6822 *J. Med. Chem.* **2009,** *52,* 6822–6834
DOI: 10.1021/jm9008746

Journal of
Medicinal
Chemistry
Article

Synthesis and Evaluation of Stable Bidentate Transition Metal Complexes of 1-(Chloromethyl)-5-hydroxy-3-(5,6,7-trimethoxyindol-2-ylcarbonyl)-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinoline (*seco*-6-azaCBI-TMI) as Hypoxia Selective Cytotoxins

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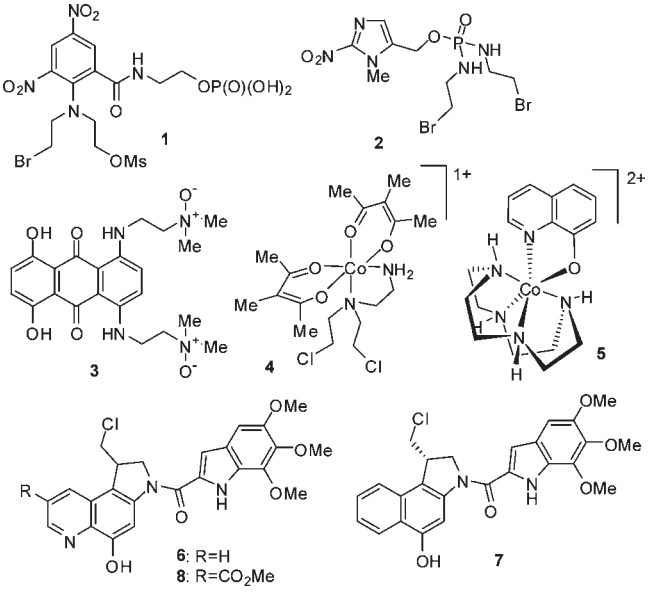
Received June 15, 2009

A series of metal complexes were prepared as potential prodrugs of the extremely toxic DNA minor groove alkylator 1-(chloromethyl)-5-hydroxy-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinoline (*seco*-6-azaCBI-TMI) and close analogues. The pyrrolo[3,2-*f*]quinoline cytotoxins were prepared from 2-methoxy-4-nitroaniline in a nine-step synthesis involving a Skraup construction of a quinoline intermediate, its appropriate functionalization, and a final radical cyclization. The metal complexes were prepared from these and the labile metal complex synthons $[Co(cyclen)(OTf)_2]^+$, $[Cr(acac)_2(H_2O)_2]^+$, and $[Co_2(Me_2dtc)_5]^+$. The cobalt complexes were considerably more stable than the free effectors and showed significant attenuation of the cytotoxicity of the latter, with IC_{50} ratios (complex/effector) of 50- to 150-fold, and substantial hypoxic cell selectivity, with IC_{50} ratios (oxic/hypoxic cells) of 20- to 40-fold. The cobalt complexes were also efficiently activated by ionizing radiation, with *G* values for loss of the compound close to the theoretical value for one-electron reduction of 0.68 μmol/J. This work extends earlier observations that cobalt cyclen complexes are suitable for both the bioreductive and radiolytic release of potent pyrrolo[3,2-*f*]quinoline effectors.

Introduction

Prodrugs capable of selective activation in hypoxic tumor cells are an attractive concept for tumor-specific chemotherapy, since hypoxia is known to be more severe and extensive in human solid tumors than normal tissues.[1] Hypoxic cells in solid tumors are resistant to radiation therapy[2] and to some chemotherapeutic drugs[3] and are thus a doubly attractive target for prodrugs.[4]

Such prodrugs can potentially be converted to an active form (effector) in hypoxic tissue by two different methods. In the first approach, endogenous cellular enzymes such as NADPH−cytochrome P-450 oxidoreductase are utilized to convert prodrugs to a transient a one-electron adduct. While this process may occur in all tissue, the intermediate can be reoxidized to the parent prodrug by the molecular oxygen present in normal tissue, but in the absence of oxygen it can be further converted or fragmented to toxic species.[5,6] A great deal of work has been reported on bioreductive enzyme prodrugs, with many different classes of compounds explored.[7] Examples include the deactivated nitrogen mustard DNA cross-linking agents PR-104 (**1**)[8] and TH-302 (**2**),[9] and banoxantrone (**3**)[10] (a prodrug of a DNA-binding topoisomerase inhibitor), all of which have reached phase II clinical trial. An earlier approach employed transition metal complexes of well-known DNA alkylating nitrogen mustards (e.g., **4**)[11,12] on the premise that the stability of cobalt(III) complexes with nitrogen-based ligands is dramatically decreased on one-electron reduction of the metal, resulting in release of the coordinated ligands.[13] While these compounds appear to have insufficient stability to work well in vivo, these studies demonstrated the potential utility of Co(III) complexes as bioreductive prodrugs.



In the second approach, the reducing equivalents from therapeutic ionizing radiation are utilized to activate prodrugs (radiation-activated prodrugs). This approach takes

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 Published on Web 10/12/2009

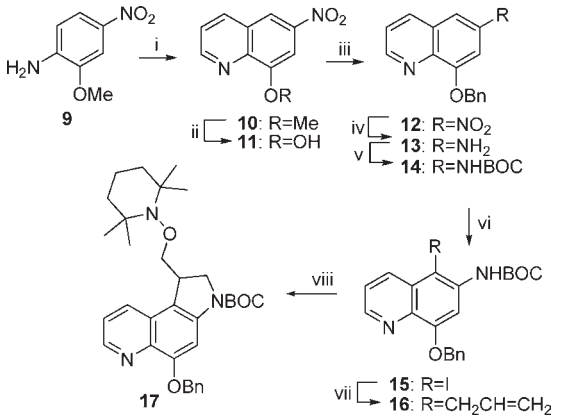
advantage of the ability of modern radiotherapy to deliver ionizing radiation selectively to the tumor field and has theoretical advantages over bioreduction.[14,15] These include the additional selectivity provided by radiation targeting, as well as hypoxia, and the lack of dependence on expression of reductive enzymes, including an ability to utilize necrotic regions devoid of enzyme activity.[14] This radiation-activated prodrug approach has been considered for activation of 2-oxopropyl prodrugs of pyrimidines.[16] We have also explored this approach in the context of cobalt(III) and chromium(III) complexes of 8-hydroxyquinoline (8-HQ) bearing a variety of different ligands at the auxiliary metal coordination positions.[17] Analogues such as **5**, with 1,4,7,10-tetraazacyclododecane (cyclen) as the auxiliary ligand, released 8-HQ very efficiently on exposure to radiation [G(8-HQ) $\geq$ 0.46 μmol/J in formate buffer, close to the theoretical value for one-electron reduction of 0.62 μmol/J in this system]. HPLC studies in high-density cell cultures showed that **5** was stable under both aerobic and hypoxic conditions and efficiently masked the cytotoxicity of the ligand in the complex, being ~1000-fold less cytotoxic than 8-HQ itself. These properties suggested the feasibility of also using such complexes as radiation-activated prodrugs for the release of more cytotoxic ligands incorporating a coordinating 8-HQ moiety that could also form stable metal complexes.

In a search for more potent effectors able to form stable Co(III) chelates, we considered the CBI-TMI class of compounds, developed following the discovery of the intensely cytotoxic natural products CC-1065 and the duocarmycins.[18] We expected that the unsubstituted aza compound **6** would be at least as cytotoxic as the "parent" compound **7**[19] (much more potent than aliphatic mustards) and, as an 8-hydroxyquinoline, would be able to form metal complexes. Previous structure−activity studies on CBI-TMI analogues have shown that electron-withdrawing groups in the benzoindole ring provide compounds (e.g., **8**) of greater stability and moderately greater potency than the parent.[20] *N*-Acyl-*O*-aminophenol prodrugs of the related duocarmycin, designed to be released by reducing nucleophiles such as thiols which are suggested to be at higher levels in hypoxic tumor environments, have been reported[21] but not evaluated for hypoxic cell selectivity.

The spirocyclic compound (+)-*N*-BOC-CPyI (**46**) has been shown to have greatly increased reactivity for adenine-N3 alkylation of DNA in the presence of various metal ions, with the relative rates of solvolysis correlating well with the stability constants of the metal complexes with 8-HQ.[22] This was attributed to metal chelation of the ketoquinoline moiety; sequential reaction of **46** with $Zn(OTf)_2$ and MeOH gave the isolated methoxy derivative **48** via the chelate **47** (Scheme 6). We considered that the aza seco compound **6** and analogues would form more stable transition metal chelates with appropriate polydentate auxiliary ligands, as we have demonstrated previously for the metal complexes of 8-hydroxyquinoline.[17] These complexes of **6** and analogues could thus potentially act as hypoxia-selective prodrugs, activated by either endogenous enzymes or ionizing radiation.

We later confirmed this with a study of the cobalt complex (**39**) of 1-(chloromethyl)-5-hydroxy-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinoline (**6**).[23] Complex **39** was shown to be relatively stable in solution and demonstrated a G value for loss of the complex in formate buffer of 0.68 μmol/J and a selectivity of about 20-fold for hypoxic over aerobic HT29 cells in culture by clonogenic assay.

Scheme 1[a]



[a] Reagents and conditions: (i) glycerol, H_2SO_4, As_2O_5; (ii) 48% HBr, reflux, 65 h; (iii) BnBr, NaI, K_2CO_3, DMF, 20 °C, 9 h; (iv) Fe, AcOH, aq EtOH, reflux, 10 min; (v) $(BOC)_2O$, dioxane, reflux, 3 h; (vi) NIS, MeCN, reflux, 1 h; (vii) $BrCH_2CH{=}CH_2$, NaH, DMF, 20 °C, 3 h; (viii) Bu_3SnH, TEMPO, benzene, under N_2, reflux, 3 h.

We report here the syntheses of **39** and related transition metal complexes of **6** and other potent 8-hydroxyquinoline derivatives and their evaluation as prodrugs for activation under hypoxia by cellular (enzymatic) reduction and by ionizing radiation.

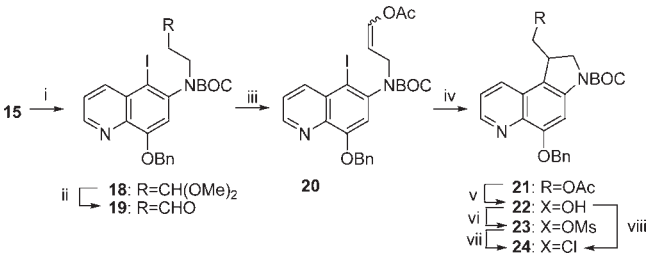
Chemistry

Our initial approach (Scheme 1) to the synthesis of the (racemic) **6** was based on Boger's 5-exo-trig radical cyclization methodology,[24] in which cyclization onto an unfunctionalized alkene and trapping of the primary radical with TEMPO produce a concise synthesis.[25]

Conversion of 2-methoxy-4-nitroaniline (**9**) by the Skraup reaction gave 8-methoxy-6-nitroquinoline (**10**); careful application of the simplest reported procedure[26] allowed the yield to be improved from the reported 68% to 80%. The methyl protecting group was then replaced with benzyl to allow for a more ready removal at the end of the synthesis. Demethylation of **10** did not require the reported[27] TFA; when the sample was heated with 48% aqueous HBr (5 equiv) for 65 h, the HBr salt of the quinolinol **11** precipitated from the cooled reaction mixture (87% yield) and was benzylated directly (BnBr, excess K_2CO_3, NaI, DMF, 99%) to give **12**. Reduction of **12** with iron dust in EtOH/AcOH/H_2O gave amine **13** quantitatively. Protection of **13** with di-*tert*-butyl dicarbonate in refluxing THF was incomplete, but in refluxing dioxane a 98% yield of **14** was achieved. Iodination of **14** with *N*-iodosuccinimide (NIS) in the presence of catalytic acid[22] was complicated by the basic center present in the quinoline, and a preferable method was treatment with NIS in refluxing acetonitrile.[28] This gave iodide **15** regiospecifically in 93% yield. This could be allylated uneventfully and the allyl derivative **16** cyclized efficiently in the presence of Bu_3SnH and TEMPO to give **17**. Unfortunately, attempts to cleave the N−O bond of **17** with a variety of reagents (Zn/THF/H_2O/AcOH,[25] Zn/NaOH/H_2O/EtOH, Na/EtOH, Al/Hg/THF,[29] or SmI_2/THF[30,31]) were unsuccessful.

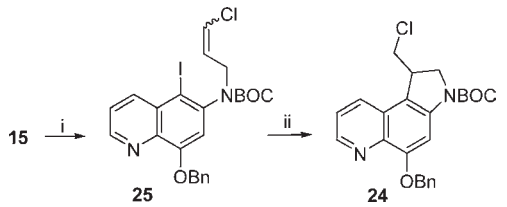
Of several possible alternative routes, the most attractive appeared to be cyclization onto a vinyl ether or ester, where the presence of oxygen on the vinyl group of the starting material allows a simple reductive cyclization (Scheme 2). The dimethoxy acetal **18**, prepared by alkylation of **15** with

Scheme 2[a]



[a] Reagents and conditions: (i) $Br(CH_2)_2CH(OMe)_2$, NaH, 20 °C, 22 h; (ii) TsOH, Me_2CO/water (10:1), reflux, 2.25 h; (iii) Ac_2O, Et_3N, DMAP, THF, reflux, 4 h; (iv) Bu_3SnH, AIBN, benzene, reflux, 5.5 h; (v) Cs_2CO_3, EtOH/water (2:1), reflux, 30 min; (vi) MsCl, Et_3N, DCM, 0 °C, 30 min; (vii) LiCl, DMF, 80 °C, 1 h; (viii) Ph_3P, CCl_4, DCM, 20 °C, 4 h.

