Acetate as a model for aspartate-based CXCR4 chemokine receptor binding of cobalt and nickel complexes of cross-bridged tetraazamacrocycles


A number of disease states including WHIM syndrome, HIV infection and cancer have been linked to the chemokine receptor CXCR4. High-affinity CXCR4 antagonist transition metal complexes of configurationally restricted bis-tetraazamacrocyclic ligands have been identified in previous studies. Recently synthesised and structurally characterised Co²⁺/Co³⁺ and Ni²⁺ acetate complexes of mono-macrocycle cross-bridged ligands have been used to mimic their known coordination interaction with the aspartate side chains on binding to CXCR4. Here, X-ray crystal structures for three Co²⁺/Co³⁺ acetate complexes and five Ni²⁺ acetate complexes are presented and demonstrate flexibility in the mode of binding to the acetate ligand concomitantly with the requisite cis-V-configured cross-bridged tetraazamacrocycle. Complexes of the smaller Co³⁺ metal ion exclusively bind acetate by chelating both oxygens of acetate. Larger Co²⁺ and Ni²⁺ metal ions in cross-bridged tetraazamacrocycles show a clear tendency to coordinate acetate in a monodentate fashion with a coordinated water molecule completing the octahedral coordination sphere. However, in unbridged tetraazamacrocycle acetate structures reported in the literature, the coordination preference is to chelate both acetate oxygens. We conclude that the short ethylene cross-bridge restricts the equatorial bulk of the macrocycle, prompting the metal ion to fill the equator with the larger monodentate acetate plus water ligand set. In unbridged ligand examples, the flexible macrocycle expands equatorially and generally only allows chelation of the sterically smaller acetate alone. These results provide insight for generation of optimised bis-macrocyclic CXCR4 antagonists utilising cobalt and nickel ions.

Introduction

The topological complexity of cross-bridged tetraazamacrocycles (Figure 1) imparts rigidity and kinetic stability to their transition metal complexes. For this reason, these complexes have been utilised in applications where complex stability is paramount, such as aqueous oxidation catalysis and medical imaging. Another important property of these ligands is that the short cross-bridge restricts the configuration of the complex to a folded, cis geometry where the macrocycle takes up axial and cis-equatorial positions of octahedral, square-pyramidal, or trigonal bipyramidal coordination geometries (Figure 1). Open coordination sites must be located cis to each other, which is important in oxidation applications and has been
exploited more recently in producing optimised protein-binding complexes.\textsuperscript{16, 17}

We have taken advantage of these properties by designing bis-linked cross-bridged tetraazamacrocycle metal complexes (\textbf{Figure 2}) that have remarkably efficient binding\textsuperscript{16, 18-20} to the aspartate side chains of the CXCR4 chemokine receptor, a trans-membrane receptor important to the fusion process of the HIV virus to leukocytes,\textsuperscript{17} the metastasis of cancer cells,\textsuperscript{21} and other biological processes.\textsuperscript{22} Most relevant to this work, we have shown that a dinickel complex, the meta analogue of ligand 7 (\textbf{Figure 3}) was similarly efficient as AMD3100 at binding CXCR4, with an IC\textsubscript{50} of 14 nM.\textsuperscript{19} As part of our CXCR4 antagonist program, we have attempted to probe the aspartate-metal ion interaction by synthesizing acetate salts of cross-bridged complexes. The main aim of this work is to study physicochemical parameters of components of compounds that are likely to be of relevance to CXCR4 antagonist design. Our goal was to produce single crystals suitable for X-ray diffraction that contain acetate ligands bound to the metal ion as a model for the aspartate-metal ion interaction occurring in the biological system. From these structures, we hoped to gain an understanding of the geometric and electronic requirements for producing strong-binding CXCR4 antagonists.

Because of the significant challenges in production of X-ray quality bis-linked tetraazamacrocycle complex crystals,\textsuperscript{18, 23} single-macrocycle transition metal complexes are often used as models.\textsuperscript{16, 18-20, 23} To provide the most accurate model for our bis-macrocycle antagonists, which are linked through a xylene linker, we have synthesised a number of monobenzyl and dibenzyl\textsuperscript{4} pendant arm containing cross-bridged tetraazamacrocycles (\textbf{Figure 3}). These ligands provide the same cross-bridged macrocycle geometric requirement around the metal ion, including the bulky benzyl group attached to one (or two) of the coordinated nitrogen atoms. In this work, we present the synthesis, characterization, and structural study of these ligands complexed to cobalt and nickel ions, which we are also evaluating in our research to determine the optimal combination of chelator and metal ion for CXCR4 antagonism. Additionally, we report here for the first time the synthesis and CXCR4 binding ability of a dicobalt bis-macroyclic antagonist (Co\textsuperscript{2+})\textsuperscript{7}, for comparison with the mono-macroyclic model complexes, AMD3100, and our known dinickel and dicopper antagonists.

\section*{Experimental}

\textbf{General}

Elemental analyses were performed by Quantitative Technologies Inc. Electrospray Mass spectra were collected at the Oklahoma University Health Sciences Center Laboratory for Molecular Biology and Cytometry Research on a Bruker-Daltonics HCT Ultra ion trap mass spectrometer. NMR spectra were obtained on a Varian Bruker AVANCE II 300 MHz NMR Spectrometer. Electronic spectra were recorded using a Beckman Coulter DU640 UV-Vis Spectrometer. Electrochemical experiments were performed on a BAS100B Electrochemical Analyzer. A button Pt electrode was used as the working electrode with a Pt-wire counter electrode and an Ag-wire pseudo-reference electrode. Scans were taken at 200 mV/s. Acetonitrile solutions of the complexes (1 mM) with tetrabutylammonium hexafluorophosphate (0.1 M) as a supporting electrolyte were used. The measured potentials were referenced to SHE using ferrocene (+0.400 V versus SHE) as an internal standard. All electrochemical measurements were carried out under N\textsubscript{2}.

\textbf{Synthesis}

Anhydrous CoCl\textsubscript{2}, Co(OAc)\textsubscript{2}, and NiCl\textsubscript{2} were purchased from Aldrich and used as received. Anhydrous Ni(OAc)\textsubscript{2} was prepared from Ni(OAc)\textsubscript{2} • 4H\textsubscript{2}O (Fluka) dried under vacuum over refluxing ethanol in an Abderhalden drying pistol until a constant weight was reached, which corresponded to the loss of four equivalents of water.

4,11-dibenzyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (1),\textsuperscript{24} 4,10-dibenzyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (2),\textsuperscript{24}
4-benzyl-11-methyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (3), and 4-benzyl-10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (4),

4,11-dimethyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (5),

4,10-dimethyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (6), and

1,4-bis([11-methyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecan-4-yl]methyl)benzene (7).

General Complexation Procedure for Chloride Complexes

1.00 mmol of the ligand (1-2) and 1.00 mmol of the anhydrous metal(II) chloride salt (Ni or Co) were added to 20 ml of dry DMF in an inert atmosphere glovebox. The reaction was stirred at room temperature for 18 h. Product NiCl₂ precipitated over the course of the reaction. The reaction mixture was removed from the glovebox, filtered to remove any trace solids, and evaporated to dryness. These crude crystals were grown by ether diffusion into the mother liquor. Product NiCl₂: Green-blue powder. Yield: 0.314 g (62%). X-ray quality crystals were obtained. Elemental analysis(%) calcd. NiC₂₆H₃₈N₄Cl₂: C 58.23, H 7.14, N 10.45; Found C 58.28, H 6.30, N 8.15; Found C 49.19, H 6.50, N 8.29. MS (ES) m/z 542.2 [Co(LCl)]⁺.

General Complexation Procedure for Mononuclear Acetate Complexes

1.00 mmol of the ligand (1-6) and 1.00 mmol of the anhydrous metal(II) acetate salt (Ni or Co) were added to 25 ml of dry DMF in an inert atmosphere glovebox. The reaction was stirred at room temperature for 18 h. The crude [ML(OAc)]·[OAc] solution was removed from the glovebox, filtered to remove any trace solids, and evaporated to dryness. These crude products were dissolved in 10 ml of dry methanol, to which was added dropwise a 5 ml dry methanol solution of 5 equivalents (0.815 g, 5.00 mmols) of NH₄PF₆. Powders of the [ML(OAc)]PF₆ salts precipitated, were collected, washed with cold methanol and ether, and dried under vacuum. Samples of [NiL(OAc)]PF₆ and [Ni5(OAc)PF₆] were synthesised as previously reported.

[Co(OAc)]PF₆: Pale pink powder. Yield: 0.416 g (64%). No X-ray quality crystals were obtained. Elemental analysis(%) calcd. [CoC₂₆H₃₈N₄(C₂H₃O₂)]PF₆ • H₂O (760.691 g/mol): C 48.91, H 6.30, N 8.15; Found C 49.19, H 6.50, N 8.29. MS (ES) m/z 524.2 [Co(LOAc)]⁺.

[Co2(OAc)](PF₆)₃: Purple powder. Oxidation to the Co³⁺ compound was again observed for the cyclen-based ligand. Yield: 0.416 g (64%). X-ray quality crystals were obtained from a cooled methanol solution. Elemental analysis(%) calcd. [CoC₂₆H₃₈N₄(C₂H₃O₂)]PF₆ • 0.6H₂O (760.691 g/mol): C 48.91, H 6.30, N 8.15; Found C 49.19, H 6.50, N 8.29. MS (ES) m/z 495.2 [Ni(LOAc)]⁺.

[Co3(OAc)]PF₆: Purple powder. Yield: 0.207 g (35%). No X-ray quality crystals were obtained. Elemental analysis(%) calcd. [CoC₂₆H₃₈N₄(C₂H₃O₂)]PF₆ • 0.4NH₄PF₆ • 1.2H₂O (680.278 g/mol): C 38.84, H 6.08, N 9.06; Found C 39.19, H 6.30, N 8.67. MS (ES) m/z 497.2 [Ni2(ML)(OAc)]²⁺.

