# Synthesis and Evaluation of CC-1065 and Duocarmycin Analogues Incorporating the Iso-CI and Iso-CBI Alkylation Subunits: Impact of Relocation of the C-4 Carbonyl

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The synthesis of 2-(*tert*-Butyloxycarbonyl)-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*f*]indol-8-one (**31**, *N*-BOC-iso-CBI) and 1-(*tert*-Butyloxycarbonyl)-4-hydroxy-3-[[(methanesulfonyl)oxy]methyl]-2,3-dihydroindole (**19**, seco-*N*-BOC-iso-CI) containing an isomeric structural modification in the CC-1065 and duocarmycin alkylation subunits and their incorporation into analogues of the natural products are detailed. The approach was based on a directed ortho metalation of an appropriately functionalized benzene (**13**) or naphthalene (**24**) precursor to regiospecifically install iodine at the C-2 position. Conversion of these respective intermediates to the dihydroindole skeleton utilized an established 5-*exo-trig* aryl radical cyclization onto an unactivated alkene with subsequent TEMPO trap or the more recent 5-*exo-trig* aryl radical cyclization onto a vinyl chloride for direct synthesis of the immediate precursors. Closure of the activated cyclopropane to complete the iso-CBI nucleus was accomplished by a selective ortho spirocyclization. The evaluation of the iso-CBI-based agents revealed a significant stability comparable to that of CC-1065 and duocarmycin A, but that it is more reactive than duocarmycin SA (6−7×) or the direct comparison CBI-based agents (5×) for which X-ray structure comparisons served to establish the basis for their inherent reaction regioselectivity and reactivity. Resolution and synthesis of a full set of natural product analogues and subsequent evaluation of their DNA alkylation properties revealed that the iso-CBI analogues, even with the relocation of the C-4 carbonyl and the most substantial structural modifications to the alkylation subunit to date, reacted at comparable rates and retain the identical and characteristic sequence selectivity of CC-1065 and the duocarmycins. This observation is inconsistent with the proposal that a sequence-dependent C-4 carbonyl protonation by strategically located DNA backbone phosphates controls the DNA alkylation selectivity but is consistent with the proposal that it is determined by the AT-rich noncovalent binding selectivity of the agents and the steric accessibility of the N3 alkylation site. Confirmation that the DNA alkylation reaction is derived from adenine N3 addition to the least substituted carbon of the activated cyclopropane, and its quantitation (95%) was established by isolation and characterization of the depurination adenine N3 adduct. Consistent with past studies and despite the deep-seated structural change in the alkylation subunit, the agents were found to exhibit potent cytotoxic activity that correlates with their inherent reactivity.

(+)-CC-1065 (**1**)[1] and the duocarmycins **2** and **3**[2,3] are the initial members of a potent class of antitumor antibiotics that derive their biological properties through alkylation of DNA (Figure 1).[4] Prior studies have shown

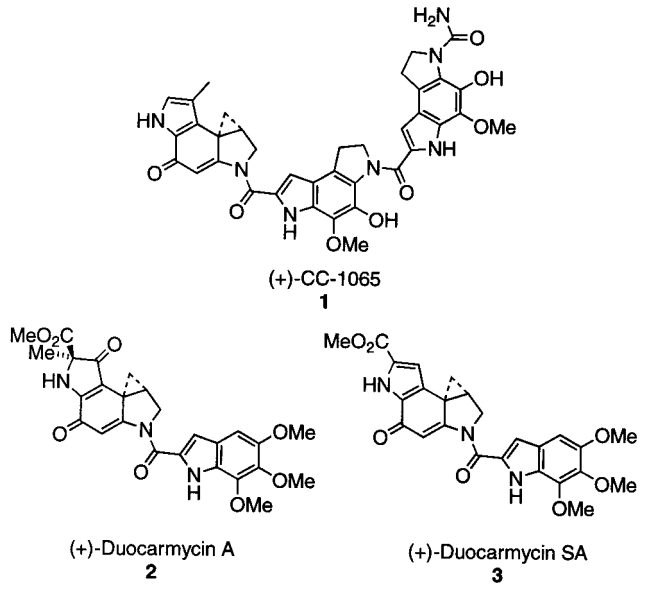


**Figure 1.**

that the natural products can withstand and may benefit from significant structural modifications to the alkylation subunit and that the resulting agents retain their ability

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to participate in the characteristic sequence-selective DNA alkylation reaction.[4,5] These structural modifications and the definition of their effects have served to advance the understanding of the origin of the sequence selectivity[4,6] and the catalysis[7,8] of the DNA alkylation reaction by **1**−**3**.

Throughout our investigations,[4,7] we have advanced the proposal that the DNA alkylation sequence selectivity is determined by the AT-rich noncovalent binding selectivity of the agents and their steric accessibility to the adenine N3 alkylation site. This noncovalent binding model accommodates and explains the reverse and offset 5 or 3.5 base-pair AT-rich adenine N3 alkylation selectivities of the natural and unnatural enantiomers of **1**[9] and **2**−**3**,[10,11] respectively, and requires that simple derivatives of the alkylation subunits exhibit alkylation selectivities distinct from the natural products. It offers a beautiful explanation for the identical alkylation selectivities of both enantiomers of such simple derivatives (5′-AA > 5′-TA), and the more extended AT-rich selectivity of the advanced analogues of **1**−**3** corresponds nicely to the length of the agent and the size of the required binding region surrounding the alkylation site. Further support of this model includes the demonstrated AT-rich noncovalent binding of the agents,[12] their preferential noncovalent binding coincidental with DNA alkylation,[13] and a demonstration that the characteristic DNA alkylation selectivity does not require the presence of the C-4 carbonyl or even the activated cyclopropane.[14,15] A more recent demonstration of the complete switch in the inherent enantiomeric DNA alkylation selectivity that accompanied the reversal of the orientation of the DNA binding subunits with reversed versus extended analogues of the duocarmycins further established the accuracy of this interpretation.[16] This model contrasts an alternative proposal in which a sequence-dependent backbone phosphate protonation of the C-4 carbonyl activates the agent for DNA alkylation and controls the sequence selectivity.[6,8,17] In efforts to further distinguish the accuracy of these two interpretations, herein we report the synthesis and evaluation of a series of analogues related to the CI and CBI alkylation subunits **4** and **5** that *isomerically* relocate the key C-4 carbonyl to the C-6 or C-8 position, now ortho to the cyclopropane (Figure 2). From structural studies of DNA−agent adducts,[17−19] the C-4 carbonyl of the natural products projects out of the minor groove lying on the outer face of the complexes potentially accessible to the phosphate backbone. In contrast, the relocated carbonyls of iso-CI and iso-CBI (**6** and **7**) would be required to project into the minor groove inaccessible to the phosphate backbone if participating in an analogous adenine N3 alkylation reaction. Thus, the examination of **6** and **7** and their related analogues of **1**−**3**, which incorporate the most substantial structural modification to the alkylation

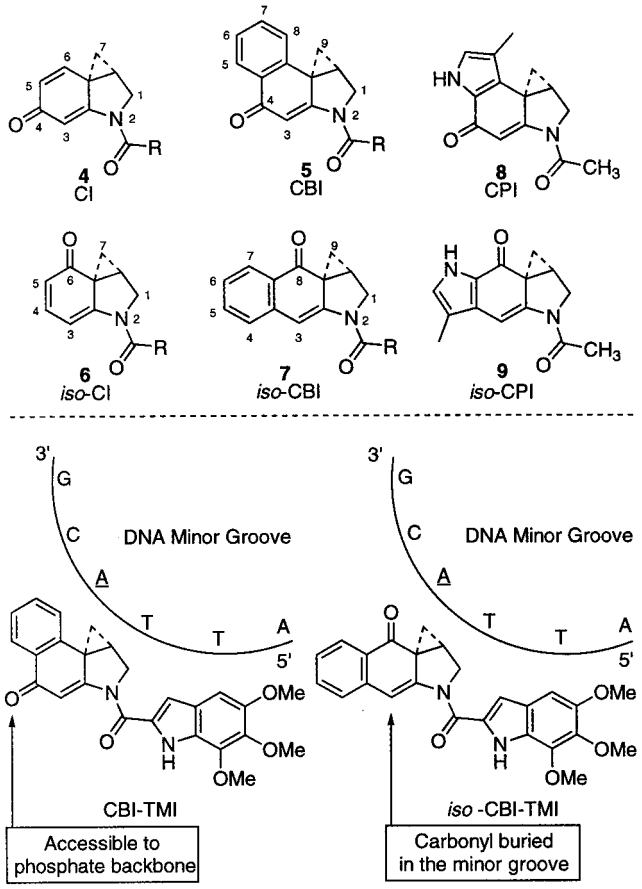


**Figure 2.**

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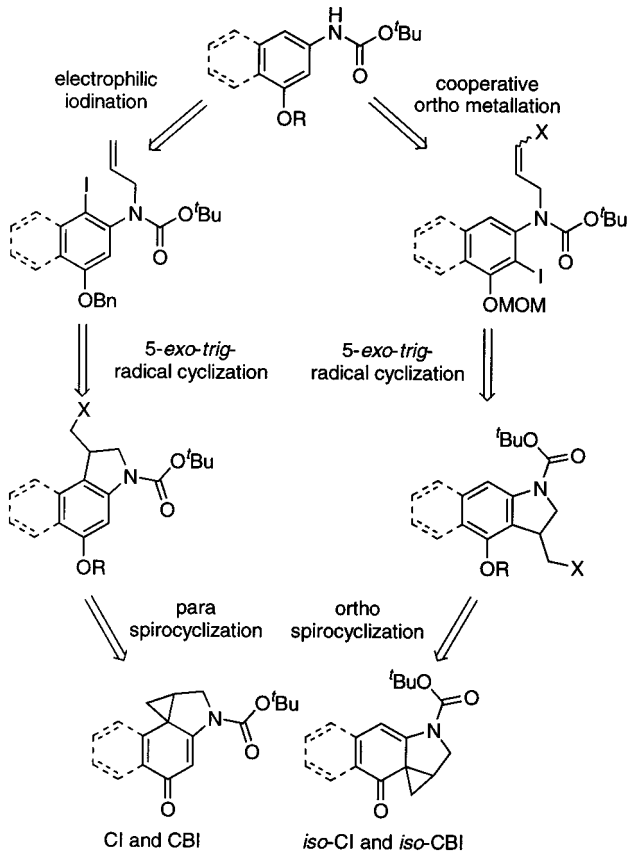
subunits to date, would permit an assessment of the importance of the C-4 carbonyl positioning to the properties of the agents.

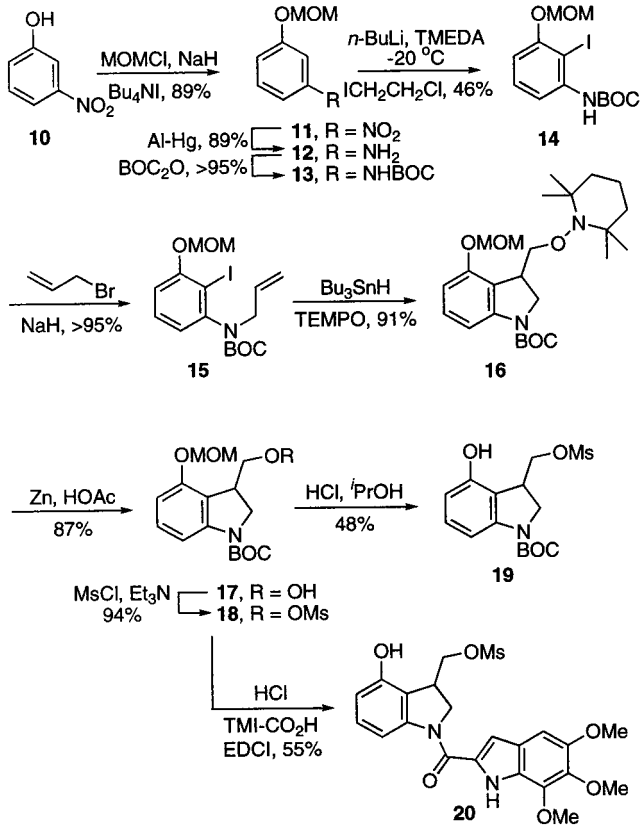
**Synthesis.** Only one report of the synthesis of such agents isomeric to the natural products has been detailed. Sundberg and co-workers[20] prepared an agent isomeric with the alkylation subunit of CC-1065 employing an intramolecular carbene insertion of an *o*-quinonediazide onto a tethered alkene (Figure 2). Presumably because of the perceived unique character of the authentic alkylation subunit at the time of the work, its chemical behavior, biological characteristics, and DNA alkylation properties were not examined. An alternative route was devised for the synthesis of the isomeric CI[21] and CBI[22] analogues (Scheme 1). The strategy is complementary to our most recent synthesis of CBI[5,22] in which the dihydroindole skeleton was constructed by a 5-*exo*-*trig* radical cyclization of an aryl radical onto a tethered alkene, and its extension to the isomeric analogues required C2 versus C4 regiocontrol in the key aromatic halogenation step. In the synthesis of CI and CBI, a regioselective electrophilic halogenation served to install iodine or bromine para to the phenol ether. For the isomeric agents, an ortho halogenation protocol was required, and a cooperative directed ortho metalation[23] was implemented to install the C2 halide. Its adoption required two easily removed cooperative directing metalation groups: OMOM[24] and NHBOC.[25] In addition, the Winstein para Ar-3′ spirocyclization,[26] utilized to close the cyclopropane in the CI and CBI synthesis, is now replaced by an ortho spirocyclization requiring *C*-alkylation with cyclopropane formation[27] rather than competitive *O*-alkylation and dihydrofuran formation.

This approach was first examined with iso-CI employing the commercially available 3-nitrophenol (**10**), Scheme 2. Protection of the phenol as the MOM ether **11** (NaH, MOMCl, $Bu_4NI$, 89%), reduction of the nitro group with

**Scheme 1**




**Scheme 2**




Al−Hg amalgam[28] ($Et_2O-H_2O$, 89%), and BOC protection of the free amine **12** ($BOC_2O$, >95%) provided **13**, a key intermediate with which to examine the directed ortho metalation. Treatment of **13** with 3.5 equiv of *n*-BuLi and TMEDA at −20 °C (2 h) in THF, and reaction of the

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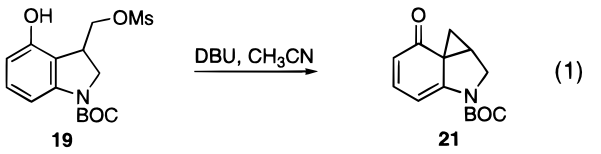
aryllithium intermediate with 1-chloro-2-iodoethane provided **14** (46%) along with recovered starting material (41%). Although not extensively examined, this conversion was not improved through use of longer reaction times, different reaction temperatures or solvents, or additional amounts of *n*-BuLi. However, the conversion did allow the synthesis to proceed, and, as detailed, this reaction proved much more effective in the iso-CBI series where it was more carefully optimized. *N*-Alkylation with allyl bromide (NaH, >95%) was followed by $Bu_3$-SnH-promoted 5-*exo-trig* free radical cyclization of **15** with in situ TEMPO trap[22] of the resulting primary radical to provide **16** (91%). Subsequent reduction of the N−O bond (Zn, HOAc, 87%) without the competitive removal of either the MOM ether or the *N*-BOC protecting groups afforded the free alcohol **17** in excellent overall yield. Activation of the primary alcohol (MsCl, $Et_3N$, 94%) afforded the key intermediate **18**.[29]

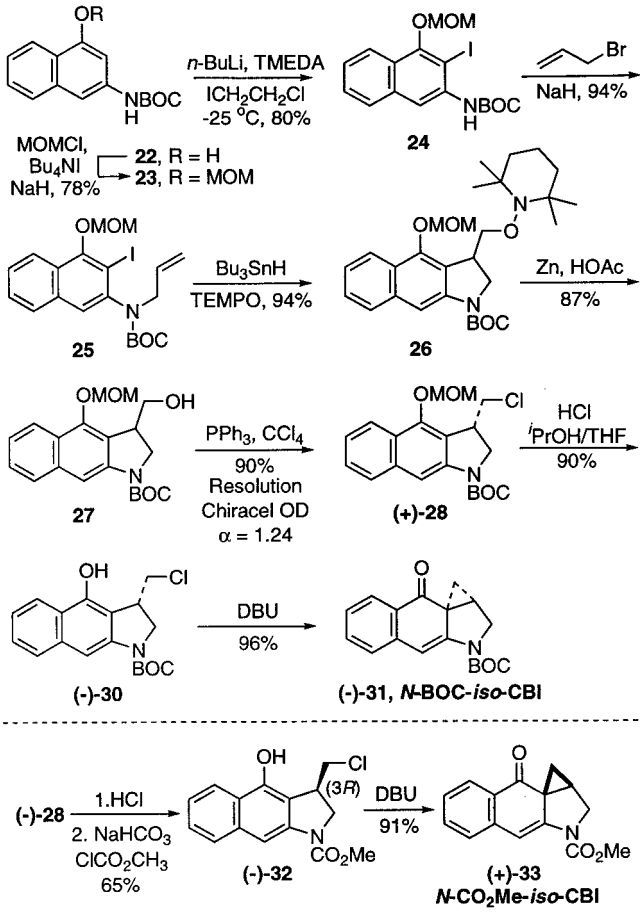
To prepare *N*-BOC-iso-CI for direct comparison with prior agents, selective removal of the MOM group in the presence of the BOC group was required. Mild acid-catalyzed deprotection (HCl, *i*-PrOH/THF, 48%) provided seco-*N*-BOC-iso-CI (**19**) accompanied by 41% recovery of starting material. Although not optimized, this selective deprotection found greater success in the synthesis of *N*-BOC-iso-CBI where it was more closely examined. Exhaustive deprotection of the MOM ether and the BOC group (3.6 M HCl/EtOAc) followed by EDCI-promoted[30] coupling with 5,6,7-trimethoxyindole-2-carboxylic acid (TMI-COOH)[10,31] provided **20** (55% overall). Consistent with expectations, spirocyclization of **19** to *N*-BOC-iso-CI (**21**) could be effected by treatment with DBU, but its exceptional reactivity precluded attempts to isolate and characterize the agent (eq 1).[32] Sufficient for our considerations, the studies revealed that *N*-BOC-iso-CI is more reactive than its counterpart *N*-BOC-CI which, although exceptionally reactive, can be isolated by quick column chromatography. Since the seco agents can be expected to behave analogous to their ring-closed cyclopropane counterparts in biological assays and DNA alkylation studies,[4] and given that the careful comparisons could be made with the more stable iso-CBI based agents, their isolation and characterization were not further pursued.




