Synthesis and Cytotoxicity of Amino-*seco*-DSA: An Amino Analogue of the DNA Alkylating Agent Duocarmycin SA

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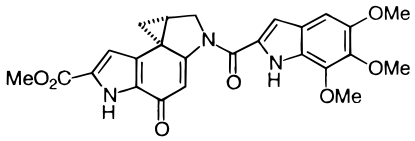
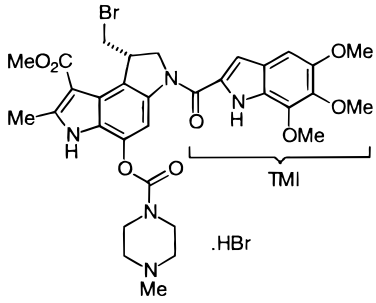
This paper describes the synthesis of methyl 5-amino-1-(chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate **8**, an amino analogue of the anticancer antibiotic and potent DNA minor groove alkylating agent *seco*-duocarmycin SA. Key points in the synthesis are sequential radical cyclization and Hemetsberger reaction steps to construct the indoline and indole rings of the target compound from a 1,2,3-trisubstituted benzene precursor. An intermediate has been resolved by chiral chromatography to provide the separate enantiomers of **8**. Racemic **8** alkylates DNA at adenine in AT rich sequences, similar to *seco*-duocarmycin SA and the previously reported amino-*seco*-CBI **7**, but is 15−60 times less potent than **7** in an in vitro cytotoxicity test. Derivatives of **8** in which the amino group is replaced by an electron-withdrawing nitro or nitrobenzylcarbamate substituent are considerably less toxic and may have application as prodrugs to be activated selectively in a tumor environment.

Introduction

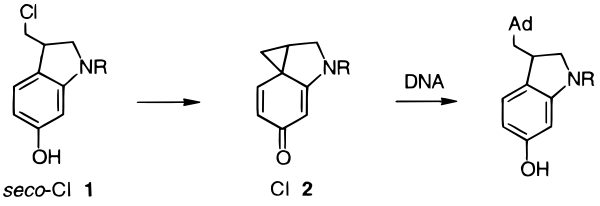
CC-1065 and the duocarmycins constitute a group of exceptionally potent antitumor antibiotics which bind to DNA in the minor groove and alkylate at N-3 of adenine.[1] The natural products and related synthetic derivatives have been extensively investigated to understand the mechanism of the alkylation step and the basis for their sequence selectivity.[2] These studies have identified structure **2** (CI) as the minimum potent pharmacophore, which can be formed by ring closure of a *seco*-CI parent **1**.[3] In fact, the toxicity of many *seco* phenolic derivatives is identical to that of the corresponding ring-closed form, suggesting that cyclization is rapid under physiological conditions.[3,4] This observation has led to the preparation of prodrugs in which the *seco* phenol is protected with an electron-withdrawing group such as a carbamate. Examples of this type are the drug candidates carzelesin[5] and KW-2189,[6] currently in clinical trial as anticancer agents. Although in these cases the prodrug is cleaved nonselectively by plasma esterases to give systemic release of the cytotoxin, the compounds clearly exhibit improved therapeutic efficacy compared to their nonprodrug analogues.[5a,6c]



(+)-Duocarmycin SA



KW-2189



seco-CI **1** CI **2**

With this background we have been investigating a series of compounds, in which the *seco* phenol is replaced by an amino group, and have earlier reported the synthesis of amino-*seco*-CI **6**[7] and amino-*seco*-CBI **7**.[8] These compounds appear to act by the same mechanism as the known phenols **3**[3] and **4**:[9] alkylation of DNA occurs

(1) Boger, D. L.; Johnson, D. S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1438, and references therein.

(2) For recent contributions, see: (a) Boger, D. L.; Santillán, A., Jr.; Searcey, M.; Jin, Q. *J. Am. Chem. Soc.* **1998**, *120*, 11554. (b) Boger, D. L.; Turnbull, P. *J. Org. Chem.* **1998**, *63*, 8004.

(3) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *J. Am. Chem. Soc.* **1990**, *112*, 8961.

(4) Boger, D. L.; Johnson, D. S.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 1635.

(5) (a) Li, L. H.; DeKoning, T. F.; Kelly, R. C.; Krueger, W. C.; McGovren, J. P.; Padbury, G. E.; Petzold, G. L.; Wallace, T. L.; Ouding, R. J.; Prairie, M. D.; Gebhard, I. *Cancer Res.* **1992**, *52*, 4904. (b) Wolff, I.; Bench, K.; Beijnen, J. H.; Bruntsch, U.; Cavalli, F.; de Jong, J.; Groot, Y.; van Tellingen, O.; Wanders, J.; Sessa, C. *Clin. Cancer Res.* **1996**, *2*, 1717. (c) van Tellingen, O.; Punt, C. J. A.; Awada, A.; Wagener, D. J. T.; Piccart, M. J.; Groot, Y.; Schaaf, L. J.; Henrar, R. E. C.; Nooijen, W. J.; Beijnen, J. H. *Cancer Chemother. Pharmacol.* **1998**, *41*, 377.

(6) (a) Kobayashi, E.; Okamoto, A.; Asada, M.; Okabe, M.; Nagamura, S.; Asai, A.; Saito, H.; Gomi, K.; Hirata, T. *Cancer Res.* **1994**, *54*, 2404. (b) Nagamura, S.; Asai, A.; Kanda, Y.; Kobayashi, E.; Gomi, K.; Saito, H. *Chem. Pharm. Bull.* **1996**, *44*, 1723. (c) Nagamura, S.; Kobayashi, E.; Gomi, K.; Saito, H. *Bioorg. Med. Chem.* **1996**, *4*, 1379.

(7) (a) Tercel, M.; Denny, W. A. *J. Chem. Soc., Perkin Trans. 1* **1998**, 509. (b) Tercel, M.; Denny, W. A.; Wilson, W. R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2735.

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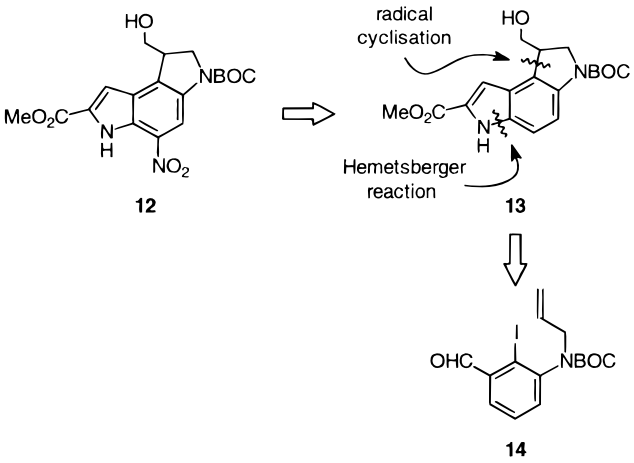
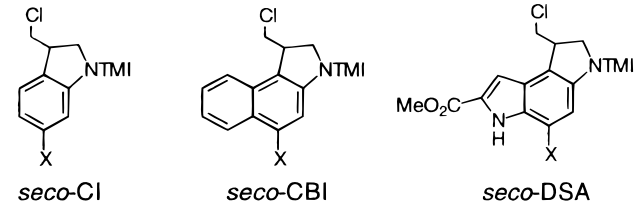
Scheme 1



at N-3 of adenine in AT rich sequences,[10] with the "natural" *S* enantiomer being the more potent of the pair.[8a,10a] We have also observed that replacing the amino group with, for example, nitro (**9**[7] and **10**[8]) or nitrobenzylcarbamate groups significantly reduces cytotoxicity. This raises the possibility that these relatively stable derivatives could be used as prodrugs, for example, with a localized nitroreductase, for tumor specific release of a cytotoxin. In this regard we have recently shown that nitro-*seco*-CI **9** is 400-fold more cytotoxic in vitro when administered in the presence of an *Escherichia coli* nitroreductase.[11]



	seco-CI	*seco*-CBI	*seco*-DSA
X = OH	**3**	**4**	**5**
NH_2	**6**	**7**	**8**
NO_2	**9**	**10**	**11**

TMI = (5,6,7-trimethoxyindol-2-yl)carbonyl

Although the amino-*seco*-CI **6** was found to be 50−100 times less cytotoxic than **3**,[7b] in the CBI series the amino and phenol forms were equitoxic, with **7** inhibiting tumor cell proliferation at subnanomolar concentrations.[8a] We now report the extension of our studies to the duocarmycin SA (DSA) alkylating agent, which in the phenol form **5** is the most potent known member of this class.[1]

