KW-2189 (8).

10 Figure 3 illustrates the structures of CBI analogs
which are based on CPI analogs.

Figure 4 illustrates the synthesis of compound 14 with
the indicated intermediates, substrates, and
15 intermediate steps.

Figures 5A-5B illustrate the synthesis of compounds 16,
20, 22, 24, 26, 28, 30, 32 and 34 with the indicated
intermediates, substrates, and intermediate steps.

Figure 6 illustrates the structures of compounds 9, 10,
11, 12, and 14 with the indicated rate constant k (s^{-1} ,
pH 3) of acid-catalyzed solvolysis, half life $t_{1/2}$ in
solution and cytotoxic activity IC_{50} (L1210 cells).
25

Figure 7 illustrates a summary of the cytotoxic
activity (IC_{50} (L1210, nM)) of the set of agents
examined and the comparison with the related CPI-based
agents. Agents are indicated as natural or unnatural.
30

Figure 8 illustrates the structures of CBI analogs 25-29

- 38 -

and the intermediate CBI analogs 30-34 with the indicated R groups.

5 Figure 9 illustrates a summary of the comparative cytotoxic activity (IC_{50} (L1210, nM)) of prior agents prepared in our studies. Agents are indicated as natural or unnatural.

10 Figure 10 illustrates the synthesis of compounds 82, 84, 86, 88, 90 and 92 with the indicated intermediates, substrates, and intermediate steps.

15 Figure 11 illustrates a summary of the results of the cytotoxic evaluations of the agents examined and the comparison with the related CPI-based agents. Agents are indicated as natural or unnatural.

20 Figure 12 illustrates the structures of CBI analogs (+)-62, 63, (+)-64 and 65, and indicates functional groups on the CDPBO and CDPBI analogs which are H-bond acceptor and donor, respectively.

25 Figure 13 illustrates a summary of the results of the cytotoxic evaluations (IC_{50} (L1210, pM)) of the agents examined and the comparison with the related CPI-based agents. Agents are indicated as natural or unnatural.

30 Figure 14 illustrates the structures of CBI intermediate analogs 33, 66, 67 and 68.

Figure 15 illustrates a summary of the relative alkylation efficiency (Rel DNA Alkylation) and representative cytotoxicity (IC_{50} (L1210, nM)) of agents

- 39 -

66-68 which contain a peripheral quaternary ammonium salt and are close analogs with 28 (values indicated).

Figure 16 illustrates the synthesis of compounds 102 and 104 with the indicated intermediates, substrates, and intermediate steps.

Figure 17 illustrates the synthesis of compound 112 with the indicated intermediates, substrates, and intermediate steps.

Figure 18 illustrates a summary of the relative alkylation efficiency (Rel DNA Alkylation) and representative cytotoxicity (IC_{50} (L1210, nM)) of agents 9, 72, 73, 78, 79, and 25.

Figures 19A-19B illustrate the 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, $1 - [(A - A_i) / (A_f - A_i)]$ versus time from which the first order solvolysis rate constant was derived.

Figures 20A-20D illustrate the results of studies acid-catalyzed solvolysis studies of 21-24 conducted at pH 3 (CH_3OH-H_2O) were 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.

The comparisons of 21-24 reveal a direct, linear relationship between the cytotoxic potency (L1210, $\log 1/IC_{50}$) and the solvolytic stability ($-\log k_{solv}$, pH 3) of

- 40 -

the agents. Similarly, a linear relationship was found between the electron-withdrawing properties of the N² substituents (Hammett ρ constant) and the solvolysis reactivity ($-\log k_{\text{sol}}, \text{pH } 3$) of the agents with the strongest electron-withdrawing substituents providing the most stable agents. This latter relationship reflects the influence of the N² substituent on the ease of C4 carbonyl protonation required for catalysis of solvolysis and cyclopropyl ring cleavage with the stronger electron-withdrawing N² substituents exhibiting slower solvolysis rates. Less obvious but more fundamental, the observations were found to follow a predictable linear relationship between the cytotoxic potency (L1210, $\log 1/\text{IC}_{50}$) and the electron-withdrawing properties of the N² substituent (Hammett ρ) with the strongest electron-withdrawing substituents providing the biologically most potent agents.

Figure 21 illustrates the 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 minutes 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-indole₂ ((+)-4, 1×10^{-4} - 1×10^{-6} M); lanes 9-11, (+)-CBI-indole₂ ((+)-27, 1×10^{-5} - 1×10^{-7} M).

Figure 22 illustrates the thermally-induced strand cleavage of 5' end-labeled duplex DNA (clone w794, 144 bp, nucleotide no 138-5238). Incubation of agent-DNA

- 41 -

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).

Figures 23A-23B represent the following: 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-indole₂ (27) and (+)-CPI-indole₂ (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.

Figures 24A-24E illustrate the data for 4-6 available classes of agents that bear five different DNA binding subunits which we have examined and although this relationship is undoubtedly a second order polynomial indicative of a parabolic relationship that will exhibit an optimal stability-reactivity/potency, the agents employed in the figure lie in a near linear range of such a plot. What is unmistakable in the comparisons, is the fundamental direct correlation

- 42 -

between functional (solvolytic) stability and cytotoxic potency.

5 Figure 25 illustrates the synthesis of compound 128 with the indicated intermediates, substrates, and intermediate steps.

10 Figure 26 illustrates the synthesis of compound 154 with the indicated intermediates, substrates, and intermediate steps.

15 Figure 27 illustrates the synthesis of compounds 160 and 162 with the indicated intermediates, substrates, and intermediate steps.

20 Figures 28A-28B illustrate the structures of CBI analogs (+)-160, intermediate 156, (+)-162 and intermediate 158, and indicates functional groups on the CDPBO and CDPBI analogs which are H-bond acceptor and donor, respectively.

25 Figure 29 illustrates the synthesis of compounds 182, 184 and 186 with the indicated intermediates, substrates, and intermediate steps.

Figure 30 illustrates the synthesis of compounds 190, 192, 194, and 196 with the indicated intermediates, substrates, and intermediate steps.

Synthetic Methods**Preparation of N-(tert-Butyloxycarbonyl)-4-benzyloxy-1-iodo-2-naphthyl-amine (4).**

- 5 **Compound 4 (Illustrated in Figure 4).** A solution of 2 (as prepared in three steps from commercially available 1,3-dihydroxynaphthalene (71% overall), by Boger et. al. *J. Org. Chem.* 1992, 2873) (1.28 g, 3.66 mmol) in 60 mL of a 1:1 mixture of tetrahydrofuran-CH₃OH was cooled to -78 °C and 20 µL of H₂SO₄ (or 20 mg P-toluenesulfonic acid H₂O) in 0.5 mL of tetrahydrofuran was added. *N*-Iodosuccinimide
- 10 (910 mg, 4.03 mmol) in 5 mL of tetrahydrofuran was then introduced by cannula over 5 min. Upon complete reaction (ca. 3 h at -78 °C), 10 mL of saturated aqueous NaHCO₃ and 50 mL of Diethyl ether were added. The reaction mixture was warmed to 25 °C and solid NaCl was added to saturate the aqueous layer. The organic layer was separated and the aqueous layer was extracted with Diethyl ether (2 x 10 mL).
- 15 The organic layers were combined, washed with saturated aqueous NaHCO₃ (1 x 10 mL) and saturated aqueous NaCl (2 x 10 mL), dried (Na₂SO₄), and concentrated. The crude product was purified by elution through a short column of SiO₂ (2 x 4 cm, 20% Ethylacetate-hexane) to provide 4 (1.48 g, 85%) as a white, crystalline solid: mp 111-112 °C; ¹H NMR (CDCl₃, 400 MHz) 8.21 (dd, 1H, *J* = 8.4, 0.8 Hz), 8.03 (s, 1H), 8.01 (d, 1H, *J* = 7.6 Hz), 7.55-7.33 (m, 7H), 7.30 (br s, 1H), 5.27 (s, 2H), 1.56 (s,
- 20 9H); ¹³C NMR (CDCl₃, 100 MHz) 155.7, 152.8, 138.3, 136.6, 134.8, 131.2, 128.7, 128.6, 128.5, 128.1, 127.8, 124.5, 123.7, 122.7, 100.0, 81.2, 70.4, 28.4; IR (film) 3384, 2974, 2923, 1739, 1617, 1598, 1567, 1515, 1494, 1444, 1392, 1366, 1332, 1226, 1152, 1107, 1082 cm⁻¹; FABHRMS (NBA-CsI) *m/z* 607.9715 (C₂₂H₂₂INO₃ + Cs⁺ requires 607.9699). Anal. Calcd for C₂₂H₂₂INO₃: C, 55.59; H, 4.67; N, 2.95.
- 25 Found: C, 55.85; H, 4.43; N, 2.97.

Preparation of 2-[N-(tert-Butyloxycarbonyl)-N-(2-propenyl)]amino-4-benzyloxy-1-iodonaphthalene (6) (Illustrated in Figure 4)

- A solution of 4 (1.38 g, 2.90 mmol) in 25 mL of dimethylformamide at 0 °C was treated with NaH (60% dispersion in oil, 139 mg, 3.5 mmol) in several portions over 15 min. After 45 min, allyl bromide
- 30

- 44 -

(1.05 g, 8.70 mmol) was added and the reaction mixture was warmed to 25 °C and stirred for 3 h. The reaction mixture was quenched by addition of 20 mL saturated aqueous NaHCO₃ and the aqueous layer was extracted with Ethylacetate (4 x 15 mL). The combined organic layers were washed with saturated aqueous NaCl (2 x 10 mL),
 5 dried (Na₂SO₄), and concentrated under reduced pressure. Centrifugal thinlayerchromatography (2 mm Chromatotron plate, 20-50% CH₂Cl₂-hexanes) provided **6** (1.24 g, 83%, typically 80 - 95%) as a colorless oil (mixture of amide rotamers in CDCl₃): ¹H NMR (CDCl₃, 400 MHz) (major rotamer) 8.30 (d, 1H, *J* = 8.2 Hz), 7.29 (d, 1H, *J* = 8.2 Hz), 7.60-7.29 (m, 7H), 6.67 (s, 1H), 5.96-5.86 (m, 1H),
 10 5.27-4.96 (m, 4H), 4.52 (dd, 1H, *J* = 15.0, 5.7 Hz), 3.79 (dd, 1H, *J* = 15.0, 7.2 Hz), 1.29 (s, 9H); ¹³C NMR (CDCl₃, 100 MHz) (major rotamer) 154.9, 153.8, 143.0, 136.4, 135.3, 133.5, 132.7, 128.7, 128.6, 128.4, 128.1, 127.2, 126.1, 122.4, 117.9, 108.0, 95.0, 80.3, 70.2, 52.1, 28.3; IR (film) 3048, 2976, 2923, 1703, 1590, 1403, 1367, 1326, 1251, 1147, 1105 cm⁻¹; FABHRMS (NBA-NaI) *m/z* 538.0855
 15 (C₂₅H₂₆INO₃ + Na⁺ requires 538.0860).

Preparation of 5-(Benzyloxy)-3-(*tert*-butyloxycarbonyl)-1-(2',2',6',6'-tetramethylpiperidiny1-*N*-oxymethyl)-1,2-dihydro-3*H*-benz[e]indole (8**)**

(Illustrated in Figure 4) A solution of **6** (1.85 g, 3.59 mmol) and Tempo (1.68 g, 10.8 mmol) in 120 mL of freshyl distilled benzene (Na/benzophenone) under N₂ was
 20 treated with Bu₃SnH (1.045 g, 3.59 mmol). The solution was warmed at 70 °C and three additional equivalents of Tempo (3 x 0.56 g) and Bu₃SnH (4 x 1.045 g) were added sequentially in four portions over the next 45 min. After 1 h, the solution was cooled to 25 °C and the volatiles were removed under reduced pressure. Centrifugal
 25 thinlayerchromatography (4 mm Chromatotron plate, 0-10% Ethylacetate-hexanes gradient elution) followed by recrystallization from hexanes provided **8** (1.71 g, 87%, typically 70 - 90%) as white needles: mp 170-172 °C; ¹H NMR (C₆D₆, 400 MHz) 8.56 (d, 1H, *J* = 8.3 Hz), 8.38 (br s, 1H), 7.77 (d, 1H, *J* = 8.4 Hz), 7.35 (ddd, 1H, *J* = 8.3, 7.6, 1.2 Hz), 7.28 (d, 2H, *J* = 7.0 Hz), 7.20 (t, 1H, *J* = 7.6 Hz), 7.15 (t, 2H), 7.07
 30 (t, 1H, *J* = 7.2 Hz), 4.36 (m, 1H), 4.12 (dd, 1H, *J* = 9.0, 4.5 Hz), 3.84 (m, 1H), 3.61 (m, 1H), 1.53 (s, 9H), 1.37-1.17 (m, 6H), 1.22 (s, 3H), 1.13 (s, 3H), 1.07 (s, 3H), 1.00

(s, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) 154.1, 151.4, 140.0, 135.8, 129.4, 127.3, 126.7, 126.3, 125.8, 121.9, 121.6, 121.4, 121.1, 114.8, 95.3, 79.2, 69.0, 58.5, 51.5, 38.4, 38.3, 37.2, 31.9, 27.3, 18.9, 15.8; IR (film) 2973, 2930, 1704, 1626, 1582, 1460, 1406, 1381, 1367, 1328, 1266, 1144, 1037, 787, 759 cm^{-1} ; FABHRMS (NBA- CsI)
5 m/z 677.2340 ($\text{C}_{34}\text{H}_{44}\text{N}_2\text{O}_4 + \text{Cs}^+$ requires 677.2355). Anal Calcd for $\text{C}_{34}\text{H}_{44}\text{N}_2\text{O}_4$: C, 74.97; H, 8.14; N, 5.14. Found: C, 74.68; H, 8.37; N, 5.19.

- Preparation of 5-(Benzyloxy)-3-(*tert*-butyloxycarbonyl)-1-(hydroxy-methyl)-1,2-dihydro-3*H*-benz[e]indole (10)** (Illustrated in Figure 4) A solution of 8 (1.61 g,
10 2.95 mmol) in 70 mL of a 3:1:1 mixture of HOAc-tetrahydrofuran- H_2O was treated with zinc powder (2.31 g, 35.4 g atoms) and the resulting suspension was warmed at 70 °C with vigorous stirring. After 2 h, the reaction mixture was cooled to 25 °C and the zinc was removed by filtration. The volatiles were removed under reduced pressure and the resulting residue was dissolved in 40 mL of Ethylacetate and filtered.
15 The solution was concentrated and subjected to centrifugal thinlayerchromatography (4 mm Chromatotron plate, 15-35% Ethylacetate-hexanes gradient elution) to provide 10 (0.96 g, 1.19 g theoretical, 80%) identical in all respects with authentic material (see Boger et. al *J. Am. Chem. Soc.* 1992, 114, 5487.
- 20 **Preparation of compound 12** (Illustrated in Figure 4) A solution of 10 (1.0 equiv.) and triphenylphosphine (2.0 equiv.) in methylene chloride (.2 Molar) at 24 °C under argon was treated with carbon tetrachloride (6.0 equiv.) and the reaction mixture was stirred for 10 h (24 °C). Flash chromatography and resolution as derived from the cooresponding mandelate ester (see Boger et. al *J. Org. Chem.*, 1990, 55, 5830, expt.
25 31), affords the enantiomerically pure compound 12.

Preparation of compound 14 (Illustrated in Figure 4) A solution of 12 (1.0 equiv.) in .05 Molar tetrahydrofuran at 0 °C under argon was treated sequentially with a 25% aqueous ammonium formate (.5 M) and 10% palladium / carbon (.10 equiv.) and the
30 reaction mixture stirred vigorously for 2.5 h (0°C). Ether was mixed with the reaction mixture, and the mixture was dried (Magnesium sulphate). The solid was removed by

- 46 -

filtration through Celite (ether wash). Concentration of the filtrate in vacuo afforded **14** as colorless needles.

Preparation of compound 16 (Illustrated in Figures 5A-5B) Compound **14** (1.0 equiv.) was treated with anhydrous 3N hydrochloric acid in ethyl acetate (.033 M) at 24 °C for 20 min. The solvent was removed in vacuo to afford crude, unstable **16** (95%-100%). The crude product was directly carried on without purification (for a related procedure see Boger et. al. *J. Org. Chem.* 1990, 23, 5831 experimental 5).

Preparation of compound 18 (Illustrated in Figures 5A-5B) A solution of **14** (1.0 equiv.) in .01 M of tetrahydrofuran-dimethylformamide (1:1) was cooled to 0 °C and treated with NaH (1.5 equiv). The reaction mixture was slowly warmed to 24 °C and stirred for 3.5 h. The mixture was placed on a flash chromatography column (SiO₂, 0.5 x 3 mm), and eluted with 5-10% CH₃OH-CHCl₃ (gradient elution) to afford **18** as a bright yellow solid.

Preparation of compound 20 (Illustrated in Figures 5A-5B) A solution of **16** (1.0 equiv.) in tetrahydrofuran (.02 M) was treated with .02 M of 5% aqueous NaHCO₃ and the two-phase mixture was stirred at 24 °C for 5 h under N₂. The reaction mixture was extracted with Ethylacetate (3 x). The organic layer was dried (Na₂SO₄) and concentrated. Flash chromatography afforded **20** as a pale yellow solid. As an alternative, one can obtain compound **20** from compound **18** in trifluoroacetic acid (.1 equiv.) and methylene chloride (.02 M) at 0 °C for 2 hours (for a related procedure see Boger et. al. *J. Org. Chem.* 1990, 23, 5831 experimental 5).

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Preparation of 1-(Chloromethyl)-5-hydroxy-3-[(methylaminocarbonyl)]-1,2-dihydro-3H-benz[e]indole (22**) (Illustrated in Figures 5A-5B)** Phenol **14** (6.9 mg, 21 μmol) was treated with anhydrous 3M HCl-Ethylacetate 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 NaHCO₃ (5.2 mg, 62 μmol, 3 equiv) in tetrahydrofuran (0.3 mL) was cooled to 0 °C and treated with CH₃NCO (2.4 μL, 41 μmol, 2 equiv). The

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reaction mixture was kept at 0 °C for 1 h under Ar before the solvent was removed under a stream of N₂. Flash chromatography (SiO₂, 0.5 x 3 cm, 50-80% Ethylacetate-hexane gradient elution) afforded **22** (5.0 mg, 6.0 mg theoretical, 83%) as a pale greenish solid: ¹H NMR (CD₃OD, 400 MHz) 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-H₂, C1-H), 3.92 (d, 1H, *J* = 11.4 Hz, CHHCl), 3.51-3.53 (m, 1H, CHHCl), 2.83 (s, 3H, CH₃); IR (film) _{max} 3816, 1624, 1585, 1522, 1384, 1339, 1250, 1121 cm⁻¹; FABHRMS (NBA) *m/e* 290.0818 (M⁺ + H, C₁₅H₁₅ClN₂O₂ requires 290.0822). Natural (1*S*)-**22**: [α]⁵ -4.5 (*c* 0.36, CH₃OH). Ent-(1*R*)-**22**: [α]⁵ +5.7 (*c* 0.11, CH₃OH).

Preparation of N²-[(Methylamino)carbonyl]-1,2,9,9a-tetrahydro-cyclo-propa-[c]benz[e]-indol-4-one (24) (Illustrated in Figures 5A-5B) A solution of **22** (3.0 mg, 10.3 μmol) in dimethylformamide (0.9 mL) was cooled to 0 °C and treated with 1,8-DIZABICYCLO[5.4.0]UNDEC-7-ENE (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 (SiO₂, 0.5 x 3 cm, 0-10% CH₃OH-Ethylacetate gradient elution) afforded **24** (2.3 mg, 2.6 mg theoretical, 90%) as a pale yellow solid: ¹H NMR (CD₃OD, 400 MHz) 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, CH₃), 1.73 (dd, 1H, *J* = 7.8, 4.2 Hz, C9-H), 1.48 (t, 1H, *J* = 4.6 Hz, C9-H); IR (neat) _{max} 3358, 2920, 1680, 1622, 1594, 1539, 1466, 1458, 1410, 1281 cm⁻¹; UV (CH₃OH) _{max} 311 (15000), 257 (7300), 217 (17000), 200 (17000) nm; UV (tetrahydrofuran) _{max} 304 (11000), 248 (7200), 216 (17000), 208 (15000) nm; FABHRMS (NBA) *m/e* 255.1140 (M⁺ + H, C₁₅H₁₄N₂O₄ requires 255.1134). Natural (+)-**24**: [α]³ +183 (*c* 0.08, CH₃OH). Ent-(-)-**24**: [α]⁵ -184 (*c* 0.13, CH₃OH).

Preparation of 1-(Chloromethyl)-5-hydroxy-3-(methoxycarbonyl)-1,2-dihydro-3*H*-benz[e]indole (26) (Illustrated in Figures 5A-5B). A solution of freshly prepared, crude **16** (52 μmol) and NaHCO₃ (13.2 mg, 157 μmol, 3 equiv) in tetrahydrofuran (0.5

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mL) was cooled to 0 °C and treated with ClCO₂CH₃ (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 (SiO₂, 1 x 10 cm, 20-40% Ethylacetate-hexane gradient elution) afforded **26** (15 mg, 15 mg theoretical, 100%) as a white solid: ¹H NMR (CDCl₃, 400 MHz) 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, CHHCl, CO₂CH₃), 3.40 (t, 1H, *J* = 10.5 Hz, CHHCl); IR (film) _{max} 3275, 2918, 1678, 1442, 1388, 1335 cm⁻¹; FABHRMS (NBA) *m/e* 291.0664 (M⁺, C₁₅H₁₄ClNO₄ requires 291.0662). Natural-(1*S*)-**26**: [α]³ -30.3 (c 0.11, CH₃OH). *Ent*-(1*R*)-**26**: [α]³ +31.8 (c 0.24, CH₃OH).

Preparation of *N*²-(Methoxycarbonyl)-1,2,9,9a-tetrahydro-cyclopropa-[c]benz[e]-indol-4-one (28) (Illustrated in Figures 5A-5B). A solution of **26** (10.0 mg, 34 μmol) in tetrahydrofuran (3 mL) was cooled to 0 °C and treated with 1,8-DIZABICYCLO[5.4.0]UNDEC-7-ENE (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 NH₄Cl (3 mL) and extracted with CH₂Cl₂ (3 x 2 mL). The combined organic layer was dried (Na₂SO₄) and concentrated. Flash chromatography (SiO₂, 1 x 10 cm, 10-50% Ethylacetate-hexane gradient elution) afforded **28** (8.1 mg, 8.7 mg theoretical, 87%) as a yellow solid: ¹H NMR (CDCl₃, 400 MHz) 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, CH₂N), 3.86 (s, 3H, CH₃), 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) _{max} 3283, 2984, 1728, 1626, 1559, 1436, 1405, 1380, 1328, 1277, 1246, 1195, 1118, 1077, 1021, 764 cm⁻¹; UV (CH₃OH) _{max} 307 (32000), 255 (24000), 216 (32000), 200 (33000) nm; UV (tetrahydrofuran) _{max} 296 (33000), 253 (23000), 217 (38000), 203 (44000) nm; FABHRMS (NBA) *m/e* 256.0986 (M⁺ + H, C₁₅H₁₃NO₃ requires 256.0974). Natural (+)-**28**: [α]³ +198 (c 0.48, CH₃OH). *Ent*-(-)-**28**: [α]⁵ -196 (c 0.14, CH₃OH).

Preparation of 1-(Chloromethyl)-5-hydroxy-3-propionyl-1,2-dihydro-3H-

benz[e]indole (30) (Illustrated in Figures 5A-5B). A solution of freshly prepared, crude **16** (45 μ mol) and NaHCO_3 (11.3 mg, 135 μ mol, 3 equiv) in tetrahydrofuran (0.4 mL) was cooled to 0 °C and treated with ClCOEt (8 μ L, 90 μ mol, 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 x 10 cm, 10-40% Ethylacetate-hexane gradient elution) afforded **30** (12.8 mg, 13 mg theoretical, 98%) as a white solid: ^1H NMR (CDCl_3 , 400 MHz) 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, CHHCl), 3.41 (t, 1H, $J = 10.8$ Hz, CHHCl), 2.59-2.72 (m, 2H, CH_2CH_3), 1.39 (t, 3H, $J = 7.4$ Hz, CH_2CH_3); IR (film) max 3170, 2918, 1628, 1582, 1427, 1389 cm^{-1} ; FABHRMS (NBA) m/e 290.0953 ($\text{M}^+ + \text{H}$, $\text{C}_{16}\text{H}_{16}\text{ClNO}_2$ requires 290.0953). Natural (1*S*)-**30**: $[\alpha]_D^{25} -54$ (c 0.08, tetrahydrofuran). *Ent*-(1*R*)-**30**: $[\alpha]_D^{25} +59$ (c 0.13, tetrahydrofuran).

Preparation of *N*²-(Propionyl)-1,2,9,9a-tetrahydro-cyclopropa[c]benz[e]-indol-4-one (32) (Illustrated in Figures 5A-5B).

A solution of **30** (5.0 mg, 17 μ mol) in tetrahydrofuran (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 Ethylacetate (3 x 3 mL). The organic layer was dried (Na_2SO_4) and concentrated. Flash chromatography (Florisil, 1 x 5 cm, 60% Ethylacetate-hexane) afforded **32** (4.2 mg, 4.3 mg theoretical, 97%) as a pale yellow solid: ^1H NMR (CDCl_3 , 400 MHz) 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, CH_2CH_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) max 2924, 1698, 1626, 1599, 1562, 1461, 1406, 1241 cm^{-1} ; UV (CH_3OH) max 311 (16000), 258 (9100), 218 (14000), 201 (19000) nm; UV (tetrahydrofuran) max 301 (15000), 253

(9400), 219 (15000), 204 (15000) nm; FABHRMS (NBA) m/e 254.1173 ($M^+ + H$, $C_{16}H_{15}NO_2$ requires 254.1181). Natural (+)-**32**: +193 (c 0.03, CH_3OH). *Ent*-(-)-**32**: -197 (c 0.12, CH_3OH).

- 5 **Preparation of N^2 -(Ethylsulfonyl)-1,2,9,9a-tetrahydro-cyclopropa[c]-benz[e]indol-4-one (34) (Illustrated in Figures 5A-5B).** NaH (1.5 mg, 60% oil dispersion, 38 μ mol, 2.5 equiv) in a flame-dried flask was treated with (+)-CBI (**20**, 3.0 mg, 15.2 μ mol) in tetrahydrofuran (0.8 mL) and the mixture was stirred for 10 min at 24 °C under N_2 . A premixed solution of Triethylamine (7 μ L, 50 μ mol, 3.3 equiv) and $ClSO_2Et$ (10 μ L, 106 μ mol, 7 equiv) in tetrahydrofuran (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 x 3 cm, 40-60% Ethylacetate-hexane gradient elution) afforded **34** (2.0 mg, 4.4 mg theoretical, 45%) as a pale yellow solid: 1H NMR ($CDCl_3$, 400 MHz) 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, CH_2CH_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) \max 2923, 1618, 1559, 1354, 1149 cm^{-1} ; UV (CH_3OH) \max 301 (12000), 248 (11000), 214 (15000) nm; UV (tetrahydrofuran) \max 293 (13000), 248 (14000), 216 (16000), 208 (14000) nm; FABHRMS (NBA-NaI) m/e 290.0850 ($M^+ + H$, $C_{15}H_{15}NO_3S$ requires 290.0851). Natural (+)-**34**: +73 (c 0.10, $CHCl_3$). *Ent*-(-)-**34**: -70 (c 0.12, $CHCl_3$).

Preparation of Solvolytic Reactivity of 24,28,32,34 (Illustrated in Figures 5A-5B).

- 25 The compounds **24,28,32,34** 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 **24,28,32** and 3 months for **34**. The UV monitoring was continued until no further change was detectable. The long-wavelength absorption at 316 nm

(24,28,32) or 306 nm (34) and short-wavelength absorption at 256 nm (24,28,34) or 248 nm (32) were monitored. The solvolysis rate constant and half-life were calculated from the data recorded at the short wavelength (256 nm for 24,28,32 and 248 nm for 34) from the least square treatment ($r = 0.995$, 24; $r = 0.997$, 28; $r = 0.985$, 32; $r =$
 5 0.994, 34) of the slopes of plots of time versus $1 - [(A - A_{\text{initial}})/A_{\text{final}} - A_{\text{initial}}]$.

Preparation of Methyl 5-Nitrobenzofuran-2-carboxylate (Illustrated in Figure 10) 5-Nitrobenzofuran-2-carboxylic acid from Transworld chemicals inc. (500 mg, 2.4 mmol) in 20 mL of CH_3OH was treated with 5 drops of H_2SO_4 . The reaction mixture
 10 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 aqueous NaHCO_3 (20 mL), and extracted with Ethylacetate (3 x 30 mL). The combined organic phase was dried (Na_2SO_4) and concentrated in vacuo. Flash chromatography (SiO_2 , 2 x 20 cm, 40-60% Ethylacetate-hexane) afforded the methyl ester (469 mg, 534 mg theoretical, 88%) as
 15 a white solid: mp > 230 °C (dec); ^1H NMR (CDCl_3 , 400 MHz) 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) 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) max 3383, 3108, 1731, 1620, 1571, 1521, 1441, 1349,
 20 1270, 1177, 827, 750 cm^{-1} ; FABHRMS (NBA) m/e 222.0405 ($\text{M}^+ + \text{H}$, $\text{C}_{10}\text{H}_7\text{NO}_5$ requires 222.0402).

Preparation of compound Methyl 5-Nitrobenzoimidazol-2-carboxylate (36) (Illustrated in Figure 10). Condensation of 3-nitrobenzaldehyde with methyl 2-
 25 azidoacetate (8 equiv, 6 equiv NaOCH_3 , CH_3OH , -23 to 0 °C, 6 h, 88%) followed by thermolysis of the resulting methyl 2-azidocinnamate (xylene, reflux, 4.5 h, 81%) provided a readily separable mixture (4:1) of methyl 5- and 7-nitroindole-2-carboxylate. For methyl 5-nitroindole-2-carboxylate: ^1H NMR
 (DIMETHYLSULFOXIDE- d_6 , 400 MHz) 12.65 (br s, 1H, NH), 8.73 (d, 1H, $J = 2.3$
 30 Hz, C4-H), 8.14 (dd, 1H, $J = 2.0, 8.0$ Hz, C6-H), 7.60 (d, 1H, $J = 8.0$ Hz, C7-H), 7.45 (d, 1H, $J = 0.7$ Hz, C3-H), 3.90 (s, 3H, CO_2CH_3); IR (film) max 3316, 1701, 1614,

1531, 1435, 1343, 1261, 1203, 992, 746 cm^{-1} . Catalytic hydrogenation of the 5- NO_2 group (1 atm H_2 , 0.1 wt equiv 10% Pd-C, Ethylacetate, 25 °C, 4-5 h) provided the corresponding amine. (36): 92%, mp 150-152 °C (CH_2Cl_2); ^1H NMR (CDCl_3 , 400 MHz) 8.72 (br s, 1H, NH), 7.23 (d, 1H, $J = 8.6$ Hz, C7-H), 7.03 (dd, 1H, $J = 1.0, 2.1$ Hz, C3-H), 6.93 (dd, 1H, $J = 1.0, 2.0$ Hz, C4-H), 6.81 (dd, 1H, $J = 2.0, 8.6$ Hz, C6-H), 3.93 (s, 3H, CO_2CH_3), 3.57 (br s, 2H, NH_2); ^{13}C NMR (CDCl_3 , 100 MHz) 160.0 (C), 150.3 (C), 145.6 (C), 143.0 (C), 127.7 (C), 117.7 (CH), 113.5 (CH), 112.6 (CH), 106.1 (CH), 52.2 (CH_3); IR (film) max 3320, 1691, 1628, 1531, 1437, 1376, 1337, 1232, 1034, 997, 766 cm^{-1} ; FABHRMS (NBA) m/e 190.0746 ($\text{M}^+ + \text{H}$, $\text{C}_{10}\text{H}_{10}\text{N}_2\text{O}_2$ requires 190.0742).

