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Modelisation, Synthesis and Anti-HIV activities of N,N,N',N'',N'''-pentakis-tetraazamacrocycles salts derivatives

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Abstract. The syntheses and the anti-HIV activities of new N,N,N',N'',N'''-pentakis-tetraazamacrocyles salts analogues are described. From computer modeling studies, it was found that zinc coordination capacity of these pentakis derivatives was larger than the one of the corresponding tetrakis analogues. This suggest that a metal chelation energy could be a criteria to design novel anti-HIV polyazamacrocyles. © 1997 Elsevier Science Ltd.

Introduction. Bicyclams[1,2,3] (class I compounds Fig. 1) have been reported to be potent inhibitors of HIV-1 and HIV-2 replication with a high selectivity. It has also been suggested that these compounds targeted a virus uncoating-associated process and that their anti-HIV activities were related to their specific zinc coordination capacity.[4] We have recently reported[5] the anti-HIV properties of a new class of compounds, the N,N',N'',N'''-tetrakis-tetraazamacrocycles (class II compounds Fig. 1). In order to establish comparison between a structure and anti-HIV activity, each class of representative compounds was studied by computer molecular modeling.

Computer molecular modeling studies. N,N',N'',N'''-tetrakis-1,4,8,11-tetraazamacrocycles derivatives adopt a "jelly-fish like" geometry in which the plane P_1 containing four endocyclic nitrogen atoms forms an angle α with the plane P_2 constituted by the four terminal nitrogen atoms of the four amino side arms.[5] Using GenMol molecular modeling program,[6,7] the geometry of the new N,N,N',N'',N'''-pentakis-1,4,8,11-tetraazamacrocycles derivatives corresponding to minimal transition state energy has been determined (class III compounds Fig. 1). From this specific geometry, several structural descriptors can be deduced : **i-** constraint energy of the molecule related to its ability to catch cations (Zn^{++}); **ii-** the distance d between the (two) possible binding centers (metal binding centers being defined as the center of the square formed by four nitrogen atoms).

It has been postulated that the anti-HIV activity exhibited by bicyclams could be related to the fact that the binding to the molecular target at the inhibitory step was transition metal-

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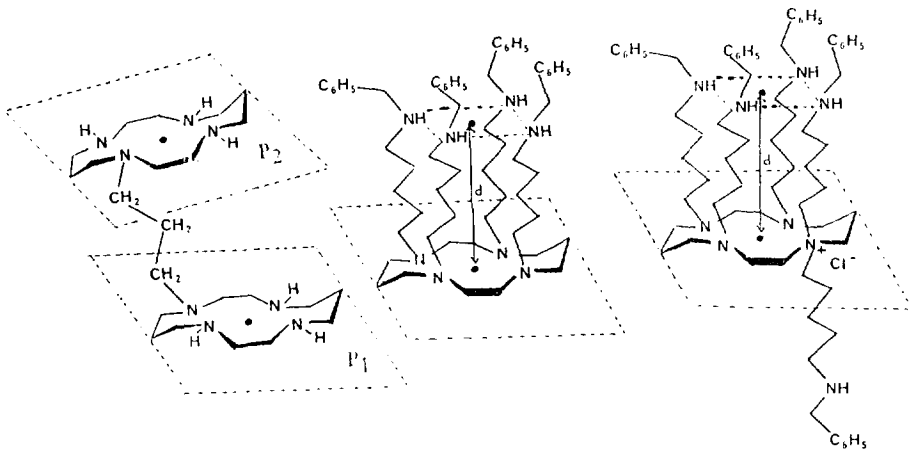
mediated.[4,8] Therefore, it was of interest to compare the values of the above mentioned structural parameters for each class of compounds.



Class I **Class II** **Class III**

Figure 1: Molecular geometry of polyazamacrocycles deduced from GenMol computer modeling software. Class I : bicyclam JM 2763 ; Class II: N,N',N'',N'''-tetrakis-1,4,8,11-tetraazacyclotetradecane derivatives; Class III : N,N,N',N'',N'''-pentakis-1,4,8,11-tetraazacyclotetradecane chloride salt analogues.

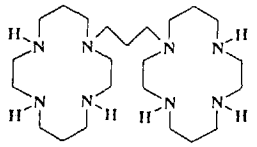
- zinc binding centers ; d : distance between the binding centers

The constraint energy (MCE) is the energy required to give a favorable geometry to the analogues with coordination to two zinc cations (see Table 1). The distances between the two binding centers are also presented in Table 1.

