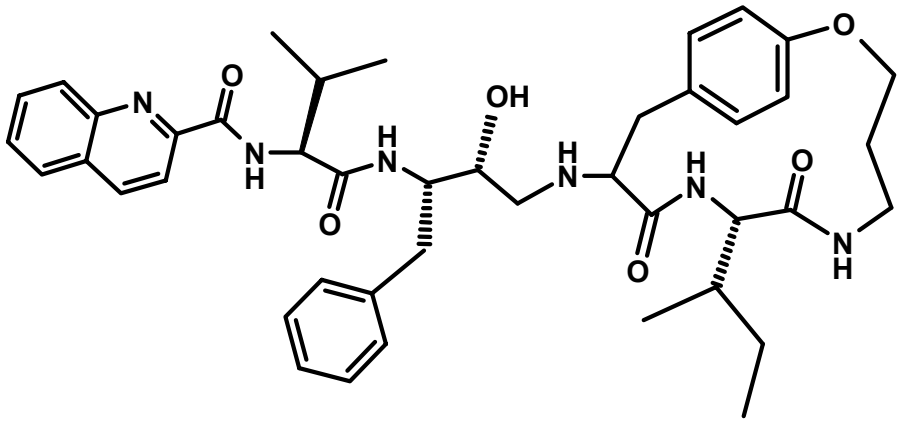
Chem 162/262 (2005) Assignment #4:

Biomolecular Interactions and Assay Development

1. Compare advantages and disadvantages of screening inhibitors for a cytoplasmic enzyme in a classical *in vitro* biochemical assay versus screening for such inhibitors in a cell-based assay.

2. In your own words, explain what "hydrophobic effect" is.

3. The free energy of interaction between the receptor and its ligand is determined both by the enthalpy and entropy changes that occur in the binding process.
   a) Discuss general strategies how to modify the structure of the lead compound such that the enthalpy of binding becomes more favorable
   b) Discuss general strategies how to modify the structure of the lead compound such that the entropy of binding becomes more favorable
   c) The following compound was synthesized (Joel Tyndall and co-workers, *J. Med. Chem.,* **43**, 3495 -3504, 2000) and showed strong inhibition of HIV protease in vitro (K_i = 0.3 nM).



   i) What are the potential advantages of this compound over existing HIV drugs?
   ii) Discuss the issues that might arise in clinical applications with drugs that are highly conformationally constrained.

Project development.

You will be submitting a drug design proposal at the end of this course. In this stage, you are expected to come up with a plan how to assay your potential drug candidates. Please discuss what the rationale behind using such an assay is and identify any potential limitations. Your assay can be either molecular-biology based (e.g. quantification of particular mRNA using molecular beacons), biochemical (e.g. measuring ligand binding to the receptor), cell-based (e.g. monitoring the formation of a fluorescent reaction product in live cells), histological (analysis of tissue appearance), clinical (determination of viral load), or end-point assay (improvement in host health). You do not need to hunt down the original research in which the assay was developed but you need to provide evidence that a particular assay or animal model is appropriate to your target and disease. Keep in mind that we are not talking about human clinical trials here yet but at most animal models of the disease.