

Oxovanadium(IV) Cyclam and Bicyclam Complexes: Potential CXCR4 Receptor Antagonists

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Received October 18, 2009

Metal complexation can have a major influence on the antiviral and coreceptor binding properties of cyclam and bicyclam macrocycles. We report the synthesis of the vanadyl cyclam complexes $[V^{(IV)}O(\text{cyclam})SO_4]$ (**1**) and $[V^{(IV)}O(\text{cyclam})Cl]Cl$ (**2**) and the analogous xylylbicyclam sulfato (**3**) and chlorido (**4**) complexes. The X-ray crystal structures of $1 \cdot 1.33CH_3OH$ and $2 \cdot CH_3OH \cdot 1.5H_2O$ show short $V=O$ bonds (1.6093(19) and 1.599(3) Å, respectively) with monodentate sulfate H-bonded to ring NH groups for **1**, but a long $V-Cl$ bond (2.650(12) Å) for **2**. The solid-state structures of **3** and **4** were compared to those of **1** and **2** using vanadium K-edge extended X-ray absorption fine structure (EXAFS) data. These suggested that complex **4** was oligomeric and contained bridging chlorido ligands. Electron paramagnetic resonance (EPR) studies suggested that the SO_4^{2-} (from **1**) and Cl^- (from **2**) ligands are readily substituted by water in solution, whereas these remain partially bound for the V^{IV} xylylbicyclam complexes **3** and **4**. The vanadyl xylylbicyclam complexes were highly active against HIV-1 (III_B) and HIV-2 (ROD) strains with IC_{50} values in the range $1-5 \mu M$ for **3** and $0.1-0.3 \mu M$ for **4**; in contrast the vanadyl cyclam complexes **1** and **2** were inactive. The factors that contribute to the activity of these complexes are discussed. Studies of vanadyl cyclam docked into a model of the human CXCR4 coreceptor revealed that the coordination of vanadium to the carboxylate of Asp171 may be accompanied by H-bonding to the macrocycle and an attractive $V=O \cdots H$ interaction involving the backbone Trp195 α -carbon proton of CXCR4. In addition, hydrophobic interactions with Trp195 are present. Both ring configuration and the xylyl linker may play roles in determining the higher activity of the bicyclam complexes.

Introduction

Macrocycles such as cyclam derivatives show promise as stem cell mobilizers and antivirals, including activity against HIV and related AIDS disease. Drug treatments used today against HIV infection are primarily associated with postinfection and classified as (i) reverse transcriptase inhibitors (RTIs), antiretroviral drugs that inhibit the enzyme reverse transcriptase, essential for successful replication of HIV; (ii) protease inhibitors that inhibit the function of HIV-1 protease, and (iii) integrase inhibitors such as Raltegravir, FDA-approved in 2007 for the treatment of HIV infections.¹ A fourth class of antiviral agents, known as entry inhibitors, target the preinfection cycle of HIV. Maraviroc is an entry

inhibitor that specifically targets the chemokine receptor, CCR5. Importantly, Pfizer's Celsentri (maraviroc), is the first new oral class of HIV treatment and was licensed by the European Agency for the Evaluation of Medicinal Products (EMA) in September 2007. Currently, all other available oral HIV medicines target HIV only after it has entered the immune cells.²

The biological targets of entry inhibitor drugs are specific protein receptors expressed on the surface of helper T-cells (a type of white blood cell or leukocyte) found in the immune system. CD4 is a glycoprotein receptor and the primary

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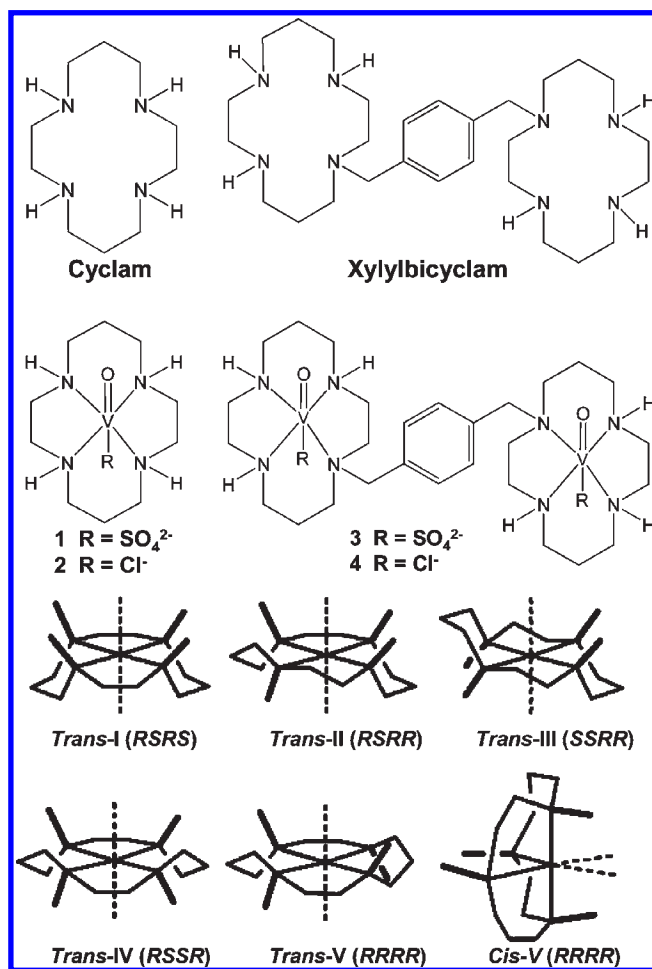
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receptor used by HIV-1 to gain entry to host cells.^{3–5} Binding of the virus to CD4 occurs through attachment of the viral envelope glycoprotein gp120, and through this association, the virus gains access to the coreceptor CXCR4, an important coreceptor for HIV-1.¹ Interaction with CXCR4 enables the virus to fuse with the cell membrane, gaining entry to the cell where it effectively discharges the viral RNA, leading to replication and the onset of infection. CXCR4 is associated with HIV infection during the later stages of AIDS disease when the immune system deteriorates rapidly.

The small molecule CXCR4 chemokine antagonist xyllylbicyclam (Chart 1; AMD3100) reached phase II clinical trials as an entry inhibitor drug against HIV ($IC_{50} < 0.1 \mu M$). It has since been further pursued as a stem cell mobilizer.⁶ The product has since been relaunched commercially under the name of *Mozobil* having completed phase III clinical trials as a stem cell mobilizer and was approved for clinical use in the US in December 2008. The same membrane protein CXCR4 that aids entry of HIV to cells also anchors stem cells in the bone marrow. Mobilization of stem cells is beneficial during, for example, transplant therapy.

Cyclam macrocycles can bind strongly to d-block metal ions.^{7–10} The specific configurations adopted by metal cyclam complexes¹¹ (Chart 1) may be important for receptor recognition and biological activity,¹² and it seems likely that the various configurations of metallo-cyclams are recognized differently by the coreceptor. Zinc(II) in particular is abundant in the blood plasma (ca. $19 \mu M$), and the binding of xyllylbicyclam to zinc(II) may play a role in its mechanism of action.^{13–17} Indeed zinc complexes with configurationally constrained bicyclam ligands⁹ can exhibit higher activity against HIV-1 than AMD3100. Recognition of metalocyclams such as Zn(II)–xyllylbicyclam by the CXCR4 coreceptor is thought to involve a combination of direct coordination to side chains (Asp, Glu), hydrogen-bonding to cyclam NH groups and hydrophobic interactions (with the periphery of the cyclam rings and the xyllyl linker).^{12,18} Here we investigate the role of axial interactions with the metal by introducing the

Chart 1. Configurations of Metalocyclams and Structures of Cyclam, Xyllylbicyclam, and Complexes 1–4^a



^a AMD3100 is the octahydrochloride salt (xyllylbicyclam · 8HCl).

vanadyl fragment (VO²⁺) into the cyclam ring. This new class of complexes introduces a different application for the bioinorganic chemistry of vanadium and complements the wealth of vanadium research involving insulin-enhancing agents, cofactors in haloperoxidases, catalysts, and nitrogenase cofactors.^{19–21} There appears to be little previously reported work on vanadyl cyclams. We have studied both cyclam and xyllylbicyclam complexes with additional chloride and sulfate ligands using a variety of techniques including X-ray crystallography, X-ray absorption near-edge spectroscopy (XANES), extended X-ray absorption fine structure (EXAFS), electron paramagnetic resonance (EPR), and molecular modeling. The anti-HIV activity of these vanadyl cyclam and xyllylbicyclam complexes was also determined.

