Configurationally Restricted Bismacrocyclic CXCR4 Receptor Antagonists

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Abstract: A zinc(II) containing configurationally restricted analogue of bismacrocyclic cyclam-type CXCR4 chemokine receptor antagonists has been synthesized and shown to adopt only one configuration in solution. The single crystal X-ray structure reveals favorable binding to acetate via a bidentate chelation that can be related to the proposed interaction with aspartate on the receptor protein surface. The zinc(II) complex is highly active against HIV infection in vitro.

AMD3100 (the octa HCl salt of 1-1′-[1,4-phenylenebis-(methylene)]-bis(1,4,8,11-tetraazacyclotetradecane)), Figure 1c, is a drug that interacts with a cell surface protein (CXCR4) via hydrogen bonding interactions or more effectively as the metal complex via coordinate bonds with aspartate residues.[1] On metal complex formation, the tetra-aza macrocyclic rings in AMD3100 show multiple configurations in solution.[2] Configurationally fixed analogues would have the advantage of presenting only one configuration in solution for coordinate bond formation on binding to the protein. Our study aims to produce a series of configurationally fixed complexes and show the key importance of the coordination interaction for drug binding. We also wish to validate the general strategy of configurational fixing as a route to improve the activity of metal-containing drugs.

The CXCR4 chemokine receptor is a seven-helix transmembrane G-protein coupled receptor with multiple critical functions in both normal and pathological physiology. It is a member of the family of 18 recognized chemokine receptors and has a sole natural ligand (CXCL12).[3] Synthetic small molecule antagonists exist, including AMD3100, and have been shown to have both a high binding specificity and an effective inhibitory action against a number of disease states.[4,5] For example, in vitro assays show that AMD3100 inhibits infection by the human immunodeficiency virus (HIV-1 and HIV-2) at micromolar concentrations.[6]

It has been demonstrated that formation of metal complexes and aza-macrocyclic ring configuration may have major effects on AMD3100−protein interactions.[1,2,7−9] In particular, it has been suggested that zinc(II) could play a key role in the biological activity of the bicyclam derivatives.[10,11] In an attempt to rationalize the effects of cyclam configuration and to produce new specific antagonists for CXCR4, we have successfully synthesized a configurationally fixed bismacrocyclic compound and its zinc(II) complex. The solution and solid-state properties of the zinc(II) complex were investigated via high field NMR (800 MHz) and X-ray crystallography. The inhibitory effect on infection by HIV-1 and HIV-2 in MT-4 cells is also presented for both the chelator, **4** ((5-5′-[1,4-phenylenebis(methylene)]-bis(1,5,8,12-tetraazabicyclo[10.2.2]hexadecane)), and the complex Zn_2**4**$(OAc)_4$.

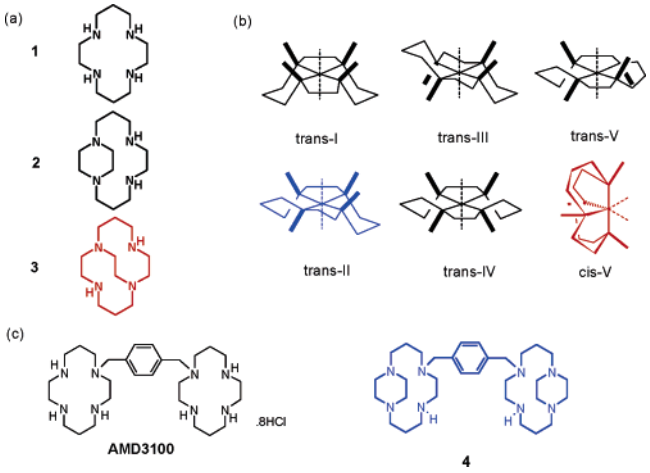

Figure 1. (a) Cyclam **1** and ethylene-bridged analogues with adjacent (**2**) or nonadjacent (**3**) nitrogens bridged. (b) Six configurations of metallo-cyclam complexes, with the sole observed configuration highlighted for **4** in blue and **3** in red. (c) AMD3100 and the constrained analog **4**.

