Summary

This thesis deals with the synthesis of substituted 4-amino-1,2,4,5-tetrahydro-2-benzazepin-3-ones. These compounds can be regarded as conformationally constrained phenylalanine or tyrosine analogues in which the aromatic side chain is anchored to the amine function of the next residue. Given the biological importance of this type of compound, versatile methods towards the preparation of substituted benzazepinones are of great interest. Different strategies were considered in this work.

The first part consists of the refinement and expansion of existing methods and the development of novel strategies for the synthesis of 4-amino-2-benzazepin-3-ones. The first examined pathway was based on the synthesis of an oxazolidinone, which serves as a *N*-acyliminium ion precursor. This methodology was developed by Flynn and coworkers and was optimized along the years. The 4-amino-2-benzazepin-3-one (Aba), a constricted Phe analogue, was obtained in improved yields, compared to literature values. Addition of the Lewis acid catalyst $TiCl_4$ to the oxazolininone induced ring closure and yielded the phthaloyl protected Aba. Similarly, the tyrosine equivalent of Aba was prepared according to the results described by Dr. R. Casimir. Whereas the constrained “Tyr-Gly” dipeptide mimic, Hba-Gly, was prepared by Casimir, the “Tyr-D-Ala” equivalent (Hba-D-Ala) was prepared analogously in this thesis. The synthesized benzazepinone dipeptidomimetics, Aba-Gly and Hba-D-Ala, were used for the preparation of constricted analogues of the opioid tetrapeptide H-Tyr-D-Ala-Phe-Gly-NH_2.

The second and third strategy considered, were also acyl iminium ion based methodologies. Two versatile syntheses of 1- and 1,2-disubstituted 2-benzazepinones were developed and published. The first methodology for 1-substitution consists of preparing benzotriazole adducts, condensing an amino acid carboxamide and an aldehyde in acid catalyzed conditions. These adducts were cyclized upon addition of $AlCl_3$. Moderate yields (~30%) were improved (~70%), provided that the aromatic ring is activated. The *cis* isomer was solely formed using this pathway. Simultaneous introduction of 1- and 2-substitutions was allowed by the *N*-acylation of an imine. The *N*-acylation, which was strongly dependent on the 2-substituent, was followed by the addition of antimony pentachloride, which resulted in the creation of the reactive iminium ion and induced the subsequent ring closure. A variety of substituted phenyl groups was introduced this way in position 1 of the Aba core. Again, improved yield were obtained after activation of the aromatic moiety. This acylation strategy, resulting in a *cis/trans* mixture of diastereoisomers, was however limited to non-enolizable aldehydes.

Finally, the strategy through an intramolecular amide bond formation, developed by Dr. Van Rompaey, is based on a reductive amination - intramolecular cyclization sequence. This method made it possible to obtain three Aba-D-Phe dipeptomimetics which were used in the preparation of new melanotropin ligands. It was this same methodology which was utilized to prepare an Aba-Lys dipeptide mimetic and a Aba-Asp dipeptide moiety. Contrary to the Lys-derivative, which was obtained without problems, the Aba-Asp synthesis

encountered the drawback of aspartimide side-product formation. The Aba-Lys and Aba-Asp dipeptomimetics were used for the preparation of novel Dmt-Aba opioid ligands.
Further derivatization of the benzazepinone scaffold was investigated using palladium catalyzed reactions. We evaluated Buchwald-Hartwig aminations, Heck reactions, Suzuki couplings and *N*-arylations of our scaffold. The substitution pattern of the starting aminobenzazepinone turned out to be crucial for the success of these transition-metal catalyzed reactions. The Pd-catalyzed methods provide access to novel substitution patterns of the Aba-scaffold. This allows a considerable expansion of the range of substituent diversity available for the priviledged template.

In the second part of this thesis, a series of Ac-Aba-Xxx-NHMe tetrapeptide models was prepared and their conformation was evaluated by NMR and molecular modeling (R. De Wachter). The aim of these small peptide models was to investigate the propensity of these Aba-bearing molecules to adopt β-turn conformations. The *N*-acetyl-*N*'-methylamide model of the Aba-Gly dipeptomimetic as well as the Ac-NMe-Aba-Gly-NHMe model showed to preferentially adopt extended conformations, in which no hydrogen bond between the acetyl C=O and the C-terminal NH was formed. The same finding was observed for both *cis* and *trans* isomers of the 1-phenyl substituted analogues. Only the spirocyclic Aba derivative, prepared by Dr. Tömböly, possessed the necessary β-turn inducing capacity.

