Synthesis and Cytotoxicity of 5-Amino-1-(chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-benz[*e*]indole (Amino-*seco*-CBI-TMI) and Related 5-Alkylamino Analogues: New DNA Minor Groove Alkylating Agents

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Received July 17, 1998

The first synthesis of *seco*-CBI-TMI alkylating agents with 5-nitrogen substituents is reported. The parent 5-amino compound was prepared in a 15-step synthesis from 1-hydroxynaphthalene-2-carboxylic acid. Reductive alkylation of the 5-amino compound gave the corresponding 5-methylamino and 5-dimethylamino analogues, while resolution of an intermediate by chiral HPLC allowed preparation of the *R* and *S* enantiomers of the 5-amino analogue. Absolute configuration was assigned by X-ray crystallography. The *S* enantiomer was about 65-fold more cytotoxic than the *R* enantiomer in cell line assays. The 5-amino and 5-methylamino compounds had in vitro cytotoxicities comparable to that of the known 5-hydroxy analogue (0.2−0.5 nM), while the 5-dimethylamino derivative was about 10-fold less potent. The high potencies of the 5-amino and 5-methylamino analogues make them of interest for the formation of relatively stable amine-based prodrugs.

Introduction

The cyclopropylindole antitumor antibiotics, exemplified by CC-1065[1] and duocarmycin SA (**1**),[2] are extremely cytotoxic DNA alkylating agents, with IC_{50}s against mammalian cell lines in the low picomolar range for some analogues.[1−3] The full complexity of the natural products is not required for high potency, with simplified analogues such as CBI-TMI (**2**) being essentially as cytotoxic as **1** in cell culture (IC_{50}s of 20 and 10 pM, respectively, in L1210 leukemia for a 72 h exposure).[4] The phenolic seco forms of these compounds (e.g., **3**) also retain essentially the full cytotoxicity of the corresponding cyclopropyldienones, indicating that ring closure to form the cyclopropane ring is rapid under cell culture conditions.[3,5] Carbamate prodrug forms of these phenolic seco compounds have been reported (e.g., carzelesin **4**) which are labile in plasma, rapidly and nonspecifically releasing the corresponding phenol.[6] While this is a desirable property for systemic release of a drug, we have been interested in the preparation of more stable prodrugs that may persist in plasma but allow specific release in tumor tissue by a localized enzyme-activation step.

We have therefore been investigating the analogous amino-*seco* compounds because of their greater potential for forming relatively stable, nontoxic prodrugs through modification of the amino function. We have recently reported the synthesis and initial evaluation of the analogues **5**−**8** in the *seco*-CI-TMI series,[7−9] and we report here an efficient synthesis of the CBI congeners **10**−**12** via the corresponding nitro analogue **9** (Chart 1 and Schemes 2−4). A preliminary account of this work has been published.[10]

Results and Discussion

Synthesis of 5-Amino-*seco*-CBI-TMI (10). Our initial approach to **10**, starting from 1-chloro-2,4-dinitronaphthalene (**13**), followed one of our recent syntheses[7,9] of the amino-*seco*-CI compound **6** (Scheme 1). Conversion of **13** to the malonate **14**, followed by nitro reduction with Na_2S, gave a good yield of the single nitroaniline isomer **15**, resulting from selective reduction of the less hindered nitro group. This is in contrast to the related amino-*seco*-CI case, where a mixture of isomers was obtained.[9] The structure of **15** was confirmed by X-ray crystallography (Supporting Information). A number of amine-protected analogues of **15** were prepared and subjected to DIBALH reduction, but only the benzyl analogue **16** gave any of the required diol (**17**), and then in only poor yield. Reduction of **16** with

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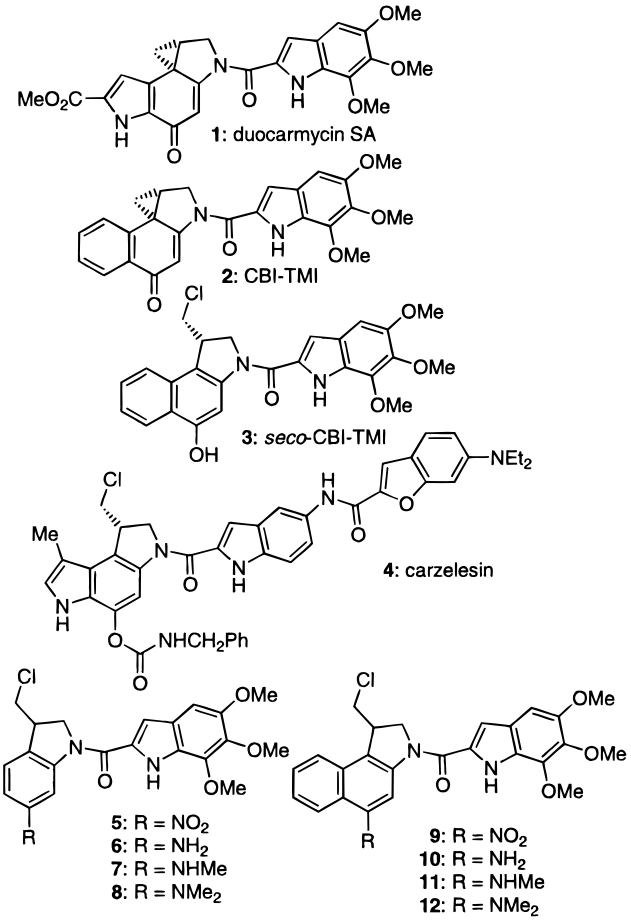
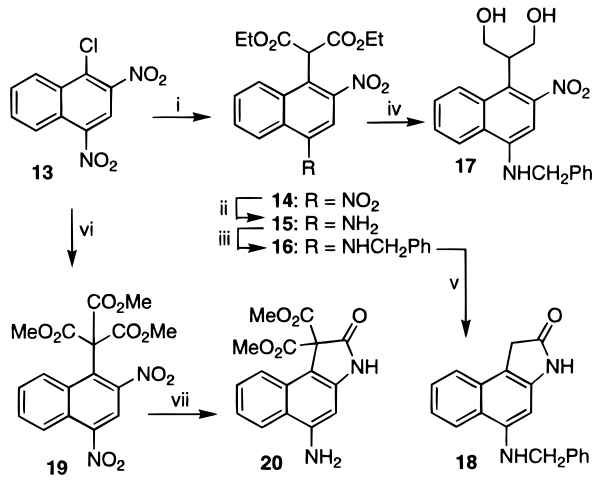
10.1021/jo981395w CCC: $15.00
Published on Web 11/20/1998

Chart 1



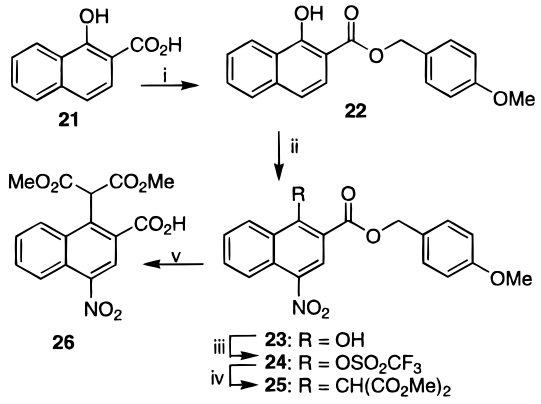
Scheme 1[a]



[a] (i) $Na/CH_2(CO_2Et)_2$, 83%; (ii) Na_2S, 69%; (iii) PhCHO/TsOH, then $NaBH_3CN/H^+$, 77%; (iv) DIBALH, 20%; (v) $Na_2S_2O_4$, 37%; (vi) $NaH/CH(CO_2Me)_3$, 37%; (vii) Fe/H^+, 3%.

