Synthesis of Stereopure Chimeric Oligonucleotides Containing PN and PS Backbone: A Systematic Evaluation of Chiral Auxiliaries

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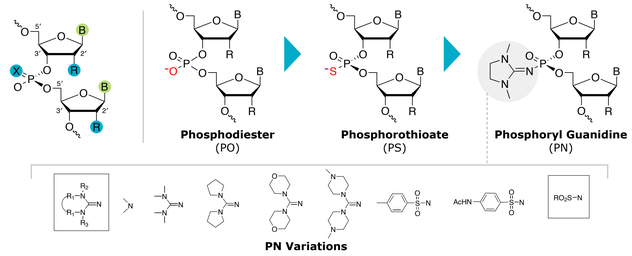
Summary

- Using PRISM™, Wave Life Sciences' proprietary discovery and drug development platform, we generate stereopure oligonucleotides—those in which the chiral configuration of backbone linkages (*Rp* or *Sp*) are controlled at each position.
- We have also expanded the repertoire of backbones we employ beyond the more traditional phosphorothioate (PS)[1] and phosphodiester (PO) backbones to include phosphoryl guanidine (PN).[2-5] All three clinical candidates in ongoing phase I clinical trials contain PN chemistry.
- We have developed a new generation chiral oxazaphospholidine (amidites), called DPSE and PSM, to support the synthesis and manufacture of various types of stereopure oligonucleotides. These chiral amidites are required to generate stereopure oligos with chimeric backbone chemistries.
- Both chiral amidites exhibit high diastereoselectivity and high coupling efficiency.
- Our optimized oligonucleotide synthesis methods support the generation of stereopure oligonucleotides at scales ranging from high-throughput to commercial manufacturing.

Introduction

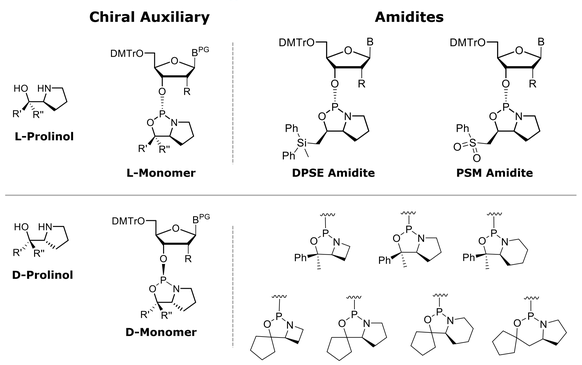
- We apply PRISM™, our discovery and drug development platform to generate rationally designed stereopure oligonucleotides (**Figure 1**).
- We have also expanded the repertoire of backbone modifications we employ beyond the more traditional PS and PO backbones to include PN and its variants (**Figure 1**).[3-5]
- The objective of this work is to identify chiral amidites which are stable, reactive with high diastereoselectivity and yields that enable large-scale manufacture of stereopure oligonucleotides with chimeric backbones.

Figure 1. Introduction to PRISM platform chemistry



Results

Figure 2. Advances in stereopure monomer synthesis and manufacturing



- Standard protecting groups
- Standard synthesis cycle
- Standard thiolation reagent
- Special synthesis cycle
- Special thiolation reagent
- Auxilliary cleaved during synthesis

- To support synthesis of stereopure oligonucleotides, we designed and synthesized several chiral auxiliaries, derived from L- or D- Proline, followed by connecting with 3'-OH group of protected nucleosides to make amidites (**Figure 2**).[6,7]
- We screened several chiral amidites 1) by changing the ring size of the chiral amino alcohol to get better reactivity and selectivity 2) by varying the R' and R'' groups to fine tune reactivity and 3) by installing the right handle to cleave the chiral auxiliary from the solid support under mild conditions.
- We optimized the process to manufacture the chiral auxiliary prolinols (DPSE and PSM), starting from low-cost L- and D-Proline in multi-kilogram scale.
- From our screen, we identified DPSE and PSM amidites which are very reactive, with high selectivity, and are stable to manufacture in multi kilo scale, with up to ~95% yield.
- We synthesized multi-kilograms of amidites as L- and D- forms of DNA and various 2'-modified RNAs including modified bases, such as 2'-F, 2'-OMe, 2'-MOE and LNA.

