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FULL PAPER

Synthesis, structures and hypoxia-selective cytotoxicity of cobalt(III) complexes containing tridentate amine and nitrogen mustard ligands

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Cobalt(III) complexes containing the tridentate nitrogen mustard ligand *N*,*N*-bis(2-chloroethyl)diethylenetriamine (DCD) and the non-alkylating analogs *N*,*N*-diethyldiethylenetriamine (DED) and diethylenetriamine (dien) have been synthesised and the mustard complexes evaluated as potential hypoxia-selective anticancer drugs. The complexes were prepared from the precursor *trans*-K_2[Co(acac)$(CO_3)(NO_2)_2$]·H_2O in a charcoal-catalyzed substitution reaction. Reaction of this precursor with dien·3HCl in water resulted in the isolation of two isomeric products, *mer*- and s-*fac*-[Co(dien)(acac)(NO_2)]$^+$. Reaction in methanol with DED·3HCl gave one product, *mer*-[Co(DED)(acac)(NO_2)]ClO_4, while from the reaction with DCD·3HCl both the tridentate mustard complex *mer*-[Co(DCD)(acac)(NO_2)]ClO_4 and, in low yield, a complex containing DCD coordinated in a bidentate fashion, *trans*-Co(η^2-DCD)(acac)$(NO_2)_2$, were isolated. The two DCD complexes were characterized by X-ray structure determinations, which confirm the tridentate coordination with a long cobalt–tertiary nitrogen bond distance in the former, and bidentate coordination with the mustard nitrogen as a pendant arm in the latter. The bidentate complex, which contains a free nitrogen mustard, had cytotoxicity similar to that of the free ligand, but cytotoxicity was successfully masked in the tridentate complex, which showed modest hypoxic selectivity (five-fold) in a clonogenic assay.

Introduction

One class of bioreductive drugs, the hypoxia-selective cytotoxins, are designed to exploit the hypoxic sub-population of cells that are present in many solid tumors, and which are resistant to radio- and chemo-therapy.[1] The basic approach is the selective conversion of a non-toxic prodrug to an active form.[2–4] The prodrug undergoes reduction by the endogenous reducing enzymes present in all cells, resulting in the formation of a transient intermediate, usually a one-electron reduction product. This can then undergo further chemical or metabolic transformation to produce the cytotoxic species in its active form. In order to be selective for hypoxic cells this reductive activation must be inhibited by O_2. In normal, well oxygenated cells, this occurs through a redox-cycling mechanism in which the one-electron reduced intermediate is reoxidised by molecular oxygen. In hypoxic cells, in which oxygen is limited, net reduction can take place and the reaction sequence leading to the active cytotoxin can proceed. There is strong evidence that redox cycling is the major mechanism by which O_2 inhibits net reduction of hypoxia selective prodrugs such as nitroaromatic compounds in cells.[5]

One of our approaches to the design of such compounds is to utilize net one-electron reduction at a transition metal center as the activation step. Nitrogen mustards (which contain the bis(2-chloroethylamine) moiety) are potent cytotoxins, utilizing the lone pair on the amine nitrogen to initiate a reaction sequence in which DNA is cross-linked by double alkylation. The normally cytotoxic nitrogen mustard can be deactivated by the formation of a coordinate bond from the mustard nitrogen to a transition metal. We have previously discussed the design and evaluation of nitrogen mustard cobalt complexes such as [Co(DCE)$(Meacac)_2$]ClO_4 **1** (Meacac = 3-methylacetylacetonate),[6–8] [Co(DCE)$(S_2CNEt_2)_2$]BPh_4[9] and [Co(DCE)$(trop)_2$]ClO_4 (trop = tropolonate).[10] Coordination of the nitrogen lone pair of the bidentate mustard ligand DCE (DCE = *N*,*N*-bis(2-chloroethyl)ethylenediamine) to cobalt(III) substantially suppresses toxicity, since the electron pair is no longer available to act as an intramolecular nucleophile. Low-spin, d^6 octahedral Co(III) complexes are kinetically very inert, whereas high spin, d^7 Co(II) complexes are much more labile.[11] It was anticipated that, provided the reduced Co(II) complex was sufficiently stable to allow reoxidation[12] in oxygenated cells to compete effectively with ligand loss, such compounds would be hypoxia-selective.[7]

We showed recently that the bidentate mustard complex **1** indeed has substantial hypoxic selectivity in disperse cell culture,[7] and outstanding activity against hypoxic cells in the interior of multicellular spheroids which have been widely used as a model for solid tumors.[8] However, selectivity does not appear to be due to redox cycling (reoxidation of the initial one-electron reduced Co(II) species by molecular oxygen). Pulse radiolysis studies[13] of **1** have allowed the kinetics of ligand substitution to be investigated, and have showed that initial reduction to the Co(II) species is followed by two other processes. The first of these, which has a first-order rate constant of 120 s^{-1}, is assigned to release of the cytotoxic DCE ligand but is not inhibited by oxygen. This suggests that reversible reoxidation of the initially-formed Co(II) species by oxygen is too slow to compete with irreversible ligand loss. It was suggested[13] that the hypoxic selectivity of **1** may be due to competition between the Co(III) species and oxygen for cellular reductants. The cobalt complexes may represent an instance where kinetic competition with O_2 for reducing species is the basis for selectivity.

The lack of importance of redox cycling in the hypoxic selectivity of **1** would suggest that the lifetime of the initial Co(II) reduction product is unlikely to be an important determinant

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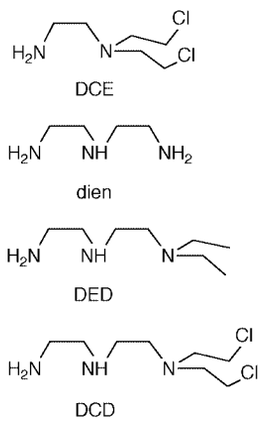
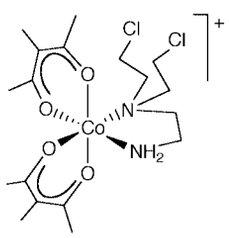
of hypoxic selectivity. However, the apparently greater hypoxic selectivity of the bidentate mustard complex **1**[7] than a related monodentate mustard[14] or aziridine[15] complex leaves open the possibility that chelate effects might be important. In order to examine this further, we have investigated incorporating the mustard functionality into a potentially tridentate ligand. We report here, the synthesis and evaluation of some Co(III) complexes containing a new tridentate nitrogen mustard, *N*,*N*-bis(2-chloroethyl)diethylenetriamine (DCD, **5**). We have also investigated cobalt(III) complexes containing the unsubstituted ligand diethylenetriamine (dien) and the non-alkylating analog *N*,*N*-diethyldiethylenetriamine (DED).





