JOC Article

Synthesis and Evaluation of Duocarmycin and CC-1065 Analogues Incorporating the 1,2,9,9a-Tetrahydrocyclopropa[*c*]benz[*e*]-3-azaindol-4-one (CBA) Alkylation Subunit

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Received July 30, 2003

An efficient eight-step synthesis (53% overall) and the evaluation of 1,2,9,9a-tetrahydrocyclopropa-[*c*]benz[*e*]-3-azaindol-4-one (CBA) and its derivatives containing an aza variant of the CC-1065/duocarmycin alkylation subunit are detailed. This unique deep-seated aza modification provided an unprecedented 2-aza-4,4-spirocyclopropacyclohexadienone that was characterized chemically and structurally (X-ray). CBA proved structurally identical with CBI, the carbon analogue, including the stereoelectronic alignment of the key cyclopropane, its bond lengths, and the bond length of the diagnostic C3a−N2 bond, reflecting the extent of vinylogous amide (amidine) conjugation. Despite these structural similarities, CBA and its derivatives were found to be much more reactive toward solvolysis and hydrolysis, much less effective DNA alkylating agents (1000-fold), and biologically much less potent (100- to 1000-fold) than the corresponding CBI derivatives.

Introduction

CC-1065 (**1**)[1] and the duocarmycins (**2** and **3**)[2,3] are the parent members of a class of potent antitumor antibiotics[4] that derive their properties through a sequence-selective alkylation of duplex DNA (Figure 1).[5,6] Extensive studies have characterized their structural features responsible for the DNA alkylation reaction and have established fundamental relationships between their structure and reactivity or activity.[5−10] Aside from the structural complexity inherent in the alkylation subunit, they possess a stability that defies intuition. This is due principally to the vinylogous amide conjugation with and stabilization of the cyclohexadienone structure, which is dominant over that activating the cross-conjugated cyclopropane.[11−13] Accordingly, disruption of this vinylogous amide conjugation leads to remarkable increases in reactivity as large as 10^4-fold[13] that we have suggested is the source of catalysis for the DNA alkylation reaction.[7−11]

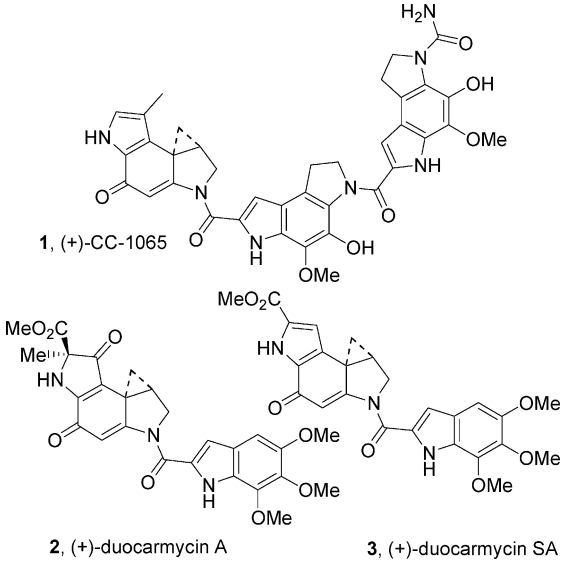

FIGURE 1. Structures of CC-1065 and the duocarmycins.

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(13) Boger, D. L.; Turnbull, P. *J. Org. Chem.* **1997**, *62*, 5849. Boger, D. L.; Turnbull, P. *J. Org. Chem.* **1998**, *63*, 8004. CSD NILJUN for *N*-CO_2Me-CBI.

10.1021/jo035119f CCC: $25.00
Published on Web 10/24/2003

The synthesis of analogues containing deep-seated structural changes have been central to these studies providing insights not accessible through examination of the natural products themselves.[14] Among those introduced, the 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI) alkylation subunit has emerged as the most extensively examined series, Figure 2.[15−59] Not only is it the most synthetically accessible alkylation subunit in a rich series, but its derivatives exhibit biological properties that surpass those of **1** and **2** while approaching those of **3**, and it exhibits a stability and inherent reaction regioselectivity that are near optimal.[15]

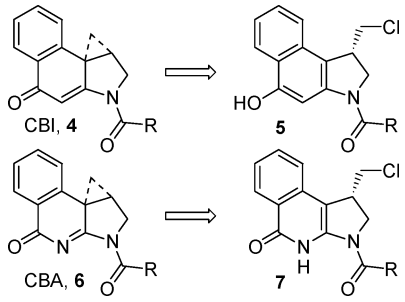

FIGURE 2. Structures of CBI and CBA.

Characteristic of this class of compounds, the cyclopropane precursors such as **5** not only stand up to prolonged storage more effectively than **4** but readily close in vitro and in vivo, displaying biological properties that are not distinguishable from the final cyclopropane-containing compounds. In a continued examination of modified alkylation subunits, we targeted the pyridone **7** as an especially storage-stable precursor to the unique 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]-3-azaindol-4-one (CBA, **6**) alkylation subunit. Although **7** was anticipated to be more stable than the CBI derivative **5**, the properties of **6**, incorporating an unprecedented 2-aza-4,4-spirocyclopropacyclohexadienone, were unknown. Herein, we provide full details[60] of the synthesis and X-ray structural characterization of this system and an examination of its chemical and biological properties.

