



INVENTION: Invention relates to a new chemical approach to the conjugation of biomolecules.

It concerns the first application of nitrile oxide cycloaddition chemistry for the conjugation of a second molecule to an oligonucleotide (DNA, RNA).

It is a technology, which could also be attractive for protein, peptide or carbohydrate ligation, applicable to the polymer and materials industry as well as drug discovery, proteomics, peptidomimetics and genomics.

The trajectory of gene therapy of which the therapeutic RNAi (RNA interference) is a major player has been a roller coaster of enthusiasm followed by depression. Whilst much research has been directed to the application of oligonucleotide therapeutics as potentially revolutionary agents for disease treatment one very significant problem remains. As early as 1999 it was recognized that this potentially explosive therapeutic required **unique delivery** of the active agent.

It has been said that “there are only three problems in gene therapy –**delivery, delivery, and delivery.**”

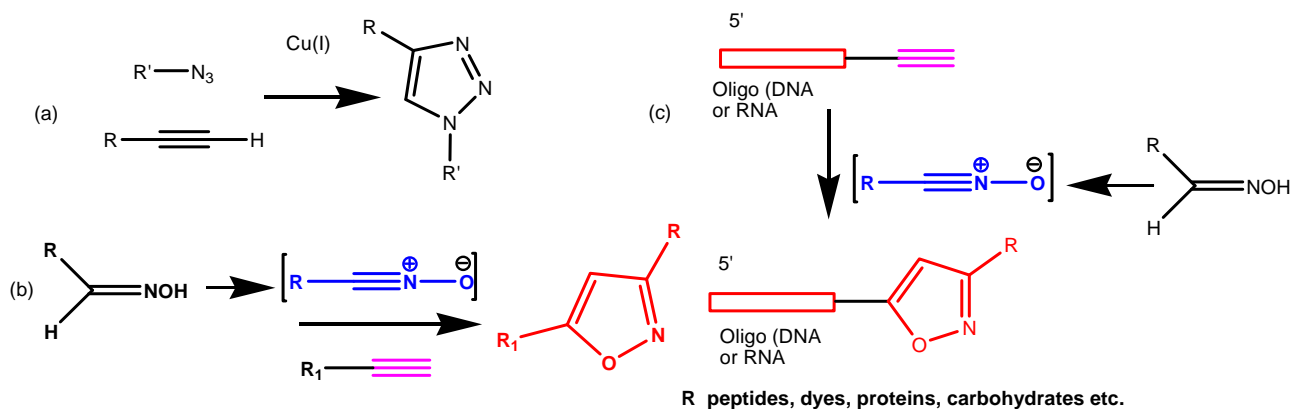


Figure 1a: Copper catalysed click chemistry

Figure 1b: Metal free click chemistry for bioconjugation (siRNA)

Figure 1c: Metal free bioconjugation on oligonucleotides for biopharmaceutical applications

The copper catalysed [3+2] azide-alkyne cycloaddition (figure 1a), considered the gold standard of click chemistry, has applications in labeling of oligonucleotides, peptides, proteins, oligosaccharides, detection of cell proliferation etc. This is an emerging technology (32 patents in last three years). The attachment of reporter groups (PEI or PEG, peptides and fluorescent tags etc) to biomolecules (oligonucleotides, DNA, RNA, proteins) is called bio conjugation. Unfortunately there are a number of limitations to the alkyne-azide click chemistry arising from the toxicity of copper and azide to living cells. Secondly, there is a requirement for inert reaction

conditions for bio-conjugation to oligonucleotide (DNA and RNA) to minimize Cu (I) mediated oxidative damage. This technical requirement makes the copper catalysed reaction unattractive to the biotech industry.

The first goal of the invention is to develop a robust copper and azide free bio-conjugation method for the post synthetic modification of oligonucleotides by conjugation, under physiological conditions, with different reporter group for example, peptides, lipids and dyes of interest. . The current invention involves conjugation by copper free click chemistry (1,3-dipolar cycloaddition) between nitrile oxides and alkynes. It as been developed with oligonucleotides (DNA and RNA) and simple nitrile oxides, future work will demonstrate the potential with peptides, lipids and polymers, (figure 1b-c). for the generation of substances having potential for applications in genomic research and genomic therapies including siRNA.

BENEFITS OF INVENTION:

The problems that the concept address is the identification of reaction conditions suitable for bio-conjugation under standard conditions and avoiding copper catalysts which are not attractive in the pharmaceutical industry

- (1) The concept will avoid the problems of handling potentially toxic and explosive organic azides by choosing the nitrile oxide as the reacting partner.
- (2) It will avoid the technically demanding requirement of most azide-alkyne reactions to be conducted under inert (air free) conditions. This requirement arises from the need for a Cu (I) catalyst in the azide-alkyne conjugation reaction.
- (3) It will avoid the need for the multi step (12 stages) synthesis of cyclic alkynes which is the only reaction to date describes copper free azide-alkyne cycloaddition. The hydrophobic nature of strained cyclic alkynes limit's the solubility in water and reducing their bioavailability.
- (4) The technology will provide pure chemically modified oligonucleotides in high chemical yield under physiological conditions by solid phase synthesis.
- (5) The products can be purified simply by washing.