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Cross-Bridged Cyclen or Cyclam Co(III) Complexes Containing Cytotoxic Ligands as Hypoxia-Activated Prodrugs

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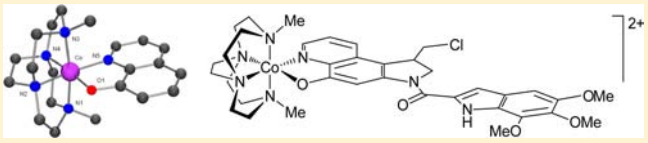
Supporting Information

ABSTRACT: A series of cobalt(III) complexes of the potent DNA minor groove alkylator (1-(chloromethyl)-5-hydroxy-1*H*-pyrrolo[3,2-*f*]quinolin-3(2*H*)-yl)(5,6,7-trimethoxy-1*H*-indol-2-yl)methanone (**3**; *seco*-CPyI-TMI), with cyclam or cyclen auxiliary ligands (**L3** and **L5**) containing a cross-bridging ethylene (CH_2CH_2) group or the *N*,*N*′-dimethyl derivatives of these (**L4** and **L6**), was prepared. Two 8-quinolinato (**2**) model complexes of these, $[Co(\mathbf{L3})(\mathbf{2})](ClO_4)_2$ and $[Co(\mathbf{L6})(\mathbf{2})](ClO_4)_2$, and the aquated derivative $[Co(\mathbf{L6})(H_2O)_2](OTf)_3$ were characterized by X-ray crystallography. Electrochemistry of the 8-quinolinato model complexes showed that the Co(III)/(II) reduction potential was lowered relative to the unsubstituted cyclen ligand. Evaluation of the cytotoxicity of the racemic *seco*-CPyI cobalt complexes in vitro showed considerable attenuation of their cytotoxicity relative to the free alkylator and marked hypoxic selectivity, especially $[Co(\mathbf{L3})(\mathbf{3})]^{2+}$ (**9**), which was 81−212-fold more potent under hypoxia than 20% oxygen in a panel of 10 human tumor cell lines. However, **9** did not elicit significant killing of hypoxic cells in HT29 tumor xenografts, suggesting possible pharmacological limitations in vivo.



INTRODUCTION

Cobalt(III) complexes of cancer drugs have been extensively studied as potential hypoxia-activated prodrugs, with a view to exploiting hypoxia to achieve tumor targeting.[1−10] The complexes are designed to undergo bioreduction to the more labile Co(II) species by ubiquitous one-electron reductases in cells. The original concept was that the Co(II) species would be reoxidized to the parent Co(III) complex by oxygen in normoxic tissue, analogous to the redox cycling of nitro-aromatics and quinones, while in hypoxic cells the Co(II) complex would dissociate to release its bioactive ligands.[2−4] Consistent with this, Co(III) complexes of bidentate nitrogen mustards with bidentate pentane-2,4-dionato (acac) auxiliary ligands (e.g., **1**) exhibited a differential cytotoxicity (HCR, hypoxic cytotoxicity ratio) of about 20-fold in hypoxic versus oxic cells in culture.[2] However, pulse radiolysis studies with these compounds,[5,6] along with limited reversibility of their electrochemical reduction,[6] suggested that hypoxic selectivity of these prototype prodrugs might be due to competition between oxygen and the Co(III) complexes for one-electron reductants in cells rather than Co(III)/Co(II) redox cycling. In addition, the limited stability of the Co(III) complexes themselves likely contributes to their lack of activity against hypoxic tumor cells in animal tumor models. These considerations indicated the need to explore ligands providing improved kinetic inertness in the Co(III) oxidation state.

An exploration of auxiliary ligands for Co(III) complexes of 8-quinolinolato (**2**) showed that the tetradentate ligand 1,4,7,10-tetraazacyclododecane (**L1**; cyclen) provided suitably inert Co(III)complexes (e.g., **4**).[7] These complexes significantly masked the potency of **2** as an inhibitor of cell proliferation. This prompted the use of the much more cytotoxic (1-(chloromethyl)-5-hydroxy-1*H*-pyrrolo[3,2-*f*]quinolin-3(2*H*)-yl)(5,6,7-trimethoxy-1*H*-indol-2-yl)methanone ligand (*seco*-CPyI-TMI, **3**) (as the racemate) in complexes such as **5**, which showed substantial hypoxic cell selectivity (Figure 1).[8] Compound **3** belongs to a larger class of compounds noted for their potent cytotoxicity,[11] and simpler analogues have been shown[12,13] to form transient transition metal complexes with acac auxiliary ligands. A recent study[10] showed that the Co(III) complexes of **3** with a variety of 1,7-dialkyl analogues of **L1** had broadly similar aqueous solubilities and solution stabilities to those of complex **5**. However, these dialkyl complexes were significantly less effective than **5** in masking the cytotoxicity of **3**.

Here, we seek to impart further stability to the complexes by a modification to the topology of the macrocycle, specifically by linking the opposing nitrogen donors of the parent macrocyles with an ethylene bridge. The bridge forces the nitrogen lone pairs to align better with the metal d orbitals, preorganizing the host site and ensuring that only complexes with *cis* geometry are formed.[14] This potentially allows a better fit around the cobalt(III) center, thereby stabilizing cobalt(III) relative to

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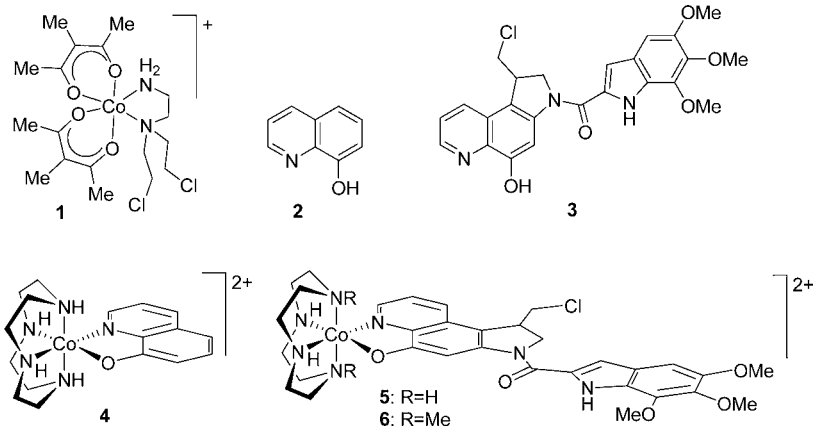


Figure 1. Examples of cobalt(III) complexes containing nitrogen mustard, 8-quinolinato (**2**), and *seco*-CPyI-TMI (**3**) ligands.

cobalt(II) and lowering the reduction potential of the Co(III) complexes. We present data on cobalt(III) complexes of the model effector **2** and cytotoxic effector **3** with a variety of macrocycles featuring cross-ring ethylene-bridged cyclams (**L3**, **L4**) and cyclens (**L5**, **L6**) (Figure 2).

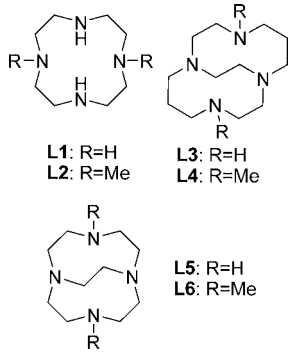


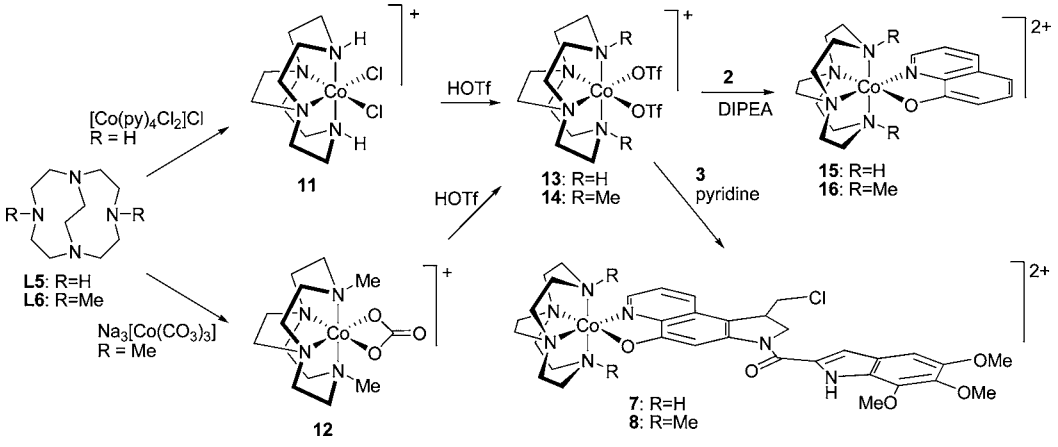
Figure 2. Tetradentate macrocyclic ligands.

SYNTHESES

Cross-ring-bridged cyclens and cyclams have been prepared by high-dilution reactions with bifunctional reagents such as oxalyl chloride[15] or propane-1,3-ditosylate,[16] but a more efficient general method for preparation of ligands **L3**–**L6** was that reported by Weisman et al.[17−19] As an example, **L3** was prepared from the bisaminal formed from condensation of cyclen and glyoxal, followed by alkylation with excess benzyl bromide to precipitate the 4,11-dialkylated bromide salt. Adding sodium borohydride in large excess and stirring for 2 weeks reduces the macrocycle selectively and forms the ethylene cross-bridge. Removal of the benzyl protecting groups by hydrogenation over a Pd/C catalyst produces **L3** as a white solid in approximately 50% yield.[19] The bis-alkylated macrocycle **L4** was prepared in two steps from the same bisaminal intermediate by reaction with excess methyl iodide followed by reduction with borohydride. The cyclen derivatives **L5** and **L6** were prepared similarly.

