Selective Metal Cation Activation of a DNA Alkylating Agent: Synthesis and Evaluation of Methyl 1,2,9,9a-Tetrahydrocyclopropa[*c*]pyrido[3,2-*e*]indol-4-one-7-carboxylate (CPyI)

Dale L. Boger* and Christopher W. Boyce

Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Received February 8, 2000

The synthesis of methyl 1,2,9,9a-tetrahydrocyclopropa[*c*]pyrido[3,2-*e*]indol-4-one-7-carboxylate (CPyI) containing a one carbon expansion of the C ring pyrrole found in the duocarmycin SA alkylation subunit and its incorporation into analogues of the natural product are detailed. The unique 8-ketoquinoline structure of CPyI was expected to provide a tunable means to effect activation via selective metal cation complexation. The synthesis of CPyI was based on a modified Skraup quinoline synthesis followed by a 5-*exo-trig* aryl radical cyclization onto an unactivated alkene with subsequent TEMPO trap or *5-exo-trig* aryl radical cyclization onto a vinyl chloride for synthesis of the immediate precursor. Closure of the activated cyclopropane, accomplished by an Ar-3′ spirocyclization, provided the CPyI nucleus in 10 steps and excellent overall conversion (29%). The evaluation of the CPyI-based agents revealed an intrinsic stability comparable to that of CC-1065 and duocarmycin A but that it is more reactive than duocarmycin SA and the CBI-based agents (3−4×). A pH−rate profile of the addition of nucleophiles to CPyI demonstrated that an acid-catalyzed reaction is observed below pH 4 and that an uncatalyzed reaction predominates above pH 4. The expected predictable activation of CPyI by metal cations toward nucleophilic addition was found to directly correspond to established stabilities of the metal complexes with the addition product ($Cu^{2+} > Ni^{2+} > Zn^{2+} > Mn^{2+} > Mg^{2+}$) and provides the opportunity to selectively activate the agents upon addition of the appropriate Lewis acid. This tunable metal cation activation of CPyI constitutes the first example of a new approach to in situ activation of a DNA binding agent complementary to the well-recognized methods of reductive, oxidative, or photochemical activation. Resolution and synthesis of a full set of natural product analogues and subsequent evaluation of their DNA alkylation properties revealed that the CPyI analogues retain identical DNA alkylation sequence selectivity and near-identical DNA alkylation efficiencies compared to the natural products. Consistent with past studies and even with the deep-seated structural change in the alkylation subunit, the agents were found to exhibit potent cytotoxic activity that directly correlates with their inherent reactivity.

(+)-Duocarmycin SA (DSA, **1**) is a remarkably potent antitumor antibiotic (IC_{50} = 10 pM, L1210) disclosed in 1990[1] that has been shown to selectively bind and alkylate DNA (Figure 1).[2] (+)-Duocarmycin SA exhibits enhanced stability and corresponding biological potency compared to its predecessors, (+)-duocarmycin A (DA, **2**)[3] and (+)-CC-1065 (**3**),[4] and therefore has been the subject of extensive investigation. Studies have shown that CC-1065 and the duocarmycins tolerate and benefit from structural modifications to the alkylation subunit and

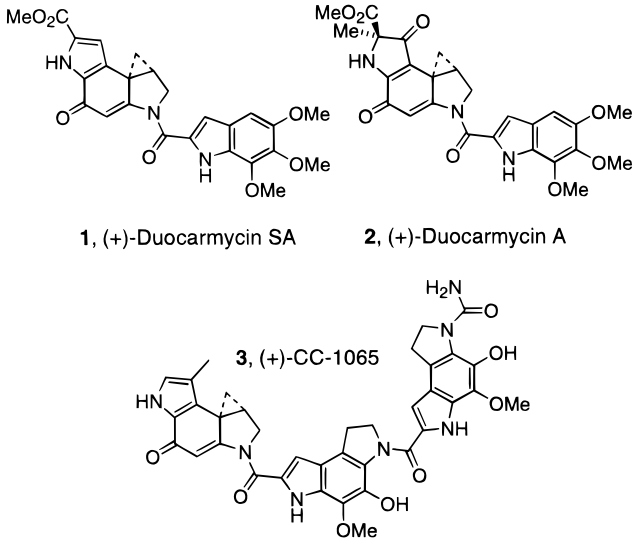


Figure 1.

(1) 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.

(2) Boger, D. L.; Machiya, K.; Hertzog, D. L.; Kitos, P. A.; Holmes, D. *J. Am. Chem. Soc.* **1993**, *115*, 9025.

(3) Ohba, K.; Watabe, H.; Sasaki, T.; Takeuchi, Y.; Kodama, Y.; Nakazawa, T.; Yamamoto, H.; Shomura, T.; Sezaki, M.; Kondo, S. *J. Antibiot.* **1988**, *41*, 1515. Takahashi, I.; Takahashi, K.; Ichimura, M.; Morimoto, M.; Asano, K.; Kawamoto, I.; Tomita, F.; Nakano, H. *J. Antibiot.* **1988**, *41*, 1915. Yasuzawa, T.; Iida, T.; Muroi, K.; Ichimura, M.; Takahashi, K.; Sano, H. *Chem. Pharm. Bull.* **1988**, *36*, 3728. Ichimura, M.; Muroi, K.; Asano, K.; Kawamoto, I.; Tomita, F.; Morimoto, M.; Nakano, H. *J. Antibiot.* **1988**, *41*, 1285. Ishii, S.; Nagasawa, M.; Kariya, Y.; Yamamoto, H.; Inouye, S.; Kondo, S. *J. Antibiot.* **1989**, *42*, 1713.

(4) Hanka, L. J.; Dietz, A.; Gerpheide, S. A.; Kuentzel, S. L.; Martin, D. G. *J. Antibiot.* **1978**, *31*, 1211. Chidester, C. G.; Krueger, W. C.; Mizsak, S. A.; Duchamp, D. J.; Martin, D. G. *J. Am. Chem. Soc.* **1981**, *103*, 7629.

10.1021/jo000177b CCC: $19.00
Published on Web 05/10/2000

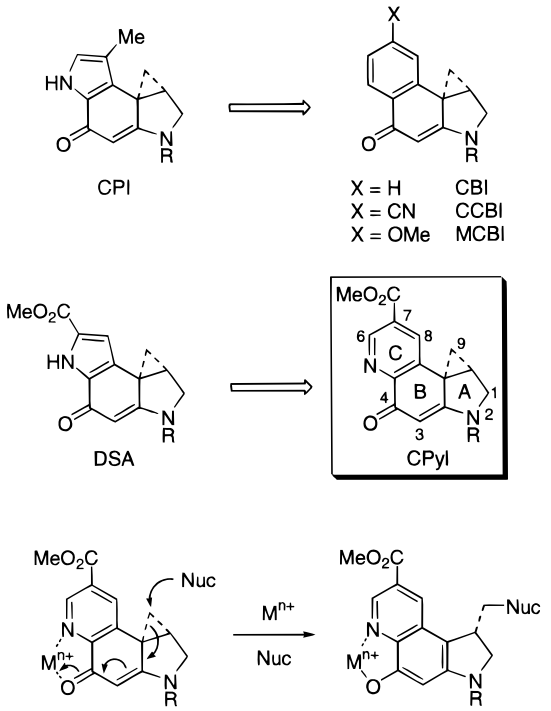


Figure 2.

that the resulting agents retain their ability to participate in the characteristic sequence selective DNA alkylation reaction.[5,6] Such structural modifications and the definition of their effects have served to advance the understanding of the origin of sequence selectivity[5,7] and catalysis[8,9] of the DNA alkylation reaction by **1**−**3.**

The substitution of the fused pyrrole C ring of CPI, the alkylating subunit of CC-1065 (**3**), with the six-membered benzene ring in CBI was shown to increase relative stability (4×) and biological potency (4×) without affecting DNA alkylation selectivity.[10] Herein, we report the preparation of methyl 1,2,9,9a-tetrahydrocyclopropa-[*c*]pyrido[3,2-*e*]indol-4-one-7-carboxylate (CPyI), which contains a similar one carbon expansion of the C ring pyrrole found in the DSA alkylation subunit but with incorporation of a pyridine (Figure 2). We anticipated that chelation of metal cations with the 8-ketoquinoline functionality in CPyI would provide a tunable means to effect Lewis acid-catalyzed functional reactivity, thereby providing a means to selectively activate the agents. Although there are several well-established methods available for selective activation of DNA binding agents including reductive activation (mitomycins), oxidative activation (aflatoxin), disulfide or trisulfide cleavage (calicheamicin), photochemical activation (psoralen), and oxidant activation of metal complexes (bleomycin), we are not aware of examples of tunable metal cation Lewis acid activation of a DNA alkylating agent. Thus, the studies represent the preparation of the first members of such a class of DNA alkylating agents.

More intriguing, comparative trace metal analyses of cancerous and noncancerous human tissue have revealed significant distinctions.[11] Although no generalizations were possible across all tumor types, within a given tumor type there were significant and potentially exploitable differences. For example, Zn was found in breast carcinoma at levels 700% higher than normal breast cells of the same type while lung carcinoma exhibited a reversed and even larger 10-fold difference. Thus, chemotherapeutic agents subject to Zn activation might exhibit an enhanced activity against breast carcinoma attributable to this difference in Zn levels.[11]

Synthesis of *N*-BOC-CPyI. The CPyI synthesis was based on a modified Skraup quinoline synthesis to provide the core structure, followed by the TEMPO trap of an aryl radical−alkene 5-*exo-trig* cyclization for introduction of the A-ring and final Ar-3′ spirocyclization (Scheme 1). Thus, 2-bromoacrolein was treated with **4** in the presence of bromine (1.0 equiv, AcOH, 100 °C, 1 h, 92%) to provide **5**.[12] Protection of phenol **5** (1.2 equiv of BnBr, 1.1 equiv of NaH, DMF, 4−25 °C, 24 h, 85%) was followed by nitro reduction with $SnCl_2$ (5.0 equiv, EtOAc, 0.5 h, 70 °C) and amine protection (4.0 equiv of $(BOC)_2O$, 2 equiv of Et_3N, dioxane, 70 °C, 1 h, 74% for two steps). The resultant 3-bromoquinoline **7** was subjected to Pd(0)-catalyzed carboxybutylation[13] (0.1 equiv of $(PPh_3)_4Pd$, CO (g), 1.2 equiv of *n*-Bu_3N, *n*-BuOH, 100 °C, 12 h, 78%) and treatment with LiOMe (1.1 equiv, MeOH, 0−25 °C, 1.5 h, 91%) to afford **9**. As detailed by Heck,[13] the use of MeOH as a reaction solvent for direct Pd-mediated carboxymethylation of **7** is limited by the achievable reaction temperatures (65 °C with MeOH versus 115 °C with *n*-BuOH) and thus requires extended reaction times (≥3 d) and generally proceeds in lower yields (≤45%). Following selective, acid-catalyzed C5 iodination[14] of **9** with *N*-iodosuccinimide (1.2 equiv, catalytic TsOH, THF−CH_3OH, 0−25 °C, 1−2 d, 88%), *N*-alkylation of the sodium salt of **10** (1.1 equiv of NaH, DMF, 4 °C, 30 min) with allyl bromide (3 equiv, DMF, 25 °C, 2.5 h, 94%) proceeded smoothly.

In contrast to related substrates,[6,14] cyclization of **11** under previously described conditions (6.0 equiv of Bu_3-SnH, 6.0 equiv of TEMPO, 70 °C, benzene, 3.5 h) did not proceed in high yield (41−54%). Although tin hydride-mediated reduction has been observed with similar compounds,[15] it had not been observed in other CC-1065/duocarmycin systems. Consequently, it was surprising to isolate significant amounts (≥30%) of the halogen-

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

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

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

(8) Boger, D. L.; Garbaccio, R. M. *Acc. Chem. Res.* **1999**, *32*, 2, 1043. Boger, D. L.; Garbaccio, R. M. *Bioorg. Med. Chem.* **1997**, *5*, 263. Boger, D. L.; Hertzog, D. L.; Bollinger, B.; Johnson, D. S.; Cai, H.; Goldberg, J.; Turnbull, P. *J. Am. Chem. Soc.* **1997**, *119*, 4977. Boger, D. L.; Bollinger, B.; Hertzog, D. L.; Johnson, D. S.; Cai, H.; Goldberg, J.; Mésini, P.; Garbaccio, R. M.; Jin, Q.; Kitos, P. A. *J. Am. Chem. Soc.* **1997**, *119*, 4987.

(9) Warpehoski, M. A.; Hurley, L. H. *Chem. Res. Toxicol.* **1988**, *1*, 315.

(10) Boger, D. L.; Ishizaki, T.; Kitos, P. A.; Suntornwat, O. *J. Org. Chem.* **1990**, *55*, 5823. Boger, D. L.; Ishizaki, T.; Wysocki, R. J., Jr.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *J. Am. Chem. Soc.* **1989**, *111*, 6461.

(11) Mulay, I. L.; Roy, R.; Knox, B. E.; Suhr, N. H.; Delaney, W. E. *J. Natl. Cancer Inst.* **1971**, *47*, 1.

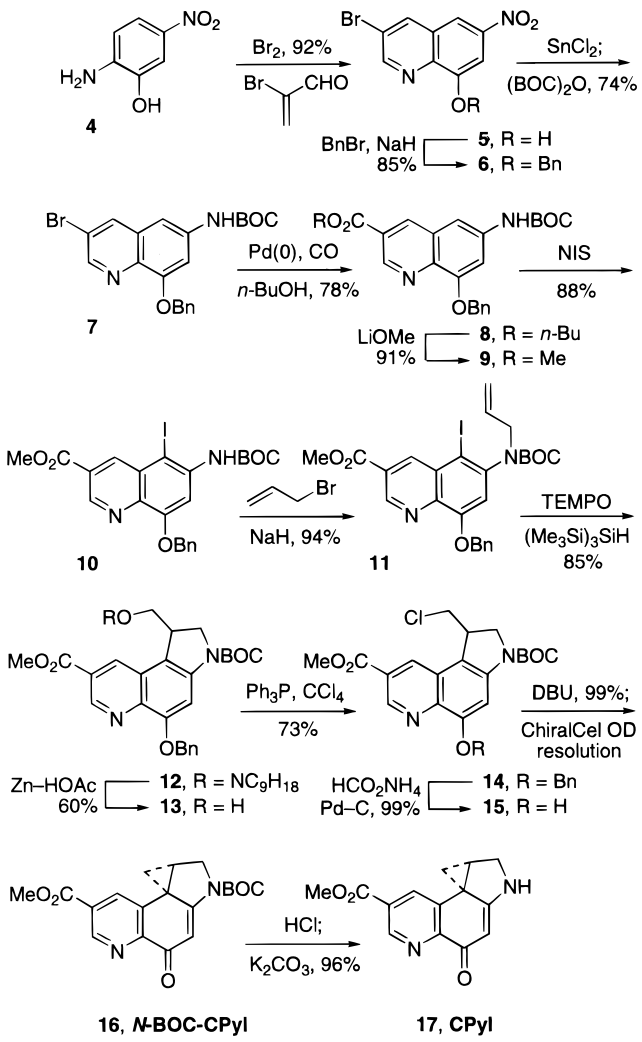
(12) Baker, R. H.; Tinsley, S. W., Jr.; Butler, D.; Riegel, B. *J. Am. Chem. Soc.* **1950**, *72*, 393. Tinsley, S. W. *J. Am. Chem. Soc.* **1955**, *77*, 4175.

