SYNTHESIS OF OLIGONUCLEOTIDES CONTAINING A 3'-*S*-PHOSPHOROTHIOLATE LINKAGE

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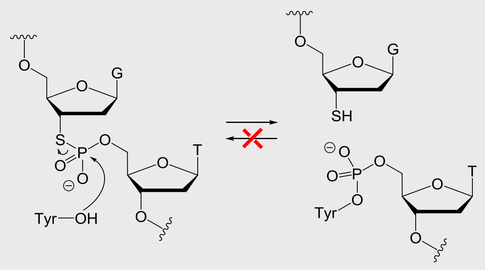
Introduction

Bacterial conjugation is a horizontal gene transfer and one way for translocation of antibiotic resistance and virulence genes. Conjugation of plasmids in bacteria includes two steps. A single stranded DNA copy is generated, which is transported to a recipient cell, catalysed by a multiprotein complex. This process is initiated by phosphodiester bond cleavage at a sequence specific site by the enzyme relaxase. Herein, the relaxase remains covalently bond to the 5'-terminus.[1] Relaxases can be classified based on their sequence and properties in families.[2] The relaxase MobM of the streptococcus plasmid pMV158 is the prototype of the MOB_V family. In contrast to the well-studied conjugation of plasmids from Gram-negative bacteria, information about the process in Gram-positive bacteria are still limited. First studies on functional properties and structural details of MobM are published.[3] But there is still a need for further investigations.

Concept

For a detailed study of the cleavage reaction, catalysed by the relaxase MobM of the streptococcus plasmid pMV158, the use of so-called "suicide oligonucleotides" can be useful. These oligonucleotides contain a phosphorothiolate linkage at the sequence specific cleavage site of the corresponding relaxase. Through this, the cleavage reaction is displaced towards the protein-DNA adduct, which can be used for biochemistry and crystallography experiments. This enables investigation of intermediate steps in relaxase reactions and was already used in studies of the relaxase TrwC.[4]



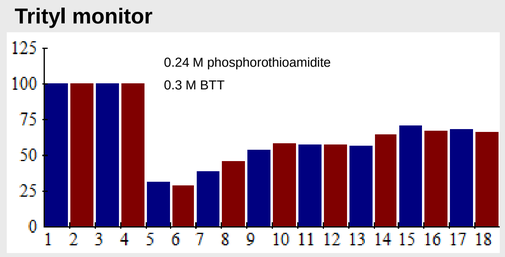
Objectives

The cleavage site of the relaxase MobM is located between a G- and a T-nucleoside of the plasmid DNA. Therefore, a suitable protected 2'-deoxyguanosine-3'-phosphorothioamidite is needed for the synthesis of the suicide oligonucleotide. One synthetic route for the formation of 2'-deoxyguanosine-3'-phosphorothioamidites was published by *Cosstick et al*.[5] We developed as well a synthetic route to obtain the needed *N*2-[(dimethylamino)-methylene]-5'-*O*-dimethoxytrityl-2'-deoxy-3'-thioguanosine-3'-*S*-[(2-cyanoethyl)-(*N*,*N*-di*iso*propyl)]-phosphoramidite.

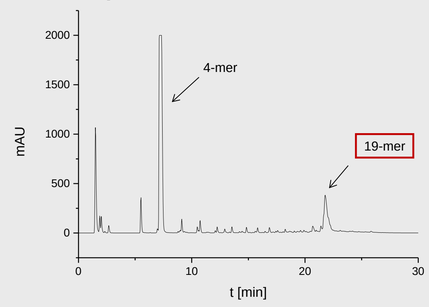
Oligonucleotide Synthesis

5'-ATAAAGTATAGTGTG*TTAT-3'

