

● USE OF RNATHIOPHOSPHORAMIDITES AND PURIFICATION GUIDELINES PHOSPHORODITHIOATED siRNAs

Usage parameters on oligo synthesizers:

Coupling: 5 – 8 minutes

Sulfurization: the EDITH reagent has been used for sulfurization with 3 minute exposure

Deprotection: no changes needed from standard RNA methods

Storage: refrigerated, dry

Stability in solution: < 3 days

Concentration on Synthesizer: 0.1 - 0.15mM

Purification of PS2-RNA oligos

Anion exchange liquid chromatography for high resolution analysis and purification at pH 8.

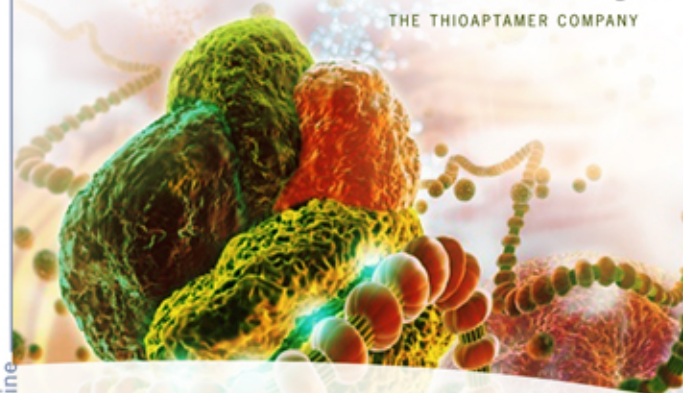
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● SEEKING COLLABORATORS:

AM Biotechnologies is actively seeking research partners who are willing to collaborate to provide data regarding the utility of PS2-modified siRNAs as therapeutics. For our research collaborators, AM Biotechnologies will provide monomers and advice regarding placement of PS2 modifications.

AM Biotechnologies also offers contract discovery of thioaptamer binding agents to customer-provided targets as well as small-scale synthesis of PS2-modified oligonucleotides.

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ENHANCED siRNA ACTIVITY WITH PHOSPHORODITHIOATED MODIFICATIONS

- :: Better Activity
- :: Achiral
- :: High Nuclease Resistance
- :: Increased Stability in vivo



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● INTRODUCTION

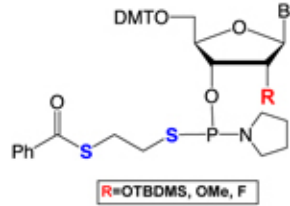
AM Biotechnologies now offers a product line of thio-phosphoramidites for the introduction of an achiral dithio backbone (phosphorodithioate) into custom synthetic RNA or siRNA oligonucleotides. We offer 2'-F, 2'-OMe, 2'-OTBDMS protected thio RNA monomers. These reagents are used in a conventional DNA/RNA synthesizer employing a slightly modified coupling protocol.

The introduction of a phosphorodithioate into a synthetic RNA oligo offers a multitude of prospective benefits:

1. Higher gene silencing activity.
2. Lower melting temperature, which may facilitate the unwinding of the siRNA by the activated RISC;
3. Increased siRNA nuclease resistance;
4. Achiral, thereby eliminating the biochemical and bioanalytical variability associated with diastereometric monothioate substitutions.
5. Increased hydrophobicity of sulfur-modified oligonucleotides may stimulate the cellular uptake of siRNA into human cells.

● **PRODUCT DESCRIPTION (structures)**

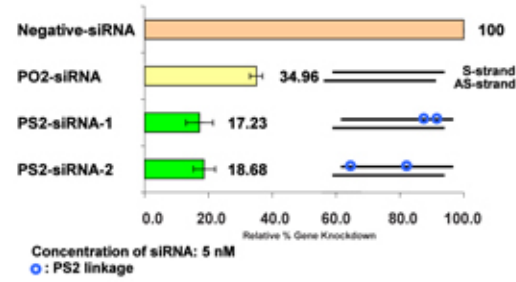
Structure of 2'-Modified-thiophosphoramidites