Chemistry. The synthesis of class I bicyclam was performed according to already published procedure.[1] N,N',N'',N'''-Tetrakis-1,4,8,11-tetraazamacrocycles **2a**, **2b**, **3a** and **3b** have been described in a submitted paper.[5] N,N,N',N'',N'''-Pentakis-tetraazamacrocycles derivatives **4a**, **4b**, **5a** and **5b** have been synthesized as follow. Heating of a mixture of tetra-N-alkylated azamacrocycle (**2a**, **2b**) with an excess of 5-aminopentyl tosylate in DMF led to the formation of the corresponding quaternary salts **4a** and **4b** in good yields (respectively 74% and 61%). The structures of **4a** and **4b** were assigned on the basis of mass spectroscopy, NMR and centesimal analysis. Two structures can be assigned to the compound **4b** : N_1,N_1,N_4,N_8,N_{12}-pentaalkylated tosylate salt or N_1,N_4,N_8,N_8,N_{12}-pentaalkylated tosylate salt. A standard deprotection led to the corresponding salts **5a** and **5b**. The compounds were characterized by ^1H NMR, ^{13}C NMR and FAB mass spectroscopy. Elemental microanalysis gave combustion

Table 1

Energetical and geometrical descriptors data and anti-HIV activities of tetrakis and pentakis tetraazamacrocycles derivatives.

Class of compounds according to fig.1	Compound	MCE[a] Kcal Mol^{-1}	d[b] Å	EC_{50}[c]	CC_{50}[d]	SI[e]
Class I		- 145	6.01	1 ± 5	>100	>100
Class II	2a	- 53	6.4	1 ± 0.5	>10	> 10
	2b	- 13.5	7.7	10 ± 5	>50	> 5
	3a	- 49.6	6.4	1 ± 0.5	10	10
	3b	- 26.5	6.7	ND*	1	ND
Class III	4a	- 56.2	7	10 ± 5	50	10
	4b	- 9.2	7.7	inactive	10	ND
	5a	- 63.2	7.6	0.1	5	50
	5b	- 33.3	7.8	inactive	10	ND

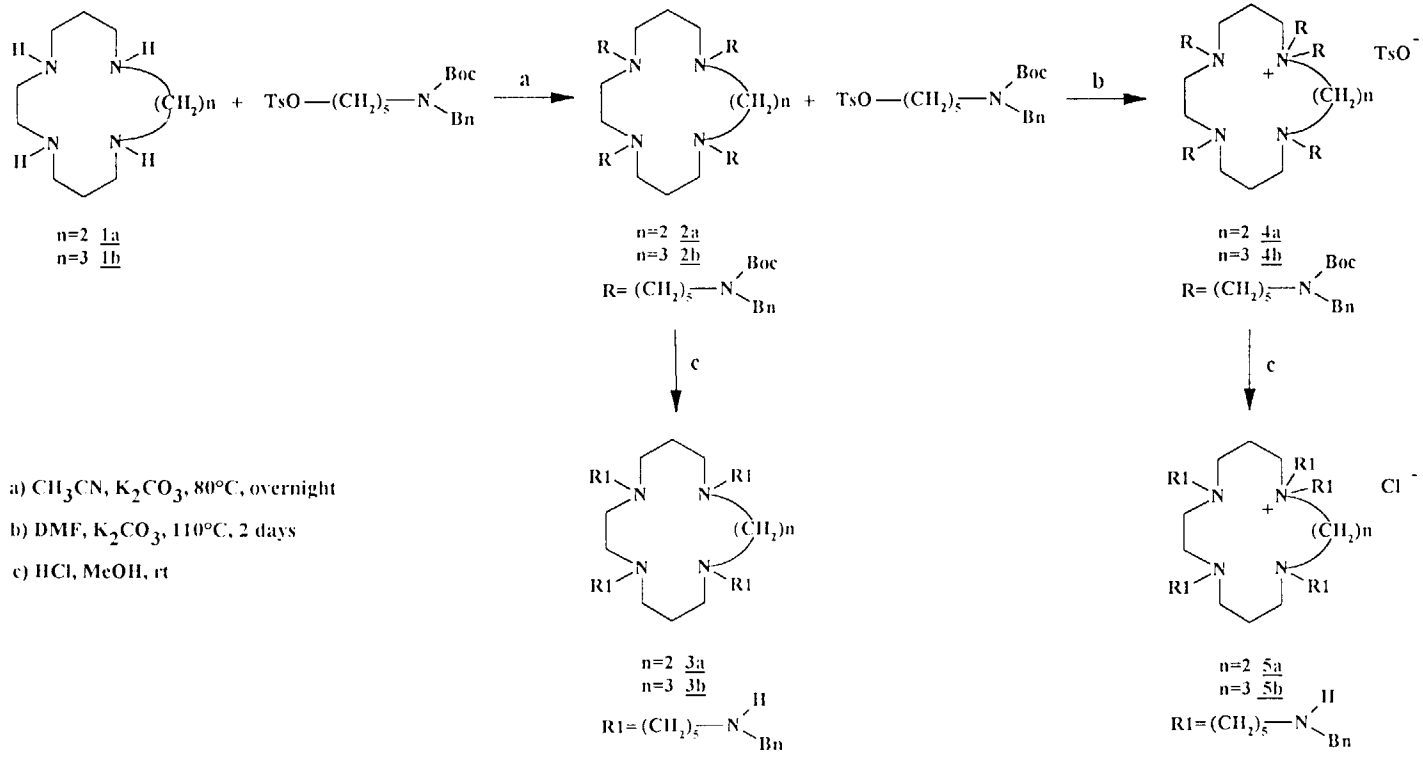
a) MCE: Molecular Constraint Energy
b) Distance between metal binding centers
c) EC_{50} values (50% antiviral effective concentration) are only determined at non cytotoxic concentrations on the basis of five compound concentrations. Data are means of four replicate samples. ND means not determined.
d) CC_{50} values (50% cytotoxic concentration). The greater than symbol (>) is used to indicate the highest concentrations at which the compounds were tested and still found to be non toxic.
e) SI (Selective Index) corresponds to the ratio CC_{50}/EC_{50}.
* Toxic at the active dose.

