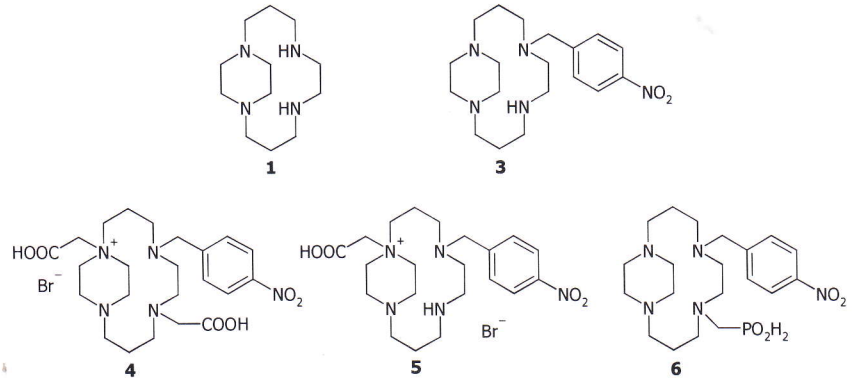
Two series of new bicyclic tetraazamacrocycles built up on skeletons of either 1,5,8,12-tetraazabicyclo[10.2.2]hexadecane (1,4-en-cyclam) **1** or 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (1,8-en-cyclam) **8** were prepared. The 1,4-en-cyclam-based unsymmetrical compounds bearing the *p*-nitrobenzyl moiety (precursor for a biomolecule-conjugation) were synthesised exploiting the low solubility of the intermediate quaternary derivative **2** in non-polar solvents. The ligands **3** and **6** exhibited the expected complexing ability towards copper(II) and zinc(II) although the complexation proceeded relatively slowly (at room temperature in order of hours). The metal complexes thus formed exhibited thermodynamic stability constants lower than those reported for similar monocyclic tetraazamacrocycles (cyclam, Me_4cyclam) probably due to inadvisability of the conformational change (chair or twisted-boat -> boat conformation of the piperazine subunit) connected to the metal ion encapsulation. On the other hand, when the stability of copper(II) complex of **3** is compared to stability constant reported for the bicyclic amine **1**, the trend in the values of these constants is the same for the couple ligand **1** – ligand **3** as for the couple cyclam – Me_4cyclam.

During the attempted synthesis of the monoacetate ligand **5a** an unusual reaction behaviour of **3** towards alkylation was observed. Reaction of the macrocyclic amine with alkyl bromoacetate agents lead (depending on the reaction solvent) to formation of either the quaternised monoacetate derivative **5** or quaternised diacetate derivative **4**. On the basis of molecular mechanics calculations, potentiometric titrations and multinuclear multidimensional NMR measurements (to establish the starting material **3** structure in solution) was this behaviour explained as a result of presence of a specific intramolecular hydrogen bond system forcing the first alkylation step on one of the piperazine ring nitrogen atoms (N1).



1 3 4 5 6

Fig. 5.1 – 1,4-en-cyclam and its derivatives mentioned in the text

The symmetrically substituted derivatives of 1,8-en-bridged cyclam were prepared either by use of common alkylating procedures (preparation of **10**) or by employing the Mannich-type reaction involving the phosphinic acid (H_3PO_2) as a substrate of this amino alkylation reaction (preparation of **13**) using the amine **8** as starting material.

The stepwise substitution on the 1,8-en-cyclam skeleton of **8** utilises the limited solubility of **9** hydrobromide in non-polar solvent (diethyl ether) which can be produced in excellent yield providing sufficient amounts of this starting material. Further substitution of **9** via alkylation using alkyl bromoacetate produces the appropriate acetate in form of its ester (**11a**) which is easily transformed to the acid (**11**). The Mannich-type reaction among **9**, phosphinic acid and paraformaldehyde produced the methylphosphinic derivative **12** in a low yield.

