Cantaurus, Vol. 12, 15-19, May 2004 © McPherson College Division of Science and Technology

Linked Cross-Bridged Cyclams as Anti-HIV Agents

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ABSTRACT

AMD3100, also known as xylyl-bicyclam, has been shown to selectively bind the CD4 co-receptor CXCR4 thus exhibiting anti-HIV properties. It has since been found that the Zn^{2+} complex of AMD3100 is ten times more active. The increased activity is thought to occur due to a folded conformation of the ligand induced by complexation with the metal. To further test this hypothesis, a conformationally restricted analog of AMD3100 has been synthesized. The analog has a 2 carbon cross-bridge imposing the folded conformation in all of its complexes. This analog's Cu^{2+} complex has also been synthesized and characterized. The compound is currently in the process of being assayed for its anti-HIV properties.

Keywords: *AMD3100, Anti-HIV, Bicyclam, cross-bridged, CXCR4, Cyclam, Xylyl-bicyclam*

INTRODUCTION

A chemical Inhibitor is defined as: "A substance which is capable of stopping or retarding a chemical reaction; to be technically useful, it must be effective in low concentration." (Access Science 2002) Biological inhibition of the HIV virus can be thought of in terms of stopping the chemical reactions used by the virus to infect the cell, thus stopping the virus from replicating. It is with this purpose in mind that this project began.

Current anti-HIV agents include drugs that target interfering directly with the reproduction of new viruses. These types of anti-HIV agents are known as reverse transcriptase and protease inhibitors. Initially these drugs did prove beneficial towards controlling the virus. However, mutations of HIV have begun to show resistance to these types of drugs. Scientists are now looking at a new target in the life cycle of HIV. One target that shows potential is blocking the site at which HIV fuses to, and thus enters the cell. (D'Souza et al. 2000) Hence, this class of anti-HIV agents has been dubbed Fusion Inhibitors. (Este` et al. 1999)

A receptor protein, CD4 has been identified as the primary receptor for the entry of HIV into the cells of the immune system. However, it was also known that CD4 alone was not responsible for infection. It wasn't until 6 years ago that the co-receptors CXCR4 and CCR5 were identified as being the doorway, along with CD4, for HIV entry into the cell. This research is concerned with the CXCR4 receptor. "CXCR4 is the natural receptor for the CXC-chemokine SDF-1α (stromal cell-derived factor 1α)", which "blocks the entry of T-tropic (X4) virus strains into the cells". (Luster A.D. 1998)

A relatively new compound, AMD3100, has been shown to selectively bind with CXCR4, thus exhibiting anti-HIV activity. (Este` et al. 1999) AMD3100, also called Xylyl-bicyclam, has two macrocyclic rings that are connected by an aromatic linker. (Gerlach et al. 2001) (Fig.1) The identical tetraazamacrocycles are called cyclam. When linked, the resulting molecule is bicyclam.

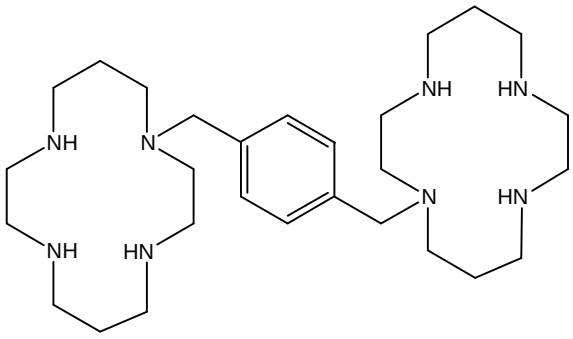

Figure 1 Xylyl-bicyclam

In a study of activity of different bicyclam derivatives against HIV through interaction with the CXCR4 receptor, it was found that the Zn^{2+} Xylyl-bicyclam complex is ten times more active than Xylyl-bicyclam alone. (Este` et al. 1999) A study in 2002 examined the likelihood that the Zn^{2+} Xylyl-bicyclam's increased activity was due to binding aspartate residues in the CXCR4 receptor. To study this, the researchers used acetate to model the aspartate residues because both have a carboxylate functional group. It was shown that the increased activity of the Zn^{2+} complex is most likely due to the change from a planar conformation to that of a folded, or cis-V, conformation upon binding with acetate, thus modeling the aspartate residues in the CXCR4 receptor. (Liang et al. 2002) A study in 2003 questioned which aspartate residues, specifically, were responsible for the increased binding affinity of the metal ion substituted Xylyl-bicyclam with the CXCR4 receptor. It was found, through mutational analysis of CXCR4 protein, that the increased binding affinity was selectively eliminated by substitution of Asp^{262}. This was achieved by only one metal ion being inserted into Xylyl-bicyclam. Thus allowing for the examination of the binding affinity for just one of the ring systems with only one aspartate residue. (Gerlach et al. 2003)