(13) Schoenberg, A.; Bartoletti, I.; Heck, R. F. *J. Org. Chem.* **1974**, *39*, 3318. Heck, R. F. *Palladium Reagents in Organic Synthesis;* Academic Press: London, Orlando. Fl, 1985.

(14) Boger, D. L.; McKie, J. A. *J. Org. Chem.* **1995**, *60*, 1271.

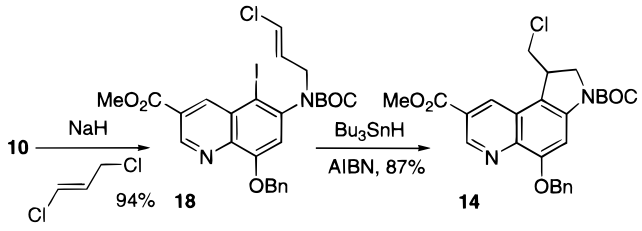
(15) Ueno, Y.; Chino, K.; Okawara, M. *Tetrahedron Lett.* **1982**, *23*, 2575. Shankaran, K.; Sloan, C. P.; Snieckus, V. *Tetrahedron Lett.* **1985**, *26*, 6001.

Scheme 1



reduced product. Characterization of this byproduct[16] was confirmed by direct comparison with an authentic sample prepared by allylation of **9** (3 equiv of allyl bromide, 1.1 equiv of NaH, DMF, 4−25 °C, 89%). The radical cyclization was improved by utilizing tris(trimethylsilyl)silane ($(CH_3Si)_3SiH$, 5 equiv, 8.0 equiv of TEMPO, toluene, 80 °C, 16 h, 85%), which has a stronger metal−hydride bond than Bu_3SnH (79 vs 74 kcal/mol)[17] and therefore a slower rate of aryl radical reduction permitting clean intramolecular 5-*exo*-*trig* cyclization. The TEMPO-trapped product **12** was reduced with activated Zn[14,18] (50 equiv, 3:1 HOAc−THF, 60 °C, 11 h, 60%) and the resulting alcohol **13** converted to the primary chloride[19] (3.0 equiv of Ph_3P, 9.0 equiv of CCl_4, CH_2Cl_2, 25 °C, 3 h, 73%). Two-phase transfer catalytic hydrogenolysis[20] of the benzyl ether **14** (10% Pd−C, 10 equiv of 25% aqueous HCO_2NH_4, 25 °C,

Scheme 2



3 h, 99%) and subsequent spirocyclization[21] by treatment of **15** with DBU (4 equiv, CH_3CN, 25 °C, 3 h, 99%) provided *N*-BOC-CPyI (**16**). Acid-catalyzed deprotection of **16** (3 M HCl−EtOAc, 25 °C, 30 min), which is accompanied by the addition of chloride to the cyclopropane, followed by treatment of the crude hydrochloride salt with K_2CO_3 (10 equiv, acetone, 25 °C, 24 h, 96%) cleanly provided CPyI (**17**).

In an improved preparation of the advanced (chloromethyl)indoline precursor **14**, adoption of a direct free radical cyclization[22] of a substrate bearing a vinyl chloride acceptor alkene provided **14** in good conversion (Scheme 2). Thus, alkylation of the sodium salt of **10** (1.1 equiv of NaH, DMF, 4 °C, 30 min) with *E*-1,3-dichloropropene (3 equiv, 25 °C, 12 h, 94%) was followed by free radical cyclization of the vinyl chloride (1.5 equiv of Bu_3SnH, catalytic AIBN, benzene, 70 °C, 6.5 h, 87%) to provide **14**. The incorporation of this improvement provided *N*-BOC-CPyI (**16**) in 10 steps and superb overall conversion (29%).

Resolution. To assess the properties of both enantiomers of the CPyI-based agents, a direct chromatographic resolution on a ChiralCel-OD semipreparative HPLC column (2 × 25 cm, 50% *i*-PrOH−hexanes eluant, 7 mL/min, $\alpha = 1.43$) provided both enantiomers of **16** (>99% ee). *N*-BOC-CPyI proved to be the only late-stage intermediate that could be effectively resolved on a ChiralCel-OD column, and similar efforts to separate **13**−**15** were not successful. The slower eluting (+)-enantiomer of **16** ($t_R = 38$ min) was assigned the natural (3*S*)-configuration, and agents derived from this (+)-enantiomer exhibited the more potent biological activity, the more effective DNA alkylation properties, and a DNA alkylation selectivity identical with the natural products. The faster eluting (−)-enantiomer of **16** ($t_R = 27$ min) was assigned the unnatural (3*R*)-configuration and agents derived from this (−)-enantiomer exhibited the corresponding less potent biological activity, the less effective DNA alkylation properties, and a DNA alkylation selectivity identical with the unnatural enantiomers of the natural products.

Synthesis of Duocarmycin and CC-1065 Analogues. The CPyI alkylation subunit was incorporated into duocarmycin and CC-1065 analogues as detailed in Scheme 3. Deprotection and concurrent ring opening of **16** (3 M HCl−EtOAc) followed by immediate coupling (5 equiv of EDCI, DMF, 25 °C) of the resulting amine hydrochloride salt with 5,6,7-trimethoxyindole-2-carboxy-

(16) Methyl 8-(benzyloxy)-6-[*N*-(*tert*-butyloxycarbonyl)-*N*-(2-propenyl)amino]quinoline-3-carboxylate: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.39 (d, $J = 2.0$ Hz, 1H), 8.70 (d, $J = 2.0$ Hz, 1H), 7.48 (m, 2H), 7.30 (m, 4H), 7.08 (br s, 1H), 5.85 (m, 1H), 5.40 (s, 2H), 5.05 (m, 2H), 4.20 (m, 2H), 3.98 (s, 3H), 1.39 (s, 9H); FABHRMS (NBA/CsI) *m*/*z* 581.1065 (M + Cs^+, $C_{26}H_{28}N_2O_5$ requires *m*/*z* 581.1053).

(17) Giese, B.; Kopping, B. *Tetrahedron Lett.* **1989**, *30*, 681. Kanabus-Kaminska, J. M.; Hawari, J. A.; Griller, D. *J. Am. Chem. Soc.* **1987**, *109*, 5267.

(18) Newman, M. S.; Evans, F. J., Jr. *J. Am. Chem. Soc.* **1955**, *77*, 946.

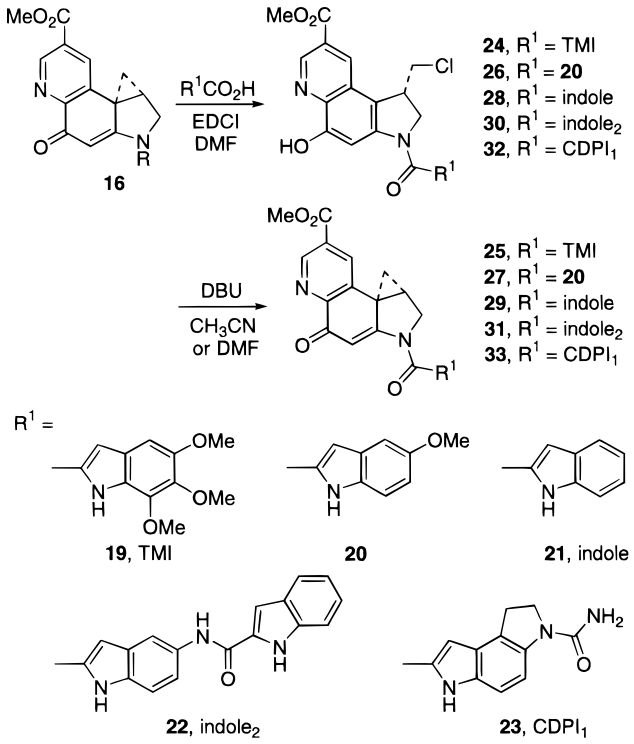
(19) Hooz, J.; Gilani, S. S. H. *Can. J. Chem.* **1968**, *46*, 86.

(20) Ram, S.; Ehrenkaufer, R. E. *Synthesis* **1988**, 91. Bieg, T.; Szeja, W. *Synthesis* **1985**, 76.

(21) Baird, R.; Winstein, S. *J. Am. Chem. Soc.* **1963**, *85*, 567. Baird, R.; Winstein, S. *J. Am. Chem. Soc.* **1962**, *84*, 788. Winstein, S.; Baird, R. *J. Am. Chem. Soc.* **1957**, *79*, 756.

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

Scheme 3



lic acid[23] (TMI, **19**, 10 h, 52%), 5-methoxyindole-2-carboxylic acid (**20**, 10 h, 59%), indole-2-carboxylic acid (**21**, 10 h, 71%), indole$_2$[24] (**22**, 16 h, 64%), and CDPI$_1$[25] (**23**, 16 h, 41%) provided **24**, **26**, **28**, **30**, and **32**, respectively. DBU (3 equiv, 3 h, 25 °C) promoted spirocyclization of **24** (DMF, 86%), **26** (CH_3CN, 90%), **28** (CH_3CN, 93%), **30** (DMF, 97%), and **32** (DMF, 67%) provided **25**, **27**, **29**, **31**, and **33**, respectively.

Solvolysis: Reactivity and Regioselectivity. Two fundamental characteristics of the alkylation subunits have proven important in past studies.[5] 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 reactivity of the agents as established by their rate of acid-catalyzed solvolysis which has been found to accurately reflect a direct relationship between intrinsic stability and in vitro cytotoxicity.[5]

Solvolysis was conducted in 50% CH_3OH−buffer mixtures (pH 3 buffer = 4:1:20 (v:v:v) 0.1 M citric acid:0.2 M Na_2HPO_4:H_2O; pH 2 buffer = 4:1:20 (v:v:v) 1.0 M citric acid:0.2 M Na_2HPO_4:H_2O) and followed spectrophotometrically by UV with the disappearance of the long-wavelength absorption of the CPyI chromophore and with the appearance of a short-wavelength absorption attributable to the solvolysis product (Figure 3 and Table 1). *N*-BOC-CPyI (**16**) proved to be reasonably stable to solvolysis even at pH 2.0 ($k = 3.72 \times 10^{-5}$ s^{-1}, $t_{1/2} = 5.2$ h) and pH 3.0 ($k = 3.81 \times 10^{-6}$ s^{-1}, $t_{1/2} = 50.5$ h) as compared to *N*-BOC-CPI (**34**, pH 3, $t_{1/2} = 37$ h) and *N*-BOC-DA (**35**, pH 3, $t_{1/2} = 11$ h) (Figure 4). However,

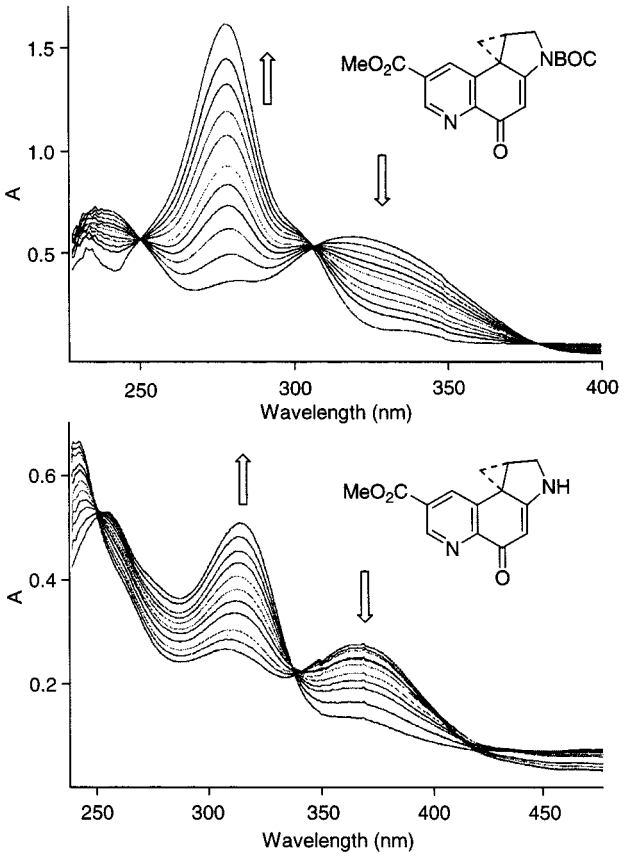


Figure 3. Solvolysis study (UV spectra) of *N*-BOC-CPyI (**16**, top) and CPyI (**17**, bottom) in 50% CH_3OH−aqueous buffer (pH 2, 4:1:20 (v:v:v) 1.0 M citric acid, 0.2 M Na_2HPO_4, and H_2O, respectively). The spectra were recorded at regular intervals, and only a few are shown for clarity. Top: 0, 1, 2, 3, 4, 5, 7, 9, 12, 16, 41 h. Bottom: 0, 5, 10, 20, 26, 35, 47, 66, 93, 144, 283 h.

Table 1. Solvolysis Rates with Phosphate Buffer[a]

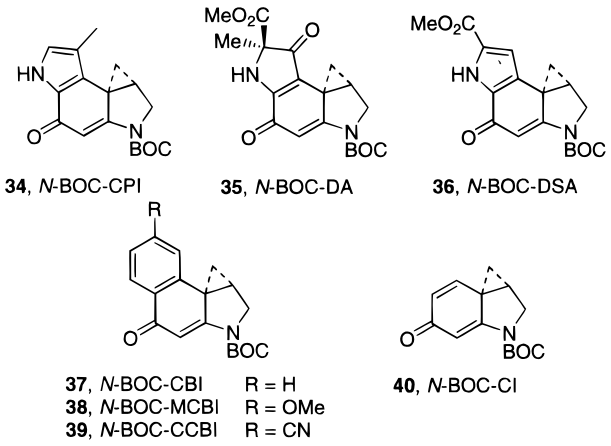
compd		k_{obs} (s^{-1})	$t_{1/2}$ (h)
16, *N*-BOC-CPyI	pH 2	3.72×10^{-5}	5
	pH 3	3.81×10^{-6}	51
17, CPyI	pH 2	4.63×10^{-6}	42
	pH 3	6.27×10^{-7}	310

[a] pH 2: 50% CH_3OH−aqueous buffer (4:1:20 (v:v:v) 1.0 M citric acid, 0.2 M Na_2HPO_4, and H_2O, respectively. pH 3: 50% CH_3OH−aqueous buffer (4:1:20 (v:v:v) 0.1 M citric acid, 0.2 M Na_2HPO_4, and H_2O, respectively).