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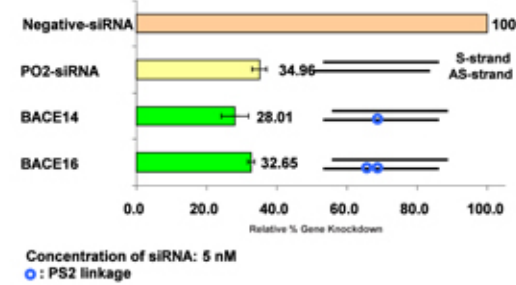
● **EVIDENCE SUPPORTING APPLICATIONS OF PS2-siRNA**

Comparative Analysis of PO2-siRNA and PS2-siRNA Silencing BACE1 Gene



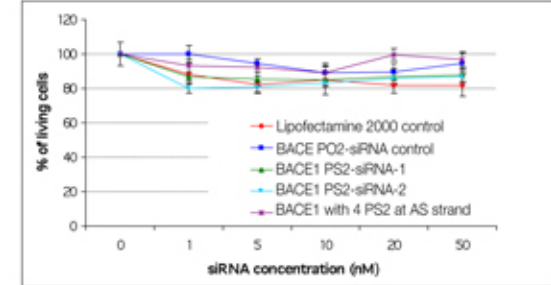
PS2 modification of the sense strand gives better gene silencing activity for the BACE1 gene.

Comparative Analysis of PO2-siRNA and PS2-siRNA Silencing BACE1 Gene



PS2 modification of the antisense strand give better gene silencing for the BACE1 gene.

Cytotoxicity of PS2-siRNAs Compared to Unmodified Positive PO2-siRNAs

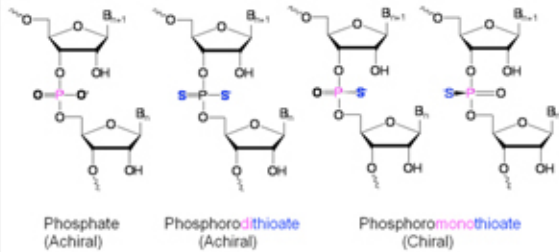


No observed cytotoxicity for PS2-siRNAs.

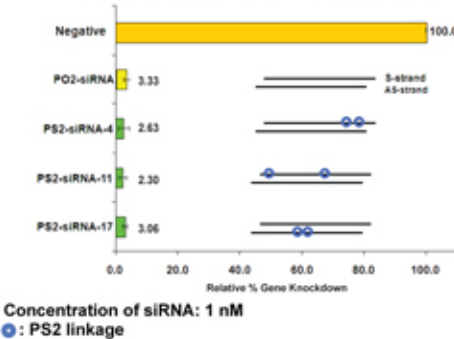
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Structure of the Different RNA Backbones

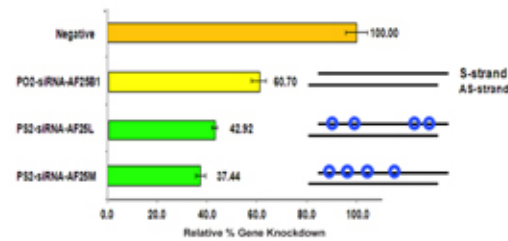


Comparative Analysis of PO2-siRNA and PS2-siRNA Silencing GFP Gene



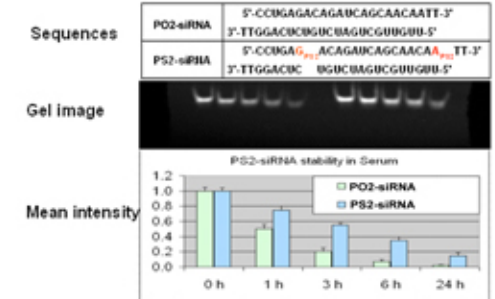
PS2 modification of either the sense or the antisense strand gives better gene silencing for the GFP gene.

Comparative Analysis of PO2-siRNA and PS2-siRNAs Silencing Luciferase Gene



PS2 modification of the sense strand give better gene silencing for the Luciferase gene.

Stability of PS2-siRNA versus PO2-siRNA in Serum



PS2 modified siRNAs have enhanced stability relative to unmodified siRNA in serum.

EXPANDING THE OLIGO MODIFICATION TOOL BOX