[Co(DCE)(Meacac)$_2$]$^+$

Experimental

General procedures

Elemental analyses were carried out by the Microanalytical Laboratory, University of Otago. Melting points were determined on an Electrothermal apparatus using the supplied stem-corrected thermometer. NMR spectra were determined on a Bruker AM-400 spectrometer. For spectra recorded using D_2O as the solvent, the sodium salt of 3-(trimethylsilyl)propionic-2,2,3,3-d_4-acid (TSP-d_4) was used as the internal reference, and spectra recorded using $CDCl_3$ or CD_3CN were referenced either to internal TMS or to the residual solvent peak. FAB mass spectra were determined on a VG 7070 spectrometer. Cyclic voltammograms were recorded with a BAS 100W electrochemical analyser using a three-electrode cell under N_2 with *iR* compensation applied. The BAS-supplied electrodes used were glassy carbon (working electrode), platinum wire (counter electrode) and Ag–AgCl gel (reference electrode). The Ag–AgCl gel reference electrode was determined to be 40 mV negative of SCE. The solvent was acetonitrile containing 0.15 mol L^{-1} $[NBu^n_4]ClO_4$ as the electrolyte. The ferrocenium–ferrocene couple (Fc^+–Fc), which was observed at *ca.* 0.43 V *vs.* Ag–AgCl, was used as an internal reference in each measurement. Potentials given in the text are reported *vs.* internal Fc^+–Fc (0.400 V *vs.* NHE).[16] Acetonitrile for electrochemistry was dried by distillation from CaH_2 under N_2. $[NBu^n_4]ClO_4$ was twice recrystallised from dry ethyl acetate and then dried *in vacuo* at 80 °C. *N*,*N*-bis(2-hydroxyethyl)ethylenediamine[7] was prepared as previously reported. The salt dien·3HCl was prepared by a method analogous to that described below for DED·3HCl (Found: C, 22.69; H, 7.73; N, 19.48; Cl, 50.24. Calc. for $C_4H_{16}N_3Cl_3$: C, 22.60; H, 7.59; N, 19.77; Cl, 50.04%). DED was purchased from Carbolabs, Inc. (New Haven, Conn.). All other chemicals and reagents were purchased from Aldrich.

HPLC analyses 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. The purity of new compounds was checked by analytical HPLC with absorption detection at 254 nm on a Bondclone C_{18} column using a linear gradient of MeCN (80% v/v) in ammonium formate (0.45 M, pH 4.5). Decomposition of compounds in culture media was determined similarly using a Waters μ-Bondapak C_{18} column and a diode array detector.

Preparations

***N*,*N*-Diethyldiethylenetriamine trihydrochloride, DED·3HCl.** DED (3.0 g) was dissolved in MeOH (20 mL) and acidified with a solution of dry HCl in MeOH and then Et_2O, added slowly until a precipitate persisted. The solution was cooled and the hygroscopic, microcrystalline product was filtered off, washed with MeOH–Et_2O (1 : 2 v/v) then Et_2O, and dried over silica gel (4.52 g, 89%). ^{1}H NMR (D_2O): δ 3.63 (m, 2 H, $CH_2CH_2NH_2$), 3.57 (m, 2 H, $CH_2CH_2NH_2$), 3.54 (t, 2 H, ^3J = 7.4 Hz, CH_2-CH_2NR_2), 3.44 (t, 2 H, ^3J = 7.4 Hz, $CH_2CH_2NR_2$), 3.33 (q, 4 H, ^3J = 7.3 Hz, CH_2CH_3), 1.33 (t, 6 H, ^3J = 7.3 Hz, CH_2CH_3). ^{13}C{^{1}H} NMR (D_2O): δ 49.86 (2 C, CH_2CH_3), 48.26 (CH_2NR_2), 46.41 ($CH_2CH_2NR_2$), 43.51 ($CH_2CH_2NH_2$), 37.04 (CH_2NH_2), 9.86 (2 C, CH_2CH_3) (Found: C, 35.50; H, 9.12; N, 15.36; Cl, 39.75. Calc. for $C_8H_{24}N_3Cl_3$: C, 35.77; H, 9.00; N, 15.64; Cl, 39.59%).

***N*-{[*N*′,*N*′-Bis(2-hydroxyethyl)]-2-aminoethyl}-*N*″-tritylglycyl carboxamide 2.** 1,1′-Carbonyldiimidazole (26.0 g, 0.16 mol) was added to a solution of *N*-tritylglycine (40.0 g, 0.13 mol) in dry DMF (200 mL). After CO_2 evolution ceased, the solution was warmed to 40 °C for 10 min, and a solution of *N*,*N*-bis(2-hydroxyethyl)ethylenediamine (19.3 g, 0.13 mol) in DMF (50 mL) was added in one portion. After 30 min, the solvent was evaporated under reduced pressure, and the residue was partitioned between ethyl acetate and saturated aqueous $NaHCO_3$. The organic layer was washed well with brine, and worked up to give an oil which was chromatographed on silica gel. Ethyl acetate eluted unidentified material, while ethyl acetate–MeOH (19 : 1) gave *N*-{[*N*′,*N*′-bis(2-hydroxyethyl)]-2-aminoethyl}-*N*″-tritylglycyl carboxamide **2** as a viscous oil (41.9 g, 72%). ^{1}H NMR ($CDCl_3$): δ 7.73 (t, 1 H, ^3J = 5.6 Hz, CONH), 7.39 (d, 6 H, ^3J = 7.3 Hz, Ar-H), 7.25 (t, 6 H, ^3J = 7.3 Hz, Ar-H), 7.17 (t, 3 H, ^3J = 7.3 Hz, Ar-H), 3.92 (br, 3 H, OH and NH), 3.51 (t, 4 H, ^3J = 5.0 Hz, NCH_2CH_2OH), 3.33 (dt, 2 H, ^3J = 5.8, 5.6 Hz, $CONHCH_2$), 2.94 (s, 2 H, $TrNHCH_2CO$), 2.60 (overlapping t + t, 6 H, CH_2NR_2 + CH_2-CH_2OH). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 172.54 (CONH), 145.30 (3 C, Ar-C_{ipso}), 128.52 (6 C, Ar-C), 128.03 (6 C, Ar-C), 126.66 (3 C, Ar-C), 70.91 (CPh_3), 59.62 (2 C, NCH_2CH_2OH), 56.89 (2 C, NCH_2CH_2OH), 54.84 ($TrNHCH_2CO$), 48.07 (NCH_2), 37.67 (NCH_2).

***N*-{[*N*′,*N*′-Bis(2-hydroxyethyl)]-2-aminoethyl}glycyl carboxamide 3.** The identity of **2** was further established by characterisation of the product following detritylation. A solution of **2** (10.0 g, 0.022 mol) in MeOH (100 mL) was treated with concentrated HCl (40 mL), and the mixture was warmed at 50 °C for 30 min then evaporated to dryness under reduced pressure. The residue was dissolved in water, and the aqueous layer was washed well with ethyl acetate and again evaporated to dryness under reduced pressure to give the dihydrochloride salt of *N*-{[*N*′,*N*′-bis(2-hydroxyethyl)]-2-aminoethyl}glycyl carboxamide **3** (5.8 g, 95%). Crystallisation from MeOH by the addition of Et_2O gave glistening plates, mp 158–160 °C. ^{1}H NMR (D_2O): δ 3.96 (t, 4 H, ^3J = 5.0 Hz, CH_2OH), 3.86 (s, 2 H, H_2NCH_2CO), 3.72 (t, 2 H, ^3J = 6.2 Hz, $CONHCH_2$), 3.53 (t, 2 H, ^3J = 6.2 Hz, CH_2NR_2), 3.48 (br t, 4 H, ^3J = 5.0 Hz, CH_2CH_2OH). ^{13}C{^{1}H} NMR (D_2O): δ 170.89 (CONH), 58.08 and 57.80 (each 2 C, CH_2CH_2OH), 55.76 (H_2NCH_2CO), 43.12 ($CONHCH_2$), 37.20 (CH_2NR_2) (Found: C, 34.5; H, 7.6; N, 15.1; Cl, 25.6. Calc. for $C_8H_{19}N_3O_3$·2HCl: C, 34.5; H, 7.6; N, 15.1; Cl, 25.5%).