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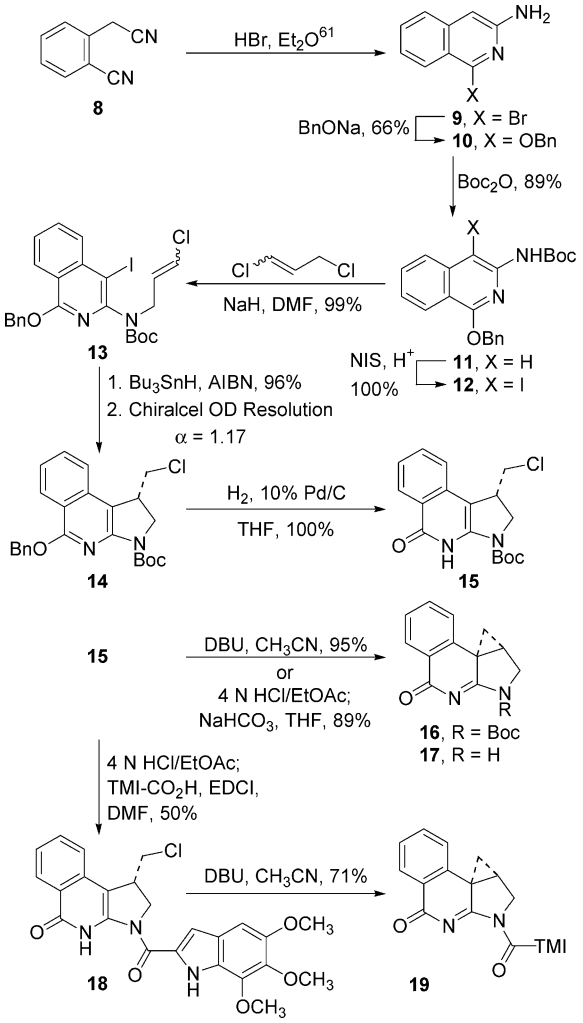
SCHEME 1



Results and Discussion

Synthesis. The synthesis of CBA began by treatment of **8** under conditions disclosed to provide 1-bromo-2-aminoisoquinoline (**9**, Scheme 1).[61] Nucleophilic introduction of the C1 benzyloxy group and subsequent Boc protection of the resulting amine **10** provided **11** (59%, 2 steps). Regioselective C4 iodination of **11** and subsequent *N*-alkylation of **12** with 1,3-dichloropropene afforded **13** and set the stage for a key 5-*exo*-*trig* aryl radical-alkene cyclization to form **14**.[62] Resolution of **14** by chromatographic separation on a semiprep Chiralcel OD column provided both enantiomers cleanly, which were then subjected to hydrogenolysis to provide **15** (*R* enantiomer not shown).[63,64] Spirocyclization of **15** was effected by treatment with DBU in anhydrous CH_3CN to give *N*-Boc-CBA (**16**) in superb yield (95%) in eight steps and 53% overall yield. Additionally, treatment of **15** with HCl and subsequent spirocyclization afforded CBA (**17**, 89%).

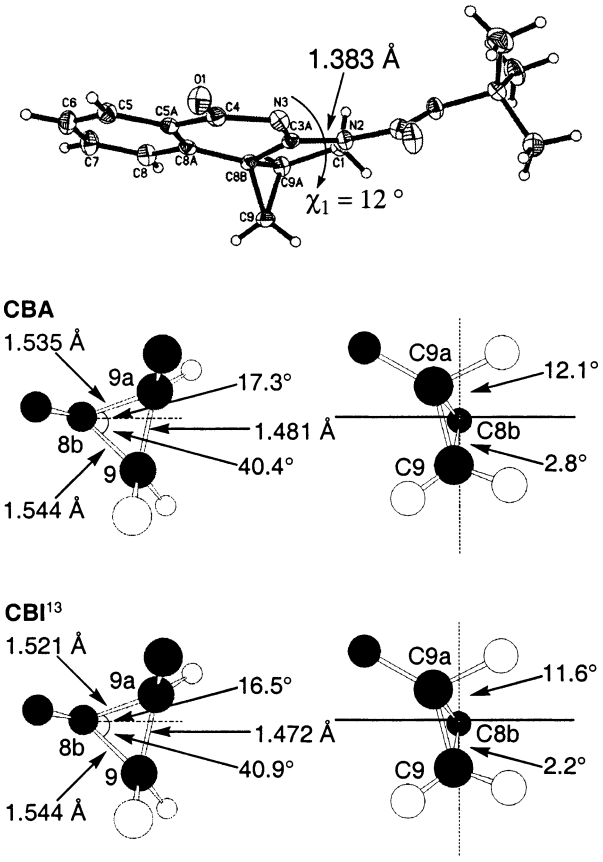


FIGURE 3. X-ray structure of **16**.

Likewise, *N*-Boc deprotection of **15** and subsequent coupling of the amine hydrochloride salt with 5,6,7-trimethoxyindole-2-carboxylic acid[65] (TMI) provided **18** (50%), which was spirocyclized to provide **19** using DBU (71%).

The assignment of the absolute configuration is confidently, but not unambiguously, assigned on the basis of the more potent biological activity of the assigned natural enantiomers, the distinguishable and readily assignable DNA alkylation selectivities of the natural and unnatural enantiomers, and the characteristic strong dextrorotatory $[\alpha]_D$ of the natural enantiomers of the final cyclopropane-containing agents.

X-ray Structure of *N*-Boc-CBA (16). With crystalline **16** in hand, its single-crystal X-ray structure determination[65] was conducted on needles obtained by recrystallization from 9:1 hexanes−CH_2Cl_2. Not only are its overall structural characteristics identical to those of *N*-CO_2Me-CBI,[13] including the stereoelectronic alignment of the key cyclopropane (Figure 3), but the two structures are nearly superimposable. Essentially all bond lengths of *N*-Boc-CBA were within ±0.02 Å of those of *N*-CO_2-Me-CBI, including not only all three cyclopropane bonds of which the reacting C8b−C9 bonds are identical (1.544 vs 1.544 Å) but also the C3a−N2 bond (1.383 vs 1.390 Å, respectively) whose length is diagnostic of the extent of vinylogous amide conjugation.[7,8] The only exceptions are the slightly more perturbed C3a−N3 versus C3a−C3

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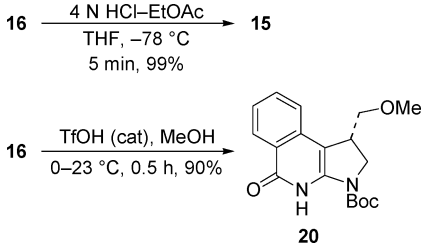
(63) Resolution was performed at this stage as a result of optimum solubility. Separation of the enantiomers of **15** (α = 1.17) and **16** (α = 1.26) is also possible.
(64) Boger, D. L.; Yun, W. *J. Am. Chem. Soc*. **1994**, *116*, 7996.
(65) The atomic coordinates for **16** have been deposited with the Cambridge Crystallographic Data Centre under deposition number CDCC 209699.

