A Novel Class of CC-1065 and Duocarmycin Analogues Subject to Mitomycin-Related Reductive Activation

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A new class of DNA alkylating agents is described that incorporate the quinone of the mitomycins, which is thought to impart tumor cell selectivity as a result of preferential reduction and activation in hypoxic tumors, into the AT-selective binding framework of the duocarmycins capable of mitomycin-like reductive activation and duocarmycin-like spirocyclization and subsequent DNA alkylation. Consistent with this design, the quinone prodrugs fail to alkylate DNA unless reductively activated and then do so with an adenine N3 alkylation sequence selectivity identical to that of the duocarmycins. Additionally, the agents exhibit a selectivity toward DT-Diaphorase (NQO1)-containing versus DT-Diaphorase-deficient (resistant) tumor cell lines, and they were shown to be effective substrates for reduction by recombinant human DT-Diaphorase. As such, the agents constitute effective duocarmycin and CC-1065 analogues subject to reductive activation. In addition, the solvolysis pH rate dependence of a series of reactive spirocyclopropanes revealed a unique and inverted order of reactivity at pH 7 versus pH 3. This behavior and the structural features responsible for it are consistent with an acid-catalyzed reaction at pH 3, but a direct uncatalyzed S_N2 reaction at pH 7 that is not subject to acid catalysis.

The duocarmycins and CC-1065 (**1**−**3**)[1,2] and the mitomycins (**4** and **5**)[3] are two families of potent antitumor antibiotics that derive their biological activity through alkylation of DNA[4] (Figure 1). Prior studies have shown that CC-1065 and the duocarmycins tolerate and benefit from structural modifications to the alkylation subunit and that the resulting agents retain their ability to participate in the characteristic sequence selective DNA alkylation reaction.[4,5] Such structural modifications and the definition of their effects have served to advance the understanding of the origin of sequence selectivity[4,6] and the catalysis[7,8] of the DNA alkylation reaction by **1**−**3**. The recent observation that *isomeric* analogues of the duocarmycins, *iso*-CBI-based agents **6**,[9] retained not only the characteristic DNA alkylation selectivity of the natural products but also comparable *in vitro* biological activity, provided the basis for the preparation of synthetic hybrids combining the DNA alkylation features of the duocarmycins and the reductive activation of the mitomycins described herein.[10]

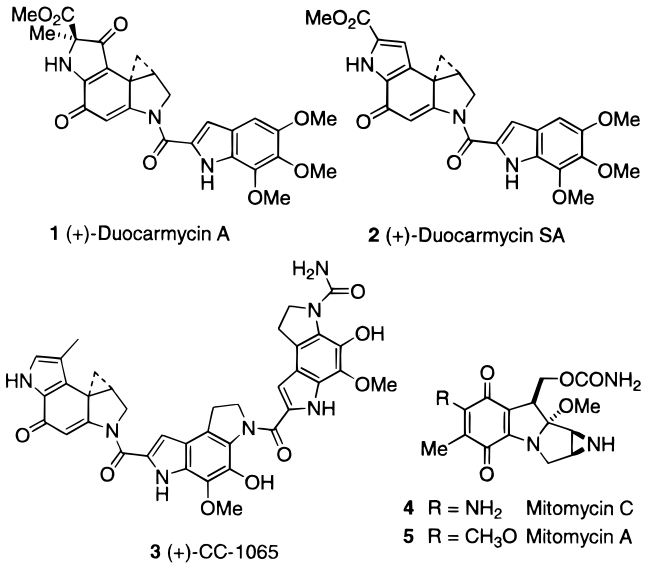

Figure 1.

One compelling feature of mitomycin C is its effective use in the clinic, either as a single agent or in combination with other agents,[11] and it is especially useful in the

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(2) Ichimura, M.; Ogawa, T.; Takahashi, K.; Kobayashi, E.; Kawamoto, I.; Yasuzawa, T.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1990**, *43*, 1037. Ichimura, M.; Ogawa, T.; Katsumata, S.; Takahashi, K.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1991**, *44*, 1045.

(3) Tomasz, M. In *Molecular Aspects of Anticancer Drug*−*DNA Interactions*; Neidle, S., Waring, M., Eds.; MacMillan: London, 1994; Vol. 2, pp 312−349.

(4) For mechanistic aspects see: Boger, D. L.; Johnson, D. S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1439.

(5) For synthetic aspects see: Boger, D. L.; Boyce, C. W.; Garbaccio, R. M.; Goldberg, J. A., *Chem Rev.* **1997**, *97*, 787.

(6) Sun, D.; Lin, C. H.; Hurley, L. H. *Biochemistry* **1993**, *32*, 4487 and references therein.

(7) Boger, D. L.; Garbaccio, R. M. *Acc. Chem. Res.*, submitted. Boger, D. L.; Garbaccio, R. M. *Bioorg. Med. Chem.* **1997**, *5*, 263.

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(10) Quyand, A., Skibo, E. B. *J. Org. Chem.* **1998**, *63*, 1893.

(11) Carter, S. K.; Crooke, S. T. *Mitomycin C: Current Status and New Developments*; Academic Press: New York, 1979. *Mitomycin C in Cancer Chemotherapy Today*; Taguchi, T., Aigner, K. R., Eds. Selected Proceedings of the 15th International Cancer Congress, Hamburg, Germany, Aug 16−22, 1990, *Excerpta Med.* **1991**.

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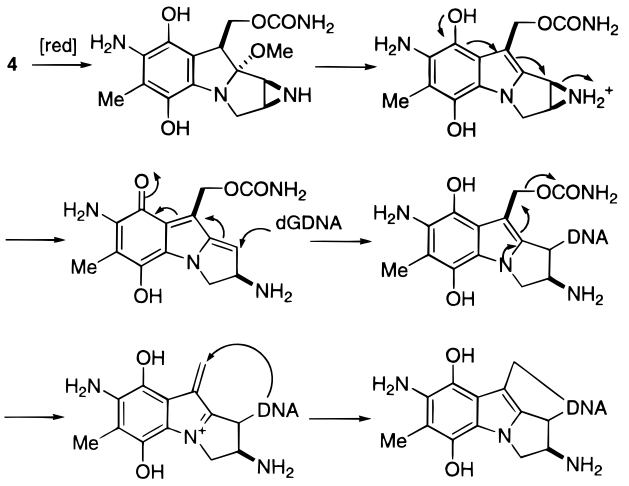
Scheme 1



treatment of solid tumors. Following reductive activation, mitomycin C is known to cross-link DNA through bifunctional alkylation (Scheme 1),[12] and it is believed that this interstrand mitomycin/DNA adduct inhibits DNA replication, resulting in inhibition of cell division.[13]

The synthesis of analogues of both these families has proven fruitful, yielding the current clinical mitomycin-like candidates KW-2149,[14] BMY-25067,[15] and the duocarmycin-like candidate KW-2189.[16] Herein, we report the incorporation of the quinone of the mitomycins, which is thought to impart tumor cell selectivity as a result of preferential reduction in the hypoxic environment of tumors, into the AT-selective binding framework of the duocarmycins to form a new hybrid agent **7** (Figure 2) capable of mitomycin-like reductive activation and duocarmycin-like DNA alkylation.

An activation cascade for such hybrid agents was envisioned to involve bioreduction, followed by in-situ spirocyclization unleashing a highly reactive DNA alkylating agent isomeric to the duocarmycins (Scheme 2). Importantly, unlike many simple mitomycin analogues, this agent is a mitosane which allows for spirocyclization and prevents the mitomycin-like release of a basic nitrogen lone pair promoting competitive elimination of chlorine. In addition, the quinone not only imparts potential tumor cell selectivity but also acts as a prodrug increasing the stability of what is predicted to be a highly reactive cyclopropylcyclohexadienone.

Synthesis. The synthetic route to the hybrid analogue **7** relied upon a cooperative directed *ortho*-metalation to install iodine at the more-hindered position on a substituted aryl precursor[9] and a subsequent 5-*exo*-*trig* radical cyclization onto a tethered vinyl chloride to complete

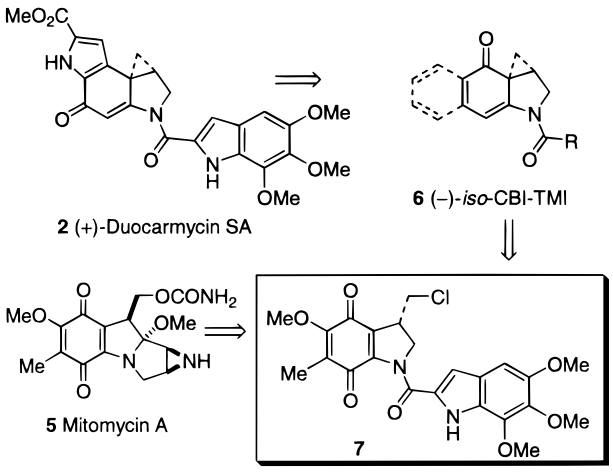
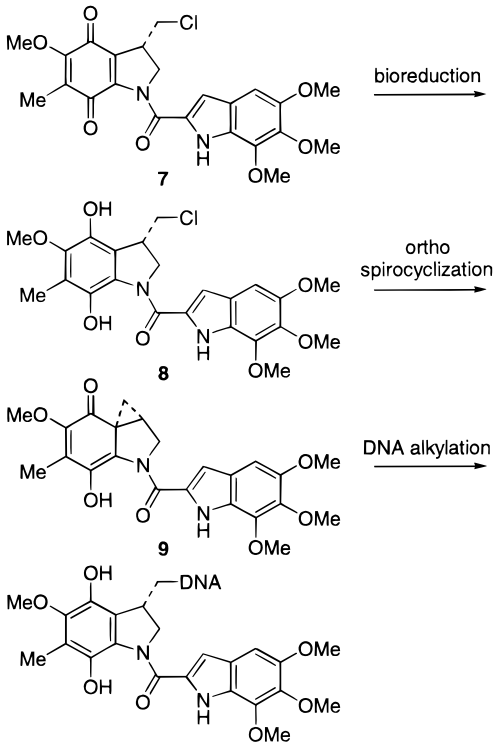


Figure 2.

Scheme 2



construction of the functionalized dihydroindole.[17] For the synthesis of the cyclopropane-containing hybrids, the *ortho*-variant[18] of the Winstein Ar-3′ spirocyclization[19] could be expected to effect cyclopropane spirocyclization. In turn, the quinone-based analogues were anticipated to be accessible from a late stage intermediate using a selective *p*-quinone oxidation protocol of a substituted 5,7-dimethoxy-4-hydroxy-2,3-dihydroindole.

The approach began with 2,4-dimethoxy-3-methylphenol (**10**), readily available from 2,6-dimethoxytoluene through the protocol described by Kishi[20] (Scheme 3). Protection of the phenol as the MOM ether **11** (NaH, MOMCl, Bu_4NI, 88%), regiospecific nitration ($Cu(NO_3)_2$·

(12) Iyer, V. N.; Szybalski, W. *Proc. Natl. Acad. Sci. U.S.A.* **1963**, *50*, 355.

(13) Iyer, V. N.; Szybalski, W. *Science* **1964**, *145*, 55. Zwelling, L. A.; Anderson, T.; Kohn, K. W. *Cancer Res.* **1979**, *39*, 365.

(14) Vyas, D. M.; Chiang, Y.; Benigni, D.; Rose, W. C.; Bradner, W. T. In *Recent Advances in Chemotherapy. Anticancer Section*; Tshigami, J., Ed.; University of Tokyo Press: Tokyo, 1985; pp 4385−486.

(15) Kono, M.; Saitoh, Y.; Kasai, M.; Sato, A.; Shirahata, K.; Morimoto, M.; Ashizawa, T. *Chem. Pharm. Bull.* **1989**, *37*, 1128−1130.

