Pergamon

Bioorganic & Medicinal Chemistry, Vol. 3, No. 6, pp. 761–775, 1995

0968-0896/95 $9.50 + .00

0968-0896(95)00066-6

CBI–CDPBO$_1$ and CBI–CDPBI$_1$: CC-1065 Analogs Containing Deep-seated Modifications in the DNA Binding Subunit

Dale L. Boger,* Weiya Yun, Hui Cai and Nianhe Han

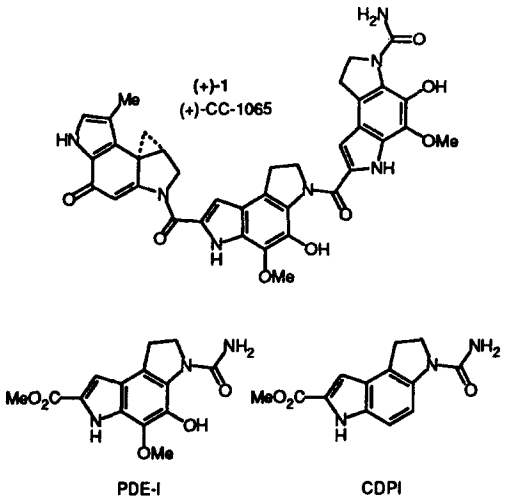
Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, U.S.A.

Abstract—The synthesis and preliminary examination of CBI–CDPBO$_1$ (**2**) and CBI–CDPBI$_1$ (**3**), CBI analogs of CC-1065 (**1**) and the duocarmycins incorporating the 3-carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]benzoxazole-7-carboxylate (CDPBO) and 3-carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]benzimidazole-7-carboxylate (CDPBI) DNA binding subunits, are detailed. The agents contain deep-seated modifications in the DNA binding subunits of the natural products with incorporation of a nitrogen capable of functioning as a hydrogen bond acceptor (CDPBO, CDPBI) or hydrogen bond donor (CDPBI) on their inside concave face which is in intimate contact with the minor groove floor. The CDPBO subunit was prepared through use of a novel and effective MnO_2-mediated oxidative coupling of 2-(benzyloxy)ethylamine with 5-hydroxyindole (**4**) to directly provide 2-[(benzyloxy)methyl]pyrrolo[3,2-*e*]benzoxazole (**6**, 48%) in a reaction cascade that initially proceeds with amine regioselective C4 nucleophilic addition to the *in situ* generated *p*-quinone monoimine **13**. Subsequent conversion of **6** to **8** (debenzylation; MnO_2–NaCN, CH_3OH) and selective reduction of the fused pyrrole (Et_3SiH–CF_3CO_2H) completed the synthesis of the 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]benzoxazole-7-carboxylate ring system. The CDPBI subunit was prepared through selective C4 nitration of **22** followed by reduction of the nitro group and acid-catalyzed closure to the corresponding 2-[(benzyloxy)methyl]pyrrolo[3,2-*e*]benzimidazole **25**. The final conversion of **25** to the 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]benzimidazole-7-carboxylate ring system (CDPBI) followed the same protocols introduced for CDPBO. The DNA alkylation efficiencies of **2** and **3** were identical and both were substantially diminished relative to that of CBI–CDPI$_1$ (**40**). Thus, the introduction of a single nitrogen atom in the DNA binding subunit of **40** has a pronounced and detrimental effect on the relative efficiency (100 ×) of DNA alkylation. Consistent with these observations, the *in vitro* cytotoxic activity of (+)-**2** and (+)-**3** were comparable (IC_{50} = 200 pM, L1210) and 40 × less potent than (+)-**40** (IC_{50} = 5 pM, L1210). In contrast to the large impact these small structural changes had on the efficiency of DNA alkylation, the selectivity of DNA alkylation by **2** and **3** was unperturbed and both agents were found to alkylate the same major sites as CBI–CDPI$_1$ (**40**). The potential origin of these effects is discussed.

Introduction

(+)-CC-1065 (**1**), disclosed in 1978,[1] and the duocarmycins, disclosed in 1988 and 1990,[2–4] represent the parent members of a class of potent antitumor antibiotics that derive their biological effects through the reversible, sequence selective alkylation of DNA.[5–13] Subsequent to their disclosure, extensive efforts have been devoted to establish their DNA alkylation selectivity and its structural origin,[5–13] to establish the link between DNA alkylation and the ensuing biological properties,[14] and to define the fundamental principles underlying the relationships between structure, chemical reactivity, and biological properties.[15–24]

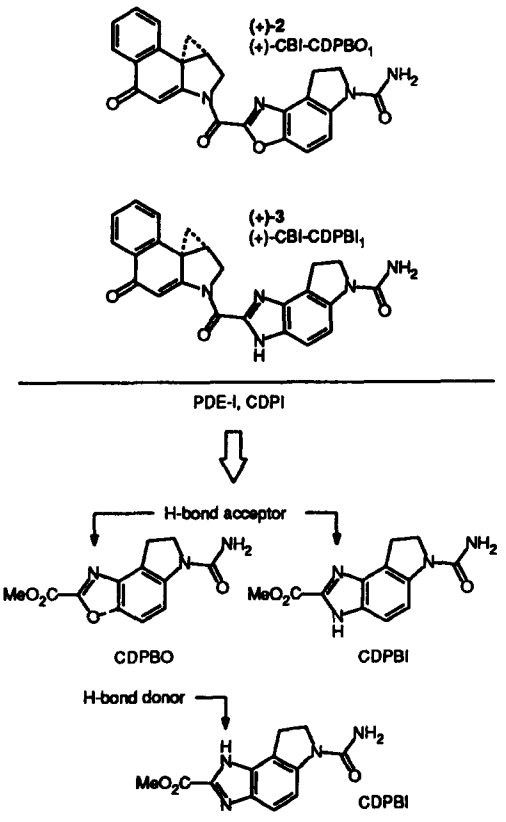
Early in the efforts, we demonstrated that the noncovalent binding affinity of CC-1065 for the DNA minor groove was derived nearly exclusively from stabilizing van der Waals contacts and hydrophobic binding. Moreover, the removal of the peripheral methoxy and hydroxy substituents (PDE-I → CDPI) had no effect on its noncovalent AT-rich binding selectivity and little effect on its noncovalent binding affinity.[9,25] This dependence on hydrophobic binding results in preferential binding in the narrower, deeper AT-rich regions of the minor groove where the stabilizing van der Waals contacts are maximized ($\Delta G°$ = 9.5–11.5 kcal mol^{-1}).[25] Moreover, we have advanced a model where this preferential AT-rich 5 base-pair noncovalent binding selectivity coupled with the steric accessibility to the adenine N3 alkylation site that is accomplished only with the deep penetration at an AT-rich site are the primary determinants controlling the sequence selectivity of the DNA alkylation reaction.[6,9,11,13,19,20] Not only does this model explain the behavior of both the natural and unnatural enantiomers of CC-1065[9] and the duocarmycins[6] but it also accommodates the behavior and DNA alkylation selectivity of the simplified structural analogs that have been disclosed to date.[6,9,10,19–22] Although these proposals have not received universal acceptance and alternative explanations have been advanced,[8,12,26] a past[27] and more recent definitive examination[28] of the proposed models for the origin of the DNA alkylation selectivity proved fully consistent with this proposal and inconsistent with other models that have been advanced.[8,12,26]



The efforts of Lown[29] and Dervan[30] 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 a hydrogen bond acceptor. Accordingly, we have been interested in determining 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 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 other words, this AT-rich binding preference of CC-1065 which is more intimately linked to the complementary shape and size of the interacting molecules than even distamycin, may not be so easily altered by a single hydrogen bond.

Herein, we report the preparation and preliminary examination of (+)- and *ent*-(–)-CBI–CDPBO$_1$ (**2**) and CBI–CDPBI$_1$ (**3**) bearing such deep-seated modifications in the DNA binding subunits of CC-1065 and the duocarmycins. The modified agents incorporate a nitrogen atom capable of functioning as a hydrogen bond acceptor (CDPBO, CDPBI) or hydrogen bond donor (CDPBI) on the inside convex face which is projected to be in intimate contact with the minor groove floor. Preceding studies of the CBI-based analogs have shown that they are chemically more stable (4 ×), biologically more potent (4 ×) and synthetically more accessible than the corresponding agents incorporating the natural CPI alkylation subunit of CC-1065.[20] Moreover, the CBI-based analogs alkylate DNA with an unaltered sequence selectivity at an enhanced rate and with a slightly greater efficiency than the corresponding CPI analog.[20] Consequently, the studies detailed herein were conducted with this synthetically more accessible and more promising CBI class of agents. In addition, the *seco* agents such as **38** and **39** which readily close to the corresponding cyclopropane containing agents have been shown in past studies to exhibit biological properties and DNA alkylation efficiencies and selectivities identical to those of the agent containing the preformed cyclopropane.[20] Consequently, for the studies detailed herein, the *seco* agents **38** and **39** were also examined.