***N*′,*N*′-Bis(2-hydroxyethyl)-*N*‴-trityldiethylenetriamine 4.** A solution of the trityl carboxamide (**2**) (10.0 g, 0.022 mol) in

THF (200 mL) was cooled to 0 °C under N_2 and borane–methyl sulfide complex (6.81 mL of 10.5 M solution in THF, 0.071 mol) was added dropwise. After gas evolution ceased, the solution was heated under reflux for 4 h, then cooled to 0 °C and the excess reagent was destroyed by the dropwise addition of MeOH. The mixture was partitioned between ethyl acetate and water, the ethyl acetate was evaporated from the organic layer and the residue was chromatographed on silica gel. Ethyl acetate eluted unidentified material, while ethyl acetate–MeOH (19 : 1) gave *N*′,*N*′-bis(2-hydroxyethyl)-*N*‴-trityldiethylenetriamine **4** (6.14 g, 64%), which crystallised from acetone as cubes, mp 154 °C. ^{1}H NMR ($CDCl_3$): δ 7.45 (br d, 6 H, ^3J = 7.3 Hz, Ar-H), 7.25 (br t, 6 H, ^3J = 7.3 Hz, Ar-H), 7.16 (br t, 3 H, ^3J = 7.3 Hz, Ar-H), 4.44 (br, 4 H, OH and NH), 3.52 (t, 4 H, ^3J = 4.9 Hz, $N(CH_2CH_2OH)_2$), 2.84 (t, 2 H, ^3J = 5.8 Hz, $NHCH_2CH_2NR_2$), 2.75, 2.66 (2 × br t, each 2 H, ^3J = 4.8 Hz, CH_2N), 2.55 (t, 4 H, ^3J = 4.9 Hz, $N(CH_2CH_2OH)_2$), 2.42 (t, 2 H, ^3J = 5.8 Hz, CH_2NR_2). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 145.7 (3 C, Ar-C), 128.67 (6 C, Ar-C), 128.86 (6 C, Ar-C), 126.37 (3 C, Ar-C), 71.01 (CPh_3), 59.50 (2 C, $N(CH_2CH_2OH)_2$), 57.31 (2 C, $N(CH_2CH_2OH)_2$), 52.26 (NCH_2), 49.08 (NCH_2), 46.58 (NCH_2), 41.43 (NCH_2).

***N*,*N*-Bis(2-chloroethyl)diethylenetriamine trihydrochloride, DCD·3HCl (5·3HCl).** A solution of the N^3-trityltriamine **4** (6.81 g, 0.016 mol) in $SOCl_2$ (200 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 ethyl acetate. The aqueous layer was evaporated to dryness under reduced pressure, and the residue was recrystallised several times from MeOH–Et_2O to give *N*,*N*-bis(2-chloroethyl)diethylenetriamine trihydrochloride (DCD·3HCl, **5**·3HCl) as hygroscopic plates, mp 138–140 °C (4.12 g, 86%). ^{1}H NMR (D_2O): δ 4.03 (t, 4 H, ^3J = 5.5 Hz, CH_2CH_2Cl), 3.79 (t, 4 H, ^3J = 5.5 Hz, CH_2CH_2Cl), 3.77 (m, 2 H, CH_2NR_2), 3.71 (m, 2 H, $CH_2CH_2NR_2$), 3.57 (t, 2 H, ^3J = 7.0 Hz, $CH_2CH_2NH_2$), 3.46 (t, 2 H, ^3J = 7.0 Hz, CH_2NH_2). ^{13}C{^{1}H} NMR (D_2O): δ 57.67 (2 C, CH_2CH_2Cl), 51.46 (CH_2N), 47.36 (CH_2N), 44.44 (CH_2N), 40.00 (2 C, CH_2CH_2Cl), 37.98 (CH_2NH_2) (Found: C, 28.2; H, 6.8; N, 12.2. Calc. for $C_8H_{19}Cl_2N_3$·3HCl: C, 28.5; H, 6.6; N, 12.5%).

Potassium *trans*-(acetylacetonato)(carbonato)dinitrocobaltate(III) monohydrate, *trans*-K_2[Co(acac)(CO_3)(NO_2)$_2$]·H_2O 6. This complex was prepared essentially as described.[17] These authors assigned *cis* stereochemistry to the complex, but it appears to actually be the *trans* isomer on the basis of NMR and reactivity data. In the absence of excess nitrite ion the complex rapidly decomposes in aqueous solution so in order to purify it the complex is rapidly recrystallised from water by the addition of an excess of KNO_2. Crude K_2[Co(acac)(CO_3)(NO_2)$_2$]·H_2O (10 g) was quickly dissolved in ice-cold water (140 mL) and then filtered rapidly (*ca*. 800 mg recovered undissolved) into a solution of KNO_2 (100 g) in water (20 mL). The filtrate was cooled in ice for 45 min then filtered and the crystals washed with cold H_2O–MeOH (1 : 2 v/v), MeOH and EtOH and dried over silica gel to give red needles (3.0 g). ^{1}H NMR (D_2O, 1.0 mol L^{-1} $NaNO_2$): δ 5.70 (s, 1H, CH), 2.16 (s, 6H, CH_3). ^{13}C{^{1}H} NMR (D_2O, 1.0 mol L^{-1} $NaNO_2$): δ 193.73 (CO), 100.91 (CH), 28.54 (CH_3) (Found: C, 17.75; H, 2.34; N, 6.70. Calc. for $C_6H_7CoK_2N_2O_9$·H_2O: C, 17.74; H, 2.23; N, 6.90%).

***mer*-(Diethylenetriamine)(acetylacetonato)nitrocobaltate(III) perchlorate, *mer*-[Co(dien)(acac)(NO_2)]ClO_4 (*mer*-7) and s-*fac*-(diethylenetriamine)(acetylacetonato)nitrocobaltate(III) perchlorate, [Co(dien)(acac)(NO_2)]ClO_4 (s-*fac*-7).** To an intimate mixture of finely ground, recrystallised *trans*-K_2[Co(acac)(CO_3)(NO_2)$_2$]·H_2O **6** (2.0 g, 4.92 mmol) and activated charcoal (0.75 g) was added a solution of dien·3HCl (0.80 g, 3.76 mmol) in water (25 mL). The mixture was stirred for 1 h then filtered through Celite and the filtrate diluted to 300 mL with water. This solution was loaded onto a Sephedex SP-C25 cation exchange column (4 × 12 cm) and the column washed with water. An aqueous solution of $LiClO_4$ (0.05 mol L^{-1}) eluted a red–orange band which broadened as it was eluted, but did not resolve into two bands. The first, major portion of the band was collected until K^+ could be detected by precipitation using an aqueous solution of $Na_3[Co(NO_2)_6]$. The remaining portion of the red–orange band was collected separately. The first fraction was concentrated to a volume of *ca*. 10 mL under reduced pressure, and cooled at 4 °C overnight. Orange crystals of *mer*-[Co(dien)(acac)(NO_2)]ClO_4 (*mer*-**7**) (0.35 g, 21.6%) were collected and washed with a small amount of ice-cold water and then extensively with Et_2O, and dried in a desiccator. A sample for analysis was recrystallised from hot water. The second fraction was collected and treated as above, to give a crystalline solid which was comprised of $KClO_4$, *mer*-**7** and s-*fac*-**7**. Successive recystallisation of this solid from water allowed removal of the less soluble *mer*-**7** and $KClO_4$. Filtration and concentration of the filtrate gave pure s-*fac*-[Co(dien)(acac)(NO_2)]ClO_4 (s-*fac*-**7**) as large red crystals (0.088 g, 5.8%).