The structural difference between a planar and folded confirmation can be seen in Figure 2.

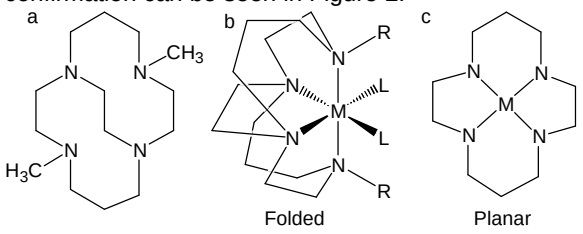

Figure 2 Conformations of cross-bridged, folded cross-bridged, and planar tetraazamacrocycles.

Cross-bridged tetraazamacrocycles are analogues of cyclam, the only difference being a short two-carbon bridge between two non-adjacent nitrogens. (Weisman et al. 1990) (Fig. 2c) In effect this bridge locks the cyclam into a very rigid folded conformation. (Fig. 2b) The goal of this research is to synthesize a permanently folded conformation of the Zn^{2+} Xylyl-bicyclam. If successfully synthesized, the resulting compound could then be assayed as an anti-HIV drug.

MATERIALS AND METHODS

cis-Decahydro-3a,5a,8a,10a-tetraaza-pyrene

note: previous students made the starting material, cyclam. A solution of cyclam (11.9 g, 5.95*10^ -2 mols) in Acetonitrile (48 mL) was flushed with N_2 gas for 15 minutes before a slight molar excess of 40% Glyoxal (9.34 g, 0.161 mols) was added. (Fig. 3) The reaction was left to stir for 3 hours at 50-65 °C under N_2 gas. The solvent was evaporated and the product extracted from the residue with chloroform (6 x 40 mL). The product was then purified by alumina chromatography (8" x 1") with 1% methanol in dichloromethane. The resulting yield of 1 was quantitative.

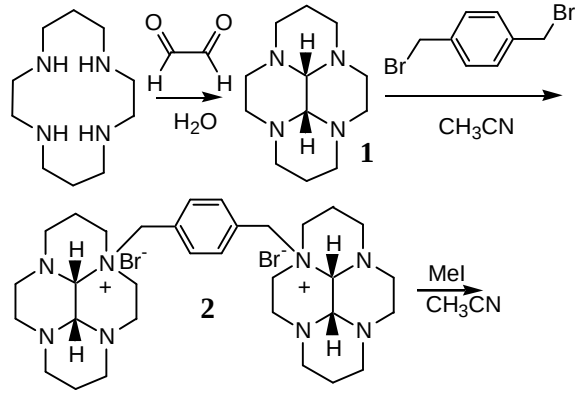

Figure 3 Glyoxal addition and linking reaction.

3a-[4-(cis-Decahydro-{5a,8a,10a-diaza-3a-azonia}-pyren-3a-ylmethyl)-benzyl]-cis-decahydro-{5a,8a,10a-diaza-3a-azonia}-pyrene. To a solution of cyclam glyoxal (7.00 g, 0.03 mols) and acetonitrile (60 mL) was added 1,4-Bis-bromomethyl-benzene (4.16 g, 0.03 mols). (Fig. 3) The mixture was left stirring at room temp for a week. The precipitate was filtered, washed with acetonitrile, and dried giving an 86.7% yield of 2 (9.68 g).

3a-[4-(8a-methyl-cis-Decahydro-{5a,10a-diaza-3a,8a-azonia}-pyren-3a-ylmethyl)-benzyl]-8a-methyl-cis-decahydro-{5a,10a-diaza-3a,8a-azonia}-pyrene. To a solution of 2 and acetonitrile (300 mL) was added Iodomethane (40 mL, 0.641 mols). (Fig. 3) The mixture was left stirring at room temperature under N_2 gas for 2 weeks. The product was filtered and washed with acetonitrile and ether giving a 75.5 % yield of 3 (11.21 g).