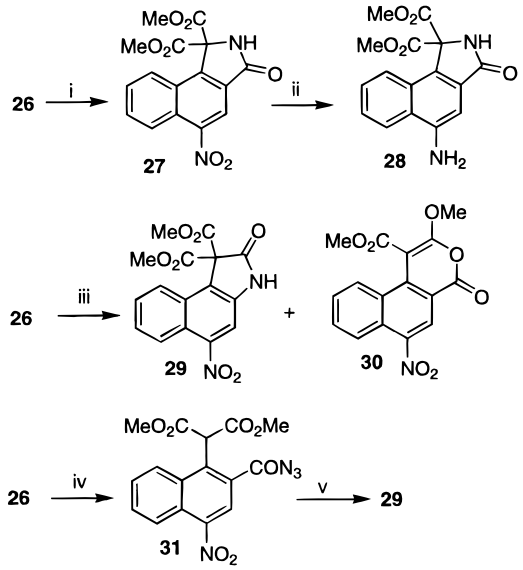
dithionite gave only the lactam **18** (in moderate yield), resulting presumably from cyclization followed by decarboxylation, and approaches via **16** were therefore abandoned. An alternative synthesis from **13**, involving an initial condensation with trimethyl methanetricarboxylate to give the triester **19**, was also explored. However, while reduction of **19** was exhaustively studied, the desired aminolactam **20** was isolated only in trace amounts.

Scheme 2[a]



[a] (i) $NaHCO_3$ (1.05 mol equiv), then 4-methoxybenzyl chloride, 66%; (ii) 70% HNO_3/AcOH, 61%; (iii) $(Tf)_2O/Et_3N$, 81%; (iv) $CH_2(COOMe)_2/K_2CO_3$, 88%; (v) TFA/$PhOCH_3$, 93%.

Scheme 3[a]

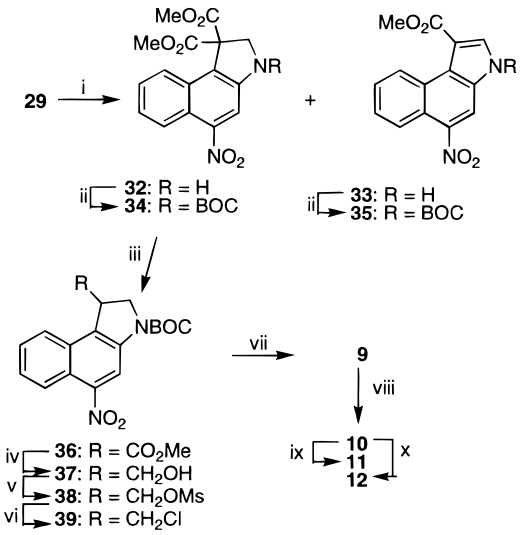


[a] (i) DPPA/Et_3N; (ii) $Pd/C/H_2$; (iii) $KHCO_3$, then NaN_3/TBAB/$PhOP(O)Cl_2$, then PhMe/reflux; (iv) NaN_3/pyridine/[$SOCl_2$/DMF (1:1)]; (v) PhMe/heat, 81% (from **26**).

The successful synthesis of **10** (Schemes 2−4) involved generation of the required amino substituent from a nitro group in the last step, a strategy successfully employed[8,9] in an alternative synthesis of **6**. Commercially available 1-hydroxynaphthalene-2-carboxylic acid (**21**) was monoprotected as the 4-methoxybenzyl ester **22**, and this was nitrated under mild conditions (70% HNO_3/AcOH) to give the 4-nitro isomer **23** in 61% isolated yield (Scheme 2). This was converted to the trifluoromethanesulfonate derivative **24**, which on reaction with dimethyl malonate anion gave the 1-malonyl derivative **25**. Selective cleavage of the 4-methoxybenzyl ester group with TFA/anisole then gave the key acid **26**.

Conversion of **26** to the desired nitrolactam **29** in an acceptable yield proved difficult (Scheme 3). Attempted Curtius rearrangement of **26** employing diphenylphosphoryl azide (DPPA) under a number of reaction conditions gave none of the desired product. In particular, conditions similar to those used[9] in the synthesis of the amino-*seco*-CI derivative **6** (DPPA/Et_3N) gave an excellent yield of a lactam, but this proved to be the isomeric compound **27**. The fact that **27** was not the desired

Scheme 4[a]



[a] (i) BH_3·Me_2S, 57%; (ii) $(BOC)_2O$/*N*-methylimidazole, 82%; (iii) NaOMe, then TFA, 98%; (iv) DIBALH, 70%; (v) MsCl, 89%; (vi) LiCl, 87%; (vii) HCl, then EDCl·HCl/TMI acid, 77%; (viii) H_2/PtO_2, 94%; (ix) $MeCO_2CHO$, then BH_3·Me_2S, 42%; (x) $NaBH_3CN$/aqueous HCl/HCHO, 68%.

product was shown initially when catalytic hydrogenation of this compound gave an aminolactam (**28**) that was different from the aminolactam (**20**) obtained in Scheme 1. The structure of the nitrolactam **27** was confirmed by X-ray crystallography (Supporting Information). This compound is presumably formed by intramolecular trapping of the intermediate carbonyl azide **31** by the malonate anion (which would be expected to be more acidic than the corresponding compound in the CI series because of increased resonance) under the basic conditions of the reaction.

Reaction of **26** with phenyl dichlorophosphate/NaN_3/pyridine followed by thermolysis of the crude product in refluxing toluene gave a low yield (16%) of the desired nitrolactam **29**, with the major product being the isochromene **30**. This is analogous to the isochromene formed in the CI series.[9] The acid **26** was finally converted successfully to **29** in excellent yield (81%) by formation of the intermediate carbonyl azide **31** with *N*,*N*-dimethyl(chlorosulfonyl)methaniminium chloride ($SOCl_2$/DMF adduct)[11] and NaN_3. Thermal rearrangement of this product under neutral conditions (refluxing toluene) then resulted in the formation of the expected product **29** (Scheme 3) via formation and trapping of the isocyanate. In contrast, treatment of azide **31** with an anhydrous base (Et_3N/CH_2Cl_2, 40 °C) gave predominantly the isomeric nitrolactam **27**, as shown by TLC analysis.

Selective reduction of the nitrolactam **29** with BH_3·Me_2S gave the desired benzindoline **32** together with ca. 5% of the benzindole **33** (Scheme 4). The mixture was not separable by chromatography, but reaction with $(BOC)_2O$ allowed separation of the unwanted product **35** from the required *N*-BOC benzindoline **34**. The latter was then treated with NaOMe to give the monoester **36**. Reduction of **36** with DIBALH gave the alcohol **37** which was converted, via the mesylate **38**, to the chloromethyl compound **39**. This key intermediate was deprotected

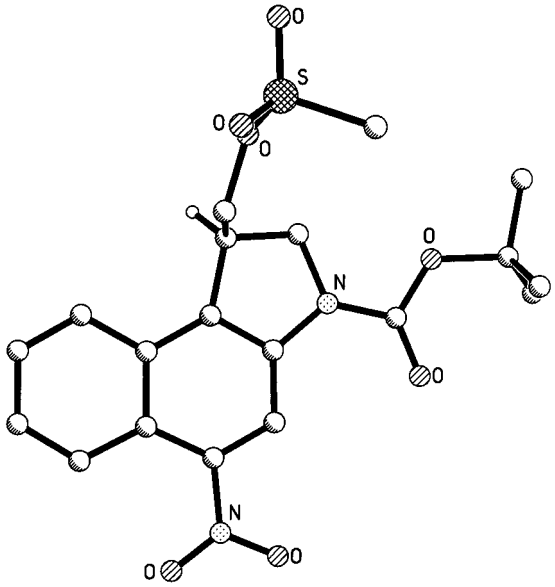


Figure 1. ORTEP drawing of (−)-(*R*)-**38**.

