Cantaurus, Vol. 13, 27-31, May 2005 © McPherson College Division of Science and Technology

Synthesis and Characterization of Cross-Bridged AMD3100 Analogs

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ABSTRACT

In 1988 AMD3100, a xylyl-bicyclam was discovered to exhibit anti-HIV activity by preventing the HIV virion from successfully interacting with the CXCR4 coreceptor and infecting the cell. AMD3100 was thus a potentially new fusion inhibitor drug and is currently undergoing clinical trials. Further research performed on AMD3100 analogs have revealed that if it is complexed with certain metals it will increase the bonding affinity to CXCR4 by causing the cyclam rings to take on a folded *cis* configuration. According to these results, an AMD3100 analog was synthesized with the addition of an ethyl cross-bridge that locked it into the *cis* folded configuration and then copper was bound to the ligand. In this complex, a metaxylyl linker was used instead of the paraxylyl linker present in AMD3100 to probe the importance of this bridging group. The copper bicyclam has been characterized and is currently being assayed for anti-HIV properties.

Keywords: *AMD3100, Anti-HIV drugs, Bi-cyclam, CXCR4, SDF-1, Xylyl-bicyclam, fusion inhibitor*

INTRODUCTION

Currently there is no cure available for AIDS, but there are a number of drugs available and currently under development to suppress the replication of the HIV virus. Most HIV treatments belong to one of four categories, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs) and fusion/uncoating inhibitors (De Clercq 2002). Fusion inhibitors specifically inhibit receptors and/or co-receptors on the cell membrane that HIV uses to attach and fuse itself to the cell and then release its RNA into the cell (De Clercq 2002). Inhibition of the HIV virus through viral entry inhibition was shown to be a legitimate focus for HIV therapy in experiments using mice (Datema et al. 1995).

The HIV virion contains glycoproteins gp120 and gp41 in its envelope that bind to the CD4 receptor on the cell membrane of the targeted immune cell (Labrosse et al. 1998). In order to enter a cell, the virus must again interact with the cell membrane, this time with co-receptors CCR5 or CXCR4, depending upon the strain (Labrosse et al. 1998). CXCR4 is a chemokine receptor belonging to the G protein class and has been found to be the the coreceptor in the T-lymphotropic (X4) HIV strain (Hatse et al. 2002). CXCR4 is only activated by one chemokine ligand and is also the only natural receptor of stromal cell-derived factor-1(SDF-1) (De Clercq 2003 and Rosenkilde et al. 2003).

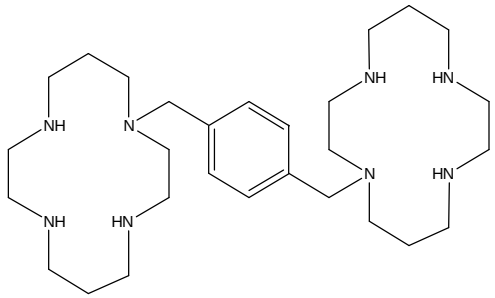

Figure 1 AMD3100

In 1988, a bicyclam compound, AMD3100, (Fig. 1) was found to exhibit anti-HIV activity through the inhibition of the CXCR4 coreceptor (De Clercq 2003). AMD3100 is composed of two cyclams (1,4,8,11-tetraazacyclotetradecane) connected by a paraxylyl linker. Bicyclams are potent and selective in blocking the entry of HIV-1 and HIV-2 into the cell after they have fused to the target cell membrane (De Clercq et al. 1994, Hatse et al 2002, and Hatse et al. 2000). Compelling evidence suggests that the aspartic acid residues Asp171 and Asp262 play a key role in the interaction between CXCR4 and AMD3100 (Hatse et al 2000). AMD3100 was sent to clinical trials but its trial was discontinued when results were unsatisfactory in phase II (Vartanian 2000). Recent developments charge that the unimpressive results from AMD3100 during clinical trials were due to unsuitable treatment methods (Archibald 2004).

