BMEG Oligonucleotides For Efficient Cellular Delivery

ChemGenes now offers BMEG Masked Oligonucleotides For Targeted Cellular Delivery of Oligonucleotides.[1,2]

- There is great demand in fields of gene therapy and molecular biology for methods or compounds that effectively deliver bio-molecules such as proteins, nucleic acids and other biologically active molecules into cells, tissues and organs.
- In order for an oligonucleotide to be useful in therapeutics, an effective amount of active bio-molecule must be delivered into the target cells or tissue. At the same time, nucleic acid delivery methods should minimize immune responses or cytotoxicity to the host.
- Current methods for delivery of negatively charged bio-molecules include viral and non-viral based delivery systems. Even though these methods have several advantages, they suffer from drawbacks such as narrow range of cell infectivity and cytotoxic to the cell. Accordingly, there exists a need for improved biomolecule delivery systems.
- ChemGenes now offers novel BMEG (S-isobutanoyl 2-(2-mercaptoethoxy)ethoxyl) phosphoramidite monomers (Figure 1). BMEG serves as irreversible masking group for efficient cellular delivery.

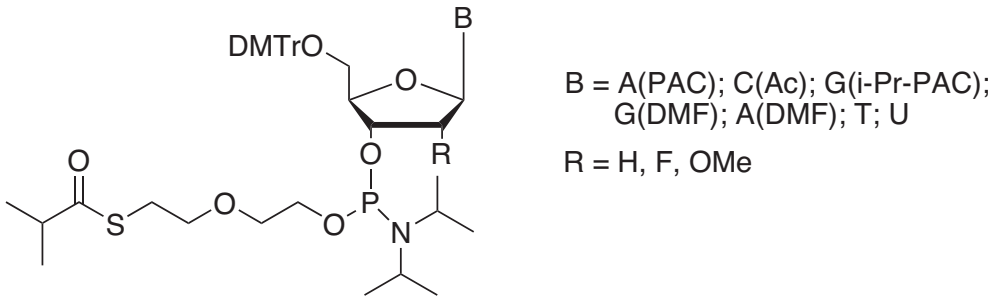

Figure 1: Structure of the various BMEG deoxy and ribo phosphoramidites.

Advantages of BMEG group:

- BMEG thioesters are thermally stable due to highly unfavored 8 membered transition state.
- Sterically bulk isopropyl group of the thioester chemically stabilizes the center to base.
- Lipophilicity of the BMEG group helps in improved cellular delivery across cell membranes *in vitro* and *in vivo*.
- BMEG group reversably masks negative charge(s) of an oligonucleotide. As a result, oligonucleotides containing these modifications have less negative total charge when compared to RNA or DNA of the same sequence.
- Since the overall reduced negative charge in these bio-polymers, they are called ''Ribo-Nucleic Neutral (RNN)'' and ''Deoxyribo-Nucleic-Neutral (DNN)''.
- BMEG phosphate protecting groups do not interfere with duplex formation.

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Oligonucleotide synthesis:

- BMEG phosphoramidite monomers are protected with ultra-mild deprotection groups such as Phenoxy-acetyl (PAC), isopropylphenoxyacetyl (iPrPAC); Acetyl (Ac) and Dimethylformamidine (DMF). It is recommended to use mild deprotecting supports such as Q-supports or oxalyl supports.
- The full length siRNN constructs of 21 nucleosides with at least 5 siRNN nucleotide insertions are sucessfully synthesized, isolated and cleanly purified.
- BMEG masked oligonucleotides are engineered to be bio-labile, such that the BMEG group(s) are cleaved from the biomolecule upon intracellular delivery and results in the Bio-compatible byproducts (Figure 2).

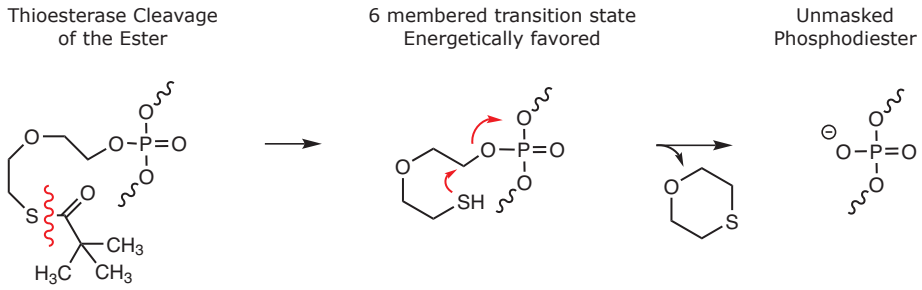

Figure 2: Schematic illustration of the cleavage mechanism of BMEG group inside a cell.

Applications of BMEG olgionucleotides in siRNA technology:

- Figure 3 shows results form knockdown of Green Fluorescence Protein (GFP) expression using siRNN that contain five BMEG monomers (BMEG 5N g2Mod/p2Mod15 nM). U2F 5N g2 Mod is a positive control SiRNA that demonstrates the maximum achievable level of reduction of dGFP expression by a construct that does not have phosphotriester protecting groups.
- The results of this experiment indicated that siRNN oligos that contain five BMEG monomers were able to load into the RISC complex and elicit a reduction in protein expression intracellularly up to 94%.

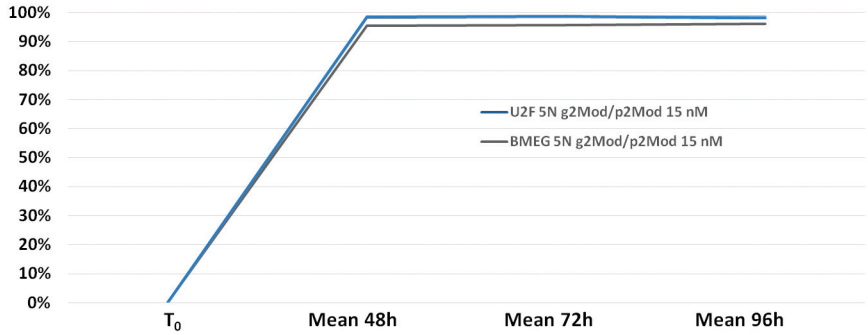

Figure 3: Green Fluorescence Protein (GFP) knockdown as response over time is shown for selected siRNN at 48 h, 72 h and 96 h.

References:

1. Petersen, S. G. Patent number **WO 2010039543 A2** "Self delivering bio-labile phosphate protected pro-oligos for oligonucleotide based therapeutics and mediating RNA interference"; 2. **World wide exclusive license to ChemGenes Corporation, USA.**

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