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Structure and Dynamics of Metallomacrocycles: Recognition of Zinc Xylyl-Bicyclam by an HIV Coreceptor

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Abstract: As platforms for the design of metal-based therapeutic and diagnostic agents, macrocycles are rigid enough to provide strong metal binding sites and orient functional groups stereoselectively, yet flexible enough to accommodate structural changes required for induced-fit recognition of biological targets. We consider the recognition of the Zn(II) complex of the bis-tetraazamacrocycle xylyl-bicyclam, a potent anti-HIV agent, by the coreceptor CXCR4, a G-protein-coupled receptor used by HIV for membrane fusion and cell entry. NMR studies show that the macrocycles of $Zn(II)_2$−xylyl-bicyclam perchlorate exist in aqueous solution as two major configurations, *trans*-I (nitrogen chirality *R,S,R,S*), and *trans*-III (*S,S,R,R*). Acetate addition induced a major structural change. X-ray crystallography shows that the acetate complex contains the unusual *cis*-V cyclam configuration (*R,R,R,R* and folded) with bidentate coordination of acetate to Zn(II) plus second-coordination-sphere double H-bond formation between diagonal NH protons on the opposite cyclam face and acetate carboxylate oxygens. Detailed 1D and 2D NMR studies show that the major configuration of $Zn(II)_2$−xylyl-bicyclam acetate in aqueous solution is *cis*-V/*trans*-I. Molecular modeling shows that an analogous *cis*-V site can be formed when $Zn(II)_2$−xylyl-bicyclam binds to CXCR4, involving the carboxylate groups of Asp262 (Zn(II) coordination) and Glu288 (double H-bonding). The second cyclam can adopt the *trans*-I (or *trans*-III) configuration with Zn(II) binding to Asp171. These interactions are consistent with the known structure−activity relationships for bicyclam anti-HIV activity and receptor mutation. Consideration of the anti-HIV activity of xylyl-bicyclam complexes of other metal ions suggests that affinity for carboxylates, configurational flexibility, and kinetic factors may all play roles in receptor recognition. For example, Pd(II) cyclam complexes interact only weakly with axial ligands and are inflexible and inactive, whereas Co(III) cyclams bind carboxylates strongly, are configurationally flexible, and yet have low activity. Our findings should aid the design of new generations of active macrocycles including highly specific chemokine receptor antagonists.

Introduction

Metal complexes are attractive for drug design[1−3] because there is a rich diversity of ways of encoding both structural (thermodynamic) and dynamic (kinetic) molecular information[4,5] that can subsequently be read out and processed at the site of action. Static information can be encoded in the metal, its oxidation state, coordination number, coordination geometry, chelate ring conformation, and second coordination sphere interactions (e.g., hydrophobic interactions and H-bonding). Dynamic information can be encoded in processes such as isomerization, ligand substitution, ligand conformational changes, chelate ring-opening, and inversion of coordinated atoms. With such high potential diversity, the major problem is to control the read-out of the molecular program in vivo under the influence of the various interaction algorithms.

Here we consider the encoding and read-out of molecular information contained in metal complexes of cyclam[6] (1,4,8,11-tetraazacyclotetradecane). Cyclams are of interest in fields as diverse as catalysis, selective metal recovery and recycling, sensors, and therapy and diagnosis.[7] There is current medical interest in the drug AMD3100, a bicyclam containing two cyclam units connected by a *p*-phenylenebis(methylene) (*p*-xylyl) linker (AMD3100, Xyl-bicyclam·8HCl, Figure 1). AMD3100 is in phase I clinical trials for stem cell transplantation used in the treatment of patients who have cancers involving the blood and immune system. It is also one of the most potent

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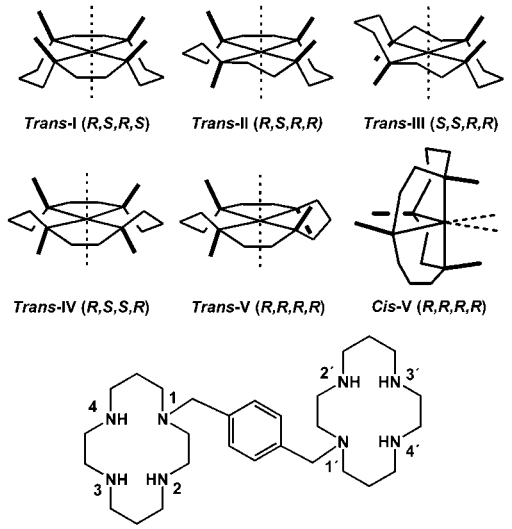

Figure 1. Cyclam complexes and bicyclam. Top: trans configurations of metal−cyclam complexes showing the chiral N atoms with NH bonds pointing up or down, together with *cis*-V, the folded form of *trans*-V. Bottom: Xyl-bicyclam, 1,1′-[1,4-phenylenebis(methylene)]-bis(1,4,8,11-tetraazacyclotetradecane). The octa-HCl salt is the anti-HIV drug AMD3100.

anti-HIV agents known[8] and has recently been in clinical trials against AIDS. AMD3100 blocks entry of T-lymphotropic HIV-1 and HIV-2 strains by specific binding to the CXCR4 coreceptor.[9] CXCR4 (receptor number 4 for natural chemotactic cytokine proteins containing a conserved Cys-X-Cys disulfide bond, mediators of white blood cell trafficking and activation) is a member of the classical G-protein-coupled receptor family of membrane proteins, which contain seven transmembrane helices. G-Protein-coupled receptors are involved in the regulation of nearly all physiological processes and ca. 40% of all therapeutic interventions.[10] On binding to diffusable extracellular ligands, they switch to active conformations capable of interacting with hundreds of G-proteins.

Figure 1 illustrates how information can be encoded in the macrocyclic frame of metal cyclam complexes. Each of the four metal-coordinated N atoms is chiral, and the N−H bonds lie above or below the mean ligand plane, giving five possible configurations.[11] The six-membered chelate rings take chair or twist-boat conformations and the five-membered rings are gauche (λ or δ) or eclipsed. Configurations with H atoms pointing in the same direction at the end of a diagonal can fold to generate a trigonal bipyramid or a cis structure. Thus, the *R,R,S,S* configuration can form only a *trans*-III complex, whereas *trans*-V can fold to *cis*-V. Energy calculations on Ni(II) complexes[12] show that *trans*-III has the lowest energy, but *trans*-I becomes more stable relative to *trans*-III on decreasing the metal coordination number from 6 (octahedral) to 5 to 4 (square-planar), in agreement with a recent survey of X-ray structures.[13] It was discovered early on for Co(III)[14] and Ni(II)[15] complexes that dynamic switching between configurations is possible under the influence of external perturbations.

