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Guidelines for the Use of Convertible Thioester Solid Supports

Version 1.3

Dear customer,

We hope that these guidelines will help you with using in oligonucleotide synthesis non-nucleosidic convertible thioester solid supports. Should you have any further questions, comments, or suggestions please feel free to contact us.

Oligonucleotides bearing functional groups at

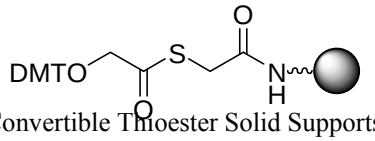


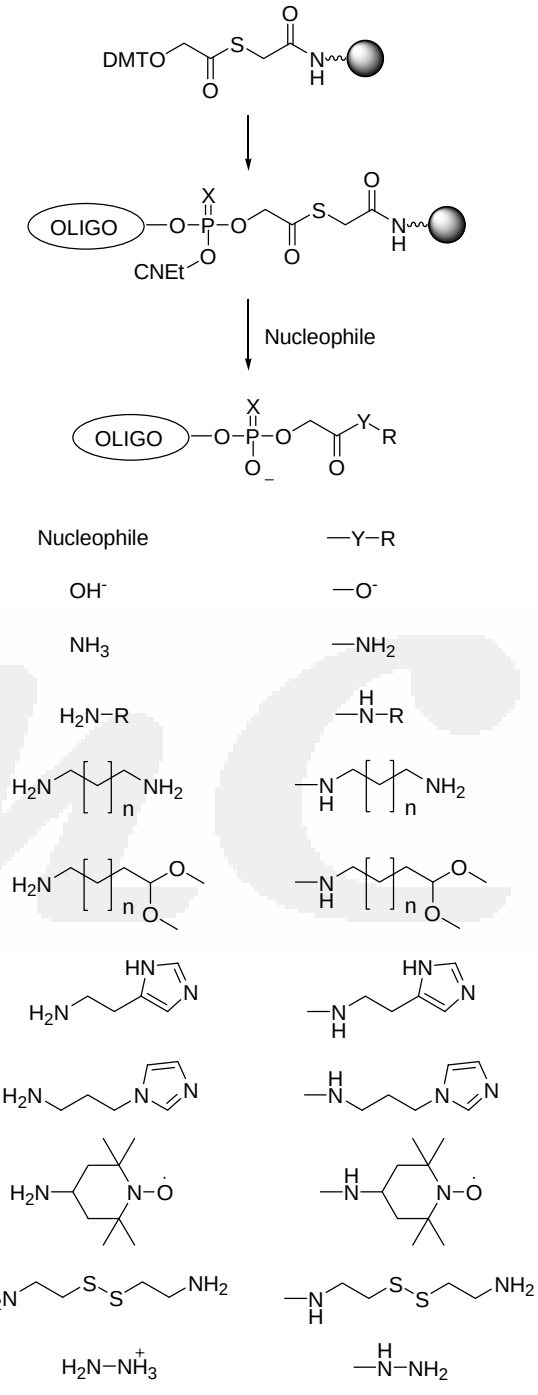
Table. Convertible Thioester Solid Supports Available from AM Chemicals.

Product #	Solid Phase Material
20211	CPG 500 Å
20212	CPG 1000 Å
20213	Polystyrene

their 3'-terminus are often used as a starting material in preparation of oligonucleotide conjugates and those labeled with a variety of reporter groups. Most often, amino-, carboxy-, and mercaproalkyl-modified oligonucleotides are used in conjugation and labeling reactions. The synthesis of these oligonucleotides is often carried out starting with a solid support designed to introduce a functional group respective for the solid support.

In our approach, a convertible solid support containing the reactive thioester group is used (Scheme).[1] Upon the completion of the oligonucleotide chain assembly, the support-bound material is treated with a nucleophile to be reacted with the solid support. For instance, when a support-bound oligonucleotide is treated with a diluted alkali, the thioester function undergoes the hydrolytic cleavage to release the oligonucleotide derivatized with the 3'-carboxylic function to solution.

In a similar fashion, the treatment with nitrogen nucleophiles i.e., ammonia, alkylamines, benzylamines, and hydrazines, introduces amido, alkylamido, and hydrazido groups, respectively.



Scheme. The Use of convertible solid supports.

A variety of substituted amines may be used for the introduction of different functions. Treatment

with diaminoalkanes results in oligonucleotides containing 3'-aminoalky group. A variety of polyamines i.e., spermine, tris(2-aminoethyl)amine, di-, tri-, and polyethylenimines gave the desired modified oligonucleotides. The reaction with histamine gave oligonucleotides 3'-derivatized with imidazole.

A mercapto group can be very efficiently introduced in an indirect manner by the reaction of the support-bound oligonucleotides with cystamine followed by the reduction of the disulfide with dithiothreitol.

A protected aldehydo group is introduced by treatment with ω,ω-dialkoxyalkylamines. Upon the completion of the base deprotection and HPLC-purification, the acetal protection is readily removed in the course of the final 5'-detritylation.

Protocols

There are no alterations to the standard protocols of oligonucleotide synthesis needed.

Upon completion of the oligonucleotide assembly, the support-bound material is typically treated with the desired nucleophilic reagent (100 µL for syntheses on 0.2 µmol scale; 200 µL/µmol of solid support for larger scales) for 30 min at room temperature.

- to introduce an amido group, the solid support is treated with concentrated aqueous ammonium hydroxide as required by the standard protocol of oligonucleotide deprotection;
- to introduce carboxylic functions, use 0.05 M aqueous NaOH;
- to form secondary amides, use 0.5 M aqueous solutions of the respective primary amines or diamines. With longer alkylamines, 4-amino-TEMPO (4-amino-2,2,6,6-tetramethyl piperidinyloxy free radical), and 3-(aminomethyl)-PROXYL (3-aminomethyl-2,2,5,5-tetramethyl-1-pyrrolidinyloxy free radical) poorly soluble in water use solutions of amines in ethyl alcohol, dioxane, or DMF.
- to prepare conjugates with polyamines, use 10% solution of the desired polyamine in 30% aqueous MeCN.
- to form hydrazides, use 0.2 M aqueous hydrazinium acetate or hydrazinium monohydrochloride.

The amines should not be used in concentrations lower that 0.2 M and higher than 1.5 M. At low concentrations, the oligonucleotide product will be contaminated with 3'-carboxylated oligonucleotides resulting from the partial hydrolysis of the thioester groups. At high concentrations of the amines, partial transamination of the cytidine nucleic bases may occur, particularly when the benzoyl protection is used.[2]

Upon completion of the derivatization, treat the reaction mixture as required by the deprotection protocol respective to the base protecting strategy used.

Reference

[1] Hovinen, J.; Guzaev, A.; Azhayeva, E.; Azhayev, A.; Lönnberg, H. Imidazole Tethered Oligodeoxyribonucleotides: Synthesis and RNA Cleaving Activity. *J. Org. Chem.* **1995**, *60*, 2205–2209.

[2] Hovinen, J.; Guzaev, A.;* Azhayev, A.; Lönnberg, H. Novel Solid Supports for the Preparation of 3'-Derivatized Oligonucleotides: Introduction of 3'–Alkylphosphate Tether Groups Bearing Amino, Carboxy, Carboxamido, and Mercapto Functionalities. *Tetrahedron* **1994**, *50*, 7203–7218.