Metal ion complexes of AMD3100 have shown a greater anti-HIV activity than AMD3100. The Zn(II) bicyclam complex in particular shows to have an increased bonding affinity to the CXCR4 coreceptor (Gerlach et al.). Research suggests that when Zn(II) –Xyl-bicyclam binds with acetate, it undergoes a configuration change and becomes *cis* folded (Gerlach et al. 2002 and Liang et al. 2002). The effectiveness of metal complexes of AMD3100 was tested using various metals. The level of anti-HIV activity expressed by these metal complexes were Zn>Ni>Cu>Co>Pd in decreasing order (Este et al.

1998). It was found that when the bicyclam binds to the CXCR4 coreceptor, it adopts a folded, cis conformation (Liang et al. 2004). In order to lock the molecule into the cis configuration, an ethyl cross-bridge can be used, connecting non-adjacent nitrogens (Hubin et al. 1999).

MATERIALS AND METHODS

All reagents and solvents were purchased from Aldrich Chemical Company and used as received.

Note: previous students synthesized cyclam using the Barefield method and tetracycle bisaminal using the Weisman method (Barefield et al. 1976 & Weisman et al. 1990) and synthesis of products **1**,**2**, and **3** were performed by Joe Blas.

3a-[3-(cis-Decahydro-{5a,8a,10a-diaza-3a-azonia}-pyren-3a-ylmethyl)-benzyl]-cis-decahydro-{5a,8a,10a-diaza-3a-azonia}-pyrene. (Synthesis 2) 10g (0.045 mols) of the tetracycle bisaminal and 5.939g (0.022 mols) of dibromo-m-xylene were added into a round-bottom flask. 200mL of CH_3CN was added to the solution under nitrogen gas (Figure 2) and stirred at room temperature for seven days. The product, a white precipitate, was then filtered, rinsed with a small amount of CH_3CN, and thoroughly washed with diethyl ether. A second crop was obtained by ether addition to the filtrate. The product was then dried under vacuum. The reaction produced 13.944g (0.020 mols) of product giving 87.7% yield.

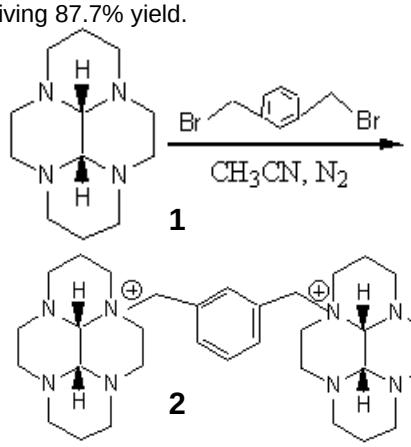

Figure 2. Glyoxal addition and linking reaction.

3a-[3-(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. (Synthesis 3) 13.85g (0.020 mols) of **2**, 20mL of CH_3I and 300mL of CH_3CN were added to a 1L round-bottom flask. The mixture was stirred under nitrogen gas for 3 weeks. The filtered product was washed with a small amount of CH_3CN and also a small amount of diethyl ether. The product was put under vacuum in a desiccator to dry. The product yield of **3** was 76%.

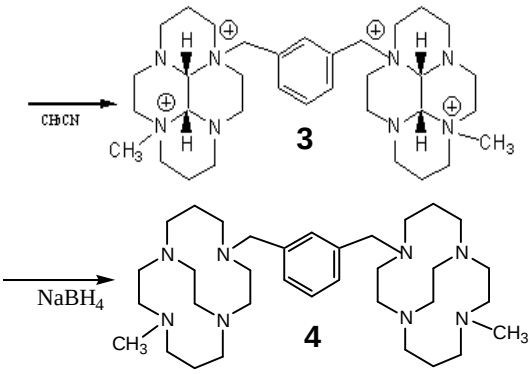

Figure 3. Methylation and reduction.

