ABSTRACT

Modified nucleic acid components (nucleotides, nucleosides, nucleobases) display a wide range of biological activities such as antiviral, cytostatic, antimicrobial or antioxidative. Many of them are successfully used in clinical practice, for instance as anti-HIV drugs or in hepatitis B treatment. The first precondition for understanding biological effects is to evaluate the chemical structure as well as the conformation of the studied compounds correctly. For this purpose, solution-state nuclear magnetic resonance (NMR) spectroscopy in combination with mass spectrometry is the most frequently used method. Not only the structure, but also physico-chemical properties can be investigated using NMR spectroscopy, for instance, conformational changes, tautomeric forms, protonation sites, acido-basic properties, non-covalent interactions, isotopic exchanges or interactions with biomolecules. Similarly to solutions, NMR spectroscopy can be employed in studies of nucleic acid components (NACs) in solid state. This technique found assertion after computational-chemistry methods development during the last decades. Different solid-state structures (polymorphic forms) or dynamics of these compounds can be established by solid-state NMR spectroscopy in combination with quantum-chemical calculations.

In this work, I dealt with the structure elucidation as well as the investigation of the physico-chemical properties of modified nucleotides, nucleosides and nucleobases using NMR spectroscopy both in solution state and solid state in combination with DFT calculations of NMR parameters. For the structure determination, 1D in combination with 2D NMR experiments were employed; the configuration and conformation of studied compounds were also investigated. During this analysis, it was noticed that NMR signals could be misassigned in substituted purine derivatives, so a new method for unambiguous NMR signal assignment in purines was developed. This method is based on the measurement of heteronuclear coupling constants in combination with DFT calculations. The same approach including correlations between experimental and calculated data was applied for development of new method for prediction of preferred tautomeric forms of purine derivatives in solution.

A non-covalent interaction essential for life is hydrogen bond (H-bond), which can be studied by NMR spectroscopy in detail. We studied nitrosopyrimidine derivatives with strong intramolecular H-bonds which stabilized two conformers, differing only in nitroso group orientation. We investigated composition of the rotamers mixture in equilibrium depending on the pyrimidine substitution. From temperature-dependent ^{1}H NMR spectra, the rotational barriers of the nitroso group were estimated. In some cases, it was possible to separate both rotamers from each other by column chromatography even at room temperature and their purity was confirmed by SS-NMR data as well as by X-ray analysis. Kinetics of the rotamer interconversion was measured

to determine rotational barriers around C5-NO single bond. Experimental data were supported by DFT calculations.

Many biologically active compounds are unable to pass across the cell membrane, therefore, more lipophilic derivatives (prodrugs) are often prepared; they are designed with the aim of active compound release after a reaction with intracellular enzymes. For confirmation that newly prepared phosphoramidate derivatives could work as prodrugs and they are decomposed by carboxypeptidase in the supposed metabolic pathway, we studied their enzymatic decomposition using ^{31}P NMR spectroscopy. The monitoring of the reaction is based on the fact that the starting phosphoramidate has a chiral center at phosphorus atom, so it has two signals in proton decoupled ^{31}P NMR spectra corresponding to two diastereoisomers. After several hours of the enzymatic reaction, a single ^{31}P signal is observed, which indicates that the final product does not contain a chiral center at the phosphorus atom. The same products were obtained after non-enzymatic hydrolysis with triethylamine and the suggested chemical structure was confirmed using ^{1}H, ^{13}C and 2D NMR experiments.

The solid-state structure of given compounds influences their biological effects and the information about their polymorphic structures is crucial for granting licenses obtained from regulatory authorities. For investigation of polymorphic crystals in this work, ^{13}C cross-polarization magic-angle spinning (CP-MAS) NMR spectroscopy was used. We prepared model polymorphic crystal forms of three pharmaceutically or industrially important compounds and characterized them using CP-MAS NMR spectroscopy as well as by Raman spectroscopy and we confirmed the determined structures by X-ray analysis. These data were supplemented by DFT calculations.

For biological properties investigation, studied compounds are usually dissolved in DMSO for a long time. We observed color changes of 5-aminopyrimidine derivatives dissolved in DMSO. We monitored these transformations by NMR and UV/Vis spectroscopy and we used series of chemical reactions with alloxan to confirm the structure of the colorful products.