Scheme 3[a]



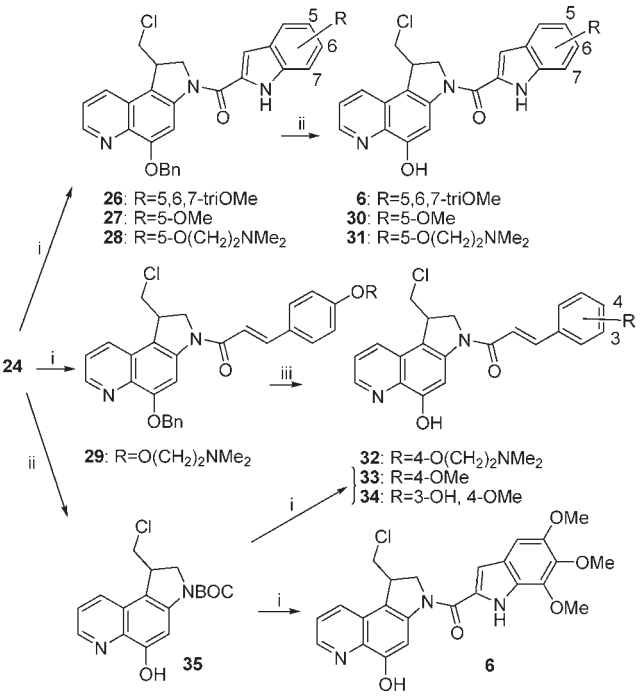
[a] Reagents and conditions: (i) NaH, DMF, then 1,3-dichloropropene, 20 °C (under N_2), 86 h; (ii) AIBN, Bu_3SnH, benzene, reflux (under N_2), 3 h.

3-bromo-1,1-dimethoxypropane,[32] could be quantitatively deprotected with TsOH (0.5 equiv) in wet acetone. Since the substrate is a quinoline, the reagent is effectively an analogue of PPTS.[33] The resulting aldehyde **19** was converted into vinyl acetate **20** (Ac_2O, DMAP, THF, reflux, 81%),[34] which underwent radical cyclization in the presence of Bu_3SnH and AIBN to give acetate **21** in good (77%) yield. Deprotection of **21** with Cs_2CO_3 gave alcohol **22**, which could be converted either directly (Ph_3P, CCl_4, 100%) or via mesylate **23** (MsCl, Et_3N, 86%; then LiCl, DMF, 89%) to the desired racemic chloromethylpyrroloquinoline **24**. An iodo substituent had initially been selected because it has been reported[24] to give better yields when TEMPO is used as the radical trap. However, in simple reductive cyclizations bromine suffices and was used here. Treatment of **14** with NBS instead of NIS gave bromoquinoline **49** in excellent yield, and yields through the remainder of the path (**49** → **50** → **51** → **52** → **21**) were similar or slightly better (Scheme S1 in Supporting Information).

At this point in the work the synthesis of oxaduocarmycin involving radical cyclization onto a vinyl chloride was reported.[35] Applied to the current work, alkylation of iodide **15** with 1,3-dichloropropene and radical cyclization of the resulting vinyl chloride **25** or the bromo equivalent gave **24** in 97% yield (Scheme 3). A similar process beginning with the corresponding bromide **49** gave **24** in 95% yield (Scheme S2 in Supporting Information).

Acid deprotection of **24**, followed by EDCI-mediated coupling with 5,6,7-trimethoxy-, 5-methoxy-, and 5-[2-(dimethylamino)ethoxy]indole-2-carboxylic acids gave amides **26**–**28**, while coupling with the requisite cinnamic acid gave **29**. The 5-benzyloxy intermediates **26**–**28** were debenzylated with Pd–C and ammonium formate, and intermediate **29** with TFA, to give the target compounds **6** and **30**–**32** (Scheme 4). For **6** the alternative route of initial debenzylation of **24** and coupling the resulting phenol **35** with TMI-2-carboxylic acid gave a lower yield, but **33** and **34** were also

Scheme 4[a]



[a] Reagents and conditions. (i) HCl(g), dioxane, 20 °C, then RCO_2H, then EDCI, DMA, 20 °C; (ii) NH_4HCO_2, Pd–C, THF (under N_2), 20 °C; (iii) TFA, 48 h, reflux.

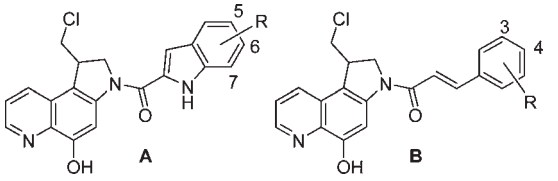
made this way for convenience. Resolution of **24** was carried out by semipreparative HPLC on a ChiralCel OD column, eluting with hexane/^{i}PrOH (9:1) (α = 1.24). The slower-eluting (−)-enantiomer was assigned the natural *S*-configuration on the basis of its conversion, via the (−)-enantiomer of **26**, to the relatively more cytotoxic (−)-enantiomer *S*-**6** (Table 1). This is consistent with related CBI analogues, where the slower-eluting enantiomers are also the more potent compounds.[36]

Preparation of Metal Complexes

Octahedral cobalt(III) complexes are relatively inert as a result of the d^6 low-spin electron configuration at the metal center and ligand substitution reactions tend to be slow. This necessitates careful design of the protocol for introducing ligands into the coordination sphere. All of the pyrrolo[3,2-*f*]-quinoline effectors are reactive in solution, undergoing spirocyclization and eventual hydrolysis and decomposition, so the substitution reaction at cobalt needs to be fast enough that these reactions cannot compete effectively with coordination to cobalt. Furthermore, **6** and its analogues are nontrivial to prepare and so ideally should be introduced into the cobalt complexes at the last step, with other ligands on cobalt already in place.

A convenient starting material for the cobalt cyclen series of complexes is $[Co(cyclen)(NO_2)_2][NO_2]$ (**37**), which has been described in the literature.[37] The nitro ligands may be replaced by more labile triflato (OTf) ligands by reaction of $[Co(cyclen)(NO_2)_2]^+$ with neat triflic acid (HOTf) (Scheme 5). The nitro groups are protonated and ultimately eliminated as NO_x gases, provided the reaction is carried out under anhydrous conditions to prevent coordination of aqua ligands. Substitution of the weakly basic, labile triflato ligands by **6** is achieved in acetonitrile or methanol, with ^{i}Pr$_2$NEt or pyridine

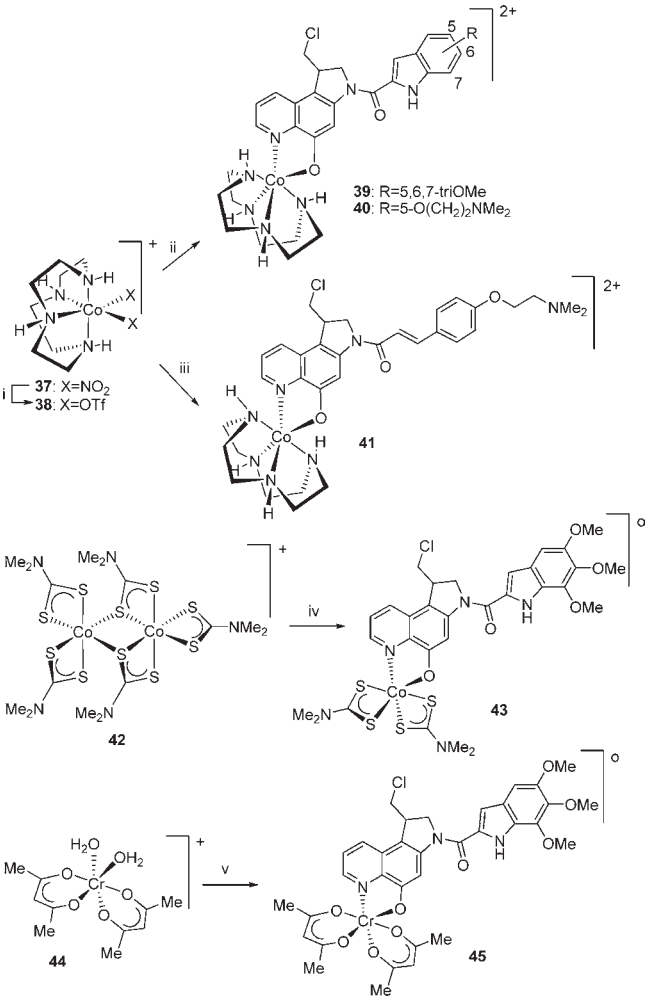
Table 1. Growth Inhibitory Properties of Pyrrolo[3,2-*f*]quinoline Analogues



				IC_{50} (nM)[c]						
compd	Fm	sol.,[a] mM	R	AA8[b]	UV4[b]	EMT6 V[b]	IC_{50} (nM)[c], SKOV[b]	HCR,[d] SKOV[b]	IC_{50} (nM), [c] A549[b]	HCR,[d] A549[b]
(±)-**6**	A	0.03	5,6,7-tri-OMe	0.14 ± 0.02	0.07 ± 0.02	0.05 ± 0.01	0.26 ± 0.03	0.69 ± 08	0.06 ± 0.01	0.76 ± 0.21
R-(−)-**6**	A		5,6,7-tri-OMe	22 ± 2	4.6 ± 0.5	7.3 ± 2.8	8.5 ± 0.3		8.8 ± 0.3	
S-(+)-**6**	A		5,6,7-tri-OMe	0.07 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.11 ± 0.02		0.04 ± 0.01	
30	A	0.110	5-OMe				0.27 ± 0.10	0.91 ± 0.30	0.08 ± 0.02	1.51 ± 0.06
31	A	0.032	5-O$(CH_2)_2NMe_2$	4.4 ± 0.8	1.7 ± 0.2	0.74 ± 0.22	6.9 ± 0.9	0.56 ± 0.10	1.3 ± 0.3	1.11 ± 0.44
32	B	0.86	4-O$(CH_2)_2NMe_2$	2.6 ± 0.2	0.73 ± 0.14	0.58 ± 0.17	1.9 ± 0.3	0.65 ± 0.21	0.92 ± 0.14	0.86 ± 0.25
33	B	0.04	4-OMe				0.29 ± 0.01		0.09 ± 0.02	
34	B	0.11	3-OH, 4-OMe				0.17 ± 0.01		0.18 ± 0.03	

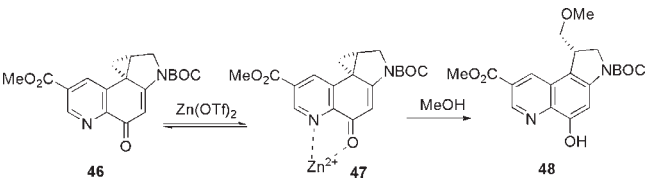
[a] Solubility in α-MEM + 5% FCS. [b] Cell lines: AA8, Chinese hamster fibroblast; UV4, AA8 mutant defective in the incision step of excision repair; EMT6V, murine breast carcinoma; SKOV3, human ovarian carcinoma; A549, human lung adenocarcinoma. [c] Concentration for 50% inhibition of cell proliferation in aerobic log phase cultures following a drug exposure time of 4 h. Results are the mean ± SEM for >2 determinations. [d] Hypoxic cytotoxicity ratio = ($IC_{50[aerobic]}/IC_{50[hypoxic]}$).

Scheme 5[a]



[a] Reagents and conditions: (i) excess CF_3SO_3H, N_2(g); (ii) **6** + 1.5 equiv of $^iPr_2NEt$, 20 °C; or **6** + pyridine, 50 °C; or **31**, 20 °C; (iii) **32**, 20 °C; (iv) **6** + 2 equiv of $^iPr_2NEt$, 20 °C; (v) **6** + 1.1 equiv of $^iPr_2NEt$, 50 °C.

Scheme 6. Solvolysis of azaCBIs through Transient Two-Coordinate Metal Complexes (from Reference 22)



added to deprotonate **6**. The product $[Co(cyclen)(\mathbf{6})]^{2+}$ (**39**) can be isolated as the perchlorate salt and purified by reverse phase HPLC. The complexes $[Co(cyclen)(\mathbf{31})][OTf]_2$ (**40**) and $[Co(cyclen)(\mathbf{32})][OTf]_2$ (**41**) were prepared similarly from $[Co(cyclen)(OTf)_2]^+$, except an added base is not required presumably because of the presence of the basic side chain on the ligands.

We have previously developed a route using the binuclear cobalt(III) precursor $[Co_2(Me_2dtc)_5]^+$ (**42**)[38] for the preparation of cobalt bis(dithiocarbamate) complexes containing reactive ligands.[39] The binuclear complex efficiently cleaves to give a neutral $Co(Me_2dtc)_3$ complex and a $[Co(Me_2dtc)_2]^+$ synthon which can coordinate the target ligand. Reaction of $[Co_2(Me_2dtc)_5][BF_4]$ with **6** proceeded smoothly with added $^iPr_2NEt$ as base (Scheme 5), and the product $[Co(Me_2dtc)_2(\mathbf{6})]$ (**43**) was purified by chromatography.

For all of the diamagnetic cobalt(III) effector complexes coordination of the effector could be confirmed by the absence of a resonance due to the OH proton in the ^1H NMR spectrum of the complex. For all three effectors this proton appears as a broad singlet close to 10.0 ppm in the spectrum of the free ligand. Integration of the peaks arising from the effector ligand in the complexes compared to those for the auxiliary ligands confirmed the presence of one effector ligand per complex.