**19** **21** (1)

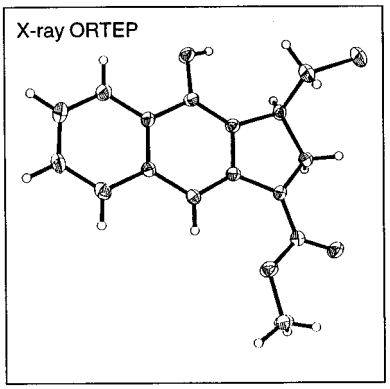
With the additional stability provided by the fused benzene ring, the CBI analogues are substantially more stable[4] and biologically more potent than the CI series. The extension of this approach to the preparation of the iso-CBI alkylation subunit is detailed in Scheme 3 and provided the opportunity to accurately document the effects of this deep-seated structural change. Starting with **22**, available in two steps (70%) from commercially available 1,3-dihydroxynaphthalene,[22] protection of the phenol (MOMCl, NaH, $Bu_4NI$, 78%) as the MOM ether provided the directed ortho metalation substrate **23**. Treatment of **23** with 3.5 equiv of *n*-BuLi and TMEDA at −25 °C in THF for 2 h and reaction of the aryllithium intermediate with 1-chloro-2-iodoethane gave the C2 iodide **24** in 80% yield, a substantial improvement over the iso-CI directed metalation. *N*-Alkylation (NaH, allyl bromide, 94%), 5-*exo-trig* free radical cyclization of **25** with in situ TEMPO trap ($Bu_3SnH$, TEMPO, 94%), and N−O bond reduction of **26** (Zn, HOAc, 87%) provided the primary alcohol **27** in excellent overall yield. Conversion to the chloride **28** upon activation of the primary alcohol under Mitsunobu conditions ($Ph_3P$, $CCl_4$, 90%) provided a resolvable intermediate that served as a penultimate precursor to all analogues. After the initial completion of this route, a report detailing the synthesis of duocarmycin SA analogues described a further improvement in this general approach.[33] A 5-*exo-trig* radical cyclization onto a tethered vinyl chloride was shown to provide the

**Scheme 3**










(29) Full experimental details and characterization for **11**−**18** is provided in the Supporting Information.

(30) EDCI = 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride.

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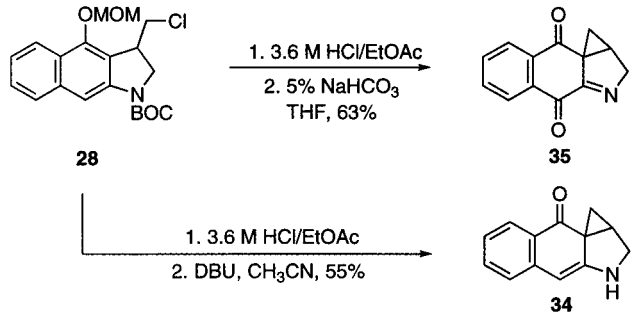
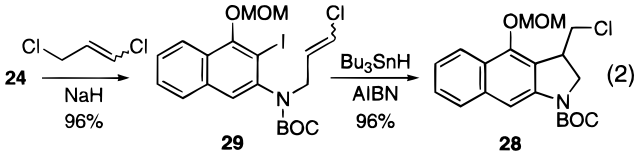
(32) Diagnostic $^1H$ NMR ($CD_3CN$, 400 MHz) signals for **21** generated in situ: $\delta$ 2.79 (dt, $J$ = 5.4, 7.8 Hz, 1H), 1.75 (dd, $J$ = 3.1, 7.8 Hz, 1H).

**Scheme 4**

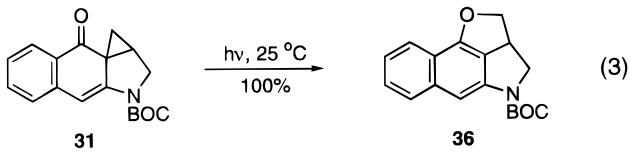



five-membered ring with a suitable leaving group already in place. This concise strategy was adopted for the synthesis of iso-CBI (eq 2). Thus, *N*-alkylation of **25** with 1,3-dichloropropene proceeded in 96% yield, providing the key radical cyclization precursor (**29**). Treatment with catalytic AIBN (0.1 equiv) and $Bu_3SnH$ (1.1 equiv) at 80 °C ($C_6H_6$) yielded the tricyclic core of iso-CBI (**28**) in 96% yield. This improved approach shortens the original synthesis by two steps, avoiding reductive removal of the TEMPO group and conversion to **28**. With this improvement, the preparation of **28** requires four steps and proceeds in 58% yield overall. Removal of the MOM ether (HCl, *i*-PrOH/THF, 90%) without competitive *N*-BOC deprotection afforded seco-*N*-BOC-iso-CBI (**30**) in superb yield. Ortho spirocyclization (DBU, $CH_3CN$, 96%) provided *N*-BOC-iso-CBI (**31**) which could be purified by standard chromatography. Similarly, exhaustive deprotection of **28** (3.6 N HCl/EtOAc) followed by *N*-acylation with methyl chloroformate provided **32** (65%), and spirocyclization (DBU, $CH_3CN$, 25 °C, 91%) afforded **33**.




Initial attempts to synthesize iso-CBI (**34**) by exhaustive deprotection (3.6 M HCl/EtOAc) and subsequent ring closure (5% aqueous $NaHCO_3$/THF) conducted in the presence of air resulted in the isolation of the quinone **35** (Scheme 4). Presumably, adventious oxidation of the intermediate iso-CBI after spirocyclization provided **35** and an interesting further modification of the iso-CBI alkylation subunit.[34] Consistent with this, spirocyclization conducted in an aprotic solvent under an inert atmosphere (2.2 equiv of DBU, $CH_3CN$, 25 °C, 20 min, Ar) provided the unstable iso-CBI (**34**, 55%), and subsequent exposure of **34** to air resulted in conversion to **35**.

Exclusive spirocyclization with cyclopropane formation was observed, and no competitive *O*-alkylation leading to **36** was detected. However, prolonged exposure of neat **31** to ambient light at 25 °C (48 h) did lead to carbonyl−cyclopropane rearrangement[35] to form **36** (eq 3). An identical neat sample of **31** protected from light by foil remained unchanged after 48 h. Thus, the handling and storage of the iso-CBI based agents were conducted, minimizing their exposure to light typically at subzero temperatures. Examination of the compounds over time showed no appreciable decomposition or rearrangement under these storage conditions.




**Resolution.** To assess the properties of both enantiomers of the iso-CBI based agents, a direct chromatographic resolution of **28** on a semipreparative ChiralCel OD column (2 × 25 cm, 3% *i*-PrOH/hexane, $\alpha = 1.24$) was utilized.[36] This procedure provided both enantiomers (>99% ee) of an advanced intermediate and avoided diastereomeric derivatization, separation, and dederivatization. The assignment of absolute configuration was based on the conversion of the slower eluting enantiomer of **28** ($t_R = 35.8$ min) to **32** and subsequent single-crystal X-ray structure determination which revealed the unnatural (3*R*)-configuration (Scheme 3).[37] Consistent with this assignment, the agents derived from the (3*S*)-enantiomer analogous to the absolute stereochemistry found in the natural products **1**−**3** exhibited the more potent biological activity, the more effective DNA alkylation properties, and a DNA alkylation selectivity identical with the natural products.

**Synthesis of Duocarmycin and CC-1065 Analogues.** The iso-CBI alkylation subunit was incorporated into CC-1065 and duocarmycin analogues as detailed in Scheme 5. Exhaustive deprotection of **28** (3.6 M HCl/EtOAc, 30 min) followed by immediate coupling (3 equiv of EDCI, DMF, 25 °C) of the amine hydrochloride salt with 5,6,7-trimethoxyindole-2-carboxylic acid[10,31] (**37**, 3 h, 91%), **38**[16] (3 h, 95%), **39** (3 h, 87%), indole$_2$[38] (**40**, 3 h, 74%), $CDPI_1$[39] (**41**, 9 h, 80%), and $CDPI_2$[39] (**42**, 12 h, 32%) provided **43**, **45**, **47**, **49**, **51**, and **53**, respectively. The poor solubility of $CDPI_2$ precluded efficient coupling and isolation, resulting in a lower yield. DBU (1.5 equiv, 25 °C) spirocyclization of **43** ($CH_3CN$, 30 min, 94%), **45** ($CH_3CN$, 30 min, 85%), **47** ($CH_3CN$, 30 min, 83%), **49** (DMF, 30 min, 88%), **51** (DMF, 60 min, 81%), and **53** (DMF, 60 min, 59%) afforded **44**, **46**, **48**, **50**, **52**, and **54**, respectively, in excellent conversions.

**Solvolysis Reactivity and Regioselectivity.** Two fundamental characteristics of the alkylation subunits have proven important in past studies.[4] The first is the stereoelectronically controlled acid-catalyzed ring opening of the activated cyclopropane which dictates preferential addition of a nucleophile to the least substituted cyclopropane carbon. The second is the relative rate of acid-catalyzed solvolysis which has been found to accurately reflect the functional reactivity of the agents and to follow a direct relationship between solvolysis stability and in vitro cytotoxicity.[4] While *N*-BOC-iso-CBI retains many of the structural characteristics of *N*-BOC-CBI, the isomeric modifications which may disrupt the vinylogous amide conjugation were anticipated to reduce the sol-

(33) Patel, V. F.; Andis, S. L.; Enkema, J. K.; Johnson, D. A.; Kennedy, J. H.; Mohamadi, F.; Schultz, R. M.; Soose, D. J.; Spees, M. M. *J. Org. Chem.* **1997**, *62*, 8868.

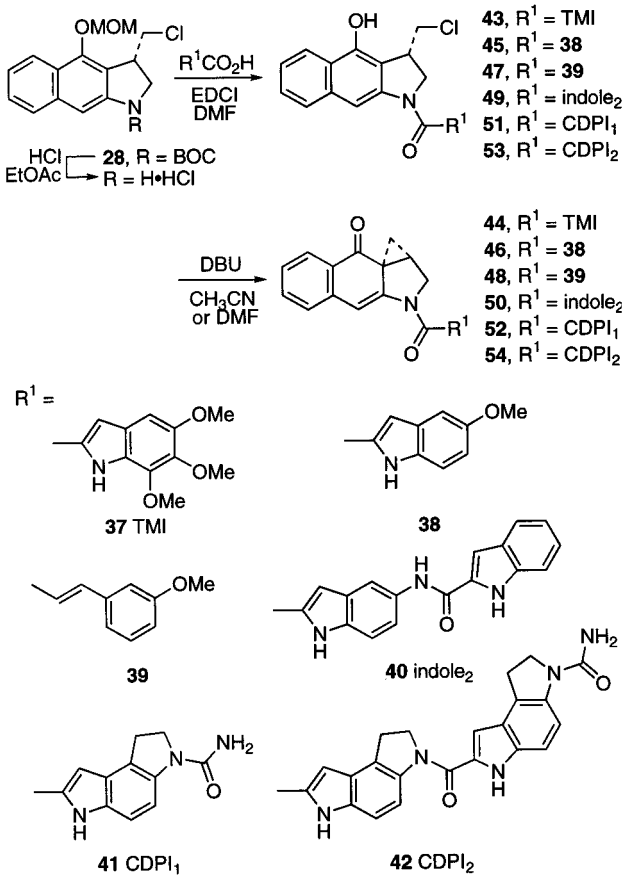
(34) Such agents may be subject to reductive activation.

(35) Wong, H. N. C.; Hon, M.-Y.; Tse, C.-W.; Yip, Y.-C.; Tanko, J.; Hudlicky, T. *Chem. Rev. (Washington, D.C.)* **1989**, *89*, 165. See also: Schweizer, E. E.; Kopay, C. M. *J. Org. Chem.* **1971**, *36*, 1489. Danishefsky, S.; Dynak, J. *Tetrahedron Lett.* **1975**, 79. Saalfrank, R. W.; Gündel, J.; Rossman, G.; Hanek, M.; Rost, W.; Peters, K.; von Schnering, H. G. *Chem. Ber.* **1990**, *123*, 1175.

(36) Boger, D. L.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 7996.

(37) The atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and may be obtained upon request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

**Scheme 5**

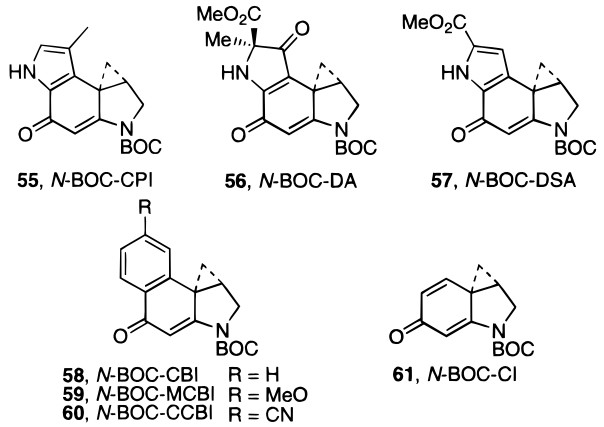



volytic stability of the agents. However, it was not clear what the magnitude of this effect might be nor whether solvolysis would still occur with the same high regioselectivity.

*N*-BOC-iso-CBI (**31**, $t_{1/2}$ = 27.6 h, $k = 6.97 \times 10^{-6}$ $s^{-1}$) and *N*-$CO_2$Me-iso-CBI (**33**, $t_{1/2}$ = 30.1 h, $k = 6.40 \times 10^{-6}$ $s^{-1}$) proved to be reasonably stable toward chemical solvolysis at pH 3, exhibiting a reactivity comparable to the CC-1065 alkylation subunit (*N*-BOC-CPI, **55**, $t_{1/2}$ = 36.7 h), but more stable than the duocarmycin A alkylation subunit (*N*-BOC-DA, **56**, $t_{1/2}$ = 11 h), Table 1. However, *N*-BOC-iso-CBI was significantly less stable than *N*-BOC-DSA (**57**, $t_{1/2}$ = 177 h) and *N*-BOC-CBI (**58**, $t_{1/2}$ = 133 h). Thus, *N*-BOC-iso-CBI (**31**) proved to be 5× more reactive than its direct comparison analogue *N*-BOC-CBI. The solvolysis was followed spectrophotometrically by UV with the disappearance of the long-wavelength absorption band of the iso-CBI chromophore (397 nm), Figure 3. The reactivity of the quinone **35** was also examined at pH 3 ($t_{1/2}$ = 5.1 h, $k = 3.80 \times 10^{-5}$ $s^{-1}$), and it proved to be 5−6× more reactive than **31** or **33**.

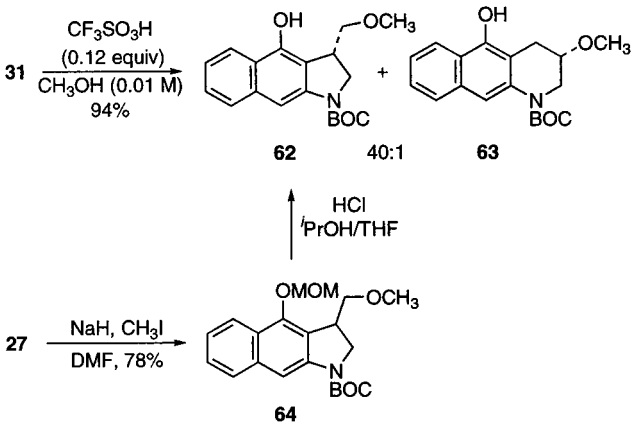
The acid-catalyzed nucleophilic addition of $CH_3OH$ to **31** was conducted on a preparative scale to establish the regioselectivity of addition and confirmed by synthesis of the expected product **62** derived from nucleophilic addition to the least substituted cyclopropane carbon. Treatment of *N*-BOC-iso-CBI with 0.1 equiv of $CF_3SO_3H$ in $CH_3OH$ (25 °C, 17 h) resulted in the clean solvolysis (94%) to provide a 40:1 mixture of **62** and **63** (Scheme 6). Consequently, the acid-catalyzed $CH_3OH$ addition to **31** occurs with near exclusive regioselectivity (40:1) analogous to *N*-BOC-CBI (**58**, >20:1)[22] which is much more selective than the natural alkylation subunits themselves (6−1.5:1).[16,40,41]

**Table 1. Solvolysis Reactivity and Regioselectivity**




| Agent | $k$ ($s^{-1}$, pH 3) | $t_{1/2}$ (h, pH 3) | Regioselectivity |
|---|---|---|---|
| **31** | $6.98 \times 10^{-6}$ | 28 h | 40 : 1 |
| **35** | $3.80 \times 10^{-5}$ | 5 h | nd |
| **55** | $5.26 \times 10^{-6}$ | 37 h | 4 : 1 |
| **56** | $1.75 \times 10^{-5}$ | 11 h | 3 : 2 |
| **57** | $1.08 \times 10^{-6}$ | 177 h | 6-4 : 1 |
| **58** | $1.45 \times 10^{-6}$ | 133 h | > 20 : 1 |
| **59** | $1.75 \times 10^{-6}$ | 110 h | > 20 : 1 |
| **60** | $0.99 \times 10^{-6}$ | 194 h | > 20 : 1 |
| **61** | $1.98 \times 10^{-2}$ | 0.01 h | nd |

**Scheme 6**




**X-ray Structure of *N*-$CO_2$Me-*iso*-CBI (33): Structural Correlation with Solvolysis Regioselectivity and Reactivity.** The single-crystal X-ray structure determination of *N*-$CO_2$Me-iso-CBI (**33**)[37] was conducted and by direct comparison with that of *N*-$CO_2$Me-CBI[42] established a structural basis for the observed properties (Figure 4). The first striking similarity between the two systems is the perpendicular orientation of the bent orbital of the cyclopropane bond extending to the least substituted C9 cyclopropane carbon. This idealized stereoelectronic alignment with the developing $\pi$-system of

(38) Boger, D. L.; Yun, W.; Han, N. *Bioorg. Med. Chem.* **1995**, *3*, 1429.
(39) Boger, D. L.; Coleman, R. S.; Invergo, B. J. *J. Org. Chem.* **1987**, *52*, 1521. Boger, D. L.; Coleman, R. S. *J. Org. Chem.* **1984**, *49*, 2240.
(40) Boger, D. L.; Goldberg, J.; McKie, J. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1955.
(41) Boger, D. L.; McKie, J. A.; Nishi, T.; Ogiku, T. *J. Am. Chem. Soc.* **1997**, *119*, 311.
(42) Boger, D. L.; Turnbull, P. *J. Org. Chem.* **1997**, *62*, 5849.