Preparation of Methyl 5-Aminobenzofuran-2-carboxylate (38) (Illustrated in Figure 10). A solution of methyl 5-nitrobenzofuran-2-carboxylate (469 mg, 2.12 mmol) in 50 mL of Ethylacetate 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 x 20 cm, 40-60% Ethylacetate-hexane) afforded 38 (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) 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) max 3359, 1725, 1562, 1488, 1434, 1331, 1301, 1222, 1158 cm^{-1} ; FABHRMS (NBA) m/e 192.0663 ($\text{M}^+ + \text{H}$, $\text{C}_{10}\text{H}_9\text{NO}_3$ requires 192.0661).

25 General Procedure for the Preparation of 46,48,50,52,54,56 (Illustrated in Figure 10).

Methyl 5-aminoindole-2-carboxylate (36), or methyl 5-aminobenzofuran-2-carboxylate (38), 1-(3-DIMETHYLAMINOPROPYL)-3-ETHYLCARBODIIMIDE HYDROCHLORIDE (EDCI) (3 equiv) and indole-2-carboxylic acid (40) Aldrich company, benzofuran-2-carboxylic acid (42) Aldrich company or benzo[*b*]thiophene-2-carboxylic acid (44) Aldrich company (1 equiv) were stirred in dimethylformamide

(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 H₂O and stirred for 30 min. The precipitate was collected by centrifugation and washed with 1N aqueous HCl, saturated aqueous NaHCO₃, and H₂O. Drying the solid in vacuo afforded desired aqueous methyl esters

5 46,48,50,52,54,56 in typical yields of 50-73%.

Methyl 5-(((1*H*-Indol-2'-yl)carbonyl)amino)-1*H*-indole-2-carboxylate (46) 5 h, 61%; mp > 270 °C (dec); ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 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, CH₃); IR (neat) _{max} 3277, 1700, 1652, 1553, 1535, 1310, 1247, 1225, 1022, 999, 742 cm⁻¹; FABHRMS (NBA-NaI) *m/e* 356.1004 (M⁺ + Na, C₁₉H₁₅N₃O₃ requires 356.1011).

15 Methyl 5-(((Benzofuro-2'-yl)carbonyl)amino)-1*H*-indole-2-carboxylate (48): 5 h, 73%; mp > 230 °C; ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 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, CH₃); IR (film) _{max} 3333, 1695, 1658, 1591, 1535, 1442, 1303, 1255, 746 cm⁻¹; FABHRMS (NBA) *m/e* 335.1036 ((M⁺ + H, C₁₉H₁₄N₂O₄ requires 335.1032).

Methyl 5-(((Benzo[*b*]thieno-2'-yl)carbonyl)amino)-1*H*-indole-2-carboxylate (50): 7 h, 62%; mp > 230 °C; ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 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, CH₃); ¹³C NMR (DIMETHYLSULFOXIDE-*d*₆, 100 MHz) 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) _{max} 3336, 1694, 1633, 1532, 1455, 1336, 1309, 1257, 1232 cm⁻¹;

FABHRMS (NBA) m/e 351.0810 ($M^+ + H$, $C_{19}H_{14}N_2O_3S$ requires 351.0803).

Methyl 5-(((1*H*-Indol-2'-yl)carbonyl)amino)benzofuran-2-carboxylate (52): 8 h, 52%; mp > 230 °C; 1H NMR (DIMETHYLSULFOXIDE- d_6 , 400 MHz) 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) \max 3346, 1712, 1643, 1577, 1543, 1308, 1289, 1234, 745 cm^{-1} ; FABHRMS (NBA) m/e 335.1040 ($M^+ + H$, $C_{19}H_{14}N_2O_4$ requires 335.1032).

10 Methyl 5-(((Benzofuro-2'-yl)carbonyl)amino)benzofuran-2-carboxylate (54): 12 h, 62%; mp > 230 °C; 1H NMR ($CDCl_3$, 400 MHz) 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) 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) \max 3382, 1729, 1663, 1562, 1541, 1475, 1431, 1291, 1204, 1151, 1103 cm^{-1} ; FABHRMS (NBA) m/e 336.0878 ($M^+ + H$, $C_{19}H_{13}NO_5$ requires 336.0872).

20 Methyl 5-(((Benzo[*b*]thieno-2'-yl)carbonyl)amino)benzofuran-2-carboxylate (56): 8 h, 50%; mp > 230 °C; 1H NMR (DIMETHYLSULFOXIDE- d_6 , 400 MHz) 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) \max 3287, 1728, 1657, 1546, 1473, 1436, 1296, 1216, 1154 cm^{-1} ; FABHRMS (NBA) m/e 352.0650 ($M^+ + H$, $C_{19}H_{13}NO_4S$, requires 352.0644).

30 General Procedure for the Preparation of 58,60,62,64,66,68 (Illustrated in Figure 10).

A solution of one the methyl esters 46,48,50,52,54 or 56 prepared as above in

tetrahydrofuran-CH₃OH-H₂O (3:1:1) was treated with 4 equiv of LiOH H₂O. The reaction mixture was stirred at 24 °C for 4-6 h. The solvent was removed and the dry residue was mixed with H₂O, acidified with 1N aqueous HCl to pH 1. The precipitate was collected by centrifugation and washed with H₂O (2x). Drying the solid in vacuo
 5 afforded the desired acid 58,60,62,64,66,68 with yields 80-100%.

5-[[[(1*H*-Indol-2'-yl)carbonyl]amino]-1*H*-indole-2-carboxylic Acid (58): 3 h, 89%; mp > 270 °C (dec); ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 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) _{max} 3413, 3354, 3315, 1665, 1596, 1532, 1463, 1444, 1409, 1306, 1222, 1159, 1080 cm⁻¹; FABHRMS (NBA) *m/e* 320.1041 (M⁺ + H, C₁₈H₁₃N₃O₃ requires 320.1035).

5-[[[(Benzofuro-2'-yl)carbonyl]amino]-1*H*-indole-2-carboxylic Acid (60): 5
 15 h, 77%; mp > 230 °C; ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 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) _{max} 3297, 1661, 1594, 1537, 1299, 1258,
 20 1229, 743 cm⁻¹; FABHRMS (NBA) *m/e* 321.0880 (M⁺ + H, C₁₈H₁₂N₂O₄ requires 321.0875).

5-[[[(Benzo[*b*]thieno-2'-yl)carbonyl]amino]-1*H*-indole-2-carboxylic Acid (62): 3 h; 80%; mp > 230 °C; ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 11.63 (s, 1H, NH), 10.46 (s, 1H, NH), 8.36 (s, 1H, C3'-H), 8.08 (s, 1H, C4-H), 8.04
 25 (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); ¹³C NMR (DIMETHYLSULFOXIDE-*d*₆, 100 MHz) 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) _{max} 3429, 3375, 1648, 1542, 1431, 1305, 1249,
 30 739 cm⁻¹; FABHRMS (NBA) *m/e* 337.0654 (M⁺ + H, C₁₈H₁₂N₂O₃S requires 337.0647).

5-(((1*H*-Indol-2'-yl)carbonyl)amino)benzofuran-2-carboxylic Acid (64): 4 h, 80%; mp > 230 °C; ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 13.54 (br s, 1H, CO₂H), 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) _{max} 3285, 1703, 1649, 1547, 1475, 1420, 1312, 1231, 1195, 1159, 744 cm⁻¹; FABHRMS (NBA) *m/e* 321.0870 (M⁺ + H, C₁₈H₁₂N₂O₄ requires 321.0875).

5-(((Benzofuro-2'-yl)carbonyl)amino)benzofuran-2-carboxylic Acid (66): 10 12 h, 100%; mp > 230 °C; ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 13.50 (br s, 1H, CO₂H), 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) _{max} 3362, 1709, 1659, 1564, 1473, 1438, 1292, 1226, 1152, 790 cm⁻¹; FABHRMS (NBA) *m/e* 322.0720 (M⁺ + H, C₁₈H₁₁NO₅ requires 322.0715).

5-(((Benzo[*b*]thieno-2'-yl)carbonyl)amino)benzofuran-2-carboxylic Acid (68): 12 h, 82%; mp > 230 °C; ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 13.61 (br s, 1H, CO₂H), 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) _{max} 3395, 1697, 1653, 1551, 1479, 1296, 1273, 1231, 1155, 1024, 991, 762 cm⁻¹; FABHRMS (NBA) *m/e* 338.0480 (M⁺ + H, C₁₈H₁₁NO₄S requires 338.0487).

25

Preparation of compounds 70,72,74,76,78,80 (Illustrated in Figure 10) - General Procedure for the Coupling of 16 with 58,60,62,64,66,68. Phenol 14 was treated with anhydrous 3M HCl-Ethylacetate at 24 °C for 30 min. The solvent was removed in vacuo to afford crude unstable 16 (quantitative). A solution of 16, one of the 30 carboxylic acids 58,60,62,64,66,68 (1 equiv), and 1-(3-DIMETHYLAMINOPROPYL)-3-ETHYLCARBODIIMIDE HYDROCHLORIDE

(EDCI) (2-3 equiv) in dimethylformamide (0.04-0.06 M) was stirred at 24 °C under N₂ for 8-12 h. The reaction mixture was concentrated under vacuum and suspended in H₂O and the precipitate was collected by centrifugation, and washed with H₂O (2x). Flash chromatography (SiO₂, 40-60% tetrahydrofuran-hexane) afforded 70 and

5 72,74,76,78,80 in yields of 60-90%.

3-[(5'-(((1*H*-Indol-2''-yl)carbonyl)amino)-1*H*-indol-2'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (70): 8.5 h, 73%; mp > 255 °C (dec); ¹H NMR (dimethylformamide-*d*₇, 400 MHz) 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), 10 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 15 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) _{max} 3258, 2923, 1659, 1624, 1578, 1512, 1411, 1395, 1233, 745 cm⁻¹; FABHRMS (NBA) *m/e* 535.1526 (M⁺ + H, C₃₁H₂₃ClN₄O₃ requires 535.1537). Natural (1*S*)-70: [α]^D₂₀ +70 (c 0.17, dimethylformamide). *Ent*-(1*R*)-70: [α]^D₂₀ -70 (c 0.17, dimethylformamide).

20 3-[(5'-(((Benzofuro-2''-yl)carbonyl)amino)-1*H*-indol-2'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (72): 14 h, 60%; mp > 230 °C; ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 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, 25 *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), 30 4.13 (dd, 1H, *J* = 3.2, 11.2 Hz, CHHCl), 3.96 (dd, 1H, *J* = 7.6, 11.2 Hz, CHHCl); ¹³C NMR (tetrahydrofuran-*d*₈, 100 MHz) 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 (CH₂), 43.9 (CH₂); IR (film)

5 \max 3272, 2954, 1610, 1585, 1513, 1408, 1253, 1135, 741 cm⁻¹; FABHRMS (NBA) *m/e* 536.1390 (M⁺ + H, C₃₁H₂₂ClN₃O₄ requires 536.1377). Natural (1*S*)-72: [α]³ +56 (*c* 0.23, tetrahydrofuran).

3-[(5'-(((Benzo[*b*]thieno-2''-yl)carbonyl)amino)-1*H*-indol-2'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (74): 11 h, 68%; mp > 10 230 °C; ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 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), 15 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) \max 3286, 1655, 1628, 1587, 1518, 1409, 1262, 1239 cm⁻¹; FABHRMS (NBA) *m/e* 552.1152 (M⁺ + H, C₃₁H₂₂ClN₃O₃S requires 552.1149). Natural (1*S*)-74: [α]³ +111 20 (*c* 0.15, dimethylformamide).

3-[(5'-(((1*H*-Indol-2''-yl)carbonyl)amino)benzofuro-2'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (76): 13 h, 80%; mp > 230 °C; ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 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), 30 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) \max 3274, 2928, 1655,

1621, 1579, 1546, 1414, 1390, 1329, 1240 cm^{-1} ; FABHRMS (NBA) m/e 536.1360 ($M^+ + H$, $C_{31}H_{22}ClN_3O_4$ requires 536.1377). Natural (1*S*)-76: $[\alpha]^{25} +26$ (c 0.36, dimethylformamide).

3-[(5'-(((Benzofuro-2''-yl)carbonyl)amino)benzofuro-2'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (78): 11 h, 88%; mp > 230 °C; ^1H NMR (DIMETHYLSULFOXIDE- d_6 , 400 MHz) 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) max 3267, 2923, 1664, 1581, 1554, 1410, 1390, 1328, 1256 cm^{-1} ; FABHRMS (NBA) m/e 537.1210 ($M^+ + H$, $C_{31}H_{21}ClN_2O_5$ requires 537.1217). Natural (1*S*)-78: $[\alpha]^{25} +26$ (c 0.28, dimethylformamide).

3-[(5'-(((benzo[*b*]thieno-2''-yl)carbonyl)amino)benzofuro-2'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (80): 18 h, 68%; mp > 230 °C; ^1H NMR (DIMETHYLSULFOXIDE- d_6 , 400 MHz) 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) max 3259, 2923, 1659, 1630, 1583, 1549, 1413, 1392, 1336, 1244, 1211 cm^{-1} ; FABHRMS (NBA) m/e 553.0985 ($M^+ + H$, $C_{31}H_{21}ClN_2O_4S$ requires 553.0989). Natural (1*S*)-80: $[\alpha]^{25} +30$ (c 0.33, dimethylformamide).

Preparation of compounds 82 and 84,86,88,90,92 (Illustrated in Figure 10)-

General Procedures for the Spirocyclization and Preparation of 82 and 84,86,88,90,92. Method A: A suspension of NaH (60% oil dispersion, 2 equiv) in

tetrahydrofuran at 0 °C under Ar was treated with a solution of 70, 72, 74, 76, 78, 80 prepared above in tetrahydrofuran-dimethylformamide (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 H₂O and dried *in vacuo*.

- 5 Flash chromatography (SiO₂, 50-70% tetrahydrofuran-hexane) afforded 82, 84, 86, 88, 90, 92 in 50-93% yield.

Method B: A solution of compounds 70, 72, 74, 76, 78, 80 in tetrahydrofuran-dimethylformamide (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
10 to warm to 24 °C and stirred for 2-4 h. The solvent was removed in vacuo, and flash chromatography (SiO₂, 50-70% tetrahydrofuran-hexane) afforded 82, 84, 86, 88, 90, 92 with 40-75% yield.

Method C: A sample of 70 (1.6 mg, 0.0030 mmol) in tetrahydrofuran (0.20 mL) was treated with the phosphazene base P₄-*t*-Bu (3.3 µL, 1 M solution in hexane,
15 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 (SiO₂, 60% tetrahydrofuran-hexane) to provide 82 (1.4 mg, 1.5 mg theoretical, 93%) as a yellow solid.

*N*²-[5'-(((1*H*-Indol-2''-yl)carbonyl)amino)-1*H*-indol-2'-yl)carbonyl]-
20 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (82, CBI-indole₂): Method A, 93%; method B, 75%; method C, 93%; mp > 240 °C; ¹H NMR (DIMETHYLSULFOXIDE-*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),
25 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 H₂O, 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) _{max} 3432, 1648, 1522, 1384, 1266, 1126, 744 cm⁻¹; UV (dimethylformamide) _{max} 316 (=
30 45000), 274 nm (25000); FABHRMS (NBA) *m/e* 499.1792 (M⁺ + H, C₃₁H₂₂N₄O₃ requires 499.1770). Natural (+)-82: [α]³ +114 (*c* 0.03, dimethylformamide), [α]⁵

+81 (*c* 0.12, tetrahydrofuran). *Ent* (-)-82: $[\alpha]_D^{25}$ -120 (*c* 0.1, dimethylformamide), $[\alpha]_D^{25}$ -81 (*c* 0.12, tetrahydrofuran).

*N*²-[(5'-(((Benzofuro-2''-yl)carbonyl)amino)-1*H*-indol-2'-yl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (84): Method A, 60%;

- 5 method B, 49%; mp > 230 °C; ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 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); ¹³C NMR (tetrahydrofuran-*d*₈, 100 MHz) 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 (CH₂), 33.2 (C), 29.9 (CH) 28.5 (CH₂); IR (film) ν_{\max} 3299, 1654, 1595, 1517, 1388, 1262, 1127, 744 cm⁻¹; FABHRMS (NBA) *m/e* 500.1610 (M⁺ + H, C₃₁H₂₁N₃O₄ requires 500.1610).
- 15 20 Natural (+)-84: $[\alpha]_D^{25}$ +91 (*c* 0.13, tetrahydrofuran).

*N*²-[(5'-(((Benzo[*b*]thieno-2''-yl)carbonyl)amino)-1*H*-indol-2'-yl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (86): Method A, 50%; method B, 46%; mp > 230 °C; ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 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 H₂O, 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/e* 516.1391 (M⁺ + H, C₃₁H₂₁N₃O₃S

25 30

requires 516.1382). Natural (+)-86: +73 (*c* 0.05, dimethylformamide).

*N*²-[(5'-(((1*H*-Indol-2''-yl)carbonyl)amino)benzofuran-2'-yl)carbonyl]-

1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (88): Method A, 63%;

method B, 45%; mp > 230 °C; ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz)

- 5 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-H₂), 3.27-3.28 (m, 1H, partially obscured by H₂O, C9a-H), 1.72-1.78 (m, 2H, C9-H₂); IR (film) _{max} 3330, 1660, 1548, 1382, 1300, 1242, 1035 cm⁻¹; FABHRMS (NBA) *m/e* 500.1600 (M⁺ + H, C₃₁H₂₁N₃O₄ requires 500.1610). Natural (+)-88: +176 (*c* 0.09, dimethylformamide).

*N*²-[(5'-(((Benzofuro-2''-yl)carbonyl)amino)benzofuro-2'-yl)carbonyl]-

- 15 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (90): Method A, 93%;

method B, 49%; mp > 230 °C; ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz)

- 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 H₂O, 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) _{max} 3369, 2921, 1660, 1600, 25 1549, 1378, 1295, 1244, 1050 cm⁻¹; FABHRMS (NBA) *m/e* 501.1470 (M⁺ + H, C₃₁H₂₀N₂O₅ requires 501.1450). Natural (+)-90: [α]³ +90 (*c* 0.10, dimethylformamide).

*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 (92): Method B,

- 30 50%; mp > 230 °C; ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 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-H₂), 3.27-3.28 (m, 1H, obscured by H₂O, C9a-H), 1.76-1.80 (m, 2H, C9-H₂); IR (film) ν_{\max} 2920, 2851, 1661, 1599, 1555, 1466, 1381, 1297, 1243 cm⁻¹; FABHRMS (NBA) m/e 517.1233 (M⁺ + H, C₃₁H₂₀N₂O₄S requires 517.1222). Natural (+)-92: $[\alpha]_D^{25} +69$ (c 0.04, dimethylformamide).

- 10 **Preparation of 2,4-Dinitrophenyl-*N*¹-[*N*²-(*tert*-Butyloxycarbonyl)-hydrazino]-carboxylate (96)** (Illustrated in Figure 16) A suspension of bis(2,4-dinitrophenyl)carbonate (94, 394 mg, 1.0 mmol) as prepared by Gray et. al. *Tetrahedron*, 1977,33,, 739, in 1.5 mL of Ethylacetate at 24 °C under N₂ was treated with a solution of *tert*-butylcarbazate from Aldrich company (132 mg, 1.0 mmol) in
- 15 Ethylacetate (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 96 (271 mg, 72% pure as a mixture with 2,4-dinitrophenol) and a second crop of crystals was obtained from the mother liquor to
- 20 afford pure 96 (12 mg) as colorless flakes: mp 105-107 °C (hexane, colorless flakes); ¹H NMR (CDCl₃, 200 MHz) 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(CH₃)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.

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- Preparation of 3-[*N*¹-[*N*²-(*tert*-Butyloxycarbonyl)hydrazino]carbonyl]-1-chloromethyl-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (98)** (Illustrated in Figure 16). Phenol 14 (6.0 mg, 18 μmol) was treated with anhydrous 3N HCl-Ethylacetate (0.5 mL) at 24 °C for 20 min. the solvent was removed in vacuo to afford crude,
- 30 unstable 16 quantitatively. A solution of 16 in tetrahydrofuran (0.4 mL) at 24 °C under Ar was treated sequentially with 96 (11 mg, 72% pure, 23.4 μmol, 1.3 equiv) and Triethylamine (2.5 μL, 18 μmol, 1 equiv), and the reaction mixture was stirred for

5.5 h (24 °C). Flash chromatography (1.5 x 15 cm SiO₂, 66% Ethylacetate-hexane) afforded **98** (6.4 mg, 7.0 mg theoretical, 91%) as a white solid: mp 221 °C; ¹H NMR (CDCl₃-dimethylformamide-*d*₇, 200 MHz) 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, CHHCl), 3.37 (t, 1H, *J* = 11 Hz, CHHCl), 1.50 (s, 9H, C(CH₃)₃); IR (KBr) ν_{\max} 3408, 2926, 1718, 1654, 1584, 1522, 1476, 1394, 1246, 1160, 862, 758 cm⁻¹; UV (tetrahydrofuran) λ_{\max} 318 (= 9500), 308 (8200), 260 (31000), 254 nm (32000); FABHRMS (DTT-DTE) *m/e* 392.1364 (M⁺ + H, C₁₉H₂₂ClN₃O₄ requires 392.1377).

Preparation of 1-(Chloromethyl)-3-(hydrazino)carbonyl-5-hydroxy-1,2-dihydro-3H-benz[*e*]indole hydrochloride (100) (Illustrated in Figure 16). A sample of **98** (1.0 mg, 2.6 μmol) was treated with anhydrous 3N HCl-Ethylacetate (1 mL) at 24 °C for 30 min. The solvent was removed in vacuo to afford **100** (0.9 mg, 0.87 mg theoretical, 100%) as a white solid: mp 225 °C (dec); ¹H NMR (CDCl₃-DIMETHYLSULFOXIDE-*d*₆, 300 MHz) 10.00 (bs s, N⁺H₃), 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, CHHCl), 3.51 (t, 1H, *J* = 10.2 Hz, CHHCl); 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 (dimethylformamide) λ_{\max} 322 (= 9300), 310 (sh, 7900), 270 nm (23000); FABHRMS (DTT-DTE) *m/e* 292.0867 (M⁺ + H, C₁₄H₁₄ClN₃O₂ requires 292.0853).

Preparation of compounds 102 and 104 (Illustrated in Figure 16). A solution of **98** or **100** (1.0 equiv) in tetrahydrofuran (0.02 molar) was treated with 0.02 molar of 5% aqueous NaHCO₃ and the two-phase mixture was stirred at 24 °C for 5 h under N₂. The reaction mixture was extracted with Ethylacetate (3 x). The organic layer was dried (Na₂SO₄) and concentrated. Flash chromatography affords **102** and

104 as a pale yellow solid.

Preparation of N^1 -[N^2 -(*tert*-Butyloxycarbonyl)hydrazino]carbonyl-CDPI₂ (108) (Illustrated in Figure 17). *N*-BOC-CDPI₂ (106, 6.2 mg, 12.8 μ mol) as previously described in Boger et. al *J. Org. Chem.* 1987, 52, 1521, was treated with CF₃CO₂H (0.5 mL) at 24 °C for 1 h. The CF₃CO₂H was removed by a stream of N₂ and the residue was dried in vacuo. A solution of the crude salt in dimethylformamide (0.2 mL) at 24 °C under Ar was treated sequentially with 96 (72% pure in 2,4-dinitrophenol, 9.1 mg, 19.3 μ mol, 1.5 equiv) and Triethylamine (1.8 μ L, 12.8 μ mol, 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 NaHCO₃ (1 mL), H₂O (0.5 mL), 10% aqueous citric acid (1 mL), and H₂O (4 x 1 mL). Drying the solid in vacuo afforded 108 (6.3 mg, 6.9 mg theoretical, 91%) as a pale yellow solid: mp 257 °C (dec); ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 300 MHz) 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-H₂), 4.02 (t, 2H, *J* = 8.3 Hz, C2'-H₂), 3.2-3.6 (m, 4H, partly obscured by H₂O, C1-H₂, C1'-H₂), 1.43 (s, 9H, C(CH₃)₃); IR (KBr) _{max} 3424, 1686, 1508, 1438, 1372, 1160, 800, 684 cm⁻¹.

Preparation of N^1 [N^2 -(*tert*-Butyloxycarbonyl)hydrazino]carbonyl-*seco*-CBI-CDPI₂ (110) (Illustrated in Figure 17). A slurry of crude 16 freshly prepared from 14 (3.7 mg, 11.1 μ mol), 1-(3-DIMETHYLAMINOPROPYL)-3-ETHYLCARBODIIMIDE HYDROCHLORIDE (EDCI) (6.4 mg, 33 μ mol, 3 equiv), and 108 (6.0 mg, 11.1 μ mol, 1 equiv) in dimethylformamide (0.2 mL) at 24 °C under Ar was vigorously stirred for 10 h. The solvent was removed in vacuo and the residue was washed with H₂O (2 x 2 mL) and dried in vacuo. Flash chromatography (0.5 x 5 cm SiO₂, 0-66% dimethylformamide-toluene gradient elution) afforded 110 (5.5 mg, 8.4 mg theoretical, 65%) as a pale yellow solid: mp 250 °C (dec); ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 300 MHz) 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'-H₂), 4.59 (d, 1H, $J = 10$ Hz, C2-H), 4.26 (m, 1H, CHHCl), 4.03 (t, 2H, $J = 8$ Hz, C2''-H₂), 3.9-4.0 (m, 2H, C1-H), CHHCl, 3.2-3.6 (m, 4H, partly obscured by H₂O, C1'-H₂, C1''-H₂), 1.44 (s, 9H, C(CH₃)₃); IR (KBr) max 3410, 3315, 2962, 2927, 1664, 1610, 1582, 1508, 1416, 1370, 1340, 1262, 1158, 1098, 802, 762, 528 cm⁻¹; UV (dimethylformamide) max 340 ($\epsilon = 43000$), 310 (44000), 270 nm (26000); FABHRMS (DTT-DTE) m/e 760.2635 ($M^+ + H$, C₄₁H₃₈ClN₇O₆ requires 760.2650).

Preparation of compound 112 (Illustrated in Figure 17) A sample of 110 (1.0 equiv.) is treated with anhydrous 3N HCl-Ethylacetate (.25 M) at 24 °C for 30 min. The solvent is then removed in vacuo to afford 112 as a white solid.

Preparation of 2-[(Benzyloxy)methyl]pyrrolo[3,2-*e*]benzoxazole (116) (Illustrated in Figure 25). A solution of 5-hydroxyindole 114 (499 mg, 3.75 mmol) from Aldrich company and 2-(benzyloxy)ethylamine (1.13 g, 7.50 mmol, 2 equiv) monobenzylated from Aldrich company in anhydrous ethylene glycol dimethyl ether (DME, 120 mL) was cooled to 0 °C and activated MnO₂ (15 g, 30 wt equiv) was added. The reaction mixture was allowed to stir at 24 °C for 14 h before filtration through a Celite pad to remove MnO₂. The solvent was removed in vacuo. Flash chromatography (SiO₂, 2.5 x 25 cm, 40-50% Ethylacetate-hexane gradient elution) afforded 116 (500 mg, 1.04 g theoretical, 48%) as a pale orange- yellow oil: ¹H NMR (CDCl₃, 400 MHz) 8.98 (br s, 1H, NH), 7.27-7.40 (m, 8H, ArH), 6.92-6.93 (m, 1H, ArH), 4.84 (s, 2H, PhCH₂), 4.70 (s, 2H, C2-CH₂); ¹³C NMR (CDCl₃, 100 MHz) 161.4 (C), 146.1 (C), 137.1 (C), 133.7 (C), 128.5 (two CH), 128.1 (two CH), 128.05 (CH), 127.8 (C), 125.3 (CH), 119.4 (C), 109.3 (CH), 104.8 (CH), 100.1 (CH), 73.2 (CH₂), 64.6 (CH₂); IR (neat) max 3265, 2862, 1671, 1452, 1367, 1212, 1089, 738, 698 cm⁻¹; FABHRMS (NBA) m/e 279.1140 ($M^+ + H$, C₁₇H₁₄N₂O₂ requires 279.1134).

Preparation of 2-(Hydroxymethyl)pyrrolo[3,2-*e*]benzoxazole (118) (Illustrated in Figure 25). A solution of 116 (67 mg, 0.24 mmol) in Ethanol (4 mL) was treated with 3 drops of conc HCl followed by 10% Pd-C (34 mg, 0.5 wt equiv). The reaction mixture was stirred at 24 °C under 1 atm of H₂ for 30 min, and neutralized with the addition of Triethylamine. The mixture was filtered through a Celite pad to remove the catalyst and the solvent was removed in vacuo. Flash chromatography (SiO₂, 1.0 x 20 cm, 60-80% Ethylacetate-hexane gradient elution) afforded 118 (31.5 mg, 45.1 mg theoretical, 70%) as a white crystalline solid: mp 169-171.5 °C (CH₃OH-CH₂Cl₂); ¹H NMR (CD₃OD, 400 MHz) 7.42 (dd, 1H, *J* = 0.8, 8.8 Hz, ArH), 7.36 (d, 1H, *J* = 3.1 Hz, C7-H), 7.32 (d, 1H, *J* = 8.8 Hz, ArH), 6.78 (dd, 1H, *J* = 0.8, 3.1 Hz, C8-H), 4.82 (s, 2H, CH₂OH); ¹³C NMR (CD₃OD, 100 MHz) 165.5 (C), 147.0 (C), 135.6 (C), 133.3 (C), 126.8 (CH), 120.3 (C), 110.6 (CH), 105.0 (CH), 99.7 (CH), 58.2 (CH₂); IR (film) _{max} 3266, 1566, 1438, 1364, 1221, 1083, 1037, 775, 735, 668 cm⁻¹; FABHRMS (NBA) *m/e* 189.0668 (M⁺ + H, C₁₀H₈N₂O₂ requires 189.0664). Anal. Calcd for C₁₀H₈N₂O₂: C, 63.81; H, 4.29; N, 14.89. Found: C, 63.50; H, 4.20; N, 14.50.