Experimental Section

Materials. Cyclam (1,4,8,11-tetraazacyclotetradecane), α, α' -dibromo-*p*-xylene, ethyl trifluoroacetate, triethylamine, barium chloride, and vanadyl sulfate hydrate were purchased from Aldrich Chemical Co. and were reagent grade unless otherwise stated.

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Synthesis of Oxovanadium(IV)–Cyclam Sulfate, 1. Cyclam, (0.91 g, 4.56 mmol) was dissolved in HPLC grade methanol (35 mL) and refluxed under N₂ for 10 min. Vanadyl sulfate hydrate (1.07 g, 4.56 mmol, 1 mol equiv) was added, and the reaction was refluxed for 24 h. On stirring, a color change was observed from blue to green after 1 min and to gray after 3 min. After 15 min, the solution turned cloudy green, and after 30 min, it was blue/gray in color. After 2.5 h, this color deepened to a dark blue/gray. The solution settled into two layers. The top layer was vivid green in color, and a blue precipitate settled out as the bottom layer. The top layer was separated by centrifugation and then filtration, and the solvent was removed under reduced pressure to give a green powder (yield 1.50 g, 4.13 mmol, 90.6%). The product was further dried under vacuum. Anal calc for C₁₀H₂₄N₄VSO₅·MeOH·0.5H₂O: %C, 32.70; %H, 6.23; %N, 13.86. Found: %C, 32.43; %H, 6.49; %N, 14.46. ESI-MS: $m/z = 363.90 [M + H]^+$; $\nu_{\max} (\text{KBr})/\text{cm}^{-1} \text{V}=\text{O}, 974; \text{SO}_4, \nu_3 1106, 1064, 1028; \nu_4 619, 598.$

Crystals of 1·1.33CH₃OH suitable for X-ray diffraction were obtained by slow diffusion of ether into a methanolic solution of the product at 277 K.

Synthesis of Oxovanadium(IV)–Cyclam Dichloride, 2. Oxovanadium(IV)–Cyclam, 1 (0.73 g, 0.21 mmol), was dissolved in distilled water (10 mL), and barium chloride (0.63 g, 0.30 mmol, 3 mol equiv excess) was added as an aqueous solution (4 mL). A white precipitate formed immediately, and the mixture was stirred at room temperature in air for 16 h. The mixture was separated using centrifugal force (4000 rpm, 1 h), and the green solution was filtered. The solvent was removed under reduced pressure and further dried under vacuum to give a crystalline green powder. Anal calc for C₁₀H₂₄N₄VOCl₂·H₂O: %C, 33.70; %H, 7.35; %N, 15.70. Found: %C, 34.04; %H, 7.22; %N, 16.05. ESI-MS: $m/z = 302.10 (\text{Cl}^{35}), 304.00 (\text{Cl}^{37}) [M]^+$; $\nu_{\max} (\text{KBr})/\text{cm}^{-1} \text{V}=\text{O}, 945.$ Crystals of 2·CH₃OH·1.5H₂O suitable for X-ray diffraction were formed by slow diffusion of ether into a methanolic solution of the product at 277 K. Yield of crystals 0.03 g, 0.08 mmol, 36.6%.

Synthesis of Oxovanadium(IV)–(Xylylbicyclam) Disulfate, 3. Xylylbicyclam (0.05 g, 0.10 mmol) was dissolved in 5 mL anhydrous methanol and refluxed gently under argon for 5 min. Vanadyl sulfate hydrate (0.05 g, 0.20 mmol, 2 mol equiv) was added, and a color change was observed, both in the solution and in the solid added. After 3 min, the initial clear solution began to turn green and the blue solid (vanadyl sulfate hydrate) turned gray. After 15 min, the reaction became more homogeneous and the color darkened to brown. On standing, the flask contents settled into two layers, and a pale green solution formed on top of the dark solid. The reaction was refluxed for 16 h, and the solution on standing was green in color. The dark solid changed color to light blue. The green solution was separated from the blue solid under centrifugal force, 4000 rpm, 1 h, and the solvent removed under reduced pressure and further dried under vacuum. The product dried to give a dark green powder (0.05 g, 0.06 mmol, 61.9%).

Anal calc for C₂₈H₅₄N₈V₂S₂O₁₀·MeOH·3H₂O: %C, 38.07; %H, 7.05; %N, 12.25. Found: %C, 37.53; %H, 7.29; %N, 11.67. ESI-MS: $m/z = 829.1 [M + H]^+$; $\nu_{\max} (\text{KBr})/\text{cm}^{-1} \text{V}=\text{O}, 945; \text{SO}_4^{2-} \nu_3 1120, 1034; \nu_4 615.$

Synthesis of Oxovanadium(IV)–(Xylylbicyclam) Tetrachloride, 4. Oxovanadium(IV)–(xylylbicyclam) sulfate 3, (0.05 g, 0.06 mmol) was dissolved in water (3 mL) and heated until fully dissolved. Barium chloride (0.03 g, 0.12 mmol, 2 mol equiv) was added as an aqueous solution (2 mL), and a cloudy solution formed immediately. The mixture was stirred for 16 h at room temperature and then separated under centrifugal force to give a green solution and a blue solid. The green solution was decanted and filtered, and the solvent was removed by freeze-drying. On drying, a color change was noted from green to brown, and on redissolving in water, a green solution was obtained (yield: 0.04 g, 4.50 mmol, 75%).

Anal calc for C₂₈H₅₄N₈V₂Cl₂O₂·5H₂O·2NaCl: %C, 36.77; %H, 7.05; %N, 12.25. Found: %C, 37.09; %H, 7.02; %N, 11.53.

ESI-MS: $m/z = 705.4 [M - 2\text{Cl}]^{2+} (100\%), 741.3 [M - \text{Cl}]^+;$ $\nu_{\max} (\text{K/Br})/\text{cm}^{-1} \text{V}=\text{O} 964.$

X-ray Crystallography. Diffraction data for compound 1 and 2 were collected at 150 K using a Bruker Smart Apex CCD diffractometer. Absorption corrections for all data sets were performed with the multiscan procedure SADABS.²² Both structures were solved by direct methods (SHELXS or SIR92).^{23,24} Refinement was against F^2 using all data (CRYSTALS).²⁵ Unless stated otherwise, H-atoms were placed in geometrically calculated positions. In complex 1, one of the molecules of methanol of solvation was disordered over 3 sites about a 3-fold axis, and no H-atoms were placed on this part of the structure. A second molecule of methanol of solvation was on a general position; the hydroxyl H-atom on this molecule was located in a difference map and refined. In complex 2, H atoms attached to oxygen were also located in a difference map. H-atom positions were initially refined subject to restraints and thereafter allowed to ride on their parent atoms. The programs Diamond 3.020,²⁶ Mercury 1.4.1,²⁷ and ORTEP 32²⁸ were used for analysis of data and production of graphics. X-ray crystallographic data have been deposited in the Cambridge Crystallographic Data Centre under the accession numbers CCDC 748340 and 748341.

IR Spectroscopy. Infrared spectra were recorded as KBr pellets in the range 4000–400 cm⁻¹ on a Perkin-Elmer Paragon 1000 Fourier-transform spectrometer.