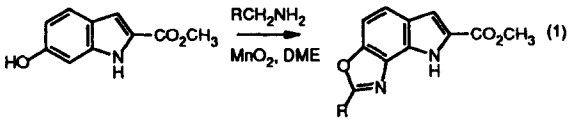
In our detailed comparisons of CC-1065 with CPI–CDPI$_n$ (n = 1–3),[9] no distinctions between (+)- or *ent*-(–)-CC-1065 and (+)- and *ent*-(–)-CPI–CDPI$_2$[9] were detected indicating that the substitution of the CDPI$_2$ for PDE-I$_2$ with removal of the hydroxy and methoxy substituents does not perturb the inherent binding and DNA alkylation selectivity (5 base-pair AT-rich selectivity). In addition, (+)- and *ent*-(–)-CPI–CDPI$_1$ and (+)- and *ent*-(–)-CPI–PDE-I$_1$ proved to be only subtly distinguishable from the natural product and exhibited a smaller 3–4 base-pair AT-rich binding and alkylation selectivity.[9] Consequently, we elected to make our initial comparisons with the agents containing a single DNA binding subunit where the single deep-seated structural modification might be expected to exert a more pronounced effect. Herein, we report that introduction of a single nitrogen into the concave face of the agent substantially alters the DNA alkylation efficiency of the resulting agents but does not significantly perturb the selectivity of DNA alkylation.



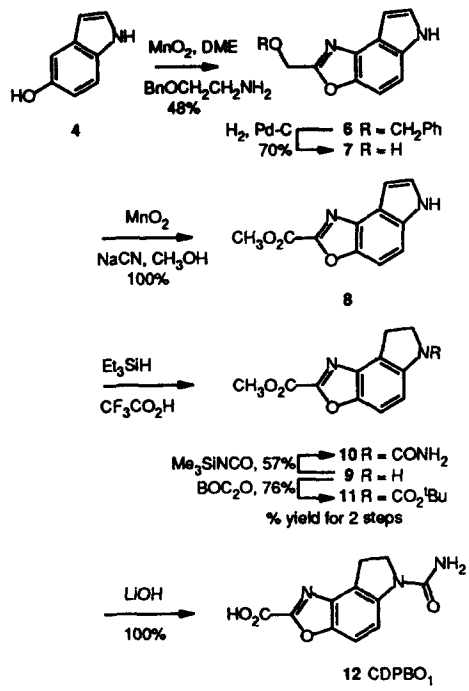
Results

Synthesis of the 3-carbamoyl-1,2-dihydro-3H-pyrrolo-[3,2-e]benzoxazole-7-carboxylic acid (CDPBO) subunit

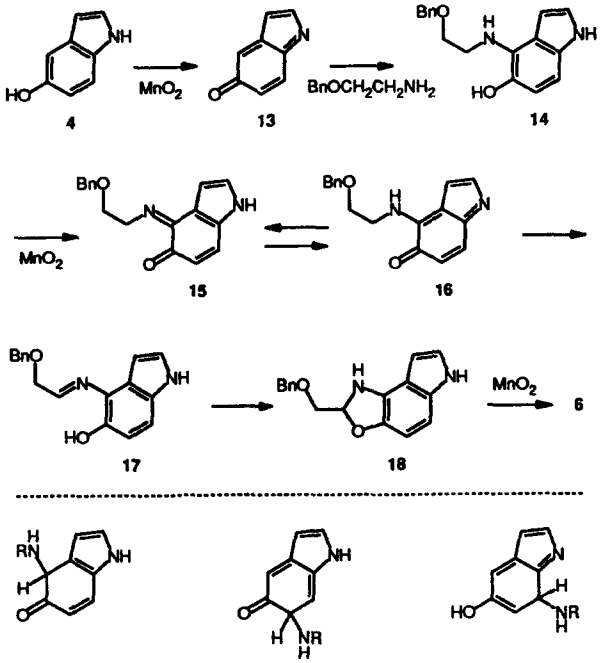
In preceding studies, we have demonstrated the general utility of an unusual oxidative coupling of methyl 6-hydroxyindole-2-carboxylate with primary amines mediated by MnO_2 in the preparation of 2-substituted methyl pyrrolo[2,3-*e*]benzoxazole-5-carboxylates (eq. 1)[31] and reported the apparent interception of the proposed intermediate *o*-quinone monoimine. Consequently, we elected to exploit this technology and potentially extend its scope in the development of a synthetic approach to the isomeric 2-substituted pyrrolo[3,2-*e*]benzoxazole nucleus (i.e. **6–8**). Notably, this requires that the more reactive 5-hydroxyindole nucleus lacking the C2 methyl ester react accordingly and that the isomeric 5-hydroxy substitution cleanly redirect the primary amine nucleophilic addition to C4 of the extended *p*-quinone monoimine **13**.



This anticipated extension of the methodology proved surprisingly successful given the number of steps and complexity of the conversion of **4** to **6**. Treatment of a mixture of 5-hydroxyindole (**4**) and 2-(benzyloxy)-ethylamine (**5**, 2 equiv.) in DME (0.03 M) with MnO_2 (30 wt equiv., 25 °C, 14 h) cleanly provided 2-[(benzyloxy)methyl]pyrrolo[3,2-*e*]benzoxazole (**6**, 48%), Scheme 1, and the quality of this transformation routinely improved as the scale of the reaction was increased.[32] Like the reaction shown in equation 1 which was examined in detail, the conversion of **4** to **6** presumably proceeds by oxidation of 5-hydroxyindole (**4**) to the *p*-quinone monoimine **13**[33] which suffers a regioselective C4 addition reaction of the added primary amine to provide **14** (Scheme 2). Subsequent MnO_2-mediated reoxidation of **14** to the *o*-quinone monoimine **15** or extended *p*-quinone monoimine **16** followed by a 1,5-hydrogen shift driven by rearomatization of **15** and **16** provides **17**. Closure of **17** to the N,O-acetal **18** and subsequent MnO_2 oxidation with aromatization of the fused oxazole provides **6**. The regioselectivity of the initial nucleophilic addition is consistent with the relative stability of the resulting adducts in which full aromatization to the fused pyrrole ring is established only with C4 addition versus the potentially competitive C6 or C7 addition. In the case of the conversion illustrated in equation 1, the intermediate *o*-quinone monoimine analogous to **15** was trapped and established to be generated en route to the fused oxazole product providing good evidence for the mechanism illustrated in Scheme 2. Of the two isomeric fused oxazoles potentially generated, the structure **6** was firmly established by ^{1}H NMR, J(H4/H5) = 8.8 Hz.



Scheme 1.



Scheme 2.

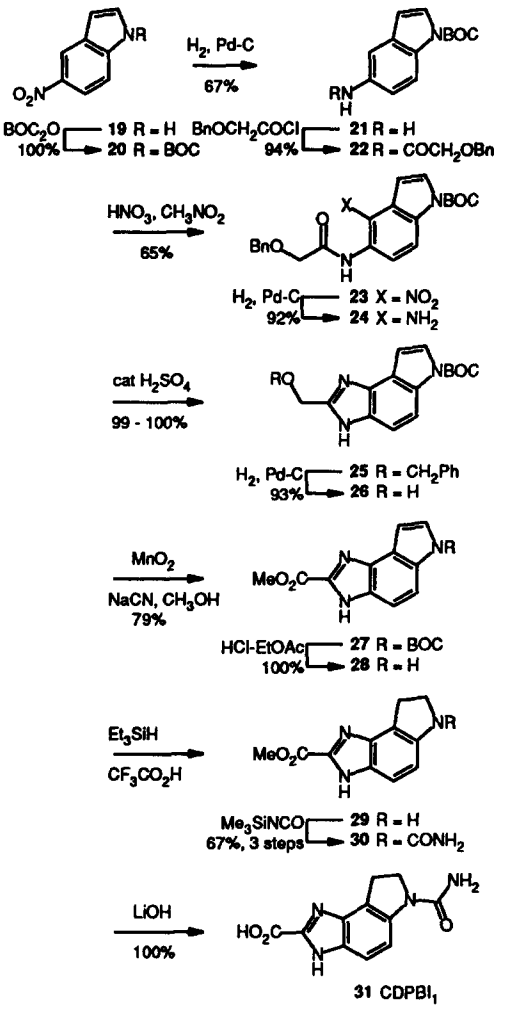
Deprotection of **6** by catalytic hydrogenolysis conducted in the presence of an acid catalyst cleanly provided **7** and competitive hydrogenolysis of the C2 benzyl center of **6** and **7** was not observed (Scheme 1). Direct conversion of **7** to the methyl ester **8** was accomplished by MnO_2 oxidation in the presence of NaCN in CH_3OH.[34] Selective reduction of the more electron-rich heteroaromatic ring of **8** was accomplished upon treatment with Et_3SiH–CF_3CO_2H.[35]

Presumably, exposure of **8** to strong acid leads to C1 protonation and reduction of the resulting iminium ion. Similarly, treatment of **8** with $NaCNBH_3$–CF_3CO_2H[36] (3.0 equiv., 15–25 °C, 2 h) provided **9** but in more modest conversions (56%) and the use of $NaCNBH_3$ in less acidic solvents (CH_3CO_2H, CF_3CH_2OH) proved unsuccessful. The free indoline **9** proved somewhat unstable to isolation and characterization and, consequently, was immediately subjected to acylation upon reaction with Me_3SiNCO or BOC_2O to provide **10** or **11**, respectively. In the reaction of **9** with Me_3SiNCO, the addition of cat. DMAP provided **10** in lower conversions and contaminated with additional byproducts presumably due to further acylation of the initial urea. Methyl ester hydrolysis of **10** provided **12** ($CDPBO_1$).