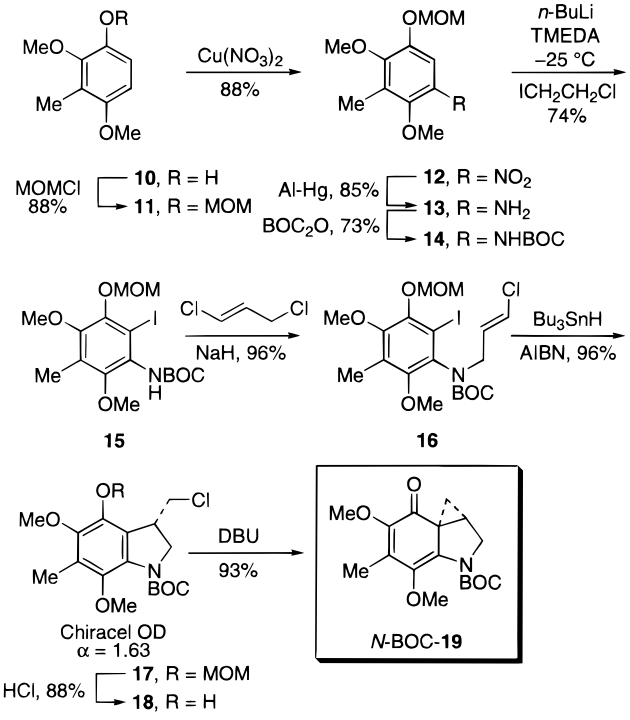
(16) Yasuzawa, T.; Muroi, K.; Ichimura, M.; Takahashi, I.; Ogawa, T.; Takahashi, K.; Sano, H. Saitoh, Y. *Chem. Pharm. Bull.* **1995**, *43*, 378. Nagamura, S.; Asai, A.; Kanda, Y.; Kobayashi, E.; Gomi, K.; Saito, H. *Chem. Pharm. Bull.* **1996**, *44*, 1723. Nagamura, S.; Kobayashi, E.; Gomi, K.; Saito, H. *Bioorg. Med. Chem.* **1996**, *4*, 1379. Asai, A.; Nagamura, S.; Kobayashi, E.; Gomi, K.; Saito, H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1215. Nagamura, S.; Kobayashi, E.; Gomi, K.; Saito, H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2147.

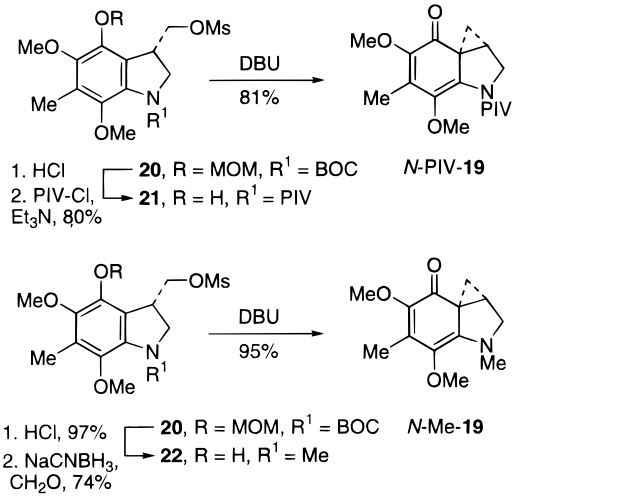
(17) Boger, D. L.; Boyce, C. W.; Garbaccio, R. M.; Searcey, M. *Tetrahedron Lett.* **1998**, *39*, 2227.

(18) Brown, R. F. C.; Matthews, B. R.; Rae, I. D. *Tetrahedron Lett.* **1981**, *22*, 2915. Smith, A. B., III; Yokoyama, Y.; Huryn, D. M.; Dunlap, N. K. *Tetrahedron Lett.* **1987**, *28*, 3659.

(19) Baird, R.; Winstein, S. *J. Am. Chem. Soc.* **1963**, *85*, 567.

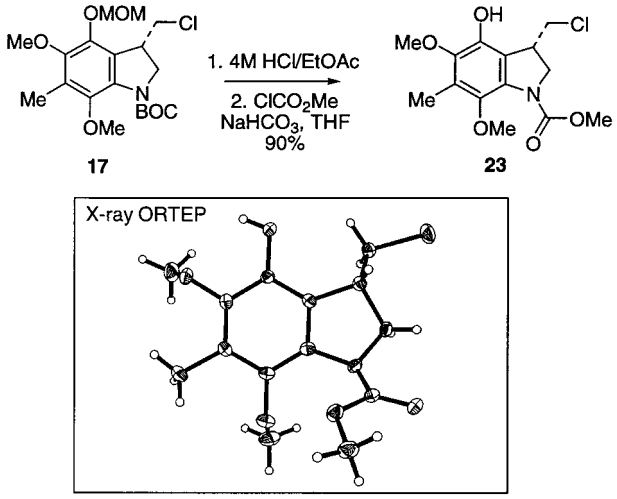
Scheme 3





2.5H_2O, 88%), reduction of the nitro group with Al−Hg amalgam[21] (Et_2O-H_2O, 85%), and BOC protection of the free amine **13** (BOC_2O, 73%) provided **14**, a key intermediate on which to examine the directed *ortho*-metalation for completion of the preparation of the hexasubstituted benzene. Treatment of **14** with 3.7 equiv of *n*-BuLi and TMEDA at −25 °C (2 h) in THF and reaction of the aryllithium intermediate with 1-chloro-2-iodoethane provided **15** in 74% yield. *N*-Alkylation of **15** with 1,3-dichloropropene proceeded in 96% yield, providing the key radical cyclization precursor **16**. Treatment of **16** with catalytic AIBN (0.1 equiv) and Bu_3SnH (1.1 equiv) at 74 °C effected 5-*exo*-*trig* cyclization and provided the dihydroindole core of the hybrid analogue in 96% yield.[17] Removal of the MOM ether (HCl, *i*-PrOH/THF, 88%) without competitive *N*-BOC deprotection afforded the seco agent in excellent yield. *Ortho* spirocyclization (DBU, CH_3CN, 88%) provided the first target *N*-BOC-**19** which, although reactive, could be purified by rapid chromatography.[22]

Scheme 4



For purposes related to establishing the stability of the novel *ortho* spirocyclopropyl-cyclohexadienones and the regioselectivity of nucleophilic addition, the corresponding *N*-pivaloyl and *N*-methyl derivatives (*N*-PIV-**19** and *N*-Me-**19**) were also prepared (Scheme 3). The *N*-pivaloyl derivative was prepared by a route analogous to that outlined above[23] or by BOC deprotection of **20** and *N*-acylation with pivaloyl chloride. Similarly, *N*-methylation was effected following BOC deprotection and reductive amination (9 equiv of CH_2O, 3 equiv of AcOH, 3 equiv of $NaCNBH_3$) to provide **22**.[23] In each case, spirocyclization effected by treatment with DBU afforded the corresponding cyclopropylcyclohexadienones in superb yield.

Resolution and Assignment of Absolute Stereochemistry. To establish the properties of both enantiomers of the agents, a direct chromatographic resolution of **17** on a semipreparative ChiralCel OD column (2 × 25 cm, 1% *i*-PrOH/hexane, $\alpha = 1.63$) was utilized.[24] This procedure provided both enantiomers (>99% ee) of an advanced intermediate and avoided diastereomeric derivatization, separation, and dederivatization. The superb separation of racemic **17** allowed 200 mg injections suitable for our preparative efforts. In addition, the carbomethoxy derivatives of these and related alkylation subunits prove to be crystalline.[25] Thus, the slower eluting enantiomer (+)-**17** was deprotected and acylated with methyl chloroformate (90%) in a one-pot procedure to yield **23**, which was recrystallized from EtOAc/hexanes (Scheme 4). Its absolute configuration was established by a single-crystal X-ray structure determination which revealed the natural (3*S*) configuration.[26]

Synthesis of the Mitomycin-Related A-Ring. A key challenge in the preparation of this set of agents was to

(20) Kishi, Y. *J. Nat. Prod.* **1979**, *42*, 549. Nakatsubo, F.; Fukuyama, T.; Cocuzza, A. J.; Kishi, Y. *J. Am. Chem. Soc.* **1977**, *99*, 8115. Fukuyama, T.; Nakatsubo, F.; Cocuzza, A. J.; Kishi, Y. *Tetrahedron Lett.*, **1977**, *49*, 4295.

(21) Meyers, A. I.; Durandetta, J. L. *J. Org. Chem.* **1975**, *40*, 2021.

(22) Even neat at subzero temperatures, **19** was subject to slow decomposition to a complex mixture.

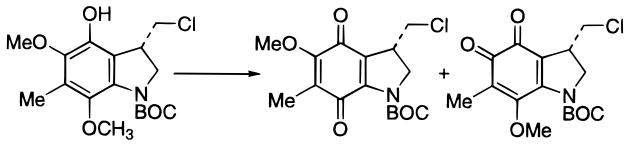
(23) See Supporting Information.

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

(25) Boger, D. L.; Turnbull, P. *J. Org. Chem.* **1997**, *62*, 5849.

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

Table 1



oxidant	solvent (25 °C)	**24**, %	**25**, %
salcomine/O_2	DMF	40	<5
CAN	1:1 CH_3CN:H_2O	21	55
CAN	1:1 CH_3CN:H_2O (5 °C)	24	60
DDQ	20:1 acetone:H_2O	25	58
$PhSeO_2H$	CH_2Cl_2	<10	86
$PhSeO_2H$	Ac_2O	0	85
$^{\bullet}ON(KSO_3)_2$	1:1 acetone/KH_2PO_4	<10	<5
$^{\bullet}ON(KSO_3)_2$	1:1 CH_2Cl_2/KH_2PO_4	25	12

selectively access the *p*-quinone present in the mitomycins. Recognizing the susceptibility of **18** to both base-catalyzed cyclization and ensuing degradation and over-oxidation to the indole, mild oxidants were examined (Table 1). Bis(salicylidene)-ethyenediiminocobalt (II) (salcomine)[27] provided the most selective oxidation to the *p*-quinone **24** (DMF, 40%) of the reagents examined and only trace amounts of the *o*-quinone **25** could be observed in the NMR of the crude reaction products. To our knowledge such an oxidative demethylation has not been observed previously with the salcomine/O_2 system. In preliminary studies, CAN[28] and DDQ[29] were found to be less successful even though they appeared to be the reagents of choice for selective oxidative demethylation in the synthesis of a number of similar mitosane analogues.[30] With **18**, both oxidants provided the corresponding *o*-quinone **25** as the major product in a 2.4:1 ratio to the desired *p*-quinone. Fremy's salt,[31] well recognized for its mild *p*-selective oxidations, provided the desired quinone **24** as the major product in a 2:1 ratio to the undesired *o*-quinone **25**, but in lower yield (20%) and was unsuccessful under the single phase (acetone−water) conditions typically employed. The mild oxidant $PhSeO_2H$[32] yielded a highly selective (9:1) and complimentary oxidation to the *o*-quinone (86%). When this oxidation was run in Ac_2O, thus forming the mixed selenic-anhydride (a known *ortho*-oxidant), none of the *p*-quinone was formed.

Preliminary indications differentiating between the *o*- and *p*-quinones were provided by the compound's color and their UV and mass spectrum. The *o*-quinone **25** was deep red and the *p*-quinone **24** was bright orange consistent with related observations.[33] Also consistent with this assignment, the *o*-quinone **25** absorbs at a higher wavelength and with a lower extinction coefficient (UV λ_{max} (CH_3OH) 332 nm $\epsilon = 6900$) than the *p*-quinone (UV λ_{max} (CH_3OH) 314 nm $\epsilon = 13600$) as is generally seen

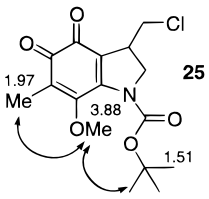


Figure 3.

even with annelated quinones. In addition, the *o*-quinone displayed a pronounced tendency, when compared to the *p*-quinone, to form the $[M + 2]^+$ peak during analysis by mass spectroscopy. Generally, *o*-quinones display key ^{13}C carbonyl resonances at higher fields than *p*-quinones, as well as a characteristic IR carbonyl stretch at a higher wavenumber, but with the case of **24** (^{13}C NMR 180.3 and 179.3 ppm, IR 1715 and 1651 cm^{-1}) and **25** (^{13}C NMR 181.6 and 175.4 ppm, IR 1728 and 1646 cm^{-1}), the key peaks were too close to be considered diagnostic. Conclusive evidence was established by 1D GOESY[34] which for **25** clearly indicated an NOE between the C-7 methoxy and the BOC group at N-1 (Figure 3) which was not observed for the *p*-quinone **24**.

Synthesis of the Duocarmycin-Mitomycin Hybrid Analogues. The hybrid subunit **24** was incorporated in to duocarmycin-like analogues. Exhaustive deprotection of **17** (3.6 M HCl/EtOAc, 30 min) followed by immediate coupling (3 equiv of EDCI,[35] DMF, 25 °C) of the amine hydrochloride salt with 5,6,7-trimethoxyindole-2-carboxylic acid (**26**)[36] produced the *seco* agent **27** (Scheme 5).[37] DBU (1.3 equiv, 25 °C, 30 min, 82%) spirocyclization of **27** afforded the labile **28**, containing the reactive cyclopropane representing a key comparison analogue.