Preparation of methyl Pyrrolo[3,2-*e*]benzoxazole-2-carboxylate (120) (Illustrated in Figure 25). A solution containing NaCN (42 mg, 0.85 mmol, 5 equiv) and activated MnO₂ (148 mg, 1.7 mmol, 10 equiv) in 10.5 mL of CH₃OH was treated with a solution of 118 (32 mg, 0.17 mmol) in CH₃OH (5.5 mL) at 0 °C under Ar. The reaction mixture was allowed to warm to 24 °C and was stirred for 4 h. The reaction mixture was filtered through a Celite pad (2 x 30 mL Ethylacetate wash) to remove MnO₂ and the combined organic layer was washed with H₂O, saturated aqueous NaCl, dried (Na₂SO₄), and concentrated in vacuo. Flash chromatography (SiO₂, 1 x 15 cm, 40% Ethylacetate-hexane) afforded 120 (37 mg, 37 mg theoretical, 100%) as an off-white solid: mp 207-208 °C (Ethylacetate-hexane); ¹H NMR (CDCl₃, 400 MHz) 8.69 (br s, 1H, NH), 7.57 (d, 1H, *J* = 8.9 Hz, ArH), 7.46 (d, 1H, *J* = 8.9 Hz, ArH), 7.39 (t, 1H, *J* = 2.8 Hz, C7-H), 7.05-7.06 (m, 1H, C-8H), 4.09 (s, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) 157.2 (C), 151.1 (C), 146.7 (C), 133.9 (C), 133.2 (C), 125.8 (CH), 119.8 (C), 113.0 (CH), 104.7 (CH), 100.4 (CH), 53.5 (CH₃); IR (film) _{max} 3356, 2921, 1738, 1537, 1437, 1371, 1148 cm⁻¹; FABHRMS (NBA) *m/e* 217.0610 (M⁺ + H,

$C_{11}H_8N_2O_3$ requires 217.0613). Anal. Calcd for $C_{11}H_8N_2O_3$: C, 61.10; H, 3.73; N, 12.96. Found: C, 60.92; H, 3.71; N, 12.79.

Preparation of Methyl 1,2-Dihydro-3*H*-pyrrolo[3,2-*e*]benzoxazole-7-carboxylate

5 (122) (Illustrated in Figure 25). Compound 120 (47.6 mg, 0.22 mmol) was dissolved in CF_3CO_2H (1 mL) and cooled to 0 °C. The mixture was stirred for 10 min before Et_3SiH (355 μL , 2.20 mmol, 10 equiv) was added to the reaction mixture. The mixture was warmed to 24 °C and stirred for 4.5 h. The solvent was removed under a stream of N_2 and the residue was dissolved in CH_2Cl_2 (10 mL), and washed with
 10 saturated aqueous $NaHCO_3$ (10 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo to afford crude 9 as a yellow solid which was used directly in the next step without further purification due to its propensity to air oxidize back to starting material. For crude 122: 1H NMR ($CDCl_3$, 400 MHz) 7.25 (d, 1H, $J = 8.7$ Hz, C5-H), 6.80 (d, 1H, $J = 8.7$ Hz, C4-H), 4.01 (s, 3H, CH_3), 3.65 (t, 2H, $J = 8.6$ Hz, C2- H_2), 3.31 (t, 2H, $J = 8.6$ Hz, C1- H_2); FABHRMS (NBA) m/e 219.0768 ($M^+ + H$, $C_{11}H_{10}N_2O_3$ requires 219.0770).

Preparation of Methyl 3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]benzoxazole-7-carboxylate (124) (Illustrated in Figure 25).

20 mmol of 120) in anhydrous CH_2Cl_2 (2 mL) was treated with 85% trimethylsilyl isocyanate (Me_3SiNCO , 174 μL , 1.10 mmol, 5 equiv) and the mixture was stirred at 24 °C under N_2 for 4 h. The resulting insoluble residue was collected by centrifugation and washed with CH_2Cl_2 (2 x 3 mL) and CH_3OH (3 mL). Drying the solid in vacuo afforded pure 124 (32.7 mg, 57.4 mg theoretical, 57% from 120) as a pale yellow
 25 solid: mp > 230 °C (dec); 1H NMR (DIMETHYLSULFOXIDE- d_6 , 400 MHz) 8.20 (d, 1H, $J = 9.0$ Hz, C4-H), 7.57 (d, 1H, $J = 9.0$ Hz, C5-H), 6.35 (br s, 2H, NH_2), 4.04 (t, 2H, $J = 8.9$ Hz, C2- H_2), 3.96 (s, 3H, CH_3), 3.38 (t, 2H, $J = 8.9$ Hz, C1- H_2); ^{13}C NMR (DIMETHYLSULFOXIDE- d_6 , 100 MHz) 156.5 (C), 155.9 (two C), 146.1 (C), 143.0 (C), 136.9 (C), 122.0 (C), 115.2 (CH), 109.3 (CH), 53.4 (CH_3), 48.2
 30 (CH_2), 25.2 (CH_2); IR (film) \max 3448, 3179, 1727, 1675, 1606, 1543, 1487, 1423, 1321, 1229, 1155, 1140, 1028, 818 cm^{-1} ; FABHRMS (NBA) m/e 262.0830 ($M^+ + H$,

$C_{12}H_{11}N_3O_4$ requires 262.0828). Anal. Calcd for $C_{12}H_{11}N_3O_4$: C, 55.16; H, 4.25; N, 16.09. Found: C, 54.93; H, 4.17; N, 15.95.

Preparation of Methyl 3-(*tert*-Butyloxycarbonyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]benzoxazole-7-carboxylate (126) (Illustrated in Figure 25). A solution of crude **122** (1.8 mg, 0.008 mmol) dissolved in tetrahydrofuran (100 μ L) was treated with di-*tert*-butyl dicarbonate (3.6 mg, 3.8 μ L, 0.016 mmol, 2 equiv). The reaction mixture was stirred at 24 °C for 2 h and 4 °C for 24 h. The solvent was removed in vacuo and flash chromatography (SiO₂, 20-40% Ethylacetate-hexane gradient elution) afforded **126** (2.0 mg, 2.6 mg theoretical, 76%) as a pale yellow solid: ¹H NMR (CDCl₃, 400 MHz) 8.17 (br s, 1H, C4-H), 7.43 (d, 1H, *J* = 9.0 Hz, C5-H), 4.13 (t, 2H, *J* = 8.8 Hz, C2-H₂), 4.07 (s, 3H, CH₃), 3.41 (t, 2H, *J* = 8.8 Hz, C1-H₂), 1.56 (s, 9H, C(CH₃)₃); FABHRMS (NBA) *m/e* 319.1290 (M⁺ + H, C₁₆H₁₈N₂O₅ requires 319.1294).

Preparation of 3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]benzoxazole-7-carboxylic Acid (128) (Illustrated in Figure 25). A suspension of **124** (27 mg, 0.103 mmol) and LiOH H₂O (8.8 mg, 0.21 mmol, 2 equiv) in tetrahydrofuran-CH₃OH-H₂O (3:1:1, 2.5 mL) was stirred at 24 °C for 4 h. The solvent was removed under a stream of N₂ and the residual solid was suspended in H₂O (2 mL) and acidified with 1N aqueous HCl to pH 1. The insoluble residue was collected by centrifugation and washed with H₂O (2 x 3 mL). Drying the solid in vacuo afforded **128** (25 mg, 25 mg theoretical, 100%) as a pale yellow powder: mp > 230 °C (dec); ¹H NMR (CF₃CO₂D, 400 MHz) 8.31 (d, 1H, *J* = 9.4 Hz, C4-H), 7.76 (d, 1H, *J* = 9.4 Hz, C5-H), 4.41 (t, 1H, *J* = 8.4 Hz, C2-H₂), 3.73 (t, 1H, *J* = 8.4 Hz, C1-H₂); ¹³C NMR (DIMETHYLSULFOXIDE-*d*₆, 100 MHz) 155.9 (C), 154.8 (two C), 145.2 (C), 142.0 (C), 136.3 (C), 120.9 (C), 112.2 (CH), 108.4 (CH), 48.1 (CH₂), 25.2 (CH₂); IR (film) _{max} 3476, 3174, 1677, 1606, 1481, 1419, 1369, 1234, 1061, 815 cm⁻¹; FABHRMS (NBA) *m/e* 248.0674 (M⁺ + H, C₁₁H₉N₃O₄ requires 248.0671).

Preparation of 1-(*tert*-Butyloxycarbonyl)-5-nitroindole (132) (Illustrated in Figure 26).

A solution of 5-nitroindole (130, 2.0 g, 12.3 mmol) from Aldrich company and 4-DIMETHYLAMINOPYRIDINE (226 mg, 1.85 mmol, 0.15 equiv) in dioxane (90 mL) was treated with di-*tert*-butyl dicarbonate (5.38 g, 24.7 mmol, 2 equiv), and the reaction mixture was stirred at 24 °C for 10-15 min before the solvent was removed in vacuo. Flash chromatography (SiO₂, 2.5 x 25 cm, 20-50% Ethylacetate-hexane gradient elution) afforded 132 (3.17 g, 3.17 g theoretical, 100%) as an off-white solid: mp 135-137 °C (CH₂Cl₂, off-white fine needles); ¹H NMR (CDCl₃, 400 MHz) 8.42 (d, 1H, *J* = 2.1 Hz, C4-H), 8.21 (d, 1H, *J* = 9.1 Hz, C7-H), 8.14 (dd, 1H, *J* = 2.1, 9.1 Hz, C6-H), 7.70 (d, 1H, *J* = 3.8 Hz, C2-H), 6.67 (d, 1H, *J* = 3.8 Hz, C3-H), 1.67 (s, 9H, C(CH₃)₃); ¹³C NMR (CDCl₃, 100 MHz) 148.9 (C), 143.6 (C), 138.2 (C), 130.2 (C), 128.8 (CH), 119.4 (CH), 117.2 (CH), 115.2 (CH), 107.8 (CH), 85.1 (C), 28.0 (three CH₃); IR (film) _{max} 2982, 1737, 1515, 1462, 1329, 1285, 1254, 1156, 1027, 904, 768, 745 cm⁻¹; FABHRMS (NBA) *m/e* 263.1043 (M⁺ + H, C₁₃H₁₄N₂O₄ requires 263.1032). Anal. Calcd for C₁₃H₁₄N₂O₄: C, 59.52; H, 5.38; N, 10.69. Found: C, 59.53; H, 5.36; N, 10.53.

Preparation of 5-Amino-1-(*tert*-butyloxycarbonyl)indole (134) (Illustrated in Figure 26). A solution of 132 (1.0 g, 3.81 mmol) in Ethylacetate (30 mL) was treated with 10% Pd-C (500 mg, 0.5 wt equiv) and the mixture was stirred under 1 atm of H₂ at 24 °C for 5 h. The catalyst was removed by filtration through Celite, and the solvent was removed in vacuo. Flash chromatography (SiO₂, 2 x 20 cm, 40-60% Ethylacetate-hexane gradient elution) afforded 134 (593 mg, 884 mg theoretical, 67%) as a pale brown oil: ¹H NMR (CDCl₃, 400 MHz) 7.90 (br s, 1H, C7-H), 7.50 (br s, 1H, C2-H), 6.82 (d, 1H, *J* = 2.3 Hz, C4-H), 6.70 (dd, 1H, *J* = 2.3, 8.7 Hz, C6-H), 6.39 (d, 1H, *J* = 3.6 Hz, C3-H), 3.43 (br s, 2H, NH₂), 1.65 (s, 9H, C(CH₃)₃); ¹³C NMR (CDCl₃, 100 MHz) 149.8 (C), 142.3 (C), 131.7 (C), 129.1 (C), 126.2 (CH), 115.7 (CH), 113.8 (CH), 106.9 (CH), 106.0 (CH), 83.2 (C), 28.3 (three CH₃); IR (neat) _{max} 3359, 1725, 1477, 1454, 1380, 1357, 1343, 1285, 1229, 1166, 1150, 1132, 1024 cm⁻¹; FABHRMS (NBA) *m/e* 232.1212 (M⁺, C₁₃H₁₆N₂O₂ requires 232.1212).

Preparation of 5-(2-Benzoyloxyacetyl)amino-1-(*tert*-butoxy-carbonyl)-indole

(136) (Illustrated in Figure 26). A solution of 134 (991 mg, 4.27 mmol) and K_2CO_3 (400 mg, 5.12 mmol, 1.2 equiv) in tetrahydrofuran (75 mL) was cooled to 0 °C and stirred for 10 min before benzyloxyacetyl chloride (851 μ L, 5.12 mmol, 1.2 equiv) was added. The reaction mixture then was allowed to warm to 24 °C and stirred under N_2 for 2 h. The mixture was diluted with H_2O (100 mL), extracted with Ethylacetate (3 x 150 mL), dried (Na_2SO_4) and concentrated. Flash chromatography (SiO_2 , 2 x 20 cm, 40-50% Ethylacetate-hexane gradient elution) afforded 136 (1.53 g, 1.62 g theoretical, 94%) as a pale yellow oil: 1H NMR ($CDCl_3$, 400 MHz) 8.37 (br s, 1H, NH), 8.06 (br d, 1H, J = 8.6 Hz, C7-H), 7.95 (d, 1H, J = 2.0 Hz, C4-H), 7.56 (d, 1H, J = 3.9 Hz, C2-H), 7.34-7.41 (m, 5H, C_6H_5), 7.27 (dd, 1H, J = 2.0, 8.6 Hz, C6-H), 6.51 (d, 1H, J = 3.9 Hz, C3-H), 4.65 (s, 2H, $PhCH_2$), 4.11 (s, 2H, $COCH_2$), 1.65 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 62.5 MHz) 167.4 (C), 149.5 (C), 136.5 (C), 132.2 (two C), 130.9 (C), 128.7 (two CH), 128.3 (CH), 128.0 (two CH), 126.6 (CH), 116.9 (CH), 115.3 (CH), 112.1 (CH), 107.3 (CH), 83.6 (C), 73.7 (CH_2), 69.6 (CH_2), 28.1 (three CH_3); IR (neat) \max 3381, 2978, 1732, 1688, 1537, 1473, 1372, 1131, 745, 699 cm^{-1} ; FABHRMS (NBA) m/e 381.1823 ($M^+ + H$, $C_{22}H_{24}N_2O_4$ requires 381.1814).

Preparation of 5-(2-Benzyloxyacetyl)amino-1-(*tert*-butyloxycarbonyl)-4-nitroindole (138) (Illustrated in Figure 26). Compound 136 (783 mg, 2.06 mmol) was dissolved in CH_3NO_2 (38 mL), cooled to 0 °C, and treated with 65% HNO_3 (1.1 mL). The mixture was warmed to 24 °C and stirred for 3 h before it was diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (3 x 40 mL). The organic layer was dried (Na_2SO_4) and concentrated. Flash chromatography (SiO_2 , 2 x 20 cm, 20-40% Ethylacetate-hexane gradient elution) afforded 138 (570 mg, 878 mg theoretical, 65%) as a bright yellow solid: mp 145-146.5 °C (CH_2Cl_2 , light yellow flakes); 1H NMR ($CDCl_3$, 400 MHz) 11.40 (br s, 1H, NH), 8.63 (d, 1H, J = 9.4 Hz, ArH), 8.40 (d, 1H, J = 9.4 Hz, ArH), 7.71 (d, 1H, J = 4.0 Hz, C2-H), 7.27-7.40 (m, 5H, C_6H_5), 7.17 (d, 1H, J = 4.0 Hz, C3-H), 4.68 (s, 2H, $PhCH_2$), 4.10 (s, 2H, $COCH_2$), 1.62 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) 168.9 (C), 148.8 (C), 136.5 (C), 132.2 (C), 130.7 (C), 129.8 (CH), 129.6 (C), 128.6 (two CH), 128.2 (CH), 128.0 (two CH), 125.7 (C), 122.2 (CH), 117.9 (CH), 107.2 (CH), 85.2 (C), 73.8 (CH_2), 69.7 (CH_2),

28.1 (three CH₃); IR (film) ν_{\max} 3319, 1737, 1701, 1491, 1372, 1323, 1288, 1152, 1108 cm⁻¹; FABHRMS (NBA) m/e 425.1605 (M⁺ + H, C₂₂H₂₃N₃O₆ requires 425.1587).

Anal. Calcd for C₂₂H₂₃N₃O₆: C, 62.09; H, 5.45; N, 9.88. Found: C, 61.84; H, 5.47; N, 9.99.

5

Preparation of 4-Amino-5-(2-benzyloxyacetyl)amino-1-(*tert*butyloxy-carbonyl)-indole (140) (Illustrated in Figure 26). Method A. Compound 138 (212 mg, 0.50 mmol) was dissolved in tetrahydrofuran (3.5 mL) and treated with a solution of Na₂S₂O₄ (870 mg, 5.0 mmol, 10 equiv) in H₂O (3.5 mL). The reaction mixture was stirred at 24 °C under N₂ for 20 h before it was diluted with H₂O (10 mL), and extracted with Ethylacetate (3 x 10 mL). The organic layer was dried (Na₂SO₄) and concentrated. Flash chromatography (SiO₂, 1.5 x 20 cm, 50-60% Ethylacetate-hexane gradient elution) afforded 140 (138 mg, 197 mg theoretical, 70%) as a pale yellow oil identical in all respects to the sample described below.

- 10 Method B. A solution of 138 (910 mg, 2.13 mmol) in Ethylacetate (45 mL) was treated with 10% Pd-C (455 mg, 0.5 wt equiv) and the mixture was stirred under 1 atm of H₂ at 24 °C for 3 h. The catalyst was removed by filtration through Celite, and the solvent was removed in vacuo. Flash chromatography (SiO₂, 2 x 25 cm, 60% Ethylacetate-hexane) afforded 140 (776 mg, 846 mg theoretical, 92%) as a pale yellow oil (no debenzoylation product was detected): ¹H NMR (CDCl₃, 400 MHz) 8.23 (br s, 1H, NH), 7.55 (d, 1H, *J* = 8.7 Hz, ArH), 7.49 (d, 1H, *J* = 3.8 Hz, C2-H), 7.34-7.39 (m, 5H, C₆H₅), 7.01 (d, 1H, *J* = 8.7 Hz, ArH), 6.50 (d, 1H, *J* = 3.8 Hz, C3-H), 4.67 (s, 2H, PhCH₂), 4.17 (s, 2H, COCH₂), 4.16 (br s, 2H, NH₂), 1.64 (s, 9H, C(CH₃)₃); ¹³C NMR (CDCl₃, 100 MHz) 168.4 (C), 149.8 (C), 136.8 (C), 134.6 (C), 134.5 (C), 128.8 (two CH), 128.4 (CH), 128.2 (two CH), 124.8 (CH), 122.7 (CH), 120.6 (C), 116.1 (C), 106.2 (CH), 103.9 (CH), 83.8 (C), 73.8 (CH₂), 69.7 (CH₂), 28.3 (three CH₃); IR (neat) ν_{\max} 3362, 2978, 1731, 1676, 1491, 1350, 1299, 1152, 1126 cm⁻¹; FABHRMS (NBA-CsI) m/e 528.0878 (M⁺ + Cs, C₂₂H₂₅N₃O₄ requires 528.0899). Anal. Calcd for C₂₂H₂₅N₃O₄: C, 66.80; H, 6.38; N, 10.63. Found: C, 66.58; H, 6.34; N, 10.39.

Preparation of 2-[(Benzyloxy)methyl]-6-(*tert*-butyloxycarbonyl)-pyrrolo-3,2-

e]benzimidazole (142) (Illustrated in Figure 26). Compound 140 (192 mg, 0.484 mmol) was dissolved in tetrahydrofuran (25 mL) and treated with a solution of tetrahydrofuran (5 mL) containing 2 drops of conc H₂SO₄. The mixture was stirred at 24 °C under N₂ for 24 h before the reaction was neutralized with the addition of saturated aqueous NaHCO₃ (20 mL). The mixture was extracted with Ethylacetate (3 x 20 mL) and the organic layer was concentrated in vacuo. Flash chromatography (SiO₂, 1.5 x 20 cm, 40-50% Ethylacetate-hexane gradient elution) afforded **142** (181 mg, 183 mg theoretical, 99%) as a pale orange oil: ¹H NMR (CDCl₃, 400 MHz) 8.15 (d, 1H, *J* = 8.8 Hz, C5-H), 7.66 (d, 1H, *J* = 3.5 Hz, C7-H), 7.45 (br d, 1H, *J* = 8.8 Hz, C4-H), 7.32-7.36 (m, 5H, C₆H₅), 6.92 (br s, 1H, C8-H), 4.91 (s, 2H, PhCH₂), 4.65 (s, 2H, C2-CH₂), 1.69 (s, 9H, C(CH₃)₃); IR (neat) _{max} 2978, 1732, 1436, 1370, 1343, 1286, 1150, 1128 cm⁻¹; FABHRMS (NBA) *m/e* 378.1826 (M⁺ + H, C₂₂H₂₃N₃O₃ requires 378.1818).

Preparation of 6-(*tert*-Butyloxycarbonyl)-2-(hydroxymethyl)pyrrolo[3,2-

e]benzimidazole (144) (Illustrated in Figure 26). A solution of **142** (677 mg, 1.79 mmol) in Ethanol (20 mL) was treated with 3 drops of conc HCl followed by 10% Pd-C (340 mg, 0.5 wt equiv). The reaction mixture was stirred at 24 °C under 1 atm of H₂ for 5 h before being quenched with the addition of several drops of Triethylamine. The catalyst was removed by filtration through Celite, and the solvent was removed in vacuo. Flash chromatography (SiO₂, 2 x 25 cm, 10-20% CH₃OH-Ethylacetate gradient elution) afforded **144** (481 mg, 516 mg theoretical, 93%) as an off-white powder: mp 152 °C (dec, CH₃OH-CH₂Cl₂); ¹H NMR (CD₃OD, 400 MHz) 8.10 (d, 1H, *J* = 9.0 Hz, C5-H), 7.67 (d, 1H, *J* = 3.7 Hz, C7-H), 7.44 (d, 1H, *J* = 9.0 Hz, C4-H), 6.93 (d, 1H, *J* = 3.7 Hz, C8-H), 4.87 (s, 2H, CH₂OH), 1.69 (s, 9H, C(CH₃)₃); ¹³C NMR (CD₃OD-CDCl₃, 400 MHz) 154.0 (C), 150.9 (C), 133.2 (C), 132.7 (C), 132.0 (C), 126.1 (CH), 119.7 (C), 111.5 (CH), 110.9 (CH), 104.9 (CH), 84.6 (C), 58.6 (CH₂), 28.4 (three CH₃); IR (film) _{max} 3179, 2920, 1729, 1676, 1365, 1342, 1289, 1150, 1126 cm⁻¹; FABHRMS (NBA-NaI) *m/e* 310.1160 (M⁺ + Na, C₁₅H₁₇N₃O₃ requires 310.1168).

Methyl 6-(*tert*-Butyloxycarbonyl)pyrrolo[3,2-*e*]benzimidazole-2-

carboxylate (146) (Illustrated in Figure 26). A solution containing NaCN (478 mg, 9.75 mmol, 5 equiv) and activated MnO₂ (1.69 g, 19.5 mmol, 10 equiv) in CH₃OH (42 mL) was treated with a solution of 144 (560 mg, 1.95 mmol) in CH₃OH (17 mL) at 0 °C under Ar. The reaction mixture was allowed to warm to 4 °C and was stirred for 8 h. The reaction mixture was filtered through a Celite pad (Ethylacetate wash) to remove MnO₂. Ethylacetate was added (150 mL total) and the combined organic layer was washed with H₂O (100 mL), saturated aqueous NaCl, dried (Na₂SO₄), and concentrated in vacuo. Flash chromatography (SiO₂, 2 x 25 cm, 60-80% Ethylacetate-hexane gradient elution) afforded 146 (485 mg, 615 mg theoretical, 79%) as a light yellow foam: ¹H NMR (CDCl₃, 400 MHz) 8.26 (d, 1H, *J* = 9.0 Hz, C5-H), 7.64 (d, 1H, *J* = 3.6 Hz, C7-H), 7.49 (d, 1H, *J* = 9.0 Hz, C4-H), 6.98 (br s, 1H, C8-H), 4.01 (s, 3H, CO₂CH₃), 1.62 (s, 9H, C(CH₃)₃); IR (film) _{max} 3378, 1729, 1364, 1341, 1319, 1243, 1146, 1127 cm⁻¹; FABHRMS (NBA) *m/e* 316.1299 (M⁺ + H, C₁₆H₁₇N₃O₄ requires 316.1297). Anal. Calcd for C₁₆H₁₇N₃O₄: C, 60.93; H, 5.44; N, 13.33. Found: C, 60.84, H, 5.53; N, 12.97.

Preparation of Methyl Pyrrolo[3,2-*e*]benzimidazole-2-carboxylate (148)

(Illustrated in Figure 26). Compound 146 (100 mg, 0.32 mmol) was treated with anhydrous 3M HCl in Ethylacetate (10 mL) at 24 °C for 5 h. The reaction then was neutralized with saturated aqueous NaHCO₃ to pH 7-8 and extracted with Ethylacetate (2 x 10 mL) and CH₃CN (2 x 15 mL). The combined organic layer was concentrated in vacuo to afford 148 as a yellow solid which was used in the next reaction without further purification. For 148: mp 182 °C (dec, CH₃OH-CH₂Cl₂, light yellow powder); ¹H NMR (CD₃OD, 400 MHz) 7.45 (d, 1H, *J* = 8.8 Hz, ArH), 7.37 (d, 1H, *J* = 8.8 Hz, ArH), 7.29 (d, 1H, *J* = 3.0 Hz, C7-H), 6.85 (d, 1H, *J* = 3.0 Hz, C8-H), 4.02 (s, 3H, CO₂CH₃); IR (film) _{max} 3380, 1716, 1631, 1518, 1434, 1387, 1314, 1238 cm⁻¹; FABHRMS (NBA) *m/e* 216.0779 (M⁺ + H, C₁₁H₉N₃O₂ requires 216.0773).

Preparation of Methyl 3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]benzimidazole-7-carboxylate (152) (Illustrated in Figure 26). Crude 148

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prepared above (0.32 mmol theoretical) was treated with $\text{CF}_3\text{CO}_2\text{H}$ (2.5 mL) and the mixture was stirred at 24 °C for 40 min. The reaction mixture was cooled to 0 °C before Et_3SiH (510 μL , 3.17 mmol, 10 equiv) was added. The reaction mixture was warmed to 24 °C and stirred for 6 h. The solvent was removed under a stream of N_2 and the dry residue was dissolved in CH_2Cl_2 (20 mL). Several drops of CH_3OH were added to help dissolve the residue. The organic solution was washed with saturated aqueous NaHCO_3 and concentrated in vacuo to afford **150** as a bright yellow solid which was used directly in the next reaction without further purification due to its propensity to air oxidize back to starting material. A solution of **150** dissolved in 10 mL of CH_2Cl_2 -dimethylformamide (10:1) was treated with 85% Me_3SiNCO (220 μL , 1.38 mmol, 5 equiv). The reaction mixture was stirred at 24 °C for 8 h. The solvent was removed in vacuo, and the dry residue was slurried in CH_2Cl_2 (5 mL). The sample was collected by centrifugation, washed with CH_2Cl_2 (2x) and CH_3OH (1x) to afford pure **152** (55.4 mg, 82.5 mg theoretical, 67% from **146**) as a light gray solid: mp > 230 °C (dec); ^1H NMR ($\text{CF}_3\text{CO}_2\text{D}$, 400 MHz) 8.42 (d, 1H, $J = 9.4$ Hz, C4-H), 7.78 (d, 1H, $J = 9.4$ Hz, C5-H), 4.35 (t, 2H, $J = 8.4$ Hz, C2- H_2), 4.21 (s, 3H, CO_2CH_3), 3.64 (t, 2H, $J = 8.4$ Hz, C1- H_2), a doubling of the ^1H NMR signals was observed when the spectrum was recorded in DIMETHYLSULFOXIDE- d_6 which we attribute to the two accessible tautomeric forms of **152**; ^{13}C NMR ($\text{CF}_3\text{CO}_2\text{D}$, 100 MHz) 160.8 (C), 156.6 (C), 145.7 (C), 139.4 (C), 130.5 (C), 130.3 (C), 121.9 (CH), 119.8 (C), 117.0 (CH), 57.5 (CH_3), 50.9 (CH_2), 27.0 (CH_2); IR (KBr) max 3406, 3187, 3027, 1727, 1664, 1441, 1394, 1209, 769 cm^{-1} ; FABHRMS (NBA) m/e 261.0993 ($\text{M}^+ + \text{H}$, $\text{C}_{12}\text{H}_{12}\text{N}_4\text{O}_3$ requires 261.0988).

25 Preparation of 3-Carbamoyl-1,2-dihydro-3H-pyrrolo[3,2-*e*]benzimidazole-7-carboxylic Acid (154**) (Illustrated in Figure 26).** A suspension of **152** (50 mg, 0.19 mmol) in 6 mL of tetrahydrofuran- CH_3OH - H_2O (3:1:1) was treated with $\text{LiOH} \cdot \text{H}_2\text{O}$ (16 mg, 0.38 mmol, 2 equiv). The reaction mixture was stirred at 24 °C under N_2 for 6 h before the solvent was removed in vacuo. The residual solid was mixed with H_2O (3 mL) and acidified with 1N aqueous HCl to pH 1. The precipitate was collected by centrifugation and washed with H_2O (2 x 2 mL). Drying the solid in vacuo afforded

154 (47 mg, 47 mg theoretical, 100%) as a pale yellow fluffy solid: mp > 230 °C (dec); ¹H NMR (CF₃CO₂D, 400 MHz) 8.45 (d, 1H, *J* = 9.2 Hz, C4-H), 7.83 (d, 1H, *J* = 9.2 Hz, C5-H), 4.40 (t, 1H, *J* = 8.4 Hz, C2-H₂), 3.69 (t, 1H, *J* = 8.4 Hz, C1-H₂); ¹³C NMR (CF₃CO₂D, 100 MHz) 160.7 (C), 157.8 (C), 145.4 (C), 140.1 (C), 130.6 (C), 130.2 (C), 121.7 (CH), 119.8 (C), 116.9 (CH), 50.8 (CH₂), 26.9 (CH₂); IR (film) _{max} 3183, 1665, 1587, 1496, 1448, 1247, 1119 cm⁻¹; FABHRMS (NBA) *m/e* 247.0838 (M⁺ + H, C₁₁H₁₀N₄O₃ requires 247.0831).

