Łódź, dn. 17.04.2018

M.Sc. Karolina Bartosik

Supervisor: prof. Elżbieta Sochacka

Co-supervisor: dr Grażyna Leszczyńska

## DOCTORAL DISSERTATION ABSTRACT

### „Studies on the synthesis of oligoribonucleotides containing 5-substituted uridines and 2-thiouridines *via* post-synthetic method of RNA modification"

5-Substituted uridines and 2-thiouridines are modified ribonucleosides present in cytosolic and mitochondrial transfer ribonucleic acids (tRNA/mt-tRNA). Among them, uridines and 2-thiouridines containing aminomethyl group at position 5 in pyrimidine base (xnm$^5$U/xnm$^5$s$^2$U) are important group of modifications as they are located at position 34 of tRNA anticodon arms (the first anticodon letter, the so-called "wobble" position) and play a crucial role in protein biosynthesis. At present, the influence of 5-aminomethyluridines and 5-aminomethyl-2-thiouridines on structure and biological functions of tRNAs is not fully defined. To understand structural requirements of tRNA activity in the cell processes, chemically synthesized oligoribonucleotides modified with xnm$^5$U/xnm$^5$s$^2$U, particularly oligomers related to the anticodon arm domains, are often used as useful model compounds. Moreover, properly designed RNA fragments containing unnatural xnm$^5$U/xnm$^5$s$^2$U have found application as potential therapeutic nucleic acids molecules with enhanced selectivity and affinity towards pathogenic RNA/DNA sequences, as well as improved bioavailability and enzymatic stability in the cell.

Presented dissertation is a contribution to the current research on development new, effective procedures for synthesis of RNA fragments modified with xnm$^5$U/xnm$^5$s$^2$U type nucleosides. The main goal of the thesis was to elaborate protocols for efficient synthesis of oligoribonucleotides containing xnm$^5$U/xnm$^5$s$^2$U *via* post-synthetic method of RNA modification. To obtain the desired xnm$^5$U-RNA/xnm$^5$s$^2$U-RNAs, precursor nucleosides - 5-pivaloyloxymethyluridine (Pivom$^5$U) and 5-pivaloyloxymethyl-2-thiouridine (Pivom$^5$s$^2$U) - were incorporated into RNA chain by phosphoramidite method. The pivaloyloxyl group at pseudobenzylic position (position C-5,1) of Pivom$^5$U/Pivom$^5$s$^2$U was supposed to serve as the good leaving group in the reaction of nucleophilic substitution with different nucleophiles.

In the first part of my PhD research, the studies on nucleophilic substitution of pivaloyloxyl group on nucleoside level were performed. For this purpose, 5-pivaloyloxymethyluridine (Pivom$^5$U) and 5-pivaloyloxymethyl-2-thiouridine (Pivom$^5$s$^2$U) were efficiently synthesized and then were reacted with selected range of nucleophilic reagents. The pivaloyloxyl group of Pivom$^5$U and Pivom$^5$s$^2$U was effectively substituted with ammonia, methylamine, isopentenylamine, diethylamine, morpholine, piperidine, tetrabutyloammonium salts of amino acids (glycine and taurine), as well as with a methoxide, a thiolate and a cyanide anion. In general, $S_N$ reactions were carried out at elevated temperature (50-60 °C) in alcohol, water or in alcohol-water mixture as the solvents for 1-20 h. Elaborated methodology was found to be efficient for preparation of 22 uridine and 2-thiouridine derivatives in 65-90% yields, including natural tRNA components (nm$^5$U/nm$^5$s$^2$U, mnm$^5$U/mnm$^5$s$^2$U, inm$^5$U/inm$^5$s$^2$U, cmnm$^5$U/cnm$^5$s$^2$U, τm$^5$U/τm$^5$s$^2$U, cnm$^5$U). The other obtained modified nucleosides are considered to be useful for nucleic acid therapeutic applications.

The second part of dissertation was focused on the incorporation of 5-pivaloyloxymethyluridine and its 2-thio analogue into oligoribonucleotides and their post-synthetic substitution with selected nuclephilic reagents in order to obtain oligomer modified with naturally existing xnm$^5$U/xnm$^5$s$^2$U. Particularly important was to develop the procedure for post-synthetic conversion of Pivom$^5$s$^2$U-RNA as 2-thiocarbonyl group of xnm$^5$s$^2$U-RNA is generally known to be prone to oxidation and/or oxidative desulfuration during standard oligomer synthesis,. Precursor Pivom$^5$U/Pivom$^5$s$^2$U nucleosides were transformed into 5'-DMTr-2'-TBDMS 3'-*O*-phosphoramidites using standard procedure and introduced into model pentamer 5'-GU**Pivom$^5$U**AC-3'/5'-GU**Pivom$^5$U**AC-3' (containing all canonical units) by phosphoramidite chemistry on solid support. For effective post-synthetic transformation three step protocols were elaborated. Support-linked precursor pentamers were divested of their β-cyanoethyl group and then were treated with nucleophiles under optimized conditions, previously used for the synthesis of modified nucleosides. The nucleophilicity of used reagents was sufficient to cleave oligomers from the support and to remove the base-labile protecting groups. Finally, the converted oligoribonucleotides were desilylated. As a result, series of model pentamers containing native 5-aminomethyluridines (nm$^5$U, mnm$^5$U, inm$^5$U, cmnm$^5$U, τm$^5$U, cnm$^5$U) and 5-aminomethyl-2-thiouridines (nm$^5$s$^2$U, mnm$^5$s$^2$U, inm$^5$s$^2$U, cmnm$^5$s$^2$U, τm$^5$s$^2$U) were obtained in 19-84% yields.

To demonstrate the applicability of the developed methodology in the preparation of longer and biologically relevant xnm$^5$U/xnm$^5$s$^2$U-RNAs, Pivom$^5$U and Pivom$^5$s$^2$U units were incorporated by phosphoramidite chemistry into 17-mers of the sequence homologous to the anticodon stem and loop of tRNA *E.coli* specific for lysine. To transform 17-mers into RNAs bearing native nucleoside derivatives such as mnm$^5$U, mnm$^5$s$^2$U, τm$^5$U and τm$^5$s$^2$U, methylamine and tetrabutylammonium salt of taurine were used as a nucleophiles and conditions previously optimized for the 5-mer analogs were adopted. The desired 17-mers modified with mnm$^5$U, mnm$^5$s$^2$U, τm$^5$U and τm$^5$s$^2$U were obtained in yields of 60-78%.

The results of the studies have been partially published. The synthesis of nucleoside units xnm$^5$U/xnm$^5$s$^2$U was presented in *Tetrahedron Lett.* **2015**, *56*, 6593 and *Org. Biomol. Chem.* **2017**, *15*, 2097, while the effective synthesis of 5-mers and 17-mers modified with 5-aminomethyluridines was published in *Org. Biomol. Chem.* **2017**, *15*, 2097.

Elaborated methodology of effective substitution of pivaloyloxyl group present at Pivom$^5$U/Pivom$^5$s$^2$U with various nucleophiles allows significantly simplify the protocol for the preparation of nucleoside units xnm$^5$U/xnm$^5$s$^2$U as well as xnm$^5$U/xnm$^5$s$^2$U-modified oligomers. Contrary to standard procedure used for oligoribonucleotide synthesis and deprotection, the 2-thiocarbonyl group of xnm$^5$s$^2$U-RNA proved to be stable under applied conditions of post-synthetic transformation. It is noteworthy that the method is convenient and much less time consuming than standard procedure based on direct incorporation employing appropriately protected phosphoramidities of modified nucleosides.

Karolina Bartosik