Chromium(III) complexes also tend to be relatively inert, and the same design strategy was employed as for cobalt. The diaqua complex $[Cr(acac)_2(H_2O)_2]^+$ (**44**) is a suitable precursor, as the aqua ligands are sufficiently labile.[40] A mixture of cis and trans $[Cr(acac)_2(H_2O)_2]ClO_4$ in dry acetonitrile reacts

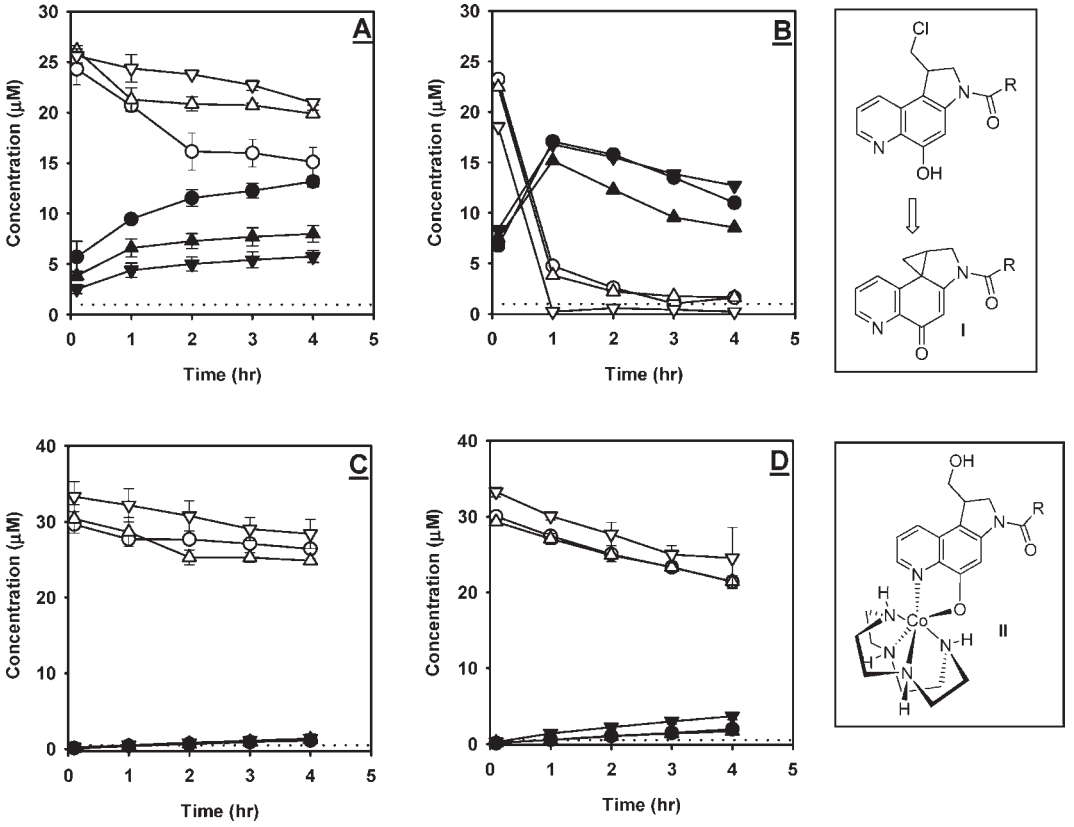


Figure 1. (A, B) Stability of effectors in DMSO at room temperature and in αMEM plus 5% FCS at 37 °C, respectively: **6** (○); **31** (△); **32** (▽). Closed symbols are the corresponding spiro-cyclized products as illustrated with general structure **I**. (C, D) Stability of the corresponding metal complexes in formate buffer at room temperature and in αMEM plus 5% FCS at 37 °C, respectively: **39** (○); **40** (△); **41**(▽). Closed symbols are the corresponding hydrolysis products as illustrated with general structure **II**. Each symbol denotes the mean ± range for the duplicate determinations, by HPLC.

with **6** and $^{i}Pr_2NEt$ to give [Cr(acac)$_2$(**6**)] (**45**) which was purified by flash chromatography on silica gel (50% MeCN/$CHCl_3$) in 75% yield (Scheme 5).

Results and Discussion

Biological results for **6** (*R*, *S*, and racemate) and a small series of racemic analogues **30**−**34** are recorded in Table 1. While **6** is a very potent cytotoxin with IC_{50} in the nanomolar range, it is considerably less cytotoxic than the reported values for the CBI **7**[19] and the related ester **8**,[22] at least with the different drug exposure time and cell lines used (the natural *S*-enantiomer of **8** has a reported IC_{50} of 0.021 nM against L1210 murine leukemia cells in culture on 72 h of exposure). The two enantiomers of **6** exhibited striking differences in cytotoxicity, with the (+)-enantiomer being on average 330-fold more toxic than the (−)-enantiomer across the five cell lines, this differential being larger than that reported for **7** and its enantiomer (67-fold for the cyclopropyl forms) or for the enantiomers of **8** (16-fold). The analogues with basic side chains (**31** and **32**) were less potent than those with neutral ones. The two different side chains (indole and cinnamate) did not result in significant changes in potency (cf. **6** and **31**−**33**). The ability of **6** and **31**−**32** to inhibit the growth of hypoxic cells was confirmed, as demonstrated by HCR values close to unity (Table 1). The use of basic side chains in effectors **31** and **32** did not give marked increases in aqueous solubility.

The physiochemical properties of these effectors and their cobalt/cyclen complexes are shown in Figure 1. The effectors were relatively unstable, converting initially to the corresponding spirocyclized compounds (**I**) as shown in Figure 1A,B. These in turn slowly decomposed in culture medium (Figure 1B). The complexes **39**−**41** were more stable in both formate buffer and culture medium, being only slowly converted to the more polar hydrolysis products (**II**) shown in Figure 1C,D. The hydrolysis and spirocyclized products were identified spectroscopically and by analogy with previous work.[22]

The biological properties of the metal complexes are given in Table 2. The cobalt bis(dithiocarbamate) complex **43** proved too insoluble to work with. The three cobalt/cyclen complexes **39**−**41** showed significant attenuation of the cytotoxicity of the corresponding free effectors (prodrug/effector [P/E] ratios of 50- to 150-fold), confirming stable complexes in each case and demonstrating marked suppression of cytotoxicity in the prodrug form. They also showed significant hypoxic cell selectivity (HCRs in the range of 20- to 40-fold, although 7.5-fold for **39** in A549 cells). The aerobic toxicities of these three complexes follow the order of toxicity of the corresponding pyrrolo[3,2-*f*]quinoline effectors, suggesting that they are governed significantly by slow release of the effector under these conditions, as previously shown[23] for **39**. A more extensive study of **39** in a range of cell lines showed HCRs between 8- and 38-fold (Figure 2). The chromium/acac complex **45** showed excellent attenuation of the cytotoxicity of the free effector **6** (P/E ratio of ∼700-fold in both of the cell lines studied) but showed no differential cytotoxicity between aerobic and hypoxic cells (HCR 1- to 2-fold). Both of these properties can likely be attributed to the very low reduction potential of chromium complexes of this type ($E_{1/2}$ values of −211 and −1180 mV, respectively, for the cobalt/cyclen and chromium/cyclen complexes of 8-HQ).[17] This results in a very

Table 2. Biological Properties of Metal Complexes

	SKOV3[a]				A549[b]					
		IC_{50} (nM)[d]				IC_{50} (nM)[d]			*G*-values	
compd	P/E[c]	oxic	hypoxic	HCR[e]	P/E[c]	oxic	anoxic	HCR[e]	*G*(−P)[f]	*G*(+E)[g]
39	152	38 ± 14	3.7 ± 1.5	18 ± 8	88	5.0 ± 0.9	1.2 ± 0.5	7.5 ± 3.2	0.68[h]	0.57[h]
40	84	580 ± 120	20 ± 2	31 ± 2	100	130 ± 14	2.9 ± 0.7	41 ± 13		
41	54	100 ± 15	8.1 ± 5.5	26 ± 12	46	43 ± 3	2.4 ± 1.5	35 ± 15	0.61	0.54
45	680	170 ± 30	230 ± 30	1.30 ± 0.13	737	42 ± 14	25 ± 4	2.0 ± 0.8		

[a] SKOV3 human ovarian carcinoma. [b] A549 human lung adenocarcinoma. [c] IC_{50}(prodrug)/IC_{50}(effector) under oxic conditions. [d] For log phase cultures with drug exposure time of 4 h. [e] Hypoxic cytotoxicity ratio (oxic IC_{50}/hypoxic IC_{50}); these are intraexperiment ratios and thus may differ from the ratios of the average oxic/hypoxic values in the table. [f] *G* value (μmol/J) for loss of prodrug (complex). [g] *G* value (μmol/J) for formation of effector (both the cyclopropyl and *seco* forms were detected and were summed to give the total yield of effector). [h] Data from ref 23.

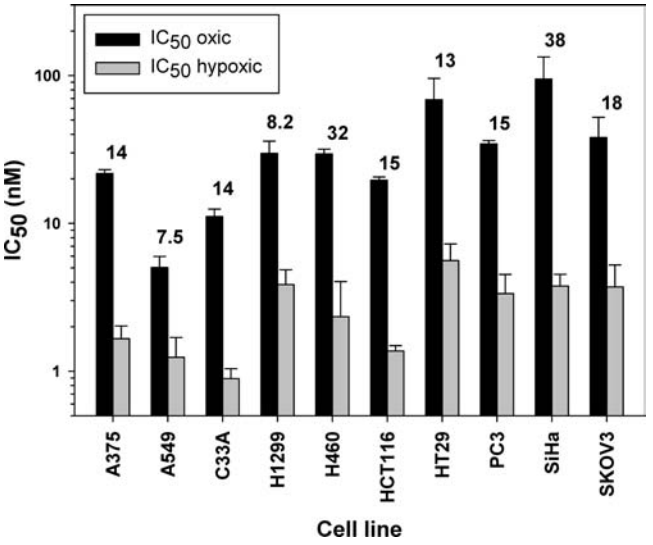


Figure 2. Cytotoxicity of **39** under oxic and hypoxic conditions across a human cell line panel, using log phase cultures and a drug exposure time of 4 h. Results are the mean ± SEM for two to eight determinations. The numbers above the bars are HCRs as defined in Table 2. Cell lines are A375 melanoma, A549 NSCLC, C33A cervical, H460 NSCLC, HCT116 colon, HT29 colon, PC3 prostate, SiHa cervical, and SKOV3 ovarian.

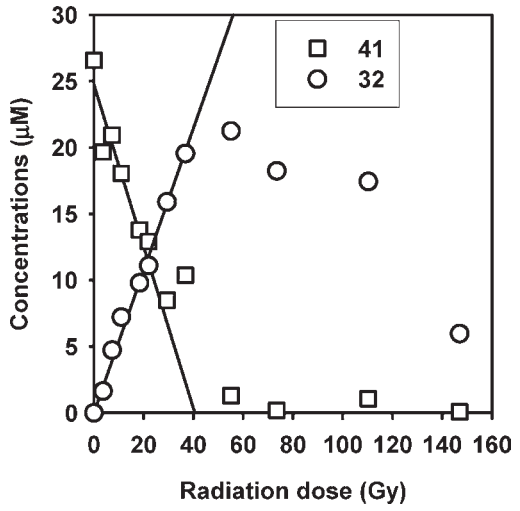


Figure 3. Radiolytic reduction of **41** in deoxygenated formate buffer (pH 7.0), showing the release of effector **32**. An equal volume of isopropanol was added immediately after the radiolysis, and **32** was quantitated as the sum of *seco*- and cyclopropyl forms.

stable complex **45**, with a very low rate of cellular reduction, as the redox potentials of possible biological reductants such as NADH or glutathione are not high enough to overcome the low potential.

It was shown previously[23] that on exposure to ionizing radiation in formate buffer the cobalt cyclen complex **39** had a *G* value for loss of the compound close to the theoretical value for one-electron reduction (0.68 μmol/J). This is similar to the value seen[17] for the corresponding cobalt cyclen complex with 8-HQ. In the case of **39**, both the cyclopropyl and seco forms of the free ligand were detected after irradiation, but if these were summed, the formation of free effector was close to quantitative. HPLC analysis of irradiated formate solutions of the cobalt cyclen complex **41** (of the "solubilized" effector **32**) showed broadly similar reductive properties (Figure 3), with a stoichiometry of reduction (*G* value for prodrug loss of 0.61 μmol/J), and efficient one-electron release on reduction (*G* value for effector formation of 0.54 μmol/J, Table 2). This extends the earlier observation[23] of the suitability of the cobalt/cyclen system for the radiolytic release of CBI-type effectors.

Conclusions

The 1*H*-pyrrolo[3,2-*f*]quinoline analogues **6** and **30**−**34** prepared here retain the characteristic high and enantiomerically selective cellular potencies of the broad class of CBI toxins. They form stable cobalt and chromium complexes with a variety of ancillary ligands. The corresponding cobalt cyclen complexes **39**−**41** were markedly less cytotoxic than the corresponding free effectors and also showed significant hypoxic cell selective toxicity (7.7- to 40-fold), demonstrating their utility as hypoxia-activated cytotoxins. Complexes **39** and **41** also showed efficient and close to quantitative release of their effectors on exposure to ionizing radiation, supporting previous work on the suitability of the cobalt cyclen 1*H*-pyrrolo[3,2-*f*]quinoline complexes for the radiolytic release of cytotoxins.

Experimental Section

All reagents used were of analytical grade. Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal 2300 melting point apparatus. NMR spectra were obtained on a Bruker Avance 400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C spectra. Spectra were obtained in $(CD_3)_2SO$ unless otherwise specified and are referenced to Me_4Si. Chemical shifts and coupling constants were recorded in units of ppm and Hz, respectively. Assignments were determined using COSY, HSQC, and HMBC two-dimensional experiments where appropriate. Mass spectra were determined on a VG-70SE mass spectrometer using an ionizing potential of 70 eV at a nominal resolution of 1000. High-resolution spectra were obtained at nominal resolutions of 3000, 5000, or 10 000 as appropriate. FAB+ spectra used *m*-nitrobenzyl alcohol as the

matrix and a xenon atom gun. Accurate mass calculations were referenced to polyethylene glycol (PEG). Solutions in organic solvents were dried with anhydrous Na_2SO_4. Solvents were evaporated under reduced pressure on a rotary evaporator. Thin-layer chromatography was carried out on aluminum-backed silica gel plates (Merck 60 F_{254}), with visualization of components by UV light (254 nm) or exposure to I_2. Column chromatography was carried out on silica gel (Merck 230−400 mesh). DCM refers to dichloromethane. DMSO refers to dimethyl sulfoxide. EtOAc refers to ethyl acetate. MeOH refers to methanol, MeCN refers to acetonitrile. Petroleum ether refers to petroleum ether, boiling range 40−60 °C. All solvents were freshly distilled.

Scheme 1. 8-Hydroxy-6-nitroquinoline Hydrobromide (11). A solution of 8-methoxy-6-nitroquinoline (**10**), prepared from 2-methoxy-4-nitroaniline (**9**) by the reported method.[26] (500 g, 0.245 mol), in 48% aqueous HBr (0.205 L, 1.22 mol) was stirred at reflux for 65 h. The mixture was cooled in ice, and the precipitate was removed by filtration and dried in a desiccator to give the hydrobromide salt of **11** (58.0 g, 87%): sublime 140 °C, mp >230 °C; ^{1}H NMR ($CDCl_3$) δ 10.69 (br s, 2 H), 9.20 (dd, J = 4.9, 1.5 Hz, 1 H), 9.11 (dd, J = 8.5, 1.5 Hz, 1 H), 8.64 (d, J = 2.4 Hz, 1 H), 8.05 (dd, J = 8.5, 4.9 Hz, 1 H), 7.90 (d, J = 2.4 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 152.0, 149.4, 146.4, 144.3, 135.4, 128.3, 124.1, 114.5, 106.5. Anal. ($C_9H_6N_2O_3 \cdot$HBr) C, H, N.