Cyclams are Strong Metal-Chelating Agents. The concentration of Zn(II) in blood plasma is ca. 20 μM,[16] and Zn(II) binds strongly to cyclam (log *K* 20.12).[17] Taking account of cyclam pK_a values, this gives a conditional dissociation constant (K_d) at blood plasma pH (7.4) of ca. 0.1 pM. Since the level of free Zn(II) in plasma is ca. 1 nM[16] and the affinity of alkyl-substituted cyclams is likely to be even higher than for cyclam itself, it is reasonable to expect that Xyl-bicyclam can exist as a Zn(II) complex in vivo. An example of the recruitment of Zn(II) by an organic drug is that of benzimidazole serine protease inhibitors,[18] and Zn(II) is responsible for the activation of bacterial enterotoxins[19] and anthrax lethal factor.[20]

The Zn(II) complex of Xyl-bicyclam is 10 times more active than AMD3100 in its interaction with the CXCR4 receptor,[21] and there is a close correlation between anti-HIV activity and CXCR4 interaction, decreasing in the order (as perchlorate salts, except chloride complex for Co(III)): $Zn(II)_2$ > AMD3100 > $Ni(II)_2$ > $Cu(II)_2$ ≫ $Co(III)_2$ ≫ $Pd(II)_2$.

This has led to the suggestion that the Zn(II) complex is an important antagonist in vivo.[21] Particularly notable is the low activity of $Co(III)_2$−Xyl-bicyclam and the virtual inactivity of the $Pd(II)_2$−Xyl-bicyclam complex.[22] Also, Zn_2−bicyclen complexes (cyclen = 1,4,7,10-tetraazacyclododecane, five-membered chelate rings) containing a xylyl linker are reported to have reduced toxicity, compared to the free macrocycle, and high anti-HIV activity.[23]

Our aim is to elucidate the nature of potential first and second coordination sphere interactions of metal (bi)cyclam complexes with the CXCR4 receptor. Our investigations include solid-state and dynamic solution studies as well as modeling. We chose to study interactions of metal bicyclams with acetate, since there is strong evidence[24] from receptor mutagenesis studies that bicyclam blocking of CXCR4 is dependent on the carboxylate side chains of Asp171 and Asp262 in trans-membrane helices

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IV and VI. Our findings provide a new basis for the improved design of receptor-targeted macrocycles.

Experimental Section

Materials. Bz-cyclam and Xyl-bicyclam were synthesized using a general reported method for the preparation of monosubstituted cyclams.[25] Zinc perchlorate, zinc acetate dihydrate, palladium acetate (Aldrich), zinc chloride (Acros), potassium tetrachloropalladate (Alfa), and NMR solvents deuterium oxide (Aldrich, 99.9%) and chloroform-*d* (Apollo, 99.8%) were used as received.

WARNING! Perchlorate salts of complexes with organic ligands are potentially explosive and should be treated with great care. Only small amounts were prepared in these experiments.

Syntheses. Zn_2(Xyl-bicyclam)$(ClO_4)_4$ (1). Bicyclam (387.1 mg, 0.77 mmol) and $Zn(ClO_4)_2 \cdot 6H_2O$ (573.4 mg, 1.54 mmol) were stirred in MeOH (10 mL) at 333 K for 1 h. The precipitate was filtered off and washed with a small amount of MeOH. Yield 89%. Anal. Calcd for $C_{28}H_{54}N_8O_{10}Cl_4Zn_2$ (%): C 32.61, H 5.28, N 10.86. Found: C 32.19, H 5.35, N 10.22.

[Zn(Bz-cyclam)Cl]Cl·2.5CDCl$_3$ (2). 1-Benzyl-(1,4,8,11-tetraazacyclotetradecane) (Bz-cyclam, 100 mg, 0.34 mmol) and dry $ZnCl_2$ (47 mg, 0.34 mmol) were refluxed in MeOH (20 mL) under N_2 for 18 h. After cooling, diethyl ether was added to cloud point and colorless crystals formed on standing at 277 K. Crystals suitable for X-ray diffraction were obtained from an NMR solution in $CDCl_3$.

[Zn_2(Xyl-bicyclam)$(OAc)_2$]$(OAc)_2 \cdot 2CH_3OH$ (3). Bicyclam (49.6 mg, 0.1 mmol) and $Zn(OAc)_2$ (43.3 mg, 0.2 mmol) in MeOH (3 mL) were heated under reflux for 1 h. The white powder obtained by rotary evaporation was recrystallized by slow diffusion of diethyl ether into a MeOH solution. Yield 58%. Anal. Calcd for $C_{38}H_{74}N_8O_{10}Zn_2$ (%) C 32.61, H 5.28, N 10.86. Found: C 32.19, H 5.35, N 10.22.

[Pd(cyclam)]$(OAc)_2 \cdot 2H_2O$ (4). $Pd(OAc)_2$ (236.3 mg, 1.05 mmol) was reacted with cyclam (200.3 mg, 1 mmol) in water (100 mL) under N_2 with stirring for 8 h and addition of CH_3CO_2H to maintain a pH of ca. 4.4. The cloudy black solution was filtered and gave a yellow powder after rotary evaporation. Yellow crystals suitable for X-ray diffraction were obtained by slow diffusion of diethyl ether into a MeOH solution of this powder. Yield 84%. Anal. Calcd for $C_{14}H_{34}N_4O_4$-Pd (%) C 36.48, H 7.44, N 12.16. Found: C 36.93, H 7.24, N 12.60.

[Pd(cyclam)]$Cl_2 \cdot 3H_2O$ (5). This was prepared by an adaptation of a literature procedure.[26] The structure (*trans*-III) was confirmed by X-ray crystallography (data not shown).