X-ray Absorption Spectroscopy. X-ray absorption spectra (XAS) were recorded at Stanford Synchrotron Radiation Laboratory (SSRL) on the 20-pole wiggler beamline 7–3, under ring operating conditions of 80–100 mA and 3 GeV. Data were collected using a Si (220) $\phi = 0^\circ$ double crystal monochromator and the signal was detected using a 30 element Ge detector array. All samples were prepared by lightly grinding 5 mg of vanadium complex with boron nitride in a mortar and pestle until a fine uniform powder was formed. XAS data were collected in the energy range from 5235 to 6485 eV using XAS Collect software. Ten scans of each compound were obtained at the V K edge, to ensure reproducibility. Energy calibration was performed using the V K-edge spectrum of a vanadium metal foil, assuming the lowest energy peak to be 5465 eV. Data processing, including averaging scans, energy calibration, background subtraction, and normalization was performed in SixPACK.²⁹ EXAFS analysis was carried out using Artemis.³⁰

EPR Spectroscopy. EPR spectra were recorded on an X-band Bruker ER200D-SCR spectrometer connected to a Datalink 486DX PC with EPR Acquisition System, version 2.42, software. Measurements were recorded in fluid solution at 298 K and in frozen solution at 77 K. Data were simulated using Bruker WINEPR Simphonia version 1.26 software by the National EPR Centre, University of Manchester, UK, and

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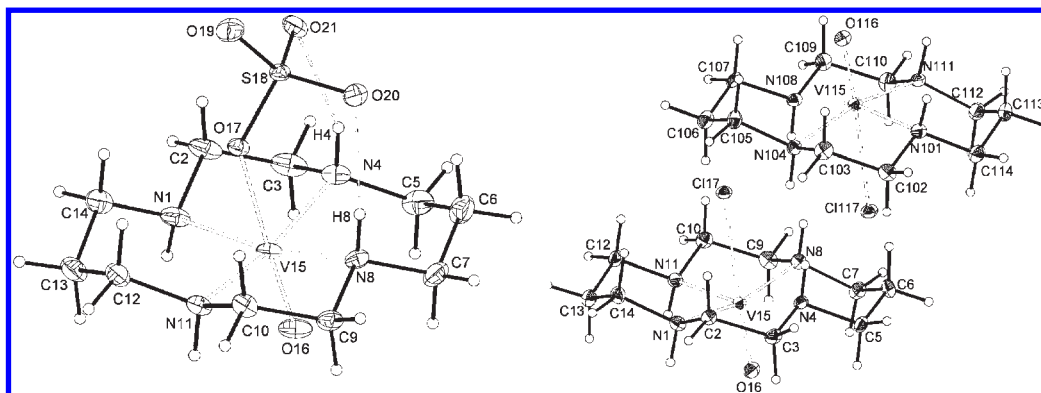


Figure 1. ORTEP (30% probability thermal ellipsoids) views of the sulfato complex **1** (a, left) and the chlorido complex **2** (b, right).

corrected as for the DPPH (2,2-diphenyl-1-picrylhydrazyl) standard.³¹

Antiviral Testing. Experimental protocols for anti-HIV testing are detailed in the Supporting Information.³²

Molecular Modeling. Our previously reported homology model of CXCR4¹² was used for manual docking of oxovanadium(IV) cyclam complex **1** under the SYBYL version 6.9 (Tripos Associates, St. Louis, MO) modeling environment. Complex **1** was first superimposed onto one ring of the previously docked [Zn₂-xylylbicyclam]–CXCR4 interaction¹² using all equivalent C α atoms (crystal structures for both vanadium bicyclams complexes **3,4** were unavailable). The vanadium cyclam complex **1** was then “merged” with the CXCR4 protein model into a separate MOL2 file using SYBYL6.9. Expected clashes were observed between Asp171 and the sulfate group that were subsequently removed and two models of possible interaction were produced. In model 1 (Figure 7a), parameters were set with a view to investigate the possibility of metal–carboxylate interactions with Asp171 in the binding site. In this case, the vanadium center was fixed relative to the receptor and distance restraints of 2.14 Å (V–sulfate bond length) with an average force constant of 500 kcal mol^{−1} Å^{−2} were applied to the postulated V–O bond with Asp171, to represent metal–carboxylate binding. A second distance (1.609 Å) was set for V=O to maintain the bond length and prevent stretching during the minimization process. Bad contacts and geometries were then relieved by energy minimizing the region of the CXCR4 receptor surrounding the oxovanadium(IV) cyclam complex **1** (all amino acid residues with a sphere radius of 5 Å from the cyclam ligand). In model 2 (Figure 7b), no distance restraint was applied between the vanadium center and carboxylate oxygens of Asp171, but docking was otherwise undertaken as in model 1.

Results

Synthesis and Structure. The synthesis of the sulfate complex **1** was achieved by addition of 1 mol equiv of VOSO₄·*x*H₂O to cyclam in methanol and refluxing for 24 h. The chloride complex **2** was synthesized by addition of 1 mol equiv BaCl₂ to **1** in aqueous solution to precipitate the sulfate ligand as BaSO₄. For complexes **3** and **4**, xylylbicyclam was synthesized according to a previously published procedure³³ and the sulfate and chloride

complexes were obtained by procedures similar to those used for the corresponding cyclam complexes.

X-ray crystallographic data for complexes **1** and **2** were obtained at 150 K. Complex **1** (Figure 1a) crystallized as [V(IV)O(cyclam)SO₄]·1.33CH₃OH as the *trans*-III isomer (space group *R3c*). A definition of the six possible configurations cyclam can adopt on complexing with a metal ion (Chart 1) was proposed over 40 years ago,¹¹ and the *trans*-III configuration is generally considered to be the most thermodynamically stable isomer. Similarly complex **2**, [V(IV)O(cyclam)Cl]Cl·CH₃OH·1.5H₂O (Figure 1b), also exhibited the *trans*-III configuration. This complex crystallized with two molecules in the asymmetric unit (space group *Cc*). Both cyclam complexes have a distorted octahedral geometry with sulfate in **1** and chloride in **2** as ligands *trans* to the axial V=O group. Complex **1** is neutral and the sulfate ligand coordinates as a monodentate ligand. Two of the remaining sulfate oxygens form hydrogen bonds to two NH protons of the cyclam ring (Figure 1a). Crystal packing along the *c* axis of the unit cell shows hydrogen bonding between adjacent macrocycles via the sulfate ligand as NH···O···HN. In complex **2**, crystal packing shows networks of hydrogen bonding between chlorine atoms, ring protons, and solvent molecules, contributing to the stability of the *trans*-III configuration. The Cl–V=O angle (176.99(12)°) is significantly smaller than the corresponding O₃SO–V=O angle in the sulfate complex, **1** (177.83(9)°). The N(1)–V(15)–N(8) angle (165.46(16)°) is also significantly smaller than the corresponding angle in complex **1**, (166.88(7)°). Selected bond lengths and angles are given in Table 1 for comparison with EXAFS data. V–N bond lengths are comparable with similar examples for vanadium complexes found on searching the CCDC. No previous examples of double H-bonding by sulfate as an axial ligand in cyclam-type macrocycles appear to have been reported.

Infrared Spectroscopy. IR spectroscopy for **1** showed a fairly high characteristic V(IV)=O vibrational stretch at 974 cm^{−1} suggesting that sulfate is weakly bound.²⁰ The V(IV)=O stretch for the chloride species **2** is found at 988 cm^{−1}. The V(IV)=O stretch for the sulfate bicyclam **3** was at 945 cm^{−1} and for chlorido bicyclam **4** at 964 cm^{−1}. Both stretches are lower for the bicyclams, consistent with stronger binding of sulfate and chloride as suggested by the EPR data (vide infra).

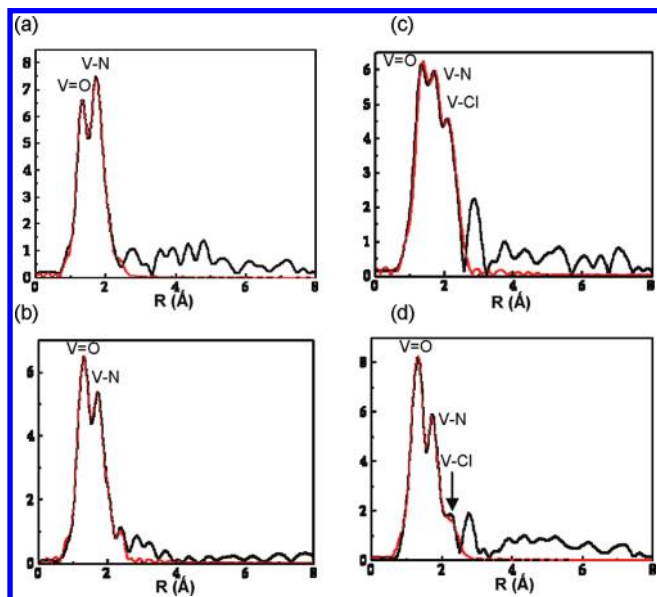
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Table 1. Selected Bond Distances (Å) and Angles (deg) for Complexes **1** and **2**

bond/angle	length (Å)/angle (deg)	
	[VO(cyclam)SO ₄] · 1.33CH ₃ OH (1)	[VO(cyclam)Cl]Cl · CH ₃ OH · 1.5H ₂ O (2)
N(1)—V(15)	2.107(2)	2.097(4)
N(4)—V(15)	2.114(2)	2.097(4)
N(8)—V(15)	2.0841(19)	2.081(4)
N(11)—V(15)	2.0976(19)	2.082(4)
V(15)—O(16)	1.6093(17)	1.599(3)
V(15)—O(17)	2.1359(16)	
V(15)—Cl(17)		2.6501(12)
O(16)—V(15)—O(17)	177.83(9)	
O(16)—V(15)—Cl(17)		176.99(12)

