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***Review Article***

# THE EFFECT OF 3'-BRIDGING SULFUR ON THE SYNTHESIS, PROPERTIES, AND STRUCTURAL STABILIZATION OF OLIGONUCLEOTIDES

***Mohamed Ibrahim Elzagheid**
*Chemical and Process Engineering Technology Department, Jubail Industrial College, Jubail Industrial City, 31961, Kingdom of Saudi Arabia*
**Author for Correspondence*

## ABSTRACT
The synthesis of 3'-bridging sulfur oligonucleotides was elaborated and its impact on the properties and structural stabilization was discussed. The 3′-PS linkages provide such oligonucleotides with unique properties. They can be cleaved by snake venom phosphodiesterase (SVPD), silver ion and also Iodine into several products, but they are resistant to cleavage by nuclease P1 and bovine spleen phosphodiesterase.

***Keywords:*** *RNA/DNA, Oligonucleotides, 3'-S-Phosphorothiolates, 3'-Bridiging Sulfur*

## INTRODUCTION
Sulfur for Oxygen substitutions within the phosphodiester backbone (Figure 1) has received great attention. Among those are the phosphorthiolates in which sulfur replaces one of the bridging phosphodiester oxygen within a linkage. The replacement can take place in the 3'- or 5'- positions. Phosphorothiolate linkages, in which sulfur replaces the 3′-bridging oxygen connected to furanose has a difficult synthetic challenge. Nevertheless, they also represent interesting synthetic targets as future potential therapeutics (Eckstein, 1985; Agrawal and Zhao, 1998).

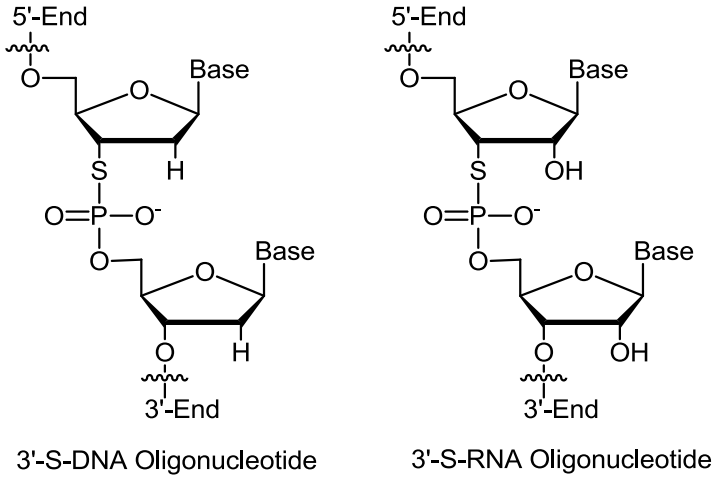
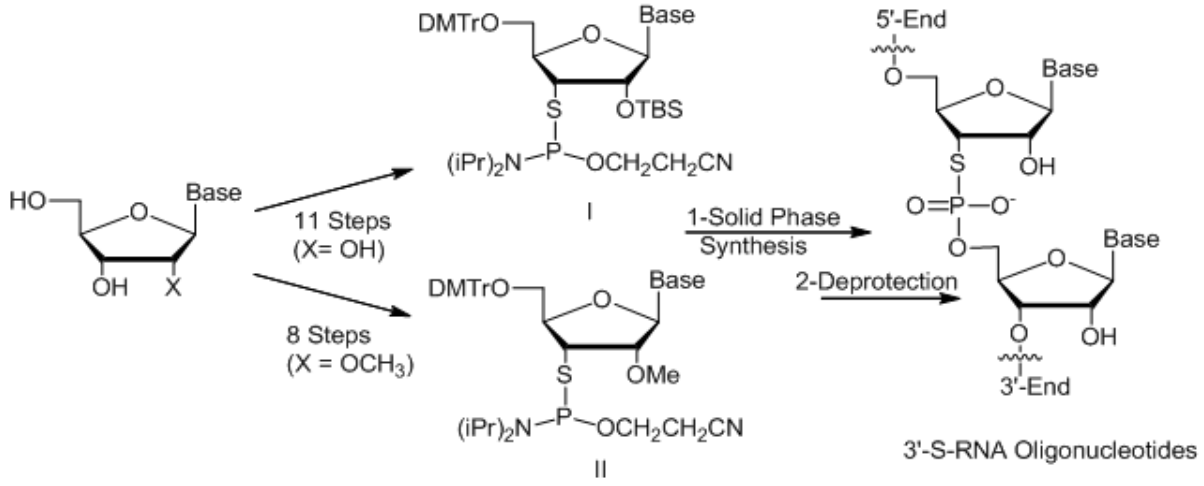



