Click chemistry and Oligonucleotides: How a simple reaction can do so much

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ABSTRACT
Copper catalyzed Alkyne Azide 1,3-dipolar cycloaddition (CuAAC) "click reaction" was applied for the construction of oligonucleotide conjugates, circular objects and phosphodiester glyco-clusters. To this end, several strategies were developed to introduce either alkyne or azide functions into an oligonucleotide.

INTRODUCTION
Oligonucleotides conjugates are widely used for various applications in biology, biotechnology and medicine. Furthermore, DNA is a versatile material for the construction of nano-objects. We have used the CuAAC or click reaction (1,2) as a versatile reaction for these applications. The main advantages of this reaction come from the chemoselectivity and the orthogonality of alkyne and azide with many other functionalities the efficiency of the reaction and the possibility to work in water or in organic solvents.

RESULTS AND DISCUSSION
We developed three strategies to introduce one or several alkyne functions into an oligonucleotide. Firstly, alkyne was introduced using an amideative oxidation of H-phosphonate diester linkages with CCl₄ in presence of propargylamine (3,4). Secondly nucleotide phosphoramidites bearing a pentynyl on the phosphorous atom were used affording phosphotriester linkage with a pentynyl group (5). Thirdly, different nucleosidic phosphoramidite and solid support derivatives bearing up to three alkynes were synthesized and introduced into an oligonucleotide (Figure 1).

Likewise, we developed a strategy to introduced an azide function into an oligonucleotide. Since azide reacts with phosphoramidite (6) it must be introduced after oligonucleotide elongation. For that purpose, we synthesized phosphoramidites bearing a 6-bromoethyl group (Figure 2) (5,7). After its incorporation and total elongation, the oligonucleotide was treated with sodium azide affording an azido oligonucleotide.

![Figure 2. Structure of phosphoramidite building blocks bearing a 6-bromoethyl group](https://academic.oup.com/nass/article-abstract/52/1/47/1106713/1)

These different building blocks were used for several constructions: Carbohydrate oligonucleotide conjugates, carbohydrate clusters and their small libraries, pseudopeptide oligonucleotide conjugates and circular oligonucleotides.

Carbohydrate oligonucleotide conjugates: Different constructions were performed providing oligonucleotide conjugated with one to ten carbohydrates with different inter-residues distance and spatial arrangements (Figure 3). They were synthesized from fluorescent DNAs with a polyalkyne scaffold which were clicked with carbohydrate azide derivatives by CuAAC under microwaves (3). They were then used for immobilization of carbohydrates on a DNA chip for further interactions with lectines (4).

![Figure 3. Structure of some fluorescent-oligonucleotide carbohydrate conjugates](https://academic.oup.com/nass/article-abstract/52/1/47/1106713/2)
Carbohydrate clusters and their small libraries: Solid supported poly-alkyne scaffolds with phosphorester linkages were synthesized by DNA chemistry. Then, they were either clicked with one or two carbohydrate azide derivatives affording respectively glyoclusters bearing the same carbohydrate (8) or small libraries of galactosyl-clusters bearing two different linkers dispatched in an aleatory manner (Figure 4).

**Figure 4.** Structure of fucosyl clusters and libraries of galactosyl clusters.

**Pseudo peptide oligonucleotide conjugates:** Starting from the same oligonucleotide polyalkyne scaffolds used for the synthesis of carbohydrate conjugates, we introduced by "click" several amine or guanidine residues affording pseudo peptide oligonucleotide conjugates (Figure 5).

**Figure 5.** Structure of pseudolysine or pseudoarginine oligonucleotide conjugates obtained by click.

Circular oligonucleotides: We introduced into the same oligonucleotide alkyne and azide functions starting from alkyne solid support and finishing the elongation with the bromohexyl phosphorylomide and eventually the azidation. Their circularization occurred during the click (5). We observed the monomolecular reaction when the reaction was performed in solution and a competitive intermolecular reaction when the reaction was performed on solid support. Thus in the latter case a mixture of cyclic mono-DNA and di-DNA was obtained. Alternatively, using the different building blocks we introduced alkyne and azide function at different positions into the sequence of the oligonucleotide that allowed circularization by click providing several cyclic objects exhibiting dangling sequences on 3'-end or on 3'- and 5'-ends (Figure 6).

**Figure 6.** Schematic representation of cyclic oligonucleotides obtained by click.

**CONCLUSION**

Oligonucleotides bearing alkyne groups were efficiently conjugated with different azide derivatives by CuAAC reaction assisted by microwaves. The conjugation was either applied on solid support or in solution. This strategy is very versatile and allows multiple labelling of oligonucleotide or polyalkyne scaffolds providing carbohydrate oligonucleotide conjugates, carbohydrate clusters, pseudo peptide oligonucleotide conjugates and circular oligonucleotides.

**REFERENCES**


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