Guanidinium Morpholinos (GMOs) as Therapeutics

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Introduction

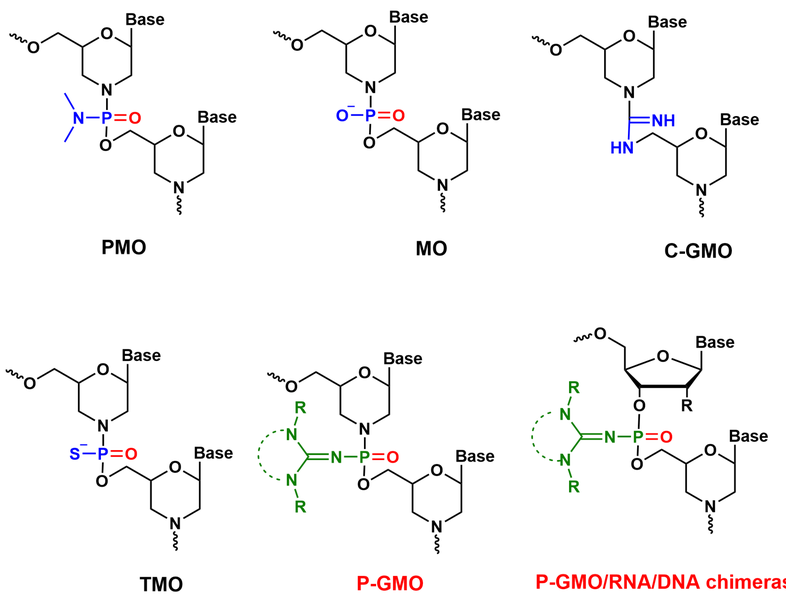
Guanidinium morpholinos (GMOs) are synthetic oligonucleotide analogs containing a modified Morpholino (PMO) backbone with a positively charged or neutral guanidinium group attached to non-bridging position of phosphorous. The resulting enhanced cellular uptake of GMOs allows direct cellular delivery.

Several therapeutic oligonucleotide drugs containing morpholino motifs have been approved, including Exondys 51® in 2016, Vyondys 53® in 2019, Viltepso® in 2020, Amondys 45® in 2021, and Elevidys in 2023. Five of these are for the treatment of Duchenne muscular dystrophy. Several other morpholino oligonucleotide drugs are currently undergoing clinical trials. Previous studies have confirmed that PMOs have a high binding affinity to target mRNA. PMOs are sequence-specific, water-soluble, and low-toxicity. Various morpholino modifications have been explored, including phosphor-diamidate morpholino (PMO), thiomorpholino (TMO), morpholino oligonucleotide (MO), positively charged guanidinium morpholinos (C-GMO) and neutral guanidinium morpholinos (P-GMO) .

Since the delivery of macromolecular drugs into cells is a major challenge in oligonucleotide therapeutics, ongoing efforts aim to identify biomolecules that facilitate the efficient uptake of therapeutic drugs into cells.

Morpholino oligonucleotides (MOs) are tools for gene silencing through RNA modulation. However, to fully realize their therapeutic potential, it is crucial to address cellular uptake and specificity challenges effectively. Enhancing MOs with peptide conjugates and fluorescent compounds can significantly improve their delivery efficiency and tracking capabilities within living systems.

Types of Morpholino Modifications



Key Applications of PMOs and GMOs

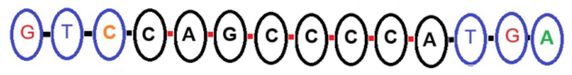
Application area	Use
Developmental Biology	Investigating gene function in embryos, for example in Zebrafish, Xenopus, and Sea Urchins, via microinjection.
Therapeutics	As exon skipping ASOs. For example, treating genetic diseases like **Duchenne Muscular Dystrophy (DMD)** by skipping faulty exons to restore a functional reading frame.
Viral Research	Antisense PMOs of GMOs for blocking highly conserved regions of viral RNA genomes to inhibit replication, for example, targeting Ebola or SARS-CoV-2

Cross linker for the conjugation of P-GMO with CPP

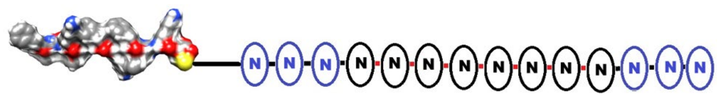
Currently, we are exploring different crosslinking methods including terminal azide conjugation with which we had success as well.