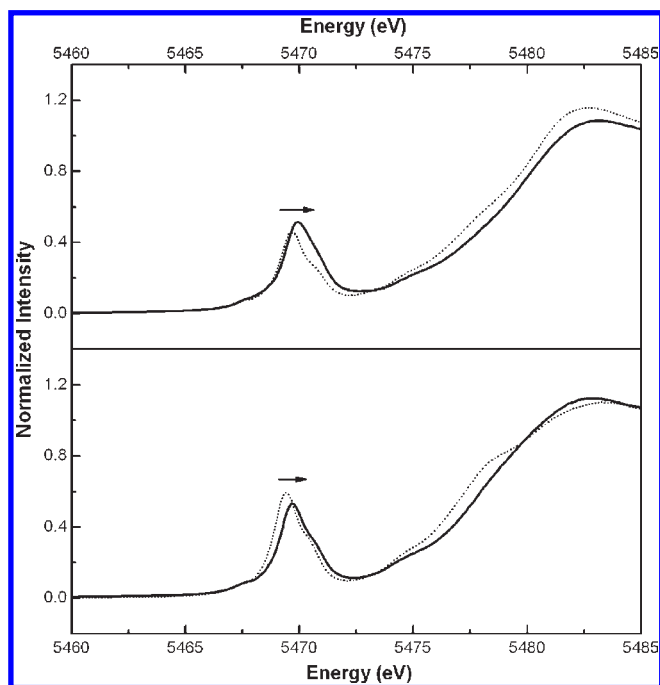
**Figure 2.** R-space (nonphase shift corrected) with calculated fits EXAFS data for (a) [V^{IV}O(cyclam)SO₄] **1**, (b) [(V^{IV}O)₂(xylylbicyclam)-(SO₄)₂] **3**, (c) [V^{IV}O(cyclam)Cl]⁺ **2**, and (d) [(V^{IV}O)₂(xylylbicyclam)-(Cl)₂]²⁺ **4**. (key) Black line, R-space; red line, R-space fit.

X-ray Absorption Spectroscopy. Vanadium K-edge EXAFS data for the two bicyclam complexes **3** and **4** are compared with those of the cyclam analogues **1** and **2** in Figure 2 which shows the R-space EXAFS and calculated fits. Data were Fourier-transformed over k-ranges of at least 12.5 Å⁻¹; EXAFS-derived parameters were obtained from R-space fits and are provided in Table 2. For the sulfate complexes **1** and **3**, two main first-coordination shells are clearly observed and are assigned as V=O and V—N/O single scattering peaks. The anticipated scattering peak for the axial V—OSO₃ single scattering path is seemingly indistinguishable from that of the intense equatorial V—N scattering feature due to similarities in scattering path length and phase. For the chlorido complexes **2** and **4**, three main first-coordination shells are observed in the data, assigned to the V=O, V—N, and V—Cl scattering pathways. The presence of the axial V—Cl scattering peak reflects the marked differences between second-row and third-row scatterers and more pronounced differences in bond distances. Statistical parameters from the best R-space fits and all k-space data for the four complexes are given in the Supporting Information; Table S2, Figure S2. The

Table 2. EXAFS Parameters for Oxovanadium(IV)—Cyclam Complexes **1** (Sulfate) and **2** (Chloride) Compared with Bicyclam Complexes **3** (Sulfate) and **4** (Chloride)

complex	scattering pair	N ^a	R (Å) ^a	σ ^{2a}	SO ₂ ^a	ΔE _o ^a	CN ^c	global SO ₂ ^d
1	V—O	1 ^b	1.63	0.00096	0.88	0.88	0.88	1.0
	V—N	4 ^b	2.11	0.0016	0.96	0.19	3.84	
	V—O	1 ^b	2.46	0.0023	0.84	1.37	0.84	
2	V—O	1 ^b	1.63	0.0034	1.23	6.94	1.23	1.0
	V—N	4 ^b	2.11	0.0017	0.81	5.69	3.24	
	V—Cl	1 ^b	2.58	0.0044	1.43	-3.38	1.43	
3	V—O	1 ^b	1.62	0.00083	1.03	-2.07	1.03	1.0
	V—N	4 ^b	2.12	0.0020	0.80 ^b	1.99	3.20	
	V—O	1 ^b	2.59	0.0073	0.80 ^b	19.81 ^e	0.80	
4 Fit 1	V—O	1 ^b	1.61	0.0011	1.31	-1.40	1.05	0.8
	V—N	4 ^b	2.13	0.0054	1.26	1.33	4.03	
	V—Cl	1 ^b	2.63	0.0052	0.59	-6.45	0.47	
4 Fit 2	V—O	1 ^b	1.61	0.0011	1.31	-1.59	1.05	0.8
	V—N	4 ^b	2.13	0.0059	1.36	1.68	4.35	
	V—Cl	1 ^b	2.63	0.011	1.20 ^b	-8.37	0.96	

^a N = path degeneracy, R = distance in Å, σ² = bond variance (related to Debye–Waller factor), SO₂ = amplitude reduction parameter, and ΔE_o = internal reference energy. ^b These parameters were fixed during the fitting procedures. ^c Estimated coordination number for each path, where CN = N*SO₂*(GSO₂). ^d Global SO₂ (GSO₂) is defined as a global amplitude reduction factor (equivalent for all paths). GSO₂ was adjusted in cases where CN for V—O deviated significantly from 1. ^e Note the ΔE_o is large for this sample; however, this provided the best possible fit. ¹ (k³, Δk = 2–13 Å⁻¹, Δr = 0.7–2.5 Å), **2** (k³, Δk = 1.6–12.5 Å⁻¹, Δr = 0.5–2.6 Å), **3** (k³, Δk = 1.7–12.5 Å⁻¹, Δr = 0.7–2.5 Å), **4** (k³, Δk = 2–13 Å⁻¹, Δr = 0.7–2.5 Å).

**Figure 3.** Normalized solid-state XANES spectra of the pre-edge region of the four vanadyl compounds. (top) [V^{IV}O(cyclam)SO₄] **1** (·····), [(V^{IV}O)₂(xylylbicyclam)(SO₄)₂] **3** (—). (bottom) [V^{IV}O(cyclam)Cl]⁺ **2** (·····), [(V^{IV}O)₂(xylylbicyclam)(Cl)₂]²⁺ **4** (—). The arrow indicates the slight increase in energy of the pre-edge feature when moving from vanadyl cyclam to bicyclam for both the Cl and SO₄ compounds.

V K-edge XANES spectra for complexes **1–4** (Figure 3) are dominated by intense pre-edge features due to formally electric-dipole-forbidden V_{3d} ← V_{1s} transitions. The

Table 3. EPR Parameters $A(G)$ and g -values for Cyclam and Bicyclam Complexes in Water at 298 K and Aqua Complexes (Axial Ligand, X = Solvent)

cyclam complexes axial ligand X trans to V=O				bicyclam complexes axial ligand X trans to V=O			
parameter/temp	sulfate (1)	chloride (2)	H ₂ O	parameter/temp	sulfate (3)	chloride (4)	H ₂ O
$A_{\text{iso}}(G)$ 298 K	88	88	88	$A_{\text{iso}}(G)$ 298 K	84 95	87 115	88
g_{iso} 298 K	1.972	1.972	1.972	g_{iso} 298 K	1.974 1.972	1.972 1.959	1.974

Table 4. EPR Parameters $A(G)$ and g -Values for Cyclam and Bicyclam Complexes in Methanol at 298 and 77 K