Figure 3. NMR spectra of stereopure amidites

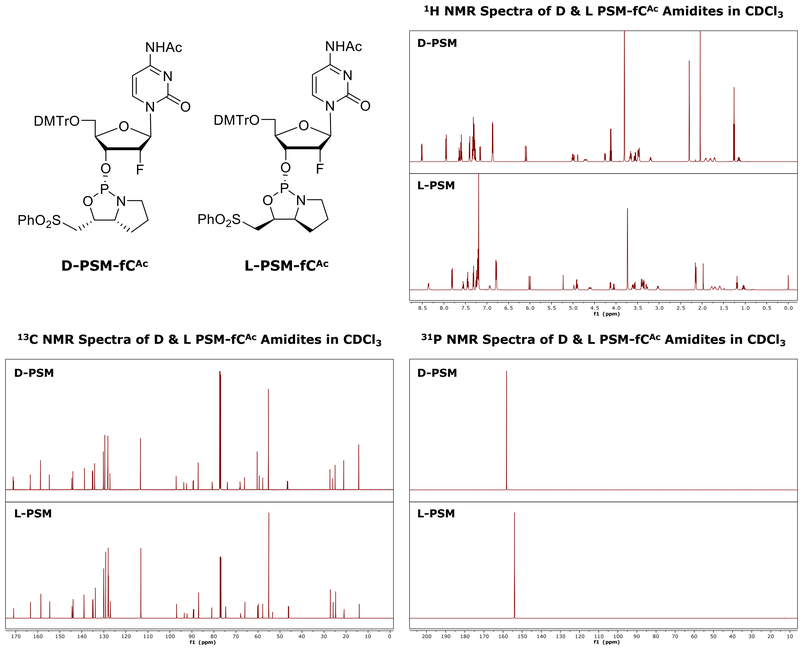
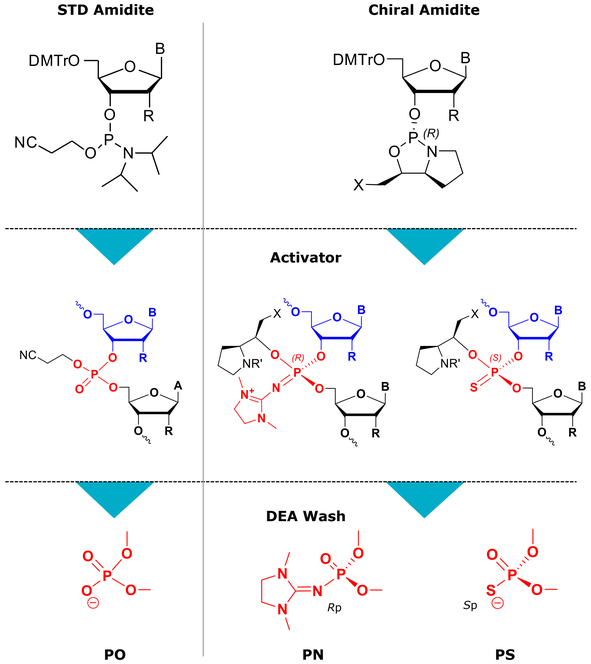


Figure 4. Efficient scalable process for oligo synthesis



- Standard CNE amidite for PO
- Chiral amidite for both PS & PN backbones
- High diastereoselectivity
- High coupling efficiency
- Standard synthesis cycle
- Standard thiolation reagent

Standard C&D Process

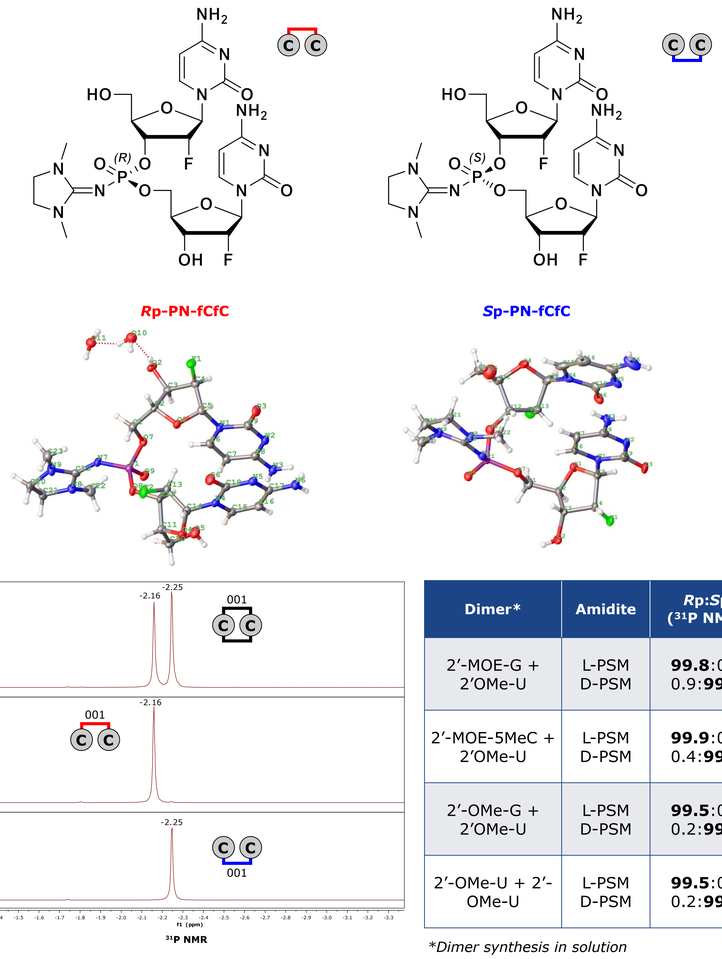
- On column DEA wash
- Ammonium hydroxide

