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Hypoxia-Selective Antitumor Agents. 7. Metal Complexes of Aliphatic Mustards as a New Class of Hypoxia-Selective Cytotoxins. Synthesis and Evaluation of Cobalt(III) Complexes of Bidentate Mustards

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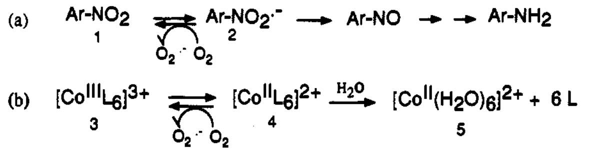
Nitrogen mustards coordinated to Co(III) are potential hypoxia-selective cytotoxins, since one-electron reduction to the Co(II) complexes greatly labilizes the Co–N bonds, causing the release of activated aliphatic mustards which can act as diffusible cytotoxins. Two series of Co(III) complexes of the bidentate bisalkylating nitrogen mustard ligands *N*,*N'*-bis(2-chloroethyl)-ethylenediamine (BCE) and *N*,*N*-bis(2-chloroethyl)ethylenediamine (DCE) have been synthesized and evaluated for their hypoxia-selective cytotoxicity against AA8 cells *in vitro*. The complexes also bear two 3-alkylpentane-2,4-dionato (acac) auxiliary ligands; cyclic voltammetry studies show that variation of the alkyl group in the auxiliary ligands alters the reduction potentials of the complexes (within a series) over a range of about 150 mV. In both series, the patterns of cytotoxicities of the cobalt complexes were broadly similar to those of the respective free ligands, suggesting that the cytotoxicity of these compounds is due to release of the free ligands. The nonsymmetrical ligand DCE and its cobalt complexes were 1 order of magnitude more cytotoxic than the corresponding BCE compounds. Although the unsubstituted acac/DCE complex showed no hypoxic selectivity against repair-deficient UV4 cells in a stirred suspension culture assay, the methyl and ethyl analogues showed substantial selectivity. The results may indicate a narrow range of acceptable reduction potential, with an optimum close to that for the methyl analogue ($E_{1/2} = -305$ mV). The methyl analogue also shows hypoxic selectivity against repair-proficient cell lines (e.g., AA8 and EMT6) and has high activity against EMT6 cells in intact spheroids, suggesting that the released DCE is capable of back-diffusion from the hypoxic core of the spheroid. This work shows that metal complexes of nitrogen mustards have significant hypoxia-selective cytotoxicity toward mammalian cells in cell culture and are a new general class of hypoxia-selective cytotoxins.

Hypoxic cells in solid tumors are resistant to ionizing radiation, and in some cases limit the ability of radiotherapy to control tumors.[1,2] The noncycling status of many hypoxic cells[3] and the difficulty of achieving adequate drug concentrations in regions distant from functional blood vessels[4] suggests that hypoxic cells may also often be resistant to conventional chemotherapeutic agents. Drugs which are selectively cytotoxic under hypoxic conditions are thus of potential value in cancer chemotherapy and radiotherapy. A particular attraction of this approach is that, because normal tissues are well oxygenated, hypoxia-selective cytotoxins (HSC) are expected to have selectivity for tumors. Although hypoxic cells are only a small subpopulation in most tumors,[5] it has been argued that repeated administration of HSC might turn hypoxia to advantage by killing tumor cells as they cycle through a hypoxic state.[6] In addition, if bioactivation of drugs in hypoxic regions generates relatively stable cytotoxins capable of limited diffusion, then tumor hypoxia could be exploited to kill surrounding tumor cells at higher oxygen concentrations.[7,8]

Three classes of drugs (nitro aromatics (1), quinones, and *N*-oxides) are known to be activated by reduction via metabolic pathways which are inhibited by oxygen.[7] In each case, selectivity for hypoxic cells is achieved by back-oxidation of the initial one-electron adduct (e.g., nitro aromatic (2) or semiquinone radical anion) by oxygen, thus suppressing metabolic activation in oxygenated cells (e.g., Scheme Ia). However, in many cases activation can also be effected by two-electron reduction (e.g., to the hydroquinone or nitroso compound) thus bypassing the oxygen-reversible intermediate. NAD(P)H:quinone oxidoreductase (DT diaphorase) is a well-known example of a two-electron reductase which can activate quinones[9] and, with lower efficiency, nitro compounds[10,11] under aerobic conditions. Other reductases such as xanthine dehydrogenase also appear to have some ability to reduce quinones[12] and nitroheterocycles[13] under aerobic conditions by two-electron reduction.

Scheme I



An attractive alternative chemistry for hypoxia-selective metabolism is the use of complexes of transition metals for which only a one-electron reduction is possible.[14] Cobalt complexes containing nitrogen mustard ligands are of particular interest. The cytotoxicity of nitrogen mustards depends on the electron density on the mustard nitrogen,[15] which controls its alkylating reactivity. Coordination of the nitrogen lone pair to Co(III) should suppress its toxicity since the electron pair is no longer available to act as a nucleophile. The d^6 low-spin electronic configuration of octahedral Co(III) complexes (3) renders them kinetically inert (for example, the half-life for the aquation of $[Co^{III}(NH_3)_6]^{3+}$ is 6×10^9 s),[16] and the nitrogen

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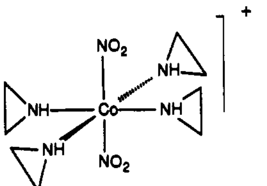
mustard ligand would be displaced only very slowly. Since the Co(III)–Co(II) reduction potential can fall in the range of cellular reductants (−200 to −400 mV *vs* NHE), chemical or metabolic one-electron reduction of the inert Co(III) complexes would be expected. The resulting labile Co(II) species (4) would undergo very facile ligand substitution by water,[17] releasing the cytotoxic free nitrogen mustard and $[Co^{II}(H_2O)_6]^{2+}$ (5) (Scheme Ib). To ensure hypoxic selectivity, the reduced Co(II) complex containing the nitrogen mustard ligand must be sufficiently stable to allow reoxidation[18,19] in oxygenated cells to compete effectively with ligand loss. The overall process is essentially irreversible, since $[Co^{II}(H_2O)_6]^{2+}$ is highly stable with respect to reoxidation ($E°$ = +1800 mV). This chemistry is analogous to that describing the oxygen-sensitive reduction of nitro aromatic compounds (Scheme Ia) in that in oxygenated cells reoxidation of the $[Co^{II}L_6]^{2+}$ intermediate provides a "futile" biochemical cycle capable of suppressing net reduction.

A number of Co(III) complexes of the monodentate monoalkylator aziridine are known,[20-22] and we have carried out a preliminary evaluation of some of these (e.g., 6).[21] A Co(III) complex (7) of the monodentate bisalkylator bis(2-chloroethyl)amine has recently been prepared and shown to be a radiosensitiser of hypoxic cells.[23] However, these complexes with monodentate alkylating ligands appear to lack selectivity for hypoxic cells as cytotoxins, probably because they provide Co(II) complexes of insufficient stability for reoxidation by free oxygen to compete with ligand release. Since the kinetic stability of Co(II) complexes is greatly increased if chelating ligands are used, we report in this paper the synthesis and characterization of a number of Co(III) complexes of bidentate bisalkylating nitrogen mustards and the evaluation of these complexes as HSCs. A preliminary account of some of this work has been published.[24]



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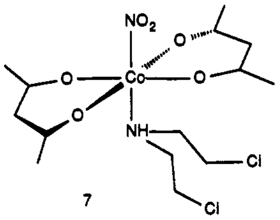

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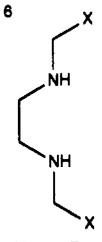
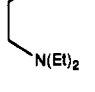
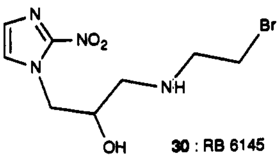


8 : X = CH3 (BEE)
10 : X = CH2Cl (BCE)



9 (DEE)



30 : RB 6145

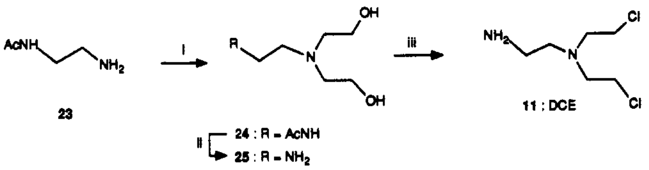
Chemistry

N,N-Bis(2-chloroethyl)ethylenediamine (DCE; 11) was prepared[25] by treatment of *N*-acetylethylenediamine (23) with excess oxirane to give the diol 24.[26] This was deacetylated with concentrated HCl and converted to the mustard with $SOCl_2$ (Scheme II). *N,N'*-Bis(2-chloroethyl)ethylenediamine dihydrochloride (BCE; 10) was prepared similarly[27] by treatment of commercially-available *N,N'*-bis(2-hydroxyethyl)ethylenediamine with $SOCl_2$.