The two ligands **L3** and **L5** contain secondary and tertiary aliphatic amines and have the potential to be very good Brønsted bases. Reaction of $HgCl_2$ with **L3** under ambient conditions was reported to result in a dimeric complex $[Hg(\mathbf{L3})Cl_2]_2$ in which the $HgCl_2$ units lie outside the **L3** coordination pocket; the internal cavity is occupied by intramolecular amine N−H···N hydrogen bonds. On the other hand, reaction of $HgCl_2$ with **L5** under anhydrous conditions produced $Hg(\mathbf{L5})Cl_2$ in which the Hg^{2+} is bound within the folded ligand pocket.[20] Brønsted basicity is even higher for **L4** and **L6**, which contain only tertiary amines and show evidence for proton sponge behavior. For $[H_2\mathbf{L4}]^{2+}$ the pK_{a1} and pK_{a2} values were reported to be 9.58 and >24, respectively.[21] Even so, a copper(II) complex of the related *N*,*N*′-dibenzyl cross-bridged cyclam ligand was prepared using

Scheme 1. Syntheses of Complexes Containing the Cyclen Ligands L5 and L6



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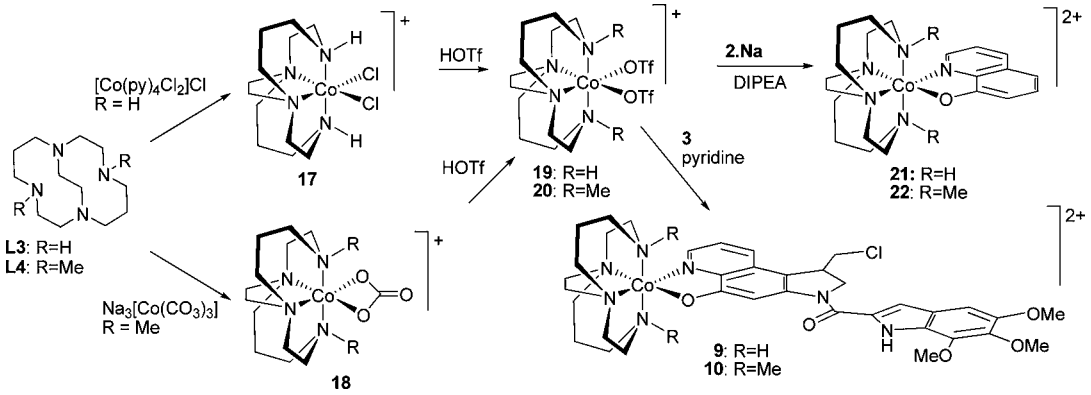
Scheme 2. Syntheses of Complexes Containing the Cyclam Ligands L3 and L4



the hydrated metal salt $CuCl_2 \cdot 2H_2O$ and bears the copper ion fully coordinated to the ligand.[18] A cobalt(III) complex of **L4** has been reported previously, $[Co(\mathbf{L4})Cl_2]Cl$, prepared by oxidation of the cobalt(II) complex $Co(\mathbf{L4})Cl_2$ in aqueous HCl solution.[22] The triprotonated ligand $[H_3\mathbf{L6}]^{3+}$ has three pK_a values (<2, 5.8, and 11.3),[23] but unlike **L4**, it is not technically a proton sponge as all its pK_a values lie within the normal pH range. Nevertheless, dry conditions were reported to be required for preparation of the $[Co(\mathbf{L6})Cl_2](PF_6)$ complex.[22]

The strategy for preparation of the cobalt complexes of **2** and **3** was to first introduce the cross-ring-bridged macrocycle (**L**) into the cobalt coordination sphere and then to prepare an intermediate triflato complex which could serve as a common intermediate for the target complexes $[Co(\mathbf{L})(\mathbf{2})]^{2+}$ and $[Co(\mathbf{L})(\mathbf{3})]^{2+}$ by displacement of the labile triflato ligands. This approach has the advantages of introducing the reactive cytotoxin **3** as the final step in the sequence, and the preparation of the complexes containing the more readily available **2** are used to develop and optimize the synthetic route.

We recently explored methods for synthesis of cobalt(III) complexes of cyclen- and cyclam-based ligands[8,10] and found that *trans*-$[Co(py)_4Cl_2]Cl$,[24] $Na_3[Co(NO_2)_6]$, and $Na_3[Co(CO_3)_2] \cdot 3H_2O$[25] are all useful for introducing a macrocyclic ligand into the coordination sphere of the inert cobalt(III) metal center. For the cross-bridged cyclen and cyclam ligands utilized in this study, *trans*-$[Co(py)_4Cl_2]Cl$ proved suitable as an entry to the **L3** and **L5** complexes while $Na_3[Co(CO_3)_3]$ was useful for the *N,N′*-dimethyl ligands **L4** and **L6** (Schemes 1 and 2). The cobalt carbonato precursor was a good match for the more basic **L4** and **L6** ligands, which are likely to bind at least one proton very tightly, and the basic carbonate helps to neutralize this. An attempt to prepare $[Co(\mathbf{L4})Cl_2]Cl$ using *trans*-$[Co(py)_4Cl_2]Cl$ as the precursor resulted in a blue-green crystalline product which was identified by a partially refined crystal structure to be the salt $[H_2\mathbf{L4}][CoCl_4]$.

Scheme 1 illustrates these two routes for formation of complexes containing the cyclen ligands **L5** and **L6**. The first step utilized the free base ligand and cobalt(III) salt, yielding **11** and **12.** Both complexes were subjected to ligand exchange using neat trifluoromethanesulfonic acid (triflic acid, HOTf) under anhydrous conditions to give the triflato complexes **13** and **14**. The labile triflato ligands were then displaced by reaction with **2** in the presence of base (DIPEA (diisopropylethylamine) or pyridine) to give the quinolinato complexes **15** and **16**. Parallel chemistry (Scheme 2) was used for preparation of the cyclam complexes **17**−**22** containing **L3** and **L4**.

All of the triflato complexes were isolated and purified by crystallization and other complexes by crystallization, gel filtration, or ion exchange chromatography. Minor modifications of conditions were required for some reactions, for example, the sodium salt of **2** was used rather than **2** in the presence of DIPEA for preparation of **21** from **19**. A cobalt(III) complex $[Co(\mathbf{L6})Cl_2](PF_6)$ containing **L6** has been reported previously,[22] prepared by an anhydrous route. An alternative route to **16** involved reaction of **L6** with *trans*-$[Co(py)_4Cl_2]Cl$ to give the dichloro complex $[Co(\mathbf{L6})Cl_2]Cl$. Reaction of this directly (without going through the triflato salt) with **2** and triethylamine (TEA) in methanol gave **16** as the perchlorate salt after purification by cation exchange chromatography. However, a difficulty with this route was that it was only effective when the ligand **L6** had been freshly prepared; otherwise, it tends to pick up protons and form the unwanted salt $[H_2\mathbf{L6}][CoCl_4]$, significantly reducing the yield. The route to **16** via **12** and **14** gives higher yields and is preferred.

Although the cross-bridged cyclen ligands **L5** and **L6** enclose a slightly smaller cavity than the cyclams **L3** and **L4**, cobalt(III) complexes formed readily with both series of ligands, and indeed, there are literature reports of complexes of cross-bridged cyclens with larger metals such as copper(II) and indium(III) ions.[18,26] Preparation of the quinolinato complexes served as models for synthesizing the corresponding complexes containing the highly cytotoxic[8] *seco*-CPyI-TMI ligand. The methodology shown in Schemes 1 and 2 was effective for preparing the set of four compounds **7**−**10**, containing ligands **L3**−**L6**, with yields in the range 49−84% for the final step, comparable to the 65−89% yields obtained for the quinolinato model compounds.

NMR SPECTROSCOPY

All complexes were characterized by ^{1}H and ^{13}C NMR spectroscopy (Figures S1−S12, Supporting Information). Spectra were assigned using a range of 1D and 2D NMR experiments (DEPT, COSY, NOESY, HSQC, and HMBC). Precursor complexes containing chloro, carbonato, and triflato ligands are symmetric. For example, **18** and **20** show seven signals and **12** shows four signals for the macrocyclic ligands in their ^{13}C NMR spectra. The ^{19}F NMR spectrum of the triflato complex **14** ($[Co(\mathbf{L6})(OTf)_2](OTf)$) in CD_3CN shows two distinct chemical shifts corresponding to the coordinated and counterion triflates. These slowly change with time as a result of displacement of the labile triflato ligands by solvent, although more slowly than observed for the simpler cyclen complex $[Co(\mathbf{L1})(OTf)_2](OTf)$,[10] suggesting that **14** is more kinetically

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inert. An attempt to crystallize **14** from methanol/diethyl ether resulted in isolation of the triflate salt of the diaqua complex $[Co(\mathbf{L6})(H_2O)_2](OTf)_3$ (**14a**), the structure of which was confirmed by X-ray crystallography (see Figure 3c).

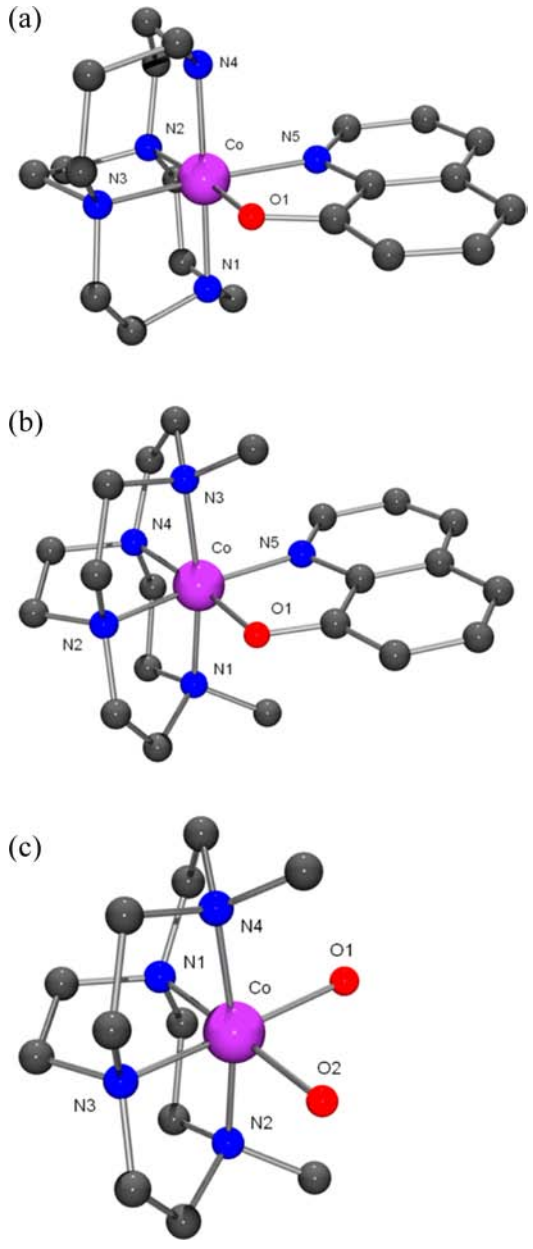

Figure 3. Molecular structures of the cations (a) $[Co(\mathbf{L3})(\mathbf{2})]^{2+}$ (**21**), (b) $[Co(\mathbf{L6})(\mathbf{2})]^{2+}$ (**16**), and (c) $[Co(\mathbf{L6})(OH_2)_2]^{3+}$ (**14a**) (hydrogen atoms omitted for clarity).