4-methyl-11-[4-(4-methyl-1, 4, 8, 11-tetraaza-bicyclo[6.6.2]hexadec-11-ylmethyl)-benzyl]-1,4,8,11-tetraaza-bicyclo[6.6.2] hexadecane. To a solution of 3 (5.99 g, 5.52*10^ -3 mols) and 95% ethanol (520 mL) was added slowly sodium borohydride (12.0 g, 0.317 mols) in a 1 liter round bottom flask. (Fig. 4) The mixture was allowed to stir at room temperature under N_2 gas for 5 days. The sodium borohydride was decomposed with 6 M HCl to a pH ~ 1-2. The solvent was then evaporated. Approximately 100 mL of deionized water was added to the residue and subsequently made basic to a pH ~ 14 by addition of 30% aqueous KOH, followed by KOH pellets. The product was then extracted with benzene (4 x 120 mL) and then dried with sodium sulfate. The benzene layer was evaporated. A 71% yield of 4 (2.28 g) was obtained.

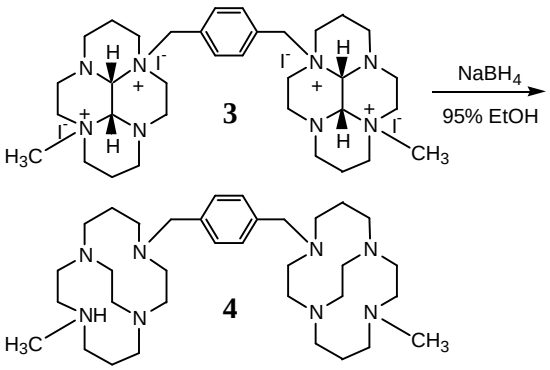

Figure 4 Methylation and reduction.

Dichloro(4-methyl-11-[4-(4-methyl-1, 4, 8, 11-tetraaza-bicyclo[6.6.2]hexadec-11-ylmethyl)-benzyl]-1,4,8,11-tetraaza-bicyclo[6.6.2] hexadecane)copper (II) hexafluorophosphate. To a solution of 4 (0.292 g, 5*10^ -4 mols) and methanol (15 mL) was added a solution of copper (II) chloride dihydrate (0.171 g, 1.27*10^ -3 mols) in dichloromethane (20 mL) and methanol (10 mL). The reaction was left to stir for one week under N_2 gas. (Fig. 5) To this solution was added a solution of NH_4PF_6 (0.815 g, 4.97*10^ -3 mols) and methanol (5 mL). A whitish precipitate formed immediately in the dark blue solution. The complex was filtered through a

frit and washed with MEOH and ether giving a 71% yield of 5 (0.381g).

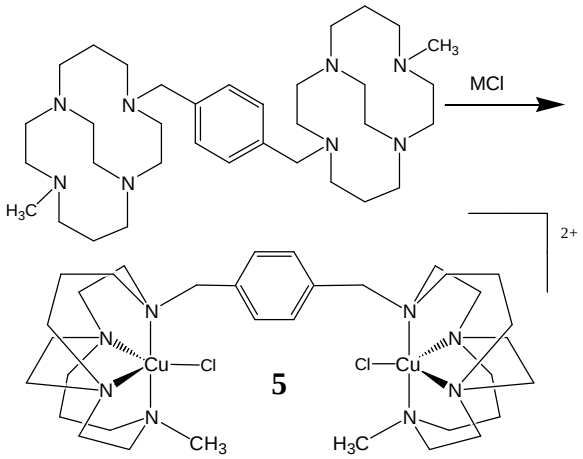

Figure 5 Metal complexation reaction.