[Co4(OAc)](OAc)PF₆: Pale purple powder. Yield: 0.356 g (60%). No X-ray quality crystals were obtained. Elemental analysis(%) calcd. [CoC₂₆H₃₈N₄(C₂H₃O₂)]PF₆ • 0.35NH₄PF₆ (650.266 g/mol): C 40.64, H 5.95, N 9.37; Found C 40.46, H 5.70, N 9.59. MS (ES) m/z 447.2 [Ni3(ML)(OAc)]³⁺.

[Co5(OAc)]PF₆: Bright pink powder. Oxidation to the Co³⁺ compound was again observed for the cyclen-based ligand. Yield: 0.350 g (49%). No X-ray quality crystals were obtained. Elemental analysis(%) calcd. [CoC₂₆H₃₈N₄(C₂H₃O₂)]PF₆ • 0.35NH₄PF₆ (650.266 g/mol): C 40.64, H 5.95, N 9.37; Found C 40.46, H 5.70, N 9.59. MS (ES) m/z 447.2 [Ni3(ML)(OAc)]³⁺.

[Co6(OAc)]PF₆: Pale purple powder. Yield: 0.488 g (86%). X-ray quality crystals were obtained from the evaporation of a methanol solution. Elemental analysis(%) calcd. [CoC₂₆H₃₈N₄(C₂H₃O₂)]PF₆ • 0.35NH₄PF₆ (650.266 g/mol): C 40.64, H 5.95, N 9.37; Found C 40.46, H 5.70, N 9.59. MS (ES) m/z 447.2 [Ni3(ML)(OAc)]³⁺.

[X] = 0.00
methanol, to which was added dropwise a 5 ml dry methanol
dryness. These crude products were dissolved in 10 ml of dry
glovebox, filtered to remove any trace solids, and evaporated to
calcld. \([\text{CoC}_12\text{H}_{26}\text{N}_4(\text{C}_2\text{H}_3\text{O}_2)]\)(PF₆)₂ (634.27 g/mol): C 26.51, H 3.946, N 8.83; Found C 26.51, H 4.48, N 8.81. MS (ES) m/z 372.2 [CoL(OAc)]²⁺.

\([\text{Ni6(OAc)}]PF_6\): Bright pink powder. Yield: 0.386 g (61%). X-ray quality crystals were obtained from diffusion of ether into dichloromethane solution. Elemental analysis(%)
calcld. \([\text{NiC}_12\text{H}_{26}\text{N}_4(\text{C}_2\text{H}_3\text{O}_2)]\)(PF₆)₂ (489.06 g/mol): C 34.38, H 5.98, N 11.46; Found C 34.15, H 5.96, N 11.37. MS (ES) m/z 344.2 [NiL(OAc)]²⁺.

\([\text{Co6(OAc)}]_2(PF_6)_2\): 1.00 mmol (0.583 g) of the ligand (7) and 2.00 mmol (0.360 g) of the anhydrous cobalt(II) acetate salt were added to 30 ml of dry CH₃CN in an inert atmosphere glovebox. The reaction was stirred at room temperature for 18 h. The crude \([\text{CoL(OAc)}]_2\) solution was removed from the glovebox, filtered to remove any trace solids, and evaporated to dryness. These crude products were dissolved in 10 ml of dry methanol, to which was added dropwise a 5 ml dry methanol solution of 5 equivalents (0.815 g, 5.00 mmols) of NH₄PF₆. A pink powder of the \([\text{Co7(OAc)}]_2(PF_6)_2\) complex precipitated, was collected, washed with cold methanol and ether, and dried under vacuum. Yield: 0.115 g (10%). Elemental analysis(%)
calcld. \([\text{CoC}_3\text{H}_5\text{N}_2(\text{C}_2\text{H}_3\text{O}_2)]\)(PF₆)₂ • 0.4NH₄PF₆ • 4 H₂O (1246.05 g/mol): C 36.63, H 6.28, N 9.44; Found C 36.89, H 6.21, N 9.07. MS (ES) m/z 410.0 [CoL(OAc)]²⁺

X-ray Crystallography

The sets of X-ray diffraction intensity data from all samples were collected in series of \(\omega\)-scans using a Stoe IPDS2 image plate diffractometer operating with MoKα radiation. Crystals were mounted at the end of a glass fiber and cooled to 150(2) \(K\) in an Oxford Cryosystems nitrogen gas cryostream. Data were scaled and merged and a multi-scan method was applied for the absorption corrections of the collected data.29 The structures were solved using dual-space methods within SHELXT and full-matrix least squares refinement was carried out within SHELXL-2014 via the WinGX program interface.30 All non-hydrogen positions were located in the direct and difference Fourier maps and refined using anisotropic displacement parameters.

The structure of \([\text{Co1Cl}_2]\) was twinned by 180° rotation about the 1 0 0 reciprocal direction. The twin fraction was 0.946:0.054(4). The crystal of \([\text{Ni1Cl}_2]\) was refined as an inversion twin with twin fraction 0.52:0.48(2). The structure of \([\text{Ni6(OAc)}]PF_6\) was twinned by 180° rotation about the 0 0 1 reciprocal direction. (Twin fraction 0.8420:0.1580(17)) A small number of reflections suspected of being partially overlapped between two twin domains were omitted from the final refinement. The crystal of \([\text{Co6(OAc)}]_2(PF_6)_2\) was refined as an inversion twin with twin fraction 0.54:0.46(4).

Anti-viral assays

Anti-HIV activity and cytotoxicity measurements in MT-4 and other cells were based on the viability of cells that had been infected or not infected with the HIV-1 strain IIIb and exposed to various concentrations of the test compound. After the cells were allowed to proliferate for 5 days, the number of viable cells was quantified by a tetrazolium-based colorimetric method as described by Pauwels et al.21, 31 The metal complexes were dissolved in water or phosphate buffer prior to addition. Initial dissolution in DMSO was required for the hexafluorophosphate salt compounds followed by dilution into aqueous solution.

Results and Discussion

Preparation of metal complexes

The initial complex formation reactions utilised anhydrous chloride salts of Ni²⁺ and Co²⁺, following procedures previously used for dimethyl cross-bridged cyclam and cyclen ligands26, 27 or more recently cross-bridged homocyclen.25 The resulting complexes, although pure and amenable to crystallisation from the reaction solution by simply cooling them, or diffusing in ether, were not comprehensively characterised for two reasons. First, they were only slightly soluble in solvents such as acetonitrile and methanol. Lack of solubility hindered the ability to obtain solution phase data such as electronic spectra and cyclic voltammetry. It had been observed in previous studies in our group that making acetate complexes rather than chloride complexes increased the solubility of the resulting complexes significantly.16, 18-20 Secondly, the complexes synthesised from acetate salts are of high interest to characterise the coordination interaction of the metal centres with carboxylate functional groups, which occurs when complexes of this type bind to the aspartate side chains of the CXCR4 chemokine receptor.20, 33 Therefore, we decided to synthesise and comprehensively characterise complexes with all six ligands starting from acetate salts of Ni²⁺ and Co²⁺.

Complexation of the ligands with the acetate salts were carried out in an inert atmosphere glovebox, primarily to protect the ligands from exposure to water, which tends to protonate these highly basic ligands and inhibit complex formation.2, 15, 34 After visible colour changes and stirring overnight to complete the complexation reactions, the reaction mixtures were removed from the glovebox to work up in air. Interestingly, all cobalt complexes with cyclen ligands air-oxidized to give Co³⁺ products, while the cyclam-based ligand complexes were air stable and gave only Co²⁺ products. This is consistent with prior work on cobalt complexes of cross-bridged cyclen ligands.26 It appears the smaller cyclen ligand cavity favours the smaller Co³⁺ ion. Cyclic voltammetry studies examining the redox behaviour of these complexes is discussed below.

Crystallography
Tables S1-S2 contains crystallographic data for the new crystal structures presented here. Table S3 contains selected bond lengths and bond angles for these structures.

Macrocycle-metal ion interactions
Due to their relevance to this work, two closely related crystal structures from one of our previous publications are included in this discussion: [Ni1(OAc)(H2O)]+ and [Ni5(OAc)(H2O)]+. Crystallographic details for these new structures, along with selected bond lengths and angles, are presented in Tables S1-S3 in the Supporting Information.

Prior to focussing on the detailed structural parameters, some general observations can be made. First, as constrained by the ligand cross-bridge, all complexes are found in the cis-V configuration. Figure 4 shows the three chloride examples characterised, using both metal ions and both macrocycle ring sizes. Figure 4a is CoCl2; Figure 4b is NiCl2; and Figure 4c is Ni2Cl2, all with six-coordinate octahedral coordination geometries. Consistent with prior work and all of the other structures presented below, changing the identity of the metal ion, the alkyl substituent, benzyl in both cases of Figure 4, or the labile additional equatorial ligands does not alter this configuration, which is a fixed feature of ethylene cross-bridged transition metal complexes.

Second, how fully engulfed the metal ion is by the ligand is dependent on the parent macrocycle ring size. Figure 5 demonstrates this tendency using the [Ni6(OAc)]+ complex from a cyclen macrocycle and the [Ni5(OAc)(H2O)]+ complex from a cyclam macrocycle. We have found that the Nα-M-Nα bond angle is a convenient measure of how far into the folded macrocyclic cavity the metal ion is found. As shown in Figure 5a, this bond angle is 163.82(14)° for the smaller cyclen parent macrocycle ring, indicating reduced ability of the complex to achieve an undistorted octahedral structure where this angle would be 180°. Figure 5b, illustrates that the same Ni2+ metal ion in the larger cyclam parent ring ligand is much closer to linear for this bond angle at 173.41(11)°.