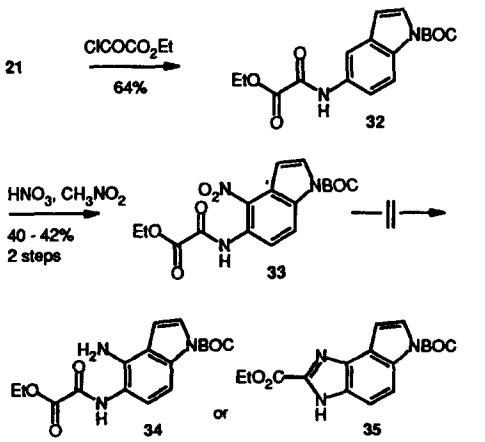
Synthesis of the 3-carbamoyl-1,2-dihydro-3H-pyrrolo-[3,2-e]benzimidazole-7-carboxylic acid (CDPBI) subunit

Catalytic hydrogenation of *N*-BOC-5-nitroindole (**20**) followed by immediate acylation of the free amine **21** with 2-(benzyloxy)acetyl chloride provided **22** (Scheme 3) and prolonged reduction periods (> 12 h versus 5 h) provided substantial amounts of the corresponding indoline.[37] Selective C4 nitration accomplished by treatment of **22** with HNO_3 in CH_3NO_2 (0 to 25 °C) cleanly provided **23**. Notably, no *N*-BOC deprotection was observed under the reaction conditions and no competitive C3 nitration was detected. Consequently, the *N*-BOC protection of **20** not only served to convey stability to the intermediates **21**–**26** but to also deactivate the pyrrole ring of **22** toward electrophilic substitution. Subsequent nitro reduction by catalytic hydrogenation at room temperature was cleanly accomplished without evidence of competitive hydrogenolysis of the benzyl ether and provided higher yields of **24** (92%) than the more conventional $Na_2S_2O_4$ reduction (70%). Mild, acid-catalyzed ring closure effected by treatment of **24** with cat. H_2SO_4 in THF (25 °C, 24 h) provided the benzimidazole **25** in exceptional yield (99–100%) without detectable *N*-BOC deprotection. Hydrogenolysis removal of the benzyl ether necessarily conducted in the presence of an acid catalyst (HCl–EtOH) provided the alcohol **26** (93%) and proved to be surprisingly selective. No competitive cleavage of the corresponding C2 benzyl center of **25**–**26** was observed. Moreover, no reaction was observed in the absence of the acid catalyst even upon prolonged reaction times or at elevated temperatures and no reaction was detected upon exposure of **25** to two-phase, transfer hydrogenolysis conditions.[38] Direct conversion of the alcohol **26** to the methyl ester **27** was accomplished by treatment with MnO_2 in the presence of NaCN in CH_3OH.[34]

An alternative and more direct preparation of **27** or **35** outlined in Scheme 4 employing the oxalate amide **32**[39] failed to close selectively to the benzimidazole upon the nitro reduction of **33**.[39] Although this was not examined in detail, at least 2 or 3 reaction products were detected upon reduction of **33** with either $Na_2S_2O_4$ (5 equiv., 1:1 THF:H_2O, 25 °C, 30 h) or Al(Hg) (10 equiv., 9:1 THF:H_2O, 25 °C, 3h) and suggested that subsequent competitive ring closure to both 5- and 6-membered heteroaromatic ring systems were observed under the reaction conditions. Similarly, reduction of **33** by catalytic hydrogenation (0.1 wt equiv. 10% Pd/C, 1 atm H_2, EtOAc, 25 °C) failed to provide **34** or **35** cleanly.



Scheme 3.



Scheme 4.

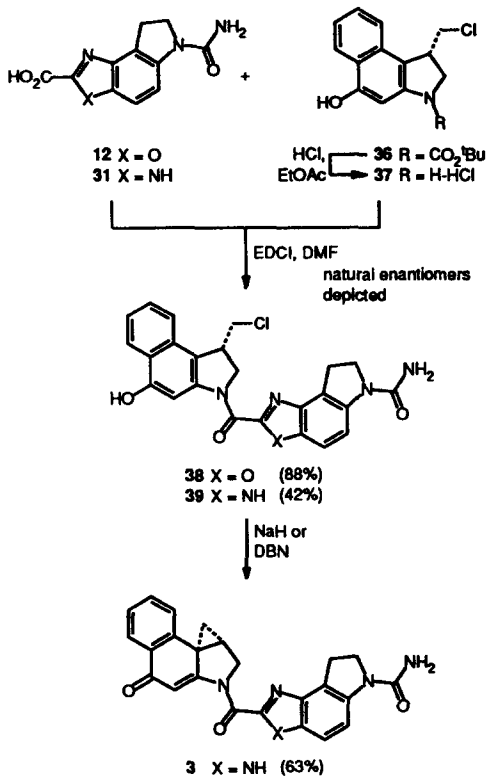
Acid-catalyzed *N*-BOC deprotection of **27** followed by selective reduction of the more electron-rich heteroaromatic ring of **28** upon treatment with Et_3SiH–CF_3CO_2H[35] followed by immediate acylation of the unstable free indoline **29** with Me_3SiNCO provided **30** in 67% overall yield from **27**. Again, the reduction of **28** to **29** with Et_3SiH–CF_3CO_2H proved much more productive than attempts to use $NaCNBH_3$–CH_3CO_2H.[36] Methyl ester hydrolysis of **30** provided **31** (CDPBI$_1$).

Synthesis of CBI–CDPBO$_1$ (2) and CBI–CDPBI$_1$ (3)

Acid-catalyzed deprotection (3 N HCl–EtOAc, 25 °C, 30–40 min, 95–100%) of natural (1*S*)- and *ent*-(1*R*)-**36**[20] followed by immediate coupling of the unstable indoline hydrochloride salt **37** with CDPBO$_1$ (**12**) and CDPBI$_1$ (**31**) deliberately conducted in the absence of added base (2 equiv. EDCI, DMF, 25 °C, 6–12 h) provided *seco*-CBI–CDPBO$_1$ (**38**) and *seco*-CBI–CDPBI$_1$ (**39**), (Scheme 5). Although this coupling proceeded uneventfully to provide **38** in excellent conversion (88%), it produced **39** in more modest yields (42%). This may be attributed to the remarkable insolubility of **31** even in DMF and the use of more polar mixed solvent systems (DMF–HMPA, DMF–DMSO) failed to improve on this conversion. Subsequent spirocyclization of **39** effected by treatment with NaH or DBN (1.5 equiv., 1:1 THF–DMF, 0–25 °C, 3.5 h, 63%) provided natural (+)- and *ent*-(–)-CBI–CDPBI$_1$ (**3**). In the case of the ring closure of **38**, the product **2** proved to be remarkably sensitive to subsequent hydrolysis. Despite our considerable experience with this spirocyclization reaction in related systems, the subsequent hydrolysis of **2** by adventitious water under the reaction conditions or upon workup and purification always led to low conversions to **2** with predominant isolation of CBI and CDPBO$_1$ (**12**). Since past studies have demonstrated that the *seco* agents such as **38** exhibit properties indistinguishable from those of agents containing the preformed cyclopropane, our initial examination of **2** was conducted with its precursor **38**. Our comparison of the analogous pairs **39/3** did not reveal significant distinctions between the agents to merit continued efforts to isolate and fully characterize CBI–CDPBO$_1$ (**2**).