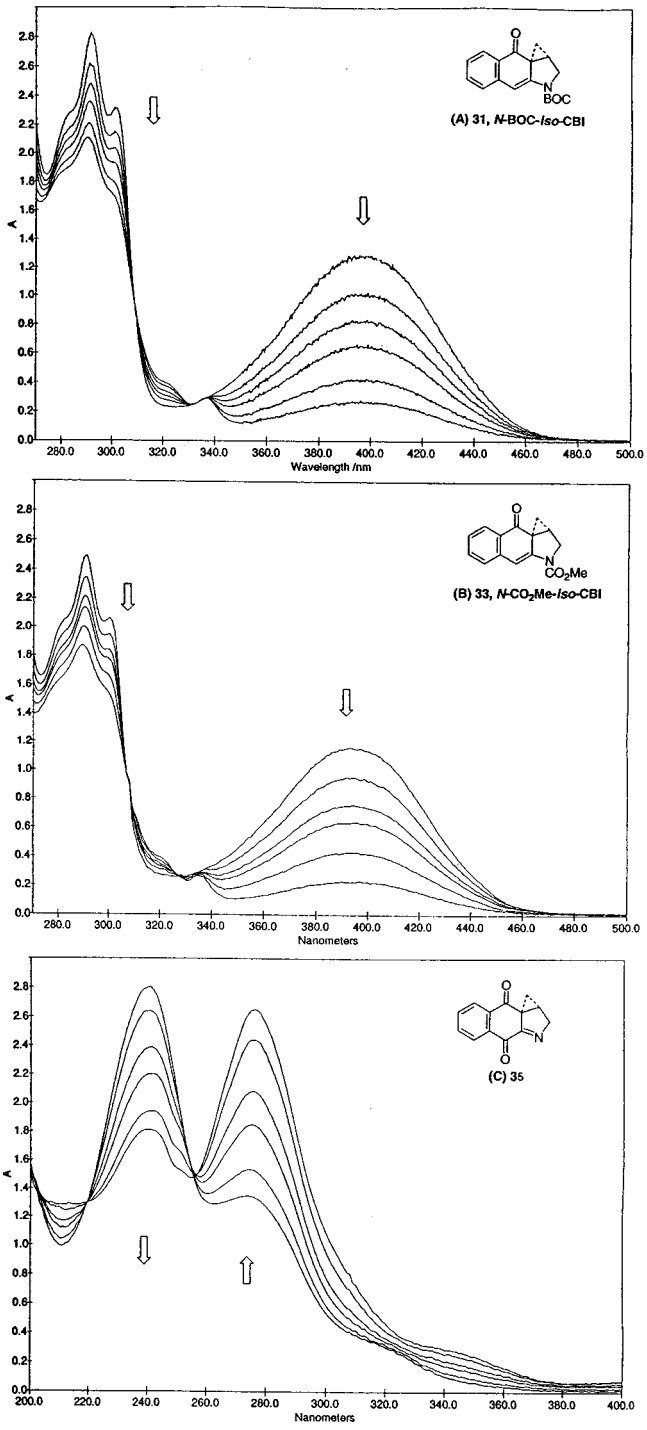



**Figure 3.** Solvolysis study (UV spectra) of *N*-BOC-iso-CBI (**31**, top), *N*-$CO_2$Me-iso-CBI (**33**, middle), and **35** (bottom) in 50% $CH_3OH$−aqueous buffer (pH 3.0, 4:1:20 (v/v/v) 0.1 M citric acid, 0.2 M $NaH_2PO_4$, and $H_2O$, respectively). The spectra were recorded at regular intervals, and only a few are shown for clarity. Top: (hours) 0, 10, 20, 28, 56, 84. Middle: (hours) 0, 10, 21, 29, 57, 86. Bottom: (hours) 0, 1, 3, 5, 10, 16.

the solvolysis product phenol imposes a preference for nucleophilic addition to the less substituted C9 cyclopropane carbon. In contrast, the cyclopropane bond extending to the tertiary C9a carbon is nearly orthogonal to the $\pi$-system of the cyclohexadienone, and $S_N2$ addition to this carbon is disfavored stereoelectronically as well as sterically. The relative cyclopropane bond lengths of iso-CBI reflect this orientation and $\pi$ conjugation in which the breaking C9−C8a cyclopropane bond (1.531 Å) is longer, and thus weaker, than the C9a−C8a bond (1.513

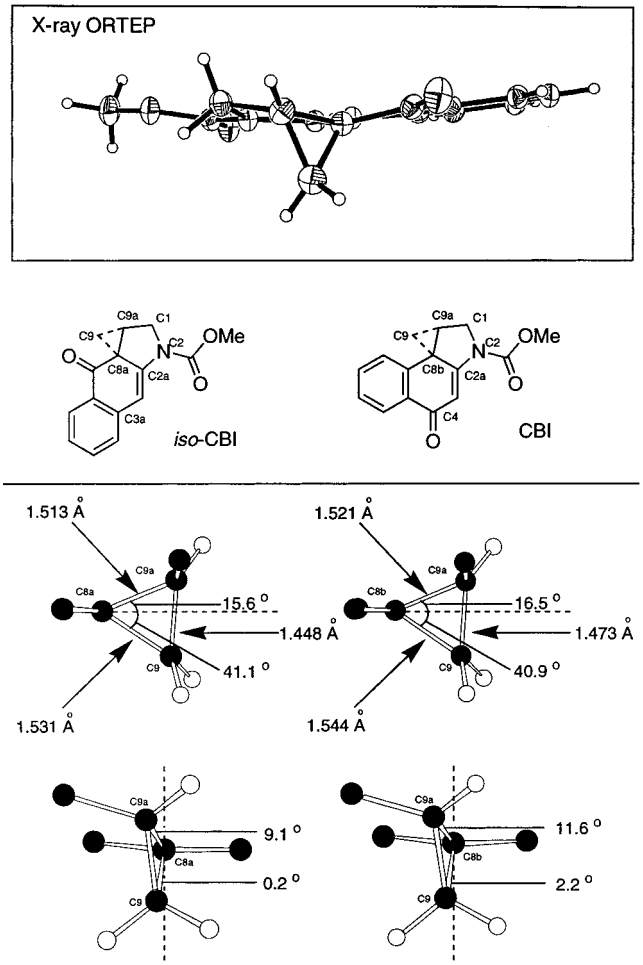



**Figure 4.** Stick models of the side view and 90° rotation view of the activated cyclopropane of *N*-$CO_2$Me-iso-CBI and *N*-$CO_2$-Me-CBI illustrating data taken from the X-ray crystal structures and highlighting the stereoelectronic and geometric alignment of the cyclopropane with the cyclohexadienone $\pi$-system.

Å) extending to the more substituted carbon. Although the ring expansion solvolysis would place a developing positive charge on a preferred secondary versus primary carbon, this inherent preference is overridden by the stereoelectronic control of the reaction regioselectivity as well as the characteristics of a $S_N2$ reaction which prefer attack at the less substituted center.

In addition, the X-ray structures have provided insights into the origin of the difference in stability between CBI and iso-CBI. The stability of CC-1065, the duocarmycins, and their analogues is a result of at least three structural features: the conjugative stability provided by the fused aromatic system, the nonideal alignment of the cyclopropane, and the strong cross-conjugative stability provided by the vinylogous amide. The first of these features, the fused aromatic ring, is present in both iso-CBI and CBI. Like the CI/CBI comparison, the iso-CI/iso-CBI reactivity comparison reveals that the diminished aromatization driving force for iso-CBI relative to iso-CI contributes significantly to the stability. In addition, the cyclopropane alignments with the $\pi$-systems in *N*-$CO_2$-Me-iso-CBI and *N*-$CO_2$Me-CBI are similar. Both are bisected by the plane of the cyclohexadienone nearly equally (41°/15° for iso-CBI and 41°/16° for CBI). In both, the cyclopropane is not only pulled down but also to the side by the constraints of the fused five-membered ring (9° for iso-CBI and 12° for CBI). Thus, both benefit in

**Table 2**

| agent | $N^2-C^{2a}$ bond length (Å) | $t_{1/2}$ (h, pH 3) |
|---|---|---|
| *N*-$CO_2$Me-CBI[42] | 1.390 | 133 |
| *N*-$CO_2$Me-iso-CBI | 1.400 | 28 |
| *N*-BOC-CBQ[43] | 1.415 | 2.1 |
| *N*-$CO_2$Me-CNA[42] | 1.428 | 0.03 |

stability from the nonideal alignment and conjugation of the cyclopropane which is imposed by the fused five-membered ring. The important distinction between the two systems is the direct cross-conjugated stability afforded the activated cyclopropane by the vinylogous amide. Diagnostic of this vinylogous amide conjugation is the shortened length of the $N^2-C^{2a}$ bond, reflecting this resonance stabilization. As this cross-conjugated vinylogous amide stabilization decreases, the conjugation and inherent reactivity of the cyclopropane correspondingly increases.[7,16,42] Consistent with this, *N*-$CO_2$Me-iso-CBI exhibits a $N^2-C^{2a}$ bond length (1.400 Å versus 1.426 Å for **32**) indicative of a diminished but not eliminated vinylogous amide conjugation relative to *N*-$CO_2$Me-CBI (1.390 Å versus 1.416 Å for seco *N*-$CO_2$Me-CBI)[42] and that follows trends established in recent studies (Table 2).[42]

**DNA Alkylation Selectivity and Efficiency.** The DNA alkylation properties of the agents were examined within w794 and w836 duplex DNA[44] for which comparative results are available for related agents. The alkylation site identification and the assessment of the relative selectivity among the available sites were obtained by thermally induced strand cleavage of the singly 5′ end-labeled duplex DNA after exposure to the agents. Following treatment of the end-labeled duplex DNA with a range of agent concentrations and temperatures in the dark, the unbound agent was removed by EtOH precipitation of the DNA. Redissolution of the DNA in aqueous buffer, thermolysis (100 °C, 30 min) to induce strand cleavage at the sites of DNA alkylation, denaturing high-resolution polyacrylamide gel electrophoresis (PAGE) adjacent to Sanger dideoxynucleotide sequencing standards, and autoradiography led to identification of the DNA cleavage and alkylation sites. The full details of this procedure have been disclosed elsewhere.[44]

A representative comparison of the DNA alkylation by (−)-iso-CBI-TMI (**44**) alongside that of (+)-duocarmycin SA and (+)-CBI-TMI is illustrated in Figure 5. There are three important conclusions that can be drawn from these comparisons. First, (−)-iso-CBI-TMI alkylates DNA in a manner identical with (+)-duocarmycin SA and (+)-CBI-TMI, exhibiting the same sequence selectivity. No new sites of alkylation were detected, and only adenine N3 alkylation was detected under the conditions of limiting agent and excess DNA. Notably, such sequencing studies only detect the higher affinity alkylation sites and minor sites of comparable affinities (1−0.01×). Under these conditions, the studies illustrate that iso-CBI-TMI, like duocarmycin SA and CBI-TMI, exhibits an exclusive preference for adenine versus guanine N3 alkylation. However, given the reactivity of iso-CBI-TMI, it is likely that a minor guanine alkylation could be expected at incubations carried out at higher agent−base pair ratios analogous to that observed with the more

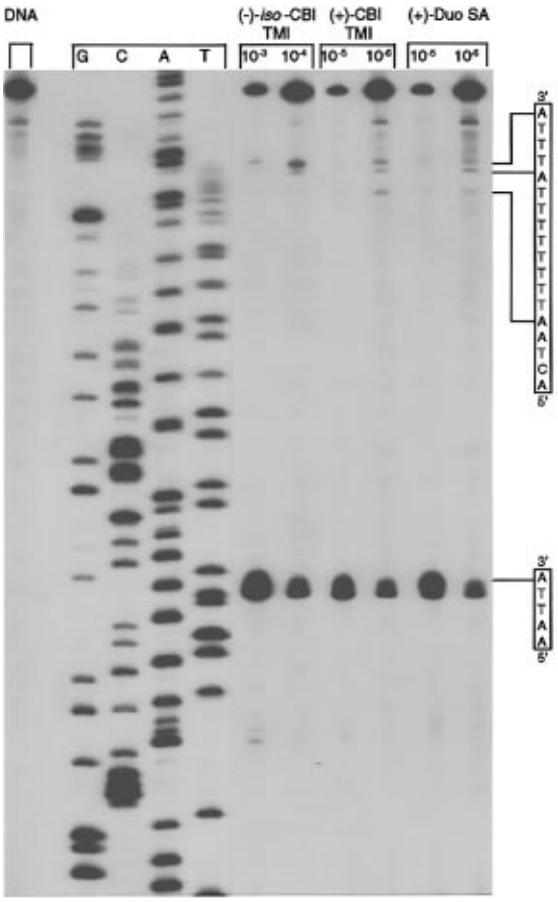



**Figure 5.** Thermally induced strand cleavage of w794 DNA (SV40 DNA segment, 144 bp, nucleotide nos. 138−5238); DNA-agent incubation for 24 h at 25 °C, removal of unbound agent and 30 min of thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography; lane 1, control DNA; lanes 2−5, Sanger G, C, A, and T sequencing reactions; lanes 6−7 (−)-iso-CBI-TMI ($1 \times 10^{-3}$ and $1 \times 10^{-4}$ M); lanes 8−9, (+)-CBI-TMI ($1 \times 10^{-5}$ and $1 \times 10^{-6}$ M); lanes 10−11, (+)-duocarmycin SA ($1 \times 10^{-5}$ and $1 \times 10^{-6}$).

reactive agents including CC-1065[45] and duocarmycin A.[46] Importantly, the identical behavior of (−)-iso-CBI-TMI and (+)-CBI-TMI illustrate that the position of the C-4 carbonyl does not influence the sequence selectivity of the DNA alkylation. This is inconsistent with the proposal of a sequence-dependent phosphate backbone protonation of the C-4 carbonyl for activation of the agent for DNA alkylation that controls the sequence selectivity.[6,8] It is, however, fully consistent with the model in which it is controlled by the AT-rich noncovalent binding selectivity of the agents and their steric accessibility to the adenine N3 alkylation sites.[4]

Second, although there are no distinctions in the sequence selectivity, there is a significant difference in the relative efficiencies of DNA alkylation. Consistent with its relative reactivity and cytotoxic potency, (−)-iso-CBI-TMI alkylated DNA 50−100× less efficiently than (+)-CBI-TMI and (+)-duocarmycin SA. Thus, (−)-iso-CBI-TMI was found to alkylate DNA with an efficiency comparable to duocarmycin A which has been shown to be ca. 10× less efficient than duocarmycin SA.[10,11] In

(43) Boger, D. L.; Mesini, P. *J. Am. Chem. Soc.* **1994**, *116*, 11335. Boger, D. L.; Mesini, P.; Tarby, C. M. *J. Am. Chem. Soc.* **1994**, *116*, 6461. Boger, D. L.; Mesini, P. *J. Am. Chem. Soc.* **1995**, *117*, 11647.

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(45) Park, H.-J.; Hurley, L. H. *J. Am. Chem. Soc.* **1997**, *119*, 629.

(46) Sugiyama, H.; Ohmori, K.; Chan, K. L.; Hosoda, M.; Asai, A.; Saito, H.; Saito, I. *Tetrahedron Lett.* **1993**, *34*, 2179. Yamamoto, K.; Sugiyama, H.; Kawanishi, S. *Biochemistry* **1993**, *32*, 1059. Asai, A.; Nagamura, S.; Saito, H. *J. Am. Chem. Soc.* **1994**, *116*, 4171.

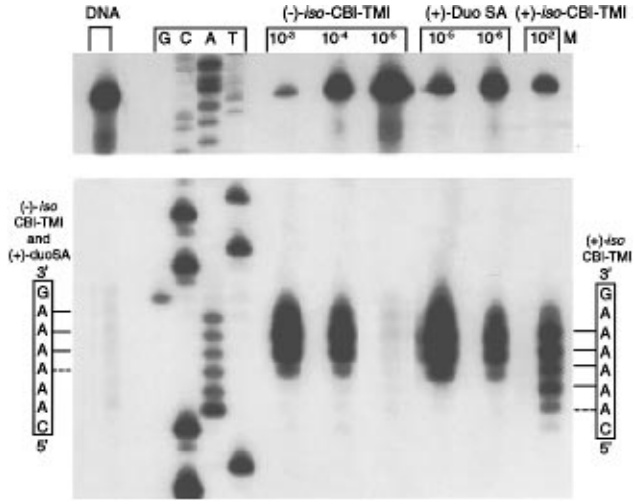



**Figure 6.** Thermally induced strand cleavage of w836 DNA (146 bp, nucleotide nos. 5189−91): DNA-agent incubation for 24 h ((−)-iso-CBI-TMI) at 25 °C, removal of unbound agent and 30 min of thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography; lane 1, control DNA; lanes 2−5, Sanger G, C, A, and T sequencing reactions; lanes 6−8 (−)-iso-CBI-TMI ($1 \times 10^{-3}$ to $1 \times 10^{-5}$ M); lanes 9−10, (+)-duocarmycin SA ($1 \times 10^{-5}$ to $1 \times 10^{-6}$ M); lanes 11−12, (+)-iso-CBI-TMI ($1 \times 10^{-2}$).