Most Co(III) complexes undergo very slow substitution at the inert metal center, rendering the synthesis of complexes containing very reactive ligands difficult. The preparation of the Co(III) complexes of the bidentate mustards required a cobalt–ligand system which undergoes relatively rapid substitution at Co(III), since the nitrogen mustards are unstable when in the required free base form. Complexes containing the isosteric but nonalkylating diamines *N,N'*-bis(2-ethyl)ethylenediamine (BEE; 8) and *N,N*-bis(2-ethyl)ethylenediamine (DEE; 9) were also prepared, both as noncytotoxic model compounds and for use as analogues of the more reactive mustards in the development of suitable synthetic routes. Suitable cobalt(III) precursor complexes are the series *trans*-Na[Co(Racac)$_2$(NO$_2$)$_2$] (26–29) (R = H,[28] Me, Et, *n*-Pr; Racac = 3-alkylpentane-2,4-dionato anion), each prepared by treatment of $Na_3[Co(NO_2)_6]$ with Na[Racac]. The reaction of the free bases of the chelating ligands with the Na[Co(Racac)$_2$(NO$_2$)$_2$] complexes in the presence of activated charcoal (Scheme III) gave good yields of the complexes of the diamines with the symmetrical mustard BCE, but the unsymmetrical mustard DCE was less stable in the free base form and gave considerably lower yields of the complexes (Table I). The Clacac complex (18) was prepared from the corresponding acac complex (14) by direct chlorination with *N*-chlorosuccinimide.[29] In most cases the complexes could be purified by direct crystallization of the perchlorate salts, though cation-exchange chromatography was necessary for the DCE complexes, 21 and 22, and for the Clacac complex, 18. When necessary, the more soluble chloride salts could be generated by anion-exchange chromatography. In some instances, final purification was effected by reverse-phase HPLC.

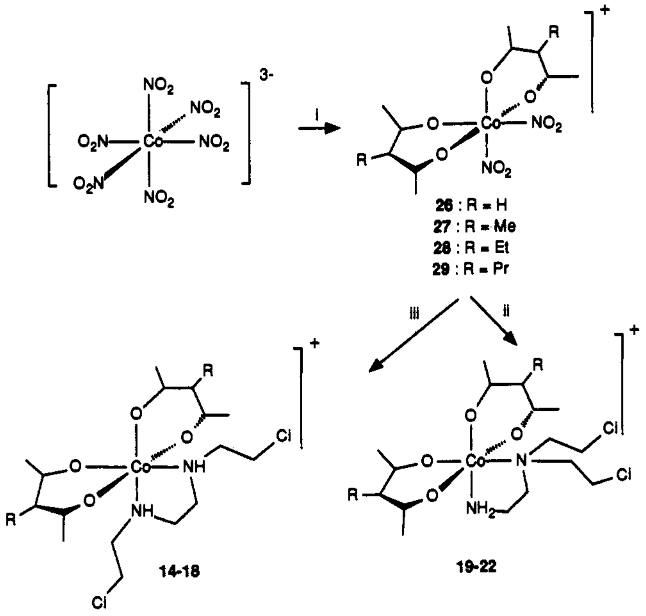
The structures of the Co(III) complexes were established by combustion elemental analysis and UV/vis, IR, and

Scheme II[a]



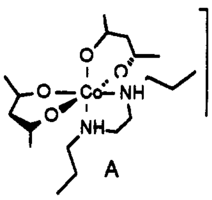
[a] (i) oxirane/H_2O/5 °C/24 h; (ii) concentrated HCl/90 °C/20 h; (iii) $SOCl_2$/20 °C/24 h.

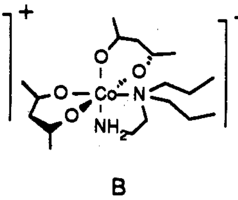
Scheme III[a]



[a] (i) Na(Racac)/H_2O/5 °C/12 h; (ii) SCE·2HCl/NaOH/H_2O/charcoal/20 °C/20 min; (iii) BCE·2HCl/NaOH/H_2O/charcoal/20 °C/20 min.

Table I. Physicochemical and Biological Data of Alkylating Ligands and Their Corresponding Co(III) Complexes


A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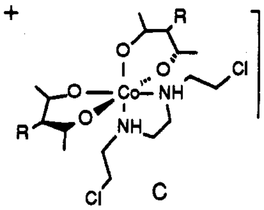
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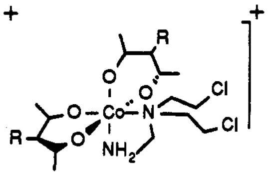
D

no.	struct	X	formula	analyses	$E_{1/2}$[a]	IC_{50} (air)[b] AA8	HF(air)[c] AA8/UV4	CT_{10}[d] (UV4)	air/N_2 ratio[e] (UV4)
8						2400 ± 600	0.7 ± 0.2		
9						19000 ± 3000	0.99 ± 0.07		
10						30 ± 4	29 ± 2		
11						1.5 ± 0.3	53 ± 7	0.18	2.0
12	A		$C_{16}H_{30}N_2O_4Co{\cdot}ClO_4$	C, H, N, Cl	−510	>5000[f,g]	ND[f]		
13	B		$C_{16}H_{30}N_2O_4Co{\cdot}ClO_4$	C, H, N	−410	34 ± 0.8	0.9 ± 0.2		
14	C	H	$C_{16}H_{28}Cl_2N_2O_4Co{\cdot}ClO_4$	C, H, N, Cl	−310	890 ± 160	14 ± 4	21500	1.6
15	C	Me	$C_{16}H_{32}Cl_2N_2O_4Co{\cdot}ClO_4$	C, H, N	−42	650 ± 170	2.7 ± 0.4	>2720	>1
16	C	Et	$C_{20}H_{36}Cl_2N_2O_4Co{\cdot}ClO_4$	C, H, N, Cl	−460	139 ± 18	4.8 ± 1.4		
17	C	Pr	$C_{22}H_{40}Cl_2N_2O_4Co{\cdot}ClO_4$	C, H, N	−500	18.5 ± 3.3	3.5 ± 0.7		
18	C	Cl	$C_{16}H_{26}Cl_4N_2O_4Co{\cdot}ClO_4$	C, H, N	−135	26 ± 2.0	13 ± 4		
19	D	H	$C_{16}H_{28}Cl_2N_2O_4Co{\cdot}ClO_4$	C, H, N	−235	3.1 ± 0.8	64 ± 15	0.64 ± 0.17	1.9 ± 0.2
20	D	Me	$C_{16}H_{32}Cl_2N_2O_4Co{\cdot}ClO_4$	C, H, N, Cl	−305	4.6 ± 0.6	48 ± 8	2.4 ± 0.6	20 ± 4
21	D	Et	$C_{20}H_{40}Cl_2N_2O_4Co{\cdot}Cl{\cdot}2H_2O$	C, H, N, Cl	−350	2.3 ± 0.4	20 ± 3	0.51 ± 0.15	4.9 ± 1.9
22	D	Pr	$C_{22}H_{40}Cl_2N_2O_4Co{\cdot}ClO_4$	C, H, N	−385	1.35 ± 0.06	31 ± 9		
30			RB 6145[g]			145	5.9	6600	50[h]

[a] $E_{1/2}$ = peak potential *vs* NHE for the reduction, from square-wave voltammetry. [b] IC_{50} = concentration of drug (μM) to inhibit the growth of AA8 cells in culture to 50% of controls, using the protocol detailed in refs 35 and 41; values are means of at least 3 determinations ± SEM. [c] HF = hypersensitivity factor = the ratio IC_{50}(AA8)/IC_{50}(UV4) under aerobic conditions. [d] Concentration (μM) × time (h) for 90% kill in aerobic UV4 stirred cell suspensions. [e] Aerobic CT_{10}/hypoxic CT_{10}. [f] Nontoxic at the solubility limit. [g] RB 6145 employed as positive control. [h] For a 1-h exposure.