4-methyl-11-[3-(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. (Synthesis 4) 8.1234g (0.007 mols) of **3** and 720mL 95%ETOH were added together in a 1L flask. It was stirred under nitrogen gas for 5 minutes after which 16.3323g (0.432 mols) of $NaBH_4$ was added. The mixture was stirred at room temperature under nitrogen gas for seven days. 90mL of 6M HCL was added slowly until the pH was 1-2 and the solvent was evaporated under reduced pressure. ~120mL of 30% KOH was slowly added until the solution had a pH of 12-14 and the product was extracted in benzene (4×225mL). The solution was dried over Na_2SO_4 for seven days. The solution was then filtered and the solvent evaporated which resulted in yellow-colored oil with some white precipitate. The product yield was 74% (6.04g, 0.010 mols). The product **4** was found to be very soluble in CH_2Cl_2 and only slightly soluble in methanol and CH_3CN.

Product **4** was purified again by slowly pippeting ~10mL 6M HCl, evaporating the solvent and put under vacuum using liquid nitrogen for a week. 75-100mL of ether was added to product **4** and stirred for seven days at room temperature. The resulting solution and precipitate was filtered and placed under vacuum in a dessicator.

Dichloro(4-methyl-11-[3-(4-methyl)-1, 4, 8, 11-tetraaza-biyclo[6.6.2]hexad-11-ylmethyl)-benzyl] 1,4,8,11-tetraaza-bicyclo[6.6.2]hexadecane) copper (II) chloride. (Synthesis 5) 0.3057g (0.0005 mols) of the product **4** was combined with ~15mL MeOH. 0.1710g (0.0013 mols) of $CuCl_2$ was dissolved in ~15mL of MeOH. The two two solutions were combined in a 50mL flask and left stirring uncovered at room temperature for seven days. The blue solution was filtered through celite and crystallization by evaporation and diffusion of ether was attempted. Both attempts failed. The fractions were recombined and evaporated.

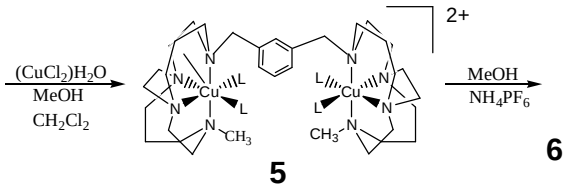

Figure 4. Metal complexation with copper(II).

Dichloro(4-methyl-11-[3-(4-methyl)-1, 4, 8, 11-tetraaza-biyclo[6.6.2]hexad-11-ylmethyl)-benzyl] 1,4,8,11-tetraaza-bicyclo[6.6.2]hexadecane) copper (II) hexafluorophosphate. (Synthesis 6) 10mL of CH_3OH was added to the product **5**. It was combined with 0.4136g (0.0025 mols) of NH_4PF_6 in 10mL of CH_3OH resulting in a pale blue precipitate which was filtered and dried under vacuum in a dessicator. The yield was 76% (0.399g) for product **6**.

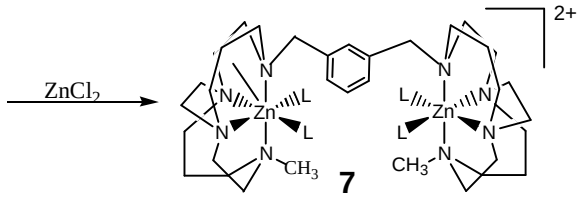

Figure 5. Metal complexation with zinc(II).

Dichloro(4-methyl-11-[3-(4-methyl)-1, 4, 8, 11-tetraaza-biyclo[6.6.2]hexad-11-ylmethyl)-benzyl] 1,4,8,11-tetraaza-bicyclo[6.6.2]hexadecane) zinc (II) chloride. (Synthesis 6) 0.4737g (0.0008 mols) of the purified product **4** was added to 15mL of H_2O. ~15mL of 30% KOH was slowly added until the solution was strongly basic, a pH of 12-14. The product was extracted using benzene (5×15mL). and dried using a copious amount of Na_2SO_4 and stirred at room temperature for four days. The drying agent was removed and the solvent was evaporated. 0.1445g (0.001) $ZnCl_2$ and 40mL DMF was added to the solution which was then stirred at reflux for ~3 hours. The solution was filtered and the filtrate dried under vacuum. Yield was 27% (0.186g, 0.0002 mols) for product **7**.