Third, the ionic radius of the metal ion also plays a role in the deviation from regular octahedral geometry for the complex. Figure 6 illustrates this trend with the three different metal ions present in the complexes discussed: low spin Co3+ (69 pm ionic radius); high spin Ni2+ (83 pm); and high spin Co2+ (89 pm). Figure 6a-b shows the comparison of low spin Co3+ and high spin Ni2+ in same coordination sphere of ligand 7, and an iso-bidentate acetate. The smaller cross-bridged cyclen ligand is more complementary for the small, low spin Co3+ ion, having an Nα-M-Nα bond angle of 171.06(19)°, while the larger high spin Ni2+ ion is not as well accommodated with a 163.82(14)° Nα-M-Nα bond angle. The significant difference in ionic radius (14 pm) results in a ~7° bond angle difference. A much smaller difference is discernible in Figure 6c-d in the larger cross-bridged cyclam system where two cations of much more similar ionic radius (high spin Co2+, 89 pm; high spin Ni2+, 83 pm) are similarly situated within the ligand 5 cavity and bind monodentate acetate anions and water molecules to complete their octahedral coordination geometries. The Nα-M-Nα bond angles are 173.0(2)° (hs Co2+) and 173.41(11)° (hs Ni2+).

Together, these three trends echo those seen for other cross-bridge tetraazamacrocyle transition metal complexes and most usefully compiled in our previous work.

Acetate Binding
In this work, we are using acetate as a model to better understand the binding of these cobalt and nickel complexes to...
the aspartate carboxylate side chain on the surface of the CXCR4 chemokine receptor.\textsuperscript{16, 18, 20} In a recent study of Cu\textsuperscript{2+} and Zn\textsuperscript{2+} CXCR4 chemokine receptor antagonists,\textsuperscript{23} we were able to discern several trends based on similar acetate-as-model crystal structures that shed light on the likely coordination environment in the antagonist/receptor interaction and rationalised our antagonist binding affinities and residence time data.\textsuperscript{23} The aim of this study is to learn similar information about our cobalt and nickel antagonists. Figure 7 shows all of the crystal structures of Ni/Co cross-bridged ligands 1-6 containing acetate bound to the metal ions. The acetate binding mode is briefly described along with the coordination sphere of these complexes prior to drawing conclusions from the structural study. Figure 8 contains additional Ni/Co complex crystal structures from the literature, where the metal ion is bound to an unbridged tetraazaamacrocycle derived from cyclam or cyclen and coordinated in a cis configuration where each metal ion is also bound to an acetate ligand. Table 3 provides the geometrical parameters for all discussed structures.

For the cross-bridged ligand 1-6 acetate complexes, there are three groups of related structures. Figure 7a [Co2(OAc)]\textsuperscript{c+} and Figure 7b [Co6(OAc)]\textsuperscript{f+} contain the first type of observed complex. These are slightly distorted octahedral complexes of Co\textsuperscript{3+} ions with four nitrogens from the cross-bridged ligand occupying the two axial and two cis-equatorial positions. The acetate ligands in these complexes are acting as iso-bidentate chelates at the remaining cis-equatorial sites. Even though Co\textsuperscript{3+} salts were used for complexation, aerobic workup of the formed complexes leads to oxidation to Co\textsuperscript{4+}. Both ligand 2 and 7 are derived from the smaller 12-membered cyclen ring, which, in our hands, always results in isolation of the smaller Co\textsuperscript{3+} ion, which is more complementary to the smaller ring size.\textsuperscript{26}

The second group of related structures are shown in Figure 7c-7f. Figure 7c contains [Co5(OAc)(H\textsubscript{2}O)]\textsuperscript{e+}, which features the larger Co\textsuperscript{3+} ion in a larger cyclam-derived ring system. Aerobic workup does not lead to oxidation, as the larger ring system is more complementary for the larger Co\textsuperscript{4+} ion.\textsuperscript{26} This Co\textsuperscript{3+} complex has common features with Figures 7d-f featuring Ni\textsuperscript{2+} ions, Figure 7d [Ni5(OAc)(H\textsubscript{2}O)]\textsuperscript{d+}, Figure 7e [Ni1(OAc)(H\textsubscript{2}O)]\textsuperscript{f+}, Figure 7f [Ni2(OAc)(H\textsubscript{2}O)]\textsuperscript{h+}. All of these M\textsuperscript{2+} complexes have slightly distorted octahedral geometries with the macrocycle nitrogens again occupying both axial and two cis-equatorial sites. However, in each of these four cases, the acetate ligand is bound equatorially in a monodentate fashion, with the uncoordinated acetate oxygen acting as a hydrogen bond acceptor from a water molecule coordinated in the final equatorial position. Only one of these complexes incorporates a cyclen-derived ligand.

The final two structures also contain cyclen-derived ligands: Figure 7g [Ni4(OAc)]\textsuperscript{g+} and Figure 7h [Ni6(OAc)]\textsuperscript{h+}. In both cases, the Ni\textsuperscript{2+} ions are in distorted octahedral geometries with the acetate ligands bound in an isobidentate manner equatorially, and the remaining coordination sites occupied by the cross-bridged ligand nitrogen donors.

Among these eight cross-bridged tetraazaamacrocycle acetate complexes, the coordination of water accompanying the monodentate coordination of the acetate ligand was an unexpected result, and could play a significant role in understanding the coordination of our cross-bridged CXCR4 antagonists to the aspartate carboxylate side-chains where they bind. Thermodynamically, bidentate coordination of the acetate chelate should be favoured over two monodentate ligands.\textsuperscript{1} However, similar behaviour observed for our Zn\textsuperscript{2+} cross-bridged complexes, as examined by crystallography and DFT calculations, revealed that the acetate-water ligand pair interacting through hydrogen bonding was energetically more...
favourable than the bidentate coordination of acetate alone.\textsuperscript{20}
The cross-bridge plays an important role in dictating the acetate coordination mode.\textsuperscript{20}

To characterise the influence of the cross-bridge for cobalt and nickel, we required examples of unbridged cis-coordinated tetraazamacrocycle complexes having similar acetate ligands. Figure 8 shows six such cyclam and cyclen derived examples that were found in the literature for comparison to our cross-bridged complexes. Table 1 lists geometrical parameters for all 14 complexes in Figures 7-8.
Table 1. X-ray structural parameters determining acetate binding mode in Co/Ni complexes.

