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5′-ALDEHYDE-MODIFIER C2 PHOSPHORAMIDITE

Introduction

Aldehyde modifiers are attractive electrophilic substitutions in oligonucleotides since they are able to react with amino groups to form a Schiff's base, with hydrazino groups to form hydrazones, and with semicarbazides to form semicarbazones. The Schiff's base is unstable and must be reduced with sodium borohydride to form a stable linkage but hydrazones and semicarbazones are very stable linkages. Similarly to activated carboxylic acids, aldehydes are generally unstable to oligonucleotide deprotection conditions. In collaboration with Epoch Biosciences, we offer the protected benzaldehyde derivative (1) as a 5′-aldehyde modifier.[1] The acetal protecting group is sufficiently hydrophobic for use in RP HPLC and cartridge purification and is readily removed after oligonucleotide synthesis under standard oligonucleotide detritylation conditions with 80% acetic acid.

Synthesis

Use regular coupling time and synthesize the oligonucleotide DMT-On.

Oligonucleotide Deprotection

Deprotect the oligonucleotide as required by nucleobases in 30% ammonium hydroxide. At this point, the oligo may either be purified by reverse phase or treated with dilute acetic acid to remove the acetal protecting group. The 5′-Aldehyde-Modifier C2 is about as hydrophobic as a dimethoxytrityl group when protected as the acetal. A Glen-Pak™ purification protocol is listed below.

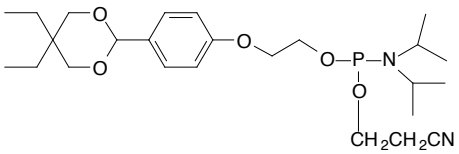
Aldehyde Deprotection

The aldehyde is released by treatment with 80% acetic acid for 1 hour at room temperature.

Intellectual Property

Reference

(1) M.A. Podyminogin, E.A. Lukhtanov, and M.W. Reed, *Nucleic Acids Res*, 2001, **29**, 5090-8.



(1) Epoch 5′-Aldehyde-Modifier C2

Glen-Pak Purification

Materials

1.	Glen-Pak DNA Purification Cartridge (60-5100-XX, 60-5200-XX)	1
2.	Vacuum manifold (96 well or 12-24 port SPE type, if appropriate)	1
3.	HPLC Grade Acetonitrile	0.5mL
4.	2.0M Triethylamine Acetate (60-4110-XX, TEAA, pH7)	1mL
5.	100 mg/mL Sodium Chloride	1mL
6.	Salt Wash Solution (5% Acetonitrile in 100mg/mL Sodium Chloride)	2mL
7.	Deionized Water	2mL
8.	50% Acetonitrile/Water	1mL

Procedure

1. Prep Glen-Pak cartridge by flushing the cartridge with 0.5 mL of ACN, followed by 1 mL of 2 M TEAA.
2. Add 1mL of 100 mg/mL Sodium Chloride solution to the deprotected oligonucleotide solution for a final volume of 2mL. Apply the oligo/salt mixture to the cartridge in 1mL aliquots (collect the eluent and save in case of loading failure or error).
3. Wash the cartridge with 2 x 1mL of Salt Wash Solution. This rinses away the remainder of the failure sequences from the cartridge.
4. Wash the cartridge with 2 x 1mL of deionized water. to rinse away the excess salts.
5. Elute the purified oligo using 1 x 1mL 50% acetonitrile in water and evaporate to dryness.
6. Treat the residue with 80% acetic acid for 1 hour at room temperature to release the aldehyde.
7. Evaporate the aqueous acetic acid to dryness.