Riding new Tides: Synthesis of oligonucleotides on PurePep® Chorus

PurePep®

Luísa Aguiar, Dominik Sarma, Robert Zitterbart

Gyros Protein Technologies, 4675 South Coach Drive, Tucson, Arizona, 85714, U.S.A.

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Introduction

Modern drug discovery incorporates novel modalities such as oligonucleotides, RNA, protein degraders, as well as next-generation peptides. Therefore, quick access to these molecules through flexible synthesis platforms is crucial for competitive drug development. This application note highlights the successful synthesis of short oligonucleotides (18–24 bases) on the PurePep® Chorus, showcasing its versatility beyond peptide synthesis.

Methods

We adopted all steps of the phosphoramidite approach on the PurePep Chorus peptide synthesizer at a 5 µmol scale to synthesize different oligonucleotides shown in Table 1.

- Coupling was performed using 0.5 mL of 0.1M nucleotide phosphoramidite in ACN and 0.5 mL of 0.5M 5-ethylthio-H-tetrazole in ACN for 2 x 2 min.
- Capping was performed with a mixture of 0.5 mL of Ac2O/Lutidine/THF 1:1:8 v/v and 0.5 mL of 20% v/v N-Me-imidazole in THF for 2 minutes after coupling and 1 min 10 s after the oxidation step.
- Oxidation was achieved with 0.1 M iodine in THF/pyridine/water 88:10:2 v/v for 2 min.
- Detritylation was performed with 3% trichloroacetic acid (TCA) in dichloromethane (DCM) for 2 x 2 min.

Cleavage was performed using a 1:1 v/v mixture of 30% aqueous NH4OH and 40% aqueous methylamine for 1 hour at RT.

Purification was performed with the DMT-on method with reversed-phase solid phase extraction using Glen-Pak DNA Purification cartridges (PN 60-5200-10) at a scale of 1 µmol.

Table 1. Oligonucleotides synthesized during this study.

ID	Name	Sequence	DMT-on (crude)	DMT-off (purified)
#1	pBluescript SK primer	5′-CGC TCT AGA ACT AGT GGA TC-3′	68.9%	86.4%
#2	Genasense G3139	5′-TCT CCC AGC GTG CGC CAT-3′	64.0%	77.7%
#3	GAL1 Forward primer	5′-AAT ATA CCT CTA TAC TTT AAC GTC-3′	66.2%	90.3%
#4	3′-pBabe-Seq Primer	5′-ACC CTA ACT GAC ACA CAT TCC-3′	68.3%	93.9%
#5	Fomivirsen	5′-GCG TTT GCT CTT CTT CTT GCG-3′	87.9%	97.3%
#6	U6 Primerc	5′-GGG CAG GAA GAG GGC CTA C-3′	65.3%	83.2%
#7	M13 Reverse* (-48)	5′-A*G*C* G*GA TAA CAA TTT CA*C* A*C-3′	13.0%	98.7%
#8	Intein Forward*	5′-CCC GC*C* G*C*T GCT TTT GCA CGT* G*A*G-3′	25.3%	98.9%
#9	pBAD Reverse* (F)	5′-G*A*T TTA ATC TGT AFC* A*G*G-3′	29.3%	92.6%

a – DMT protected oligo nucleotide; b – fully deprotected oligo nucleotide; c – mutated: first T changed to C; '*' indicates multiple phosphorothioate linkages; F – 2′-Fluoro-dU

Results & Discussion

- During the synthesis of #1, the growing oligonucleotide chain has been monitored by LC/MS after each extension up to the length of 8–10 nucleotides (Figure 1).
- The chromatograms demonstrate an efficient elongation of the target sequence through automated oligo synthesis on the PurePep Chorus synthesizer.

