Abstract

The aim of this thesis was to study the steric influence of the base-modified nucleoside triphosphates (**NTPs**) on the enzymatic incorporation into RNA, as well as to study their inhibitory effect on different viral RNA polymerases *in vitro*. Their parent nucleosides and prodrug derivatives were also prepared and their antiviral activity evaluated.

In the first part of the thesis, **NTPs** bearing groups varying in size from small methyl and ethynyl substituents via medium-size phenyl and benzofuryl groups, up to large dibenzofuran ring were prepared. Aromatic substituents were installed via Suzuki coupling on iodinated triphosphates or, in the case of modified guanosines, by the phosphorylation of modified nucleosides. Methyl and ethynyl **NTPs** were prepared via Pd-catalyzed coupling with $AlMe_3$ and Sonogashira coupling, respectively, followed by the phosphorylation of modified nucleoside. To examine their incorporation into RNA by T7 RNA polymerase, templates coding for 35mer RNA containing one, three or seven modifications were designed. Modified pyrimidine triphosphates worked well for all the sequences, while the biggest dibenzofuryl group was not accepted in the difficult sequence with seven modifications. In the case of **A^RTPs** dibenzofuryl modification did not incorporate at all, while other modifications were well tolerated. To incorporate modified **G^RTPs**, transcription was performed in the presence of **GMP** that served as an initiator, while the nascent chain was then elongated with modified **G^RTPs** with similar efficiency to modified **ATPs**.

In the second part of the thesis, small library of modified nucleoside triphosphates was also synthesized and used in an *in vitro* inhibition assay against different viral polymerases. In vitro assays indicate inhibition of the zika virus polymerase in nanomolar range for benzofuryl, benzothienyl and naphthyl derivatives and inhibition of JEV and WNV polymerases in micromolar range for smaller aromatic and ethynyl derivatives. Nucleosides and ProTide and SATE-class nucleoside monophosphate prodrugs were prepared. ProTides were prepared either by organometallic coupling on the iodinated ProTide or by 5'-*O*-phosphorylation of modified nucleosides. Later approach was used for the synthesis of mixed SATE prodrug, while the bis(SATE) prodrugs were prepared by reacting modified H-Phosphonate with *S*-acylthioethanol. Nucleosides and ProTides show moderate to no antiviral activity, while activity of SATE derivatives remains to be determined. Metabolic studies show that SATE prodrug efficiently

penetrates and deprotects to monophosphate inside the cell, while there are no traces of parent ProTide in the cell, although exact metabolic fate requires further examination.