NMR spectroscopy. ^1H and ^{13}C NMR was an important tool for distinguishing the three diastereomers formed when the symmetrical BCE is coordinated to Co(III). Upon coordination each nitrogen atom becomes chiral, producing *RR*, *SS*, and *meso-RS* isomers. In general the more stable form (*RS*) was enriched by fractional crystallization, and NMR analysis was used to confirm the isomeric purity. An X-ray structure determination on [Co(Clacac)$_2$(BCE)]ClO$_4$ (**18**) confirmed the expected structure.[24] The stereochemistry at each chiral nitrogen atom confirmed the stereochemical assignments made by NMR. Assignments of ^1H and ^{13}C NMR spectra were established from chemical shift considerations together with 2-dimensional (^1H–^1H and ^1H–^{13}C) NMR experiments on selected compounds.

Results and Discussion

Physicochemical Properties. The bidentate Co(III) complexes prepared in this work are listed in Table I. The electrochemical properties of these complexes were investigated by cyclic and square-wave voltammetry. As observed previously[21] for Co(III) aziridine complexes, reduction to Co(II) in aqueous solution is generally an irreversible process that is ascribed to rapid ligand loss from the labile Co(II) center.[30] As a result, the Co(III)/Co(II) reduction as measured by cyclic voltammetry is usually electrochemically irreversible. However, it was found that electrochemical measurements of the reduction processes of the bidentate mustard complexes in CH_2Cl_2 (0.15 M tetrabutylammonium perchlorate) at a platinum electrode showed quasi-reversible behavior. This is consistent with slower ligand substitution at Co(II), relating to both the presence of bidentate ligands on cobalt and the use of the nonnucleophilic solvent, CH_2Cl_2. Similar behavior has been reported for other tris(β-diketonato)cobalt(III)/(II) couples.[31] Reduction potentials for the nitrogen mustard complexes were determined by square-wave voltammetry, using the peak potential of the reduction wave referenced to the internal ferrocinium/ferrocene couple.

The Co(III)/Co(II) reduction potentials of the complexes (**12**, **13**) with the nonalkylating ligands showed the significant effect of varying the structure of the amine ligand, with the complex (**12**) of the symmetrical diamine BEE having a much lower potential (−510 mV versus the normal hydrogen electrode; NHE) than that of the complex (**13**) of the asymmetrical diamine DEE (−410 mV). This structural difference was reflected in the reduction potentials of the analogous alkylating complexes, with [Co(acac)$_2$(BCE)]$^+$ (**14**) having a potential (−310 mV) approximately 125 mV lower than that of [Co(acac)$_2$(DCE)]$^+$ (**19**). A useful way to fine tune the reduction potentials within each series was by variation of the substituent R in the 3-position of the auxiliary Racac ligands. More electron-donating groups (R = Cl < H < Me < Et < Pr) increase the effective charge on the oxygen atoms of the Racac ligand coordinated to Co(III), which in turn stabilizes the higher oxidation state with respect to reduction to Co(II). This can be seen in the trend in $E_{1/2}$ values in the series (**14**–**18**) (Table I). Although the lipophilicities of the complexes were not measured, variation of the 3-substituent also allows significant variation of this parameter.

We have previously shown that coordination of reactive nitrogen species such as aziridine to Co(III) stabilizes the ligand, even in the presence of strong acid. The bidentate Co(III) complexes showed similar stability in neutral solution, showing no detectable change (as monitored by ^1H NMR) over periods of weeks at room temperature.

Biological Activity. The cytotoxicity of the ligands and their corresponding complexes were evaluated in a growth-inhibition assay against both the CHO-derived cell line AA8[32] and the subline UV4 (Table I). The latter is a DNA repair mutant with a marked hypersensitivity to alkylating agents which cause cell killing by bulky DNA monoadducts or interstrand cross-links,[33] and the ratio of IC_{50} values in these two lines (the hypersensitivity factor HF = IC_{50}(AA8)/IC_{50}(UV4)) thus provides valuable information about the mechanism of cytotoxicity.[34,35] The

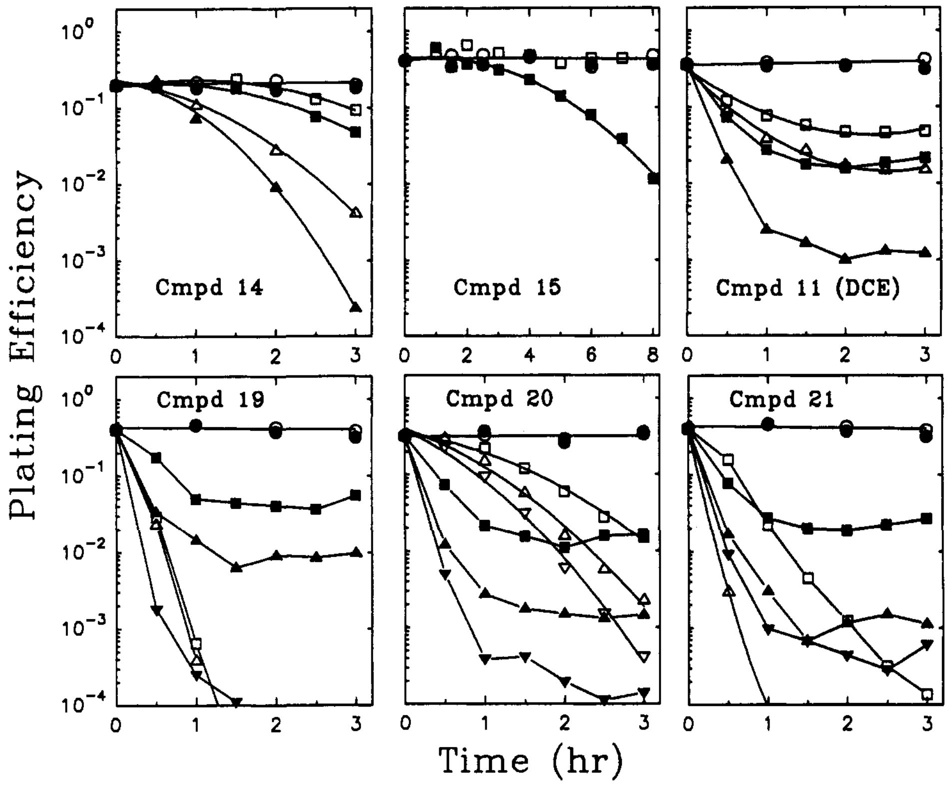

Figure 1. Cytotoxicity of selected compounds against UV4 cells under aerobic (open symbols) and hypoxic (filled symbols) conditions in stirred, continuously-gassed cell suspensions. Symbols: (○, ●) controls; **14** (□, ■) 2000 μM; (△, ▲) 10 000 μM; **15** (□, ■) 340 μM; **11** (□, ■) 0.1 μM, (△, ▲) 0.15 μM; **19** (□) 1.25 μM, (△) 1.8 μM, (■) 0.3 μM, (▲) 0.5 μM, (▼) 0.8 μM; **20** (□) 1 μM, (△) 1.5 μM, (▽) 2 μM, (■) 0.15 μM, (▲) 0.2 μM, (▼) 0.25 μM; **21** (□) 1 μM, (△) 1.8 μM, (■) 0.2 μM, (▲) 0.3 μM, (▼) 0.4 μM.

two nonalkylating diamines (**8**, **9**) were relatively nontoxic as expected and had HF values of essentially unity, indicating that their cytotoxic effects are probably not due to DNA adduct formation. The cobalt complexes of these nonalkylating ligands showed very different aerobic cytotoxicities in AA8 cell cultures. The BEE complex (**12**) was relatively nontoxic as expected, but the high toxicity of **13** was a surprise. The latter compound also showed an HF of about unity, indicating no direct DNA-alkylating activity.

