




Experimental Procedures

Steps	Procedure Details
	<p>Treatment Scheme</p> $\text{HO}-\text{C}_6\text{H}_{13}-\text{S}-\text{S}-\text{C}_6\text{H}_{13}-\text{O}-\text{P}(=\text{O})(\text{OH})-\text{O}-\text{Oligonucleotide} \xrightarrow{\text{DTT}} \text{HS}-\text{C}_6\text{H}_{13}-\text{O}-\text{P}(=\text{O})(\text{OH})-\text{O}-\text{Oligonucleotide} + \text{HO}-\text{C}_6\text{H}_{13}-\text{SH}$ <p>Structure delivered</p> <p>Structure after treated</p> <p>Ethyl acetate Extraction</p> <p>Remove</p> <p>DTT</p>
<p>1</p>  <p>Dissolve dried oligonucleotides</p>	<p>1. Dissolve the dried thiol-modified oligonucleotide (5 O.D. based) with 50 µl of nuclease-free water or an appropriate buffer, e.g., 0.1 M triethylammonium acetate (TEAA), pH 7.5.</p>
 <p>Incubate the mixture</p>	<p>2. Add 10 µl of 1 N dithiothreitol (DTT)* into an oligonucleotide tube and mix by vortexing and briefly spin down.</p> <p>* 1 N DTT: Dissolve 1.545 g of DTT in 20 ml of 0.01 M Sodium acetate, pH 5.2 and filter with 0.22 µm syringe filter.</p> <p>3. Incubate the mixture for 15 min at room temperature.</p>
<p>2</p>  <p>Remove excess DTT with ethyl acetate</p>	<p>4. After the incubation, add 50 µl of ethyl acetate to remove excess DTT and unwanted thiol fragments from the thiol-modified oligonucleotide mixture and mix by vortexing.</p> <p>5. The mixture will separate into two layers, discard the upper layer of the mixture after it is separated.</p> <p>* Note: Upper layer contains DTT with ethyl acetate and lower layer contains oligonucleotides in the aqueous phase.</p> <p>6. Proceed immediately to the next step since the free sulfhydryl group becomes unstable after the removal of DTT.</p> <p>7. Repeat step 4-6, 3 times.</p>