<table>
<thead>
<tr>
<th>Complex</th>
<th>M\textsuperscript{II}</th>
<th>r(pm)	extsuperscript{36}</th>
<th>N\textsubscript{ax}-M-N\textsubscript{ax} Angle (°)</th>
<th>N\textsubscript{ax}-M-N\textsubscript{eq} Angle (°)</th>
<th>O-M-O Angle (°)</th>
<th>OAc Binding Mode</th>
<th>M-N Bond Distance (Å)</th>
<th>M-O Bond Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Co\textsubscript{2}(OAc)]\textsuperscript{2+}</td>
<td>Co\textsuperscript{3+}</td>
<td>69</td>
<td>170.77(16)</td>
<td>90.42(17)</td>
<td>68.16(18)</td>
<td>iso-bidentate</td>
<td>Co-N\textsubscript{ax} = 2.033(3)</td>
<td>Co-N\textsubscript{eq} = 1.898(3)</td>
</tr>
<tr>
<td>[Co\textsubscript{6}(OAc)]\textsuperscript{2+}</td>
<td>Co\textsuperscript{3+}</td>
<td>69</td>
<td>171.06(19)</td>
<td>90.85(19)</td>
<td>67.57(19)</td>
<td>iso-bidentate</td>
<td>Co-N\textsubscript{ax} = 2.006 (avg)</td>
<td>Co-N\textsubscript{eq} = 1.903 (avg)</td>
</tr>
<tr>
<td>[Co\textsubscript{5}(OAc)(H\textsubscript{2}O)]\textsuperscript{+}</td>
<td>Co\textsuperscript{2+}</td>
<td>89</td>
<td>173.0(2)</td>
<td>83.39(19)</td>
<td>88.18(18)</td>
<td>monodentate/H\textsubscript{2}O</td>
<td>Co-N\textsubscript{ax} = 2.179 (avg)</td>
<td>Co-N\textsubscript{eq} = 2.126 (avg)</td>
</tr>
<tr>
<td>\textsuperscript{19}[Ni\textsubscript{5}(OAc)(H\textsubscript{2}O)]\textsuperscript{+}</td>
<td>Ni\textsuperscript{2+}</td>
<td>83</td>
<td>173.41(11)</td>
<td>84.02(10)</td>
<td>88.35(10)</td>
<td>monodentate/H\textsubscript{2}O</td>
<td>Ni-N\textsubscript{ax} = 2.192 (avg)</td>
<td>Ni-N\textsubscript{eq} = 2.097 (avg)</td>
</tr>
<tr>
<td>\textsuperscript{19}[Ni\textsubscript{1}(OAc)(H\textsubscript{2}O)]\textsuperscript{+}</td>
<td>Ni\textsuperscript{2+}</td>
<td>83</td>
<td>175.41(10)</td>
<td>85.50(12)</td>
<td>88.18(11)</td>
<td>monodentate/H\textsubscript{2}O</td>
<td>Ni-N\textsubscript{ax} = 2.183 (avg)</td>
<td>Ni-N\textsubscript{eq} = 2.132 (avg)</td>
</tr>
<tr>
<td>[Ni\textsubscript{2}(OAc)(H\textsubscript{2}O)]\textsuperscript{+}</td>
<td>Ni\textsuperscript{2+}</td>
<td>83</td>
<td>163.52(6)</td>
<td>85.59(7)</td>
<td>87.61(6)</td>
<td>monodentate/H\textsubscript{2}O</td>
<td>Ni-N\textsubscript{ax} = 2.1532 (avg)</td>
<td>Ni-N\textsubscript{eq} = 2.0580 (avg)</td>
</tr>
<tr>
<td>[Ni\textsubscript{4}(OAc)]\textsuperscript{+}</td>
<td>Ni\textsuperscript{2+}</td>
<td>83</td>
<td>163.71(13)</td>
<td>87.69(14)</td>
<td>63.01(12)</td>
<td>iso-bidentate</td>
<td>Ni-N\textsubscript{ax} = 2.150 (avg)</td>
<td>Ni-N\textsubscript{eq} = 2.025 (avg)</td>
</tr>
<tr>
<td>[Ni\textsubscript{6}(OAc)]\textsuperscript{+}</td>
<td>Ni\textsuperscript{2+}</td>
<td>83</td>
<td>163.82(14)</td>
<td>87.08(14)</td>
<td>62.78(13)</td>
<td>iso-bidentate</td>
<td>Ni-N\textsubscript{ax} = 2.145 (avg)</td>
<td>Ni-N\textsubscript{eq} = 2.023 (avg)</td>
</tr>
<tr>
<td>\textsuperscript{37}[Co(Bn\textsubscript{2}Cyclam)(OAc)]\textsuperscript{+}</td>
<td>Co\textsuperscript{2+}</td>
<td>89</td>
<td>171.54(10)</td>
<td>97.16(11)</td>
<td>61.51(9)</td>
<td>iso-bidentate</td>
<td>Co-N\textsubscript{ax} = 2.254 (avg)</td>
<td>Co-N\textsubscript{eq} = 2.107 (avg)</td>
</tr>
<tr>
<td>\textsuperscript{37}[Ni(Bn\textsubscript{2}Cyclen)(OAc)]\textsuperscript{+}</td>
<td>Ni\textsuperscript{2+}</td>
<td>83</td>
<td>160.84(9)</td>
<td>101.83(10)</td>
<td>62.31(8)</td>
<td>iso-bidentate</td>
<td>Ni-N\textsubscript{ax} = 2.180 (avg)</td>
<td>Ni-N\textsubscript{eq} = 2.033 (avg)</td>
</tr>
<tr>
<td>\textsuperscript{37}[Ni(Bn\textsubscript{2}Cyclen)(OAc)(H\textsubscript{2}O)]\textsuperscript{+}</td>
<td>Ni\textsuperscript{2+}</td>
<td>83</td>
<td>160.29(7)</td>
<td>96.99(7)</td>
<td>88.19(6)</td>
<td>monodentate/H\textsubscript{2}O</td>
<td>Ni-N\textsubscript{ax} = 2.161 (avg)</td>
<td>Ni-N\textsubscript{eq} = 2.066 (avg)</td>
</tr>
<tr>
<td>\textsuperscript{38}[Ni(Me\textsubscript{4}Cyclen)(OAc)]\textsuperscript{+}</td>
<td>FODTAV</td>
<td>83</td>
<td>158.44(16)</td>
<td>108.64(17)</td>
<td>61.92(14)</td>
<td>iso-bidentate</td>
<td>Ni-N\textsubscript{ax} = 2.154 (avg)</td>
<td>Ni-N\textsubscript{eq} = 2.104 (avg)</td>
</tr>
<tr>
<td>\textsuperscript{39}[Ni(Cyclen)(OAc)]\textsuperscript{+}</td>
<td>XADMAR</td>
<td>83</td>
<td>160.5(2)</td>
<td>102.1(2)</td>
<td>61.47(15)</td>
<td>iso-bidentate</td>
<td>Ni-N\textsubscript{ax} = 2.098(4)</td>
<td>Ni-N\textsubscript{eq} = 2.050 (avg)</td>
</tr>
<tr>
<td>\textsuperscript{40}[Ni(Bn\textsubscript{1}Cyclam)(OAc)]\textsuperscript{+}</td>
<td>NEXQEN</td>
<td>83</td>
<td>173.54(6)</td>
<td>100.36(6)</td>
<td>62.01(6)</td>
<td>iso-bidentate</td>
<td>Ni-N\textsubscript{ax} = 2.149 (avg)</td>
<td>Ni-N\textsubscript{eq} = 2.073 (avg)</td>
</tr>
</tbody>
</table>
Analysis of the geometric parameters

The identity of the metal ion is not a good predictor of the acetate binding mode for these metals ions. Both coordination modes, isobidentate and monodentate with water binding, were characterised for the divalent ions, Co\(^{2+}\) and Ni\(^{2+}\). However, oxidation state was more predictive; for Co\(^{3+}\), only isobidentate coordinated acetate was observed. This observation may be most related to the size of the metal ion. Co\(^{3+}\) in an octahedral geometry has a 69 pm ionic radius, while the Ni\(^{2+}\) and Co\(^{2+}\) ionic radii are much larger, at 83 pm and 89 pm, respectively. The small Co\(^{3+}\) ion has short Co-N\(_{eq}\) bonds (~1.90 Å) in both complexes, which contributes to the large 90.42(17°) and 90.85(19°) N\(_{eq}\)-Co-N\(_{eq}\) bond angles, the largest of any of the cross-bridged complexes. Below, we discuss how this latter parameter is the most accurate predictor of coordination mode.

N\(_{eq}\)-M-N\(_{eq}\) bond angles varied significantly, from 158.44(16)° for unbridged [Ni(Me\(_{4}\)Cyclen)(OAc)]\(^+\) to 175.41(10)° for cross-bridged cyclam complex [Ni\(_{1}\)(OAc)(H\(_{2}\)O)]\(^+\). However, the value of this parameter does not correlate well with the acetate binding mode. For example, both observed coordination modes are found for N\(_{eq}\)-M-N\(_{eq}\) bond angles near both extremes for this parameter: largest iso-bidentate value 173.54(6)° for [Ni(Bn\(_{1}\)Cyclam)(OAc)]\(^+\); smallest iso-bidentate value 158.44(16)° for [Ni(Me\(_{4}\)Cyclen)(OAc)]\(^+\); largest monodentate/H\(_{2}\)O value 175.41(10)° for [Ni\(_{1}\)(OAc)(H\(_{2}\)O)]\(^+\); smallest monodentate/H\(_{2}\)O value 160.29(7)° for [Ni(Bn\(_{2}\)Cyclen)(OAc)(H\(_{2}\)O)]\(^+\).

N\(_{eq}\)-M-N\(_{eq}\) bond angles varied significantly as well, from 83.39(19)° for [Co(5)(OAc)(H\(_{2}\)O)]\(^+\) to 108.64(17)° for [Ni(Me\(_{4}\)Cyclen)(OAc)]\(^+\). As a general rule, if a complex had an N\(_{eq}\)-M-N\(_{eq}\) angle > 85.59°, its coordination mode was iso-bidentate, and if the N\(_{eq}\)-M-N\(_{eq}\) angle < 85.59°, the coordination mode was monodentate/H\(_{2}\)O. This result is similar to what we found in our recent study of Cu\(^{2+}\) and Zn\(^{2+}\) complexes of related ligands. An explanation for this trend is that the short ethylene cross-bridge often restricts the N\(_{eq}\)-M-N\(_{eq}\) angle to less than the ideal 90°, which causes an abundance of space on the opposite equatorial side, which can best be filled by the more sterically demanding pair of cis ligands consisting of a monodentate acetate hydrogen bonded to a water molecule, which demonstrate O-M-O bond angles all near 88° (Table 1). Even a modest increase in the N\(_{eq}\)-M-N\(_{eq}\) angle begins to restrict this space, allowing the smaller iso-bidentate coordination mode (O-M-O bond angles 61.47°-68.16°, Table 1) on the opposite equatorial side to adequately fill the smaller equatorial space.
From Table 1, it is apparent that the monodentate/H\textsubscript{2}O coordination mode is much more prevalent among the cross-bridged ligand complexes. Excluding the small Co\textsuperscript{3+} ion complexes, only two complexes of cross-bridged ligands bind acetate in the iso-bidentate mode: [Ni(4(OAc))\textsuperscript{2+}] and [Ni(6(OAc))\textsuperscript{2+}]. Both ligands are cyclen-derived, but this is not a general rule as 2 forms a Ni\textsuperscript{2+} complex which coordinates acetate in the monodentate plus H\textsubscript{2}O mode. Evidently, although the ethylene cross-bridge favours monodentate acetate/H\textsubscript{2}O coordination, it does not dictate it in all cases.

From the previously published unbridged ligand structures used for comparison, five out of six bind acetate in the iso-bidentate coordination mode. The lack of an ethylene cross-bridge allows much more flexibility in the macrocycle, most clearly represented by the variation in the $N_{eq}$-$M$-$N_{eq}$ angles, ranging from 96.99\textdegree to 108.64\textdegree. In contrast to the cross-bridged complexes discussed above, the larger $N_{eq}$-$M$-$N_{eq}$ angles restrict the equatorial space available on the opposite equatorial side, so that coordination of the iso-bidentate acetate is then favoured. However, even in this group, there is one example of the monodentate/H\textsubscript{2}O coordination mode [Ni(Bn\textsubscript{2}Cyclen)(OAc)(H\textsubscript{2}O)]\textsuperscript{2+} (Figure 8c).

Interestingly, for these chelators, the same metal-ligand combination can produce both coordination modes: [Ni(Bn\textsubscript{2}Cyclen)(OAc)]\textsuperscript{2+} (Figure 8b) is iso-bidentate with $N_{eq}$-$M$-$N_{eq}$ angle 101.83(10)$^\text{o}$ and O-Ni-O angle 62.31(8)$^\text{o}$, values clearly in line with the other iso-bidentate complexes of unbridged ligands. However, [Ni(Bn\textsubscript{2}Cyclen)(OAc)(H\textsubscript{2}O)]\textsuperscript{2+} (Figure 8c) is monodentate/H\textsubscript{2}O with $N_{eq}$-$M$-$N_{eq}$ angle 96.99(7)$^\text{o}$ and O-Ni-O angle 88.19(6)$^\text{o}$. This latter complex is the greatest outlier in Table 3, with $N_{eq}$-$M$-$N_{eq}$ angle much greater than the 85.59$^\text{o}$ cut-off identified above for this coordination mode. Yet, this complex still manages an O-Ni-O angle of 88.19(6)$^\text{o}$, in line with the monodentate/H\textsubscript{2}O structures of the cross-bridged ligand complexes. This may indicate that precise prediction is not warranted, or may be a feature of the more flexible non-bridged ligands. In general terms, cross-bridged ligand complexes appear to favour iso-bidentate acetate coordination in their Ni\textsuperscript{2+} and Co\textsuperscript{2+} complexes.

