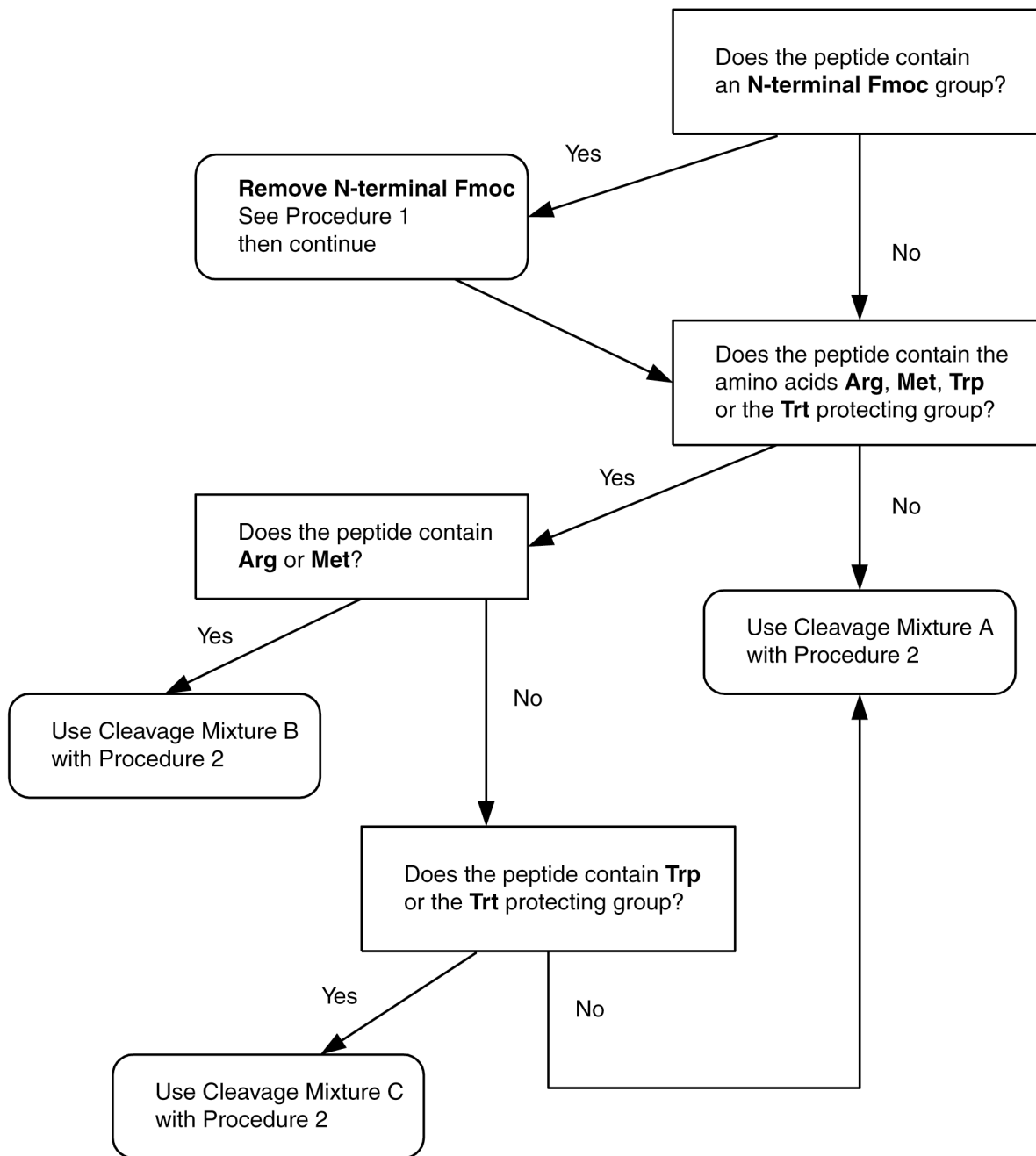


Fmoc Cleavage Flow Chart



Procedure 2: TFA Cleavage Procedure

WARNING *TFA is an extremely dangerous and corrosive liquid. Perform this procedure in an efficient, properly functioning fume hood. Wear appropriate eye protection, lab coat and gloves.*

Remove the Fmoc group from the peptide-resin BEFORE beginning this procedure. (See Procedure 1)

In the first three steps of the TFA cleavage procedure presented here, the peptide is deprotected and removed from the resin support. Following step 3, you may use either filtration (method I), centrifugation (method II), or diethyl ether extraction (method III) to isolate the peptide from the TFA solution.

The following procedures are designed for cleavage of 0.1 to 1.5 g of peptide-resin. We strongly suggest that you first use only 20-50 mg of peptide-resin to perform a small-scale trial run with any of these procedures. Analyze the crude material that results from the small-scale trial run to determine the optimum cleavage conditions for your peptide, then apply them to the remaining peptide-resin.

Cleavage mixtures (for 0.1-1.5 g peptide-resin):

A: 0.5 mL deionized H₂O
9.5 mL TFA

B¹: 0.75 g crystalline phenol
0.25 mL EDT
0.5 mL thioanisole
0.5 mL deionized H₂O
10 mL TFA

C: 0.25 mL EDT
0.25 mL deionized H₂O
9.5 mL TFA

Cleavage steps

Recommended Equipment and Chemicals:

round-bottom flask (10-50 mL) with stopper
micro stir bar
stir plate
ice bath
micropipettor
pipet tips

TFA
appropriate cleavage mixture

1. Prepare the appropriate cleavage mixture — either A, B, or C — as described above and indicated by the Fmoc Cleavage Flow Chart.

Note *Cleavage mixture B has been used successfully with peptides containing up to four Arg(Pmc). With five or more Arg(Pmc), more deprotection time may be required. However, do not extend this reaction to more than three hours.*

2. Place the dried peptide-resin in a round-bottom flask that contains a micro stir bar. Cool the flask in an ice bath.
3. Cool the cleavage mixture in an ice bath, then add it to the cooled peptide-resin to give a total reaction volume of 10 mL per 0.1-1.5 g of peptide-resin. After all the cleavage mixture has been added, remove the flask from the ice bath and allow it to warm to room temperature. Stopper the flask and stir the reaction mixture at room temperature for 1.5 hours. To isolate the peptide from the TFA solution, continue with either method I, method II, or method III.