Results and Discussion

Synthesis. Preparation of the BOC protected nitro-*seco*-DSA **12** was planned on the basis of nitration of an advanced intermediate **13** (Scheme 1). This option to introduce the 5-substituent at a late stage allows for a more concise synthetic plan than many of the previously reported DSA syntheses.[12] The indoline ring of **13** was envisioned as being constructed via Boger's radical cyclization of an aryl radical onto a tethered alkene,[13] while the indole could be prepared using a Hemetsberger cyclization (see below). Although the indole and indoline rings could be constructed in either order, the more logical sequence places the generally lower-yielding Hemetsberger reaction first. The strategy is thus reduced to the preparation of a suitably functionalized trisubstituted benzene **14**. Such a compound should be readily prepared from 2-iodo-3-nitrobenzoic acid **15**, itself easily available from 3-nitrophthalic acid.[14]

Proceeding with this plan clearly requires reduction of the acid and nitro groups of **15** without reductive removal of the iodine (Scheme 2). The acid was first reduced with BH_3·DMS and the alcohol protected as the acetate **17**.[15] The nitro group was then reduced with Fe/HOAc, which fortunately gave no significant deiodination.[16] Protecting **18** proved surprisingly difficult, presumably due to the bulky ortho iodo substituent. Reaction with BOC_2O in dioxane proceeded cleanly but only to 50% completion, even in the presence of excess BOC_2O and following solvent evaporation to remove *t*-BuOH from the reaction mixture. More forcing conditions (e.g., DMAP) gave several products, while NaHMDS[17] led to acyl group migration (or competing formation of the $NBOC_2$ derivative when the alcohol protecting group was TBDMS). The best procedure was simply to recycle recovered **18** which eventually provided **19** in ca. 90% yield. This was then converted to the allyl compound **20**, the acetate was cleaved, and the alcohol was oxidized to provide **14**, the substrate for the first of the proposed cyclization steps.

In the Hemetsberger reaction a benzaldehyde is condensed with the anion of methyl azidoacetate, and the resulting azidocinnamate pyrolyzed to give a substituted indole (Scheme 3).[18] Normally the pyrolysis proceeds in good yield, but the condensation step can be problematic, particularly since the anion of methyl azidoacetate is unstable with respect to loss of nitrogen. The usual procedure is to add the aldehyde and excess methyl azidoacetate to methoxide at 0 °C and collect the azidocinnamate which precipitates from solution. However, **14**

(8) (a) Atwell, G. J.; Tercel, M.; Boyd, M.; Wilson, W. R.; Denny, W. A. *J. Org. Chem*, **1998**, *63*, 9414. (b) Atwell, G. J.; Wilson, W. R.; Denny, W. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1493.

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

(10) (a) For amino-*seco*-CI, see: Tercel, M.; Gieseg, M. A.; Milbank, J. B.; Boyd, M.; Fan, J.-Y.; Tan, L. K.; Wilson, W. R.; Denny, W. A. *Chem. Res. Toxicol.* **1999**, *12*, in press. (b) For amino-*seco*-CBI, Gieseg, M. A.; Matejovic, J.; Denny, W. A. *Anti-Cancer Drug Des.* **1999**, *14*, in press.

(11) Tercel, M.; Denny, W. A.; Wilson, W. R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2741.

(12) Previous syntheses of duocarmycin SA: (a) Boger, D. L.; Machiya, K.; Hertzog, D. L.; Kitos, P. A.; Holmes, D. *J. Am. Chem. Soc.* **1993**, *115*, 9025. (b) Muratake, H.; Abe, I.; Natsume, M. *Chem. Pharm. Bull.* **1996**, *44*, 67. (c) Muratake, H.; Tonegawa, M.; Natsume, M. *Chem. Pharm. Bull.* **1998**, *46*, 400. (d) Muratake, H.; Matsumura, N.; Natsume, M. *Chem. Pharm. Bull.* **1998**, *46*, 559. (e) Fukuda, Y.; Terashima, S. *Tetrahedron Lett.* **1997**, *38*, 7207. For a concise synthesis of an oxaduocarmycin SA analogue, see: (f) 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. See also: (g) Muratake, H.; Okabe, K.; Takahashi, M.; Tonegawa, M.; Natsume, M. *Chem. Pharm. Bull.* **1997**, *45*, 799.

(13) Boger, D. L.; McKie, J. A. *J. Org. Chem.* **1995**, *60*, 1271. A recent modification involving cyclization onto a vinyl chloride has been described.[12f]

(14) Culhane, P. J. *Organic Syntheses*; Wiley & Sons: New York, 1967; Collect. Vol. 1, p 125.

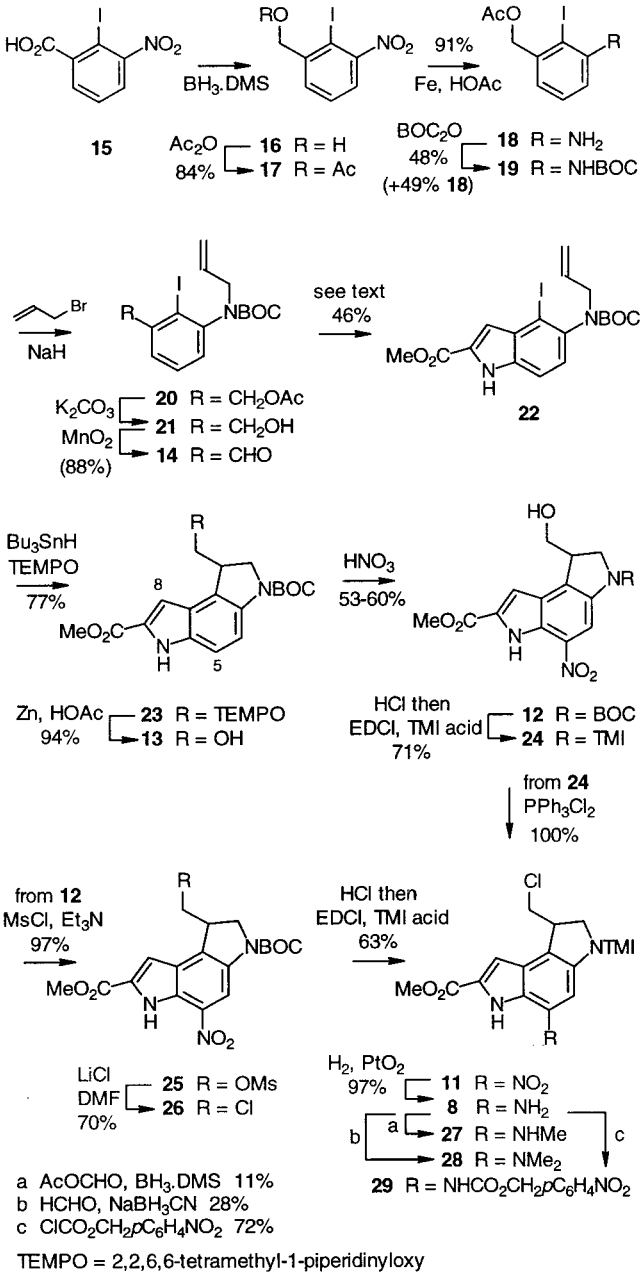
(15) Attempts to reduce the nitro group and protect with BOC_2O without prior alcohol protection led to selective formation of the carbonate.

(16) Ni_2B has been recommended as a reagent for the selective reduction of iodonitroaromatics, but in this case gave lower yields and more deiodination than the simple metal−acid reduction. Seltzman, H. H.; Berrang, B. D.; *Tetrahedron Lett.* **1993**, *34*, 3083.

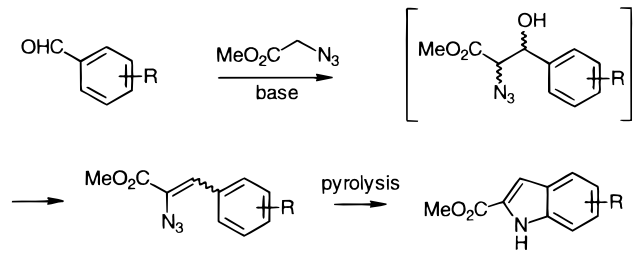
(17) Kelly, T. A.; McNeil, D. W. *Tetrahedron Lett.* **1994**, *35*, 9003.