**Electronic Properties**

The electronic spectra of nickel and cobalt complexes have been used to compare the properties of their ligands with those currently in the literature. In addition to their use as structural models for the binding of cross-bridged metal complexes to aspartate in the CXCR4 receptor protein, these new Ni\textsuperscript{2+}, Co\textsuperscript{2+}, and Co\textsuperscript{3+} complexes can provide insight into the general properties of cross-bridged tetraazamacrocyclic ligand complexes through comparison with other related bridged derivatives and to unbridged macrocyclic ligands.

In addition, transition metal complexes introduced into biological systems are challenged by a number of redox active compounds that may oxidize or reduce the metal ion, which may result in inactivation or metal ion release. Characterising the redox behaviour of a potential inorganic medicinal compounds is therefore important for understanding their biological stability.41, 42

The electronic spectra of nickel and cobalt complexes have been used to compare the properties of their ligands with those currently in the literature. In addition to their use as structural models for the binding of cross-bridged metal complexes to aspartate in the CXCR4 receptor protein, these new Ni\textsuperscript{2+}, Co\textsuperscript{2+}, and Co\textsuperscript{3+} complexes can provide insight into the general properties of cross-bridged tetraazamacrocyclic ligand complexes through comparison with other related bridged derivatives and to unbridged macrocyclic ligands.

In addition, transition metal complexes introduced into biological systems are challenged by a number of redox active compounds that may oxidize or reduce the metal ion, which may result in inactivation or metal ion release. Characterising the redox behaviour of a potential inorganic medicinal compounds is therefore important for understanding their biological stability.41, 42
in Table 2. The electronic spectra of octahedral nickel(II) complexes are useful for determining ligand field strengths.27, 43, 44 \( \Delta_o \) is given directly by the energy of the lowest energy absorption band. For the six octahedral acetate complexes, this gives the following results: \( \Delta_o = 10,215 \text{ cm}^{-1} \) for [Ni1(OAc)]\(^{2+} \); \( \Delta_o = 10,515 \text{ cm}^{-1} \) for [Ni2(OAc)]\(^{2+} \); \( \Delta_o = 10,604 \text{ cm}^{-1} \) for [Ni3(OAc)]\(^{2+} \); \( \Delta_o = 10,638 \text{ cm}^{-1} \) for [Ni4(OAc)]\(^{2+} \); \( \Delta_o = 11,236 \text{ cm}^{-1} \) for [Ni5(OAc)]\(^{2+} \); and \( \Delta_o = 11,403 \text{ cm}^{-1} \) for [Ni6(OAc)]\(^{2+} \).

Of note are the higher extinction coefficients for the cyclen-based complexes (~10-40 M\(^{-1}\)cm\(^{-1}\)) compared to the cyclam-based complexes (~5-17 M\(^{-1}\)cm\(^{-1}\)). This behaviour is likely due to greater distortion away from octahedral for the smaller ligand ring, which can’t engulf the metal ion as fully. This greater distortion would make the transitions that are forbidden in the truly octahedral geometry, more likely to occur, giving the higher extinction coefficients observed.27

Interestingly, the trend of increasing ligand field strength in these complexes is with a decrease in macrocycle size, from the 14-membered cyclam-based ligands to the 12-membered cyclen-based ligands. This is the opposite of the trend that was observed for similar Ni\(^{2+}\) dichloride complexes with dimethyl cross-bridged ligands.27 In that series of octahedral dichloro complexes, the observation was decreasing ligand field strength with decreasing macrocycle ring size, which was attributed to poorer orbital overlap as the octahedron became more distorted with the ligand size decrease. The change from methyl substituents to at least one benzyl group, as well as the change from two chloro ligands to one acetate ligand, appears to sufficiently effect the electronic properties to reverse the trend. For example, \( \Delta_o = 11,236 \text{ cm}^{-1} \) for cyclam-based [Ni5(OAc)]\(^{2+} \); and \( \Delta_o = 11,403 \text{ cm}^{-1} \) for cyclen-based [Ni6(OAc)]\(^{2+} \). The smaller ring system appears to enforce a stronger ligand field on the Ni\(^{2+}\) cation in these acetate complexes.

What did not change, however, is the similarity in \( \Delta_o \) between these cross-bridged ligand complexes and those of cis-binding unbrided macrocycles. For example, the value for cis-Ni(13(ane)N4)Cl2 is \( \Delta_o = 11,111 \text{ cm}^{-1} \) and cis-Ni(TACD)(NO3)\(_2\) is \( \Delta_o = 9,756 \text{ cm}^{-1} \) (TACD = 1,4,7,10-tetrazenyl-1,4,7,10-tetraazacyclododecane).46 The ethylene cross-bridge does not greatly change the ligand field strength of the macrocyclic ligand if both are bound in a cis configuration. However, the cross-bridge does topologically prohibit trans configurations, which have much higher ligand field strengths for Ni\(^{2+}\). The value of Dq, used to measure the ligand field strength of such tetragonally distorted complexes47 can be significantly larger. For example, for the unbridged cyclam ligand, [Ni(cyclam)Cl2] is in a trans configuration and the value of Dq is \( 14,870 \text{ cm}^{-1} \).47

Finally, the effect of N-substitution on the cross-bridged ligands can be considered. The smooth increase in \( \Delta_o \) from ligand 1 to ligand 6 indicates the presence of benzyl groups lessens the ligand field strength as, for both cyclam (ligands 1, 3, and 5) and cyclen (ligands 2, 4, and 6) series: dibenzyl < monobenzyl < dimethyl. The steric requirements of the benzyl pendant arms may disrupt the preferred ligand conformation, resulting in a weaker ligand field strength.

Electronic Spectra of cobalt complexes

As noted above, the cyclen-based complexes oxidised upon workup in air, resulting in Co\(^{3+}\) complexes of ligands 2, 4, and 6. However, the cyclam-based complexes were air stable and allowed Co\(^{2+}\) complexes to be isolated. Therefore, some of the comparisons that could be made for the Ni\(^{3+}\) complexes aren’t possible. However, CoCl2 (Co\(^{2+}\)) and [CoCl2]\(^{+}\) (Co\(^{3+}\)) complexes with ligands 5 and 6 have all been previously synthesised and spectroscopically characterised,26 so several meaningful comparisons can be made. Figure 10 shows representative spectra and Table 2 lists the relevant numerical parameters.

The electronic spectra in acetonitrile of the cyclam-based, d\(^7\) Co\(^{2+}\) complexes are typical of high spin Co\(^{2+}\) complexes, having a single major absorption band centred between 500 and 600 nm and low extinction coefficients.44 Interestingly, the spectrum for the [Co3(OAc)]\(^{3+}\) complex (not pictured, \( \lambda_{\max} = 550 \text{ nm, } \varepsilon = 74 \text{ M}^{-1} \text{cm}^{-1} \)) has only a single smooth peak with no fine structure but an increased \( \varepsilon \), while in the spectrum for each of the [Co1(OAc)]\(^{3+}\) and [Co5(OAc)]\(^{3+}\) complexes (see Figure 10a) the major absorption peak is split with one two shoulders on the maximum absorption peak and \( \varepsilon \sim 15-20 \text{ M}^{-1} \text{cm}^{-1} \). These latter spectra are similar to those observed for the CoCl2 complexes with ligands 5 and 6 previously published, which all have this major peak split in the same way and similarly small extinction coefficients.26 For CoCl2 \( \lambda_{\max} = 540 (24 \text{ M}^{-1} \text{cm}^{-1}) \) and 558 nm (21 M\(^{-1}\)cm\(^{-1}\)) and for Co(6)Cl2 \( \lambda_{\max} = 546 (34 \text{ M}^{-1} \text{cm}^{-1}) \) and 568 nm (35 M\(^{-1}\)cm\(^{-1}\)). The change in splitting pattern and extinction
coefficient for \([\text{Co3(OAc)}]^+\) indicate that the asymmetry of the single benzyl group of \([\text{Co3(OAc)}]^+\) results in a different structure from all of the other \([\text{Co}^{2+}]\) complexes. Unfortunately, we were unable to produce X-ray quality crystals of this sample to better understand what this structural change is.

The cyclen-based \([\text{Co}^{3+}]\) complexes are confirmed as the expected low spin \(d^6\) according to sharp proton and carbon NMR spectra. Their electronic spectra are typical of octahedral \([\text{Co}^{3+}]\) amine complexes.\(^{48}\) These spectra show the usual two absorption bands between 300 and 700 nm (with much higher extinction coefficients than the \([\text{Co}^{2+}]\) complexes) that are generally associated with cis configuration \([\text{CoN}_4]\).\(^{44}\) Figure 9b shows the spectrum for \([\text{Co2(OAc)}]^+\), which is representative of all three \([\text{Co}^{3+}]\) complexes.

As with \([\text{Ni}^{2+}]\), the electronic spectra of octahedral \([\text{Co}^{3+}]\) complexes can be used to estimate the ligand field strengths of the ligands, expressed as \(\Delta_0 \).\(^{49,50}\) In this method, the energy of the lowest energy absorption band plus the Racah parameter (3800 cm\(^{-1}\) for \([\text{Co}^{3+}]\))\(^{49-51}\) equals \(\Delta_0 \). Since both \([\text{Co2(OAc)}]^+\) and \([\text{Co4(OAc)}]^+\) have their lowest energy absorption at 523 nm, they have the identical \(\Delta_0 = 22,920\) cm\(^{-1}\). This similarity in \(\Delta_0\) for both of these cyclen-based ligands was not quite as apparent in the \([\text{Ni}^{2+}]\) complexes above, where the values of \(\Delta_0\) differed by 123 cm\(^{-1}\). However, the value of \(\Delta_0\) for \([\text{Co6(OAc)}]^+\), \(\Delta_0 = 23,524\) cm\(^{-1}\), is somewhat larger, as was observed for the replacement of benzyl with methyl substituents for the \([\text{Ni}^{2+}]\) complexes, above. Again, disruption of the preferred ligand configuration by the bulky benzyl groups may be explain the lower values of \(\Delta_0\) in benzylated ligands.