16 was less stable than *N*-BOC-DSA (**36**, pH 3, $t_{1/2} = 177$ h) and *N*-BOC-CBI (**37**, pH 3, $t_{1/2} = 133$ h). The rate of solvolysis was found to be independent of the phosphate buffer countercation (Na^+, NH_4^+, or K^+) within experimental error.

The acid-catalyzed nucleophilic addition of CH_3OH to **16** was conducted on a preparative scale to establish the regioselectivity of addition and was confirmed by synthesis of the expected product **41** derived from nucleophilic addition to the least substituted cyclopropane carbon. Treatment of *N*-BOC-CPyI (**16**) with catalytic CF_3SO_3H (0.3 equiv, CH_3OH, 25 °C, 20 h, 91%) resulted in the clean addition to provide a single product **41** (Scheme 4). Similarly, treatment of **16** with HCl−EtOAc (2 equiv, THF, −78 °C, 2 min, 96%) provided **15** as a single product. Clean cleavage of the C8b−C9 bond with S_N2 addition of CH_3OH or HCl to the least substituted C9 cyclopropane carbon was observed, and no cleavage

(23) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *J. Am. Chem. Soc.* **1990**, *112*, 8961.
(24) Boger, D. L.; Yun, W.; Han, N. *Bioorg. Med. Chem.* **1995**, *3*, 1429.
(25) 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.



34, *N*-BOC-CPI **35**, *N*-BOC-DA **36**, *N*-BOC-DSA

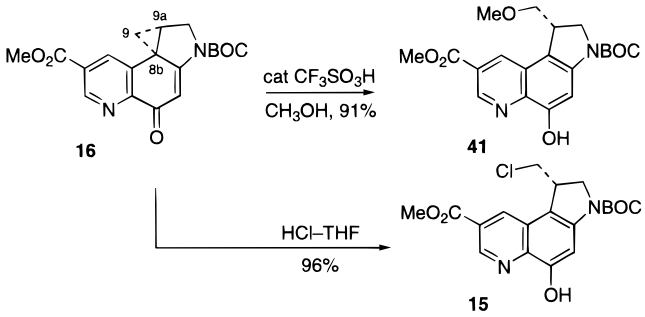
37, *N*-BOC-CBI R = H
38, *N*-BOC-MCBI R = OMe
39, *N*-BOC-CCBI R = CN

40, *N*-BOC-CI

Agent	k (s^{-1}, pH 3)	$t_{1/2}$ (h, pH 3)	Regioselectivity
16	3.81×10^{-6}	51	> 20 : 1
34	5.26×10^{-6}	37	4 : 1
35	1.75×10^{-5}	11	3 : 2
36	1.08×10^{-6}	177	6–4 : 1
37	1.45×10^{-6}	133	> 20 : 1
38	1.75×10^{-6}	110	> 20 : 1
39	0.99×10^{-6}	194	> 20 : 1
40	1.98×10^{-2}	0.01	nd

Figure 4.

Scheme 4



of the C8b−C9a bond with ring expansion was detected (>20:1). This is in sharp contrast to the natural products where substantial amounts of the alternative ring expansion addition products have been observed (6−1.5:1).[26] Nonetheless, the observations are consistent with prior studies with *N*-BOC-CBI (**37**), *N*-BOC-MCBI (**38**), and *N*-BOC-CCBI (**39**), where no (>20:1) ring expansion solvolysis product was detected (Figure 4).[23,27]

Solvolysis pH Dependence. Duocarmycin SA (**1**) is known to be exceptionally stable at neutral conditions, and it was interesting to observe that *N*-BOC-CPyI (**16**) possessed measurable solvolytic reactivity in 50% aqueous CH_3OH (pH 7). A full pH−rate profile in 50% CH_3−OH−universal buffer (pH 2−12, $B(OH)_3$−citric acid−Na_3PO_4)[28] demonstrated a near-first-order rate

Table 2. Solvolysis Rates of *N*-BOC-CPyI with Universal Buffer[a]

pH	k_{obs} (s^{-1})	$t_{1/2}$ (h)
2.0	3.45×10^{-5}	5.5
3.0	4.79×10^{-6}	40
4.0	1.15×10^{-6}	165
5.0	8.41×10^{-7}	230
6.0	7.88×10^{-7}	245
7.0	8.29×10^{-7}	230
8.0	7.27×10^{-7}	265
9.0	6.73×10^{-7}	290
10.0	6.97×10^{-7}	275
50% CH_3OH-H_2O	9.80×10^{-7}	195
CH_3OH	stable	stable

[a] 50% CH_3OH−aqueous buffer ($B(OH)_3$−citric acid−Na_3PO_4).

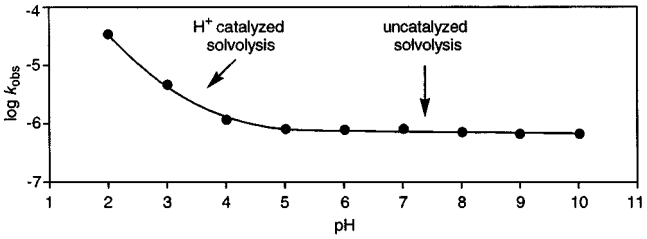


Figure 5. Plot of pH versus log k_{obs} for solvolysis of *N*-BOC-CPyI in universal buffer (pH 2−10).

dependenceon acid at pH 2−4 (Table 2 and Figure 5). Above pH 4, the dependence on acid concentration disappeared indicating a change in mechanism from acid-catalyzed solvolysis to one which is uncatalyzed. A loss of isobestic behavior in the UV trace at pH 11 indicated a second reaction event at this pH, presumably arising from hydrolysis of either the BOC or methyl ester. From a regression analysis best fit plot of the k_{obs} versus pH, rate constants of 3.37×10^{-3} $M^{-1}s^{-1}$ and 8.36×10^{-7} s^{-1} for the acid-catalyzed and uncatalyzed reactions, respectively, were established. This, plus the demonstration that related reactions above pH 4 are not only not specific acid-catalyzed, but also not general acid-catalyzed,[29] suggests this is simply an uncatalyzed S_N2 nucleophilic addition. The demonstration of an uncatalyzed solvolysis above pH 4 is consistent with the observation that the DNA alkylation reaction at the physiological pH of 7.6 is not acid-catalyzed and that catalysis must come from an alternative source.[8,29]

Solvolysis: Metal Catalysis. Central to the projected use of the CPyI agents and their potential of metal cation activation was their reactivity in the presence of metal cations.[30] Treatment of *N*-BOC-CPyI (**16**) with $Zn(OTf)_2$ (1.1 equiv) in CH_3OH (25 °C, 4 h, 92%) on a preparative scale resulted in clean addition to provide a single product **41** (Scheme 5). In contrast, *N*-BOC-CBI (**37**) did not exhibit this metal-catalyzed reactivity in the presence of $Zn(OTf)_2$ (1.5 equiv, CH_3OH, 27 h, 25 °C, 100% recovery), confirming that the CPyI 8-ketoquinoline structure is key to metal chelation, activation, and Lewis acid-catalyzed reaction.

(26) Warpehoski, M. A.; Harper, D. E. *J. Am. Chem. Soc.* **1994**, *116*, 7573. Warpehoski, M. A.; Harper, D. E. *J. Am. Chem. Soc.* **1995**, *117*, 2951. Boger, D. L.; Goldberg, J.; McKie, J. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1955. Boger, D. L.; McKie, J. A.; Nishi, T.; Ogiku, T. *J. Am. Chem. Soc.* **1997**, *119*, 311.

(27) Boger, D. L.; McKie, J. A.; Cai, H.; Cacciari, B.; Baraldi, P. G. *J. Org. Chem.* **1996**, *61*, 1710. Boger, D. L.; Han, N.; Tarby, C. M.; Boyce, C. W.; Cai, H.; Jin, Q.; Kitos, P. *J. Org. Chem.* **1996**, *61*, 4894.

(28) Perrin, D. D.; Dempsey, B. *Buffers for pH and Metal Ion Control;* Chapman and Hall: London, 1979; p 156.

(29) Boger, D. L.; Garbaccio, R. M. *J. Org. Chem.* **1999**, *64*, 5666. Boger, D. L.; Turnbull, P. *J. Org. Chem.* **1998**, *63*, 8004. Boger, D. L.; Turnbull, P. *J. Org. Chem.* **1997**, *62*, 5849. Boger, D. L.; Boyce, C. W.; Johnson, D. S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 233.

(30) Boger, D. L.; Cassidy, K. C.; Nakahara, S. *J. Am. Chem. Soc.* **1993**, *115*, 10733. Yoshida, K.; Ishiguro, M.; Honda, H.; Yamamoto, M.; Kubo, Y. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 4335. Yoshida, K.; Yamamoto, M.; Ishiguro, M. *Chem. Lett.* **1986**, 1059. Pratt, Y. T. *J. Org. Chem.* **1962**, *27*, 3905.

Scheme 5

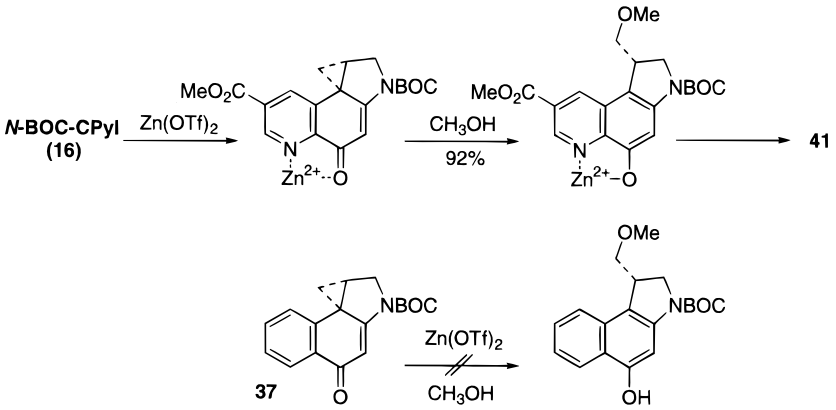


Table 3. Metal-Catalyzed Addition to *N*-BOC-CPyI[a]

metal	equiv	k_{obs} (s^{-1})	$t_{1/2}$ (h)
$Cu(acac)_2$	1.0	5.57×10^{-5}	3.5
$Ni(acac)_2$	1.0	3.51×10^{-5}	5.5
$Zn(acac)_2$	1.0	1.67×10^{-5}	11.5
$Mn(acac)_2$	1.0	9.79×10^{-7}	200
$Mg(acac)_2$	1.0	$<7 \times 10^{-7}$	>250
$Fe(acac)_2$	1.0	4.09×10^{-6}	47
$Cr(acac)_2$	1.0	9.73×10^{-6}	20
$Zn(OTf)_2$	1.0	2.48×10^{-4}	0.8
$Zn(OTf)_2$	0.2	9.19×10^{-6}	21
$Zn(OTf)_2$[b]	1.0	5.86×10^{-6}	33
$Ti(O^iPr)_4$	1.0	1.23×10^{-6}	16
$Cu(OMe)_2$	1.0	5.53×10^{-5}	3.5

[a] Solvolysis run in CH_3OH except where noted. [b] Solvolysis run in 50% CH_3OH-H_2O.

The rates of metal-catalyzed (Cu^{2+}, Ni^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+}, Fe^{3+}, Cr^{3+}, and Ti^{4+}) addition to *N*-BOC-CPyI (**16**) in CH_3OH were measured spectrophotometrically by UV with the disappearance of the long-wavelength absorption of the CPyI chromophore and with the appearance of a short-wavelength absorption (Table 3). Beautifully, within a series of divalent metals with acetylacetonate (acac) ligands, the relative rates of solvolysis (rate: $Cu^{2+} > Ni^{2+} > Zn^{2+} > Mn^{2+} > Mg^{2+}$) corresponded directly with established stability constants of the metal complexes with 8-hydroxyquinoline (stability: $Cu^{2+} > Ni^{2+} > Zn^{2+} > Mn^{2+} > Mg^{2+}$).[31] Higher valence metals including Fe^{3+}, Cr^{3+}, and Ti^{4+} also demonstrated an analogous activation of CPyI for nucleophilic addition. Notably, no apparent catalysis was observed for $Mg(acac)_2$ over and beyond the background rate indicating that this endogenous metal cation, like Na^+, does not activate CPyI effectively. Stronger Lewis acids were found to provide a faster reaction rate ($Zn(OTf)_2$, $t_{1/2} = 0.8$ h, versus $Zn(acac)_2$, $t_{1/2} = 11.5$ h) and incorporation of H_2O in the solvent slowed the reaction ($Zn(OTf)_2$, 50% aqueous CH_3OH, $t_{1/2} = 33$ h). *N*-BOC-CBI (**37**) demonstrated no detectable reaction in the $Zn(OTf)_2-CH_3OH$ system after 7 d, further confirming the role of metal cation catalysis for CPyI. Thus, the well-behaved reactivity of CPyI predictably tunable by choice of the metal cation provides the opportunities to selectively activate the agents.

DNA Alkylation Selectivity and Efficiency. The DNA alkylation properties of the agents were examined within w794 duplex DNA[32] for which comparative results are available for related agents.[5] 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.[32] A representative comparison of the DNA alkylation properties of both enantiomers of *N*-BOC-CPyI (**16**) alongside both enantiomers of *N*-BOC-DSA (**36**) is presented in Figure 6. Both natural enantiomers exhibited approximately the same efficiencies of DNA alkylation detectable at 10^{-3} M (37 °C, 48 h) and prominent at 10^{-2} M. (+)-*N*-BOC-CPyI was slightly less efficient than the unnatural enantiomer, (−)-*N*-BOC-CPyI, at both 24 and 48 h incubations which is also reflected in the relative cytotoxic activities of the two enantiomers. Like the preceding BOC derivatives examined,[5] **16** alkylated DNA much less efficiently than **24**−**33** ($10^4\times$), providing detectable alkylation at $10^{-2}-10^{-3}$ M only under vigorous conditions (37 °C, 24−72 h) and much less selectively than **24**−**33**, exhibiting a two-base pair AT-rich alkylation selectivity (5′-A*A* > 5′-T*A*). This unusual behavior of the two enantiomers alkylating the same sites is analogous to past observations.[5] It is a natural consequence of the reversed binding orientation of the two enantiomers and the diastereomeric relationship of the two adducts that result in the two enantiomers covering the exact same binding site surrounding the alkylated adenine. This has been discussed in detail illustrated elsewhere, and *N*-BOC-CPyI (**16**) conforms nicely to these past observations and models.[5]

A representative comparison of the DNA alkylation by (+)-CPyI−TMI (**25**), (+)-CPyI−indole$_2$ (**31**), and (+)-CPyI−CDPI$_1$ (**33**) alongside that of (+)-duocarmycin SA (**1**) and (+)-CC-1065 (**3**) within w794 DNA is illustrated in Figure 7. (+)-CPyI−TMI and (+)-duocarmycin SA alkylate DNA with identical selectivity and near identical efficiency with the latter agent being slightly more effective. This is nicely illustrated in Figure 7 where the two agents detectably alkylate the same high affinity site

(31) Phillips, J. P. *Chem. Rev.* **1956**, *56*, 271. Irving, H.; Williams, R. J. P. *J. Chem. Soc.* **1953**, 3192.