(18) (a) Hemetsberger, H.; Knittel, D.; Weidmann, H. *Monatsh. Chem.* **1969**, *100*, 1599. (b) Hemetsberger, H.; Knittel, D.; Weidmann, H. *Monatsh. Chem.* **1970**, *101*, 161. (c) Knittel, D. *Synthesis* **1985**, 186.

Scheme 2

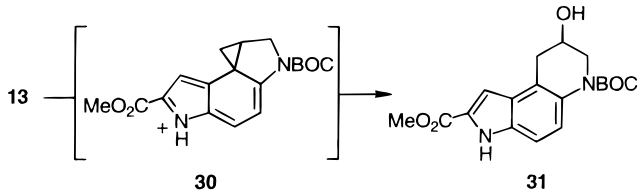


Scheme 3



gave incomplete reaction and poor recovery of material by this method, the corresponding azidocinnamate being produced in only 20−35% yield as an oil. After some experimentation it was found that NaHMDS at −78 °C allowed isolation of the intermediate azido alcohol (as a 1:1 mix of diastereoisomers) in good yield (64%) along with recovered **14**. Reaction with MsCl/Et_3N then produced the azidocinnamate, which was heated in xylene to give indole **22** in 46% yield from **14**. Following the reported procedure,[13] this compound was reacted with Bu_3SnH/TEMPO and the TEMPO group cleaved with Zn/HOAc, which provided indoline **13** in a straightforward manner.

Nitration of **13** (HNO_3/CH_3NO_2/0 °C) produced a 2:1 mixture of the 5- and 8-NO_2 isomers, with the desired product **12** isolated in 53−60% yield. The des(hydroxymethyl) analogue of **13** has been nitrated under identical conditions to give a >8:1 mixture in favor of the 5-NO_2 isomer,[19] suggesting that the hydroxymethyl group of **13** is directing nitration to the 8-position. However, nitration of the mesylate of **13** gave the same 2:1 isomer ratio, while nitration of **23** (i.e., with the TEMPO group intact) resulted in oxidation of the indoline ring as the predominant reaction. Other nitration reagents that are also compatible with the acid-sensitive BOC group (NO_2BF_4, $AcONO_2$) did not improve the yield of **12**. The nitration products were also quite sensitive to the reaction conditions: at 20 °C **12** was isolated in the same yield but with the 5,8-dinitro compound as byproduct, while under dilute conditions (<20 mM) at 0 °C no nitration took place. The starting material recovered from this reaction was found to contain 10% of the ring-expanded alcohol



31.[20] Presumably this product is formed via a cyclopropyl iminium intermediate **30**, analogous to the mechanism proposed for the alkylation of DNA by the prodrug KW-2189.[21]

The alcohol **12** was next converted to the mesylate **25** and treated with LiCl, but conditions that were appropriate in the CBI series (DMF, 80 °C) here gave elimination and rearrangement of the initial exomethylene product to the corresponding 3-methylindole. While milder conditions (20 °C) did yield **26**, this product was particularly insoluble, and it was more convenient to reverse the order of the next steps. Thus, **12** was deprotected and coupled with 5,6,7-trimethoxyindole-2-carboxylic acid under the standard conditions[3,7a] to give **24**, and the alcohol converted to chloride **11** (PPh_3Cl_2, pyridine) in excellent yield. The synthesis was completed by hydrogenation of the nitro group over PtO_2, which proceeded without any significant reductive cleavage of the chloro substituent, and produced the target compound **8** in 14 steps and 8% overall yield. Finally, in the same way as described for the amino CI and CBI analogues,[7,8] **8** was converted to the methyl, dimethyl, and nitrobenzylcarbamate derivatives **27**, **28**, and **29**.

Resolution of Enantiomers. Of the intermediates examined, mesylate **25** gave the highest solubility and best resolution on a Daicel Chiracel OD column ($\alpha = 1.37$, 1:1 *i*-PrOH/hexane). Although this compound is prone to elimination, when the resolution, chromatography, and

(19) Boger, D. L.; Sakya, S. M. *J. Org. Chem.* **1992**, *57*, 1277.

(20) **31** was separated from the co-eluting **13** by selective acylation of the primary alcohol. Particularly diagnostic features of the ^1H NMR spectrum of **31** were the OH doublet and large (17.5 Hz) geminal coupling constant of the benzylic protons.

(21) Asai, A.; Nagamura, S.; Saito, H. *J. Am. Chem. Soc.* **1994**, *116*, 4171.

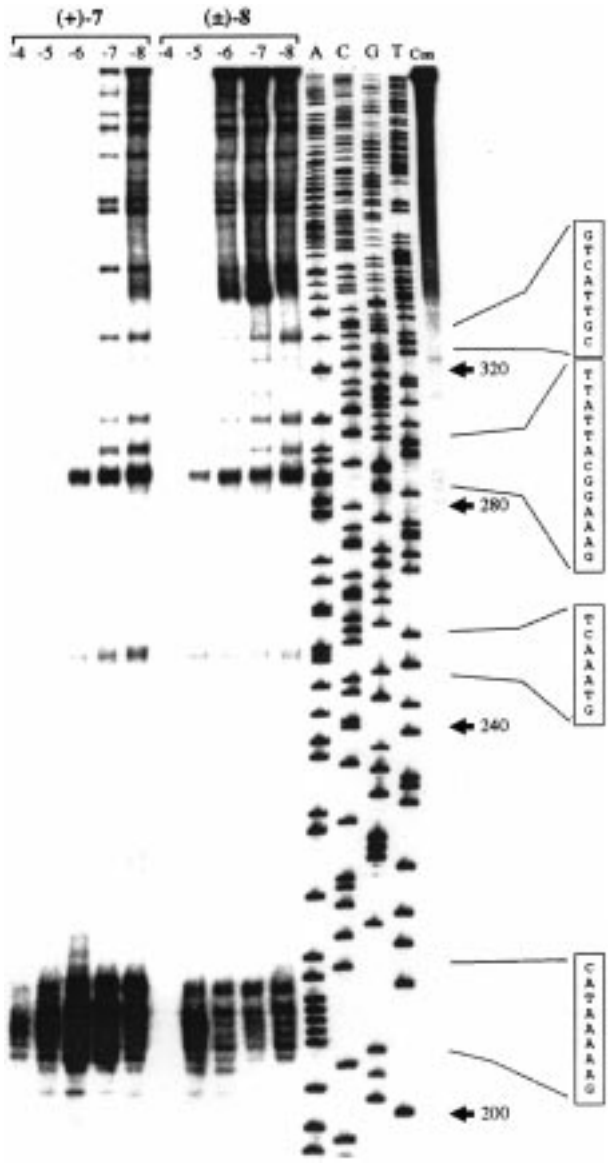


Figure 1. Cleavage products from DNA alkylated with **7** and **8** resolved using 6% denaturing polyacrylamide gel electrophoresis. The compounds were incubated with 50 ng of end-labeled DNA for 6 h at 37 °C and then heated at 100 °C for 30 min to generate the thermal cleavage products. The molar concentration of each compound is given at the top of the lanes as 1×10^x where x is the value shown. Dideoxy sequencing lanes are shown on the right with the position numbering following the GenBank entry for the *gpt* gene (×00221). The untreated control is labeled Con. The smear visible toward the top of the control and alkylation lanes is an artifact of the preparation of the end-labeled DNA and has no effect on the alkylation specificity.[10b]

solvent evaporation were carried out at room temperature, the separate enantiomers were obtained in 70% recovery and 92 and 88% ee, respectively. Each enantiomer was converted to optically active **11** and **8** by the route shown in Scheme 2. The slower eluting (+)-**25** has been tentatively assigned the natural *S* configuration on the basis of the greater cytotoxicity of the derived enantiomer of **8**.[22]

DNA Alkylation and in Vitro Cytotoxicity. A preliminary examination of DNA alkylation by racemic **8** was carried out with a 5′ end-labeled 502 bp fragment from the bacterial *gpt* gene, using the previously described thermal cleavage and gel electrophoresis assay.[10b,23] As shown in Figure 1, alkylation was confined to adenine in AT rich sequences, with **8** alkylating the same sites, at similar relative intensities and at the same low (10^{-8} M) concentration as previously observed for amino-*seco*-CBI **7**.[10b] However, this activity did not translate to equivalent cytotoxicity: IC_{50}'s determined in a cell line panel (AA8 Chinese hamster ovary, EMT6 murine mammary carcinoma, and SKOV3 human ovarian cancer cell lines) showed **8** to be 15−60-fold *less* toxic than **7** (Table 1). The relative order of toxicity for phenol *seco* agents CI < CBI < DSA,[1] is thus altered in the amino series to CI < DSA < CBI. Further IC_{50} values in Table 1 provide information of relevance to the formation of amino-*seco*-DSA prodrugs. Monomethylation to give **27** provides an analogue of slightly enhanced potency (as observed previously with amino-*seco*-CI and CBI derivatives). Dimethylation to give **28** has a similar effect on toxicity, a surprising result (also observed in the amino-*seco*-CI series) since **28** clearly cannot ring close to a cyclic intermediate. In contrast, derivatives bearing electron-withdrawing groups, the nitro compound **11** and nitrobenzylcarbamate **29**, show a significant loss of potency of several 100-fold (up to 1200-fold for **29**) when compared to the parent compound **8**.