values for C, H, N within 0.4% of the theoretical values. The overall synthesis is summarized on Scheme 1.

Antiviral activity and discussion. Compounds **2a**, **2b**, **3a**, **3b**, **4a**, **4b**, **5a** and **5b** were tested for their inhibitory effects upon HIV-1 replication in MT-4 cells according to known procedures.[9,10,11] Additional cellular toxicities caused by these compounds were also determined for all assayed cell cultures. The results of antiviral activity and cytotoxicity assays are shown in Table 1. From these results, it can be observed that : **i-** compounds **3a**, **3b**, **5a**, **5b** displayed potent inhibitory effects on HIV-1. **ii-** analogues **3a**, **3b** and **5a** exhibited substantially increased cytotoxicity to MT-4 cells in comparison to the bicyclam which has been taken as a reference compound (Table 1). **iii-** furthermore, compounds **2a**, **2b**, **4a** and **4b** bearing *t*-Boc protecting groups on the N-terminal amino group exhibited substantially reduced activity against HIV-1 or were inactive. Taking into account the computer modeling results, the following observations may help in the setting up of correlations between anti-HIV activity and the defined descriptors used in modeling studies (i.e. constraint energy required for metal binding, and metal binding distance) : **i-** compounds that elicited the most potent activities (i.e. **2a**, **4a**, **5a**) are represented by a negative constraint energy for metal binding ranging from -49.6 to -63.2 kcal.mol^{-1}. **ii-** pentakis analogues **4a** and **5a** that constraint energy values are respectively -56.2 and -63.2 kcalmol^{-1} are more active than the corresponding tetrakis derivatives **2a** and **3a**. **iii-** compounds including in their structure terminal N-Boc group displayed low affinity for metal binding (higher constraint energy values) and therefore are less active, or inactive against HIV cytopathogenicity. **iiii-** As previously reported,[5] the size of the macrocycle should be 14 or 15 membered rings and the metal binding distance should range between 6.5 to 7.5 Å.

In summary, the synthesis and the evaluation of an anti HIV activity of a serie of new N,N,N',N'',N'''-pentakis-tetraazamacrocycles salts have been performed. These compounds appeared to be more active than the corresponding tetrakis analogues. From computer modeling study it has been calculated that the zinc coordination capacity of the pentakis derivatives was larger than the one of the corresponding tetrakis derivatives. We observed a striking structure-activity relationship between the zinc coordination capacity and anti-HIV potency. One of the reasons could be that N,N,N',N'',N'''-Pentakis-tetraazamacrocycles derivatives have a more adapted molecular shape for binding to the target. Further work is in progress to provide more evidence that transition metal chelation can be used as thermodynamical descriptor to design anti-HIV inhibitors.

Scheme 1



a) CH_3CN, K_2CO_3, 80°C, overnight

b) DMF, K_2CO_3, 110°C, 2 days

c) HCl, MeOH, rt

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