(HCl/dioxane) and coupled with 5,6,7-trimethoxyindole-2-carboxylic acid (TMI acid) to give **9**. Catalytic reduction of this over platinum oxide gave the target racemic amino-*seco*-CBI analogue **10** in excellent yield. Racemic **10** was mono- and dimethylated by formylation/borane reduction[9,12] and reductive amination[9] to give respectively racemic **11** and **12**.

Resolution of Enantiomers. The alcohol, mesylate, and chloro intermediates **37**−**39** were all resolvable on a Diacel Chiralcel OD semipreparative column, as described[4] for related phenolic CBI derivatives [α values 1.14 in *i*-PrOH/hexane (1:1), 1.40 in *i*-PrOH/hexane (1:1), and 1.10 in *i*-PrOH/hexane (7:3), respectively]. The mesylate **38** not only was the most soluble of the three but also showed an unusually large α value, allowing baseline resolution of the enantiomers. An X-ray crystal structure determination (Figure 1) of the faster-running mesylate showed it to be the *R* enantiomer (Flack parameter[13] of −0.0054). Both enantiomers were converted to the corresponding 5-nitro-*seco*-CBI-TMI derivatives (−)-(*R*)-**9** and (+)-(*S*)-**9**, and these in turn were converted to the amino compounds (−)-(*R*)-**10** and (+)-(*S*)-**10**.

In Vitro Cytotoxicity. The cytotoxicities of the amino compounds **10**−**12** were evaluated[14] in a panel of three cell lines. AA8 is a Chinese hamster ovary cell line, UV4 is a subline of AA8 that is deficient in excision repair and hypersensitive to drugs that produce bulky DNA adducts or cross-links,[15] and EMT6 is a murine mammary carcinoma cell line. Results are recorded in Table 1 for 4 h drug exposures. Under these conditions, the natural enantiomer of the known *seco*-phenol **3** had an IC_{50} of 0.1−0.2 nM across the cell line panel. The reported[4] IC_{50} of **2** (the ring-closed form of **3**) is somewhat lower than that of **3** (0.03 nM) but in a different cell line (L1210) for a longer exposure time (72 h). The corresponding 5-amino analogue (+)-(*S*)-**10** showed toxicity

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Table 1. Cytotoxicity (IC_{50}s in nM, 4 h Exposure, ±SE) for Nitrogen-Substituted *seco*-CBI-TMI Analogues[a]

compound	AA8	UV4	EMT6
3	0.21 ± 0.06	0.09 ± 0.004	0.13 ± 0.01
(+)-(*S*)-**10**	0.21 ± 0.03	0.14 ± 0.01	0.13 ± 0.01
(−)-(*R*)-**10**	13.6 ± 3.2	2.7 ± 0.21	7.0 ± 0.66
(±)-**10**	0.46 ± 0.05	0.29 ± 0.02	0.27 ± 0.03
(±)-**11**	0.17 ± 0.02	0.12 ± 0.01	0.11 ± 0.01
(±)-**12**	5.6 ± 0.56	3.7 ± 0.01	3.9 ± 1.25

[a] Average of two or more determinations.

almost identical to that of **3**. This is in marked contrast to the CI series, where the (racemic) amino compound **6** is 50−120-fold less cytotoxic than the corresponding (racemic) phenol in the same panel.[7] In the 5-amino-CBI series, the *R* enantiomer was much less cytotoxic than the *S* enantiomer (65-fold in AA8, 19-fold in UV4, and 54-fold in EMT6). This follows the pattern seen with **2** and its enantiomer where the ratio was 90-fold.[3] We have also investigated the sequence specificity of DNA alkylation by (+)-(*S*)-**10** and (−)-(*R*)-**10**[16] and have found exclusive adenine alkylation with the strongest cleavage at polyA sequences. The *S* enantiomer was at least 10-fold more effective than the *R* enantiomer, showing strong alkylation at a drug concentration as low as 10^{-8} M.

The (racemic) 5-methylamino analogue **11** was slightly more potent than (±)-**10**, but the (racemic) dimethylamino compound **12** was at least 10-fold less cytotoxic. One possible explanation for this loss of potency is that **12** (unlike **10** or **11**) is unable to ring-close to a cyclic intermediate (i.e., similar to the loss in potency on alkylating a *seco*-CI phenol[17]). However, in the amino-CI series, **8** was no less toxic than **6** or **7**.[8] Thus the most likely reason is that steric interaction of the peri hydrogen with the bulky dimethylamino group of **12** rotates the latter significantly out of plane, resulting in considerable deconjugation.

All of the compounds were slightly more cytotoxic in the repair-deficient UV4 cell line, although the differentials (ca. 2-fold) were less than those usually observed,[15] including those with analogous amino-CI alkylators.[7]

Conclusions

Although care must be taken with the key Curtius rearrangement of the malonate **26** to form the indolone **29**, the described method offers a robust synthesis of the 5-amino-*seco*-CBI alkylating agent **10** (15 steps in 6% overall yield from **22**), suitable for multigram synthesis. Both the 5-amino and 5-methylamino derivatives (**10** and **11**) have subnanomolar in vitro cytotoxicities, comparable to that of the corresponding phenol **3**. Like **3** and its enantiomer, the enantiomers of **10** show large differentials in cytotoxicity, with the "natural" *S* enantiomer being the more potent. The high potencies of the 5-amino analogues make this class of compounds attractive for use in prodrug strategies.[18]

Experimental Section

Analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on a digital melting point apparatus and are as read. NMR spectra were measured at 400 MHz (^{1}H) or 100 MHz (^{13}C) and are referenced to Me_4Si. Mass spectra were recorded at nominal 5000 resolution. All compounds tested for cytotoxicity were >96% pure by HPLC. Compound **3** was made by a reported[4] method.

4-Methoxybenzyl 1-Hydroxynaphthalene-2-carboxylate (22). 4-Methoxybenzyl chloride (98%, 46.7 g, 0.29 mol) was added to a suspension of the powdered sodium salt of 1-hydroxynaphthalene-2-carboxylic acid **(21)** (61.3 g, 0.29 mol; prepared from the acid and 1.05 mol equiv of aqueous $NaHCO_3$) in DMSO (205 mL) at 20 °C, and the mixture was stirred at 70 °C for 1 h. After cooling, the mixture was poured into dilute aqueous $KHCO_3$ (3.5 L), and the resulting precipitate was collected, washed with water, and dried. The solid was extracted with boiling petroleum ether (bp 90−95 °C, 1.1 L), and the hot extracts were treated with decolorizing charcoal, filtered, and cooled for a prolonged period at 0 °C to provide crude **22** (59.4 g, 66%), suitable for further use. A sample was recrystallized from *i*-Pr_2O/petroleum ether: mp 92−93 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.91 (s, 1 H), 8.31 (d, J = 8.2 Hz, 1 H), 7.92 (d, J = 8.1 Hz, 1 H), 7.73 (d, J = 8.8 Hz, 1 H), 7.71 (t, J = 7.5 Hz, 1 H), 7.61 (t, J = 7.6 Hz, 1 H), 7.48 (d, J = 8.6 Hz, 2 H), 7.42 (d, J = 8.9 Hz, 1 H), 6.99 (d, J = 8.6 Hz, 2 H), 5.40 (s, 2 H), 3.78 (s, 3 H). Anal. Calcd for $C_{19}H_{16}O_4$: C, 74.01; H, 5.23. Found: C, 73.72; H, 5.22.