Design Examples of PN Morpholino modified oligonucleotides

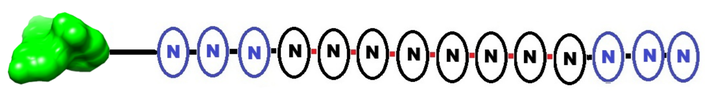
[1] PN Morpholino Gapmer



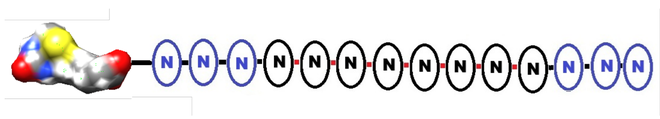
[2] PN Morpholino Gapmer with 5'- CPP Conjugate



[3] PN Morpholino Conjugate with 5'-FAM



[4] PN Morpholino Conjugate with 5'-Biotin



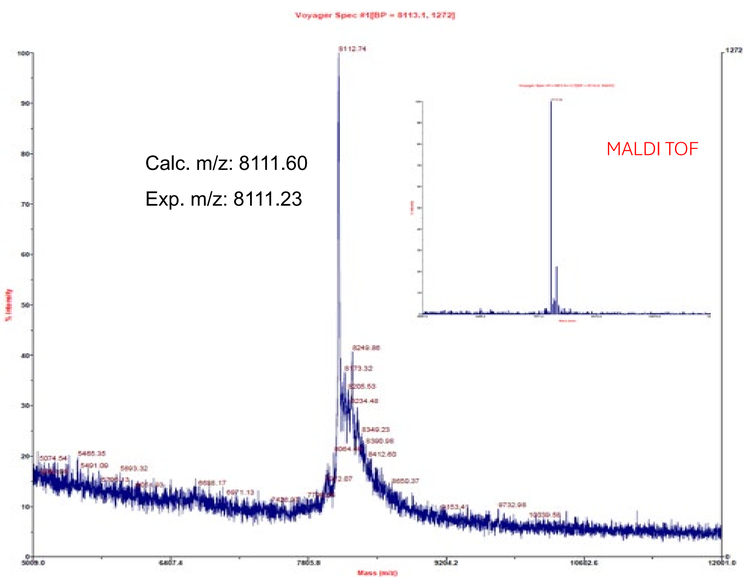
■ = PO ■ = PS (N) = PN Morpolino (N) = dN

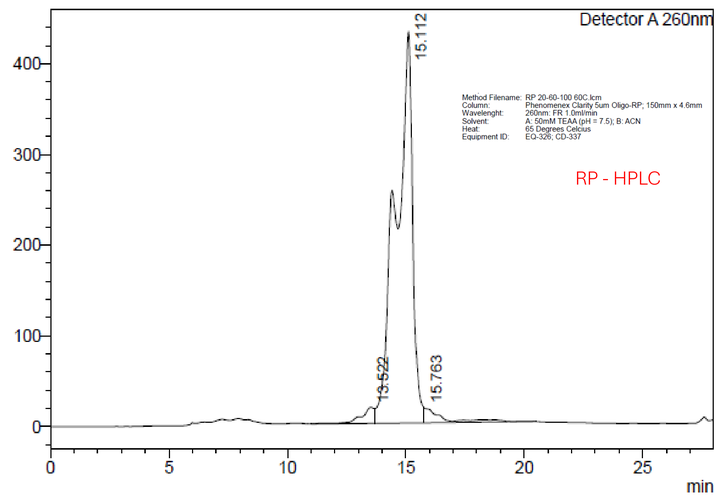
Conjugation of morpholino guanidinium modified oligonucleotide with CPP

PKKKRKVEDPYC[Linker][mrNpn][mrNpn][mrNpn][dNs][dNs] [dNss] [dNss] [dNs] [dNs] [dNs] [dNs] [dNs] [dNs] [dNs] [dNpn] [mrNpn][mrNpn][mrN]; PKKKRKVEDPYC = CPP

mr = morpholino; pn = guanidinium; s = thio;

N = nucleobase, green letters = CPP





References

Summerton J. Biochim Biophys Acta. 1999 Dec 10;1489(1):141-58.

Bhadra J et al.; Curr Protoc Nucleic Acid Chem. 2015 Sep 1;62:4.65.1-4.65.26.

Corey & Abrams; Genome Biol. 2001;2(5): REVIEWS1015.

Langner et al.; J Am Chem Soc. 2020 Sep 23;142(38):16240-16253.

