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SYNTHESIS USING METHYL PHOSPHONAMIDITES

Since methyl (Me) phosphonate linkages are uncharged and nuclease resistant, oligonucleotides containing them are interesting to researchers.[1] The synthesis of these oligos is quite straightforward but changes are necessary from regular deprotection procedures because the linkages are more base-labile. To help in purification and isolation of these oligos, as many phosphodiester linkages (prepared from regular cyanoethyl (CE) phosphoramidites) as possible should be included in each oligo.

Synthesis

Due to solubility differences, dissolve the Me phosphonamidites at the normal monomer concentration as follows:

dA	in anhydrous acetonitrile
Ac-dC[2,3]	in anhydrous acetonitrile
dG	in anhydrous tetrahydrofuran
dT	in anhydrous acetonitrile

Me phosphonamidites have been used successfully with all popular DNA synthesizers following the manufacturer's protocol for CE phosphoramidite synthesis. A reaction time of 5 minutes is recommended for syntheses of 1 µmoles and below. The use of the acetyl protected dC (Ac-dC) monomer is preferred to avoid base modification of dC residues during deprotection with ethylenediamine.[2,3] Trityl monitors may understate the coupling efficiency, presumably due to a difference in the rate of release of the trityl group.

Cleavage and Deprotection

A one-pot procedure for cleavage and deprotection has been described.[4,5] This is preferred to the procedure[6] we have used in the past since it leads to less cleavage of the methyl phosphonate backbone during the ammonium hydroxide cleavage step and evaporation of the ethylene-diamine.

1. Air-dry the support in the synthesis column, open the column and transfer the support to a deprotection vial.
2. Add 0.5mL of an ammonium hydroxide solution consisting of acetonitrile/ethanol/ammonium hydroxide (45:45:10) to the support. Seal the vial and leave it at room temperature for 0.5 hours.
3. Add 0.5mL of ethylenediamine to the vial and reseal it. Leave it at room temperature for a further 6 hours.
4. Decant the supernatant and wash the support twice with 0.5mL acetonitrile/water (1:1).
5. Dilute the combined supernatant and washes to 15mL with water.
6. Adjust the pH to 7 with 6M hydrochloric acid in acetonitrile/water (1:9) (~2mL).
7. Desalt using standard Poly-Pak™ or RP cartridge procedures.

Desalting Procedure

This procedure is a variation on the one described in the User Guide to DNA Purification, provided with Poly-Pak cartridges. Please refer to the User Guide for further details.

1. Pre-condition the cartridge with acetonitrile and 2M TEAA, as usual.
2. Load the neutralized solution on to the cartridge a minimum of two times. Retain the eluted solution for later analysis, if necessary.
3. Wash the cartridge with 10mL of water.
4. Elute with at least two 2mL aliquots of

acetonitrile/water (1:1). Measure A_{260} units to confirm that the oligo has been eluted.

5. Combine the aliquots containing oligo and evaporate to dryness.

HPLC Purification

Regular purification procedures can be used if the oligo contains several phosphodiester linkages. If the oligo contains a very high percentage Me phosphonate linkages, RP HPLC purification may work. However, these oligos have poor solubility characteristics and may precipitate in the sample loop, HPLC column, or other surfaces. More detailed descriptions of HPLC purification have been published.[4,5]

Note

These procedures have been shown to work well for small scale synthesis (1 μmole or below). For larger synthesis scales, changes may be required in the capping and oxidation steps. See reference 6 for details.

References

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