mer-[Co(dien)(acac)(NO_2)]ClO_4 (*mer*-**7**): ^{1}H NMR (D_2O): δ 5.75 (s, 1 H, CH), 3.20 (m, 4 H, CH_2NHCH_2), 3.04 (m, 2 H, CH_2NH_2), 2.64 (m, 2 H, CH_2NH_2), 2.17 (s, 3H, CH_3CO), 2.132 (s, 3H, CH_3CO). ^{13}C{^{1}H} NMR (D_2O): δ 195.10, 194.09 (CO), 101.91 (CH), 51.99 (CH_2NHCH_2), 49.04 (CH_2NH_2), 29.20, 28.91 (CH_3CO) (Found: C, 26.55; H, 4.96; N, 13.76; Cl, 8.73. Calc. for $C_9H_{20}N_4O_8ClCo$: C, 26.58; H, 4.96; N, 13.78; Cl, 8.72%).

s-*fac*-[Co(dien)(acac)(NO_2)]ClO_4 (s-*fac*-**7**): ^{1}H NMR (D_2O) δ 5.75 (s, 1 H, CH), 3.20 (m, ^2J = 13.0 Hz, 4 H, CH_2NHCH_2), 3.04 (d, ^3J = 7.2 Hz, 2 H, CH_2NH_2), 2.64 (d, ^3J = 7.2 Hz, 2 H, CH_2NH_2), 2.16 (s, 6 H, CH_3CO). ^{13}C{^{1}H} NMR (D_2O): δ 193.73 (CO), 100.82 (CH), 54.36 (CH_2NHCH_2), 45.37 (CH_2NH_2), 28.91 (CH_3CO) (Found: C, 26.68; H, 5.15; N, 13.66; Cl, 8.72. Calc. for $C_9H_{20}ClCoN_4O_8$: C, 26.59; H, 4.96; N, 13.78; Cl, 8.72%).

***mer*-(*N*,*N*-Diethyldiethylenetriamine)(acetylacetonato)nitrocobaltate(III) perchlorate, *mer*-[Co(DED)(acac)(NO_2)]ClO_4 8.** To an intimate mixture of ground, recrystallised *trans*-K_2[Co(acac)(CO_3)(NO_2)$_2$]·H_2O **6** (0.30 g, 0.738 mmol) and activated charcoal (0.12 g) was added a solution of DED·3HCl (0.20 g, 0.744 mmol) in MeOH (13 mL). The mixture was stirred for 2 h, $NaClO_4$·H_2O (210 mg) was added to precipitate most of the K^+, then filtered through Celite and the filtrate diluted to 300 mL with water. This solution was loaded onto a Sephedex SP-C25 cation exchange column and the column washed with water. An aqueous solution of $NaClO_4$ (0.05 M) eluted a magenta colored band which was collected, concentrated to a volume of *ca*. 10 mL under reduced pressure, and cooled at 4 °C overnight. Magenta colored crystals of *mer*-[Co(DED)(acac)(NO_2)]ClO_4 **8** were collected and washed with a small amount of ice-cold water and then extensively with Et_2O, and dried in a desiccator (0.14 g, 41%). ^{1}H NMR (D_2O): δ 5.91 (s, 1H, CH), 3.43 (dt, ^2J = 13.0, ^3J = 3.8 Hz, 1H, CH_2NR_2), 3.08 (dt, ^2J = 13.5, ^3J = 3.9 Hz, 1H, $CH_2CH_2NR_2$), 3.02 (dt, ^2J = 12.5, ^3J = 3.7 Hz, 1H, CH_2NR_2), 2.92 (dd, ^2J = 13.5, ^3J = 3.8 Hz, 1H, $CH_2CH_2NR_2$), 2.69 (m, 4H, $CH_2CH_2NH_2$), 2.59 (dd, ^2J = 12.8, ^3J = 7.1 Hz, 1H, CH_2CH_3), 2.52 (m, 2H, CH_2CH_3), 2.39 (dq, ^2J = 14.0, ^3J = 7.2 Hz, 1H, CH_2CH_3), 2.28 (s, 3H, CH_3CO), 2.23 (s, 3H, CH_3CO), 1.06 (t, ^3J = 7.2 Hz, 3H, CH_3), 0.92 (t, ^3J = 7.1 Hz, 3H, CH_3). ^{13}C{^{1}H} NMR (D_2O): δ 195.96, 193.39 (CO), 102.85 (CH), 63.64 (CH_2NR_2), 55.85, 52.04 (CH_2NHR); 50.12 (CH_2NH_2); 48.79, 48.74 (CH_2CH_3); 29.14, 29.07 (CH_3CO); 11.11, 9.32 (CH_3) (Found: C, 33.57; H, 6.02; N, 11.82; Cl, 7.96. Calc. for $C_{13}H_{28}ClCoN_4O_8$: C, 33.74; H, 6.10; N, 12.11; Cl, 7.66%). The compound was determined by analytical HPLC to be 99.5% pure.

Table 1 Crystal data and structure refinement for *mer*-[Co(DCD)(acac)(NO_2)]ClO_4 **10** and *trans*-[Co(η^2-DCD)(acac)(NO_2)$_2$] **9**

	mer-[Co(DCD)(acac)(NO_2)]ClO_4 **10**	*trans*-[Co(η^2-DCD)(acac)(NO_2)$_2$] **9**
Formula	$C_{13}H_{26}Cl_3CoN_4O_8$	$C_{13}H_{26}Cl_2CoN_5O_6$
M	531.66	478.22
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$
T/K	293(2)	293(2)
λ/Å	0.71069	0.71069
a/Å	15.7714(3)	19.242(14)
b/Å	11.4969(2)	7.475(3)
c/Å	12.75700(10)	14.545(6)
α/°	90	90
β/°	108.74(1)	103.42(5)
γ/°	90	90
V/Å^3	2191.35(6)	2034.9(19)
Z	4	4
D_c/g cm^{-3}	1.611	1.561
μ/mm^{-1}	1.195	1.145
Measured reflections	13351	3592
Unique reflections (R_{int})	4817 (0.026)	3572 (0.026)
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0618$, $wR_2 = 0.1545$	$R_1 = 0.0790$, $wR_2 = 0.1912$
R indices (all data)	$R_1 = 0.0852$, $wR_2 = 0.1726$	$R_1 = 0.1915$, $wR_2 = 0.2353$

***trans*-[η^2-*N*,*N*-Bis(2-chloroethyl)diethylenetriamine](acetylacetonato)dinitrocobaltate(III), *trans*-[Co(η^2-DCD)(acac)(NO_2)$_2$] 9.** To a stirred suspension of recrystallised *trans*-K_2[Co(acac)(CO_3)(NO_2)$_2$]·H_2O **6** (0.30 g, 0.746 mmol) in dry MeOH (16 mL) was added DCD·3HCl (0.25 g, 0.744 mmol) and activated charcoal (0.11 g). The mixture was stirred for 45 min at 20 °C then $NaClO_4$·H_2O (0.21 g.) was added (to precipitate most of the K^+ as $KClO_4$) and then filtered through Celite. The charcoal and Celite were washed with small amounts of MeOH and water, and the washings were added to the filtrate. The filtrate was diluted to 250 mL with water and cooled whereupon a yellow–orange microcrystalline solid slowly formed. Filtration gave *trans*-[Co(η^2-DCD)(acac)(NO_2)$_2$] **9** (15 mg, 4.2%) which was washed with cold water and then repeatedly with dry Et_2O and dried over silica gel. ^{1}H NMR ($CDCl_3$): δ 5.7 (br s, 1H, NH_2), 5.49 (s, 1H, CH), 5.2 (br s, 1H, NH_2), 3.68 (m, 4H, CH_2Cl), 3.38 (br m, 2H, C$H_2CH_2NH_2$), 3.04 (m, 4H, CH_2CH_2Cl), 2.86 (m, 2H, CH_2NH_2), 2.55 (br m, 2H, CH_2NR_2), 2.21 (s, 3H, CH_3), 2.2 (br m, 2H, C$H_2CH_2NR_2$), 2.06 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 98.17 (CH), 55.73 (CH_2CH_2Cl), 52.09 (CH_2NR_2), 50.37 (C$H_2CH_2NH_2$), 47.93 (C$H_2CH_2NR_2$), 42.66 (CH_2NH_2), 41.91 (CH_2Cl), 26.98, 26.30 (CH_3CO). FAB^+ mass spectrum (*m*-NBA) *m*/*z* (%): 435, 433, 431 ($M^+ - NO_2$, 15, 65, 100; $^{37}Cl_2$, $^{37}Cl^{35}Cl$, $^{35}Cl_2$); 389, 387, 385 ($M^+ - 2NO_2$, 15, 60, 100; $^{37}Cl_2$, $^{37}Cl^{35}Cl$, $^{35}Cl_2$) (Found: C, 32.23; H, 5.57; N, 14.61. Calc. for $C_{13}H_{26}Cl_2CoN_5O_6$: C, 32.65; H, 5.48; N, 14.64%). The compound was determined by analytical HPLC to be 95.6% pure.