The *seco* agent **27** was treated with $PhSeO_2H$, and the labile *o*-quinone analogue **29** was isolated in 80% yield (Scheme 5). Treatment of **27** with salcomine/O_2 in DMF led to a complex mixture of products, probably arising from competitive oxidation of the trimethoxyindole subunit. Thus, an alternative pathway to the *p*-quinone was developed utilizing **24**. Reduction of **24** (H_2, 10 wt % Pd/C, CH_3OH, 5 min, 100%, Scheme 6) provided the air-stable hydroquinone **30** which was subsequently deprotected (3.6 M HCl/EtOAc, 30 min) and immediately coupled with **26** (1.2 equiv, 3 equiv of EDCI, DMF, 25 °C) to give the suprisingly air-stable hydroquinone **31** in 44% yield. The hydroquinone was then easily oxidized (air, 1.5 wt equiv Pd/C, EtOAc, 5 h, 100%)[38] without competitive oxidation of the indole binding subunit to provide **7**, the duocarmycin-mitomycin hybrid (Scheme 6).

Solvolysis Reactivity and Regioselectivity. Two fundamental characteristics of the alkylation subunits have proven important in past studies.[4] The first is the stereoelectronically-controlled 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

(27) Van Dort, H. M.; Geursen, H. J. *Recl. Trav. Chim. Pays-Bas* **1967**, *86*, 520. Hibino, S.; Weinreb, S. M. *J. Org. Chem.* **1977**, *42*, 332. Yoshida, K.; Nakajima, S.; Ohnuma, T.; Ban, Y.; Shibasaki, M. *J. Org. Chem.* **1988**, *53*, 5355.

(28) CAN = Ceric ammonium nitrate $Ce(NH_4)_2(NO_3)_6$.

(29) DDQ = 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone.

(30) Remers, W. A.; Iyengar, B. S. Synthesis of Mitomycins In *Recent Progress in the Chemical Synthesis of Antibiotics*; Springer-Verlag: Berlin, 1990; 415−445.

(31) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229.

(32) Barton, D. H. R.; Finet, J.-P.; Thomas, M. *Tetrahedron* **1988**, *44*, 6397.

(33) Berger, S.; Rieker, A. In *The Chemistry of the Quinonoid Compounds*; Patai, S., Ed.; Wiley: London, 1974; pp 163−229.

(34) Stonehouse, J.; Adell, P.; Keeler, J.; Shaka, A. J. *J. Am. Chem. Soc.* **1994**, *116*, 6037.

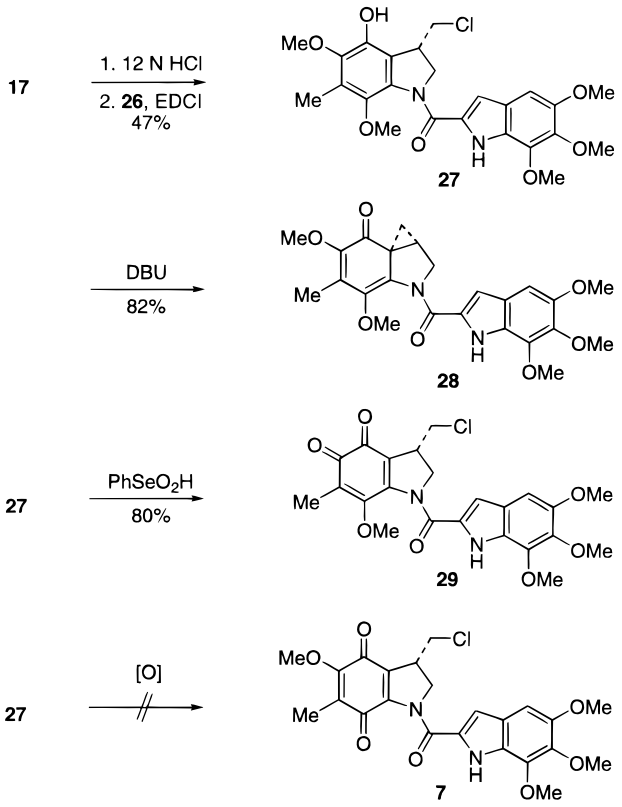
(35) EDCI = 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

(36) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Kitos, P. A.; Suntornwat, O. *J. Org. Chem.* **1990**, *55*, 4499.

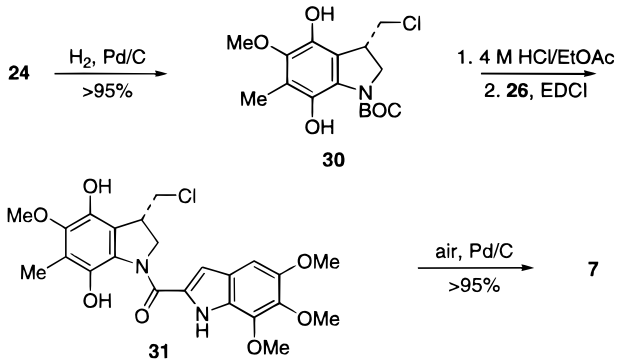
(37) The low yield is believed to arise from the propensity for the *seco* agent to cyclize under the reaction conditions and then quickly decompose during reaction and slow chromatography.

(38) Luly, J. R.; Rapoport, H. *J. Org. Chem.* **1984**, *49*, 1671.

Scheme 5



Scheme 6



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 biological activity.[4] Although *N*-BOC-**19** is highly reactive, it was sufficiently stable to purify and characterize and its solvolysis behavior may be considered representative of that expected of **9**, **28**, and related intermediates. Its solvolysis at pH 3 and pH 7, as well as that for *N*-PIV-**19** and *N*-Me-**19**, were followed spectrophotometrically by UV with the disappearance of the long-wavelength absorption band (Figure 4).

The agents *N*-BOC-**19** ($t_{1/2}$ = 95 s, $k = 7.28 \times 10^{-3}$ s^{-1}), *N*-PIV-**19** ($t_{1/2}$ = 108 s, $k = 6.41 \times 10^{-3}$ s^{-1}), and *N*-Me-**19** ($t_{1/2}$ = 77 s, $k = 9.00 \times 10^{-3}$ s^{-1}) proved to be slightly more stable than *N*-BOC-CI (**35**, $t_{1/2}$ = 35 s, $k = 1.98 \times 10^{-2}$ s^{-1}) and much more reactive than a related isomeric system *N*-BOC-*iso*-CBI (**6**, $t_{1/2}$ = 28 h, $k = 6.98 \times 10^{-6}$ s^{-1}) (Table 2). From past studies,[4] it is known that the lack of a fused aromatic ring and the corresponding increased aromatization energy is the source of the substantial increase in reactivity relative to *iso*-CBI (**6**)

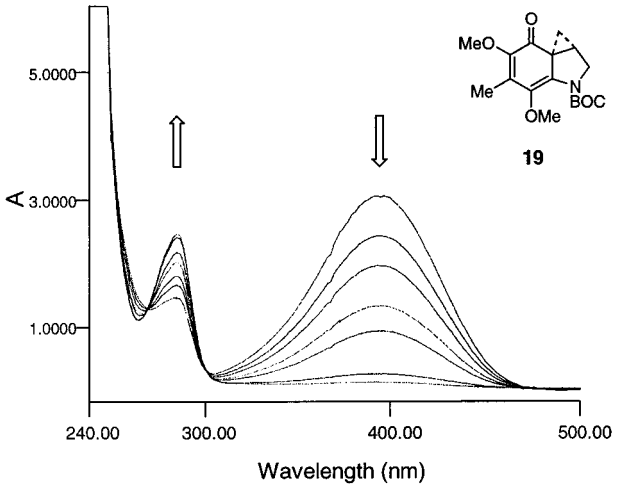
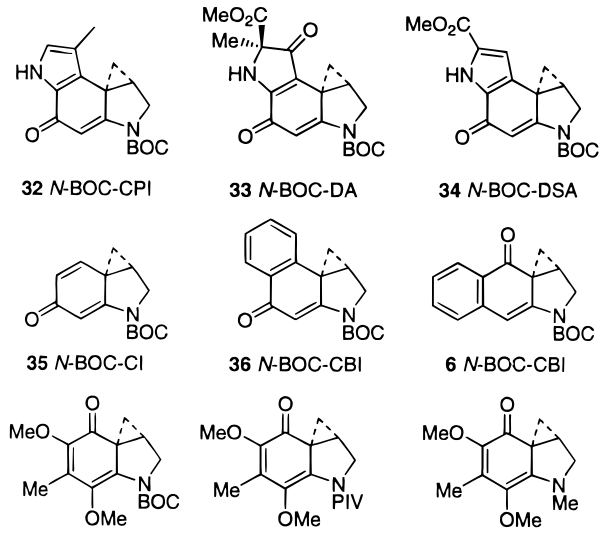


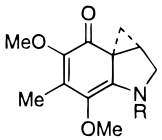
Figure 4.

Table 2. Solvolysis Reactivity and Regioselectivity



Agent	k (s^{-1}, pH 3)	$t_{1/2}$ (h, pH 3)	Regioselectivity
32	5.26×10^{-6}	37 h	4 : 1
33	1.75×10^{-5}	11 h	3 : 2
34	1.08×10^{-6}	177 h	6-4 : 1
35	1.98×10^{-2}	35 s	nd
36	1.44×10^{-6}	133 h	>20 : 1
6	6.98×10^{-6}	28 h	40 : 1
BOC-**19**	7.28×10^{-3}	95 s	>20 : 1
PIV-**19**	6.41×10^{-3}	108 s	nd
Me-**19**	9.00×10^{-3}	77 s	>20 : 1

Agent	k (s^{-1}, pH 7)	$t_{1/2}$ (h, pH 7)	Regioselectivity
BOC-**19**	6.79×10^{-4}	17 min	>20 : 1
PIV-**19**	8.88×10^{-4}	13 min	nd
Me-**19**	4.20×10^{-5}	275 min	>20 : 1

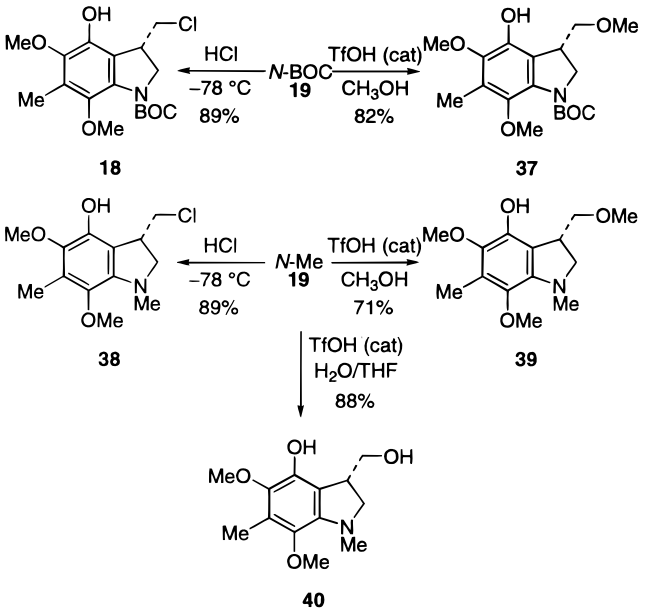


Reactivity:

R = Me > BOC > PIV (pH 3, H^+ catalyzed)
R = Me < BOC < PIV (pH 7, uncatalyzed)

and the increased stability of **19** relative to the analogous CI (3×) was a welcomed property. Interestingly, the order of stability for *N*-BOC-**19** ($t_{1/2}$ = 17 min, $k = 6.79 \times 10^{-4}$

Scheme 7



s^{-1}), *N*-PIV-**19** ($t_{1/2}$ = 13 min, $k = 8.88 \times 10^{-4}$ s^{-1}), and *N*-Me-**19** ($t_{1/2}$ = 275 min, $k = 4.20 \times 10^{-5}$ s^{-1}) was reversed at pH = 7. Recent studies[39] enlisting pH rate profiles have shown that closely related nucleophilic addition reactions of modified alkylation subunits are acid-catalyzed at pH = 3, and it is the uncatalyzed reactions that dominate at pH = 7. In agreement with these studies, *N*-PIV-**19** (108 s) was the most stable derivative at pH = 3 (acid-catalyzed) followed in order by *N*-BOC-**19** (95 s) and *N*-Me-**19** (77 s). Thus, the most easily protonated compound exhibits the greater reactivity at pH 3. At pH = 7, where the uncatalyzed reaction dominates the reaction rate, the order is reversed and *N*-Me-**19** (275 min) is the most stable agent with the greatest degree of vinylogous amide stabilization of the reactive spirocyclopropylcyclohexadienone, followed in order by *N*-BOC-**19** (17 min) and *N*-PIV-**19** (13 min) in which the nitrogen lone pair conjugation is partially and progressively diminished by carbamate and amide conjugation, respectively. This simple observation has very significant ramifications. The preceding studies of the solvolysis pH rate profiles of reactive alkylation subunits did not show a rate dependence on the buffer concentration above pH 6. This indicated that the solvolysis reactions at pH > 6 were not only not specific acid-catalyzed, but that they also were not general acid-catalyzed. Though surprising, these counterintuitive conclusions are confirmed with this reverse order of reactivity at pH 7 which is only consistent with the switch from an acid-catalyzed reaction to one which is now uncatalyzed.

