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5'-DMS(0)MT-AMINO-MODIFIER

INTRODUCTION

5'-Amino-Modifiers are designed for use in automated synthesizers to functionalize the 5'-terminus of a target oligonucleotide. The primary amine can be used to attach a variety of products to the oligonucleotide. 5'-DMS(0)MT-Amino-Modifier C6 is an alternative to 5'-Amino-Modifier. This product is optimized for use in cartridge purification procedures and the trityl protecting group can be removed on the cartridge.

USE OF 5'-AMINO-MODIFIERS

Diluent: Anhydrous Acetonitrile

Add fresh diluent to product vial to recommended concentration and swirl vial occasionally over several minutes until product is completely dissolved. (Some oils may require between 5 and 10 minutes.) Use care to maintain anhydrous conditions. In case of transfer to an alternate vial type, ensure recipient vial has been pre-dried. For more information, see:

http://www.glenresearch.com/Technical/TB_ ABITransfer.pdf.

Coupling: No changes needed from standard method recommended by synthesizer manufacturer.

Deprotection: Deprotect as required by nucleobases.

WARNING: Drying down the oligo after cleavage and deprotection without addition of a non-volatile base (for example, TRIS) will lead to loss of the Trityl protecting group. For more information, see: http://www.glenresearch.com/GlenReports/GR21-19. html

Storage: Freezer storage, -10 to -30°C, dry

Stability in Solution: 1-2 days

DEPROTECTION

In the past, we have recommended carrying out deprotection for at least 17 hours at approximately 40°C due to the increased potential for thermally initiated side reactions. While this procedure is still acceptable, we have found it to be unnecessary and now recommend deprotection as required by the nucleobases.

FIGURE 1: STRUCTURES OF 5'-DMS(0)MT-AMINO-MODIFIER



 Do not remove the DMS(0)MT group on the synthesis column unless you plan to conjugate the amine while the oligo is still on the support.

 Do not dry down the solution of DMS(0)MT-on oligo if you plan to do DMS(0)MT-on purification without adding a base like TRIS base to avoid DMS(0)MT loss.

If the 5'-amine is required for on-column conjugation, the DMS(O)MT protecting group of this 5'-Amino-Modifier can be removed on the synthesizer by deblocking until the color elutes totally (typically 5 min.). However, for maximum amine reactivity, it is preferable to retain the DMS(O)MT group for later removal with aqueous acid.

PURIFICATION

The modified oligonucleotide may be purified using a reverse phase cartridge, e.g., Glen-PakTM or Poly-PakTM, HPLC or gel electrophoresis. Cartridge purification is accomplished using the trityl-on procedure. Reverse Phase (RP) HPLC may be performed either before or after attachment of the label. If purification is desired prior to label attachment, the DMS(O)MT group should not be removed from the oligonucleotide as the lipophilic character of the DMS(O)MT group aids in HPLC purification. RP HPLC purification is best accomplished using a C18 column.

If a cartridge is used for purification, the DMS(0) MT can be removed efficiently on the cartridge with 4% aqueous trifluoroacetic acid over a 5 minute period.

After HPLC purification, the DMS(0)MT group may be removed by treating the purified oligonucleotide with acetic acid:water (20:80) at room temperature for 1 hour. The solution will become hazy due to the release of DMS(0)MT alcohol which is only slightly soluble in this solution. To remove the DMS(0)MT alcohol, extract 3X with ethyl acetate.