**Figure 1: Structures of DNA/RNA Oligonucleotides containing 3'-S-Bridging Sulfur Atom**

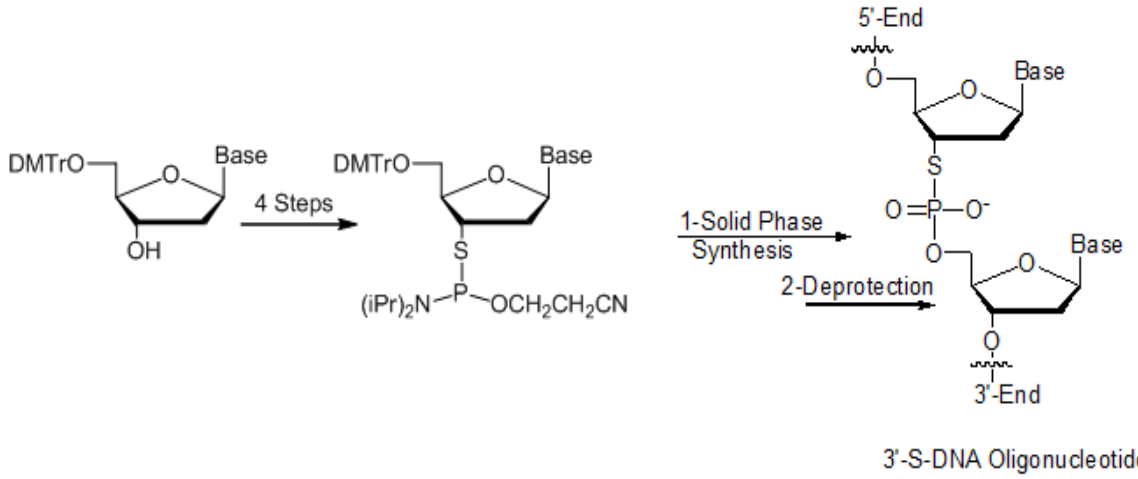
### *Synthesis*
Reese and his team initiated the synthesis of 3′-*S*-phosphorothiolate RNA dinucleotides (Liu and Reese, 1995; Liu and Reese, 1996) and Cosstick and his colleagues applied the phosphoramidite chemistry to the construction of 3′-PS linkages (Cosstick and Vyle, 1989). This phosphoramidite chemistry was implemented in the synthesis of oligonucleotides containing an RNA dinucleotide 3′-PS linkage. For example, the 2′-*O*-TBS-3′-*S*-phosphoramidites I was prepared from the corresponding 3′-*S*-thiol-nucleosidesin high yields (Sun *et al.*, 1997) and similarly 2′-*O*-TBS-3′-*S*-phosphoramidites was

synthesized in 11 steps starting from guanosine derivative (Matulic-Adamic and Beigelman, 1999). Another similar strategy was used to prepare the 2′-*O*-methyl-3′-*S*-thioguanosine phosphoramidite II in eight steps starting from 2′-*O*-methylguanosine (Scheme 1) (Lu *et al.*, 2008).

The 3′-S-thionucleoside phosphoramidites are then incorporated into RNAs via solid-phase synthesis. Although, coupling of 2′-deoxy-3′-S-phosphoramidites (Scheme 2) can be accomplished via a fully automated protocol (Gaynor *et al.*, 2007), the3′-S-ribonucleoside phosphoramidites are less reactive and require manual coupling (using p-nitrophenyltetrazole as an activator) (Sun *et al.*, 1997; Lu *et al.*, 2008).




**Scheme 1:** The synthesis of 3′-S-Ribonucleoside phosphoramidites and their oligonucleotides, TBS= tertiary butyldimethylsilyl, DMTr= 4, 4'-dimethoxytrityl, Me= methyl, iPr= isopropyl, Base= any nucleic acids base




**Scheme 2:** The synthesis of 3′-S-nucleoside phosphoramidites and their oligonucleotides, DMTr= 4, 4'-dimethoxytrityl, Me= methyl, iPr= isopropyl, Base= any nucleic acids base.

***Properties***

3′-PS linkages provide nucleic acids with unique properties, as summarized in Figure 2 (Liu and Reese, 2000; Weinstein *et al*., 1996; Lara *et al*., 1996). Snake venom phosphodiesterase (SVPD), silver ion and also Iodine cleaves the P-S bond to yield several products. On the other hand, dinucleotides having 3'PS

linkages are resistant to cleavage by nuclease P1 and bovine spleen phosphodiesterase. The P-O bond can be cleaved by sodium hydroxide, hydrochloric acid and RNase T1 and T2.