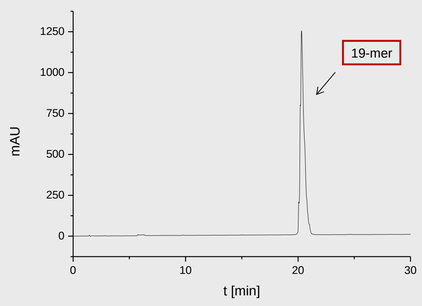
Trityl monitor

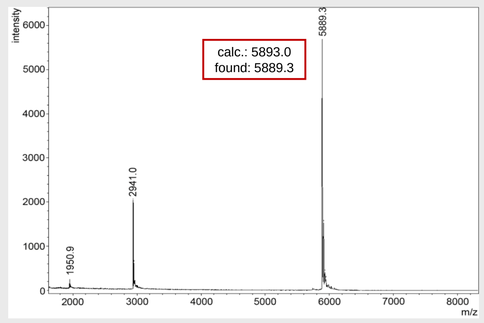


HPLC purification



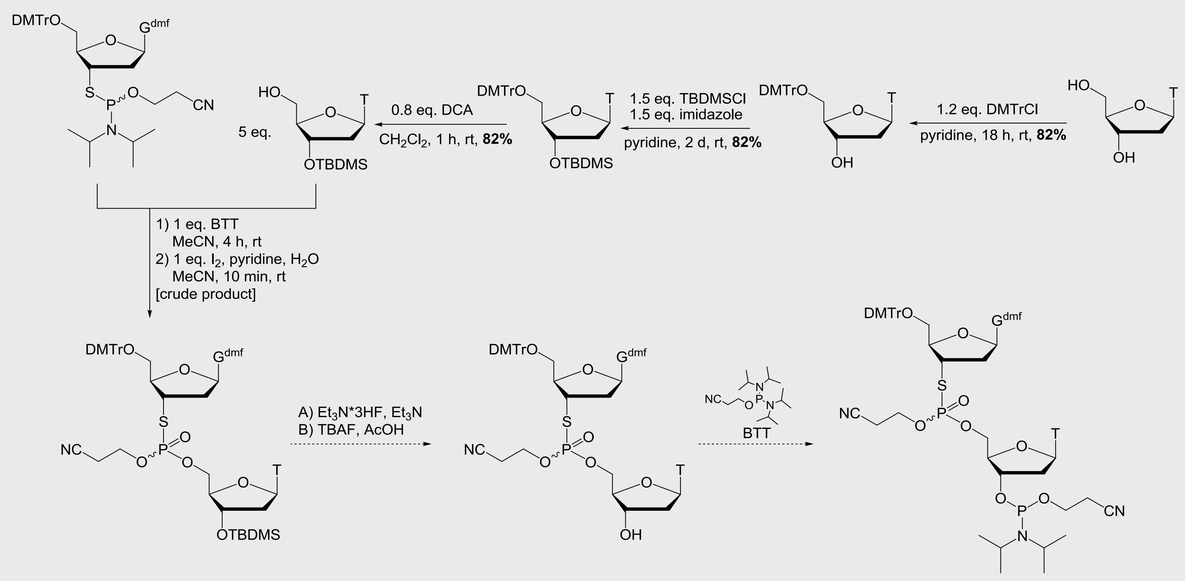
Purified oligonucleotide



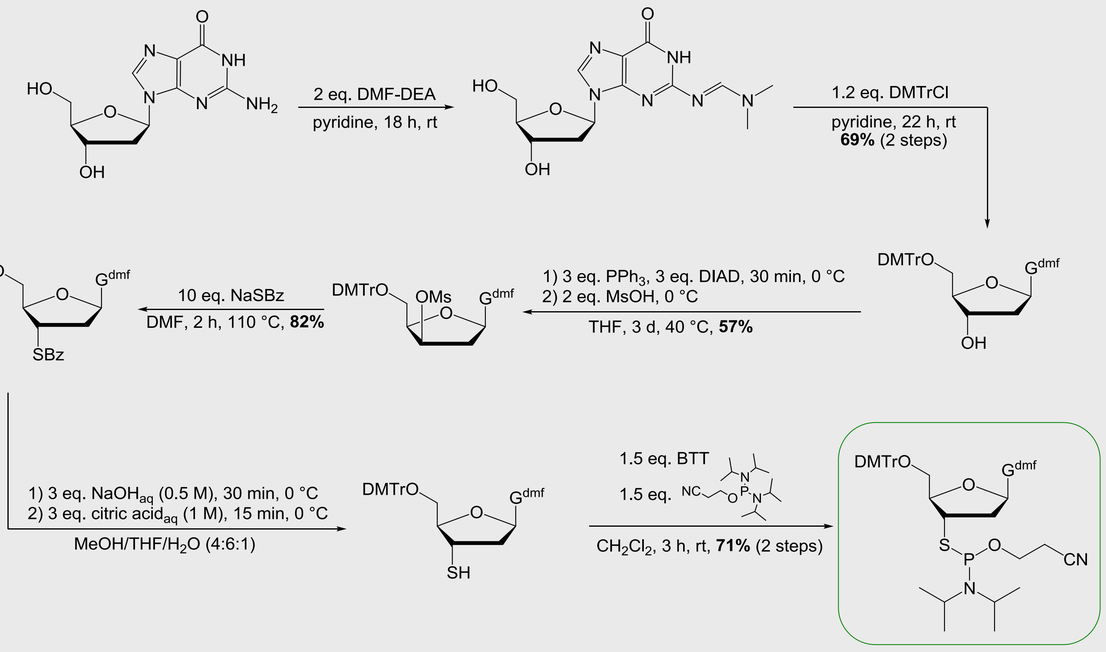


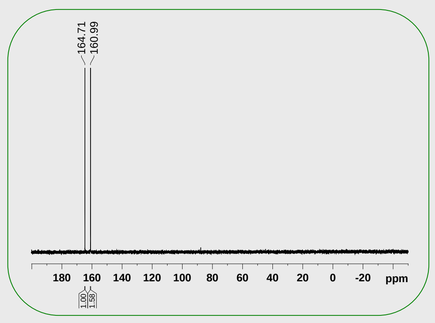
Dimer Synthesis

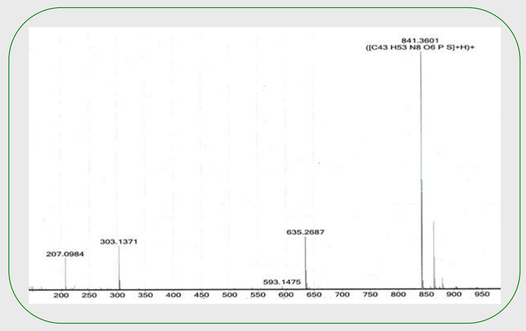
To overcome the problem of low coupling yields of the phosphorothioamidite in the oligonucleotide synthesis, a dimer containing the phosphorothiolate linkage could be synthesised at first. Subsequently, this should be used in solid phase synthesis.



Synthesis of the 3'-*S*-Phosphorothioamidite







Conclusion

We synthesised a 2'-deoxyguanosine-3'-phosphorothioamidite and applied it successfully in oligonucleotide synthesis of a 19-mer. Now, with the oligonucleotide, mechanistic studies of the cleavage reaction of relaxase MobM will be performed. To overcome the problem of the low coupling yields of the phosphorothioamidite