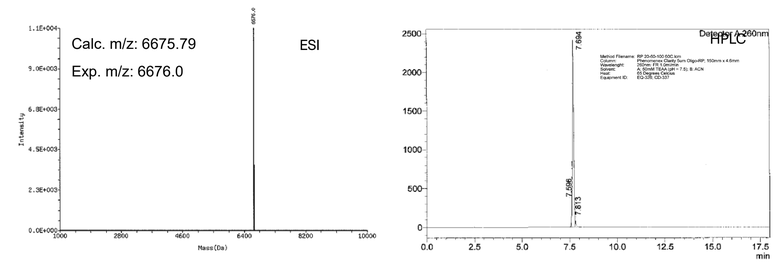
Sinha et al.; Mol Ther Nucleic Acids. 2023 Mar 21;32:203-228.

Sinha et al.; J Am Chem Soc. 2024 Sep 23;146(48): 32989-33001.

Fluorescense tag on morpholino guanidinium modified sequence

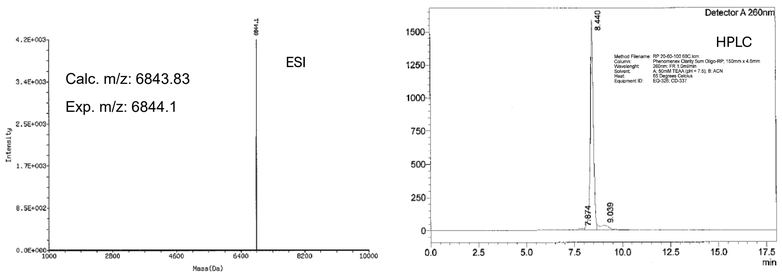
➢ Fully modified morpholino with FAM labeling

Seq. 1: 5'-[FAM][mrT] [mrA] [mrT] [mrT] [mrT] [mrC] [mrT] [mrGpn] [mrGpn] [mrCpn] [mrCpn] [mrG] [mrG] [mrT] [mrC] [mrG] [dC]-3'



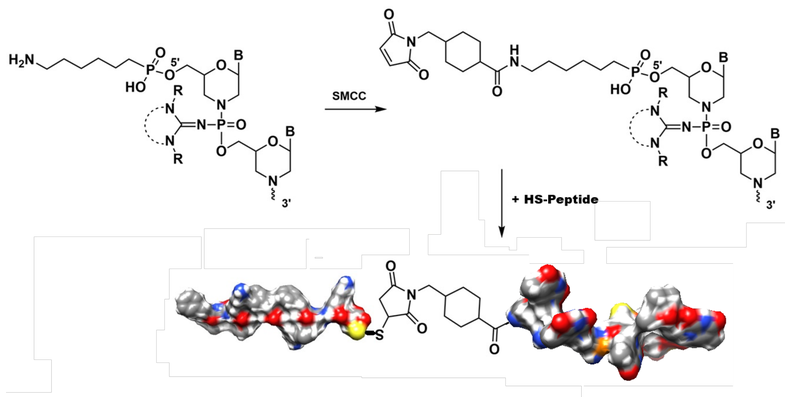
➢ Morpholino/DNA chimera with FAM labeling

Seq. 2: 5'-[FAM][mrTpn] [mrApn][mrTpn][mrTpn] [dTs] [dCs] [dTs] [dGs] [dGs] [dCs] [dCs] [dCs] [dGs] [dGs] [mrTpn] [mrCpn][mrGpn][dC]-3'



ESI and HPLC profile for RP HPLC purified fluorescence tagged morpholino guanidinium modification

Example of cross linker chemistry for the conjugation of PGMO with CPP



Peptide-oligo conjugation via SMCC links amine-modified oligonucleotides to cysteine-containing peptides using a heterobifunctional crosslinker in solution phase.

The NHS ester end of SMCC reacts with the oligo-nucleotide's primary amine to form a maleimide-activated intermediate, followed by Michael addition of the peptide's thiol group.

Conclusion

Chimeric antisense oligonucleotides that incorporate phosphoryl guanidinium morpholino (PGMO) backbones and phosphorothioate (PS)-modified DNA/RNA nucleotides can be successfully synthesized, fluorescently labeled, and conjugated to cell-penetrating peptides (CPPs). The conjugation with CPPs enhances cellular uptake and facilitates intracellular delivery of the PGMO constructs. This approach can significantly improve the bioavailability and biological activity of morpholino oligonucleotides, representing a promising platform for efficient and targeted antisense therapy and precise modulation of RNA function in therapeutic applications.

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