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

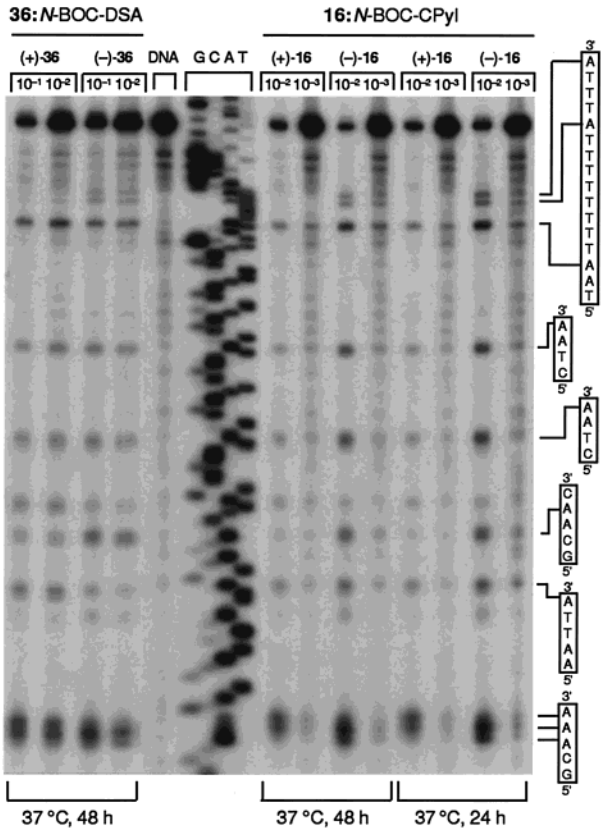


Figure 6. Thermally induced strand cleavage of w794 DNA (SV40 DNA segment, 144 bp, nucleotide nos. 138−5238), with DNA−agent incubation for 24 or 48 h, as indicated, at 37 °C, removal of unbound agent, and 30 min of thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography: lanes 1 and 2, (+)-*N*-BOC-DSA (1×10^{-1} and 1×10^{-2}); lanes 3 and 4, (−)-*N*-BOC-DSA (1×10^{-1} and 1×10^{-2}); lane 5, control DNA; lanes 6−9, Sanger G, C, A, and T sequencing reactions; lanes 10 and 11, (+)-*N*-BOC-CPyI (1×10^{-2} and 1×10^{-3}); lanes 12 and 13, (−)-*N*-BOC-CPyI (1×10^{-2} and 1×10^{-3}); lanes 14 and 15, (+)-*N*-BOC-CPyI (1×10^{-2} and 1×10^{-3}); lanes 16 and 17, (−)-*N*-BOC-CPyI (1×10^{-2} and 1×10^{-3}).

of 5′-AATT*A* at 10^{-6}−10^{-7} M (25 °C, 24 h). This is analogous to the observations made in comparisons of duocarmycin SA and CBI−TMI.[33] Like the preceding agents, the CPyI-based agents exhibit AT-rich adenine N3 alkylation selectivities that start at the 3′ adenine N3 alkylation site with agent binding in the minor groove in the 3′ to 5′ direction covering 3.5 or 5 base pairs (data not shown).

A representative comparison of the DNA alkylation by (−)-CPyI−TMI (**25**), (−)-CPyI−indole$_2$ (**31**), and (−)-CPyI−CDPI$_1$ (**33**) alongside the unnatural enantiomer of duocarmycin SA (**1**) and the natural enantiomer of CC-1065 (**3**) within w794 DNA is illustrated in Figure 8. The unnatural enantiomer DNA alkylation is considerably slower, and the results shown in Figure 8 were obtained only with their incubation for 72 h (25 °C) versus incubation for 24 h (25 °C, Figure 7) for the natural enantiomers. Despite the longer reaction times, the extent of alkylation by the unnatural enantiomers is lower, requiring higher agent concentrations to detect. The DNA alkylation selectivity and efficiency observed with *ent*-(−)-CPyI−TMI (**25**) and *ent*-(−)-duocarmycin SA

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

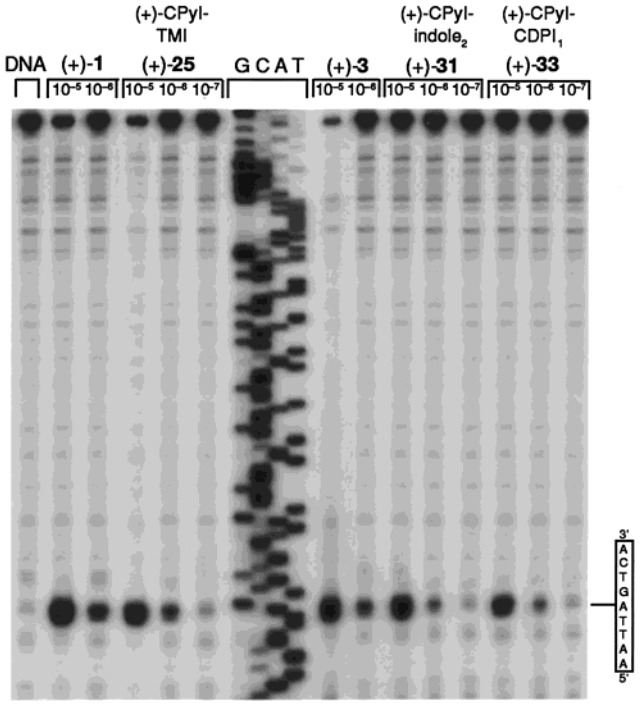


Figure 7. Thermally induced strand cleavage of w794 DNA (SV40 DNA segment, 144 bp, nucleotide nos. 138−5238), with DNA−agent incubation for 24 h at 25 °C, removal of unbound agent, and 30 min of thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography: lane 1, control DNA; lanes 2 and 3, (+)-duocarmycin SA (**1**, 1×10^{-5} and 1×10^{-6}); lanes 4−6, (+)-CPyI−TMI (**25**, 1×10^{-5}−1×10^{-7}); lanes 7−10, Sanger G, C, A, and T sequencing reactions; lanes 11 and 12, (+)-CC-1065 (**3**, 1×10^{-5} and 1×10^{-6}); lanes 13−15, (+)-CPyI−indole$_2$ (**31**, 1×10^{-5}−1×10^{-7}); lanes 16−18, (+)-CPyI−CDPI$_1$ (**33**, 1×10^{-5}−1×10^{-7}).

(**1**) were indistinguishable with the latter agent being slightly more effective. The alkylation sites for the unnatural enantiomers proved consistent with adenine N3 alkylation with agent binding in the minor groove in the reverse 5′ to 3′ direction across a 3.5 or 5 base-pair AT-rich site surrounding the alkylation site (data not shown). This is analogous to the natural enantiomer alkylation selectivity except that it extends in the reverse 5′ to 3′ direction in the minor groove and, because of the diastereomeric nature of the adducts, is offset by one base pair relative to the natural enantiomers.

In Vitro Cytotoxic Activity. Past studies with agents in this class have defined a direct correlation between inherent stability and cytotoxic potency.[5] Consistent with their relative reactivity, the CPyI-based agents exhibited cytotoxic activity that closely followed this relationship (Table 4 and Figure 9).[5] The results, which also follow trends established in the DNA alkylation studies, demonstrate that the (+)-enantiomer of the analogues possessing the configuration of the natural products is the more potent enantiomer by 3−30×. The exceptions to this trend are the simple alkylation subunits **16** and **17** themselves which, like others in the series, typically exhibit comparable activities. 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 in the binding subunit of the duocarmycins,[8] CPyI−TMI (**25**) and **27** were found to be

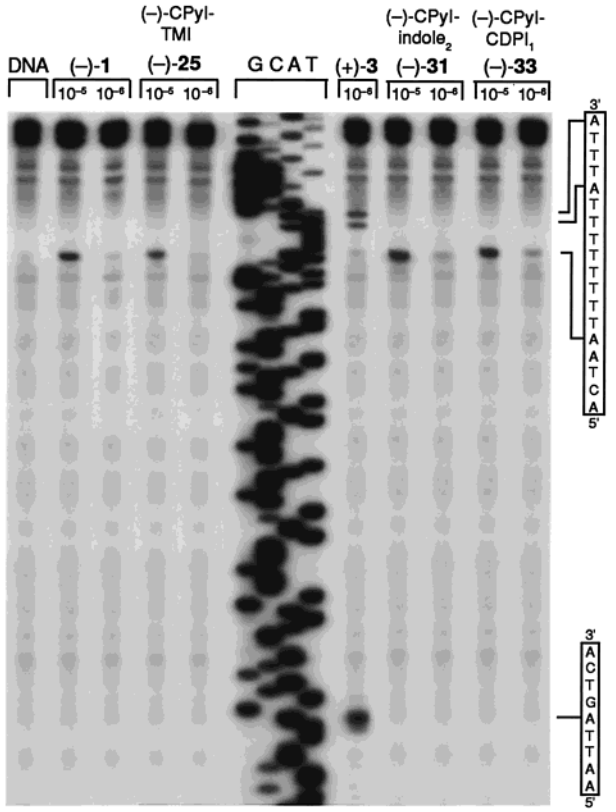


Figure 8. Thermally induced strand cleavage of w794 DNA (SV40 DNA segment, 144 bp, nucleotide nos. 138−5238), with DNA−agent incubation for 72 h at 25 °C, removal of unbound agent, and 30 min of thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography: lane 1, control DNA; lanes 2 and 3, (−)-duocarmycin SA (**1**, 1×10^{-5} and 1×10^{-6}); lanes 4 and 5, (−)-CPyI−TMI (**25**, 1×10^{-5} and 1×10^{-6}); lanes 6−9, Sanger G, C, A, and T sequencing reactions; lane 10, (+)-CC-1065 (**3**, 1×10^{-6}); lanes 11 and 12, (−)-CPyI−indole$_2$ (**31**, 1×10^{-5} and 1×10^{-6}); lanes 13 and 14, (−)-CPyI−CDPI$_1$ (**33**, 1×10^{-5} and 1×10^{-6}).

Table 4. in Vitro Cytotoxic Activity

agent	configuration	IC_{50} (L1210)[a]
16, (+)-*N*-BOC-CPyI	natural	110 nM
16, (−)-*N*-BOC-CPyI	unnatural	70 nM
17, (+)-CPyI	natural	1 μM
17, (−)-CPyI	unnatural	2 μM
25, (+)-CPyI−TMI	natural	27 pM (21 pM)
25, (−)-CPyI−TMI	unnatural	850 pM (350 pM)
27, (+)-**27**	natural	30 pM (40 pM)
27, (−)-**27**	unnatural	500 pM (500 pM)
29, (+)-CPyI−indole	natural	400 pM (800 pM)
29, (−)-CPyI−indole	unnatural	1000 pM (800 pM)
31, (+)-CPyI−indole$_2$	natural	8 pM (7 pM)
31, (−)-CPyI−indole$_2$	unnatural	45 pM (30 pM)
33, (+)-CPyI−CDPI$_1$	natural	15 pM (10 pM)
33, (−)-CPyI−CDPI$_1$	unnatural	40 pM (30 pM)

[a] The value in parentheses corresponds to the IC_{50} value determined for the corresponding seco precursor. The assays were conducted as detailed in ref 24.

equipotent illustrating that the C6 and C7 methoxy groups of **25** are not contributing to its cytotoxic potency. The indole derivative **29** was found to be less potent (10×) than both **25** and **27**, further demonstrating the importance of the C5 methoxy, which we have suggested is derived from extending the rigid length of the agents and contributing to the alkylation catalysis.[8] Finally, the longer agents, CPyI−indole$_2$ (**31**) and CPyI−CDPI$_1$ (**33**),

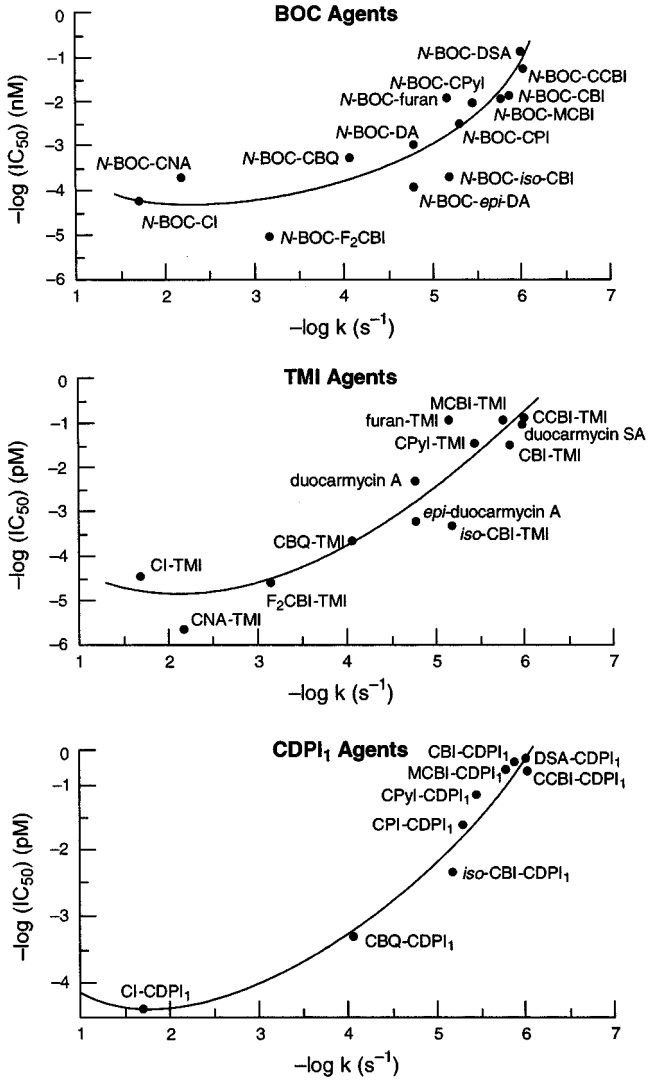


Figure 9.

displayed the most potent cytotoxic activity reflecting their longer length and greater adduct stability.

