Development in Potential Anti-HIV & Antimetastatic Drugs: C_3-Symmetric Tris-Linked Bridged Tetraazamacrocycles as Potential CXCR4 Antagonists

1. Societal Impact:

CXCR4 chemokine receptors are found on the surface of immune, and other, cells, and together with the specific natural ligand, CXCL12,have been revealed to play a role in a number of disease states. CXCR4 expression has also been reported in at least 23 different cancers. Target organs for breast metastases such as liver, lung, and bone have high levels of CXCL12. Due to the wide-ranging potential biomedical applications that might result, our aim is to develop new antagonists for the CXCR4 co-receptor.

2. Objectives:

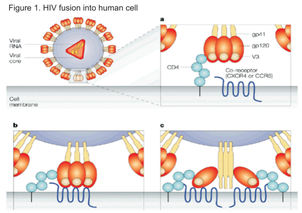
Our objectives were to synthesize C3-symmetric tris-linked analogues of our most effective bis-tetraazamacrocycle metal complexes and to characterize their chemical and physical properties in preparation for determining if the added macrocycle enhances their antagonism of CXCR4.

3.Methods:

Synthetic routes extending our bis-linked ligand syntheses to use the C 3-symmetric linker 1,3,5-tris(bromomethyl)benzene were developed. Copper(II), nickel(II), cobalt(II), and zinc(II) complexes were made using our previous methods. Electrospray mass spectra, UV-Visible spectra, cyclic voltammograms, magnetic moments, X-Ray crystal structures, and 1H and 13C NMR spectra were collected to characterize the complexes.

RECEPTOR-HIV ACTIVITY

Figure 1. HIV fusion into human cell



Nature Reviews Drug Discovery 2003, 2, 581-587.

ANTI-CANCER ACTIVITY Invasion assays

- Cell invasion assays in response to chemokine gradient.
- Initially used SJSA cells.
- Experiments run in presence and absence of antagonist.

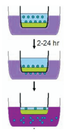
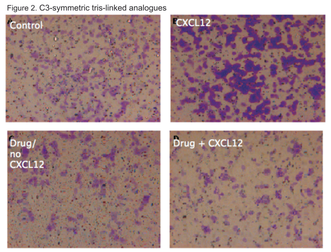
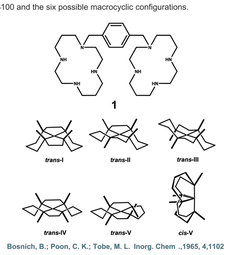


Figure 2. C3-symmetric tris-linked analogues



19

Figure 3. AMD3100 and the six possible macrocyclic configurations.



Bosnich, B.; Poon, C. K.; Tobe, M. L. Inorg. Chem .,1965, 4,1102

Restrict to one configuration

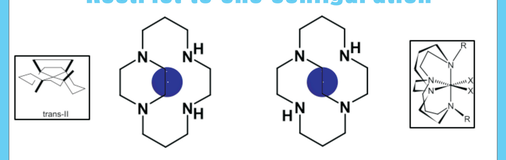
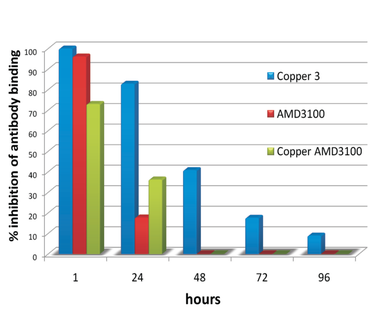