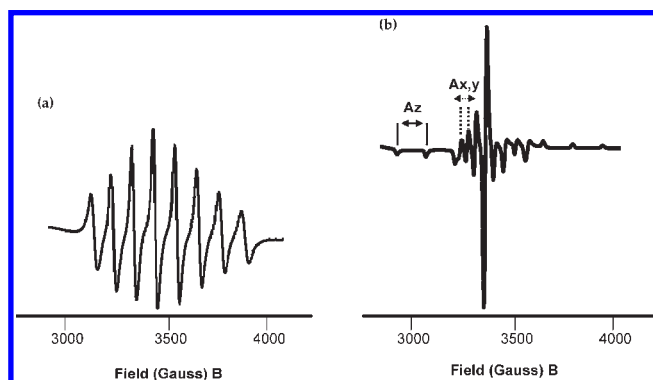
cyclam complexes axial ligand X trans to V=O				bicyclam complexes axial ligand X trans to V=O		
parameter/temp	sulfate (1)	chloride (2)	MeOH	parameter/temp	sulfate (3)	chloride (4)
$A_{\text{iso}}(G)$ 298 K	88	88	88	$A_{\text{iso}}(G)$ 298 K	90	90
g_{iso} 298 K	1.972	1.972	1.972	g_{iso} 298 K	1.967	1.973
A^{\parallel} 77 K	167 <i>zz</i>	167 <i>zz</i>	167 <i>zz</i>	A^{\parallel} 77 K	167 <i>zz</i> A_1	167 <i>zz</i> A_1 174 <i>zz</i> A_2
A^{\perp} 77 K	53 <i>xx</i>	53 <i>xx</i>	53 <i>xx</i>	A^{\perp} 77 K	53 <i>xx</i> A_1	53 <i>xx</i> A_1 53 <i>xx</i> A_2
g 77 K	1.968 <i>zz</i> 1.983 <i>xx</i>	1.968 <i>zz</i> 1.983 <i>xx</i>	1.968 <i>zz</i> 1.983 <i>xx</i>	g 77 K	1.957 <i>zz</i> A_1 1.972 <i>xx</i> A_1	1.957 <i>zz</i> A_1 1.971 <i>xx</i> A_1 1.950 <i>zz</i> A_2 1.971 <i>xx</i> A_2

^a A_1 and A_2 represent $A(G)$ parameters for the overlapping sets of signals seen for oxovanadium(IV)–xylylbicyclam **3** (1 set) and **4** (2 sets) in methanol at 77 K.

noncentrosymmetric environment at the V center, enforced primarily by the presence of the short V=O bond, allows for large 4p/3d mixing and thus intense pre-edge features for all complexes. The overall nature of the ligand field is very similar for all cases, with subtle differences in both transition intensities and energies (vide infra).

EPR Spectroscopy. EPR spectroscopy revealed 8-line spectra associated with the ⁵¹V ($I = 7/2$, 99.76% natural abundance) quadrupolar nucleus, as expected for these 3d¹ complexes. Calculated A and g -values in spectra of room temperature and frozen solutions are shown in Tables 3 and 4 for cyclam and bicyclam complexes, in water and methanol respectively, and spectra for the cyclam complexes are shown in Figure 4. Spectra for the bicyclam complexes are shown in Figures 5 (room temperature) and 6 (frozen solutions). Simulated EPR spectra for all samples are shown in Figure S3 of the Supporting Information. Data were simulated using Bruker WINEPR Simphonia version 1.25 software and corrected as for the DPPH standard.³¹ Spectra were obtained in water and methanol and compared with a reference complex where the sixth axial ligand X is expected to be a solvent molecule. Synthesis procedures for solvent-exchanged complexes (solvent ligand denoted X) are detailed in the Supporting Information (S4). These complexes were synthesized for EPR study by removing the chloride ligands from complexes **2** and **4**.

Antiviral Testing. All four compounds were tested for antiviral activity, and results are shown in Supporting Information Table S1 (cyclam complexes) and Table 5 (bicyclam complexes). The cyclam complexes **1** and **2** were inactive (IC₅₀ values > 340 and > 360 μM, respectively). The xylylbicyclam complexes **3** and **4** were highly active, although less active than AMD3100 and the Zn-complex, Zn₂-xylylbicyclam. Cytotoxicity levels are low, but slightly higher than for AMD3100 and Zn₂-xylylbicyclam.

**Figure 4.** EPR spectra of sulfato oxovanadium(IV) cyclam **1**: (a) in water at 298 K (the aqua complex, see Table 3) and (b) in frozen methanol at 77 K (sulfato complex).

Coreceptor Docking. Oxovanadium(IV)–cyclam complex **1** was docked into an homology model of CXCR4, and two models of possible interaction were produced, in keeping with experimental observations from EPR data. Differences in bonding interactions for the two models were noticed.

In model 1 (Figure 7a), parameters were set with a view to investigate the possibility of metal–carboxylate interactions with Asp171 in the binding site. The vanadium center was fixed relative to the receptor and distance restraints of 2.14 Å (V–sulfate bond length) with an average force constant of 500 kcal mol⁻¹ Å⁻² were applied to the postulated V–O bond with Asp171. A second distance (1.609 Å) was set for V=O to maintain the bond length and prevent stretching during the minimization process. Energy minimization was performed for the region of the CXCR4 receptor surrounding the oxovanadium(IV) cyclam complex **1**, i.e. all amino acid residues with a sphere radius of 5 Å from the cyclam ligand.

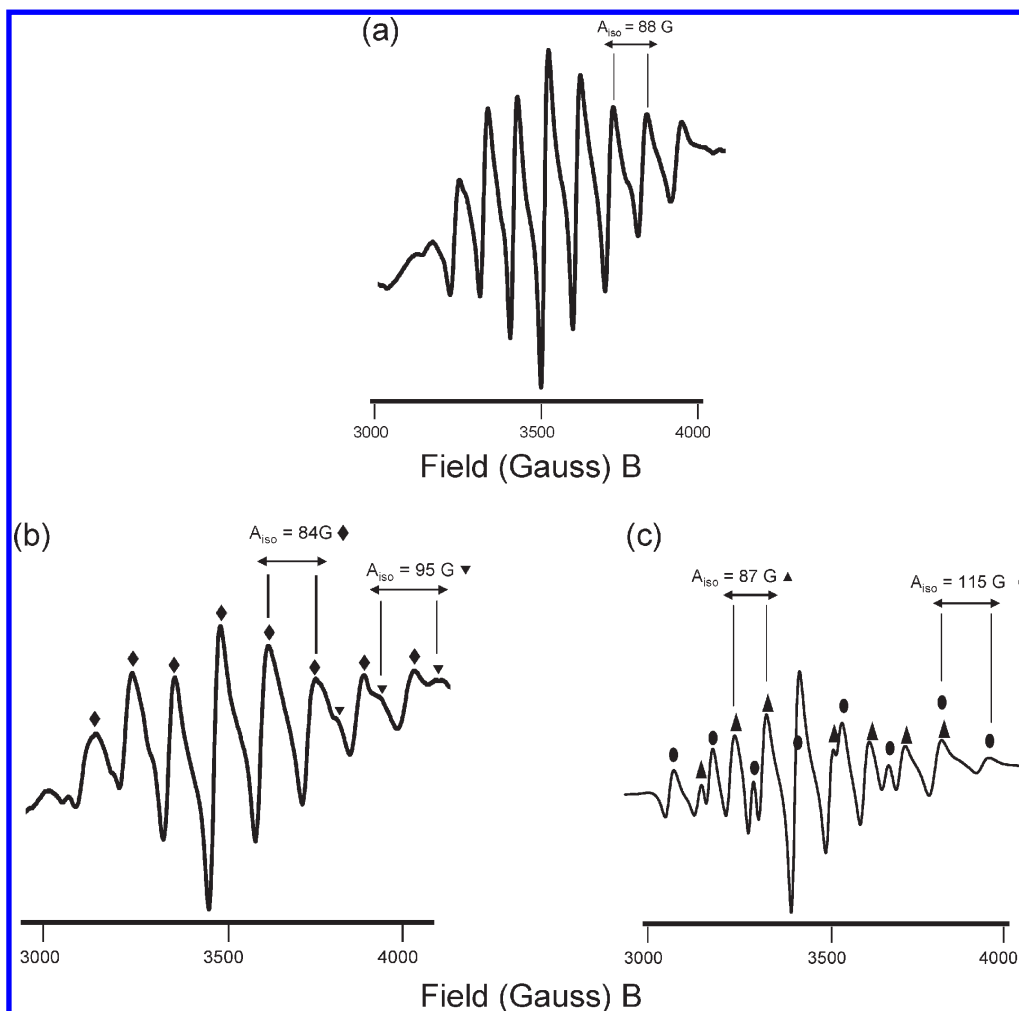


Figure 5. EPR solution spectra at 298 K of oxovanadium(IV) bicyclam complexes $[(VO)_2(\text{bicyclam})X_2]$ where X = solvent, sulfate, or chloride: (a) aqua complex, (b) sulfato complex **3**, and (c) chlorido complex **4**. The spectra clearly show two different environments for vanadium for both the sulfato and chlorido complexes. The chlorido complex in particular shows significant overlapping signals which may be due to different ring configurations in solution or replacement of one chlorido ligand by H_2O .