Conclusions. A short and efficient 10 step (29% overall) synthesis of CPyI featuring a modified Skraup quinoline synthesis followed by a *5-exo-trig* aryl radical cyclization onto a vinyl chloride is detailed and constitutes a one carbon expansion of the C ring pyrrole found in the duocarmycin SA alkylation subunit. CPyI was found to be 3−4× less stable than CBI and duocarmycin SA but possesses a superior stability to CC-1065 and duocarmycin A. Nucleophilic addition occurred at the least substituted cyclopropane carbon with a regioselectivity (>20:1) comparable to that of CBI but which exceeds that of the natural products themselves (6−1.5:1). A pH−rate profile of the addition of nucleophiles to CPyI demonstrated that it is an acid-catalyzed reaction below pH 4, but an uncatalyzed reaction above pH 4 consistent with the observation that the DNA alkylation reaction at physiological pH is not acid-catalyzed.[8,29] The tunable activation of CPyI by metal cations toward nucleophilic addition, which directly follows established stabilities of the resulting metal complexes with the addition product ($Cu^{2+} > Ni^{2+} > Zn^{2+} > Mn^{2+} > Mg^{2+}$), provides the opportunity to selectively and predictably initiate reactions of the agent simply by addition of the appropriate Lewis acid. This novel activation arises from

chelation to the CPyI 8-ketoquinoline core, a unique structural feature that is not found in the natural products or alkylation subunit analogues disclosed to date. Resolution and incorporation of CPyI into a full set of duocarmycin and CC-1065 analogues allowed for examination of their cytotoxic and DNA alkylation properties. The CPyI analogues were 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 (+)-enantiomers possessing the absolute configuration of **1**−**3** proved to be more potent (3−30×) than the unnatural (−)-enantiomers. DNA alkylation studies revealed that the CPyI analogues exhibited identical DNA alkylation sequence selectivity and near-identical DNA alkylation efficiencies compared to the natural products. Thus, this set of analogues, which contain a unique structural modification in the alkylation subunit, retain full DNA alkylation and cytotoxic properties of the natural products, while possessing a novel capability for predictable and tunable activation by chelation of Lewis acids. Although this Lewis acid activation is of limited use for agents which already display effective DNA alkylation properties, its use is especially effective when applied to CPyI members which are poor at alkylating DNA. This includes *N*-BOC-CPyI (**16**) for which the alkylation efficiency is increased 10^3× and reversed analogues of CPyI in which the DNA binding subunits are attached through the C-terminus methyl ester rather than N-terminus secondary amine.[34] These studies will be disclosed in due course.

Experimental Section

3-Bromo-8-hydroxy-6-nitroquinoline (5). A solution of 2-bromoacrolein (5.0 g, 37 mmol, 1.0 equiv) in glacial acetic acid (110 mL) at 25 °C was titrated to the appearance of a faint reddish color with bromine (ca. 5.9 g, 37 mmol, 1.0 equiv). 2-Hydroxy-4-nitroaniline (**4**, 5.7 g, 37 mmol, 1.0 equiv) was added, and the solution was gradually heated to 100 °C. The solution was cooled to 25 °C after 1 h. Filtering and neutralization of the precipitate with sodium phosphate buffer (1 M, pH 7, Na_2HPO_4−NaH_2PO_4) afforded 9.2 g (92%) of **5** as a light yellow solid: mp 240−241 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.93 (d, J = 2.0 Hz, 1H), 8.50 (d, J = 2.0 Hz, 1H), 8.23 (d, J = 2.2 Hz, 1H), 8.18 (s, 1H), 7.92 (s, J = 2.3 Hz, 1H); ^{13}C NMR (DMSO-d_6, 62.5 MHz) δ 155.1, 152.1, 146.3, 139.5, 139.1, 128.7, 119.1, 113.5, 105.1; IR (film) ν_{max} 3408 (br), 3089, 1587 cm^{-1}. Anal. Calcd for $C_9H_5BrN_2O_3$: C, 40.18; H, 1.87; N, 10.41. Found: C, 40.21; H, 1.91; N, 9.98.

8-(Benzyloxy)-3-bromo-6-nitroquinoline (6). A solution of **5** (13.7 g, 51.0 mmol, 1.0 equiv) in anhydrous DMF (150 mL) was cooled to 4 °C under N_2 and treated with KI (1.70 g, 10.0 mmol, 0.2 equiv) and NaH (60% dispersion in oil, 2.24 g, 56.0 mmol, 1.1 equiv). Benzyl bromide (7.30 mL, 6.10 mmol, 1.2 equiv) was added after 30 min, and the reaction was allowed to warm to 25 °C. After 24 h, the reaction volume was reduced by two-thirds in vacuo and EtOAc was added. The reaction mixture was poured onto H_2O and extracted with EtOAc. The combined organic extracts were washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated. Flash chromatography (SiO_2, 5.5 × 20 cm, 50−100% CH_2Cl_2−hexane gradient) afforded **6** (15.6 g, 85%) as a yellow solid: mp 170 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.06 (d, J = 2.2 Hz, 1H), 8.44 (d, J = 2.2 Hz, 1H), 8.25 (d, J = 2.2 Hz, 1H), 7.83 (d, J = 2.2 Hz, 1H), 7.52 (apparent d, J = 7.4 Hz, 2H), 7.38 (m, 2H), 7.32 (m, 1H), 5.47 (s, 2H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 155.4, 153.3, 146.4, 140.3, 138.8, 135.0, 128.7 (2C), 128.6, 128.4 (2C), 127.5, 119.9, 115.1, 103.3, 71.4; IR (film) ν_{max} 3082, 3055, 2933, 2871, 1519 cm^{-1}; FABHRMS (NBA/NaI) m/z 359.0040 (M + H$^+$, $C_{16}H_{11}BrN_2O_3$ requires m/z 359.0031). Anal. Calcd for $C_{16}H_{11}BrN_2O_3$: C, 53.50; H, 3.09; N, 7.80. Found: C, 53.81; H, 3.23; N, 7.48.

8-(Benzyloxy)-3-bromo-6-(*N*-(*tert*-butyloxycarbonyl)-amino)quinoline (7). A solution of **6** (0.20 g, 0.56 mmol, 1.0 equiv) in EtOAc (1.1 mL) at 25 °C was treated with $SnCl_2$·$2H_2O$ (0.63 g, 2.8 mmol, 5.0 equiv). The reaction mixture was heated to 70 °C under N_2 until an orange slurry formed (ca. 0.5 h). After being cooled to 25 °C, the reaction mixture was poured on ice and neutralized with 1 N aqueous NaOH. The aqueous layer was extracted with EtOAc, and the combined organic layers were filtered, washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated. The yellow solid was placed under vacuum for 0.5 h and then dissolved in anhydrous dioxane (5.0 mL) and treated with di-*tert*-butyl dicarbonate (0.49 g, 2.3 mmol, 4.0 equiv) and Et_3N (0.16 mL, 1.1 mmol, 2.0 equiv). The reaction mixture was warmed to 70 °C under Ar for 1 d. After cooling of the mixture to 25 °C, the solvent was removed in vacuo. Chromatography (SiO_2, 3 × 13 cm, 25% EtOAc−hexane) afforded **7** (0.18 g, 74%) as a light yellow solid: mp 162 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.77 (d, J = 2.0 Hz, 1H), 8.13 (d, J = 2.5 Hz, 1H), 7.47 (apparent d, J = 7.5 Hz, 2H), 7.42 (d, J = 2.0 Hz, 1H), 7.35 (m, 2H), 7.28 (m, 1H), 7.01 (d, J = 2.0 Hz, 1H), 6.61 (s, 1H), 5.37 (s, 2H), 1.51 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.9, 152.4, 148.3, 137.9, 136.3, 136.2, 135.4, 131.0, 128.6 (2C), 128.0, 127.3 (2C), 118.6, 104.7, 103.6, 81.1, 70.9, 28.3 (3C); IR (film) ν_{max} 3354, 2971, 2919, 1807, 1766, 1724 cm^{-1}; FABHRMS (NBA/CsI) m/z 429.0825 (M + H$^+$, $C_{21}H_{21}BrN_2O_3$ requires m/z 429.0814).

***n*-Butyl 8-(Benzyloxy)-6-(*N*-(*tert*-butyloxycarbonyl)-amino)quinoline-3-carboxylate (8).** A solution of **7** (4.4 g, 10 mmol, 1.0 equiv) in *n*-BuOH (85 mL) was degassed with N_2. $Pd(PPh_3)_4$ (1.2 g, 1.0 mmol, 0.1 equiv) and *n*-Bu_3N (2.9 mL, 12 mmol, 1.2 equiv) were added, and the solution was again purged with N_2. The reaction mixture was flushed with CO and then slowly heated to 100 °C under a CO atmosphere. Upon complete reaction (ca. 12 h), H_2O and saturated aqueous NH_4Cl were added. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated. Chromatography (SiO_2, 5.5 × 20 cm, 25% EtOAc−hexane) afforded **8** (3.6 g, 78%) as a yellow solid: mp 135−136 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.31 (d, J = 2.0 Hz, 1H), 8.66 (d, J = 2.1 Hz, 1H), 7.61 (d, J = 1.8 Hz, 1H), 7.49 (apparent d, J = 7.4 Hz, 2H), 7.35 (apparent t, J = 7.2 Hz, 2H), 7.29 (m, 1H), 7.11 (d, J = 2.1 Hz, 1H), 6.64 (br s, 1H), 5.39 (s, 2H), 4.38 (t, J = 6.6 Hz, 2H), 1.78 (m, 2H), 1.52 (s, 9H), 1.49 (m, 2H, buried under 1.52 ppm), 0.98 (t, J = 7.4 Hz, 3H); ^{13}C NMR (acetone-d_6, 100 MHz) δ 165.7, 155.7, 153.5, 146.8, 139.8, 139.5, 137.8, 137.7, 129.5, 129.1 (2C), 128.6, 128.4 (2C), 124.8, 107.1, 106.9, 80.4, 71.2, 65.5, 31.3, 28.3 (3C), 19.8, 13.9. IR (film) ν_{max} 3222, 3049, 2958, 2930, 2876, 1717, 1617 cm^{-1}; FABHRMS (NBA/CsI) m/z 451.2249 (M + H$^+$, $C_{26}H_{30}N_2O_5$ requires m/z 451.2233).

Methyl 8-(Benzyloxy)-6-(*N*-(*tert*-butyloxycarbonyl)-amino)quinoline-3-carboxylate (9). A solution of **8** (2.9 g, 6.4 mmol, 1.0 equiv) in CH_3OH (70 mL) was cooled to 4 °C under N_2 and treated with LiOMe (0.28 g, 7.1 mmol, 1.1 equiv). The reaction mixture was allowed to warm to 25 °C after 20 min. Upon complete reaction (ca. 1.5 h), H_2O was added. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The organic layers were combined, washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated. Chromatography (SiO_2, 5 × 19 cm, 25−30% EtOAc−hexane gradient) afforded **9** (2.4 g, 91%) as a yellow solid: mp 173−174 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.29 (d, J = 2.0 Hz, 1H), 8.66 (d, J = 2.1 Hz, 1H), 7.56 (d, J = 1.8 Hz, 1H), 7.45 (m, 2H), 7.30 (m, 2H), 7.25 (m, 1H), 7.18 (d, J = 2.0 Hz, 1H), 6.85 (s, 1H), 5.36 (s, 2H), 3.98 (s, 3H), 1.50 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.9, 154.6, 152.6, 146.9, 138.6,

(34) Boger, D. L.; Wolkenberg, S. E.; Boyce, C. W. *J. Am. Chem. Soc.*, in press.

137.9, 137.8, 136.0, 128.5, 128.4 (2C), 127.9, 127.3 (2C), 123.8, 106.5, 105.5, 80.8, 70.8, 52.4, 28.2 (3C); IR (film) ν_{max} 3333, 3241, 2974, 1723, 1621 cm^{-1}; FABHRMS (NBA/CsI) *m/z* 409.1773 (M + H$^+$, $C_{23}H_{24}N_2O_5$ requires *m/z* 409.1763). Anal. Calcd for $C_{23}H_{24}N_2O_5$: C, 67.63; H, 5.92; N, 6.86. Found: C, 68.00; H, 5.98; N, 6.75.

Methyl 8-(Benzyloxy)-6-(*N*-(*tert*-butyloxycarbonyl)-amino)-5-iodoquinoline-3-carboxylate (10). A solution of **9** (2.1 g, 5.2 mmol, 1.0 equiv) in a 1:1 mixture of THF−CH_3OH (85 mL) was cooled to 4 °C and treated with catalytic TsOH (40 mg) in THF (0.5 mL). *N*-Iodosuccinimide (1.4 g, 6.2 mmol, 1.2 equiv) in THF (10 mL) was slowly added over 10 min. After 1.5 h, the reaction mixture was warmed to 25 °C and stirred 45 h. Upon complete reaction, saturated aqueous $NaHCO_3$, Et_2O, and H_2O were added. The organic layer was separated, and the aqueous layer was extracted with Et_2O and EtOAc. The organic layers were combined, washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated. Chromatography (SiO_2, 5 × 19 cm, hexanes and then 30% EtOAc−hexane) provided **10** (2.3 g, 84%, typically 80−88%) as a yellow solid: mp 182−183 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.27 (d, J = 1.9 Hz, 1H), 8.96 (d, J = 1.9 Hz, 1H), 8.40 (s, 1H), 7.58 (m, 2H), 7.36 (m, 2H), 7.27 (m, 1H), 7.26 (s, 1H), 5.43 (s, 2H), 4.01 (s, 3H), 1.55 (s, 9H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 165.4, 155.0, 152.3, 147.6, 141.9, 139.8, 139.7, 135.8, 129.6, 128.5 (2C), 128.1, 128.0 (2C), 125.1, 105.6, 81.7, 78.4, 71.1, 52.6, 28.2 (3C); IR (film) ν_{max} 3384, 2974, 1723 cm^{-1}; FABHRMS (NBA/CsI) *m/z* 535.0743 (M + H$^+$, $C_{23}H_{23}IN_2O_5$ requires *m/z* 535.0730).