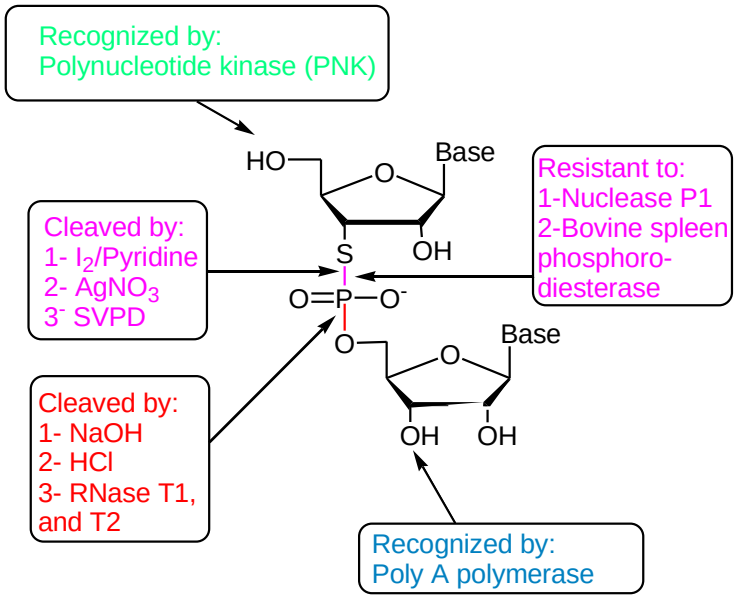



**Figure 2: Summary of the Unique Properties of Dinucleotides having 3'PS Linkages**

***Structural Stabilization***

Most nucleosides exhibit sugar puckers that equilibrate between C3'-endo (North, or A-form RNA) and C2'-endo (South, or B-form DNA) conformations (Figure 3). Preference for a North or South conformations has great impact on nucleic acid structure and biological function (Ken *et al*., 2015; Nan-Sheng *et al*., 2011).

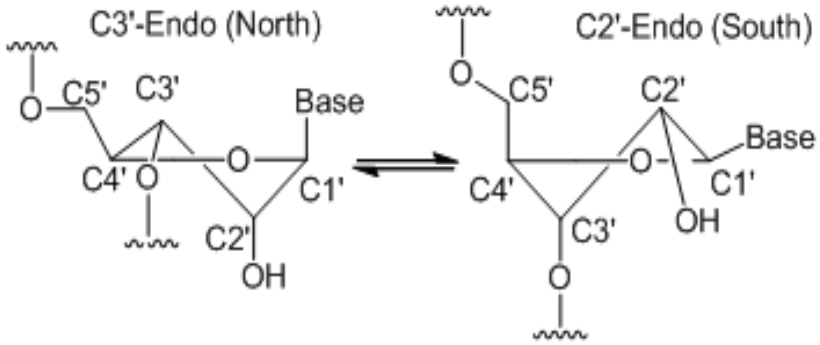



**Figure 3: Equilibrium between C3'-Endo and C2'-Endo Conformations in RNA**

The 3′-PS linkage has a great influence on the conformation of the sugar moiety. In particular, 3′-*S*-phosphorothiolates reinforce the "north" conformation (Figure 4) not only for their own sugar, but also (to a lesser extent) for the sugar of the subsequent nucleotide.

This effect is thought to be caused by the favorable stacking of the heterocyclic bases (Jayakumar *et al*., 2007; Kathryn *et al*., 2015).

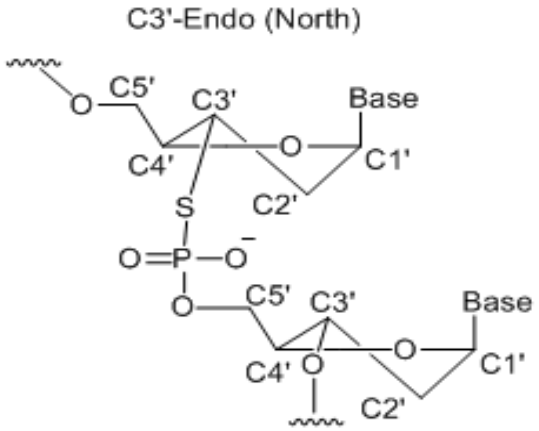



**Figure 4: The Sugar Pucker of the DNA -3'-S-Phosphorothiolate Exhibits C3'-Endo Conformation**

The adaption of 3′-PS sugars of the north conformation has increased the interest in these substitutions as stabilizers of A-form helical structural geometry of the nucleic acids for therapeutic applications. For example, by forcing antisense DNA to adapt an RNA-like conformation, 3′-*S*-phosphorothiolates increase the $T_m$ of DNA: RNA duplexes by two degree Celsius per each modification (Beevers *et al*., 2004). Due to this effect they can be considered as potential modulators of transcriptional repression induced by siRNAs (Gaynor *et al*., 2010).

## CONCLUSION

Oligonucleotides containing these linkages remain difficult to synthesize and manipulate. As described herein, chemists have succeeded in constructing the desired oligonucleotides, but in comparison to unmodified oligonucleotides, their synthesis generally requires much more effort and experience. Once synthesized, 3′-PS oligonucleotides need special precautions during purification and storage to avoid cleavage at the modified linkages due to their greater liability.

Both chemical and enzymatic approaches for preparing these oligonucleotides have improved greatly over the years since the linkages were first described. However, significant synthetic challenges remain, notably due to the lack of an efficient fully automated solid-phase protocol. Ongoing research should continue to expand the stock of available synthetic methods, and applications for these unique modified oligonucleotides.

## ACKNOWLEDMENT

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