Table 1. In Vitro Cytotoxicity (IC_{50}'s in nM, 4 h Exposure, ±SE[a])

	compound	AA8	EMT6	SKOV3
CI	**6**	347 ± 23	269 ± 37	628 ± 40
CBI	**7**	0.46 ± 0.05	0.27 ± 0.03	1.04 ± 0.11
DSA	**8**	27 ± 7	7.2 ± 0.9	15.8 ± 1.3
	(S)-**8**[b]	14.9 ± 3.3	5.3 ± 1.6	10.7 ± 3.2
	(R)-**8**[b]	49 ± 2	15.6 ± 7.1	23 ± 1
	11	13100 ± 8350	2480 ± 860	11300 ± 3100
	27	2.4 ± 0.5	0.88 ± 0.19	1.98 ± 0.12
	28	12.5 ± 0.9	5.3 ± 0.7	11.6 ± 1.4
	29	3030 ± 420	10200 ± 4800	19600 ± 2500

[a] Average of two or more determinations. [b] Absolute configuration assigned on the basis of the "natural" *S*-enantiomer being the more potent.

Conclusions

The synthesis described in this paper makes available both enantiomers of a further amino-*seco* analogue of the cyclopropylindole class of alkylating agents. Like the previously described amino-*seco*-CI and CBI compounds, amino-*seco*-DSA **8** has been found to alkylate DNA at adenine in a sequence-selective manner. Although **8** is unexpectedly less cytotoxic than the CBI analogue **7**, it remains a potent cytotoxin with IC_{50}'s in the nanomolar range. Further, the observation that analogues **11** and **29** are considerably less cytotoxic than **8** provides the basis for the future investigation of these and similar compounds as prodrugs of these potent DNA alkylating agents.

Experimental Section

2-Iodo-3-nitrobenzyl Alcohol (16). BH_3·DMS (34 mL, 0.34 mmol) was added to a solution of 2-iodo-3-nitrobenzoic

(22) Suitable crystals for a determination of absolute configuration by X-ray crystallography have not been obtained. However this assignment is consistent with the previous pattern of nitro-*seco*-CI and CBI properties: in each case the slower eluting mesylate enantiomer is dextrorotatory and gives rise to the more cytotoxic amino derivative.

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

acid[14] (82.3 g, 0.28 mol) and $B(OMe)_3$ (64 mL, 0.56 mol) in dry THF (400 mL) under nitrogen, and the mixture stirred at reflux for 90 min. The solution was cooled, MeOH and then H_2O were added, and the mixture was evaporated. Aqueous NaCl was added to the residue, and the mixture was extracted with EtOAc (×3). The extracts were washed with aqueous NaCl, dried (Na_2SO_4), and evaporated to give crude **16** as a yellow-orange solid suitable for use in the next step. A sample was filtered through a short column of silica, eluting with CH_2Cl_2, and recrystallized from PhH as pale yellow needles: mp 91−91.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.72 (dd, J = 7.7, 1.1 Hz, 1 H), 7.59 (dd, J = 8.1, 1.5 Hz, 1 H), 7.50 (t, J = 7.8 Hz, 1 H), 4.78 (s, 2 H), 2.14 (br s, 1 H). Anal. Calcd for $C_7H_6INO_3$: C, 30.13; H, 2.17; N, 5.02. Found: C, 30.42; H, 2.05; N, 4.91.

2-Iodo-3-nitrobenzyl Acetate (17). Ac_2O (37 mL, 0.39 mol) was added to a solution of **16** from the previous reaction, Et_3N (59 mL, 0.42 mol) and DMAP (0.25 g, 2 mmol) in CH_2Cl_2 (400 mL) at 0 °C. The ice-bath was removed, and the yellow solution was stirred for 10 min and then evaporated. The residue was dissolved in EtOAc, washed with aqueous HCl (2 N, ×2) and aqueous $NaHCO_3$ (×2), dried (Na_2SO_4), and evaporated. The resulting orange oil crystallized from aqueous MeOH to give **17** as a pale yellow solid (75.8 g, 84% for two steps): mp 64−65 °C; ^{1}H NMR ($CDCl_3$) δ 7.60 (dd, J = 7.9, 1.7 Hz, 1 H), 7.57 (dd, J = 7.9, 1.7 Hz, 1 H), 7.48 (t, J = 7.8 Hz, 1 H), 5.22 (s, 2 H), 2.18 (s, 3 H). Anal. Calcd for $C_9H_8INO_4$: C, 33.67; H, 2.51; N, 4.36. Found: C, 33.88; H, 2.43; N, 4.32.

3-Amino-2-iodobenzyl Acetate (18). 17 (8.00 g, 24.9 mmol) was dissolved in EtOH (100 mL) at reflux, and H_2O (100 mL) and AcOH (20 mL) were added. Fe powder (5.57 g, 97 mmol) was washed with HCl (2 N) and then H_2O and added to the hot solution. The mixture was stirred vigorously at reflux for a further 15 min and then cooled to 20 °C. Concentrated NH_3 (40 mL) was added and the mixture was filtered through Celite, eluting with EtOAc. The EtOH was evaporated, and the residue was diluted with aqueous NaCl and extracted with EtOAc (×2). The extracts were dried (Na_2SO_4) and evaporated, and the resulting solid was recrystallized from MeOH to give **18** as a cream powder (3.72 g, 51%): mp 80−81 °C. The mother liquor was evaporated and the residue purified by chromatography (30% EtOAc/petroleum ether) to give more **18** (2.93 g, 40%). ^{1}H NMR ($CDCl_3$) δ 7.12 (t, J = 7.7 Hz, 1 H), 6.76 (dd, J = 7.2, 1.3 Hz, 1 H), 6.72 (dd, J = 7.9, 1.4 Hz, 1 H), 5.11 (s, 2 H), 4.24 (br s, 2 H), 2.14 (s, 3 H). Anal. Calcd for $C_9H_{10}INO_2$: C, 37.14; H, 3.46; N, 4.81. Found: C, 37.42; H, 3.41; N, 4.78.

3-(*tert*-Butyloxycarbonyl)amino-2-iodobenzyl Acetate (19). A solution of **18** (26.9 g, 92 mmol) and BOC_2O (40.3 g, 184 mmol) in dioxane (200 mL) was stirred at reflux for 2 days. The solution was evaporated and the residue separated by chromatography (10−20% EtOAc/petroleum ether) to give recovered **18** (13.2 g, 49%) and **19** as a pale yellow oil (17.5 g, 48%). A sample crystallized from petroleum ether as a white solid: mp 62−63 °C; ^{1}H NMR ($CDCl_3$) δ 8.00 (dd, J = 8.2, 1.0 Hz, 1 H), 7.31 (t, J = 7.9 Hz, 1 H), 7.08 (dd, J = 7.7, 1.4 Hz, 1 H), 6.99 (br s, 1 H), 5.14 (s, 2 H), 2.15 (s, 3 H), 1.53 (s, 9 H). Anal. Calcd for $C_{14}H_{18}INO_4$: C, 42.98; H, 4.64; N, 3.58. Found: C, 43.27; H, 4.70; N, 3.83.