SCHEME 2



bond (1.30 vs 1.35 Å) and the adjacent N3−C4 vs C3−C4 bond (1.40 vs 1.44 Å) reflecting the intrinsically shorter C=N vs C=C bond lengths.

Notably, the cyclopropane alignment of **16**, like that of CBI, is such that the bent orbitals of the longer C8b−C9 cyclopropane bond are aligned with the conjugated π-system, whereas those of the alternative and shorter C8b−C9a bond are nearly orthogonal. Additionally remarkable is the near identical relative orientation of the N2 carbamate. With **16**, this places the carbamate carbonyl proximal to N3, introducing what is likely a destabilizing electrostatic repulsion between the N3 lone pair and the carbamate carbonyl lone pair. Despite this interaction, the χ1 dihedral angle (12°) exhibits no significant distortion in efforts to relieve the destabilizing electrostatic interaction, nor does it adopt the alternative transoid versus cisoid conformation.

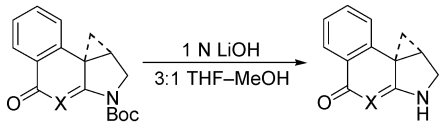
The stability of **16** and its unprecedented 2-aza-4,4-spirocyclopropacyclohexadienone may be attributed to the geometrical constraints of the fused five-membered ring that prevents an ideal, bisected conjugation of the cyclopropane with the π-system[13,40] and, most importantly, the N2 amidine cross-conjugation.

Reaction Regioselectivity. Consistent with the stereoelectronic alignment of the cyclopropane, acid-catalyzed nucleophilic addition proceeded exclusively (>20:1) at the least substituted cyclopropane carbon with cleavage of the reactive and aligned C8b−C9 bond (Scheme 2). Notably, this stereoelectronic control overrides any intrinsic preference for ring expansion ring opening with partial positive charge development on the more substituted cyclopropane carbon and may benefit from the preference for S_N2 nucleophilic attack to occur at the least substituted carbon. Thus, treatment of **16** with anhydrous HCl resulted in exclusive formation of **15** in 5 min at −78 °C (99%). Similarly, treatment of **16** with CF_3SO_3H−MeOH (0.02 M CF_3SO_3H, 0−23 °C, 30 min) led to exclusive formation of **20** (90%) derived from MeOH addition to the least substituted cyclopropane carbon.

Significantly, **16** was much more sensitive to base treatment under protic conditions than CBI. Thus, rapid hydrolysis (1 N LiOH, 3:1 THF−MeOH) of the carbamate of **16** to provide CBA (**17**) is observed at room temperature (≤5 min, 98%) or 0 °C (30 min, >95%). In contrast, an identical treatment of *N*-Boc-CBI led to a much slower hydrolysis at room temperature (6 h) and no reaction at 0 °C, indicating that it is roughly 100-fold less reactive (Scheme 3). Similarly, CBA-TMI (**19**) proved very sensitive to protic base, undergoing rapid and quantitative hydrolysis to CBA (**17**) upon treatment with LiOH (23 °C, 15 min).

This latter reactivity proved to be key to understanding the behavior of the CBA system. This hydrolytic reactivity of **16** and **19** indicates that there is little, if any, stabilizing conjugation of the N2 lone pair with the Boc carbonyl. Rather, the N2 lone pair is intimately tied into the amidine conjugation. Thus, unlike CBI, which prefers to maintain the linking amide conjugation and relinquish its vinylogous amide conjugation if perturbed, CBA in structures such as CBA-TMI (**19**) prefers to relinquish the linking amide (an acyl amidine) rather than disrupt the more intimate amidine conjugation. Notably, this is analogous to the behavior observed with modifications in the CBI-TMI linking amide.[42]

SCHEME 3



	temp	time (% overall)
16, X = N	23 °C	5 min (98%)
	0 °C	30 min (96%)
	–78 °C	stable
X = CH	23 °C	6 h (92%)
	0 °C	stable
	–78 °C	not done

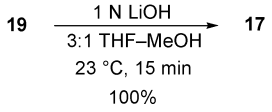


TABLE 1. Solvolysis Reactivity[a]

	CBA (**17**)	CBI[13]
k, s^{-1} (pH 3)	6.44×10^{-5}	2.07×10^{-7}
$t_{1/2}$, h (pH 3)	3.0	903

[a] pH = 3: 50% CH_3OH−buffer, buffer is 4:1:20 (v/v/v) 0.1 M citric acid, 0.2 M Na_2HPO_4, and deionized H_2O.

Solvolysis Reactivity. The chemical solvolysis of **16** at pH = 3 (50% CH_3OH−buffer, buffer is 4:1:20 (v/v/v) 0.1 M citric acid, 0.2 M Na_2HPO_4, and deionized H_2O) and pH = 7 (50% H_2O−CH_3OH) was not observed and led instead to the hydrolysis of the *N*-Boc group, Thus, complete hydrolysis of the Boc was observed to provide **17** at both pH = 3 and 7 in 0.25 and 1.3 h, respectively. In contrast, the apparent solvolysis of CBA (**17**) was measurable and was found to be >100-fold more reactive than CBI (Table 1). This reactivity was assumed, but not confirmed, to represent cyclopropane solvolysis. Regardless of the origin of this increased reactivity, this behavior was found to correlate with a corresponding loss in biological potency.