Preparation of 3-[(3'-Carbamoyl-1',2'-dihydro-3'*H*-pyrrolo[3',2'-*e*]benzoxazol-7'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (156) (Illustrated in Figure 27). Phenol 14 (5.3 mg, 0.0159 mmol) was treated with anhydrous 3M HCl-Ethylacetate (2 mL) at 24 °C for 30 min. The solvent was removed in vacuo to afford crude unstable 16 (quantitative). A mixture of 16, [3-(dimethylamino)propyl]-ethylcarbodiimide hydrochloride (1-(3-DIMETHYLAMINOPROPYL)-3-ETHYLCARBODIIMIDE HYDROCHLORIDE (EDCI), 6.1 mg, 0.032 mmol, 2 equiv), and CDPBO₁ 128 (3.7 mg, 0.015 mmol, 0.95 equiv) was stirred in dimethylformamide (400 μL) at 24 °C under Ar for 12 h. The solvent was removed in vacuo and the dry residue was mixed with H₂O (1 mL) and stirred for 10 min. The precipitate was collected by centrifugation, and washed with H₂O (2 x 1 mL) and dried in vacuo. Flash chromatography (SiO₂, 0.5 x 10 cm, 0-10% CH₃OH-CHCl₃ gradient elution) afforded 156 (6.4 mg, 7.3 mg theoretical, 88%) as a pale greenish powder: mp > 230 °C (dec); ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 10.57 (s, 1H, OH), 8.21 (d, 1H, *J* = 9.0 Hz, C4'-H), 8.14 (d, 1H, *J* = 8.3 Hz, C6-H), 8.02 (s, 1H, C4-H), 7.86 (d, 1H, *J* = 8.4 Hz, C9-H), 7.61 (d, 1H, *J* = 9.0 Hz, C5'-H), 7.55 (t, 1H, *J* = 7.7 Hz, C8-H), 7.40 (t, 1H, *J* = 7.8 Hz, C7-H), 6.36 (br s, 2H, NH₂), 4.90 (d, 1H, *J* = 10.5 Hz, C2-H), 4.78 (dd, 1H, *J* = 8.8, 11.9 Hz, C2-H), 4.23-4.25 (m, 1H, C1-H), 3.99-4.08 (m, 3H, CHHCl and C2'-H₂), 3.86 (dd, 1H, *J* = 7.9, 10.9 Hz, CHHCl), 3.38-3.43 (m, 2H, C1'-H₂); ¹³C NMR (DIMETHYLSULFOXIDE-*d*₆, 100 MHz) 156.1, 155.9, 154.4, 154.1, 145.4, 142.9, 141.3, 136.7, 129.8, 127.6, 123.8, 123.2, 123.1, 122.7, 121.8, 116.2, 114.5, 109.0, 100.0, 55.3, 48.2, 47.5, 41.2, 25.3; IR (film) _{max} 3359, 3225, 1650, 1583, 1488, 1424,

1258, 1120, 1024, 764 cm^{-1} ; FABHRMS (NBA) m/e 463.1182 ($M^+ + H$, $C_{24}H_{19}ClN_4O_4$ requires 463.1173). Natural (1*S*)-156: $[\alpha]_D^{25} +44$ (c 0.12, dimethylformamide). *Ent*-(1*R*)-156: $[\alpha]_D^{25} -41$ (c 0.09, dimethylformamide).

- 5 Preparation of 3-[(3'-Carbamoyl-1',2'-dihydro-3'*H*-pyrrolo[3',2'-
e]benzimidazol-7'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-
 benz[*e*]indole (158) (Illustrated in Figure 27). Phenol 14 (4.6 mg, 0.0138 mmol)
 was treated with anhydrous 3M HCl-Ethylacetate (2 mL) at 24 °C for 40 min. The
 solvent was removed in vacuo to afford crude unstable 16 (quantitative). A mixture of
 10 16, [3-(dimethylamino)propyl]-ethylcarbodiimide hydrochloride (1-(3-
 DIMETHYLAMINOPROPYL)-3-ETHYLCARBODIIMIDE HYDROCHLORIDE
 (EDCI), 5.3 mg, 0.028 mmol, 2 equiv) and CDPBI₁ 154 (3.4 mg, 0.14 mmol, 1 equiv)
 was stirred in dimethylformamide (400 μL) at 24 °C under N_2 for 6 h. The solvent
 was removed in vacuo. The dry residue was dissolved in 10% $\text{CH}_3\text{OH}-\text{CHCl}_3$ and
 15 loaded on a flash chromatography column (SiO_2 , 0.8 x 10 cm) and eluted with 0-10%
 $\text{CH}_3\text{OH}-\text{CHCl}_3$ gradient elution to afford 158 (5.2 mg, 12.6 mg theoretical, 42%) as a
 light gray solid: mp > 230 °C (dec); ^1H NMR (DIMETHYLSULFOXIDE- d_6 , 400
 MHz) 13.16 (br s, 1H, NH), 10.49 (br s, 1H, OH), 8.09-8.14 (m, 2H, ArH), 8.03 (d,
 1H, $J = 9.0$ Hz, ArH), 7.86 (d, 1H, $J = 8.3$ Hz, C9-H), 7.52-7.56 (apparent t, 2H, $J =$
 20 8.7 Hz, ArH), 7.38 (t, 1H, $J = 7.4$ Hz, C7-H), 6.25 and 6.21 (two s, 2H, NH_2), 5.17
 (d, 1H, $J = 10.4$ Hz, C2-H), 4.83 (apparent t, 1H, $J = 9.2$ Hz, C2-H), 4.24 (m, 1H,
 C1-H), 4.02 (t, 3H, $J = 8.7$ Hz, CHHCl , C2'- H_2), 3.83-3.88 (m, 1H, CHHCl), 3.27-
 3.37 (m, 2H, obscured by H_2O , C1'- H_2); IR (film) max 3355, 3212, 2925, 1620, 1584,
 1499, 1446, 1423, 1333, 1257, 1122, 1019 cm^{-1} ; FABHRMS (NBA) m/e 462.1345
 25 ($M^+ + H$, $C_{24}H_{20}ClN_5O_3$ requires 462.1333) Natural (1*S*)-158: $[\alpha]_D^{25} +49$ (c 0.19,
 dimethylformamide). *Ent*-(1*R*)-158: $[\alpha]_D^{25} -48$ (c 0.04, dimethylformamide).

- Preparation of N^2 -[(3'-carbamoyl-1',2'-dihydro-3'*H*-pyrrolo[3',2'-
e]benzimidazol-7'-yl)carbonyl]-1,2,9,9a-tetrahydro-cyclopropa-[*c*]-enz[*e*]-indol-4-
 30 one (162) or COMPOUND (160) (Illustrated in Figure 27). A solution of 156 or
 158 (1.4 mg, 3 μmol) in 300 μL of tetrahydrofuran-dimethylformamide (1:1) was

cooled to 0 °C and treated with DBN (0.5 μ L, 4.5 μ mol, 1.5 equiv). The reaction mixture was slowly warmed to 24 °C and stirred for 3.5 h. The mixture was placed on a flash chromatography column (SiO₂, 0.5 x 3 mm), and eluted with 5-10% CH₃OH-CHCl₃ (gradient elution) to afford **160** or **162** (0.8 mg, 1.3 mg theoretical, 5 63%) as a bright yellow solid. Selected representative data for **162**: mp > 230 °C; ¹H NMR (dimethylformamide-*d*₇, 400 MHz) 13.40 (br s, 1H, NH), 8.21 (d, 1H, *J* = 8.9 Hz, C4'-H), 8.10 (d, 1H, *J* = 7.8 Hz, C5-H), 7.64 (t, 1H, *J* = 7.5 Hz, C7-H), 7.55 (d, 1H, *J* = 8.9 Hz, C5'-H), 7.48 (t, 1H, *J* = 8.0 Hz, C6-H), 7.31 (s, 1H, C4-H), 7.29 (d, 1H, *J* = 7.8 Hz, C8-H), 6.29 (br s, 2H, NH₂), 5.33 (d, 1H, *J* = 11.8 Hz, C1-H), 4.77 10 (dd, 1H, *J* = 5.0, 11.8 Hz, C1-H), 4.20 (t, 1H, *J* = 8.8 Hz, C2'-H₂), 3.44 (t, 2H, partially obscured by H₂O, *J* = 8.8 Hz, C1'-H₂), 3.31-3.35 (m, 1H, C9a-H), 1.76-1.80 (m, 2H, C9-H₂); IR (film) ν_{\max} 1656, 1620, 1589, 1495, 1442, 1406, 1272, 1125 cm⁻¹; FABHRMS (NBA) *m/e* 426.1545 (M⁺ + H, C₂₄H₁₉N₅O₃ requires 426.1566). Natural (+)-**162**: [α]³ +95 (*c* 0.04, dimethylformamide). *Ent*-(-)-**162**: [α]⁴ -94 (*c* 0.05, 15 dimethylformamide).

DNA Alkylation Studies. General procedures, the preparation of singly ³²P 5' end-labeled double-stranded DNA, the agent binding studies, gel electrophoresis, and autoradiography were conducted according to procedures described in full detail elsewhere. (Boger et. al *Tetrahedron* 1991, 47, 2661). Eppendorf tubes containing 20 the 5' end-labeled DNA (9 μ L, w794 and w836) in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) were treated with the agent DIMETHYLSULFOXIDE (1 μ L at the specified concentration). The solution was mixed by vortexing and brief centrifugation and subsequently incubated at 25 °C for 3 days. The covalently modified DNA was separated from unbound agent by Ethanol precipitation and resuspended in TE buffer 25 (10 μ L). The solution of DNA in an Eppendorf tube sealed with parafilm was heated at 100 °C for 30 min to induce cleavage at the alkylation sites, allowed to cool to 25 °C and centrifuged. Formamide dye (0.33% xylene cyanol FF, 0.03% bromophenol blue, 8.7% Na₂EDTA 250 mM) was added (5 μ L) to the supernatant. Prior to electrophoresis, the sample was denatured by warming at 100 °C for 5 min, placed in 30 an ice bath, and centrifuged, before the supernatant (3 μ L) was loaded directly onto the gel. Sanger dideoxynucleotide sequencing reactions were run as standards adjacent

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to the reaction samples. Polyacrylamide gel electrophoresis (PAGE) was run on an 8% sequencing gel under denaturing conditions (8 M urea) in TBE buffer (100 mM Tris, 100 mM boric acid, 0.2 mM Na₂EDTA) followed by autoradiography.

- 5 **Preparation of compounds 164,166,168 (Illustrated in Figure 29)** Condensation of 3-nitrobenzaldehyde with methyl 2-azidoacetate (8 equiv, 6 equiv NaOCH₃, CH₃OH, -23 to 0 °C, 6 h, 88%) both reagents commercially available from Aldrich, followed by thermolysis of the resulting methyl 2-azidocinnamate (xylene, reflux, 4.5 h, 81%) provided a readily separable mixture (4:1) of methyl 5- and 7-nitroindole-2-
- 10 carboxylate. For methyl 7-nitroindole-2-carboxylate (164): mp 122-125 °C (CH₂Cl₂, light yellow fine needles); ¹H NMR (CDCl₃, 400 MHz) 10.37 (br s, 1H, NH), 8.31 (d, 1H, *J* = 8.0 Hz, C4-H), 8.06 (d, 1H, *J* = 8.0 Hz, C6-H), 7.36 (d, 1H, *J* = 2.4 Hz, C3-H), 7.28 (t, 1H, *J* = 8.0 Hz, C5-H), 3.99 (s, 3H, CO₂CH₃); IR (film) _{max} 3372, 1727, 1531, 1445, 1344, 1298, 1249, 1188, 1107, 830, 763 cm⁻¹; FABHRMS (NBA) *m/e*
- 15 221.0560 (M⁺ + H, C₁₀H₈N₂O₄ requires 221.0562). For methyl 5-nitroindole-2-carboxylate (168): ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 12.65 (br s, 1H, NH), 8.73 (d, 1H, *J* = 2.3 Hz, C4-H), 8.14 (dd, 1H, *J* = 2.0, 8.0 Hz, C6-H), 7.60 (d, 1H, *J* = 8.0 Hz, C7-H), 7.45 (d, 1H, *J* = 0.7 Hz, C3-H), 3.90 (s, 3H, CO₂CH₃); IR (film) _{max} 3316, 1701, 1614, 1531, 1435, 1343, 1261, 1203, 992, 746 cm⁻¹. Similarly,
- 20 condensation of 4-nitrobenzaldehyde with methyl 2-azidoacetate (8 equiv, 6 equiv NaOCH₃, CH₃OH, -23 to 0 °C, 7 h, 84%) followed by thermolysis (xylene, reflux, 4 h, 83%) provided methyl 6-nitroindole-2-carboxylate (166): ¹H NMR (CDCl₃, 400 MHz) 9.27 (br s, 1H, NH), 8.39 (d, 1H, *J* = 2.0 Hz, C7-H), 8.04 (dd, 1H, *J* = 2.0, 8.0 Hz, C5-H), 7.78 (d, 1H, *J* = 8.0 Hz, C4-H), 7.28 (d, 1H, *J* = 2.3 Hz, C3-H), 4.00 (s, 3H,
- 25 CO₂CH₃).

- Preparation of compounds 170,172,174 (Illustrated in Figure 29)** Catalytic hydrogenation of 164,166 or 168 (1 atm H₂, 0.1 wt equiv 10% Pd-C, Ethylacetate, 25 °C, 4-5 h) provided the corresponding amines. For methyl 7-aminoindole-2-
- 30 carboxylate (170): 79%; mp 184 °C (dec, pale green crystals); ¹H NMR (CDCl₃, 400 MHz) 9.47 (br s, 1H, NH), 7.21 (s, 1H, C3-H), 7.20 (d, 1H, *J* = 7.4 Hz, C6-H), 6.99

- (t, 1H, $J = 7.5$ Hz, C5-H), 6.67 (d, 1H, $J = 7.4$ Hz, C4-H), 3.97 (s, 3H, CO_2CH_3), 2.30 (br s, 2H, NH_2); IR (film) max 3205, 2815, 1693, 1547, 1437, 1345, 1247, 1211, 1112, 997, 827, 783, 734 cm^{-1} ; FABHRMS (NBA) m/e 190.0747 ($\text{M}^+ + \text{H}$, $\text{C}_{10}\text{H}_{10}\text{N}_2\text{O}_2$ requires 190.0742). For methyl 6-aminoindole-2-carboxylate (172): 76%, ^1H NMR (5 CDCl_3 , 400 MHz) 8.58 (br s, 1H, NH), 7.45 (d, 1H, $J = 8.4$ Hz, C4-H), 7.11 (d, 1H, $J = 2.1$ Hz, C3-H), 6.62 (d, 1H, $J = 1.9$ Hz, C7-H), 6.59 (dd, 1H, $J = 1.9, 8.4$ Hz, C5-H), 3.89 (s, 3H, CO_2CH_3), 3.79 (br s, 2H, NH_2); IR (film) max 3351, 2922, 1694, 1629, 1528, 1440, 1271, 1206, 1130, 999, 834, 736, 668 cm^{-1} ; FABHRMS (NBA) m/e 190.0740 ($\text{M}^+ + \text{H}$, $\text{C}_{10}\text{H}_{10}\text{N}_2\text{O}_2$ requires 190.0742). For methyl 5-aminoindole-2-
- 10 carboxylate (174): 92%, mp 150-152 °C (CH_2Cl_2); ^1H NMR (CDCl_3 , 400 MHz) 8.72 (br s, 1H, NH), 7.23 (d, 1H, $J = 8.6$ Hz, C7-H), 7.03 (dd, 1H, $J = 1.0, 2.1$ Hz, C3-H), 6.93 (dd, 1H, $J = 1.0, 2.0$ Hz, C4-H), 6.81 (dd, 1H, $J = 2.0, 8.6$ Hz, C6-H), 3.93 (s, 3H, CO_2CH_3), 3.57 (br s, 2H, NH_2); ^{13}C NMR (CDCl_3 , 100 MHz) 160.0 (C), 150.3 (C), 145.6 (C), 143.0 (C), 127.7 (C), 117.7 (CH), 113.5 (CH), 112.6 (CH), 106.1 (CH), 52.2 (CH_3); IR (film) max 3320, 1691, 1628, 1531, 1437, 1376, 1337, 1232, 1034, 997, 766 cm^{-1} ; FABHRMS (NBA) m/e 190.0746 ($\text{M}^+ + \text{H}$, $\text{C}_{10}\text{H}_{10}\text{N}_2\text{O}_2$ requires 190.0742).
- 15
- 20 **General Method for the Preparation of Trimethylammonium Substituted Indole-2-carboxylate Methyl Esters: Methyl 5-(Trimethylammonio)indole-2-carboxylate Iodides (176-180) (Illustrated in Figure 29).** Compound 174 (76 mg, 0.4 mmol) was dissolved in dimethylformamide (3 mL) and treated with NaHCO_3 (168 mg, 2.0 mmol, 5 equiv) and CH_3I (568 mg, 248 μL , 4.0 mmol, 10 equiv). The reaction
- 25 mixture was stirred at 24 °C under N_2 for 4 h before the solvent was removed in vacuo. The dry residue was slurried in H_2O and precipitate was collected by centrifugation. Recrystallization from CH_3CN afforded 180 (129 mg, 144 mg theoretical, 90%) as a pale yellow solid: mp 228 °C (dec); ^1H NMR (DIMETHYLSULFOXIDE- d_6 , 400 MHz) 12.37 (br s, 1H, NH), 8.26 (d, 1H, $J = 2.6$
- 30 Hz, C4-H), 7.89 (dd, 1H, $J = 2.6, 9.3$ Hz, C6-H), 7.64 (d, 1H, $J = 9.3$ Hz, C7-H), 7.29 (s, 1H, C3-H), 3.86 (s, 3H, CO_2CH_3), 3.65 (s, 9H, $\text{N}(\text{CH}_3)_3$); ^{13}C NMR

(DIMETHYLSULFOXIDE- d_6 , 100 MHz) 161.4 (C), 140.6 (C), 136.4 (C), 129.7 (C), 125.9 (C), 116.9 (CH), 114.1 (CH), 113.9 (CH), 108.9 (CH), 56.9 (three CH₃), 52.2 (CH₃); IR (film) \max 3446, 1708, 1537, 1437, 1339, 1259, 1205, 995, 937, 770, 742 cm⁻¹; FABHRMS (NBA) m/e 233.1290 (M⁺ - I, C₁₃H₁₇IN₂O₂ requires 233.1290).

5 Anal. Calcd for C₁₃H₁₇IN₂O₂: C, 43.35; H, 4.76; N, 7.78. Found: C, 42.99; H, 4.62; N, 7.51.

Methyl 7-(trimethylammonio)indole-2-carboxylate Iodide (176) procedure as

above except with 170: mp 151.5 °C (dec, pale green fine needles); ¹H NMR

(CD₃CN, 400 MHz) 10.27 (br s, 1H, NH), 8.07 (d, 1H, J = 8.0 Hz, C4-H), 7.81 (d, 10
1H, J = 8.0 Hz, C6-H), 7.53 (s, 1H, C3-H), 7.41 (t, 1H, J = 8.0 Hz, C5-H), 4.05 (s, 3H, CO₂CH₃), 3.89 (s, 9H, N(CH₃)₃); ¹³C NMR (CD₃OD, 100 MHz) 162.8 (C), 133.5 (C), 133.2 (C), 131.5 (C), 128.0 (C), 127.1 (CH), 121.7 (CH), 117.8 (CH), 111.2 (CH), 56.7 (three CH₃), 52.8 (CH₃); IR (film) \max 3188, 1717, 1614, 1467, 1438, 1306, 1254, 1204, 1149, 944, 833, 731 cm⁻¹; FABHRMS (NBA) m/e 233.1297
15 (M⁺ - I, C₁₃H₁₇IN₂O₂ requires 233.1290). Anal. Calcd for C₁₃H₁₇IN₂O₂: C, 43.35; H, 4.76; N, 7.78. Found: C, 43.37; H, 4.73; N, 7.78.

Methyl 6-(Trimethylammonio)indole-2-carboxylate Iodide (178) procedure as

above except with 172: mp 209 °C (dec, colorless crystals); ¹H NMR

(DIMETHYLSULFOXIDE- d_6 , 400 MHz) 12.48 (br s, 1H, NH), 7.91 (d, 1H, J = 9.0
20 Hz, C4-H), 7.80 (d, 1H, J = 2.0 Hz, C7-H), 7.75 (dd, 1H, J = 2.1, 9.1 Hz, C5-H), 7.25 (s, 1H, C3-H), 3.86 (s, 3H, CO₂CH₃), 3.68 (s, 9H, N(CH₃)₃); ¹³C NMR
(DIMETHYLSULFOXIDE- d_6 , 100 MHz) 153.5 (C), 124.2 (C), 123.9 (C), 122.2 (C), 118.7 (C), 117.8 (CH), 112.4 (CH), 108.5 (CH), 101.9 (CH), 47.4 (three CH₃), 43.5 (CH₃); IR (film) \max 3409, 1716, 1605, 1564, 1489, 1433, 1325, 1226, 1005, 942
25 cm⁻¹; FABHRMS (NBA) m/e 233.1290 (M⁺ - I, C₁₃H₁₇IN₂O₂ requires 233.1290). Anal. Calcd for C₁₃H₁₇IN₂O₂: C, 43.35; H, 4.76; N, 7.78. Found: C, 43.36; H, 4.72; N, 7.81.

General Method for the Preparation of Trimethylammonium Substituted Indole-2-carboxylic Acids: 5-(Trimethylammonio)indole-2-carboxylic Acid

30 (182,184,186) (Illustrated in Figure 29). A solution of 180 (100 mg, 0.28 mmol) in tetrahydrofuran-CH₃OH-H₂O (3:1:1, 2.6 mL) was treated with LiOH-H₂O (35 mg,

- 0.83 mmol, 3 equiv), and the reaction mixture was stirred at 24 °C for 6 h. The solvent was removed and the dry residue was mixed with H₂O (10 mL) and saturated aqueous NaCl (5 mL). The solution was acidified to pH 1 with the addition of 1N aqueous HCl and extracted with CH₃CN (10 mL each) until no UV active material was detected in aqueous solution. The extracts were combined, dried (Na₂SO₄) and concentrated. Recrystallization from CH₃CN afforded **186** (73.8 mg, 96.2 mg theoretical, 77%) as a pale yellow solid: ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 13.31 (br s, 1H, CO₂H), 12.19 (br s, 1H, NH), 8.23 (d, 1H, *J* = 1.8 Hz, C4-H), 7.88 (d, 1H, *J* = 9.2 Hz, C6-H or C7-H), 7.59 (d, 1H, *J* = 9.2 Hz, C6-H or C7-H), 7.19 (d, 1H, *J* = 1.1 Hz, C3-H), 3.65 (s, 9H, N(CH₃)₃); ¹³C NMR (DIMETHYLSULFOXIDE-*d*₆, 100 MHz) 162.4 (C), 140.5 (C), 136.3 (C), 131.3 (C), 126.1 (C), 116.5 (CH), 133.8 (two CH), 108.2 (CH), 56.8 (three CH₃); IR (film) _{max} 3342, 3016, 1697, 1538, 1469, 1419, 1339, 1226, 1194, 938, 852, 772 cm⁻¹; FABHRMS (NBA) *m/e* 219.1143 (M⁺ - Cl, C₁₂H₁₅ClN₂O₂ requires 219.1134).
- 15 7-(Trimethylammonio)indole-2-carboxylic Acid (182)** procedure as above except use **176**: mp > 198 °C (dec, white solid); ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 13.41 (br s, 1H, CO₂H), 12.23 (br s, 1H, NH), 7.94 (d, 1H, *J* = 7.9 Hz, C4-H), 7.74 (d, 1H, *J* = 8.0 Hz, C6-H), 7.38 (s, 1H, C3-H), 7.26 (t, 1H, *J* = 8.0 Hz, C5-H), 3.79 (s, 9H, N(CH₃)₃); ¹³C NMR (DIMETHYLSULFOXIDE-*d*₆, 100 MHz) **20** 162.2 (C), 132.2 (C), 131.5 (C), 131.3 (C), 126.6 (C), 125.3 (CH), 120.1 (CH), 116.9 (CH), 109.5 (CH), 55.5 (three CH₃); IR (film) _{max} 3327, 1694, 1477, 1444, 1416, 1388, 1328, 1173, 1141, 940, 730 cm⁻¹; FABHRMS (NBA) *m/e* 219.1141 (M⁺ - Cl, C₁₂H₁₅ClN₂O₂ requires 219.1134).
- 6-(Trimethylammonio)indole-2-carboxylic Acid (184)** procedure as above except **25** use **178**: mp > 195 °C (dec, off-white needles); ¹H NMR (DIMETHYLSULFOXIDE-*d*₆, 400 MHz) 13.12 (br s, 1H, CO₂H), 12.33 (br s, 1H, NH), 7.88 (d, 1H, *J* = 9.0 Hz, C4-H), 7.81 (d, 1H, *J* = 2.2 Hz, C7-H), 7.73 (d, 1H, *J* = 2.2, 9.0 Hz, C5-H), 7.17 (d, 1H, *J* = 1.7 Hz, C3-H), 3.66 (s, 9H, N(CH₃)₃); ¹³C NMR (DIMETHYLSULFOXIDE-*d*₆, 100 MHz) 162.3 (C), 143.7 (C), 135.7 (C), 131.6 (C), 126.9 (C), 123.6 (CH), **30** 112.5 (CH), 107.0 (CH), 104.5 (CH), 56.5 (three CH₃); IR (film) _{max} 3260, 1689, 1530, 1328, 1222, 1131, 835, 778 cm⁻¹; FABHRMS (NBA) *m/e* 219.1142 (M⁺ - Cl,

$C_{12}H_{15}ClN_2O_2$ requires 219.1134).

Preparation of 3-[(Indol-2'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3H-benz[e]indole (*seco*-CBI-indole, 190) (Illustrated in Figure 30). A

5 sample of 14 (4.0 mg, 0.012 mmol) was treated with anhydrous 4 M HCl-Ethylacetate (1 mL) at 25 °C for 30 min. The solvent was removed in vacuo to afford crude unstable 16 (quantitative). A mixture of 16, [3-

(dimethylamino)propyl]ethylcarbodiimide hydrochloride (1-(3-

DIMETHYLAMINOPROPYL)-3-ETHYLCARBODIIMIDE HYDROCHLORIDE

10 (EDCI), 5.8 mg, 0.030 mmol, 2.5 equiv), and indole-2-carboxylic acid (188, 2.9 mg, 0.018 mmol, 1.5 equiv) from Aldrich company in 0.2 mL of dimethylformamide was stirred at 25 °C under Ar for 16 h. The mixture was diluted with 0.3 mL of H₂O and extracted with Ethylacetate (0.4 mL x 4). The combined organic layer was concentrated. Chromatography (SiO₂, 40% Ethylacetate-hexane) afforded 190 (3.4

15 mg, 4.3 mg theoretical, 79%) as a pale yellow solid: ¹H NMR (tetrahydrofuran-*d*₈, 400 MHz) 11.04 (br s, 1H, NH), 9.31 (s, 1H, OH), 8.21 (d, 1H, *J* = 8.3 Hz, C6-H), 8.02 (br s, 1H, C4-H), 7.78 (d, 1H, *J* = 8.3 Hz, C9-H), 7.67 (d, 1H, *J* = 7.9 Hz, C4'-H), 7.48 (dd, 1H, C8-H partially obscured by overlapping C7'-H), 7.47 (d, 1H, *J* = 8.3 Hz, C7'-H), 7.30 (dd, 1H, *J* = 8.0, 8.3 Hz, C7-H), 7.22 (dd, 1H, *J* = 7.1, 8.3 Hz, C6'-H),

20 7.17 (s, 1H, C3'-H), 7.06 (dd, 1H, *J* = 7.1, 7.9 Hz, C5'-H), 4.78 (m, 2H, C2-H₂), 4.17 (m, 1H, C1-H), 4.00 (dd, 1H, *J* = 3.2, 11.1 Hz, CHHCl), 3.61 (m, 1H, CHHCl); IR (film) _{max} 3427, 3225, 3056, 2965, 2865, 1608, 1578, 1512, 1417, 1394, 1363, 1338, 1316, 1252, 1140, 1058, 850, 804, 743 cm⁻¹; FABHRMS (NBA) *m/e* 377.1065 (M⁺ + H, C₂₂H₁₇ClN₂O₂ requires 377.1057). Natural (1*S*)-2: [α]³ +8.8 (*c* 0.17,

25 tetrahydrofuran).

General Method for the Coupling of *seco*-N-BOC-CBI (14) with 15-17: 3-[7'-((Trimethylammonio)indol-2'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-

dihydro-3H-benz[e]indoles (190,192,194,196) (Illustrated in Figure 30). Phenol

30 14 (9.0 mg, 0.027 mmol) was treated with anhydrous 3M HCl-Ethylacetate (2 mL) at 24 °C for 30 min. The solvent was removed in vacuo to afford crude unstable 16

(quantitative). A mixture of **16**, [3-(dimethylamino)propyl]ethylcarbodiimide hydrochloride (1-(3-DIMETHYLAMINOPROPYL)-3-ETHYLCARBODIIMIDE HYDROCHLORIDE (EDCI), 10.3 mg, 0.054 mmol, 2.0 equiv), and **182** (9.3 mg, 0.027 mmol, 1.0 equiv) in 0.5 mL of dimethylformamide was stirred at 24 °C under
 5 Ar for 12 h. The solvent was removed in vacuo and the dry residue was mixed with H₂O (3 mL) and saturated aqueous NaCl (2 mL). The mixture was extracted with CH₃CN (5 mL x 3). The organic layer was dried (Na₂SO₄) and concentrated.

Chromatography (SiO₂, *n*-Butanol-H₂O-Ethylacetate-HOAc, 5:5:5:3) afforded **192**

(10.3 mg, 15.2 mg theoretical, 68%) as a pale yellow solid: mp > 152 °C (dec); ¹H

10 NMR (CD₃OD, 400 MHz) 8.22 (d, 1H, *J* = 8.3 Hz, C6-H), 8.02 (d, 1H, *J* = 7.9 Hz, C4'-H), 7.97 (br s, 1H, C4-H), 7.81 (d, 1H, *J* = 8.6 Hz, C6'-H or C9-H), 7.79 (d, 1H, *J* = 8.4 Hz, C6'-H or C9-H), 7.55 (t, 1H, *J* = 8.2 Hz, C8-H), 7.43 (s, 1H, C3'-H), 7.39 (t, 1H, *J* = 8.2 Hz, C7-H), 7.35 (t, 1H, *J* = 8.0 Hz, C5'-H), 4.73-4.77 (m, 1H, C2-H), 4.65 (dd, *J* = 1.7, 11.0 Hz, C2-H), 4.17-4.21 (m, 1H, C1-H), 4.00 (dd, 1H, *J* = 3.1,
 15 11.2 Hz, CHHCl), 3.90 (s, 9H, N(CH₃)₃), 3.69 (apparent t, 1H, *J* = 10.6 Hz, CHHCl); IR (film) _{max} 3354, 1624, 1584, 1466, 1414, 1326, 1259 cm⁻¹; FABHRMS (NBA) *m/e* 434.1648 (M⁺ - Cl, C₂₅H₂₅Cl₂N₃O₂ requires 434.1635). Natural (1*S*)-3: [³] -9.9 (*c* 0.10, CH₃OH).