There are six possible configurations that a cyclam ring can adopt on complexation to a metal ion, as defined by Bosnich and co-workers and shown in Figure 1b, where *trans*-III is generally the most thermodynamically stable.[12] The addition of an ethylene bridge is one strategy to restrict the adopted configuration. A configurationally restricted target CXCR4 antagonist, **4**, is shown in Figure 1c. Side-bridged or piperazinocyclams, **2**, restrict the configuration adopted by the cyclam ring via the provision of an ethylene bridge between adjacent nitrogen positions in the macrocyclic ring. A configurational equilibrium exists in solution for cyclam complexes, however, we have shown that the presence of the bridge results in a single configuration.[13] The macrocycles **2** and **3**, shown in Figure 1a, are known but have not previously been incorporated into a xylyl-bridged structure.[14,15] There is a recent report of the synthesis of xylyl-bridged biscyclen compounds.[16]

The bismacrocyclic chelator, **4**, was synthesized via modified routes based on those of Kolinski used in the formation of piperazine-containing cyclam macrocycles (Scheme 1).[17] Monofunctionalization of a hydropyrene derivative followed by subsequent reduction with $NaBH_4$ results in the cleavage of the quaternary nitrogen to carbon bond, and the resulting monoaminal is either reductively cleaved or hydrolyzed on aqueous workup. The resulting bis(piperazinocyclam) compound, **4**, contains a *para*-xylyl bridge with one secondary amine nitrogen in each macrocycle. These high-yielding routes reflect the increasing exploitation of hydropyrene derivatives in macrocycle syntheses.[18−20]

Site-directed mutagenesis studies of the CXCR4 receptor have demonstrated a role for two outer surface aspartate residues in the interaction with AMD3100 complexes.[21] The zinc acetate

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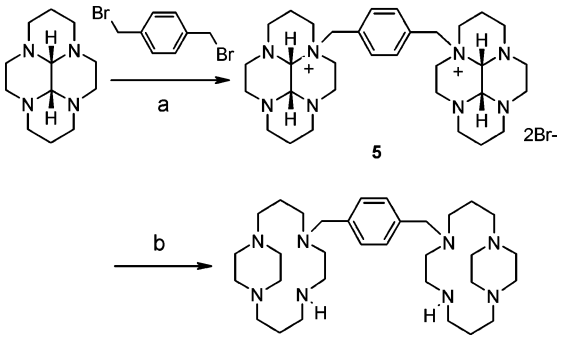
Scheme 1[a]



[a] Reagents: (a) acetonitrile, rt, 24 h (89%); (b) $NaBH_4$, EtOH, reflux, 1 h (65%).

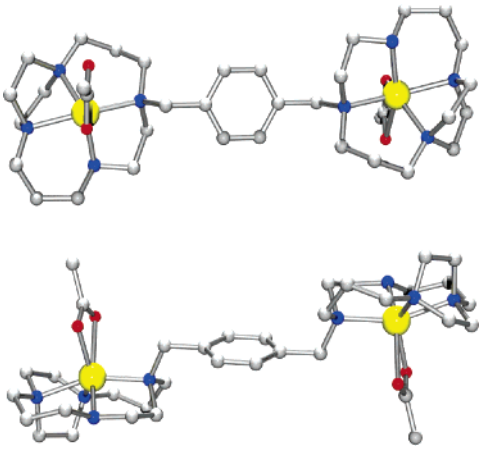


Figure 2. Ball and stick representations of the single crystal X-ray structure of $[Zn_2\mathbf{4}(OAc)_2]^{2+}$. Hydrogens have been omitted for clarity. Selected bond lengths [Å]: Zn−O(1) 2.112(3), Zn−O(2) 2.407(4), Zn−N(1) 2.166(2), Zn−N(2) 2.106(3), Zn−N(3) 2.228(3), Zn−N(4) 2.091-(3).

complex of **4** was synthesized to provide a structural model of the interaction between the metal center and the aspartate residues of the CXCR4 receptor. The complex was prepared in good yield by heating **4** with zinc acetate in acetonitrile at reflux. Single crystals were grown by slow evaporation of a methanolic solution.