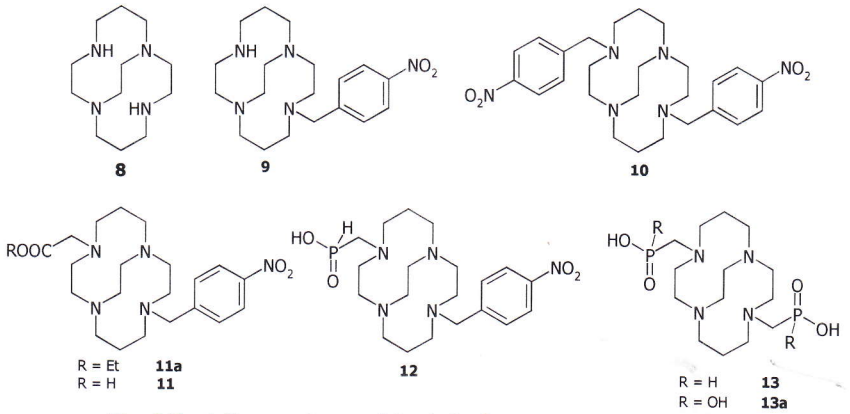


Fig. 5.2 – 1,8-en-cyclam and its derivatives mentioned in the text

All the prepared ligands can form coordination compounds with copper(II) but only few of them were isolated in the solid state, usually the complexes could be only isolated in form of intensively coloured glassy solids. The solid-state structures of copper(II) complexes with ligands **3**, **9** and **13a** have been determined using the single crystal X-ray structure determination method and the complexes exhibit the structural properties expected for this class of compounds.

Comparing the thermodynamic stability constants of copper(II) complexes of 1,4-en-cyclam-based ligands to the constants of similar complexes of 1,8-H_4te2p (Appendix I) results in a discovery that the new constrained macrocycles form copper(II) complexes with significantly lower thermodynamic stability constants than these cyclam-based ones. This observation can be explained involving energetics of the piperazine ring transformation during metal complex formation and the stabilisation effects of the methyl-phosphonate groups present in the H_4te2p ligand.

In addition, the kinetic inertness of Cu^{II} complexes with ligands **3** and **6** is not better than of the H_4te2p complex. The $[Cu(\mathbf{3})Br]^{2+}$ complex completely decomposed in acidic solution (1M $HClO_4$) during 24 hours at ambient temperature. Decomplexation of the ligand **6** from the appropriate Cu^{II} complex proceeded slower, after 10 d under the same conditions some complex (~20 %) was still present in the solution.

On the other hand, the 1,8-en-cyclam-based ligands represent a group of compounds with higher kinetic inertness expected. The ability and willingness of these ligands to form copper(II) complexes is probably strongly pH-dependent as no complex formation have been observed to pH ~5 at ambient temperature (the complex formation proceeds in a sealed ampoule at 80 °C in order of days) and a relatively good complexation proceeds at pH > 5 at slightly elevated temperature (50 °C, in order of hours) in the case of ligand **9**. A similar behaviour is supposed for ligands **11**, **12** and **13**.

All the presented results show that the ligands built either on the skeleton of 1,4-en-cyclam or 1,8-en cyclam are well suitable for copper(II) complexes formation. However, the ligands of the former groups, although they form copper(II) complexes very easily, seem not to be useful for the *in vivo* applications due to low stability of their copper(II) complexes. On the other hand, the ligands of the latter group form Cu^{II} complexes less readily and mostly under conditions not suitable for radioabelling of modified biomolecules but their kinetic inertness is supposed to be sufficient for the application into a living organism. For the purpose of the non-specific radiopharmaceuticals preparation is the 1,8-H_4te2p ligand of choice; for preparation of radiopharmaceuticals with more specific effect the bridged ligands could possibly represent a better alternative. Obviously, some more investigation, especially on copper(II) complexes formation / decomposition of the bridged macrocyclic ligands, is required to decide whether and with what limitations are the compounds of the invention useful for the radiomedicine.