The acid-catalyzed nucleophilic addition of HCl and CH_3OH to the *N*-BOC derivative **19** was conducted on a preparative scale to establish the regioselectivity of addition. Treatment of **19** with HCl (1.2 equiv, −78 °C) resulted in clean addition to provide the *seco*-agent **18** (Scheme 7) as did CH_3OH (cat. TfOH) to give **37**. Additionally, similar observations were made with the *N*-Me-**19** in which chloride (**38**, HCl, 89%), methanol (**39**, cat. TfOH, 71%), and water (**40**, cat. TfOH, 88%) all added to the least-substituted carbon of the cyclopropane under acidic conditions (Scheme 7). Consequently, the nucleophilic additions to *N*-BOC-**19** and *N*-Me-**19** occur with exclusive regioselectivity (>20:1) analogous to *N*-BOC-*iso*-CBI,[9] which are much more selective than the natural alkylation subunits themselves **32**−**34** (6−1.5:1).[4]

DNA Alkylation Selectivity and Efficiency. The DNA alkylation properties of the agents were examined within w794 duplex DNA[40] for which comparative results are available for related duocarmycin-based 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, 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 electrophoesis (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.[40]

A representative comparison of DNA alkylation by the hybrid agents **7**, and **31** alongside that of (+)-duocarmycin SA (**2**) is illustrated below (Figure 5). There are three important conclusions that can be drawn from these comparisons. First, the quinone-based analogue **7**, under nonreductive conditions, did not alkylate DNA even at 10^{-2} M concentration. This is consistent with expectations that this agent, in its oxidized form, cannot undergo in situ spirocyclization to form the reactive cyclopropane and thus alkylate DNA efficiently. Second, the reduced form of **7**, the hydroquinone **31**, alkylates DNA in a sequence-specific manner identical to the alkylation pattern of the natural product (+)-duocarmycin SA and does so with approximately only 100× less efficiency. Duocarmycin SA is the most efficient DNA alkylating agent of the natural products in this class, and despite this 100-fold difference in efficiency, **31** represents a very effective agent. For example, duocarmycin A (**1**) is 20× less efficient than duocarmycin SA (**2**) and only 5× more efficient than **31**. No new sites of DNA alkylation were detected with **31**, and only adenine N3 alkylation was observed under the conditions of limiting agent and excess DNA. Notably, such sequencing studies only detect the high affinity alkylation sites and minor sites of comparable affinity (1−0.01×). Under these conditions, the studies illustrate that despite the similarity of the alkylation subunit to that of the guanine-selective mitomycin A, the binding selectivity of the full structure of **31** including the trimethoxyindole subunit of the hybrid dominates the observed sequence selectivity retaining the adenine alkylation. Last, it was demonstrated that $Na_2S_2O_4$ mediated reduction of the quinone **7** to presumably produce the hydroquinone which alkylated DNA with the same selectivity and near identical efficiency as the synthetic hydroquinone, supporting a reaction cascade in which DNA alkylation is only observed following reduction (see Scheme 2).

Although the data are not shown herein, the *seco*-agent

(39) Boger, D. L.; Garbaccio, R. M. *J. Org. Chem.* **1999**, *64*, 5666.

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

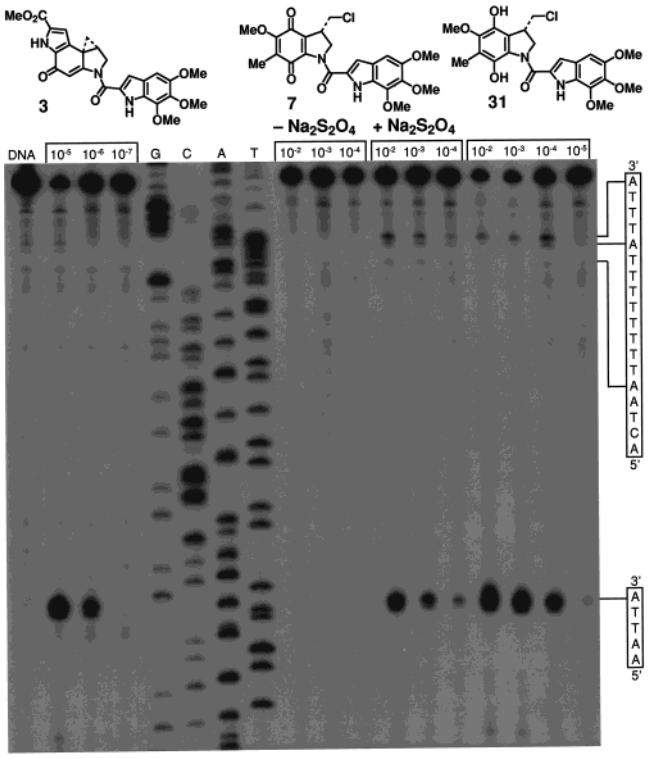


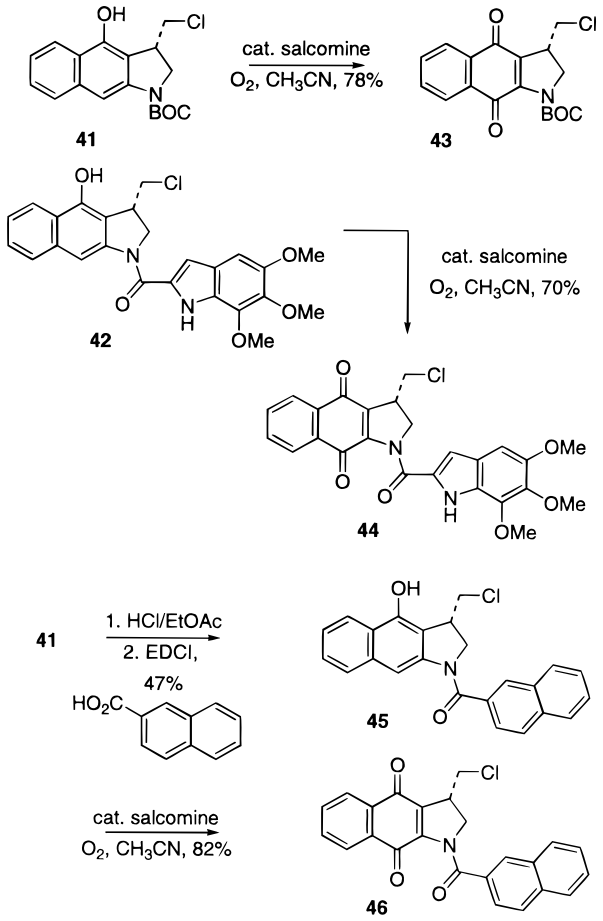
Figure 5. Thermally induced strand cleavage of w794 DNA (SV40 DNA segment, 144 bp, nucleotide nos. 138-5238); DNA–agent incubation for 24 h at 25 °C, removal of unbound agent, and 30 min of thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography; lane 1, control DNA; lanes 2–4, (+)-duocarmycin SA (1×10^{-5} to 1×10^{-7}); lanes 5–8, Sanger G, C, A, and T sequencing reactions; lanes 9–11, **7** (1×10^{-2} to 1×10^{-4}); lanes 12–14, **7** + 1 μL 100 mM $Na_2S_2O_4$ (1×10^{-2} to 1×10^{-4}), lanes 15–18, **31** (1×10^{-2} to 1×10^{-5}).

27 and the corresponding analogue **28** containing the preformed activated cyclopropane also alkylated DNA in a manner exactly analogous to **31** and duocarmycin SA (**2**). Thus, only adenine N3 alkylation was observed, and it occurred with a selectivity that was identical to that of duocarmycin SA. This indicates that a likely intermediate in the alkylation of DNA by the reduced hydroquinone **31** is the cyclopropane-containing intermediate **9**. In addition and although not an objective of the present work, neither **27** nor its cyclopropane-containing analogue **28** can be physically subject to a DNA phosphate backbone protonation of the C6 carbonyl for activation of the DNA alkylation reaction. Thus, such an activation event cannot be contributing to or controlling the sites of DNA alkylation. Finally, both **27** and **28** alkylated DNA with an efficiency that was 10× lower than the hydroquinone **31** and 100–1000× less efficient than duocarmycin SA.

Second Generation Analogues. The remarkable stability of the *iso*-CBI based analogues relative to the *iso*-CI based structures[9] and the corresponding low stability of **19** suggested a benzannulated set of analogues to be examined in parallel. These agents, following bioreduction and cyclization, would be more stable and possibly more potent than **7** or **24**.

Our prior efforts entailing the preparation of *iso*-CBI-TMI had provided the key precursors **41** and **42**.[9] Naphthols **41** and **42** were oxidized to the bright yellow naphthoquinones **43** (78%) and **44** (70%), respectively, by the action of salcomine/O_2 (cat., CH_3CN, Scheme 8).

Scheme 7



In addition to the trimethoxyindole derivative **44**, a naphthalene-linked naphthoquinone **46** was also synthesized. *N*-BOC deprotection of **41** (3.6 M HCl/EtOAc) followed by immediate coupling with 2-naphthalenecarboxylic acid (3 equiv of EDCI, DMF, 25 °C) produced the *seco* agent **45** (47%). Oxidation of **45** (cat. salcomine, O_2, CH_3CN) provided the final naphthoquinone **46** in excellent yield (82%).

Reduction by DT-Diaphorase. To confirm that the agents were substrates for enzymatic reduction, recombinant human DT-Diaphorase[41] (NQO1) was acquired and an established HPLC assay[42] was enlisted to measure the rate of substrate reduction and cofactor oxidation. Mitomycin C (**4**, MMC) and mitomycin A (**5**, MMA) were used as control agents as the former is a poor substrate for DT-diaphorase, and the latter is a good substrate at pH 7.4. The simple BOC-protected quinone **24** was an excellent substrate for DT-diaphorase and was reduced at a greater rate than mitomycin A (Table 3). Nitrogen lone pair conjugation with the BOC carbamate makes **24** easier to reduce than mitomycin A, and this is consistent with the observed rates of metabolism. Naphthoquinone analogues **43** and **46** were also good substrates for DT-diaphorase. Again, a correlation between the expected reduction potential and DT-diaphorase metabolism was observed with **43** and **46** where the amide in **46** is more strongly conjugated with the nitrogen lone pair than the carbamate in **43**. Thus **24**, **43**, and **46**

(41) Beall, H. D.; Mulcahy, R. T.; Siegel, D.; Traver, R. D.; Gibson, N. W.; Ross, D. *Cancer Res.* **1994**, *54*, 3196.
(42) Winski, S. L.; Hargreaves, R. H. J.; Butler, J.; Ross, D. *Clin. Canc. Res.* **1998**, *4*, 3083.

Table 3. Reduction Rates of Quinones by Recombinant NQO1[a]

5, mitomycin A	1.05 ± 0.03
4, mitomycin C	not detected[b]
24	1.14 ± 0.08
43	0.53 ± 0.04
46	0.85 ± 0.05

[a] Metabolism of quinones (50 μM) at 23 °C in pH 7.4 buffer by human recombinant NQO1 (0.1−1.0 μg) was measured as irreversible NADH oxidation (200 μM) by HPLC,[43] rates of reduction are expressed as μmol/min/mg NQO1. [b] MMC does reduce at pH 5.8 (0.15 mmol/min/mg).[40]

Table 4. In Vitro Cytotoxic Activity

		IC_{50} (μM)			
agent	configuration	L1210	H460	H596	selectivity
(−)-**18**	natural	85	70	50	
(+)-**18**	unnatural	85	70	70	
(−)-**30**	natural	9	5	10	
(+)-**30**	unnatural	9	6	10	
(−)-**25**	natural	9	12	15	
(+)-**25**	unnatural	9	10	14	
(−)-**24**	natural	9	4	12	3
(+)-**24**	unnatural	9	2	6	3
(−)-**27**	natural	0.9	3	9	
(+)-**27**	unnatural	>20	>20	>20	
(+)-**31**	natural	0.6	0.07	0.08	
(−)-**31**	unnatural	1	5	9	
(+)-**7**	natural	1	0.8	3	3.8
(−)-**7**	unnatural	8	6	10	1.7
43	racemic	0.3	0.9	14	16
44	racemic	0.1	0.2	1	5
46	racemic	0.01	0.08	2	25
5, MMA	natural	nd	0.01	2	200

were all found to be good substrates for DT-diaphorase with comparable rates of reaction to mitomycin A, confirming their ability to undergo bioreduction in reductase enriched cell lines and consistent with results obtained in the cytotoxic assays below.