4-Methoxybenzyl 1-Hydroxy-4-nitronaphthalene-2-carboxylate (23). A warm, vigorously stirred solution of **22** (25.4 g, 0.082 mol) in AcOH (290 mL) was cooled to 25 °C and treated in one portion with a solution of HNO_3 (70% w/w, 18.6 g, 0.20 mol) in AcOH (25 mL). The temperature rose to 35 °C (controlled with external cooling), and a solid was separated. After being stirred for a further 10 min at 30 °C, the mixture was cooled to 0 °C. The precipitate was collected, washed with cold AcOH and *i*-Pr_2O, and recrystallized from CH_2Cl_2/*i*-Pr_2O to give **23** (17.9 g, 61%): mp 163−164 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 12.50 (br s, 1 H), 8.60 (s, 1 H), 8.60 (d, J = 8.6 Hz, 1 H), 8.47 (d, J = 8.3 Hz, 1 H), 7.97 (ddd, J = 8.5, 7.2, 1.2 Hz, 1 H), 7.79 (t, J = 7.7 Hz, 1 H), 7.50 (d, J = 8.6 Hz, 2 H), 7.00 (d, J = 8.7 Hz, 2 H), 5.43 (s, 2 H), 3.78 (s, 3 H). Anal. Calcd for $C_{19}H_{15}NO_6$: C, 64.58; H, 4.28; N, 3.97. Found: C, 64.66; H, 3.97; N, 4.24.

4-Methoxybenzyl 4-Nitro-1-(trifluoromethanesulfonyloxy)naphthalene-2-carboxylate (24). A suspension of **23** (12.90 g, 36.5 mmol) in CH_2Cl_2 (180 mL) was treated with Et_3N (6.58 mL, 47.5 mmol), and the resulting solution was cooled to 0 °C and treated dropwise with trifluoromethanesulfonic anhydride (7.82 mL, 43.8 mmol). The mixture was stirred at 0 °C for 30 min and then treated with additional Et_3N (1.00 mL, 7.2 mmol) followed by trifluoromethanesulfonic anhydride (1.19 mL, 6.7 mmol). After being stirred for a further 2 h at 20 °C, the mixture was washed twice with water, then dried (Na_2SO_4), and concentrated under reduced pressure. The residue was extracted with boiling petroleum ether (bp 90−95 °C, 450 mL) in the presence of decolorizing charcoal, and the filtered solution was then cooled to 65 °C and refiltered through a Celite pad. Following prolonged cooling, the separated solid was collected and washed with petroleum ether to give crude **24** (14.29 g, 81%), suitable for further use. A sample was recrystallized from *i*-Pr_2O/petroleum ether: mp 74−75 °C; ^{1}H NMR ($CDCl_3$) δ 8.71 (s, 1 H), 8.57 (d, J = 8.7 Hz, 1 H), 8.30 (d, J = 8.4 Hz, 1 H), 7.92 (ddd, J = 8.6, 7.1, 1.4 Hz, 1 H), 7.84 (ddd, J = 8.5, 7.2, 1.2 Hz, 1 H), 7.44 (d, J = 8.7 Hz, 2 H), 6.93 (d, J = 8.8 Hz, 2 H), 5.43 (s, 2 H), 3.82 (s, 3 H). Anal. Calcd for $C_{20}H_{14}F_3NO_8S$: C, 49.49; H, 2.91; N, 2.89; S, 6.61. Found: C, 49.69; H, 2.79; N, 2.86; S, 6.56.

4-Methoxybenzyl 1-[Di(methoxycarbonyl)methyl]-4-nitronaphthalene-2-carboxylate (25). A stirred solution of **24** (13.20 g, 27.2 mmol) and dimethyl malonate (5.39 g, 40.8 mmol) in DMF (85 mL) was cooled to −10 °C and treated with powdered K_2CO_3 (22.53 g, 163 mmol). The mixture warmed to 20 °C over 4 h, and after being stirred for a further 12 h at 20 °C, it was poured slowly into cold, stirred 0.5 N HCl (1750 mL). The resulting solid was dissolved in CH_2Cl_2, and the solution was washed twice with water, dried (Na_2SO_4), and

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evaporated to dryness. The residue was crystallized from CH_2Cl_2/*i*-Pr_2O to give **25** (11.21 g, 88%), suitable for further use. A sample was recrystallized from MeOH: mp 124−125 °C; ^{1}H NMR ($CDCl_3$) δ 8.62 (s, 1 H), 8.45 (d, J = 8.6 Hz, 1 H), 8.21 (d, J = 8.7 Hz, 1 H), 7.79 (ddd J = 8.4, 7.0, 1.2 Hz, 1 H), 7.69 (ddd, J = 8.6, 7.1, 1.4 Hz, 1 H), 7.41 (d, J = 8.7 Hz, 2 H), 6.94 (d, J = 8.7 Hz, 2 H), 6.62 (s, 1 H), 5.37 (s, 2 H), 3.83 (s, 3 H), 3.69 (s, 6 H). Anal. Calcd for $C_{24}H_{21}NO_9$: C, 61.67; H, 4.53; N, 3.00. Found: C, 61.64; H, 4.67; N, 3.14.

1-[Di(methoxycarbonyl)methyl]-4-nitronaphthalene-2-carboxylic Acid (26). TFA (36 mL) was added in one portion to a mixture of **25** (9.20 g, 19.7 mmol) and anisole (2.16 g, 20 mmol), and the resulting solution was stirred at 20 °C for 10 min and then diluted with cold water (800 mL). The precipitated semisolid was collected and dissolved in EtOAc, and the solution was washed with water, dried (Na_2SO_4), and concentrated under reduced pressure and below 30 °C until the appearance of a crystalline solid. Addition of *i*-Pr_2O completed the precipitation of the product, which was recrystallized from EtOAc/*i*-Pr_2O/petroleum ether/AcOH (1 drop) to give **26** (6.37 g, 93%): mp 153−154 °C (dec); ^{1}H NMR [$(CD_3)_2$SO] δ 14.1 (br s, 1 H), 8.62 (s, 1 H), 8.36 (d, J = 8.2 Hz, 1 H), 8.30 (d, J = 8.7 Hz, 1 H), 7.92 (t, J = 7.6 Hz, 1 H), 7.84 (ddd, J = 8.4, 7.0, 1.1 Hz, 1 H), 6.65 (s, 1 H), 3.63 (s, 6 H); ^{13}C NMR δ 167.25, 167.23, 146.3, 137.8, 133.0, 130.7, 129.0, 128.6, 126.3, 125.2, 123.1, 122.8, 52.7, 51.2. Anal. Calcd for $C_{16}H_{13}NO_8$: C, 55.34; H, 3.77; N, 4.03. Found: C, 55.36; H, 3.80; N, 3.74.

