BIOCATALYTIC APPROACHES TO THERAPEUTIC OLIGONUCLEOTIDE MANUFACTURE

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Therapeutic oligonucleotides bind to mRNA to modulate the production of disease related proteins and have emerged as a new drug modality for the treatment of a range of disease areas including genetic disorders and viral infections. Until recently, approved therapeutics were limited to the treatment of rare diseases, however in 2020 Inclisiran was approved for the treatment of atherosclerotic cardiovascular disease, which affects approximately 30 million people in the USA alone. This emergence of oligonucleotide products for more common diseases, creates a significant manufacturing challenge as existing methods of oligonucleotide synthesis are restricted to 10Kg batches and are not suitable for large scale applications (>100Kg). Current chemical approaches rely on iterative deprotection, coupling, capping and oxidation steps to extend sequences on a solid support. They use an excess of synthetically complex building blocks with protecting groups which must be removed during the synthesis with washing steps, resulting in poor atom efficiency. The process also uses prohibitively large volumes of acetonitrile (1000Kg per Kg of oligonucleotide) and chromatographic purification of the final product. Due to limitations of existing synthetic methods, current marketed RNA-based therapeutics are typically supplied with ~90% purity and as complex mixtures of diastereoisomers (resulting from phosphorothioate modifications). Here we present a biocatalytic strategy for therapeutic oligonucleotide synthesis inspired by the polymerase chain reaction, which has the potential to address the limitations of current manufacturing processes. Target sequences containing pharmaceutically relevant modifications are produced in a single operation, using a catalytic template and unprotected nucleotide triphosphate building blocks, in aqueous media. To showcase the approach, we have synthesized the first approved therapeutic oligonucleotide Vitravene as a single diastereoisomer in 63% yield and 85% purity, without the need for chromatographic purification.

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