RESULTS AND DISCUSSION

Ligand Synthesis

(**Synthesis 1) *cis*-Decahydro-3a,5a,8a,10a-tetraazapyrene.** The glyoxal condensate was synthesized by previous students using a well documented condensation reaction (Weisman et al. 1990 and Blas, 2004). Due to the documented success of this reaction, analytical testing was not performed.

(Synthesis 2) This step results in the bis-alkylation at the non-adjacent nitrogens on the macrocycle. The reaction causes two macrocyles to be joined together by the aromatic linker, 1,3-Bis-bromomethyl-benzene. This product formed a white precipitate in the solution. The calculated elemental analysis for $C_{32}H_{52}N_8Br_2{\cdot}HBr$ is C, 48.68%; H, 6.77%; N, 14.19%. The actual analysis found C, 50.36%; H, 7.04%; N, 13.51%. Although not analytically pure, the product was deemed pure enough to proceed. The FAB+ mass spectrum showed two peaks at 547.5 m/z and 629.4 m/z corresponding to **2** losing two bromine ions and one bromine ion respectively.

(Synthesis 3) Methyl groups are added onto the non-adjacent nitrogens of each macrocycle that were not involved in the linking alkylation. This results in a positive charge present at the non-adjacent nitrogens. The calculated elemental analysis for $C_{34}H_{58}N_8I_4$ ·1.33H_20 is C, 36.77%; H, 5.51%; N, 10.09%. The actual analysis found C, 37.17%; H, 5.34%; N, 9.68% for the tetracation. The FAB+ mass spectrum exhibited a peak at 577.4 m/z corresponding to the actual mass of 578g/mol. These results were acceptable and the synthesis proceeded.

(Synthesis 4) In order to neutralize the positively charged non-adjacent nitrogens, $NaBH_4$ was used in a reduction reaction which created the desired cross-bridged macrocycle (Weisman et al. 1990). The calculated elemental analysis for the HCl salt $C_{34}H_{66}N_8Cl_4$ ·8HCl·4H_2O is C, 43.13%; H, 8.30%; N, 11.84%. The actual analysis found C, 43.68%; H, 8.28%; N, 11.45%. A FAB^+ mass spec was then obtained before the ligand was purified as a salt. There was a large peak at 583.5 m/z corresponding to the mass of the compound which is 582.920g/mol (see Figure 6).

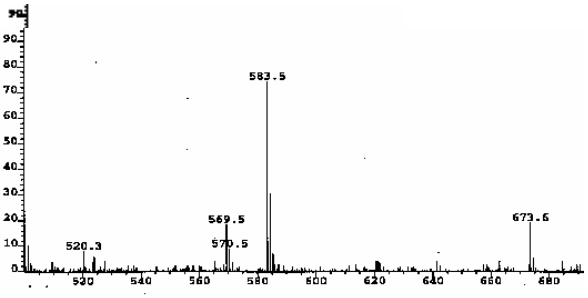

Figure 6 FAB+ Mass Spec of **4**

Synthesis of Copper Complex

(Synthesis 5) This compound turned a dark blue mixed with some brown. Basic attempts were made to crystallize the compound, all of which failed. The metal complex was also very hygroscopic and thus was further reacted with NH_4PF_6 in an anion metathesis reaction in an attempt to produce a c more easily purified compound.

(Synthesis 6) Upon being dried under vacuum, the compound exhibited a medium teal color and it

did not exhibit hygroscopic properties. The calculated elemental analysis for $Cu_2C_{34}H_{62}N_8Cl_2P_2F_{12}\cdot H_2O$ is C, 37.50%; H, 5.92%; N 10.29% which are within 0.4% of the analysis found which was C, 37.59%; H, 5.62%; N 10.01% The FAB+ mass spec exhibited a large peak at 925.2 m/z which corresponds to the calculated mass. The IR shows peak at 1070cm^{-1} confirming the presence of PF_6.

