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5'-MMT-AMINO-MODIFIERS

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. The shorter carbon chain linkers may be used to attach compounds where proximity to the oligonucleotide poses no problem. The longer carbon chain linkers have specific applications in affinity chromatography where the oligonucleotide must be adequately spaced from the surface, and for labelling with biotin or fluorescent tags where interaction with the oligonucleotide, or the duplex it forms, may quench some of the fluorescence. The use of trityl-protected amino-modifiers is recommended when purification is required prior to use.

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.

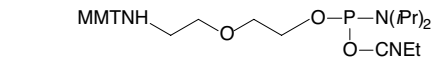
Deprotection: Deprotect as required by nucleobases.

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

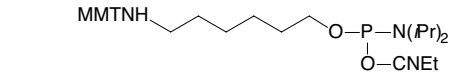
Storage: Freezer storage, -10 to -30°C, dry

Stability in Solution: 2-3 days

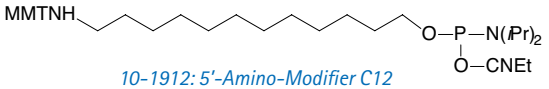
***FIGURE 1:* STRUCTURES OF 5'-MMT-AMINO-MODIFIERS**



10-1905: 5'-Amino-Modifier 5



10-1906: 5'-Amino-Modifier C6



10-1912: 5'-Amino-Modifier C12

KEY POINTS

- Do not remove the MMT 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 MMT-on oligo if you plan to do MMT-on purification without first adding a non-volatile base like TRIS base to avoid MMT loss.
- Do not remove the MMT group during cartridge purification since it will predominantly reattach. Rather, remove the MMT with aqueous acetic acid after purification. Using 20% glacial acetic acid/80% water allows extraction of MMT-OH using ethyl acetate before drying the solution. Or dry the oligo and desalt by your preferred method.

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.

If the 5'-amine is required for on-column conjugation, the MMT protecting group of the 5'-Amino-Modifiers can be removed on the synthesizer by deblocking until the yellow color elutes totally (typically 5 min.). The solution of MMT cation produced by acid deprotection is yellow and is not well quantified by trityl monitors. The method used to determine coupling efficiency is described below. However, for

maximum amine reactivity, it is preferable to retain the MMT group during deprotection and remove it later with 20% acetic acid in water.

PURIFICATION

The modified oligonucleotide may be purified using a reverse phase cartridge, e.g., Glen-Pak™ or Poly-Pak™, 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 MMT group should not be removed from the oligonucleotide as the lipophilic character of the MMT group aids in HPLC purification. RP HPLC purification is best accomplished using a C18 column.

If the cartridge technique is used for purification, the MMT removal on the cartridge with 2% aqueous trifluoroacetic acid is not efficient since the reaction is reversible and the trityl cation is not removed from the cartridge during the procedure. For cartridge or HPLC purification, the MMT 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 MMT alcohol which is only slightly soluble in this solution. To remove the MMT alcohol, extract 3X with ethyl acetate. The upper ethyl acetate layer containing the MMT alcohol is separated and discarded. The oligo remains in aqueous solution.

DETERMINATION OF COUPLING EFFICIENCY

This procedure is designed specifically for the determination of coupling efficiency. It should not be used for routine deprotection. The determination is based on a comparison of the absorbance of MMT cation at 472nm against the absorbance of the previous DMT cation at 497nm. The ratio factor (RF) of the absorbance of standard solutions is:

$$RF = \frac{\text{A-DMT(497)}}{\text{A-MMT(472)}} = 1.33$$

Procedure

1. Carry out the synthesis (1µmole) in the trityl-on mode.
2. Collect the last DMT solution in a 100mL volumetric flask and make up to the mark with 0.1M toluenesulfonic acid in anydrous acetonitrile (TSA). Measure the absorbance (A1) at 497nm, after zeroing the instrument at 600nm with TSA.
3. After the synthesis is complete, remove the synthesis column and manually deblock the MMT group with aliquots of the normal deblocking mix until all yellow color has eluted. This process takes up to 15 minutes. Collect the solution in a 100mL volumetric flask and make up to the mark with TSA. Measure the absorbance (A2) at 472nm.
4. Calculate the coupling efficiency using the formula:

$$\text{Coupling (\%)} = \frac{A2}{A1} \times RF \times 100$$

Note: Due to incomplete deblocking of the MMT group, this procedure yields a coupling efficiency determination about 5% below the actual coupling efficiency.