Methods and Instrumentation. X-ray Crystallography. Diffraction data for compounds were collected on a Bruker Smart APEX diffractometer equipped with an Oxford Cryosystems low-temperature device. Absorption corrections were based on the multiscan procedure SADABS. A summary of X-ray crystallographic data for complexes **2**, **3**, and **4** is given in Table S1, and their structures have been deposited in the Cambridge Crystallographic Data Center under the accession numbers CCDC-188718, 188719, and 188720, respectively. H-bonding was determined using PLATON.[27]

Complex 2. The structure was solved by Patterson methods using DIRDIF.[28] The phenyl group is disordered over two positions; the different components were refined as rigid hexagons with occupancies fixed at 0.5. All the chloroform molecules of crystallization are disordered. The molecule labeled "S" in the tables is disordered over two orientations; that labeled "T" is disordered about a common C−Cl vector, while molecule "U" lies on a crystallographic inversion center. H-atoms were placed in calculated positions and all atoms except for part-weight carbon sites were refined anisotropically. The final model (334 parameters) was refined against F^2 (SHELXL-97)[29] to wR2 = 0.131 based on all 7449 data and $R_1 = 0.044$, based on 5726 data with $I \geq 2\sigma(I)$, GOF = 1.123, max Δ/σ on last cycle 0.02, max residual electron density = 0.78 e Å^{-3}.

Complex 3. The structure was solved by Patterson methods, using DIRDIF,[28] which gave an automatic solution and expansion to show all 27 atoms in the asymmetric unit. Refinement of 292 parameters by full-matrix least squares on F^2 using SHELX97[29] gave wR2 = 0.116 based on all data and $R_1 = 0.047$, based on 3995 data with $I \geq 2\sigma(I)$, GOF = 0.929, max Δ/σ on last cycle 0.08, max residual electron density = 1.5 e Å^{-3}. All non-hydrogen atoms refined anisotropically; hydrogen atoms of the complex were placed in calculated positions and allowed to ride. Other hydrogen atoms were located, idealized, and also allowed to ride.

Complex 4. The structure was solved by direct methods and refined by full-matrix least squares against F^2 (SHELXTL). The asymmetric unit contains two half-cyclam complexes in which the Pd atoms lie on crystallographic inversion centers. Methyl groups were modeled using the Sheldrick rotating rigid group method; H-atoms in amine and hydroxyl groups were located in Fourier maps and refined freely. Refinement of 263 parameters by full-matrix least squares against F^2 using SHELXL-97[29] gave wR2 = 0.101 based on all data and $R_1 = 0.043$ based on 3088 data with $I > 2\sigma(I)$, GOF = 1.038, max $\Delta/\sigma < 0.001$, max residual density = 1.51 e Å^{-3}. Other refinement details were as described for **3**.

NMR Spectroscopy. NMR data were acquired on Bruker DMX500 and Varian Unity INOVA spectrometers operating at 500.13 and 599.82 MHz for ^{1}H and using TBI [^{1}H, ^{13}C, X] or triple resonance [^{1}H, ^{13}C, ^{15}N] probeheads, respectively, equipped with *z*-field gradients. Standard pulse sequences were used for two-dimensional (2D) heteronuclear single quantum coherence (HSQC), total correlation spectroscopy (TOCSY; spin-lock time 70 ms), double-quantum-filtered correlated spectroscopy (DQF−COSY), and nuclear Overhauser enhancement spectroscopy (NOESY, typical mixing time 750 ms). The water resonance was suppressed via the WET method (water suppression enhanced through T_1 effects).[30] All data were converted to Xwinnmr (Version 2.0, Bruker U.K. Ltd) prior to Fourier transformation.

Typically data were acquired on 5−16 mM solutions in 10% D_2O/90% H_2O or $CDCl_3$ in 5 mm tubes at 298 K. ^{1}H and ^{13}C shifts were referenced to sodium trimethylsilylpropionate (TSP), and ^{15}N shifts to 1 M $^{15}NH_4Cl$ in 1.5 M HCl (external).

Kinetic Analyses. Rate constants were obtained by fitting the appropriate rate equations using the program Scientist (Version 2.0, MicroMath, Inc.). The configurational changes were assumed to occur by parallel first-order reactions.

Molecular Modeling. An homology model of human CXCR4 (sequence SWISS_PROT:CCR4_HUMAN, P30991) based on the X-ray structure of bovine rhodopsin (PBD accession code 1F88)[31] was built using the Homology module of Insight II (Version 2000, Biosym Technologies). The sequence alignment is shown in Table S2. The loops were generated de novo and selected so as to avoid clashes with Zn_2-bicyclam, which had been incorporated into the rhodopsin template. The Discover simulation package (Biosym Technologies) with the consistent valence force field was employed for energy minimization. The final step employed 500 cycles of the steepest descent method until the rms derivative of the energy was <0.001.

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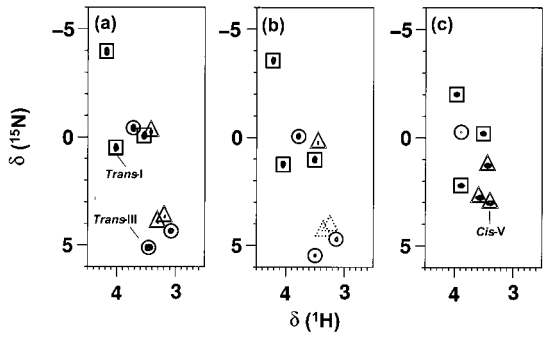

Figure 2. Comparison of [^{1}H,^{15}N] HSQC spectra of (a) Zn_2−Xyl-bicyclam perchlorate (**1**), (b) Zn−Bz-cyclam chloride (**2**), and (c) Zn_2−Xyl-bicyclam acetate (**3**) in 10% D_2O/90% H_2O. In each case, three sets of NH peaks can be identified (due to their connectivities in COSY and TOCSY spectra and by integration) corresponding to three major cyclam configurations, *trans*-I, *trans*-III, and *cis*-V. Peaks for the latter are strong only for complex **3**.

