Design and synthesis of novel ASOs for Myotonic Dystrophy Type 1 (DM1)

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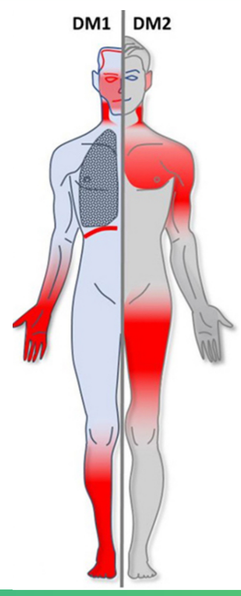
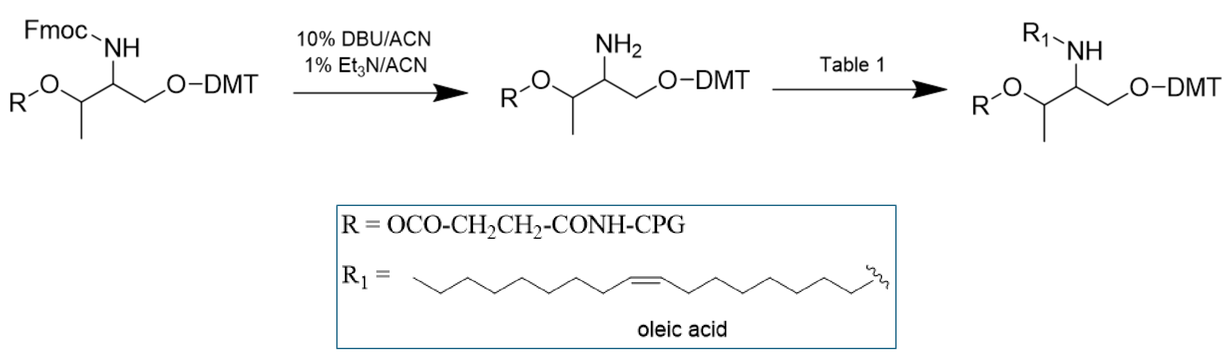
1 - INTRODUCTION

Myotonic dystrophy type 1 (DM1) is a multisystemic autosomal dominant progressive neuromuscular disorder caused by CTG repeat expansion in the 3'-UTR of the DMPK gene. This mutation causes formation of toxic RNA transcripts that accumulate in nuclear foci and sequester RNA-binding proteins such as MBNL, resulting in widespread defects in alternative splicing and cellular dysfunction (1).

Notwithstanding significant progress in studying pathogenesis of DM1, currently there is a limitation remaining in the field of effective therapies. Antisense oligonucleotides (ASOs) have emerged as a promising strategy to selectively target and modulate disease-causing RNA. However, their clinical application is still challenged by several limitations, including inefficient delivery to affected tissues, limited stability, potential off-target effects, and reduced intracellular uptake (2).