The X-ray structure (Figure 2) reveals an asymmetric unit containing one macrocyclic ring complexed to a six-coordinate zinc ion. The two zinc centers in one molecule are identical and related by crystallographic symmetry. Analysis of the macrocyclic configuration reveals the equivalent of a *trans*-II-type arrangement. The coordination of the bound acetate corresponds to the proposed interaction of each of the macrocyclic units with CXCR4 aspartate residues, Asp-171 and Asp-262. The acetate ligand is bound in an anisobidentate fashion with Zn−O bond lengths of 2.112(3) Å and 2.407(4) Å. The zinc ion is significantly displaced from the plane of the four nitrogen atoms and protrudes from the cavity, enhancing the interaction of the zinc ion with the bound acetate. The "unbound" acetate is present in disordered positions in the asymmetric unit and shows no interaction with the bismacrocyclic cation. We initially postulated that the antiviral activity of the "*trans*-fixed" (**2**) compounds would be lower than that of the "*cis*-fixed" (**3**) due to the lack of two available binding sites to bind a chelating acetate group. However, a comparison of the bond lengths and angles surrounding the metal center in $[Zn_2\mathbf{4}(OAc)_2]^{2+}$ shows them to be similar to those observed in the single-crystal X-ray structure of Zn_2AMD3100$(OAc)_4$.[7] In the $[Zn_2\mathbf{4}(OAc)_2]^{2+}$ cation, zinc sits out of the plane of the

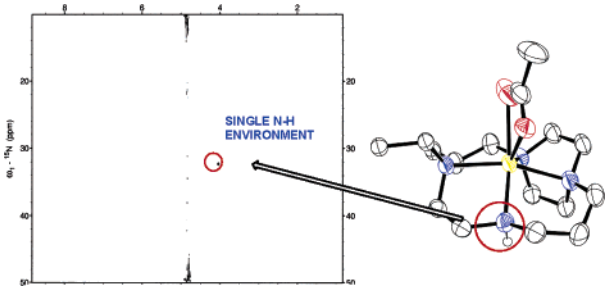


Figure 3. The [^{1}H, ^{15}N] HSQC spectrum of $Zn_2\mathbf{4}(OAc)_4$ in 10% H_2O/90% D_2O and an ORTEP representation of the solid state structure showing the secondary amine position corresponding to the single peak observed in the NMR spectrum.

macrocycle, effectively forming a distorted octahedral geometry with the acetate group, which may be of key importance to the interaction with the chemokine receptor. Hence, the "*trans*-fixed" compounds could be effective inhibitors of HIV infection if the configuration is maintained in solution.

High-field NMR studies were carried out to confirm that the single macrocyclic ring configuration observed in the solid state is also the sole configuration observed in solution. Geometric constriction of the macrocycle increases the complexity of the proton NMR spectrum, giving multiple overlapping peaks and necessitating the use of high field NMR to fully assign the ^{1}H spectrum. Two-dimensional (2D) homonuclear [^{1}H, ^{1}H] NOESY and COSY NMR data sets and natural abundance 2D heteronuclear [^{1}H, ^{13}C] and [^{1}H, ^{15}N] HSQC NMR data were acquired. Only one peak was observed in the [^{1}H, ^{15}N] HSQC NMR at room temperature, corresponding to the single NH from each piperazinocyclam and indicative of a sole configuration (Figure 3). The zinc complex of AMD3100 shows multiple peaks for the N−H protons representing the multiple configurations in the equilibrium.[7] This equilibrium can be shifted by varying the concentration of acetate. Varying the anion bound to the metal center in the $Zn_2\mathbf{4}$ complex did not result in any observable change in the proton NMR spectrum. The single configuration we observed by NMR appears to be consistent with the solid-state structure where both piperazinocyclam rings are identical and the equivalent of the *trans*-II configuration.

To probe the ability of **4** and $[Zn_2\mathbf{4}]^{4+}$ to bind to the CXCR4 chemokine receptor and inhibit infection by HIV, the compounds were tested in an assay monitoring viral infection. The chemokine receptors are cofactors for the entry of HIV into $CD4^+$ cells. The virus gains entry to the cell by first interacting with the CD4 receptor and then binding to either the CCR5 or CXCR4 chemokine receptor, leading to membrane fusion. It is known that the inhibition of HIV infection by AMD3100 is a result of the bicyclam compound binding to the CXCR4 receptor and preventing viral entry.[21] We have confirmed that **4** and $[Zn_2\mathbf{4}]^{4+}$ bind to the CXCR4 receptor by flow cell cytometry displacement studies with an anti-CXCR4 antibody. While it is possible that $[Zn_2\mathbf{4}]^{4+}$ could have other modes of anti-HIV action, such as binding to viral RNA sites, it is unlikely the selectivity would be as high.[22] The inhibition of cytopathic effects of HIV-1 (III_B) and HIV-2 (ROD) viral strains by the piperazino cyclam compounds were determined, and the results presented in Table 1. The induced cytotoxicity of the compounds was also measured in MT-4 cells in parallel. In an identical assay, the EC_{50} values for AMD3100 and its zinc complex are 0.011 and 0.008 μM, respectively, for infection by HIV-1 (III_B) in MT-4.[5] The comparison between the free bismacrocycles shows a marked contrast with **4** considerably less-active than AMD3100 in these assays. This can be rationalized by the