DNA Alkylation Properties. A comparison of DNA alkylation efficiency and selectivity of (+)-CBA-TMI (**19**) and (+)-duocarmycin SA (**3**) was conducted following protocols previously detailed[66] (Figure 4). These comparisons revealed that the alkylation selectivity for (+)-**19** was indistinguishable from that of (+)-**3**, and this is illustrated nicely in Figure 4 where both compounds exclusively alkylate the same 5′-AATTA-3′ high affinity site. This selectivity confirmed the tentatively assigned

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

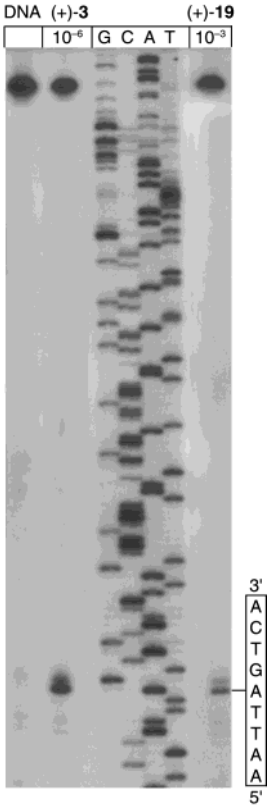


FIGURE 4. Thermally induced strand cleavage of w794 DNA (SV40 DNA segment, 144 bp, nucleotide nos. 138−5238). DNA-agent incubation at 25 °C for (+)-duocarmycin SA (24 h) and 37 °C for (+)-CBA-TMI (120 h), removal of unbound agent and 30 min thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography. Lane 1, control DNA; lane 2, (+)-duocarmycin SA ((+)-**3**, 1×10^{-6} M); lanes 3−6, Sanger G, C, A, and T reactions; lane 7, (+)-CBA-TMI ((+)-**19**, 1×10^{-3} M).

natural absolute configuration of (+)-**19**. In contrast to (+)-**3** and (+)-CBI-TMI, the rate and efficiency of DNA alkylation by (+)-CBA-TMI (**19**) was low. The detectable alkylation for **19** was observed only at high agent concentrations (10^{-3} vs 10^{-6} M), at increased reaction times (120 vs 2−24 h), and at elevated reaction temperatures (37 vs 5−23 °C). Potentially, this may be attributed to either competitive hydrolysis of **19** under the pH 8 incubation conditions or the lack of alkylation catalysis upon DNA binding. The former may well be expected on the basis of the enhanced sensitivity of **19** to aqueous base hydrolysis (Scheme 3) and the latter anticipated to arise from a DNA binding induced conformation change that would preferentially relinquish the acyl amidine conjugation over that of the amidine itself. Regardless of the origin of the effect, the less effective DNA alkylation by (+)-**19** correlates well with its less potent cytotoxic activity (1000-fold vs (+)-CBI-TMI and 1000- to 10 000-fold vs (+)-**3**).

Cytotoxic Activity. The in vitro cytotoxic activity of the CBA-based analogues is summarized in Table 2. In general, the CBA-based analogues were found to be 100- to 1000-fold less active than the corresponding CBI-based agents. For example, *N*-Boc-CBA (**16**) was 50-fold less potent than *N*-Boc-CBI. Similarly, CBA-TMI (**19**) was 1000-fold less potent than CBI-TMI. Removing the N2-acyl group with **17** ($IC_{50} = 5\ \mu M$) did not result in a large decrease in potency relative to **16**, but both **16** and **17** were roughly 100-fold less potent than **19**, which is consistent with past observations. As expected, the unnatural enantiomers exhibited reduced cytotoxic activity compared to that of the natural enantiomers and the seco precursors **15** and **18** exhibited cytotoxic activity indistinguishable from that of the cyclopropane-containing final products.

Conclusion

An efficient eight-step synthesis (53% overall) of CBA and its derivatives is detailed constituting the preparation of an unprecedented CC-1065/duocarmycin alkylation subunit aza analogue. Although an X-ray comparison of *N*-Boc-CBA (**16**) with *N*-Boc-CBI revealed nearly identical structures, the solvolysis and hydrolysis reactivity of CBA was much greater than that of CBI. Presumably this is a consequence of a CBA preference to relinquish the linking acyl conjugation over that of the imbedded amidine itself. Characteristic of this behavior, the CBA derivatives displayed a DNA alkylation rate and efficiency that was 1000- to 10 000-fold lower than the corresponding CBI derivatives while exhibiting the same DNA alkylation sequence selectivity. Similarly, the cytotoxic activity of the CBA derivatives was 100- to 1000-fold less potent than the corresponding CBI-based agents.

Experimental Section

3-Amino-1-benzyloxyisoquinoline (10). A solution of benzyl alcohol (0.70 mL, 6.72 mmol) in DMF (8 mL) cooled to 0 °C was treated with NaH (60% mineral oil dispersion, 0.269 g, 6.72 mmol) in portions and stirred at 0 °C for an additional 0.5 h. A solution of 3-amino-1-bromoisoquinoline[61] (0.500 g, 2.24 mmol) in DMF (4 mL) was treated dropwise with this solution. The deep red mixture was warmed to 25 °C and stirred for 1.5 h. The reaction was quenched with the addition of saturated aqueous $NaHCO_3$ (25 mL) and extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with H_2O (3 × 20 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (SiO_2, 10% EtOAc−hexanes) afforded 3-amino-1-benzyloxyisoquinoline (**10**, 0.372 g, 66%) as a tan oil: $R_f = 0.61$ (33% EtOAc−hexanes); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.15 (d, $J = 8.2$ Hz, 1H), 7.55 (d, $J = 7.0$ Hz, 2H), 7.49 (m, 2H), 7.43 (t, $J = 7.0$ Hz, 2H), 7.36 (t, $J = 7.3$ Hz, 1H),

TABLE 2. In Vitro Cytotoxic Activity

compound	IC_{50} (nM)[a]	compound	IC_{50} (nM)[a]
(+)-*N*-Boc-CBI	80	(−)-*N*-Boc-CBI	900
(+)-CBI-TMI	0.03	(−)-CBI-TMI	2
(+)-*N*-Boc-CBA, **16** (**15**)	4000 (4000)	(−)-*N*-Boc-CBA, **16** (**15**)	9000 (10 000)
(+)-CBA, **17**	5000	(−)-CBA, **17**	60000
(+)-CBA-TMI, **19** (**18**)	50 (40)	(−)-CBA-TMI, **19** (**18**)	3000 (5000)

[a] L1210 cytotoxic activity, average of 2−7 determinations in triplicate.

