6 Summary

Starting with the CD4 binding peptide NMWQKVGTPL **2**, peptidomimetic structures have been designed, synthesized and analyzed. All have better pharmacological properties than the lead. In detail, these compounds have better dissociation constants, a higher proteolytic stability and a lower molecular weight.

First of all, it was tested if the program Flexidock can be used to calculate the binding energies of peptides and peptidomimetics binding to CD4. Therefore, an in silico alanine scan was conducted, replacing each amino acid of NMWQKVGTPL **2** subsequentially with alanine and calculating the binding energies. The results of this matched with the findings of a STD epitope mapping.

These data resulted in the design of a template for the peptidomimetics. All non binding subunits were removed, while keeping or replacing conservatively the binding parts and introducing peptidomimetic replacements. With a MW = ~800 g/mol, the peptidomimetics have a lower molecular weight than the lead (MW =~1200 g/mol) and they show a lower calculated binding energy.

The synthesis of the compounds is partially a solid phase and partially a solution phase synthesis. It starts with an amino alcohol bound to a 2'chloro trityl resin, to which several subunits are coupled, using a Fmoc protocol to form the amide bonds. Afterwards, mild acid treatment results in cleavage from the resin, retaining all side chain protecting groups. The primary hydroxyl function is reacted with an isocyanate to give a carbamate. At last, the side chain protecting groups were removed and the product is purified on a RP-HPLC.

The two synthesized compounds **I** and **II** showed K_D-values of 40 and 20 µM (SPR) and 31 and 45 µM (STD), respectively, much stronger binding to CD4 than the lead (K_D = 6 mM). They have a 4-5 times longer half-life in a proteolytic pronase digestion assay. The STD epitope mapping shows a similar binding mode of the peptidomimetics and the lead.

The design concept allowed an easy access to similar compounds. To improve on the binding affinity and to get structure acitivity relationship information, 16

peptidomimetics with single substitutions have been synthesized in parallel. The compounds were fully characterized with MALDI-TOF-MS and 2D-NMR experiments. SPR analysis shows K_D-values of 6 to 146 µM for these compounds, the best (Ligand **IV**) having a 1000 fold lower dissociation constant than the lead. Furthermore, 5 compounds combining the best subunits have been synthesized in parallel. They have K_D-values of 11-120 µM, not showing a further increase in binding affinity.

The peptidomimetics containing a prolinol subunit show a equilibrium between two conformers, differing at the threonine prolinol amide bond (*cis* and *trans* conformations). Modeling and analytical data show the trans form to be more stable and to bind better to CD4. The stabilization of the *trans* form will be an important task for the further development of these substances.

A direct correlation between the binding energies calculated by Flexidock and the dissociation constants determined by SPR could not be found. However, the general trend of a correlation can be seen.

The importance of a glycosylation site in GP120, directly neighbouring the binding decapeptide was clearified with molecular modelling, since the synthesis of the glycopeptidomimetics could not be finished. While the saccharide is not showing any direct contact to CD4, all glycosylated peptides and peptidomimetics show a better calculated binding energy than the corresponding nonglycosylated compounds. This is due to the structure stabilizing effect of the saccharide, mediated via hydrogen bonds.