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Synthetic trimethylguanosine cap analogs modified in triphosphate bridge as tools for studies of snurportin and optimization of nuclear localization signal for therapeutic factors

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Abstract of doctoral dissertation

A trimethylguanosine (TMG) cap structure is present at the 5' end of several small nuclear and nucleolar RNAs. It consists of N^2,N^2,N^7-trimethylguanosine connected *via* the 5',5'-triphosphate bridge to the first transcribed nucleotide of the RNA chain ($m_3^{2,2,7}$GpppN). In higher organisms, including humans, the TMG cap structure is involved in nuclear import. In the cytoplasm, it is recognized by an adaptor protein, snurportin 1, and then after recruitment of Importin β the construct can be translocated through the nuclear pore complex. The interaction between TMG cap and snurportin is essential for efficient nuclear import of snRNPs. Recently, it has been reported that the TMG cap is a potential nuclear import signal for nucleus-targeting therapeutic nucleic acids and proteins. However, TMG cap signal is prone to degradation by hydrolases, e.g. the NUDIX family pyrophosphatases such as Nudt.

The aim of my work was the synthesis and biophysical characterization of a series of dinucleotide TMG cap ($m_3^{2,2,7}$GpppG) analogs modified within the 5',5'-triphosphate bridge as tools to study TMG cap-dependent biological processes. The bridge was altered at different positions by introducing either bridging (imidodiphosphate, O to NH and methylenebisphosphonate, O to CH_2) or non-bridging (phosphorothioate, O to S and boranophosphate, O to BH_3) modifications, or by elongation to tetraphosphate. We expected

those analog to be resistant against enzymatic degradation and to maintain their affinity to snurportin 1.

The affinity of synthesized TMG cap analogs to snurportin 1 was determined using fluorescence titration assay based on the intrinsic protein fluorescence quenching by cap analogs. It has been found that the modification with imidodiphosphate group in α-β position of triphosphate bridge as well as phosphorothioate group in both α or β positions of triphosphate bridge does not decrease the affinity of an analog to human snurportin 1 in comparison to non-modified TMG cap analog. Moreover, diastereoisomer D1 of the analog modified with phosphorothiote group in α position of triphosphate bridge ($m_3^{2,2,7}Gppp_SG$ D1) has the highest affinity to snurportin 1.

The stability studies of novel analogs in blood serum revealed that the α-β-bridging O to NH substitution ($m_3^{2,2,7}GppNHpG$) confers the highest resistance. Due to favorable properties in all assays, $m_3^{2,2,7}GppNHpG$ and $m_3^{2,2,7}Gppp_SG$ D1 were selected as promising candidates for further studies on the efficiency of the TMG cap as a nuclear import signal.