3-[6'-((Trimethylammonio)indol-2'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-

20 dihydro-3*H*-benz[e]indole (**194**) procedure as above except use **186**: ¹H NMR (CD₃OD, 400 MHz) 8.21 (d, 1H, *J* = 8.3 Hz, C6-H), 7.99 (d, 1H, *J* = 8.8 Hz, C4'-H), 7.87 (br s, 1H, C4-H), 7.80 (d, 1H, *J* = 8.3 Hz, C9-H), 7.65 (dd, 1H, *J* = 2.3, 9.3 Hz, C5'-H), 7.64 (s, 1H, C7'-H), 7.54 (t, 1H, *J* = 8.2 Hz, C8-H), 7.36-7.45 (m, 1H, C7-H), 7.30 (s, 1H, C3'-H), 4.75-4.82 (m, 1H, C2-H), 4.70 (dd, 1H, *J* = 1.8, 10.9 Hz, C2-H), 4.19-4.23 (m, 1H, C1-H), 4.00 (dd, 1H, *J* = 3.2, 11.2 Hz, CHHCl), 3.75 (s,
 25 9H, N(CH₃)₃), 3.69 (dd, 1H, *J* = 3.0, 11.2 Hz, CHHCl); IR (film) _{max} 3373, 1625, 1577, 1558, 1519, 1409, 1342, 1256 cm⁻¹; FABHRMS (NBA) *m/e* 434.1722 (M⁺ - Cl, C₂₅H₂₅Cl₂N₃O₂ requires 434.1714). Natural (1*S*)-4: [³] +53 (*c* 0.04, CH₃OH).

3-[5'-((Trimethylammonio)indol-2'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-

30 dihydro-3*H*-benz[e]indole (**196**) procedure as above except use **188**: ¹H NMR (CD₃OD, 400 MHz) 8.27 (d, 1H, *J* = 2.6 Hz, C4'-H), 8.21 (d, 1H, *J* = 8.3 Hz, C6-

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H), 7.83 (br s, 1H, C4-H), 7.81 (dd, 1H, $J = 2.8, 9.3$ Hz, C6'-H), 7.80 (d, 1H, $J = 8.3$ Hz, C9-H), 7.73 (d, 1H, $J = 9.2$ Hz, C7'-H), 7.53 (t, 1H, $J = 8.2$ Hz, C8-H), 7.37 (t, 1H, $J = 8.4$ Hz, C7-H), 7.33 (s, 1H, C3'-H), 4.68-4.76 (m, 2H, partially obscured by H₂O, C2-H₂), 4.17-4.21 (m, 1H, C1-H), 3.99 (dd, 1H, $J = 3.2, 11.2$ Hz, CHHCl), 3.73
 5 (s, 9H, N(CH₃)₃), 3.65 (dd, 1H, $J = 8.8, 11.2$ Hz, CHHCl); IR (film) ν_{\max} 3374, 1557, 1416, 1342, 1265, 1232, 758 cm⁻¹; FABHRMS (NBA) m/e 434.1619 (M⁺ - Cl, C₂₅H₂₅Cl₂N₃O₂ requires 434.1635). Natural (1*S*)-5: [α]³ +64 (c 0.10, CH₃OH).

DNA Alkylation Studies of 2-5: Selectivity and Efficiency. Eppendorf tubes containing singly ³²P 5'-end-labeled double-stranded DNA¹⁰ (9 μ L) in TE buffer (10
 10 mM Tris, 1 mM EDTA, pH 7.5) were treated with the agents 190-196 in DIMETHYLSULFOXIDE (1 μ L, at the specified concentrations). The solutions were mixed by vortexing and brief centrifugation and subsequently incubated at 4 °C for 24 h. The covalently modified DNA was separated from unbound agent by Ethanol precipitation of the DNA. The Ethanol precipitations were carried out by adding *t*-
 15 RNA as a carrier (1 μ L, 10 μ g/ μ L), a buffer solution containing salt (0.1 volume, 3 M NaOAc in TE) and -20 °C Ethanol (2.5 volumes). The solutions were mixed and chilled at -78 °C in a REVCO freezer for 1 h or longer. The DNA was reduced to a pellet by centrifugation at 4 °C for 15 min, washed with -20 °C 70% Ethanol (in TE containing 0.2 M NaCl) and recentrifuged briefly. The pellets were dried in a Savant
 20 Speed Vac concentrator and resuspended in TE buffer (10 μ L). The solutions of alkylated DNA were warmed at 100 °C for 30 min to induce cleavage at the adenine N3 alkylation sites. After brief centrifugation, formamide dye solution (5 μ L) was added. Prior to electrophoresis, the samples were denatured by warming at 100 °C for 5 min, placed in an ice bath, centrifuged briefly, and the supernatant (2.8 μ L) was
 25 loaded onto a gel. Sanger dideoxynucleotide sequencing reactions were run as standards adjacent to the agent treated DNA reaction samples. Polyacrylamide gel electrophoresis (PAGE) was run on an 8% sequencing gel under denaturing conditions (19:1 acrylamide: N,N'-methylenebisacrylamide, 8 M urea) in TBE buffer (100 mM Tris, 100 mM boric acid, 0.2 mM Na₂EDTA). PAGE was pre-run for 30 min with
 30 formamide dye solution prior to loading the samples. Autoradiography of dried gels was carried out at -78 °C using Kodak X-Omat AR film and a Picker Spectra™

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PCT/US96/16481

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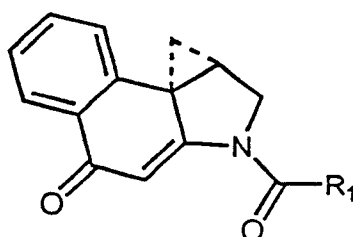
intensifying screen.

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What is claimed:

1. A compound represented by the following structure:

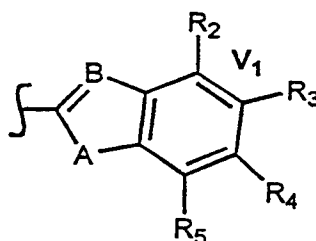
5



wherein

R_1 is selected from the group consisting of $-\text{CH}_2\text{CH}_3$ (alkyl), $-\text{NHCH}_3$ ($-\text{N}$ -alkyl), $-\text{OCH}_3$ (O -alkyl), $-\text{NH}_2$, $-\text{NHNH}_2$, $-\text{NHNHCO}_2^t\text{Bu}$, and a radical represented by the following structure:

10



wherein:

A is selected from the group consisting of NH and O;

15

B is selected from the group consisting of C and N;

R_2 is selected from the group consisting of hydrogen, hydroxyl, O -alkyl (C1-C6), N -alkyl (C1-C6), and a first N -substituted pyrrolidine ring;

20

R_3 is selected from the group consisting of hydrogen, hydroxyl, O -alkyl (C1-C6), N -alkyl

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(C1-C6)₃, the first N-substituted pyrrolidine ring;

R₄ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), and N-alkyl (C1-C6)₃;

R₅ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), and N-alkyl (C1-C6)₃; and

V₁ represents a first vinylene group between R₂ and R₃;

with the following provisos:

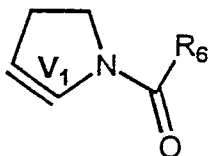
if R₂ participates in the first N-substituted pyrrolidine ring, then R₃ also participates in the first N-substituted pyrrolidine ring;

if R₃ participates in the first N-substituted pyrrolidine ring, then R₂ also participates in the first N-substituted pyrrolidine ring;

if R₂ and R₃ participate in the first N-substituted pyrrolidine ring, then R₄ and R₅ are hydrogen;

if R₂ is hydrogen, then R₄ and R₅ are hydrogen and R₃ is N-alkyl (C1-C6)₃; and

wherein the first N-substituted pyrrolidine ring is fused to the first vinylene group between R₂ and R₃ and is represented by the following structure:



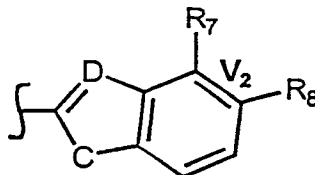
wherein:

V₁ represents the first vinylene group between R₂

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and R_3 ;

R_6 is selected from the group consisting of $-\text{CH}_2\text{CH}_3$ (alkyl), $-\text{NHCH}_3$ ($-\text{N-alkyl}$), $-\text{OCH}_3$ (O-alkyl), $-\text{NH}_2$, $-\text{NHNH}_2$, $-\text{NHNHCO}_2^t\text{Bu}$, and a radical represented by the following structure:



wherein:

C is selected from the group consisting of NH and O;

D is selected from the group consisting of C and N;

R_7 is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, and a second N-substituted pyrrolidine ring;

R_8 is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, the second N-substituted pyrrolidine ring;

V_2 represents the second vinylene group between R_7 and R_8 ;

with the following provisos:

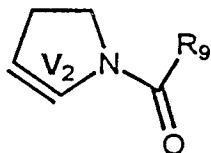
if R_7 participates in the N-substituted pyrrolidine ring, then R_8 also participates in the N-substituted pyrrolidine ring;

if R_8 participates in the N-substituted pyrrolidine ring only if R_7 also participates in the N-substituted pyrrolidine ring; and

wherein the second N-substituted pyrrolidine ring is fused to the second vinylene group between R_7 and R_8 and is represented by the following

- 90 -

structure:



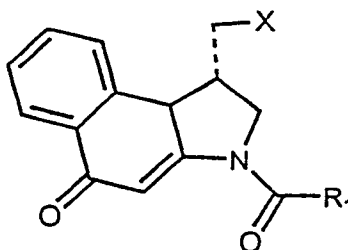
5 wherein:

V₂ represents the second vinylene group between R₇ and R₈;

10 R₉ is selected from the group consisting of -CH₂CH₃ (alkyl), -NHCH₃ (-N-alkyl), -OCH₃ (O-alkyl), -NH₂, -NHNH₂, and -NHNHCO₂^tBu.

- 91 -

2. A compound represented by the following structure:

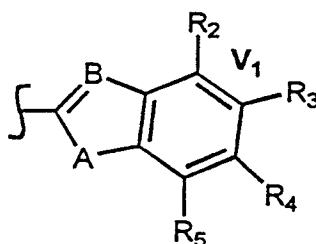


wherein

5 X is selected from the group consisting of chlorine, bromine, iodine, and OTOS; and

R₁ is selected from the group consisting of -CH₂CH₃ (alkyl), -NHCH₃ (-N-alkyl), -OCH₃ (O-alkyl), -NH₂, -NHNH₂, -NHNHCO₂^tBu, and a radical

10 represented by the following structure:



wherein:

A is selected from the group consisting of NH and O;

15 B is selected from the group consisting of C and N;

R₂ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6), and a first N-substituted pyrrolidine ring;

20 R₃ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl

- 92 -

(C1-C6)₃, the first N-substituted pyrrolidine ring;

R₄ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), and N-alkyl (C1-C6)₃;

R₅ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), and N-alkyl (C1-C6)₃; and

V₁ represents a first vinylene group between R₂ and R₃;

with the following provisos:

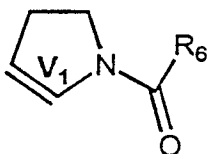
if R₂ participates in the first N-substituted pyrrolidine ring, then R₃ also participates in the first N-substituted pyrrolidine ring;

if R₃ participates in the first N-substituted pyrrolidine ring, then R₂ also participates in the first N-substituted pyrrolidine ring;

if R₂ and R₃ participate in the first N-substituted pyrrolidine ring, then R₄ and R₅ are hydrogen;

if R₂ is hydrogen, then R₄ and R₅ are hydrogen and R₃ is N-alkyl (C1-C6)₃; and

wherein the first N-substituted pyrrolidine ring is fused to the first vinylene group between R₂ and R₃ and is represented by the following structure:



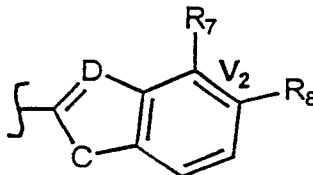
wherein:

V₁ represents the first vinylene group between R₂

- 93 -

and R₃;

R₆ is selected from the group consisting of -CH₂CH₃ (alkyl), -NHCH₃ (-N-alkyl), -OCH₃ (O-alkyl), -NH₂, -NHNH₂, -NHNHCO₂^tBu, and a radical represented by the following structure:



wherein:

C is selected from the group consisting of NH and O;

D is selected from the group consisting of C and N;

R₇ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, and a second N-substituted pyrrolidine ring;

R₈ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, the second N-substituted pyrrolidine ring;

V₂ represents the second vinylene group between R₇ and R₈;

with the following provisos:

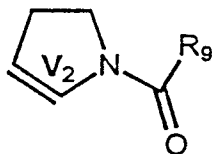
if R₇ participates in the N-substituted pyrrolidine ring, then R₈ also participates in the N-substituted pyrrolidine ring;

if R₈ participates in the N-substituted pyrrolidine ring only if R₇ also participates in the N-substituted pyrrolidine ring; and

wherein the second N-substituted pyrrolidine ring is fused to the second vinylene group between R₇ and R₈ and is represented by the following

- 94 -

structure:

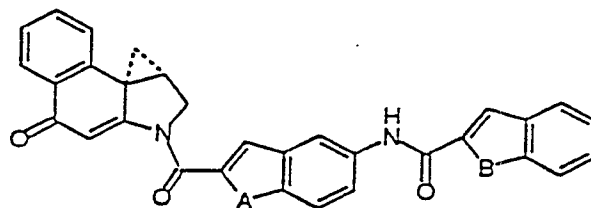


5 wherein:

V_2 represents the second vinylene group between R_7 and R_8 ;

R_9 is selected from the group consisting of $-CH_2CH_3$ (alkyl), $-NHCH_3$ ($-N$ -alkyl), $-OCH_3$ (O -alkyl), $-NH_2$, $-NHNH_2$, and $-NHNHCO_2^tBu$.

3. A compound represented by the following structure:



57-61

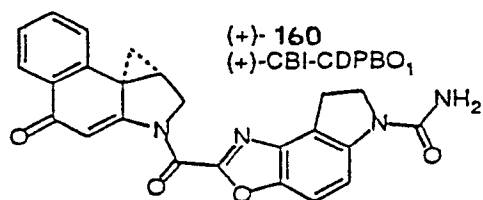
wherein A is selected from the group consisting of NH and O and B is selected from the group consisting of NH, O, and S.

- 95 -

4. A compound compound represented by the following structure:

5

62

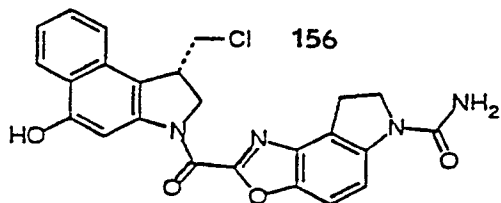


10

5. A compound compound represented by the following structures:

15

63



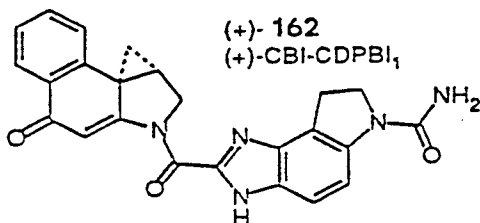
20

6. A compound compound represented by the following structure:

25

30

64

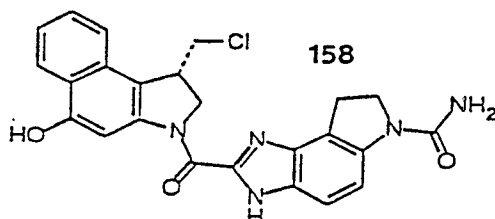


- 96 -

7. A compound compound represented by the following structure:

5

65

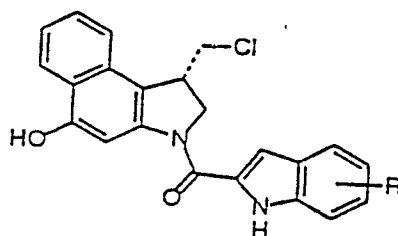


10

8. A compound compound represented by the following structure:

15

66-68



20

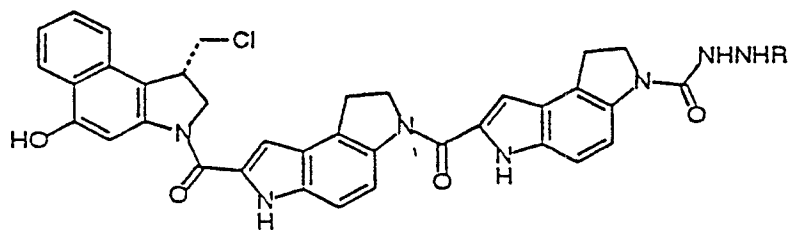
where R is selected from the group comprising of: H, 5-NMe₃⁺, 6-NMe₃⁺, 7-NMe₃⁺.

9. A compound compound represented by the following structure:

25

78, 79

30



where R is selected from the group comprising of:
CO₂^tBu, H-HCl.

35

WO 97/12862

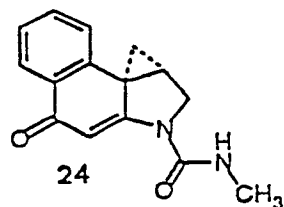
PCT/US96/16481

- 97 -

10. A compound compound represented by the following structure:

5

21

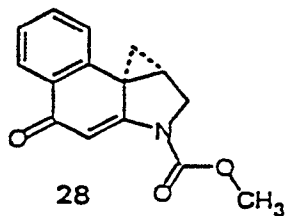


10

11. A compound compound represented by the following structure:

15

22

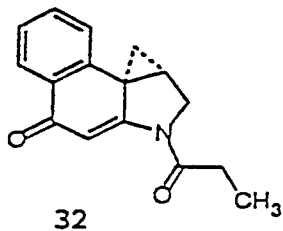


20

12. A compound compound represented by the following structure:

25

23



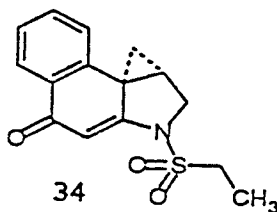
30

- 98 -

13. A compound compound represented by the following structure:

5

24

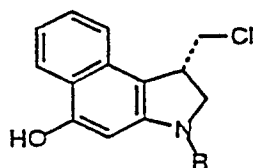


10

14. A compound compound represented by the following structure:

15

16, 18-20



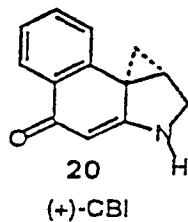
20

where R is selected from the group comprising of: H-HCl, CONHMe, CO₂CH₃, COEt.

15. A compound compound represented by the following structure:

30

17



35

WO 97/12862

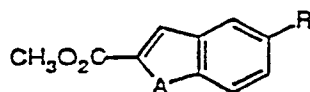
PCT/US96/16481

- 99 -

16. A compound compound represented by the following structure:

5

36



10

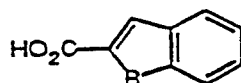
wherein A is selected from the group consisting of O and R is selected from the group consisting of NO₂ and NH₂.

15

17. A compound compound represented by the following structure:

20

38, 39



25

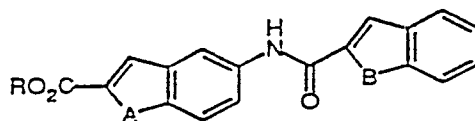
wherein B is selected from the group consisting of O and S.

- 100 -

18. A compound compound represented by the following structure:

5

41-51



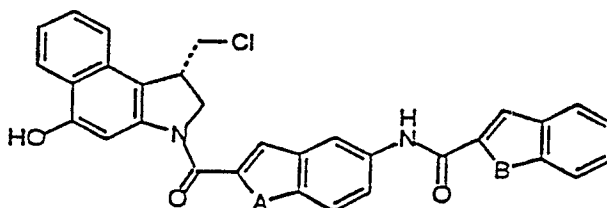
10 wherein A is selected from the group consisting of NH and O and B is selected from the group consisting of NH, O, and S and R is selected from the group consisting of H and CH₃.

15

19. A compound compound represented by the following structure:

20

52-56



25 wherein A is selected from the group consisting of NH and O and B is selected from the group consisting of NH, O, and S.

WO 97/12862

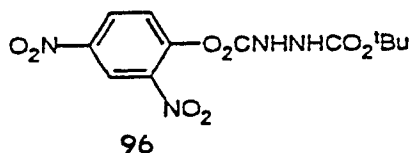
PCT/US96/16481

- 101 -

20. A compound compound represented by the following structure:

5

71

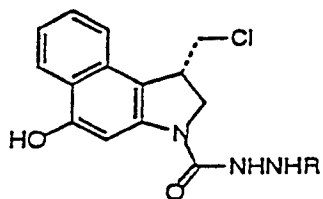


21. A compound compound represented by the following structure:

10

72-73

15



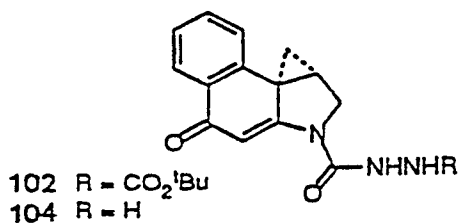
where R is selected from the group comprising of:
CO₂^tBu, H-HCl.

20

22. A compound compound represented by the following structure:

25

74-75



where R is selected from the group comprising of:
CO₂^tBu, H.

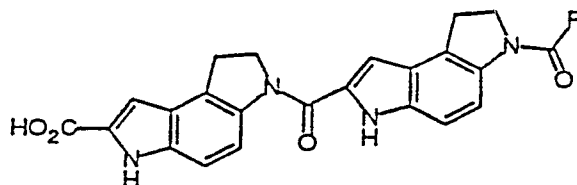
30

- 102 -

23. A compound compound represented by the following structure:

5

76-77

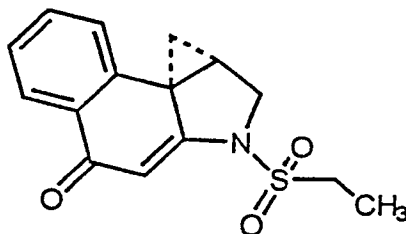


10

where R is selected from the group comprising of: O^tBu ,
 $NHNHCO_2^tBu$.

15

24. A compound compound represented by the following structure:



1 / 34

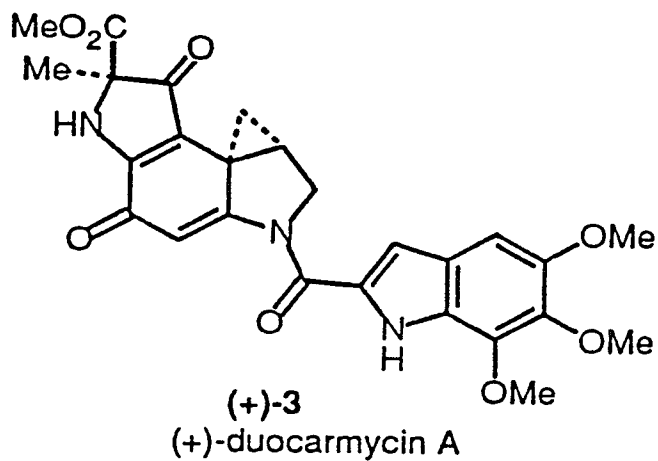
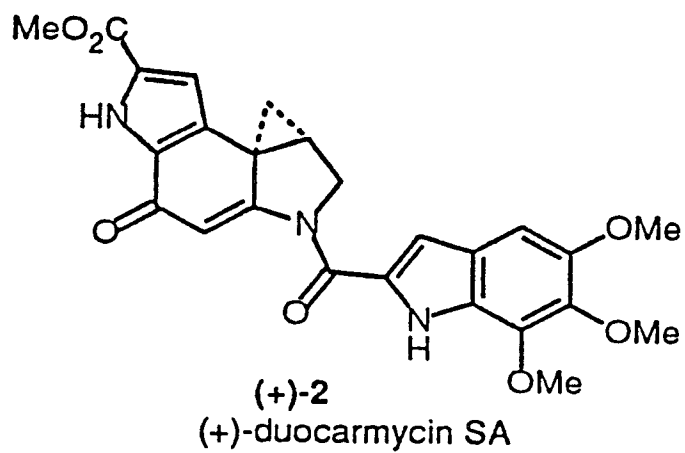
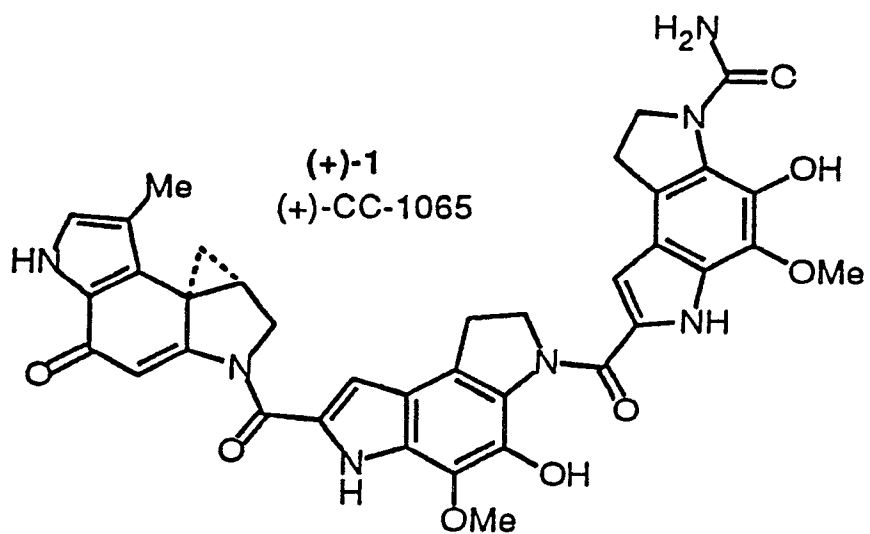
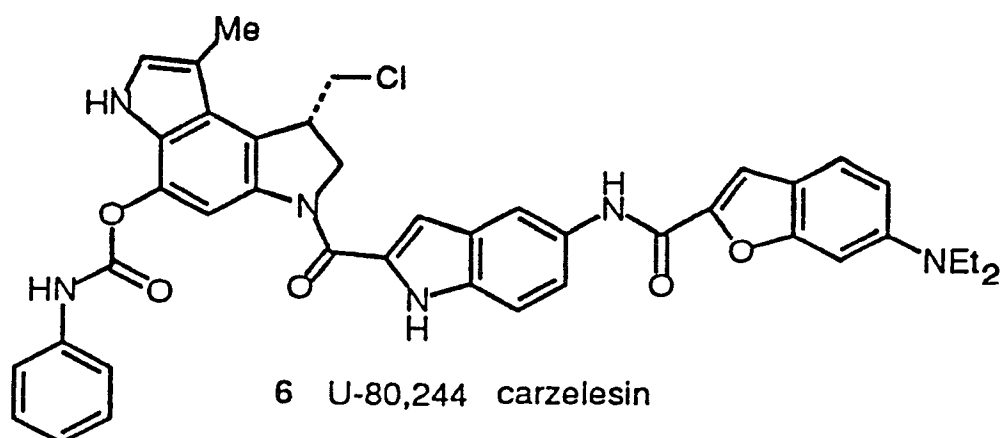


FIG. 1
SUBSTITUTE SHEET (RULE 26)

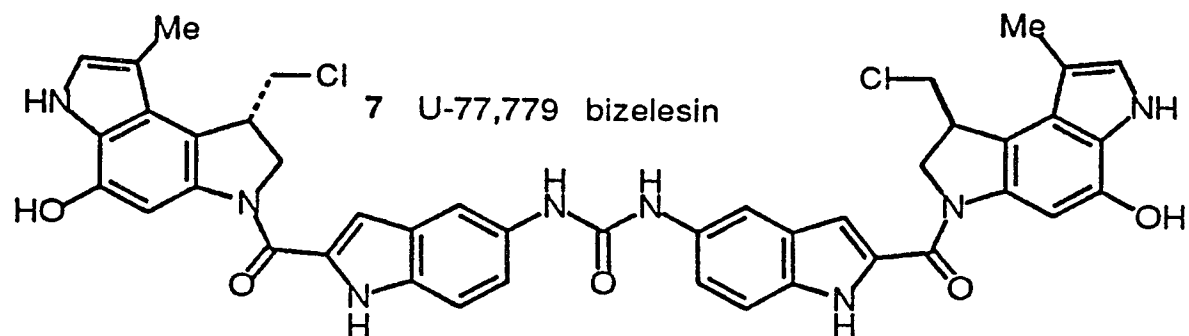
2 / 34

The chemical structure shows a tryptophan derivative (left) linked to a tryptophan derivative (middle) via an amide bond, which is further linked to a tryptophan derivative (right) via another amide bond. The tryptophan derivative on the left has a methyl group (Me) at the 5-position and a hydrogen atom (HN) at the 1-position. The tryptophan derivative in the middle has a hydrogen atom (NH) at the 1-position. The tryptophan derivative on the right has a substituent X at the 3-position. The structure is labeled 2 / 34.