The 8-quinolinato complexes **15** and **16** containing cyclen ligands retain a plane of symmetry, but the cyclam complexes **21** and **22** have lower symmetry, and a unique signal is observed for each carbon atom in their NMR spectra. The racemic *seco*-CPyI-TMI **3** has a stereocenter where the chloromethyl group links to the indoline ring; as a result, the cobalt complexes **7** and **8** each occur as two enantiomers while **9** and **10** each form as two diastereomers, indicated by the doubling of some signals in both the ^1H and the ^{13}C NMR spectra.

X-RAY CRYSTALLOGRAPHY

Figure 3a shows one of the two enantiomers in the unit cell of racemic **21** which contains the cyclam derivative **L3**. The good matching of the metal center to the ligand is seen from the axial N4−Co−N1 bond angle of 178.64(9)° and the equatorial N2−Co−N3 bond angle of 89.04(9)°, which indicate little distortion from octahedral geometry around the metal. The axial Co−N bond lengths are slightly shorter than the equatorial Co−N lengths (in the same plane as the 8-quinolinato ligand). Complexes **16** and **14a** (Figure 3b and 3c) both contain the cyclen ligand **L6** in which there is not quite such a good fit around the cobalt(III) center with the axial N−Co−N angles both close to 170°, although the equatorial N−Co−N bond angles are both close to 89°. The structure of **16** can be compared to one published structure containing **L6**, $[Co(\mathbf{L6})Cl_2]PF_6$,[22] and to two related structures recently published by our group,[10] $Co(R_2cyclen)(\mathbf{2})$ (R = $CH_2CH_2CH_2SO_3^-$ or $CH_2CH_2CH_2CH_2P(OH)O_2^-$) in which the R groups are attached to the axial nitrogen donors of the cyclen ligand in the same positions as the methyl groups in **L6**. A key conformational difference is that the R_2cyclen ligands contain NH groups in the equatorial positions, allowing different configurations at these nitrogens, and in both structures these are oriented so one N−H is directed toward the 8-quinolinato N donor while the other N−H is directed away, designated as the anti-O,syn-N configuration. The cross-bridge in **L6** effectively locks the macrocycle in the anti,anti configuration. These related structures share with **16** and **14a** axial Co−N bond lengths that are slightly longer (1.99−2.01 Å) than the equatorial Co−N lengths (1.91−1.94 Å) and axial N−Co−N angles (close to 170°) distorted away from the ideal 180°.

ELECTROCHEMISTRY

The half-wave reduction potentials of the more soluble model complexes **15**, **16**, and **21** were measured in 0.1 M $NaNO_3$ aqueous solution by cyclic voltammetry (Figure S13, Supporting Information). For example, complex **21** shows cathodic and anodic peak potentials at −540 and −480 mV (relative to Ag/AgCl reference), respectively (scan rate 100 mV s^{-1}). Potentials remain approximately the same as the scan rate is varied over the range 20, 50, 100, and 500 mV s^{-1}, and the ratios of the cathodic (i_{pc}) and anodic (i_{pa}) currents at different scan rates are close to 1. The value of ΔE_p (at 100 mV s^{-1} scan rate) is 60 mV, and there is a direct proportional relationship between i_{pc} and i_{pa} with the square root of the scan rate, all of which are consistent with reversible one-electron redox behavior.

Data in Table 1 show that introducing the cross-bridge into the macrocycles has shifted the reduction potentials of the complexes to significantly more negative values. The $E_{1/2}$ of **15**

Table 1. Redox Potentials[a] of the 8-Quinolinato Complexes (0.1 M $NaNO_3$ solution, scan rate 100 mV s^{-1})

	compound	E_{pc}/mV	E_{pa}/mV	ΔE_p/mV	$E_{1/2}$/mV
4	$[Co(\mathbf{L1})(\mathbf{2})](ClO_4)_2$	−195	−136	59	−166
16	$[Co(\mathbf{L6})(\mathbf{2})](ClO_4)_2$	−220	−154	66	−187
21	$[Co(\mathbf{L3})(\mathbf{2})](ClO_4)_2$	−331	−271	60	−301
15	$[Co(\mathbf{L5})(\mathbf{2})](OTf)_2$	−393	−306	87	−349

[a]Potentials reported vs NHE (Ag/AgCl (3 M NaCl) 0.209 V vs NHE).

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(−349 mV) compares with $E_{1/2}$ (−166 mV) for the nonbridged analogue **4**, confirming the hypothesis that introducing the cross-bridge would lead to more negative reduction potentials. However, the $E_{1/2}$ of **16** (−187 mV) is comparable with that of **4**, indicating that alkylation on both axial nitrogens nearly counteracts the effect of the cross-bridge. *N*-Alkylation is known to induce more positive reduction potentials,[27] as observed in the complexes [Co(R_2cyclen)(**2**)] (R = CH_3, $CH_2CH_2CH_2SO_3^-$ or $CH_2CH_2CH_2CH_2P(OH)O_2^-$).[7,10] On the basis of these trends, the $E_{1/2}$ of **22** would be expected to be approximately 162 mV more positive than **21**, or about 48 mV more positive than **16**. Both estimates give an $E_{1/2}$ value of −139 mV for **22**.

RADIOLYTIC RELEASE OF LIGAND

A sample of **9** in anoxic formate buffer (0.1 M) at pH 7.0 was irradiated using a ^{60}Co γ radiation source to provide a model one-electron reduction system (Figure 4). Decreases in the concentration of **9** and increase in the concentration of free **3** were directly proportional to the radiation dose up to about 40 Gy. The *G* values calculated from the slopes of lines for the concentrations of **3** and **9** are 0.438 and 0.588 μM Gy^{-1}, respectively. These values are close to the theoretical[28] reductant yield (e_{aq}^- + $CO_2^{-\bullet}$) of 0.59 μM Gy^{-1} and indicate release of **3** from the cobalt complex with one-electron stoichiometry, as observed previously[3] for the unbridged cyclen complex **5**.

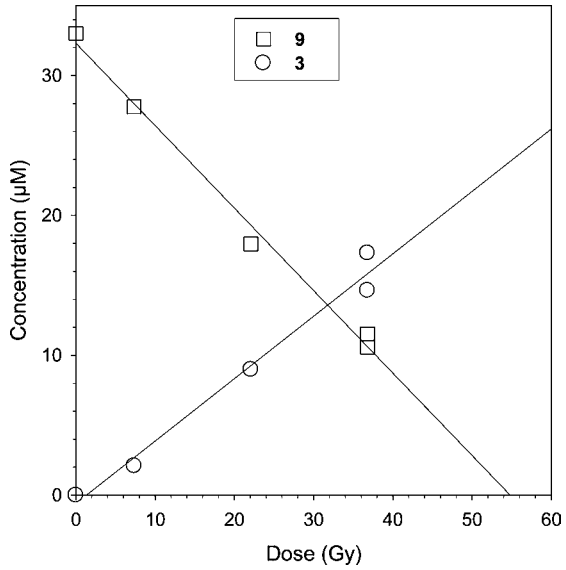

Figure 4. Radiolytic reduction of **9** (30 μM) in 0.1 M anoxic formate buffer at pH 7.0. Concentrations were analyzed by HPLC. Lines are linear regressions.

CELL LINE STUDIES

Complexes **5**−**10** containing the cytotoxin **3** were evaluated for antiproliferative activity under both oxic and hypoxic conditions in two human tumor cell lines, SKOV3 ovarian carcinoma and HT29 human colon adenocarcinoma (Table 2). As previously noted,[8,9] complexation of **3** to give **5** and **6** lowered cytotoxicity considerably. Complex **5** had P/E (IC_{50} prodrug/IC_{50} effector) ratios of 146 and 110 in the SKOV3 and HT29 cell lines, respectively, while the dimethylated analogue **6** had less substantial attenuation, with P/E values of 30 and 22. The bridged cyclen complex **7** provided similar levels of attenuation in the SKOV3 and HT29 cell lines (P/E values 169 and 105, respectively), and the corresponding dimethylated analogue **8** was deactivated almost as well (P/E 135 and 48). The loss in cell culture medium of **5** and **6** (46% and 81%, respectively, remaining after 24 h at 37 °C) and the corresponding bridged complexes **7** and **8** (29% and 73%) suggest that *N*-methyl groups in the ligand provide additional stability. The 8-quinolinato complex **21** showed a low reduction potential (Table 1), and the corresponding complex containing the effector, **9**, was clearly superior in terms of toxicity attenuation in the SKOV3 cell line, with a P/E ratio of 423, although was less attenuated in the HT29 line (P/E ratio 100). Cytotoxicity was less well masked in the corresponding bridged Me_2C_2cyclam complex [Co(**L4**)(**3**)](OTf)$_2$ (**10**).