8-Benzyloxy-6-nitroquinoline (12). A mixture of **11** (58.0 g, 0.214 mol), DMF (400 mL), K_2CO_3 (103.5 g, 0.75 mmol), and NaI (1.60 g, 10.7 mmol) was stirred at room temperature, while benzyl bromide (25.4 mL, 0.214 mmol) was added in four portions at half hourly intervals. A total of 9 h after the first addition, the mixture was poured onto ice (1.5 kg) and the precipitate was removed by filtration, washed with water, and dried. The crude material was dissolved in DCM, and the solution was filtered through alumina to give **12** (59.55 g, 99%): mp (EtOH) 152−153 °C; ^{1}H NMR ($CDCl_3$) δ 9.13 (dd, J = 4.2, 1.8 Hz, 1 H), 8.35 (d, J = 2.3 Hz, 1 H), 8.29 (dd, J = 8.4, 1.8 Hz, 1 H), 7.83 (d, J = 2.3 Hz, 1 H), 7.59 (dd, J = 8.4, 4.2 Hz, 1 H), 7.56 (d, J = 7.6 Hz, 2 H), 7.40 (dd, J = 7.6, 7.2 Hz, 2 H), 7.33 (t, J = 7.2 Hz, 1 H), 5.50 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 155.4, 152.5, 145.6, 142.6, 137.9, 135.4, 128.8, 128.4, 127.8, 127.5, 123.3, 116.3, 103.1, 71.4. Anal. ($C_{16}H_{12}N_2O_3$) C, H, N.

6-Amino-8-benzyloxyquinoline (13). Iron dust (16.0 g, 0.285 mol) was added to a solution of **12** (8.00 g, 28.5 mmol) and AcOH (16 mL, 0.285 mol) in EtOH−water (5:1, 240 mL) at reflux. After 10 min, the mixture was carefully poured into saturated aqueous $NaHCO_3$ (300 mL). The mixture was filtered through Celite, and the filter cake was washed with water (100 mL), EtOH (3 × 50 mL), and DCM (3 × 100 mL). The combined filtrates were diluted with water (300 mL), and the aqueous layer was separated and extracted with DCM (2 × 50 mL). The combined extracts were washed with water, dried (Na_2SO_4), and evaporated to give **13** (7.13 g, 100%) as a tan solid: mp 183−185 °C; ^{1}H NMR ($CDCl_3$) δ 8.66 (dd, J = 4.2, 1.6 Hz, 1 H), 7.84 (dd, J = 8.3, 1.6 Hz, 1 H), 7.48 (dd, J = 8.1, 1.7 Hz, 2 H), 7.23−7.39 (m, 3 H), 7.28 (dd, J = 8.3, 4.2 Hz, 1 H), 6.51, 6.48 (2 × d, J = 2.3 Hz, 2 H), 5.36 (s, 2 H), 3.85 (br s, 2 H); ^{13}C NMR ($CDCl_3$) δ 155.2, 155.7, 144.8, 136.8, 135.9, 133.5, 130.8, 128.6, 127.8, 127.0, 122.0, 102.6, 100.0, 70.6. Anal. ($C_{16}H_{14}N_2O$) C, H, N.

8-Benzyloxy-6-(*tert*-butyloxycarbonylamino)quinoline (14). A mixture of **13** (7.63 g, 30.5 mmol), BOC_2O (8.65 g, 39.6 mmol), and dioxane (70 mL) was stirred at reflux for 2 h. Further BOC_2O (0.86 g, 4.0 mmol) was added, and the mixture was heated at reflux for another 1 h. The dioxane was evaporated, the remaining oil was triturated with pentane, and the resulting solid was removed by filtration, dissolved in DCM, and filtered through alumina to give **14** (10.42 g, 98%) as a cream solid: mp 180−181 °C; ^{1}H NMR ($CDCl_3$) δ 8.77 (dd, J = 4.2, 1.6 Hz, 1 H), 7.98 (dd, J = 8.3, 1.6 Hz, 1 H), 7.55 (d, J = 2.1 Hz, 1 H), 7.41 (dd, J = 7.4, 2.2 Hz, 2 H), 7.34 (dd, J = 8.3, 4.2 Hz, 1 H), 7.20−7.29 (m, 3 H), 7.02 (d, J = 2.1 Hz, 1 H), 5.28 (s, 2 H), 1.49 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 154.6, 152.7, 147.4, 137.2, 136.8, 136.3, 135.2, 129.9, 128.4, 127.7, 127.2, 122.0, 105.8, 103.5, 80.6, 70.6, 28.2. Anal. ($C_{21}H_{22}N_2O_3$) C, H, N.

8-Benzyloxy-6-(*tert*-butyloxycarbonylamino)-5-iodoquinoline (15). A mixture of **14** (1.04 g, 3.0 mmol), NIS (0.70 g, 3.1 mmol), and CH_3CN (10 mL) was stirred at reflux for 30 min. Further NIS (40 mg, 0.18 mmol) was added, and the mixture stirred at reflux for a further 30 min. The CH_3CN was evaporated, and the residue was taken up in EtOAc (30 mL) and washed with a solution of $Na_2S_2O_5$ and Na_2CO_3 in water (×3). The aqueous washes were back-extracted with EtOAc (×2). The combined organic extracts were washed with water, dried (brine, $MgSO_4$), filtered through silica gel, and evaporated to give **15** (1.33 g, 93%), which crystallized from hexane as tan needles: mp 118−119 °C; ^{1}H NMR ($CDCl_3$) δ 8.79 (dd, J = 4.2, 1.4 Hz, 1 H), 8.32 (dd, J = 8.6, 1.4 Hz, 1 H), 8.29 (s, 1 H), 7.59 (dd, J = 8.0, 1.7 Hz, 2 H), 7.43 (dd, J = 8.6, 4.2 Hz, 1 H), 7.25−7.39 (m, 3 H), 7.24 (br s, 1 H), 5.43 (s, 2 H), 1.57 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 155.2, 152.4, 148.1, 139.5, 138.9, 138.3, 136.2, 130.7, 128.5, 128.0, 123.4, 103.9, 81.5, 78.1, 71.0, 28.3. Anal. ($C_{21}H_{21}IN_2O_3$) C, H, N.

8-Benzyloxy-6-[*N*-(*tert*-butyloxycarbonyl)-*N*-(2-propenyl)amino]-5-iodoquinoline (16). NaH (60% in oil, 0.185 g, 4.62 mmol) was washed with pentane (2 × 2 mL) and then treated with a solution of **14** (2.00 g, 4.20 mmol) in DMF (20 mL) over 5 min. After 30 min, the effervescence had ceased and the solution had become deep-yellow. Allyl bromide (0.45 mL, 5.0 mmol) was added, and the mixture was stirred for 3.25 h. The mixture was poured into water (100 mL) and extracted with EtOAc (4 × 20 mL). The combined extracts were washed with water (×2), dried (brine, $MgSO_4$), and evaporated. The residue was triturated with pentane and the precipitate was collected by filtration and dried to give **16** (2.02 g, 93%) as a cream solid: mp 121−122 °C; ^{1}H NMR ($CDCl_3$) major rotamer δ 8.94 (dd, J = 4.1, 1.3 Hz, 1 H), 8.50 (dd, J = 8.6, 1.3 Hz, 1 H), 7.52 (dd, J = 8.6, 4.1 Hz, 1 H), 7.48 (d, J = 7.3 Hz, 2 H), 7.35 (dd, J = 7.3, 7.2 Hz, 2 H), 7.30 (t, J = 7.2 Hz, 1 H), 6.83 (br s, 1 H), 5.77 (dddd, J = 16.8, 10.4, 7.2, 5.7 Hz, 1 H), 5.49, 5.41 (2 × d, J = 13.4 Hz, 1 H each), 4.92 (d, J = 10.4 Hz, 1 H), 4.87 (d, J = 16.8 Hz, 1 H), 4.45 (dd, J = 15.0, 5.7 Hz, 1 H), 3.78 (dd, J = 15.0, 7.2 Hz, 1 H), 1.26 (s, 9 H); ^{13}C NMR ($CDCl_3$) major rotamer δ 154.5, 153.5, 150.0, 143.4, 141.1, 140.0, 136.2, 133.0, 131.1, 128.7, 128.0, 126.9, 123.3, 118.3, 112.7, 93.6, 80.6, 70.9, 51.8, 28.2. Anal. ($C_{24}H_{25}IN_2O_3$) C, H, N, I.

5-Benzyloxy-3-(*tert*-butyloxycarbonyl)-1-[(2,2,6,6-tetramethylpiperidin-1-yl)oxymethyl]-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinoline (17). A solution of **16** (1.03 g, 1.99 mmol) and 2,2,6,6-tetramethylpiperidinyloxyl (1.56 g, 10.0 mmol) in benzene (60 mL) under nitrogen at reflux was treated with Bu_3SnH (2.14 mL, 8.0 mmol) over 1.75 h. The mixture was heated at reflux for a further 1.25 h before the solvent was evaporated. The residue was taken up in Et_2O and extracted with 0.2 M HCl (×8). The combined extracts were washed with Et_2O, neutralized with $NaHCO_3$, and extracted with DCM (×3). These extracts were dried (brine, $MgSO_4$), evaporated, and triturated with pentane. The precipitate was collected by filtration, washed with pentane, and dried to give **17** (0.786 g, 72%) as cream crystals: mp 162−164 °C; ^{1}H NMR ($CDCl_3$) δ 8.79 (dd, J = 4.1, 1.4 Hz, 1 H), 8.10 (dd, J = 8.4, 1.4 Hz, 1 H), 8.06 (br s, 1 H), 7.56 (br s, 2 H), 7.33−7.40 (m, 3 H), 7.30 (t, J = 7.3 Hz, 1 H), 5.43, 5.38 (2 × d, J = 12.3 Hz, 1 H each), 4.17 (dd, J = 11.2, 2.2 Hz, 1 H), 4.06 (dd, J = 11.2, 8.9 Hz, 1 H), 3.97 (dd, J = 8.4, 5.8 Hz, 1 H), 3.84 (dd, J = 8.4, 7.2 Hz, 1 H), 3.76 (dddd, J = 8.9, 7.2, 5.8, 2.2 Hz, 1 H), 1.55 (s, 9 H), 1.20−1.50 (m, 6 H), 1.08 (s, 3 H), 1.04 (s, 3 H), 0.99 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 154.9, 152.4, 146.9, 141.5 (br), 137.3, 136.6, 131.3, 128.5, 127.9, 127.8, 126.1, 121.7, 115.5 (v br), 100.4 (br), 80.8 (br), 78.5, 70.7, 59.8, 52.7, 39.6, 37.8 (br), 33.0, 28.4, 20.2, 17.0. Anal. ($C_{33}H_{43}N_3O_4$) C, H, N.

Scheme 2. 8-Benzyloxy-6-[*N*-(*tert*-butyloxycarbonyl)-*N*-(3,3-dimethoxypropyl)amino]-5-iodoquinoline (18). NaH (60% in oil, 92 mg, 2.3 mmol) under nitrogen was washed with pentane (2 × 2 mL), cooled (ice−water), and treated with a solution of **15**

(1.00 g, 2.10 mmol) in DMF (10 mL) over 5 min. The mixture was allowed to warm to room temperature and stirred for 30 min, over which time it became bright-yellow and effervescence ceased. A solution of 3-bromo-1,1-dimethoxypropane (0.69 g, 3.77 mmol) in DMF (0.5 mL) was added, and the mixture was stirred at room temperature for 22 h. The mixture was poured into pH 7.4 phosphate buffer (50 mL) and extracted with EtOAc (3 × 20 mL). The combined extracts were washed with water (2 × 50 mL), dried (brine, Na_2SO_4), evaporated, and purified by dry flash column chromatography (SiO_2, 10−90% EtOAc/hexane) to give **18** (1.00 g, 83%) as a cream powder: mp 120−121 °C; ^{1}H NMR ($CDCl_3$) major rotamer δ 8.94 (br d, J = 2.9 Hz, 1 H), 8.52 (dd, J = 8.6, 1.5 Hz, 1 H), 7.45−7.58 (m, 3 H), 7.25−7.40 (m, 3 H), 6.96 (br s, 1 H), 5.46 (s, 2 H), 4.40 (t, J = 4.7 Hz, 1 H), 3.84 (br ddd, J = 14.6, 7.3, 7.3 Hz, 1 H), 3.33 (ddd, J = 14.6, 8.2, 5.8 Hz, 1 H), 3.28, 3.25 (2 × s, 3 H each), 1.65−1.95 (m, 2 H), 1.23 (br s, 9 H); ^{13}C NMR ($CDCl_3$) major rotamer δ 154.6, 153.6, 149.9, 143.8, 141.3, 139.8, 136.0, 131.2, 128.7, 128.0, 127.0, 123.4, 112.3, 102.9, 93.3, 80.3, 70.9, 53.1, 52.7, 45.4, 31.2, 28.1. HRMS: $C_{26}H_{31}IN_2O_5$ requires $M^{+\bullet}$ 578.1278. Found 578.1257.

8-Benzyloxy-6-[*N*-(*tert*-butyloxycarbonyl)-*N*-(3-oxopropyl)-amino]-5-iodoquinoline (19). A solution of **18** (0.75 g, 1.30 mmol), $TsOH \cdot H_2O$ (0.12 g, 0.65 mmol), and water (3.75 mL) in acetone (38 mL) was stirred at reflux for 2.25 h. Most of the acetone was evaporated, and the residue was diluted with water (50 mL) and saturated aqueous $NaHCO_3$ (5 mL) and extracted with EtOAc (3 × 20 mL). The combined extracts were washed with water (2 × 50 mL), dried (Na_2SO_4), and evaporated to give **19** (0.68 g, 99%) as a pale-yellow foam: ^{1}H NMR ($CDCl_3$) major rotamer δ 9.68 (s, 1 H), 8.97 (dd, J = 4.2, 1.5 Hz, 1 H), 8.51 (dd, J = 8.6, 1.5 Hz, 1 H), 7.53 (dd, J = 8.6, 4.2 Hz, 1 H), 7.47−7.55 (m, 2 H), 7.25−7.40 (m, 3 H), 6.87 (br s, 1 H), 5.49 (s, 2 H), 4.17 (br dt, J = 14.5, 7.1 Hz, 1 H), 3.59 (dt, J = 14.5, 6.5 Hz, 1 H), 2.57 (br dd, J = 7.1, 6.5 Hz, 2 H), 1.23 (s, 9 H); ^{13}C NMR ($CDCl_3$) major rotamer δ 200.3, 154.8, 153.4, 150.0, 143.0, 141.0, 139.7, 135.9, 131.0, 128.6, 127.9, 127.0, 123.4, 112.1, 93.1, 80.7, 70.7, 42.9, 42.5, 27.9. HRMS: $C_{24}H_{25}IN_2O_4$ requires $M^{+\bullet}$ 532.0859. Found 532.0862.