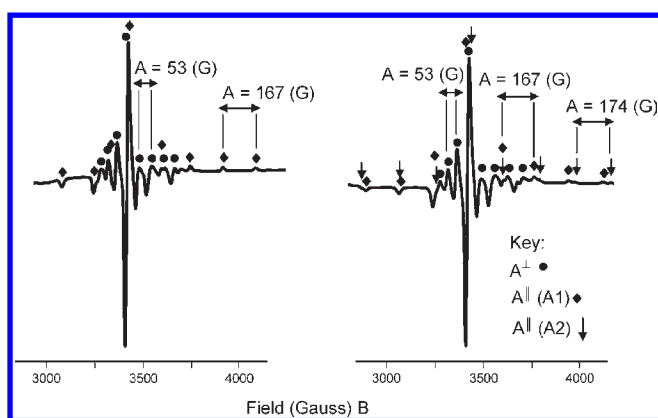


Figure 6. EPR spectra of oxovanadium(IV) bicyclam complexes $[(VO)_2(\text{bicyclam})X_2]$, where X = sulfate (complex **3**, left) and chloride (complex **4**, right), as frozen methanolic solutions at 77 K. Hyperfine couplings (A_{\parallel} , z-axis) are seen for two sets of signals from complex **4** (A_1 and A_2) and one set of signals from complex **3** (A_1). These signals cannot all be attributed to the solvent-exchanged complex $[(VO)_2(\text{bicyclam})X_2]$ where X = methanol, as only one set of signals would be seen and both complexes would give rise to signals with the same A and g values. It seems likely that only one V center of the bicyclam complexes undergoes exchange.

In the energy-minimized model 1 (Figure 7a), hydrogen bonds are observed between carboxylate oxygen atoms of Asp171 and two NH protons of the cyclam ring (~ 2.0 and 2.2 \AA) and a weak attraction is observed between $V=O \cdots H$ (2.3 \AA) of the α -carbon proton from Trp195 in the CXCR4 protein backbone. It is now recognized that $CH \cdots O$ hydrogen bonding can play an important role in stabilizing protein structures.³⁴ Additionally, Trp195 is involved in a putative stacking (hydrophobic) interaction with the hydrocarbon backbone of the cyclam ring, as previously suggested for the Zn_2 -bicyclam adduct.¹⁸ An optimum distance between the vanadium center and the carboxylate oxygens of Asp171 gives rise to the possibility of a coordinative bond.

The EPR data suggest that complexes **1** and **2** (unlike complexes **3** and **4**) may have the sixth (axial) ligand replaced by solvent. In light of these findings, model 2 (Figure 7b) was produced, such that no distance restraint was applied between the vanadium center and carboxylate oxygens of Asp171, but docking was otherwise

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Table 5. Anti-HIV Activity and Cytotoxicity Evaluation in MT-4 Cell Cultures for Bicyclam Complexes **3** (Sulfate) and **4** (Chloride)

complex	strain	IC ₅₀ μM ^a	CC ₅₀ μM ^b	SI ^c	avg IC ₅₀ μM	avg CC ₅₀ μM	SI
3	HIV-1 (III _B)	1.149	> 151	> 131	1.365	> 151	> 111
		1.57	> 151	> 96			
	HIV-2 (ROD)	4.457	> 151	> 34	3.297	> 151	> 46
4	HIV-1 (III _B)	0.119	> 161	> 1351	0.116	> 161	> 1365
		0.117	> 161	> 1379			
	HIV-2 (ROD)	0.236	> 161	> 683	0.193	> 161	> 853
		0.142	> 161	> 1139			

^a IC₅₀: 50% inhibitory concentration of the compound required to protect 50% of the cells against the cytopathicity of HIV. For details of the method, see ref 32. ^b CC₅₀: 50% cytotoxic concentration of the compound required to reduce viability of uninfected MT-4 cells by 50%. ^c SI: Selectivity index or ratio of CC₅₀ to IC₅₀.

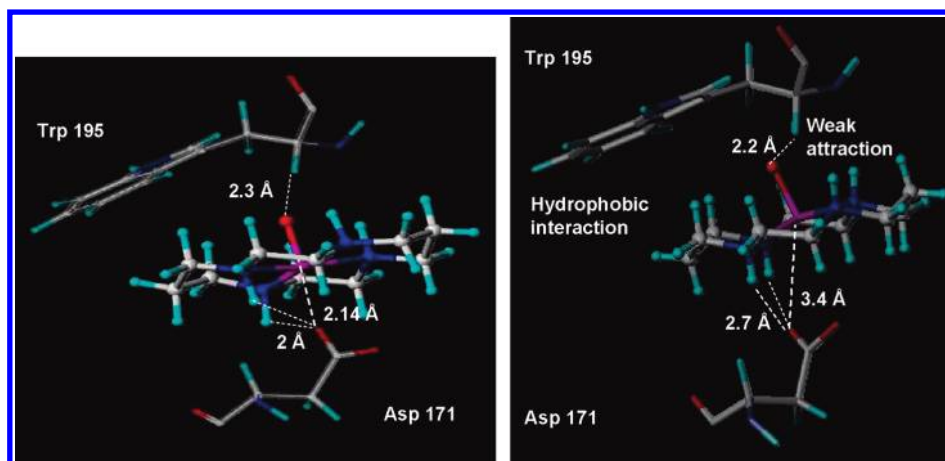


Figure 7. Molecular model of {V^{IV}O(cyclam)} bound to the coreceptor CXCR4, based on the *trans*-III configuration of [V^{IV}O(cyclam)SO₄] **1** found in its X-ray structure. (a) Model 1 (left), vanadyl cyclam forms a coordinative bond (2.14 Å) and other H-bonds with Asp171. A weak V=O...H (2.3 Å) attraction is also seen between the α-carbon proton from Trp195 and the vanadyl oxygen atom. (b) Model 2 (right), which lacks the coordination bond to the carboxylate of Asp171 (V...O 3.4 Å).

undertaken as in model 1. In the energy-minimized model 2, potential hydrogen bonds are again observed between the carboxylate oxygens and NH protons on the cyclam ring. Another weak attraction between the oxygen of V=O and the proton of the α-carbon of Trp195 is also seen, but the distance between the vanadium center and carboxylate oxygens of Asp171 (3.4 Å) is not compatible with formation of a coordination bond.

Discussion

Crystallographic Studies. There appear to be no previous reports of the structures of 6-coordinate oxovanadium(IV) complexes of cyclam or xylylbicyclam, although several vanadium structures do exist with ligands such as chloride trans to the V=O group. A five-coordinate square-pyramidal oxo-vanadium(IV) cyclam has been reported,³⁵ but no crystal structure is available for comparison of V—N bond lengths. The V=O bonds in both **1** and **2** are short (1.6093(19) Å and 1.599(3) Å, respectively)³⁶ and somewhat intermediate between reported bond lengths for V^{IV} and V^V. A search of the Cambridge Crystallographic Database (CCDC) revealed 21 six-coordinate complexes with V^(IV)=O groups and an additional 10 containing the V^(V)=O group. Bond lengths

varied between 1.575 and 1.623 Å for V^{IV}=O complexes and between 1.614 and 1.640 Å for V^V=O complexes. The V=O bond lengths of **1** and **2** are comparable with the V=O bond in the square-pyramidal oxovanadium(IV) complex [V^{IV}O(acac)₂] (1.58 Å).³⁷ The V—O(sulfate) bond length of 2.1359(16) Å, is slightly longer than the V—O bond in [V^(IV)O(acac)₂] (1.97 Å).³⁷ In complex **2**, the V—Cl bond length (2.6501(12) Å) is significantly longer than in examples of octahedral vanadium(IV) complexes with a chloride ligand trans to V=O (2.465 Å,³⁸ 2.468 Å³⁹) and suggests that this ligand is weakly bound. A CCDC search for similar vanadyl macrocyclic complexes with sulfate and chloride ligands in the sixth axial position did not reveal any reported examples. Three vanadyl non-macrocyclic complexes with a sulfate ligand were found and their bond lengths are compared in Table 6. In **1**, the V=O bond length is comparable with the *trans*-V=O(SO₄) complex **a**³⁶ *trans*-[V^{IV}-O(SO₄)(C₂₃H₃₄N₄O₂)]·5H₂O, but significantly shorter than the *trans*-V=O(SO₄) complex **b**³⁸ *trans*-[V^{IV}O(SO₄)-(bipy)₂] and longer than the *cis*-V=O(SO₄) complex **c**⁴⁰