The cytotoxicities of the *seco*-CPyI-TMI complexes were compared under both aerobic and hypoxic conditions to determine their potential as hypoxia-activated prodrugs (Table 2). The free effector **3** is known[8] to be equitoxic under both aerobic and hypoxic conditions, as expected from its mechanism of action, and showed hypoxic cytotoxicity ratios (HCR = IC_{50}[oxic]/IC_{50}[hypoxic]) in both cell lines close to unity. Cobalt complexes **5** and **6** have been shown previously[8,9]

Table 2. Solubility, Stability, and Cytotoxicity of *seco*-CPyI-TMI Co(III) Complexes and Tirapazamine (TPZ)

				IC_{50} (nM)[c]							
				SKOV3				HT29			
no.	compound	solubility (μM)[a]	stability (%)[b]	oxic	P/E[d]	hypoxic	HCR[e]	oxic	P/E	hypoxic	HCR
3	*seco*-CPyI-TMI	37		0.26 ± 0.03		0.40 ± 0.04	0.69 ± 0.08	0.63 ± 0.15		0.69 ± 0.05	0.90 ± 0.18
5[f]	[Co(**L1**)(**3**)]$^{2+}$	3000	46	38 ± 14	146	3.7 ± 1.5	18 ± 8	69 ± 27	110	5.6 ± 1.7	13 ± 3
6[f]	[Co(**L2**)(**3**)]$^{2+}$	>4700	81	7.9 ± 0.1	30	0.40 ± 0.07	20 ± 3	14 ± 4	22	0.67 ± 0.09	22 ± 9
7	[Co(**L5**)(**3**)]$^{2+}$	>4600	29	44 ± 1	169	0.67 ± 0.07	67 ± 7	66 ± 8	105	1.2 ± 0.1	54 ± 6
8	[Co(**L6**)(**3**)]$^{2+}$	>4700	73	35 ± 3	135	0.52 ± 0.04	67 ± 1	30 ± 1	48	0.85 ± 0.11	36 ± 4
9	[Co(**L3**)(**3**)]$^{2+}$	4600	61	110 ± 17	423	0.52 ± 0.04	212 ± 36	63 ± 8	100	0.71 ± 0.04	89 ± 10
10	[Co(**L4**)(**3**)]$^{2+}$	3500	1	<3.7	<15	0.40	<9				
	TPZ			310 ± 60[g]		4.9 ± 1.1[g]	72 ± 14	352 ± 12[g]		5.1 ± 0.2[g]	75 ± 1

[a]Solubility (μM) in culture medium at 20 °C. [b]Stability (% remaining after 24 h at 37 °C in culture medium). [c]IC_{50} for inhibition of cell proliferation following 4 h drug exposure. Values are the means and errors are SEM for 3−6 determinations. [d]P/E: IC_{50} prodrug/IC_{50} effector (compound **2** or **3**). [e]HCR (hypoxic cytotoxicity ratio) is the intraexperiment ratio: oxic IC_{50}/hypoxic IC_{50}. [f]IC_{50} data from refs 8 and 9. [g]Units for TPZ IC_{50} values are μM.

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Table 3. Cytotoxicity of Co Complexes 5 and 9 and Tirapazamine (TPZ) in a Panel of Human Tumor Cell Lines

	TPZ			5[a]			9		
	IC_{50} (μM)[b]			IC_{50} (nM)			IC_{50} (nM)		
cell line	aerobic	hypoxic	HCR[c]	aerobic	hypoxic	HCR	aerobic	hypoxic	HCR
A375	584 ± 118	3.40 ± 1.38	223 ± 125	22 ± 1	1.66 ± 0.36	14 ± 2	21 ± 4	0.112 ± 0.009	188 ± 23
A-549	260 ± 53	4.31 ± 0.47	67 ± 10	5.0 ± 0.9	1.24 ± 0.45[d]	7.5 ± 3.2	9.8 ± 1.7	0.137 ± 0.049	81 ± 10
A549/POR	19.2 ± 3.1	0.66 ± 0.11	32 ± 5	6.9 ± 1.3	1.62 ± 0.30[d]	4.3 ± 1.8	9.6 ± 1.1	0.142 ± 0.032	76 ± 18
C-33A	47 ± 3	1.2 ± 0.2	46 ± 7	11 ± 1	0.89 ± 0.1	14 ± 4	9.7 ± 0.6	0.094 ± 0.011	106 ± 12
H1299	268 ± 26	2.67 ± 0.28	110 ± 15	30 ± 6	3.86 ± 0.99	8.2 ± 1.3	37 ± 5	0.262 ± 0.050	151 ± 28
H460	170 ± 15	1.58 ± 0.22	127 ± 13	30 ± 2	2.34 ± 1.70	32 ± 15	37 ± 2	0.258 ± 0.083	200 ± 88
HCT116	106 ± 7	2.12 ± 0.22	56 ± 5	20 ± 1	1.37 ± 0.12	15 ± 2	14 ± 2	0.110 ± 0.006	125 ± 13
PC3	967 ± 80	14.4 ± 1.0	73 ± 8	34 ± 2	3.35 ± 1.16	15 ± 7	40 ± 7	0.300 ± 0.083	166 ± 61
SiHa	203 ± 14	2.41 ± 0.18	97 ± 6	95 ± 39	3.77 ± 0.75	38 ± 19	38 ± 9	0.277 ± 0.049	169 ± 56

[a]See also ref 8. [b]Footnote c of Table 2. [c]Footnote e of Table 2.

to have moderate HCR values of around 13−22. The bridged C_2cyclen and Me_2C_2cyclen complexes [Co(**L5**)(**3**)](OTf)$_2$ (**7**) and [Co(**L6**)(**3**)](OTf)$_2$ (**8**) showed significantly greater HCRs (36−67). The bridged cyclam complex **9**, which had the highest P/E value in the SKOV3 cell line, showed the highest HCRs in both cell lines (212 in SKOV3 and 89 in HT29). These hypoxic selectivities were at least as high as for the reference hypoxia-activated prodrug tirapazamine (TPZ), but **9** had dramatically (~10^4-fold) higher potency than TPZ. The parent complex **5** and the most selective bridged complex **9** were evaluated for aerobic and hypoxic cytotoxicity, along with TPZ, in a larger panel of human tumor cell lines (Table 3), to see if the superiority of the bridged complex was maintained. This proved to be the case; the unbridged complex **5** had an average HCR of 16, whereas the bridged analogue **9** had a much superior average HCR of 148. This higher HCR reflected the greater potency of **9** than **5** under hypoxia. In 9 of the 10 cell lines tested (Tables 2 and 3) the hypoxic selectivity of **9** was superior to that of TPZ.

The antiproliferative activity of **5** and **9** was also compared with TPZ in A549/POR, an A549 cell line with 9-fold overexpression of NADPH:cytochrome P450 oxidoreductase (POR) relative to the parental line[4] (Table 3). POR overexpression increased the potency of TPZ 13.5-fold under aerobic conditions and 6.5-fold under hypoxia but had no significant effect ($p > 0.05$) on the IC_{50} of **9** under either condition. The latter finding is consistent with the lack of hypersensitivity of A549/POR cells to **5**[29] and the unchanged rate of metabolism of **5** in A549 and A549/POR cells.[4]

MOUSE TOXICITY AND ANTITUMOR ACTIVITY

To determine whether **9** had therapeutic activity in vivo, the complex was formulated in 5% DMSO/saline. In a pilot toxicity study in male C3H/HeN mice, the maximum tolerated i.p. dose was ~24 μmol kg^{-1}, with 7% body weight loss at the nadir (5 days after dosing). Antitumor activity was then assessed by dosing CD-1 nude mice bearing HT-29 tumors with **9** at 17.8 μmol kg^{-1}, either alone or in combination with a single 20 Gy dose of ionizing radiation (used to sterilize aerobic tumor cells). Eighteen hours later, the tumors were excised and surviving clonogens assessed (Figure 5). No significant tumor cell killing was observed following dosing with **9** alone, and administration of **9** either 5 min before or 30 min after the radiation dose did not significantly increase cell killing compared to radiation alone ($p > 0.05$), indicating a lack of activity against the hypoxic tumor cell population. In contrast, combination of radiation with the reference hypoxia-activated prodrug TPZ clearly caused increased cell killing compared to radiation alone.

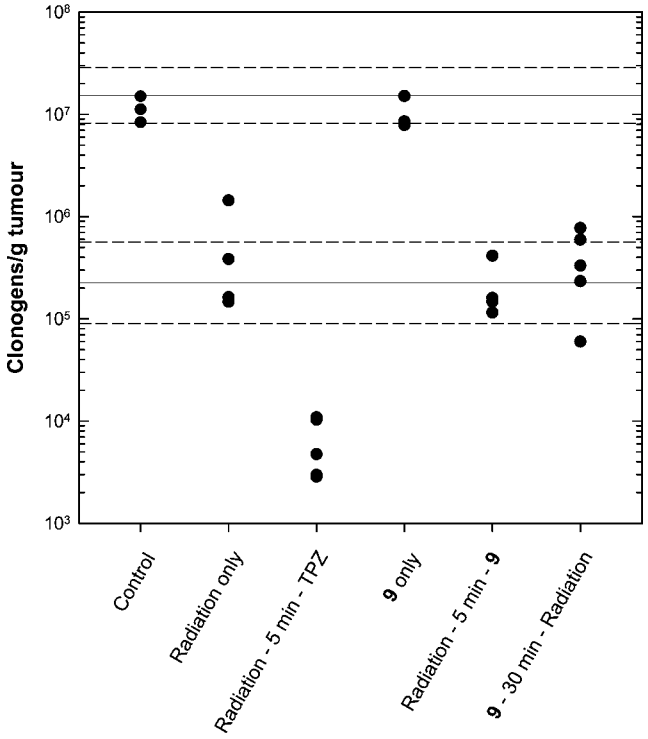

Figure 5. Antitumor activity of **9** in HT-29 human tumor xenografts assayed by tumor excision and clonogenic assay 18 h after i.p. dosing with **9** (17.8 μmol/kg) or TPZ (270 μmol/kg) either alone (groups of 3 mice) or in combination with γ irradiation (20 Gy, groups of 5 mice). Symbols represent tumors from individual animals; upper and lower lines represent historical controls from previous experiments for controls (65 tumors) and radiation treatment alone (113 tumors), ±1 standard deviation (dashed lines). Data are also shown for tirapazamine (TPZ) plus γ irradiation (20 Gy); TPZ alone has no effect (not shown).

