Design and Synthesis of Cobalt(III) Nitrogen Mustard Complexes as Hypoxia Selective Cytotoxins. The X-Ray Crystal Structure of bis(3-Chloropentane-2,4-dionato)(*RS-N,N'*-bis(2-chloroethyl)ethylenediamine)cobalt(III) Perchlorate, $[Co(Clacac)_2(bce)]ClO_4$

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The design, synthesis and biological evaluation of $[Co(Meacac)_2(dce)]^+$, a cobalt(III) nitrogen mustard complex that is effective as a hypoxia selective cytotoxin for potential anti-cancer use, is reported.

The majority of solid tumours contain cells which are either chronically or transiently hypoxic.[1] These cells tend to be resistant to chemotherapy for reasons including their low proliferative activity[2] and inaccessibility to drugs,[3] and thus represent a considerable clinical problem in the chemotherapy of solid tumours.[4] The fact that the hypoxic environment is essentially restricted to solid tumours, since dose-limiting normal tissues are well-perfused, provides a further incentive to develop drugs (hypoxia-selective cytotoxins; HSCs) which would be activated only in hypoxic regions, since such compounds might be truly specific to solid tumours. To date, the majority of such HSCs have been nitroaromatic compounds, in which reduction of the nitro group provides the hypoxia-selective bioactivation.[5]

This communication describes the design, synthesis, characterisation and biological evaluation of a series of cobalt(III) nitrogen mustard complexes, some of which are effective HSCs in cell culture. Molecules containing bis(2-chloroethyl)-amine functionality (nitrogen mustards) are well known DNA cross-linking agents, with potent cytotoxic activity. Their cytotoxicity depends on the electron density on the mustard nitrogen, which controls its alkylating reactivity. Coordination of the nitrogen lone pair of a nitrogen mustard to Co^{III} should suppress its toxicity since the electron pair is no longer available. Such Co^{III} complexes are kinetically very inert, and the nitrogen mustard ligand would be displaced only very slowly, unless the complex is reduced to the much more labile Co^{II} state. Since the Co^{III}–Co^{II} reduction potential falls in the range of cellular reductants −0.20 to −0.40 V *vs.* normal hydrogen electrode (NHE)], net chemical or metabolic one-electron reduction of the inert Co^{III} complexes would be expected. The resulting labile Co^{II} species would undergo very facile ligand substitution by water, releasing the cytotoxic free nitrogen mustard.

To ensure hypoxic selectivity, the reduced Co^{II} complex containing the nitrogen mustard ligand must be sufficiently stable to allow reoxidation in oxygenated cells to compete effectively with ligand loss. Monodentate alkylating ligands appear not to provide complexes of sufficient stability, since Co^{III} complexes bearing either aziridine[6] or bis(2-chloro-ethyl)amine[7] ligands do not show hypoxic selectivity. However, the kinetic stability of the reduced Co^{II} complexes is greatly increased if chelating ligands are used. We have therefore prepared and studied the Co^{III} complexes of the chelating nitrogen mustard ligands *N,N'*-bis(2-chloro-ethyl)ethylenediamine (bce)[8] and *N,N*-bis(2-chloro-ethyl)ethylenediamine (dce).[9]

The preparation of these complexes 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. Suitable cobalt(III) complexes are *trans*-$Na[Co(acac)_2(NO_2)_2]$[10] **1a** (acac = pentane-2,4-dionato anion) and the new compound *trans*-$Na[Co(Meacac)_2(NO_2)_2]$ **1b** (Meacac = 3-methylpentane-2,4-dionato anion), each prepared by treatment of $Na_3[Co(NO_2)_6]$ with Na(acac) or Na(Meacac), respectively. Reaction of the free base of bce or dce with **1** produces the desired Co^{III} nitrogen mustard complexes **2** and **3**, respectively (Scheme 1). Elaboration of **3** to produce **4** (Clacac = 3-chloropentane-2,4-dionato anion) was achieved by chlorination of the 3-position on the acac ligand with *N*-chlorosuccinimide[11] (Scheme 1), which provides an analogue with a higher reduction potential.

The mustard complexes **2**, **3** and **4** were isolated as perchlorate salts by metathesis with $NaClO_4$, or as chloride salts after ion exchange chromatography. The complexes were characterised by elemental analysis, IR, and ^1H and ^{13}C NMR spectroscopy.† Three diastereoisomers are possible for each complex of the bce ligand, because the two nitrogen donor atoms become chiral upon coordination to cobalt. When the products are precipitated rapidly from solution three isomers arising from the stereochemistry at nitrogen can be detected by ^1H and ^{13}C NMR. However, slow crystallisation produces a single isomer in high yield as a stereochemically pure product.