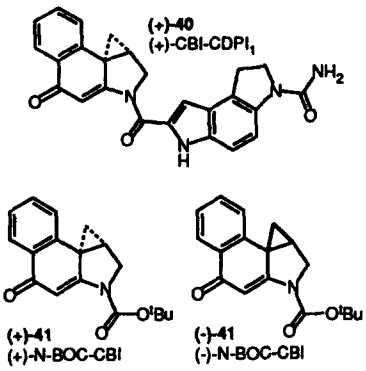
DNA alkylation efficiency and selectivity

The DNA alkylation properties of the agents were examined within two 150 base-pair segments of duplex DNA for which comparative results are available for related agents. Two clones of phage M13mp10 were selected for study and contain SV40 nucleosomal DNA inserts: w794 (nucleotide no. 5238-138) and its complement w836 (nucleotide no. 5189-91).[40] The alkylation site identification and the assessment of the relative selectivity among the available sites was obtained by thermally-induced strand cleavage of the singly 5' end-labeled duplex DNA after exposure to the agents. Following treatment of the end-labeled duplex DNA with a range of agent concentrations, the unbound agent was removed by EtOH precipitation of the DNA. Redissolution of the DNA in aqueous buffer, thermolysis (100 °C, 30 min) to induce strand cleavage at the sites of DNA alkylation, denaturing high resolution polyacrylamide gel electrophoresis (PAGE) adjacent to Sanger dideoxynucleotide sequencing standards,[41] and autoradiography led to identification of the DNA cleavage and alkylation sites. It has been established in our prior studies that rapid thermolysis at 100 °C at neutral to low pH (pH 6–7) and at low salt concentration minimize a potentially competitive reversible DNA alkylation reaction and optimize thermal depurination leading to stoichiometric strand cleavage at the sites of DNA alkylation.[6] Thermal depurination reactions conducted at lower temperatures (50–90 °C), at higher pH (8.0), and to a lesser extent at high ionic strength favor reversal of the DNA alkylation reaction and result in less efficient strand cleavage. This may be attributed to base-catalyzed phenol deprotonation required for retroalkylation observed at the higher pH or maintenance of duplex (favors retroalkylation) versus denatured (favors depurination) DNA observed at the lower reaction temperatures or at the higher ionic strength. The full details of this procedure have been disclosed and discussed elsewhere.[9] Consistent with past observations, the precursor *seco* agent **39** was found to be indistinguishable from **3** in the DNA alkylation assays and in the cytotoxic assays which may be attributed to the ease with which the ring closure occurs even under the conditions of the assays. Consequently, the examination of **2** detailed below was conducted with the precursor agent **38**.



Scheme 5.

The most relevant comparisons made in our examination of **2** and **3** were those with (+)-and *ent*-(−)-CBI–CDPI$_1$ (**40**), which lacks the internal nitrogen imbedded in the DNA binding subunits of **2** and **3**, and (+)- and *ent*-(−)-*N*-BOC-CBI (**41**) which lacks a DNA binding subunit altogether. The DNA alkylation selectivities and efficiencies illustrated in Figure 1 within w794 DNA are representative of the comparisons.



Most notably, the DNA alkylation selectivities and efficiencies of the natural enantiomers of **2** and **3** were

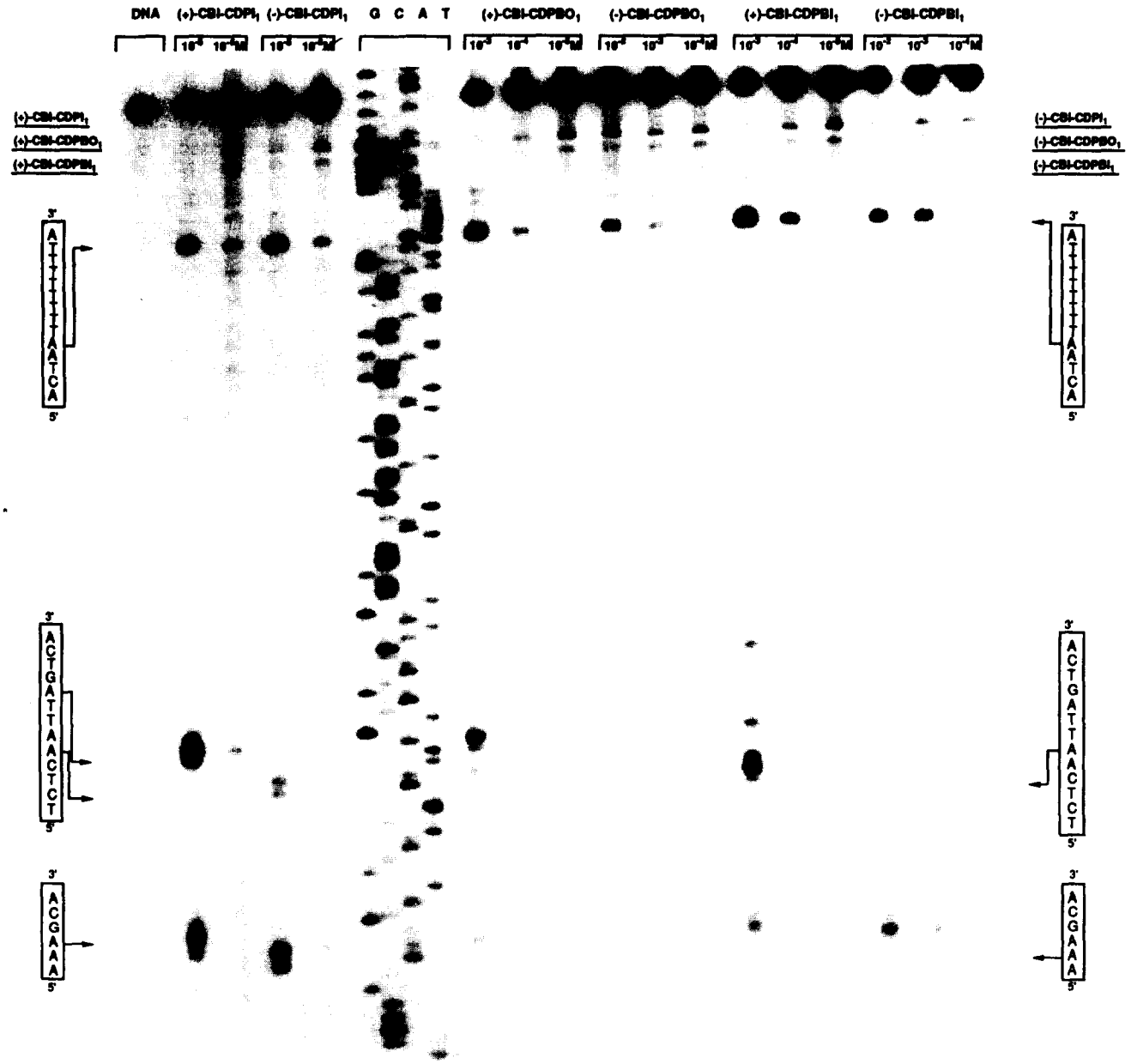


Figure 1. Thermally-induced strand cleavage of double-stranded DNA (SV40 DNA fragment, 144 bp, nucleotide no. 5238-138, clone w794) after 72 h incubation of agent–DNA at 25 °C followed by removal of unbound agent and 30 min incubation at 100 °C; 8% denaturing PAGE and autoradiography. Lane 1, control DNA; lanes 2 and 3, (+)-CBI–CDPI$_1$ (**40**, 1×10^{-5} and 1×10^{-6} M); lanes 4 and 5, *ent*-(−)-CBI–CDPI$_1$ (**40**, 1×10^{-5} and 1×10^{-6} M); lanes 6–8, Sanger G, C, A and T reactions; lanes 9–11, *seco*-(+)-CBI–CDPBO$_1$ (**38**, 1×10^{-3} to 1×10^{-5} M); lanes 12–14, *seco-ent*-(−)-CBI–CDPBO$_1$ (**38**, 1×10^{-2} to 1×10^{-4} M); lanes 15–17, (+)-CBI–CDPBI$_1$ (**3**, 1×10^{-3} to 1×10^{-5} M); lanes 18–20, (−)-CBI–CDPBI$_1$ (**3**, 1×10^{-2} to 1×10^{-4} M).

essentially identical. Moreover, both were approximately 100 × less efficient at alkylating DNA than (+)-CBI–CDPI$_1$ (**40**). This is illustrated nicely in Figure 1 where detectable alkylation by the natural enantiomers of **2** and **3** is observed at 10^{-4} M concentration while that of (+)-CBI–CDPI$_1$ is observed at 10^{-6} M concentration. Notably, the DNA–agent incubations were conducted for 72 h, 25 °C for Figure 1 where all of the DNA alkylation reactions have been run to completion.[42] The differences were even more pronounced at shorter, more conventional reaction periods (4 or 25 °C, 24 h) indicating that both the rate and efficiency of DNA alkylation by (+)-**2** and (+)-**3** are considerably diminished relative to that of (+)-**40**. Thus, the simple incorporation of a single nitrogen into **3** versus **40** 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,[20] the unnatural enantiomers of **2** and **3** proved to be 10–100 × less efficient at alkylating DNA than the corresponding natural enantiomers. This is illustrated nicely in Figure 1 where both *ent*-(–)-**2** and (–)-**3** exhibit detectable DNA alkylation at 10^{-2}–10^{-3} M versus 10^{-4} M for the corresponding natural enantiomers. Interestingly, *ent*-(–)-**2** was perceptibly less efficient at alkylating DNA than *ent*-(–)-**3**. Moreover, since the enantiomeric purity of the agents was ≥ 99.9%, this DNA alkylation detected at 10–100 × higher concentrations for the unnatural enantiomers may be attributed to the agent and not contaminant natural enantiomer in the sample. Under the conditions of incubation at 25 °C for 72 h, the two enantiomers of CBI–CDPI$_1$ (**40**) alkylated DNA with the same relative efficiency (10^{-6} M detectable alkylation). However, at 25 °C (24 h), the natural enantiomer alkylation was complete and approximately 10 × more intense than the unnatural enantiomer alkylation indicating that the latter occurs at a slower rate.