3-[*N*-(*tert*-Butyloxycarbonyl)-*N*-(2-propenyl)]amino-2-iodobenzyl Acetate (20). NaH (4.48 g of a 60% dispersion in oil, 113 mmol) was washed with petroleum ether (×3) and suspended in DMF (80 mL) under nitrogen at 0 °C. A solution of **19** (29.2 g, 75 mmol) and allyl bromide (19.4 mL, 225 mmol) in DMF (100 mL) was added in a single portion. After 5 min the mixture was allowed to warm to 20 °C and stirred for a further 1 h. H_2O was added, and the DMF was evaporated. The residue was diluted with H_2O and extracted with CH_2Cl_2 (×3), and the extracts were dried (Na_2SO_4) and evaporated. This gave crude **20** as a colorless oil, suitable for use in the next step: ^{1}H NMR ($CDCl_3$) δ 7.36−7.24 (m, 2 H), 7.19 (br d, J = 7.1 Hz, ca. 0.4 H, minor rotamer), 7.08 (br d, J = 7.1 Hz, ca. 0.6 H, major rotamer), 6.00−5.90 (m, 1 H), 5.18 (s, 2 H), 5.24−5.05 (m, 2 H), 4.59−4.43 (m, 1 H), 3.75−3.58 (m, 1 H), 2.16 (s, 3 H), 1.53 (s, ca. 3 H, minor rotamer), 1.34 (s, ca. 6 H, major rotamer); MS (CI, NH_3) m/z 449 (10%, M + NH_4), 432 (3%, M + H), 393 (100%, M − C_4H_8 + NH_4), 376 (30%, M − C_4H_8 + H). HRMS Calcd for $C_{17}H_{23}INO_4$ 432.0672. Found 432.0665.

3-[*N*-(*tert*-Butyloxycarbonyl)-*N*-(2-propenyl)]amino-2-iodobenzyl Alcohol (21). Crude **20** from the previous reaction was dissolved in MeOH (400 mL), and a solution of K_2CO_3 (12.4 g, 90 mmol) in H_2O (80 mL) was added. The mixture was stirred at 20 °C for 15 min, and the MeOH was evaporated. The aqueous residue was extracted with EtOAc (×2), and the extracts were dried (Na_2SO_4) and evaporated to give crude **21** as a pale yellow oil, suitable for use in the next step. A sample crystallized from petroleum ether as white prisms: mp 91.5−92.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.39−7.29 (m, 2 H), 7.16 (br d, J = 6.3 Hz, ca. 0.4 H, minor rotamer), 7.06 (br d, J = 7.2 Hz, ca. 0.6 H, major rotamer), 6.00−5.89 (m, 1 H), 5.14−5.03 (m, 2 H), 4.75−4.68 (m, 2 H), 4.57−4.42 (m, 1 H), 3.74−3.59 (m, 1 H), 2.12 (br s, 1 H), 1.53 (s, ca. 3 H, minor rotamer), 1.34 (s, ca. 6 H, major rotamer). Anal. Calcd for $C_{15}H_{20}INO_3$: C, 46.29; H, 5.18; N, 3.60. Found: C, 46.51; H, 5.35; N, 3.58.

3-[*N*-(*tert*-Butyloxycarbonyl)-*N*-(2-propenyl)]amino-2-iodobenzaldehyde (14). Crude **21** from the previous reaction was dissolved in EtOAc (300 mL), MnO_2 (40 g, 0.46 mol) was added, and the mixture was stirred at reflux for 20 h. The mixture was filtered through Celite, eluting with EtOAc. More MnO_2 (40 g, 0.46 mol) was added, and the mixture was stirred at reflux for a further 6 h. The filtration and oxidation were repeated once more with fresh MnO_2 (40 g, 0.46 mol), and after a further 6 h at reflux TLC (25% EtOAc/petroleum ether) indicated that the oxidation was complete. The mixture was filtered through Celite, eluting with EtOAc, and the filtrate was evaporated to give **14** as a pale yellow oil (25.3 g, 88% for three steps): ^{1}H NMR ($CDCl_3$) δ 10.17 (s, 1 H), 7.80−7.75 (m, 1 H), 7.49−7.34 (m, 2 H), 6.01−5.89 (m, 1 H), 5.18−5.03 (m, 2 H), 4.62−4.46 (m, 1 H), 3.77−3.64 (m, 1 H), 1.55 (s, ca. 4 H, minor rotamer), 1.34 (s, ca. 5 H, major rotamer); MS (CI, NH_3) m/z 405 (4%, M + NH_4), 388 (4%, M + H), 349 (100%, M − C_4H_8 + NH_4), 332 (40%, M − C_4H_8 + H). HRMS Calcd for $C_{15}H_{19}INO_3$ 388.0410. Found 388.0399.

Methyl 5-[*N*-(*tert*-Butyloxycarbonyl)-*N*-(2-propenyl)]-amino-4-iodoindole-2-carboxylate (22). NaHMDS (38.4 mL of a 2 M solution in THF, 77 mmol) was added dropwise over 45 min to a solution of **14** (7.43 g, 19.2 mmol) and methyl azidoacetate (11.0 g, 96 mmol) in THF (80 mL) under nitrogen at −78 °C. The brown solution was stirred at this temperature for 1 h and then was poured into H_2O (300 mL) containing aqueous HCl (2 N, 43 mL). The mixture was extracted with EtOAc (×2), and the extracts were dried (Na_2SO_4) and evaporated. Chromatography (10−20% EtOAc/petroleum ether) gave recovered **14** (0.83 g, 11%) and crude azido alcohol as a pale yellow foam (6.18 g, 64%).

This azido alcohol (6.34 g, 12.6 mmol) was dissolved in CH_2Cl_2 (50 mL) at 0 °C, and Et_3N (4.40 mL, 32 mmol) and MsCl (1.2 mL, 15 mmol) were added. The mixture was stirred at 0 °C for 30 min and then diluted with H_2O and extracted with CH_2Cl_2 (×2). The extracts were dried (Na_2SO_4) and evaporated, and the residue was purified by chromatography (8% EtOAc/petroleum ether) to give crude azidocinnamate as a pale yellow oil (5.11 g, 84%).

A solution of this azidocinnamate (5.11 g, 10.6 mmol) in xylene (80 mL) was added dropwise over 40 min to xylene (70 mL) at reflux under nitrogen. The solution was stirred at reflux for a further 10 min and then evaporated. Chromatography (20% EtOAc/petroleum ether) gave **22** as a cream solid (4.09 g, 85%, 46% overall from **14**): mp 178−179 °C (MeOH); ^{1}H NMR ($CDCl_3$) δ 9.32 (br s, 1 H), 7.35−7.04 (m, 3 H), 6.03−5.92 (m, 1 H), 5.13−5.02 (m, 2 H), 4.58−4.46 (m, 1 H), 3.97 (s, 3 H), 3.90 (dd, J = 14.6, 5.9 Hz, ca. 0.7 H, major rotamer), 3.75 (dd, J = 15.5, 6.5 Hz, ca. 0.3 H, minor rotamer), 1.56 (s, ca. 3 H, minor rotamer), 1.33 (s, ca. 6 H, major rotamer). Anal. Calcd for $C_{18}H_{21}IN_2O_4$: C, 47.38; H, 4.64; N, 6.14. Found: C, 47.32; H, 4.78; N, 6.30.

Methyl 3-(*tert*-Butyloxycarbonyl)-1-[(2,2,6,6-tetramethylpiperidino)oxy]methyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]-indole-7-carboxylate (23). A solution of Bu_3SnH (13.8 mL, 51 mmol) in toluene (150 mL) was added dropwise over 3 h to

a solution of **22** (5.86 g, 12.8 mmol) and TEMPO (2,2,6,6-tetramethylpiperidinyloxy, 10.0 g, 64 mmol) in toluene (400 mL) under nitrogen at 70−80 °C, during which time the red TEMPO color faded to pale yellow. TLC analysis (30% EtOAc/petroleum ether) showed some unreacted starting material. More TEMPO (3.0 g, 19 mmol) was added in a single portion, followed by a solution of Bu_3SnH (3.5 mL, 13 mmol) in toluene (80 mL) added dropwise over 2 h. The mixture was cooled and evaporated. Chromatography ($CHCl_3$ and then 5−20% EtOAc/petroleum ether) gave a pink solid (ca. 10 g) which was recrystallized from MeOH to give **23** as a white solid (3.65 g, 59%): mp 191.5−193 °C. The mother liquor was evaporated and purified by chromatography (5−20% EtOAc/petroleum ether) followed by recrystallization (MeOH) to give a second crop (1.12 g, 18%). ^{1}H NMR ($CDCl_3$) δ 8.84 (s, 1 H), 8.05 (br s, ca. 0.7 H, major rotamer), 7.63 (br s, ca. 0.3 H, minor rotamer), 7.26 (d, J = 8.8 Hz, 1 H), 7.13 (s, 1 H), 4.20−4.05 (m, 3 H), 3.94 (s, 3 H), 3.91−3.74 (m, 2 H), 1.58 (br s, 9 H), 1.48−1.27 (m, 6 H), 1.21 (s, 3 H), 1.11 (s, 3 H), 1.08 (s, 3 H), 1.06 (s, 3 H); ^{13}C NMR (one peak not observed) δ 162.2, 152.7, 137.2, 134.0, 127.9, 124.4, 114.5, 110.9, 106.3, 80.1, 78.4, 59.9, 52.2, 52.0, 39.7, 33.1, 28.5, 20.2, 17.1. Anal. Calcd for $C_{27}H_{39}N_3O_5$: C, 66.78; H, 8.09; N, 8.65. Found: C, 66.79; H, 8.16; N, 8.66.