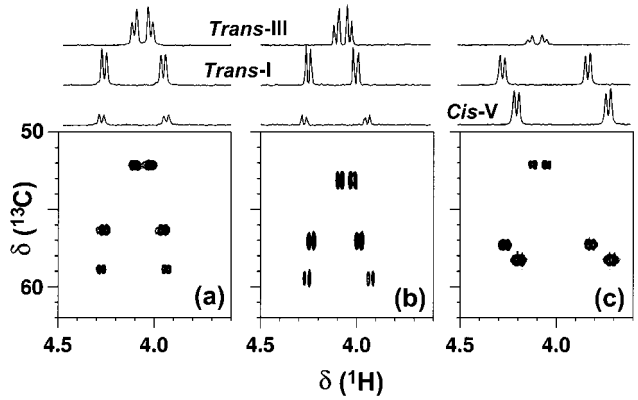

Figure 3. Detection of cyclam configurations in aqueous solution. Bottom: 2D [^{1}H,^{13}C] HSQC NMR spectra of the aromatic ring linker CH_2 region for (a) Zn_2−Xyl-bicyclam perchlorate (**1**), (b) Zn−Bz-cyclam chloride (**2**), and (c) Zn_2−Xyl-bicyclam acetate (**3**). Top: slices taken at each of the three ^{13}C chemical shifts showing the two nonequivalent geminal protons. The CH_2 resonances are sensitive only to the attached cyclam unit, unlike the peaks for the aromatic xylyl linker (see Figure 4). Peaks assignable to the *cis*-V configuration are weak, except when acetate is present. Full aliphatic regions of the spectra are shown in Figures S1, S3, and S7.

Since the NMR studies indicated that the major solution configuration of Zn_2−Xyl-bicyclam acetate is *cis*-V/*trans*-I, this was built from the X-ray coordinates of **3** with modification of the second ring to *trans*-I (based on the published X-ray structure[32] of [Zn(II)(1,4,8,11-tetramethyltetraazacyclotetradecane(CH_3OCO_2)]ClO_4) followed by energy minimization using Sybyl (Version 6.7, Tripos Inc.). Zn_2-Xyl-bicyclam was manually docked with the receptor so as to bring the *cis*-V ring near to Asp262 and the *trans*-I ring close to Asp171. Following docking, the ligand was fixed relative to the receptor, and distance restraints of 2.2 Å with an average force constant of 500 kcal mol^{-1} Å^{-2} were applied to the Zn−O bonds to Asp 171 and Asp 262. Bad contacts between the protein and metal bicyclam were then relieved by energy-minimizing the protein while the original structure for the metal bicyclam was maintained. During the minimization, the side chains of Asp171 and Asp262 moved into more favorable positions for binding to Zn without additional restraints. A similar procedure was used to optimize the H-bonds between the carboxylate oxygens of Glu288 and the diagonal NH protons on the *cis*-V ring using distance restraints of 2.0 Å and an average force constant of 200 kcal mol^{-1} Å^{-2}. WebLab ViewerPro 4 (Molecular Simulations Inc.) and MOLMOL (Version 2K.1)[33] were also used for modeling and graphics.

Results and Discussion

Configuration of Zn(II)$_2$−Xyl-Bicyclam Perchlorate (1) in Aqueous Solution. Complex **1** probably adopts the *trans*-III configuration in the solid state, as does [Zn(cyclam)-$(ClO_4)_2$],[34] but we did not obtain crystals. We studied the configuration of **1** in solution using NMR methods. The spectra were very complicated due not only to the presence of more than one configuration for each cyclam unit of the bicyclam, but also because of time-dependent changes.

Analysis of the NMR spectra of **1** was aided by the assignment of the NMR peaks for complex **2** (see Figures 2b and S1), the monocyclam complex Zn(II)−Bz-cyclam chloride (**2**), effectively complex **1** with one methylene-Zn(II)-cyclam removed. The X-ray crystal structure of [Zn(Bz-cyclam)Cl]Cl·2.5$CDCl_3$ (**2**) (Figure S2) showed it to be 5-coordinate with an axial Cl ligand, and NMR data for a $CDCl_3$ solution were consistent with the presence of a single configuration, *trans*-III, as in the crystal. Over a period of 3 h after dissolution of **2** in water at 298 K, the initially intense set of peaks assignable to *trans*-III gave rise to three sets of peaks (Figure 3b) assignable to *trans*-III (43%), *trans*-I (42%; planar five-membered rings, Figure 1), and a minor configuration (15%, possibly *cis*-V). Moore et al.[35] reported slow formation of different conformers after dissolution of (polymeric, chloride-bridged) [Zn(cyclam)-Cl]$^+$ in water.

The 2D [^{1}H,^{15}N] HSQC NMR spectra of equilibrium aqueous solutions of complexes **1** and **2** (Figure 2a,b) and the aliphatic region of their 2D [^{1}H,^{13}C] HSQC NMR spectra (Figure 3a,b, and Figures S1 and S3) were almost identical. This suggests that the cyclam units in the bicyclam complex **1** have little influence on each other in aqueous solution.

Aqueous solutions of **1** attained equilibrium in ca. 6 h and consisted of ca. 45% *trans*-I, 34% *trans*-III, and 21% *cis*-V. These configurational changes had half-lives of 14−87 min at 298 K (Figure S4a) and require two NH inversions (Figure 1). Analysis of 2D NMR [^{1}H,^{1}H] NOESY and DQF−COSY cross-peak connectivities allowed "walks" from one cyclam ring through the aromatic linker onto the second cyclam unit and therefore to the assignment of specific cyclam configurations to each end of the bicyclam ligand (Figure S5). This analysis suggested that the *cis*-V/*trans*-I configuration predominates in aqueous solutions of **1**.

Acetate Triggers a Rapid Configurational Change of Zinc Xyl-bicyclam Perchlorate (1). Addition of acetate to aqueous solutions of **1** produced dramatic changes in the NMR spectra, as illustrated in Figure 4 for the aromatic linker resonances. Equilibria were reached within 1 h (at 298 K). The proportion of the *cis*-V/*cis*-V configuration increased from 0% to ca. 19% on addition of 4 mol equiv of acetate, and that of the *cis*-V/*trans*-I configuration increased from 26% to 38% (Figure 5).