Methyl 8-(Benzyloxy)-6-[(*N*-(*tert*-butyloxycarbonyl)-*N*-(2-propenyl))amino]-5-iodoquinoline-3-carboxylate (11). A solution of **10** (0.40 g, 0.75 mmol, 1.0 equiv) in anhydrous DMF (6.2 mL) at 4 °C in a flamed-dried round-bottom flask was treated with NaH (60% dispersion in oil, 33 mg, 0.82 mmol, 1.1 equiv) and stirred under Ar. After 30 min, allyl bromide (94 μL, 2.3 mmol, 3.0 equiv) was added, and the reaction mixture was warmed to 25 °C and stirred 2.5 h. Saturated aqueous $NaHCO_3$ and EtOAc were added, and the reaction was then poured on H_2O. The aqueous layer was extracted with EtOAc, and the combined organic extract was washed with H_2O and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 2.5 × 16 cm, 20−25% EtOAc−hexane gradient) afforded **11** (0.40 g, 94%) as a gold solid: mp 128−129 °C; major rotamer ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.40 (s, 1H), 9.12 (s, 1H), 7.43 (apparent d, J = 7.5 Hz, 2H), 7.34 (apparent t, J = 7.5 Hz, 2H), 7.26 (m, 1H), 6.89 (s, 1H), 5.74 (m, 1H), 5.43 (m, 2H), 4.86 (m, 2H), 4.41 (dd, J = 5.5, 14.5 Hz, 1H), 4.02 (s, 3H), 3.76 (dd, J = 7.0, 14.8 Hz, 1H), 1.23 (s, 9H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 165.2, 154.4, 153.3, 149.6, 144.3, 143.7, 141.6, 135.8, 132.8, 130.2, 128.8 (2C), 128.1 (2C), 126.9, 125.2, 118.5, 114.7, 94.7, 80.8, 71.0, 52.7, 51.7, 28.1 (3C); IR (film) ν_{max} 3060, 2972, 2922, 1729, 1700 cm^{-1}; FABHRMS (NBA/CsI) *m/z* 575.1036 (M + H$^+$, $C_{26}H_{27}IN_2O_5$ requires *m/z* 575.1043).

Methyl 5-(Benzyloxy)-3-(*tert*-butyloxycarbonyl)-1-[[(2′,2′,6′,6′-tetramethylpiperidino)oxy]methyl]-1,2-dihydro-3*H*-pyrido[3,2-*e*]indole-8-carboxylate (12). A solution of **11** (20 mg, 35 μmol, 1.0 equiv) in anhydrous toluene (1.2 mL) was treated with a solution of TEMPO (16 mg, 0.11 mmol, 3.0 equiv) in toluene (0.11 mL) and $(TMS)_3SiH$ (11 μL, 37 μmol, 1.05 equiv). The solution was warmed to 80 °C, and 5 equiv of TEMPO (2 × 14 mg in 0.29 mL toluene) and 4 equiv $(TMS)_3SiH$ (4 × 11 μL) were added in portions over the next 4 h. After 16 h, the reaction mixture was cooled to 25 °C and the volatiles were removed in vacuo. Chromatography (SiO_2, 1.5 × 12 cm, 20% EtOAc−hexane) provided **12** (18 mg, 85%) as a gold solid: mp 157−158 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.27 (d, J = 2.0 Hz, 1H), 8.84 (d, J = 2.0 Hz, 1H), 8.15 (br s, 1H), 7.55 (br s, 2H), 7.35 (apparent t, J = 7.2 Hz, 2H), 7.28 (m, 1H), 5.42 (d, J = 12.4 Hz, 1H), 5.38 (d, J = 12.3 Hz, 1H), 4.09 (m, 2H), 3.95 (s, 3H), 3.92 (m, 2H), 3.80 (m, 1H), 1.54 (s, 9H), 1.30 (m, 6H), 1.06 (s, 3H), 1.01 (s, 3H), 0.95 (s, 3H), 0.85 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.0, 154.9, 152.4, 146.4, 142.2, 138.5, 136.3, 134.6, 128.5 (2C), 128.0 (3C), 125.0, 123.3, 117.3, 102.4, 79.1, 70.9, 59.8, 52.8, 52.5, 39.6, 39.5, 37.7, 33.0, 32.8, 28.4 (3C), 20.1 (4C), 17.0; IR (film) ν_{max} 2969, 2927, 2876, 1729, 1703, cm^{-1}; FABHRMS (NBA/CsI) *m/z* 726.2383 (M + Cs$^+$, $C_{35}H_{45}N_3O_6$ requires *m/z* 736.2363). Anal. Calcd for $C_{35}H_{45}N_3O_6 \cdot H_2O$: C, 67.61; H, 7.62; N, 6.76. Found: C, 67.70; H, 7.11; N, 6.27.

Methyl 5-(Benzyloxy)-3-(*tert*-butyloxycarbonyl)-1-(hydroxymethyl)-1,2-dihydro-3*H*-pyrido[3,2-*e*]indole-8-carboxylate (13). A solution of **12** (20 mg, 33 μmol, 1.0 equiv) in a 3:1 mixture of THF−H_2O (0.90 mL) was treated with activated zinc powder (54 mg, 0.80 mmol, 25 equiv) and HOAc (0.20 mL), and the resulting suspension was warmed to 60 °C with vigorous stirring. After 7 h, additional Zn (54 mg) was added and the reaction was stirred for 4 h. The Zn powder was removed by filtration through Celite with a CH_2Cl_2 wash, and the mixture was concentrated in vacuo. The resulting residue was dissolved in EtOAc and filtered through Celite, and the solution was concentrated in vacuo. Chromatography (SiO_2, 1 × 12 cm, 20−50% EtOAc−hexane) provided **13** (9.2 mg, 60%): mp 186 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.05 (s, 1H), 8.65 (s, 1H), 8.06 (br s, 1H), 7.55 (br s, 2H), 7.36 (apparent t, J = 7.1 Hz, 2H), 7.30 (apparent t, J = 7.2 Hz, 1H), 5.28 (s, 2H), 4.16 (m, 1H), 4.09 (m, 1H), 4.00 (s, 3H), 3.76 (m, 3H), 1.55 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.9, 154.8, 152.3, 146.0, 138.1, 136.2, 133.6, 128.5 (2C), 128.0 (2C), 127.9, 124.8, 123.5, 115.0, 102.4, 82.0, 70.9, 64.9, 52.5, 52.3, 41.0, 28.4 (3C); IR (film) ν_{max} 3214, 2961, 2924, 2850, 1717, 1701 cm^{-1}; FABHRMS (NBA/CsI) *m/z* 465.2032 (M + H$^+$, $C_{26}H_{28}N_2O_6$ requires *m/z* 465.2026).

Methyl 5-(Benzyloxy)-3-(*tert*-butyloxycarbonyl)-1-(chloromethyl)-1,2-dihydro-3*H*-pyrido[3,2-*e*]indole-8-carboxylate (14). Method A. A solution of **13** (6.7 mg, 14 μmol, 1.0 equiv) in anhydrous CH_2Cl_2 (0.14 mL) under Ar was treated sequentially with Ph_3P (13 mg, 48 μmol, 3.0 equiv) and CCl_4 (14 μL, 0.15 mmol, 9.0 equiv). The reaction mixture was stirred at 25 °C for 3 h. The solvent was then evaporated under a stream of N_2. Radial chromatography (SiO_2, 1.0 mm, 20% EtOAc−hexane) afforded **14** (5.1 mg, 73%) as a gold solid: mp 194 °C (EtOAc−hexane); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.29 (d, J = 2.0 Hz, 1H), 8.62 (d, J = 1.9 Hz, 1H), 8.16 (br s, 1H), 7.53 (br s, 2H), 7.37 (m, 2H), 7.30 (m, 1H), 5.41 (m, 2H), 4.24 (d, J = 11.1 Hz, 1H), 4.13 (m, 1H), 4.00 (s, 3H), 3.98 (m, 1H, buried under 4.00 ppm), 3.82 (dd, J = 3.3, 11.1 Hz, 1H), 3.47 (t, J = 10.5 Hz, 1H), 1.56 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.7, 155.6, 152.2, 146.3, 143.0, 138.7, 136.0, 132.6, 128.5 (2C), 128.0 (3C), 124.4, 123.8, 114.5, 102.3, 81.6, 70.9, 53.0, 52.6, 46.8, 40.8, 28.3 (3C); IR (film) ν_{max} 2976, 2954, 2942, 1723, 1702 cm^{-1}; FABHRMS (NBA/NaI) *m/z* 483.1675 (M + H$^+$, $C_{26}H_{27}ClN_2O_5$ requires *m/z* 483.1687). Anal. Calcd for $C_{26}H_{27}ClN_2O_5$: C, 64.66; H, 5.63; N, 5.80. Found: C, 64.78; H, 5.73; N, 5.66.

Methyl 3-(*tert*-Butyloxycarbonyl)-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-pyrido[3,2-*e*]indole-8-carboxylate (15). A slurry of **14** (0.9 g, 1.9 mmol, 1.0 equiv) and 10% Pd−C (0.36 g) in THF (6.2 mL) under N_2 was cooled to −78 °C and degassed under vacuum. The reaction was warmed to 25 °C and treated with 25% aqueous HCO_2NH_4 (1.2 g in 4.7 mL H_2O, 19 mmol, 10 equiv). After 3 h, the catalyst was removed by filtration through Celite and the solvent was evaporated under reduced pressure to afford **15** (0.72 g, 99%) as a bright yellow solid: mp 162−163 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.09 (d, J = 1.8 Hz, 1H), 8.62 (d, J = 1.7 Hz, 1H), 8.28 (br s, 1H), 8.00 (br s, 1H), 4.22 (m, 1H), 4.13 (dd, J = 8.9, 11.6 Hz, 1H), 4.00 (s, 3H), 3.94 (m, 1H), 3.78 (dd, J = 3.5, 11.1 Hz, 1H), 3.47 (dd, J = 9.9, 11.0 Hz, 1H), 1.53 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.6, 153.5, 152.1, 144.9, 143.7, 136.2, 133.1, 124.4, 123.6, 113.6, 103.0, 81.7, 52.9 52.7, 46.6, 40.7, 28.3 (3C); IR (film) ν_{max} 3385, 2978, 2919, 1701 cm^{-1}; FABHRMS (NBA/NaI) *m/z* 393.1228 (M + H$^+$, $C_{19}H_{21}ClN_2O_5$ requires *m/z* 393.1217).

Methyl 2-(*tert*-Butyloxycarbonyl)-1,2,9,9a-tetrahydrocyclopropa[*c*]pyrido[3,2-*e*]indol-4-one-7-carboxylate (16, *N*-BOC-CPyI). A solution of **15** (81 mg, 0.21 mmol, 1.0 equiv) in CH_3CN (6.9 mL) at 25 °C under Ar was treated with DBU (0.12 mL, 0.83 mmol, 4.0 equiv) and stirred 3 h. Flash chromatography was applied directly to the reaction mixture

(SiO_2, 2.5 × 8 cm, 2% MeOH−CH_2Cl_2) and furnished **16** as a light yellow-white solid (68 mg, 93%, typically 90−99%): mp 255 °C (dec); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.28 (d, J = 1.9 Hz, 1H), 7.84 (d, J = 1.9 Hz, 1H), 6.98 (br s, 1H), 4.04 (m, 2H), 3.97 (s, 3H), 2.87 (dt, J = 4.0, 8.0 Hz, 1H), 1.68 (dd, J = 4.8, 7.9 Hz, 1H), 1.55 (m, 1H, buried under 1.53), 1.53 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 182.9, 165.0, 159.3, 151.3, 150.9, 149.1, 135.8, 131.4, 127.0, 110.0, 83.9, 53.0, 52.8, 33.1, 28.7, 28.1 (3C), 23.4; IR (film) ν_{max} 2974, 1728 cm^{-1}; UV (CH_3OH) λ_{max} 318 (ϵ = 9000), 240 (shoulder, ϵ = 11 000), 218 (ϵ = 15 500); FABHRMS (NBA/NaI) m/z 379.1282 (M + Na^+, $C_{19}H_{20}N_2O_5$ requires m/z 379.1270).

Resolution of *N*-BOC-CPyI. Samples of racemic **16** were resolved by semipreparative HPLC chromatography on a Daicel ChiralCel OD column (10 μm, 2 × 25 cm) using 50% *i*-PrOH−hexane eluant (7 mL/min). The enantiomers eluted with retention times of 27.3 and 37.8 min (α = 1.43). (+)-(8b*R*,9a*S*)-**16**: $[\alpha]^{22}_D$ +120 (*c* 0.46, THF). (−)-(8b*S*,9a*R*)-**16**: −118 (*c* 0.47, THF).

Methyl 1,2,9,9a-Tetrahydrocyclopropa[*c*]pyrido[3,2-*e*]-indol-4-one-7-carboxylate (17, CPyI). A solution of **16** (4.0 mg, 11 μmol, 1.0 equiv) in 3 M HCl−EtOAc (0.37 mL) 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. The residue was taken up in acetone (0.28 mL) and treated with K_2CO_3 (16 mg, 0.11 mmol, 10 equiv). After being stirred for 24 h at 25 °C, the reaction mixture was filtered through Celite to provide **17** as a yellow solid film (2.7 mg, 96%): ^{1}H NMR (acetone-d_6, 400 MHz) δ 9.07 (d, J = 2.0 Hz, 1H), 7.94 (d, J = 2.0 Hz, 1H), 7.06 (br s, 1H), 5.72 (s, 1H), 3.93 (s, 3H), 3.91 (m, 1H), 3.72 (apparent d, J = 10.8 Hz, 1H), 3.28 (m, 1H), 1.78 (dd, J = 4.0, 8.0 Hz, 1H), 1.43 (t, J = 4.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.9, 168.9, 165.4, 152.6, 148.7, 135.2, 130.9, 126.3, 98.8, 52.7, 50.7, 32.5, 29.4, 26.1; IR (film) ν_{max} 3163, 2923, 2853, 1727 cm^{-1}; UV (CH_3OH) λ_{max} 360 (ϵ = 7000), 298 (shoulder, ϵ = 5000), 226 (ϵ = 15 000); FABHRMS (NBA/NaI) m/z 257.0927 (M + H^+, $C_{14}H_{12}N_2O_3$ requires m/z 257.0926). (+)-(8b*R*,9a*S*)-**17**: $[\alpha]^{22}_D$ +41 (*c* 0.14, CH_2Cl_2). (−)-(8b*S*,9a*R*)-**17**: −45 (*c* 0.09, CH_2Cl_2).