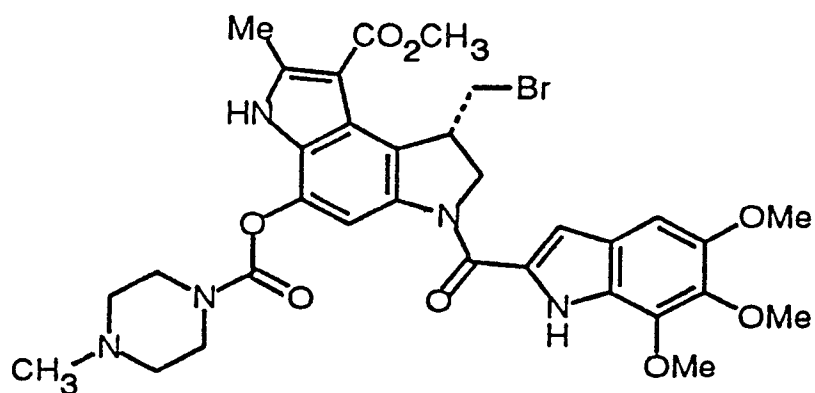
4 X = NH U-71,184
5 X = O U-73,975 adozelesin



6 U-80,244 carzelesin



7 U-77,779 bizelesin



8 KW-2189 **FIG.2**
SUBSTITUTE SHEET (RULE 26)

3 / 34

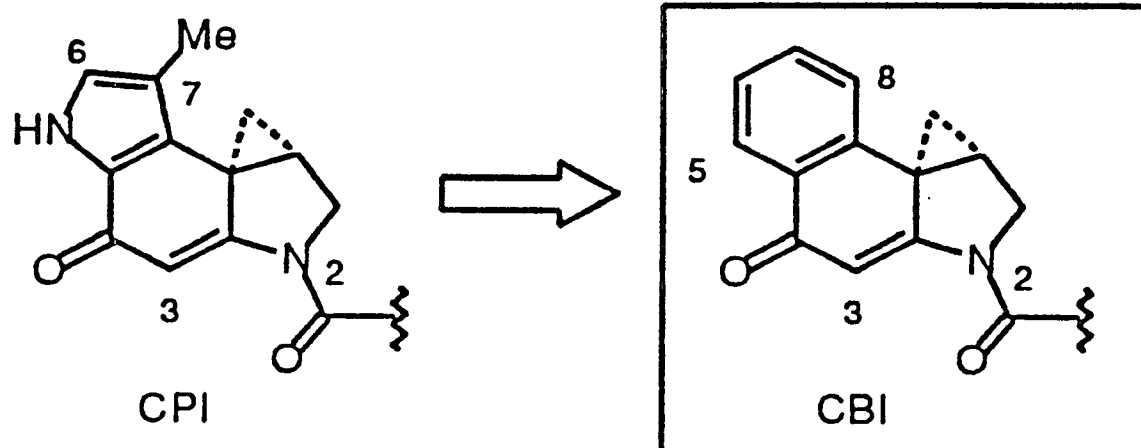
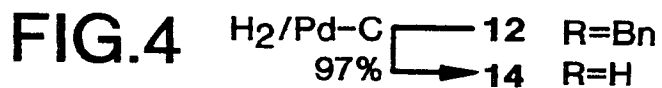
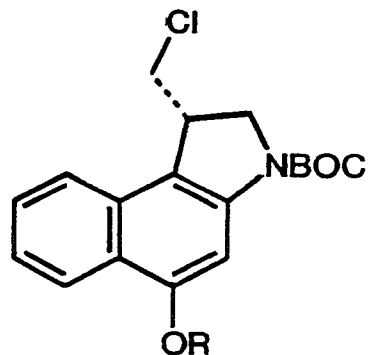
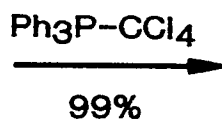
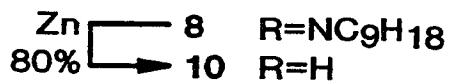
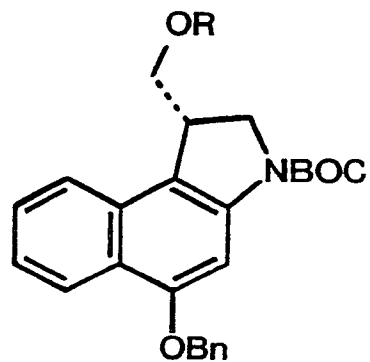
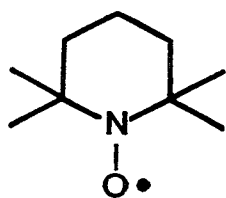
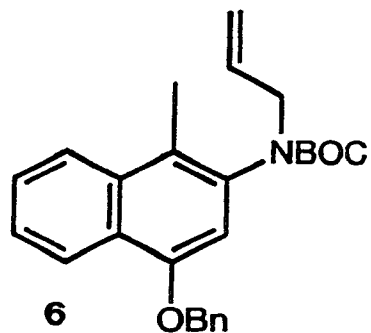
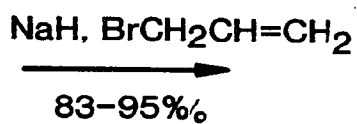
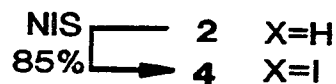
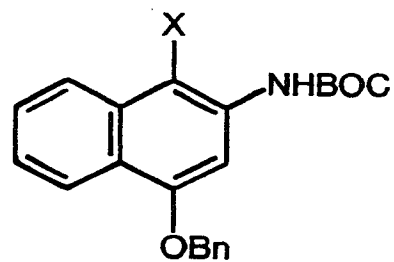
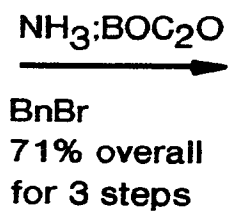
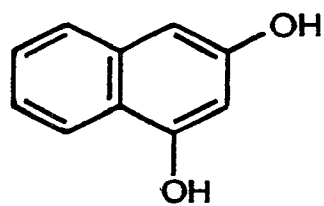


FIG.3

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5/34

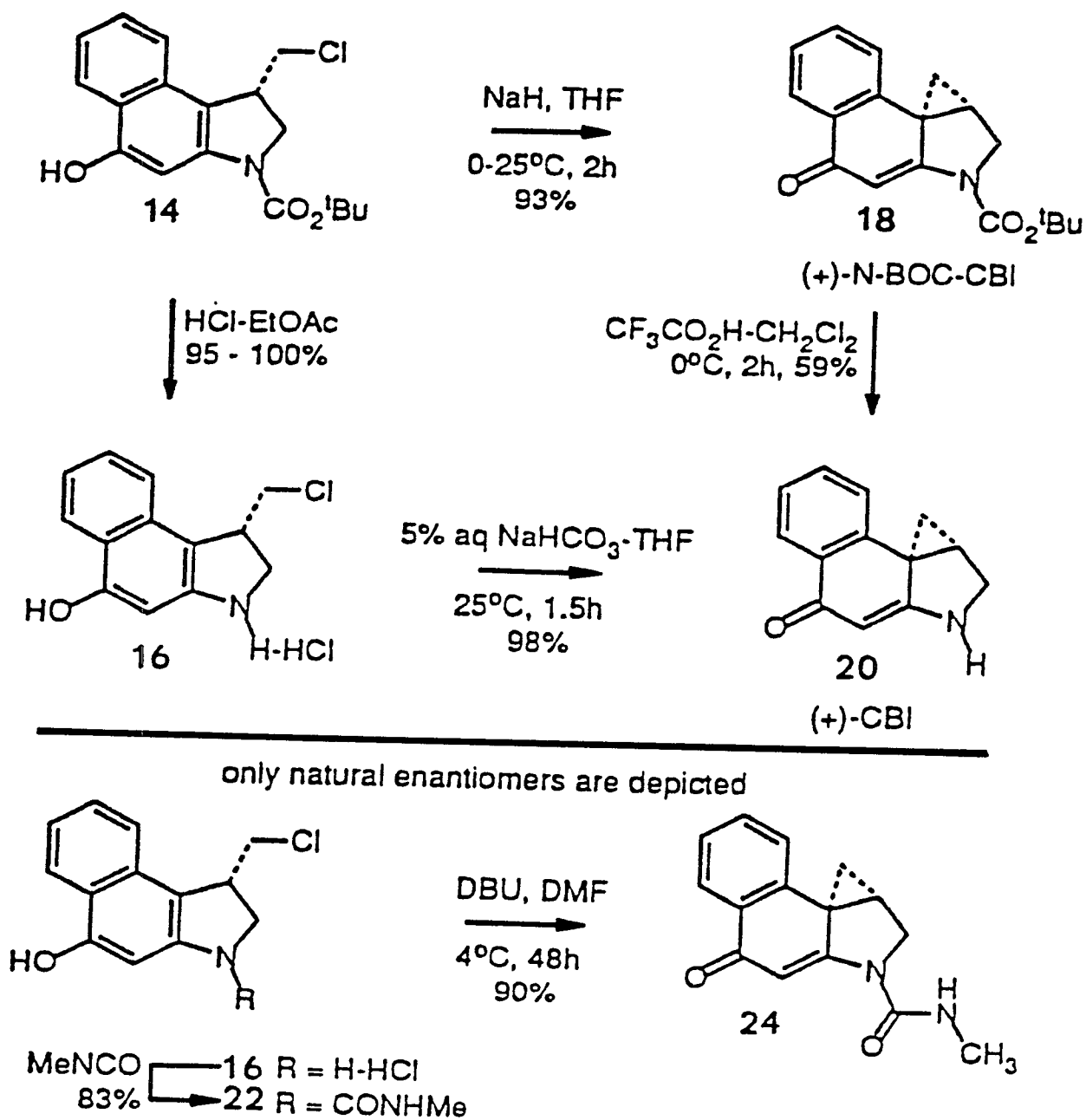


FIG.5A

6 / 34

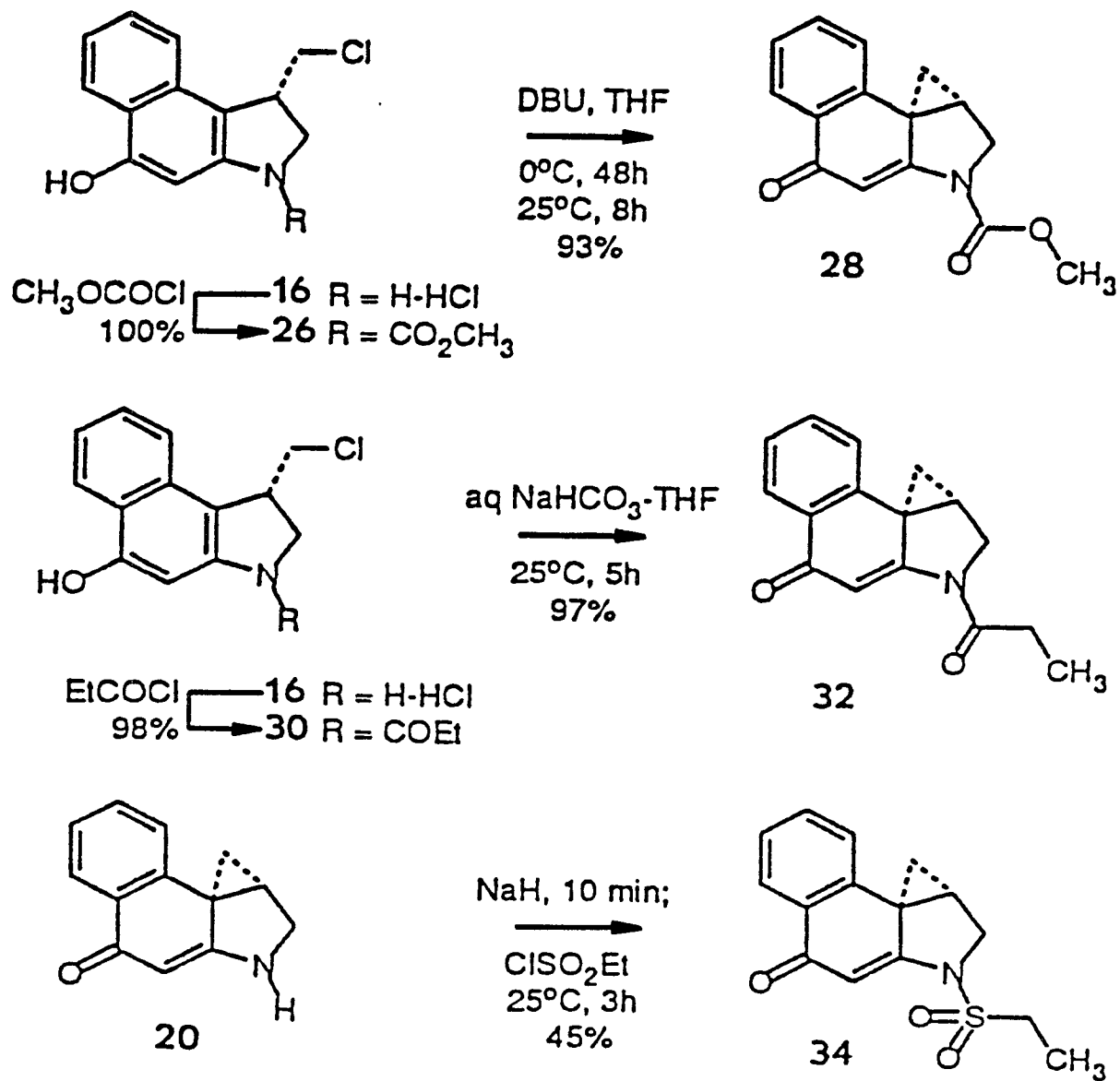
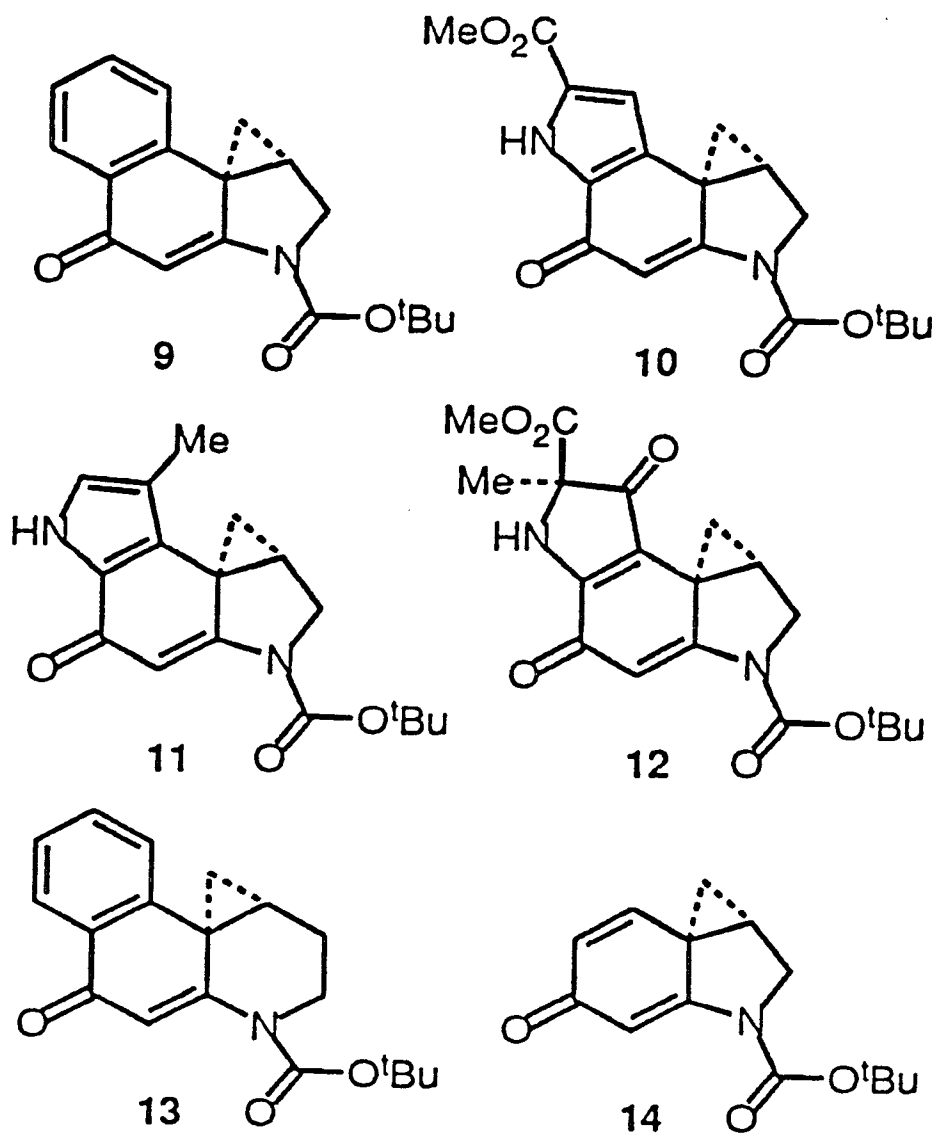


FIG.5B

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	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

FIG.6

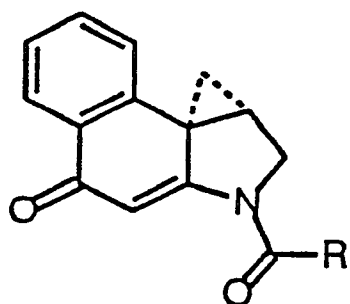
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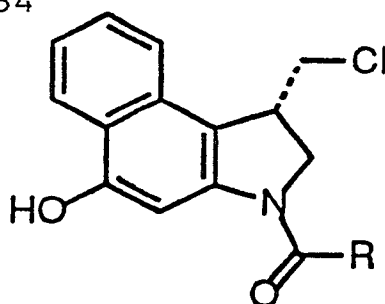
Agent	Configuration	IC ₅₀ (L1210, nM)
9, (+)-N-BOC-CBI	natural	80
9, (-)-N-BOC-CBI	unnatural	1000
11, (+)-N-BOC-CPI	natural	330
17, (+)-CBI	natural	
17, (-)-CBI	unnatural	
(+)-15	natural	80
(-)-15	unnatural	1000
(+)-21	natural	200
(+)-22	natural	140
(+)-23	natural	110
(+)-24	natural	25
(-)-21	unnatural	
(-)-22	unnatural	
(-)-23	unnatural	
(-)-24	unnatural	
18	natural	200
18	unnatural	
19	natural	140
19	unnatural	
20	natural	110
20	unnatural	

FIG.7

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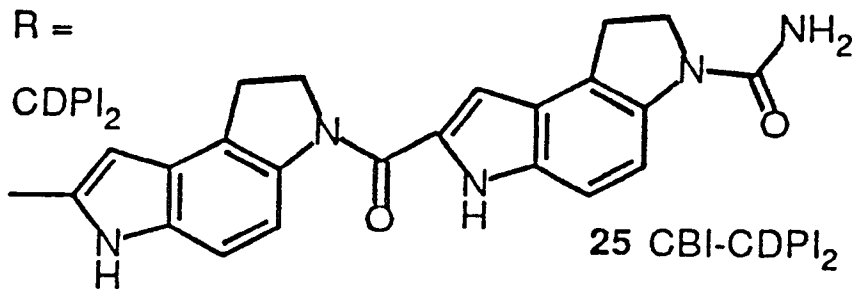
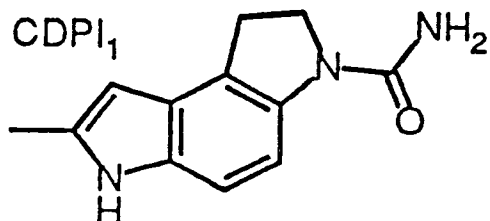
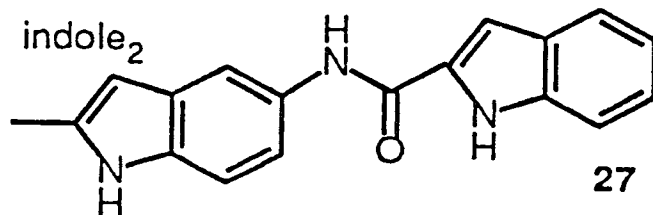
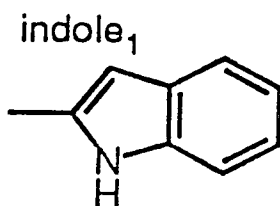
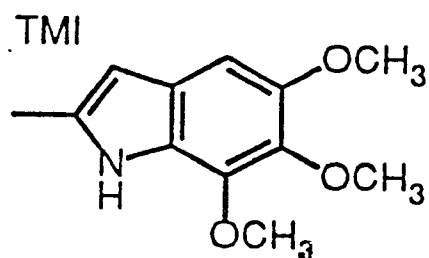
25 - 29



30 - 34

only natural enantiomers depicted

R =

25 CBI-CDPI₂26 CBI-CDPI₁27 CBI-indole₂28 CBI-indole₁

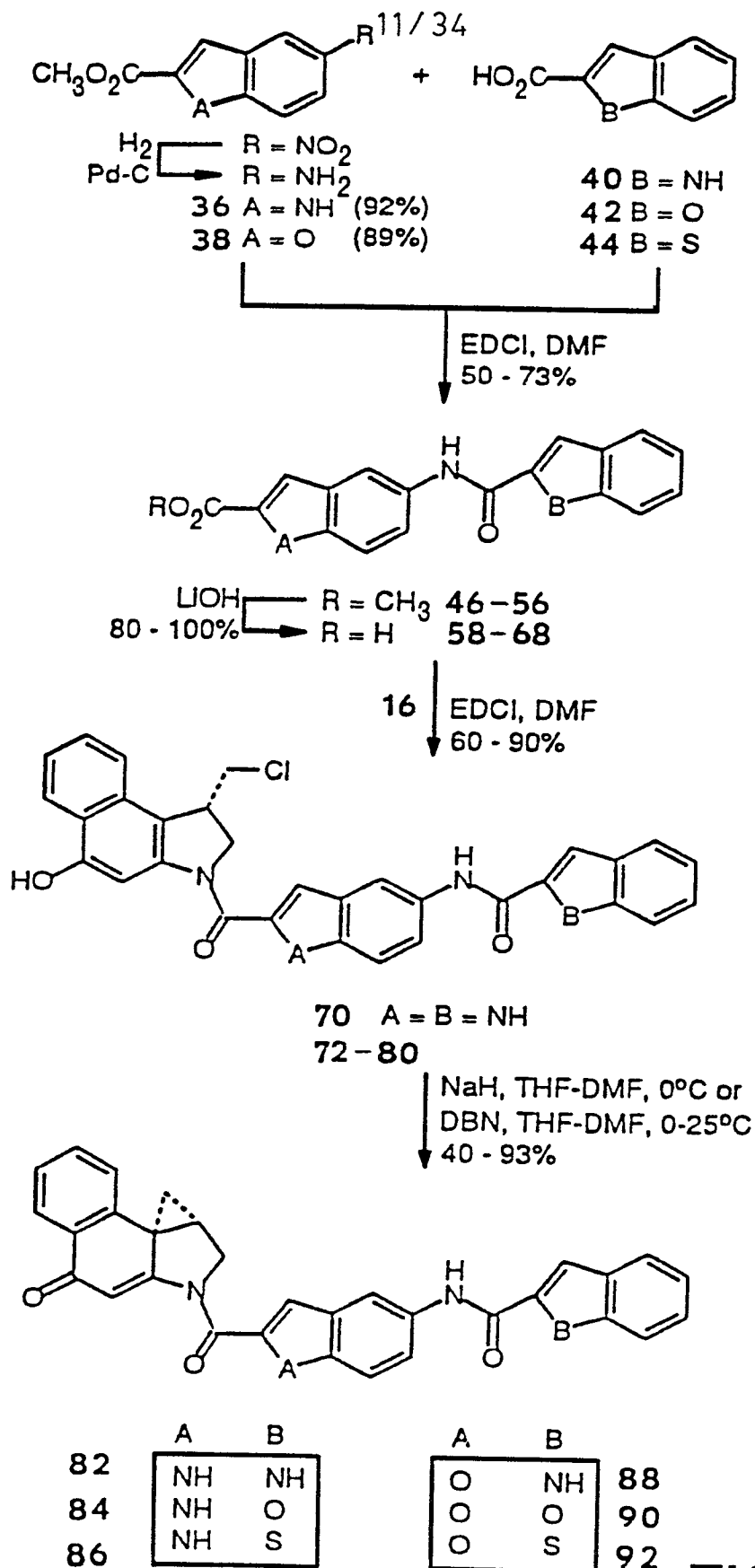
29 CBI-TMI

FIG.8

10/34

FIG.9

Agent	Configuration	IC ₅₀ (L1210, pM)
1, (+)-CC-1065	natural	20
1, <i>ent</i> -(-)-CC-1065	unnatural	20
2, (+)-duocarmycin SA	natural	10
2, <i>ent</i> -(-)-duocarmycin SA	unnatural	100
3, (+)-duocarmycin A	natural	500
3, <i>ent</i> -(-)-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	?
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



only natural enantiomers are depicted

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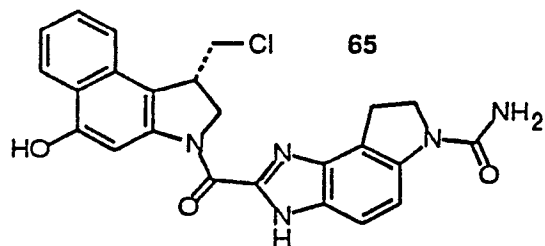
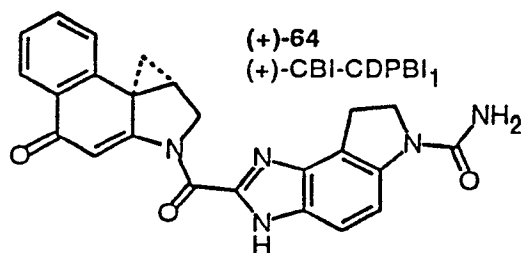
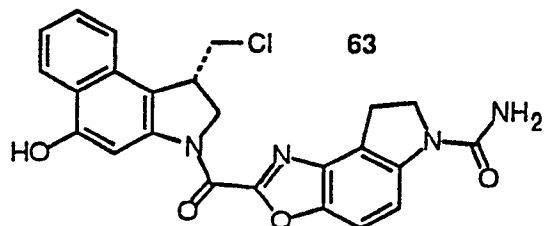
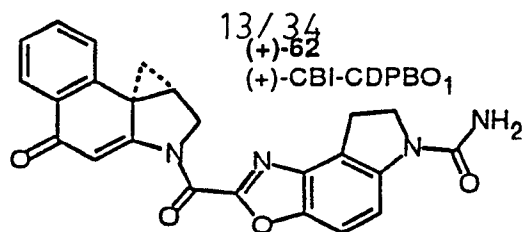
FIG. 10

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Agent	Configuration	IC ₅₀ (L1210, pM)
(+)-CPI-indole ₂	natural	40
(-)-CPI-indole ₂	unnatural	1000 ^x Upjohn Value
	<u>A</u>	<u>B</u>
(+)-27	NH	NH
(+)-57	NH	O
(+)-58	NH	S
(+)-59	O	NH
(+)-60	O	O
(+)-61	O	S
(-)-27	NH	NH
(-)-57	NH	O
(-)-58	NH	S
(-)-59	O	NH
(-)-60	O	O
(-)-61	O	S
		? 300 Upjohn Value
	unnatural	30,000
	unnatural	15,000 ^b
	unnatural	NT ^c
	unnatural	1300
	unnatural	1200 ^b

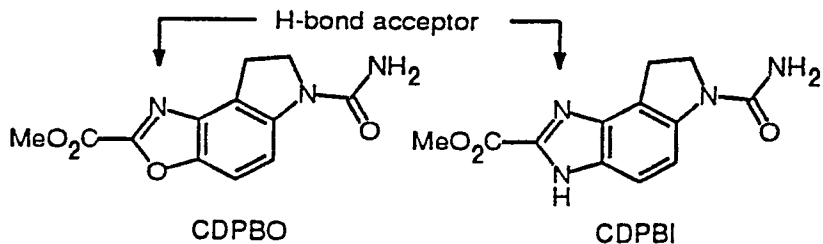
^aThe corresponding CPI analogs of 27, 57, 59, and 60 exhibited IC₅₀ values of 40, 40, 30, and 30 pM, respectively. ^bTested as the seco derivative 53 or 56. ^cNot tested.

FIG.11



only natural enantiomers are depicted

PDE-I, CDPI



H-bond donor

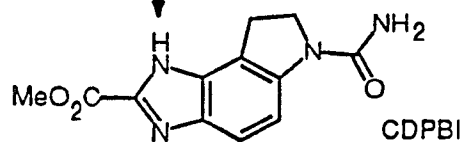


FIG. 12

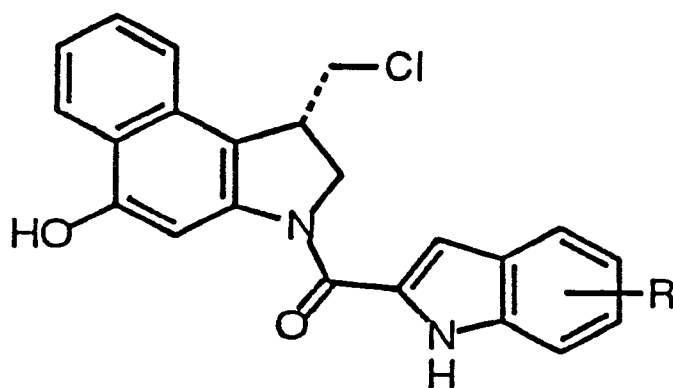
SUBSTITUTE SHEET (RULE 26)

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Agent	Configuration	IC ₅₀ (L1210, pM)
26, (+)-CBI-CDPI ₁	natural	5
26, (-)-CBI-CDPI ₁	unnatural	380
62, (+)-CBI-CDPBO ₁	natural	200
62, (-)-CBI-CDPBO ₁	unnatural	17000
64, (+)-CBI-CDPBI ₁	natural	200
64, (-)-CBI-CDPBI ₁	unnatural	2000
63,	natural	200
63,	unnatural	17000
65,	natural	200
65,	unnatural	2000
1, (+)-CC-1065	natural	20
1, (-)-CC-1065	unnatural	20
2, (+)-duocarmycin SA	natural	10
2, (-)-duocarmycin SA	unnatural	100

FIG.13

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33 R = H

66 R = 5-NMe₃⁺

67 R = 6-NMe₃⁺

68 R = 7-NMe₃⁺

FIG. 14

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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

^aRelative efficiency for alkylation of w794 DNA (4 °C, 24 h)**FIG. 15**

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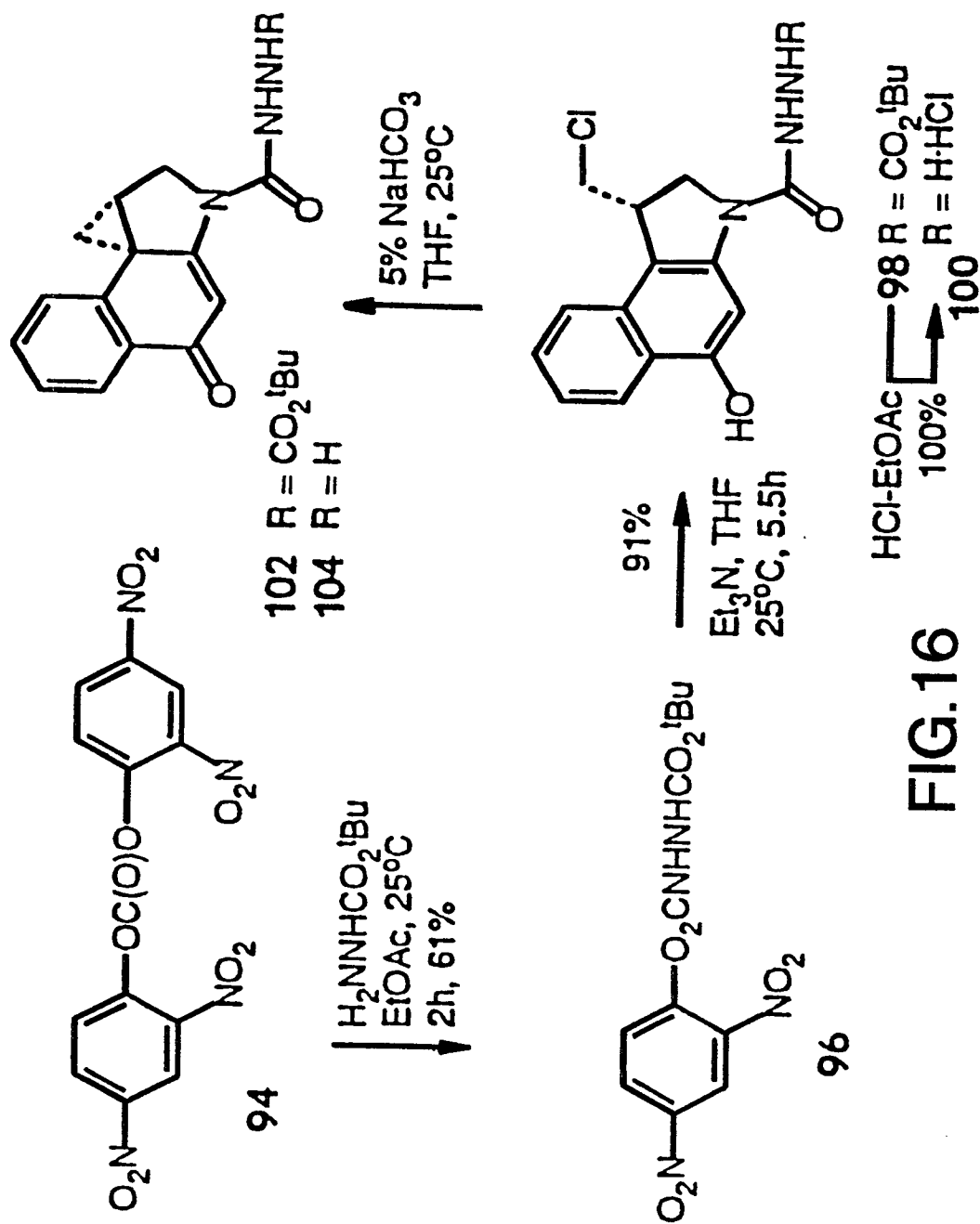


FIG.16

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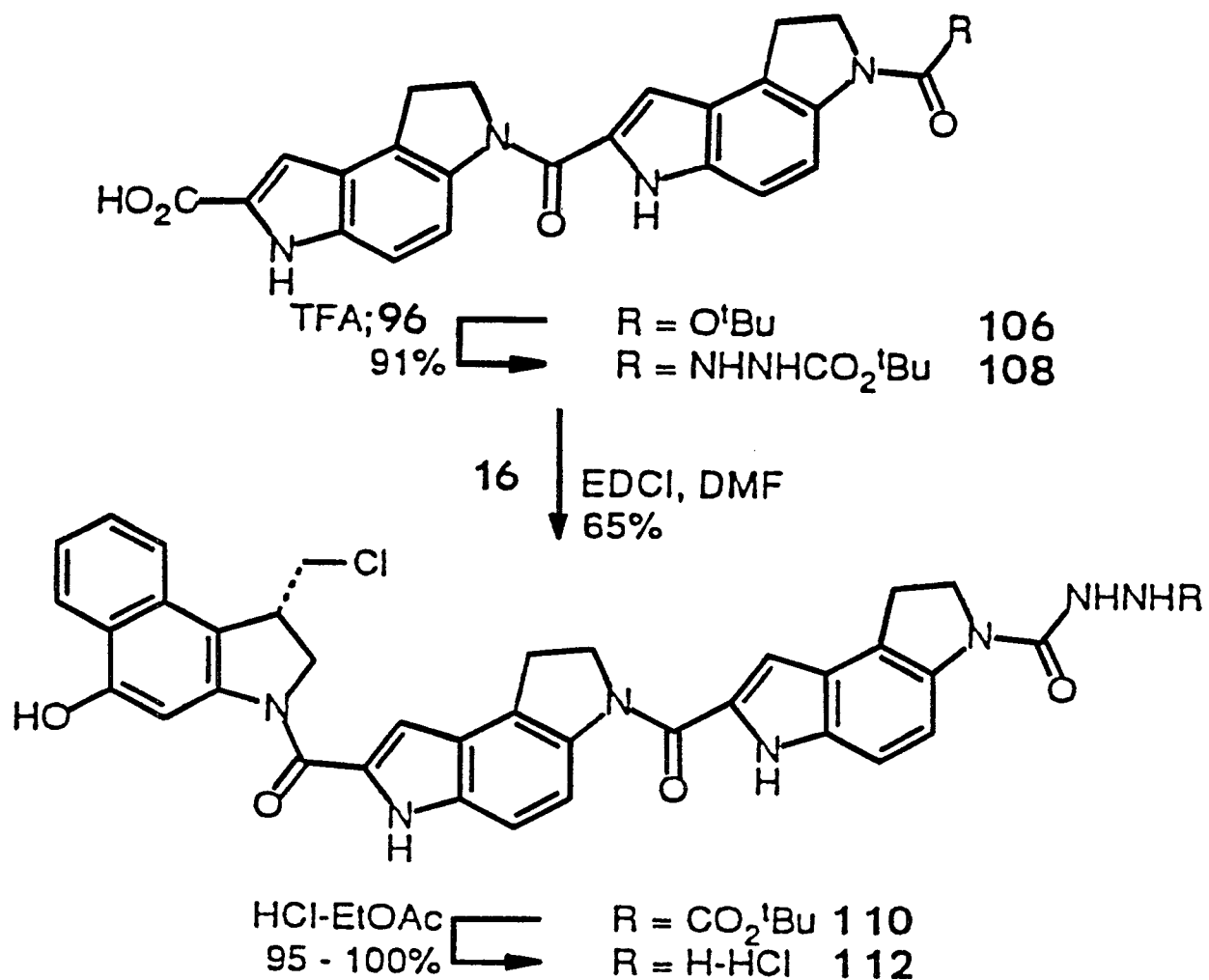


FIG. 17

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Agent	IC ₅₀ (L1210, nM)	rel DNA alkylation ^a
9, (+)- <i>N</i> -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

^aRelative efficiency of alkylation of w794 DNA; 37 °C, 24 h, reference 31.