CONCLUSIONS

Complexes using bridged cyclen and cyclam macrocycles as tetradentate auxiliary ligands were designed to lower the reduction potentials of the complexes from those of the previously studied cyclen complexes by constraining the macrocycle and thus increasing the electron density at the cobalt center. The cobalt(III) complexes thus become more

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difficult to reduce and less prone to loss of the effector. In addition, the macrocycle is partially preorganized by the cross-bridge into the preferred *cis* conformation for metal binding. Cyclic voltammetry measurements showed the bridged complexes had substantially lower reduction potentials while retaining one-electron reduction stoichiometry.

The nonalkylated ligands **L3** and **L5** formed the desired dichloro and (more reactive) bis-triflato intermediate complexes readily. In contrast, the much more basic "proton sponge"-like *N*-methylated ligands **L4** and **L6** gave primarily the protonated ligands on reaction with $[Co(py)_4Cl_2]Cl{\cdot}6H_2O$, and formation of the desired complexes required use of cobalt(III) precursors and intermediates containing the basic carbonato ligand. The cross-bridged 8-quinolinato complex $[Co(\mathbf{L3})(\mathbf{2})](ClO_4)_2$ (**21**) (a model for **9**) had an $E_{1/2}$ value more than 100 mV more negative than the corresponding cyclen 8-quinolinato complex $[Co(\mathbf{L1})(\mathbf{2})](ClO_4)_2$ (**4**), which likely contributed to the improved solution stability and improved hypoxic selectivity in culture of **9** relative to **4**.

The hypoxic selectivity of **9** in a panel of 10 human tumor cell lines was markedly superior to **5** and at least as great as the widely studied benzotriazine di-*N*-oxide TPZ. However, unlike the latter compound, cytotoxicity of **5** and **9** was not enhanced by overexpression of the one-electron reductase POR, which is well known to activate nitroaromatic, quinone, and benzotriazine di-*N*-oxide prodrugs under hypoxia.[30] Thus, the identity of the reductases (or nonenzymatic reductants) responsible for activation of these Co(III) complexes is unknown. Despite the ~10^4-fold greater molar potency of **9** than TPZ in hypoxic cell cultures, when compared in a xenograft model at just a 15-fold difference in molar dose (Figure 5), TPZ was clearly more effective than **9** in killing hypoxic (radioresistant) tumor cells. This finding suggests there is a major pharmacological limitation for activity of **9** as a hypoxia-selective cytotoxin in vivo.

EXPERIMENTAL SECTION

Sodium triscarbonatocobaltate(III) trihydrate[25] and *trans*-dichlorotetrapyridinecobalt(III) chloride hexahydrate[31] were prepared as described previously. Ligands **L3**–**L6** were prepared by literature methods: **L3**,[19] **L4**,[19] **L5**,[32] **L6**.[32] Chemicals and solvents were purchased from commercial sources and used as received unless specified otherwise. Deionized Milli Q water was utilized where mentioned. Cation exchange chromatography was performed on a Sephadex SP-C25 gel in the sodium form. Gel permeation chromatography was performed on Sephadex LH-20 gel either in water or in methanol. Amersham Bioscience supplied all resins. Analytical-grade solvents were used in chromatography. Final products were analyzed by reverse-phase HPLC (Alltima C18 5 μm column, 150 × 3.2 mm; Alltech Associated, Inc., Deerfield, IL) using an Agilent HP1100 equipped with a diode-array detector. Mobile phases were gradients of 80% CH_3CN/20% H_2O (v/v) in 45 mM NH_4O_2CH at pH 3.5 and 0.5 mL min^{-1}. Purity was determined by monitoring at 330 ± 50 nm and ≥95% unless stated.

High-resolution fast atom bombardment mass spectra (FAB^+) were recorded on a VG 70-SE mass spectrometer. Spectra were measured in *m*-nitrobenzyl alcohol as the matrix under argon and referenced to poly(ethylene glycol). Low-resolution mass spectra ($APCI^+$) were run on a Surveyor MSQ mass spectrometer using a methanol solution of the samples. Microanalyses (C, H, N, and Cl) were carried out at the Campbell Microanalysis Laboratory, University of Otago. ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 or a Bruker Avance-300 spectrometer at 400 and 100 MHz or 300 and 75 MHz, respectively. Spectra recorded in $CDCl_3$, D_2O, CD_3CN, CD_3OD, and DMSO-d_6 were referenced to TMS (tetramethylsilane), TSP-d_4 (3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt), or their respective residual solvent peaks. Full assignments of the ^1H and ^{13}C NMR spectra of the complexes are given in the Supporting Information.

***cis*-Dichloro(1,4,7,10-tetraaza-bicyclo[5.5.2]tetradecane)-cobalt(III) Chloride $[Co(L5)Cl_2]Cl$ (11).** A mixture of *trans*-dichlorotetrapyridinecobalt(III) chloride hexahydrate (1.42 g, 2.40 mmol) and 1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (**L5**) (0.480 g, 2.42 mmol) in MeOH (50 mL) was stirred at 50 °C overnight (~15 h). The resulting solution was concentrated, and Et_2O was added to precipitate out **11** (0.754 g, 86%) as a blue solid, which was used directly for the next step. ^1H NMR (CD_3OD, 400 MHz): 2.82 (m, 4H), 3.36 (m, 4H), 3.58 (s, 4H, $2NCH_2$), 3.92 (m, 8H), 7.38 (br, 2H, 2NH). HRMS (FAB+): found, *m*/*z* 327.05490/329.05188 $[M - Cl]^+$; calcd for $C_{10}H_{22}{}^{35/37}ClCoN_4$, 327.05535/329.05240.

***cis*-Bis(triflato)(1,4,7,10-tetraaza-bicyclo[5.5.2]tetradecane)-cobalt(III) Triflate $[Co(L5)(Otf)_2]Otf$ (13).** Solid **11** (0.600 g, 1.65 mmol) was cooled in an ice bath and treated with trifluoromethanesulfonic acid (5 mL) with stirring. The mixture was warmed to 40 °C for 15 min; then N_2 gas was bubbled through for 15 min to remove the HCl. Addition of Et_2O gave a pink precipitate which was collected by filtration and washed with Et_2O to give **13** (1.05 g, 90%) as a pink solid. ^1H NMR (D_2O, 400 Hz) $[Co(L5)(D_2O)_2](Otf)_3$: δ 2.95 (m, 4H, $2NCH_2$), 3.37 (m, 4H), 3.63 (m, 4H), 3.70 (s, 4H, $2NCH_2$), 4.09 (m, 4H), (exchangeable NH not observed). HRMS (ESI): found, *m*/*z* 405.0620 $[M - 2Otf - H]^+$; calcd for $C_{11}H_{21}CoF_3N_4O_3S$, 405.0618. Anal. Calcd for $C_{13}H_{22}CoF_9N_4O_9S_3$: C, 22.17; H, 3.15; N, 7.95. Found: C, 22.24; H, 3.44; N, 7.68.

8-Quinolinolato(1,4,7,10-tetraaza-bicyclo[5.5.2]-tetradecane)cobalt(III) Triflate $[Co(L5)(2)](Otf)_2$ (15). A mixture of **13** (0.113 g, 0.16 mmol), **2** (0.044 g, 0.30 mmol), and DIPEA (58 μL, 0.33 mmol) in MeOH (6 mL) was stirred at 45 °C overnight (~17 h). Et_2O (30 mL) was then added to precipitate a brown solid, which was collected by decanting and washed with Et_2O to give **15** (0.100 g, 89%). ^1H NMR (D_2O, 400 MHz): 3.09 (m, 2H), 3.42 (m, 6H), 3.59 (m, 2H), 3.73 (m, 4H), 3.85 (m, 2H), 4.02 (m, 4H), 7.27 (q, 2H), 7.56 (t, 1H), 7.72 (q, 1H), 8.55(d, 1H), 9.04 (d, 1H) exchangeable NH not observed). ^{13}C NMR (D_2O, 100 MHz): 57.31, 57.91, 61.99, 62.67, 63.31, 66.89, 111.10, 115.79, 123.61, 130.86, 141.28, 150.95. HRMS (FAB+): found, *m*/*z* 550.11510 $[M - Otf]^+$; calcd for $C_{20}H_{28}CoF_3N_5O_4S$, 550.11461. Anal. Calcd for $C_{21}H_{28}CoF_6N_5O_7S_2{\cdot}0.5Et_2O$: C, 37.50; H, 4.52; N, 9.51. Found: C, 37.86; H, 4.15; N, 9.97. HPLC purity: 95%.

1-(Chloromethyl)-3-(5,6,7-trimethoxyindol-2-ylcarbonyl)-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinolin-5-ato(1,4,7,10-tetraaza-bicyclo[5.5.2]tetradecane)cobalt(III) Triflate $[Co(L5)(3)](Otf)_2$ (7). A mixture of **13** (0.211 g, 0.30 mmol), **3** (0.154 g, 0.33 mmol), and pyridine (72 uL, 0.90 mmol) in MeOH (30 mL) and CH_2Cl_2 (10 mL) was stirred at 45–50 °C for 1 week. Volatiles were evaporated, the residue was dissolved in MeOH, and the resulting solution was filtered through Celite. Filtrate was concentrated and purified by a LH-20 column twice (2 × 40 cm) using MeOH as eluent to give **7** (0.200 g, 65%) as a brown solid. ^1H NMR (D_2O, 400 MHz): 3.05–4.20 (m, 34H including protons of three MeO groups at 3.77, 3.79, and 4.04 ppm, respectively), 4.54 (m, 1H, one of H-v), 6.98 (s, 1H), 7.25 (s, 1H), 7.70 (2d, 2H), 8.44 (d, 1H), 8.91 (d, 1H) (exchangeable NH not observed). ^{13}C NMR (D_2O, 100 MHz,): δ 40.8, 48.2, 56.3, 56.5, 56.9, 57.6, 57.8, 58.2, 61.7, 62.3, 62.4, 62.4, 63.2, 63.3, 67.3, 98.7, 106.3, 108.34, 112.3, 118.6, 124.2, 125.5, 126.2, 130.3, 135.9, 138.4, 140.0, 143.5, 144.6, 148.8, 149.3, 161.2, 165.4. HRMS (ESI): found, *m*/*z* 722.2277/724.2270 $[M - 2Otf + e]^+$; calcd for $C_{34}H_{42}{}^{35/37}ClCoN_7O_5$, 722.2268/724.2239. Anal. Calcd for $C_{36}H_{43}ClCoF_6N_7O_{11}S_2$: C, 42.30; H, 4.24; N, 9.59. Found: C, 42.60; H, 4.54; N, 9.62. HPLC purity: 92%.