7.19 (m, 1H), 6.33 (s, 1H), 5.55 (s, 2H), 4.26 (br s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.3, 152.2, 141.3, 137.7, 130.8, 128.6 (2C), 128.0 (2C), 127.9, 124.6, 124.3, 122.4, 114.6, 92.6, 67.9; IR (film) ν_{max} 3473, 3382, 1633, 1336, 1156 cm^{-1}; MALDIFT-HRMS (DHB) *m/z* 251.1182 (M + H$^+$, $C_{16}H_{14}N_2O$ requires 251.1179).

1-Benzyloxy-3-[(*tert*-butyloxycarbonyl)amino]isoquinoline (11). A solution of **10** (0.372 g, 1.48 mmol) in *t*-BuOH (4.2 mL) was treated with Boc_2O (0.581 g, 2.66 mmol) and stirred for 24 h at 25 °C during which time the yellow solution turned light orange and a precipitate formed. The mixture was filtered, and the solid was washed with hexanes and collected. The filtrate was concentrated in vacuo and purified by flash chromatography (SiO_2, 10% EtOAc−hexanes). The precipitate was determined to be pure product and was combined with the product isolated by chromatography to afford **11** (0.462 g, 89%) as an orange solid: mp 109−110 °C; R_f = 0.65 (33% EtOAc−hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.18 (d, *J* = 8.0 Hz, 1H), 7.77 (s, 1H), 7.68 (d, *J* = 8.2 Hz, 1H), 7.57 (m, 1H), 7.51 (d, *J* = 7.0 Hz, 2H), 7.41 (t, *J* = 7.6 Hz, 2H), 7.34 (t, *J* = 7.0 Hz, 2H), 7.07 (br s, 1H) 5.51 (s, 2H), 1.57 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.7, 152.5, 146.9, 143.9, 140.5, 137.4, 131.0, 128.7 (2C), 128.0 (2C), 127.9, 126.3, 124.2, 116.9, 99.1, 85.4, 81.0, 28.5 (3C); IR (film) ν_{max} 2980, 1809, 1631, 1331, 1156 cm^{-1}; MALDIFT-HRMS (DHB) *m/z* 351.1705 (M + H$^+$, $C_{21}H_{22}N_2O_3$ requires 351.1703).

1-Benzyloxy-3-(*tert*-butyloxycarbonyl)amino-4-iodoisoquinoline (12). A solution of **11** (50 mg, 0.14 mmol) in THF (1.5 mL) at −20 °C was treated with *N*-iodosuccinimide (38 mg, 0.17 mmol) and *p*-TsOH (10 mg, 0.053 mmol). The mixture was stirred at −20 °C for 0.5 h and then warmed to 25 °C for 24 h. The THF was removed by a stream of N_2, and the mixture was diluted with EtOAc (5 mL) and treated with aqueous saturated Na_2SO_3 (5 mL). The aqueous layer was extracted with EtOAc (3 × 25 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (SiO_2, 10% EtOAc−hexanes) afforded **12** (67 mg, 100%) as a white solid: mp 93−94 °C; R_f = 0.48 (20% EtOAc−hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.19 (d, *J* = 8.2 Hz, 1H), 7.93 (d, *J* = 8.5 Hz, 1H), 7.68 (t, *J* = 8.2 Hz, 1H), 7.58 (d, *J* = 7.6 Hz, 2H), 7.46 (t, *J* = 7.6 Hz), 7.42 (t, *J* = 7.6 Hz, 2H), 7.36 (t, *J* = 7.1 Hz, 1H), 7.10 (s, 1H), 5.63 (s, 2H), 1.60 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.4, 152.0, 144.9, 140.3, 137.1, 132.3, 130.5, 128.7 (2C), 128.6 (2C), 128.2, 126.3, 124.8, 118.6, 81.1, 77.1, 68.8, 28.6 (3C); IR (film) ν_{max} 3279, 1721, 1574, 1162 cm^{-1}; MALDIFT-HRMS (DHB) *m/z* 499.0483 (M + Na$^+$, $C_{21}H_{21}IN_2O_3$ requires 499.0489).

3-[*N*-(*tert*-Butyloxycarbonyl)-*N*-(3-chloroprop-2-en-1-yl)amino]-1-benzyloxy-4-iodoisoquinoline (13). A solution of **12** (0.264 g, 0.554 mmol) in DMF (2.5 mL) cooled to 0 °C was treated with NaH (60% suspension in mineral oil, 33.2 mg, 0.831 mmol) in portions and stirred for 0.5 h. A catalytic amount of Bu_4NI (10 mg) and 1,3-dichloropropene (0.154 mL, 1.66 mmol) were added sequentially, and the solution was warmed to 25 °C and stirred for 3 h before being treated with saturated $NaHCO_3$ (5 mL). The aqueous layer was extracted with Et_2O (3 × 25 mL), and the combined organic layers were washed with H_2O (3 × 20 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (SiO_2, 10% EtOAc−hexanes) afforded **13** (0.306 g, 99%) as a tan oil as a mixture of *E*- and *Z*-olefin isomers, amide rotamers: R_f = 0.65 (10% EtOAc−hexanes); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.28 (br s, 1H), 8.09 (d, *J* = 8.5 Hz, 1H), 7.75 (br s, 1H), 7.59 (m, 1H), 7.51 (d, *J* = 7.0 Hz, 2H), 7.41 (t, *J* = 7.0 Hz, 2H), 7.35 (t, *J* = 7.0 Hz, 1H), 5.91−6.10 (m, 2H), 5.57 (s, 2H), 4.66 and 4.46 (two d, *J* = 12.7 and 9.6 Hz, 1H), 4.13−4.33 (two m, 1H), 1.60, 1.39 and 1.37 (three s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 140.6, 137.2 and 137.1 (C), 132.4 and 132.3 (C), 131.9 and 131.8 (C), 129.3, 128.7 (2C), 128.6, 128.23 and 128.20 (C), 128.17 (2C), 127.9, 127.8, 124.7, 121.0, 119.8, 81.1, 77.4, 68.6, 48.1 and 45.4 (C), 28.5 (3C); IR (film) ν_{max} 3445, 1704, 1568, 1162 cm^{-1}; MALDIFT-HRMS (DHB) *m/z* 573.0429 (M + Na$^+$, $C_{24}H_{24}ClIN_2O_3$ requires 573.0412).