The two alkylating ligands BCE (**10**) and DCE (**11**) were orders of magnitude more cytotoxic than the corresponding nonalkylating amines and had high HF values indicative of DNA cross-linking activity, as expected. The nonsymmetrical DCE (**11**) was 15-fold more potent in the IC$_{50}$ assay; the most likely reason for this is higher reactivity rather than geometrical factors. In tissue culture medium at 37 °C, **11** had a half-life of ca. 15 min (measured by bioassay),[36] whereas **10** was much more stable (little loss after 2 h). The biological activities of the two series of cobalt complexes of the alkylating ligands (BCE and DCE) provide considerable insight into the mechanism of action and required properties of this type of compound. In both series, the HF values of the cobalt complexes were broadly similar to those of the respective free ligands, those of the DCE complexes (**19**–**22**) being much larger (20–64) than those of the BCE complexes (**14**–**18**) (3–14). This suggests that the cytotoxicity of both series of compounds is due to release of the free ligands.

The cobalt–BCE complex (**14**) was much less toxic than the parent ligand toward aerobic AA8 cells (IC$_{50}$ = 890 versus 30 μM), suggesting both that complexation does inactivate the mustard and that **14** is reasonably stable under aerobic conditions in cell culture despite quite a high reduction potential. Other members of the series have higher aerobic cytotoxicities, which are not likely to be due to lower stability as the alkyl derivatives (**15**–**17**) have lower reduction potentials and may be related instead to higher lipophilicity. The Clacac complex (**18**) was as cytotoxic as the free ligand. Since it is likely that the mustard will be equally deactivated in this complex, the higher aerobic toxicity may be due to its more ready reduction, as signaled by its high reduction potential (−135 mV). The DCE complexes (**19**–**22**) proved much more cytotoxic in aerobic AA8 cultures, with IC$_{50}$ values little different from that of the free ligand. This is likely due to the more positive reduction potentials of this series ($E_{1/2}$ for the DCE complex (**19**) is −235 mV, compared with −310 mV for the BCE complex (**14**)).

The hypoxic selectivities of selected compounds (those with reduction potentials in the appropriate range, approximately −300 to −450 mV) were determined in UV4 cell cultures, using the stirred suspension culture assay (Table I and Figure 1). The concentration × time to reduce cell survival to 10% (CT$_{10}$) was used as a measure of cytotoxic potency under aerobic and hypoxic conditions (Table I).

The parent BCE complex (**14**) showed very low potency in this assay (CT$_{10}$ = 21 500 μM-h), as it did in the IC$_{50}$ assay. However, it showed some preferential toxicity in hypoxic cultures, with the ratio of CT$_{10}$ values (air/N$_2$) being 1.6 (Figure 1). The methyl analogue (**15**) was selectively toxic to hypoxic cells but was not sufficiently soluble to determine aerobic cytotoxicity so the extent of its selectivity could not be determined. In contrast, the DCE complexes (**19**–**22**) were much more potent (CT$_{10}$ values ca. 1 μM-h), although in each case potency was less than for the free DCE ligand. DCE itself showed weak hypoxic selectivity, as has been reported for other nitrogen mustards.[36,37] The parent DCE complex (**19**) showed hypoxic selectivity no greater than that of the free ligand, but the methyl analogue (**20**) showed substantial selectivity

((20 ± 4)-fold over three independent experiments),[38] and the ethyl analogue (**21**) was also significantly selective, although with a lower ratio (Table I and Figure 1).

The results may indicate a narrow range of acceptable reduction potential, with an optimum close to that for the methyl analogue (**20**) ($E_{1/2}$ = −305 mV), although the relationship between the (nonthermodynamic) $E_{1/2}$ values and rates of reduction (or its reversibility by oxygen) has not yet been established for compounds of this type. The decrease in rates of cell killing with time seen in Figure 1 suggests that all three DCE complexes investigated are metabolically labile under hypoxic (but not oxic) conditions, with no obvious dependence of this on $E_{1/2}$. No such lability is evident for the BCE complexes (**14**, **15**), although the stability of the released BCE ligand would preclude detection of metabolic reduction of these complexes by this method.

On the basis of this limited range of examples, the DCE complexes appear to have hypoxic selectivity superior to that of the BCE compounds. The reasons for this are not clear but may relate to the much higher cytotoxicity of the DCE ligand, which may ensure that ligand release rather than redox cycling is the dominant mode of cytotoxicity. Studies in progress indicate that the hypoxic selectivity of **20** is not restricted to the repair-deficient UV4 cell line, since it shows similar selectivity for AA8 and EMT6 cells under hypoxic conditions. It also has high activity against intact EMT6 spheroids, suggesting that the released DCE is capable of back-diffusion from the hypoxic core of the spheroid.[39] This compound is currently under investigation *in vivo* as the lead compound of this new class.

Conclusions

The cobalt complexes of nitrogen mustards offer an attractive alternative chemistry to nitro aromatic compounds as a design for HSCs, since obligate one-electron reduction to the Co(II) species results in the rapid release of very reactive aliphatic mustards. The critical point was whether the reduced $Co(II)L_6$ complex could be made sufficiently stable to allow reoxidation in aerobic cells to compete with ligand release. Previous work[22,23] suggested that monodentate alkylating nitrogen ligands could not provide sufficient stability. The results shown here provide valuable insight into this and other questions relating to the design of metal complexes as HSCs and suggest that chelating diamines can provide complexes of sufficient stability to allow reoxidation of the labile Co(II) species to compete with ligand release. The pattern of hypoxic selectivities shown by the DCE series of complexes (**19**–**22**) suggests the critical importance of the redox potential, which presumably controls the rate of bioreduction, and implies a quite narrow optimal range.

This work demonstrates that metal complexes of nitrogen mustards have significant hypoxia-selective cytotoxicity toward mammalian cells in cell culture. The compounds have the capability of releasing mustard ligands which are reactive enough to be highly cytotoxic and yet possess a long enough half-life to diffuse from the hypoxic cells where they are activated to surrounding cells at higher levels of oxygen tension.[7] This work is a first step toward defining the parameter limits (particularly metal redox potential and released mustard reactivity) for this new general class of hypoxia-selective cytotoxins.

Experimental Section

Reagents and Physical Measurements. *N,N'*-Bis(2-hydroxyethyl)ethylenediamine and *N*-acetylethylenediamine were obtained from Aldrich. Cation-exchange chromatography was performed on SP Sephadex C-25 (Pharmacia) in the Na^+ form. HPLC purifications were performed using a Waters 600 quaternary pump with WISP injector and Gilson 202 fraction collector controlled by an HP chemstation and using an HP 1040A diode-array spectrometer as detector. ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on a Bruker AM 400 spectrometer. Chemical shifts are reported relative to internal Me_4Si. High-resolution mass spectra (FAB^+) were recorded from a 3-nitrobenzyl alcohol matrix on a VG 70-SE mass spectrometer using argon gas. Electrochemical measurements were performed on a Bioanalytical Systems BAS 100A using the software packages provided for cyclic voltammetry and Osteryoung square-wave voltammetry. Tetra-*n*-butylammonium perchlorate electrolyte was twice recrystallized from EtOAc and dried in vacuo at 80 °C. Melting points are uncorrected. Elemental analyses were performed by the Microchemical Laboratory at the University of Otago, Dunedin, New Zealand.

***N,N'*-Bis(2-chloroethyl)ethylenediamine Dihydrochloride (BCE·2HCl, 10).** A solution of *N,N'*-bis(2-hydroxyethyl)ethylenediamine (5.40 g, 0.036 mol) in $SOCl_2$ (60 mL) was heated at 90 °C for 1 h and then left at room temperature for 24 h. Excess $SOCl_2$ was removed under reduced pressure, and the residue was triturated with 2-propanol. Recrystallization from boiling 2-propanol (800 mL) to which enough H_2O (*ca.* 5 mL) had been added to effect complete dissolution of the solid gave *N,N'*-bis(2-chloroethyl)ethylenediamine dihydrochloride (5.95 g, 63%) (BCE·2HCl; **10**) as plates. ^1H NMR (D_2O): δ 3.93 (m, 4 H, CH_2-Cl), 3.58 (s, 4 H, CH_2NHR), 3.58 (m, 4 H, CH_2CH_2Cl). ^{13}C NMR (D_2O): δ 51.96 (CH_2CH_2Cl), 45.56 (CH_2NHR), 41.74 (CH_2Cl).