An X-ray crystal structure of $[Co(Clacac)_2(bce)]ClO_4$ **4** (Fig. 1) confirms the *cis* geometry and *RS* stereochemistry,‡ which is also presumed for **3**. The geometry about the cobalt atom in **4** is a distorted octahedral arrangement of four oxygen and two nitrogen donor atoms. The structure confirms the complete chlorination at the 3-position of the pentane-2,4-dionato moiety. The slight deviations from planarity observed for the Clacac ligand are also seen in the structure of a Co^{III}

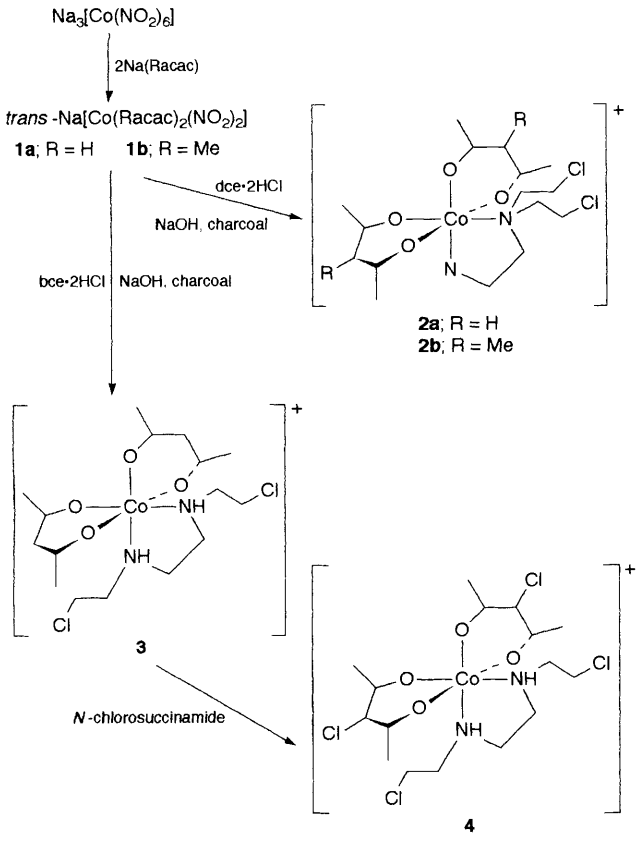
† *Selected data* for **2a**: ^1H NMR (400 MHz, $CDCl_3$) δ 5.60, 5.53 (s, 1 H, C*H*); 4.39, 4.22 (br m, 1 H, NH_2), 3.93, 3.69, 3.59, 3.50 (m, 1 H, CH_2Cl), 3.07 (m, 2 H, CH_2NH$_2$), 2.79 (t, 2 H, $^3J_{H,H}$ 6.32 Hz, CH_2NR$_2$), 3.02, 2.61, 2.38, 2.26 (m, 1 H, CH_2CH$_2$Cl) and 2.21, 2.19, 2.10, 1.97 (s, 3 H, CH_3CO). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 191.51, 191.46, 191.12, 189.91 (*C*O); 99.81, 98.46 (*C*H), 61.40 (*C*H$_2$NR$_2$), 55.71, 53.77 (*C*H$_2$Cl), 42.11 (*C*H$_2$NH$_2$), 37.97, 35.97 (*C*H$_2$CH$_2$Cl) and 26.68, 26.64, 26.26, 25.71 (*C*H$_3$CO).

For **2b**: Resonances similar to **2a**. ^1H NMR (400 MHz, $CDCl_3$) δ 2.35, 2.33 (s, 3 H, CH_3). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 103.83, 102.16 (*C*CH$_3$) and 14.89, 14.69 (*C*H$_3$).

For **3**: ^1H NMR (400 MHz, $[^2H_6]Me_2SO$) δ 5.97 (br, 2 H, N*H*), 5.66 (s, 2 H, C*H*), 4.03, 3.93 (m, 2 H, CH_2Cl), 2.86, 2.75 (m, 2 H, CH_2NHR), 2.71, 2.58 (m, 2 H, CH_2CH$_2$Cl) and 2.14, 2.08 (s, 3 H, CH_3CO). $^{13}C\{^1H\}$ (100 MHz, $[^2H_6]Me_2SO$) 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) and 26.38, 26.25 (*C*H$_3$CO).