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Table 6. Distribution of V=O Bond Lengths for CN = 6 Complexes with Sulfate as an Axial Ligand (Data from the CCDC)

bond	[V=O cyclam] (Å)	range (Å)	[V=O SO ₄] ^a (Å) ³⁶	range (Å)	[V=O SO ₄] ^b (Å) ³⁸	[V=O SO ₄] ^c (Å) ⁴⁰
V=O	1.6093(17)	1.6144–1.6042	1.602(5)	1.617–1.587	1.638	1.585
V–O	2.1359(16)	2.1407–2.1311	2.255(5)	2.27–2.24	1.981	1.959
O–S	1.5050(15)	1.5095–1.5005	1.483(5)	1.498–1.468	1.830	1.523

^a *trans*-[V^{IV}O(SO₄)-(C₂₃H₃₄N₄O₂)]·5H₂O. ^b *trans*-[V^{IV}O(SO₄)(bipy)]₂. ^c *cis*-[V^{IV}O(SO₄)(phen)]₂.

cis-[V^{IV}O(SO₄)(phen)]₂. In **1**, the V–O bond of the metal–sulfate interaction is shorter than that found for complex **a** where the bond length is thought to be due to a significant trans influence of the vanadyl group. The V–O bond length in **1** is found to be significantly longer than for **b** and **c**. In **1**, the corresponding O–S bond length is longer than that found in **a**, but shorter than in **b** and **c**.

Single crystals suitable for X-ray diffraction studies were not obtained for either of the oxovanadium(IV) xylylbicyclam complexes **3** or **4**, although the latter did afford a polycrystalline material.

Spectroscopic Studies. In the absence of crystallographic data, we have used V K-edge XAS to characterize structurally and compare the solid-state structures of the cyclam and bicyclam complexes. Most notably, a drastic change in the intensity of the V–Cl feature from the cyclam complex **2** to the bicyclam complex **4** is seen (see Figure 2c and d) with a concomitant increase in the V–Cl distance from 2.58 to 2.63 Å, respectively. The observed drop in intensity for the bicyclam complex suggests either the loss of chloride-ligand occupancy in the solid state structure of **4** or a large increase in disorder of the chloride ligand. To explore these two possibilities, different fits to the EXAFS data were attempted. The best fit to the first coordination sphere yielded a coordination environment consistent with VON₄Cl_{0.5} corresponding to the loss of chloride from one of the vanadyl units in the bicyclam complex. Enforcing the anticipated VON₄Cl coordination environment for each ring yielded a significantly poorer fit with a concomitant doubling of σ^2 for the V–Cl pathway (Table 2). Comparisons of these fits and their associated goodness-of-fit parameters suggests that partial loss of Cl ligation provides the most reasonable interpretation of the data. The VON₄Cl_{0.5} formulation could indicate partial occupancy of the sixth coordination position or result from bridging chloride ligands to generate oligomeric species in the solid state. This interpretation is consistent with the observed lengthening of the V–Cl bonds by 0.05 Å and provides a rationale for problems encountered during the synthetic workup and recrystallization process, where precipitation of insoluble products was observed. This is further supported by the near-edge XAS spectra (see Figure 3), which show only subtle changes in the 3d manifold. The most notable change is a small shift to higher energy (0.1–0.2 eV) in the pre-edge features of the bicyclam complexes as compared to their cyclam analogues. Partial loss of the axial chloride ligand in **4** as compared to **2** would be expected to result in a significant increase in the electric dipole-allowed component of the 1s → 3d_{z²} transition

associated with the short V=O bond.^{41,42} The fact that the overall intensity and relative energy distribution of the 1s → 3d transitions remains essentially unchanged on going from the cyclam to the bicyclam implies that the overall ligand field is not perturbed significantly.

EPR data for water and methanol solutions show that the *g*-values are close to 2 in all cases as expected, as the unpaired electron is in a nondegenerate orbital. Any orbital angular momentum is subsequently quenched resulting in a “spin-only” system. In addition, there should be little mixing in of an orbital component from excited states through spin–orbit coupling, leading to typical *g*-values close to, but slightly less than, 2. The unpaired electron is in the d_{xy} orbital and oriented between the N atoms in the cyclam plane. As d_{xy} is essentially nonbonding, any interaction with the nitrogen orbitals is very small and no resolved coupling to nitrogen is seen. The cyclam complexes **1**, **2** show very similar spectra to the X = H₂O aqua complex [VO(cyclam)-(H₂O)]²⁺ and may suggest that the sixth ligand has been replaced by solvent. As the pH values of the solutions were acidic (pH = 3), it seems likely that the axial ligand is water rather than hydroxide. This proposed ligand exchange is consistent with the X-ray crystal structure data (Table 1) where bond lengths of the axial ligand trans to the V=O group suggest that the ligand is weakly bound. For the sulfate **3** and chloride **4** complexes, significant differences are observed. Neither set of peaks for complex **3** show values of *g* and *A* similar to that of the aqua complex, which suggests there is little substitution of the sixth ligand by solvent unlike that proposed for the cyclam complexes. The low *A*_{iso} value of 84 (G) is at the lower end of those reported for other vanadium(IV) species with O-donor ligands (85–110 G), although not the lowest reported (71 G).^{43–45} The values of *g*_{iso} (1.985, 1.983) fall within reported ranges.^{44,45} The separate set of signals observed and confirmed by simulation may also suggest that each vanadium center is in a different environment, indicating that the macrocyclic rings may adopt different configurations. The possibility of greater delocalization onto the sulfate ligand may also result in the observed low *A*_{iso} value. Complex **4** clearly shows two sets of peaks in the room temperature spectrum. Some degree of partial substitution of chloride by water is indicated by the similar *g*-value and *A*_{iso} value (87 G) shown in Table 3. A larger *A*_{iso} value was found for the second set of peaks for complex **4** (*A*_{iso} = 115 G) and suggests that a stronger interaction exists between the unpaired electron and the vanadium center. The *A*-value, the

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hyperfine coupling constant, is related to the unpaired electron density at the vanadium center. For this particular configuration, the unpaired electron may be more localized on the vanadium than for the other configuration where $A_{\text{iso}} = 87$ G. An interesting observation is that the average g and A values used to describe the frozen solution spectra should approximate to the isotropic g and A values used to describe the fluid solution spectra. There are some differences between these values in the complexes described which may be explained by different solvent interactions of the complexes with water and methanol or may indicate that certain configurations are being “frozen out” in the frozen solution spectra. Large deviations from square-pyramidal geometry and also the nature of the sixth ligand can influence the A -value, and π -donation into the d_{xy} orbital can have a large effect. In the light of these observations, it would appear that for the bicyclam complexes the retention of, or alternatively slow exchange of the sixth axial ligand, appears to affect the value of A . Previously, 2D NMR studies on zinc xylylbicyclam¹² revealed an equilibrium mixture of *trans*-I, *trans*-III, and *cis*-V configurations in solution, but such experiments were not possible with the paramagnetic vanadyl complexes **1–4**.