***N,N*-Bis(2-chloroethyl)ethylenediamine Dihydrochloride (DCE·2HCl, 11).** Oxirane (27.0 g, 0.60 mol) was added to a cooled (5 °C) solution of *N*-acetylethylenediamine (**23**) (25.0 g, 0.24 mol) in water (50 mL). The solution was stirred for 4 h at 5 °C and then overnight at room temperature before being concentrated under reduced pressure. The residue was chromatographed on SiO_2, and elution with EtOAc/MeOH (9:1) gave *N*-acetyl-*N',N'*-bis(2-hydroxyethyl)ethylenediamine (**24**) as a viscous oil (35.7 g, 78%). This was used directly, the entire sample being dissolved in concentrated HCl (250 mL), warmed to 90 °C for 20 h, and then concentrated under reduced pressure to give the dihydrochloride salt of *N,N*-bis(2-hydroxyethyl)ethylenediamine (**25**) as a syrup which slowly crystallized, mp 116–120 °C (lit.[26] mp 116–118 °C). This was dissolved in MeOH, and the solution was neutralized with powdered $KHCO_3$, filtered, and evaporated. The residue was triturated with Me_2CO/MeOH (1:1), and the triturate was evaporated to give the corresponding free base as a straw-colored liquid, which was used without further characterization.[26] A solution of this (2.96 g, 0.02 mol) in $SOCl_2$ (150 mL) was stirred at room temperature for 48 h. Excess $SOCl_2$ was then removed under reduced pressure, and the residue was dissolved in water and washed several times with EtOAc. The aqueous layer was evaporated to dryness under reduced pressure, and the resulting crude residue was crystallized from MeOH to give *N,N*-bis(2-chloroethyl)ethylenediamine dihydrochloride (2.64 g, 49%) (DCE·2HCl; **11**) as hygroscopic white plates, mp 136 °C (lit.[25] mp 139–140 °C). ^1H NMR (D_2O): δ 4.03 (t, J = 5.7 Hz, 4 H, CH_2Cl), 3.81 (t, J = 5.7 Hz, 4 H, CH_2CH_2Cl), 3.74 (t, J = 7.7 Hz, 2 H, CH_2NR_2), 3.55 (t, J = 7.7 Hz, 2 H, CH_2NH_2). ^{13}C NMR (D_2O): δ 57.55 (CH_2CH_2Cl), 52.54 (CH_2NR_2), 39.82 (CH_2Cl).

$Na[Co(Meacac)_2(NO_2)_2]\cdot 0.5H_2O$ (27). $Na_3[Co(NO_2)_6]$ (3.27 g, 8.11 mmol) was dissolved in H_2O (11 mL) and added to a mixture of NaOH (0.70 g, 17.5 mmol) and 3-methyl-2,4-pentanedione (2.0 g, 17.5 mmol) in H_2O (11 mL) which had been cooled in an ice bath. Rapid formation of red-brown crystals occurred after 10 min, and after cooling at 5 °C for 12 h these were collected by filtration and washed with Me_2CO and Et_2O and dried in air to give **27** (2.81 g, 82.9%). This was recrystallized by dissolving 1 g in H_2O (35 mL) and filtering into $NaNO_2$ solution (5 g in 15 mL of H_2O). The resulting crystalline product was washed with EtOH/Me_2CO (2:1) and dried in air to give $Na[Co(Meacac)_2(NO_2)_2]\cdot 0.5H_2O$ (**27**) (0.40 g, 40%). Anal. ($C_{12}H_{18}N_2O_8NaCo\cdot 0.5H_2O$) C, H, N.

Sodium Bis(3-ethyl-2,4-pentanedionato)dinitrocobaltate(III) Hydrate $Na[Co(Etacac)_2(NO_2)_2]\cdot H_2O$ (28). Prepared as described for **27** using $Na_3[Co(NO_2)_6]$ (3.28 g, 8.12 mmol), 3-ethyl-2,4-pentanedione (2.37 g, 18.5 mmol), and NaOH (0.74 g, 18.5 mmol) to give Na[Co(Etacac)$_2$(NO$_2$)$_2$]·H$_2$O (**28**) (2.91 g, 80.3%).

Sodium Bis(3-*n*-propyl-2,4-pentanedionato)dinitrocobaltate(III) Hydrate Na[Co(Pracac)$_2$(NO$_2$)$_2$]·H$_2$O (29). Prepared as described for **27** by mixing Na[Co(NO$_2$)$_6$] (1.0 g, 2.48 mmol) in H$_2$O (2.5 mL) and Na[Pracac]·H$_2$O (0.94 g, 5.16 mmol) in H$_2$O (5 mL). After 5 min the solution was filtered to remove a brown insoluble impurity, MeOH (2 mL) was added, and the solution was left at room temperature for 48 h to give Na[Co(Pracac)$_2$(NO$_2$)$_2$]·H$_2$O (**29**) (0.23 g, 19.6%). Anal. ($C_{16}H_{26}N_2O_8$-NaCo·H$_2$O) C, H, N.

Bis(2,4-pentanedionato)(*N*,*N'*-bis(2-chloroethyl)ethylenediamine)cobalt(III) Perchlorate [Co(acac)$_2$(BCE)]ClO$_4$ (14). Na[Co(acac)$_2$(NO$_2$)$_2$]·H$_2$O (**26**)[28] (0.40 g, 1.025 mmol) was dissolved with stirring in a mixture of H$_2$O (6 mL) and MeOH (2 mL). An ice-cooled solution of BCE·2HCl (**10**) (0.279 g, 1.081 mmol) dissolved in H$_2$O (2 mL) was neutralized by the addition of 2.0 mL of a solution of NaOH (0.43 g) in MeOH (10 mL). Immediately, activated charcoal (0.25 g) was added to the stirred solution of cobalt complex (**26**), followed rapidly by the solution of deprotonated BCE. The mixture was stirred for 20 min and then filtered through Celite, and the charcoal was washed once with water and once with MeOH. The washings were added to the filtrate, followed by NaClO$_4$·H$_2$O (3.2 g) in H$_2$O (3 mL), and the mixture was cooled in an ice bath. After 2 h the purple crystalline mass was filtered and washed twice with cold H$_2$O and three times with Et$_2$O and dried in air to give [Co(acac)$_2$(BCE)]ClO$_4$ (**14**) (0.475 g, 81%). ^{1}H NMR ((CD$_3$)$_2$SO): δ 5.97 (br, 2 H, N*H*), 5.66 (s, 2 H, C*H*), 4.03, 3.93 (m, 2 H, C*H*$_2$Cl), 2.86, 2.75 (m, 2 H, C*H*$_2$NHR), 2.71, 2.58 (m, 2 H, C*H*$_2$CH$_2$Cl), 2.14, 2.08 (s, 3 H, C*H*$_3$CO). ^{13}C NMR ((CD$_3$)$_2$SO): δ 189.47, 189.31 (*C*O), 97.93 (*C*H), 50.72 (*C*H$_2$Cl), 49.70, (*C*H$_2$NHR), 39.60 (*C*H$_2$CH$_2$Cl), 26.38, 26.25 (*C*H$_3$CO). Anal. ($C_{16}H_{28}Cl_2N_2O_4$Co·ClO$_4$) C, H, N, Cl.

Bis(3-methyl-2,4-pentanedionato)(*N*,*N'*-bis(2-chloroethyl)ethylenediamine)cobalt(III) Perchlorate [Co(Meacac)$_2$(BCE)]ClO$_4$ (15). Reaction of Na[Co(Meacac)$_2$(NO$_2$)$_2$]·H$_2$O (**27**) (0.50 g, 1.196 mmol) with BCE·2HCl (**10**) (0.34 g, 1.318 mmol) as described for **14** gave [Co(Meacac)$_2$(BCE)]ClO$_4$ (**15**) (0.40 g, 58.7%). ^{13}C NMR (CDCl$_3$) (major isomer): δ 188.65, 188.20 (*C*O), 102.25 (*C*Me), 50.70 (*C*H$_2$Cl), 49.70 (*C*H$_2$NHR), 40.07 (*C*H$_2$CH$_2$Cl), 26.65, 26.33 (*C*H$_3$CO), and 14.95 (*C*H$_3$). Anal. ($C_{16}H_{32}Cl_2N_2O_4$Co·ClO$_4$) C, H, N.