For **4**: Resonances similar to **3**. ^1H NMR (400 MHz, $CDCl_3$) no C*H* peak near δ 5.66. $^{13}C\{^1H\}$ (100 MHz, $CDCl_3$): δ 107.06 (*C*Cl).

‡ *Crystal data* for $[Co(Clacac)_2(bce)]ClO_4$ **4**: $C_{16}H_{26}Cl_5CoN_2O_8$, M = 610.18, orthorhombic, space group, *Pbca*, a = 8.282(9), b = 19.852(5), c = 30.170(6) Å, U = 4960.3 Å^3, $F(000)$ = 2496, D_c = 1.634 g cm^{-3}, Z = 8, μ(Mo-Kα, λ = 0.71069 Å) = 12.3 cm^{-1}. Intensity data were collected to a 2θ limit of 40° on a Nonius CAD-4 diffractometer and corrected for Lorentz, polarisation and absorption effects. The structure was solved from Patterson and heavy-atom electron density maps and refined by full-matrix least-squares analysis. All atoms were allowed to assume anisotropic motion with hydrogen atoms riding on the atom to which they were attached with fixed thermal parameters. Refinement converged to R = 0.078 (R_w = 0.084) for 1355 reflections for which $I > 3\sigma(I)$. The data are limited by crystal quality leading to a low diffraction limit and consequent poor standard deviations. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.



Scheme 1

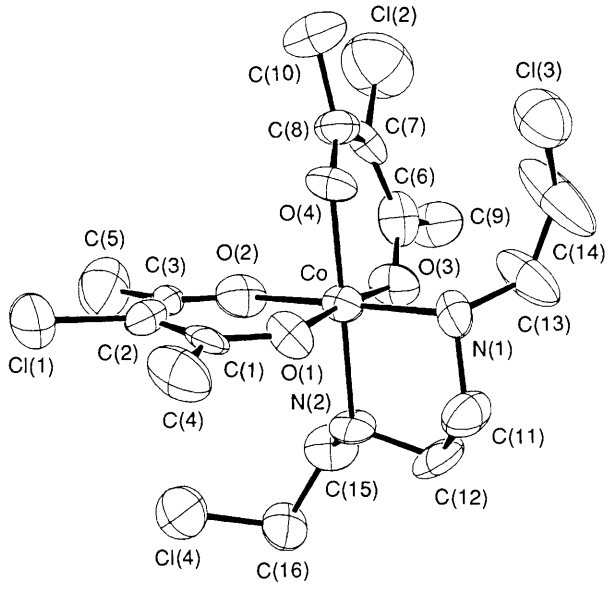


Fig. 1 Molecular structure of $[Co(Clacac)_2(bce)]ClO_4$ **4**. Important bond lengths (Å) and bond angles (°): Co–O(1) 1.894(9), Co–O(2) 1.889(13), Co–O(3) 1.848(11), Co–O(4) 1.883(11), Co–N(1) 1.988(14), Co–N(2) 1.961(12), O(1)–Co–O(2) 93.5(5), O(3)–Co–O(4) 95.3 (5); O(1)–Co–O(4) 89.2(5), O(2)–Co–O(4) 88.6(5), O(1)–Co–O(3) 174.9(5), O(2)–Co–O(3) 89.1(6), O(1)–Co–N(1) 84.6(5), O(1)–Co–N(2) 88.6(5), O(2)–Co–N(1) 178.1(6), O(3)–Co–N(1) 92.7(6), O(3)–Co–N(2) 86.9(6), O(4)–Co–N(1) 91.7(5), O(4)–Co–N(2) 177.7(5), N(1)–Co–N(2) 87.6(6).