The third part of the discussed work covers the biological applications in which the prepared benzazepinones were integrated and biologically evaluated *in vitro* as well as *in vivo*.
The introduction of the 4-amino-2-benzazepin-3-one scaffolds in the *N*-terminal tetrapeptide of dermorphin, an opioid peptide, yielded the favorable side-chain topography for interaction with the μ and δ opioid receptors. The conformational constraints imposed by the 4-amino-1,2,4,5-tetrahydro-2-benzazepin-3-one-ring on the side chains of Tyr^1 and Phe^3, and on the peptide backbone are well tolerated by the μ opioid receptor (MOR), and are favoured for the δ opioid receptor (DOR). Additionally, the highly constrained analogue H-Hba-D-Ala-Aba-Gly-NH_2 shows potent *in vivo* antinociception after i.t. administration and a prolonged duration of action. Intravenous administration of this same peptidomimetic proved that passage through the BBB is realized with a potency comparable to morphine.

Replacement of the H-Dmt-Tic pharmacophore by H-Dmt-Aba, resulted in novel low nanomolar ligands for the MOR and DOR. Aba-Gly analogues containing -NH-CH_2-Ph, -NH-Ph or -Bid (benzoimidazole) C-termini, were examined. Their receptor affinity/activity profiles were determined and led to remarkable results. δ-Opioid receptor affinities were considerably weaker than the reference Dmt-Tic compounds. However, μ-opioid receptor affinity was maintained and selectivity increased. Assessment of the importance of ionic charges in the pharmacophore was done by preparing Aba-Lys and Aba-Asp dipeptidic scaffolds and led to interesting data. Comparing Dmt-Aba-Lys-NH-Bn, to its Aba-Asp counterpart, we established that a negative charge was detrimental for both μ and δ

affinity. The positive charge of the aminoalkyl side-chain of L-Lys^3 was tolerated for binding at the μ receptor, yielding a low nanomolar ligand (i.e. 10.1 nM).

The introduction of the dipeptidomimetic D-BT ((*S*)-[3-amino-4-oxo-2,3-dihydro-5*H*-benzo[b][1,4]thiazepin-5-yl] acetic acid) in BK or in the HOE 140 sequence, produced potent and selective B_2 receptor ligands. The structural resemblance between the D-BT moiety and the 4-amino-1,2,4,5-tetrahydro-2-benzazepin-3-one (Aba) scaffolds urged us to examine the effects of introducing this type of structure into the bradykinin and bradykinin-related sequences. Incorporation of these dipeptidomimetics into the HOE 140 sequence, indicated that mimetics adopting extended conformations were less potent than the spiro-Aba mimetics, which adopt a turn conformation. These replacements in HOE 140 provided two new potent analogues with a K_i = 25 nM (BK9) and a K_i = 3.2 nM (BK10), which maintained the B_2 antagonist character.

Replacement of the His^6-*D*-Phe^7 dipeptide in MT-II and His^6-*D*-Nal(2')^7 in SHU9119 by Aba-*D*-Phe/Aba-pCl-*D*-Phe and Aba-*D*-Nal(2'), respectively was expected to provide new structure activity relationships in the search for selective and potent ligands for the *h*MC1- to *h*MC5R. The α-MSH analogues Aba-1 (Ac-Nle-Asp-Aba-D-Phe-Arg-Trp-Lys-NH_2), Aba-2 (Ac-Nle-c[Asp-Aba-D-Phe-Arg-Trp-Lys]-NH_2), Aba-3 (Ac-Nle-c[Asp-Aba-pCl-D-Phe-Arg-Trp-Lys]-NH_2) and Aba-4 (Ac-Nle-c[Asp-Aba-D-Nal(2')-Arg-Trp-Lys]-NH_2) were synthesized by N^{α}-Fmoc solid phase methodology. Competition binding experiments, combined with the adenylate cyclase assay, were used to evaluate the activities of these peptides at the human melanotropin receptors to reveal a new highly selective high affinity *h*MC3R antagonist (Aba-2) and an *h*MC3R/*h*MC5R antagonist (Aba-4). These results, in conjunction with earlier SAR work on cyclic α- and γ-MSH analogues, suggest that the unique conformational and sterical attributes of the Aba mimetic may be responsible for the observed antagonist activities, and high *h*MC3R receptor selectivity against the *h*MC1R and *h*MC4R. The newly developed melanotropin peptides will be used to clarify the exact biological functions of the physiologically important melanocortin-3 receptor.