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TECHNICAL BULLETIN - 5-FORMYL-dC III-CE PHOSPHORAMIDITE

INTRODUCTION

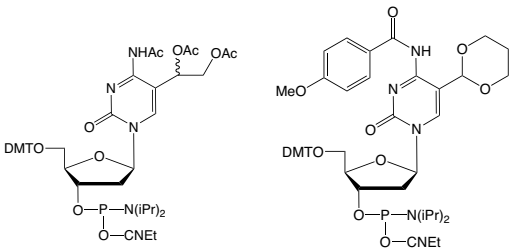
Epigenetics is the study of heritable changes in gene expression and regulation that are not due to variations in the DNA sequence itself. Methylation of deoxycytidine to form 5-methyl deoxycytidine (5-Me-dC) is considered one mechanism involved in the regulation of gene expression. Oxidation of 5-Me-dC is proposed as a mechanism in the active demethylation pathway. 5-Me-dC is oxidized stepwise to 5-hydroxymethyl-dC (5-hm-dC), 5-formyl-dC (5-f-dC) and 5-carboxy-dC (5-ca-dC).

Is it possible to produce an oligo containing all of these bases? Before the introduction of an alternative 5-f-dC monomer in 2013, the answer was – no – but why?

First, ca-dC is not compatible with deprotection using ammonium hydroxide or AMA which would lead to a mixture of the desired carboxylic acid and incorrect amides. So, an oligo containing all four of these methylated analogues has to be deprotected using sodium hydroxide in aqueous methanol to be compatible with ca-dC. Fortunately, hm-dC II is also compatible with sodium hydroxide deprotection, as indeed is f-dC (1).

However, the structure of the f-dC monomer (1) requires that the fully deprotected oligo be treated with 50mM aqueous sodium periodate to generate the formyl group. This treatment has been reported to be incompatible with hm-dC.[1]

The Carell group in Munich was able to design a monomer for f-dC which seems to meet all of the requirements to prepare an oligo containing all of the methylated variants.[1] The structure of the f-dC III monomer (2) is shown. The aldehyde is protected as an acetal group and the exocyclic amino group is protected with the 4-methoxy-benzoyl group.



(1) 5-Formyl-dC *(2) 5-Formyl-dC III*

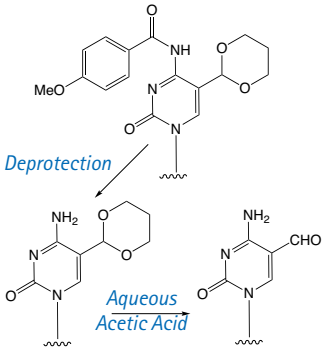


Figure 1: Deprotection and Oxidation to 5-Formyl-dC

SYNTHESIS

Use a 3 minute coupling time. No other changes are required.

BASE DEPROTECTION

Ammonium hydroxide at room temperature for 17 hours can be used for the deprotection of oligos containing f-dC III and other modifications compatible with standard ammonium hydroxide deprotection.

However, if the oligo contains hm-dC II and/or ca-dC, the deprotection must be carried out using 0.4M sodium hydroxide in methanol/water 4:1 (v/v) for 17 hours at room temperature.[2] *(Please note that this deprotection scheme is not compatible with the use of dmf-protected dG in the oligo. However, iBu-dG is fully deprotected under these conditions.)* After deprotection with sodium hydroxide, the oligo must not be isolated by simple evaporation. Instead, the sodium hydroxide can be neutralized using a suitable buffer or the oligo can be ethanol precipitated.

PURIFICATION

These modified oligos can be simply purified by Glen-Pak purification. The normal procedure can be followed with the exception that the removal of the 5'-DMT group with 2% aqueous trifluoroacetic acid should be omitted. The DMT group is then removed during the acetic acid/water procedure described below for removal of the acetal protecting groups.

ACETAL DEPROTECTION

To remove the acetal protecting group the oligo is treated with acetic acid/water 80:20 (v/v) for 6 hours at 20 °C. It should be noted that both time and temperature are critical for complete removal of the acetal protecting group with absence of side reactions.

Reference:

1. T. Carell, et al., *Angew. Chem.-Int. Edit.*, 2013, In press.
2. http://www.glenresearch.com/Technical/TB_NaOH_Deprotection.pdf