Chapter 6

General Conclusions

From the analysis of the conformational profiles of two active and two inactive farnesyltransferase inhibitors characterized using computational methods, only one conformation common to the active inhibitors and not present in the inactive peptides was identified by cross-comparisons among the different classes of conformations. This structure, was identified as the bioactive conformation, and can be characterized by a C14 pseudo-ring stabilized by a hydrogen bond between the amino group of Cys^1 and the carboxylate group of Met^4 together with a C11 pseudo-ring involving the residues Cys^1 and Tic^3.

The analysis of the density of states of a model tetrapeptide demonstrates the superior efficacy of the iterative simulated annealing procedure used to characterize a subset of low-energy conformations in regard to a random strategy. Furthermore, whereas in the random search the density exhibits a bimodal distribution, the more efficient SA procedure only yields structures within the lower peak of the distribution. The origin of the bimodal distribution can be explained in simplistic terms, as due to the result of the superimposition of two separate distributions of different kinds of conformations that differentiate upon the hydrogen bonds that are found in the structure.

The preferred conformation of Hoe 140, a bradykinin (BK) antagonist studied by the iterative simulated annealing (SA) procedure, exhibits a type II' β-turn, motif that had been proposed in the literature as a requisite for BK B_2 antagonism. Based on this hypothesis the pharmacophore for BK B_2 antagonism has been redefined using the values of the type II' β-turn structures obtained in the SA procedure conducted on Hoe 140. An exclusion volume for the pharmacophore was also defined following the hypothesis that the β-turn orient the side chains of residues at positions *i+1* and *i+2* towards the side chains of the receptor.

Based on the β-turn inducing capacity of the restrained residue cyclopropane phenylalanine (c_3Phe), peptides of sequence Thi^1-Ser^2-c_3Phe^3-Pro^4-Arg^5 and Thi^1-Ser^2-Pro^3-c_3Phe^4-Arg^5 including all possible disateroisomers of c_3Phe were studied as possible antagonists of BK. Although all compounds studied exhibit β-turn conformations, peptides with sequence Pro^3-c_3Phe^4 appear to be better inducers of β-turn than peptides with sequence c_3Phe^3-Pro^4. Compounds containing the sequence Pro^3-c_3Phe^4 exhibit different ratios of type I to type II β-turns, in agreement with previous experimental results obtained for dipeptides of sequence Pro-c_3Phe available in the literature. Fulfillment of the pharmacophore requirements yields that only one of the compounds is predicted to exhibit activity. However, the replacement of Pro for Oic could be an appropriate strategy in order to better fulfill the distance requirement between the aromatic ring of c_3Phe and the hydrophobic moiety in the *i+2* position of the β-turn.

Comparison between the conformational profile of substance P deduced from a simulated annealing study and that using MD, suggests first, that structures derived from the SA procedure

are more extended and second, in contrast to MD simulations, the SA procedure samples all the different values locally attainable by the different dihedrals. As a consequence the structures obtained by SA are much more diverse than those obtained in the MD simulations. However, MD simulations sample other conformations that exhibit larger secondary structure motifs that are not sampled by the SA procedure. These differences can be attributed to either cooperative effects between neighboring residues in the peptide chain, or to an effect of the different treatment of the solvent. Furthermore, the SA procedure does not sample all the range of conformations with a high content of secondary structure that is obtained by long MD trajectories.

A novel method of clustering structures based on information theory, described in the present work, efficiently groups structures upon their structural similarity. Using this tool, it has been shown that conformations structurally related tend to fluctuate among themselves along the MD trajectories. In the SA procedure, consecutive conformations are not structurally related and therefore the method is not conditioned by the previous structures obtained and constitutes an efficient tool for obtaining a diverse set of structures.

^{1}H-NMR experiments of SPOH in water and methanol suggest that the peptide does not adopt a rigid conformation. On the other hand, MD studies suggest that both SP in water and SPOH in water and methanol adopt an ensemble of conformations characterized by the presence of an extended N-terminus affecting the 3 first residues and from that point onwards the peptide would adopt a flexible helix that would interconvert between α- and 3_{10}-helical conformations. These results are in agreement with the results that had been previously obtained for SP in a TFE/water mix and for SP in methanol.

The absence of a unique conformation in water of the peptide cannot be interpreted as the peptide lacking of structure. On the contrary, the peptide adopts a restricted group of conformations presenting flexible motifs that interconvert among them easily. Indeed, the preferred conformation of the peptide in solution can be justified in terms of its sequence, as it has been discussed in chapter 5.

When SPOH is placed within a box of explicit DMSO molecules the peptide adopts an extended and rigid structure. This is consistent with studies that point out to DMSO as responsible for the disruption of unstable hydrogen bonds and the reduction in the number of backbone conformations.

The methodology for classification (CLASICO) and clustering (CLUSTERIT) of peptide conformations has proven useful for the analysis of the different behavior of the peptide within the different solvents providing information on the variable rigidity of the peptide and allowing for the comparison of the MD trajectories. This methodology is mostly automated and it does not present

any restriction on the size of the peptide or protein under study. Accordingly, it is proposed the use of this method to suitably tackle the complexity that arises in the analysis of the folding of peptides and proteins by molecular dynamics studies.