Methyl 8-(Benzyloxy)-6-[*N*-(*tert*-butyloxycarbonyl)-*N*-(*E*-3-chloro-2-propenyl)amino]-5-iodoquinoline-3-carboxylate (18). A solution of **10** (1.2 g, 2.2 mmol, 1.0 equiv) in anhydrous DMF (20 mL) was cooled to 4 °C in a flamed-dried round-bottom flask under Ar and was treated with NaH (60% dispersion in oil, 98 mg, 2.5 mmol, 1.1 equiv). After 30 min, *E*-1,3-dichloropropene (0.61 mL, 6.7 mmol, 3.0 equiv) was added and the reaction mixture was gradually warmed to 25 °C and stirred 12 h. The reaction was poured on H_2O and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with EtOAc, and the combined organic extract was washed with saturated aqueous $NaHCO_3$, H_2O, and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 5.5 × 14 cm, 20−30% EtOAc−hexane gradient) afforded **18** (1.2 g, 85%, typically 84−94%) as a yellow foam (mixture of amide rotamers in $CDCl_3$). Major rotamer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.43 (s, 1H), 9.12 (s, 1H), 7.47 (d, J = 7.4 Hz, 2H), 7.35 (t, J = 7.2 Hz, 2H), 7.30 (t, J = 7.3 Hz, 1H), 6.87 (s, 1H), 5.92 (m, 1H), 5.85 (m, 1H), 5.45 (s, 2H), 4.34 (dd, J = 6.8, 14.9 Hz, 1H), 4.04 (s, 3H), 3.79 (dd, J = 7.6, 15.0 Hz, 1H), 1.22 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.2, 154.7, 153.2, 149.8, 144.0, 143.7, 141.6, 135.7, 130.2, 128.9 (2C), 128.3 (2C), 128.1, 127.0, 125.4, 122.3, 114.3, 94.8, 81.2, 71.2, 52.8, 48.8, 28.1 (3C); IR (film) ν_{max} 3062, 2971, 1729, 1704 cm^{-1}; FABHRMS (NBA/CsI) m/z 740.9646 (M + Cs^+, $C_{26}H_{26}ClN_2O_5I$ requires m/z 740.9629).

Methyl 5-(Benzyloxy)-3-(*tert*-butyloxycarbonyl)-1-(chloromethyl)-1,2-dihydro-3*H*-pyrido[3,2-*e*]indole-8-carboxylate (14). Method B. A solution of **18** (0.65 g, 1.1 mmol, 1.0 equiv) in benzene (20 mL) under Ar was treated with Bu_3SnH (0.15 mL, 0.50 mmol, 0.5 equiv) and catalytic AIBN (18 mg) and stirred at 70 °C. Additional Bu_3SnH (0.29 mL, 1.1 mmol, 1.0 equiv in 2 portions) was added over the next 1 h. After 3 h, the reaction mixture was concentrated in vacuo. Chromatography (SiO_2, 4 × 20 cm, 20−30% EtOAc−hexane gradient) provided **14** (0.46 g, 87%).

Methyl 1-(Chloromethyl)-5-hydroxy-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-pyrido[3,2-*e*]indole-8-carboxylate (*seco*-CPyI−TMI, 24). A solution of **16** (10.0 mg, 28.1 μmol, 1.0 equiv) in 3 M HCl−EtOAc (0.935 mL) 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. The residue was dissolved in anhydrous DMF (0.300 mL) and treated with 5,6,7-trimethoxyindole-2-carboxylic acid (**19**, 10.6 mg, 42.1 μmol, 1.5 equiv) and EDCI (27.0 mg, 140 μmol, 5.0 equiv). After being stirred for 10 h at 25 °C under Ar, the reaction mixture was concentrated in vacuo and suspended in H_2O. The precipitate was collected by centrifugation and washed with H_2O (4 mL). Flash chromatography (SiO_2, 0.7 × 7 cm, 1−5% MeOH−$CHCl_3$ gradient) afforded **24** (7.7 mg, 52%) as a light yellow solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.39 (br s, 1H), 9.20 (d, J = 1.7 Hz, 1H), 8.74 (d, J = 1.7 Hz, 1H), 8.39 (s, 1H), 8.33 (br s, 1H), 7.00 (d, J = 2.0 Hz, 1H), 6.86 (s, 1H), 4.78 (dd, J = 2.0, 10.5 Hz, 1H), 4.70 (t, J = 9.0 Hz, 1H), 4.16 (m, 1H), 4.08 (s, 3H), 4.04 (s, 3H), 3.93 (s, 3H), 3.90 (s, 3H), 3.88 (m, 1H, buried under 3.90 ppm), 3.52 (t, J = 10.0 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 165.3, 160.2, 154.3, 149.3, 145.0, 143.9, 140.1, 139.1, 137.4, 133.8, 130.6, 125.6, 123.7, 123.6, 123.2, 116.2, 106.6, 105.4, 98.0, 61.6, 61.2, 61.0, 56.0, 55.3, 52.6, 48.0; IR (film) ν_{max} 3332, 2926, 2838, 1725, 1621 cm^{-1}; FABHRMS (NBA/CsI) m/z 526.1397 (M + H^+, $C_{26}H_{24}ClN_3O_7Cl$ requires m/z 526.1381). (+)-(1*S*)-**24**: $[\alpha]^{25}_D$ +6 (*c* 0.33, $CHCl_3$). (−)-(1*R*)-**24**: $[\alpha]^{25}_D$ −6 (*c* 0.33, $CHCl_3$).

Methyl 2-[(5,6,7-Trimethoxyindol-2-yl)carbonyl]-1,2,9,-9a-tetrahydrocyclopropa[*c*]pyrido[3,2-*e*]indol-4-one-7-carboxylate (25, CPyI−TMI). A solution of **24** (1.5 mg, 2.8 μmol, 1.0 equiv) in anhydrous DMF (0.10 mL) at 25 °C was treated with DBU (1.3 μL, 8.6 μmol, 3.0 equiv) and stirred 3 h under Ar. Direct chromatography of the reaction mixture (0.7 × 3 cm, 5% MeOH−CH_2Cl_2) furnished **25** as a light yellow solid (1.2 mg, 86%): ^{1}H NMR (acetone-d_6, 400 MHz) δ 10.50 (br s, 1H), 9.15 (d, J = 1.7 Hz, 1H), 8.12 (d, J = 1.7 Hz, 1H), 7.18 (d, J = 2.6 Hz, 1H), 7.14 (s, 1H), 6.94 (s, 1H), 4.69 (dd, J = 4.7, 9.9 Hz, 1H), 4.58 (d, J = 10.3 Hz, 1H), 4.00 (s, 3H), 3.96 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H), 3.43 (m, 1H), 1.98 (dd, J = 5.8, 10.0 Hz, 1H), 1.82 (t, J = 5.7 Hz, 1H); IR (film) ν_{max} 3342, 2923, 2846, 1727, 1632 cm^{-1}; FABHRMS (NBA/NaI) m/z 490.1629 (M + H^+, $C_{26}H_{23}N_3O_7$ requires m/z 490.1614). (+)-(8b*R*,9a*S*)-**25**: $[\alpha]^{25}_D$ +42 (*c* 0.085, CH_2Cl_2). (−)-(8b*S*,9a*R*)-**25**: $[\alpha]^{25}_D$ −44 (*c* 0.045, CH_2Cl_2).

Methyl 1-(Chloromethyl)-5-hydroxy-3-[(5-methoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-pyrido[3,2-*e*]indole-8-carboxylate (26). Flash chromatography (SiO_2, 0.7 × 6 cm, 1−5% MeOH−$CHCl_3$ gradient) afforded **26** (59%) as a yellow solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.30 (br s, 1H), 9.21 (d, J = 1.7 Hz, 1H), 8.74 (d, J = 1.8 Hz, 1H), 8.39 (s, 1H), 8.27 (br s, 1H), 7.36 (d, J = 8.8 Hz, 1H), 7.12 (d, J = 2.2 Hz, 1H), 7.03 (m, 2H), 4.81 (dd, J = 2.0, 10.8 Hz, 1H), 4.72 (t, J = 8.8 Hz, 1H), 4.17 (m, 1H), 4.04 (s, 3H), 3.89 (m, 1H, buried under 3.86 ppm), 3.86 (s, 3H), 3.53 (t, J = 10.3 Hz, 1H); IR (film) ν_{max} 3340, 2924, 2857, 1718, 1603 cm^{-1}; FABHRMS (NBA/NaI) m/z 466.1186 (M + H^+, $C_{24}H_{20}ClN_3O_5$ requires m/z 466.1170). (+)-(1*S*)-**26**: $[\alpha]^{25}_D$ +13 (*c* 0.16, $CHCl_3$). (−)-(1*R*)-**26**: $[\alpha]^{25}_D$ −12 (*c* 0.27, $CHCl_3$).

Methyl 2-[(5-Methoxyindol-2-yl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[*c*]pyrido[3,2-*e*]indol-4-one-7-carboxylate (27). Flash chromatography (0.7 × 3 cm, 5% MeOH−CH_2Cl_2) furnished **27** as a light yellow solid (90%): ^{1}H NMR (acetone-d_6, 400 MHz) δ 10.88 (br s, 1H), 9.15 (d, J = 1.8 Hz, 1H), 8.13 (d, J = 1.8 Hz, 1H), 7.48 (d, J = 9.2 Hz, 1H), 7.24 (s, 1H), 7.21 (s, 1H), 7.14 (d, J = 2.2 Hz, 1H), 6.97 (dd, J = 2.4, 9.2 Hz, 1H), 4.74 (dd, J = 4.9, 10.0 Hz, 1H), 4.65 (d, J = 10.1 Hz, 1H), 3.95 (s, 3H), 3.81 (s, 3H), 3.46 (m, 1H), 1.98 (dd, J = 4.6, 7.9 Hz, 1H), 1.85 (t, J = 4.6 Hz, 1H); IR (film) ν_{max} 3205, 2951, 1719, 1624 cm^{-1}; FABHRMS (NBA/NaI) m/z 452.1209 (M + Na^+, $C_{24}H_{19}N_3O_5$ requires m/z 452.1222). (+)-(8b*R*,9a*S*)-**27**: $[\alpha]^{25}_D$ +49 (*c* 0.07, CH_2Cl_2). (−)-(8b*S*,9a*R*)-**27**: $[\alpha]^{25}_D$ −44 (*c* 0.055, CH_2Cl_2).

Methyl 1-(Chloromethyl)-5-hydroxy-3-[(indol-2-yl)carbonyl]-1,2-dihydro-3*H*-pyrido[3,2-*e*]indole-8-carboxylate (*seco*-CPyI−indole, 28). Flash chromatography (SiO_2,

0.7 × 7 cm, 1−5% MeOH−$CHCl_3$ gradient) afforded **28** (71%) as a yellow solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.38 (br s, 1H), 9.21 (d, J = 1.6 Hz, 1H), 8.75 (d, J = 1.7 Hz, 1H), 8.41 (s, 1H), 8.30 (br s, 1H), 7.73 (d, J = 8.2 Hz, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.35 (t, J = 7.5 Hz, 1H), 7.18 (t, J = 7.2 Hz, 1H), 7.13 (s, 1H), 4.83 (dd, J = 2.0, 11.0 Hz, 1H), 4.75 (t, J = 8.7 Hz, 1H), 4.18 (m, 1H), 4.04 (s, 3H), 3.89 (dd, J = 3.5, 11.1 Hz, 1H), 3.55 (t, J = 10.3 Hz, 1H); IR (film) ν_{max} 3331, 2923, 2853, 1723, 1614 cm^{-1}; FABHRMS (NBA/NaI) m/z 436.1052 (M + H$^+$, $C_{23}H_{18}ClN_3O_4I$ requires m/z 436.1064). (+)-(1*S*)-**28**: $[\alpha]^{25}_D$ +3 (*c* 0.35, $CHCl_3$). (−)-(1*R*)-**28**: $[\alpha]^{25}_D$ −3 (*c* 0.29, $CHCl_3$).

Methyl 2-[(Indol-2-yl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[*c*]pyrido[3,2-*e*]indol-4-one-7-carboxylate (CPyI−Indole, 29). Flash chromatography (0.7 × 3 cm, 5% MeOH−CH_2Cl_2) furnished **29** as a light yellow solid (93%): ^{1}H NMR (acetone-d_6, 400 MHz) δ 10.98 (br s, 1H), 9.15 (d, J = 1.6 Hz, 1H), 8.13 (d, J = 2.0 Hz, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.31 (m, 2H), 7.26 (s, 1H), 7.12 (t, J = 8.0 Hz, 1H), 4.77 (dd, J = 4.8, 10.0 Hz, 1H), 4.68 (d, J = 10.0 Hz, 1H), 3.96 (s, 3H), 3.47 (m, 1H), 1.99 (dd, J = 4.8, 8.0 Hz, 1H), 1.85 (t, J = 4.8 Hz, 1H); IR (film) ν_{max} 3217, 2922, 2847, 1728, 1637 cm^{-1}; FABHRMS (NBA/NaI) m/z 400.1310 (M + H$^+$, $C_{23}H_{17}N_3O_4$ requires m/z 400.1297). (+)-(8b*R*, 9a*S*)-**29**: $[\alpha]^{25}_D$ +48 (*c* 0.065, CH_2Cl_2). (−)-(8b*S*, 9a*R*)-**29**: $[\alpha]^{25}_D$ −43 (*c* 0.065, CH_2Cl_2).

Methyl 1-(Chloromethyl)-5-hydroxy-3-{[5-[*N*−(indol-2-yl)carbonyl]aminoindol-2-yl]carbonyl}-1,2-dihydro-3*H*-pyrido[3,2-*e*]indole-8-carboxylate (*seco*-CPyI−Indole$_2$, 30). Flash chromatography (SiO_2, 0.7 × 6 cm, 10% DMF−$CHCl_3$) afforded **30** (64%) as a yellow solid: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.82 (s, 1H), 11.73 (s, 1H), 10.45 (s, 1H), 10.20 (s, 1H), 9.14 (s, 1H), 8.89 (s, 1H), 8.23 (m, 2H), 7.67 (d, J = 7.8 Hz, 1H), 7.59 (d, J = 8.9 Hz, 1H), 7.47 (m, 2H), 7.42 (s, 1H), 7.28 (s, 1H), 7.21 (t, J = 7.2 Hz, 1H), 7.06 (t, J = 7.2 Hz, 1H), 4.87 (t, J = 10.0 Hz, 1H), 4.62 (m, 1H), 4.46 (m, 1H), 4.02 (dd, J = 2.8, 11.1 Hz, 1H), 3.97 (s, 3H), 3.91 (m, 1H); IR (film) ν_{max} 3282, 2921, 2840, 1702, 1620 cm^{-1}; FABHRMS (NBA/CsI) m/z 726.0548 (M + Cs$^+$, $C_{32}H_{24}ClN_5O_5$ requires m/z 726.0520). (+)-(1*S*)-**30**: $[\alpha]^{25}_D$ +26 (*c* 0.105, DMF). (−)-(1*R*)-**30**: $[\alpha]^{25}_D$ −28 (*c* 0.06, DMF).

