Synthesis of an universal linker to label oligonucleotides via Click Chemistry

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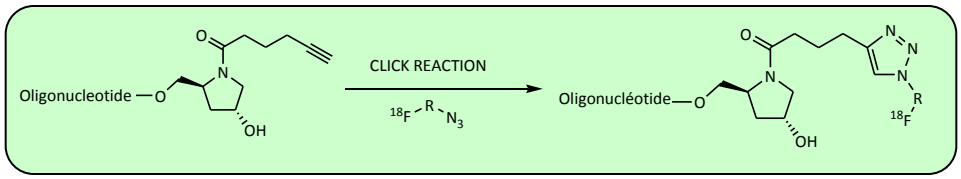
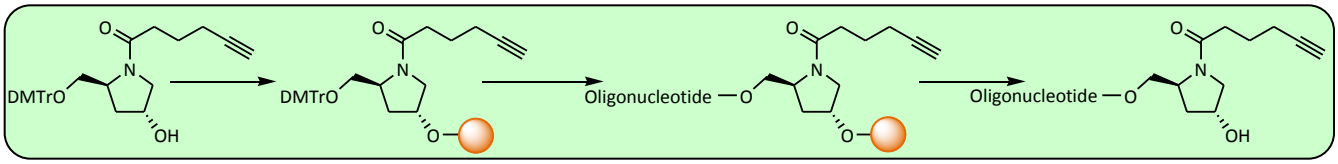
Introduction:

For more than 3 decades, oligonucleotides have been used for therapies, imaging and diagnostics. They are known to hybridize specifically with RNA of a complementary sequence on tissue sections and more recently to block the expression of target mRNA when administered in vivo (1). Positron emission Tomography (PET), with fluorine-18 ($t_{1/2}$ = 109.8 min) as a positron-emitting nuclide, is the most advanced technology currently available for studying in vivo molecular interactions and to assess the pharmacokinetics of new therapeutic agents such as modified oligonucleotides. One of the methods to connect the oligonucleotide and fluorine-18 by using prosthetic groups is Click chemistry, and more particulaly, the Huisgen 1,3 -dipolar cycloaddition (scheme 1).



Scheme 1. *"Click reaction" between our modified oligonucleotide and a synthon*

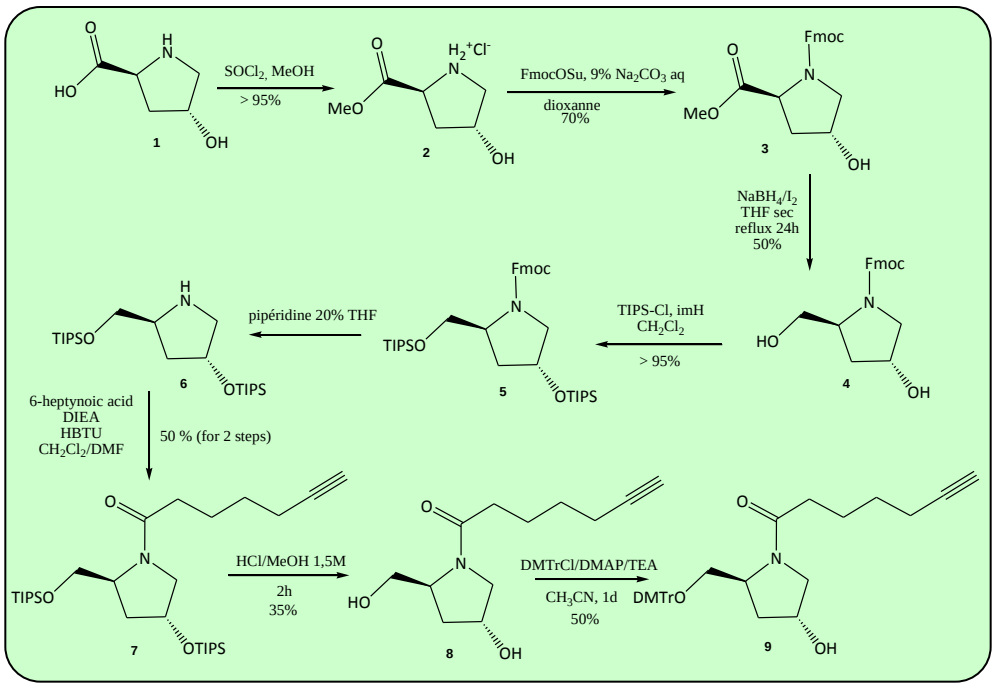
The originality of our strategy is the use of a universal linker diverted from the trans-4-hydroxy-proline which mimics a sugar of the oligonucleotide sequence and should improve their resistance to exonucleases. It owns an alkyne group which can be react by Click chemistry with the azide group on the prosthetic group. For the synthesis of the oligonucleotide on solid support, this linker has to have a secondary alcohol protected by a DMTr group and also a free primary alcohol as described in Scheme 2.



Scheme 2. *Synthesis of the modified oligonucleotide*

Synthesis :

The synthesis of this new linker is described in scheme 3. The total yield is 3% and all the compounds have been characterized by HPLC and ESI-MS and analyzed by RMN ^{1}H and ^{13}C. Crystallographic results for the compound 8 are exposed in figure 4 and 5. The crystal system is orthorhombic P212121, non centrosymmetric. These analyses proof the optical purity of the enantiomer.



Scheme 3. *Synthesis of the universal linker diverted from the trans-4-hydroxy-proline*

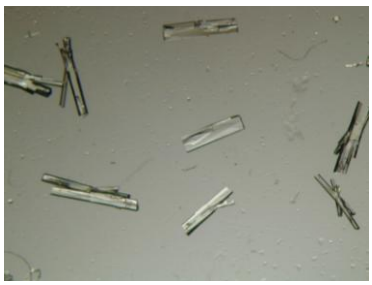


Figure 4: *Crystals of 8.*

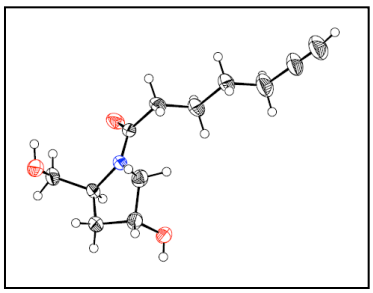


Figure 5: *Crystal structure (ORTEP) of the compound 8.*

Conclusion :

A new linker has been synthesized on a gram scale. The new 4-trans-hydroxyproline derivative is an universal linker and can be fixed to a number of different resins from which any oligonucleotide sequence can be synthesized.

Acknowledgements :

The authors wish to acknowledge the Laboratory of Structural Biological Chemistry, University of Namur, FUNDP for cristallographic analyses and the Région Wallone for financial support (OligoPET).

References :

(1) Viel, T., Kuhnast B.; Hinnen, F.; Boisgard, R.; Tavitian, B.; Dollé E.; "Fluorine-18 labelling of small interfering RNAs (siRNAs) for PET imaging." J. Label. Compd. Radiopharm. 2007, 50, 1159-1168.