Methyl 3-(*tert*-Butyloxycarbonyl)-1-(hydroxymethyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (13). Zinc powder (7.39 g, 113 mmol) was added to a solution of **23** (6.86 g, 14.1 mmol) in THF (150 mL), HOAc (150 mL), and H_2O (50 mL). The mixture was stirred at reflux for 40 min, cooled, and filtered through Celite eluting with EtOAc. The filtrate was evaporated, and the residue was diluted with H_2O and extracted with EtOAc (×2). The extracts were washed with H_2O and aqueous $NaHCO_3$, dried (Na_2SO_4), and evaporated. Recrystallization from MeOH gave **13** as a white solid (3.86 g, 79%): mp 189.5−191 °C dec. The mother liquor was purified by chromatography (40% EtOAc/petroleum ether) to give more **13** (0.74 g, 15%). ^{1}H NMR (d_6-DMSO) δ 11.88 (s, 1 H), 7.87 (v br s, 1 H), 7.29 (d, J = 8.9 Hz, 1 H), 7.11 (d, J = 1.4 Hz, 1 H), 4.95 (t, J = 5.2 Hz, 1 H), 4.03 (t, J = 10.5 Hz, 1 H), 3.92−3.86 (m, 1 H), 3.87 (s, 3 H), 3.82−3.75 (m, 1 H), 3.67 (br s, 1 H), 3.55−3.48 (m, 1 H), 1.51 (s, 9 H); ^{13}C NMR δ 161.6, 151.8, 136.2, 134.6, 127.8, 123.7, 122.6, 113.3, 111.3, 105.5, 79.3, 63.1, 51.7, 51.2, 42.2, 28.1. Anal. Calcd for $C_{18}H_{22}N_2O_5$: C, 62.42; H, 6.40; N, 8.09. Found: C, 62.31; H, 6.56; N, 8.32.

Methyl 3-(*tert*-Butyloxycarbonyl)-1-(hydroxymethyl)-5-nitro-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (12). 13 (447 mg, 1.29 mmol) was dissolved in CH_3NO_2 (50 mL) by heating, and the solution cooled in an ice bath. As the starting material began to precipitate (internal temperature ca. 5 °C) concentrated HNO_3 (0.16 mL, 2.6 mmol) was added dropwise, giving an orange-red mixture. The suspension was stirred at 0 °C for 40 min, then poured into cold H_2O, and extracted with CH_2Cl_2 (×3). The extracts were washed with aqueous $NaHCO_3$, dried (Na_2SO_4), and evaporated. The residue was recrystallized from MeOH to give **12** as an orange powder (229 mg, 45%): mp 200 °C dec; ^{1}H NMR (d_6-DMSO) δ 11.22 (d, J = 1.5 Hz, 1 H), 8.72 (br s, ca. 0.8 H, major rotamer), 8.42 (br s, ca. 0.2 H, minor rotamer), 7.47 (d, J = 2.0 Hz, 1 H), 5.02 (t, J = 5.2 Hz, 1 H), 4.14 (t, J = 10.5 Hz, 1 H), 3.95 (dd, J = 11.2, 4.7 Hz, 1 H), 3.92 (s, 3 H), 3.89−3.80 (m, 1 H), 3.73 (t, J = 5.2 Hz, 2 H), 1.54 (s, 9 H). Anal. Calcd for $C_{18}H_{21}N_3O_7$: C, 55.24; H, 5.41; N, 10.74. Found: C, 55.35; H, 5.35; N, 10.65.

The mother liquor was purified by chromatography (40% EtOAc/petroleum ether) to give more **12** (70 mg, 14%) and methyl 3-(*tert*-butyloxycarbonyl)-1-(hydroxymethyl)-8-nitro-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate as an orange solid (139 mg, 28%): mp 151−155 °C dec, PhH/petroleum ether; ^{1}H NMR (d_6-DMSO) δ 13.3 (v br s, 1 H), 8.04 (br s, 1 H), 7.46 (d, J = 9.0 Hz, 1 H), 4.93 (s, 1 H), 4.05 (dd, J = 11.2, 2.3 Hz, 1 H), 3.99−3.93 (m, 1 H), 3.95 (s, 3 H), 3.88−3.82 (m, 1 H), 3.48−3.40 (m, 1 H), 3.19−3.11 (m, 1 H), 1.53 (s, 9 H). Anal. Calcd for $C_{18}H_{21}N_3O_7$: C, 55.24; H, 5.41; N, 10.74. Found: C, 55.48; H, 5.10; N, 10.57.

Methyl 3-(*tert*-Butyloxycarbonyl)-5,8-dinitro-1-(hydroxymethyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate. When the nitration was conducted at 20 °C, chromatography (30% EtOAc/petroleum ether) gave, in addition to **12** (55%), the 5,8-dinitro product as an orange solid (4%): mp 187−189 °C (MeOH); ^{1}H NMR (d_6-DMSO) δ 13.47 (s, 1 H), 8.84 (s, ca. 0.8 H, major rotamer), 8.54 (s, ca. 0.2 H, minor rotamer), 4.95 (s, 1 H), 4.18−4.12 (m, 1 H), 4.10−4.04 (m, 2 H), 3.98 (s, 3 H), 3.48 (dd, J = 10.4, 4.7 Hz, 1 H), 3.32 (dd, J = 10.6, 6.5 Hz, 1 H), 1.55 (s, 9 H). Anal. Calcd for $C_{18}H_{20}N_4O_9$: C, 49.54; H, 4.62; N, 12.84. Found: C, 49.77; H, 4.61; N, 12.74.

Methyl 6-(*tert*-Butyloxycarbonyl)-8-hydroxy-6,7,8,9-tetrahydro-3*H*-pyrrolo[3,2-*f*]-quinoline-2-carboxylate (31). When the nitration was attempted at 0 °C under dilute conditions [concentrated HNO_3 (0.72 mL) added dropwise to a solution of **13** (1.97 g) in CH_3NO_2 (290 mL)], TLC analysis showed that no nitration occurred, but recovered **13** (1.90 g) contained (^{1}H NMR) ca. 10% of another co-eluting product. Recrystallization from MeOH gave pure **13**, and from the mother liquor a 1:1 mixture of **13** and the second product. This mixture was partially acetylated (Ac_2O, pyridine, catalytic DMAP, THF, 20 °C, 1 h), and the unreacted starting material was recovered by chromatography (40% EtOAc/petroleum ether) and recrystallized from PhH to give **31** as a cream solid: mp 186−187 °C; ^{1}H NMR (d_6-DMSO) δ 11.90 (s, 1 H), 7.40 (d, J = 9.0 Hz, 1 H), 7.21 (d, J = 9.0 Hz, 1 H), 7.10 (d, J = 1.2 Hz, 1 H), 5.19 (d, J = 4.1 Hz, 1 H), 4.09−4.01 (m, 1 H), 3.82 (dd, J = 12.5, 2.7 Hz, 1 H), 3.40 (dd, J = 12.5, 7.6 Hz, 1 H), 3.38 (s, 3 H), 3.22 (dd, J = 17.5, 6.4 Hz, 1 H), 2.75 (dd, J = 17.5, 6.2 Hz, 1 H), 1.45 (s, 9 H). Anal. Calcd for $C_{18}H_{22}N_2O_5 \cdot {}^1/_4$PhH: C, 64.00; H, 6.47; N, 7.66. Found: C, 63.73; H, 6.62; N, 7.75.