RESULTS AND DISCUSSION

Synthesis of Ligand

(Synthesis 1) ***cis-Decahydro-3a,5a,8a,10a-tetraaza-pyrene.*** The first step in the synthesis of a cross-bridged cyclam is a condensation reaction between glyoxal and cyclam, which forms a four-bonded ethyl bridge, one bond to each nitrogen. This step is the same whether one is trying to synthesize cross-bridged cyclam or whether one is attempting to link two cross-bridged cyclams together, but the next step is where their paths diverge

Note: Because the glyoxal addition is a simple reaction and has been documented as being a very successful one, it was decided to forgo analytical characterization of the product. (Weisman et al. 1990)

(Synthesis 2) **3a-[4-(cis-Decahydro-{5a,8a,10a-diaza-3a-azonia}-pyren-3a-ylmethyl)-benzyl]-cis-decahydro-{5a,8a,10a-diaza-3a-azonia}-pyrene.** The fundamental step in the synthesis of cross-bridged cyclam is bis-alkylation at non-adjacent nitrogens on the macrocycle. This is usually accomplished with methyl groups on the non-adjacent nitrogens of both macrocycles. The fundamental step in the synthesis of linked cross-bridged cyclam is first alkylating one nitrogen, on two separate macrocycles, thus linking them. This was accomplished in reaction with the aromatic linker 1,4-Bis-bromomethyl-benzene. (Baccon et al. 2001) At this point it was decided that documentation of the product from lab results was needed and subsequently sent samples off for analysis.

Analysis of product: ^{13}C NMR (500 MHz, D_2O) showed peaks (ppm): 133.919, 128.534, 82.128, 69.406, 61.475, 53.792, 53.119, 51.765, 51.175, 46.396, 41.794, 18.225, 17.813. The ^{1}H NMR (500MHz, D_2O) data was complex, but consistent with molecule. The calculated elemental analysis for $C_{32}H_{52}N_8Br_2$ is C, 54.23%; H, 7.40%; N, 15.81%. The actual analysis found these percentages to be C, 44.78%; H, 7.17%; N, 11.81%. ES+ mass spectrometry in 90% MeOH exhibited peaks at m/z = L^+ (630) and m/z = L^{++} (274), which is consistent with the calculated mass of this molecule.

The elemental analysis was received first and by no means did it indicate that the correct product had been obtained. One alternative calculation, however, of $C_{32}H_{61}N_8Br_3O_4$ accounted for C, 44.61%; H, 7.14%; N, 13.01%. The mass spec was received shortly there after with strong peaks at the correct m/z values. Because the peaks were strong at the right m/z values it was decided to continue with the synthesis.

(Synthesis 3) **3a-[4-(8a-methyl-cis-Decahydro-{5a,10a-diaza-3a,8a-azonia}-pyren-3a-ylmethyl)-benzyl]-8a- methyl-cis-decahydro-{5a,10a-diaza-3a,8a-azonia}-pyrene.** As previously mentioned the linking reaction with 1,4-Bis-bromomethyl-benzene already alkylated one nitrogen on each macrocycle. The second, non-adjacent nitrogen on each macrocycle was alkylated in this reaction with Iodomethane.

Analysis of the product: ^{13}C NMR (500 MHz, D_2O) showed peaks (ppm): 134.186, 127.970, 76.992, 76.685, 65.130, 61.179, 60.557, 51.082, 50.874, 49.410, 48.065, 46.374, 46.314, 56.254, 18.231, 17.892. The ^{1}H NMR (500MHz, D_2O) data was complex, but consistent with molecule. The calculated elemental analysis for $C_{34}H_{58}N_8I_4$ is C, 37.59%; H, 5.38%; N, 10.31%. The actual analysis found these percentages to be C, 35.98%; H, 5.30%; N, 9.14%. ES+ mass spectrometry in 90% MeOH exhibited peaks at m/z = L^+ (959) and m/z = L^{++} (416), which is consistent with the calculated mass of this molecule.

Again the elemental analysis did not return satisfactory percentages. However, the mass spec showed yet again correct m/z values. It was decided to continue on, as before, with the synthesis.