Scalable Process

- 96-well plate to GMP manufacturing
- Comparable yield to stereorandom oligonucleotides

- The chiral amidites are suitable for the synthesis of linkages with either PS or PN backbone (**Figure 4**).
- Over the past few years, we have optimized a process where we use our new generation chiral auxiliaries for generating stereopure backbones. We employ the standard phosphoramidite synthesis cycle, cleavage and deprotecting conditions, and yields for stereopure oligonucleotides are comparable to stereorandom oligonucleotides.
- We have optimized the process for high-throughput (96-well plate format) stereopure oligo synthesis with chimeric backbone to support high-throughput screening and SAR studies.
- Using our optimized process, we have successfully synthesized chimeric stereopure oligonucleotides that execute various mechanisms, including silencing by RNase H and RNAi, exon skipping, and RNA base editing, for our preclinical and clinical programs.

Figure 5. Characterization of stereopure dimers

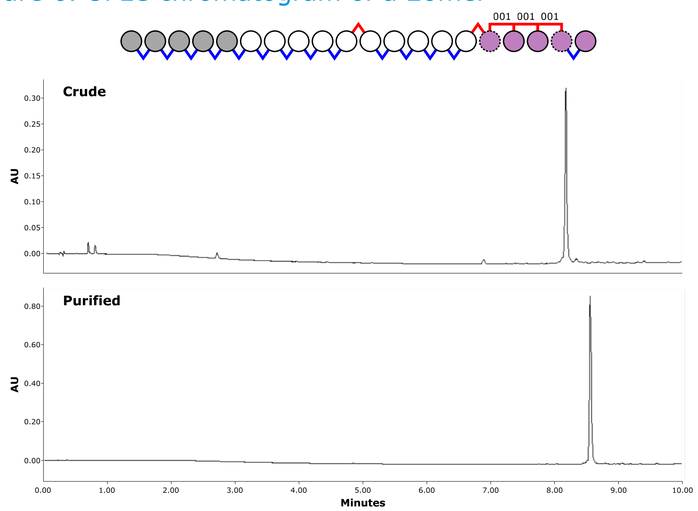


Dimer*	Amidite	*Rp*:*Sp* (³¹P NMR)
2'-MOE-G + 2'OMe-U	L-PSM D-PSM	**99.8**:0.2 0.9:**99.1**
2'-MOE-5MeC + 2'OMe-U	L-PSM D-PSM	**99.9**:0.1 0.4:**99.6**
2'-OMe-G + 2'OMe-U	L-PSM D-PSM	**99.5**:0.5 0.2:**99.8**
2'-OMe-U + 2'-OMe-U	L-PSM D-PSM	**99.5**:0.5 0.2:**99.8**

**Dimer synthesis in solution*

- We characterized stereopure dimers by X-ray crystallography and ^{31}P NMR (**Figures 5**).
- These methods confirm the configuration of the PN linkage in the dimer and show high diastereoselectivity for multiple types of dimers.

Figure 6. UPLC chromatogram of a 20mer



- By employing our new generation of chiral auxiliaries, we have developed a scalable process for synthesis of stereopure oligonucleotides with chimeric backbone (PS/PO/PN) (**Figure 6**). Our simplified process provides yields comparable to stereorandom oligonucleotides.

References: 1. Iwamoto, N. et al., 2017. *Nat. Biotech.* 35:845-851; 2. Kupryushkin, M.S. et al., *Acta Naturae* 2014. 6:116–118; 3. Kandasamy, P. et al., 2022, *Nucleic Acids Res.* 50:5443-5466; 4. Kandasamy, P. et al., 2022, *Nucleic Acids Res.* 50:5401-5423. 5. Monian, P. et al., 2022, *Nat. Biotech.* 40:1093-1102; 6. Wada, T. & Shimizu, M., 2010, WO2010064146; 7. Kandasamy, P. et al., 2020, WO 2020/191252 A1.
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