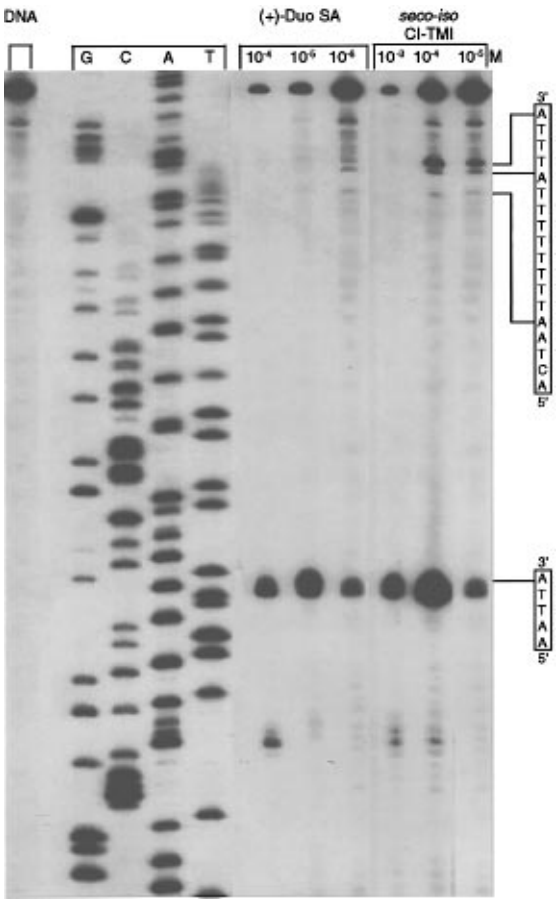



**Figure 7.** Thermally induced strand cleavage of w794 DNA (SV40 DNA segment, 144 bp, nucleotide nos. 138−5238); DNA-agent incubation for 24 h at 25 °C, removal of unbound agent and 30 min of thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography; lane 1, control DNA; lanes 2−5, Sanger G, C, A, and T sequencing reactions; lanes 6−8 (+)-duocarmycin SA ($1 \times 10^{-4}$ to $1 \times 10^{-6}$ M); lanes 9−11, seco-iso-CI-TMI ($1 \times 10^{-3}$ to $1 \times 10^{-5}$ M).

addition and analogous to the observations made with the agents containing the more reactive alkylation subunits including duocarmycin A[10,21,43,46] but unlike the more stable agents,[4] the alkylation efficiency of (−)-iso-CBI-TMI was found to increase as the incubation temperature was decreased from 25 °C to 4 °C. This suggests that the differences may be attributed in part to the nonproductive solvolysis of iso-CBI-TMI which competes with alkylation and lowers the overall efficiency of DNA alkylation.

Third, although the rate of DNA alkylation by **44** was not accurately quantitated, it is qualitatively similar to those of CBI-TMI and duocarmycin SA and much faster than agents we have examined which exhibited substantially diminished rates (e.g. reversed analogues of duocarmycin SA).[16] Thus, the relocation of the C-4 carbonyl did not impact the rate of DNA alkylation in a manner that would be consistent with a phosphate backbone protonation (or cation Lewis acid complexation) required of catalysis in the alkylation site model.

Similar results were obtained within w836 DNA (Figure 6). The natural enantiomer (−)-iso-CBI-TMI alkylated the same sites as (+)-duocarmycin SA but did so with a 50−100 fold lower efficiency. Within the segment illustrated, the two natural enantiomers alkylated the first three 3′ adenines in the sequence 5′-AAAAAA and less effectively the fourth 3′ adenine corresponding to alkylation and 3′→5′ binding across a 3−4 base-pair AT-rich site (i.e., 5′-AAAA > 5′-CAAA). Like the unnatural enantiomer of CBI-TMI, the unnatural enantiomer, (+)-iso-CBI-TMI, alkylated DNA 50−100× less effectively than the natural enantiomer and did so with a selectivity identical with that of *ent*-(−)-duocarmycin SA. Within w836, this constitutes the central adenines within the sequence 5′-AAAAAA and corresponds to alkylation of 3−4 base-pair AT-rich sites with binding in the reverse 5′→3′ direction. Because of the diastereomeric nature of the adducts, the unnatural enantiomer alkylates the second 5′ base (adenine) within the sequence (i.e., 5-AAAA > 5′-CAAAA). This has been discussed in detail and illustrated elsewhere[4] and both enantiomers of iso-CBI-TMI conform nicely to the models. Thus, the relocation of the C-4 carbonyl from the outer face of a bound complex potentially proximal to the phosphate backbone to a position deep in the minor groove inaccessible to the phosphates had no impact on the DNA alkylation selectivity of either enantiomer.

In addition, the DNA alkylation properties of **20**, seco iso-CI-TMI, were examined alongside (+)-duocarmycin SA, and a representative comparison is illustrated in Figure 7 with w794 DNA. In past studies, such seco agents have behaved in a manner indistinguishable from their cyclopropane ring-closed counterparts exhibiting identical DNA alkylation selectivities, efficiencies, and cytotoxic activity, indicating that ring closure is not limiting under the assay conditions.[4] Since we were not able to isolate the ring closed iso-CI agents because of their exceptional reactivity, the examination was conducted with the seco precursor **20**. Analogous to the observations made with iso-CBI-TMI, **44** alkylated DNA in a manner identical with duocarmycin SA alkylating only adenine and exhibiting the same sequence selectivity. Although no new sites of alkylation were detected, the relative selectivity among the available sites was slightly lower with **20**. This is illustrated nicely in Figure 7 where **20** alkylates the minor sites more prominently than does (+)-duocarmycin SA. Interestingly and im-

**Table 3. Calf Thymus DNA Alkylation**

| conditions | base-pair equiv | **66** | **65** | **44** | solvolysis |
|---|---|---|---|---|---|
| 4 °C, 72 h | 75[a] | 94% | <5% | nd | nd |
| 4 °C, 72 h | 150[a] | 95% | <5% | nd | nd |
| 4 °C, 72 h | 150[b] | 92% | <5% | nd | nd |
| 4 °C, 120 h | 150[b] | 95% | <5% | nd | nd |

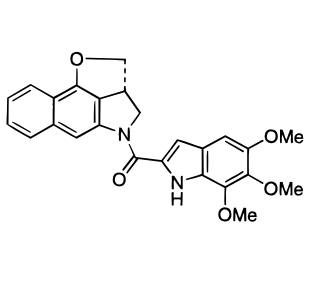
[a] Analytical scale, HPLC separation and UV quantitation. [b] Preparative scale, isolation and weight quantitation. [c] nd = not detected.

portantly, **20** alkylated DNA only 10× less efficiently than (+)-duocarmycin SA being far more effective than the ring closed iso-CI-TMI might be projected to be. To date, this observation is unique to the iso-CI series and, as yet, has not been observed in the iso-CBI series or with other analogues incorporating modified alkylation subunits. Whether this stems from the use of the mesylate versus chloro seco precursor for **20** or is unique to the iso-CI series remains to be established. However, it does suggest that in selected instances the seco precursors may exhibit productive properties that exceed those of the corresponding cyclopropane ring-closed materials especially with the more reactive agents. Unlike the more stable agents which readily undergo cyclopropane ring closure, the ring closure of **20** to iso-CI-TMI under the conditions of the assay is unlikely. Rather, the DNA alkylation most likely occurs directly with **20** without the intermediate generation of the free cyclopropane agent. Thus, not only did the relocation of the C-4 carbonyl not alter the DNA alkylation selectivity, but its removal altogether may be possible,[14] providing a class of agents which, depending on the nature of the electrophile, also exhibit comparable DNA alkylation selectivities, efficiencies, and rates. This is consistent with early observations that related electrophiles that lack the capabilities for ring closure to an activated cyclopropane exhibit DNA alkylation selectivities identical with the corresponding natural products.[14]

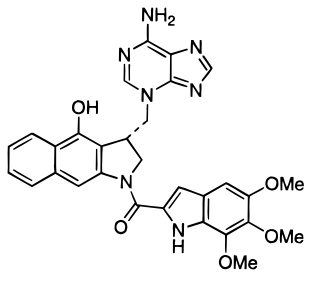
**Quantitation, Isolation, and Characterization of the (−)-*iso*-CBI-TMI Adenine Adduct.** The initial alkylation studies established that (−)-iso-CBI-TMI alkylated adenine within the minor groove in a manner identical with (+)-duocarmycin SA. The thermal cleavage of DNA used to identify the alkylation sites in these studies only detects adducts susceptible to thermal glycosidic bond cleavage (adenine N3, guanine N3, or guanine N7 alkylation), and potential alkylation events involving other nucleophilic centers in DNA may not be detected in this assay. To confirm that (−)-iso-CBI-TMI alkylates DNA in a manner identical with (+)-duocarmycin SA, and in efforts that established the relative extent of adenine N3 versus alternative alkylation events, the quantitation of the adenine N3 alkylation reaction and confirmation of the structure of the product of the reaction were established through isolation and characterization of the thermally released adenine adduct.

This was addressed through a study of the alkylation of calf thymus DNA. Optimized conditions for the alkylation were established for (−)-**44** on an analytical scale (100 $\mu$g of agent). For this purpose, the long-wavelength UV absorption of the agent and the adduct provided a useful quantitative measure of the adenine adduct, unreacted starting material, and any side products (solvolysis or rearrangement), Table 3. Analytical HPLC analysis was used to confirm the identity of the products through correlation of retention times and UV spectral data with authentic materials. The preparative DNA alkylation reaction and subsequent isolation of (+)-**66** was carried out under conditions determined to provide complete consumption of the agent in the presence of a large excess of DNA. Thus, extraction (EtOAc) of the aqueous buffer solution containing calf thymus DNA following alkylation (4 °C, 72 h, 150 bp) afforded no recovered (−)-**44**, and only a small amount (<5%) of the rearranged product **65**. Conducting the reaction at 4 °C in the presence of a large excess of DNA precluded competitive solvolysis. Thermal treatment of the alkylated DNA in aqueous 10 mM sodium phosphate buffer (100 °C, 30 min, pH 7.0) followed by EtOAc extraction provided **66** in 90−95% conversion, ⩾95% purity (by HPLC). Repeating this thermal treatment provided little or no additional adduct. No trace of a competing guanine adduct could be detected. This high conversion to a single adduct established that **44** participates exclusively in the adenine N3 alkylation reaction under the conditions examined.

Full characterization of **66** unambiguously established its structure, and the spectral characteristics showed strong homology to the duocarmycin SA[11] and A[47] adenine N3 adducts and N3 methyl adenine (Table 4). In the $^1$H NMR, the C3-H of adduct **66** was observed as a single proton (1H) at a characteristic chemical shift of 4.36−4.37. The C3-H NMR signal for the benzylic center resulting from alternative adenine N3 addition to the more substituted cyclopropane carbon would be readily distinguishable appearing as two protons (3.5−3.6 ppm, 2H) with a large geminal coupling constant (19.5 Hz).[2] Additionally diagnostic of the structure were the chemical shifts and coupling constants for C2-$H_2$ and C4-$H_2$. Characteristic of an adenine N3 alkylation product, the adenine C2-H and C8-H were readily distinguishable. The $^1$H NMR of the protonated base was taken in DMSO/1% TFA, and again a strong correlation to the spectral data obtained for the duocarmycin SA adduct was observed. The two adenine C6-$NH_2$ protons were seen as separate signals indicating restricted rotation about the $^6$C=$NH_2^+$ bond as is present in protonated *N*-methyl adenine. In addition, a downfield shift as a result of protonation of both the adenine C8-H and adenine C2-H was also observed. The $^{13}$C NMR of **66** was also found to be in excellent agreement with that of the duocarmycin SA[11] and A[47] adducts. The key distinguishing signals are found within or proximal to the fused five- versus six-membered ring with **66** exhibiting chemical shifts consistent only with the former, i.e. C3 at $\delta$ 39.7 consistent with duocarmycin SA ($\delta$ 41.1) and inconsistent with the six-membered ring in duocarmycin $B_1$/$C_1$ ($\delta$ 33−34).




**65**

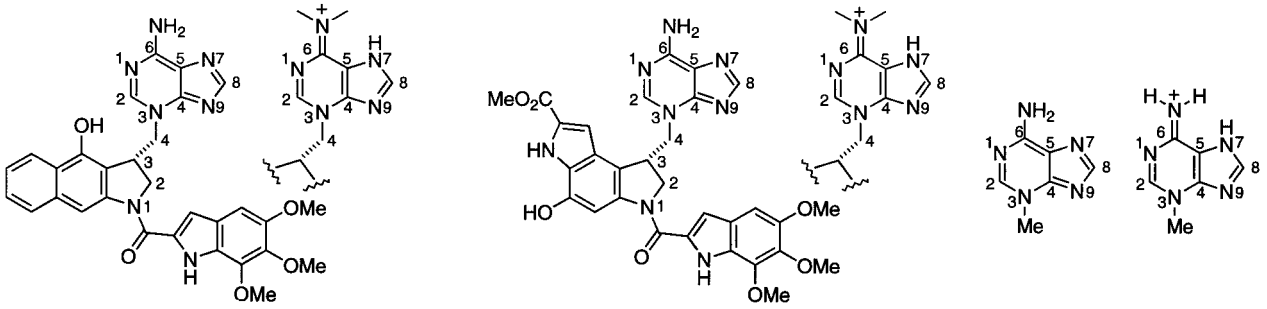



**66**

Thus, the adenine N3 addition to the least substituted cyclopropane carbon of (−)-iso-CBI-TMI, as with (+)-

(47) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H. *J. Am. Chem. Soc.* **1991**, *113*, 6645.

**Table 4. $^1$H (400 MHz) and $^{13}$C NMR (150 MHz)**



| | **66**, (−)-iso-CBI-TMI-adenine N3 adduct | | (+)-duocarmycin SA-adenine N3 adduct | | N3-Me adenine | |
|---|---|---|---|---|---|---|
| assignment | acetone-$d_6$ | DMSO-$d_6$ + 1% *d*-TFA | acetone-$d_6$ | DMSO-$d_6$ + 1% *d*-TFA | acetone-$d_6$ | DMSO-$d_6$ + 1% *d*-TFA |
| Ade-C8-H (1H, s) | 8.22 | 8.45 | 8.12 | 8.55 | 8.22 | 8.72 |
| Ade-C2-H (1H, s) | 8.06 | 8.39 | 7.98 | 8.46 | 7.79 | 8.65 |
| N3-$CH_3$ (3H, s) | n/a | n/a | n/a | n/a | 3.99 | 3.97 |
| C6-$NH_2$ (2H) | | 9.27 (br s) | | 9.25 (br s) | | 9.20 (br s) |
| | | 8.78 (br s) | | 8.94 (br s) | | 9.17 (br s) |
| C4-H (1H) | 4.92 (dd, *J* = 10.8, 1.9 Hz) | 4.72 (dd, *J* = 10.8, 5.6 Hz) | 4.89 (dd, *J* = 13.6, 4.9 Hz) | 4.71 (dd, *J* = 13.6, 5.4 Hz) | | |
| C4-H (1H) | 4.73 (dd, *J* = 10.8, 8.5 Hz) | 4.68 (dd, *J* = 10.8, 5.6 Hz) | 4.67 (dd, *J* = 13.6, 7.4 Hz) | 4.60 (dd, *J* = 13.6, 8.0 Hz) | | |
| C3-H (1H, m) | 4.37 | 4.36 | 4.55 | 4.24 | | |
| C2-H (1H) | 4.89 (dd, *J* = 14.5, 7.0 Hz) | 4.55 (dd, *J* = 9, 9 Hz) | 4.82 (br d, *J* = 11.0 Hz) | 4.52 (br d, *J* = 11.0 Hz) | | |
| C2-H (1H) | 4.73 (dd, *J* = 14.5, 7.6 Hz) | 4.54 (br d, *J* = 9 Hz) | 4.61 (dd, *J* = 11.0, 8.6 Hz) | 4.47 (dd, *J* = 11, 10 Hz) | | |
| | | | | | ($CD_3OD$) | |
| Ade-C2 | 152.2 | | 153.0 | | 152.9 | |
| Ade-C4 | 151.0 | | 149.9 | | 151.1 | |
| Ade-C5 | 123.0 | | 122.7 | | 120.8 | |
| Ade-C6 | 156.9 | | 155.6 | | 156.8 | |
| Ade-C8 | 143.9 | | 146.4 | | 146.0 | |
| N3-$CH_3$ | n/a | | n/a | | 36.9 | |
| C2 | 56.4 | | 53.9 | | | |
| C3 | 39.7 | | 41.4 | | | |
| C4 | 57.5 | | 58.9 | | | |

duocarmycin SA, was found to account for 90−100% of the consumption of the agent in the presence of duplex DNA and confirmed that it binds and alkylates DNA in a manner identical with the natural products despite the relocation of the C-4 carbonyl.

**In Vitro Cytotoxic Activity.** Past studies with agents in this class have defined a direct correlation between solvolysis stability and cytotoxic potency.[4] Consistent with their relative reactivity, the iso-CBI based agents exhibited cytotoxic activity that closely followed this relationship despite the deep-seated structural modification (Table 5, Figure 8).[4] The results, which also follow trends established in the DNA alkylation studies, demonstrate that the (−)-enantiomer of the analogues possessing the (*S*)-configuration analogous to the natural products is the more potent enantiomer by 10−50×. The exception to this generalization is *N*-BOC-iso-CBI where the two enantiomers were not readily distinguishable and the unnatural enantiomer was consistently slightly more potent (1−2×). The seco precursors, which lack the preformed cyclopropane but possess the capabilities of ring closure, were found to possess cytotoxic activity that was indistinguishable from the final ring-closed agents. Consistent with the unique importance of the C5 methoxy group of the duocarmycins, iso-CBI-TMI (**44**) and **46** were found to be equipotent, illustrating that the C6 and C7 methoxy groups of **44** are not contributing to its cytotoxic potency. The cinnamate derivative **48** was found to be substantially less potent (40−50×), suggestive of the requirement for a rigid $N^2$ DNA binding subunit.[7] Finally, the agents exhibited a smooth trend of increasing cytotoxic potency as the size and length of the DNA binding subunits increased with iso-CBI-$CDPI_1$ and iso-CBI-$CDPI_2$ displaying the most potent cytotoxic activity in the series exhibiting $IC_{50}$ values of 200 and 50 pM, respectively.[48]

**Table 5. In Vitro Cytotoxic Activity**

| agent | configuration | $IC_{50}$ (L1210)[a] |
|---|---|---|
| **31**, (−)-*N*-BOC-iso-CBI | natural | 5 $\mu$M |
| **31**, (+)-*N*-BOC-iso-CBI | unnatural | 3 $\mu$M |
| **34**, (±)-**34** | racemic | 12 $\mu$M |
| **35**, (±)-**35** | racemic | 15 $\mu$M |
| **44**, (−)-iso-CBI-TMI | natural | 2 nM |
| **44**, (+)-iso-CBI-TMI | unnatural | 50 nM |
| **46**, (−)-**46** | natural | 2 nM (2 nM) |
| **46**, (+)-**46** | unnatural | 110 nM (60 nM) |
| **48**, (−)-**48** | natural | 90 nM (40 nM) |
| **48**, (+)-**48** | unnatural | 350 nM |
| **50**, (−)-iso-CBI-$indole_2$ | natural | 3 nM (6 nM) |
| **50**, (+)-iso-CBI-$indole_2$ | unnatural | 20 nM (60 nM) |
| **52**, (−)-iso-CBI−$CDPI_1$ | natural | 1 nM (0.2 nM) |
| **52**, (+)-iso-CBI−$CDPI_1$ | unnatural | 70 nM (6 nM) |
| **54**, (−)-iso-CBI−$CDPI_2$ | natural | 0.05 nM (0.03 nM) |
| **54**, (+)-iso-CBI−$CDPI_2$ | unnatural | 0.5 nM (0.5 nM) |

[a] The value in parentheses corresponds to the $IC_{50}$ value determined for the corresponding seco precursor.