1,1-Di(methoxycarbonyl)-5-nitro-1,2-dihydro-3*H*-benz[*e*]isoindol-3-one (27). A solution of **26** (3.00 g, 8.64 mmol) in THF (25 mL) was treated at 0 °C with DPPA[19] (4.76 g, 17.28 mmol) followed by Et_3N (2.41 mL, 17.28 mmol), and the mixture was stirred at 20 °C under N_2 for 12 h. The reaction was quenched by pouring it into excess 0.1 N HCl, and the precipitated semisolid was collected and dissolved in CH_2Cl_2. The solution was washed with water, dried (Na_2SO_4), diluted with an equal volume of EtOAc, and filtered through a column of silica gel. Removal of solvent followed by trituration of the residue with *i*-Pr_2O gave a crystalline solid that was recrystallized from CH_2Cl_2/*i*-Pr_2O to give **27** (2.52 g, 85%): mp 192−194 °C; ^{1}H NMR [$(CD_3)_2$SO] δ 10.42 (s, 1 H), 8.41 (s, 1 H), 8.38−8.30 (m, 2 H), 8.01−7.91 (m, 2 H), 3.78 (s, 6 H); ^{13}C NMR δ 167.5, 166.4, 149.3, 142.0, 131.0, 129.2, 129.1, 128.5, 126.1, 126.0, 123.3, 116.2, 71.6, 54.1; HRMS (EI) [M^+] calcd for $C_{16}H_{12}N_2O_7$ (M^+) 344.0645, found 344.0647. Anal. Calcd for $C_{16}H_{12}N_2O_7$: C, 55.82; H, 3.51; N, 8.14. Found: C, 55.76; H, 3.38; N, 8.15.

5-Amino-1,1-di(methoxycarbonyl)-1,2-dihydro-3*H*-benz[*e*]isoindol-3-one (28). Hydrogenation of **27** in THF/MeOH (1:1) over Pd/C at 50 psi for 1.5 h gave **28** (91%): mp (EtOAc) 228−231 °C; ^{1}H NMR [$(CD_3)_2$SO] δ 9.69 (s, 1 H), 8.22 (d, J = 8.2 Hz, 1 H), 8.02 (dd, J = 8.3, 1.0 Hz, 1 H), 7.59 (ddd, J = 8.2, 7.0, 1.2 Hz, 1 H), 7.54 (ddd, J = 8.3, 6.9, 1.4 Hz, 1 H), 6.86 (s, 1 H), 6.35 (s, 2 H), 3.71 (s, 6 H); ^{13}C NMR δ 170.4, 168.0, 148.1, 131.1, 128.6, 127.0, 125.5, 125.2, 124.7, 124.2, 123.3, 98.9, 70.7, 53.3; HRMS (EI) calculated for $C_{16}H_{14}N_2O_5$ (M^+) 314.0903, found 314.0902.

Methyl 2-Methoxy-6-nitro-4-oxo-4*H*-benz[*f*]isochromene-1-carboxylate (30). A mixture of **26** (500 mg, 1.44 mmol) and $KHCO_3$ (158 mg, 1.58 mmol) in MeOH (20 mL) and water (20 mL) was stirred at 20 °C until homogeneous and then evaporated under reduced pressure and below 30 °C. The residue was shaken with CH_2Cl_2 (20 mL), and the resulting suspension was treated sequentially with NaN_3 (187 mg, 2.88 mmol), tetrabutylammonium bromide (TBAB) (46 mg, 0.15 mmol), and phenyl dichlorophosphate (330 mg, 1.56 mmol). The mixture was stirred at 20 °C for 3 h and then filtered through a short column of silica gel, eluting with further CH_2Cl_2. After evaporation of the solvent, the resulting solid was heated with stirring in dry toluene (10 mL) at reflux for 15 min. The mixture was concentrated under reduced pressure, and the residue was chromatographed on silica gel. Elution with CH_2Cl_2 gave a solid that was recrystallized from EtOAc/*i*-Pr_2O to give **30** (198 mg, 42%): mp 178.5−179 °C; ^{1}H NMR [$(CD_3)_2$SO] δ 8.63 (s, 1 H), 8.47 (d, J = 8.6 Hz, 1 H), 7.97 (ddd, J = 8.5, 7.0, 1.3 Hz, 1 H), 7.90 (d, J = 8.1 Hz, 1 H), 7.82 (ddd, J = 8.5, 7.0, 1.3 Hz, 1 H), 4.14 (s, 3 H), 3.87 (s, 3 H); ^{13}C NMR δ 165.8, 160.9, 157.6, 143.3, 142.2, 132.2, 128.1, 127.12, 127.08, 126.4, 123.5, 122.7, 110.6, 89.9, 57.4, 52.8; HRMS (EI) calcd for $C_{16}H_{11}NO_7$ (M^+) 329.0536, found 329.0533. Anal. Calcd for $C_{16}H_{11}NO_7$: C, 58.36; H, 3.37; N, 4.25. Found: C, 58.30; H, 3.38; N, 3.93.

Further elution with CH_2Cl_2/EtOAc (6:1) gave the lactam **29** (79 mg, 16% crude yield) (characterized below).

1,1-Di(methoxycarbonyl)-5-nitro-1,3-dihydro-2*H*-benz[*e*]indol-2-one (29). A stirred suspension of **26** (7.00 g, 20.16 mmol) and powdered NaN_3 (3.28 g, 50.44 mmol) in CH_2Cl_2 (100 mL) was treated with pyridine (3.99 g, 50.44 mmol), then cooled to −5 °C, and treated in one portion with *N*,*N*-dimethyl(chlorosulfonyl)methaniminium chloride [$SOCl_2$/DMF adduct][11] (4.26 g, 22.18 mmol). After being stirred at 20 °C for 2 h, the mixture was washed twice with water, dried (Na_2SO_4), and filtered through a short column of silica gel, eluting with further CH_2Cl_2 (400 mL). Removal of the solvent under reduced pressure and below 30 °C gave the crude carbonyl azide **31**, which was immediately heated with stirring in dry toluene (65 mL) under reflux for 8 min. The mixture was cooled to 0 °C to complete precipitation of the product, which was collected and washed with toluene. This solid was stirred as a suspension in CH_2Cl_2 (25 mL) for 10 min at 20 °C and diluted with *i*-Pr_2O, and the resulting solid was collected and washed with *i*-Pr_2O to give **29** (5.60 g, 81%). A sample was crystallized from EtOAc/*i*-Pr_2O: mp 219−221 °C (dec); ^{1}H NMR [$(CD_3)_2$SO] δ 11.59 (s, 1 H, NH), 8.21 (d, J = 8.7 Hz, 1 H, H-6), 7.95 (s, 1 H, H-4), 7.87 (d, J = 8.5 Hz, 1 H, H-9), 7.74 (t, J = 7.6 Hz, 1 H, H-8), 7.65 (t, J = 7.7 Hz, 1 H, H-7), 3.72 (s, 6 H, 2 × CO_2CH_3); ^{13}C NMR δ 168.4 (C-2), 164.1 (CO_2CH_3), 148.4 (C-5), 140.5 (C-3a), 129.6 (C-9a), 129.2 (C-8), 127.1 (C-7), 123.3 (C-6), 123.1 (C-9), 121.7 (C-5a), 120.7 (C-9b), 108.8 (C-4), 66.8 (C-1), 53.9 (OCH_3). Signal assignments were confirmed by HMQC, HMBD, and COSY spectra. HRMS (EI) calcd for $C_{16}H_{12}N_2O_7$ (M^+) 344.0645, found 344.0642. Anal. Calcd for $C_{16}H_{12}N_2O_7$: C, 55.82; H, 3.51; N, 8.14. Found: C, 56.01; H, 3.55; N, 8.12.