***mer*-[*N*,*N*-Bis(2-chloroethyl)diethylenetriamine](acetylacetonato)nitrocobaltate(III) perchlorate *mer*-[Co(DCD)(acac)(NO_2)]ClO_4 10.** The tridentate DCD complex was isolated from the filtrate in the above preparation of **9**. The filtrate was loaded onto a Sephedex column (2 × 16 cm) and washed with water. Elution was begun with 0.025 mol L^{-1} $NaClO_4$ (*ca.* 200 mL) and then with 0.05 mol L^{-1} $NaClO_4$ until the first band was collected. In this way, the residual K^+ remaining after the precipitation of $KClO_4$ was not eluted (no precipitate with aqueous $Na_3[Co(NO_2)_6]$ solution) with the product which precluded difficulties with cocrystallisation of $KClO_4$. The magenta coloured solution (*ca.* 100 mL) of the first band eluted from the column was evaporated to small volume (*ca.* 8 ml) under reduced pressure and cooled at 4 °C for a week. The crystals that formed were filtered off and washed with a little ice-cold water then repeatedly with Et_2O and dried over silica gel to give *mer*-[Co(DCD)(acac)(NO_2)]ClO_4 **10** (0.040 g, 10.1%). From the filtrate and water wash was isolated a further 25 mg (6.3%). Total yield = 16.4%. ^{1}H NMR (acetone-d_6): δ 6.65 (br t, 1H, NH), 5.95 (s, 1H, CH), 4.62, 4.28 (br, 1H, NH_2); (4.10, 3.85), (3.95, 3.70) (m, 1H, CH_2Cl); 3.74, 3.05 (m, 1H, C$H_2CH_2NR_2$); 3.38, 2.70 (m, 1H, CH_2NR_2); 3.20, 2.85 (m, 1H, C$H_2CH_2NH_2$); (3.12, 2.85), (3.10, 2.75) (m, 1H, CH_2CH_2Cl), 2.80, 2.78 (m, 1H, CH_2NH_2); 2.312, 2.307 (s, 3H, CH_3). ^{13}C NMR (acetone-d_6): δ 194.24, 191.72 (CO); 101.02 (CH), 62.66 (CH_2NR_2), 60.27, 54.01 (CH_2CH_2Cl); 50.62 (CH_2NH_2), 48.83, 48.11 (CH_2NHCH_2); 38.00, 37.35 (CH_2Cl); 27.55, 27.47 (CH_3CO). FAB^+ mass spectrum (*m*-NBA) *m*/*z* (%): 435, 433, 431 (M^+, 15, 65, 100; $^{37}Cl_2$, $^{37}Cl^{35}Cl$, $^{35}Cl_2$); 389, 387, 385 ($M^+ - NO_2$, 13, 67, 100; $^{37}Cl_2$, $^{37}Cl^{35}Cl$, $^{35}Cl_2$). High resolution FAB^+ mass spectrum (*m*-NBA) *m*/*z*: M^+($^{37}Cl_2$): calc. 435.0604; found 435.0596; M^+($^{35}Cl^{37}Cl$): calc. 433.0634; found 433.0651; M^+($^{35}Cl_2$): calc. 431.0663; found 431.0666.

X-Ray crystal structure determinations

A summary of crystal data, structure solutions and refinements is given in Table 1. After initial isotropic refinement, anisotropic thermal parameters were refined for all non-hydrogen atoms. Hydrogen atoms were refined using the riding model, except for the positions of the hydrogens on N(1) and N(2) in **10** which were refined individually.

CCDC reference number 186/1832.

See http://www.rsc.org/suppdata/dt/a9/a909447d/ for crystallographic files in .cif format.

Cytotoxicity testing

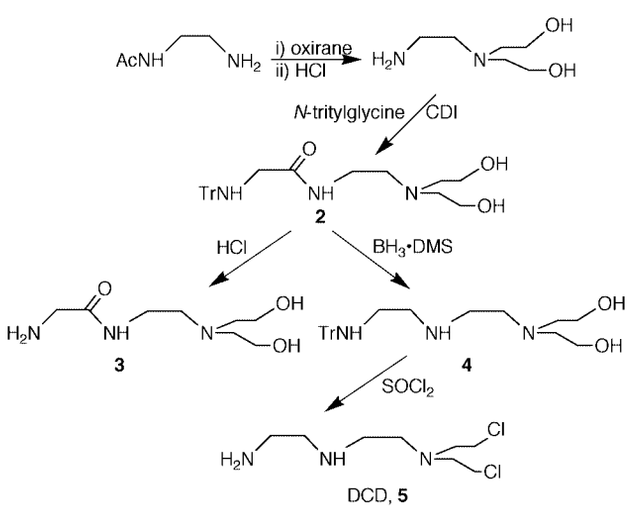
Cell lines were maintained and tested in αMEM with 5% fetal bovine serum. Stock solutions of compounds were prepared in acetone and stored at −80 °C (**9**,**10**), or were dissolved in culture medium immediately before use. Inhibition of cell proliferation was tested using log phase cultures in 96 well trays, using 18 h exposure to drugs and growth for a further 4 days before staining with methylene blue as previously.[18] The IC_{50} was defined as the drug concentration required to lower cell density to 50% of controls on the same plate. Kinetics of cell killing, as assessed by clonogenic assay, was compared under aerobic and anoxic (<10 ppm O_2) conditions by gassing stirred cell suspensions with 5% CO_2 in air or N_2, respectively, as described previously.[19] Drug concentrations were chosen that gave similar rates of killing, and the drug concentration × time to reduce cell survival to 10% of controls (CT_{10}) was determined as an inverse measure of cytotoxic potency.

Results and discussion

Syntheses

The preparation of cobalt(III) complexes containing multidentate nitrogen mustard ligands presents several synthetic challenges. Firstly, the inert nature of the cobalt(III) center, crucial to the design of the complexes for cancer chemotherapy, results in slow substitution reactions and consequent difficulties when the incoming ligand is reactive. This is the case for the mustard ligands which are susceptible to hydrolysis or solvolysis in their free base forms, and are therefore stored and handled as their hydrochloride salts. Deprotonation of these salts is an integral part of the coordination process. The mustard ligands themselves are not trivial to prepare, and ideally should be introduced into the coordination sphere at the last step of the preparation procedure. The unsubstituted parent tridentate triamine dien and the non-alkylating analog DED were used to develop the synthetic routes and refine the preparative conditions prior to synthesizing the mustard complexes containing the DCD ligand. Although DED and dien do not undergo corresponding decomposition reactions to DCD in their free base forms, the ligands were used as the trihydrochloride salts in the syntheses in order to model the reaction conditions for the much more reactive mustard DCD. Complexes of the non-toxic analogs are also useful as controls in the biological evaluation.