Comparison of \(\Delta_0\) for these three complexes is appropriate with the \([\text{CoCl}_4]^+\) complexes of ligands 5 and 6,\(^{26}\) \(\Delta_0 = 19,430\) cm\(^{-1}\) for \([\text{Co}(5)\text{Cl}_4]^+\); and \(\Delta_0 = 21,130\) cm\(^{-1}\) for \([\text{Co}(6)\text{Cl}_4]^+\). Of course, the most appropriate comparison is the latter one, because all three of these ligands are based on the 12-membered cyclen ring. The increase in \(\Delta_0\) in the present ligand 2, 4, and 6 cases may be due to two factors. The first is the change of the equatorial ligands from \(\text{Cl}\) to \(\text{O}\) donors; these \(\text{O}\) donors should be slightly stronger field ligands.\(^{52}\) The unbridged cyclen complex cis-[Co(cyclen)CO]\(^{+}\) has been reported to have \(\Delta_0 = 22,670\) cm\(^{-1}\).\(^{51}\) Here the macrocyclic ligand is forced to be cis by the chelating carbonate ligand. This complex has a very similar coordination environment to \([\text{Co2(OAc)}]^+\), \([\text{Co4(OAc)}]^+\), and \([\text{Co6(OAc)}]^+\).

The second reason for the higher \(\Delta_0\) values for the present acetate complexes is the fact that the lowest energy band in the present ligand 2, 4, and 6 complexes is actually an overlapping pair \(E(1\text{T}_{1g})\) and \(A(1\text{T}_{1g})\) absorptions, which is why cis \(\text{CoN}_2\text{X}_2\) complexes only appear to have two total absorbance bands.\(^{44}\) \([\text{Co6Cl}_2]\) \(^+\) surprisingly, exhibits all three bands in a cis configuration complex, most likely due to larger-than-normal distortion of the octahedral geometry.\(^{56}\) Changing the two chloro ligands to an acetate ligand together with the addition of the one or two benzyl groups at the macrocycle N-donors leads to less severe distortion and a return to the usual two absorption bands. In terms of \(\Delta_0\) calculations, the values for \([\text{Co2(OAc)}]^+\), \([\text{Co4(OAc)}]^+\), and \([\text{Co6(OAc)}]^+\) will be high compared to \([\text{Co6Cl}_2]\)\(^+\), because the \(\Delta_0\) values are calculated for a broad peak which mixes in a higher energy absorbance, while the unique lowest energy absorbance peak was used for \([\text{Co6Cl}_2]\)\(^+\).

### Electrochemical studies of nickel complexes

![Figure 11. Cyclic voltammograms for (a) \([\text{Ni4(OAc)}]^+\) and (b) \([\text{Ni6(OAc)}]^+\)](image)

<table>
<thead>
<tr>
<th>Complex</th>
<th>(E_{\text{pa}}) (V) ([\text{Ni}^{2+}/\text{Ni}^{3+}])</th>
<th>(E_{\text{pc}}) (V) ([\text{Ni}^{2+}/\text{Ni}^{3+}])</th>
<th>(E_{\text{c}}) (V) ([\text{Ni}^{2+}/\text{Ni}^{3+}])</th>
<th>(E_{\text{pc}}-E_{\text{c}}) mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Ni3(OAc)}]^+)</td>
<td>+1.255</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>([\text{Ni2(OAc)}]^+)</td>
<td>---</td>
<td>+1.177</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>([\text{Ni3(OAc)}]^+)</td>
<td>+1.277</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>([\text{Ni4(OAc)}]^+)</td>
<td>---</td>
<td>+1.077</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>([\text{Ni5(OAc)}]^+)</td>
<td>+1.193</td>
<td>78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>([\text{Ni6(OAc)}]^+)</td>
<td>+1.062</td>
<td>92</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The cyclic voltammograms in acetonitrile of (a) \([\text{Ni4(OAc)}]^+\) [representative also of \([\text{Ni2(OAc)}]^+\), \([\text{Ni5(OAc)}]^+\), and \([\text{Ni6(OAc)}]^+\)] and (b) \([\text{Ni1(OAc)}]^+\) [representative also of \([\text{Ni3(OAc)}]^+\)] are shown in Figure 11. The redox potentials and peak separations of all six \([\text{Ni}^{2+}]\) acetate complexes are listed in Table 3. The cyclam ligands 1 and 3 surprisingly give only irreversible oxidations to \([\text{Ni}^{3+}]\) (Table 3, Figure 11b). This was unexpected since all three of the \([\text{NiCl}_2]\) complexes where \(L = 5-6\) have reversible \([\text{Ni}^{2+}/\text{Ni}^{3+}]\) couples as well as irreversible reductions assigned to \([\text{Ni}^{2+}/\text{Ni}^{3+}]\). The substitution of benzyl for methyl groups (for example changing ligand 5 to ligand 1) on cross-bridged ligand complexes of iron and manganese had minimal effects on the cyclic voltammetry of those complexes,\(^2,4\) so significant changes were not expected for these nickel complexes. However, all of the iron and manganese complexes referred to were dichloro complexes and so there may be some influence of the acetato ligand on the nickel complex properties in this work. Perhaps there is reactivity of the bound acetate ligand upon oxidation of the mono- and di-benzyl-cyclam nickel complexes that leads to

---

\(2, 4\)

---

\(2, 4\)
the irreversible behaviour. In support of this idea is the known oxidation catalyst behaviour of the iron\(^{53}\) and manganese\(^{2,9}\) complexes of cross-bridged cyclams. Oxidative Ni\(^{3+}\) coordination to carbon ligands is also well-known\(^{34,59}\) so perhaps modification of the benzyl pendant arms is occurring. The anodic peaks are nearly identical, +1.255 V for [Ni(1OAc)]\(^{+}\) and +1.277 V for [Ni(3OAc)]\(^{+}\), suggesting that the presence of either one or two benzyl groups has little effect on the ability of the metal ion to be oxidized.

In contrast, the cyclen-based complexes (ligands 2, 4, and 6) and the dimethyl cyclam complex (ligand 5) all give reversible oxidation cycles for Ni\(^{3+/2+}\) (Table 3, Figure 11a). Clearly, the smaller cyclen ring better stabilizes the small Ni\(^{3+}\) ion in these complexes, as oxidation occurs more easily than for the cyclam-based complexes. Apparently, the lower oxidation potential does not activate the process that leads to the irreversible nature of the cyclam-based benzyl-containing complexes. Among cyclen-based ligand complexes, the difference in oxidation potential between the mono- and di-benzyl ligands and the dimethyl ligand is minimal. Even though reversible oxidation is present, the irreversible reduction common to all three of the NiIICl\(_2\) complexes where \(L = 5-6\) are not seen. An explanation could be that the hard oxygen donors of the acetate ligand do not stabilise soft Ni\(^{3+}\) as well as chloride does.

### Electrochemical studies of cobalt complexes

The cyclic voltammograms in acetonitrile of (a) [Co2(OAc)]\(^{+}\) (representative also of [Co4(OAc)]\(^{+}\)) and (b) [Co3(OAc)]\(^{+}\) (representative also of [Co1(OAc)]\(^{+}\)) are shown in Figure 12. The redox potentials and peak separations of all four cobalt acetate complexes can be found in Table 4. The cyclen-based compounds are initially in the Co\(^{3+/2+}\) oxidation state, while the cyclam-based compounds are initially in the Co\(^{2+/3+}\) oxidation state.

The Co\(^{3+}\) cyclen-based complex voltammograms are relatively simple, with quasi-reversible reductions. For [Co2(OAc)]\(^{+}\) two such reductions occur at \(E_{1/2} = +0.014\) V (\(\Delta E = 109\) mV) and \(E_{1/2} = -0.640\) V (\(\Delta E = 178\) mV). For [Co4(OAc)]\(^{+}\) the corresponding reductions occur at \(E_{1/2} = +0.040\) V (\(\Delta E = 185\) mV) and \(E_{1/2} = -0.758\) V (\(\Delta E = 104\) mV). For Co(6)(OAc)\(^{2+}\), only one reduction is observed at \(E_{1/2} = -0.144\) V (\(\Delta E = 107\) mV). The reductions can be assigned as the Co\(^{3+/2+}\) and Co\(^{2+/1+}\) couples, with the spacing between them in the order of 650-800 mV, which corresponds well to the spacing of MnIICl\(_2\) (\(L = 5\) and 6) which both have reversible Mn\(^{2+/3+}\) and Mn\(^{3+/4+}\) couples nearly 750 mV apart.\(^2\) The Co\(^{3+/2+}\) reduction of [Co6(OAc)]\(^{2+}\) complex occurs at the most negative potential, -0.144 V, and uniquely among this set of cyclen complexes, a second reduction is not observed. The benzyl pendant arms of 2 and 4 help stabilise and/or enclose Co\(^{3+}\), whereas this stabilization is not present in [Co6(OAc)]\(^{2+}\), which does not reach the Co\(^{3+}\) oxidation state in our experiments. Interestingly, the CoIICl\(_2\) (\(L = 5-6\)) complexes all have similar Co\(^{3+/2+}\) redox couples with \(E_{1/2}\) values near 0.00 V vs. SHE. However, these dichloro complexes have only irreversible reductions to Co\(^{2+}\) at much lower potentials, below -2.00 V.\(^{26}\) The presence of only one negatively charged acetate, rather than two negatively charged chlorides, as well as at least one benzyl pendant arm, makes the reduction to Co\(^{2+}\) both more accessible and more reversible for the ligand 2 and 4 complexes.