***cis*-Dichloro(1,4,8,11-tetraazabicyclo[6.6.2]hexadecane)-cobalt(III) Chloride $[Co(L3)Cl_2]Cl$ (17).** A solution of 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (**L3**) (0.089 g, 0.393 mmol) in MeOH (3 mL) was treated with a (dark green) solution of *trans*-dichlorotetrapyridinecobalt(III) chloride hexahydrate (0.238 g, 0.404 mmol) in MeOH, and the mixture was stirred and heated under nitrogen at 45 °C. After 1 h a red solution with a blue precipitate was

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obtained. The precipitate was collected by filtration and washed with Et_2O to give **17** as a blue solid (0.109 g, 71%). HRMS (FAB+): found, m/z 355.08704, 357.08356, and 359.08207 $[M - Cl]^+$; calcd for $CoC_{12}H_{26}{}^{35}Cl_2CoN_4$ 355.08665, for $C_{12}H_{26}{}^{35}Cl^{37}ClCoN_4$ 357.08370, and for $C_{12}H_{26}{}^{37}Cl_2CoN_4$ 359.08075.

***cis*-Bis(triflato)(1,4,8,11-tetraazabicyclo[6.6.2]hexadecane)-cobalt(III) Triflate [Co(L3)(Otf)$_2$]Otf (19).** Under a nitrogen atmosphere, solid **17** (0.041 g, 0.105 mmol) was treated dropwise at 0 °C with neat trifluoromethanesulfonic acid (1 mL, 11.254 mmol). The mixture was heated on a water bath at 45 °C for 30 min while bubbling with N_2 to remove HCl. The solution was allowed to cool to room temperature, and ice-cold diethyl ether (250 mL) was then added very slowly to induce precipitation while the flask was constantly flushed with nitrogen. The resulting precipitate was collected and washed with cold Et_2O to give **19** (0.070 g, 91.0%). The hygroscopic product was stored in a desiccator and used directly for the next step.

8-Quinolinolato(1,4,8,11-tetraazabicyclo[6.6.2]hexadecane)-cobalt(III) Perchlorate [Co(L3)(2)](ClO$_4$)$_2$ (21). To a pink solution of **19** (0.037 g, 0.051 mmol) in MeOH (4 mL) was added the sodium salt of **2** (0.011 g, 0.066 mmol) in MeOH (1.5 mL). The resulting orange solution was was heated at 50 °C under nitrogen with stirring for 5 h to give a brown solution containing suspended precipitates. Solvent was removed on a rotary evaporator, and the solid was redissolved in water (50 mL) to give a brown/yellow solution with a brown suspension. The solution was filtered and the filtrate diluted to 250 mL with water before loading onto a Sephadex SP-C25 cation exchange column. Two column volumes of water were allowed to pass through the column. The column was eluted with aqueous sodium perchlorate solution beginning with 0.05 M concentration. Pale pink and pale green/yellow minor bands were elutued at 0.05 and 0.1 M eluent concentration, respectively. A major brown band was collected when 0.2 M eluent was applied. The volume of this fraction was reduced by one-half on a rotary evaporator, and the flask was left to stand overnight. The resulting dark black/brown crystals were collected, washed with cold ether, and dried in a desiccator to give **21** (0.023 g, 70%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.91 (m), 1H), 1.93 (m, 1H), 1.95 (m, 1H), 2.05 (m, 1H), 2.08 (m, 1H), 2.33 (m, 2H), 2.43 (m, 1H), 2.48 (m, 1H), 2.53 (m, 1H), 2.61 (m, 1H), 2.74 (m, 1H), 2.78 (m, 1H), 2.87 (m, 1H), 2.88 (m, 1H), 3.11 (m, 1H) 3.18 (m, 1H), 3.25 (m, 1H), 3.26 (m, 3H), 3.32 (m, 1H), 3.67 (m, 2H), 6.65 (bt, 1H), 6.85 (bt, 1H), 7.20 (m, 1H), 7.22 (m, 1H), 7.50 (t, J = 7.9 Hz, 1H), 7.68 (dd, J = 5.2, 8.3 Hz, 1H), 8.59 (d, J = 8.2 Hz, 1H), 8.75 (d, J = 5.2 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 19.2, 19.4, 43.0, 43.3, 45.9, 46.2, 56.7, 57.1, 57.7, 60.3, 65.9, 66.8, 112.7, 115.3, 123.3, 129.8, 130.2, 139.7, 144.8, 151.7, 164.1. Single crystals were produced by concentration of an aqueous $NaClO_4$ solution of **21**.

1-(Chloromethyl)-3-(5,6,7-trimethoxyindol-2-ylcarbonyl)-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinolin-5-ato(1,4,8,11-tetraazabicyclo[6.6.2]hexadecane)cobalt(III) Trifluoroacetate [Co(L3)(3)](O$_2$CCF$_3$)$_2$ (9). To a suspension of **3** (0.026 g, 0.055 mmol) in predried MeOH (10 mL) under nitrogen was added a solution of **19** (0.167 g, 0.145 mmol) in MeOH (10 mL), followed by TEA (6 mg, 0.063 mmol). The mixture was heated with stirring at 50 °C for 72 h to give a dark brown solution, which was evaporated under reduced pressure to give a dark brown oil. This was redissolved in MeOH (2 mL) and filtered, and the filtrate was loaded onto a Sephadex LH-20 gel filtration column. Elution with MeOH gave a major brown band which was collected, and the solvent was removed to give the crude product as brown crystalline material containing the TEA salt as the major impurity. Preparative HPLC [Column Synergi-Max RP (250 × 21.2 mm); mobile phase, gradient mixture of A TFA/H_2O, pH 2.5 and B TFA/MeCN; flow rate 15 mL/min, R_t 17.39 min] gave **9** (0.028 g, 49%). ^{1}H NMR (DMSO-d_6, 400 MHz): 1.92 (m, 4H), 2.10 (m, 4H), 2.33 (m, 4H), 2.44 (m, 4H), 2.61 (m, 4H), 2.77 (m, 4H), 3.11 (m, 2H), 3.34 (m, 16H), 3.67 (m, 6H), 3.81 (s, 6H), 3.83 (s, 6H), 3.86 (m, 2H), 3.95 (s, 3H), 3.96 (s, 3H), 4.02 (m, 2H), 4.23 (m, 2H), 4.53 (td, J = 2.0, 11.2 Hz, 2H), 4.78 (m, 2H), 6.57 (bs, 1H), 6.85 (bs, 1H), 6.94 (bs, 1H), 7.13 (bs, 1H), 6.96 (s, 2H), 7.09 (s, 1H), 7.10 (s, 1H), 7.64 (dd, J = 5.2, 8.0 Hz, 2H), 8.05 (s, 1H), 8.06 (s, 1H), 8.62 (m, 4H), 11.38 (d, J = 2.0 Hz, 1H), 11.42 (d, J = 1.6 Hz, 1H). ^{13}C NMR (DMSO-id$_6$, 100 MHz): δ 19.3, 19.4, 40.1, 43.0, 43.3/43.4, 45.8/45.9, 46.1/46.2, 47.4, 55.5/55.6, 55.9, 56.7, 57.0/57.1, 57.8, 60.4, 60.9, 61.0, 106.0/106.1, 106.4/106.5, 110.8/111.0, 123.0, 123.7/123.9, 125.4/125.5, 125.8/125.9, 130.5, 135.2/135.3, 138.9/139.0, 139.9/140.0, 142.3/142.5, 145.3/145.4, 149.2/149.3, 149.5/149.6, 157.6/157.9, $^2J_{CF}$ = 32.3 Hz (CO of the $O_2CCF_3^-$ counterion), 160.3/160.4, 165.3/165.4.

(4,10-Dimethyl-1,4,7,10-tetraaza-bicyclo[5.5.2]tetradecane)-(carbonato)cobalt(III) Chloride [Co(L6)(CO$_3$)]Cl (12). To a suspension of 4,10-dimethyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (**L6**) (0.259 g, 1.144 mmol) in water (30 mL) was added sodium triscarbonatocobaltate trihydrate (1.178 g, 3.254 mmol), and the mixture was heated for 24 h at 60 °C in air resulting in a dark pink solution containing a brown precipitate. The solution was filtered, and the filtrate was diluted with water (150 mL) and loaded onto a Sephadex SP-C25 cation exchange column. A dark pink band was retained on the top of the column, which was washed with water and then eluted with aqueous sodium chloride solution, beginning with 0.05 M concentration. A major dark pink band moved down the column using 0.05 M eluent and was collected using 0.1 M eluent. The fraction was reduced to dryness on a rotary evaporator to give pink and white crystalline solid to which MeOH was added, giving a dark pink solution with white precipitate. The solution was filtered and reduced to dryness on a rotary evaporator. This extraction process was repeated several times until the product is almost free of the white sodium chloride salt. The final desalting process was carried out on a Sephadex LH-20 column using water as the eluent. A single pink/purple band was collected, and the solvent was removed under reduced pressure to give **12** as a dark red-purple crystalline solid (0.397 g, 91%). ^{1}H NMR (D_2O/TSP, 400 MHz): δ 2.38 (CH_3, s, 6H), 3.29 (dd, J = 12.8, 5.2 Hz, 4H), 3.39 (dd, J = 14.2, 6.8 Hz, 4H), 3.56 (m, 4H), 3.70 (m, 4H), 3.76 (s, 4H). ^{13}C NMR (D_2O/TSP, 100 MHz): δ 50.6, 64.1, 65.0, 70.7. HRMS (FAB+): found, m/z 345.13446 $[M - Cl]^+$; calcd for $C_{13}H_{26}CoN_4O_3$, 345.13369.