Electronic Structure of the Copper Complex

The U.V.-Vis results can be seen at different concentrations in Figures 7 and 8. At a concentration of 5×10^{-4} M in CH_3CN, the sample exhibited a $?_{MAX}$ = 674nm (e = 2170 M^{-1}cm^{-1}). This is consistent with a forbidden d-d transition and also corresponds with the elemental analysis which shows that one chloro ligand is bound and there is likely a 5-coordinate Cu(II) center (Musker et al. 1987). Another peak was defined at a lower concentration of 5×10^{-5} M in CH_3CN. This U.V.-Vis showed a $?_{MAX}$ = 290nm (e = 10600 M^{-1}cm^{-1}). This may be due to a ligand to metal charge transfer bond from the chloro ligand to the Cu(II) center. The large e extinction coefficient is consistent with this conclusion because the proposed transition is one allowed by the selection rules.

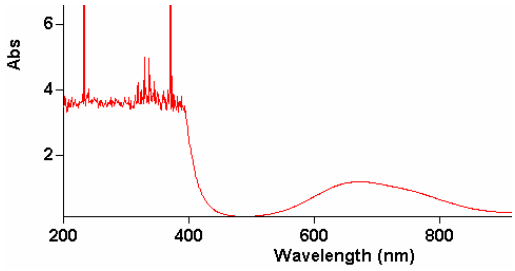

Figure 7 U.V.-Vis of **6** at 5×10^{-4} M in CH_3CN

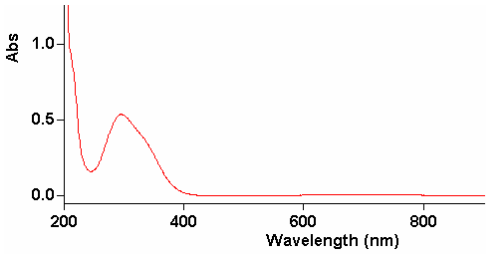

Figure 8 U.V.-Vis of **6** at 5×10^{-5} M in CH_3CN

The first time the magnetic moment characterization was performed, the results were extremely low, possibly due to the hygroscopic property of **6**. The copper complex was then sufficiently dried under vacuum and the test was performed again. This time the results yielded μ_{ef} = 2.73. Typical Cu^{2+} μ_{ef} is 1.70 – 2.20 and for two uncoupled copper ions the value is 3.40 – 4.40. The experimental value of 2.73 is considerably lower than this (Huheey et al. 1993, and Carlin et al. 1976). One possible explanation is antiferromagnetic coupling of the two Cu^{2+} ions, possibly through a bridging chloro ligand. EPR spectroscopy would test this hypothesis and is currently underway.

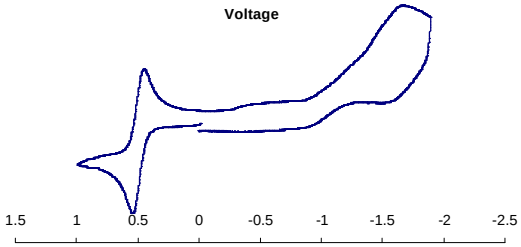

Figure 9 Electrochemical analysis of **6** and ferrocene.

Electrochemical characterization of **[ClCu(6)CuCl][PF$_6$]$_2$·4H$_2$O** shows one irreversible reduction which is probably the reduction of one Cu(II) center to Cu(I), followed by the loss of a chloro ligand. The related paraxylyl complex showed two irreversible reductions (Ullom, 2004). The difference may be due to bridging of the two copper ions which is more likely in the metaxylyl case.

Zinc Complex Synthesis

(Sythesis 7) 7 was synthesized under conditions that were successful according to the Cu compound synthesis. The compound was a very hygroscopic light yellow powder, however once it was completely dried under N_2, it lost its hygroscopic property. **7** was sent off for a mass spec and an elemental analysis. The mass spec did not contain any peaks in the expected range, suggesting that the synthesis had failed. This was confirmed by the elemental analysis which was considerably unlike the expected analysis.

In conclusion, a novel cross-bridged bicyclam was synthesized and characterized. Copper metals were then incorporated into the ligand resulting in a novel copper complex that was characterized. Anti-HIV testing of these compounds is anticipated in the near future.

ACKNOWLEDGEMENTS

I would like to thank Dr. Tim Hubin for the opportunity to work on this research and for all of his support, guidance and help. I also thank Joe Blas for the synthesis of bicyclam.

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