(48) While we were unable to isolate *N*-BOC-iso-CI (**21**) and iso-CI-TMI for direct comparison, their seco precursors (±)-**19** and (±)-**20** exhibited surprisingly potent cytotoxic activity ($IC_{50}$, L1210 = 10 $\mu$M and 6 nM, respectively) that likely exceeds that of the ring closed materials themselves.

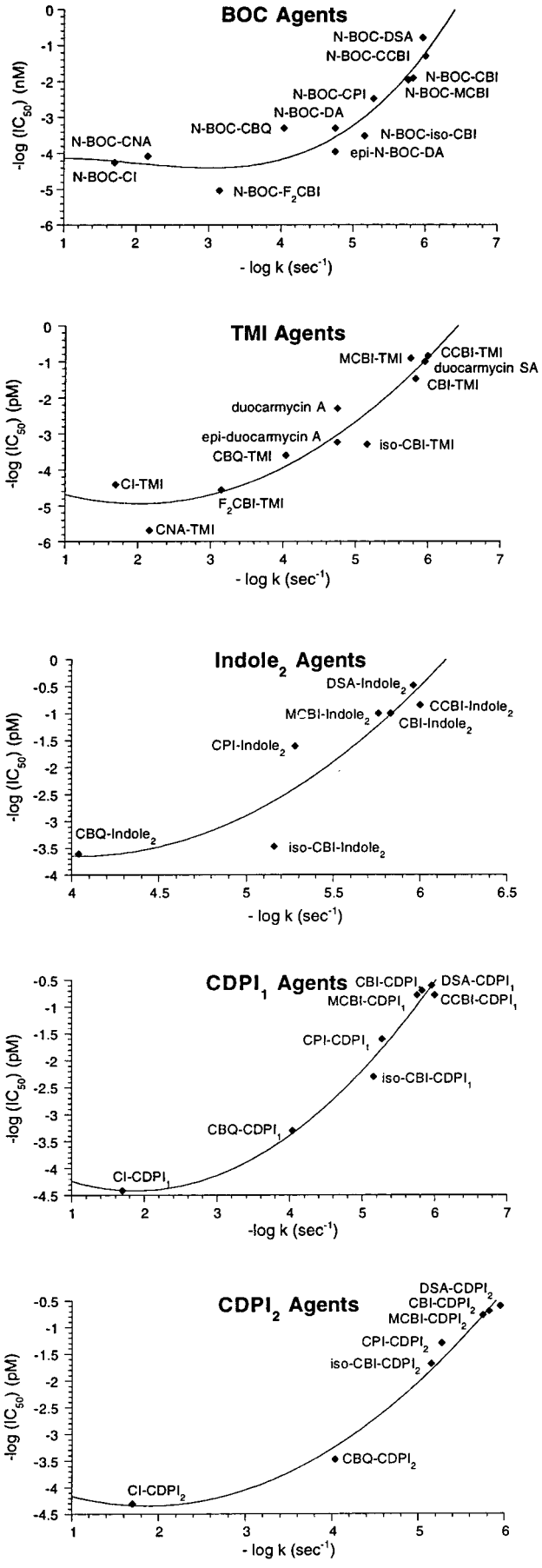



**Figure 8.**

**Conclusions.** In summary, iso-CI and iso-CBI were designed to test a key element of the different proposals for the origin of the DNA alkylation selectivity with the duocarmycins and CC-1065. These agents were prepared by application of a directed ortho metalation and ortho spirocyclization in an improved synthetic scheme complementary to that reported for CI and CBI. Iso-CBI was found to be 5× less stable than CBI and possess a reactivity comparable with CC-1065 and duocarmycin A. In addition, nucleophilic addition occurred at the least substituted cyclopropane carbon with a regioselectivity (40:1) comparable to that of CBI but which exceeds that of the natural products themselves (6−1.5:1). Comparison of the X-ray structures of iso-CBI and CBI revealed the near identical nonideal conjugation of the cyclopropanes and the exclusive stereoelectronic alignment of the cleaved cyclopropane bond. Consistent with recent observations, the lower stability of iso-CBI relative to CBI itself can be attributed to a diminished cross-conjugated vinylogous amide stabilization for which the $N^2-C^{2a}$ bond length is diagnostic.[7,42] Resolution and incorporation of iso-CBI into a full set of duocarmycin and CC-1065 analogues allowed for comparison of their properties and a further distinguishing test for the origin of the DNA alkylation sequence selectivity. The iso-CBI analogues were highly potent cytotoxic agents exhibiting picomolar $IC_{50}$'s which correlated with their relative stability. In addition to smoothly following this correlation, the analogues displayed a smooth trend of increasing cytotoxic potency with the increasing length in the DNA binding subunit. Analogous to the natural products, the (*S*)-enantiomer possessing the absolute configuration of **1**−**3** proved to be more potent (10−50×) than the (*R*)-enantiomer. DNA alkylation studies revealed a strong correlation between DNA alkylation efficiency and cytotoxic potency as (−)-iso-CBI-TMI was approximately 50−100× less efficient than (+)-CBI-TMI and (+)-duocarmycin SA. In addition, both the iso-CI and iso-CBI analogues, with the repositioned C-4 carbonyl, exhibited the identical sequence selectivity and alkylated the same sites as CBI-TMI and duocarmycin SA derived from adenine N3 addition to the least substituted cyclopropane carbon at comparable reaction rates. This is inconsistent with a proposal that the DNA alkylation selectivity is controlled by a sequence-dependent DNA backbone phosphate protonation of the C-4 carbonyl for activation of alkylation but is consistent with the noncovalent binding and steric accessibility model. Finally, this set of isomeric analogues contains the most significant structural modification to the alkylation subunit to date, yet remain effective DNA alkylating agents with properties comparable to the natural products themselves.

## Experimental Section[29]

**1-(*tert*-Butyloxycarbonyl)-4-hydroxy-3[[(methanesulfonyl)oxy]methyl]-2,3-dihydroindole (19).** A solution of **18**[29] (22 mg, 0.057 mmol) in 2 mL of 1:1 *i*-PrOH/THF was treated with 12 N HCl (33 $\mu$L, 0.4 mmol) and stirred for 48 h at 25 °C. The reaction solution was concentrated under reduced pressure. Flash chromatography ($SiO_2$, 1.5 × 10 cm, 40% EtOAc/hexane) afforded recovered **18** (8 mg, 41%) and **19** (9.5 mg, 48%) as a white film: $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 7.43 (m, 1H), 7.07 (m, 1H), 6.39 (d, $J$ = 8.6 Hz, 1H), 5.44 (br s, 1H), 4.55 (dd, $J$ = 4.5, 9.8 Hz, 1H), 4.26 (dd, $J$ = 8.1, 9.8 Hz, 1H), 4.00 (m, 2H), 3.79 (m, 1H); IR (film) $\nu_{max}$ 3347, 2977, 1682, 1622, 1602, 1467, 1394, 1172, 1145, 948 cm$^{-1}$; FABHRMS (NBA/CsI) *m/z* 476.0156 ($C_{15}H_{21}NO_6S + Cs^+$ requires 476.0144).

**4-Hydroxy-3[[(methanesulfonyl)oxy]methyl]-1-[5,6,7-trimethoxyindol-2-yl)carbonyl]-2,3-dihydroindole (20).** A sample of **18**[29] (9.0 mg, 0.023 mmol) was dissolved in 3.6 N

HCl/EtOAc (2.4 mL), and the solution was stirred for 30 min at 25 °C. The solvents were removed by a stream of $N_2$, and the residual salt was thoroughly dried under high vacuum. The salt was dissolved in anhydrous DMF (1.2 mL) and treated with **37**[10] (7 mg, 0.028 mmol) and EDCI (13 mg, 0.07 mmol). The resulting solution was stirred at 25 °C for 3 h under Ar. The reaction mixture was then diluted with $H_2O$ (5 mL) and extracted with EtOAc (3 × 5 mL). The combined organic layer was dried ($Na_2SO_4$) and concentrated under reduced pressure. Flash chromatography ($SiO_2$, 1.5 × 10 cm, 50% EtOAc/hexane) yielded pure **20** (6.0 mg, 55%) as a white film: $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 9.33 (br s, 1H), 7.87 (d, $J$ = 8.0 Hz, 1H), 7.19 (m, 1H), 6.92 (d, $J$ = 2.4 Hz, 1H), 6.84 (s, 1H), 6.52 (dd, $J$ = 0.5, 8.0 Hz, 1H), 4.65 (dd, $J$ = 4.3, 10.1 Hz, 1H), 4.58 (dd, $J$ = 9.2, 10.8 Hz, 1H), 4.51 (dd, $J$ = 3.9, 10.8 Hz, 1H), 4.31 (dd, $J$ = 8.4, 10.1 Hz, 1H), 4.05 (s, 3H), 4.01 (m, 1H), 3.92 (s, 3H), 3.89 (s, 3H), 2.95 (s, 3H); IR (film) $\nu_{max}$ 3261, 2938, 1659, 1611, 1462, 1352, 1306, 1282, 1174, 1107 cm$^{-1}$; FABHRMS (NBA/CsI) $m/z$ 609.0319 ($C_{22}H_{24}N_2O_8S$ + $Cs^+$ requires 609.0308).

***N*-(*tert*-Butyloxycarbonyl)-4-(methoxymethoxy)-2-naphthylamine (23).** A solution of **22**[22] (6.67 g, 25.0 mmol) in 125 mL of anhydrous DMF at 0 °C was treated with NaH (1.13 g, 28.0 mmol) in several portions over 5 min. After 10 min, $Bu_4NI$ (0.925 g, 2.5 mmol) was added followed by the dropwise addition of $ClCH_2OCH_3$ (2.9 mL, 38 mmol). The reaction mixture was stirred at 25 °C for 5 h before the reaction was quenched by the slow addition of 100 mL of $H_2O$. The aqueous layer was extracted with EtOAc (3 × 100 mL). The organic layers were combined, washed with 10% aqueous $NaHCO_3$ (100 mL) and $H_2O$ (4 × 50 mL), dried ($Na_2SO_4$), and concentrated under reduced pressure. Flash chromatography ($SiO_2$, 4 × 15 cm, 0−15% EtOAc/hexane gradient) provided **23** (6.0 g, 78%) as a peach colored solid: mp 64−66 °C; $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 8.15 (d, $J$ = 7.8 Hz, 1H), 7.67 (m, 2H), 7.38 (m, 2H), 7.05 (d, $J$ = 1.9 Hz, 1H), 6.87 (br s, 1H), 5.34 (s, 2H), 3.51 (s, 3H), 1.54 (s, 9H); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 153.3, 152.8, 136.0, 134.8, 127.0, 126.9, 123.7, 122.5, 121.6, 108.0, 101.8, 94.6, 80.5, 56.1, 28.2; IR (film) $\nu_{max}$ 3334, 2977, 1713, 1634, 1538, 1392, 1367, 1248, 1160, 1057 cm$^{-1}$; FABHRMS (NBA) $m/z$ 303.1463 ($C_{17}H_{21}NO_4$ requires 303.1471). Anal. Calcd for $C_{17}H_{21}NO_4$: C, 67.31; H, 6.98; N, 4.62. Found: C, 67.13; H, 7.18; N, 4.89.

***N*-(*tert*-Butyloxycarbonyl)-3-iodo-4-(methoxymethoxy)-2-naphthylamine (24).** A solution of **23** (0.435 g, 1.43 mmol) in 5.7 mL of anhydrous THF was cooled to −25 °C and treated with TMEDA (0.758 mL, 5.0 mmol) followed by *n*-BuLi (2.29 mL of a 2.5 M solution in hexane, 5.0 mmol) in a slow dropwise manner. The resulting gold solution was stirred for 2 h at −25 °C. The reaction mixture was treated with 1-chloro-2-iodoethane (0.37 mL, 5.0 mmol) and stirred for 15 min at 25 °C. The reaction was diluted with $H_2O$ (40 mL) and extracted with $Et_2O$ (3 × 20 mL), and the combined organic extracts were washed with saturated aqueous NaCl, dried ($Na_2SO_4$), and concentrated under reduced pressure. Flash chromatography ($SiO_2$, 2.5 × 15 cm, 0−7% EtOAc/hexane gradient) yielded **24** (490 mg, 80%) as a yellow oil: $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.35 (s, 1H), 8.03 (d, $J$ = 12.8 Hz, 1H), 7.78 (d, $J$ = 12.5 Hz, 1H), 7.42 (m, 2H), 7.14 (br s, 1H), 5.20 (s, 2H), 3.74 (s, 3H), 1.56 (s, 9H);$^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 154.7, 152.6, 135.1, 134.8, 127.6, 127.4, 125.1, 125.0, 122.2, 113.0, 100.5, 87.7, 81.1, 58.5, 28.4; IR (film) $\nu_{max}$ 3391, 2977, 2933, 1732, 1524, 1367, 1340, 1276, 1228, 1157 cm$^{-1}$; FABHRMS (NBA/NaI) $m/z$ 430.0507 ($C_{17}H_{20}INO_4$ + $H^+$ requires 430.0515).

**2-[[*N*-(*tert*-Butyloxycarbonyl)-*N*-(2-propen-1-yl)]amino]-3-iodo-4-(methoxymethoxy)naphthalene (25).** A solution of **24** (0.490 g, 1.1 mmol) in 36 mL of anhydrous DMF was cooled to −10 °C and treated with NaH (69 mg, 1.7 mmol) in small portions. The resulting suspension was stirred 15 min and treated with neat allyl bromide (0.49 mL, 5.7 mmol) in a slow dropwise manner. The reaction mixture was warmed to 25 °C and stirred for 1 h. The reaction mixture was quenched with the addition of 5% aqueous $NaHCO_3$ (50 mL), and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with $H_2O$ (5 × 10 mL), dried ($Na_2SO_4$), and concentrated under reduced pressure to yield **25** as a 2:1 mixture of amide rotamers as a yellow oil. Flash chromatography ($SiO_2$, 2.5 × 15 cm, 10% EtOAc/hexane) yielded **25** (503 mg, 94%) as a colorless oil: $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 8.16 (m, 1H), 7.77 (m, 1H), 7.50 (m, 3H), 5.97 (m, 1H), 5.23 (m, 2H), 5.07 (m, 2H), 4.56 (m, 1H), 3.80 (m, 1H), 3.72 (s, 3H), 1.55 and 1.33 (s, 9H); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 155.6 and 155.4, 154.1 and 153.9, 141.3 and 141.1, 133.8 and 133.5, 127.7 and 127.6, 127.1, 126.8, 125.2, 124.5, 122.4, 117.8 and 117.3, 100.6, 95.7, 80.6 and 80.3, 58.3, 53.6, 52.4, 28.3 and 28.2; IR (film) $\nu_{max}$ 2975, 2930, 1703, 1581, 1566, 1385, 1366, 1159, 1047 cm$^{-1}$; FABHRMS (NBA/CsI) $m/z$ 601.9825 ($C_{20}H_{24}INO_4$ + $Cs^+$ requires 601.9804).

**1-(*tert*-Butyloxycarbonyl)-4-(methoxymethoxy)-3-[[(2′,2′,6′,6′-tetramethyl-piperidino)oxy]methyl]-2,3-dihydro-1*H*-benzo[*f*]indole (26).** A solution of **25** (470 mg, 1.00 mmol) and TEMPO (468 mg, 3.0 mmol) in 43 mL of anhydrous benzene was treated with $Bu_3SnH$ (0.283 mL, 1.05 mmol). The solution was warmed at 50 °C, and an additional 1.05 equiv of $Bu_3SnH$ (0.283 mL, 1.05 mmol) was added twice during the next 30 min. Another 3.0 equiv of TEMPO (468 mg, 3.0 mmol) was added in 10 mL of anhydrous benzene, along with an additional 1.05 equiv of $Bu_3SnH$ added twice during the next 45 min. After 1.5 h total, the solution was cooled to 25 °C, and the volatiles were removed under reduced pressure. Flash chromatography ($SiO_2$, 2.5 × 15 cm, 0−12% EtOAc/hexane gradient) provided **26** (470 mg, 94%) as a yellow oil: $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 8.05 (br s, 1H), 7.97 (d, $J$ = 8.0 Hz, 1H), 7.74 (d, $J$ = 7.7 Hz, 1H), 7.36 (m, 2H), 5.24 (d, $J$ = 5.9 Hz, 1H), 5.16 (d, $J$ = 5.9 Hz, 1H), 4.26 (m, 1H), 4.15 (m, 1H), 4.01 (m, 1H), 3.81 (m, 2H), 3.63 (s, 3H), 1.61 (s, 9H), 1.23 (s, 3H), 1.17 (s, 3H), 1.04 (s, 3H), 1.04 (s, 3H), 0.99 (s, 3H), 1.48−0.89 (m, 6H); $^{13}$C NMR ($CDCl_3$, 62.5 MHz) $\delta$ 152.0, 149.3, 141.1, 135.3, 127.2, 125.7, 124.4, 123.4, 122.6, 121.0, 107.0, 99.1, 80.2, 76.0, 59.3, 57.1, 51.2, 39.1, 32.6, 27.9, 19.6, 16.6; IR (film) $\nu_{max}$ 2974, 2931, 1709, 1634, 1446, 1374, 1352, 1332, 1147 cm$^{-1}$; FABHRMS (NBA/CsI) $m/z$ 631.2168 ($C_{29}H_{42}N_2O_5$ + $Cs^+$ requires 631.2148).