Binding of acetate was confirmed by ^{13}C NMR studies using $CH_3{}^{13}CO_2^-$ (Figure S6). Fast exchange of acetate was observed on the NMR time scale. From the binding curve, we estimated a binding constant of log $K = 2.75 \pm 0.15$, assuming formation of 1:1 complexes of acetate with Zn−cyclam units and that the two Zn−cyclam units are independent. This value can be

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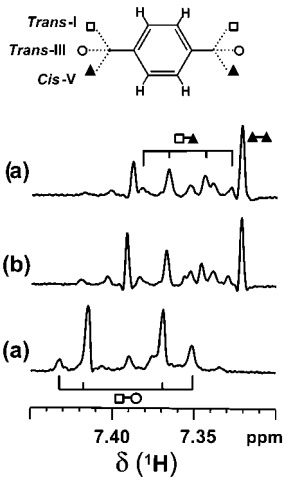

Figure 4. Effect of acetate on the configurations of the cyclam rings of Zn_2−Xyl-bicyclam. The aromatic linker region of resolution-enhanced ^1H NMR spectra of aqueous solutions of (a) Zn_2−Xyl-bicyclam perchlorate (**1**), (b) as in part a but after addition of 4 mol equiv of acetate, and (c) crystalline Zn_2−Xyl-bicyclam acetate (**3**). The solvent was 10% D_2O/90% H_2O, and all samples were at equilibrium. The xylyl aromatic resonances are sensitive to both of the attached cyclam units, being singlets when the two units are the same but AB quartets when they are different. Acetate induces a marked increase in the proportion of *cis*-V/*cis*-V and *cis*-V/*trans*-I species (see Figure 5). Labels: □, *trans*-I; ○, *trans*-III; ▲, cis-V.

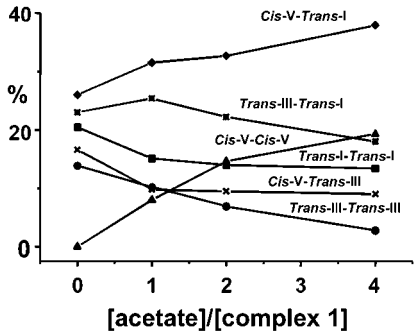

Figure 5. Effect of acetate on the distribution of cyclam configurations of Zn_2−Xyl-bicyclam as determined by integration of the ^1H NMR resonances for the aromatic linker (see Figure 4). NaOAc was added stepwise to a 5 mM solution of (Zn_2−Xyl-bicyclam)$(ClO_4)_4$ in 10% D_2O/90% H_2O at 298 K to give molar ratios (Zn_2−Xyl-bicyclam)$(ClO_4)_4$:acetate of 1:1, 1:2, and 1:4. The pH of the solutions was within the range 6.6−7.4, and in each case, equilibrium was reached within 1 h. The proportions of species present at the 1:4 mol ratio are similar to those for a solution of crystalline **3**.

compared to log $K = 2.6$ reported for acetate binding to the Zn(II) complex of 1,5,9-trazacyclododecane ([12]aneN_3).[36]

We also prepared the acetate complex in crystalline form by reacting zinc acetate with Xyl-bicyclam in methanol and determined its X-ray structure.

$[Zn_2$(Xyl-bicyclam)$(OAc)_2](OAc)_2\cdot 2CH_3OH$ (3) Adopts the Unusual *cis*-V Configuration in the Solid State. The crystal structure of complex **3** comprises $[Zn_2$(Xyl-bicyclam)$(OAc)_2]^{2+}$ cations, acetate anions, and MeOH molecules as solvent of crystallization. The aromatic linker symmetrically bridges the cyclam rings. Each monocyclam ring resides on a center of inversion, is folded diagonally about N(4)/N(11), and adopts a *cis*-V configuration with the 5- and 6-membered rings in gauche and chair conformations, respectively. Zn(II) has a distorted octahedral geometry (Figure 6a). Cyclam nitrogens N(1) and

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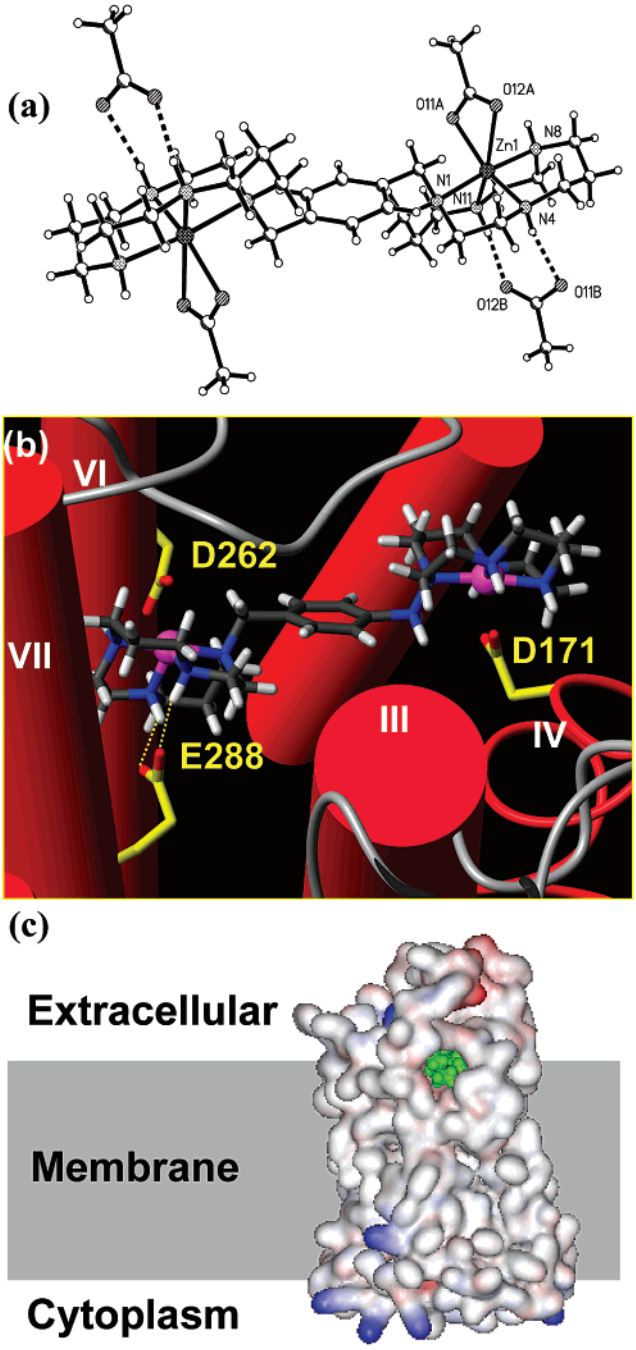