Bis(3-ethyl-2,4-pentanedionato)(*N*,*N'*-bis(2-chloroethyl)ethylenediamine)cobalt(III) Perchlorate [Co(Etacac)$_2$(BCE)]ClO$_4$ (16). This was prepared as described for **14** using Na[Co(Etacac)$_2$(NO$_2$)$_2$]·H$_2$O (**28**) (0.80 g, 1.868 mmol), BCE·2HCl (0.48 g, 1.868 mmol), and NaOH (0.14 g, 3.6 mmol). Crystals of [Co(Etacac)$_2$(BCE)]ClO$_4$ (**16**) (0.585 g, 52.4%) were isolated by slow evaporation of the MeOH/H$_2$O solution. ^{1}H NMR (CDCl$_3$): δ 4.88 (br s, 2 H, N*H*), 3.89, 3.78 (m, 2 H, C*H*$_2$Cl), 3.08 (br q, 2 H, C*H*$_2$NHR), 2.76 (t, 2 H, C*H*$_2$NHR), 2.40, 2.19 (m, 2 H, C*H*$_2$CH$_2$Cl), 2.35, 2.17 (s, 3 H, C*H*$_3$CO), 2.32 (q, 4 H, *J* = 7.4 Hz, C*H*$_2$CH$_3$), 1.02 (t, 6 H, *J* = 7.4 Hz, C*H*$_3$CH$_2$). ^{13}C NMR (CDCl$_3$): δ 189.26, 189.13 (*C*O), 110.49 (*C*Et), 50.91 (*C*H$_2$Cl), 49.82 (*C*H$_2$NHR), 39.69 (*C*H$_2$CH$_2$Cl), 25.56, 25.00 (*C*H$_3$CO), 22.37 (*C*H$_2$CH$_3$), 15.17 (*C*H$_3$CH$_2$). Anal. ($C_{20}H_{36}Cl_2N_2O_4$Co·ClO$_4$) C, H, N, Cl.

Bis(3-*n*-propyl-2,4-pentanedionato)(*N*,*N'*-bis(2-chloroethyl)ethylenediamine)cobalt(III) Perchlorate [Co(Pracac)$_2$(BCE)]ClO$_4$ (17). This complex was prepared as described for **14** using Na[Co(Pracac)$_2$(NO$_2$)$_2$]·H$_2$O (**29**) (0.500 g, 1.054 mmol), BCE·2HCl (0.272 g, 1.054 mmol), and NaOH (0.084 g, 2.108 mmol). After the reaction mixture was filtered to remove the charcoal, the solution was acidified with 0.1 N HCl and extracted with CHCl$_3$. The CHCl$_3$ solution was evaporated to dryness, and the residue was dissolved in MeOH/H$_2$O (1:3), loaded on to a Sephadex-SP-C25 column in the Na$^+$ form, washed with H$_2$O, and eluted with aqueous NaCl solution (0.15 mol L^{-1}). The eluant containing the product was extracted with CHCl$_3$, and the resulting solution was evaporated to dryness, giving [Co(Pracac)$_2$(BCE)]Cl (0.365 g, 60.1%). This was converted to the ClO$_4^-$ salt (95% yield) by addition of NaClO$_4$ to a MeOH/H$_2$O solution of the complex, followed by slow evaporation to give dark green-purple dichroic needles of [Co(Pracac)$_2$(BCE)]ClO$_4$ (**17**). ^{1}H NMR (CDCl$_3$): δ 4.87 (br s, 2 H, N*H*), 3.88, 3.77 (m, 2 H, C*H*$_2$Cl), 3.08 (dd, *J* = 7.8, 3.7 Hz, 2 H, C*H*$_2$NHR), 2.76 (dd, *J* = 8.3, 11.0 Hz, 2 H, C*H*$_2$NHR), 2.39, 2.16 (m, 2 H, C*H*$_2$CH$_2$Cl), 2.10, 2.16 (s, 3 C*H*$_3$CO), 2.26 (t, *J* = 7.8 Hz, 4 H, C*H*$_2$CH$_2$CH$_3$), 1.38 (m, 4 H, C*H*$_2$CH$_3$), 0.95 (t, *J* = 7.3 Hz, 6 H, C*H*$_3$). ^{13}C NMR (CDCl$_3$): δ 189.39, 189.24 (*C*O), 109.00 (*C*Pr), 50.89 (*C*H$_2$Cl), 49.80 (*C*H$_2$NHR), 39.67 (*C*H$_2$CH$_2$Cl), 31.19 (*C*H$_2$CH$_2$CH$_3$), 25.80, 25.28 (*C*H$_3$CO), 24.21 (*C*H$_2$CH$_3$), 13.86 (*C*H$_3$). Anal. ($C_{22}H_{40}Cl_2N_2O_4$Co·ClO$_4$) C, H, N.

Bis(3-chloro-2,4-pentanedionato)(*N*,*N'*-bis(2-chloroethyl)ethylenediamine)cobalt(III) Chloride [Co(Clacac)$_2$(BCE)]Cl (18). *N*-Chlorosuccinimide (0.204 g, 1.53 mmol) was dissolved in MeOH (60 mL). [Co(acac)$_2$(BCE)]ClO$_4$ (**14**) (0.24 g, 0.443 mmol) was added portionwise, and the solution was stirred for 6 h at 20 °C. The solvent volume was reduced to 30 mL, and H$_2$O (50 mL) was added. The solution was loaded on to a Sephadex-SP-C25 cation-exchange column (2.5 × 10 cm) prepared in the Na$^+$ form. The column was washed with water, and the complex was eluted with 0.1 N NaCl. The eluted band was extracted five times with CH$_2$Cl$_2$, and the combined extracts were evaporated. Toluene (5 mL) was added to the residue, and the solution was further evaporated to give a magenta-colored oil. Addition of Me$_2$CO (3 mL) produced a mass of fine needles of [Co(Clacac)$_2$(BCE)]Cl (**18**) (0.15 g, 55.5%), which were filtered and washed quickly with Me$_2$CO followed by Et$_2$O and dried in air in a desiccator. ^{1}H NMR (CDCl$_3$): δ 5.86 (br, 2 H, N*H*), 3.97 (m, 4 H, C*H*$_2$Cl), 3.00 (m, 4 H, C*H*$_2$NHR), 2.95 (m, 4 H, C*H*$_2$CH$_2$Cl), 2.49, 2.42 (s, 3 H, C*H*$_3$CO). ^{13}C NMR (CDCl$_3$): δ 188.74, 188.45 (*C*O), 107.06 (*C*Cl), 51.10 (*C*H$_2$Cl), 50.16 (*C*H$_2$NHR), 39.92 (*C*H$_2$CH$_2$Cl), 28.26, 27.60 (*C*H$_3$CO). Anal. ($C_{16}H_{26}Cl_4N_2O_4$Co·Cl) C, H, N.

Bis(2,4-pentanedionato)(*N*,*N'*-diethylethylenediamine)cobalt(III) Perchlorate [Co(acac)$_2$(BEE)]ClO$_4$ (12). This complex was prepared as described for **14** using Na[Co(acac)$_2$(NO$_2$)$_2$]·H$_2$O (**26**) (0.70 g, 1.794 mmol) and BEE (0.25 g, 2.151 mmol) to give [Co(acac)$_2$(BEE)]ClO$_4$ (**12**) (0.75 g, 88.4%). ^{1}H NMR (CDCl$_3$): δ 5.51 (s, 2 H, C*H*), 4.57 (br, 2 H, N*H*), 3.07 (br q, *J* = 3.8 Hz, 2 H, C*H*$_2$NHR), 2.62 (m, 2 H, C*H*$_2$NHR), 2.40, 1.88 (m, 2 H, C*H*$_2$CH$_3$), 2.20, 2.09 (s, 3 H, C*H*$_3$CO), 1.32 (t, *J* = 7.3 Hz, 6 H, C*H*$_3$CH$_2$). ^{13}C NMR (CDCl$_3$): δ 190.55, 190.37 (*C*O), 98.63 (*C*H), 49.97 (*C*H$_2$NHR), 44.63 (*C*H$_2$CH$_3$), 26.36, 26.27 (*C*H$_3$CO), 12.22 (*C*H$_3$CH$_2$). Anal. ($C_{16}H_{30}N_2O_4$Co·ClO$_4$) C, H, N, Cl.