**1-(*tert*-Butyloxycarbonyl)-3-(hydroxymethyl)-4-(methoxymethoxy)-2,3-dihydro-1*H*-benzo[*f*]indole (27).** A solution of **26** (220 mg, 0.44 mmol) in 15 mL of 3:1:1 HOAc/$H_2O$/THF was treated with Zn powder (1.15 g, 17.6 mmol), and the resulting suspension was warmed at 70 °C under a reflux condenser and with vigorous stirring for 1 h. The reaction mixture was cooled to 25 °C, and the Zn was removed by filtration through Celite with a 25 mL $CH_2Cl_2$ wash. The volatiles were removed under reduced pressure, and the resulting residue was dissolved in 25 mL of EtOAc and filtered. The solution was concentrated under reduced pressure. Flash chromatography ($SiO_2$, 2.5 × 10 cm, 30−40% EtOAc/hexane gradient) provided **27** (138 mg, 87%) as a colorless oil: $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 8.05 (br s, 1H), 7.91 (d, $J$ = 8.0 Hz, 1H), 7.72 (d, $J$ = 7.5 Hz, 1H), 7.36 (m, 2H), 5.23 (d, $J$ = 6.0 Hz, 1H), 5.16 (d, $J$ = 6.0 Hz, 1H), 4.10 (m, 1H), 3.94−3.78 (m, 4H), 3.63 (s, 3H), 2.04 (d, $J$ = 8.4 Hz, 1H), 1.58 (s, 9H); $^{13}$C NMR ($CDCl_3$, 125 MHz) $\delta$ 152.5, 149.7, 142.0, 136.0, 127.9, 126.4, 124.6, 124.1, 123.1, 121.3, 107.8, 100.0, 81.1, 65.1, 57.8, 51.3, 40.7, 28.3; IR (film) $\nu_{max}$ 3447, 2975, 1704, 1634, 1447, 1353, 1336, 1149 cm$^{-1}$; FABHRMS (NBA) $m/z$ 360.1821 ($C_{20}H_{25}NO_5$ + $H^+$ requires 360.1811).

**1-(*tert*-Butyloxycarbonyl)-3-(chloromethyl)-4-(methoxymethoxy)-2,3-dihydro-1*H*-benzo[*f*]indole (28). From 27**: A solution of **27** (55 mg, 0.16 mmol) in 3 mL of anhydrous $CH_2Cl_2$ was treated with $CCl_4$ (155 $\mu$L, 1.6 mmol) and $Ph_3P$ (212 mg, 0.81 mmol), and the mixture was stirred at 25 °C for 2 h. The solution was concentrated in vacuo. Flash chromatography ($SiO_2$, 1.5 × 15 cm, 0−12% EtOAc/hexane gradient) provided **28** (52 mg, 90%) as a white solid: mp 99−101 °C; $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 8.03 (br s, 1H), 7.89 (d, $J$ = 8.1 Hz, 1H), 7.72 (d, $J$ = 7.6 Hz, 1H), 7.37 (m, 2H), 5.24 (d, $J$ = 6.1 Hz, 1H), 5.15 (d, $J$ = 6.1 Hz, 1H), 4.06 (m, 4H), 3.65 (s, 3H), 3.51 (app t, $J$ = 10.1 Hz, 1H), 1.59 (s, 9H); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 152.3, 150.5, 141.1, 136.2, 127.9, 126.6, 124.6, 124.1, 122.4, 121.4, 107.7, 100.0, 81.2, 57.5, 52.1, 45.9, 40.8, 28.4; IR (film) $\nu_{max}$ 2976, 1704, 1634, 1449, 1336, 1147, 1055, 983 cm$^{-1}$; FABHRMS (NBA) $m/z$ 378.1463 ($C_{20}H_{24}Cl\ NO_4$ + $H^+$ requires 378.1472). Anal. Calcd for $C_{20}H_{24}INO_4$: C, 63.75; H, 6.40; N, 3.71. Found: C, 63.42; H, 6.11; N, 3.41.

**From 29**: A solution of **29** (500 mg, 1.0 mmol) in 10 mL of anhydrous benzene was treated with AIBN (15.7 mg, 0.1 mmol) and $Bu_3SnH$ (295 $\mu$L, 1.1 mmol) and warmed at 80 °C for 2 h. The reaction mixture was concentrated under reduced pressure, and flash chromatography ($SiO_2$, 2.5 × 15 cm, 0−20% EtOAc/hexanes gradient) yielded **28** (360 mg, 96%) as a white solid identical with that described above.

**Resolution of 28.** A sample of **28** (4.0 mg) in 0.9 mL of 5% *i*-PrOH/hexane was resolved on a semipreparative Daicel Chiralcel OD column (10 $\mu$m, 2 × 25 cm, 7.0 mL/min flow rate, 3% *i*-PrOH/hexane). The effluent was monitored at 254 nm, and the enantiomers were eluted with retention times of 29.0 and 35.8 min ($\alpha = 1.25$). The first enantiomer (*S*) was found to be >99% enantiomerically pure. The second fraction (*R*) was reinjected in order to obtain a sample of >99% ee. The fractions containing the separated enantiomers were collected and concentrated to afford (+) and (−)-**28**. (+)-(3*S*)-**28**: $[\alpha]^{25}_D$ +29 (*c* 0.50, $CH_2Cl_2$); (−)-(3*R*)-**28**: $[\alpha]^{25}_D$ −30 (*c* 0.50, $CH_2Cl_2$).

**2-[*N*-(*tert*-Butyloxycarbonyl)-*N*-(3-chloro-2-propen-1-yl)amino]-3-iodo-4-(methoxymethoxy)naphthalene (29).** A solution of **24** (0.480 g, 1.1 mmol) in 11 mL anhydrous DMF was cooled to 0 °C, and treated with NaH (67 mg, 2.2 mmol) in small portions. The resulting suspension was stirred 15 min and treated with neat 1,3-dichloropropene (0.52 mL, 5.5 mmol) in a slow dropwise manner, followed by catalytic $Bu_4NI$ (40 mg, 0.1 mmol). The reaction mixture was warmed to 25 °C and stirred for 12 h. The reaction mixture was quenched with the addition of 5% aqueous $NaHCO_3$ (50 mL), and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with $H_2O$ (5 × 10 mL), dried ($Na_2SO_4$), and concentrated under reduced pressure to yield **29** as a mixture of rotamers and *E* and *Z* alkenes as a yellow oil. Flash chromatography ($SiO_2$, 2.5 × 15 cm, 0−20% EtOAc/hexanes gradient) yielded **29** (540 mg, 96%) as a yellow oil: $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 8.16 (m, 1H), 7.80 (m, 1H), 7.55 (m, 2H), 7.44 (s, 1H), 6.11 (m, 2H), 5.25 (d, $J$ = 5.6 Hz, 1H), 5.20 (d, $J$ = 5.6 Hz, 1H), 4.51 (m, 1H), 3.75 (m, 1H), 3.73 (s, 3H), 1.55 and 1.31 (s, 9H); $^{13}$C NMR ($CDCl_3$, 62.5 MHz) $\delta$ 156.2, 153.9, 140.7, 134.0, 128.7, 127.9, 127.7, 127.4, 127.1, 124.7, 122.5, 121.7, 100.7, 80.8, 77.2, 58.5, 49.5, 28.2; IR (film) $\nu_{max}$ 2975, 1699, 1565, 1387, 1366, 1328, 1294, 1254, 1162 cm$^{-1}$; FABHRMS (NBA/NaI) *m*/*z* 504.0424 ($C_{20}H_{23}ClINO_4$ + $H^+$ requires 504.0439).

**1-(*tert*-Butyloxycarbonyl)-3-(chloromethyl)-4-hydroxy-2,3-dihydro-1*H*-benzo[*f*]indole (30).** A solution of **28** (18 mg, 47.5 $\mu$mol) in 1.5 mL of 1:1 *i*-PrOH/THF was treated with 12 N HCl (0.20 mL, 0.38 mmol), and the mixture was stirred at 25 °C for 6 h before the volatiles were removed in vacuo. Flash chromatography ($SiO_2$, 1.5 × 15 cm, 0−20% EtOAc/hexane gradient) provided **30** (14.5 mg, 90%) as a pale yellow oil: $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 7.85 (d, $J$ = 8.0 Hz, 1H), 7.71 (d, $J$ = 7.8 Hz, 1H), 7.43−7.30 (m, 2H), 5.67 (s, 1H), 4.09−3.86 (m, 4H), 3.65 (dd, $J$ = 8.0, 10.0 Hz, 1H), 1.59 (s, 9H); $^{13}$C NMR ($CDCl_3$, 62.5 MHz) $\delta$ 147.9, 135.8, 132.7, 128.8, 128.0, 126.6, 123.7, 121.2, 119.5, 119.5, 104.4, 77.2, 52.6, 46.4, 34.7, 28.5; IR (film) $\nu_{max}$ 3368, 2976, 1668, 1477, 1450, 1369, 1145, 978, 746 cm$^{-1}$; FABHRMS (NBA/CsI) *m*/*z* 466.0197 ($C_{18}H_{20}ClNO_3$ + $Cs^+$ requires 466.0186). (−)-(3*S*)-**30**: $[\alpha]^{25}_D$ −54 (*c* 0.25, $CH_3OH$); (+)-(3*R*)-**30**: $[\alpha]^{25}_D$ +51 (*c* 0.30, $CH_3OH$).

**2-(*tert*-Butyloxycarbonyl)-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*f*]indol-8-one (*N*-BOC-iso-CBI, 31).** A solution of **30** (5.0 mg, 0.015 mmol) in 0.6 mL of $CH_3CN$ was treated with DBU (2.7 $\mu$L, 0.018 mmol), and the mixture was stirred at 25 °C for 15 min. Flash chromatography ($SiO_2$, 1.5 × 10 cm, 10% EtOAc/hexane) afforded **31** (4.3 mg, 96%) as a light golden oil: $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 7.98 (d, $J$ = 7.8 Hz, 1H), 7.50 (dt, $J$ = 1.4, 7.8 Hz, 1H), 7.28 (d, $J$ = 7.8 Hz, 1H), 7.21 (dt, $J$ = 1.1, 7.8 Hz, 1H), 6.97 (br s, 1H), 3.88 (br d, $J$ = 11.2 Hz, 1H), 3.81 (dd, $J$ = 4.9, 11.1 Hz, 1H), 2.88 (dt, $J$ = 5.5, 7.8 Hz), 1.87 (dd, $J$ = 3.2, 7.8 Hz, 1H), 1.53 (s, 9H), 1.41 (dd, $J$ = 3.2, 5.5 Hz, 1H); $^1$H NMR ($C_6D_6$, 400 MHz) $\delta$ 8.34 (dd, $J$ = 0.6, 7.8 Hz, 1H), 7.10 (m, 2H), 6.92 (m, 1H), 3.18 (br m, 1H), 2.32 (m, 1H), 1.49 (dd, $J$ = 3.1, 7.8 Hz, 1H), 1.39 (s, 9H), 0.66 (dd, $J$ = 3.1, 5.4 Hz, 1H); $^{13}$C NMR ($CDCl_3$, 62.5 MHz) $\delta$ 194.7, 154.3, 142.0, 134.3, 127.7, 127.3, 125.9, 125.2, 103.2, 77.2, 51.5, 46.1, 32.0, 28.2, 25.7; IR (film) $\nu_{max}$ 2973, 2927, 1716, 1670, 1635, 1472, 1410, 1324, 1143, 1009 cm$^{-1}$; UV (THF) $\lambda_{max}$ ($\epsilon$) 387 (2700), 302 (6000 sh), 293 (6900), 250 (20400) nm; FABHRMS (NBA/NaI) *m*/*z* 298.1450 ($C_{18}H_{19}NO_3 + H^+$ requires 298.1443). (−)-(8a*S*,9a*S*)-**31**: $[\alpha]^{25}_D$ −172 (*c* 0.08, $CH_3OH$); (+)-(8a*R*,9a*R*)-**31**: $[\alpha]^{25}_D$ +179 (*c* 0.12, $CH_3OH$).

**3-Chloro-4-hydroxy-1-(methoxycarbonyl)-2,3-dihydro-1*H*-benzo[*f*]indole (32).** A solution of **28** (11 mg, 29.1 $\mu$mol) was treated with 1.0 mL of 3.6 N HCl/EtOAc, and the resulting solution was stirred for 30 min at 25 °C. The solvent was removed by a stream of $N_2$, and the residual salt was dried under vacuum. This salt was suspended in 0.6 mL of anhydrous THF and treated with $NaHCO_3$ (5.4 mg, 64.1 $\mu$mol, 2.2 equiv) and $ClCO_2CH_3$ (4.5 $\mu$L, 58.2 $\mu$mol, 2.0 equiv) and the mixture was stirred for 3 h at 25 °C. Upon completion, the reaction mixture was diluted with 10 mL of $H_2O$, extracted with EtOAc (3 × 10 mL), dried ($Na_2SO_4$), and concentrated in vacuo. Flash chromatography ($SiO_2$, 1.5 × 15 cm, 0−40% EtOAc/hexane gradient) provided pure **32** (5.5 mg, 65%) as a white solid: $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 7.86 (d, $J$ = 8.2 Hz, 1H), 7.86 (br s, 1H), 7.73 (d, $J$ = 8.0 Hz, 1H), 7.42 (m, 1H), 7.35 (m, 1H), 5.80 (s, 1H), 4.11 (m, 2H), 4.01−3.87 (m, 5H), 3.63 (app t, $J$ = 9.4 Hz, 1H); IR (film) $\nu_{max}$ 3379, 2956, 1673, 1435, 1372, 1283, 1218 cm$^{-1}$; FABHRMS (NBA/NaI) *m*/*z* 291.0656 ($M^+$, $C_{15}H_{14}ClNO_3$ requires 291.0662).

The structure and absolute configuration of (3*R*)-**32** derived from the slower eluting enantiomer of **28** ($t_R$ = 35.8 min, $[\alpha]^{25}_D$ −9 (*c* 0.10, $CH_3OH$)) was obtained from a single-crystal X-ray structure determination conducted on prisms grown from EtOAc/hexane.[37]

**2-(Methoxycarbonyl)-1,2,9,9a-tetrahydrocyclopropa[*c*]-benzo[*f*]indol-8-one (*N*-$CO_2$Me-iso-CBI, 33).** A solution of **32** (5.0 mg, 0.017 mmol) in 0.5 mL of $CH_3CN$ was treated with DBU (3.9 $\mu$L, 0.025 mmol), and the mixture was stirred at 25 °C for 15 min. Flash chromatography ($SiO_2$, 1.5 × 10 cm, 0−40% EtOAc/hexane gradient) afforded **33** (4.0 mg, 91%) as a pale yellow solid:$^1$H NMR ($C_6D_6$, 400 MHz) $\delta$ 8.33 (d, $J$ = 6.0 Hz, 1H), 7.40 (br s, 1H), 7.10 (app t, $J$ = 6.0 Hz, 1H), 7.03 (d, $J$ = 6.0 Hz, 1H), 6.91 (m, 1H), 3.36 (s, 3H), 3.17 (m, 1H), 2.93 (m, 1H), 2.30 (dt, $J$ = 4.0, 6.0 Hz, 1H), 1.49 (dd, $J$ = 2.4, 6.4 Hz, 1H), 0.64 (dd, $J$ = 2.8, 4.4 Hz, 1H); IR (film) $\nu_{max}$ 2954, 1731, 1633, 1445, 1323, 1196, 1018 cm$^{-1}$; UV (THF) $\lambda_{max}$ ($\epsilon$) 386 (2300), 302 (4500 sh), 293 (5100), 250 (18000) nm; FABHRMS (NBA/NaI) *m*/*z* 255.0901 ($M^+$, $C_{15}H_{13}NO_3$ requires 255.0895). (+)−(8a*R*,9a*R*)-**33**: $[\alpha]^{25}_D$ +31 (*c* 0.07, $CH_3OH$).

The structure of **33** was confirmed with a single-crystal X-ray structure determination conducted on plates grown from EtOAc/hexane.[37]

**1,2,9,9a-Tetrahydrocyclopropa[*c*]benzo[*f*]indol-8-one (iso-CBI, 34).** A solution of **28** (10.6 mg, 28.1 $\mu$mol) was treated with 3.6 M HCl/EtOAc and the mixture was stirred at 25 °C for 30 min. The volatiles were removed by a stream of $N_2$ and the residual salt was suspended in 1.0 mL of degassed $CH_3CN$. The suspension was treated with DBU (9.3 $\mu$L, 61.8 $\mu$mol, 2.2 equiv) and stirred under Ar for 20 min at 25 °C in the dark. Flash chromatography ($SiO_2$, 1.5 × 15 cm, 40% EtOAc/hexane) provided pure **34** (3.0 mg, 55%) as an unstable bright yellow oil: $^1$H NMR ($C_6D_6$, 400 MHz) $\delta$ 8.38 (d, $J$ = 8.0 Hz, 1H), 7.18 (m, 1H), 6.99 (d, $J$ = 8.0 Hz, 1H), 6.87 (m, 1H), 5.27 (s, 1H), 2.60 (m, 1H), 2.52 (m, 1H), 2.45 (m, 1H), 1.70 (m, 1H), 1.00 (m, 1H); IR (film) $\nu_{max}$ 3358, 1682, 1594, 1470, 1284, 1262, 1223 cm$^{-1}$; FABHRMS (NBA/NaI) *m*/*z* 198.0926 ($C_{13}H_{11}NO$ + $H^+$ requires 198.0919).

**9,9a-1*H*-Dihydrocyclopropa[*c*]benzo[*f*]indol-3,8-dione (35).** A solution of **28** (10.6 mg, 28.1 $\mu$mol) was treated with 4 M HCl/EtOAc, and the mixture was stirred at 25 °C for 30 min. The volatiles were removed by a stream of $N_2$, and the residual salt was suspended in 1.0 mL of THF. The suspension was treated with 1.0 mL of 5% aqueous $NaHCO_3$ and stirred exposed to the air for 2 h at 25 °C. The bright orange solution was extracted with EtOAc (3 × 5 mL), washed with $H_2O$ (2 × 5 mL), dried ($Na_2SO_4$), and concentrated in vacuo. Flash chromatography ($SiO_2$, 1.5 × 15 cm, 60% EtOAc/hexane) provided pure **35** (3.5 mg, 63%) as an orange film: $^1$H NMR ($C_6D_6$, 400 MHz) $\delta$ 8.14 (m, 1H), 8.05 (m, 1H), 7.00 (m, 2H), 3.62 (dd, $J$ = 6.2, 19.4 Hz, 1H), 3.52 (ddt, $J$ = 0.6, 2.2, 19.4 Hz, 1H), 2.55 (ddt, $J$ = 2.2, 6.0, 8.3 Hz, 1H), 1.54

(ddd, $J$ = 0.6, 3.3, 8.4 Hz, 1H), 0.30 (dd, $J$ = 3.3, 5.8 Hz, 1H); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 191.6, 182.0, 168.9, 135.8, 135.6, 135.0, 134.6, 128.4, 127.0, 63.8, 49.7, 35.0, 30.4; IR (film) $\nu_{max}$ 2923, 1692, 1680, 1585, 1361, 1262, 1223, 1082 cm$^{-1}$; UV ($CH_3OH$) $\lambda_{max}$ ($\epsilon$) 271 (5200), 239 (13300) nm; FABHRMS (NBA/NaI) $m/z$ 212.0715 ($C_{13}H_9NO_2 + H^+$ requires 212.0712).