In Vitro Cytotoxic Activity. Past studies with agents in this class have defined a direct correlation between solvolysis stability and cytotoxic potency. Consistent with their reactivity, the hybrid agents exhibited cytotoxic activity against L1210 that closely mirrored this relationship even with the deep-seated structural modifications (Table 4). These results, which also parallel trends established in the DNA alkylation studies, demonstrated that the enantiomer with the natural (3*S*) configuration corresponding to that of the natural product, is the more potent enantiomer by about 1−10× for the extended agents **7**, **27**, **31**, and little to no difference in the enantiomers was observed for the simple BOC-substituted agents **18**, **24**, **25**, **30**. The *p*-quinones bearing the mitomycin A substitution (**7** and **24**) exhibited cytotoxic potencies comparable to the corresponding air-stable hydroquinones (**30** and **31**), both of which were typically more potent than the methyl ether precursors (**18** and **27**). Consistent with expectations, the intrinsically more stable naphthoquinone analogues (**43**, **44**, and **46**) were found to be more potent.

A second set of assays were conducted in order to evaluate the efforts to impart tumor cell selectivity on a duocarmycin−mitomycin hybrid. It has been reported that a variety of tumor types have elevated DT-diaphorase (NQO1) activities relative to normal tissue.[43] DT-Diaphorase is a two-electron reductase that uses either NADH or NADPH as a cofactor[44] to catalyze the two-electron reduction of quinones and can protect cells against their toxic effects. Paradoxically, DT-Diaphorase is also involved in the reductive activation of mitomycin C (**4**) and related analogues.[45] Central to the design of the mitomycin−duocarmycin hybrid would be a differential activity against H_{460} and H_{596} nonsmall cell lung cancer (NSCLC) cell lines which display high DT-Diaphorase activity (1360 nmol min^{-1} mg^{-1}) and no DT-Diaphorase activity, respectively.[46] Increased potency in the H_{460} cell line versus the H_{596} cell line would be consistent with their preferential activation by DT-Diaphorase. The results indicate a modest (3−6×) increase in cytotoxic potency for the quinones **7** and **24** against the H_{460} cell line, which has high DT-diaphorase activity, compared to the H_{596} cell line which has no measurable DT-diaphorase activity. In addition, the benzannelated naphthoquinone analogues (**43**, **44**, and **46**) displayed even greater levels of selectivity with **46** exhibiting a 24× increased potency against H_{460} consistent with reductive activation by DT-Diaphorase.

Conclusions. A new class of bioreductively activated DNA alkylating agents were designed and synthesized, incorporating the mitomycin-like quinone structure into the framework of the duocarmycins. The first generation analogues were studied and found to be highly reactive in their cyclopropane form, and a second generation set of analogues were synthesized in parallel to achieve increased potency. Both these sets of analogues were found to alkylate DNA only upon quinone reduction and to do so with the same characteristic sequence selectivity as the duocarmycins and with only 100× less efficiency. Biological activity assays revealed potency of a similar magnitude. A cell-based assay established that these analogues, like the mitomycins, were more active in a DT-Diaphorase-enriched cell line versus a DT-Diaphorase-deficient (resistant) cell line. Further studies confirmed that these analogues are good substrates for human recombinant DT-Diaphorase, establishing the viable potential for these and related agents to be tumor-selective DNA-alkylating agents subject to bioreductive alkylation.

Experimental Section

2,4-Dimethoxy-3-methyl-1-(methoxymethoxy)benzene (11). A solution of 2,4-(dimethoxy)-3-methylphenol (1.0 g, 5.95 mmol) in 60 mL of anhydrous DMF at 0 °C was treated with NaH (357 mg, 9.91 mmol) in several portions over 5 min. After 10 min, Bu_4NI (219 mg, 0.60 mmol) was added followed by the dropwise addition of $ClCH_2OCH_3$ (0.68 mL, 8.91 mmol). The reaction mixture was stirred at 25 °C for 36 h before the reaction was quenched by the slow addition of 30 mL of H_2O. The aqueous layer was extracted with EtOAc. The organic layers were combined, washed with 10% aqueous $NaHCO_3$ and H_2O, dried (Na_2SO_4), and concentrated under

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(44) Ernster, L. *Methods Enzymol.* **1967**, *10*, 309. Ernster, L. *Chim. Scripta* **1987**, *27A*, 1.

(45) Beall, H. D.; Hudnott, A. R.; Winski, S.; Siegel, D.; Swann, E.; Ross, D.; Moody, C. J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 545. Beall, H. D.; Mulcahy, T.; Siegel, D.; Traver, R. D.; Gibson, N. W.; Ross, D. *Cancer Res.* **1994**, *54*, 3196. Ross, D.; Siegel, D.; Beall, H.; Prakash, A. S.; Mulcahy, R. T.; Gibson, N. W. *Cancer Metastasis Rev.* **1993**, *12*, 83. Ross, D.; Beall, H.; Traver, R. D.; Siegel, D.; Phillips, R. M.; Bibson, N. W. *Oncol. Res.* **1994**, *6*, 493.

(46) Traver, R. D.; Siegel, D.; Beall, H. D.; Phillips, R. M.; Gibson, N. W.; Franklin, W. A.; Ross, D. *Br. J. Cancer* **1997**, *75*, 69.

reduced pressure. Flash chromatography (SiO_2, 3 × 10 cm, 10% EtOAc/hexane) provided **11** (1.11 g, 88%) as a light yellow oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 6.92 (d, J = 8.8 Hz, 1H), 6.51 (d, J = 8.8 Hz, 1H), 5.13 (s, 2H), 3.79 (s, 3H), 3.76 (s, 3H), 3.50 (s, 3H), 2.13 (s, 3H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 153.5, 149.2, 144.3, 121.0, 114.4, 105.3, 96.0, 60.4, 56.0, 55.7, 8.9; IR (film) ν_{max} 2937, 2833, 1595, 1487, 1440, 1420 cm^{-1}; FABHRMS (NBA) m/z 212.1040 ($C_{11}H_{16}O_4$ requires 212.1049).

2,4-Dimethoxy-3-methyl-5-nitro-1-(methoxymethoxy)benzene (12). A solution of **11** (1.11 g, 5.21 mmol) in 18 mL of freshly distilled Ac_2O at 0 °C was treated with $Cu(NO_3)_2 \cdot 2.5H_2O$ (2.41 g, 10.4 mmol) in several portions over 5 min. The reaction mixture was stirred for 2 h at 0 °C, and 1 h at 25 °C before the reaction was poured over H_2O and extracted with EtOAc. The combined organic layers were washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated under reduced pressure. The crude light yellow oil (1.18 g, 88%) was carried on to the next transformation: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.54 (s, 1H), 5.18 (s, 2H), 3.87 (s, 3H), 3.81 (s, 3H), 3.48 (s, 3H), 2.21 (s, 3H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 153.0, 147.8, 145.8, 138.9, 128.2, 110.5, 95.3, 61.8, 60.5, 56.2, 9.5; IR (film) ν_{max} 2942, 2829, 1522, 1481 cm^{-1}; FABHRMS (NBA) m/z 258.0977 ($C_{11}H_{15}NO_6 + H^+$ requires 258.0978).

5-Amino-2,4-dimethoxy-3-methyl-1-(methoxymethoxy)benzene (13). A solution of **12** (1.18 g, 4.57 mmol) in 90 mL of moist Et_2O (8:2:1 Et_2O:EtOH:H_2O) was cooled to 0 °C, and treated with freshly prepared Al−Hg[19] (1.23 g Al, 45.7 mmol) which had been prepared from small 1 × 1 cm pieces of Al. The reaction mixture was stirred vigorously for 0.5 h at 0 °C and 1 h at 25 °C. The reaction mixture was filtered through Celite, and the Celite was washed thoroughly with Et_2O. The solution was washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated under reduced pressure to afford **13** (0.88 g, 85%) as a crude brown oil, which was immediately carried on to the next step: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 6.42 (s, 1H), 5.11 (s, 2H), 3.70 (s, 3H), 3.66 (s, 3H), 3.56 (m, 2H), 3.47 (s, 3H), 2.16 (s, 3H); ^{13}C NMR ($CDCl_3$, 250 MHz) δ 147.0, 140.4, 140.3, 135.8, 125.4, 102.0, 95.4, 60.6, 59.4, 56.0, 9.34; IR (film) ν_{max} 3446, 3359, 2935, 2826, 1617, 1492 cm^{-1}; ESIMS m/z 228 ($C_{11}H_{17}NO_4 + H^+$ requires 228).

[*N*-(*tert*-Butyloxycarbonyl)amino]-2,4-dimethoxy-5-(methoxymethoxy)-3-methylbenzene (14). A solution of crude **13** (0.88 g, 3.85 mmol) in 40 mL of anhydrous THF was treated with BOC_2O (1.73 g, 7.72 mmol), and the reaction mixture was warmed at reflux (65 °C) for 18 h. The solvents were removed under reduced pressure, and flash chromatography (SiO_2, 3 × 10 cm, 10% EtOAc/hexane) provided pure **14** as a yellow oil (0.96 g, 76%): ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.72 (br s, 1H), 6.86 (br s, 1H), 5.18 (s, 2H), 3.75 (s, 3H), 3.67 (s, 3H), 3.51 (s, 3H), 2.19 (s, 3H), 1.50 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.7, 146.4, 143.6, 141.6, 127.8, 124.8, 105.3, 95.5, 80.4, 60.5, 60.4, 56.4, 28.3, 9.5; IR (film) ν_{max} 3437, 3341, 2977, 2935, 1731, 1519, 1454 cm^{-1}; FABHRMS (NBA/CsI) m/z 460.0723 ($C_{16}H_{25}NO_6 + Cs^+$ requires 460.0736).

[*N*-(*tert*-Butyloxycarbonyl)amino]-2,4-dimethoxy-6-iodo-5-(methoxymethoxy)-3-methylbenzene (15). A solution of **14** (0.55 g, 1.67 mmol) in 6.6 mL of anhydrous THF was cooled to −25 °C and treated with TMEDA (0.94 mL, 6.18 mmol) followed by *n*-BuLi (2.5 mL of a 2.5 M solution in hexane, 6.18 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 (0.45 mL, 6.18 mmol) and stirred for 15 min at 25 °C. The reaction was diluted with H_2O and extracted with Et_2O, 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 × 10 cm, 20% EtOAc/hexane) yielded **15** (560 mg, 74%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.99 (br s, 1H), 5.10 (s, 2H), 3.75 (s, 3H), 3.69 (s, 3H), 3.65 (s, 3H), 2.17 (s, 3H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.6, 151.8, 150.5, 146.9, 129.3, 126.7, 99.1, 95.8, 80.6, 60.5, 60.3, 58.5, 28.3, 9.8; IR (film) ν_{max} 3321, 2975, 2936, 1722, 1485, 1455 cm^{-1}; FABHRMS (NBA/CsI) m/z 585.9688 ($C_{16}H_{24}INO_6 + Cs^+$ requires 585.9703).

