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„Synthesis and conformational studies of selected pyrimidinone and thiopyrimidinone nucleosides."

Heterobase-modified nucleosides, including compounds studied in this PhD research, are analogues of natural components of nucleic acids (mainly tRNA), and they play significant role in modulation of their stability and biological functions. The presence of modification in nucleobase moiety influences conformational parameters of nucleoside unit, mainly due to steric and stereoelectronic interactions. As a result, more interest is put in determining the relationship between the structure of modification and the conformation of the nucleoside, especially the puckering of sugar ring.

The main goal of the presented research was to extend the knowledge on the influence of the position of modification within a pyrimidine nucleobase ring on the conformational properties of nucleoside. It was achieved by the synthesis of model compounds, conformational studies in a solution (using high-resolution NMR methods) and in the solid state - for compounds which obtained crystals were suitable for X-ray studies.

Synthetic part of the dissertation relates to the development of the procedures for the preparation of selected pyrimidinone and thiopyrimidinone nucleosides: **H^{4}s^{2}U**, **H^{2,4}s^{6}U**, **m^{2}o^{4}U**, **m^{6}H^{2}o^{4}U**, **dH^{2}o^{4}U**, **dH^{2}s^{4}U**. Synthetic procedures based on the silyl method of N-glycosdic bond formation or by the modification of the nucleobase moiety in the precursor nucleoside units. The remaining group of model compounds (**s^{4}U**, **H^{2}s^{4}U**, **sm^{2}U**, **ges^{2}U**) and nucleosides used for comparative studies (**U**, **s^{2}U**, **H^{4}o^{2}U**, **H^{2}o^{4}U**, **H^{2,4}o^{6}U**) were obtained by the slightly modified procedures described in the literature.

Conformational parameters of nucleosides: sugar ring puckering (C2′-*endo*/C3′-*endo* or S/N, respectively), orientation of the heterobase (*syn*/*anti*) and conformation of the 5′-hydroxymethylene group were determined for all analyzed compounds.

Conformational analysis of model pairs of compounds: zebularine (**H^{4}o^{2}U**) - 2 thiozebularine (**H^{4}s^{2}U**), nucleoside 6 pyrimidinone (**H^{2,4}o^{6}U**) - 6-thiopyrimidinone (**H^{2,4}s^{6}U**) and comparison of the results with the data available for uridine (**U**) and 2 thiouridine (**s^{2}U**) showed an increase of population of C3′*endo* (Ntype) conformers in C2/C6 thiocarbonyl analogues. It results from the steric hindrance in the interaction between the substituent in the base moiety and 2′-hydroxyl group of ribose ring. In addition, changes in electron distribution in 2-thiopyrimidinone or 6thiopyrimidinone moieties, enhance steric and stereoelectronic forces and lead to a total (100%) preference of the sugar ring in the C3′-*endo* conformation in **H^{4}s^{2}U** and **H^{2,4}s^{6}U**, in comparison with 71% of the N-type conformers for **s^{2}U**. Significant influence of the steric interactions on conformational properties of nucleosides, was also confirmed by structural studies of a set of 2′-deoxyribonucleosides, including 2′deoxyzebularine (**dH^{4}o^{2}U**), 2′-deoxy-2-thiozebularine (**dH^{4}s^{2}U**), 2′-deoxyuridine (**dU**) and 2′deoxy-2-thiouridine (**ds^{2}U**).

In contrast to the corresponding ribonucleosides, sugar ring in a 2′deoxyribonucleosides, preferentially adopts C2′-*endo* (S-type) conformation due to reduced steric effect (caused by the lack of 2′-hydroxyl function) and the influence of O4′-C4′-C3′-O3′ gauche effect.

Results obtained from the analysis of model compounds allowed to determine that C2′-*endo* (S-type) conformation of the sugar ring, which is rare among the RNA nucleosides, is associated with changes in electron nature of the aglycon, which is the effect of transformation of the nucleobase to the 4pyrimidinone structure (**H^2o^4U**). The lack of characteristic urea/thiourea function affects the sugar ring puckering, what resulted from studies performed for **m^2o^4U**, **sm^2U**, **ges^2U** and **$m^6H^2o^4U$**. Even though a steric hindrance associated with the presence of the substituents at C2/C6 position (with different structure and size) in heterobase moiety was present, only small shift of the equilibrium toward C3'-*endo* conformation was observed (47% N for **m^2o^4U**, 59% N **sm^2U**, 38% **ges^2U**, 54% **$m^6H^2o^4U$** in comparison with 38% N for **H^2o^4U**).

The results of the studies have been partially published. Synthesis and conformational studies of 2thiopyrimidinone nucleoside (**H^4s^2U**) were presented in *Coll. Czech. Chem. Commun.* **2011**, *76 (9)*, 1103-1119, while the efficient synthesis of 2′-deoxyzebularine and conformational analysis of its both anomers were published in *Carbohydr. Res.* **2014**, *392*,715.

A separate part of my PhD research concerned the conformational studies of 4-pyrimidinone ribofuranosides with different substituents at C5 position (**$x^5H^2o^4U$**, where x: OCH_3, CH_2COOH, CH_2COOCH_3, CH_2CONH_2, CH_2NHCH_3, CH_2NHCH_2COOH). These nucleosides are formed as a result of oxidative desulfurization of corresponding 5-substituted 2-thiouridines (**x^5s^2U**), naturally occurring in the tRNA. Conformational analysis in aqueous solutions and comparison of the obtained parameters with the data for the corresponding derivatives of 2-thiouridine (**x^5s^2U**) and uridine (**x^5U**) confirmed, that the loss of the 2-thiocarbonyl group in the 5-substituted heterobase strongly influences the conformation of the sugar ring and significantly shift the equilibrium toward S-type (C2′*endo*). The obtained results also indicate that the structure of the substituent at C5 position of **$x^5H^2o^4U$** heterobases only slightly influences the conformation of the ribose ring. This part of the study was published in *Bioorg. Med. Chem.* **2015**, *23*, 5587-5594.

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