**4-(*tert*-Butyloxycarbonyl)-1,2,2a,3-tetrahydrofurano-[4,3,2-*c*,*d*]benzo[*f*]indole (36).** A neat sample of **31** (6.0 mg, 16 $\mu$mol) was allowed to sit under room light at 25 °C for 48 h to yield **36** (6.0 mg, 100%): $^1$H NMR ($CD_3CN$, 500 MHz) $\delta$ 7.80 (d, $J$ = 8.0 Hz, 1H), 7.67 (d, $J$ = 7.7 Hz, 1H), 7.35 (m, 1H), 7.29 (m, 1H), 7.05 (br s, 1H), 5.22 (dd, $J$ = 8.0, 8.5 Hz, 1H), 4.64 (dd, J = 9.0, 11.0 Hz, 1H), 4.40 (dd, $J$ = 8.5, 10.5 Hz, 1H), 4.22 (m, 1H), 3.88 (m, 1H), 1.55 (br s, 9H); IR (film) $\nu_{max}$ 2975, 2932, 1699, 1475, 1456, 1418, 1353, 1166, 1132 cm$^{-1}$; FABHRMS (NBA/NaI) $m/z$ 297.1451 ($M^+$, $C_{18}H_{19}NO_3$ requires 297.1365).

**3-(Chloromethyl)-4-hydroxy-1-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*f*]indole (43).** A sample of **28** (8.4 mg, 0.022 mmol) was treated with 1.0 mL of 3.6 N HCl/EtOAc, and the resulting solution was stirred for 30 min at 25 °C. The solvent was removed by a stream of $N_2$, and the residual salt was dried under vacuum. This salt was dissolved in 0.9 mL of anhydrous DMF and treated with 5,6,7-trimethoxyindole-2-carboxylic acid[10] (6.8 mg, 0.026 mmol) and EDCI (12.7 mg, 0.067 mmol), and the mixture was stirred for 3 h at 25 °C. Upon completion, the reaction mixture was diluted with 20 mL of EtOAc, washed with $H_2O$ (5 × 3 mL), dried ($Na_2SO_4$), and concentrated in vacuo. Flash chromatography ($SiO_2$, 1.5 × 15 cm, 0−40% EtOAc/hexane gradient) provided **43** (11.2 mg, 91%) as a white solid: $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 9.41 (s, 1H), 8.35 (s, 1H), 7.88 (d, $J$ = 8.4 Hz, 1H), 7.82 (d, $J$ = 7.8 Hz, 1H), 7.45 (m, 1H), 7.41 (m, 1H), 6.96 (d, $J$ = 2.3 Hz, 1H), 6.85 (s, 1H), 5.95 (br s, 1H), 4.58 (m, 2H), 4.07 (m, 2H), 4.07 (s, 3H), 3.94 (s, 3H), 3.90 (s, 3H), 3.64 (dd, $J$ = 10.3, 12.0 Hz, 1H); $^{13}$C NMR (acetone-$d_6$, 400 MHz) $\delta$ 193.6, 161.1, 151.0, 149.7, 143.4, 141.5, 136.7, 131.6, 128.8, 127.3, 126.5, 124.7, 124.5, 124.0, 121.9, 115.3, 107.6, 107.2, 99.0, 61.44, 61.37, 56.4, 54.9, 46.6, 41.7; IR (film) $\nu_{max}$ 3405, 3301, 2937, 1594, 1446, 1312, 1228, 1106 cm$^{-1}$; FABHRMS (NBA/CsI) $m/z$ 467.1358 ($C_{25}H_{23}ClN_2O_5 + H^+$ requires 467.1374). (−)-(3*S*)-**43**: $[\alpha]^{25}_D$ −4 ($c$ 0.22, $CHCl_3$); (+)-(3*R*)-**43**: $[\alpha]^{25}_D$ +4 ($c$ 0.35, $CHCl_3$).

**2-[(5,6,7-Trimethoxyindol-2-yl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*f*]indol-8-one (iso-CBI-TMI, 44).** A solution of **43** (5.7 mg, 0.012 mmol) in 0.5 mL of $CH_3CN$ was treated with DBU (2.2 $\mu$L, 0.015 mmol) and stirred at 25 °C for 15 min. Flash chromatography ($SiO_2$, 1.5 × 10 cm, 50% EtOAc/hexane) provided pure **44** (4.2 mg, 94%) as a light golden oil: $^1$H NMR ($C_6D_6$, 400 MHz) $\delta$ 9.65 (s, 1H), 8.36 (m, 1H), 7.76 (s, 1H), 7.10 (m, 1H), 7.03 (m, 1H), 6.94 (m, 1H), 6.77 (s, 1H), 6.45 (d, $J$ = 2.2 Hz, 1H), 3.78 (s, 3H), 3.70 (s, 3H), 3.54 (s, 3H), 3.52 (d, $J$ = 9.9 Hz, 1H), 3.26 (dd, $J$ = 5.2, 9.9 Hz, 1H), 2.43 (m, 1H), 1.57 (dd, $J$ = 3.3, 7.8 Hz, 1H), 0.70 (dd, $J$ = 3.3, 5.4 Hz, 1H); $^{13}$C NMR (acetone-$d_6$, 400 MHz) $\delta$ 194.5, 191.8, 161.7, 151.4, 147.1, 143.1, 142.0, 140.4, 135.6, 131.3, 129.4, 128.9, 127.3, 126.9, 126.7, 125.0, 112.7, 108.1, 107.6, 99.3, 63.7, 63.6, 57.0, 54.3, 40.6; IR (film) $\nu_{max}$ 3295, 2934, 1667, 1651, 1409, 1306, 1279, 1109 cm$^{-1}$; UV ($CH_3OH$) $\lambda_{max}$ 390 ($\epsilon$ 7100), 332 ($\epsilon$ 14100), 245 nm ($\epsilon$ 19100); FABHRMS (NBA/NaI) $m/z$ 431.1615 ($C_{25}H_{22}N_2O_5 + H^+$ requires 431.1607). (−)-(8a*S*,9a*S*)-**44**: $[\alpha]^{25}_D$ −41 ($c$ 0.13, $CH_3OH$); (+)-(8a*R*,9a*R*)-**44**: $[\alpha]^{25}_D$ +38 ($c$ 0.08, $CH_3OH$).

**3-(Chloromethyl)-4-hydroxy-1-[(5-methoxyindol-2-yl)-carbonyl]-2,3-dihydro-1*H*-benzo[*f*]indole (45).** Flash chromatography ($SiO_2$, 1.5 × 10 cm, 0−40% EtOAc/hexane gradient) afforded pure **45** (95%) as a clear oil: $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 9.38 (br s, 1H), 8.35 (s, 1H), 7.93 (d, J = 7.6 Hz, 1H), 7.82 (d, $J$ = 7.9 Hz, 1H), 7.41 (m, 2H), 7.36 (d, $J$ = 9.0 Hz, 1H), 7.11 (d, $J$ = 2.2 Hz, 1H), 7.01 (m, 2H), 6.43 (s, 1H), 4.63 (d, $J$ = 5.4 Hz, 2H), 4.11 (m, 2H), 3.86 (s, 3H), 3.64 (m, 1H); IR (film) $\nu_{max}$ 3287, 2927, 1665, 1596, 1518, 1445, 1402, 1389, 1290, 1231, 1167 cm$^{-1}$; FABHRMS (NBA/NaI) $m/z$ 406.1071 ($C_{25}H_{19}ClN_2O_3$ requires 406.1084). (−)-(3*S*)-**45**: $[\alpha]^{25}_D$ −26 ($c$ 0.07, $CH_3OH$); (+)-(3*R*)-**45**: $[\alpha]^{25}_D$ +25 ($c$ 0.04, $CH_3OH$).

**2-[(5-Methoxyindol-2-yl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*f*]indol-8-one (46).** Flash chromatography ($SiO_2$, 1.5 × 5 cm, 40% EtOAc/hexane) afforded **46** (85%) as a light golden oil: $^1$H NMR ($C_6D_6$, 400 MHz) $\delta$ 9.15 (s, 1H), 8.38 (m, 1H), 7.80 (s, 1H), 7.12−7.05 (m, 4H), 6.94 (ddd, $J$ = 1.6, 6.8, 8.3 Hz, 1H), 6.85 (app dt, $J$ = 0.8, 8.6 Hz, 1H), 6.39 (d, $J$ = 1.6 Hz, 1H), 3.51 (s, 3H), 3.48 (d, $J$ = 10.0 Hz, 1H), 3.21 (dd, $J$ = 5.2, 10.0 Hz, 1H), 2.42 (dt, $J$ = 5.3, 7.8 Hz, 1H), 1.56 (dd, $J$ = 3.3, 7.8 Hz, 1H), 0.68 (dd, $J$ = 3.3, 5.3 Hz, 1H); IR (film) $\nu_{max}$ 3303, 2927, 1667, 1643, 1597, 1518, 1408, 1277, 1030, 1013 cm$^{-1}$; FABHRMS (NBA/NaI) $m/z$ 371.1388 ($C_{23}H_{18}N_2O_3 + H^+$ requires 371.1396). (−)-(8a*S*,9a*S*)-**46**: $[\alpha]^{25}_D$ −66 ($c$ 0.04, $CH_3OH$); (+)-(8a*R*,9a*R*)-**46**: $[\alpha]^{25}_D$ +71 ($c$ 0.03, $CH_3OH$).

**3-(Chloromethyl)-4-hydroxy-1-[((*E*)-3-(2-methoxyphenyl)propenyl)carbonyl]-2,3-dihydro-1*H*-benzo[*f*]indole (47).** Flash chromatography ($SiO_2$, 1.5 × 10 cm, 0−40% EtOAc/hexane gradient) afforded **47** (87%) as a pale yellow solid: $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.40 (br s, 1H), 7.87 (m, 3H), 7.40 (m, 2H), 7.31 (m, 1H), 7.22 (m, 1H), 7.20 (s, 1H), 6.93 (m, 2H), 5.81 (m, 1H), 4.36 (m, 2H), 4.09 (m, 2H), 3.85 (s, 3H), 3.65 (m, 1H); IR (film) $\nu_{max}$ 3266, 2963, 1659, 1598, 1445, 1377, 1269, 1158, 1048 cm$^{-1}$; FABHRMS (NBA/NaI) $m/z$ 394.1217 ($C_{25}H_{20}ClNO_3 + H^+$ requires 394.1210). (−)-(3*S*)-**47**: $[\alpha]^{25}_D$ −18 ($c$ 0.15, $CH_3OH$); (+)-(3*R*)-**47**: $[\alpha]^{25}_D$ +22 ($c$ 0.04, $CH_3OH$).

**2-[((*E*)-3-(2-Methoxyphenyl)propenyl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*f*]indol-8-one (48).** Flash chromatography ($SiO_2$, 1.5 × 5 cm, 40% EtOAc/hexane) afforded **48** (83%) as a light golden oil: $^1$H NMR ($C_6D_6$, 400 MHz) rotamers $\delta$ 8.39 (d, $J$ = 7.5 Hz, 1H), 8.09 (d, $J$ = 15.4 Hz, 1H), 7.15−6.95 (m, 8H), 6.76 (ddd, $J$ = 1.0, 2.5, 8.1 Hz, 1H), 3.70−3.50 (m, 1H), 3.31 (s, 3H), 3.00−2.80 (m, 1H), 2.35 (m, 1H), 1.63 (m, 1H), 0.73 (m, 1H); IR (film) $\nu_{max}$ 2926, 1673, 1626, 1471, 1408, 1383, 1265, 1156, 1092, 1044, 1009 cm$^{-1}$; FABHRMS (NBA/NaI) $m/z$ 357.1360 ($M^+$, $C_{25}H_{19}NO_3$ requires 357.1365). (−)-(8a*S*,9a*S*)-**48**: $[\alpha]^{25}_D$ −21 ($c$ 0.08, $CH_3OH$); (+)-(8a*R*,9a*R*)-**48**: $[\alpha]^{25}_D$ +23 ($c$ 0.03, $CH_3OH$).

**3-(Chloromethyl)-4-hydroxy-1-{[5-[*N*-(indol-2-yl)carbonyl]aminoindol-2-yl]carbonyl}-2,3-dihydro-1*H*-benzo[*f*]-indole (49).** Flash chromatography ($SiO_2$, 1.5 × 10 cm, 40−80% EtOAc/hexane gradient) afforded **49** (74%) as a light brown solid: $^1$H NMR (DMF-$d_7$, 400 MHz) $\delta$ 11.76 (s, 2H), 10.30 (s, 1H), 10.28 (s, 1H), 8.41 (s, 1H), 8.29 (s, 1H), 8.25 (d, $J$ = 8.0 Hz, 1H), 7.81 (d, $J$ = 8.4 Hz, 1H), 7.71 (dd, $J$ = 2.8, 9.2 Hz, 1H), 7.69 (d, $J$ = 9.6 Hz, 1H), 7.59 (d, $J$ = 8.6 Hz, 2H), 7.52 (s, 1H), 7.46 (m, 1H), 7.40 (m, 1H), 7.30 (d, $J$ = 1.6 Hz, 1H), 7.25 (m, 1H), 7.09 (m, 1H), 4.83 (m, 1H), 4.70 (dd, $J$ = 2.4, 10.4 Hz, 1H), 4.30 (m, 1H), 4.16 (dd, $J$ = 2.8, 11.2 Hz, 1H), 3.98 (dd, $J$ = 8.8, 11.2 Hz, 1H); IR (film) $\nu_{max}$ 3280, 2929, 1660, 1650, 1594, 1519, 1443, 1389, 1314, 1250, 1098, 749 cm$^{-1}$; FABHRMS (NBA/CsI) $m/z$ 667.0529 ($C_{31}H_{23}ClN_4O_3 + Cs^+$ requires 667.0513). (−)-(3*S*)-**49**: $[\alpha]^{25}_D$ −18 ($c$ 0.10, $CH_3OH$); (+)-(3*R*)-**49**: $[\alpha]^{25}_D$ +21 ($c$ 0.06, $CH_3OH$).

**2-{[5-[[*N*-(indol-2-yl)carbonyl]amino]indol-2-yl]carbonyl}-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*f*]indol-8-one (iso-CBI-indole$_2$, 50).** Flash chromatography ($SiO_2$, 1.5 × 10 cm, 10% DMF/toluene) afforded **50** (88%) as a light golden solid: $^1$H NMR (DMF-$d_7$, 400 MHz) $\delta$ 11.75 (s, 1H), 11.72 (s, 1H), 10.28 (s, 1H), 8.37 (s, 1H), 7.96 (d, $J$ = 7.9 Hz, 1H), 7.73−7.64 (m, 3H), 7.58 (t, $J$ = 8.1 Hz, 2H), 7.50 (m, 2H), 7.35 (m, 2H), 7.23 (m, 2H), 7.08 (t, $J$ = 7.3 Hz, 1H), 4.57 (m, 2H), 3.09 (dt, $J$ = 5.5, 7.7 Hz, 1H), 1.86 (dd, $J$ = 3.1, 7.7 Hz, 1H), 1.77 (dd, $J$ = 3.2, 5.5 Hz, 1H); IR (film) $\nu_{max}$ 3289, 2927, 1668, 1652, 1557, 1520, 1409, 1313 cm$^{-1}$; FABHRMS (NBA/CsI) $m/z$ 499.1752 ($C_{31}H_{22}N_4O_3 + H^+$ requires 499.1770). (−)-(8a*S*,9a*S*)-**50**: $[\alpha]^{25}_D$ −21 ($c$ 0.10, DMF); (+)-(8a*R*,9a*R*)-**50**: $[\alpha]^{25}_D$ +21 ($c$ 0.06, DMF).

**1-[(3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indol-7-yl)carbonyl]-3-(chloromethyl)-4-hydroxy-2,3-dihydro-1*H*-benzo[*f*]indole (51).** Flash chromatography ($SiO_2$, 1.5 × 10 cm, 10−50% DMF/toluene gradient) afforded pure **51** (80%) as a yellow solid: $^1$H NMR (DMF-$d_7$, 400 MHz) $\delta$ 11.64 (s, 1H), 10.32 (s, 1H), 8.28 (s, 1H), 8.25 (d, $J$ = 8.4 Hz, 1H), 8.16 (d, $J$ = 8.9 Hz, 1H), 7.81 (d, $J$ = 8.1 Hz, 1H), 7.46 (m, 1H), 7.39 (m, 2H), 7.15 (d, $J$ = 1.7 Hz, 1H), 6.13 (s, 2H), 4.82 (m, 1H), 4.68

(dd, $J$ = 2.7, 10.8 Hz, 1H), 4.30 (m, 1H), 4.15 (m, 3H), 3.97 (dd, $J$ = 8.3, 10.8 Hz, 1H), 3.39 (m, 2H); IR (film) $\nu_{max}$ 3337, 2924, 1662, 1652, 1513, 1456, 1441, 1400, 1344, 1272 cm$^{-1}$; ESIMS $m/z$ 461 (M + H$^+$, $C_{25}H_{21}ClN_4O_3$ requires 461). (−)-(3*S*)-**51**: $[\alpha]^{25}_D$ −21 (*c* 0.18, DMF); (+)-(3*R*)-**51**: $[\alpha]^{25}_D$ +22 (*c* 0.13, DMF).

