Selective functionalization of vitamin B_{12} at the *meso* position and within the nucleotide loop

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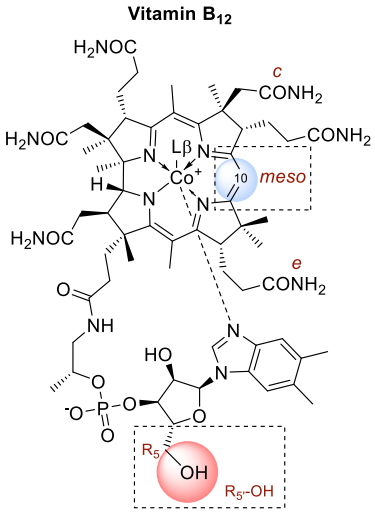

Vitamin B_{12} (cobalamin) is an essential nutrient for mammals and certain bacteria. As an exogenous compound, cobalamin reaches mammalian cells via a system of transport proteins and this fact makes it an attractive candidate for the delivery of cargoes into cells. Development of conjugates requires suitably designed building blocks and vitamin B_{12} possesses a number functional groups susceptible to chemical modifications, however complex nature of cobalamin makes them extremely challenging.

The goal of my studies was to design an efficient methodology allowing for the preparation of cobalamin conjugates in a reversible manner at the $R_{5'}$ position. Moreover, I decided to functionalize cobalamin at the *meso* position – which has not been considered so far for the synthesis of conjugates. I have decided to use newly developed and already established methodologies to prepare vitamin B_{12} conjugates with synthetic oligonucleotides, namely peptide nucleic acid (PNA) and fluorescent dyes.

At the first stage of my research, I have developed a three-step synthesis leading to vitamin B_{12} derivative highly reactive toward thiols and proved that disulfide bond formed during preparation of conjugates with this molecule, reduces in the presence of glutathione (a thiol present in eukaryotic cells). Functionalizations at the *meso* position involved nitration and subsequent reduction leading to *meso*-aminocobalamin. The obtained compound can be successfully reacted with aldehydes and acid anhydrides. In addition, I investigated the effect of the electronic properties of C10 substituents on the structural, photophysical and electrochemical properties of cobalamin.

Another part of my research focused on the development of methodologies leading to vitamin B_{12} conjugates with synthetic oligonucleotides (PNA) appended at the $R_{5'}$, *c*, *e*, *meso* position and cobalt, with the use of spacers of various character and length. I have also developed a method for attaching cobalamin to fluorescent dyes at the $R_{5'}$ position using copper(I)-catalyzed 1,3-dipolar cycloaddition of azides to terminal alkynes (CuAAC).

My studies demonstrate that vitamin B_{12} can serve as an efficient carrier for synthetic oligonucleotides (PNA) into bacterial cells, which opens new routes for developing antibacterial agents. Additionally, cobalamin conjugates with fluorescent dyes can be applied as molecular probes to track mRNA and small noncoding RNA in live mammalian cells.