6-[*N*-(3-Acetoxy-2-propenyl)-*N*-(*tert*-butyloxycarbonyl)amino]-8-benzyloxy-5-iodoquinoline (20). A mixture of **19** (0.62 g, 1.16 mmol), Et_3N (0.40 mL, 2.87 mmol), Ac_2O (0.25 mL, 2.65 mmol), DMAP (14 mg, 0.11 mmol), and THF (12 mL) was stirred at reflux for 2 h. Further Et_3N (0.80 mL, 5.74 mmol), Ac_2O (0.50 mL, 5.3 mmol), and DMAP (10 mg, 0.08 mmol) were added, and heating was continued for a further 2 h. The solvent was evaporated, and the residue was diluted with pH 7.4 phosphate buffer (50 mL) and extracted with EtOAc (3 × 20 mL). The combined extracts were washed with water (50 mL), dilute aqueous $NaHCO_3$ (50 mL), and water (50 mL) before being dried (brine, Na_2SO_4), and evaporated. The residue was purified by dry flash column chromatography (SiO_2, 10−80% EtOAc−hexane) to give **20** (0.54 g, 81%) as a white foam, which contained a 1:4 mixture of Z and E isomers: ^{1}H NMR ($CDCl_3$) major rotamer δ 8.94 (br s, 1 H), 7.45−7.55 (m, 3 H), 7.27−7.40 (m, 3 H), 6.84−7.12 (m, 2 H), 5.36−5.58 (m, 2.8 H), 4.91 (ddd, J = 7.6, 6.5, 5.9 Hz, 0.2 H), 4.57 (dd, J = 15.0, 5.9 Hz, 0.2 H), 4.39 (dd, J = 14.7, 6.8 Hz, 0.8 H), 4.06 (dd, J = 15.0, 7.6 Hz, 0.2 H), 3.86 (dd, J = 14.7, 7.9 Hz, 0.8 H), 2.08 (s, 2.4 H), 1.88 (s, 0.6 H), 1.57 (br s, 1.8 H), 1.26 (br s, 7.2 H); ^{13}C NMR ($CDCl_3$) major rotamer δ 167.4, 167.0, 154.5, 149.8, 154.3, 149.8, 153.3, 153.1, 142.8, 140.9, 139.7, 138.8, 139.7, 143.1, 135.8, 130.9, 136.0, 127.8, 126.8, 126.7, 128.4, 123.2, 112.1, 112.0, 109.1, 108.2, 93.5, 93.1, 80.9, 80.4, 70.8, 70.7, 46.4, 42.7, 27.9, 28.1, 20.3, 20.1. HRMS: $C_{26}H_{27}IN_2O_5$ requires $M^{+\bullet}$ 574.0965. Found 574.0962.

1-(Acetoxymethyl)-5-benzyloxy-3-(*tert*-butyloxycarbonyl)-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinoline (21). A solution of **20** (0.54 g, 0.94 mmol), AIBN (15 mg, 0.09 mmol), and Bu_3SnH (0.32 g, 1.13 mmol) in benzene (45 mL) was stirred at reflux under nitrogen for 5.5 h. The solvent was evaporated, the residue was triturated with pentane, and the precipitate was collected by filtration to give **21** (0.32 g, 77%), which crystallized from MeOH as fluorescent pale-yellow rectangular plates: mp 172−173 °C; ^{1}H NMR ($CDCl_3$) δ 8.82 (dd, J = 4.1, 1.4 Hz, 1 H), 8.14 (dd, J = 8.4, 1.4 Hz, 1 H), 8.07 (br s, 1 H), 7.55 (br s, 2 H), 7.41 (dd, J = 8.4, 4.1 Hz, 1 H), 7.36 (dd, J = 7.3, 7.3 Hz, 2 H), 7.30 (tt, J = 7.3, 2.4 Hz, 1 H), 5.44, 5.39 (2 × d, J = 12.5 Hz, 1 H each), 4.42−4.52 (m, 1 H), 4.05−4.14 (m, 2 H), 3.82−3.93 (m, 2 H), 2.08 (s, 3 H), 1.57 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 171.0, 155.2, 152.3, 146.9, 142.0 (br), 137.0, 136.3, 131.1, 128.5, 127.9, 127.7, 126.0, 122.1, 113.3 (v br), 100.4 (br), 81.4 (br), 70.7, 65.8, 52.6, 37.7, 28.4, 20.9. Anal. ($C_{26}H_{28}N_2O_5$) C, H, N.

5-Benzyloxy-3-(*tert*-butyloxycarbonyl)-1-(hydroxymethyl)-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinoline (22). A mixture of **21** (0.22 g, 0.50 mmol), Cs_2CO_3 (0.42 g, 1.29 mmol), and EtOH−water (2:1, 6 mL) was stirred at reflux for 30 min. The mixture was diluted with EtOAc (30 mL) and dilute aqueous $NaHCO_3$ (50 mL), and the separated aqueous phase was extracted with EtOAc (30 mL). The combined extracts were washed with water (3 × 50 mL), dried (brine, Na_2SO_4), and evaporated to give **22** (0.19 g, 95%), which crystallized from MeOH as tiny white needles: mp 156−157 °C; ^{1}H NMR ($CDCl_3$) δ 8.54 (br s, 1 H), 7.99 (br d, J = 8.0 Hz, 1 H), 7.91 (br s, 1 H), 7.55 (d, J = 6.6 Hz, 2 H), 7.20−7.40 (m, 4 H), 5.29 (s, 2 H), 4.00−4.22 (m, 2 H), 3.65−3.78 (m, 3 H, H-1), 3.23 (br s, 1 H), 1.56 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 154.4, 152.5 (br), 146.2 (br), 142.2 (v br), 136.3, 136.2, 131.3, 128.5, 128.0 (v br), 127.9, 125.9, 121.6, 114.7 (v br), 100.4 (br), 81.0 (br), 70.7, 64.6, 52.3, 40.9 (br), 28.4. Anal. ($C_{24}H_{26}N_2O_4 \cdot H_2O$) C, H, N.

5-Benzyloxy-1-(methylsulfonyloxymethyl)-3-(*tert*-butyloxycarbonyl)-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinoline (23). MsCl (0.06 mL, 0.7 mmol) was added to a cooled (ice−water) solution of **22** (0.17 g, 0.41 mmol) and Et_3N (0.2 mL, 1.4 mmol) in DCM (3 mL), and the mixture was stirred for 30 min. The DCM was evaporated, and the residue was stirred with water (25 mL) for 10 min. The mixture was extracted with EtOAc (2 × 25 mL). The combined extracts were washed with water (2 × 50 mL), dried (Na_2SO_4), and evaporated to give **23** (0.17 g, 86%), which crystallized from MeOH as tiny cream needles: mp 156−157 °C; ^{1}H NMR ($CDCl_3$) δ 8.80 (dd, J = 4.2, 1.4 Hz, 1 H), 8.02 (dd, J = 8.7, 1.4 Hz, 1 H), 7.97 (br s, 1 H), 7.55 (br d, J = 6.9 Hz, 2 H), 7.41 (dd, J = 8.7, 4.2 Hz), 7.25−7.38 (m, 3 H), 5.40 (s, 2 H), 4.46 (dd, J = 9.8, 3.7 Hz, 1 H), 3.93−4.24 (m, 4 H), 2.90 (s, 3 H), 1.57 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 155.6, 152.1, 147.0, 141.0 (v br), 137.1, 136.1, 130.5, 128.4, 127.9, 127.6 (br), 125.7, 122.3, 112.7 (v br), 100.3, 81.6 (br), 70.7, 69.9, 52.0, 38.2 (br), 37.4, 28.3. Anal. ($C_{25}H_{28}N_2O_6S$) C, H, N, S.

5-Benzyloxy-3-(*tert*-butyloxycarbonyl)-1-(chloromethyl)-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinoline (24). Method 1. A mixture of **23** (50 mg, 0.10 mmol), LiCl (25 mg, 0.59 mmol), and DMF (0.25 mL) was stirred at 80 °C for 1 h before ice (3 g) was added. The precipitate was removed by filtration, washed with water, and taken up in EtOAc (20 mL). This solution was washed with water (20 mL), dried (Na_2SO_4), and evaporated to give **24** (39 mg, 89%), which crystallized from MeOH as fluorescent cream needles: mp 178−179 °C; ^{1}H NMR ($CDCl_3$) δ 8.82 (dd, J = 4.2, 1.5 Hz, 1 H), 8.05 (br s, 1 H), 7.99 (br d, J = 8.4 Hz, 1 H), 7.55 (br s, 2 H), 7.41 (dd, J = 8.4, 4.2 Hz, 1 H), 7.35 (dd, J = 7.3, 7.3 Hz, 2 H), 7.30 (tt, J = 7.3, 2.4 Hz, 1 H), 5.42, 5.38 (2 × d, J = 12.4 Hz, 1 H each), 4.23 (br d, J = 11.7 Hz, 1 H), 4.12 (dd, J = 11.7, 8.9 Hz, 1 H), 3.92 (dddd, J = 10.1, 8.9, 3.2, 2.6 Hz, 1 H), 3.81 (dd, J = 11.1, 3.2 Hz, 1 H), 3.45 (dd, J = 11.1, 10.1 Hz, 1 H), 1.56 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 155.5, 152.3, 146.9, 141.9 (br), 137.1, 136.3, 130.3, 128.5, 127.9, 127.7 (br), 125.6, 122.2, 113.4 (v br), 100.4 (br), 81.6 (br), 70.8, 53.0, 46.3, 41.1, 28.4. Anal. ($C_{24}H_{25}ClN_2O_3$) C, H, N, Cl.

Method 2. CCl_4 (0.05 mL, 0.52 mmol) was added to a mixture of **22** (19 mg, 0.047 mmol), Ph_3P (37 mg, 0.14 mmol), and DCM (0.4 mL), and the mixture was stirred under nitrogen for 4 h. The mixture was diluted with dilute aqueous $NaHCO_3$ (5 mL) and extracted with EtOAc (3 × 5 mL). The combined extracts were

dried (Na_2SO_4), evaporated, and purified by dry flash column chromatography (SiO_2, 10−90% EtOAc/hexane) to give **24** (20 mg, 100%) identical with the material prepared above.

Scheme 3. 8-Benzyloxy-6-[*N*-(*tert*-butyloxycarbonyl)-*N*-(3-chloro-2-propenyl)amino]-5-iodoquinoline (25). NaH (60% dispersion in oil, 0.26 g, 6.5 mmol) under nitrogen was washed with pentane (3 × 2 mL), cooled (ice−water), and treated with a solution of **15** (2.80 g, 5.88 mmol) in DMF (28 mL) over 5 min. The cooling bath was removed and the mixture was allowed to stir for 30 min, by which time the solution was deep-yellow and effervescence had ceased. 1,3-Dichloropropene (0.98 g, 8.82 mmol) was added, and the mixture was stirred for 86 h. The mixture was diluted with water (150 mL) and extracted with EtOAc (4 × 25 mL). The combined extracts were washed with water (3 × 100 mL), dried (brine, Na_2SO_4), and evaporated. The residue was triturated with pentane and the precipitate was collected by filtration to give **25** (3.02 g, 93%) as a tan powder: mp 115−135 °C consisting of a 1:1 mixture of *Z* and *E* isomers; ^{1}H NMR ($CDCl_3$) major rotamer δ 8.95 (br s, 1 H), 8.50 (dd, J = 8.4, 2.5 Hz, 1 H), 7.46−7.55 (m, 3 H), 7.27−7.41 (m, 3 H), 6.79−6.96 (m, 1 H), 5.30−6.03 (m, 4 H), 4.54 (dd, J = 15.5, 5.6 Hz, 0.5 H), 4.38 (dd, J = 14.8, 6.8 Hz, 0.5 H), 4.18 (dd, J = 15.5, 6.9 Hz, 0.5 H), 3.79 (dd, J = 14.8, 7.8 Hz, 0.5 H), 1.23−1.82 (m, 9 H); ^{13}C NMR ($CDCl_3$) major rotamer δ 154.7, 155.2, 153.6, 153.3, 150.2, 150.1, 143.2, 142.8, 141.2, 140.2, 136.2, 136.0, 131.13, 131.08, 128.79, 128.73, 128.12, 127.99, 127.2, 126.6, 126.98, 126.90, 123.5, 123.4, 122.0, 121.1, 112.2, 111.9, 93.65, 93.58, 80.90, 80.85, 71.0, 70.9, 48.8, 45.4, 28.4, 28.1. HRMS: $C_{24}H_{24}ClIN_2O_3$ requires $M^{+\bullet}$ 550.0520, 552.0491. Found 550.0536, 552.0503. Purification of the mother liquors by dry flash column chromatography (silica gel, 10−60% EtOAc−hexane) gave further **25** (0.14 g, 4%).

5-Benzyloxy-3-(*tert*-butyloxycarbonyl)-1-(chloromethyl)-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinoline (24). A solution of **25** (3.00 g, 5.45 mmol), AIBN (89 mg, 0.54 mmol), and Bu_3SnH (1.75 g, 6.0 mmol) in benzene (270 mL) was heated at reflux under nitrogen for 3 h. The benzene was evaporated, the residue was triturated with pentane, and the precipitate was collected by filtration to give **24** (2.21 g, 95%), identical with the material prepared above.

