Novel Purification Method for Oligonucleotides

MITSUBISHI GAS CHEMICAL

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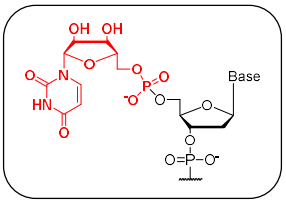
Introduction

Oligonucleotides are typically synthesized using the solid-phase phosphoramidite method. However, because the coupling efficiency is less than 100%, base-deletion byproducts are inevitably generated. Single-base deletion byproducts (N-1 mer) must be removed during the purification process, as they may cause off-target effects. Nevertheless, achieving complete removal of N-1mer using conventional purification methods remains challenging due to their physicochemical properties being highly similar to those of the target oligonucleotides.
In this study, we present an innovative approach to separating base-deletion byproducts by leveraging the specific interaction between diols and boronic acids.

Method

The separation of synthesized N mer and terminal diol-modified N-1 mer was evaluated under the analytical conditions shown in Table 1. The synthesis and treatment of each sample were performed as follows:

- **N mer**: Synthesized in a 1 µmol scale using a standard solid-phase synthesis protocol.
- **Terminal diol-modified N-1 mer**: A 0.1 M solution of 2',3'-diacetyl uridine CED phosphoramidite (purchased from ChemGenes) was prepared in anhydrous acetonitrile and reacted for 96 seconds.
- **Cleavage and deprotection**: After synthesis, cleavage was performed with aqueous ammonia at room temperature for 1 hour, followed by deprotection at 60°C for 5 hours. The deprotected oligonucleotide was obtained by centrifugation and re-dissolved in water.

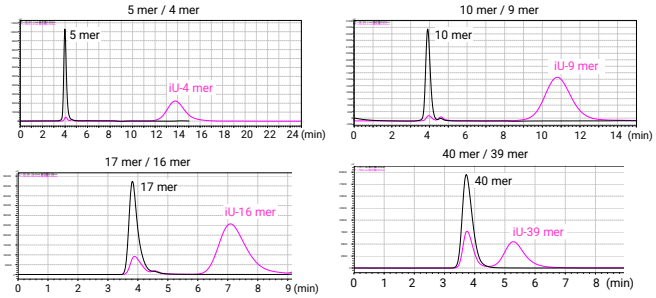


Diol with modified terminal (inverted uridine)

Table 1 Analytical conditions	
Instrument	LC-20AD (Shimadzu)
Flow rate	0.7 ml/min
Mobile phase	1000 mM TEAA, pH 9.0
Column	TSKgel Boronate-5PW (Tosoh)
Oven temp.	29 ℃
Detection	UV at 254 nm

Result

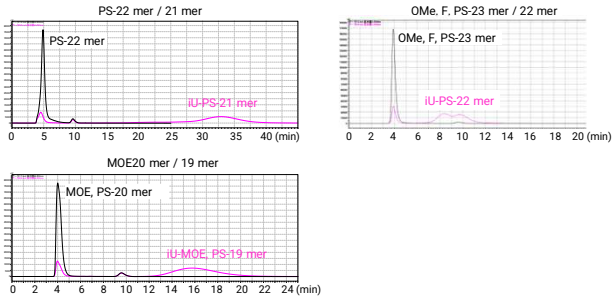
1. The effect of base length (Natural DNA sequence)



Mer	Sequence (5' - 3')
5	TTTTT
4	iUTTTT
10	TTTTTTTTTT
9	iUTTTTTTTTT
17	GTAAAACGACGGCCAGT
16	iUTAAAACGACGGCCAGT
40	GAAGCACTTGTCTCCCTTGCGAAGCACTTGTCTCCCTTGC
39	iUAAGCACTTGTCTCCCTTGCGAAGCACTTGTCTCCCTTGC

iU = inverted uridine

2. The effect of types of nucleic acid bases



Modification	Mer	Sequence (5' - 3')
PS	22	T^G^A^C^T^G^T^G^A^A^C^G^T^T^C^G^A^G^A^T^G^A (ODN 1018 VacciGrade™)
	21	iU^G^A^C^T^G^T^G^A^A^C^G^T^T^C^G^A^G^A^T^G^A
*2'-O-Me, 2'F, PS	23	U^a^agAuGaGaCaCuCuUuCuG^G^U (Givosiran™ - antisense)
	22	iU^a^agAuGaGaCaCuCuUuCuG^G^U
**2'-O-MOE, PS	20	g^c^c^t^c^A^G^T^C^T^G^C^T^T^C^g^c^a^c^c (Mipomersen™)
	19	iU^c^c^t^c^A^G^T^C^T^G^C^T^T^C^g^c^a^c^c

iU = inverted uridine, ^ = PS linkage
*Upper case = 2'-O-Me-RNA, Lower case = 2'deoxy-2'Fluoro
**Upper case = DNA, Lower case = 2'-O-MOE-RNA

Conclusion

- The target molecule (N mer) and single-base deletion byproduct (N-1 mer) were efficiently separated using the interaction between boronic acid and diol.
- The novel purification method was found to be applied to long natural DNA sequences (40 mer/39 mer) and various modified nucleic acids.
- Separation was achieved with neither organic solvents such as acetonitrile nor complex separation conditions (e.g., gradient elution).

Ongoing and future works

- We are investigating the synthesis of oligonucleotides with diol amidites as capping agents and developing a purification method for base-deletion byproducts.
- Verification of therapeutic outcome using this purification method is planed as a future work.

Acknowledgement

The patent for this purification method has already been applied.