**2-[(3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indol-7-yl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*f*]-indol-8-one (iso-CBI-CDPI$_1$, 52).** Flash chromatography ($SiO_2$, 1.5 × 10 cm, 30−50% DMF/toluene gradient) afforded **52** (81%) as a brown solid: $^1$H NMR (DMF-$d_7$, 400 MHz) $\delta$ 11.60 (s, 1H), 8.15 (d, $J$ = 8.8 Hz, 1H), 7.95 (d, $J$ = 7.6 Hz, 1H), 7.66 (t, $J$ = 7.4 Hz, 1H), 7.49 (d, $J$ = 7.8 Hz, 1H), 7.35 (m, 3H), 7.10 (s, 1H), 6.13 (s, 2H), 4.55 (m, 2H), 4.13 (t, $J$ = 8.8 Hz, 2H), 3.36 (t, $J$ = 9.1 Hz, 2H), 3.09 (m, 1H), 1.85 (dd, $J$ = 2.9, 7.6 Hz, 1H), 1.76 (dd, $J$ = 3.3, 5.4 Hz, 1H); IR (film) $\nu_{max}$ 3346, 2927, 1667, 1651, 1505, 1435, 1408, 1343, 1279, 1098 cm$^{-1}$; ESIMS $m/z$ 425 (M + H$^+$, $C_{25}H_{20}N_4O_3$ requires 425). (−)-(8a*S*,9a*S*)-**52**: $[\alpha]^{25}_D$ −13 (*c* 0.06, DMF); (+)-(8a*R*,9a*R*)-**52**: $[\alpha]^{25}_D$ +12 (*c* 0.07, DMF).

**1-{[3-[*N*-(3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indol-7-yl)carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indol-7-yl]carbonyl}-3-chloromethyl-4-hydroxy-2,3-dihydro-1*H*-benzo[*f*]indole (53).** Flash chromatography ($SiO_2$, 1.5 × 15 cm, 10−50% DMF/toluene gradient) afforded pure **53** (32%) as a red solid: $^1$H NMR (DMF-$d_7$, 400 MHz) $\delta$ 11.85 (s, 1H), 11.54 (s, 1H), 10.33 (s, 1H), 8.39 (m, 1H), 8.30 (s, 1H), 8.26 (d, $J$ = 8.4 Hz, 1H), 8.13 (d, $J$ = 8.9 Hz, 1H), 7.82 (d, $J$ = 8.0 Hz, 1H), 7.48 (m, 2H), 7.38 (m, 2H), 7.31 (s, 1H), 7.08 (s, 1H), 6.10 (s, 2H), 4.86 (m, 1H), 4.73 (m, 3H), 4.31 (m, 1H), 4.15 (m, 3H), 3.99 (dd, $J$ = 8.2, 10.7 Hz, 1H), 3.55 (m, 2H), 3.39 (m, 2H); IR (film) $\nu_{max}$ 3338, 2956, 2927, 1727, 1659, 1650, 1604, 1510, 1402, 1365, 1286 cm$^{-1}$; ESIMS $m/z$ 645/647 (M + H$^+$, $C_{36}H_{29}ClN_6O_4$ requires 645/647). (−)-(3*S*)-**53**: $[\alpha]^{25}_D$ −19 (*c* 0.10, DMF); (+)-(3*R*)-**53**: $[\alpha]^{25}_D$ +21 (*c* 0.08, DMF).

**2-{[3-[*N*-(3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indol-7-yl)carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indol-7-yl]carbonyl}-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*f*]-indol-8-one (iso-CBI-CDPI$_2$, 54).** Flash chromatography ($SiO_2$, 1.5 × 10 cm, 50% DMF/toluene) afforded **53** (59%) as a brown solid: $^1$H NMR (DMF-$d_7$, 400 MHz) $\delta$ 11.80 (s, 1H), 11.53 (s, 1H), 8.37 (m, 1H), 8.13 (d, $J$ = 8.9 Hz, 2H), 7.65 (m, 1H), 7.49 (d, $J$ = 7.8 Hz, 1H), 7.47 (d, $J$ = 9.1 Hz, 1H), 7.36 (m, 3H), 7.24 (d, $J$ = 1.5 Hz, 1H), 7.07 (s, 1H), 6.12 (s, 2H), 4.74 (t, $J$ = 8.4 Hz, 2H), 4.58 (m, 2H), 4.14 (t, $J$ = 8.9 Hz, 2H), 3.52 (m, 2H), 3.38 (t, $J$ = 8.8 Hz, 2H), 3.11 (m, 1H), 1.87 (dd, $J$ = 3.1, 7.8 Hz, 1H), 1.78 (dd, $J$ = 3.1, 5.5 Hz, 1H); IR (film) $\nu_{max}$ 3293, 2926, 1665, 1612, 1581, 1503, 1431, 1409, 1363, 1344, 1278, 1185 cm$^{-1}$; ESIMS $m/z$ 607 (M − H$^+$, $C_{36}H_{28}N_6O_4$ requires 607). (−)-(8a*S*,9a*S*)-**54**: $[\alpha]^{25}_D$ −34 (*c* 0.05, DMF); (+)-(8a*R*,9a*R*)-**54**: $[\alpha]^{25}_D$ +33 (*c* 0.03, DMF).

**Solvolysis Regioselectivity: 1-(*tert*-Butyloxycarbonyl)-4-hydroxy-3-(methoxymethyl)-2,3-dihydro-1*H*-benzo[*f*]-indole (62).** A solution of **31** (7.7 mg, 0.026 mmol) in 2.5 mL of $CH_3OH$ was cooled to 0 °C, and $CF_3SO_3H$ in $CH_3OH$ (311 μL, 0.01 N, 0.12 equiv) was added. After slowly warming to 25 °C over 17 h, the reaction was quenched by the addition of $NaHCO_3$ (2.1 mg), filtered through Celite, and concentrated under reduced pressure. Flash chromatography ($SiO_2$, 1.5 × 15 cm, 0−40% EtOAc/hexane gradient) yielded the major isomer **62** (8.0 mg, 94%) as a white film: $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 9.05 (s, 1H), 8.12 (d, $J$ = 6.4 Hz, 1H), 7.80 (m, 1H), 7.65 (s, 1H), 7.37 (m, 1H), 7.29 (m, 1H), 4.17 (m, 1H), 3.83 (m, 1H), 3.77 (dd, $J$ = 2.8, 6.4 Hz, 1H), 3.60 (dd, $J$ = 6.8, 8.8 Hz, 1H), 3.54 (s, 3H), 3.49 (m, 1H), 1.56 (s, 9H); IR (film) $\nu_{max}$ 3233, 2975, 1704, 1644, 1435, 1348, 1148, 1026, 956 cm$^{-1}$; FABHRMS (NBA/NaI) $m/z$ 352.1535 ($C_{19}H_{23}NO_4$ + Na$^+$ requires 352.1525).

For the minor isomer **63** (<2% by $^1$H NMR of the crude reaction mixture): $^1$H NMR ($CDCl_2$, 500 MHz) $\delta$ 8.10 (m, 1H), 7.95 (d, $J$ = 6.5 Hz, 1H), 7.73 (m, 1H), 7.38 (m, 1H), 7.33 (m, 1H), 7.24 (m, 1H), 4.13 (m, 1H), 3.98 (s, 3H), 3.92 (m, 1H), 3.81 (m, 2H), 3.50 (m, 1H), 1.57 (s, 9H); FABHRMS (NBA/NaI) $m/z$ 352.1534 ($C_{19}H_{23}NO_4$ + Na$^+$ requires 352.1525).

**Preparation of Authentic 62.** A solution of **27** (17.3 mg, 0.05 mmol) in 1.6 mL of anhydrous DMF was treated with NaH (4.3 mg, 0.14 mmol) in small portions, and the resulting suspension was stirred for 15 min at 0 °C. Methyl iodide (18 μL, 0.29 mmol) was added neat and the resulting mixture warmed to 25 °C over 2 h. The reaction mixture was quenched with the addition of 5% aqueous $NaHCO_3$ (10 mL), and the aqueous layer was extracted EtOAc (3 × 5 mL). The combined organic extracts were washed with $H_2O$ (5 × 5 mL), dried ($Na_2$-

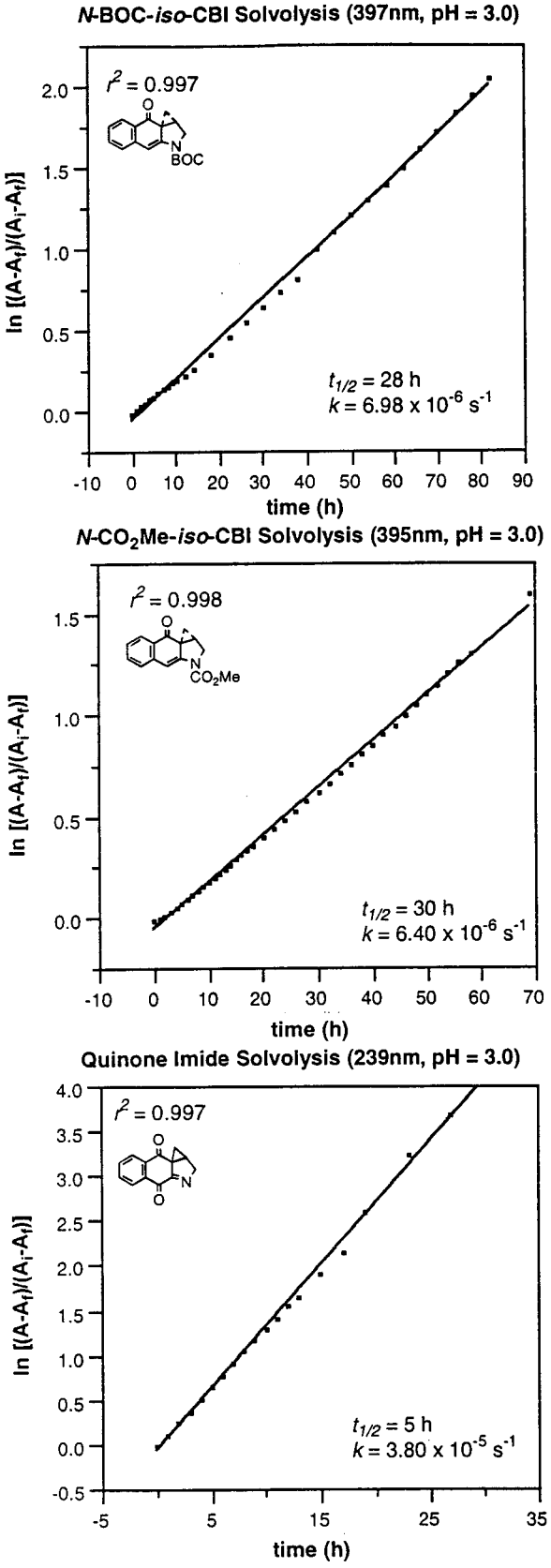



**Figure 9.**

$SO_4$), and concentrated under reduced pressure. The crude mixture was subjected to flash chromatography ($SiO_2$, 1.5 × 15 cm, 10% EtOAc−hexanes) to yield the intermediate methyl ether **64** (14.0 mg, 78%) as a colorless oil. **64** was subjected to the selective MOM deprotection reaction and purification as detailed for **30** to yield the major solvolysis product **62**, identical in all aspects.

**Solvolysis Reactivity.** *N*-BOC-iso-CBI (**31**, 0.2 mg) was dissolved in $CH_3OH$ (1.5 mL) and mixed with pH 3 aqueous buffer (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. The solvolysis solution was sealed and kept at 25 °C protected from light. The UV spectrum was measured at regular intervals every 1 h during the first 24 h, every 4 during the next 72 h, and every 12 h for an additional week. The decrease in the long wavelength absorption at 397 nm was monitored, Figure 3. The solvolysis rate constant ($k = 6.98 \times 10^{-6}$ $s^{-1}$) and the half-life ($t_{1/2} = 28$ h) were calculated from data recorded at the long wavelength from the least-squares treatment ($r = 0.99$) of the slope of the plot of time versus $\ln[(A_f - A_i)/(A_f - A)]$ (Figure 9).

Similarly, *N*-$CO_2$Me-iso-CBI (**33**, 0.2 mg) was dissolved in $CH_3OH$ (1.5 mL) and mixed with pH 3 aqueous buffer (1.5 mL). The solvolysis solution was sealed and kept at 25 °C protected from light. The UV spectrum was measured at regular intervals every 1 h during the first 24 h, every 4 h during the next 72 h, and every 12 h for an additional week. The decrease in the long wavelength absorption at 395 nm was monitored, Figure 3. The solvolysis rate constant ($k = 6.40 \times 10^{-6}$ $s^{-1}$) and the half-life ($t_{1/2} = 30$ h) were determined as detailed above ($r = 0.99$).

Similarly, **35** (0.2 mg) was dissolved in $CH_3OH$ (1.5 mL) and mixed with pH 3 aqueous buffer (1.5 mL). The solvolysis solution was sealed and kept at 25 °C protected from light. The UV spectrum was measured at regular intervals every 1 h during the first 12 h, every 2 h for the next 24 h, and every 4 h for an additional day. The decrease in the short wavelength absorption at 239 nm was monitored, Figure 9. The solvolysis rate constant ($k = 3.80 \times 10^{-5}$ $s^{-1}$) and the half-life ($t_{1/2} = 5$ h) were determined as detailed above ($r = 0.99$).

**Isolation, Characterization, and Quantitation of the Thermally Released (−)-iso-CBI-TMI Adenine Adduct 66.** (−)-iso-CBI-TMI (**44**, 1.0 mg, 2.32 μmol) in 500 μL of DMSO was added to a solution of calf thymus DNA (Sigma, 220 mg, ca. 150 bp) in 10 mM sodium phosphate buffer (13 mL, pH 7.0) in a 50 mL centrifuge tube. The mixture was cooled at 4 °C for 72 h and then extracted with EtOAc (3 × 10 mL) to remove hydrolyzed, rearranged (**65**), or unreacted **44**. UV and HPLC assay of the EtOAc extracts revealed no unreacted **44** (0%) and <5% rearranged **65**. The centrifuge tube containing the aqueous DNA layer was sealed with Teflon tape and warmed at 100 °C for 30 min. The resulting solution was cooled to 25 °C and extracted with EtOAc (3 × 10 mL). The combined organic layer was dried ($Na_2SO_4$) and concentrated under reduced pressure to afford a yellow solid. Chromatography ($SiO_2$, 0.5 × 3 cm, 0−7% $CH_3OH/CHCl_3$ gradient elution) afforded (+)-**66** as a pale yellow solid (1.25 mg, 1.31 mg theoretical, 95%, 90−95% in four runs) contaminated with a small impurity (<5% by HPLC). For **66**: $[\alpha]^{23}_D$ +28 (*c* 0.06, $CH_3OH$); $R_f$ = 0.3 ($SiO_2$, 10% $CH_3OH/CHCl_3$); $^1$H NMR (acetone-$d_6$, 400 MHz) δ 10.37 (s, 1H, N1′-H), 8.58 (s, 1H, ArOH), 8.31 (d, *J* = 7.9 Hz, 1H, ArH), 8.22 (s, 1H, Ade-C8−H), 8.07 (s, 1H, Ade-C2−H), 7.78 (d, *J* = 7.9 Hz, 1H, ArH), 7.49 (br s, 1H, ArH), 7.45 (ddd, *J* = 1.3, 6.8, 7.9 Hz, 1H, ArH), 7.38 (ddd, *J* = 1.3, 6.8, 7.9 Hz, 1H, ArH), 7.10 (d, *J* = 2.3 Hz, 1H, C3′-H), 6.93 (s, 1H, C5′-H), 4.92 (dd, *J* = 10.6, 1.9 Hz, 1H, C*H*H-Ade), 4.89 (dd, *J* = 14.5, 7.0 Hz, 1H, C*H*HNCO), 4.74 (dd, *J* = 14.5, 7.6 Hz, 1H, CH*H*NCO), 4.73 (dd, *J* = 10.8, 2.2 Hz, 1H, CH*H*-Ade), 4.37 (m, 1H, $CH_2CHCH_2$), 4.04 (s, 3H, $OCH_3$), 3.87 (s, 3H, $OCH_3$), 3.86 (s, 3H, $OCH_3$); $^1$H NMR (DMSO-$d_6$ + 1% *d*-TFA, 400 MHz) δ 11.40 (s, 1H, N1′-H), 9.28 (br s, 1H, N*H*H), 8.78 (br s, 1H, NH*H*), 8.45 (s, 1H, Ade-C8−H), 8.40 (s, 1H, Ade-C2−H), 8.07 (d, *J* = 7.2 Hz, 1H, Ar−H), 8.05 (br s, 1H, ArOH), 7.76 (d, *J* = 7.2 Hz, 1H, ArH), 7.43 (dd, *J* = 6.4, 6.4 Hz, 1H, ArH), 7.35 (dd, *J* = 6.4, 6.4 Hz, 1H, ArH), 6.95 (s, 1H, C3′-H), 6.90 (s, 1H, C5′-H), 4.72 (dd, *J* = 10.8, 5.6 Hz, 1H, C*H*H-Ade), 4.68 (dd, *J* = 10.8, 5.6 Hz, 1H, C*H*H-Ade), 4.55 (dd, *J* = 9.0, 9.0 Hz, 1H, C*H*HNCO), 4.54 (br d, *J* = 9.0 Hz, 1H, CH*H*NCO), 4.36 (m, 1H), 3.93 (s, 3H, $OCH_3$), 3.80 (s, 3H, $OCH_3$), 3.79 (s, 3H, $OCH_3$); $^{13}$C NMR (150 MHz, acetone-$d_6$) δ 170.5, 161.0, 156.9, 152.2, 151.2, 151.0, 150.0, 143.9, 142.9, 141.4, 140.0, 136.7, 131.8, 128.4, 127.2, 126.4, 124.7, 124.6, 124.1, 123.0, 114.2, 107.3, 106.1, 98.9, 61.5, 61.4, 57.5, 56.5, 56.4, 39.8; IR (film) $\nu_{max}$ 3251, 2911, 2850, 1684, 1647, 1458, 1312, 1200, 1024 $cm^{-1}$; UV ($CH_3OH$) $\lambda_{max}$ 331 (ε 12400), 300 (ε 12600), 240 nm (ε 17700); FABHRMS (NBA) *m/z* 566.2074 (M + $H^+$, $C_{30}H_{27}N_7O_5$ requires 566.2083).

HPLC $t_R$ (4 × 250 mm column, 1.0 mL/min, 35% $CH_3CN$/0.05 N aqueous $HCO_2NH_4$) were **44** (21.6 min), **65** (39.0 min), solvolysis product (24.0 min), and **66** (17.5 min).

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**Supporting Information Available:** Experimental procedures and characterization for **11**−**18**, experimental for the DNA alkylation studies, tables of data employed to prepare Figure 8, copies of $^1$H NMR spectra for **12**, **13**, **15**−**20**, **24**−**27**, **29**−**36**, **43**−**54**, **62**, **63**, and **66**, and details of the X-ray structure determinations of **32** and **33** are provided (70 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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