FIG.18

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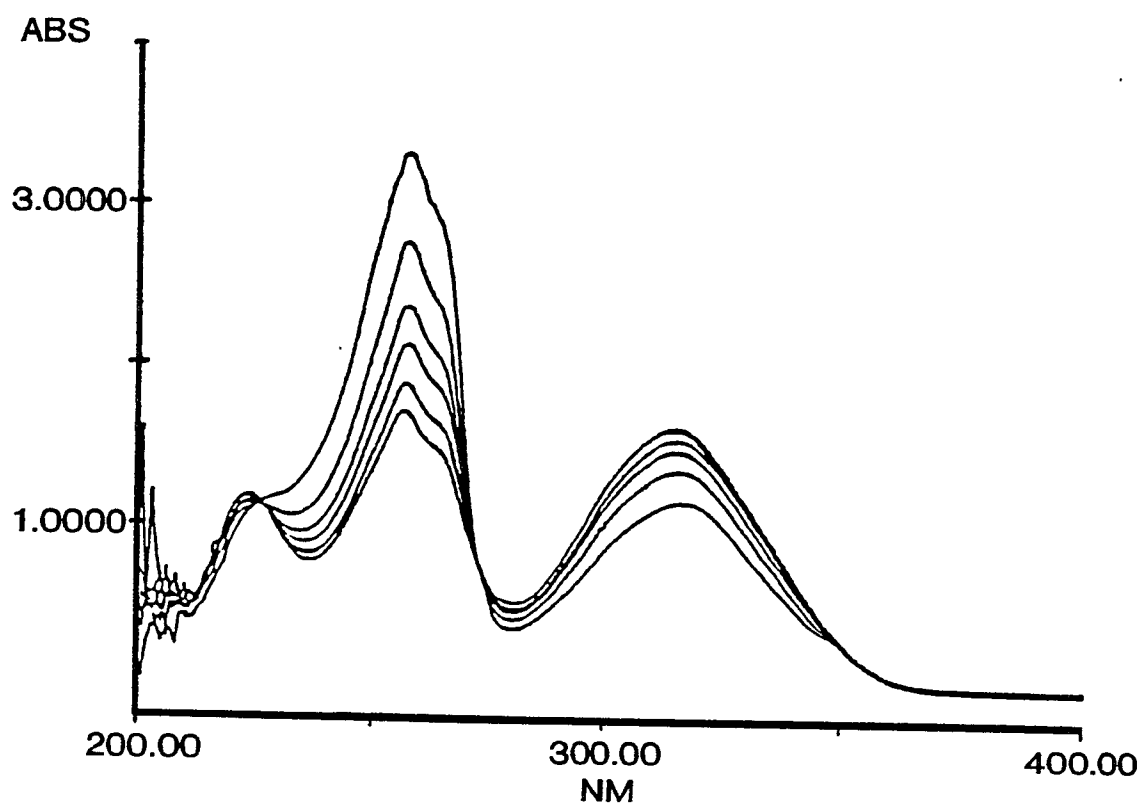


FIG. 19A

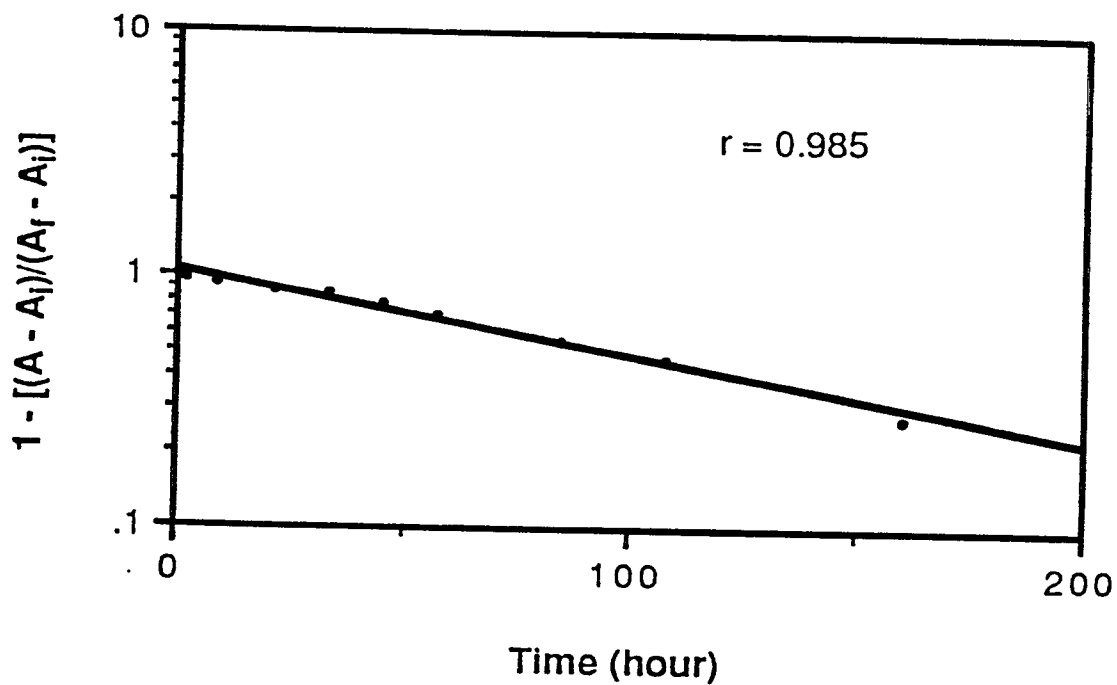
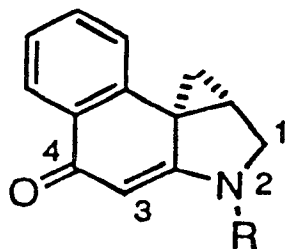


FIG. 19B

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R	k (s ⁻¹ , pH 3)	t _{1/2}	IC ₅₀ (L1210)	sigma
21 CONHMe	5.4 x 10 ⁻⁶	36 h	200 nM	0.36
22 CO ₂ Me	3.4 x 10 ⁻⁶	57 h	140 nM	0.45
23 COEt	2.0 x 10 ⁻⁶	96 h	110 nM	0.48
24 SO ₂ Et	0.5 x 10 ⁻⁶	383 h	24 nM	0.72

FIG.20A

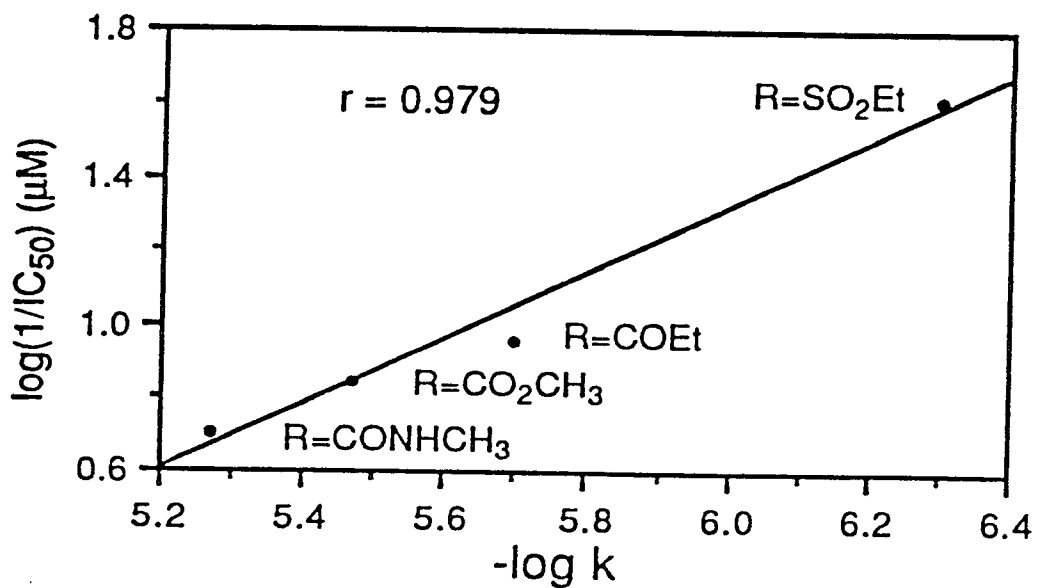


FIG.20B

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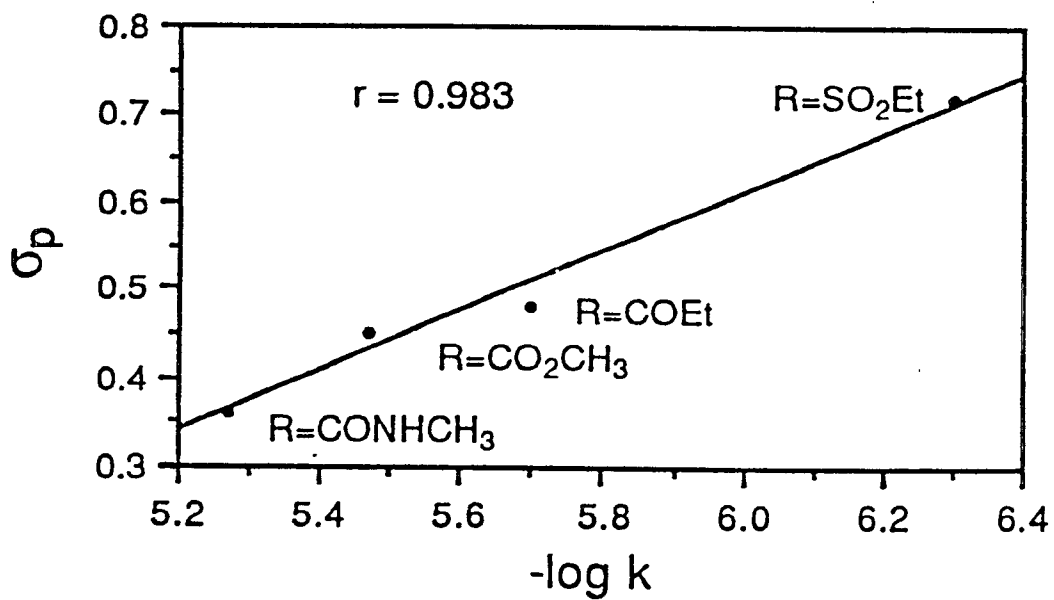


FIG.20C

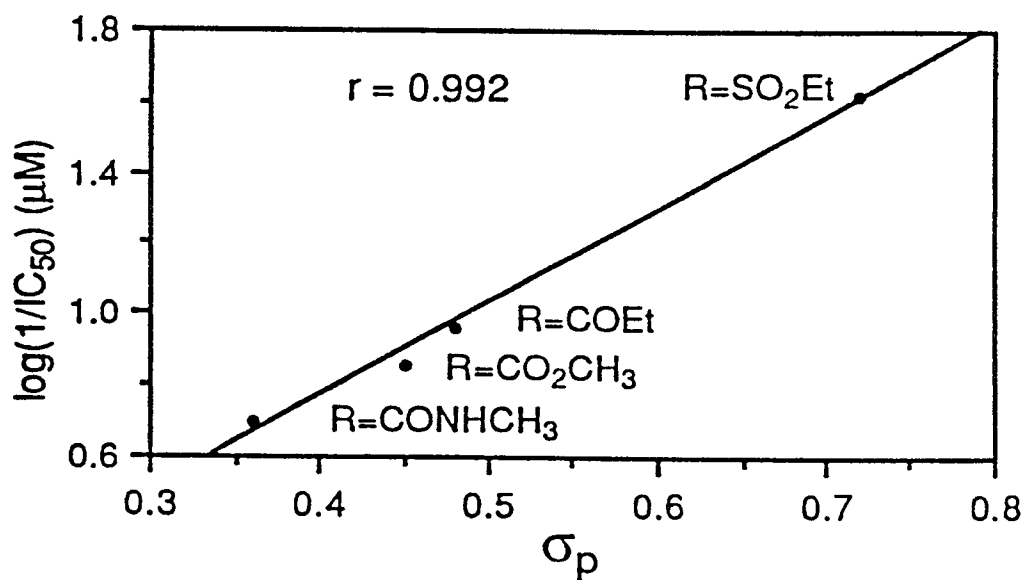


FIG.20D

WO 97/12862

PCT/US96/16481

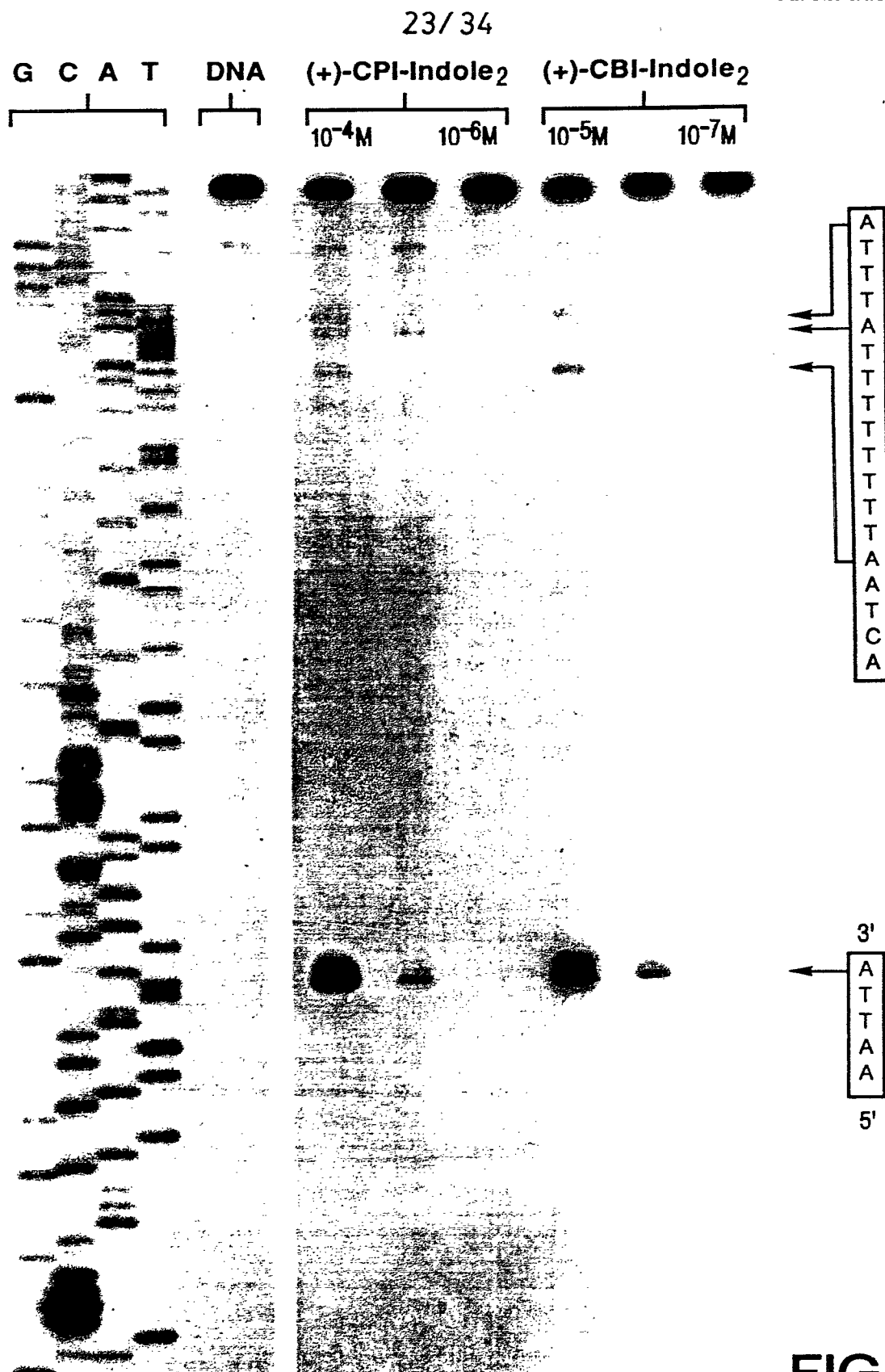


FIG.21

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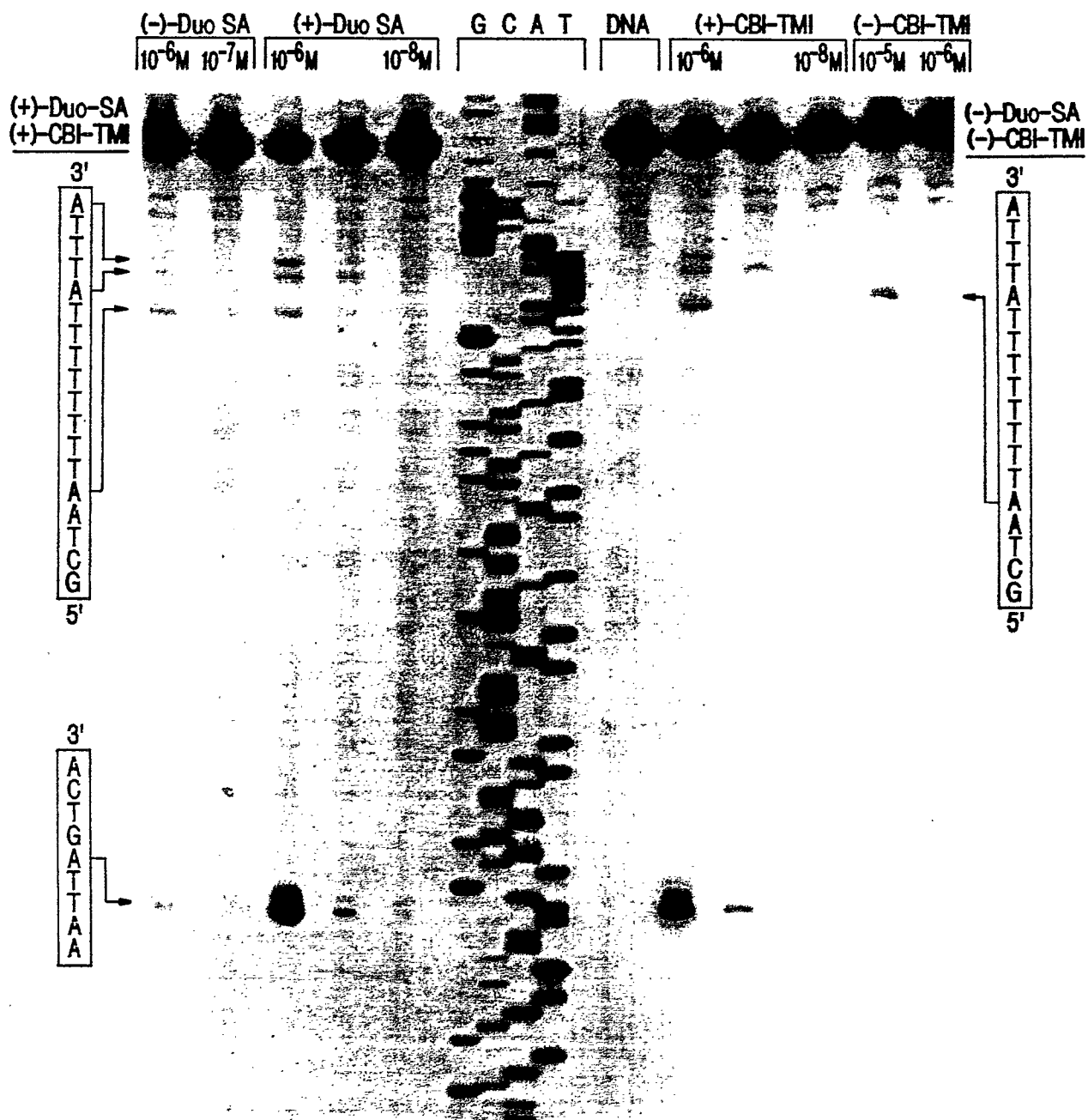


FIG.22

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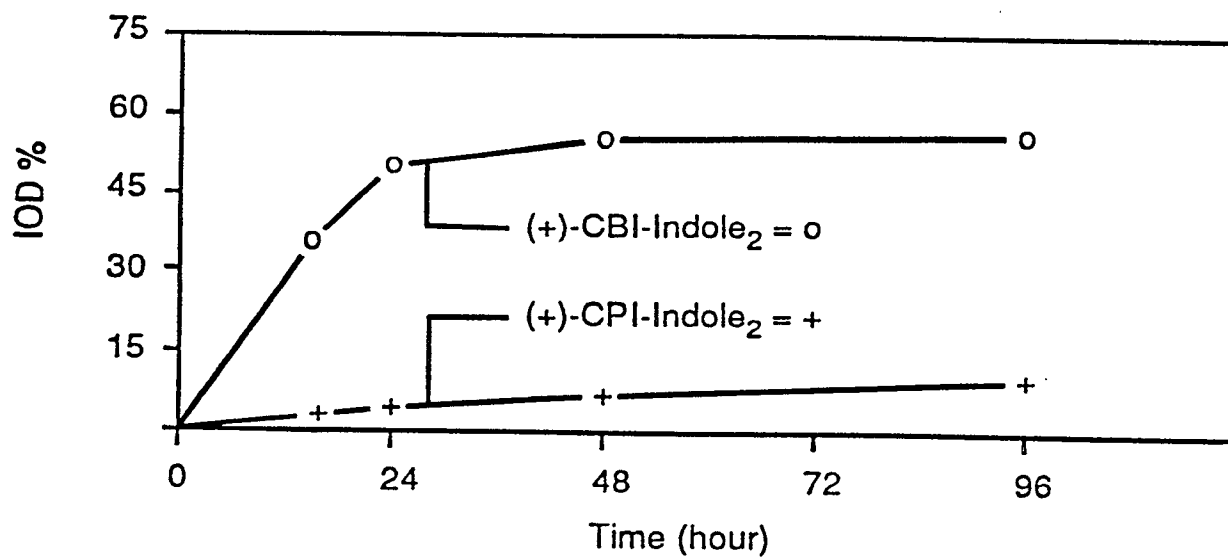


FIG.23A

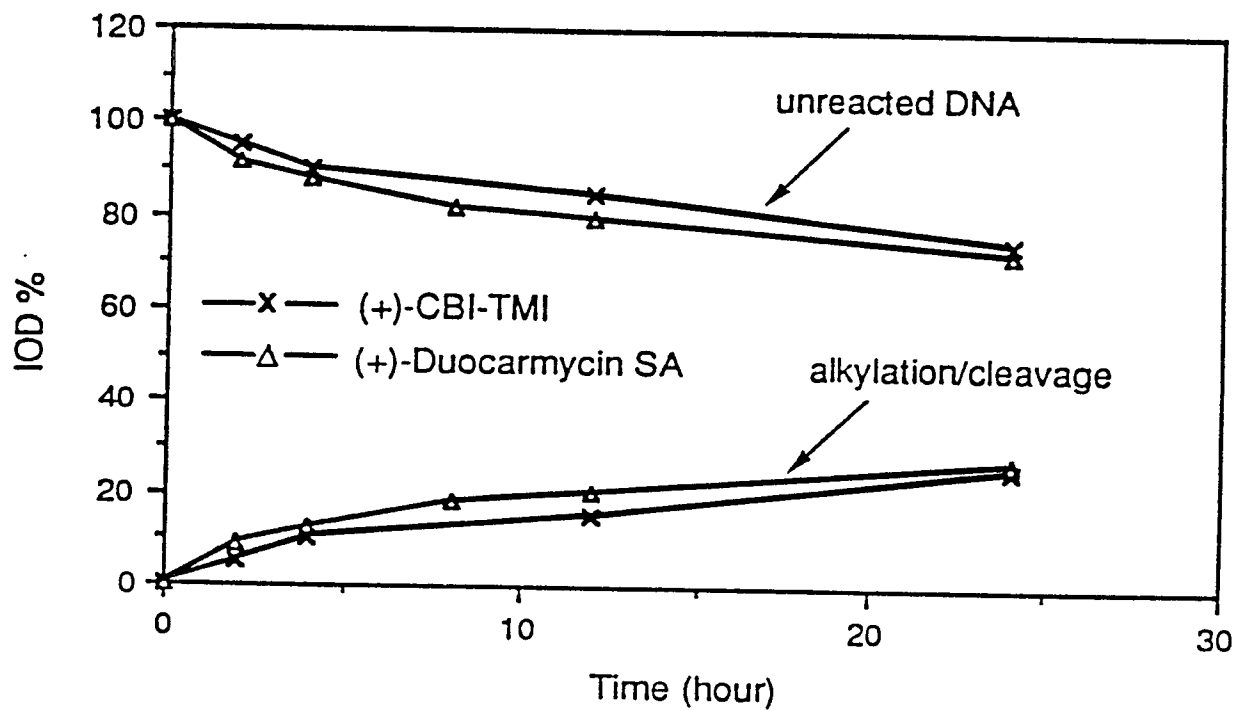


FIG.23B

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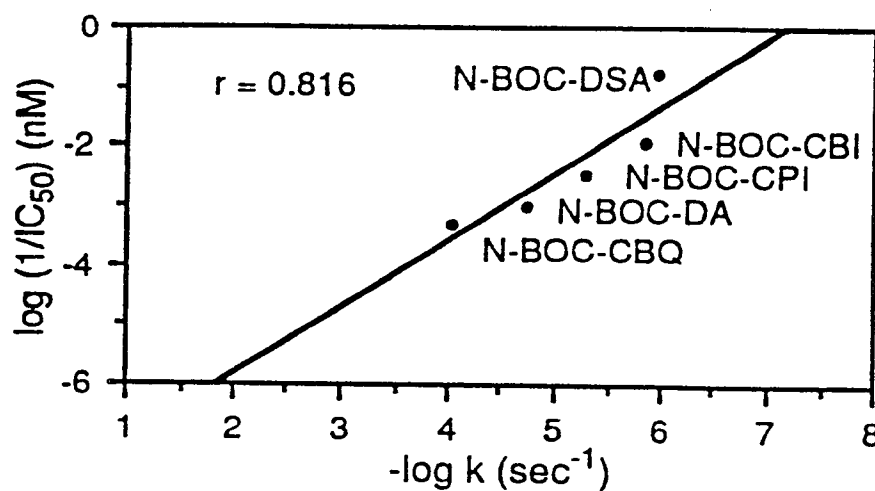