[*N*-(*tert*-Butyloxycarbonyl)-*N*-(3-chloro-2-propen-1-yl)amino]-2,4-dimethoxy-6-iodo-5-(methoxymethoxy)-3-methylbenzene (16). A solution of **15** (0.610 g, 1.34 mmol) in 13.4 mL of anhydrous DMF was cooled to 0 °C and treated with NaH (60% dispersion in oil, 121 mg, 4.03 mmol) in small portions. The resulting suspension was stirred for 15 min and treated with neat 1,3-dichloropropene (0.52 mL, 5.5 mmol) dropwise, followed by catalytic *n*-Bu_4NI (50.0 mg, 0.13 mmol). The reaction mixture was warmed to 25 °C and stirred for 3 h. The reaction mixture was quenched with the addition of saturated aqueous $NaHCO_3$, and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. Flash chromatography (SiO_2, 3 × 10 cm, 0−20% EtOAc/hexane gradient) yielded **16** (0.681 g, 96%) as a colorless oil as a mixture of rotamers: ^{1}H NMR ($CDCl_3$, 400 MHz) 2:1 rotamers δ 6.15−6.03 (m, 1H), 6.00−5.90 (m, 1H), 5.11−5.03 (m, 2H), 4.17−3.87 (m, 2H), 3.77 and 3.74 (s, 3H), 3.65 and 3.63 (s, 3H), 3.627 and 3.622 (s, 3H), 2.14 and 2.13 (s, 3H), 1.50 and 1.34 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) rotamers δ 153.65 and 153.62, 152.8 and 152.1, 151.0 and 150.7, 147.0 and 146.7, 134.5 and 134.0, 129.5 and 129.0, 127.0 and 126.7, 121.0 and 120.6, 99.0, 97.7 and 97.3, 80.8 and 80.6, 60.5 and 60.4, 60.3 and 60.2, 58.5 and 58.4, 50.4, 48.8, 28.3, and 28.2, 9.9; IR (film) ν_{max} 2973, 2936, 1704, 1456 cm^{-1}; FABHRMS (NBA/CsI) m/z 659.9655 ($C_{19}H_{27}ClINO_6 + Cs^+$ requires 659.9626).

1-(*tert*-Butyloxycarbonyl)-3-(chloromethyl)-5,7-dimethoxy-4-(methoxymethoxy)-6-methyl-2,3-dihydroindole (17). A solution of **16** (580 mg, 1.09 mmol) in 10.9 mL of anhydrous benzene was treated with AIBN (17.0 mg, 0.11 mmol) and Bu_3SnH (311 μL, 1.15 mmol) and warmed at 75 °C for 2 h. The reaction mixture was concentrated under reduced pressure. Flash chromatography (SiO_2, 2.5 × 15 cm, 0−20% EtOAc/hexane gradient) yielded **17** (430 mg, 96%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.10 (d, J = 5.9 Hz, 1H), 5.04 (d, J = 5.9 Hz, 1H), 4.22 (dd, J = 2.6, 11.8 Hz, 1H), 4.02−3.89 (m, 2H), 3.68 (s, 3H), 3.62 (s, 3H), 3.53 (s, 3H), 3.51 (m, 1H), 3.42 (dd, J = 9.8, 18.8 Hz, 1H), 2.12 (s, 3H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.6, 147.8, 144.9, 142.3, 130.1, 126.3, 125.0, 99.1, 81.0, 60.2, 58.3, 57.2, 54.4, 44.9, 42.7, 28.1, 9.8; IR (film) ν_{max} 2935, 1703, 1471, 1417 cm^{-1}; FABHRMS (NBA/NaI) m/z 424.1490 ($C_{19}H_{28}ClNO_6 + Na^+$ requires 424.1503).

Resolution of 17. A sample of **17** (200 mg) in 1.0 mL of 1% *i*-PrOH/hexane was resolved on a semipreparative

Daicel Chiralcel OD column (10 mm, 2 × 25 cm, 7.0 mL/min flow rate, 1% *i*-PrOH/hexane). The effluent was monitored at 254 nm, and the enantiomers were eluted with retention times of 18.0 (*R*) and 31.0 (*S*) min ($\alpha = 1.63$). Both enantiomers were found to be >99% enantiomerically pure by analytical HPLC. The fractions containing the separated enantiomers were collected and concentrated to afford (+) and (−)-**17**. (−)-(3*R*)-**17**: $[\alpha]^{25}_D$ −13.9 (*c* 1.0, CH_2Cl_2); (+)-(3*S*)-**17**: $[\alpha]^{25}_D$ +13.1 (*c* 1.0, CH_2Cl_2).

1-(*tert*-Butyloxycarbonyl)-3-(chloromethyl)-5,7-dimethoxy-4-hydroxy-6-methyl-2,3-dihydroindole (18). A solution of **17** (88.0 mg, 0.22 mmol) in 11.0 mL 1:1 *i*-PrOH/THF was treated with 12 N HCl (0.13 mL, 1.53 mmol), and the mixture was stirred for 44 h at 25 °C before the volatiles were removed in vacuo. Flash chromatography (SiO_2, 1.5 × 15 cm, 0−20% EtOAc/hexane gradient) provided **18** (62 mg, 82%) as a white solid: mp 95−97 °C (white needles, EtOAc); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 5.49 (s, 1H), 4.15 (dd, J = 3.9, 11.8 Hz, 1H), 4.05 (dd, J = 7.7, 11.8 Hz, 1H), 3.93 (dd, J = 3.3, 10.7 Hz, 1H), 3.71 (s, 3H), 3.61 (s, 3H), 3.59 (m, 1H), 3.48 (dd, J = 9.4, 10.7 Hz, 1H), 2.19 (s, 3H), 1.50 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.5, 142.4, 141.6, 141.0, 131.0, 124.8, 117.0, 80.1, 61.0, 58.6, 54.7, 44.8, 42.0, 28.1, 10.0; IR (film) ν_{max} 3386, 2978, 2937, 1699, 1476 cm^{-1}; FABHRMS (NBA/NaI) *m*/*z* 357.1334 ($C_{17}H_{24}ClNO_5$ requires 357.1343); (+)-(3*R*)-**18**: $[\alpha]^{25}_D$ +7.1 (*c* 1.0, CH_2Cl_2); (−)-(3*S*)-**18**: $[\alpha]^{25}_D$ −7.2 (*c* 1.0, CH_2Cl_2).

***N*-(*tert*-Butyloxycarbonyl)-3,5-dimethoxy-4-methyl-1,2,7,7a-tetrahydrocycloprop [1,2-*c*]indol-6-one (19).** A solution of **18** (3.1 mg, 8.30 μmol) in 0.5 mL of CH_3CN was treated with DBU (1.6 μL, 10.9 μmol) and stirred for 20 min at 25 °C. The reaction solution was loaded onto a 1% Et_3N/hexane-slurried silica gel column, and quick flash chromatography (SiO_2, 1.5 × 10 cm, 50% EtOAc/hexane) afforded **19** (2.5 mg, 93%) as a golden oil: ^{1}H NMR (C_6D_6, 400 MHz) δ 3.73 (s, 3H), 3.71 (d, J = 11.4 Hz, 1H), 3.26 (s, 3H), 3.06 (dd, J = 4.5, 11.4 Hz, 1H), 2.07 (s, 3H), 2.05 (m, 1H), 1.69 (dd, J = 3.2, 7.5 Hz, 1H), 1.42 (s, 9H), 0.81 (dd, J = 3.2, 5.8 Hz, 1H); IR (film) ν_{max} 2978, 2933, 1699, 1633 cm^{-1}; FABHRMS (NBA/CsI) *m*/*z* 454.0645 ($C_{17}H_{23}NO_5$ + Cs^+ requires 454.0631).

3-(Chloromethyl)-5,7-dimethoxy-4-hydroxy-6-methyl-1-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-2,3-dihydroindole (27). A sample of **17** (17.4 mg, 0.038 mmol) was dissolved in 3.6 N HCl/EtOAc (3.8 mL) and stirred for 30 min at 25 °C. The solvents were removed by a stream of N_2, and the residual salt was thoroughly dried on the pump. The salt was then dissolved in DMF (1.5 mL) and treated with **26**[29] (11.5 mg, 0.045 mmol) and EDCI (21.5 mg, 0.11 mmol). The resulting mixture was stirred at 25 °C for 3 h under Ar. The reaction was then diluted with H_2O and extracted with EtOAc. The collected organic layer was dried (Na_2SO_4) and condensed under reduced pressure to yield crude **27**. Flash chromatography (40−60% EtOAc/hexanes gradient) yielded pure **27** (9.8 mg, 47%) as a white film: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.15 (s, 1H), 6.90 (d, J = 2.4 Hz, 1H), 6.82 (s, 1H), 5.52 (s, 1H), 4.53 (m, 2H), 4.06 (s, 3H), 3.97 (dd, J = 3.3, 11.0 Hz, 1H), 3.92 (s, 3H), 3.89 (s, 3H), 3.76 (s, 3H), 3.73 (m, 1H), 3.58 (s, 3H), 3.50 (dd, J = 9.8, 11.0 Hz, 1H), 2.24 (s, 3H); IR (film) ν_{max} 3296, 2937, 1634, 1462 cm^{-1}; FABHRMS (NBA/CsI) *m*/*z* 491.1601 ($C_{24}H_{27}N_2O_7Cl$ + H^+ requires 491.1585). (+)-(3*R*)-**27**: $[\alpha]^{25}_D$ +10.0 (*c* 0.12, CH_2Cl_2); (−)-(3*S*)-**27**: $[\alpha]^{25}_D$ −10.7 (*c* 0.075, CH_2Cl_2).

3,5-Dimethoxy-4-methyl-1-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2,7,7a-tetrahydrocycloprop[1,2-*c*]-indol-6-one (28). A solution of **27** (1.0 mg, 2.0 μmol) in 0.5 mL of CH_3CN was treated with DBU (0.5 μL, 4.0 μmol) and stirred for 20 min at 25 °C. The reaction solution was then loaded onto a 1% Et_3N/EtOAc-slurried silica gel column, and quick flash chromatography (SiO_2, 1.5 × 3 cm, EtOAc) afforded **28** (0.76 mg, 82%) as a golden oil: ^{1}H NMR (C_6D_6, 400 MHz) δ 9.20 (s, 1H), 6.81 (s, 1H), 6.64 (s, 1H), 3.81 (s, 3H), 3.80 (m, 1H), 3.75 (s, 3H), 3.66 (s, 3H), 3.44 (s, 3H), 3.33 (m, 1H), 3.18 (s, 3H), 2.13 (m, 1H), 1.81 (dd, J = 3.5, 7.7 Hz, 1H), 1.63 (s, 3H), 0.97 (dd, J = 3.5, 5.8 Hz, 1H); IR (film) ν_{max} 3291, 2931, 1623, 1464 cm^{-1}; FABHRMS (NBA/NaI) *m*/*z* 455.1711 ($C_{24}H_{26}N_2O_7$ + H^+ requires 455.1740).

1-(*tert*-Butyloxycarbonyl)-3-(chloromethyl)-5-methoxy-6-methyl-2,3-dihydroindole-4,7-dione (24). A solution of **17** (48.0 mg, 0.13 mmol) in 6.7 mL of anhydrous DMF was treated with salcomine (22.0 mg, 67.0 μmol), and O_2 was bubbled through the dark brown solution for 20 h at 25 °C. The reaction mixture was filtered through Celite, and the Celite was washed thoroughly with EtOAc. The filtrate was washed with H_2O and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated under reduced pressure. Flash chromatography (SiO_2, 1.5 × 10 cm, 10% EtOAc/hexane) provided **24** (18.0 mg, 40%) as a bright orange oil: ^{1}H NMR (C_6D_6, 400 MHz) δ 3.75 (dd, J = 6.0, 11.9 Hz, 1H, C*H*HCl), 3.62 (s, 3H, OCH_3), 3.56 (dd, J = 10.6, 11.9 Hz, 1H, CH*H*Cl), 3.36 (dd, J = 6.0, 11.1 Hz, 1H, C*H*HN), 3.16 (dd, J = 3.1, 11.1 Hz, 1H, CH*H*N), 2.81 (m, 1H, C3−H), 1.84 (s, 3H, C6−CH_3), 1.44 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.3 (C-4), 179.3 (C-7), 155.0 (C-5), 151.9 (*C*O_2*t*-Bu), 148.7 (C-3b), 127.5 (C-6), 125.1 (C-3a), 82.2 (*C*$(CH_3)_3$), 60.5 (OCH_3), 53.8 (CH_2Cl), 45.2 (C2), 40.5 (C3), 27.8 (C(*C*H_3)$_3$), 9.0 (C6−CH_3); IR (film) ν_{max} 2931, 1716, 1651, 1590 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 313 (13600), 215 (37400) nm; FABHRMS (NBA/NaI) *m*/*z* 342.1121 ($C_{16}H_{20}NO_5Cl$ + H^+ requires 342.1108); (+)-(3*R*)-**24**: $[\alpha]^{25}_D$ +29 (*c* 0.45, CH_2Cl_2); (−)-(3*S*)-**24**: $[\alpha]^{25}_D$ −30 (*c* 0.42, CH_2Cl_2).