DED and dien are commercially available, and were converted to hydrochloride salts prior to their use in complexation reactions. The novel mustard DCD was prepared as outlined in Scheme 1. CDI-induced coupling of *N*-tritylglycine and



Scheme 1

N,*N*-bis(2-hydroxyethyl)ethylenediamine gave a good yield of the carboxamide **2** (characterized in part as the deprotected carboxamide **3**) which was reduced with borane–methyl sulfide complex to give the *N*-trityltriaminediol **4**. Reaction of this with thionyl chloride hydrolyzed the trityl group and converted the diol to the mustard DCD **5**, isolated as the trihydrochloride salt DCD·3HCl.

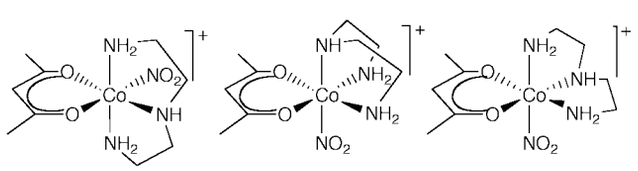
Stereochemistry is a further important consideration in the preparation of mixed ligand cobalt(III) complexes. An octahedral complex containing a flexible tridentate triamine ligand such as diethylenetriamine (dien) may adopt *fac* or *mer* geometry, Δ or Λ configuration at the cobalt center and, for the *mer* isomer, (*R*) or (*S*) configuration at the secondary amine. In addition, the unsymmetrical nature of the substituted triamines DCD and DED means that additional diastereomers are potentially possible, especially when the other three ligands on cobalt are not identical.

There are a few reported examples of cobalt(III) complexes containing N-substituted dien derivatives as ligands. The conventional synthesis of cobalt(III) complexes, oxidation of cobalt(II) in the presence of the ligands, can lead to oxidative dealkylation of the susbstituents on nitrogen, and in any case can give rise to mixtures when mixed-ligand complexes are desired. We investigated the reaction of $[Co(NO_2)_6]^{3-}$ with dien, but the rate of substitution was too slow and, when the mustard ligand was used, the reaction could not compete with decomposition of the mustard.

The precursor complex we have previously utilized for the preparation of cobalt complexes containing bidentate mustard ligands was *trans*-$[Co(Racac)_2(NO_2)_2]^+$, which undergoes relatively fast substitution reactions for a cobalt(III) center.[6,7] Reactions with the neutralized hydrochloride salts of bidentate mustards (L) gave products $[Co(Racac)_2(L)]^+$, including the lead compound $[Co(DCE)(Meacac)_2]^+$ **1**. A closely related precursor complex is $K_2[Co(acac)(CO_3)(NO_2)_2]\cdot H_2O$ **6** which had been reported in the literature and assigned as the isomer containing *cis*-NO_2 ligands.[17] In the original preparation the product was isolated from the reaction mixture and characterized without further purification. Like $Na[Co(acac)_2(NO_2)_2]\cdot H_2O$, the complex is subject to decomposition in aqueous solution but is stabilized by the presence of excess NO_2^- ions, and can be recrystallised under these conditions. We observed that the acac ligand exhibits two equivalent CH_3 groups in the NMR spectrum, consistent with *trans* stereochemistry rather than the *cis* geometry proposed in the original report.

trans-$K_2[Co(acac)(CO_3)(NO_2)_2]\cdot H_2O$ **6** also exhibits relatively rapid substitution chemistry, and as such proved to be useful for the preparation of the target tridentate amine and nitrogen mustard complexes. The amines are utilized as the trihydrochloride salts, and the reactions proceed without the requirement for added base since the carbonato ligand acts as a base, neutralising two equivalents of acid per mustard ligand, and is itself labilised in the process. The precursor complex is somewhat soluble in MeOH, allowing the substitution reactions to proceed in this medium, so long as the amine hydrochloride salts are also sufficiently soluble which is the case for DED·3HCl and DCD·3HCl, but not dien·3HCl. Another factor which results in improved yields of the mustard complexes is that solvolysis of the free mustard ligand is slower in MeOH than in aqueous solution. In addition, the substitution reactions are expected to proceed by a dissociative mechanism, and the MeOH ligand in an intermediate solvato complex would be more readily displaced by the incoming mustard nitrogen than an aqua ligand.

trans-$K_2[Co(acac)(CO_3)(NO_2)_2]\cdot H_2O$ **6** reacts with dien·3HCl in the presence of a charcoal catalyst to produce the cationic complex $[Co(dien)(acac)(NO_2)]^+$ **7**. Purification by cation exchange chromatography serves to separate the monocations from other products, but does not allow complete separation of the one *mer* and two *fac* isomers possible for this complex. ^1H NMR spectroscopy of the product after chromatography shows a mixture of isomers, which could be assigned (after subsequent isolation of the pure diastereomers) to the *mer* isomer (the predominant product), the s-*fac* isomer and a very small amount of a third compound, presumably the u-*fac* isomer. The perchlorate salts of the *mer* and s-*fac* isomers can be separated by fractional crystallization, with the less soluble isomer *mer*-$[Co(dien)(acac)(NO_2)]ClO_4$ (*mer*-**7**) crystallizing first. Pure s-*fac*-$[Co(dien)(acac)(NO_2)]ClO_4$ (s-*fac*-**7**) can be isolated as the second fraction to crystallize. The *mer*, s-*fac* and u-*fac* isomers



Isomers of $[Co(dien)(acac)(NO_2)]^+$

are clearly distinguished by ^{1}H and ^{13}C NMR spectroscopy on the basis of the symmetry differences. The *mer* isomer contains the acac, NO_2 and secondary nitrogen of the dien ligand in a plane of symmetry, and has inequivalent acac methyl groups. In the s-*fac* isomer the plane of symmetry contains the secondary nitrogen but bisects the acac group, and thus the two acac methyl groups are equivalent. The u-*fac* isomer has no symmetry planes or axes and thus has no equivalent resonances in the NMR spectra.

The reaction of DED·3HCl with *trans*-K_2[Co(acac)-$(CO_3)(NO_2)_2]\cdot H_2O$ **6** proceeds under very mild conditions, in MeOH for 2 h at room temperature in the presence of charcoal. The product comprises a single stereoisomer, *mer*-[Co(DED)-$(acac)(NO_2)]^+$ **8**, isolated in 41% yield as the ClO_4^- salt following cation exchange chromatography. The assignment of the *mer* stereochemistry was made by comparison of its NMR data with that of the corresponding DCD complex (*vide infra*). DED contains a tertiary amine donor and is a more sterically demanding ligand than dien, and presumably this results in significant destabilisation of the *fac* isomers.

The corresponding reaction of DCD·3HCl with *trans*-K_2[Co(acac)$(CO_3)(NO_2)_2]\cdot H_2O$ **6** was carried out in a shorter reaction time (45 min). Dilution of the reaction mixture with water prior to cation exchange chromatography resulted in crystallization of a neutral complex in low yield, identified as the bidentate DCD complex *trans*-Co(η^2-DCD)$(acac)(NO_2)_2$ **9**, which contains the triamine ligand coordinated through only the primary and secondary nitrogen donor atoms. Cation exchange chromatography of the material remaining in solution and elution with 0.05 mol L^{-1} $NaClO_4$ solution gave the tridentate complex *mer*-[Co(DCD)$(acac)(NO_2)]ClO_4$ **10** containing the η^3-DCD ligand coordinated through all three nitrogens.

