PROCEDURE FOR ULTRAMILD DEPROTECTION OF OLIGODEOXYNUCLEOTIDES

The synthesis of labelled oligonucleotides has become a standard procedure in many laboratories and many labelling reagents, e.g., biotin and fluorescein, are now available as ß-cyanoethyl (CE) phosphoramidites. Labels which are currently available as CE phosphoramidites have one common property - they must be stable to the strongly alkaline conditions required for removal of the base protecting groups. This property is lacking in several interesting dyes and labels. We sought an alternative protecting scheme for the normal CE phosphoramidites which would allow UltraMILD deprotection and would not react with a wider variety of sensitive monomers, tags and labels. A set of monomers using phenoxyacetyl (Pac) protected dA and 4-isopropyl-phenoxyacetyl (iPr-Pac) protected dG, along with acetyl protected dC, met the desired criteria for UltraMILD deprotection.

We recommend the use of phenoxyacetic anhydride (Pac_2O) in Cap A. This modification removes the possibility of exchange of the iPr-Pac protecting group on the dG with acetate from the acetic anhydride capping mix. Cleavage and deprotection can be carried out in 2 hours at room temperature with ammonium hydroxide or 4 hours with 0.05M potassium carbonate in methanol. If regular Cap A using acetic anhydride is used, overnight deprotection is required to remove acetyl protected dG formed by exchange.

Note that 0.05M potassium carbonate in methanol can not be evaporated to dyness without damaging the oligonucleotide. The solution must be neutralized prior to drying using 6 µL of glacial acetic acid per mL of 0.05M potassium carbonate in methanol.

UltraMild deprotection can be especially useful with sensitive labelling reagents such as TAMRA, Cyanine 5 and HEX since cleavage and deprotection can be carried out in 4 hours at room temperature with 0.05M potassium carbonate in anhydrous methanol as described below.

PROCEDURE

1. Carry out the synthesis of oligonucleotides containing labile bases or tags using recommended procedures.
2. Open the synthesis column and transfer the support to a suitable reaction vial.
3. Treat the support with 1 mL of 0.05M potassium carbonate in anhydrous methanol for a minimum of 4 h at room temperature.
4. Pipette the supernatant from the support and neutralize with 1.5mL of 2M triethylammonium acetate.

Either:

5. Desalt the oligonucleotide using normal procedures, lyophilize the resulting product and store the oligonucleotide at -20°C.

Or:

5a. Dilute the neutralized solution with 13.5 mL of water (to bring the methanol content to about 5%). Apply the diluted oligonucleotide solution to a prepared purification cartridge and carry out the standard purification scheme. (If the oligonucleotide is 5'-labelled and contains no DMT group, skip the 2% TFA wash.)

5b. Elute the purified oligonucleotide and lyophilize the resulting product. Store the oligonucleotide at -20°C.