in the oligonucleotide synthesis, we will synthesise a dimer containing a 3'-*S*-phosphorothiolate linkage for the use in solid phase synthesis.

References

[1] Llosa, M., Gomis-Rüth, F.X., Coll, M., de la Cruz, F. *Mol. Microbiol*., **2002**, *45*, 1-8. [2] Garcillán-Barcia, M.P., Francia, M.V., de la Cruz, F. *FEMS Microbiol. Rev*., **2009**, *33*, 657-687. [3] Lorenzo-Díaz, F., Dostál, L., Coll, M., Schildbach, J.F., Menéndez, M., Espinosa, M. *Nucl. Acids Res*., **2011**, *39*, 4315-4329; Fernández-López, C., Pluta, R., Pérez-Luque, R., Rodríguez-González, L., Espinosa, M., Coll, M., Lorenzo-Díaz, F., Boer, D.R. *J. Bacteriol*., **2013**, *195*, 3000-3008. [4] Gonzalez-Perez, B., Lucas, M., Cooke, L.A., Vyle, J.S., de la Cruz, F., Moncalián, G. *EMBO J*., **2007**, *26*, 3847-3857. [5] Piperakis, M.M., Gaynor, J.W., Fisher, J., Cosstick, R. *Org. Biomol. Chem*., **2013**, *11*, 966-974.

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