1,1-Di(methoxycarbonyl)-5-nitro-1,2-dihydro-3*H*-benz[*e*]indole (32). BH_3·Me_2S (9.2 mL, 92 mmol) was added to a solution of **29** (17.60 g, 51 mmol) in THF (150 mL), and the mixture was stirred under reflux for 2 h. After the mixture had cooled, MeOH (15 mL) was slowly added followed by water (30 mL), and the mixture was then concentrated under reduced pressure and below 30 °C to a small volume. Addition of water provided a semisolid, which was collected and dissolved in CH_2Cl_2. The solution was washed twice with water, dried (Na_2SO_4), and concentrated under reduced pressure to provide a solid which was chromatographed on silica gel. Elution with CH_2Cl_2/EtOAc (10:1) provided the crude product which was recrystallized from *i*-Pr_2O and, following cooling, was collected to give **32** (9.62 g, 57%), which was contaminated with ca. 3% 1-(methoxycarbonyl)-5-nitro-3*H*-benz[*e*]indole (**33**). Multiple recrystallizations of a sample from *i*-Pr_2O gave pure **32**: mp 141 °C; ^{1}H NMR ($CDCl_3$) δ 8.25 (d, J = 8.7 Hz, 1 H), 7.82 (d, J = 8.6 Hz, 1 H), 7.59 (s, 1 H), 7.51 (ddd, J = 8.5, 7.0, 1.2 Hz, 1 H), 7.42 (ddd, J = 8.6, 7.1, 1.3 Hz, 1 H), 4.36 (d, J = 2.3 Hz, 2 H), 4.23 (br s, 1 H), 3.79 (s, 6 H); ^{13}C NMR δ 169.9, 149.1, 148.4, 131.9, 128.1, 125.2, 123.6, 122.1, 120.6, 109.9, 64.64, 56.6, 53.4. Anal. Calcd for $C_{16}H_{14}N_2O_6$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.23; H, 4.02; N, 8.57.

3-(*tert*-Butyloxycarbonyl)-1,1-di(methoxycarbonyl)-5-nitro-1,2-dihydro-3*H*-benz[*e*]indole (34). A mixture of crude **32** (9.35 g, 28.3 mmol), di-*tert*-butyl dicarbonate (97%, 8.28 g, 36.8 mmol), and 1-methylimidazole (3.02 g, 36.8 mmol) in THF (100 mL) was stirred at 45 °C for 1 h and then concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 and 0.1 N AcOH, and the organic layer was washed twice with water, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was stirred with CH_2Cl_2 (40 mL), then cooled, and filtered to remove some

(19) Ninomiya, K.; Shioiri, T.; Yamada, S. *Tetrahedron* **1974**, *30*, 2151.

of the minor product 3-(*tert*-butyloxycarbonyl)-1-(methoxycarbonyl)-5-nitro-3*H*-benz[*e*]indole (**35**): mp (EtOAc) 247−249 °C; ^{1}H NMR ($CDCl_3$) δ 9.73−9.66 (m, 1 H), 9.18 (s, 1 H), 8.57−8.51 (m, 1 H), 8.55 (s, 1 H), 7.77−7.68 (m, 2 H), 4.01 (s, 3 H), 1.75 (s, 9 H); HRMS (EI) calcd for $C_{19}H_{18}N_2O_6$ (M^+) 370.1165, found 370.1164. Anal. Calcd for $C_{19}H_{18}N_2O_6$: C, 61.61; H, 4.90; N, 7.57. Found: C, 61.64; H, 4.94; N, 7.27.

The CH_2Cl_2 solution was evaporated, and the residue was chromatographed on silica gel. Elution with CH_2Cl_2/petroleum ether (1:1) provided a further quantity of **35**, and continued elution with CH_2Cl_2 gave a solid which was triturated with *i*-Pr_2O/petroleum ether to give **34** (9.98 g, 82%). A sample was recrystallized from *i*-Pr_2O: mp 151 °C; ^{1}H NMR ($CDCl_3$) δ 8.85 (br s, 1 H), 8.28 (br s, 1 H), 7.93 (d, J = 7.8 Hz, 1 H), 7.61−7.51 (m, 2 H), 4.69 (s, 2 H), 3.80 (s, 6 H), 1.61 (s, 9 H). Anal. Calcd for $C_{21}H_{22}N_2O_8$: C, 58.60; H, 5.15; N, 6.51. Found: C, 58.65; H, 5.38; N, 6.42.

3-(*tert*-Butyloxycarbonyl)-1-(hydroxymethyl)-5-nitro-1,2-dihydro-3*H*-benz[*e*]indole (37). NaOMe (30.46 mL of a 0.913 M solution in MeOH, 27.81 mmol) was added dropwise to a stirred solution of **34** (7.98 g, 18.54 mmol) in THF (120 mL) at 10 °C. After 30 min at 20 °C, TFA (2.34 mL, 30.58 mmol) was added in one portion, causing dissipation of the deep purple color. The reaction mixture was diluted with saturated NaCl and extracted twice with CH_2Cl_2. The extracts were washed twice with water, dried (Na_2SO_4), and concentrated under reduced pressure and below 30 °C. The resulting oil was refrigerated (−20 °C), providing a solid that was triturated with *i*-Pr_2O/petroleum ether to give crude (*tert*-butyloxycarbonyl)-1-(methoxycarbonyl)-5-nitro-1,2-dihydro-3*H*-benz[*e*]indole **(36)** (6.76 g, 98%), which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 8.89 (br s, 1 H), 8.38 (br s, 1 H), 7.87 (d, J = 8.3 Hz, 1 H), 7.63−7.51 (m, 2 H), 4.61 (dd, J = 10.5, 3.9 Hz, 1 H), 4.56−4.44 (m, 1 H), 4.32 (t, J = 11.1 Hz, 1 H), 3.72 (s, 3 H), 1.61 (s, 9 H).

Crude **36** (6.76 g, 18.15 mmol) was dissolved in THF (120 mL) and added dropwise over 20 min to a stirred solution of DIBALH (81.7 mL of a 1 M solution in hexanes, 81.7 mmol) in THF (200 mL) under N_2 at 0 °C. The mixture was stirred for a further 30 min at 5 °C, then poured into ice-cold 2 N HCl (400 mL), and extracted twice with EtOAc. The combined extracts were washed once with water, dried (Na_2SO_4), and concentrated under reduced pressure and below 25 °C. The residue was dissolved in CH_2Cl_2/EtOAc (2:1) and filtered through a column of silica gel. The solvent was removed under reduced pressure and below 25 °C, and the resulting solid was dissolved in the minimum volume of hot CH_2Cl_2. Prolonged cooling at −20 °C provided a crystalline product that was collected and washed with a small volume of cold CH_2Cl_2 and then *i*-Pr_2O to give **37** (3.96 g). A further 0.41 g was obtained from the mother liquor following chromatography on silica gel, elution with CH_2Cl_2/EtOAc (4:1), and crystallization from CH_2Cl_2; total yield 4.37 g, 70%. A sample was recrystallized from *i*-Pr_2O/petroleum ether: mp 176 °C; ^{1}H NMR ($CDCl_3$) δ 8.90 (br s, 1 H), 8.43 (br s, 1 H), 7.88 (d, J = 7.9 Hz, 1 H), 7.62−7.51 (m, 2 H), 4.34−4.24 (m, 1 H), 4.17 (dd, J = 11.4, 9.5 Hz, 1 H), 4.04−3.93 (m, 2 H), 3.83−3.74 (m, 1 H), 1.61 (s, 9 H). Anal. Calcd for $C_{18}H_{20}N_2O_5$: C, 62.78; H, 5.85; N, 8.13. Found: C, 62.94; H, 6.13; N, 8.00.