Figure 4. Restriction to one configuration



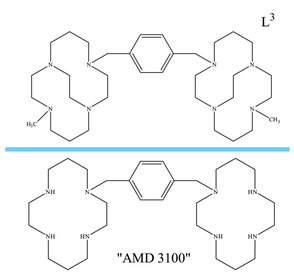
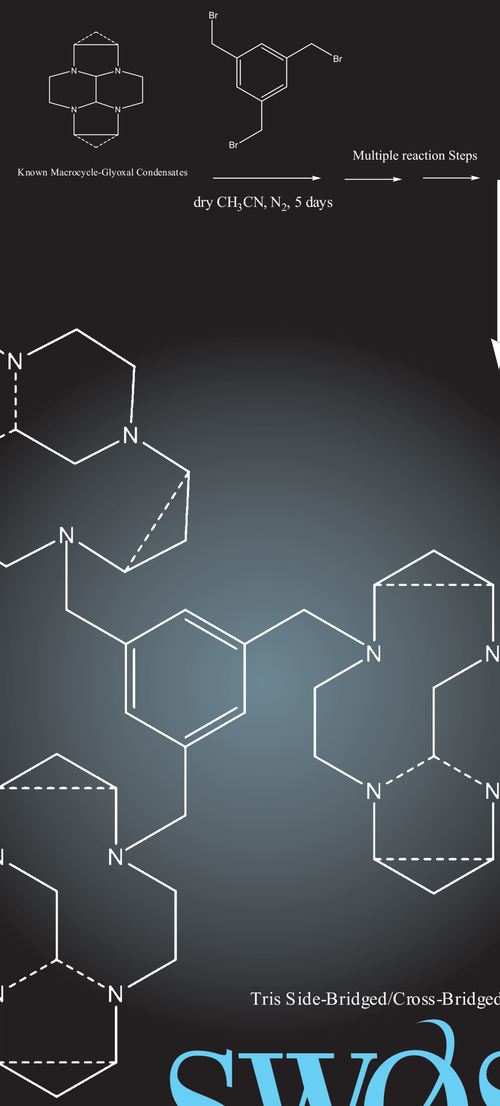


Figure 5. The inhibition of anti-CXCR4 antibody binding over time after exposure to 32nM of the drug. A population of 100,000 cells was isolated for each data point and analyzed by flow cell cytometry using a secondary fluorescein tagged IgG antibody (negative values are not shown).

Synthesis of C_3-symmetric tris-linked analogues



Characterization using NMR

Figure 6. NMR Spectrometer

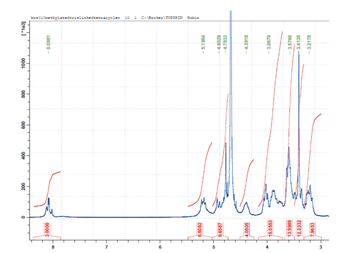


Figure 7.Tris Methylated Tetracyclen Proton NMR Spectra

11

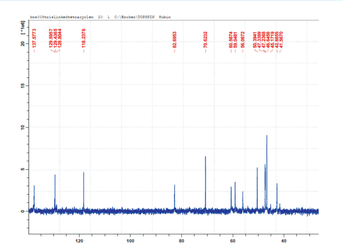
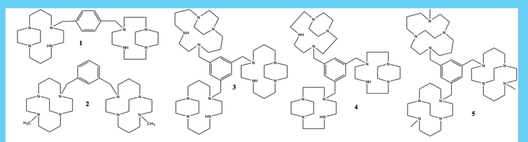


Figure 8. Tris Tetracyclen Carbon NMR Specta

10



IC_{50} (μg/ml) values calculated from Ca-signaling experiments		
	U87.CD4.CXCR4	U87.CD4.CCR5
Zn_2-**1**	0.05	1.79
Ni_2-**2**	0.22	10.3
Zn_3-**3**	0.07	6.7
Co_3-**3**	3.93	15.74
Co_3-**4**	5.12	15.89
Cu_3-**5**	0.35	14.84
Zn_3-**5**	0.44	17.78
AMD3100	0.011	---
maraviroc	---	0.00209
AMD3451	>1	>1

Figure 9. Binding Experiment CXCR4 & CCR5

4. Results:

The ligand syntheses of the side-bridged and cross-bridged C3-symmetric ligands proceeded similarly to the previously developed bis-ligand routes. Complexation with the desired metal ions proceeded as expected. Characterization of the metal complexes resulted in publishable quality of purity in each step of synthesis. Experiments investigating the Calcium release have shown that the C3-symmetric compounds are highly potent as CXCR4 antagonists, just as in the bis-linked compounds.

5. Conclusions:

C3-symmetric tris-linked bridged tetraazamacrocycles are easily produced, using an appropriate linker and following synthetic methods adapted from the bis-linked analogues. Metal ion complexation proceeds smoothly following known procedures. Calcium ion release is observed when the natural ligand for CXCR4, CXCL12, binds. Preventing Calcium release is evidence of strong antagonism by the potential drug molecule. Also, several of the C3-symmetric compounds have demonstrated excellent antagonism of a related chemokine receptor, CCR5, as well. This exciting result may lead to a new class of dual chemokine receptor antagonists.

6. Future plans:

Experimental data on the specific disease states of HIV infection and cancer with the resulting complexes will inform our understanding of the requirements for producing even more efficient CXCR4 antagonists of this class.

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