More interesting was the observed DNA alkylation selectivities of **2** and **3**. The DNA alkylation selectivity of (+)-**2** and (+)-**3** were essentially identical and both were comparable to the selectivity observed with (+)-**40**. Although the DNA alkylation selectivity of (+)-**2** and (+)-**3** 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. This is illustrated nicely in Figure 1. Not only did (+)-**2** and (+)-**3** alkylate DNA with the near identical selectivity of (+)-**40**, but the unnatural enantiomer selectivity for **2** and **3** proved essentially identical to that of *ent*-(–)-**40**. A subtle and perceptible loss of alkylation selectivity for (+)-**3** was detected with the minor sites observed at the highest agent concentration (10^{-3} M) which correspond to alkylation of sites detected with (+)-*N*-BOC-CBI (**41**) at comparable (10^{-2}–10^{-3} M) concentrations.[20] We attribute these minor sites to alkylation of DNA through a bound conformation of (+)-**3** in which the CDPBI$_1$ subunit is not bound in the minor groove but extends out into solution with the agent covering an alkylation site region comparable to that observed with (+)-**41**. Thus, in a manner essentially identical to (+)- and *ent*-(–)-**40** which exhibit distinct alkylation selectivities (5'-A/TA/TA/TA versus 5'-A/TAA/TA/T, respectively)[9] characteristic of the reverse binding orientations and offset 3.5 base-pair AT-rich binding sites surrounding the alkylation site,[9] the two enantiomers of **2** and **3** alkylated essentially the same sites as the corresponding enantiomers of **40** within duplex DNA. Moreover, this was observed to occur not with the increasing tolerance for incorporation of GC base-pairs in the alkylation sequence, but rather with a diminished DNA alkylation efficiency (100 ×) relative to that of (+)- and *ent*-(–)-CBI–CDPI$_1$.

Discussion

Although the origin of the diminished DNA alkylation efficiency of **2** and **3** was not unambiguously established, at least one contributing factor is the preferred conformations of **2** and **3** relative to that of **40**. Heterocycles including benzoxazole-2-carboxylates and benzimidazole-2-carboxylates which contain a carbonyl flanked by two heteroatoms in the aromatic ring preferentially adopt conformations in which the carbonyl is perpendicular to the plane of the adjacent aryl ring. This is often clear from the chemistry of the carbonyl group which, by virtue of the lost conjugation, is more reactive than a typical aryl carboxylate[43] and approaches the chemical reactivity of an α-dicarbonyl system. For systems such as that found in **2**, this may be attributed in part to the destabilizing electrostatic interactions between the carbonyl lone pair and the heteroatom lone pairs that are present when the carbonyl adopts either of the in plane, conjugated conformations (Figure 2). Consistent with these

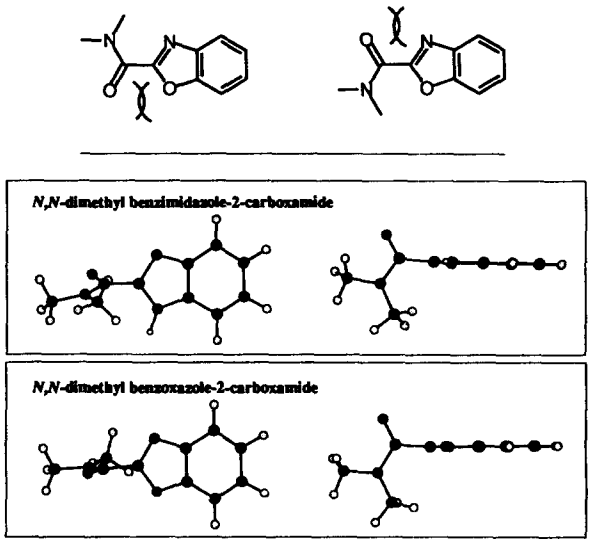


Figure 2. Top: lone-pair interactions which destabilize the carbonyl in plane (conjugated) conformation of benzoxazole-2-carboxamides. Bottom: models of the MNDO low energy conformations of *N,N*-dimethyl benzimidazole-2-carboxamide and *N,N*-dimethyl benzoxazole-2-carboxamide illustrating the nonconjugated perpendicular carbonyl conformations. AM1 gave comparable results.

expectations, the MNDO and AM1[44] low energy conformations for both *N,N*-dimethyl benzoxazole-2-carboxamide and *N,N*-dimethyl benzimidazole-2-carboxamide constitute the perpendicular, out of plane (deconjugated) carbonyl conformation (Figure 2) while that of *N,N*-dimethyl indole-2-carboxamide more closely approximates the in plane, conjugated (eclipsed) conformation.

Consequently, **2** and **3** may be expected to preferentially adopt a conformation in which the CDPBO or CDPBI subunit is perpendicular to the CBI alkylation subunit rather than the near planar conformation required for minor groove binding. Consistent with this interpretation, **2** proved extraordinarily sensitive to hydrolysis of the linking amide. In part, the diminished DNA alkylation efficiency of **2** and **3** may be attributed to either their conformational preference for the perpendicular conformation about the linking amide or their enhanced instability leading to rapid hydrolysis of the linking amide. Both are the result of destabilization of the linking amide in plane, conjugated carbonyl conformation of **2** and **3**.

The introduction of the concave face nitrogen atom into the agents **2** and **3** did not impart increasingly more tolerant GC-rich binding or alkylation selectivity and it diminished the apparent AT-rich binding affinity as well, as judged by the reduced DNA alkylation efficiency. These observations are consistent with the more recent interpretation emerging from the studies of the modified distamycins in which their increasing GC-rich binding is also accompanied by preferential 2:1 agent:DNA stoichiometry at the binding sites.[29,30] The diminished van der Waals contacts or hydrophobic binding of a 1:1 complex within the wider minor groove containing a GC base pair is satisfactorily compensated by incorporating an additional ligand while simultaneously satisfying the guanine C2–amine hydrogen bond capabilities of one or both of the bound ligands. In the absence of the compensating van der Waals contacts derived from the 2:1 complexes, the AT-rich DNA binding affinity is simply reduced and that of GC-rich binding is minimally increased. The net result being increasingly more selective, but not specific, GC-rich binding agents which exhibit an overall diminished DNA binding affinity. Whether this diminished DNA binding affinity within AT-rich regions is the result of the loss of stabilizing van der Waals contacts or the result of destabilizing interactions derived from the newly introduced nitrogen remains to be established. Importantly, the behavior of **2** and **3** qualitatively follow the overall binding affinity observations made with such 1:1 complexes and, to date, no evidence of 2:1 complexes has been detected with this series of agents.

In vitro *cytotoxic activity of 2 and 3*

The *in vitro* cytotoxic activities of **2** and **3** and the relevant comparison agents are summarized in Table 1. Consistent with their relative efficiencies of DNA alkylation, the natural enantiomers of **2** and **3** were essentially indistinguishable (200 pM, L1210) and 40 × less potent than (+)-CBI–CDPI$_1$. Thus, the introduction of the single nitrogen atom in the DNA binding subunit of **3** reduced the biological potency 40-fold. Consistent with prior observations, the natural enantiomers of **2** and **3** were 10–100 × more potent than the corresponding unnatural enantiomers. Further, consistent with the relative efficiencies of DNA alkylation, not only were the natural enantiomers more potent than the unnatural enantiomers but *ent*-(−)-**3** was more potent than *ent*-(−)-**2**.

Table 1. *In vitro* cytotoxic activity

Agent	Configuration	IC_{50} (L1210, pM)[a]
(+)-**2**, (+)-CBI–CDPBO$_1$	natural	200[b]
(−)-**2**, (−)-CBI–CDPBO$_1$	unnatural	17000[b]
(+)-**3**, (+)-CBI–CDPBI$_1$	natural	200
(−)-**3**, (−)-CBI–CDPBI$_1$	unnatural	2000
(+)-**39**	natural	200
(−)-**39**	unnatural	2000
(+)-**40**, (+)-CBI–CDPI$_1$	natural	5
(−)-**40**, (−)-CBI–CDPI$_1$	unnatural	380
(+)-CC-1065	natural	20
(−)-CC-1065	unnatural	20
(+)-duocarmycin SA	natural	10
(−)-duocarmycin SA	unnatural	100

[a]Conducted as detailed in Ref. 20. [b]Tested using (+)- and *ent*-(−)-**38**.