Figure 6. First and second coordination sphere interactions of Zn_2−Xyl-bicyclam with carboxylate groups. (a) X-ray crystal structure of [Zn_2−Xyl-bicyclam$(OAc)_2](OAc)_2\cdot 2CH_3OH$ (**3**) (CH_3OH not shown). Both cyclam units adopt the *cis*-V configuration with chelation by acetate on one cyclam face and double H-bonding on the other. A space-filling model of the cation is shown in Figure S8a. (b) Model of Zn_2−Xyl-bicyclam bound to the CXCR4 coreceptor. Transmembrane helices are red cylinders, except helix IV, which is depicted as a ribbon for clarity. One of the Zn(II)−cyclams has axial coordination to the oxygens of Asp262 and double H-bonds between two of its NH groups and the oxygens of Glu288 on the opposite cyclam face and is in the *cis*-V configuration, resembling that in part a above. The second Zn(II)−cyclam is *trans*-I with axial coordination to Asp171. (c) View of the model of CXCR4 showing the location of the proposed Zn_2−Xyl-bicyclam binding site. The *cis*-V cyclam is visible through a channel on one side of the protein. The likely position of the membrane is indicated in gray, Zn_2−Xyl-bicyclam is green, and electrostatic potentials are colored blue-positive, red-negative, and white-neutral. The unstructured N-terminus (residues 1−38) and C-terminus (residues 320−352) have been omitted.

Table 1. Selected Bond Distances (Å) and Angles (deg) for Complexes **2**−**4**[a]

[Zn(Bz-cyclam)Cl]Cl·2.5CDCl$_3$ (**2**)			
Zn(1)−N(1)	2.220(2)	N(4)−Zn(1)−N(11)	153.71(9)
Zn(1)−N(4)	2.082(2)	N(4)−Zn(1)−N(1)	82.76(8)
Zn(1)−N(8)	2.129(2)	N(11)−Zn(1)−N(1)	93.88(8)
Zn(1)−N(11)	2.094(2)	N(8)−Zn(1)−N(11)	83.33(8)
Zn(1)−Cl(1)	2.3059(7)	N(8)−Zn(1)−N(1)	155.50(8)
		N(1)−Zn(1)−Cl(1)	104.73(6)
[Zn$_2$(Xyl-bicyclam)(OAc)$_2$](OAc)$_2$·2CH$_3$OH (**3**)			
Zn(1)−N(1)	2.252(2)	N(4)−Zn(1)−N(11)	105.32(10)
Zn(1)−N(4)	2.087(3)	N(4)−Zn(1)−N(1)	83.73(9)
Zn(1)−N(8)	2.161(3)	N(11)−Zn(1)−N(1)	93.94(9)
Zn(1)−N(11)	2.071(2)	N(8)−Zn(1)−N(11)	83.49(10)
Zn(1)−O(11A)	2.089(2)	N(8)−Zn(1)−N(1)	174.67(4)
Zn(1)−O(12A)	2.407(2)	O(11A)−Zn(1)−O(12A)	58.34(8)
[Pd(cyclam)](OAc)$_2$·2H$_2$O (**4**)			
Pd(1)−N(1)	2.038(4)	N(1)−Pd(1)−N(2)	95.05(15)
Pd(1)−N(2)	2.043(3)	N(1)−Pd(1)−N(2A)[b]	84.95(15)
Pd(2)−N(3)	2.036(4)	N(3)−Pd(2)−N(3B)[c]	180.0
Pd(2)−N(4)	2.037(3)	N(3)−Pd(2)−N(4)	95.28(15)
N(1)−Pd(1)−N(1A)[b]	180.0	N(3)−Pd(2)−N(4B)[c]	84.72(15)

[a] For atom labels, see Figures S2, 6, and S9. [b,c] Symmetry transformations used to generate equivalent atoms: $b = -x + 1, -y, -z$; $c = -x, -y + 1, -z + 1$.

Table 2. Distances (Å) and Angles (deg) for Possible Hydrogen Bonds in Complexes **2**−**4**[h]

D−H···A	H···A (Å)	D···A (Å)	∠D−H···A (deg)
[Zn(Bz-cyclam)Cl]Cl·2.5CDCl$_3$ (**2**)			
N4−H4···Cl2[a]	2.537	3.312(2)	141.1
N8−H8···Cl1[b]	2.724	3.440(2)	134.4
N11−H11A···Cl2[c]	2.556	3.446(2)	160.4
[Zn$_2$(Xyl-bicyclam)(OAc)$_2$](OAc)$_2$·2CH$_3$OH (**3**)			
N4−H4···O11B[d]	2.06	2.953(3)	162
N8−H8B···O1C[e]	2.06	2.972(4)	168
N11−H11B···O12B[d]	1.90	2.831(4)	174
O1C−H1C···O11B[f]	1.81(2)	2.697(4)	168(4)
[Pd(cyclam)](OAc)$_2$·2H$_2$O (**4**)			
O2W−H2WA···O3	1.93(6)	2.805(5)	167(6)
O1W−H1WB···O1	2.00(6)	2.791(5)	162(5)
N1−H1N···O2	2.10(5)	2.819(4)	168(6)
N2G[g]−H2NG[g]···O1	2.19(4)	2.871(4)	156(4)
N3−H3N···O3	2.06(5)	2.812(4)	168(5)

[a−g] Symmetry transformations used to generate equivalent atoms: $a = -x + 1, y + 1/2, -z + 3/2$; $b = x, -y + 3/2, z + 1/2$; $c = -x + 1, -y + 1, -z + 1$; $d = -x + 1, -y + 1, -z$; $e = x, y + 1, z - 1$; $f = -x, -y, z + 1$; $g = -x + 1, -y, -z$. [h] For atom labels, see Figures S2, 6, and S9.