5-Benzyloxy-3-*N*-(*tert*-butyloxycarbonyl)-1-(chloromethyl)-1,2-dihydropyrrolo[2,3-*c*]isoquinoline (14). A solution of **13** (0.0623 g, 0.113 mmol) in C_6H_6 (1.5 mL) was degassed by three freeze−pump−thaw cycles and treated with Bu_3SnH (37 μL, 0.136 mmol) and AIBN (3.7 mg, 0.0227 mmol). A stream of Ar was bubbled through the solution for 10 min before the reaction vessel was closed and warmed at 80 °C for 2 h. The mixture was cooled to 25 °C and concentrated in vacuo. Flash chromatography (SiO_2, 0−10% EtOAc−hexanes gradient) afforded **14** (0.0461 g, 96%) as a white solid: mp 116−117 °C; R_f = 0.57 (25% EtOAc−hexanes); ^{1}H NMR (acetone-d_6, 500 MHz) δ 8.25 (d, *J* = 8.4 Hz, 1H), 7.58 (m, 4H), 7.43 (app t, *J* = 6.6 Hz, 2H), 7.35 (m, 2H), 5.64 (d, *J* = 12.5 Hz, 1H), 4.28 (d, *J* = 2.2 Hz, 1H), 4.17 (m, 1H), 3.84 (m, 2H), 3.51 (t, *J* = 10.2 Hz, 1H), 1.64 (s, 9H); ^{13}C NMR (CD_2Cl_2, 150 MHz) δ 161.8, 152.1, 137.8, 136.4, 131.9, 129.0, 128.9, 128.8, 128.5 (2C), 128.4 (2C), 126.0, 124.3, 121.7, 116.2, 107.1, 81.3, 68.8, 52.3, 47.3, 39.6, 28.8 (3C), 28.2; IR (film) ν_{max} 3446, 1723, 1682, 1564, 1349, 1154 cm^{-1}; MALDIFT-HRMS (DHB) *m/z* 447.1449 (M + Na$^+$, $C_{24}H_{25}ClN_2O_3$ requires 447.1446).

Resolution of 14. The enantiomers of **14** were resolved on a HPLC semipreparative Daicel Chiralcel OD column (10 μm, 2 × 25 cm) using 2% *i*-PrOH−hexane eluant (7 mL/min). The enantiomers eluted with retention times of 14.6 min (natural enantiomer) and 12.5 min (unnatural enantiomer, α = 1.17). (1*S*)-**14**: $[\alpha]^{23}_D$ −13 (*c* 0.047, CH_2Cl_2). (1*R*)-**14**: $[\alpha]^{23}_D$ +14 (*c* 0.047, CH_2Cl_2).

3-*N*-(*tert*-Butyloxycarbonyl)-1-(chloromethyl)-1,2-dihydropyrrolo[2,3-*c*]isoquinolin-5-one (15, *seco*-*N*-Boc-CBA). A solution of **14** (22.8 mg, 0.0538 mmol) in THF (0.4 mL) was treated with a catalytic amount of 10% Pd/C (5 mg) and placed under 1 atm H_2. The mixture was stirred for 2 h at 25 °C, filtered through a Celite plug, and concentrated in vacuo. The residue was dissolved in a minimal amount of $CHCl_3$ and then precipitated by the slow addition of hexanes to afford **15** (18.0 mg, 100%) as a white solid: mp 134−136 °C (dec); R_f = 0.38 (25% EtOAc−hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.61 (br s, 1H), 8.35 (d, *J* = 8.1 Hz, 1H), 7.62 (t, *J* = 7.7 Hz, 1H), 7.30 (m, 2H), 4.09 (m, 2H), 3.91 (m, 1H) 3.79 (m, 1H), 3.49 (t, *J* = 10.0 Hz, 1H) 1.57 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 162.0, 152.6, 140.4, 135.8, 133.5, 129.4, 124.3, 122.7, 120.9, 96.3, 83.6, 52.2, 46.4, 40.4, 28.5 (3C); IR (film) ν_{max} 3323, 1697, 1636, 1410, 1144 cm^{-1}; MALDIFT-HRMS (DHB) *m/z* 357.0975 (M + Na$^+$, $C_{17}H_{19}ClN_2O_3$ requires 357.0976). (1*S*)-**15**: $[\alpha]^{23}_D$ −58 (*c* 0.1, CH_2Cl_2). (1*R*)-**15**: $[\alpha]^{23}_D$ +56 (*c* 0.1, CH_2Cl_2).

3-*N*-(*tert*-Butyloxycarbonyl)-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]-3-azaindol-4-one (16, *N*-Boc-CBA). A sample of **15** (2.5 mg, 7.5 μmol) was dissolved in freshly distilled CH_3CN (100 μL) under Ar. Anhydrous DBU (5.7 μL, 37 μmol) was added at 23 °C, and the mixture was stirred for 1 h. The reaction mixture was then concentrated under a stream of N_2 and applied directly to PTLC (SiO_2, 10 × 20 cm, EtOAc) to afford *N*-Boc-CBA (**16**, 2.1 mg, 95%) as a white solid: mp 157−160 °C (dec); R_f = 0.38 (EtOAc); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.23 (d, *J* = 7.9 Hz, 1H), 7.52 (t, *J* = 7.7 Hz, 1H), 7.42 (t, *J* = 7.7 Hz, 1H), 6.83 (d, *J* = 7.4 Hz, 1H), 4.05 (m, 2H), 2.79 (m, 1H), 1.68 (app dd, *J* = 4.9, 2.2 Hz, 1H), 1.62 (app t, *J* = 4.9 Hz, 1H), 1.57 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 176.1, 174.2, 150.2, 138.6, 133.4, 129.9, 129.5, 128.1, 121.1, 84.8, 51.8, 31.4, 28.5 (3C), 23.5, 1.9; IR (film) ν_{max} 2915, 1748, 1601, 1160 cm^{-1}; UV (MeOH) 270 nm (ϵ 11000), 202 nm (ϵ 21000); MALDIFT-HRMS (DHB) *m/z* 321.1211 (M + Na$^+$, $C_{17}H_{18}N_2O_3$ requires 321.1210). Natural-(8b*A*,9a*S*)-**16**: $[\alpha]^{23}_D$ +30 (*c* 0.06, CH_2Cl_2). *ent*-(8b*S*,9a*R*)-**16**: $[\alpha]^{23}_D$ −32 (*c* 0.06, CH_2Cl_2). The sample for X-ray analysis was obtained by recrystallization from 10% CH_2Cl_2−hexanes, which provided colorless needles.