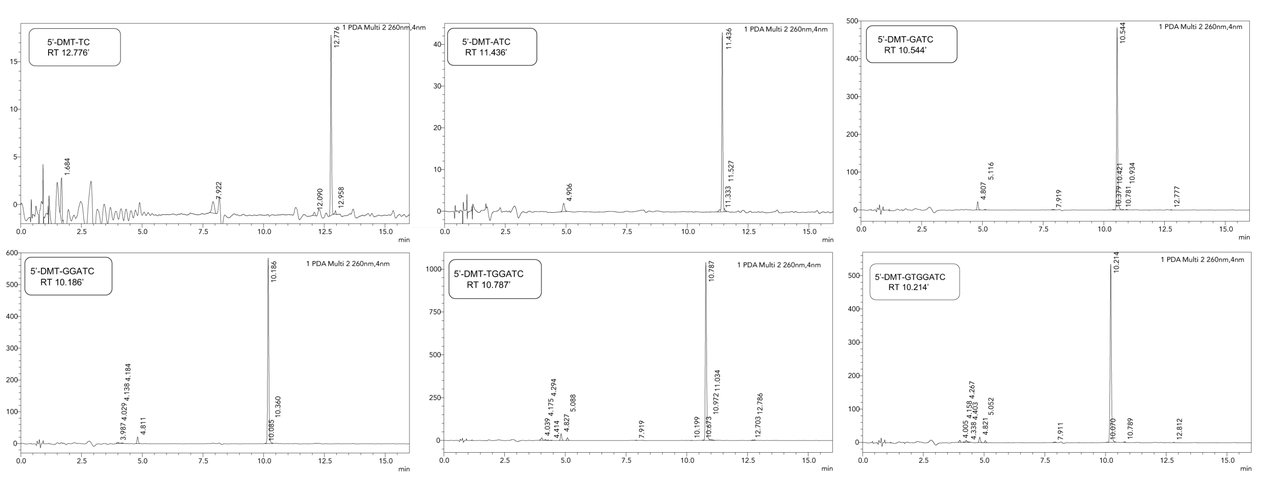

Figure 1. UV260 nm chromatogram of the universal pBluescript SK primer synthesis progress, steps 1 through 6 (crude, microcleavage).

- Encouraged by the initial results from our coupling studies, we continued the synthesis of oligos #2. In this example we highlight how impurities can easily be removed with the DMT-on method.
- Figure 2 shows the crude DMT-on oligo before the purification (A and C) and DMT-off after purification (B and D).
- While the improvement becomes apparent from the UV chromatogram, remaining truncation sequences are observed with MS analysis (D).

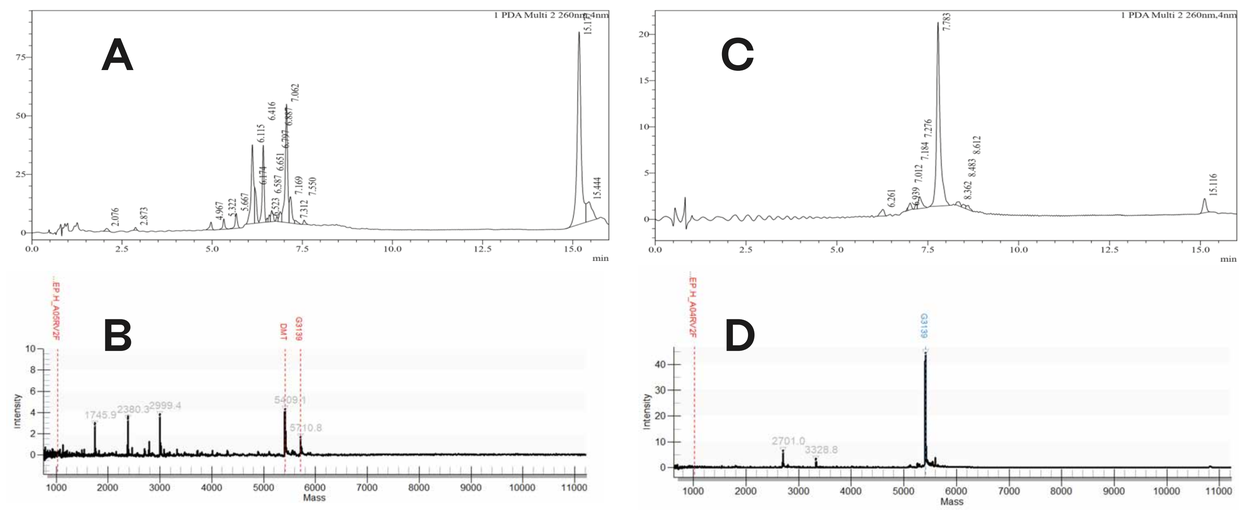

Figure 1. UV260 nm chromatogram of crude Genasense G3139 (DMT on) [A] and desalted Genasense G3139 (DMT off) [B] and their respective MALDI-TOF mass-spectra [C] and [D].

- Oligonucleotides #3 to #7 were synthesized automatically on the PurePep Chorus and purified with the DMT-on method in very good purity as depicted in Table 1.
- The PurePep® Chorus peptide synthesizer allows the successful synthesis of several short (18–24 bases) oligonucleotides due to the operation under inert conditions.
- We have also demonstrated the synthesis of phosphorothioates and other labeled and specially substituted nucleotide-containing oligonucleotides with the same platform.

Conclusions

- Expand the synthesis capabilities from peptides to oligonucletides on the PurePep Chorus
- Efficiently optain high-quality short oligonucleotide sequences with DMT-on purification
- Apply sulfurization in the automated synthesis process to expand the chemical flexibility

PurePep Chorus

- PurePep Chorus is a versatile instrument for the synthesis of peptides, peptide-nucleic acids, peptoids, oligonucleotides, and other polymers assembled on the solid phase
- The PurePep Pathway features zero cross-contamination for maximum purity
- Upgradable design for 2, 4 or 6 reaction vessels and independent induction heating and UV-monitoring
- Save reagent cost with Single-Shot™ delivery with no dead volume and zero priming

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