**"Studies on a new method for the synthesis of oligoribonucleotides modified with 5 aminomethyluridines (xnm$^5$U) and 5-aminomethyl-2-thiouridines (xnm$^5$s$^2$U) *via* post-synthetic nucleophilic substitution of 5-pivaloyloxymethyluridine/2-thiouridine"**

Uridines and 2-thiouridines containing 5-aminomethyl group (xnm$^5$U/xnm$^5$s$^2$U) are present in transfer RNA (tRNA) sequences in both prokaryotic and eukaryotic organisms.[1-5] These modifications, located at 34 position ("wobble" position, the first anticodon letter) of cytosilic and mitochondrial tRNA (mt-tRNA) anticodon arm domains, play a crucial role in decoding of genetic information in the cells. They arrange appropriate conformation of anticodon arm and stabilization of ribosomal mRNA-tRNA complex, and determine the precise recognition of the purine-ending codons (codons NNG/NNA). The absence of these modifications often causes total inhibition of the protein biosynthesis resulting in the occurrence of disease symptoms.[6,7] Deficiency of the 5-taurinomethyl-2-thiouridine in the sequence of human mt-tRNA$^{Lys}$ leads to mitochondrial encephalomyopathic disease MERRF.[6] In this context, chemically synthesized modified tRNA fragments, in particular anticodon arm sequences, represent important tools for the studies on structural aspects of tRNA functionality in the cell processes.[1,2] In many cases, because of the insufficient availability of tRNA/mt-tRNA molecules from biological material and synthetic limitation, the relation between the character of nucleoside modification and tRNA function was not recognized.

Moreover, oligoribonucleotides modified with 5-substituted uridines/2-thiouridines xnm$^5$U/xnm$^5$s$^2$U may be useful in designing of new therapeutic nucleic acid molecules.[8-11] In this aspect, the nitrogen atom present at xnm$^5$U/xnm$^5$s$^2$U modification plays the crucial role. The protonation of the amine moieties, neutralizing the negative charge of the oligomer phosphate groups, can significantly increase their bioavailability. The presence of amine group(s) enhances the selectivity and affinity of oligomer to phatogenic RNA/DNA molecules as well as the resistance to enzymatic degradation in the cell. It is also important that 2-thiocarbonyl function present in the modified unit (xnm$^5$s$^2$U) can stabilize helical structure of oligonucleotides.[12]

Although, there are many opportunities for application of oligoribonucleotides modified with uridines/2-thiouridines xnm$^5$s$^2$U/xnm$^5$s$^2$U, there are no many reports describing their effective synthesis.[1,13-16] Among the three described methods (chemical, enzymatic and semienzymatic), only the chemical procedures enable large-scale synthesis of oligomer and incorporation of 2-thiopyrimidine nucleosides xnm$^5$s$^2$U into RNA sequences.

The aim of presented project is to develop an efficient synthesis of oligoribonucleotides modified with 5-substituted uridines and 2-thiouridines xnm$^5$U/xnm$^5$s$^2$U *via* post-synthetic transformation of 5-pivaloyloxymethyluridine (Pivom$^5$U) and 5-pivaloyloxymethyl-2-thiouridine (Pivom$^5$s$^2$U). The proposed methodology assumes the use of pivaloyloxy group present at pseudobenzylic position of Pivom$^5$U/Pivom$^5$s$^2$U as a leaving group in reaction of nucleophilic substitution with series of diverse nitrogen nucleophiles. As a result, one precursor oligomer modified with Pivom$^5$U or Pivom$^5$s$^2$U enables to obtain many various oligonucleotide products depending on the character of the applied nucleophile. The following nucleophiles: ammonium, primary amines, secondary amines including secondary cyclic amines and tetra-*n*-butyloammonium salts of amino acids will be tested.
The conditions of post-synthetic transformation Pivom$^5$U/Pivom$^5$s$^2$U → xnm$^5$U/xnm$^5$s$^2$U will be elaborated using model oligoribonucleotide with the sequence: 5'-GUPivom5UAC-3' / 5'-GUPivom$^5$s$^2$UAC-3', containing all canonical units. Pivom$^5$U- and Pivom$^5$s$^2$U-modified precursor oligoribonucleotides will be obtained using manual protocol of RNA synthesis by phosphoramidite method on solid support.

To investigate the potential of our new elaborated methodology for modified RNA preparation we plan to synthesize the longer 17-mer oligonucleotide with the sequence of anticodon stem and loop of tRNA$^{Lys}_{E.coli}$ (modified with 5-methylaminomethyl-2-thiouridine (mnm$^5$s$^2$U) at the position related to 34 position in tRNA molecule) *via* post-synthetic transformation of Pivom$^5$s$^2$U-modified precursor with methylamine. In addition, attempts to introduce several Pivom$^5$U units into oligoribonucleotide chain will be also undertaken. Their post-synthetic transformation with selected nucleophile, allows to apply the proposed methodology for the preparation of oligomers with enhanced therapeutic properties as compared to single modified RNA fragments.

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