1,2,9,9a-Tetrahydrocyclopropa[*c*]benz[*e*]-3-azaindol-4-

one (17, CBA). A sample of **15** (3.3 mg, 9.9 μmol) was treated with 4 N HCl/EtOAc (500 μL) and stirred at 23 °C for 1 h. The solvent was removed under a steam of N_2 and dried under high vacuum for 30 min. The gray residue was dissolved in THF (500 μL) and treated with saturated aqueous $NaHCO_3$ (500 μL), and the mixture was stirred for 3 h at 23 °C. The mixture was extracted with EtOAc (3 × 10 mL), and the organic layers were combined, dried over Na_2SO_4, and concentrated. The solid was dissolved in THF and purified by PTLC (SiO_2, 10 × 20 cm, 50% EtOAc−EtOH) to afford CBA (**17**, 1.7 mg, 89%) as a pale yellow solid: R_f = 0.15 (50% EtOAc−EtOH); ^{1}H NMR (acetone-d_6, 500 MHz) δ 8.07 (d, J = 8.1 Hz, 1H), 7.53 (t, J = 7.7 Hz, 1H), 7.37 (t, J = 7.7 Hz, 1H), 7.04 (d, J = 7.7 Hz, 1H), 3.86 (dd, J = 8.1, 5.5 Hz, 1H), 3.72 (d, J = 13.6 Hz, 1H), 3.10 (m, 1H), 1.62 (dd, J = 8.5, 4.1 Hz, 1H), 1.28 (t, J = 3.7 Hz, 1H); IR (film) ν_{max} 2923, 1739, 1595, 1550 cm^{-1}; MALDIFT-HRMS (DHB) m/z 199.0868 (M + H$^+$, $C_{12}H_{10}N_2O$ requires 199.0866). Natural-(8b*R*,9a*S*)-**17**: [α]$^{23}_D$ +165 (*c* 0.03, CH_2Cl_2). *ent*-(8b*S*,9a*R*)-**17**: [α]$^{23}_D$ −167 (*c* 0.03, CH_2Cl_2).

***seco*-CBA-TMI (18).** A sample of **15** (9.7 mg, 29.0 μmol) was treated with 4 N HCl/EtOAc (300 μL) and stirred at 23 °C for 1 h. The solvent was removed under a stream of N_2 and dried under high vacuum for 30 min. The gray residue was dissolved in DMF (300 μL), and 5,6,7-trimethoxyindole-2-carboxylic acid[64] (7.3 mg, 29.0 μmol) and EDCI (16.8 mg, 87.0 μmol) were added. The mixture was stirred under Ar at 23 °C for 18 h in the absence of light before the solvent was removed under a stream of N_2. Flash chromatography (SiO_2, 3 × 17 cm, 10−50% EtOAc−hexanes gradient) afforded *seco*-CBA-TMI (**18**, 6.7 mg, 50%) as a yellow solid: R_f = 0.51 (EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.89 (br s, 1H), 8.44 (d, J = 8.1 Hz, 1H), 7.67 (t, J = 8.1 Hz, 1H), 7.40 (m, 2H), 7.03 (d, J = 2.2 Hz, 1H), 6.84 (br s, 1H), 4.67 (m, 2H), 4.12 (s, 3H), 4.00 (m, 2H), 3.97 (s, 3H), 3.92 (s, 3H), 3.56 (app t, J = 10.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.9, 164.0, 161.9, 144.7, 142.3, 135.7, 134.1, 131.3, 130.2, 127.2, 125.8, 124.4, 122.1, 108.4, 98.3, 86.5, 62.4, 62.1, 57.1, 55.0, 46.9, 42.3; IR (film) ν_{max} 3238, 1646, 1312 cm^{-1}; MALDIFT-HRMS (DHB) m/z 468.1324 (M + H$^+$, $C_{24}H_{22}ClN_3O_5$ requires 468.1321). (1*S*)-**18**: [α]$^{23}_D$ −14 (*c* 0.09, CH_2Cl_2). (1*R*)-**18**: [α]$^{23}_D$ +16 (*c* 0.09, CH_2Cl_2).

CBA-TMI (19). A sample of **18** (2.1 mg, 4.5 μmol) was dissolved in freshly distilled CH_3CN (50 μL) under Ar. Anhydrous DBU (3.4 μL, 22.4 μmol) was added at 23 °C, and the mixture was stirred for 1 h. The reaction mixture was then concentrated under a stream of N_2. PTLC (SiO_2, 20 × 20 cm, EtOAc) afforded CBA-TMI (**19**, 1.5 mg, 71%) as a white solid: R_f = 0.49 (EtOAc); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.33 (d, J = 7.9 Hz, 1H), 7.54 (t, J = 7.9 Hz, 1H), 7.46 (t, J = 7.9 Hz, 1H), 7.01 (d, J = 7.9 Hz, 1H), 6.85 (s, 1H), 6.80 (s, 1H), 6.31 (s, 1H), 4.16 (d, J = 11.8 Hz, 1H), 3.87 (s, 3H), 3.71 (s, 3H), 3.87 (d, J = 11.8 Hz, 1H), 2.98 (s, 3H), 2.00 (t, J = 7.9 Hz, 1H), 1.59 (br s, 1H), 0.99 (t, J = 6.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 163.4, 151.8, 140.9, 140.6, 138.2, 133.9, 133.0, 129.6, 129.5, 129.3, 128.9, 127.5, 123.6, 122.4, 101.6, 98.8, 95.3, 62.0, 60.4, 57.0, 44.4, 37.7, 31.4; IR (film) ν_{max} 3257, 1713, 1683 cm^{-1}; MALDIFT-HRMS (DHB) m/z 432.1558 (M + H$^+$, $C_{24}H_{21}N_3O_5$ requires 432.1554). Natural-(8b*R*,9a*S*)-**19**: [α]$^{23}_D$ +51 (*c* 0.05, CH_2Cl_2). *ent*-(8b*S*,9a*R*)-**19**: [α]$^{23}_D$ −49 (*c* 0.04, CH_2Cl_2).