N(8) can be considered to occupy axial Zn(II) coordination sites and N(4) and N(11) and two acetate oxygens the equatorial positions. The equatorial Zn−N bond lengths (2.07, 2.09 Å, Table 1) are normal for Zn−cyclam complexes, but the axial bonds are long (Zn−N(1) 2.25 Å, Zn−N(8) 2.16 Å). One of the Zn−O bonds is also long (2.41 Å) compared to the other (2.09 Å), which is normal in length.[32]

A notable feature of the structure is the pair of relatively strong H-bonds, N(4)−H(4)···O(11B) (2.06 Å, 162°) and N(11)−H(11)···O(12B) (1.90 Å, 174°), between the oxygens of a noncoordinated acetate anion and the cyclam NH protons on the cyclam face opposite to the coordinated acetate (Figure 6a, Table 2). On the coordination side, N(8)−H(8B) forms an H-bond to solvent MeOH (2.06 Å, 168°). An interesting parallel is provided by the complex acetato-*C*-*rac*-(5,7,7,12,14,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane)Ni(II) perchlorate, which has a folded structure, bidentate acetate, and all NH groups H-bonded to perchlorate oxygen atoms.[37] In the X-ray structure of the host−guest complex between 4-*tert*-butyl benzoic acid and cyclam, there is double H-bond formation between diagonal NH groups of protonated cyclam and carboxylate groups on each side of the cyclam ring.[38] Host−guest assemblies of metal complexes and carboxylates are being used for the synergistic extraction of metal ions such Ni(II) and Cu(II) into organic phases, an area that parallels the membrane processes of interest in the present study.[39]

The *cis*-V configuration is unusual for Zn−cyclam complexes. In a search of the Cambridge Structural Database, we found 46 structures for Zn(II)−cyclam complexes: 4 *trans*-I, 33 *trans*-III, 1 *cis*-IV, 1 *trans*-V, and 7 *cis*-V, but of the latter, 6 contain highly constrained cyclams (e.g., an *N*,*N*-linker across the macrocycle) and the seventh is highly substituted on the periphery. No *cis*-V Zn−cyclams with carboxylates as ligands appear to have been reported previously. It is interesting to note that the N(4)−Zn−N(11) bond angle of 105.3°, which is opposite to the O−Zn−O angle, is larger than the analogous N−Co−N angle of 103.0° reported previously for [Co(III)-(cyclen)(CO$_3$)]ClO$_4$·H$_2$O.[40] Chelation becomes more favorable as the O−M−O angle decreases, and this can be achieved by increasing the N−M−N opposite angle.[41,42]

***cis*-V Configurations of [Zn$_2$(Xyl-bicyclam)(OAc)$_2$](OAc)$_2$ (3) Are Dominant in Aqueous Solution.** After dissolution of crystalline **3** in 10% D$_2$O/90% H$_2$O, equilibrium was reached rapidly (<20 min, Figure S4b), and ^{1}H, ^{13}C, and ^{15}N NMR peaks assignable to the three configurations *cis*-V (58%), *trans*-I (35%), and *trans*-III (7%) were detectable (Figures 2, 3, 4, and S7), with the *cis*-V/*trans*-I configuration predominating (44%) and *cis*-V/*cis*-V, the crystal configuration, accounting for ca. 29% of the total bicyclam configurations. This distribution is very similar to that obtained after addition of 4 mol equiv of acetate to complex **1** (Figure 5). It is evident that acetate stabilizes the folded *cis*-V configuration in solution.

Recognition of Zinc Xyl-Bicyclam by the Viral Coreceptor CXCR4. We docked *cis*-V/*trans*-I Zn$_2$−Xyl-bicyclam onto an homology model of CXCR4 based on the 7-helical transmembrane structure of bovine rhodopsin.[24,31] In the energy-minimized model (Figure 6b,c), the *cis*-V cyclam is coordinated to Asp262 with Zn(II)−carboxylate bonds of similar length (2.27 Å) to the average for **3**, and via double NH H-bonds on the opposite face to the oxygens of Glu288 (COO···H−N, 2.01 Å). The *trans*-I cyclam is bound to the oxygens of Asp171 (Zn−O 2.28 Å) (Figure S8b).

Thus the high anti-HIV potency of Zn$_2$−Xyl-bicyclam may be related to its ability to block the CXCR4 coreceptor via these specific interactions. Asp171 and Asp262 in trans-membrane helices IV and VI, respectively, have already been identified by receptor mutagenesis studies as key sites for bicyclam blocking.[24] Mutation of Glu288 in helix VII, which is involved in stromal cell-derived factor 1α (SDF-1α) binding and signaling

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and is relatively close to the cell surface, impairs coreceptor activity, and Glu288 may be involved in interactions with the V3 loop of the HIV envelope protein gp120.[43] The hydrophobic para-substituted *p*-phenylenebis(methylene) linker is known to be important for the high anti-HIV activity of cyclams,[44] and it is notable that there is a high population of aromatic amino acid side chains in the extracellular loop regions of our homology model of CXCR4. Aromatic $\pi-\pi$ stacking interactions may be important for stabilizing intermediate binding steps along the pathway through the extracellular loops. Lack of loop flexibility prevented docking of Zn_2−bicyclam into a similar site in a CXCR4 model with a C109−C186 disulfide bond analogous to that found in rhodopsin. This might suggest that in vivo docking will be enhanced under reducing conditions, a postulate which could be explored in cell systems and which might have implications for combination therapy.

Inactive Pd(II)−Cyclams Do Not Bind Axial Carboxylates or Adopt the *cis*-V Configuration. We found the structures of nine complexes of Pd(II) with cyclam or cyclam derivatives in the Cambridge Structural Database. Seven of these have the *trans*-III configuration, and two are *trans*-I. We sought to determine whether Pd(II)−cyclam can bind axial carboxylates or adopt the *cis*-V configuration.