Table 1. Anti-HIV Activities, Cytotoxicity and Selectivity Index in MT-4 Cells

compd	HIV strain	Av EC_{50}[a] (μM)	SD[b]	Av CC_{50}[c] (μM)	SD[b]	SI[d]
4	HIV-1 (III_B)	6.98	1.84	>225		>32
	HIV-2 (ROD)	23.2	6.40	>225		>10
$Zn_2(OAc)_4$**4**	HIV-1 (III_B)	0.0025	0.0010	60.56	4.73	24 225
	HIV-2 (ROD)	0.0040	0.0013	60.56	4.78	15 140
AMD3100[5]	HIV-1 (III_B)	0.011		>225		>20 455
Zn_2AMD3100[5]	HIV-1 (III_B)	0.008		>225		>28 125

[a] Average effective concentration to reduce the HIV-induced cytopathic effect by 50% in MT-4 cells. [b] Standard deviation over three assays. [c] Concentration required to have a cytotoxic effect reducing MT-4 cell viability by 50%. [d] Selectivity index based on μM conversion.

interaction with the two aspartate residues via H-bonding. AMD3100 has three secondary amines on each macrocyclic ring and has greater flexibility to find the optimal arrangement to maximize these interactions. Compound **4** has only one secondary amine per macrocycle and is configurationally restricted, hence, may not adopt a configuration idealized for H-bonding interactions. The tertiary amines are likely to be protonated, and the piperazino ring may adopt the lower energy chair conformation with a N−H pointing out of the macrocyclic cavity, however, this may not be correctly oriented for optimized H-bonding.

The zinc complex, $[Zn_2\mathbf{4}]^{4+}$, has a lower EC_{50} value than $[Zn_2AMD3100]^{4+}$ and AMD3100. Most striking, however, is the major variation on binding the metal ion to **4**, an enhancement of 3 orders of magnitude in the anti-HIV activity is observed. This demonstrates the importance of the coordination interaction to the chemokine receptor binding and validates the strategy of configurational restriction. Additional interactions such as hydrogen bonding have also been proposed to occur in the binding site. These are likely to be disfavored for $[Zn_2\mathbf{4}]^{4+}$ due to the increased steric bulk of the ethyl bridge. However, the antiviral activity is still at least as good as the zinc AMD3100 complex, suggesting that the major contribution to receptor binding stems from an optimized coordination interaction with the metal center. This is highly relevant to the development of new metallo-based chemokine receptor antagonists and of interest in the general development of metal compounds for protein interaction.

In conclusion, we have reported the synthesis of a configurationally fixed cyclam derivative that forms a zinc complex with one configurational isomer observed in solution by NMR. This compound is highly active against HIV, and its antiviral action is expected to occur via the same mechanism as that of the bicyclams, a binding interaction with the chemokine receptor CXCR4. It is demonstrated that configurational fixing may be a route to optimization of the interactions of the antagonist with the receptor. A further desirable property of ethyl-bridged complexes could come from increased kinetic stability in vivo.[23,24] Studies of the bismacrocyclic compounds of *cis*-V restricted geometry macrocycles, **3**, and unsymmetric bismacrocylic compounds with configurational restriction are underway. We also aim to further characterize the binding of the complex to the receptor using anti-CXCR4 antibody competition assays.

AMD3100 has been in clinical trials against HIV and is now in advanced clinical trials for stem cell mobilization (as the drug Mozobil). The CXCR4 receptor is also implicated in both angiogenesis-driven and inflammatory disease states, such as cancer and rheumatoid arthritis, and it follows that configurationally fixed antagonists may have therapeutic value.

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Note Added after ASAP Publication. There was an error in the units given for the EC_{50} values for AMD3100 and its zinc complex listed in the text in the version published ASAP September 2, 2006; the corrected version was published ASAP September 12, 2006.

Supporting Information Available: Experimental protocols, synthetic procedures, crystallographic parameters, a preliminary CXCR4 binding competition study and full NMR assignments. This material is available free of charge via the Internet at http://pubs.acs.org.

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