The DED complex (**8**) and the bidentate (**9**) and tridentate (**10**) DCD complexes were characterised by ^{1}H and ^{13}C NMR spectroscopy and FAB mass spectrometry. The identities of the two DCD complexes were further established by X-ray crystal structure determinations. These confirmed for *trans*-[Co(η^2-DCD)$(acac)(NO_2)_2]$ **9** the *trans*-NO_2 ligands and the bidentate nature of the DCD ligand with the tertiary mustard nitrogen not coordinated to cobalt (Fig. 1), and for *mer*-[Co(DCD)-$(acac)(NO_2)]ClO_4$ **10** the *mer* arrangement and stereochemistry at the secondary nitrogen of the DCD ligand (Fig. 2). The crystal structure of the tridentate DCD complex (**10**) shows a hydrogen bonding interaction between the hydrogen on the secondary nitrogen of the DCD ligand and one of the oxygen atoms of the NO_2 ligand, which may explain why only this diastereomer is observed.

X-Ray crystal structure determinations

The Co–N bond lengths to the primary and secondary nitrogens in **9** and **10** are similar, averaging 1.937 Å for *mer*-[Co(DCD)$(acac)(NO_2)]ClO_4$ **10** and 1.948 Å for *trans*-[Co(η^2-DCD)$(acac)(NO_2)_2]$ **9**. However the tertiary amine Co–N(3) distance of 2.114(4) Å in **10** is exceptionally long. Related complexes which contain a coordinated terminal tertiary amine (*i.e.* not constrained as part of a macrocycle) are [Co(DCE)-$(Meacac)_2]ClO_4$ (**1**)[20] and $[Co(L)]PF_6$,[21] which contains an acylic hexadentate ligand (L) incorporating a diethylamine donor. The tertiary amine Co–N distances in these molecules are 2.092(4) and 2.082(4) Å, respectively. The long Co–N distances arise as a consequence of the steric bulk of the coordinated tertiary amine. This is also evident from the N(3)–Co–O(2) angle in **10** which is 97.3(1)°.

The plane of the NO_2 ligand is not constrained to a particular orientation for electronic reasons. However, in *mer*-[Co(DCD)$(acac)(NO_2)]ClO_4$ **10** it is oriented such that it is aligned with the O(2)–Co–N(2) plane. The hydrogen atom H(2NA) on N(2), the orientation of which is determined by the configuration at N(2), was located crystallographically and points towards O(4) of the NO_2 ligand, indicative of an N(2)–H(2NA)–O(4) hydrogen bond. The N(2)–H(2NA) and H(2NA)···O(4) distances of 0.85 and 2.16 Å, respectively, are consistent with this. A similar situation occurs for the orientation of one NO_2 ligand in **9**, with an N(1)–H(1NA)–O(3) hydrogen bond (corresponding distances are 0.90 and 2.17 Å).

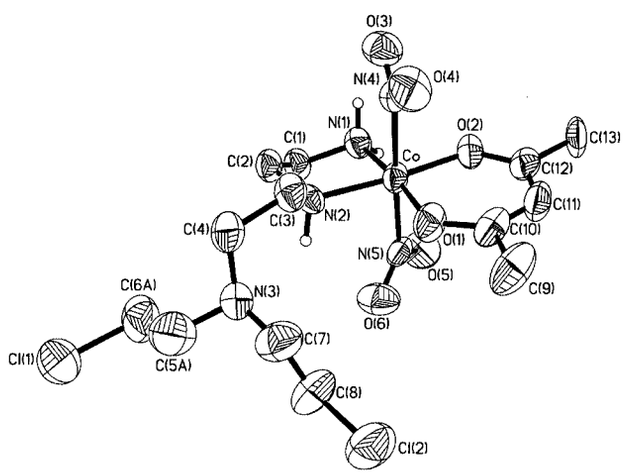


Fig. 1 ORTEP view and atomic labeling scheme of the *trans*-[Co(η^2-DCD)$(acac)(NO_2)_2]$ cation (**9**). Selected bond lengths (Å): Co–O(1) 1.869(7), Co–O(2) 1.887(6), Co–N(1) 1.932(8), Co–N(5) 1.946(8), Co–N(4) 1.954(8), Co–N(2) 1.965(7).

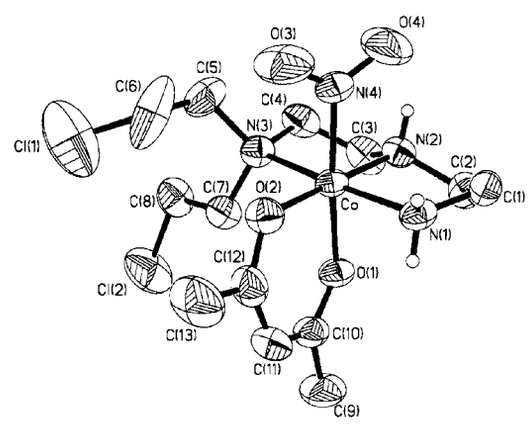
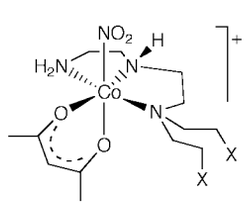
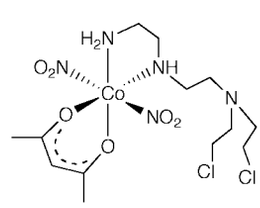


Fig. 2 ORTEP view and atomic labeling scheme of the *mer*-[Co-(DCD)$(acac)(NO_2)]ClO_4$ cation (**10**). Selected bond lengths (Å): Co–O(2) 1.885(3), Co–O(1) 1.913(3), Co–N(4) 1.923(3), Co–N(2) 1.927(4), Co–N(1) 1.946(4), Co–N(3) 2.114(4).





8 X = H, *mer*-[Co(DED)$(acac)(NO_2)]ClO_4$ **9** X = H, *trans*-[Co(η^2–DCD)$(acac)(NO_2)_2]$
10 X = Cl, *mer*-[Co(DCD)$(acac)(NO_2)]ClO_4$

Table 2 Biological activity of cobalt mustard complexes and the corresponding free mustard ligands

No.	Compound	Growth inhibition assay: IC_{50}[a]/μM AA8	Growth inhibition assay: IC_{50}[a]/μM UV4	Clonogenic assay: CT_{10}(air)[b]/μM h^{-1}	Clonogenic assay: Air/N_2 ratio[c]
Mustard ligands					
	DCE	1.44 ± 0.26	0.055 ± 0.003		
5	DCD	50.0 ± 1	2.67 ± 0.02		
Co complexes					
8	$[Co(DED)(acac)(NO_2)]ClO_4$	3710 ± 410	3150 ± 20	44800	1.0
10	$[Co(DCD)(acac)(NO_2)]ClO_4$	750 ± 100	35.4 ± 9	262	5.2
9	$[Co(\eta^2\text{-DCD})(acac)(NO_2)_2]$	27	1.5		
1	$[Co(DCE)(Meacac)_2]ClO_4$	4.6 ± 0.6	0.113 ± 0.019	2.34	20.3

[a] IC_{50} values determined against aerobic cells, using an exposure time of 18 h. Values are means ± SEM (standard error of the mean). Values without SEM are for a single determination only. [b] CT_{10}: drug concentration (μM) × time (h) required to reduce cell survival to 10% of controls under hypoxic conditions, using UV4 cells in a clonogenic assay (see text). [c] Ratio of CT_{10} values in air and N_2 [CT_{10}(air)/CT_{10}(nitrogen)].