Scheme 4. 1-(Chloromethyl)-5-hydroxy-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinoline (6). A suspension of **24** (0.65 g, 1.53 mmol) in dioxane (40 mL) was saturated with HCl, allowed to stand for 1 h, and evaporated. 5,6,7-Trimethoxyindole-2-carboxylic acid (0.38 g, 1.53 mmol), EDCI (0.88 g, 4.6 mmol), and DMA (25 mL) were added to the remaining green-yellow solid, and the red mixture was stirred at room temperature for 39 h. The mixture was poured into a mixture of ice (60 g) and pH 7.4 phosphate buffer (60 mL). The precipitate was removed by filtration, washed with water, and taken up in EtOAc (60 mL). This solution was washed with water (3 × 50 mL), dried (brine, Na_2SO_4), and evaporated. The remaining oil was triturated with Et_2O. The precipitate was collected by filtration, purified by flash column chromatography (silica gel, EtOAc), and triturated with Et_2O to give 5-benzyloxy-1-(chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl)-carbonyl]-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinoline (**26**) (0.38 g, 44%) as a pale-yellow solid: mp 182−184 °C; ^{1}H NMR ($CDCl_3$) δ 9.59 (s, 1 H), 8.84 (dd, J = 4.2, 1.6 Hz, 1 H), 8.37 (s, 1 H), 7.95 (dd, J = 8.5, 1.6 Hz, 1 H), 7.58 (br d, J = 7.2 Hz, 2 H), 7.38 (dd, J = 8.5, 4.2 Hz, 1 H), 7.36 (dd, J = 7.3, 7.2 Hz, 2 H), 7.30 (t, J = 7.3 Hz, 1 H), 6.93 (d, J = 2.2 Hz, 1 H), 6.84 (s, 1 H), 5.48, 5.42 (2 × d, J = 12.5 Hz, 1 H each), 4.69 (dd, J = 10.8, 1.9 Hz, 1 H), 4.57 (dd, J = 10.8, 8.5 Hz, 1 H), 4.06, 3.93, 3.90 (3 × s, 3 H each), 4.02 (dddd, J = 10.3, 8.5, 3.2, 1.9 Hz, 1 H), 3.83 (dd, J = 11.4, 3.2 Hz, 1 H), 3.42 (dd, J = 11.4, 10.3 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 160.5, 155.3, 147.8, 150.2, 142.3, 140.6, 138.8, 138.2, 129.5, 125.1, 123.5, 136.4, 130.4, 128.6, 128.0, 127.7, 125.6, 122.3, 115.3, 106.7, 102.3, 97.6, 70.8, 61.4, 61.1, 56.2, 55.1, 45.9, 42.5. $C_{31}H_{28}ClN_3O_5$ requires M + H 558.1796, 560.1766. Found (FAB) 558.1770, 560.1786. Anal. ($C_{31}H_{28}ClN_3O_5$) C, H, N.

THF (10 mL) and then 25% aqueous HCO_2NH_4 (1.1 mL) were added to a cooled (ice−water) mixture of **26** (0.25 g, 0.45 mmol) and 10% Pd/C (0.13 g) under nitrogen. The mixture was stirred at 0 °C for 7.5 h and was then filtered through Celite. The Celite was washed with a solution of concentrated HCl (2 mL) and MeOH (40 mL) and then with DCM−MeOH (3:1, 40 mL). The combined filtrates were diluted with water (40 mL) and DCM (30 mL) and neutralized with pH 7.4 phosphate buffer. The lower layer was separated and then diluted with MeOH (20 mL) and warmed to dissolve the suspended solid. The aqueous phase was extracted with DCM (2 × 20 mL). The extracts were combined, washed with water (100 mL), dried (Na_2SO_4), and concentrated to a volume of 20 mL. The concentrate was diluted with MeOH (20 mL) and was concentrated to a volume of 10 mL. The precipitate was removed by filtration and washed with MeOH to give **6** (0.14 g, 66%) as a pale-yellow microcrystalline solid: mp >230 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.50 (d, J = 2.1 Hz, 1 H), 10.03 (br s, 1 H), 8.76 (dd, J = 4.1, 1.3 Hz, 1 H), 8.40 (dd, J = 8.4, 1.3 Hz, 1 H), 7.97 (s, 1 H), 7.56 (dd, J = 8.4, 4.1 Hz, 1 H), 7.09 (d, J = 2.1 Hz, 1 H), 6.97 (s, 1 H), 4.77 (dd, J = 11.0, 9.3 Hz, 1 H), 4.48 (dd, J = 11.0, 2.0 Hz, 1 H), 4.25 (dddd, J = 9.3, 3.9, 3.3, 2.0 Hz, 1 H), 4.03 (dd, J = 10.6, 3.3 Hz, 1 H), 3.93, 3.82, 3.80 (3 × s, 3 H each), 3.89 (dd, J = 10.6, 3.9 Hz, 1 H); ^{13}C NMR ($(CD_3)_2SO$) δ 160.3, 153.9, 146.3, 149.1, 142.7, 139.9, 139.0, 136.0, 130.7, 125.4, 124.8, 123.1, 131.6, 122.4, 114.6, 106.2, 102.8, 98.0, 61.0, 60.9, 55.9, 55.0, 47.6, 40.5. Anal. ($C_{24}H_{22}ClN_3O_5$) C, H, N.

1-(Chloromethyl)-3-[(5-methoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinolin-5-ol (30). A suspension of **24** (0.10 g, 0.24 mmol) in dioxane (15 mL) was saturated with HCl, stirred for 5 h, and evaporated. 5-Methoxy-1-*H*-indole-2-carboxylic acid (0.054 g, 0.28 mmol), EDCI (0.23 g, 1.17 mmol), and DMA (5 mL) were added to the remaining yellow solid, and the red mixture was stirred for 52 h. The mixture was partitioned between DCM and cold 5% $KHCO_3$ solution. The aqueous layer was extracted with DCM (×3). The organic extracts were dried (brine, Na_2SO_4). Flash chromatography (EtOAc/petroleum ether 7:3) gave 5-(benzyloxy)-1-(chloromethyl)-3-[(5-methoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinoline (**27**) (0.11 g, 98%) as a yellow solid: mp 186−189 °C; ^{1}H NMR ($CDCl_3$) δ 9.55 (s, 1 H), 8.88 (dd, J = 4.2, 1.7 Hz, 1 H), 8.37 (s, 1 H), 7.99 (dd, J = 8.3, 1.6 Hz, 1 H), 7.56 (d, J = 7.3 Hz, 2 H), 7.42 (dd, J = 8.3, 4.1 Hz, 1 H), 7.33 (m, 4 H), 7.10 (d, J = 2.3 Hz, 1 H), 6.99 (m, 2 H), 5.48 (d, J = 12.5 hz, 1 H), 5.42 (d, J = 12.6 Hz, 1 H), 4.74 (dd, J = 10.9, 2.0 Hz, 1 H), 4.61 (dd, J = 10.6, 8.7 Hz, 1 H), 4.05 (m, 1 H), 3.85 (s, 3 H), 3.84 (dd, J = 11.2, 4.1 Hz, 1 H), 3.45 (dd, J = 11.0, 10.5 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 160.7, 155.4, 154.7, 147.9, 142.4, 138.4, 136.4, 131.4, 130.5, 130.2, 128.6, 128.2, 128.0, 127.7, 125.2, 122.4, 117.0, 115.4, 112.7, 106.2, 102.5, 102.4, 70.9, 55.7, 55.2, 45.9, 42.6. Anal. ($C_{29}H_{24}ClN_3O_3$) C, H, N.

THF (6 mL) and then HCO_2NH_4 (0.14 g, 2.21 mmol) in H_2O (0.7 mL) were added to a cooled (0 °C) mixture of **27** (0.11 g, 0.22 mmol) and 10% Pd/C (0.05 g) under N_2. The mixture was stirred at 0 °C for 5 h and was then filtered through Celite. The Celite was washed with DCM and water. The aqueous layer was extracted with DCM (×3). The organic extracts were dried (brine, Na_2SO_4) and evaporated. Precipitation of the residue from DCM/MeOH gave **30** (0.077 g, 89%) as a gray solid: mp 224−227 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.66 (s, 1 H), 10.02 (br s, 1 H), 8.77 (dd, J = 4.1, 1.3 Hz, 1 H), 8.41 (dd, J = 8.4, 1.4 Hz, 1 H), 8.07 (s, 1 H), 7.57 (dd, J = 8.4, 4.1 Hz, 1 H), 7.40 (d, J = 9.0 Hz, 1 H), 7.16 (d, J = 2.4 Hz, 1 H), 7.12 (d, J = 1.6 Hz, 1 H), 6.92 (dd, J = 8.9, 2.3 Hz, 1 H), 4.82 (dd, J = 10.8, 9.4 Hz, 1 H), 4.57 (dd, J = 11.0, 2.3 Hz, 1 H), 4.30 (m, 1 H), 4.04 (dd, J = 11.1, 3.3 Hz, 1 H), 3.91 (dd, J = 11.1, 7.2 Hz, 1 H), 3.78 (s, 3 H). HRMS FAB [M + H] calcd for $C_{22}H_{19}{}^{35}ClN_3O_3$ = 408.1115. Found, 408.1101. Calcd for $C_{22}H_{19}{}^{37}ClN_3O_3$ = 410.1085. Found, 410.1092.

1-(Chloromethyl)-3-({5-[2-(dimethylamino)ethoxy]-5-hydroxyindol-2-yl}carbonyl)-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinoline (31). A suspension of **24** (0.20 g, 0.47 mmol) in cooled (0 °C) dioxane (5 mL) was saturated with HCl, allowed to warm to room temperature over 2 h, and evaporated. 5-[2-(Dimethylamino)-ethoxy]-1-*H*-indole-2-carboxylic acid hydrochloride[41] (0.13 g, 0.47 mmol), EDCI (0.27 g, 1.42 mmol), and DMA (3 mL) were added to the remaining yellow solid, and the red mixture was stirred at room temperature for 20 h. The mixture was partitioned between EtOAc and 5% $NaHCO_3$ solution. The aqueous layer was extracted with EtOAc (×3), and the EtOAc extracts were dried (brine, Na_2SO_4). Flash chromatography (alumina, EtOAc/MeOH, 49:1 then 9:1) gave (5-(benzyloxy)-1-(chloromethyl)-1*H*-pyrrolo[3,2-*f*]quinolin-3(2*H*)-yl)(5-(2-(dimethylamino)-ethoxy)-1*H*-indol-2-yl)methanone (**28**) (0.22 g, 84%): mp 176−179 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.68 (s, 1 H), 8.79 (dd, J = 4.1, 1.5 Hz, 1 H), 8.41 (dd, J = 8.6, 1.5 Hz, 1 H), 8.29 (s, 1 H), 7.56 (m, 3 H), 7.40 (m, 4 H), 7.17 (d, J = 2.3 Hz, 1 H), 7.11 (d, J = 1.5 Hz, 1 H), 6.92 (dd, J = 9.0, 2.4 Hz, 1 H), 5.32 (s, 2 H), 4.82 (dd, J = 10.7, 9.6 Hz, 1 H), 4.58 (dd, J = 10.9, 2.1 Hz, 1 H), 4.32 (m, 1 H), 4.05 (t, J = 5.7 Hz, 2 H), 4.04 (m, 1 H), 3.93 (dd, J = 11.2, 6.9 Hz, 1 H), 2.65 (t, J = 5.8 Hz, 2 H), 2.23 (s, 6 H); ^{13}C NMR [$(CD_3)_2SO$] δ 160.3, 154.5, 153.0, 147.3, 142.3, 137.4, 136.7, 131.6, 131.3, 130.6, 128.4, 127.9, 127.7, 127.4, 125.1, 122.4, 116.2, 116.0, 113.1, 105.5, 103.1, 102.0, 70.0, 66.9, 66.2, 57.8, 54.9, 47.7, 45.5, 40.7. HRMS FAB [M + H] calcd for $C_{32}H_{37}{}^{35}ClN_3O_3$ = 542.2210. Found, 542.2214. Calcd for $C_{32}H_{33}{}^{37}ClN_3O_3$ = 544.2181. Found, 544.2188.

THF (8 mL) and then HCO_2NH_4 (0.23 g, 3.6 mmol) in H_2O (1 mL) were added to a cooled (0 °C) mixture of **28** (0.20 g, 0.36 mmol) and 10% Pd/C (0.1 g) under N_2. The mixture was stirred at 0 °C for 14 h, and was then filtered through Celite. The Celite was washed with DCM/H_2O. The aqueous layer was extracted with DCM (×3). The DCM extracts were dried (brine, Na_2SO_4) and passed through a short plug of silica gel to give **31** (0.16 g, 93%): mp 209−215 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.66 (s, 1 H), 10.02 (br s, 1 H), 8.76 (dd, J = 4.1, 1.4 Hz, 1 H), 8.41 (dd, J = 8.5, 1.3 Hz, 1 H), 8.07 (s, 1 H), 7.56 (dd, J = 8.5, 4.1 Hz, 1 H), 7.40 (d, J = 8.9 Hz, 1 H), 7.17 (d, J = 2.2 hz, 1 H), 7.11 (d, J = 1.2 Hz, 1 H), 6.93 (dd, J = 8.9, 2.3 Hz, 1 H), 4.82 (dd, J = 10.7, 9.6 Hz, 1 H), 4.57 (dd, J = 11.0, 2.1 Hz, 1 H), 4.29 (m, 1 H), 4.06 (t, J = 5.9 Hz, 2 H), 4.04 (m, 1 H), 3.91 (dd, J = 11.1, 7.2 Hz, 1 H), 2.64 (t, J = 5.8 Hz, 2 H), 2.28 (s, 6 H); ^{13}C NMR [$(CD_3)_2SO$] δ 160.3, 153.9, 153.0, 146.4, 142.8, 136.1, 131.6, 130.7, 127.4, 124.8, 124.7, 122.5, 116.0, 114.6, 113.1, 105.5, 103.1, 103.0, 66.1, 57.8, 54.9, 47.7, 45.5, 40.7. Anal. ($C_{25}H_{25}ClN_4O_3\cdot$0.5DCM) C, H, N.