Table 1 Electrochemical and biological data

Compound		$E_{\frac{1}{2}}$[a]/V	AA8 IC_{50} air[b] /μmol dm^{-3}	Air/N_2 IC_{50} ratio AA8	UV4	HF[c] air
Ligands						
bce			31	0.98	0.62	29.4
dce			1.1	0.67	1.17	53.0
Complexes						
3	$[Co(acac)_2(bce)]^+$	−0.31	893	0.42	3.76	14.0
4	$[Co(Clacac)_2(bce)]^+$	−0.13	26.1	0.54	0.92	12.8
2a	$[Co(acac)_2(dce)]^+$	−0.23	1.7	1.33	0.85	54.8
2b	$[Co(Meacac)_2(dce)]^+$	−0.31	2.7	2.87	2.01	48.5

[a] $E_{\frac{1}{2}}$ values measured vs. NHE by square wave voltammetry at a platinum disc electrode in 0.15 mol dm^{-3} $(Bu^n_4N)ClO_4$ in CH_2Cl_2, with ferrocene (0.55 V *vs.* NHE) as an internal reference. [b] Concentration giving 50% inhibition of cell proliferation following an 18 h drug exposure under aerobic conditions. [c] AA8 IC_{50}/UV4 IC_{50} under aerobic conditions.

complex containing the analogous 3-bromopentane-2,4-dionato ligand.[12]

The cytotoxicity and hypoxic selectivity of the compounds were evaluated initially in a growth inhibition assay using the Chinese hamster ovary fibroblast subline AA8, and the derived mutant line UV4 which is deficient in the repair of DNA adducts and thus hypersensitive to DNA cross-linking agents[13] (Table 1). The unsymmetrical DCE was the more cytotoxic of the two free ligands, but both had high HF values [HF = IC_{50}(AA8)/IC_{50}(UV4)], consistent with DNA cross-linking.[13] All four cobalt complexes had HF values similar to

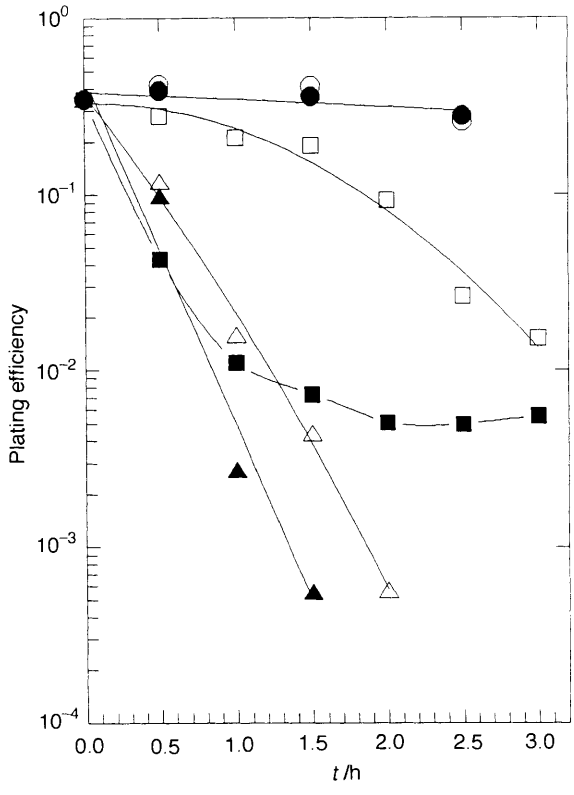


Fig. 2 Plating efficiency of UV4 cells during treatment of stirred suspension cultures with $[Co(Meacac)_2(dce)]ClO_4$ **2b** at 0.25 (■), 0.5 (▲), 1.0 (□) or 2.0 (△) μmol dm^{-3} compared with non-drug-treated controls (○, ●). Open symbols are for aerobic and filled symbols for hypoxic conditions.

those of the corresponding free ligands, indicating that toxicity was indeed due to release of the respective ligand. However, the acac complex **3** was much less toxic than the free ligand, suggesting both that complexation does deactivate the mustard and that **3** is stable under aerobic conditions. The higher aerobic toxicity of **4** may be due to its more facile reduction, as signalled by its higher reduction potential (−0.13 *vs.* −0.31 V).

The Meacac dce complex **2b** showed hypoxic selectivity in both the AA8 and UV4 lines in the growth inhibition assay. It was therefore also evaluated by determining the colony-forming ability of UV4 cells treated as stirred suspensions during gassing with air or nitrogen, this assay being more suitable for the determination of compounds of short half-life.[14] On the basis of the concentration × time required for 90% cell kill at concentrations giving similar rates of kill (2.5 μmol dm^{-3} aerobic and 0.5 μmol dm^{-3} hypoxic), complex **2b** was about sixfold more cytotoxic under hypoxic conditions in this assay (Fig. 2). Complex **2b** is the first metal complex to show significant hypoxia-selective cytotoxicity towards mammalian cells in cell culture through reductive release of toxic ligands. This finding suggests that metal complexes of nitrogen mustards constitute a new class of HSCs.

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