We prepared Pd_2−Xyl-bicyclam in solution but did not isolate it. Addition of 2 mol equiv of acetate at pH 7 caused little change to the ^{1}H NMR resonances. We were able to crystallize the acetate adduct of Pd(II) cyclam, [Pd(cyclam)](OAc)$_2$·2H$_2$O (**4**). In the X-ray structure of **4**, cyclam has the *trans*-III (*meso*-*R*,*R*,*S*,*S*) configuration with gauche $\delta\lambda$ en chelate rings and Pd(II) is square-planar (Figure S9). There is no axial interaction between acetate and Pd(II) (shortest Pd−O distance 4.0 Å), although acetate is H-bonded to NH protons. We assigned fully 1D and 2D ^{1}H and ^{15}N NMR spectra of [Pd(cyclam)]Cl$_2$ (**5**) in 10% D_2O/90% H_2O, and these did not change over a period of several days. Notable was the presence of only one ^{1}H/^{15}N correlation for NH protons, and further analysis of the spectra confirmed that only the *trans*-III configuration was present in solution. Addition of acetate (1−4 mol equiv) to an aqueous solution of **5** at pH 7 had no effect on the spectrum, even after several weeks at 298 K. We conclude that acetate does not induce configurational changes of Pd(II) cyclam or Pd(II) Xyl−bicyclam, and that Pd(II)−cyclams cannot readily adopt a *cis*-V configuration. The lack of direct coordination of Pd(II)−cyclam to carboxylates may be a major factor in determining its anti-HIV inactivity[21] through lack of strong binding to the CXCR4 receptor. However, since the free ligand Xyl−bicyclam does bind to the receptor, stereospecific H-bond interactions are also likely to play a role.

Conclusions−Relevance to in Vivo Activity

Xyl-bicyclam octahydrochloride, the drug AMD3100, is a potent CXCR4 coreceptor antagonist. The Zn(II)−Xyl-bicyclam perchlorate complex is slightly more active in vitro than the ligand itself, and AMD3100 may be active in vivo as a Zn(II) complex.[21] It is therefore of interest to ask whether our findings from model systems are likely to be applicable under in vivo conditions.

We have shown that acetate binding to Zn_2−Xyl-bicyclam induces the rapid formation of the unusual *cis*-V configuration via chelation and second coordination sphere double H-bonding to diagonal cyclam NH protons. Molecular modeling suggests that the carboxylate groups of Asp262 and Glu288 can stabilize a similar *cis*-V configuration for one Zn(II)−cyclam unit of Zn_2−Xyl-bicyclam bound to CXCR4. The second Zn(II)−cyclam unit can bind to Asp171 with the bicyclam connecting across the main coreceptor binding site. Such a network of interactions allows a strong induced-fit recognition and is consistent with the high antagonistic potency of Zn(II)$_2$−Xyl-bicyclam.

Although binding of acetate to a Zn(II)−cyclam unit in Zn_2−Xyl-bicyclam (log K = 2.75) is only moderately strong in aqueous solution, we estimate that binding to an aspartate carboxylate in CXCR4 will be enhanced by the decrease in dielectric constant inside the protein. For a dielectric constant of 30,[45] the stability enhancement would be about 1.5 log units, which gives a stability constant of ca. 4.3 log units for a Zn−cyclam−carboxylate interaction in the coreceptor. Further stabilization of the interaction of Zn_2−Xyl-bicyclam with CXCR4 is presumably brought about by hydrogen bonding and hydrophobic interactions, the magnitude of which we have not attempted to determine in our studies. However, a simplistic approach, taking into account Zn−carboxylate binding only, yields a cumulative stability constant of ca. log K = 8.5. This value is in line with the reported strengths of binding of xylyl-bicyclams to the CXCR4 coreceptor in COS-7 cells.[24] Therefore, both solution studies as well as pharmacological studies yield nano- to micromolar dissociation constants, which are consistent with the reported serum concentrations of AMD3100 in treated human subjects.[46,]

Direct binding of the metal ions in metallobicyclam complexes to CXCR4 carboxylate groups is likely to be a major factor in determining the strength of the interaction. Thus Pd(II)−cyclams, which adopt only square-planar configurations, would be expected to be inactive, since all four coordination positions are occupied by cyclam nitrogen atoms. Indeed, this has been reported to be the case.[21] Our X-ray and NMR studies confirmed the lack of direct interaction of both Pd(II)−cyclam and Pd(II)$_2$−Xyl-bicyclam with acetate and also the lack of ability of acetate to induce a configurational change.

In contrast, Co(III)−cyclams are known to bind strongly to carboxylates and, moreover, are capable of undergoing isomerization from trans to cis configurations.[14] It might be expected, therefore, that Co(III)$_2$−Xyl-bicyclam would bind strongly to the CXCR4 coreceptor and be highly active as an anti-HIV agent. However, this is not the case: Co(III)−cyclams are reported to have very low activity.[21,22] This may be attributable,

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at least in part, to the kinetic inertness of Co(III)−cyclams.[47] Our ^{1}H and ^{13}C NMR studies (data not shown) of the interaction of $[Co(III)(cyclam)Cl_2]^+$ with acetate show that equilibrium takes several days to be reached (298 K) and that binding is indeed strong (slow exchange NMR regime). Configurational interconversions may also be relatively slow for Cu(II)− and Ni(II)−cyclam complexes, which may contribute to their lower activity in comparison to Zn(II)−cyclams.

It should be possible to modify the design of (bi)cyclam drugs to increase their selectivity even further by controlling the readout of both static and dynamic information encoded in metallocyclam complexes. In particular, it is important to optimize inhibition of HIV recognition without affecting chemokine receptor activity. Since we detected at least six configurations for $Zn(II)_2$−Xyl-bicyclam in aqueous solution, consideration could be given to the administration of preformed metal−(bi)-cyclam complexes with configurational locking of one cyclam unit into the potentially preferred *cis*-V configuration. These new insights into the general principles governing the configurational control of cyclams are also likely to be of use in the design of macrocycles for many other specific purposes.

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Supporting Information Available: Table S1 listing X-ray crystallographic data for complexes **2**−**4**, Table S2 showing sequence alignment of human CXCR4 and bovine rhodopsin, Figures S1−S9 showing ^{1}H, ^{13}C NMR data for complexes **1**−**3**, dependence of the distribution of configurations of Zn_2−Xyl-bicyclam complexes on time after dissolving in aqueous solution, 2D [^{1}H,^{1}H] NOESY and DQF COSY NMR spectra for complexes **1** and **2**, plot of ^{13}C chemical shift versus the molar ratio of **1:**$CH_3{}^{13}COONa$, space-filling models of Zn_2−Xyl-bicyclam in the X-ray structure of complex **3** and bound to the CXCR4 coreceptor, and X-ray crystal structures of complexes **2** and **4** (PDF, CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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