Methyl 1-(Hydroxymethyl)-5-nitro-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (24). 12 (1.52 g, 3.87 mmol) was stirred in HCl-saturated dioxane (120 mL) for 100 min (until TLC indicated complete reaction), and the suspension was evaporated. EDCI (1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, 1.48 g, 7.74 mmol) and 5,6,7-trimethoxyindole-2-carboxylic acid (0.97 g, 3.87 mmol) in DMA (12 mL) were added, and the mixture was stirred at 20 °C for 16 h. Dilute aqueous $NaHCO_3$ (100 mL) was added, and the precipitated solid was filtered off, washed with H_2O, and dried. Trituration with hot MeOH gave **24** as an orange powder (1.45 g, 71%): mp 239−240.5 °C dec; ^{1}H NMR (d_6-DMSO) δ 11.46 (d, J = 1.6 Hz, 1 H), 11.30 (s, 1 H), 9.19 (s, 1 H), 7.54 (s, 1 H), 7.09 (s, 1 H), 6.95 (s, 1 H), 5.11 (t, J = 5.3 Hz, 1 H), 4.71 (t, J = 10.1 Hz, 1 H), 4.47 (dd, J = 10.6, 4.1 Hz, 1 H), 4.02−3.95 (m, 1 H), 3.94 (s, 3 H), 3.93 (s, 3 H), 3.82 (s, 3 H), 3.80 (s, 3 H), 3.81−3.75 (m, 2 H). Anal. Calcd for $C_{25}H_{24}N_4O_9 \cdot {}^1/_2H_2O$: C, 56.28; H, 4.72; N, 10.50. Found: C, 56.43; H, 4.39; N, 10.28.

Methyl 1-(Chloromethyl)-5-nitro-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (11). PPh_3Cl_2 (1.65 g, 5.1 mmol) was added to a solution of **24** (1.34 g, 2.55 mmol) in pyridine (75 mL) and the solution stirred at 20 °C. After 10 min more PPh_3Cl_2 (2.06 g, 6.4 mmol) was added, and after a further 10 min the solution was poured into H_2O and the mixture stirred for 5 min. The precipitated solid was filtered off, washed with H_2O, and redissolved in CH_2Cl_2 (400 mL). This solution was filtered through Celite, eluting with CH_2Cl_2, and the filtrate was dried (Na_2SO_4) and evaporated. The resulting orange solid was recrystallized from CH_2Cl_2 to give **11** as an orange powder (0.88 g, 64%): mp 246−247.5 °C. The mother liquor was evaporated onto silica and purified by chromatography (50% EtOAc/petroleum ether) to give more **11** (0.50 g, 36%). ^{1}H NMR ($CDCl_3$) δ 10.39 (s, 1 H), 9.42 (s, 1 H), 9.39 (s, 1 H), 7.31 (d, J = 2.2 Hz, 1 H), 6.97 (d, J = 2.4 Hz, 1 H), 6.86 (s, 1 H), 4.80 (t, J = 10.0 Hz, 1 H), 4.69 (dd, J = 10.8, 4.4 Hz, 1 H), 4.32−4.23 (m, 1 H), 4.09 (s, 3 H), 4.05 (dd, J = 11.4, 3.9 Hz, 1 H), 4.02 (s, 3 H), 3.95 (s, 3 H), 3.91 (s, 3 H), 3.77 (dd, J = 11.4, 8.8 Hz, 1 H); ^{13}C NMR (d_6-DMSO) δ 160.5, 160.0, 149.2, 140.0, 139.0, 137.5, 133.6, 132.1, 131.3, 130.2, 127.7, 126.1, 125.5, 123.2, 111.3, 107.5, 106.4, 97.9, 61.1, 60.9, 55.9, 54.1, 52.3, 47.0, 42.0. Anal. Calcd for $C_{25}H_{23}ClN_4O_8$: C, 55.31; H, 4.27; N, 10.32. Found: C, 55.39; H, 4.12; N, 10.26.

Methyl 5-Amino-1-(chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (8). A solution of **11** (559 mg, 1.0 mmol) in THF (100 mL) with PtO_2 (0.10 g, 0.44 mmol) was hydrogenated at 50 psi for 30 min. The catalyst was filtered off, the filtrate was evaporated, and the residue was triturated with EtOAc to give **8** as a yellow-orange solid (404 mg, 76%): mp 190−196 °C (dec). The mother liquor was evaporated to give more **8** (111 mg, 21%). ^{1}H NMR (d_6-DMSO) δ 11.62 (d, J = 1.7 Hz, 1 H), 11.30 (d, J = 1.6 Hz, 1 H), 7.51 (br s, 1 H), 7.21 (s, 1 H), 6.95 (s, 2 H), 5.63 (br s, 2 H), 4.62 (dd, J = 10.7, 9.4 Hz, 1 H), 4.29 (dd, J = 11.0, 4.0 Hz, 1 H), 4.05 (dd, J = 10.8, 3.6 Hz, 1 H), 4.01−3.94 (m, 1 H), 3.93 (s, 3 H), 3.89 (s, 3 H), 3.83 (dd, J = 10.8, 7.6 Hz, 1 H), 3.81 (s, 3 H), 3.79 (s, 3 H). Anal. Calcd for $C_{25}H_{25}ClN_4O_6 \cdot {}^1/_2$EtOAc: C, 58.22; H, 5.24; N, 10.06. Found: C, 58.20; H, 5.09; N, 10.06.

Methyl 1-(Chloromethyl)-5-(methylamino)-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (27). Freshly prepared AcOCHO [40 μL of a solution prepared from HCO_2H (1.22 mL, 32 mmol) and Ac_2O (2.45 mL, 26 mmol)] was added to a solution of **8** (94 mg, 0.18 mmol) in THF (10 mL) under nitrogen at 20 °C. The solution was stirred for 2 h and then evaporated. The residue was redissolved in THF (8 mL) under nitrogen, BH_3·DMS (45 μL, 0.45 mmol) added, and the yellow solution was stirred at reflux for 30 min. The mixture was cooled, MeOH and H_2O were added, and the mixture was evaporated. The residue was diluted with H_2O and extracted with EtOAc (×2). The extracts were washed with H_2O, dried (Na_2SO_4), and evaporated. Chromatography (50−60% EtOAc/petroleum ether) followed by recrystallization from PhH gave **27** as a yellow solid (11 mg, 11%): mp 201−205 °C dec; ^{1}H NMR (d_6-DMSO) δ 11.61 (s, 1 H), 11.34 (s, 1 H), 7.42 (v br s, 1 H), 7.23 (s, 1 H), 6.96 (s, 2 H), 6.06 (s, 1 H), 4.64 (t, J = 9.6 Hz, 1 H), 4.33 (dd, J = 11.0, 3.6 Hz, 1 H), 4.06 (dd, J = 10.5, 3.4 Hz, 1 H), 4.05−3.96 (m, 1 H), 3.91 (s, 3 H), 3.88 (s, 3 H), 3.88−3.83 (m, 1 H), 3.81 (s, 3 H), 3.78 (s, 3 H), 2.81 (s, 3 H). Anal. Calcd for $C_{26}H_{27}ClN_4O_6 \cdot H_2O$: C, 57.30; H, 5.36; N, 10.28. Found: C, 57.16; H, 5.41; N, 10.35.

Methyl 1-(Chloromethyl)-5-(dimethylamino)-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-pyrrolo-[3,2-*e*]indole-7-carboxylate (28). $NaBH_3CN$ (40 mg, 0.6 mmol) and then aqueous HCl (2 N, 0.4 mL) were added to a solution of **8** (111 mg, 0.22 mmol) and HCHO (0.17 mL of a 40% w/v aqueous solution, 2.3 mmol) in THF (15 mL), and the pale orange solution was stirred at 20 °C for 100 min. The THF was evaporated, and the residue was diluted with H_2O and extracted with CH_2Cl_2 (×2). The extracts were dried (Na_2SO_4) and evaporated. Chromatography (1% MeOH/$CHCl_3$) followed by crystallization from PhH−petroleum ether gave **28** as a cream powder (33 mg, 28%): mp 200−202 °C dec; ^{1}H NMR (d_6-DMSO) δ 11.64 (s, 1 H), 11.38 (s, 1 H), 7.92 (v br s, 1 H), 7.34 (s, 1 H), 6.99 (s, 1 H), 6.97 (s, 1 H), 4.67 (t, J = 9.6 Hz, 1 H), 4.37 (dd, J = 11.0, 3.5 Hz, 1 H), 4.15−4.08 (m, 2 H), 3.96 (dd, J = 11.8, 8.2 Hz, 1 H), 3.92 (s, 3 H), 3.87 (s, 3 H), 3.82 (s, 3 H), 3.79 (s, 3 H), 2.78 (s, 6 H). Anal. Calcd for $C_{27}H_{29}ClN_4O_6$: C, 59.94; H, 5.40; N, 10.36. Found: C, 60.25; H, 5.61; N, 10.25.