In addition, the results are consistent with similar observations made with the lexitropsins where diminished biological potency accompanied the introduction of structural changes that enhance GC-rich binding selectivity but which also diminish the inherent AT-rich binding affinity.[45]

Experimental

2-[(Benzyloxy)methyl]pyrrolo[3,2-e]benzoxazole (6)

A solution of **4** (499 mg, 3.75 mmol) and 2-(benzyloxy)ethylamine (**5**, 1.13 g, 7.50 mmol, 2 equiv.) in anhydrous ethylene glycol dimethyl ether (DME, 120 mL) was cooled to 0 °C and activated MnO_2 (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 MnO_2. The solvent was removed *in vacuo*. Flash chromatography (SiO_2, 2.5 × 25 cm, 40–50% EtOAc–hexane gradient elution) afforded **6** (500 mg, 1.04 g theoretical, 48%) as a pale orange–yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.98 (*br s*, 1H, NH), 7.27–7.40 (*m*, 8H, ArH), 6.92–6.93 (*m*, 1H, ArH), 4.84 (*s*, 2H, $PhCH_2$), 4.70 (*s*, 2H, C2-CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 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 (CH_2), 64.6 (CH_2); IR (neat) ν_{max} 3265, 2862, 1671, 1452, 1367, 1212, 1089, 738, 698 cm^{-1}; FAB-HRMS (NBA) m/z 279.1140 (M^+ + H, $C_{17}H_{14}N_2O_2$ requires 279.1134).

2-(Hydroxymethyl)pyrrolo[3,2-e]benzoxazole (7)

A solution of **6** (67 mg, 0.24 mmol) in EtOH (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 H_2 for 30 min, and neutralized with the addition of Et_3N. The mixture was filtered through a Celite pad to remove the catalyst and the solvent was removed *in vacuo*. Flash chromatography (SiO_2, 1.0 × 20 cm, 60–80% EtOAc–hexane gradient elution) afforded **7** (31.5 mg, 45.1 mg theoretical, 70%) as a white crystalline solid: mp 169–171.5 °C (CH_3OH–CH_2Cl_2); ^{1}H NMR (CD_3OD, 400 MHz) δ 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, CH_2OH); ^{13}C NMR (CD_3OD, 100 MHz) δ 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 (CH_2); IR (film) ν_{max} 3266, 1566, 1438, 1364, 1221, 1083, 1037, 775, 735, 668 cm^{-1}; FAB-HRMS (NBA) m/z 189.0668 (M^+ + H, $C_{10}H_8N_2O_2$ requires 189.0664). Anal. calcd for $C_{10}H_8N_2O_2$: C, 63.81; H, 4.29; N, 14.89. Found: C, 63.50; H, 4.20; N, 14.50.

Methyl pyrrolo[3,2-e]benzoxazole-2-carboxylate (8)

A solution containing NaCN (42 mg, 0.85 mmol, 5 equiv.) and activated MnO_2 (148 mg, 1.7 mmol, 10 equiv.) in 10.5 mL of CH_3OH was treated with a solution of **7** (32 mg, 0.17 mmol) in CH_3OH (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 × 30 mL EtOAc wash) to remove MnO_2 and the combined organic layer was washed with H_2O, saturated aqueous NaCl, dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (SiO_2, 1 × 15 cm, 40% EtOAc–hexane) afforded **8** (37 mg, 37 mg theoretical, 100%) as an off-white solid: mp 207–208 °C (EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 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, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 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 (CH_3); IR (film) ν_{max} 3356, 2921, 1738, 1537, 1437, 1371, 1148 cm^{-1}; FAB-HRMS (NBA) m/z 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.

Methyl 1,2-dihydro-3H-pyrrolo[3,2-e]benzoxazole-7-carboxylate (9)

Compound **8** (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 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 **9**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 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); FAB-HRMS (NBA) m/z 219.0768 (M^+ + H, $C_{11}H_{10}N_2O_3$ requires 219.0770).

Methyl 3-carbamoyl-1,2-dihydro-3H-pyrrolo[3,2-e]-benzoxazole-7-carboxylate (10)

A solution of crude **9** (from 0.22 mmol of **8**) 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 × 3 mL) and CH_3OH (3 mL). Drying the solid *in vacuo* afforded pure **10** (32.7 mg, 57.4 mg theoretical, 57% from **8**) as a pale yellow solid: mp > 230 °C (dec.); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 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 (DMSO-d_6, 100 MHz) δ 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 (CH_2), 25.2 (CH_2); IR (film) ν_{max} 3448, 3179, 1727, 1675, 1606, 1543, 1487, 1423, 1321, 1229, 1155, 1140, 1028, 818 cm^{-1}; FAB-HRMS (NBA) m/z 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.

Methyl 3-(tert-butyloxycarbonyl)-1,2-dihydro-3H-pyrrolo-[3,2-e]benzoxazole-7-carboxylate (11)

A solution of crude **9** (1.8 mg, 0.008 mmol) dissolved in THF (100 μL) was treated with di-*tert*-butyl dicarbonate (3.6 mg, 3.8 μL, 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 (SiO_2, 20–40% EtOAc–hexane gradient elution) afforded **11** (2.0 mg, 2.6 mg theoretical, 76%) as a pale yellow solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 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-H_2), 4.07 (*s*, 3H, CH_3), 3.41 (*t*, 2H, *J* = 8.8 Hz, C1-H_2), 1.56 (*s*, 9H, $C(CH_3)_3$); FAB-HRMS (NBA) m/z 319.1290 (M^+ + H, $C_{16}H_{18}N_2O_5$ requires 319.1294).

3-Carbamoyl-1,2-dihydro-3H-pyrrolo[3,2-e]benzoxazole-7-carboxylic acid (12)

A suspension of **10** (27 mg, 0.103 mmol) and $LiOH·H_2O$ (8.8 mg, 0.21 mmol, 2 equiv.) in THF:CH_3OH:H_2O (3:1:1, 2.5 mL) was stirred at 24 °C for 4 h. The solvent was removed under a stream of N_2 and the residual solid was suspended in H_2O (2 mL) and acidified with 1 N aqueous HCl to pH 1. The insoluble residue was

collected by centrifugation and washed with H_2O (2 × 3 mL). Drying the solid *in vacuo* afforded **12** (25 mg, 25 mg theoretical, 100%) as a pale yellow powder: mp > 230 °C (dec.); ^{1}H NMR (CF_3CO_2D, 400 MHz) δ 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-H_2), 3.73 (*t*, 1H, *J* = 8.4 Hz, C1-H_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 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 (CH_2), 25.2 (CH_2); IR (film) ν_{max} 3476, 3174, 1677, 1606, 1481, 1419, 1369, 1234, 1061, 815 cm^{-1}; FAB-HRMS (NBA) m/z 248.0674 (M^+ + H, $C_{11}H_9N_3O_4$ requires 248.0671).

*1-(*tert*-Butyloxycarbonyl)-5-nitroindole (20)*

A solution of 5-nitroindole (**19**, 2.0 g, 12.3 mmol) and DMAP (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 (SiO_2, 2.5 × 25 cm, 20–50% EtOAc-hexane gradient elution) afforded **20** (3.17 g, 3.17 g theoretical, 100%) as an off-white solid: mp 135–137 °C (CH_2Cl_2, off-white fine needles); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 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, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 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 CH_3); IR (film) ν_{max} 2982, 1737, 1515, 1462, 1329, 1285, 1254, 1156, 1027, 904, 768, 745 cm^{-1}; FAB-HRMS (NBA) m/z 263.1043 (M^+ + H, $C_{13}H_{14}N_2O_4$ requires 263.1032). Anal. calcd for $C_{13}H_{14}N_2O_4$: C, 59.52; H, 5.38; N, 10.69. Found: C, 59.53; H, 5.36; N, 10.53.

*5-Amino-1-(*tert*-butyloxycarbonyl)indole (21)*

A solution of **20** (1.0 g, 3.81 mmol) in EtOAc (30 mL) was treated with 10% Pd/C (500 mg, 0.5 wt equiv.) and the mixture was stirred under 1 atm of H_2 at 24 °C for 5 h. The catalyst was removed by filtration through Celite, and the solvent was removed *in vacuo*. Flash chromatography (SiO_2, 2 × 20 cm, 40–60% EtOAc–hexane gradient elution) afforded **21** (593 mg, 884 mg theoretical, 67%) as a pale brown oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 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, NH_2), 1.65 (*s*, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 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 CH_3); IR (neat) ν_{max} 3359, 1725, 1477, 1454, 1380, 1357, 1343, 1285, 1229, 1166, 1150, 1132, 1024 cm^{-1}; FAB-HRMS (NBA) m/z 232.1212 (M^+, $C_{13}H_{16}N_2O_2$ requires 232.1212).