Bis(2,4-pentanedionato)(*N*,*N*-diethylethylenediamine)cobalt(III) Perchlorate [Co(acac)$_2$(DEE)]ClO$_4$ (13). This complex was prepared as described for **14** using Na[Co(acac)$_2$(NO$_2$)$_2$]·H$_2$O (**26**) (0.70 g, 1.794 mmol) and DEE (0.24 g, 2.065 mmol) to give [Co(acac)$_2$(DEE)]ClO$_4$ (**13**) (0.52 g, 61.3%). ^{1}H NMR (CDCl$_3$): δ 5.60, 5.55 (s, 1 H, C*H*), 4.36, 3.99 (br s, 1 H, N*H*$_2$), 3.22, 2.50, 2.23, 1.91 (q, 1 H, *J* = 6.8 Hz, C*H*$_2$CH$_3$), 3.13 (br s, 2 H, C*H*$_2$NH$_2$), 2.74 (br d, 2 H, *J* = 4.7 Hz, C*H*$_2$NR$_2$), 2.22, 2.18, 2.11, 2.02 (s, 3 H, C*H*$_3$CO), 1.17, 0.95 (t, 3 H, *J* = 6.8 Hz, C*H*$_3$CH$_2$). ^{13}C NMR (CDCl$_3$): δ 190.82, 190.75, 190.29, 189.78 (*C*O), 99.56, 98.32 (*C*H), 60.87 (*C*H$_2$NR$_2$), 47.92, 26.61 (*C*H$_2$CH$_3$), 41.71 (*C*H$_2$NH$_2$), 26.52, 26.49, 26.41, 25.78 (*C*H$_3$CO), 8.64, 7.58 (*C*H$_3$CH$_2$). Anal. ($C_{16}H_{30}N_2O_4$Co·ClO$_4$) C, H, N.

Bis(2,4-pentanedionato)(*N*,*N*-bis(2-chloroethyl)ethylenediamine)cobalt(III) Perchlorate [Co(acac)$_2$(DCE)]ClO$_4$ (19). This complex was prepared as described for **14**, using a Sephadex-SP-C25 column to purify the product. Alternatively, the free base of DCE was prepared by suspending DCE·2HCl (0.364 g, 1.41 mmol) in MeOH (5 mL) and adding NaOH (0.113 g, 2.82 mmol) in MeOH (2 mL). The resulting solution was immediately added to a solution of Co(acac)$_3$ (0.457 g, 1.28 mmol) in MeOH (45 mL), followed by activated charcoal (0.1 g). The solution was stirred for 1 h and then filtered through Celite. The combined green-red filtrate and washings were evaporated to small volume under reduced pressure, and H$_2$O (50 mL) was added. Green crystals of unreacted Co(acac)$_3$ which formed were filtered off, and the filtrate was then loaded on to a Sephadex-SP-C25 column (60 mL) in the Na$^+$ form. The column was washed with water, and elution was performed with a gradient of 0.05–0.1 N NaCl. The eluted band was extracted with CHCl$_3$ four times, and the combined extracts were evaporated to dryness under reduced pressure. The residue was taken up in water, and NaClO$_4$·H$_2$O (1 g) in MeOH was added. After cooling at 5 °C for 2 days, the dark crystals of [Co(acac)$_2$(DCE)]ClO$_4$ (**19**) which had formed (0.03 g, 4.3%) were filtered and washed with H$_2$O and dried in air in a desiccator. ^{1}H NMR (CDCl$_3$): δ 5.60, 5.53 (s, 1 H, C*H*), 4.39, 4.22 (br m, 1 H, NH_2), 3.93, 3.69, 3.59, 3.50 (m, 1 H, CH_2Cl), 3.07 (m, 2 H, CH_2NH_2), 2.79 (t, 2 H, J = 6.3 Hz, CH_2NR_2), 3.02, 2.61, 2.38, 2.26 (m, 1 H, CH_2CH_2Cl), 2.21, 2.19, 2.10, 1.97 (s, 3 H, CH_3CO). ^{13}C NMR ($CDCl_3$): δ 191.51, 191.46, 191.12, 189.91 (CO), 99.81, 98.46 (CH), 61.40 (CH_2NR_2), 55.71, 53.77 (CH_2CH_2Cl), 42.11 (CH_2NH_2), 37.97, 35.97 (CH_2Cl), and 26.68, 26.64, 26.26, 25.71 (CH_3CO). Anal. ($C_{16}H_{28}Cl_2N_2O_4Co{\cdot}ClO_4$) C, H, N.

Bis(3-methyl-2,4-pentanedionato)(*N,N*-bis(2-chloroethyl)ethylenediamine)cobalt(III) Perchlorate [Co(Meacac)$_2$(DCE)]ClO$_4$ (20). Na[Co(Meacac)$_2$(NO$_2$)$_2$]·H$_2$O (**27**) (1.50 g, 3.587 mmol) was dissolved in a mixture of MeOH (46 mL) and H_2O (27 mL). A solution of NaOH (0.330 g, 8.248 mmol) dissolved in MeOH (8 mL) was added to a solution of DCE·2HCl (1.064 g, 4.124 mmol), in H_2O (1 mL) which had been cooled in an ice bath. After 30 s, activated charcoal (0.42 g) was added to the Na[Co(Meacac)$_2$(NO$_2$)$_2$]·H$_2$O solution, followed immediately by the deprotonated DCE solution, and the mixture was stirred for 1 h. The charcoal was filtered off through Celite and washed with MeOH, which was added to the filtrate. The filtrate was acidified with 3 mol L^{-1} HCl (1.5 mL) and extracted with three portions of $CHCl_3$. The combined extracts were evaporated to dryness under reduced pressure, and the residue was taken up in H_2O (20 mL) and decanted from some insoluble material. MeOH (20 mL) was then added to the supernatant, and the solution was left open to the air at 20 °C for slow evaporation of the MeOH. After 1 week, the resulting green crystals of [Co(Meacac)$_2$(DCE)]ClO$_4$ (**20**) (0.205 g, 10%) were collected by filtration and washed with 20% MeOH/H_2O, H_2O, and finally Et_2O. The product was air-dried in a desiccator. ^1H NMR ($CDCl_3$): δ 4.30, 4.01 (br, 1 H, NH_2), 4.07, 3.78, 3.60, 3.59 (m, 1 H, CH_2Cl), 3.10 (m, 2 H, CH_2NH_2), 2.98, 2.76 (m, 1 H, CH_2NR_2), 2.61, 2.50, 2.25 (m, 1 H, CH_2CH_2Cl), 2.35, 2.33 (s, 3 H, CH_3), 2.24, 2.09, 1.98, 1.90 (s, 3 H, CH_3CO). ^{13}C NMR ($CDCl_3$): δ 189.42, 189.20, 188.87, 187.71 (CO), 103.83, 102.16 (CMe), 61.15 (CH_2NR_2), 55.67, 53.60 (CH_2CH_2Cl), 41.83 (CH_2NH_2), 38.17, 36.03 (CH_2Cl), 26.46, 26.38, 26.24, 25.64 (CH_3CO), 14.89, 14.69, (CH_3). Anal. ($C_{16}H_{32}Cl_2N_2O_4Co{\cdot}ClO_4$) C, H, N, Cl.