1-(*tert*-Butyloxycarbonyl)-3-(chloromethyl)-7-methoxy-6-methyl-2,3-dihydroindole-4,5-dione (25). A solution of **17** (6.5 mg, 18.2 μmol) in 1.1 mL of CH_2Cl_2 was treated with $PhSeO_2H$ (4.1 mg, 21.8 μmol) in 0.9 mL of CH_2Cl_2, and the resulting deep red solution was stirred for 20 min at 25 °C. The reaction mixture was quenched with the addition of saturated aqueous $NaHCO_3$, and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried (Na_2SO_4), and concentrated under reduced pressure. Flash chromatography (SiO_2, 1.5 × 5 cm, 40% EtOAc/hexane) yielded **25** (5.4 mg, 87%) as a red/purple oil: ^{1}H NMR (C_6D_6, 400 MHz) δ 3.69 (dd, J = 5.0, 11.8 Hz, 1H, C*H*HCl), 3.48 (dd, J = 10.0, 11.8 Hz, 1H, CH*H*Cl), 3.44 (dd, J = 5.8, 11.1 Hz, 1H, C*H*HN), 3.23 (s, 3H, OCH_3), 3.22 (dd, J = 3.1, 11.1 Hz, 1H, CH*H*N), 2.84 (m, 1H, C3−H), 1.70 (s, 3H, C6−CH_3), 1.33 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.6 (C-5), 175.4 (C-4), 157.7 (C-7), 153.0 (C-3b), 152.3 (*C*O_2*t*-Bu), 123.6 (C-6), 120.6 (C-3a), 82.4 (*C*$(CH_3)_3$), 60.3 (OCH_3), 54.5 (CH_2Cl), 45.2 (C2), 40.5 (C3), 27.7 (C(*C*H_3)$_3$), 9.8 (C6−CH_3); IR (film) ν_{max} 2979, 2930, 1728, 1646, 1575, 1445 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 332 (6900), 223 (10200) nm; ESIMS (NBA/NaI) *m*/*z* 342 ($C_{16}H_{20}NO_5Cl$ + H^+ requires 342).

1-(*tert*-Butyloxycarbonyl)-3-(chloromethyl)-4,7-dihydroxy-5-methoxy-6-methyl-2,3-dihydroindole (30). A solution of **24** (10.0 mg, 32.2 μmol) in 1.5 mL anhydrous

CH_3OH was treated with Pd/C (1.0 mg, 10 wt %) and stirred under H_2 for 10 min at 25 °C. The reaction mixture was filtered through Celite, and the Celite was washed thoroughly with CH_3OH. The combined organic layers were then washed with H_2O and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated under reduced pressure to yield pure **30** as a clear oil: ^{1}H NMR (C_6D_6, 400 MHz) δ 10.63 (s, 1H), 5.23 (s, 1H), 3.98 (m, 3H), 3.71 (s, 3H), 3.68 (m, 1H), 3.49 (dd, J = 9.5, 10.7 Hz, 1H), 2.16 (s, 3H), 1.52 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.8, 142.9, 137.9, 136.6, 124.5, 120.3, 114.8, 83.0, 61.1, 53.4, 45.6, 40.7, 28.1, 10.0; IR (film) ν_{max} 3422, 2980, 1667, 1471 cm^{-1}; FABHRMS (NBA/NaI) m/z 366.1078 ($C_{16}H_{22}NO_5Cl + Na^+$ requires 366.1084); (+)-(3*R*)-**30**: $[\alpha]^{25}_D$ +6.5 (*c* 0.40, CH_2Cl_2); (−)-(3*S*)-**30**: $[\alpha]^{25}_D$ −6.1 (*c* 0.38, CH_2Cl_2).

3-(Chloromethyl)-4,7-dihydroxy-5-methoxy-6-methyl-1-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-2,3-dihydroindole (31). A sample of **30** (6.9 mg, 20.0 μmol) was dissolved in 3.6 N HCl/EtOAc (1.5 mL), and the solution was stirred for 30 min at 25 °C. The solvents were removed under reduced pressure, and the residual salt was thoroughly dried under high vacuum. The salt was dissolved in anhydrous DMF (1.0 mL) and treated with **26**[29] (5.6 mg, 22.0 μmol) and EDCI (11.4 mg, 60.0 μmol). The resulting solution was stirred at 25 °C for 3 h under Ar. The reaction mixture was then diluted with H_2O, and extracted with EtOAc. The combined organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Flash chromatography (SiO_2, 1.5 × 10 cm, 0−50% EtOAc/hexane gradient) yielded **31** (4.2 mg, 44%) as a yellow oil: ^{1}H NMR (C_6D_6, 400 MHz) δ 10.87 (s, 1H), 9.32 (br s, 1H), 6.63 (d, J = 2.4 Hz, 1H), 6.62 (s, 1H), 5.06 (s, 1H), 4.23 (m, 1H), 3.86 (m, 2H), 3.79 (s, 3H), 3.69 (s, 3H), 3.53 (m, 1H), 3.51 (s, 3H), 3.21 (s, 3H), 3.18 (m, 1H), 2.41 (s, 3H); ^{13}C NMR (C_6D_6, 125 MHz) δ 160.5, 151.2, 145.1, 139.7, 139.5, 138.3, 129.0, 126.6, 126.4, 123.9, 121.6, 115.6, 108.2, 98.2, 61.1, 60.7, 60.5, 56.1, 56.0, 45.3, 42.2, 10.7; IR (film) ν_{max} 3425, 2939, 1574, 1432 cm^{-1}; FABHRMS (NBA/NaI) m/z 476.1338 ($C_{23}H_{25}N_2O_7Cl$ requires 476.1350); (−)-(3*R*)-**31**: $[\alpha]^{25}_D$ −30 (*c* 0.21, CH_2Cl_2); (+)-(3*S*)-**31**: $[\alpha]^{25}_D$ +31 (*c* 0.13, CH_2Cl_2).

3-(Chloromethyl)-5-methoxy-6-methyl-1-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-2,3-dihydroindol-4,7-dione (7). A solution of **31** (3.0 mg, 6.2 μmol) in 1.5 mL of anhydrous EtOAc was treated with Pd/C (4.5 mg, 150 wt %) and stirred under air for 6 h at 25 °C. The reaction mixture was filtered through Celite, and the Celite was washed thoroughly with EtOAc. The solution was concentrated under reduced pressure to yield pure **7** (2.9 mg, 97%) as an orange oil: ^{1}H NMR (C_6D_6, 400 MHz) δ 9.10 (s, 1H), 6.69 (d, J = 2.3 Hz, 1H), 6.61 (s, 1H), 3.91 (dd, J = 5.4, 11.2 Hz, 1H), 3.76 (m, 1H), 3.76 (s, 3H), 3.66 (s, 3H), 3.63 (s, 3H), 3.49 (dd, J = 6.2, 11.2 Hz, 1H), 3.47 (s, 3H), 3.27 (dd, J = 3.2, 11.2 Hz, 1H), 3.01 (m, 1H), 1.80 (s, 3H); ^{13}C NMR (C_6D_6, 100 MHz) δ 180.4, 179.4, 162.3, 155.5, 151.3, 150.1, 141.7, 139.6, 130.0, 127.9, 127.2, 126.3, 123.5, 108.7, 98.1, 61.1, 60.65, 60.64, 56.3, 55.9, 45.1, 42.0, 9.0; IR (film) ν_{max} 3298, 2938, 1651, 1586 cm^{-1}; FABHRMS (NBA/NaI) m/z 607.0227 ($C_{23}H_{23}N_2O_7Cl + Cs^+$ requires 607.0248); (−)-(3*R*)-**7**: $[\alpha]^{25}_D$ −81 (*c* 0.15, CH_2Cl_2); (+)-(3*S*)-**7**: $[\alpha]^{25}_D$ +80 (*c* 0.10, CH_2Cl_2).

1-(*tert*-Butyloxycarbonyl)-3-(chloromethyl)-2,3-dihydro-1*H*-benzo[*f*]indole-4,9-dione (43). A solution of **41** (8.0 mg, 26.6 μmol) in 1.5 mL of anhydrous CH_3CN was treated with salcomine (catalytic), and O_2 was bubbled through the solution for 1 h at 25 °C. The reaction mixture was filtered through Celite, and the Celite was washed thoroughly with EtOAc. The solution was then concentrated under reduced pressure and subjected directly to flash chromatography (SiO_2, 1.5 × 5 cm, 20% EtOAc/hexane) to provide **43** (7.2 mg, 78%) as a bright yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99 (m, 2H), 7.67 (m, 2H), 4.15 (dd, J = 10.5, 11.9 Hz, 1H), 4.06 (dd, J = 5.7, 11.9 Hz, 1H), 3.87 (m, 2H), 3.81 (m, 1H), 1.51 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.2, 177.2, 151.5, 150.1, 133.6, 133.4, 133.3, 132.2, 130.0, 126.7, 125.7, 83.0, 53.3, 45.3, 41.1, 27.9; IR (film) ν_{max} 2978, 2929, 1721, 1644, 1597, 1403 cm^{-1}; FABHRMS (NBA/NaI) m/z 370.0820 ($C_{18}H_{18}NO_4Cl + Na^+$ requires 370.0822).

3-(Chloromethyl)-1-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*f*]indole-4,9-dione (44). A solution of **42** (2.5 mg, 5.34 μmol) in 1.0 mL of anhydrous CH_3CN was treated with salcomine (catalytic), and O_2 was bubbled through the solution for 1 h at 25 °C. The reaction mixture was filtered through Celite, and the Celite was washed thoroughly with EtOAc. The solution was then concentrated under reduced pressure and subjected HPLC purification. Semipreparative reverse-phase HPLC (Waters Bondapak C-18 column, 300 Å, 25 × 100 mm, gradient 10−90% CH_3CN/H_2O over 30 min, 10 mL/min) afforded **44** (t_R = 25 min, 1.8 mg, 70%) as a bright yellow oil: ^{1}H NMR (C_6D_6, 400 MHz) δ 9.12 (s, 1H), 8.01 (d, J = 7.3 Hz, 1H), 7.78 (d, J = 7.3 Hz, 1H), 6.97 (m, 1H), 6.88 (m, 1H), 6.71 (d, J = 2.1 Hz, 1H), 6.55 (s, 1H), 3.93 (dd, J = 5.4, 11.0 Hz, 1H), 3.78 (m, 1H), 3.76 (s, 3H), 3.63 (s, 3H), 3.57 (dd, J = 6.2, 11.0 Hz, 1H), 3.44 (s, 3H), 3.38 (dd, J = 3.2, 11.4 Hz, 1H), 3.15 (m, 1H); IR (film) ν_{max} 3320, 2926, 1682, 1644, 1596, 1463 cm^{-1}; FABHRMS (NBA/NaI) m/z 481.1153 ($C_{25}H_{21}N_2O_6Cl + H^+$ requires 481.1166).

3-(Chloromethyl)-4-hydroxy-1-[(naphth-2-yl)carbonyl]-2,3-dihyrdo-1*H*-benzo[*f*] indole (45). A sample of **41** (11.8 mg, 31.2 μmol) was dissolved in 3.6 N HCl/EtOAc (2.0 mL), and the solution was stirred for 30 min at 25 °C. The solvents were removed under reduced pressure, and the residual salt was thoroughly dried under high vacuum. The salt was dissolved in anhydrous DMF (1.0 mL) and treated with naphthalene-2-carboxylic acid (6.4 mg, 37.0 μmol) and EDCI (17.8 mg, 93.0 μmol). The resulting solution was stirred at 25 °C for 3 h under Ar. The reaction mixture was then diluted with H_2O and extracted with EtOAc. The combined organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Flash chromatography (SiO_2, 1.5 × 10 cm, 0−30% EtOAc/hexane gradient) yielded **45** (5.7 mg, 47%) as a colorless film: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.88 (s, 1H), 8.20 (m, 2H), 8.03 (m, 4H), 7.74 (dd, J = 1.6, 8.4 Hz, 1H), 7.70 (br s, 1H), 7.63 (m, 2H), 7.43 (m, 1H), 7.37 (m, 1H), 4.39 (dd, J = 9.2, 11.4 Hz, 1H), 4.19 (m, 1H), 4.08−4.01 (m, 2H), 3.85 (dd, J = 8.9, 10.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.9, 150.3, 143.3, 137.0, 136.3, 135.4, 132.2, 130.6, 130.0, 129.7, 129.2, 129.1, 128.7, 128.3, 127.8, 126.8, 125.7, 125.0, 122.6, 116.2, 56.0, 46.9, 41.6; IR (film) ν_{max} 2923, 1688, 1598, 1468 cm^{-1}; FABHRMS (NBA/NaI) m/z 388.1097 ($C_{24}H_{18}NO_2Cl + H^+$ requires 388.1104).