Methyl 1-(Chloromethyl)-5-[(4-nitrobenzyloxycarbonyl)amino]-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (29). 4-Nitrobenzyl chloroformate (0.10 g, 0.46 mmol) was added to a solution of **8** (214 mg, 0.42 mmol) in pyridine (7 mL) and the mixture stirred at 20 °C. More 4-nitrobenzyl chloroformate (2 × 0.10 g) was added after 30 and 90 min, and after a further 1 h the mixture was diluted with H_2O and stirred for 30 min. The solid was filtered off, dried, and triturated with hot EtOAc to give **29** as a cream solid (209 mg, 72%): mp 257−258.5 °C; ^{1}H NMR (d_6-DMSO) δ 11.86 (s, 1 H), 11.37 (s, 1 H), 9.82 (s, 1 H), 8.89 (s, 1 H), 8.29 (d, J = 8.6 Hz, 2 H), 7.75 (d, J = 8.6 Hz, 2 H), 7.38 (s, 1 H), 7.01 (s, 1 H), 6.96 (s, 1 H), 5.37 (s, 2 H), 4.71 (t, J = 10.2 Hz, 1 H), 4.38 (dd, J = 11.0, 4.2 Hz, 1 H), 4.19−4.12 (m, 1 H), 4.11 (dd, J = 10.9, 3.3 Hz, 1 H), 4.01 (dd, J = 10.8, 6.6 Hz, 1 H), 3.93 (s, 3 H), 3.91 (s, 3 H), 3.82 (s, 3 H), 3.79 (s, 3 H). Anal. Calcd for $C_{33}H_{30}ClN_5O_{10}$: C, 57.27; H, 4.37; N, 10.12. Found: C, 57.43; H, 4.40; N, 10.09.

Resolution of Enantiomers. (+)- and (−)-Methyl 3-(*tert*-Butyloxycarbonyl)-1-[(methanesulfonyloxy)methyl]-5-nitro-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (25). MsCl (0.21 mL, 2.8 mmol) was added to a solution of **12** (540 mg, 1.38 mmol) and Et_3N (0.58 mL, 4.2 mmol) in CH_2Cl_2 (150 mL) at 0 °C, and the mixture was stirred at this temperature for 10 min. The mixture was diluted with H_2O and extracted with CH_2Cl_2 (×2), and the extracts were dried (Na_2SO_4) and evaporated at 20 °C onto silica. Chromatography (50% EtOAc/petroleum ether) gave **25** as a yellow solid (627 mg, 97%): mp 179−180 °C (dec, EtOAc/petroleum ether); ^{1}H NMR (d_6-DMSO) δ 11.34 (s, 1 H), 8.74 (s, ca. 0.8 H, major rotamer), 8.44 (s, ca. 0.2 H, minor rotamer), 7.59 (s, 1 H), 4.62−4.55 (m, 2 H), 4.29−4.18 (m, 2 H), 4.00−3.94 (m, 1 H), 3.93 (s, 3 H), 3.13 (s, 3 H), 1.54 (s, 9 H). Anal. Calcd for $C_{19}H_{23}N_3O_9S$: C, 48.61; H, 4.94; N, 8.95. Found: C, 48.46; H, 4.69; N, 8.75.

25 was resolved by HPLC on a Daicel Chiralcel OD semi-preparative column (10 μm, 2 × 25 cm). Samples were dissolved in CH_3CN and eluted in *i*-PrOH/hexane (1:1) at a flow rate of 6.75 mL/min, giving R_T's of 45.8 min for (−)-**25** and 62.8 min for (+)-**25** (α = 1.37). The pooled samples of each enantiomer were evaporated at room temperature and purified by chromatography (50% EtOAc/petroleum ether), and a sample of each was reinjected on the chiral column to establish optical purity:

(−)-(*R*)-**25**: mp 180−181 °C; $[\alpha]_D$ −21.2° (*c* 0.259, THF); ee 92%

(+)-(*S*)-**25**: mp 180−181 °C; $[\alpha]_D$ +18.9° (*c* 0.233, THF); ee 88%

(+)- and (−)-Methyl 3-(*tert*-Butyloxycarbonyl)-1-(chloromethyl)-5-nitro-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (26). (−)-(*R*)-**25** (214 mg, 0.46 mmol) and LiCl (0.10 g, 2.4 mmol) were dissolved in DMF (20 mL), and the solution was stirred at 20 °C for 6 days. H_2O was added, the solid was filtered off and redissolved in CH_2Cl_2, and the solution was dried (Na_2SO_4) and evaporated onto silica. Chromatography (30% EtOAc/petroleum ether) and trituration with hot EtOAc gave (−)-(*R*)-**26** as a pale yellow solid (124 mg, 66%): mp 210 °C dec; $[\alpha]_D$ −17.5° (*c* 0.292, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 10.34 (s, 1 H), 8.98 (br s, ca. 0.7 H, major rotamer), 8.61 (br s, ca. 0.3 H, minor rotamer), 7.26 (s, 1 H coincident with $CHCl_3$, signal appears at δ 7.66 in d_6-DMSO), 4.30−4.03 (m, 3 H), 4.00 (s, 3 H), 3.96 (dd, J = 11.2, 3.7 Hz, 1 H), 3.72 (dd, J = 10.9, 8.6 Hz, 1 H), 1.61 (s, 9 H). Anal. Calcd for $C_{18}H_{20}ClN_3O_6 \cdot {}^3/_4H_2O$: C, 51.07; H, 5.12; N, 9.93. Found: C, 50.93; H, 4.83; N, 9.64.

By the same procedure (+)−(*S*)-**25** gave (+)−(*S*)-**26** (74%): mp 208 °C dec; $[\alpha]_D$ +16.8° (*c* 0.298, $CHCl_3$).

(+)- and (−)-11. (−)-(*R*)-**26** (72 mg, 0.18 mmol) was stirred in HCl-saturated dioxane (10 mL) for 2 h (until TLC indicated complete reaction), and the suspension was evaporated. EDCI (77 mg, 0.4 mmol) and 5,6,7-trimethoxyindole-2-carboxylic acid (44.5 mg, 0.18 mmol) in DMA (2.5 mL) were added, and the mixture was stirred at 20 °C for 16 h. Aqueous NaCl was added and the mixture was extracted with CH_2Cl_2 (×4). The extracts were dried (Na_2SO_4), evaporated onto silica, and purified by chromatography (50% EtOAc/petroleum ether) to give (−)-(*R*)-**11** as an orange powder (60 mg, 63%): mp 236−237 °C; $[\alpha]_D$ −46.5° (*c* 0.342, $CHCl_3$).

By the same procedure (+)−(*S*)-**26** gave (+)−(*S*)-**11** (63%): mp 237−238 °C; $[\alpha]_D$ +45.9° (*c* 0.331, $CHCl_3$).

(*R*)- and (*S*)-8. By the same procedure as described above (−)-(*R*)-**11** and (+)−(*S*)-**11** were converted to (*R*)-**8** and (*S*)-**8**, each mp 210−220 °C dec. Neither enantiomer possessed measurable optical activity, but HPLC on a Daicel Chiralcel OD column (30% EtOH/hexane, R_T 58 and 66 min, respectively), confirmed that epimerisation had not occurred and the ee values were no lower than those of the corresponding

enantiomers (−)- and (+)-**25** from which they were derived.

In Vitro Cytotoxicity Assay. Growth inhibitory potency under aerobic conditions was determined using log-phase cultures in 96-well plates, as described previously.[8a] Compounds were freshly dissolved in DMSO or acetone. IC_{50} values were calculated as the drug concentration providing 50% inhibition of growth relative to the controls.

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