Electrochemistry

The substitutional lability of cobalt(II) often leads to irreversible electrochemical behavior of cobalt(III) complexes due to ligand loss from the reduced Co(II) form. Non-aqueous solvents are often employed in an attempt to slow solvolysis reactions of the reduced Co(II) complex in order to enhance chemical reversibility for the Co(III)–Co(II) couple. However, even in MeCN solution, irreversible behavior is observed for $[Co(DCE)(Meacac)_2]ClO_4$ **1** and the related tropolonate complexes.[7,10] The chelate effect can also improve electrochemical reversibility, with multidentate ligands slowing down the rate of ligand loss from Co(II). However, when the electrochemistry of *mer*-$[Co(dien)(acac)(NO_2)]ClO_4$ (*mer*-**7**), *mer*-$[Co(DED)(acac)(NO_2)]ClO_4$ **8** and *mer*-$[Co(DCD)(acac)(NO_2)]ClO_4$ **10** were investigated in MeCN, both cyclic voltammetry (CV) and Osteryoung square wave voltammetry (OSWV, step $E = 4$ mV, SW amplitude = 25 mV, SW frequency = 15 Hz) showed completely irreversible behavior ($i_a \approx 0$, *i.e.* no reoxidation wave observed by CV), even though **7**, **8** and **10** contain tridentate rather than bidentate ligands. For each complex the cathodic peak potentials (E_{pc}) were similar in both the CV and OSWV experiments, using both glassy carbon and platinum working electrodes. OSWV at glassy carbon gave somewhat more reproducible values for E_{pc} and are reported here: for *mer*-**7**, $E_{pc} = -1.11$ V (*versus* ferrocenium–ferrocene, Fc), for **8**, $E_{pc} = -810$ mV (Fc), and for **10**, $E_{pc} = -670$ mV (Fc). We have found for irreversible couples that while E_{pc} is not thermodynamically meaningful, it does nonetheless have predictive value and can be used to compare similar complexes.[7,9,10] The shift of E_{pc} from −1.11 V for the dien complex *mer*-**7** to −810 mV for the DED complex (**8**) can be attributed to the weaker ligand field of the tertiary nitrogen donor in DED. The shift of 140 mV to a more positive potential on going from the diethyl-substituted complex **8** to the bis(chloroethyl)-substituted complex **10** can be attributed to both electronic and steric effects, probably with the former predominating. The shift to more positive potential is consistent with the shifts observed for the bidentate diamine complexes containing a number of different ancillary ligand sets, including those based on dithiocarbamates, examples of which display nearly reversible electrochemistry. For complexes in which L = DCE or DEE (*N*,*N*-diethylethylenediamine), $\Delta E_p = 175$ mV for $[Co(L)(acac)_2]^+$, $\Delta E_p = 162$ mV for $[Co(L)(trop)_2]^+$, $\Delta E_{\frac{1}{2}} = 155$ mV for $[Co(L)(S_2CNMe_2)_2]^+$ and $\Delta E_{\frac{1}{2}} = 180$ mV for $[Co(L)(S_2CNEt_2)_2]^+$.[7,9,10]

The E_p value for $[Co(DCE)(Meacac)_2]^+$ **1** under these conditions was found to be −780 mV (*vs.* Fc) similar to the reported value in CH_2Cl_2 (−853 mV)[8] and much more negative than the value observed for the DCD complex **10** (−670 mV).

Biological evaluation

The free mustard DCD **5**, the DED complex **8**, and the bidentate and tridentate DCD complexes **9** and **10** were evaluated for growth inhibitory properties (IC_{50} values) in two cell lines in culture, and the results are summarized in Table 2. AA8 is a Chinese hamster ovary fibroblast line, and the related mutant subline UV4 is deficient in nucleotide excision repair, and it is therefore hypersensitive to agents which form bulky DNA monoadducts or crosslinks.[22] The ratio of IC_{50} values in these two lines, (IC_{50}(AA8)/IC_{50}(UV4)) therefore provides some information about the mechanism of cytotoxicity, with ratios significantly > 10 implying DNA-crosslinking as the primary cytotoxic event.

The tridentate mustard DCD proved to be much less cytotoxic than the corresponding bidentate analogue DCE used previously (see Table 2). The reason for this is not known; while both would be expected to have similar chemical reactivity, the (tris-cationic) tridentate analogue DCD may have slower kinetics of cell uptake. However, both had similar AA8/UV4 IC_{50} ratios (19- *vs.* 26-fold), suggesting that DNA crosslinking is the major mode of cytotoxicity in both cases. As expected, the model non-mustard complex **8** had very low cytotoxicity (IC_{50} > 3 mM), and showed little differential between AA8 and UV4. The tridentate DCD complex **10** was significantly less toxic than the free mustard DCD in both cell lines, showing that complexation of the mustard nitrogen results in significant deactivation. Complex **10** also had a high AA8/UV4 IC_{50} ratio (21-fold), indicating that aerobic cytotoxicity was due to a DNA cross-linking event; probably release of the free mustard to a small extent. The bidentate DCD complex **9**, where the mustard nitrogen is not complexed to the metal, retained high cytotoxicity (comparable to that of the free mustard DCD).

The hypoxic selectivities of **1**, **8** and **10** (sufficient quantities of **9** were not available) were evaluated in stirred suspension cultures of UV4 cells, using a clonogenic assay following a 4 h drug exposure under either aerobic (5% CO_2 in air) or hypoxic (5% CO_2 in N_2) conditions[23] (Table 2). Under these conditions, the bidentate mustard complex $[Co(DCE)(Meacac)_2]^+$ **1** showed an air/N_2 ratio of *ca.* 20 (Table 2).[6–8] As expected, the tridentate non-mustard complex **8** was very non-potent, and had a ratio of unity. In contrast, the tridentate mustard complex **10** showed a 5-fold selectivity for hypoxic conditions (Table 2).

All of the bidentate mustard complexes containing substituted acac ligands show hypoxia selectivity, with the maximum value observed for the Meacac derivative **1**. The tridentate mustard complex **10** continues this trend, and although the hypoxia selectivity is not as high as that measured for **1**, it is better than the series of bidentate mustard complexes containing dithio-

carbamate or tropolonate ligands (for example [Co(DCE)-$(S_2CNEt_2)_2$]BPh_4 and [Co(DCE)$(trop)_2$]ClO_4) for which no hypoxia selectivity was observed.[9,10]

Conclusions

A cobalt(III) complex containing the tridentate mustard ligand DCD was successfully prepared, although the difficulty in coordinating the bulky tertiary amine moiety in the ligand is demonstrated by the isolation, in low yield, of a complex containing the DCD ligand coordinated in a bidentate fashion, bearing the mustard nitrogen atom as a pendant arm. Cell culture studies show that the cytotoxicity of the mustard is greatly diminished in the tridentate complex, where the mustard is deactivated through coordination to the metal, but not in the bidentate complex where the mustard nitrogen remains uncomplexed. The cytotoxicity of the tridentate mustard DCD is more effectively masked upon complexation to cobalt(III) than is observed for the bidentate mustard DCE. However the tridentate mustard complex **10** is less selective under hypoxic conditions. A possible reason for this is that the higher potential of **10** relative to **1** might reduce the ability of O_2 to compete against **10** for reducing equivalents.[13]

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