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Convergent biocatalytic mediated synthesis of siRNA

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PURPOSE OF THE ABSTRACT

The application of biocatalysis in pharmaceutical development continues to grow with the strong technology base being applied to develop enzymatic methods for oligonucleotide synthesis resulting in improved yields and purity profiles compared to traditional methods. Biocatalytic approaches are alleviating the pressures on existing solid phase capacity and resulting in more convergent syntheses.

Herein we report a convergent biocatalytic synthesis strategy for an Alnylam model siRNA. The siRNA chemical structure includes several of the unnatural modifications and conjugations typical of siRNA drug substances. Using Almac's 3-2-3-2 hybrid RNA ligase enzyme strategy that sequentially ligates short oligonucleotide fragments (blockmers), the target siRNA was produced to high purity at 1 mM concentration. Additional strategies were investigated including the use of polynucleotide kinase phosphorylation and the use of crude blockmer starting materials without chromatographic purification1. These findings highlight a path towards a convergent synthesis of siRNAs for large scale manufacture marrying both enzymatic liquid and classical solid phase synthesis.

FIGURES

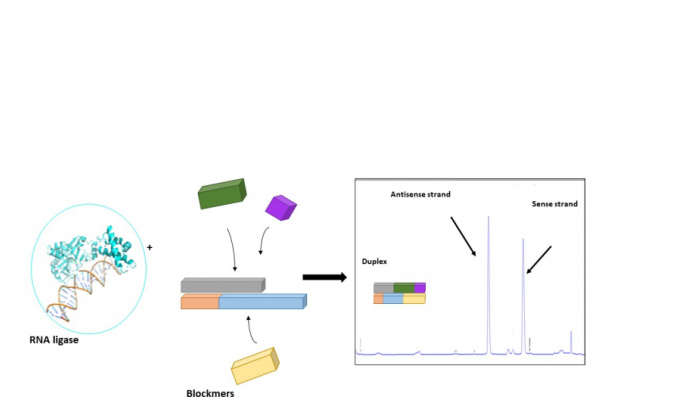

FIGURE 1

Almacs 3-2-3-2 hybrid RNA ligase enzyme strategy

Almac□s 3-2-3-2 hybrid RNA ligase enzyme strategy used to produce 1 mM of high purity siRNA.

FIGURE 2

KEYWORDS

RNA ligase | oligonucleotide synthesis | biocatalysis | process development

BIBLIOGRAPHY

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