### Table 4. Redox potentials (vs. SHE) with peak separations for cobalt acetate complexes.

<table>
<thead>
<tr>
<th>Co(^{n+}) Complex</th>
<th>(E_{\text{ox}}) (V)</th>
<th>(E_{1/2}) ((\text{Co}^{n+}/\text{Co}^{n-}))</th>
<th>((E_{E}-E_{\text{ox}})) mV</th>
<th>(E_{1/2}) ((\text{Co}^{n-}/\text{Co}^{n+}))</th>
<th>((E_{E}-E_{\text{ox}})) mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Co1(OAc)](^{+})</td>
<td>+1.226</td>
<td>+0.648</td>
<td>75</td>
<td>+0.392</td>
<td>167</td>
</tr>
<tr>
<td>[Co3(OAc)](^{+})</td>
<td>+1.159</td>
<td>+0.564</td>
<td>100</td>
<td>+0.293</td>
<td>177</td>
</tr>
<tr>
<td>[Co5(OAc)](^{+})</td>
<td>+1.415</td>
<td>+0.536</td>
<td>205</td>
<td>+0.246</td>
<td>220</td>
</tr>
<tr>
<td>[Co2(OAc)](^{2+})</td>
<td>+0.014</td>
<td>109</td>
<td>-0.640</td>
<td>178</td>
<td></td>
</tr>
<tr>
<td>[Co4(OAc)](^{2+})</td>
<td>+0.040</td>
<td>185</td>
<td>-0.758</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>[Co6(OAc)](^{2+})</td>
<td>-0.144</td>
<td>107</td>
<td>-----</td>
<td>-----</td>
<td></td>
</tr>
</tbody>
</table>

The Co\(^{2+}\) cyclam-based complexes have quite different cyclic voltammograms (see Figure 11). Starting from Co\(^{2+}\), the expected oxidation to Co\(^{3+}\) is seen for all three ligand complexes (1, 3, and 5). This initial oxidation is at \(E_{1/2} = +0.392\) V (\(\Delta E = 167\) mV) for [Co3(OAc)]\(^{+}\); at \(E_{1/2} = +0.293\) V (\(\Delta E = 177\) mV) for [Co3(OAc)]\(^{+}\); and at \(E_{1/2} = +0.246\) V (\(\Delta E = 220\) mV) for [Co5(OAc)]\(^{+}\). These potentials occurring 200-300 mV more positive than the Co\(^{2+/3+}\) redox couples of the equivalent cyclen-based ligands (2, 4, and 6, see above) complexes makes sense, as the larger cyclam ring would not stabilise the smaller Co\(^{3+}\) ion as well, resulting in a less favoured oxidation.

The cobalt(II) cyclam-based complex voltammograms also contain two additional waves. First, there is an additional reversible oxidation approximately only ~250 mV more positive for all three complexes: \(E_{1/2} = +0.648\) V (\(\Delta E = 75\) mV) for [Co1(OAc)]\(^{+}\); \(E_{1/2} = +0.564\) V (\(\Delta E = 100\) mV) for [Co3(OAc)]\(^{+}\); and \(E_{1/2} = +0.536\) V (\(\Delta E = 208\) mV) for [Co5(OAc)]\(^{+}\). The proximity to

---

This journal is © The Royal Society of Chemistry 20xx

**J. Name.,** 2018, 00, 1-3 | 13

---

Figure 12. Cyclic Voltammograms for (a) [Co2(OAc)]\(^{+}\) and (b) [Co3(OAc)]\(^{+}\).
the initial oxidation of this additional redox couple makes it unlikely to be the result of a Co$^{3+/4+}$ oxidation. More likely is the oxidation from Co$^{2+}$ to Co$^{3+}$ of a second species with a different ligand set in the same solution. This behaviour has been observed for the Co$^{3+}$ complexes CoLC$_2$(L = 5-6) in acetonitrile, where cyclic voltammetry in TBAPF$_6$ supporting electrolyte gave complex features assigned to multiple species in solution which differ by the number of bound chloride ligands. It is possible that either the coordination mode of the acetate ligand is changing, producing two different species for the [Co(1(OAc))$^+$], [Co(3(OAc))$^+$], and [Co(2(OAc))$^+$] complexes, or there is an equilibrium mixture of bound and free acetate ligand complexes. These different species would have different redox potentials and might give rise to the two closely spaced complexes. These different species would have different redox potentials and might give rise to the two closely spaced redox couples observed. Finally, an additional feature for all these cyclem-based complexes is an irreversible oxidation at greater than 1.1 V vs. SHE. This feature has not been fully assigned, but might either be due to an oxidation to Co$^{4+}$, or a ligand-based oxidation process.

The free chelators showed IC$_{50}$ values of greater than 100 µM indicating no measurable anti-HIV activity for these compounds. This is consistent with previously analysed free macrocyclic chelators in which the hydrogen-bonding potential of the chelator has been disrupted by alkylation and they are only activated on inclusion of the metal centre to give the potential for coordinate bond formation.

AMD3100 metal complexes have anti-HIV activity in this order: Zn$^{2+}$ > Ligand = Ni$^{2+}$ > Co$^{2+}$ > Pd$^{2+}$ according to the literature. In our study of cross-bridged analogues of AMD3100, we have shown that Cu$^{2+}$, Zn$^{2+}$, and Ni$^{2+}$ complexes all have low nanomolar IC$_{50}$ values against the HIV1 viral strain, with the exact ordering depending on how the specific ligand is designed. Here, we extend our studies to include cobalt. For the nickel(II) complexes previously studied, bis-macro cyclic bridged complexes with ethylene bridged structures were generally of lower anti-HIV potency than the AMD3100 complex. It was also shown that the binding of nickel(II) can be used to increase potency of unbridged AMD3100 derivatives that are functionalised at the linking xylyl group (increasing anti-HIV potency from 295 nM to 95 nM in one case).

As described above, the smaller cyclen cross-bridged ligands select for Co$^{3+}$, while the large cyclam cross-bridged ligands appear to stabilise the larger Co$^{2+}$. Unlike Cu$^{2+}$, Zn$^{2+}$, and Ni$^{2+}$ complexes, the Co$^{3+}$/Co$^{4+}$ complexes synthesised and screened here, do not appear to have very strong affinity for the CXCR4 receptor, by analogy with the X4 strain anti-HIV activity reported in Table 5. The only mononuclear cobalt complex with a measurable IC$_{50}$ was [Co(1(OAc))$^+$] with IC$_{50}$ = 1.82 µM, which is not particularly potent compared to our previous Cu$^{2+}$, Zn$^{2+}$, and Ni$^{2+}$ complexes. Of course, we do anticipate losing some potency as a result of having only one metal centre in the monomacrocyclic ligand, but all of the Ni$^{2+}$ complexes show affinity in the low or sub micromolar range.

### Table 5. Anti-HIV IC$_{50}$ values of the evaluated compounds using HIV-1 (viral strain IIIB)

<table>
<thead>
<tr>
<th>Complex</th>
<th>Ligand</th>
<th>Anti-HIV activity (IC$_{50}$) [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co$^{3+}$1</td>
<td><img src="image" alt="Co$^{3+}$1 ligand" /></td>
<td>1.82</td>
</tr>
<tr>
<td>Ni$^{2+}$1</td>
<td><img src="image" alt="Ni$^{2+}$1 ligand" /></td>
<td>0.49</td>
</tr>
<tr>
<td>Co$^{3+}$2</td>
<td><img src="image" alt="Co$^{3+}$2 ligand" /></td>
<td>&gt;100</td>
</tr>
<tr>
<td>Ni$^{2+}$2</td>
<td><img src="image" alt="Ni$^{2+}$2 ligand" /></td>
<td>0.59</td>
</tr>
<tr>
<td>Co$^{3+}$3</td>
<td><img src="image" alt="Co$^{3+}$3 ligand" /></td>
<td>&gt;100</td>
</tr>
<tr>
<td>Ni$^{2+}$3</td>
<td><img src="image" alt="Ni$^{2+}$3 ligand" /></td>
<td>0.80</td>
</tr>
<tr>
<td>Co$^{3+}$4</td>
<td><img src="image" alt="Co$^{3+}$4 ligand" /></td>
<td>&gt;100</td>
</tr>
<tr>
<td>Ni$^{2+}$4</td>
<td><img src="image" alt="Ni$^{2+}$4 ligand" /></td>
<td>0.55</td>
</tr>
<tr>
<td>Co$^{3+}$5</td>
<td><img src="image" alt="Co$^{3+}$5 ligand" /></td>
<td>&gt;100</td>
</tr>
<tr>
<td>Ni$^{2+}$5</td>
<td><img src="image" alt="Ni$^{2+}$5 ligand" /></td>
<td>3.48</td>
</tr>
<tr>
<td>Co$^{3+}$6</td>
<td><img src="image" alt="Co$^{3+}$6 ligand" /></td>
<td>&gt;100</td>
</tr>
<tr>
<td>Ni$^{2+}$6</td>
<td><img src="image" alt="Ni$^{2+}$6 ligand" /></td>
<td>7.69</td>
</tr>
<tr>
<td>(Co$^{3+}$)$_7$</td>
<td><img src="image" alt="Complex" /></td>
<td>0.690</td>
</tr>
</tbody>
</table>

**Anti-HIV activity**

AMD3100$^{17, 60-62}$ and the high potency CXCR4 antagonists that we have developed$^{16, 18-20}$ are bismacro cyclic with an aryl (xylyl) linker. Our previously collected data indicates that monomacrocyclic compounds will also have affinity for the receptor which can result in anti-HIV activity, but this will be lower than for the bismacro cyclic derivatives. The discussion relates the anti-HIV activity to CXCR4 binding as this link has been borne out in all of our previous research. The main reason for synthesising the monomacrocyclic compounds (metal complexes of 1–6) was to utilise them as simpler structural analogues to allow us to obtain X-ray structural data that models aspartate or glutamate coordination to the metal centre. We do not anticipate taking these compounds into further biological evaluation or in vivo studies as they have greater potential for off-target binding. However, it is still of interest to determine their antiviral activity and investigate the structure activity relationships for this subset of compounds.