*5-(2-Benzyloxyacetyl)amino-1-(*tert*-butyloxycarbonyl)-indole (22)*

A solution of **21** (991 mg, 4.27 mmol) and K_2CO_3 (400 mg, 5.12 mmol, 1.2 equiv.) in THF (75 mL) was cooled to 0 °C and stirred for 10 min before benzyloxyacetyl chloride (851 μL, 5.12 mmol, 1.2 equiv.) was added. The reaction mixture was then 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 EtOAc (3 × 150 mL), dried (Na_2SO_4) and concentrated. Flash chromatography (SiO_2, 2 × 20 cm, 40–50% EtOAc–hexane gradient elution) afforded **22** (1.53 g, 1.62 g theoretical, 94%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 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) δ 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) ν_{max} 3381, 2978, 1732, 1688, 1537, 1473, 1372, 1131, 745, 699 cm^{-1}; FAB-HRMS (NBA) m/z 381.1823 (M^+ + H, $C_{22}H_{24}N_2O_4$ requires 381.1814).

*5-(2-Benzyloxyacetyl)amino-1-(*tert*-butyloxycarbonyl)-4-nitroindole (23)*

Compound **22** (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 × 40 mL). The organic layer was dried (Na_2SO_4) and concentrated. Flash chromatography (SiO_2, 2 × 20 cm, 20–40% EtOAc–hexane gradient elution) afforded **23** (570 mg, 878 mg theoretical, 65%) as a bright yellow solid: mp 145–146.5 °C (CH_2Cl_2, light yellow flakes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 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) δ 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 CH_3); IR (film) ν_{max} 3319, 1737, 1701, 1491, 1372, 1323, 1288, 1152, 1108 cm^{-1}; FAB-HRMS (NBA) m/z 425.1605 (M^+ + H, $C_{22}H_{23}N_3O_6$ requires 425.1587). Anal. calcd for $C_{22}H_{23}N_3O_6$: C, 62.09; H, 5.45; N, 9.88. Found: C, 61.84; H, 5.47; N, 9.99.

*4-Amino-5-(2-benzyloxyacetyl)amino-1-(*tert*-butyloxycarbonyl)indole (24)*

Method A. Compound **23** (212 mg, 0.50 mmol) was dissolved in THF (3.5 mL) and treated with a solution of $Na_2S_2O_4$ (870 mg, 5.0 mmol, 10 equiv.) in H_2O (3.5 mL). The reaction mixture was stirred at 24 °C under N_2 for 20 h before it was diluted with H_2O (10 mL), and

extracted with EtOAc (3 × 10 mL). The organic layer was dried (Na_2SO_4) and concentrated. Flash chromatography (SiO_2, 1.5 × 20 cm, 50–60% EtOAc–hexane gradient elution) afforded **24** (138 mg, 197 mg theoretical, 70%) as a pale yellow oil identical in all respects to the sample described below.

Method B. A solution of **23** (910 mg, 2.13 mmol) in EtOAc (45 mL) was treated with 10% Pd/C (455 mg, 0.5 wt equiv.) and the mixture was stirred under 1 atm of H_2 at 24 °C for 3 h. The catalyst was removed by filtration through Celite, and the solvent was removed *in vacuo*. Flash chromatography (SiO_2, 2 × 25 cm, 60% EtOAc–hexane) afforded **24** (776 mg, 846 mg theoretical, 92%) as a pale yellow oil (no debenzylation product was detected): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 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, C_6H_5), 7.01 (*d*, 1H, *J* = 8.7 Hz, ArH), 6.50 (*d*, 1H, *J* = 3.8 Hz, C3-H), 4.67 (*s*, 2H, $PhCH_2$), 4.17 (*s*, 2H, $COCH_2$), 4.16 (*br s*, 2H, NH_2), 1.64 (*s*, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 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 (CH_2), 69.7 (CH_2), 28.3 (three CH_3); IR (neat) ν_{max} 3362, 2978, 1731, 1676, 1491, 1350, 1299, 1152, 1126 cm^{-1}; FAB-HRMS (NBA–CsI) m/z 528.0878 (M^+ + Cs, $C_{22}H_{25}N_3O_4$ requires 528.0899). Anal. calcd for $C_{22}H_{25}N_3O_4$: C, 66.80; H, 6.38; N, 10.63. Found: C, 66.58; H, 6.34; N, 10.39.

*2-[(Benzyloxy)methyl]-6-(*tert*-butyloxycarbonyl)pyrrolo-[3,2-e]benzimidazole (25)*

Compound **24** (192 mg, 0.484 mmol) was dissolved in THF (25 mL) and treated with a solution of THF (5 mL) containing 2 drops of conc. H_2SO_4. The mixture was stirred at 24 °C under N_2 for 24 h before the reaction was neutralized with the addition of saturated aqueous $NaHCO_3$ (20 mL). The mixture was extracted with EtOAc (3 × 20 mL) and the organic layer was concentrated *in vacuo*. Flash chromatography (SiO_2, 1.5 × 20 cm, 40–50% EtOAc–hexane gradient elution) afforded **25** (181 mg, 183 mg theoretical, 99%) as a pale orange oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 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, C_6H_5), 6.92 (*br s*, 1H, C8-H), 4.91 (*s*, 2H, $PhCH_2$), 4.65 (*s*, 2H, C2-CH_2), 1.69 (*s*, 9H, $C(CH_3)_3$); IR (neat) ν_{max} 2978, 1732, 1436, 1370, 1343, 1286, 1150, 1128 cm^{-1}; FAB-HRMS (NBA) m/z 378.1826 (M^+ + H, $C_{22}H_{23}N_3O_3$ requires 378.1818).

*6-(*tert*-Butyloxycarbonyl)-2-(hydroxymethyl)pyrrolo[3,2-e]benzimidazole (26)*

A solution of **25** (677 mg, 1.79 mmol) in EtOH (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 H_2 for 5 h before being quenched with the addition of several drops of Et_3N. The catalyst was removed by filtration through Celite, and the solvent was removed *in vacuo*. Flash chromatography (SiO_2, 2 × 25 cm, 10–20% CH_3OH–EtOAc gradient elution) afforded **26** (481 mg, 516 mg theoretical, 93%) as an off-white powder: mp 152 °C (dec., CH_3OH–CH_2Cl_2); ^{1}H NMR (CD_3OD, 400 MHz) δ 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, CH_2OH), 1.69 (*s*, 9H, $C(CH_3)_3$); ^{13}C NMR (CD_3OD–$CDCl_3$, 400 MHz) δ 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 (CH_2), 28.4 (three CH_3); IR (film) ν_{max} 3179, 2920, 1729, 1676, 1365, 1342, 1289, 1150, 1126 cm^{-1}; FAB-HRMS (NBA–NaI) m/z 310.1160 (M^+ + Na, $C_{15}H_{17}N_3O_3$ requires 310.1168).

*Methyl 6-(*tert*-butyloxycarbonyl)pyrrolo[3,2-e]benzimidazole-2-carboxylate (27)*

A solution containing NaCN (478 mg, 9.75 mmol, 5 equiv.) and activated MnO_2 (1.69 g, 19.5 mmol, 10 equiv.) in CH_3OH (42 mL) was treated with a solution of **26** (560 mg, 1.95 mmol) in CH_3OH (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 (EtOAc wash) to remove MnO_2. EtOAc was added (150 mL total) and the combined organic layer was washed with H_2O (100 mL), saturated aqueous NaCl, dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (SiO_2, 2 × 25 cm, 60–80% EtOAc–hexane gradient elution) afforded **27** (485 mg, 615 mg theoretical, 79%) as a light yellow foam: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 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, CO_2CH_3), 1.62 (*s*, 9H, $C(CH_3)_3$); IR (film) ν_{max} 3378, 1729, 1364, 1341, 1319, 1243, 1146, 1127 cm^{-1}; FAB-HRMS (NBA) m/z 316.1299 (M^+ + H, $C_{16}H_{17}N_3O_4$ requires 316.1297). Anal. calcd for $C_{16}H_{17}N_3O_4$: C, 60.93; H, 5.44; N, 13.33. Found: C, 60.84, H, 5.53; N, 12.97.

Methyl pyrrolo[3,2-e]benzimidazole-2-carboxylate (28)

Compound **27** (100 mg, 0.32 mmol) was treated with anhydrous 3 M HCl in EtOAc (10 mL) at 24 °C for 5 h. The reaction was then neutralized with saturated aqueous $NaHCO_3$ to pH 7–8 and extracted with EtOAc (2 × 10 mL) and CH_3CN (2 × 15 mL). The combined organic layer was concentrated *in vacuo* to afford **28** as a yellow solid which was used in the next reaction without further purification. For **28**: mp 182 °C (dec., CH_3OH–CH_2Cl_2, light yellow powder); ^{1}H NMR (CD_3OD, 400 MHz) δ 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, CO_2CH_3); IR (film) ν_{max} 3380, 1716, 1631, 1518, 1434, 1387, 1314, 1238 cm^{-1}; FAB-HRMS (NBA) m/z 216.0779 (M^+ + H, $C_{11}H_9N_3O_2$ requires 216.0773).

