Synthesis and evaluation of novel antisense oligonucleotides for Myotonic Dystrophy Type 1

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1 - INTRODUCTION

Background

- DM1 is a multisystemic, autosomal dominant, progressive neuromuscular disorder
- Caused by CTG repeat expansion in the 3'-UTR of the DMPK gene
- Toxic RNA transcripts accumulate in nuclear foci → sequester MBNL (RNA-binding proteins)
- Result: widespread defects in alternative splicing and cellular dysfunction (1)

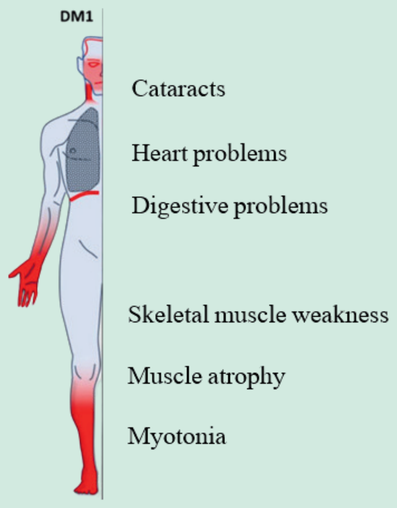
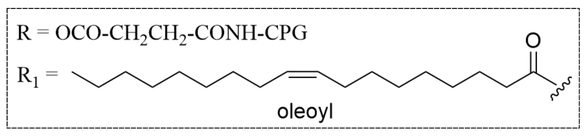


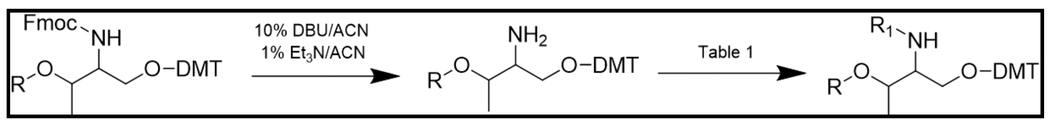
Figure 1: Multisystemic manifestations of DM1

The therapeutic gap

- No effective therapies currently exist despite research progress
- Antisense oligonucleotides (ASOs) are a promising strategy to selectively target disease-causing RNA
- Clinical translation still limited by (2):
  1. Inefficient delivery to affected tissues
  2. Limited stability
  3. Off-target effects
  4. Reduced intracellular uptake

2 - Methods of fatty acid post-synthetic conjugation

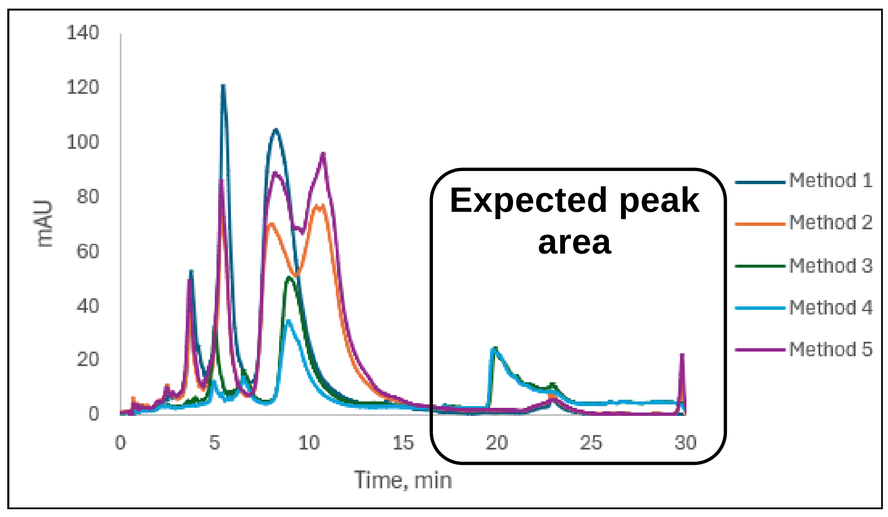




Resin	Method	Components
50 mg	1	20 eq **HATU**, 20 eq Oleic Acid, 40 eq DIPEA
50 mg	2	20 eq **PyBOP**, 20 eq Oleic Acid, 40 eq DIPEA
50 mg	3	10 eq **Oleoyl Chloride**, 20 eq TEA, DMF
50 mg	4	20 eq **HOBt**, 20 eq **EDC**, 20 eq Oleic Acid, 20 eq DIPEA
50 mg	5	12 eq **TPP**, 13 eq DMAP, 1 eq Oleic acid, 1 eq **DTNB**

3 - HPLC Characterization

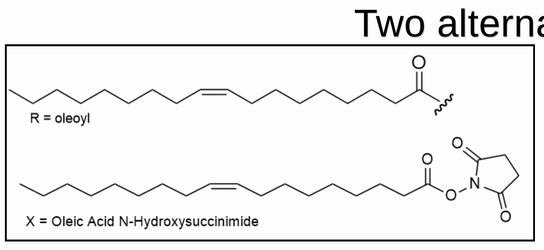
HPLC profiles of ASOs conjugated via Methods 1–5:



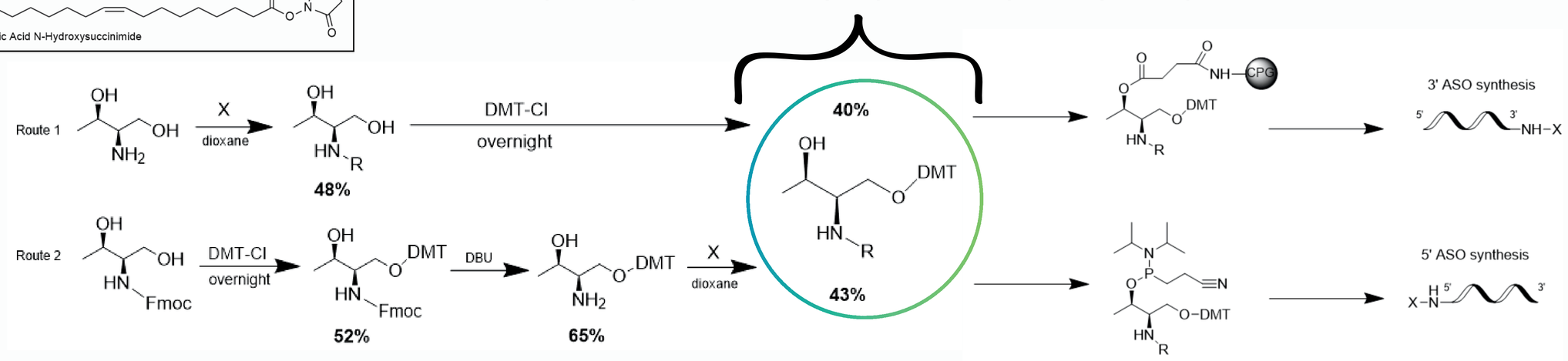
Poor coupling efficiency.

4 - Fatty acid conjugation: different approach

Two alternative routes were developed to access the same lipid-modified building block.

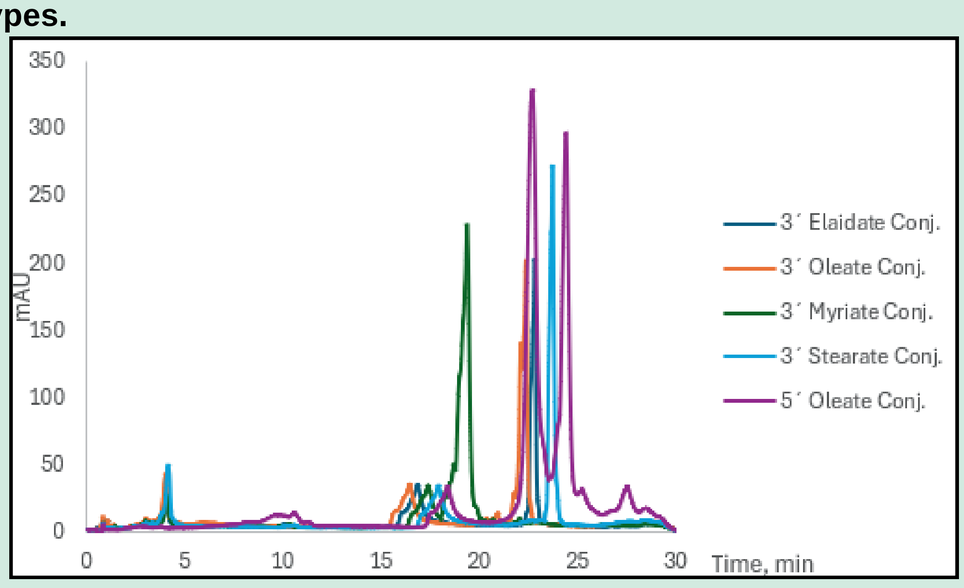


The intermediate is coupled either to solid support (3'-ASO synthesis) or converted to a phosphoramidite (5'-ASO synthesis)



5 - Sequence Characterization HPLC

HPLC profiles of 3'- and 5'-modified ASOs are presented on the plot below. Well-resolved peaks confirm successful conjugation across fatty acid types.



6 - MALDI

MALDI-TOF mass spectrometry confirmed successful conjugation of oligonucleotides with each fatty acid derivative.

Sequence	Mass, expected (Da)	Mass, measured (Da)
GTACTC 3' Oleate	2198	2193
GTACTC 3' Stearate	2200	2196
GTACTC 3' Elaidate	2198	2192
GTACTC 3' Myristate	2144	2140
X- 5' Oleate	6179	6174

7 - Conclusions

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

8 - 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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