Methyl2-{[5-[*N*−(Indol-2-yl)carbonyl]aminoindol-2-yl]-carbonyl}-1,2,9,9a-tetrahydrocyclopropa[*c*]pyrido[3,2-*e*]-indol-4-one-7-carboxylate (CPyI−Indole$_2$, 31). Flash chromatography (SiO_2, 0.7 × 2 cm, 10% DMF−CH_2Cl_2) afforded **31** (97%) as a yellow solid: ^{1}H NMR (DMF-d_7, 600 MHz) δ 11.85 (s, 1H), 11.75 (s, 1H), 10.29 (s, 1H), 9.19 (d, J = 2.0 Hz, 1H), 8.41 (s, 1H), 8.29 (d, J = 2.0 Hz, 1H), 7.75 (dd, J = 2.0, 8.7 Hz, 1H), 7.70 (d, J = 8.2 Hz, 1H), 7.60 (m, 2H), 7.53 (s, 1H), 7.38 (s, 1H), 7.26 (t, J = 7.7 Hz, 1H), 7.22 (s, 1H), 7.10 (t, J = 7.7 Hz, 1H), 4.81 (dd, J = 4.6, 10.2 Hz, 1H), 4.68 (d, J = 10.2 Hz, 1H), 3.99 (s, 3H), 3.60 (m, 1H), 2.08 (dd, J = 4.6, 7.7 Hz, 1H), 1.93 (t, J = 5.1 Hz, 1H); IR (film) ν_{max} 3275, 2921, 2851, 1718, 1636 cm^{-1}; MALDIHRMS (DHB) m/z 558.1757 (M + H$^+$, $C_{32}H_{23}ClN_5O_5$ requires m/z 558.1778). (+)-(1*S*)-**31**: $[\alpha]^{25}_D$ +50 (*c* 0.04, DMF). (−)-(1*R*)-**31**: $[\alpha]^{25}_D$ −46 (*c* 0.05, DMF).

Methyl 3-[(3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]-indol-7-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-pyrido[3,2-*e*]indole-8-carboxylate (*seco*-CPyI−CDPI$_1$, 32). Flash chromatography (SiO_2, 0.7 × 7 cm, 5% MeOH−10% DMF−$CHCl_3$) afforded **32** (41%) as a yellow solid: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.70 (s, 1H), 10.45 (s, 1H), 9.13 (d, J = 2.0 Hz, 1H), 8.88 (d, J = 2.0 Hz, 1H), 8.20 (s, 1H), 8.01 (d, J = 8.8 Hz, 1H), 7.23 (d, J = 8.1 Hz, 1H), 7.07 (s, 1H), 6.13 (s, 2H), 4.84 (t, J = 11.0, 1H), 4.58 (m, 1H), 4.45 (m, 1H), 4.00−3.91 (m, 4H, obscured by 3.97 ppm), 3.97 (s, 3H), 3.26 (m, 2H, obscured by H_2O); IR (film) ν_{max} 3344, 2923, 2841, 1715, 1660 cm^{-1}; MALDIHRMS (DHB) m/z 520.1383 (M + H$^+$, $C_{26}H_{22}ClN_5O_5$ requires m/z 520.1388). (+)−(1*S*)-**32**: $[\alpha]^{25}_D$ +23 (*c* 0.04, DMF). (−)-(1*R*)-**32**: $[\alpha]^{25}_D$ −20 (*c* 0.06, DMF).

Methyl 2-[(3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]-indol-7-yl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa[*c*]pyrido[3,2-*e*]indol-4-one-7-carboxylate (CPyI−CDPI$_1$, 33). Flash chromatography (SiO_2, 0.7 × 4 cm, 5% MeOH−10% DMF−CH_2Cl_2) afforded **33** (67%) as a yellow solid: ^{1}H NMR (DMF-d_7, 500 MHz) δ 11.73 (br s, 1H), 9.19 (d, J = 1.8 Hz, 1H), 8.28 (d, J = 1.7 Hz, 1H), 8.20 (d, J = 9.0 Hz, 1H), 7.37 (d, J = 8.5 Hz, 1H), 7.24 (s, 1H), 7.20 (s, 1H), 6.15 (br s, 2H), 4.79 (dd, J = 4.7, 9.8 Hz, 1H), 4.68 (d, J = 10.3 Hz, 1H), 4.15 (t, J = 8.6 Hz, 2H), 3.98 (s, 3H), 3.59 (m, 1H), 3.39 (m, 2H, obscured by H_2O), 2.08 (dd, J = 4.7, 8.2 Hz, 1H), 1.92 (t, J = 5.1 Hz, 1H); IR (film) ν_{max} 3338, 2917, 2848, 1718, 1653 cm^{-1}; MALDIHRMS (DHB) m/z 484.1613 (M + H$^+$, $C_{26}H_{21}N_5O_5$ requires m/z 484.1621). (+)-(1*S*)-**33**: $[\alpha]^{25}_D$ +50 (*c* 0.03, DMF). (−)-(1*R*)-**33**: $[\alpha]^{25}_D$ −53 (*c* 0.03, DMF).

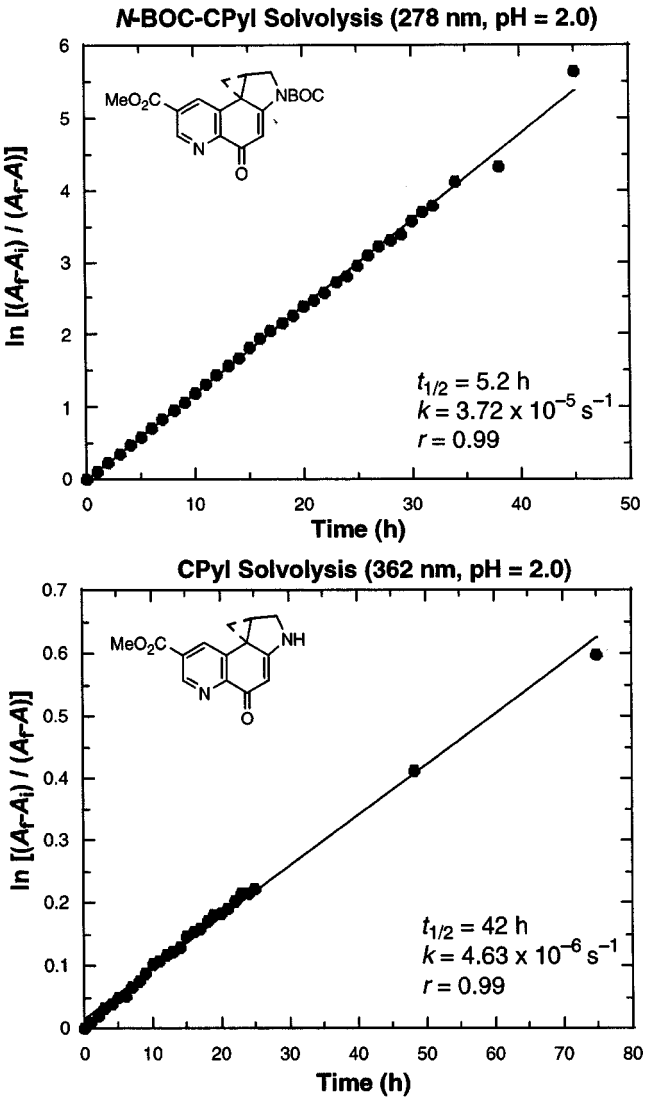


Figure 10.

Aqueous Solvolysis of *N*-BOC-CPyI and CPyI (pH 2 and pH 3, Phosphate Buffer). Samples of **16** (0.15 mg) and **17** (0.05 mg) were dissolved in CH_3OH (1.5 mL) and mixed with pH 3.0 buffer (1.5 mL, 4:1:20 (v:v:v) 0.1 M citric acid, 0.2 M Na_2HPO_4, and H_2O, respectively). Similarly, samples of **16** (0.1 mg) and **17** (0.05 mg) were dissolved in CH_3OH (1.5 mL) and mixed with pH 2.0 buffer (1.5 mL, 4:1:20 (v:v:v) 1.0 M citric acid, 0.2 M Na_2HPO_4, and H_2O, respectively). After mixing of the reagents, the UV spectra of the solution were measured against a reference solution containing CH_3OH (1.5 mL), and the appropriate aqueous buffer (1.5 mL) and these readings were used for the initial absorbance values (A_i). The UV spectrum was measured at regular intervals for 30 d (**16** at pH 3), 14 d (**16** at pH 2), 40 d (**17** at pH 3), and 8 d (**17** at pH 2). For **16**, the decrease in the long-wavelength absorption at 315 nm and increase in the short-wavelength absorption at 278 nm were monitored. The solvolysis rate constant and half-life (pH 3, $k = 3.81 \times 10^{-6}$ s^{-1}, $t_{1/2}$ = 51 h, r = 0.99; pH 2, $k = 3.72 \times 10^{-5}$ s^{-1}, $t_{1/2}$ = 5.2 h, r = 0.99) were calculated from the least-squares treatment of the slope of the plot of time versus $\ln[(A_f - A_i)/(A_f - A)]$ (Figure 10). For **17**, the decrease

in the long-wavelength absorption at 362 nm and increase in the short-wavelength absorption at 290 nm were monitored. The solvolysis rate constant and half-life were calculated by the same treatment providing $k = 6.27 \times 10^{-7}$ s^{-1} ($t_{1/2} = 310$ h, $r = 0.99$) for pH 3 and $k = 4.63 \times 10^{-6}$ s^{-1} ($t_{1/2} = 42$ h, $r = 0.99$) for pH 2.

Acid-Catalyzed Addition of CH_3OH to *N*-BOC-CPyI: Methyl 3-(*tert*-Butyloxycarbonyl)-5-hydroxy-1-(methoxymethyl)-1,2-dihydro-3*H*-pyrido[3,2-*e*]indole-8-carboxylate (41). A solution of **16** (3.0 mg, 8.4 μmol, 1.0 equiv) in CH_3OH (0.43 mL) was treated with CF_3SO_3H (0.13 mL, 2.5 μmol, 0.3 equiv) at 25 °C. After 20 h, the reaction was quenched by the addition of $NaHCO_3$ (9 mg), filtered through Celite, and concentrated in vacuo. Chromatography (SiO_2, 0.7 × 7 cm, 5−50% EtOAc−$CHCl_3$ gradient) afforded **41** (3.0 mg, 91%) as a yellow solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.10 (d, $J = 1.9$ Hz, 1H), 8.80 (s, 1H), 8.28 (br s, 1H), 8.07 (br s, 1H), 4.09 (m, 2H), 4.00 (s, 3H), 3.86 (m, 1H), 3.61 (dd, $J = 5.3$, 9.0 Hz, 1H), 3.37 (m, 1H), 3.36 (s, 3H), 1.58 (s, 9H); IR (film) ν_{max} 3386, 2979, 2926, 1721, 1708, 1624 cm^{-1}; FABHRMS (NBA/NaI) *m*/*z* 389.1706 (M + H$^+$, $C_{20}H_{24}N_2O_6$ requires *m*/*z* 389.1713).

Addition of HCl to *N*-BOC-CPyI. A solution of **16** (2.3 mg, 6.5 μmol, 1.0 equiv) in THF (0.15 mL) was cooled to −78 °C and treated with 4 M HCl−EtOAc (3.0 μL, 11 μmol, 1.7 equiv). The mixture was stirred for 2 min before the solvent was removed in vacuo. Chromatography (SiO_2, 0.7 × 7 cm, 10% EtOAc−$CHCl_3$) afforded **15** (2.4 mg, 96%).

Aqueous Solvolysis of *N*-BOC-CPyI (pH 2−11, Universal Buffer). Samples of **16** (0.025 mg) were dissolved in CH_3OH (1.0 mL), and the resulting solutions were mixed with a universal aqueous buffer[28] (pH 2−11, 1.0 mL, $B(OH)_3$−citric acid−Na_3PO_4). After mixing of the reagents, the UV spectra of the solution were measured against reference solutions and these readings were used for the initial absorbance values (A_i). The UV spectrum was measured at regular intervals (pH 2−4, every 1 h for 1 d and then every 24 h; pH 4−10, every 24 h) until no further change in absorbance was observed (A_f). The decrease in the long-wavelength absorption at 320 nm and increase in the short-wavelength absorption at 278 nm were monitored. The solvolysis rate constants and half-lives were calculated from the least-squares treatment of the slope of the plot of time versus $\ln[(A_f - A_i)/(A_f - A)]$.

Lewis Acid-Catalyzed Addition of CH_3OH to *N*-BOC-CPyI. A solution of **16** (1.2 mg, 3.4 μmol, 1.0 equiv) in CH_3OH (0.14 mL) was treated with $Zn(OTf)_2$ (1.4 mg, 3.9 μmol, 1.1 equiv) at 25 °C. After 4 h, the solvent was removed with a stream of N_2. Flash chromatography (SiO_2, 0.7 × 7 cm, 20−50% EtOAc−$CHCl_3$ gradient) afforded **41** (1.2 mg, 92%).

Metal-Catalyzed Solvolysis of *N*-BOC-CPyI. Samples of **16** (0.025 mg) were dissolved in CH_3OH (1.9 mL), and the resulting solutions were treated with 125 μL (1.0 equiv) or 25 μL (0.2 equiv) of a 0.56 mM solution (CH_3OH) of the desired metal ($Cu(acac)_2$, $Mg(acac)_2$, $Ni(acac)_2$, $Zn(acac)_2$, $Mn(acac)_2$, $Mg(acac)_2$, $Fe(acac)_3$, $Cr(acac)_3$, $Zn(OTf)_2$, $Ti(O\textit{i}\text{-}Pr)_4$, or $Cu(OMe)_2$). The solvolysis solution was sealed and kept at 25 °C protected from light. After mixing of the reagents, the UV spectra of the solution were measured against reference solutions, and these readings were used for the initial absorbance values (A_i). The UV spectrum was measured at regular intervals until no further change in absorbance was observed (A_f). The decrease in the long-wavelength absorption at 330 nm and increase in the short-wavelength absorption at 270 nm were monitored. The solvolysis rate constants and half-lives were calculated from the least-squares treatment of the slope of the plot of time versus $\ln[(A_f - A_i)/(A_f - A)]$.

Acknowledgment. We gratefully acknowledge the financial support of the National Institutes of Health (Grant CA41986), the Skaggs Institute for Chemical Biology, and the award of an ACS Medicinal Division fellowship sponsored by Bristol-Myers Squibb (1998-1999, C.W.B.). We thank Michael Hedrick and Qing Jin for conducting the cytotoxicity studies. Cover art prepared by Scott Wolkenberg and Jiyong Hong.

Supporting Information Available: ^{1}H NMR spectra for **5**−**18**, **24**−**33**, and **41**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO000177B