(Synthesis 4) **4-methyl-11-[4-(4-methyl-1, 4, 8, 11-tetraaza-bicyclo[6.6.2]hexadec-11-ylmethyl)-benzyl]-1,4,8,11-tetraaza-bicyclo[6.6.2] hexadecane.** The two alkylated nitrogens now have four bonds to them, which makes them highly active. The only remaining step for cross-bridged cyclam was a simple sodium borohydride reduction at the non-adjacent ammines. (Weisman et al. 1990) (Fig 4a) The product being linked cross-bridged cyclam ligands. During the extraction the white product was hard to confine to one layer; continued anyway. (Hubin T.J. 2002)

Analysis of the product: ^{13}C NMR (Fig. 6) showed peaks (ppm): 128.882, 128.831, 128.521, 77.483, 77.433, 77.229, 76.975, 59.939, 59.452, 58.137, 58.106, 57.191, 56.921, 56.897, 56.788, 56.745, 56.442, 56.196, 55.101, 54.303, 54.273, 52.471, 52.238, 43.191, 28.039, 28.003, 27.114, 26.537, 1.217. The ^{1}H NMR (500MHz, D_2O) data was complex but consistent with the molecule. FAB+ mass spectrometry in MeOH exhibited peaks at m/z = L^+ (583), which is consistent with the calculated mass of this molecule. The infrared spectrum (KBr) showed

peaks (cm^{-1}): 3420.58, 2960.29, 2606.15, 1629.72, 1458.14,
1384.31,1083.10,1050.90,733.62,560.61.

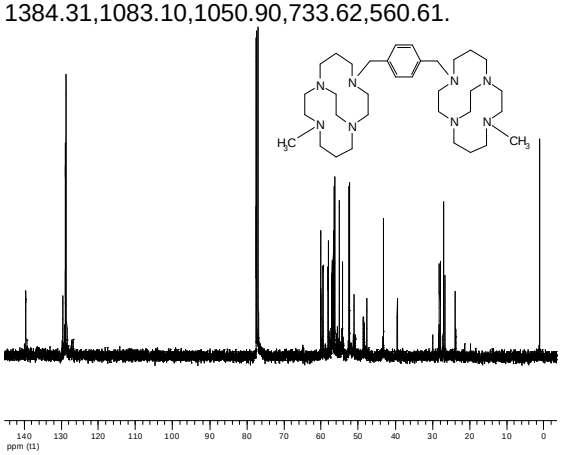

Figure 6 ^{13}C NMR (500 MHz, $CDCL_3$)

Synthesis of Metal Complexes

(Synthesis 5) **Dichloro(4-methyl-11-[4-(4-methyl-1, 4, 8, 11-tetraaza-bicyclo[6.6.2]hexadec-11-ylmethyl)-benzyl]-1,4,8,11-tetraaza-bicyclo[6.6.2] hexadecane)copper (II) hexafluorophosphate.** Upon complexation of Cu^{2+}, 5 was difficult to dry and upon contact with air showed signs of being hygroscopic. In an attempt to rid 5 of it's hygroscopic properties a metathesis reaction was done, exchanging Cl^- ions for PF_6^- ions. Upon addition of NH_4PF_6 to 5 a whitish precipitate formed immediately. When dried, 5 showed a light blue color. Also 5 did not show signs of being hygroscopic.

Complexation of the Zn^{2+} complex was attempted under similar conditions described in the materials and methods section for 5, but no complex was formed.

Analysis of the product: The calculated elemental analysis for $Cu_2C_{34}H_{62}N_8Cl_2P_2F_{12}\bullet 3H_2O$ is C, 36.30%; H, 5.55%; N, 9.96%. The actual analysis found these percentages to be C, 35.93%; H, 5.57%; N, 9.98%. The infrared spectrum (Fig. 7) (KBr) showed peaks (cm^{-1}): 3435.35, 2924.09, 1637.50, 1458.91, 1384.36, 1088.42, 1039.97, 840.22, 558.25.

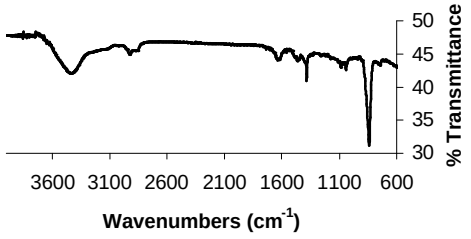

Figure 7 I.R. of complex 5

FAB+ mass spectrometry (Fig. 8) in MeOH exhibited peaks at m/z = $[Cu_2L\ Cl_2\ ][\ PF_6\ ]^+$ (926), which is consistent with the calculated mass of this molecule.