***cis*-Bis(triflato)(4,10-dimethyl-1,4,7,10-tetraaza-bicyclo-[5.5.2]tetradecane)cobalt(III) Triflate [Co(L6)(Otf) $_2$](Otf) (14).** To the dark pink solid of **12** (0.046 g, 0.121 mmol) at 4 °C was added neat trifluoromethanesulfonic acid (0.13 mL, 1.464 mmol) under nitrogen, forming a pink slurry which was heated in a water bath at 50 °C for 2 h. It was then allowed to cool to room temperature, and cold ether (25 mL) was added very slowly. Fuming was observed and a pink precipitate formed which was collected by filtration, washed with cold ether, and dried under vacuum to give **14** (0.058 g, 65%). ^{1}H NMR (D_2O/TSP, 400 MHz) [Co(L6)(D_2O) $_2$](Otf)$_3$: δ 2.84 (s, 6H), 2.96 (dd, ^2J = 13.5, 5.9 Hz, 4H), 3.57 (m, 4H), 3.71 (m, 8H), 3.92 (m, 4H). ^{13}C NMR (D_2O/TSP, 100 MHz): δ 53.8, 65.8, 67.0, 70.9, 122.3 (q, J_{CF} = 310.0 Hz). ^{19}F NMR (CD_3CN, 282 MHz): δ −80.27 (CF_3SO_3−Co), δ −80.43 ($CF_3SO_3^-$). HRMS (FAB+): found, m/z 583.05403 $[M - Otf]^+$; calcd for $C_{14}H_{26}CoF_6N_4O_6S_2$, 583.05300. Slow diffusion of ether into a methanol solution of **14** resulted in single crystals of the diaqua complex [Co(L6)(H_2O) $_2$](Otf)$_3$, **14a**.

8-Quinolinolato(4,10-dimethyl-1,4,7,10-tetraaza-bicyclo-[5.5.2]tetradecane)cobalt(III) Diperchlorate [Co(L6)(3a)](ClO$_4$)$_2$ (16). Complex **14** (0.040 g, 0.055 mmol) was dissolved in MeOH (2 mL) and treated with a solution of **2** (9 mg, 0.062 mmol) in methanol (3 mL) and pyridine (9 mg, 0.114 mmol). The mixture was heated and stirred under nitrogen at 50 °C. A green/yellow mixture was observed after 90 min and a dark green solution after 15 h. The volume of the reaction mixture was reduced to about 2 mL on a rotary evaporator and then loaded onto a Sephadex LH-20 gel filtration column, eluting with MeOH. A minor red/orange band (I), a major dark green band (II), and a minor gray band (III) were observed moving down the column. Bands I and II were not resolved and collected together. This mixture was reduced to dryness and then redissolved in water (100 mL). The solution was loaded onto a Sephadex SP-C25 cation exchange column. The column was washed with water (200 mL) and eluted using sodium perchlorate starting from 0.05 M strength. A major dark green/brown band was collected when 0.2 M strength

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eluent was applied. The volume was reduced till crystals were just observed in the flask. The flask was then kept at 4 °C over 3 days. Dark black/green crystals of **16** (0.022 g, 65%) were obtained, filtered off, washed by ice-cold ether, and stored in a desiccator. ^{1}H NMR (CD_3CN, 300 MHz): δ 1.67 (s, 6H, 7), 2 × Nme), 2.98 (dd, J = 12.3, 5.4 Hz, 2H), 3.18 (m, 2H), 3.22 (m, 2H), 3.46 (m, 2H), 3.56 (m, 2H), 3.59 (m, 2H), 3.67 (t, J = 8.9 Hz, 2H), 3.83 (t, J = 8.3 Hz, 2H), 4.09 (m, 2H), 4.19 (m, 4H), 7.26 (d, J = 8.1 Hz, 1H), 7.41 (d, J = 8.1 Hz, 1H), 7.61 (t, J = 8.1 Hz, 1H), 7.80 (dd, J = 5.4, 8.1 Hz, 1H), 8.58 (d, J = 8.1 Hz, 1H), 9.08 (d, J = 5.1 Hz, 1H). ^{13}C NMR (CD_3CN, 75 MHz): δ 51.4, 62.6, 63.5, 63.9, 68.5, 68.9, 69.3, 115.0, 118.1, 124.8, 131.6, 131.9, 142.0, 145.9, 151.8, 165.2. HRMS (FAB+): found, m/z 528.14306/530.13944 $[M - ClO_4]^+$; calcd for $C_{21}H_{32}{}^{35/37}ClCoN_5O_5$, 528.14239/530.14140. Single crystals were obtained by Et_2O diffusion into a MeOH solution of **16**.

1-(Chloromethyl)-3-(5,6,7-trimethoxyindol-2-ylcarbonyl)-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinolin-5-ato(4,10-dimethyl-1,4,7,10-tetraaza-bicyclo[5.5.2]tetradecane)cobalt(III) Triflate [Co(L6)(3)](Otf)$_2$ (8). A mixture of **3** (0.025 g, 0.054 mmol) and pyridine (0.014 g, 0.174 mmol) was stirred in MeOH (5 mL) under nitrogen for 15 min to give a yellow suspension, to which partially dissolved **14** (0.087 g, 0.118 mmol) in MeOH (5 mL) was added. The mixture was heated at 50 °C with stirring for 9 days to give a dark brown-green solution. The solution volume was reduced to ca. 2 mL, filtered, and loaded onto a Sephadex LH-20 gel filtration column using MeOH as the eluent. Five bands were observed, colored pale brown, pink, orange-pink, green (major), and yellow. The major fraction was the fourth, green band, which was collected, and the solvent was removed to give a crude product (0.051 mg) that was redissolved in MeOH and chromatographed a second time on a longer column to ensure only the green band was collected. Solvent was removed under reduced pressure to give **8** as a green solid (0.048 g, 84%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.67 (s, 3H), 1.76 (s, 3H), 3.01(m, 1H), 3.05 (m, 2H), 3.24 (m, 3H), 3.43 (m, 2H), 3.81 (s, 3H), 3.83 (s, 3H), 3.96 (m, 5H), 4.27 (bt, 1H), 4.50 (dd, J = 1.8, 11.1 Hz, 1H), 4.80 (t, J = 10.8 Hz, 1H), 6.97 (s, 1H), 7.10 (d, J = 2.1 Hz, 1H), 3.64 (m, 5H), 3.85−3.91 (m, 2H), 4.08 (m, 5H), 7.77 (dd, J = 4.8, 8.4 Hz, 1H), 8.23 (s, 1H), 8.71 (d, J = 8.4 Hz, 1H), 9.19 (d, J = 5.1 Hz, 1H), 11.39 (bs, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 40.5, 48.3, 50.7, 56.1, 56.5, 60.3, 61.4, 61.6, 61.7, 61.8, 62.8, 67.5, 67.7, 68.0, 68.1, 68.4, 98.5, 107.0, 107.5, 112.0, 123.6, 124.9, 125.9, 126.2, 131.1, 136.5, 139.5, 140.5, 142.5, 146.4, 149.8, 149.9, 161.0, 166.0. HRMS (FAB+): found, m/z 900.21882/902.21767 $[M - Otf]^+$; calcd for $C_{37}H_{47}{}^{35/37}ClCoF_3N_7O_8S$, 900.21794/902.21499. Anal. Calcd for $C_{38}H_{47}ClCoF_6N_7O_{11}S_2 \cdot 3H_2O$: C, 41.33; H, 4.84; N, 8.88. Found: C, 41.06; H, 4.66; N, 8.86.

Carbonato(4,11-dimethyl-1,4,8,11-tetraazabicyclo[6.6.2]-hexadecane)cobalt(III) Chloride [Co(L4)(CO$_3$)]Cl (18). A solution of 4,11-dimethyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (**L4**) (0.181 g, 0.623 mmol) in water (10 mL) was treated with sodium triscarbonatocobaltate trihydrate (0.410 g, 1.13 mmol), and the mixture was heated in air at 50 °C with stirring for 20 h. A purple solution with brown precipitate resulted and was filtered through a Celite pad. The purple filtrate was diluted to 200 mL with water and then loaded onto a Sephadex SP-C25 cation exchange column. A major purple band was observed adhering to the top of the column. The column was washed with two column volumes of water. The major purple band was eluted using a 0.1 M sodium chloride solution; the fraction was collected and reduced to dryness on a rotary evaporator to give a purple and white crystalline solid. The product was extracted with MeOH (predried over 3 Å molecular sieves). The white sodium chloride was filtered off, and the filtrate was concentrated to about 2 mL. The final desalting process was performed on a Sephadex LH-20 gel filtration column using water as the eluent. A single purple band was collected from the column, reduced to dryness, and dried under vacuum to give **18** as a purple crystalline solid (0.088 g, 35%). ^{1}H NMR (D_2O/TSP, 400 MHz): δ 1.61 (m, 2H), 1.78 (m, 2H), 2.42 (s, 6H), 2.62 (m, 2H), 2.73 (m, 2H), 2.91 (m, 16H). ^{13}C NMR (D_2O/TSP, 100 MHz): δ 25.5, 44.8, 53.5, 55.0, 55.1, 60.0, 60.2, 169.5. HRMS (FAB+): found, m/z 373.16459 $[M - Cl]^+$; calcd for $C_{15}H_{30}CoN_4O_3$, 373.16499