1-(Chloromethyl)-3-((2*E*)-3-{4-[2-(dimethylamino)ethoxy]phenyl}-2-propenoyl)-5-hydroxy-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinoline (32). Similar reaction of **24** (0.20 g, 0.47 mmol) with HCl/dioxane followed by treatment with (*E*)-4-[2-(dimethylamino)ethoxy]-cinnamic acid hydrochloride[42] (0.13 g, 0.47 mmol), EDCI (0.27 g, 1.42 mmol), and DMA (3 mL), and flash chromatography of the product (alumina, EtOAc/MeOH; 49:1 then 24:1) gave (*E*)-1-(5-(benzyloxy)-1-(chloromethyl)-1*H*-pyrrolo[3,2-*f*]quinolin-3(2*H*)-yl)-3-(4-(2-(dimethylamino)ethoxy)phenyl)prop-2-en-1-one (**29**) (0.18 g, 70%): mp 172−175 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.76 (dd, J = 4.1, 1.4 hz, 1 H), 8.47 (br s, 1 H), 8.35 (dd, J = 8.5, 1.4 Hz, 1 H), 7.76 (d, J = 8.7 Hz, 2 H), 7.67 (d, J = 15.3 Hz, 1 H), 7.58 (d, J = 7.3 Hz, 2 H), 7.54 (dd, J = 8.5, 4.1 Hz, 1 H), 7.44 (t, J = 7.2 Hz, 2 H), 7.37 (t, J = 7.2 Hz, 1 H), 7.08 (d, J = 15.3 Hz, 1 H), 7.02 (d, J = 8.7 Hz, 2 H), 5.31 (s, 2 H), 4.55 (dd, J = 10.7, 9.5 Hz, 1 H), 4.44 (dd, J = 10.9, 2.5 Hz, 1 H), 4.30 (m, 1 H), 4.11 (t, J = 5.8 Hz, 2 H), 3.99 (dd, J = 11.0, 3.0 Hz, 1 H), 3.91 (dd, J = 11.2, 7.2 Hz, 1 H), 2.64 (t, J = 5.7 Hz, 2 H), 2.23 (s, 6 H); ^{13}C NMR [$(CD_3)_2SO$] δ 164.1, 160.1, 154.6, 147.1, 142.6, 142.2, 137.2, 136.7, 131.1, 130.1, 128.3, 127.83, 127.78, 127.3, 125.1, 122.3, 116.9, 115.7, 114.7, 101.6, 70.0, 65.9, 57.5, 52.9, 47.8, 45.4, 40.1. Anal. ($C_{32}H_{32}ClN_3O_3$) C, H, N.

A solution of **29** (0.56 g, 1.03 mmol) was dissolved in CF_3CO_2H (15 mL) and refluxed for 48 h. Solvent was evaporated, and the residue was partitioned between DCM and cold 5% $NaHCO_3$ solution. The aqueous layer was extracted with DCM (×3). The DCM extracts were dried (brine, Na_2SO_4). Flash chromatography (DCM/MeOH/NH_3, 95:5:trace) gave **32** (0.16 g, 34%): mp 174−180 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.96 (br s, 1 H), 8.73 (dd, J = 4.0, 1.3 Hz, 1 H), 8.36 (dd, J = 8.4, 1.3 Hz, 1 H), 8.18 (br s, 1 H), 7.77 (d, J = 8.7 Hz, 2 H), 7.66 (d, J = 15.2 Hz, 1 H), 7.54 (dd, J = 8.5, 4.1 Hz, 1 H), 7.08 (d, J = 15.4 Hz, 1 H), 7.02 (d, J = 8.7 Hz, 2 H), 4.54 (dd, J = 10.7, 9.5 Hz, 1 H), 4.44 (dd, J = 11.0, 2.5 Hz, 1 H), 4.28 (m, 1 H), 4.11 (t, J = 5.7 Hz, 2 H), 4.00 (dd, J = 11.1, 3.1 Hz, 1 H), 3.88 (dd, J = 11.0, 7.4 Hz, 1 H), 2.64 (t, J = 5.8 Hz, 2 H), 2.22 (s, 6 H). Anal. ($C_{25}H_{26}ClN_3O_3\cdot$0.75EtOAc) C, H, N.

Alternative Synthesis of 6 from 24. A cooled (ice−water) mixture of **24** (0.11 g, 0.27 mmol), 10% Pd/C (55 mg), and THF (5 mL) under nitrogen was treated with 25% aqueous HCO_2NH_4 (0.67 mL). The mixture was stirred at 0 °C for 6 h and was then diluted with EtOAc (20 mL), dried (Na_2SO_4), filtered through Celite, evaporated, and purified by dry flash column chromatography (silica gel, 10−50% EtOAc/hexane) to give 3-(*tert*-butyloxycarbonyl)-1-(chloromethyl)-5-hydroxy-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinoline (**35**) (39 mg, 44%) as a white solid: mp 148−149 °C; ^{1}H NMR ($CDCl_3$) δ 8.61 (dd, J = 4.2, 1.2 Hz, 1 H), 8.01 (dd, J = 8.5, 1.2 Hz, 1 H), 7.83 (br s, 1 H), 7.41 (dd, J = 8.5, 4.2 Hz, 1 H), 4.26 (dd, J = 11.8, 2.2 Hz, 1 H), 4.14 (dd, J = 11.8, 8.5 Hz, 1 H), 3.93 (dddd, J = 9.8, 8.5, 3.2, 2.2 Hz, 1 H), 3.80 (dd, J = 11.1, 3.2 Hz, 1 H), 3.46 (dd, J = 11.1, 9.8 Hz, 1 H), 1.61 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 153.5, 152.3, 145.3, 142.4 (br), 135.0, 130.6, 124.9, 122.6, 112.4 (v br), 100.0, 81.7 (br), 53.0, 46.5, 40.9, 28.4. HRMS: $C_{17}H_{19}ClN_2O_3$ requires $M^{+\bullet}$ 334.1084, 336.1055. Found 334.1081, 336.1058.

A solution of **35** (0.14 g, 0.43 mmol) in dioxane (9 mL) was saturated with HCl, allowed to stand for 1 h, and evaporated. 5,6,7-Trimethoxyindole-2-carboxylic acid (0.11 g, 0.43 mmol), EDCI (0.25 g, 1.28 mmol), and DMA (5 mL) were added to the remaining yellow solid, and the red mixture was stirred at room temperature for 22 h. The mixture was poured into a mixture of ice (20 g) and pH 7.4 phosphate buffer (20 mL). The precipitate was removed by filtration, washed with water, and taken up in DCM/MeOH (2:1, 30 mL). Most of the solvent was boiled off, the remaining mixture was cooled in ice, and the precipitate was removed by filtration to give **6** (18 mg, 9%) identical to the material prepared above.

1-(Chloromethyl)-3-[(2*E*)-3-(4-methoxyphenyl)-2-propenoyl]-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinolin-5-ol (33). A suspension of **35** (0.10 g, 0.30 mmol) in dioxane (5 mL) was saturated with HCl, stirred for 5 h, and evaporated. 4-Methoxycinnamic acid (0.064 g, 0.36 mmol), EDCI (0.29 g, 1.50 mmol), and DMA (3 mL) were added to the remaining yellow solid, and the red mixture was stirred for 3 h. The mixture was partitioned between DCM and cold 5% $KHCO_3$ solution. The aqueous layer was extracted with DCM (×3), and the organic extracts were dried (brine, Na_2SO_4) and evaporated. Flash chromatography (DCM/MeOH, 93:7) gave **33** (0.02 g, 17%) as a yellow solid: mp (DCM/Et_2O) 208−211 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.96 (br s, 1 H), 8.73 (d, J = 3.3 Hz, 1 H), 8.35 (d, J = 7.7 Hz, 1 H), 8.18 (br s, 1 H), 7.78 (d, J = 8.7 Hz, 2 H), 7.67 (d, J = 15.3, 1 H), 7.54 (dd, J = 8.5, 4.1, 1 H), 7.08 (d, J = 15.4, 1 H), 7.01 (d, J = 8.7, 2 H), 4.54 (dd, J = 10.3, 9.5 Hz, 1 H), 4.45 (m, 1 H), 4.27 (m, 1 H), 3.99 (dd, J = 11.1, 3.2 Hz, 1 H), 3.88 (dd, J = 11.1, 7.3 Hz, 1 H), 3.82 (s, 3 H). $C_{22}H_{20}ClN_2O_3$ requires M+H 395.1163, 397.1133. Found (FAB) 395.1161, 397.1169. Anal. ($C_{22}H_{19}ClN_2O_3\cdot$ 0.1DCM) C, H, N.

1-(Chloromethyl)-3-[(2*E*)-3-(3-hydroxy-4-methoxyphenyl)-2-propenoyl]-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinolin-5-ol (34). A suspension of **35** (0.10 g, 0.30 mmol) in dioxane (5 mL) was saturated with HCl, stirred for 5 h, and evaporated. 3-Hydroxy-4-methoxycinnamic acid (0.070 g, 0.36 mmol), EDCI (0.29 g, 1.50 mmol), and DMA (3 mL) were added to the remaining yellow solid, and the red mixture was stirred for 3 h. The mixture was partitioned between DCM and cold 5% $KHCO_3$ solution. The aqueous layer

was extracted with DCM (×3). The organic extracts were dried (brine, Na_2SO_4). Flash chromatography (DCM/MeOH, 93:7) gave **34** (0.01 g, 8%) as a yellow solid: mp (DCM/Et_2O) 215−218 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.96 (br s, 1 H), 9.13 (s, 1 H), 8.73 (dd, J = 4.1, 1.4 Hz, 1 H), 8.36 (dd, J = 8.5, 1.4 Hz, 1 H), 8.17 (br s, 1 H), 7.57 (d, J = 15.3 Hz, 1 H), 7.54 (dd, J = 8.5, 4.1 Hz, 1 H), 7.25 (d, J = 2.0 Hz, 1 H), 7.20 (dd, J = 8.4, 2.0 Hz, 1 H), 6.99 (d, J = 8.1 Hz, 1 H), 6.96 (d, J = 15.0 Hz, 1 H), 4.54 (dd, J = 10.5, 9.4 Hz, 1 H), 4.44 (dd, J = 11.1, 2.6 Hz, 1 H), 4.00 (dd, J = 11.2, 3.3 Hz, 1 H), 3.88 (dd, J = 11.1, 7.5 Hz, 1 H), 3.83 (s, 3 H). HRMS FAB [M+H] calcd for $C_{22}H_{19}{}^{35}ClN_2O_4$ = 411.1112. Found, 411.1127. Anal. ($C_{22}H_{19}ClN_2O_4 \cdot 0.5$DCM) C, H, N.

Resolution of Enantiomers of 6. The benzyl intermediate **24** was resolved on a ChiralCel OD semipreparative HPLC column (2 cm × 25 cm) in hexane/^{i}PrOH (9:1) (α = 1.24). The slower-eluting (−)-enantiomer of **24** (mp 159−160 °C; $[\alpha]_D$ −7 (*c* 0.45, $CHCl_3$)) was assigned the natural *S*-configuration on the basis of its conversion via the (−)-enantiomer of **26** (mp 200−201 °C; $[\alpha]_D$ −2 (*c* 0.40, $CHCl_3$)) to the relatively more cytotoxic (−)-enantiomer *S*-**6** (mp 224−225 °C; $[\alpha]_D$ −16 (*c* 0.30, $CHCl_3$)). Similarly, the faster-eluting (+)-enantiomer of **24** (mp 159−160 °C; $[\alpha]_D$ +7 (*c* 0.45, $CHCl_3$)) was assigned the unnatural *R*-configuration on the basis of its conversion via the (+)-enantiomer of **26** (mp 200−201 °C; $[\alpha]_D$ +2 (*c* 0.40, $CHCl_3$)) to the relatively less cytotoxic (+)-enantiomer *R*-**6** (mp 224−225 °C; $[\alpha]_D$ +15 (*c* 0.30, $CHCl_3$)). The relative cytotoxicities of the enantiomers are given in Table 1.

Preparation of Metal Complexes (Scheme 5). [Co(cyclen)(6)](ClO$_4$)$_2$ (39). Solid [Co(cyclen)$(NO_2)_2$][NO_2] (**37**) (1.03 g, 2.79 mmol)[37] was cautiously added with stirring to neat triflic acid (10 mL) cooled in an ice bath. The solution was bubbled with N_2 to remove NO_x gas and warmed briefly at 40−50 °C until reaction was complete. Dry Et_2O (250 mL) was added slowly to the above cold solution (ice bath) with vigorous stirring, and the resulting precipitate was filtered off, washed with Et_2O (×4), and dried in a desiccator to give [Co(cyclen)-$(OTf)_2$][OTf] (**38**) (1.95 g, 100%). Anal. ($C_{11}H_{24}CoF_9N_4O_{11}S_3$) C, H, N. HRMS FAB [M − OTf]$^+$ calcd $C_{10}H_{20}CoF_6N_4O_6S_2$ = 529.006 05. Found: 529.004 06.

A solution of **38** (90 mg, 0.132 mmol) in dry CH_3CN (3 mL) was treated with **6** (62 mg, 0.132 mmol), and ^{i}Pr$_2$NEt (25 mg, 1.5 equiv) was then added to the stirred solution. This resulted in rapid darkening of the solution to a brown color but with significant amounts of suspended yellow solid (presumed to be unreacted/undissolved **6**) present. The mixture was stirred at room temperature for 11 days, during which time nearly all the suspended solid disappeared. The small amount remaining was removed by filtration through a 0.45 μm membrane filter and the filtrate made slightly acidic with dilute aqueous $HClO_4$. Excess aqueous 1 M $NaClO_4$ was added, and the solution was extracted with CH_3NO_2 (4 × 5 mL). The combined extracts were evaporated to dryness, the residue was resuspended in dry Et_2O (15 mL), and again evaporated to dryness (first on a rotary evaporator, finally on a vacuum line) below 20 °C to give crude product as brown flakes of glassy material (103 mg, 86%). HRMS FAB [M − ClO_4]$^+$. This material was further purified by reverse-phase HPLC, (C-18 column, TFA in CH_3CN/H_2O), and the pooled pure fractions were concentrated under reduced pressure, then combined with excess aqueous 1 M $NaClO_4$ and extracted five times with DCM. The combined organic extracts were treated as above to give complex [Co(cyclen)(**6**)][$(ClO_4)_2$] (**39**) as brownish flakes (70 mg, 59% yield). HRMS FAB [M − 2ClO_4 − H]$^+$ calcd for $C_{32}H_{41}{}^{35}ClCoN_7O_5$ = 697.218 97. Found, 697.213 27. Calcd for $C_{32}H_{41}{}^{37}ClCoN_7O_5$ = 699.216 02. Found, 699.216 01.