3-(*tert*-Butyloxycarbonyl)-1-[(methanesulfonyloxy)-methyl]-5-nitro-1,2-dihydro-3*H*-benz[*e*]indole (38). A stirred solution of **37** (0.42 g, 1.22 mmol) in pyridine (1.5 mL) was treated dropwise at 0 °C with MsCl (113 μL, 1.46 mmol) and then stirred at 20 °C for a further 2 h. The mixture was diluted with water, and the resulting solid was collected, washed with water, and dried. This product was dissolved in CH_2Cl_2, and the solution was filtered through a short column of silica gel, eluting with further CH_2Cl_2. The resulting product was triturated with *i*-Pr_2O/petroleum ether to give **38** (0.46 g, 89%): mp 145−146 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 8.75 (br s, 1 H), 8.32 (d, J = 8.5 Hz, 1 H), 8.12 (d, J = 8.2 Hz, 1 H), 7.77−7.63 (m, 2 H), 4.54 (dd, J = 10.0, 3.7 Hz, 1 H), 4.43 (dd, J = 10.0, 6.4 Hz, 1 H), 4.42−4.33 (m, 1 H), 4.25 (t, J = 10.3 Hz, 1 H), 4.14 (dd, J = 11.4, 2.5 Hz, 1 H), 3.11 (s, 3 H), 1.56 (s, 9 H). Anal. Calcd for $C_{19}H_{22}N_2O_7S$: C, 54.02; H, 5.25; N, 6.63; S, 7.59. Found: C, 54.25; H, 5.36; N, 6.87; S, 7.39.

3-(*tert*-Butyloxycarbonyl)-1-(chloromethyl)-5-nitro-1,2-dihydro-3*H*-benz[*e*]indole (39). A mixture of **38** (0.51 g, 1.21 mmol) and LiCl (0.21 g, 5 mmol) in DMF (2.5 mL) was stirred at 80 °C for 30 min, then cooled, and diluted with water. The precipitated solid was dissolved in CH_2Cl_2 and filtered through a column of silica gel. The eluate was concentrated to a small volume under reduced pressure and diluted with *i*-Pr_2O/petroleum ether to give **39** (0.38 g, 87%). A sample was recrystallized from *i*-Pr_2O: mp 168−169 °C; ^{1}H NMR ($CDCl_3$) δ 8.88 (br s, 1 H), 8.42 (br s, 1 H), 7.80 (d, J = 8.1 Hz, 1 H), 7.67−7.51 (m, 2 H), 4.34 (br s, 1 H), 4.20 (t, J = 10.2 Hz, 1 H), 4.17−4.08 (m, 1 H), 3.92 (dd, J = 11.2, 2.5 Hz, 1 H), 3.54 (t, J = 10.3 Hz, 1 H), 1.62 (s, 9 H). Anal. Calcd for $C_{18}H_{19}ClN_2O_4$: C, 59.59; H, 5.28; N, 7.72; Cl, 9.77. Found: C, 59.84; H, 5.23; N, 7.70; Cl, 9.73.

1-(Chloromethyl)-5-nitro-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-benz[*e*]indole (9). A solution of **39** (170 mg, 0.47 mmol) in dioxane (5 mL) was saturated with dry HCl, stirred at 20 °C for 2 h, and then evaporated under reduced pressure and below 30 °C. [1-(3-Dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl)[20] (226 mg, 1.18 mmol), 5,6,7-trimethoxyindole-2-carboxylic acid (124 mg, 0.49 mmol), and DMA (2.0 mL) were then added, and the mixture was stirred at 20 °C for 2.5 h. Addition of dilute $KHCO_3$ precipitated a crude product that was recrystallized from CH_2Cl_2/EtOAc (decolorizing charcoal) to give **9** (178 mg, 77%): mp 243−245 °C; ^{1}H NMR ($CDCl_3$) δ 9.44 (s, 1 H), 9.24 (s, 1 H), 8.43 (dd, J = 7.9, 1.2 Hz, 1 H), 7.87 (dd, J = 7.7, 1.5 Hz, 1 H), 7.70−7.60 (m, 2 H), 7.03 (d, J = 2.5 Hz, 1 H), 6.87 (s, 1 H), 4.88 (dd, J = 10.8, 2.1 Hz, 1 H), 4.74 (dd, J = 10.4, 8.9 Hz, 1 H), 4.36−4.26 (m, 1 H), 4.10 (s, 3 H), 3.99 (dd, J = 11.4, 3.1 Hz, 1 H), 3.95 (s, 3 H), 3.92 (s, 3 H), 3.58 (dd, J = 11.4, 9.9 Hz, 1 H); ^{13}C NMR δ 160.5, 150.4, 147.8, 140.8, 140.5, 138.9, 130.0, 129.8, 128.9, 128.5, 127.8, 125.9, 124.4, 123.5, 122.9, 122.8, 115.5, 106.9, 97.6, 61.5, 61.2, 56.3, 54.7, 45.5, 43.6. Anal. Calcd for $C_{25}H_{22}ClN_3O_6$: C, 60.55; H, 4.47; N, 8.48. Found: C, 60.14; H, 4.53; N, 8.42.

5-Amino-1-(chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-benz[*e*]indole (10). A solution of **9** (60 mg, 0.12 mmol) in THF (15 mL) was hydrogenated over PtO_2 (15 mg) at 50 psi for 2 h. The catalyst was removed by filtration, the solution was concentrated to a small volume under reduced pressure and below 30 °C, and *i*-Pr_2O was added. The resulting solid was purified by precipitation from a THF solution with *i*-Pr_2O at 20 °C to give **10** (53 mg, 94%): mp 199−204 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.41 (d, J = 1.2 Hz, 1 H, NH), 8.07 (d, J = 8.5 Hz, 1 H, H-6), 7.75 (d, J = 8.3 Hz, 1 H, H-9), 7.63 (br s, 1 H, H-4), 7.45 (t, J = 7.6 Hz, 1 H, H-8), 7.28 (t, J = 7.7 Hz, 1 H, H-7), 7.03 (d, J = 2.0 Hz, 1 H, H-3′), 6.96 (s, 1 H, H-4′), 5.98 (s, 2 H, NH_2), 4.67 (dd, J = 10.8, 8.9 Hz, 1 H, H-2), 4.41 (dd, J = 10.9, 1.4 Hz, 1 H, H-2), 4.12−4.02 (m, 1 H, H-1), 3.96 (dd, J = 11.0, 3.1 Hz, 1 H, C*H*HCl), 3.94 (s, 3 H, 7′-OCH_3), 3.82 (s, 3 H, 5′-OCH_3), 3.80 (s, 3 H, 6′-OCH_3), 3.71 (dd, J = 10.9, 8.2 Hz, 1 H, CH*H*Cl); ^{13}C NMR δ 160.1 (CO), 149.1 (C-5′), 146.1 (C-5), 142.5 (C-3a), 139.7 (C-6′), 139.0 (C-7′), 131.3 (C-2′), 130.0 (C-9a), 126.7 (C-8), 125.2 (C-7a′), 123.3 (C-6), 123.1 (C-3a′), 122.9 (C-9), 122.0 (C-7), 120.3 (C-5a), 111.9 (C-9b), 105.8 (C-3′), 98.5 (C-4), 97.9 (C-4′), 61.0 (7′-OCH_3), 60.9 (6′-OCH_3), 55.9 (5′-OCH_3), 55.0 (C-2), 47.3 (CH_2Cl), 41.2 (C-1). Signal assignments were confirmed by HMQC, HMBD, and COSY spectra and by comparison with literature values for related compounds. Anal. Calcd for $C_{25}H_{24}ClN_3O_4$: C, 64.44; H, 5.19; N, 9.02; Cl, 7.61. Found: C, 64.76; H, 5.31; N, 8.76; Cl, 7.60.