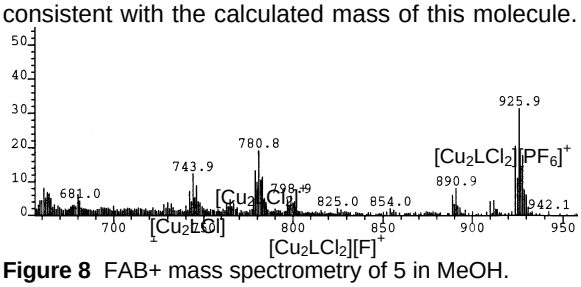

Figure 8 FAB+ mass spectrometry of 5 in MeOH.

Electronic Structure Characterization of $[ClCu(4)CuCl][PF_6]_2$

The U.V.-Vis spectrum (0.1 mmol in CH_3CN) exhibited a λ_{MAX} = 294 nm (ε = 14,100 $M^{-1}cm^{-1}$) and λ_{MAX} = 673 nm (ε = 340 $M^{-1}cm^{-1}$). Figures 9 & 10, respectively. A 5-coordinate Cu^{2+} with N_4Cl donor atoms exhibits a λ_{MAX} = 617 nm (ε = 209 $M^{-1}cm^{-1}$). (Musker W.K. 1967) λ_{MAX} = 294 nm is a ligand to metal charge transfer band, thus reducing the metal. λ_{MAX} = 673 nm is a d-d forbidden transition.

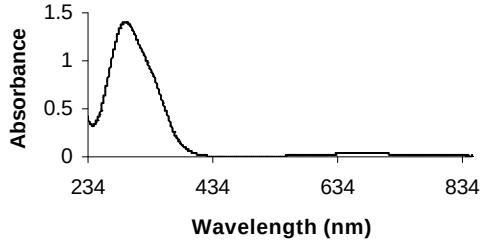

Figure 9 U.V.-Vis of 5 at 0.1 mmol, λ_{MAX} = 294 nm.

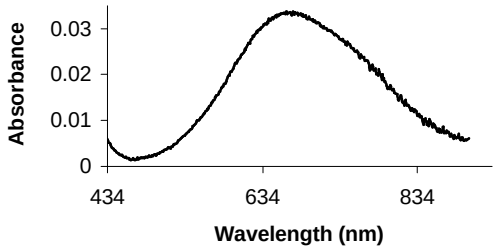

Figure 10 U.V.-Vis of 5 at 0.1 mmol, λ_{MAX} = 673 nm.

Magnetic Moment Characterization of $[ClCu(4)CuCl][PF_6]_2$

A typical Cu^{2+} d^9 with one unpaired electron shows a μ_{eff} = 2.0-2.3. (Carlin R.L. 1986) The dicopper complex, 5, showed a μ_{eff} = 3.37. (Fig. 11) This is slightly lower than predicted for 2 un-coupled Cu^{2+} ions, which would give twice the value for a μ_{eff} = 4.0-4.6. However, similar mono-copper complexes of cross-bridged tetraazamacrocycles have exhibited values as low as μ_{eff} = 1.85. (Carlin R.L. 1986) Twice this value would give a μ_{eff} = 3.70. It is

possible that the 2 Cu^{2+} ions in this complex are coupled in some manner accounting for the 3.37 value. Investigation of complex magnetic behavior is beyond the scope of current investigation.

Electrochemical Characterization of $[ClCu(4)CuCl][PF_6]_2$

An irreversible reduction from Cu^{2+} to Cu^{+} was observed with a visible return oxidation. Upon reduction the Cu^{+} is most likely losing the Cl^{-} ligand.

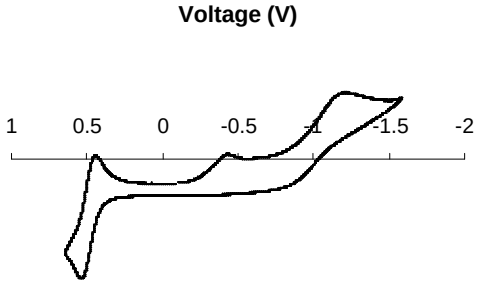

Figure 11 Complex 5 with ferrocene.

ACKNOWLEDGEMENTS

I would like to thank Dr. Hubin for his guidance, insight, and encouragement throughout the project. I would also like to thank Dr. van Asselt for his support and feedback as well.

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