3-(Chloromethyl)-1-[(naphth-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*f*]indole-4,9-dione (46). A solution of **45** (2.0 mg, 5.17 μmol) in 1.0 mL of anhydrous CH_3CN was treated with salcomine (catalytic), and O_2 was

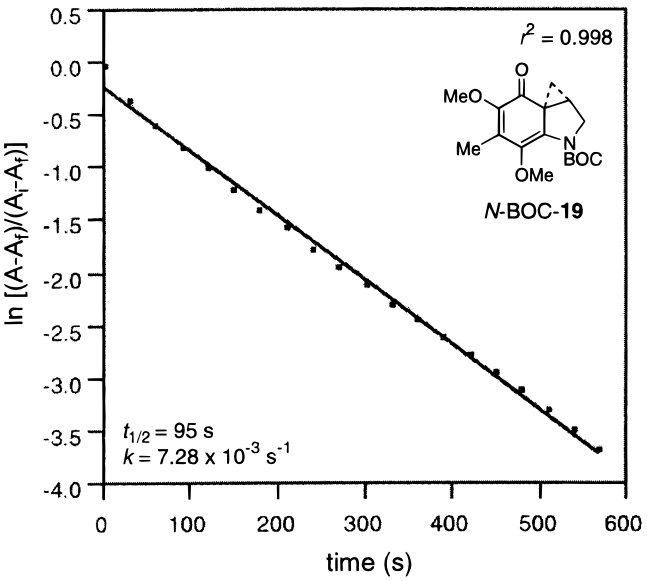

Figure 6.

bubbled through the solution for 1 h at 25 °C. The reaction mixture was then filtered through Celite, and the Celite was washed thoroughly with EtOAc. The solution was then concentrated under reduced pressure and subjected to HPLC purification. Semipreparative reverse-phase HPLC (Waters Bondapak C-18 column, 300 Å, 25 × 100 mm, gradient 10−100% CH_3CN/H_2O over 20 min, 10 mL/min afforded **46** (t_R = 17.0 min, 1.7 mg, 82%) as a bright yellow oil: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.31 (s, 1H), 8.05 (dd, J = 1.1, 7.8 Hz, 1H), 7.96 (m, 3H), 7.85−7.78 (m, 2H), 7.72 (m, 1H), 7.65−7.60 (m, 2H), 7.55 (m, 1H), 4.55 (dd, J = 10.0, 11.6 Hz, 1H), 4.28 (dd, J = 5.2, 11.6 Hz, 1H), 4.18 (dd, J = 6.5, 11.3 1H), 4.12−4.08 (m, 2H); IR (film) ν_{max} 2925, 2854, 1687, 1650, 1596 cm^{-1}; FABHRMS (NBA/NaI) m/z 402.0813 ($C_{24}H_{16}NO_3Cl$ + H^+ requires 402.0819).

Representative Solvolysis Reactivity. A sample of *N*-BOC-**19** (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 30 s until completion. The decrease in the long wavelength absorption at 397 nm was monitored, as was the absorption at 285 nm, Figure 6. The solvolysis rate constant (k = 7.28 × 10^{-3} s^{-1}) and the half-life ($t_{1/2}$ = 95 s) were calculated from data recorded at the long wavelength from the least-squares treatment (r^2 = 0.99) of the slope of the plot of time versus ln [(A_f − A_i)/(A_f − A)].

The same procedure was also carried out at pH 7, and for *N*-PIV-**19** (390 nm) and *N*-Me-**19** (445 nm) at both pH 3 and pH 7.

Solvolysis Regioselectivity: 1-(*tert*-Butyloxycarbonyl)-3-(chloromethyl)-5,7-dimethoxy-4-hydroxy-6-methyl-2,3-dihydroindole (18). A solution of *N*-BOC-**19** (3.6 mg, 15.0 μmol) in THF (1.5 mL) under Ar at −78 °C was treated with 3.6 N HCl/EtOAc (85 μL, 30.0 μmol) and stirred 10 min. The solvents were removed under reduced pressure to yield pure **18** (3.7 mg, 89%).

Solvolysis Regioselectivity: 1-(*tert*-Butyloxycarbonyl)-5,7-dimethoxy-4-hydroxy-3-(methoxymethyl)-6-methyl-2,3-dihydroindole (37). A solution of *N*-BOC-**19** (2.5 mg, 7.8 μmol) in CH_3OH (1.0 mL) under Ar at 0 °C was treated with 0.01 N TfOH (93 μL, 0.12 equiv) and allowed to warm to 25 °C for 2 h. The reaction was directly subjected to flash chromatography (SiO_2, 1.0 × 3 cm, 30% EtOAc/hexane) to provide pure **37** (2.2 mg, 82%) as a colorless oil as the only detectable reaction product: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62 (s, 1H), 4.10 (dd, J = 8.1, 11.6 Hz, 1H), 3.76 (s, 3H), 3.66 (dd, J = 5.4, 11.6 Hz, 1H), 3.60 (m, 1H), 3.59 (s, 3H), 3.54 (m, 2H), 3.44 (s, 3H), 2.16 (s, 3H), 1.49 (s, 9H); IR (film) ν_{max} 3332, 2933, 1682, 1470; MALDIFTMS (NBA) m/z 376.1733 ($C_{18}H_{27}NO_6$ + Na^+ requires 376.1736).

Solvolysis Regioselectivity: 5,7-Dimethoxy-1,6-dimethyl-4-hydroxy-3-(methoxy methyl)-2,3-dihydroindole (39). A solution of *N*-Me-**19** (3.5 mg, 0.015 mmol) in CH_3OH (1.5 mL) under Ar at 0 °C was treated with 0.01 N TfOH (180 μL, 0.12 equiv) and allowed to warm to 25 °C for 5 h. The reaction was quenched with $NaHCO_3$ (2.5 mg, 0.03 mmol), diluted with EtOAc, filtered through Celite, and concentrated. Flash chromatography (SiO_2, 1.0 × 3 cm, 30% EtOAc/hexane) provided pure **39** (2.8 mg, 71%) as a colorless oil as the only detectable reaction product: ^{1}H NMR (C_6D_6, 250 MHz) δ 7.70 (s, 1H), 3.78 (s, 3H), 3.45 (s, 3H), 3.45 (s, 3H), 3.38 (m, 1H), 3.15 (dd, J = 8.4, 9.6 Hz, 1H), 2.95 (m, 2H), 2.82 (s, 3H), 2.80 (s, 3H), 2.52 (dd, J = 7.1, 9.6 Hz, 1H), 2.35 (s, 3H); IR (film) ν_{max} 3254, 2930, 2827, 1474, 1452 cm^{-1}; FABHRMS (NBA) m/z 267.1480 (M^+, $C_{14}H_{21}NO_4$ requires 267.1471).

Solvolysis Regioselectivity: 3-(Chloromethyl)-5,7-dimethoxy-1,6-dimethyl-4-hydroxy-2,3-dihydroindole (38). A solution of *N*-Me-**19** (3.6 mg, 0.015 mmol) in THF (1.5 mL) under Ar at −78 °C was treated with 3.6 N HCl/EtOAc (85 μL, 0.03 mmol) and stirred 10 min. The solvents were removed under reduced pressure, and the residue was dissolved in H_2O (3 mL), treated with $NaHCO_3$ (2.5 mg, 0.03 mmol), and quickly extracted with Et_2O (10 mL). The organic layer was dried (Na_2SO_4), and concentrated under reduced pressure to yield pure **38** (3.7 mg, 89%) as a colorless oil as the only detectable reaction product: ^{1}H NMR (C_6D_6, 250 MHz) δ 5.08 (s, 1H), 3.96 (dd, J = 3.4, 10.1, 1H), 3.66 (m, 1H), 3.49 (t, J = 10.1 Hz, 1H), 3.35 (s, 3H), 3.29 (dd, J = 4.4, 9.8 Hz, 1H), 3.15 (s, 3H), 3.02 (t, J = 9.5 Hz, 1H), 2.72 (s, 3H), 2.14 (s, 3H); IR (film) ν_{max} 3383, 2939, 2850, 1476, 1456 cm^{-1}; FABHRMS (NBA) m/z 271.0985 (M^+, $C_{13}H_{18}ClNO_3$ requires 267.1471).

Solvolysis Regioselectivity: 5,7-Dimethoxy-1,6-dimethyl-4-hydroxy-3-(hydroxymethyl)-2,3-dihydroindole (40). A solution of *N*-Me-**19** (2.1 mg, 9 μmol) in 1:1 THF/H_2O (1.8 mL) under Ar at 0 °C was treated with 0.01 N TfOH (107 μL, 0.12 equiv) and stirred 3 days at 25 °C. The reaction mixture was then extracted with Et_2O, dried (Na_2SO_4), and subjected to flash chromatography (SiO_2, 1.0 × 5 cm, 70% EtOAc/hexane) which yielded pure **40** (2.0 mg, 88%) as a colorless oil as the only detectable reaction product: ^{1}H NMR (C_6D_6, 400 MHz) δ 7.11 (s, 1H), 3.60 (s, 3H), 3.42 (t, J =10.5 Hz, 1H), 3.25 (m, 2H), 2.92 (t, J = 9.2 Hz, 1H), 2.77 (s, 3H), 2.50 (dd, J = 6.6, 9.2 Hz, 1H), 2.29 (s, 3H); IR (film) ν_{max} 3359, 3251, 2933, 2862, 2830, 1462, 1453 cm^{-1}; FABHRMS (NBA) m/z 253.1321 (M^+, $C_{13}H_{19}NO_4$ requires 253.1314).

Substrate Reduction by DT Diaphorase (NQO1): HPLC Assay. To measure the ability of NQO1 to reduce the synthetic quinones, metabolism was followed by

HPLC measuring NADH consumption, loss of the parent quinone peak, and appearance of the hydroquinone peak.[37] NADH and the quinones were separated on a Waters HPLC system (W-600 Pumps and Controller, 996 PDA detector (340 nm)) with a Waters Bondapak C-18 column (125 Å, 3.9 × 300 mm). Separation was accomplished using a linear gradient of 5−95% CH_3OH-buffer over 12 min (with 10 mM potassium phosphate (pH 6.0) as the aqueous buffer) followed by 95% CH_3OH for 8 min. Retention times were as follows: mitomycin A (**5**) (14.0 min), mitomycin C (**4**) (12.3 min), **24** (17.1 min), **43** (17.4 min), and **46** (17.3 min). Relative rates of metabolism were calculated through autointegration as loss of NADH, as the quinones were reversibly reduced and were determined from the integration of the corresponding peaks at $t = 0$ min and $t = 35$ min. A representative trace depicting the oxidation of NADH and the reduction of **24** in the presence of mitomycin C (**4**) is shown below (Figure 7).

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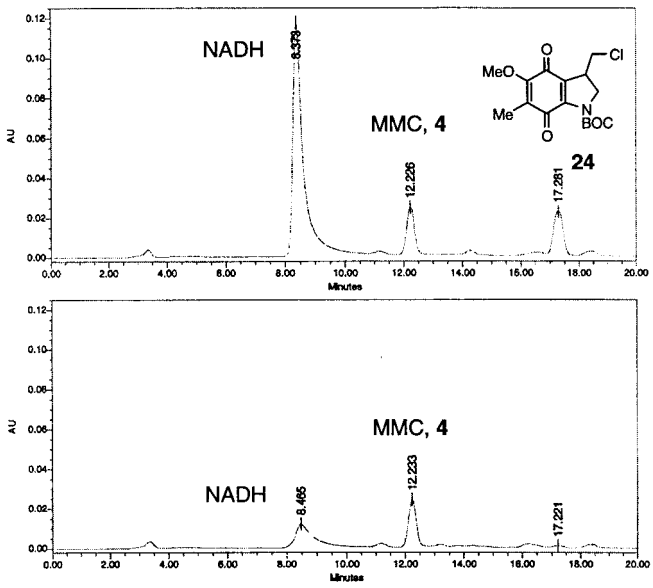

Figure 7.

Supporting Information Available: Full details of an alternative synthesis of *N*-BOC-**19** and the preparation and cyclization of *N*-PIV-**19** and *N*-Me-**19** and copies of ^1H NMR spectra of the new compounds disclosed herein. This material is available free of charge via the Internet at http://pubs.acs.org.

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