SUMMARY

This thesis discusses the preparation of metabolically stable Ang IV analogues, selective for IRAP versus AP-N and the AT_1 receptor. The research is based on the modification of the main chain and the use of conformational constraints.

In the first part modifications in the main chain by a systematic replacement of all the amino acids of Ang IV, except His, by both β^2-and β^3-homo-amino acids are performed. We discovered interesting effects on the metabolic stability and on IRAP versus AP-N and AT_1 receptor selectivity. A combination of (*R*)-β^2hVal and β^3hPhe substitution allowed the development of **AL-11** ((*R*)-β^2hVal-Tyr-Ile-His-Pro-β^3hPhe), a metabolically stable Ang IV analogue with high affinity and selectivity for IRAP. It is therefore an ideal candidate for further studies to unravel the molecular action mechanism of Ang IV.

A second approach consisted of side chain modifications with conformational constraints of the aromatic amino acids in Ang IV. The replacements of Tyr^2 resulted in a large drop in IRAP inhibitory potency, indicating the indispensable role of this residue. The next focus was the replacement of the His^4-Pro^5 dipeptide sequence by residues that have a side chain constrained in the *gauche* (+), *trans* or *gauche* (-) conformation. The Aia-Gly dipeptide, containing a constrained Trp residue as a replacement for His^4-Pro^5 combined with a (*R*)-β^2hVal substitution in position 1 affords the new ligand H-(*R*)-β^2hVal-Tyr-Ile-Aia-Gly-Phe-OH (**AL-40**). This analogue displays improved potency, compared to **AL-11,** along with protection against metabolic degradation. Furthermore, we designed two ligands in which Pro was replaced by 2-aminocyclopentane carboxylic acid. These analogues had an affinity and IRAP versus AP-N selectivity similar to native Ang IV. They differ from Ang IV by binding only to the AT_4 and not to the AT_1 receptor. Finally, changes have been made in the Phe^6 residue. The replacement by *erythro*-β-MePhe resulted in an analogue **AL-27b** (H-Val-Tyr-Ile-His-Pro-*e*βMePhe-OH) that was very selective for IRAP versus AP-N and the AT_1 receptor, though it was not protected against metabolic degradation.

The two approaches described above were combined with attempts to reduce Ang IV to the shortest possible sequence. A potent and IRAP versus AP-N selective ligand H-(*R*)-β^2hVal-Tyr-Ile-Tic-OH (**AL-35**) has been discovered. This analogue is metabolically stable by incorporation of (*R*)-β^2hVal in position 1 and selective for the AT_4 receptor by a *C*-terminal modification. Additionally the deletion of the His-Pro residues from the peptide sequence renders this ligand less peptidic.

Another goal was to prepare a fluorescently labelled analogue. Since the *N*-terminus of Ang IV cannot be substituted, fluorescein has been attached to the *C*-terminus through a spacer and also on the side chain of Lys placed in position 1.

Furthermore, a tritiated ligand of one of the most interesting analogues (**[^{3}H]AL-11**) and of Ang IV (**[^{3}H]Ang IV**) was prepared by incorporation of a 3,4-dehydro-Pro precursor. The tritiation was performed in the lab of G. Toth (Biological Research Center, Szeged, Hungary). So far, radioligand binding studies have been performed with [^{125}I]Ang IV in the presence of metal chelators EDTA and 1,10-phenanthrolin to prevent metabolic degradation. These chelators also remove the zinc from the catalytic site of the IRAP. Therefore, these radioligand binding studies only provide information about the IRAP apo-enzyme. **[^{3}H]AL-11** shows a high potency and selectivity for both the apo- and native forms of IRAP. Its metabolic stability makes it the first radioligand suitable for the labelling of native IRAP under physiologically relevant conditions.

The biological properties of the designed Ang IV analogues indicated that (*R*)-β^2hVal^1 substitution was vital for high potency along with preventing metabolic degradation. Initially racemic β^2hVal^1 was used, but the need for the (*R*)-enantiomer became apparent. Additionally, β^2hNle was investigated as an alternative for β^2hVal. Two approaches have been pursued for the preparation of the enantiomers: resolution and asymmetric synthesis.

Our first attempts for the resolution of racemic β^2hVal were based on generating chiral amides or esters followed by chromatographic separation. Because the results were not satisfactory we used the ChiroSolv™ resolving kit – base series. Here the resolution process relies on crystallization with a variety of chiral resolving agents. Also this time

no resolution was achieved. Finally, liquid-liquid extraction with a chiral cobalt^{III} salen complex was used, which was successfully used for the resolution of β^3-homo-amino acids, though without any results in our case.

Finally asymmetric synthesis was performed according to two pathways. In the first synthesis acylation of Oppolzer's sultam by isovaleryl chloride was the first step. Subsequently the silylenol ether was generated by treatment with trimethylsilyl triflate. This intermediate reacted with the aminomethylating reagent to give Bn_2-(*R*)-β^2hVal-camphorsultam. The benzyl protection was removed with ammonium formate in the presence of Pd/C, followed by saponification and Boc protection to give Boc-(*R*)-β^2hVal. In the second synthesis the procedure began with *N*-acylation of the lithium salt of the DIOZ chiral auxiliary with isovaleryl chloride. Next the electrophile generated *in situ* by the action of the Lewis acid on benzyl isopropoxymethyl carbamate reacts with the Ti-enolate of the *N*-acyl-DIOZ to give an *N*-amino-acid derivative with a diastereoselectivity of 9:1. Following the stereoselective aminomethylation, the chiral auxiliary was removed with lithium hydroperoxide and the Cbz group was changed to Boc.