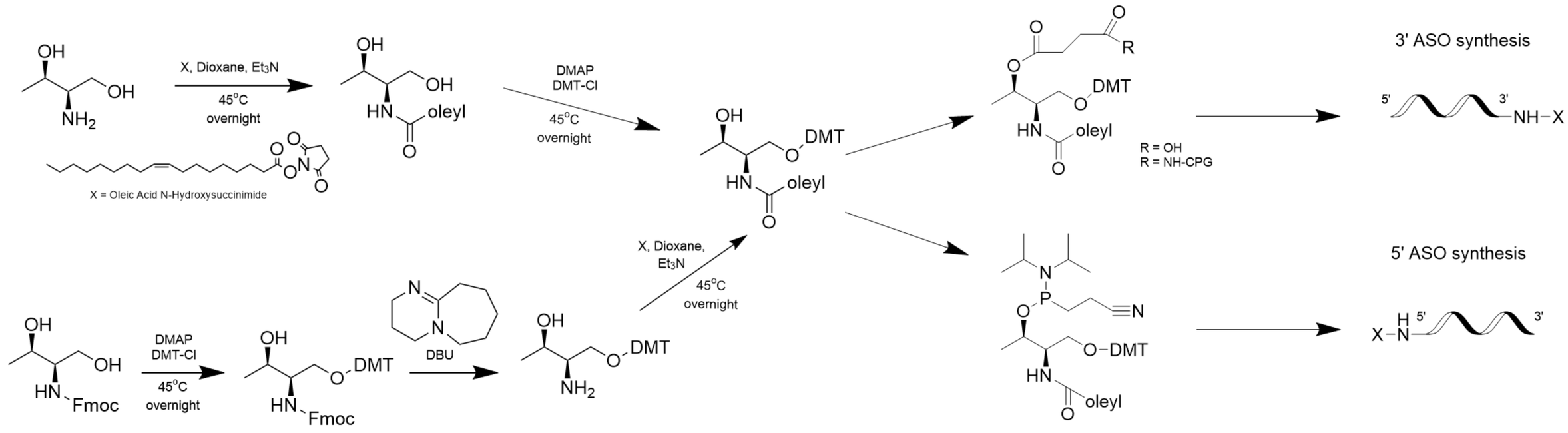


2 - Methods of fatty acid post-synthetic conjugation



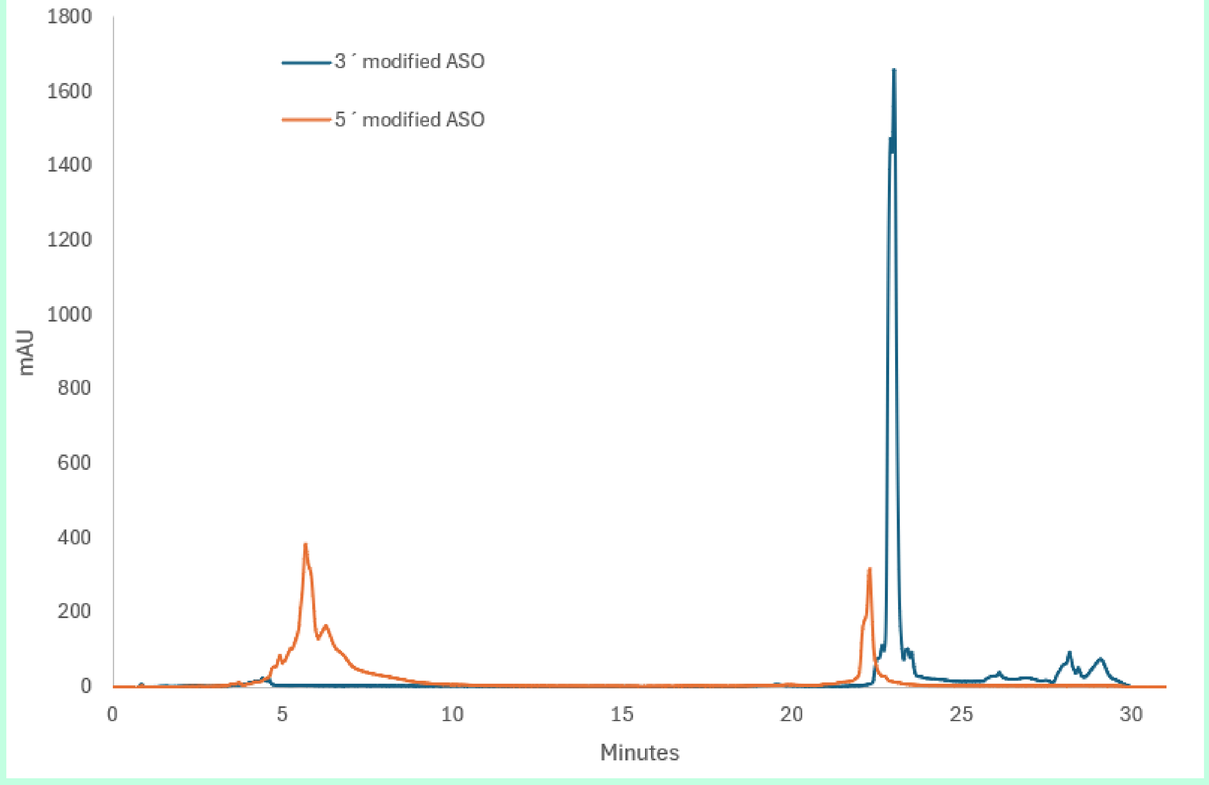
Resin	Components	HPLC
50 mg	20 eq **HATU**, 20 eq Oleic Acid, 40 eq DIPEA	x
50 mg	20 eq **PyBOP**, 20 eq Oleic Acid, 40 eq DIPEA	x
50 mg	10 eq **Oleoyl Chloride**, 20 eq TEA, DMF	x
50 mg	45 eq **HoBt**, 45 eq EDC, 45 eq DIPEA	x
50 mg	12 eq $TpPh_3$, 13 eq DMAP, 1 eq Oleic acid, 1 eq 2,2'–dithiobis-5-(nitropyridine)	x

3 - Fatty acid conjugation: different approach



4 - HPLC Characterization

HPLC profiles of 3'- and 5'-modified ASO are presented on the plot below.



5 - Conclusions

During the initial phase of the project, the synthesis of a DMT-protected Fmoc-Threoninol derivative was optimized and conjugated with oleic acid. The resulting compound was characterized by ^{1}H and ^{13}C NMR and HPLC, converted into the corresponding phosphoramidite and incorporated into an oligonucleotide.

6 - Future directions

- **generating a library of fatty acid–conjugated ASOs with 5′, 3′, and dual-end modifications**
- **synthesizing corresponding phosphoramidites**
- **evaluating their efficacy and stability *in vitro* and *in vivo*.**

7 - References

1. Chen, Z., et al. (2024) Repeat expansion disorders. Pract. Neurol., pn-2023- 003938.

2. Overby SJ, Cerro-Herreros E, Llamusi B, Artero R. RNA-mediated therapies in myotonic dystrophy. Drug Discov Today. 2018 Dec;23(12):2013-2022.

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ENTRY-DM MSCA Doctoral Network

Universitat de Barcelona

NACX Nucleic Acid Chemistry

iQAC

CSIC Consejo Superior de Investigaciones Científicas

ciber BBN Centro de Investigación Biomédica en Red Bioingeniería, Biomateriales y Nanomedicina