Anti-HIV activity measurements in MT-4 cells were based on the viability of cells that had been infected or not infected with the HIV-1 (strain IIIB) and exposed to various concentrations of the test compound. Data are presented in Table 5. Viral strain IIIB is an X4 viral strain that solely uses the CXCR4 receptor as a co-receptor for cell entry, it does not use CCR5. Our previous studies show that activity in the anti-HIV assays usually indicates CXCR4 binding.

The free chelators showed IC$_{50}$ values of greater than 100 µM indicating no measurable anti-HIV activity for these compounds. This is consistent with previously analysed free macrocyclic chelators in which the hydrogen-bonding potential of the chelator has been disrupted by alkylation and they are only activated on inclusion of the metal centre to give the potential for coordinate bond formation.
Dinuclear (Co\textsuperscript{2+})\textsubscript{2}, as expected, shows a more potent anti-HIV activity than any of the mononuclear cobalt complexes. Its IC\textsubscript{50} = 0.690 \(\mu\)M, which is about 2.5-fold more efficient than [CoI\textsubscript{4}(OAc)]\textsuperscript{4-}. This gain in efficiency for the dinuclear complex is actually somewhat lower than expected. For example, our published dinuclear nickel complex,\textsuperscript{19} most analogous with Ni\textsuperscript{2+}\textsubscript{3}, exhibited an anti-HIV IC\textsubscript{50} = 0.014 \(\mu\)M. This value is 57-fold more efficient than Ni\textsuperscript{2+}\textsubscript{3}. Clearly, cobalt complexes, even in dinuclear compounds, are not as favourable for continued development of anti-HIV CXCR4 antagonists.

It is well-known that the substitution kinetics of low spin, octahedral Co\textsuperscript{3+} complexes are very slow, which would explain the poor binding of the Co\textsuperscript{3+} complexes. An explanation for the reduced activity of the Co\textsuperscript{2+} complexes may simply lie in the Irving-Williams series for the binding strength of first row transition metals that predict weaker binding from right to left on the first transition row.\textsuperscript{65}

Consistent with the Irving-Williams series is the enhanced binding affinity of the Ni\textsuperscript{2+} complexes over those of Co\textsuperscript{2+}. Each Ni\textsuperscript{2+} complex tested gave a measurable anti-HIV activity, with the ligand 1-4 complexes demonstrating sub-micromolar activity, with the ligand 5-6 complexes approximately an order of magnitude less potent. Perhaps this disparity can be attributed to the presence of at least one benzyl group in ligands 1-4, which has been shown to be crucial to the high CXCR4 affinity of AMD3100.\textsuperscript{17}

Relating the anti-HIV activity of the Ni\textsuperscript{2+} complexes to the coordination modes observed in the crystal structures above, is not clear-cut, as ligand sets (1-4 and 5-6) give structures of both the iso-bidentate and monodentate/H\textsubscript{2}O coordination modes. However, we should keep in mind that water molecules and the aspartate carboxylate groups would be available at the site of CXCR4 binding, which would allow a given complex to select its most favourable mode in the protein environment.

Conclusions

Six mono-macrocylic cross-bridged tetraazamacroyclic ligands have been complexed to Co\textsuperscript{2+}/Co\textsuperscript{3+} and Ni\textsuperscript{2+} concurrently with an acetate anion, which serves as a model carboxylate ligand for the aspartate side chains shown to interact with xylyl-bridged bis-cyclam CXCR4 antagonists on binding to the receptor. X-ray crystal structures of three of the Co\textsuperscript{2+}/Co\textsuperscript{3+} and five of the Ni\textsuperscript{2+} complexes were obtained, to complement recently published analogues. All of these structures were examined to learn about preferences for Co\textsuperscript{2+}/Co\textsuperscript{3+} and Ni\textsuperscript{2+} macrocycle complexes in binding carboxylate ligands, which could potentially be applied to CXCR4 antagonist design.

The cross-bridged Co\textsuperscript{3+} complexes studied were all complexed to the smaller cyclen-based ligands and were found only to coordinate to acetate in a chelating iso-bidentate manner, which is likely due the short M-N bond lengths and the resulting slightly distorted octahedral coordination geometries have near 90\(^\circ\) N\textsubscript{eq}-M-N\textsubscript{eq} bond angles that allow only room for a single chelated acetate opposite the cross-bridge. As antagonists, these complexes are poor, potentially due to the slow substitution kinetics of Co\textsuperscript{3+}. These complexes were diamagnetic and had electronic spectra typical of a low spin, octahedral Co\textsuperscript{3+} ion. Their cyclic voltammograms were simple, with quasi-reversible reductions to Co\textsuperscript{2+} and Co\textsuperscript{3+}.

The cross-bridged Co\textsuperscript{2+} complexes studied were all complexed to the larger cavity cyclam-based ligands and the only structurally characterised example, [Co5(OAc)(H2O)]\textsuperscript{8+}, binds acetate in a monodentate fashion with a water molecule completing the coordination sphere. The larger Co\textsuperscript{2+} cation formed a much smaller N\textsubscript{eq}-M-N\textsubscript{eq} bond angle of ~83\(^\circ\), which allowed the monodentate acetate/water ligands room to bind equatorially. The only measurable affinity to CXCR4 of any of the cobalt complexes was from this group as perhaps the faster substitution kinetics of high spin Co\textsuperscript{2+} allow binding. This high spin nature was confirmed for all three cyclam-based ligand complexes by electronic spectra typical of this species. Cyclic voltammetry revealed complex behaviour with multiple oxidations which is consistent with the high spin species leading to multiple different complexes in acetonitrile solution incorporating bound solvent molecule(s).

Stable Ni\textsuperscript{2+} complexes were formed with both cross-bridged cyclam- and cyclen-based ligands. Most of these complexes were found to include a monodentate acetate/water ligand pair equatorially opposite of the ligand cross-bridge, as was observed for the larger Co\textsuperscript{3+} ion. Here, the N\textsubscript{eq}-M-N\textsubscript{eq} bond angles were always <85.6\(^\circ\). However, coordination sphere flexibility was observed as two complexes, both of the smaller cyclen-based ligands 4 and 6, demonstrated iso-bidentate chelation of acetate and N\textsubscript{eq}-M-N\textsubscript{eq} bond angles >87\(^\circ\). In comparison to unbridged tetraazamacrocycle Ni\textsuperscript{2+} complexes of acetate, it was discovered that the more flexible non-bridged macrocycles can expand equatorially and produce larger N\textsubscript{eq}-M-N\textsubscript{eq} bond angles (up to ~108\(^\circ\)) which strongly selected for the equatorial coordination of acetate in the bidentate chelating mode. Electronic spectra of all Ni\textsuperscript{2+} cross-bridged complexes gave typical distorted octahedral behaviour, allowing for the calculation of \(\Delta\omega\), which was larger for cyclen-based ligands and was reduced as benzyl substituents were added. Cyclic voltammograms of the cross-bridged Ni\textsuperscript{2+} complexes mostly showed reversible Ni\textsuperscript{2+}/Ni\textsuperscript{3+} redox couples, although cyclam-based ligands with at least one benzyl substituent made the oxidation irreversible.

All of the Ni\textsuperscript{2+} complexes showed micromolar activity as CXCR4 antagonists, showing that this ion is a better choice than Co\textsuperscript{2+}/Co\textsuperscript{3+} for this application. Only one mononuclear cobalt(II) complex exhibited measurable CXCR4 activity. However, the mono-macroyclic antagonists investigated in this work do not rival the bis-macroyclic antagonists we have previously identified which can bind CXCR4 through interaction with two aspartate residue side chains.\textsuperscript{19} The first dicobalt(II) bis-macroyclic complex we have prepared showed only a slight improvement over its mononuclear analogue, a surprisingly small improvement based on data from other metal ions. This result will be checked with the synthesis of other dicobalt analogues, but suggests that cobalt(II) will not be the metal of choice for out high-efficiency bismacroyclic CXCR4 antagonist.
program. Our future work will involve further investigation of a wider range of Ni$^{2+}$ bis-macrocyclic compounds as CXCR4 antagonists.

**Conflicts of interest**

There are no conflicts to declare.

**Acknowledgements**

T.J.H. acknowledges the Health Research award for project number HR13-157, from the Oklahoma Center for the Advancement of Science and Technology. This project was supported by the National Center for Research Resources and the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20MD001834. T.J.H. acknowledges the Research Corporation (CC6505) for funding. T.J.H. also acknowledges the Henry Dreyfus Teacher-Scholar Awards Program for support of this work. We gratefully acknowledge the Daisy Appeal Charity for funding (Grant: DAllu0211) and fellowship funding for BPB, and the University of Hull for infrastructure support. This work, in part, was supported by funding of the KU Leuven (GOA/10/014, PF/10/018 and C22/17/008) and the Foundation of Scientific advancement of Science and Technology. This project was supported by the National Center for Research Resources and the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20MD001834. T.J.H. acknowledges the Research Corporation (CC6505) for funding. T.J.H. also acknowledges the Henry Dreyfus Teacher-Scholar Awards Program for support of this work. We gratefully acknowledge the Daisy Appeal Charity for funding (Grant: DAllu0211) and fellowship funding for BPB, and the University of Hull for infrastructure support. This work, in part, was supported by funding of the KU Leuven (GOA/10/014, PF/10/018 and C22/17/008) and the Foundation of Scientific Research (FWO no. G.0485.08 and G.0528.12).

**Notes and references**