Bis(3-ethyl-2,4-pentanedionato)(*N,N*-bis(2-chloroethyl)ethylenediamine)cobalt(III) Perchlorate [Co(Etacac)$_2$(DCE)]ClO$_4$ (21). A freshly-prepared solution of the free base of DCE (**11**) (0.856 g, 3.319 mmol) was added to a solution of Na[Co(Etacac)$_2$(NO$_2$)$_2$]·H$_2$O (**28**) (1.288 g, 2.886 mmol) in H_2O (10 mL) and MeOH (20 mL) to which activated charcoal (0.28 g) had been added. The mixture was stirred for 1 h and then filtered through Celite, and the charcoal was washed with water and MeOH which were added to the filtrate. HCl (3 N) was added to the filtrate until the solution was acidic, and it was then extracted three times with $CHCl_3$. The combined extracts were evaporated under reduced pressure to a thick oil, which was dissolved in a mixture of MeOH (10 mL) and H_2O (10 mL) and extracted three times with $CHCl_3$ (10 mL). The combined extracts were once again evaporated to an oil and then taken up in MeOH (15 mL) and H_2O (15 mL) and loaded on to a Sephadex-SP-C25 cation-exchange resin (Na^+ form) column and eluted with 0.15 N NaCl in 10% MeOH/H_2O. The green eluant was extracted five times with $CHCl_3$, and the combined extracts were evaporated under reduced pressure to give an oil, which was dissolved in Et_2O and then evaporated to dryness to give [Co(Etacac)$_2$(DCE)]Cl·2H$_2$O as a green solid (455 mg, 29.5%). This was converted to the ClO_4^- salt **21** as described for **17**, extracted into CH_2Cl_2, and isolated by evaporation of the solvent to dryness. ^1H NMR ($CDCl_3$): δ 5.54, 4.46 (br, 1 H, NH_2), 4.02, 3.76 (m, 1 H, CH_2Cl), 3.53 (m, 2 H, CH_2Cl), 3.43, 2.69, 2.61, 2.30 (m, 1 H, CH_2CH_2Cl), 3.17 (br s, CH_2NH_2), 2.93 (br m, 2 H, CH_2NR_2), 2.40, 2.35, 2.23, 2.09 (s, 3 H, CH_3CO), 2.37, 2.29 (q, 2 H, J = 7.4 Hz, CH_2CH_3), 1.05 (t, 6 H, J = 7.4 Hz, CH_3CH_2). ^{13}C NMR ($CDCl_3$): δ 189.78, 189.09, 189.00, 188.21 (CO), 111.59, 109.32 (CEt), 61.57 (CH_2NR_2), 55.43, 53.73 (CH_2CH_2Cl), 41.84 (CH_2NH_2), 37.65, 36.17 (CH_2Cl), 25.82, 25.70, 25.30, 24.55 (CH_3CO), 22.38, 22.32 (CH_2CH_3), 15.28, 14.79 (CH_3CH_2). High-resolution MS (FAB): *m/z* 497.1359 (calcd for $C_{20}H_{36}{}^{35}Cl_2CoN_2O_4$, 497.1384).

Bis(3-*n*-propyl-2,4-pentanedionato)(*N,N*-bis(2-chloroethyl)ethylenediamine)cobalt(III) Perchlorate [Co(Pracac)$_2$(DCE)]ClO$_4$ (22). This complex was prepared as described for (**21**), using Na[Co(Pracac)$_2$(NO$_2$)$_2$]·H$_2$O (**29**) (0.80 g, 1.687 mmol), DCE-2HCl (0.53 g, 2.054 mmol), and NaOH (0.16 g, 4.0 mmol) to give [Co(Pracac)$_2$(DCE)]ClO$_4$ (**22**) (0.18 g, 17.0%) after evaporating the solution to dryness. ^1H NMR ($CDCl_3$): δ 4.21, 4.09 (br, 1 H, NH_2), 4.04, 3.78, 3.62, 3.57 (m, 1 H, CH_2Cl), 3.57, 2.57, 2.52, 2.19 (m, 1 H, CH_2CH_2Cl), 3.12 (m, 2 H, CH_2NH_2), 3.04 (m, 1 H, CH_2NR_2), 2.84 (br dt, ^2J = 12.2 Hz, ^3J = 3.4 Hz, 1 H, CH_2NR_2), 2.35, 2.35, 2.23, 2.09 (s, 3 H, CH_3CO), 2.30, 2.22 (m, 2 H, $CH_2CH_2CH_3$), 1.40 (m, 4 H, CH_2CH_3), 0.95, 0.93 (t 3 H, J = 7.4 Hz, CH_3CH_2). ^{13}C NMR ($CDCl_3$): δ 189.97, 189.66, 189.19, 188.19 (CO), 110.01, 108.13 (CH), 61.27 (CH_2NR_2), 55.62, 53.59 (CH_2CH_2Cl), 41.78 (CH_2NH_2), 37.98, 36.05 (CH_2Cl), 31.22, 31.20 ($CH_2CH_2CH_3$), 25.87, 25.54, 25.47, 24.72 (CH_3CO), 24.27, 23.68 (CH_2CH_3), 14.00, 13.87 (CH_3CH_2). Anal. ($C_{22}H_{40}N_2O_4Cl_2Co{\cdot}ClO_4$) C, H, N. High-resolution MS (FAB): *m/z* 525.1677 (calcd for $C_{22}H_{40}{}^{35}Cl_2CoN_2O_4$, 525.1697).

HPLC Purification of Cobalt Complexes. Solutions of the complexes (**15**, **19–21**) in CH_3CN were chromatographed on a semipreparative C18 μ-Bondapak column (25 × 100 mm; 45-μL injection), using a mobile phase of CH_3CN/0.37 mol L^{-1} ammonium formate, pH 4.5 (52:48 v/v), at a flow rate of 3.6 mL/min. Detection was by UV absorption at 254 nm. For example, pooled eluates from 250 mg of **20**, which were loaded in ca. 1.8-mg injections, were cooled and the upper, green CH_3CN-rich phase was separated. The CH_3CN was evaporated, and to the residual aqueous solution was added 1 mol L^{-1} NaClO$_4$ (2 mL) which was then extracted with CH_2Cl_2 (5 × 20 mL). After evaporation of the CH_2Cl_2 and dissolution in MeOH (15 mL), 1 mol L^{-1} NaClO$_4$ (7 mL) was added and the solution was allowed to evaporate slowly for several days. The green crystals (210 mg) were washed with H_2O, MeOH/H_2O, and Et_2O. This procedure gave compound **20** of 99.7% purity based on peak area.

Determination of Reduction Potentials. The Co(III)/Co(II) redox potentials were determined by Osteryoung square-wave voltammetry in CH_2Cl_2 solutions approximately 10^{-3} mol L^{-1} in cobalt complex and containing *n*-Bu$_4$NClO$_4$ (0.15 mol L^{-1}) as the electrolyte. A three-electrode configuration was used, with a Pt disk as the working electrode, a Pt wire as the auxiliary electrode, and a quasi-reference Ag/AgCl electrode prepared from Ag wire electrolytically coated with AgCl. The ferrocenium/ferrocene couple was used as internal reference (0.548 V *vs* NHE).

Cell Line Studies. AA8 and UV4 cells were maintained in logarithmic-phase growth in 25-cm^2 tissue culture flasks, using antibiotic-free α-MEM with 10% v/v heat-inactivated (56 °C, 40 min) fetal calf serum. Doubling times were approximately 14 h for AA8 and 15 h for UV4 cells. Cultures were tested for mycoplasma contamination frequently, using a cytochemical staining method.[40] Bulk cultures of AA8 cells were prepared in spinner flasks, using the above growth medium plus penicillin (100 IU/mL) and streptomycin (100 μg/mL). Growth inhibition studies were performed as described in detail elsewhere,[34,41] using 200 viable AA8 or 300 viable UV4 cells plus 5000 lethally-irradiated AA8 feeder cells per well in 96-well tissue culture dishes. The IC_{50} was determined as the drug concentration needed to reduce the cell mass (protein content, measured after 72–78 h by staining with methylene blue and measuring absorbance in a microplate photometer) to 50% of the mean value for 8 control cultures on the same 96-well plate.

The stirred suspension culture assay used has also been described in detail elsewhere.[34] Clonogenic assays were carried out with magnetically-stirred 10-mL suspension cultures of late log phase UV4 cells at 2 × 10^6 cells/mL. Samples were removed periodically during continuous gassing with 5% CO_2 in air or N_2 at 37 °C. A range of drug concentrations were studied to identify those concentrations which gave approximately the same rate of cell killing under both aerobic and hypoxic conditions. The ratios of the concentration × time for cell survival of 10% (CT_{10}) for these two survival curves was used as the measure of hypoxic selectivity.

Stability of BCE and DCE Ligands. The stabilities of the nitrogen mustard ligands (**10**, **11**) in culture medium (α-MEM containing 5% v/v fetal calf serum) at pH 7.0 and 37 °C were investigated by bioassay against UV4 cells, essentially as described previously.[36] Stock solutions of the mustards were prepared in 0.01 N HCl on ice and diluted into medium which was maintained under an atmosphere of 5% CO_2. Samples were withdrawn at 15-min intervals for 2 h, and suitable dilutions were titrated against logarithmic-phase UV4 cultures in 96-well plates to determine IC_{50} values. The fraction of the parent compound remaining at time *t* was determined as the ratio $IC_{50,t=0}/IC_{50,t}$. Pseudo-first-order half-lives were determined by logarithmic regression.

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