Methyl 3-carbamoyl-1,2-dihydro-3H-pyrrolo[3,2-e]benzimidazole-7-carboxylate (30)

Crude **28** prepared above (0.32 mmol theoretical) was treated with CF_3CO_2H (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 **29** 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 **29** dissolved in 10 mL of CH_2Cl_2:DMF (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 (2 ×) and CH_3OH (1 ×) to afford pure **30** (55.4 mg, 82.5 mg theoretical, 67% from **27**) as a light gray solid: mp > 230 °C (dec); ^{1}H NMR (CF_3CO_2D, 400 MHz) δ 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 ^{1}H NMR signals was observed when the spectrum was recorded in DMSO-d_6 which we attribute to the two accessible tautomeric forms of **30**; ^{13}C NMR (CF_3CO_2D, 100 MHz) δ 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) ν_{max} 3406, 3187, 3027, 1727, 1664, 1441, 1394, 1209, 769 cm^{-1}; FAB-HRMS (NBA) m/z 261.0993 (M^+ + H, $C_{12}H_{12}N_4O_3$ requires 261.0988).

3-Carbamoyl-1,2-dihydro-3H-pyrrolo[3,2-e]benzimidazole-7-carboxylic acid (31)

A suspension of **30** (50 mg, 0.19 mmol) in 6 mL of THF:CH_3OH:H_2O (3:1:1) was treated with LiOH·H_2O (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 1 N aqueous HCl to pH 1. The precipitate was collected by centrifugation and washed with H_2O (2 × 2 mL). Drying the solid *in vacuo* afforded **31** (47 mg, 47 mg theoretical, 100%) as a pale yellow fluffy solid: mp > 230 °C (dec.); ^{1}H NMR (CF_3CO_2D, 400 MHz) δ 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-H_2), 3.69 (*t*, 1H, *J* = 8.4 Hz, C1-H_2); ^{13}C NMR (CF_3CO_2D, 100 MHz) δ 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 (CH_2), 26.9 (CH_2); IR (film) ν_{max} 3183, 1665, 1587, 1496, 1448, 1247, 1119 cm^{-1}; FAB-HRMS (NBA) m/z 247.0838 (M^+ + H, $C_{11}H_{10}N_4O_3$ requires 247.0831).

3-[(3'-Carbamoyl-1',2'-dihydro-3'H-pyrrolo[3',2'-e]benzoxazol-7'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3H-benz[e]indole (38)

Phenol **36** (5.3 mg, 0.0159 mmol) was treated with anhydrous 3 M HCl–EtOAc (2 mL) at 24 °C for 30 min. The solvent was removed *in vacuo* to afford crude unstable **37** (quantitative). A mixture of **37**, [3-(dimethylamino)propyl]ethylcarbodiimide hydrochloride (EDCI, 6.1 mg, 0.032 mmol, 2 equiv.), and $CDPBO_1$ **12** (3.7 mg, 0.015 mmol, 0.95 equiv.) was stirred in DMF (400 μL) at 24 °C under Ar for 12 h. The solvent was removed *in vacuo* and the dry residue was mixed with H_2O (1 mL) and stirred for 10 min. The precipitate was collected by centrifugation, and washed with H_2O (2 × 1 mL) and dried *in vacuo*. Flash chromatography (SiO_2, 0.5 × 10 cm, 0–10% CH_3OH–$CHCl_3$ gradient elution) afforded **38** (6.4 mg, 7.3 mg theoretical, 88%) as a pale greenish powder: mp > 230 °C (dec.); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 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, NH_2), 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, C<u>H</u>HCl and C2'-H_2), 3.86 (*dd*, 1H, *J* = 7.9, 10.9 Hz, CH<u>H</u>Cl), 3.38–3.43 (*m*, 2H, C1'-H_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 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) ν_{max} 3359, 3225, 1650, 1583, 1488, 1424, 1258, 1120, 1024, 764 cm^{-1}; FAB-HRMS (NBA) m/z 463.1182 (M^+ + H, $C_{24}H_{19}ClN_4O_4$ requires 463.1173). Natural (1*S*)-**38**: $[\alpha]_D^{23}$ +44 (*c* 0.12, DMF). *ent*-(1*R*)-**38**: $[\alpha]_D^{23}$ –41 (*c* 0.09, DMF).

3-[(3'-Carbamoyl-1',2'-dihydro-3'H-pyrrolo[3',2'-e]benzimidazol-7'-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3H-benz[e]indole (39)

Phenol **36** (4.6 mg, 0.0138 mmol) was treated with anhydrous 3 M HCl–EtOAc (2 mL) at 24 °C for 40 min. The solvent was removed *in vacuo* to afford crude unstable **37** (quantitative). A mixture of **37**, [3-(dimethylamino)propyl]ethylcarbodiimide hydrochloride (EDCI, 5.3 mg, 0.028 mmol, 2 equiv.) and $CDPBI_1$ **31** (3.4 mg, 0.14 mmol, 1 equiv.) was stirred in DMF (400 μL) at 24 °C under N_2 for 6 h. The solvent was removed *in vacuo*. The dry residue was dissolved in 10% CH_3OH–$CHCl_3$ and loaded on a flash chromatography column (SiO_2, 0.8 × 10 cm) and eluted with 0–10% CH_3OH–$CHCl_3$ gradient elution to afford **39** (5.2 mg, 12.6 mg theoretical, 42%) as a light gray solid: mp > 230 °C (dec.); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 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* = 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, C<u>H</u>HCl, 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) ν_{max} 3355, 3212, 2925, 1620, 1584, 1499, 1446, 1423, 1333, 1257, 1122, 1019 cm^{-1}; FAB-HRMS (NBA) m/z 462.1345 (M^+ + H, $C_{24}H_{20}ClN_5O_3$ requires 462.1333). Natural (1*S*)-**39**: $[\alpha]_D^{23}$ +49 (*c* 0.19, DMF). *ent*-(1*R*)-**39**: $[\alpha]_D^{23}$ –48 (*c* 0.04, DMF).

N^2-[(3'-Carbamoyl-1',2'-dihydro-3'H-pyrrolo[3',2'-e]-benzimidazol-7'-yl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[c]benz[e]indol-4-one (3)

A solution of **39** (1.4 mg, 3 μmol) in 300 μL of THF:DMF (1:1) was cooled to 0 °C and treated with DBN (0.5 μL, 4.5 μmol, 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 (SiO_2, 0.5 × 3 mm), and eluted with 5–10% CH_3OH–$CHCl_3$ (gradient elution) to afford **3** (0.8 mg, 1.3 mg theoretical, 63%) as a bright yellow solid: mp > 230 °C; ^1H NMR (DMF-d_7, 400 MHz) δ 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, NH_2), 5.33 (*d*, 1H, *J* = 11.8 Hz, C1-H), 4.77 (*dd*, 1H, *J* = 5.0, 11.8 Hz, C1-H), 4.20 (*t*, 1H, *J* = 8.8 Hz, C2'-H_2), 3.44 (*t*, 2H, partially obscured by H_2O, *J* = 8.8 Hz, C1'-H_2), 3.31–3.35 (*m*, 1H, C9a-H), 1.76–1.80 (*m*, 2H, C9-H_2); IR (film) ν_{max} 1656, 1620, 1589, 1495, 1442, 1406, 1272, 1125 cm^{-1}; FAB-HRMS (NBA) m/z 426.1545 (M^+ + H, $C_{24}H_{19}N_5O_3$ requires 426.1566). Natural (+)-**3**: $[\alpha]_D^{23}$ +95 (*c* 0.04, DMF). *ent*-(–)-**3**: $[\alpha]_D^{24}$ –94 (*c* 0.05, DMF).

DNA alkylation studies

General procedures, the preparation of singly ^{32}P 5' end-labeled double-stranded DNA, the agent binding studies, gel electrophoresis, and autoradiography were conducted according to procedures described in full detail elsewhere.[10] Eppendorf tubes containing the 5' end-labeled DNA (9 μL, w794 and w836) in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) were treated with the agent DMSO (1 μL 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 EtOH precipitation and resuspended in TE buffer (10 μL). 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% Na_2EDTA 250 mM) was added (5 μL) to the supernatant. Prior to electrophoresis, the sample was denatured by warming at 100 °C for 5 min, placed in an ice bath, and centrifuged, before the supernatant (3 μL) was loaded directly onto the gel. Sanger dideoxynucleotide sequencing reactions[41] were run as standards adjacent to the reaction samples. Polyacrylamide gel electrophoresis (PAGE) was run on an 8% sequencing gel under denaturing conditions (8 M urea) in TBE buffer (100 mM Tris, 100 mM boric acid, 0.2 mM Na_2EDTA) followed by autoradiography.

Acknowledgments

We gratefully acknowledge the financial support of the National Institutes of Health (CA 41986) and we wish to thank Christine M. Tarby for providing Figure 2, Professor Paul A. Kitos (University of Kansas), Dr Wolfgang Wrasidlo and R. Merlock (The Scripps Research Institute) for conducting the *in vitro* cytotoxic assays.

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(*Received in U.S.A.* 1 *November* 1994; *accepted* 19 *December* 1994)