Acid-Catalyzed Addition of CH_3OH to *N*-Boc-CBA. 3-*N*-(*tert*-Butyloxycarbonyl)-1-methoxymethyl-4*H*-5-oxo-1,2-dihydropyrrolo[2,3-*c*]isoquinoline (20). A solution of **16** (1.0 mg, 3.4 μmol) in CH_3OH (70 μL) was treated with CF_3SO_3H−CH_3OH (17 μL, 0.02 M, 0.34 μmol) at 0 °C. The ice bath was removed, and the reaction mixture was stirred for 30 min at 23 °C before being quenched by the addition of $NaHCO_3$ (2.0 mg), filtered through Celite, and concentrated in vacuo. PTLC (SiO_2, 10 × 20 cm, 50% EtOAc−hexanes) afforded **20** (1.0 mg, 90%) as a white solid: R_f = 0.71 (50% EtOAc−hexanes); ^{1}H NMR (acetone-d_6, 500 MHz) δ 10.43 (br s, 1H), 8.20 (d, J = 7.0 Hz, 1H), 7.64 (t, J = 6.0 Hz, 1H), 7.56 (d, J = 7.0 Hz, 1H), 7.29 (t, J = 6.2 Hz, 1H), 4.12 (m, 1H), 4.00 (d, J = 9.5 Hz, 1H), 3.72 (m, 2H), 3.45 (app t, J = 8.0 Hz, 1H), 3.33 (s, 3H), 1.57 (s, 9H); IR (film) ν_{max} 3333, 1692, 1667, 1333 cm^{-1}; MALDIFT-HRMS (DHB) m/z 353.1473 (M + Na$^+$, $C_{18}H_{22}N_2O_4$ requires 353.1472).

Addition of HCl to *N*-Boc-CBA. A solution of **16** (1.5 mg, 5.0 μmol) in THF (50 μL) was cooled to −78 °C and treated with 4 N HCl−EtOAc (50 μL). The mixture was stirred for 2 min before the solvent was removed in vacuo. PTLC (SiO_2, 10 × 10 cm, 25% EtOAc−hexanes) afforded **15** (1.7 mg, 99%).

Hydrolysis of *N*-Boc-CBA with LiOH. A solution of **16** (800 μg, 2.7 μmol) in 3:1:1 (v/v/v) THF−CH_3OH−H_2O (30 μL) was treated with LiOH (310 μg, 7.4 μmol). The mixture was stirred for 5 min before the solvent was removed with a stream of N_2. PTLC (SiO_2, 10 × 10 cm, 10% EtOH−EtOAc) of the residue afforded **17** (520 μg, 98%).

Aqueous Solvolysis Reactivity, pH 3. Compounds **16** and **17** (50 μg) were 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 deionized H_2O, respectively. Immediately after mixing, the UV spectra of the solution was measured against a reference solution containing CH_3OH (1.5 mL) and the aqueous buffer (1.5 mL), and this reading was used as the initial absorbance value. The solution was stoppered, protected from light, and allowed to stand at 25 °C. The UV spectra were recorded at regular intervals until a constant value was obtained for the long-wavelength absorbance. The increase of the absorbance at 300 nm was monitored. The solvolysis rate constant for **17** was determined from the slope of the line obtained from the least-squares treatment (r^2 = 0.959) of the plot of $\ln[(A_f - A_i)/(A_f - A)]$ versus time; k = 6.44 × 10^{-5} s^{-1}, $t_{1/2}$ = 3.0 h (Table 1).

DNA Alkylation Studies. Selectivity and Efficiency. General procedures, the preparation of singly ^{32}P 5′ end-labeled double-stranded DNA, the compound binding studies, gel electrophoresis, and autoradiography were conducted according to procedures described elsewhere.[66] Eppendorf tubes containing the 5′ end-labeled DNA (w794, 9 μL) in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) were treated with the agent in DMSO (1 μL at the specified concentration). The solution was mixed by vortexing and brief centrifugation and subsequently incubated at 4−37 °C for 24−120 h. The covalently modified DNA was separated from unbound agent by EtOH precipitation and resuspended in TE buffer (10 μL). The solution of DNA in an Eppendorf tube sealed with parafilm was warmed to 100 °C for 30 min to induce cleavage at the alkylation sites, allowed to cool to 25 °C, and centrifuged. Formamide dye (0.03% xylene cyanol FF, 0.03% bromophenol blue, 8.7% Na_2EDTA 250 mM) was added (5 μL) to the supernatant. Prior to electrophoresis, the sample was denatured by warming at 100 °C for 5 min, placed in an ice bath, and centrifuged, and the supernatant was loaded directly onto the gel. Sanger dideoxynucleotide sequencing reactions were run as standards adjacent to the reaction samples. Polyacrylamide gel electrophoresis (PAGE) was run on an 8% sequencing gel under denaturing conditions (8 M urea) in TBE buffer (100 mM Tris base, 100 mM boric acid, 0.2 mM Na_2EDTA) followed by autoradiography.

Acknowledgment. We gratefully acknowledge the financial support of NIH (CA41986) and the Skaggs Institute for Chemical Biology. We especially thank Dr. R. Chadha for the X-ray structure determination of **16** and H. Schmitt for initial studies. D.B.K. is a Skaggs Fellow.

Supporting Information Available: ^{1}H NMR spectra of **10**−**20** are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

JO035119F