Antiviral Testing. Transition-metal complexes show varying levels of anti-HIV activity which may arise partly from a relationship between the metal–cyclam geometry and the number of binding site interactions possible. Those favoring square-planar geometries are found predominantly in the *trans*-I configuration while octahedral complexes favor *trans*-III. Analysis of all nickel(II) cyclam complexes in the Cambridge Structural Database (CSD) shows the most common configuration to be *trans*-III, so deemed the most stable configuration. Less than 23% of octahedral nickel(II) complexes with a 1,4,8,11-substituted cyclam backbone adopted the *trans*-I configuration compared with more than 75% preferring *trans*-III.⁴⁶ This situation is reversed for square-planar, square-pyramidal, and trigonal-bipyramidal complexes with less than 30% adopting *trans*-III and the majority showing a preference for the *trans*-I configuration. Repeated analyses for copper(II) complexes resulted in similar findings.⁴⁷ A study of protein recognition of macrocycles has shown how the affinity of xylylbicyclam for CXCR4 is enhanced further by binding to Ni(II) or Zn(II).⁴⁸ Anti-HIV activity is very much dependent upon the bound metal and a Zn^{II}–xylylbicyclam complex showed slightly higher biological activity than the metal-free ligand.

Our oxovanadium(IV)–xylylbicyclam complexes **3** and **4** possess high anti-HIV activity against HIV-1 (III_B) and HIV-2 (ROD) strains, with IC₅₀ values in the range 1–5 μM for **3** and 0.1–0.3 μM for **4**. The latter values are comparable with that of xylylbicyclam itself (AMD3100; 0.01 μM) but higher than that of the Zn₂–xylylbicyclam complex (0.003–0.009 μM). The reported activity of metal xylylbicyclams follows the order:

Zn(II) > xylylbicyclam \sim Ni(II) > Cu(II) > Co(III) \gg Pd(II), with the Pd(II) complex being virtually inactive.^{14,17,49} The oxovanadium(IV)–cyclam complexes **1** and **2** are also inactive. By exploring the structures of protein–cyclam adducts, insight into specific hydrophilic and hydrophobic interactions involved in the binding of bicyclam to CXCR4 can be gained. In addition to interactions with aspartate and glutamate residues, there is a possibility that the hydrocarbon backbone of the bicyclam rings is involved in hydrophobic interactions with tryptophan residues, Trp195 and Trp283.⁵⁰ This type of interaction is comparable to that of the hydrophobic contact of the side-chain of proline with tryptophan found at some protein–protein interfaces, commonly described as a Trp-Pro-Trp sandwich.⁵¹ Recently, a configurationally restricted bismacrocylic CXCR4 receptor antagonist was reported to have very high activity against HIV.⁹ The zinc complex of an ethylene-bridged analogue of AMD3100 was found to have a lower EC₅₀ value than AMD3100 and [Zn₂–AMD3100]⁴⁺ and the highest reported activity against HIV (0.0025 μM vs 0.08 μM). It is expected that particular ring configurations may play a crucial part in determining the antiviral activity of macrocyclic complexes. Metal complexes of cyclam and bicyclam in solution give rise to several different configurational isomers, each dependent on the chirality of the four ring nitrogens.⁸ The *trans*-I, II, III, and *cis*-V configurations may all have a significant influence on the bioactivity of metallo-macrocyclic antiviral compounds.^{9,10,12} The inactivity of the cyclam complexes **1** and **2** suggests that the aromatic linker of the bicyclam complexes may play an important role in the coreceptor binding of complexes **3** and **4** and the anti-HIV potency. Antiviral activity of cyclam and its derivatives is influenced by metal complexation and subsequent binding to carboxylate oxygens of acidic (Glu and Asp) amino acid residues in the binding site of CXCR4. Like complexes **1** and **2**, Pd(II)–cyclam exhibits a *trans*-III configuration in the solid state.¹⁸ Varying the counterion had no effect on ring configuration and addition of acetate did not induce any configurational change in vanadyl cyclam. Our experiments show that additional axial ligands do not bind or bind only weakly to **1** and **2** (see the Supporting Information S5, for solution IR and conductivity measurements). Our studies of these vanadyl cyclam complexes in aqueous solution suggest that they are stable, although it would be interesting to carry out further studies in the presence of potential competitor biomolecules. Our model (Figure 7) suggests that a weak attraction between the vanadyl group oxygen (V=O) and the protein backbone may result in a new binding position for these cyclam complexes. Another study has shown that Co(III)–cyclam can adopt the *trans*-III configuration in the solid state with axial acetate ligands, but solution studies show the existence of three different configurations (*trans*-III, *trans*-I, and a further unidentified *trans* configuration).¹⁸ This species is also inactive as the axial ligands exchange too slowly. The bicyclam analogue formed intermediate species on reacting with acetate that equilibrated to the *trans*-III isomer with time.^{13,18} In terms of anti-HIV activity, it would appear that

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for $V^{(IV)}=O$ complexes one exchangeable ligand is enough providing there is a second cyclam linked.

Coreceptor Docking. Molecular modeling can offer a representation of possible binding interactions in the CXCR4 active site (see Figure 7). As bicyclam complexes **3** and **4** are active against HIV, their activity may result from binding in an analogous fashion to zinc bicyclam,¹² through metal–carboxylate interactions involving aspartate residues, Asp171 and Asp272, found in two separate binding sites. However, no crystal structures were available for the oxovanadium(IV)–xylylbicyclam complexes **3**, **4**, and modeling was carried out using the oxovanadium(IV)–cyclam complex **1**. Following manual docking and minimization, interactions were found that differed from those in the Zn_2 –xylylbicyclam–CXCR4 model studied previously¹² (i.e., $Zn(II)$ –carboxylate 2.28 Å compared with $V(IV)$ –carboxylate 2.14 Å in model 1 and the absence of this interaction in model 2). An additional H-bonding interaction is also seen (Figure 7), specifically a weak attraction between the oxygen of $V=O$ and the α -carbon proton of Trp195 of CXCR4. A similarly docked oxovanadium(IV)–cyclam in the *cis*-V configuration in the second binding site could assist in further accounting for the activity of the bicyclam complexes **3** and **4**. An attempt was made to crystallize a single oxovanadium(IV)–cyclam with a phenyl substituent in order to investigate the effect of such a side arm on the macrocycle configuration but was unsuccessful.

Conclusions

We have reported here the synthesis and characterization of four new vanadyl macrocyclic complexes. The X-ray structures of the monocyclam complexes **1** and **2** appear to be the first for vanadyl cyclams. The important role of cyclam NH groups in H-bonding is illustrated by the double H-bonds formed in **1** with the uncoordinated oxygens of the axial sulfate ligand, effectively locking the macrocycle in the *trans*-III configuration. EXAFS data suggest that the xylylbicyclam chlorido complex **4** has a structure in which only one of the vanadyl centers has an axial chlorido ligand or that there is a bridging ligand creating a chlorido-bridged oligomeric species, consistent with difficulties in the synthesis. These structural differences may contribute to the differences in the

biological activity of the chlorido and sulfato complexes. EPR data for aqueous solutions revealed two different environments for the vanadium metal center in the xylylbicyclam complexes **3** and **4** and some exchange of axial sulfato and chloride ligands with solvent. Complete exchange of the axial ligands for solvent was seen for the monocyclam complexes **1** and **2**. Modeling showed that a weak attraction between the $V=O$ group and the CXCR4 backbone may result in a longer distance between the vanadium center and carboxylate oxygens of Asp171 and consequent loss of a coordination bond, which may account for the inactivity of complexes **1** and **2**. For the bicyclam complexes, these data indicate the possibility that one ring is locked in a less-favorable binding mode with the second ring bound in an alternative configuration providing further bonding interactions elsewhere, giving rise to the level of bioactivity seen for complexes **3** and **4**. This, in addition to the potential role of the aromatic xylyl-linker in the bicyclam complex may contribute to the anti-HIV activity.

Acknowledgment. We thank the BBSRC for a studentship for A.R. and support for the interdisciplinary center RASOR at the University of Edinburgh. Portions of this research were carried out at the Stanford Synchrotron Radiation laboratory, a National user facility operated by Stanford University of behalf of the US Department of Energy, Office of Basic Energy Sciences. The SSRL Structural Biology Molecular Program is supported by the Department of Energy, Office of Biological and Environmental Research, and by the National Institutes of Health, National Centre for Research Resources, Biomedical Technology Programme. We thank Professors Len Lindoy (University of Sydney), David Garner (University of Nottingham), and Dr David Collison for helpful discussion, and Dr Daniel Stone for EPR simulation work at the EPSRC National EPR Centre, University of Manchester, U.K.

Supporting Information Available: Experimental protocols for antiviral testing (and results for inactive cyclam complexes), crystallographic parameters, solution IR, conductivity tests, and EPR simulations. This material is available free of charge via the Internet at <http://pubs.acs.org>.