Thiol Modifier Amidites and Supports

Acyclic Disulfide Thiol Modifiers & Cyclic Dithiolane Disulfide Thiol[1] Modifiers:

In the recent years, development of nanometer sized structures has received much attention for various molecular biological applications. Gold is probably the most suited element because it exhibits a high chemical stability (noble metal), is characterized by its ability to strongly absorb the visible light at definite wavelengths and is intrinsically not toxic. The thiol (R-SH) modified oligonucleotides serve as attractive tools with a vast number of potential applications in the field of nucleic acid chemistry such as it enables covalent attachment of variety of ligands that contain a) α,β-unsaturated ketone; b) maleimide c) other Michael acceptor groups or d) cysteines in proteins to make disulfide bonds. In addition to this, thiol has a strong specific interaction with gold surface to form reversible covalent bond with gold.

Nuzzo and Allara have discovered that reactive thiol group adsorb on gold surface and forms ordered mono layers.[2] After this, oligonucleotides with thiol group are very much used to generate self assembled monolayers (SAMs) on the gold surfaces. Although different molecules can be immobilized (silanes, carboxylic acids, pyridines, sulphites and thiols) on different surfaces (gold, silver, platinum, copper, mercury and glass), chemisorption of thiols on gold is a common and simple procedure to immobilize probes on a surface. DNA functionalized gold nanoparticles have since become widely used building blocks in key nucleic acid based assembly strategies and serve as unique probes for recognizing specific sequences in DNA segments.[3] as a building blocks for assembling novel structures and materials[4] and bio diagnostics and nano technology based therapeutics[5] It's been proven that formation of these monolayers is influenced by several factors such as temperature, solvent, buffer concentration, chain length of the adsorbate, cleanliness of the substrate, and rate of reaction with the surface and the reversibility of adsorption of the components of the monolayer. These applications depend on the reversible association of gold and sulfur bond between the attached oligonucleotide and nano particle.

ChemGenes offers a wide variety of acyclic & cyclic disulfide modifiers (Figure 1 & 2) for generation of single or multiple reactive thiol groups for labeling oligonucleotides or forming Gold-oligonucleotide conjugates.

Acyclic Disulfide Thiol Modifiers

- The most popular acyclic disulfide thiol modifiers **1**-**4** (Figure 1) are either with a C6 or C3 spacer arm.
- Efficient synthesis of C3 disulfide amidite **1** is not reported.[6]
- ChemGenes has developed an efficient reproducible, multi gram scale synthesis and purification of C3 disulfide amidite **1** in high purity (>94%) by ^{31}P NMR.[7]

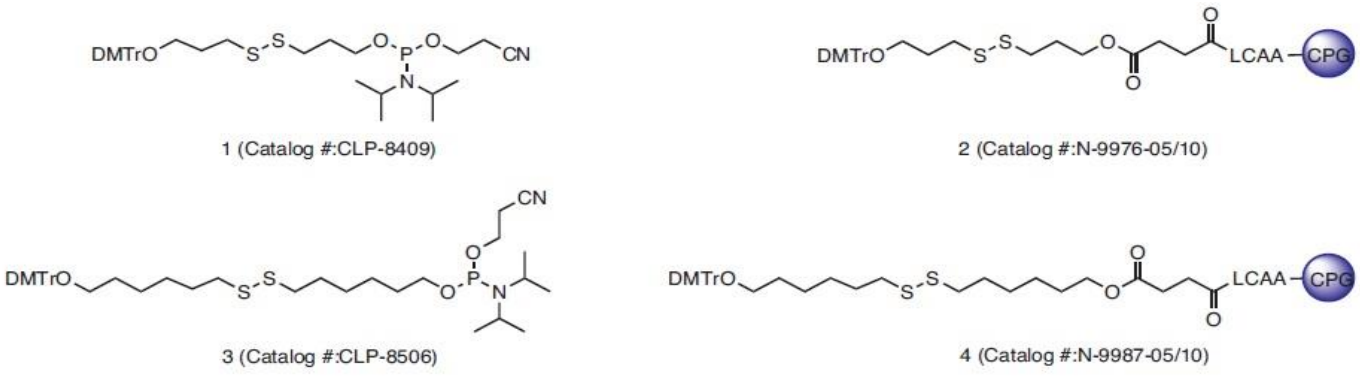
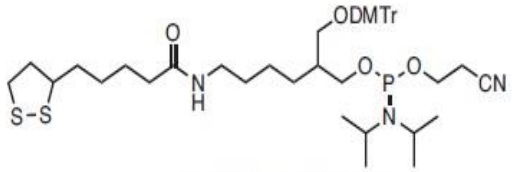


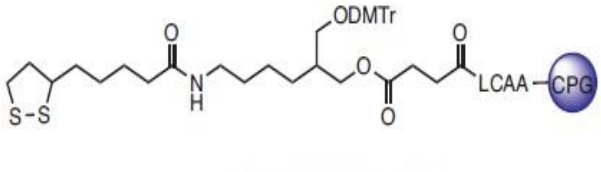
Figure 1: Chemical structures of acyclic disulfide modifiers.

Cyclic Dithiolane Disulfide Thiol Modifiers:[1]

We have developed novel dithiolane based thiol modifiers (**5**, **6** Figure 2) for introduction of thiol groups.



5 (Catalog #:CLP-8407)



6 (Catalog #:N-8408-05/10)

Figure 2: Chemical structures of cyclic disulfide modifiers.

The oligonucleotides attached with single thiol group are unstable during the washing steps and formation of stable attachment of oligonucleotides is very important property for its success in applications such as for DNA chip technology. The covalent bond between gold and sulfur is in the order of magnitude from 30 to 40 Kcal/mol, which is relatively weak in order to anchor a biopolymer onto a surface.[8]

It's been reported that oligonucleotides that are conjugated with mono functional thiol group are slowly lost at higher temperatures and also in the presence of high salt concentration buffers.[9] The stability studies by Letsinger *et. al.* on SAMs of oligonucleotides that are conjugated to gold surface by mono thiol group revealed 20 that these are completely displaced by treating with the buffers containing DTT.[10] This feature limits applications of these probes in solutions containing thiols such as a PCR solution that has DTT as a stabilizer for the polymerase enzyme.

Advantages of Cyclic Dithiolane Derivatives:

- Each incorporation introduces two thiol groups.
- Dithiolane modifier has long spacer arm with symmetrical branching chain.
- These modifiers can be used to introduce at any desired site of an oligonucleotide
- With two thiol groups, Dithiolane derivatives can potentially form very stable self assembled monolayers with gold nano-particles and these are schematically represented in Figure 3.