In 1978, Zamecnik and Stephenson opened a new era of molecular biology inhibiting the translation of RNA present in the avian Rous Sarcoma Virus with a synthetic DNA oligonucleotide. It was possible because the Watson–Crick base pairing rules allow for design of an oligonucleotide being able to recognize a selected part of the target cellular molecule. Later research performed over many years documented the existence of several cellular mechanisms, where hybridization with the delivered probe makes the original role of the target molecule very difficult or impossible to occur. These methods allow for inhibition of gene expression either during the transcription (DNA→mRNA) or translation (mRNA→protein) steps. To be therapeutically effective the oligonucleotides should be stable towards degradation (in cytoplasm or in extracellular milieu) by the nuclease, which hydrolyze DNA and RNA oligonucleotides recognized dangerous by the organism. In order to increase their stability it is necessary to make modifications within either a nucleobase or ribose (deoxyribose), or the phosphate moiety, but these changes should not decrease the thermodynamic stability of the expected probe/DNA or probe/mRNA complexes. Among numerous chemical modifications, a very early one including replacement of the non-bridging oxygen atoms in the phosphate moiety with a sulfur atom, which furnishes phosphorothioate oligonucleotides (PS-Oligos), has been found really an excellent one. PS-Oligos are isoelectronic with the unmodified precursors and exert quite good hybridization properties. However, each phosphorus atom in PS-Oligos becomes a stereogenic centre and for many years chemically synthesized PS-Oligos have been obtained as mixtures of hundreds or thousends of P-diastereomers. Their P-stereocontrolled synthesis became possible with the so called oxathiaphospholane method, which was developed in our Department. Many biological proceses involving PS-Oligos were found to be P-stereodependent.

The goal of this project is synthesis and characteristics of P-stereodefined phosphorothioate analogs of nucleic acids containing an additional modification, in which the five-membered ribose ring is replaced with a six-membered morpholine ring, and this change leads to the presence of thiophosphoramidate linkages instead of thiophosphate ones. These compounds are called here (and in the full project description) **P-stereodefined Thiophosphoramidate Morpholino Oligonucleotides** (**sTMO**). My proposal was inspired by the results of research published by Professor Marvin Caruthers from University of Colorado Boulder, where P-stereorandom TMO were synthesized using the phosphoramidite approach. Because these mixtures of P-diastereomeric TMO constructs exhibited interesting biological properties and considerable therapeutic potential I became curious whether those biological activities might be elicited by oligomer(s) with proper absolute configuration of the phosphorus atoms. If so, the unwanted side-effects resulting from the presence of inactive isomers might be limited. To verify this hypothesis 4-6 sTMO oligonucleotides as well as a few *gapmers* R_P- and S_P-[sTMO/DNA/sTMO] of 10-12 nucleotide in length will be synthesized using the oxathiaphospholane approach. Within the first part of the project the morpholine nucleosides will by synthesized, followed by their conversion into the oxathiaphospholane derivatives and determination of chromatographic conditions suitable for isolation of pure P-epimers. At this point the absolute configuration of the P-atoms in separated compounds will be hopefully determined by X-Ray analysis. Diastereomerically pure monomers will be used in synthesis of the above mentioned oligomers. In the second part, a correlation between the stereochemistry and hybridizing properties towards DNA and RNA templates will be assessed (T_m mesurements), as well as the conformational properties of the complexes will be determined by CD spectroscopy. The biological properties of the sTMO constructs and *gapmers* will be compared with those observed for TMO obtained by the phosphoramidite method. Also stability of the sTMO in human plasma will be determined.

A concept of use of R_P- and S_P-sTMO and the R_P- and S_P-[sTMO/DNA/sTMO] *gapmers* as the sequence-specific probes for making stable complexes with target oligonucleotides is a part of the general idea of search for molecular tools suitable for tuning of the physico-chemical and biological properties of cellular oligonucleotides. In a more future perspective they may be perhaps used in therapy and diagnostics. This field is intensively explored and brings important results in the form of registered drugs of the oligonucleotide origin, like those of the PS-Oligo class (*Vitravene*) or morpholine based compounds (*Eteplirsen, Golodirsen*).