FIG.24A

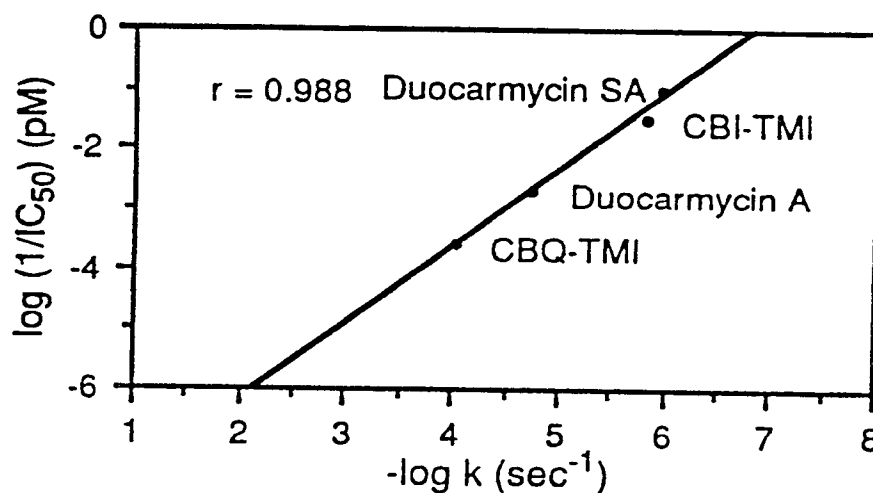


FIG.24B

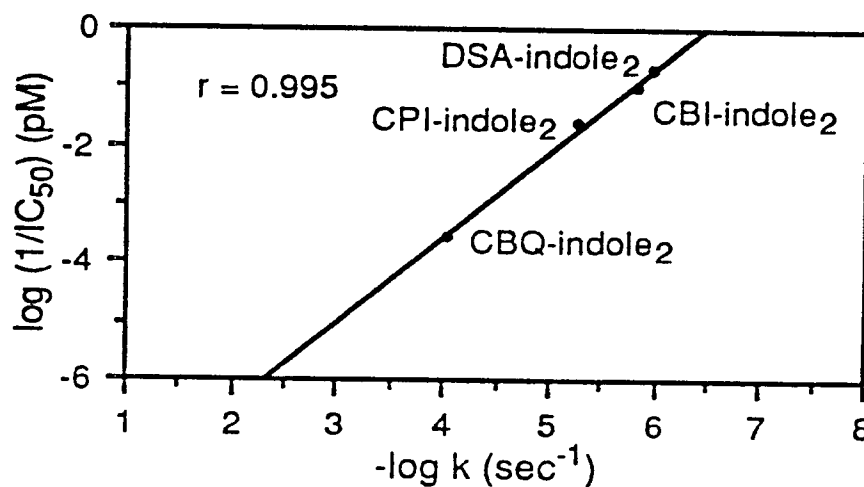


FIG.24C

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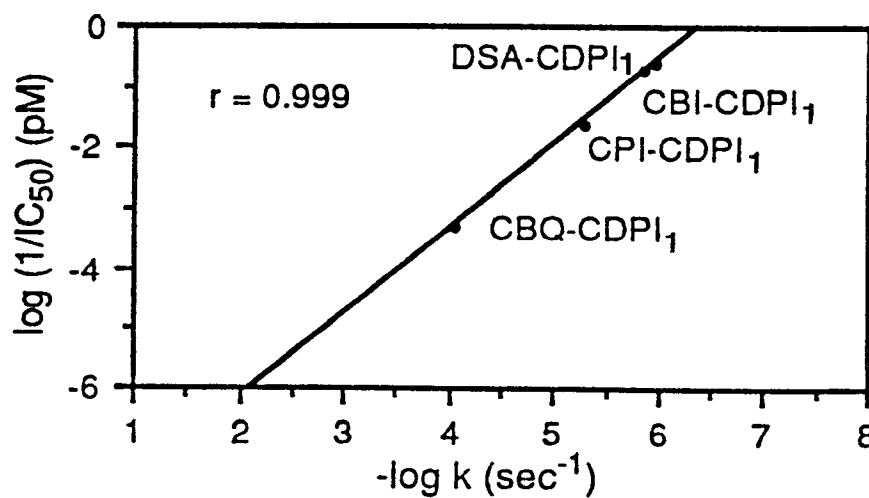


FIG.24D

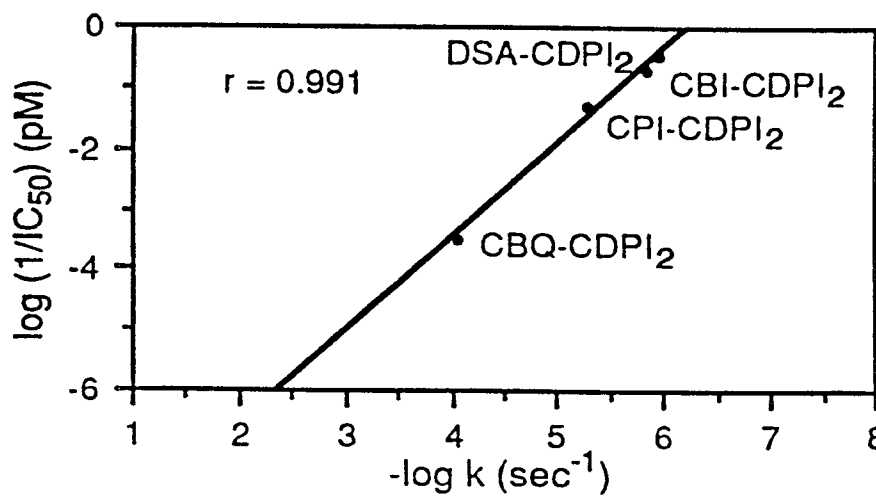


FIG.24E

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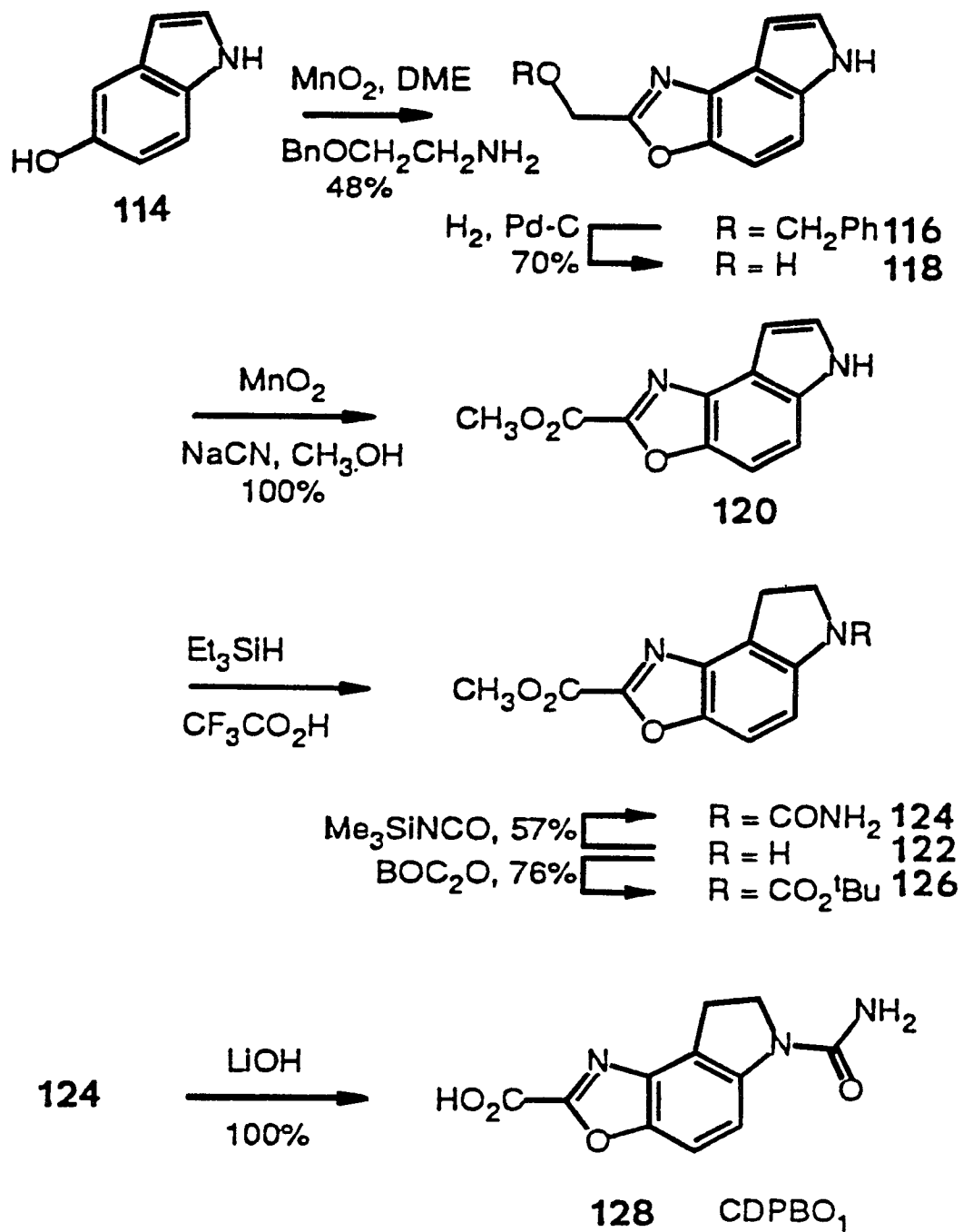


FIG.25

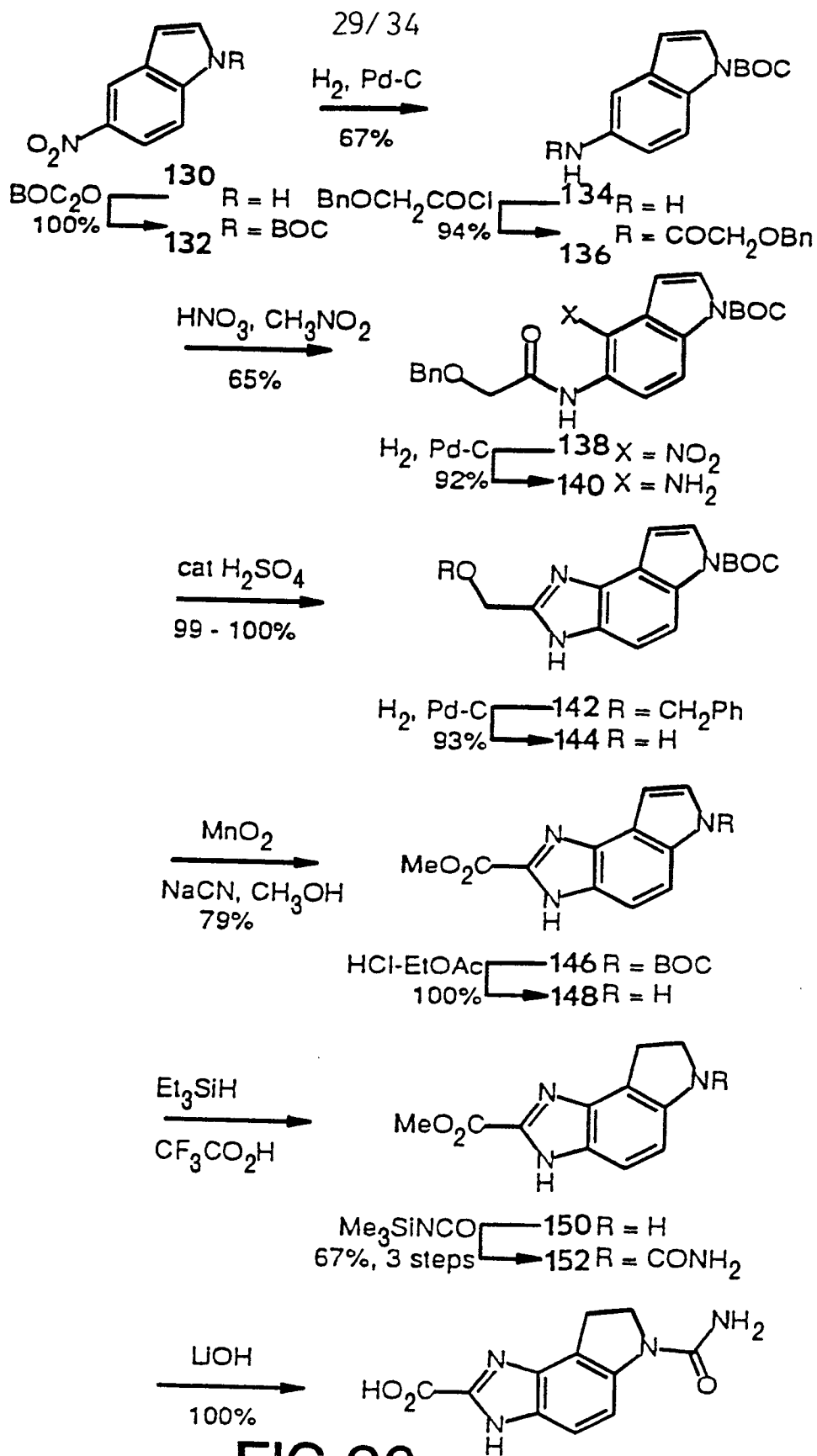


FIG.26

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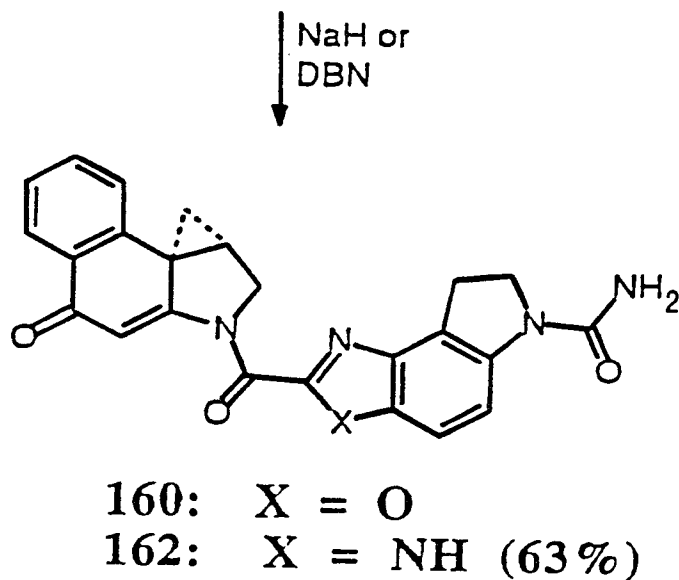
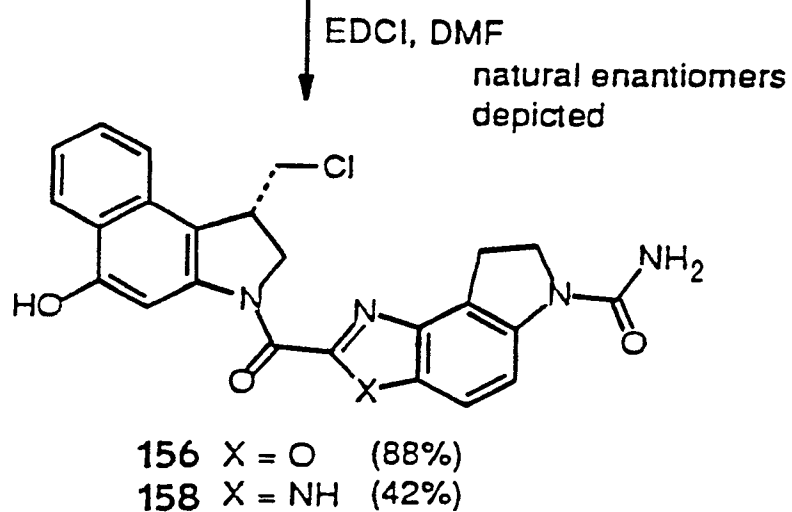
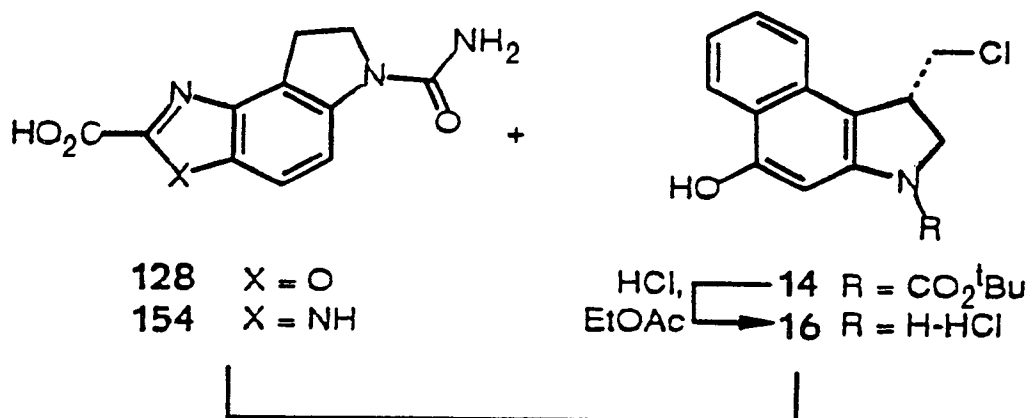
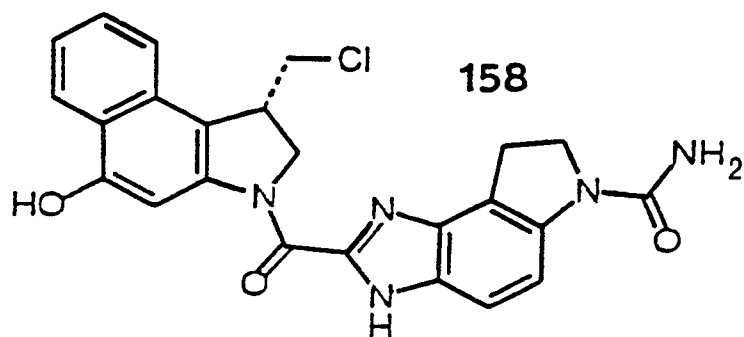
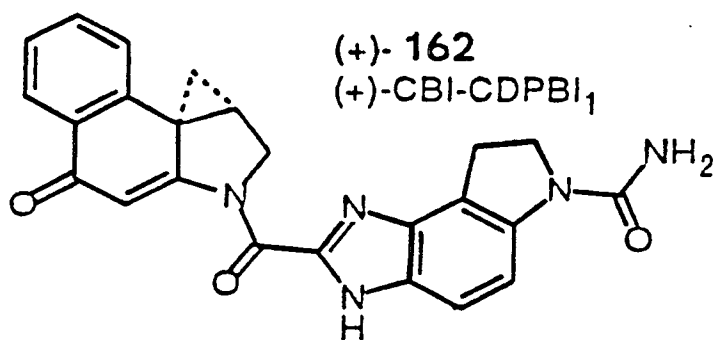
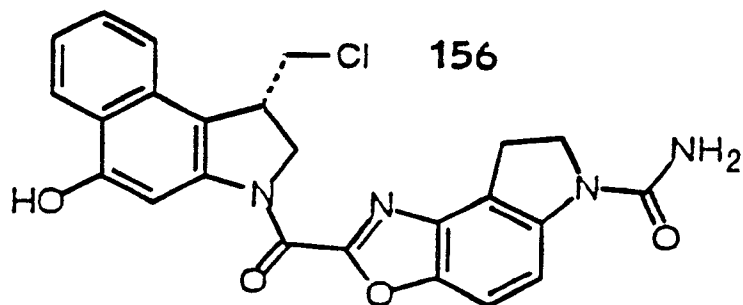
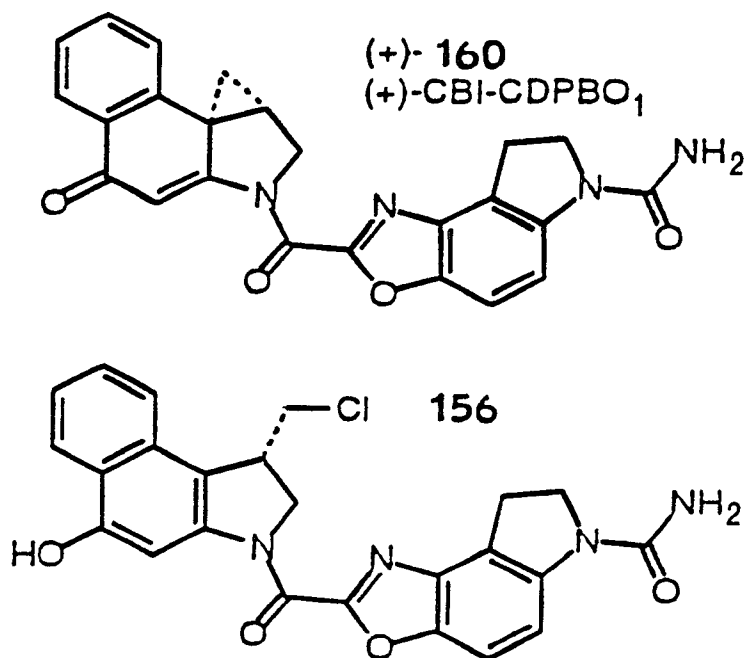


FIG.27

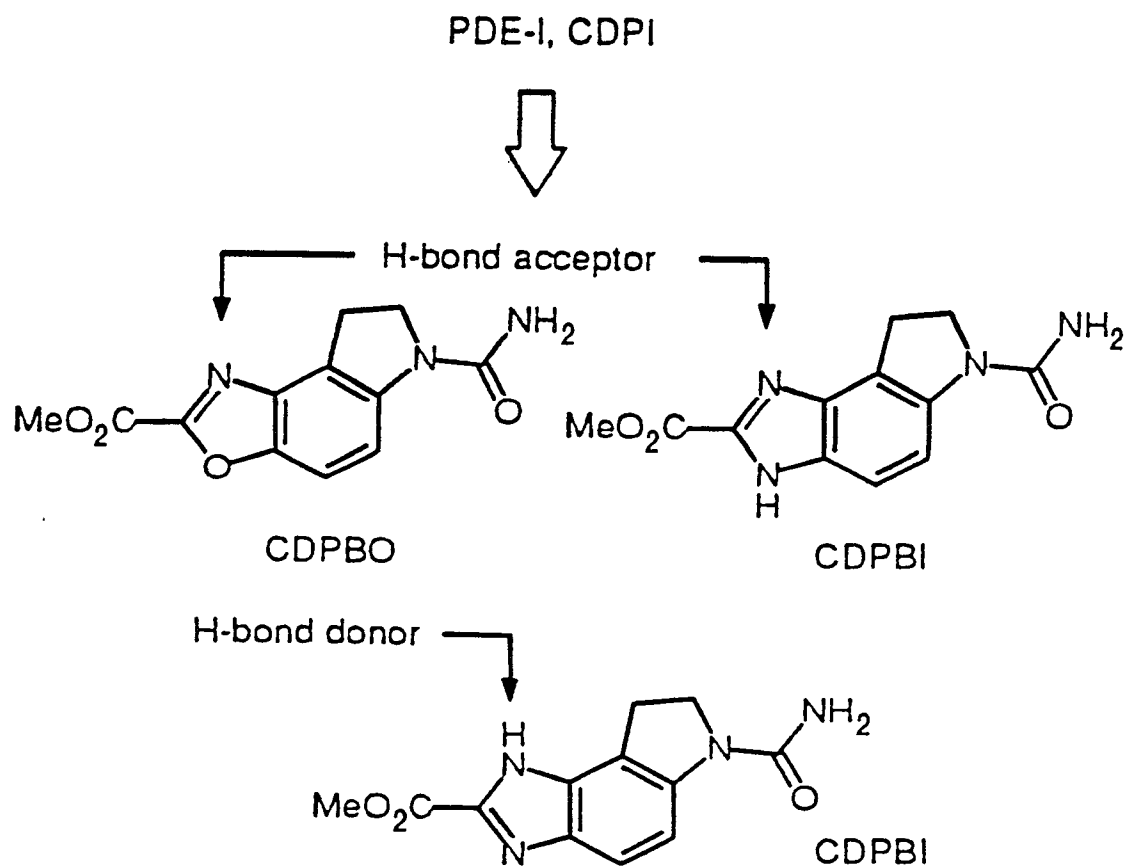
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only natural enantiomers are depicted

FIG.28A
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**FIG.28B**

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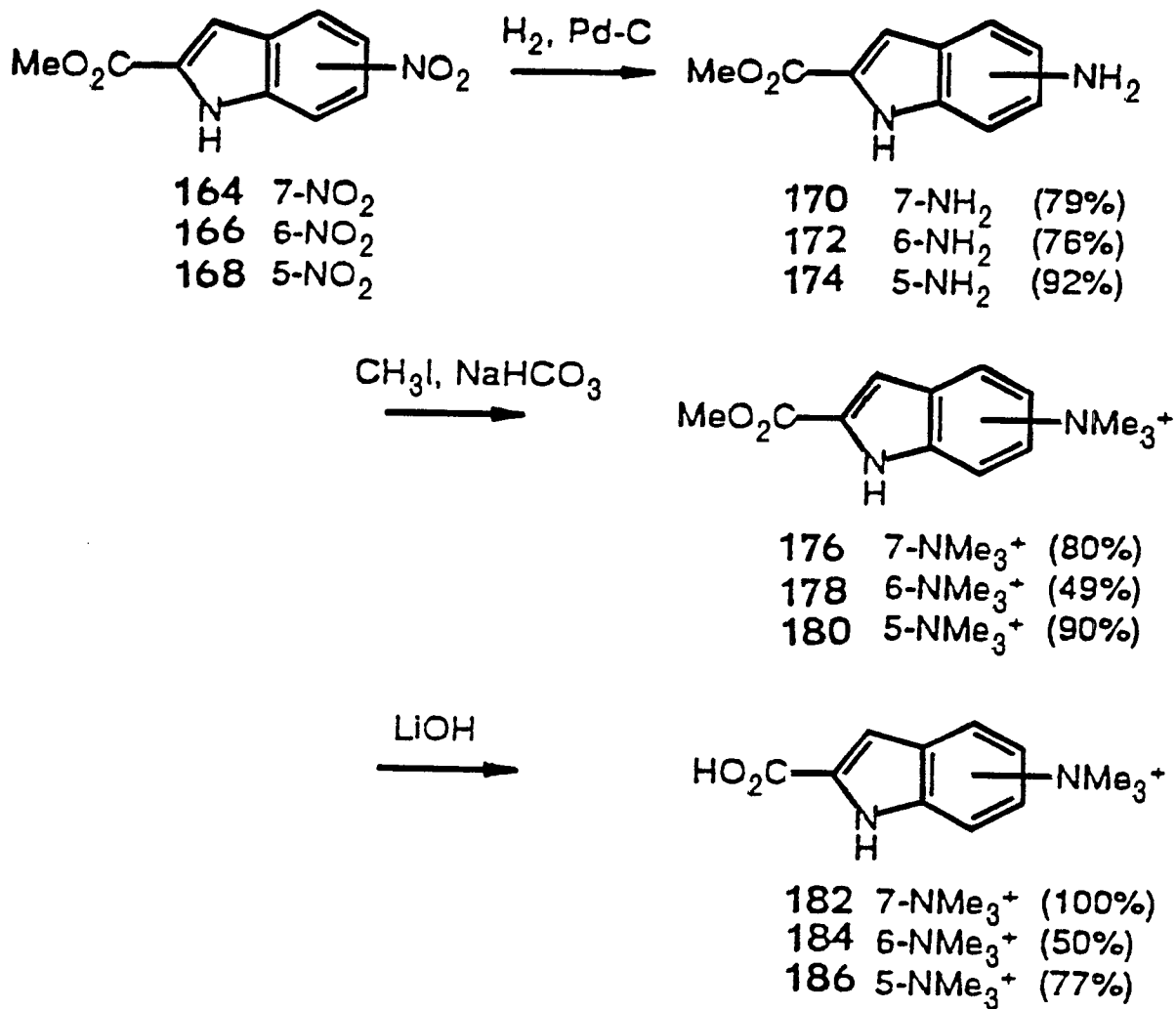


FIG.29

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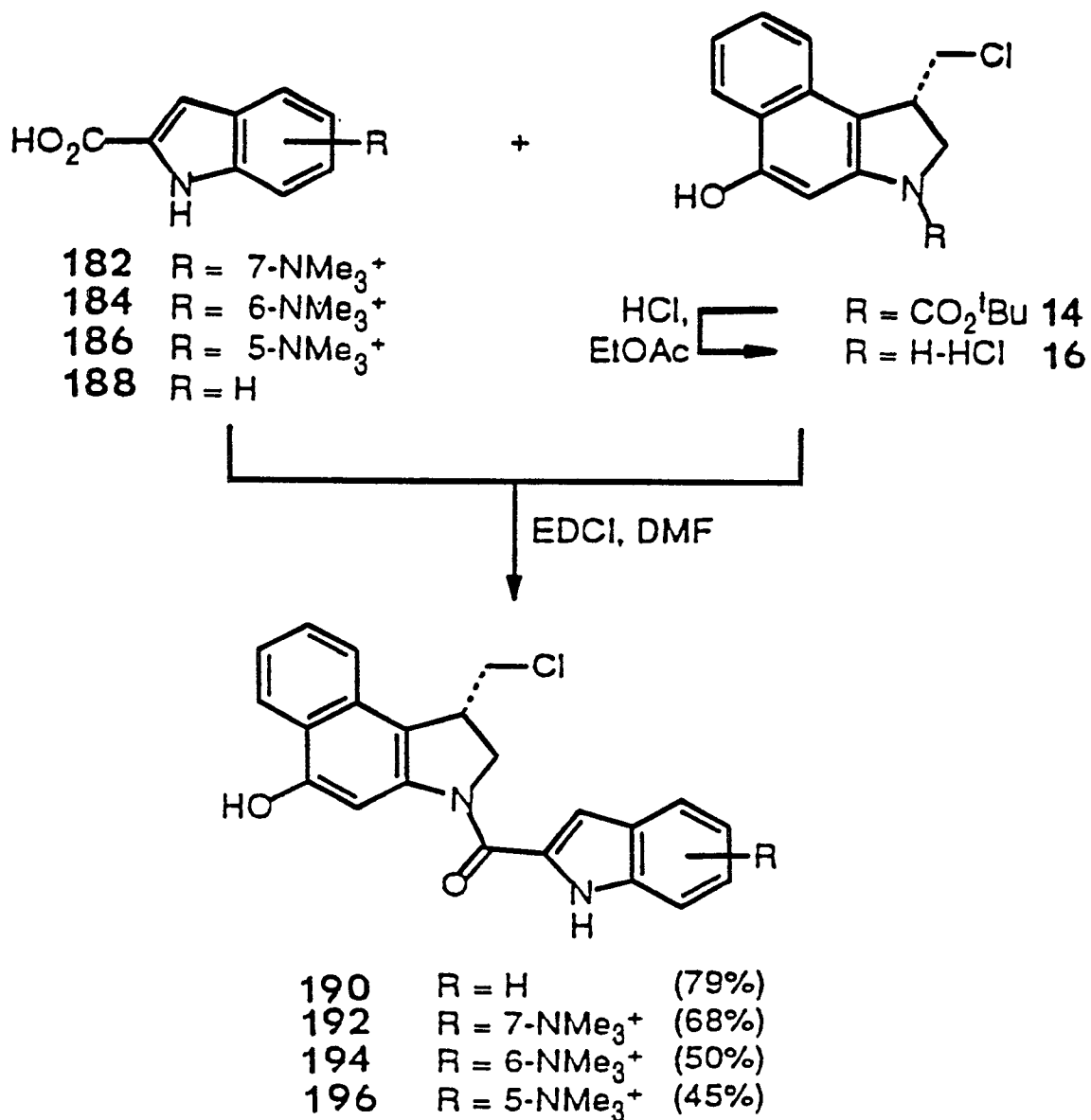


FIG.30