1-(Chloromethyl)-5-methylamino-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-benz[*e*]indole (11). Acetic−formic anhydride [60 μL of a solution prepared from formic acid (1.25 mL, 33 mmol) and acetic anhydride (2.5 mL, 27 mmol)] was added to a solution of **10** (206 mg, 0.44 mmol)

(20) Fukuda, Y.; Itoh, H.; Nakatani, K.; Terashima, S. *Tetrahedron* **1994**, *50*, 2793.

in THF (20 mL) at 0 °C under N_2. After the mixture was stirred for 30 min at 0 °C, additional acetic−formic anhydride (60 μL) was added to the heterogeneous mixture, and stirring was continued for 2.5 h at 0 °C. The mixture was then evaporated to dryness under very low pressure. THF (35 mL) was added, and the suspension was treated with BH_3·Me_2S (0.15 mL, 1.5 mmol), and then stirred at reflux for 45 min. The reaction was cooled, MeOH (2 mL) followed by 2 N HCl (10 mL) was added, and the mixture was stirred at 20 °C for 15 min. Volatiles were removed under reduced pressure, and the residue was shaken with aqueous $KHCO_3$ and extracted twice with EtOAc. The combined extracts were washed with water, dried, and concentrated under reduced pressure. Chromatography of the residue on silica gel and elution with CH_2Cl_2/EtOAc (5:1) followed by precipitation from an EtOAc solution with *i*-Pr_2O at 20 °C gave **11** (89 mg, 42%): mp 122−125 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.45 (d, J = 1.4 Hz, 1 H), 8.09 (d, J = 8.5 Hz, 1 H), 7.78 (d, J = 8.1 Hz, 1 H), 7.48 (t, J = 7.6 Hz, 1 H), 7.32 (t, J = 7.6 Hz, 1 H), ca. 7.3 (underlying s, 1 H), 7.04 (d, J = 1.8 Hz, 1 H), 6.97 (s, 1 H), 6.53 (q, J = 4.6 Hz, 1 H), 4.67 (t, J = 9.9 Hz, 1 H), 4.46 (dd, J = 11.0, 1.5 Hz, 1 H), 4.17−4.07 (m, 1 H), 3.98 (dd, J = 11.0, 3.0 Hz, 1 H), 3.92 (s, 3 H), 3.82 (s, 3 H), 3.80 (s, 3 H), 3.77 (dd, J = 11.0, 8.2 Hz, 1 H), 2.80 (br s, 3 H). Anal. Calcd for $C_{26}H_{26}ClN_3O_4$: C, 65.06; H, 5.46; N, 8.76; Cl, 7.39. Found: C, 65.28; H, 5.55; N, 8.53; Cl, 7.11.

1-(Chloromethyl)-5-(dimethylamino)-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-benz[*e*]indole (12). A mixture of **10** (181 mg, 0.39 mmol) and formaldehyde (0.30 mL of ca. 40% w/v, 4 mmol) in THF (5 mL) was treated with solid $NaBH_3CN$ (63 mg, 1.0 mmol) followed by 2 N HCl (0.7 mL). The mixture was stirred at 20 °C for 2 h, then diluted with water, and extracted three times with CH_2Cl_2. The combined extracts were washed with water, dried, and concentrated under reduced pressure, and the residue was chromatographed on silica gel. Elution with CH_2Cl_2/EtOAc (4:1) gave a gum which was triturated with EtOAc/*i*-Pr_2O, and the resulting crude product was purified by precipitation from a CH_2Cl_2 solution with *i*-Pr_2O at 20 °C to give **12** (130 mg, 68%): mp 174−175 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.48 (d, J = 1.6 Hz, 1 H), 8.14 (d, J = 8.3 Hz, 1 H), ca. 8.0 (underlying s, 1 H), 7.92 (d, J = 8.2 Hz, 1 H), 7.54 (t, J = 7.5 Hz, 1 H), 7.44 (t, J = 7.6 Hz, 1 H), 7.07 (d, J = 1.8 Hz, 1 H), 6.98 (s, 1 H), 4.73 (t, J = 9.9 Hz, 1 H), 4.51 (dd, J = 11.1, 1.8 Hz, 1 H), 4.30−4.20 (m, 1 H), 4.05 (dd, J = 11.1, 3.1 Hz, 1 H), 3.93 (s, 3 H), 3.88 (dd, J = 11.1, 7.6 Hz, 1 H), 3.82 (s, 3 H), 3.80 (s, 3 H), 2.80 (s, 6 H). Anal. Calcd for $C_{27}H_{28}ClN_3O_4$: C, 65.65; H, 5.78; N, 8.51; Cl, 7.18. Found: C, 65.73; H, 5.98; N, 8.61; Cl, 7.13.

Resolution of Enantiomers. 38 was resolved by HPLC on a Diacel Chiralcel OD semipreparative column (10 μm, 2 × 25 cm). Samples (15 mg, maximum permitted by solubility) were dissolved in CH_3CN and diluted to give a solution comprising CH_3CN/*i*-PrOH/hexane (25:37.5:37.5). Aliquots (0.8 mL) were injected and eluted in *i*-PrOH/hexane (50:50) at a flow rate of 6.75 mL/min. This gave baseline separation of the enantiomers (α value of 1.40), with the (−)-**38** enantiomer having an R_T of 30 min and the (+)-**38** enantiomer an R_T of 42 min. The absolute configuration of the enantiomers was determined by an X-ray crystal structure determination of the faster eluting (−)-**38** enantiomer, which showed the *R* configuration (Figure 1). (−)-**38** and (+)-**38** were converted via (*R*)-**39** and (*S*)-**39** to the corresponding nitro-*seco*-CBI-TMI enantiomers (−)-**9** and (+)-**9**, which were in turn reduced to the amino-*seco*-CBI-TMI enantiomers (−)-**10** and (+)-**10**.

(−)-(*R*)-**38**: mp 147−148 °C (dec); $[\alpha]_D$ −60° (*c* 0.31, THF)

(+)-(*S*)-**38**: mp 147−148 °C (dec); $[\alpha]_D$ +61° (*c* 0.31, THF)

(−)-(*R*)-**39**: mp 133−134 °C; $[\alpha]_D$ −53° (*c* 0.34, THF)

(+)-(*S*)-**39**: mp 133−134 °C; $[\alpha]_D$ +54° (*c* 0.35, THF)

(−)-(*R*)-**9**: mp 223−225 °C; $[\alpha]_D$ −55° (*c* 0.23, THF)

(+)-(*S*)-**9**: mp 223−225 °C; $[\alpha]_D$ +54° (*c* 0.23, THF)

(−)-(*R*)-**10**: mp 229−231 °C; $[\alpha]_D$ −10° (*c* 0.20, THF)

(+)-(*S*)-**10**: mp 229−231 °C; $[\alpha]_D$ +10° (*c* 0.20, THF)

The ^{1}H NMR spectra of these enantiomers were identical to those of the corresponding racemates.

In Vitro Cytotoxicity Assay. Growth inhibitory potency under aerobic conditions was determined using log-phase cultures in 96-well plates, as described previously.[13,21] IC_{50} values were calculated as the drug concentration providing 50% inhibition of growth relative to the controls.

Acknowledgment. The authors thank Drs. John Matejovic and Ho Lee for the preparation of **3** and Karin Tan, Susan Pullen, and Donna Murray for technical assistance. This work was supported by the Auckland Division of the Cancer Society of New Zealand, the Health Research Council of New Zealand, and NCI Contract NO1-CM 47019.

Supporting Information Available: Details of the synthesis of **14**−**20** and the X-ray structures of **15**, **27**, and (−)-(*R*)-**38** (22 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO981395W

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