***cis*-Bis(triflato)(1,4,8,11-tetraazabicyclo[6.6.2]hexadecane)-cobalt(III) Triflate [Co(L4)(Otf)$_2$]Otf (20).** To solid **18** (0.088 g, 0.215 mmol) in a Schlenk flask placed in an ice bath under nitrogen was added neat trifluoromethanesulfonic acid (3 mL, 33.904 mmol) with stirring. The mixture was allowed to warm to room temperature and then heated in a water bath for 2 h at 50 °C to give a dark green solution. While the flask was still constantly flushed with nitrogen, cold ether (250 mL) was added slowly to produce a green precipitate, the color of which then gradually changed from green to pink-purple. The pink-purple precipitate was filtered, washed with small amounts of cold ether, and dried under reduced pressure to give **20** (0.106 g, 65%). ^{1}H NMR (D_2O/TSP, 400 MHz) $[Co(\mathbf{L4})(D_2O)_2](Otf)_3$: δ 1.96 (m, 2H), 2.09 (m, 2H), 2.17 (s, 2H), 2.40 (dd, J = 14.0, 3.6 Hz, 2H). 2.50 (m, 2H), 2.58 (s, 6H). 2.66 (m, 4H), 2.88 (dd, J = 13.6, 3.2 Hz, 2H), 3.06 (td, J = 13.6, 3.6 Hz, 2H), 3.54 (td, J = 14.0, 4.0 Hz, 2H), 3.66 (td, J = 14.0, 3.6 Hz, 2H), 3.86 (m, 2H). ^{13}C NMR (D_2O/TSP, 100 MHz): δ 23.1, 53.8, 58.2, 59.7, 60.9, 62.7, 71.2, 122.3 (q, $^1J_{CF}$ = 316.9 Hz). HRMS (FAB+): found, m/z 611.08585 $[M - Otf]^+$; calcd for $C_{16}H_{30}CoF_6N_4O_6S_2$, 611.08430.

8-Quinolinolato(4,11-dimethyl-1,4,8,11-tetraazabicyclo-[6.6.2]hexadecane)cobalt(III) Diperchlorate [Co(L4)(2)](ClO$_4$)$_2$ (22). To a purple solution of **20** (0.030 g, 0.039 mmol) in MeOH (1 mL) was added a colorless solution of **2** (0.005 g, 0.034 mmol) and pyridine (0.004 g, 0.051 mmol) in MeOH (2 mL). An immediate color change from purple to green was observed upon mixing of the two solutions, and a dark green solution was obtained after 1 h. The mixture was heated under nitrogen at 45 °C with stirring for 20 h. Methanol was removed on a rotary evaporator to give green-yellow oil, which was dissolved in water (50 mL) and filtered, and the filtrate was loaded onto a Sephadex SP-C25 cation exchange column. The column was washed with water and eluted with sodium perchlorate starting from 0.05 M concentration. A major green band was eluted when 0.2 M eluent was applied. The band was concentrated to one-third of its volume and allowed to stand, resulting in dark green microcrystals which were collected, washed with cold ether, and dried under vacuum to give **22** (0.017 g, 74%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20 (s, 3H), 1.52 (s, 3H), 1.59 (d, 1H, J = 12.4 Hz, 1H), 1.75 (d, J = 13.9 Hz, 1H), 1.90 (m, 2H), 1.98 (m, 1H), 2.03 (m, 1H), 2.19 (m, 2H), 2.44 (m, 4H), 2.77 (m, 2H), 2.89 (m, 1H), 2.93 (m, 1H), 3.13 (d, J = 11.2 Hz, 1H), 3.46 (m, 4H), 3.72, (t, J = 10.6 Hz, 2H), 3.95, (m, 2H), 7.26, (dd, J = 8.1, 0.8 Hz, 1H), 7.40, (dd, J = 7.7, 0.8 Hz, 2H), 7.56 t, J = 7.8 Hz, 1H), 7.83 dd, J = 5.2, 8.2 Hz, 1H), 8.68 (d, J = 8.2 Hz, 1H), 9.10 (d, J = 5.2 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 20.1, 20.5, 48.5, 50.8, 55.0, 55.4, 55.6, 56.6, 56.5, 56.9, 66.1, 66.7, 57.2, 61.0, 114.0, 116.6, 123.9, 129.5, 130.4, 140.8, 145.6, 153.9, 163.7. HRMS (FAB+): found, m/z 556.17482/558.17234 $[M - ClO_4]^+$; calcd for $C_{23}H_{36}{}^{35/37}ClCoN_5O_5$, 556.17370/558.17074.

1-(Chloromethyl)-3-(5,6,7-trimethoxyindol-2-ylcarbonyl)-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinolin-5-ato(4,11-dimethyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane)cobalt(III) Triflate [Co(L4)(3)](Otf)$_2$ (10). A purple solution of **20** (0.041 g, 0.054 mmol) in MeOH (4 mL) was added to a yellow suspension of **3** (0.024 g, 0.052 mmol) in MeOH (5 mL) and pyridine (0.008 g, 0.105 mmol) under nitrogen. The mixture was heated at 45 °C with stirring for 10 days. A second portion of **20** (0.020 g, 0.026 mmol) was added, and the reaction continued for a further 3 h until no white suspension was observed and a green-yellow solution was obtained. The solution volume was reduced to 3 mL, filtered, and then loaded onto a Sephadex LH-20 gel filtration column which was eluted with MeOH. A minor orange band, a major green band, and a minor yellow band were observed of which the major green band was collected and reduced to dryness under reduced pressure to give **10** as a dark green solid (0.025 g, 49%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.27 (s, 3H), 1.33 (s, 3H), 1.57 (s, 3H), 1.64 (s, 3H), 1.80 (m, 3H), 1.93 (m, 6H), 2.04 (m, 4H), 2.22 (m, 5H), 2.45 (m, 6H), 2.73 (m, 6H), 2.92 (m, 5H), 3.13 (m, 3H), 3.52 (m, 5H), 3.60 (m, 2H), 3.71 (m, 3H), 3.80 (s, 6H), 3.82 (s, 6H), 3.90 (m, 2H), 3.95 (s, 6H), 4.02 (m, 2H), 4.27 (m, 2H), 4.50 (d, J = 10.8 Hz, 2H), 4.81 (t, J = 9.2 Hz, 2H), 6.96 (s, 2H),

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7.10 (s, 2H), 7.77 (dd, J = 5.2, 8.0 Hz, 2H), 8.28,8.29 (s, 2H), 8.71 (d, J = 8.4 Hz, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 20.1, 20.5, 40.1, 47.7/47.8, 48.6/48.7, 50.7, 55.4/55.6, 55.9, 56.4, 56.5, 56.6, 56.7, 57.0, 57.2, 60.8, 61.0, 61.1, 66.1, 66.7, 97.9, 106.5, 107.2, 112.1/112.2, 123.0, 124.3, 125.2, 125.3, 130.4, 136.2/136.3, 138.9, 139.9, 143.0/143.1, 145.7, 149.2, 151.6/151.7, 160.5, 164.9. HRMS (FAB+): found, m/z 779.29832/781.29511 [M − 2Otf + e]$^+$; calcd for $C_{38}H_{51}{}^{35/37}ClCoN_7O_5$, 779.29722/781.29427.

Electrochemistry. Cyclic voltammograms were recorded using a BAS 100W electrochemical analyzer. BAS electrodes: static mercury drop electrode (SMDE) working electrode, platinum wire counter electrode, and Ag/AgCl (3 M NaCl) reference electrode. Potentials reported vs NHE (Ag/AgCl (3 M NaCl) 0.209 V vs NHE). Supporting electrolyte: 0.1 M sodium nitrate solution.

X-ray Crystallography. Crystallographic data were collected on a Siemens SMART CCD diffractometer with a Mo Kα radiation source. Structures were solved and refined using the SHELX97 software package. Details are given in the Supporting Information.

Solubility and Stability. These were determined using the previously reported protocols.[9]

Radiolytic Reduction. Compound **9** was dissolved at 30 μM in 0.1 M sodium formate containing 5 mM sodium phosphate, pH 7.0. Samples were deoxygenated by evacuation and irradiated with a cobalt-60 source at a dose rate of 20 Gy min^{-1}. Immediately after irradiation, an equal volume of isopropanol containing 1% formic acid was added to assist solubilization of **3**. Solutions were frozen until analysis by HPLC, diluted with 0.2 M ammonium formate pH 4.5, and analyzed using an Agilent 1100 with a 150 × 3.2 mm Altima C8 column (Alltech, Chicago IL) and a gradient of acetonitrile in 0.2 M ammonium formate pH 4.5. Absorbance was monitored with a photodiode array detector at 338 nm for **9** (retention time 15.9 min) and 314 nm for **3** (retention time 19.8 min). Concentrations were determined with reference to calibration curves of both compounds, which were stable for at least 14 h in the initial mobile phase.

Antiproliferative Assay (IC_{50} Values). Sources of the human tumor cell lines and culture conditions have been reported elsewhere.[33] The origin of the A459/POR line has been reported previously.[3] Antiproliferative potency of compounds was determined as detailed previously.[29] Briefly, log phase cultures in 96-well plates were exposed to the compounds for 4 h under either aerobic (20% O_2) or anoxic conditions, the latter using a Pd/H_2 catalyzed anaerobic chamber with plasticware equilibrated in the chamber for at least 3 days to remove residual oxygen. Cell density was determined by sulforhodamine B staining after growing the cultures for a further 5−6 days under standard oxic conditions. Concentrations for 50% reduction in cellularity (IC_{50}) were interpolated by 4-parameter logistic regression.

In Vivo Studies. Animal studies were approved by the University of Auckland Animal Ethics Committee. **9** was formulated in 5% DMSO/95% saline and administered i.p. to C3H/HeN or CD-1 nude mice. HT-29 tumor xenografts were grown in CD-1 nude mice by subcutaneous inoculation of 10^7 cells, and mice were randomized to treatment groups when tumors reached a mean diameter of 9−11 mm. Tumors were collected 18 h after treatment and dissociated to determine clonogenic survivors per gram of tumor as previously described.[34]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Full assignments of the ^{1}H and ^{13}C NMR spectra of the Co complexes; details of the crystal data on complexes **14a**, **16**, and **21**; cyclic voltammetry data. This material is available free of charge via the Internet at http://pubs.acs.org. CCDC 905150, 905152, and 905154 contain supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Notes

The authors declare no competing financial interest.

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