Alternative Preparation of [Co(cyclen)(6)](OTf$_2$) (39). A suspension of **6** (17.2 mg, 0.037 mmol) in MeOH (5 mL) was treated with a solution of pyridine (9.5 mg, 0.120 mmol) in MeOH (1 mL). The resulting creamy yellow mixture was purged with nitrogen and stirred under nitrogen for 5 min. Then a solution of **38** (32.5 mg, 0.048 mmol) in MeOH (5 mL) was added. The mixture was heated and stirred under nitrogen at 50 °C for 4 days until the mixture was almost free of suspended solid. The volume of the mixture was reduced to about 1 mL on a rotary evaporator. Then the suspension was filtered and the filtrate loaded onto a Sephadex LH-20 gel filtration column with MeOH as the eluent. The major dark-brown band was collected and reduced to dryness on a rotary evaporator, followed by drying on a high vacuum line to give [Co(cyclen)(**6**)][$(OTf)_2$] (**39**) as a dark-brown crystalline solid (34.5 mg, 96%): ^{1}H NMR [CD_3OD] δ 8.85 (br s, 1 H), 8.62 (d, J = 4.4 Hz, 1 H), 8.44 (d, J = 8.4 Hz, 1 H), 8.06 (s, 1 H), 7.47 (dd, J = 8.4, 5.2 Hz, 1 H), 7.00 (s, 1 H), 6.92 (s, 1 H), 7.05, 6.69, 6.63, 5.50 (br s, 4 H), 4.60 (t, J = 8.4 Hz, 1 H), 4.52 (d, J = 10.8 Hz, 1 H), 4.24 (m, 1 H), 4.04 (s, 3 H), 3.90 (m, 8 H), 3.75 (2 H), 3.64 (2 H), 3.52 (1 H), 3.30 (3 H), 3.12 (2 H), 3.01 (4 H), 2.84 (1 H), 2.66 (1 H), (m, 16 H); ^{13}C NMR [CD_3OD] δ 168.2, 162.4, 151.3, 148.0, 147.0, 146.0, 141.6, 140.1, 136.4, 131.1, 127.2, 125.1, 124.4, 112.5, 109.0, 108.4, 99.3, 62.0, 61.9, 57.9, 57.4, 56.8, 56.3, 51.1, 48.5, 48.4, 42.0. HRMS FAB$^+$ [M − OTf]$^+$ calcd for $C_{37}H_{47}{}^{35}ClCoF_3N_7O_8S$ = 900.217 94. Found, 900.215 81. Calcd for $C_{37}H_{47}{}^{37}ClCoF_3N_7O_8S$ = 902.214 99. Found. 902.214 62.

[[Co(cyclen)(31)][(OTf$_2$)] (40). A solution of **38** (0.101 g, 0.149 mmol) in dry CH_3CN (4 mL) was treated with **31** (0.055 g, 0.118 mmol), and the mixture was stirred at room temperature for 8 h and then cooled overnight at 5 °C. A small amount of unreacted **31** was removed by filtration, and the bright-yellow solid was washed with cold CH_3CN and the washes added to the filtrate. This dark-brown solution was reduced to ~2 mL by evaporation of solvent under reduced pressure at room temperature and then chromatographed on a short (3.3 mm × 40 mm) flash silica gel column (0.32−0.60 μm). Elution was started with MeOH/CH_3NO_2 (5%), which was stepwise enriched with MeOH up to 50%. At this concentration the main band was eluted first followed closely by a small yellow-brown band. Removal of the solvent from the main band on a rotary evaporator and then on a vacuum line gave [Co(cyclen)(**31**)][$(OTf)_2$] (**40**) as a brown glassy residue (0.078 g, 67%): ^{1}H NMR [CD_3CN] δ 10.08 (br s, 1 H), 8.71 (d, J = 5.2 Hz, 1 H), 8.48 (d, J = 8.8 Hz, 1 H), 8.16 (s, 1 H), 7.68 (dd, J = 8.4, 5.2 Hz, 1 H), 7.51 (d, J = 9.2 Hz, 1 H), 7.25 (s, 1 H), 7.11 (s, 1 H), 7.09 (m, 1 H), 6.42 (br s, 1 H), 5.29 (br s, 2 H), 5.06 (br s, 1 H), 4.76 (td, J = 10.8, 2.0 Hz, 1 H), 4.69 (dd, J = 10.8, 2.4 Hz, 1 H), 4.35 (m, 2 H), 4.26 (m, 1 H), 3.95 (m, 1 H), 3.82 (m, 1 H), 3.56 (m, 6 H), 3.29 (m, 2 H), 3.13 (m, 2H), 2.95 (m, 8 H), 2.89 (m, 4 H), 2.63 (m, 2 H); ^{13}C NMR [CD_3CN] δ 168.5, 161.6, 153.4, 147.8, 147.1, 145.9, 136.1, 133.1, 132.2, 129.0, 127.0, 124.3, 117.3, 114.1, 111.7, 107.4, 107.1, 105.0, 62.9, 57.7, 57.4, 56.6, 55.9, 50.6, 48.3, 44.2, 41.9. HRMS FAB [M − 2OTf + e]$^+$ calcd for $C_{33}H_{44}{}^{35}ClCoN_8O_3$ = 694.255 69. Found, 694.253 05. Calcd, for $C_{33}H_{44}{}^{37}ClCoN_8O_3$ = 696.252 74. Found, 696.254 01.

[[Co(cyclen)(32)][(OTf$_2$)] (41). This was prepared from **38** (0.087 g, 0.128 mmol) and **32** (0.052 g, 0.115 mmol), as for **40** above, to give after flash chromatography on silica gel (20% MeOH/CH_3NO_2) [[Co(cyclen)(**32**)][$(OTf)_2$] (**41**) as a brown glass (0.089 g, 79%): ^{1}H NMR [CD_3CN] δ 8.70 (d, J = 5.0, 1 H), 8.38 (d, J = 8.4 Hz, 1 H), 8.06 (s, 1 H), 7.68 (d, J = 8.7 Hz, 2 H), 7.51 (dd, J = 8.4, 5.2 Hz, 1 H), 7.38 (d, J = 15.2 Hz, 1 H), 7.08 (d, J = 8.8 Hz, 2 H), 6.72 (d, J = 15.2 Hz, 1 H), 6.53 (br s, 1 H), 5.86 (br s, 1 H), 5.35 (br s, 1 H), 5.15 (br s, 1 H), 4.40−4.36 (m, 4 H), 4.23 (m, 1 H), 3.90 (dd, J = 11.2, 3.6 Hz, 1 H), 3.72 (dd, J = 11.2, 7.6 Hz, 1 H), 3.61 (m, 2 H), 3.54 (m, 4 H), 3.35 (m, 2 H), 3.13 (m, 2 H), 2.93 (m, 12 H), 2.72 (dd, J = 13.2, 2.4 Hz, 1 H), 2.62 (d, J = 12.4 Hz, 1 H); ^{13}C NMR [CD_3CN] δ 168.4, 165.7, 160.2, 147.5, 146.5, 145.7, 143.6, 135.9, 131.2, 129.3, 126.9, 124.2, 118.1, 116.1, 111.3, 107.4, 62.6, 57.5, 57.4, 56.0, 54.8, 50.7, 48.5, 48.3, 44.3, 41.2. HRMS FAB$^+$ [M − 2OTf + e]$^+$ calcd for $C_{33}H_{45}{}^{35}ClCoN_7O_3$ = 681.260 44. Found, 681.260 64. Calcd for $C_{33}H_{45}{}^{37}ClCoN_7O_3$ = 683.257 49. Found, 683.260 86.

[Co(Me$_2$dtc)$_2$(6)] (43). Solid [$Co_2(Me_2dtc)_5$][BF_4] (105 mg, 0.1303 mmol) (**42**)[38] was added to a suspension of **6** (46 mg,

0.0983 mmol) in 5% MeOH/DCM (4 mL). $^{i}Pr_2NEt$ (25 mg, 0.19 mmol) was added to the stirred suspension in two portions with the second added 1 day after the first. Stirring was continued at room temperature for 8 days, by which time very little suspended/unreacted **6** was evident, and the color of the solution was the deep-green of the coproduct $Co(Me_2dtc)_3$. The solution was filtered and the filtrate evaporated under reduced pressure. The residue was taken up in DCM (2 mL) and chromatographed on a flash silica gel column. Elution began in DCM, and a large green band of $Co(Me_2dtc)_3$ was eluted. Stepwise enrichment with CH_3CN in increments of 10% was carried out until the product $[Co(Me_2dtc)_2(\mathbf{6})]$ (**43**) was eluted (with ~50% CH_3CN/DCM). The main yellow-green band was collected, and solvent was removed under reduced pressure to give the product as a brownish-green amorphous residue (48 mg, 63%) free of the cytotoxic ligand **6**: ^{1}H NMR [$CDCl_3$] δ 9.36 (br s, 2 H), 8.76 (d, J = 4.9 Hz, 2 H), 8.15 (s, 2 H), 7.95 (dt, J = 8.4 Hz, 2 H), 7.37 (m, 2 H), 6.94 (s, 2 H), 6.84 (s, 2 H), 4.69 (m, 2 H), 4.58 (m, 2 H), 4.06 (s, 6 H), 3.92 (s, 8 H), 3.90 (s, 6 H), 3.80 (dt, J = 11.0, 3.3 Hz, 2 H), 3.43 (td, J = 11.0, 2.5 Hz, 2 H), 3.36 (s, 3 H), 3.34 (s, 3 H), 3.28 (s, 3 H), 3.27 (s, 3 H), 3.24 (s, 3 H), 3.21 (s, 6 H), 3.19 (s, 3 H); ^{13}C NMR [$CDCl_3$] δ 204.0, 203.9, 171.9, 160.2, 150.0, 147.1, 146.1, 145.8, 140.4, 138.9, 131.1, 130.2, 125.4, 125.3, 123.5, 122.6, 107.2, 107.0, 106.3, 97.6, 61.5, 61.2, 56.2, 55.7, 56.8, 46.2, 42.2, 38.4, 38.2, 38.0, 37.7. HRMS FAB$^+$ $[M - e]^+$ calcd for $C_{30}H_{33}{}^{35}ClCoN_5O_5S_4$ = 765.038 50. Found, 765.038 56. Calcd. for $C_{30}H_{33}{}^{35}ClCoN_5O_5S_4$ = 767.035 55. Found, 767.037 30.

[[Cr(acac)$_2$(6)] (45). Solid **6** (20 mg, 0.0427 mmol) was added to a solution of $[Cr(acac)_2(H_2O)_2]ClO_4 \cdot 2H_2O$ (mixture of cis and trans isomers) (**44**) (0.03 g, 0.071 mmol)[39] in dry CH_3CN (3 mL). The mixture was stirred, and a solution of $^{i}Pr_2NEt$ (6 mg, 0.0464 mmol) in CH_3CN (0.5 mL) was added gradually over 1 h. The solution was warmed in an oil bath at 50 °C for 0.5 h, then stirred at ambient temperature for 2 weeks. During this period undissolved **6** gradually disappeared as the complexation reaction proceeded, giving a clear red-brown solution. The solvent was removed under reduced pressure, and the residue was dissolved in $CHCl_3$ (1.0 mL) and purified by flash chromatography on silica gel. Elution with a $CH_3CN/CHCl_3$ gradient from 0 to 50% CH_3CN eluted a single yellow-brown band that trailed somewhat near the bottom of the column. The trailing material was eluted separately with 100% CH_3CN. A small amount of green irreversibly absorbed material was left at the top. The main band and tailing fraction were evaporated to dryness under reduced pressure to give yellow-brown powders of $[Cr(acac)_2(\mathbf{6})][(ClO_4)_2]$ (**45**) (18 mg, 59%, and 5 mg, 16%, respectively). These two samples gave identical accurate mass spectral results, approximately equal amounts of both $[M]^+$ and $[M + H]^+$ ions observed with relative intensities consistent with one ^{35}Cl or ^{37}Cl per molecule. HRMS FAB$^+$: $[M]^+$ calcd for $C_{34}H_{35}{}^{35}Cl^{52}CrN_3O_9$ = 716.146 69. Found, $[M]^+$ = 716.146 42. $[M + H]^+$ calcd for $C_{34}H_{36}{}^{37}Cl^{52}CrN_3O_9$ = 719.151 57. Found, $[M+H]^+$ = 719.151 22. Fragments corresponding to loss of acac ligand are observed, and the base peak corresponds to Cr(acac)$_2$. Analytical HPLC on an RP C-18 column using gradient elution starting from a 1:1 (v/v) mixture of 80% aqueous CH_3CN and phosphate buffer (pH 7.4, 0.04 M) showed one major peak (96.7%) with a prominent UV absorption band at 339 nm. A small amount (0.45%) of uncomplexed **6** could be detected and its identity was confirmed by spiking.

Stability of Compounds in Solution. Compounds were dissolved in DMSO or formate buffer (0.1 M sodium formate containing 5 mM phosphate, pH 7.0) or αMEM culture medium containing 5% fetal calf serum, typically at ~30 μM. Solutions were incubated at room temperature or 37 °C and sampled at intervals for HPLC analysis. The HPLC system comprised an Agilent MSD LC/MS with diode array absorbance and positive mode electrospray ionization detectors as previously described.[22]

Cytotoxicity in Cell Culture. The cytotoxicity of the effectors and their metal complexes was determined by assessing inhibition of cell proliferation following 4 h of exposure under oxic and hypoxic conditions, using attached cells in 96-well plates, as previously.[2] Compounds were formulated in DMSO and diluted to < 1% DMSO in the cultures.

Irradiation of Metal Complexes. Solutions (30 μM) of complex **41** in 5 mM phosphate buffer (pH 7.0) containing 0.1 M sodium formate were irradiated using a ^{60}Co source (dose rate determined by NaCl-modified Fricke dosimetry), as previously reported[22] and analyzed by LC/MS as above.

Acknowledgment. We thank Susan Pullen for undertaking the IC_{50} assays and the Auckland Division of the Cancer Society of New Zealand and the Foundation for Research, Science and Technology, New Zealand (Grant UOAX0211), for financial support.

Supporting Information Available: Synthesis details for Schemes S1 and S2; combustion analysis results for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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