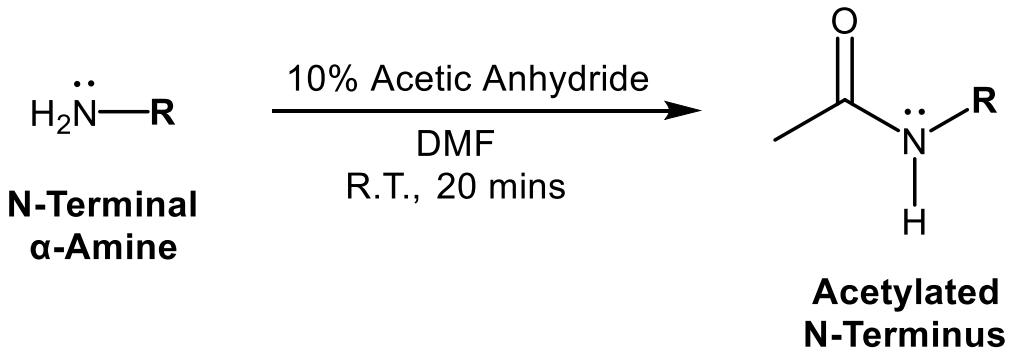
N-Terminus Acetylation Protocol

About

For certain applications it is valuable to acetylate, or cap, the N-terminus of a peptide. The following protocol details the reaction of an amine, typically on the N-terminus of peptides, with acetic anhydride to yield an acetylated cap. This is often necessary when making modifications to side-group amines, like those protected by an allyloxycarbonyl (Alloc) group. Capping ensures that after deprotection of the alloc group the only amine handle for subsequent reactions is that on the deprotected internal residue. If modifications should be completed at the N-terminus, it is often best to keep the reactive amine. Finally, acetylating can help with some solubility issues, depending on the sequence. Acetylation does remove one positive charge from the peptide since the amine is often protonated. The following protocol is based on work from Amblard et. al.[1]

Reaction Scheme



Note: **R** represents the rest of the peptide, connected to the resin.

Glassware and Equipment

- 1 x Coarse Fritted Peptide Synthesis Reaction Vessel with Rubber Stopper
- 1 x Red Peptide Synthesis Reaction Vessel Cap
- 1 x 250 mL Erlenmeyer Flask with Side-Arm Connected to Vacuum Pump
- 1 x Vortex Mixer with Tube Foam Insert or Stir Bar, Clamp, and Stir Plate
- 1 x Fume Hood
- 1 x 25-mL Graduated Cylinder or Adjustable 1000-µL Micropipette

Materials

The materials needed for this protocol are provided below. The Fisher Scientific catalog numbers are provided in parentheses.

- Dichloromethane (AC610050040)
- Methanol (A412-4)
- 10% Acetic Anhydride (A10-100) in N,N-Dimethylformamide (BP1160-4)

Reagent Tables

Table 1 – The reagent table for making the 10% acetic anhydride in DMF solution for a 0.10 mmol peptide synthesis. The highlighted cells represent the volume of the given component that should be combined.

Component	Moles (mmol)	Molecular Weight (g/mol)	Density (g/mL)	Volume (mL)
Peptide Resin	0.10			
Acetic Anhydride		102.09	1.080	0.4
Dimethylformamide (DMF)		73.09	0.944	3.6

Table 2 – The reagent table for making the 10% acetic anhydride in DMF solution for a 0.25 mmol peptide synthesis. The highlighted cells represent the volume of the given component that should be combined.

Component	Moles (mmol)	Molecular Weight (g/mol)	Density (g/mL)	Volume (mL)
Peptide Resin	0.25			
Acetic Anhydride		102.09	1.080	0.8
Dimethylformamide (DMF)		73.09	0.944	7.2

Safety Measures

When performing this protocol, users must wear safety glasses, laboratory gloves, pants, closed-toe shoes, and a fire-retardant laboratory coat. Everything should be performed in an efficient fume hood. These chemicals have the following hazard identifications:

Dichloromethane (DCM):

Methanol:

N,N-Dimethylformamide (DMF):

Acetic Anhydride:

Procedures

1. Begin by ensuring the resin has been added to a coarse fritted peptide synthesis reaction vessel.
2. Perform a dichloromethane (DCM) wash of the resin for one minute. This is done by adding DCM to the reaction vessel, placing a red cap on the reaction vessel, and placing the reaction vessel either in the tube foam insert of a vortex mixer or adding a stir bar to the reaction vessel and holding the reaction vessel with a clamp above a stir plate.
    a. Add ~2 mL of DCM for a 0.10 mmol scale synthesis.
    b. Add ~4 mL of DCM for a 0.25 mmol scale synthesis.
3. Remove the reaction vessel from the stirring, and place the black rubber stopper on the bottom of the reaction vessel in the top of the Erlenmeyer flask connected to the vacuum. Drain the reaction vessel by taking the red cap off, opening the stopcock on the reaction vessel, and turning on the vacuum.
4. Close the reaction vessel and remove from the vacuum.
5. Repeat steps 2-4 (washing and draining the reaction vessel) for one- to two-minute washes with the following solvents:
    a. 1 x DCM
    b. 1 x Methanol
    c. 2 x DCM
6. Add the 10% acetic anhydride to the reaction vessel. Place the red cap on the reaction vessel, and let the vessel shake on the vortex mixer or on the stir plate for 20 minutes.
    a. 0.10 mmol: 4-mL 10% acetic anhydride in DMF

   b. 0.25 mmol: 8-mL 10% acetic anhydride in DMF
7. After 20 minutes, drain the reaction vessel by placing the reaction vessel rubber stopper into the top of the Erlenmeyer flask vacuum setup, taking the red cap off, opening the stopcock on the reaction vessel, and turning on the vacuum.
8. Once the solution has been removed from the reaction vessel, perform the following washes, shaking for one to two minutes per wash:
   a. 3 x DCM
   b. 1 x Methanol
   c. 3 x DCM
9. After completing those washes, repeat steps 6 and 7, once more, to perform a second coupling.
10. After the second coupling, drain the reaction vessel as before. Perform the following washes, shaking for two minutes per wash:
   a. 3 x DCM
   b. 1 x Methanol
   c. 3 x DCM
   d. 1 x Methanol
   e. 3 x DCM
11. The N-terminus acetylation, or capping, is now completed. Further modifications can be performed, or the peptide can be cleaved from resin.

Reaction Mechanism

The reaction mechanism for N-terminus acetylation is given in **Figure 1**. The free amine begins the reaction through nucleophilic attack of a carbonyl carbon on acetic anhydride. This leads to the formation of a tetrahedral intermediate that forces the conjugate acid of acetic acid to act as the leaving group. The conjugate acid then steals a hydrogen atom from the positively charged nitrogen, which finishes the reaction. This leaves acetic acid and the acetylated amine.

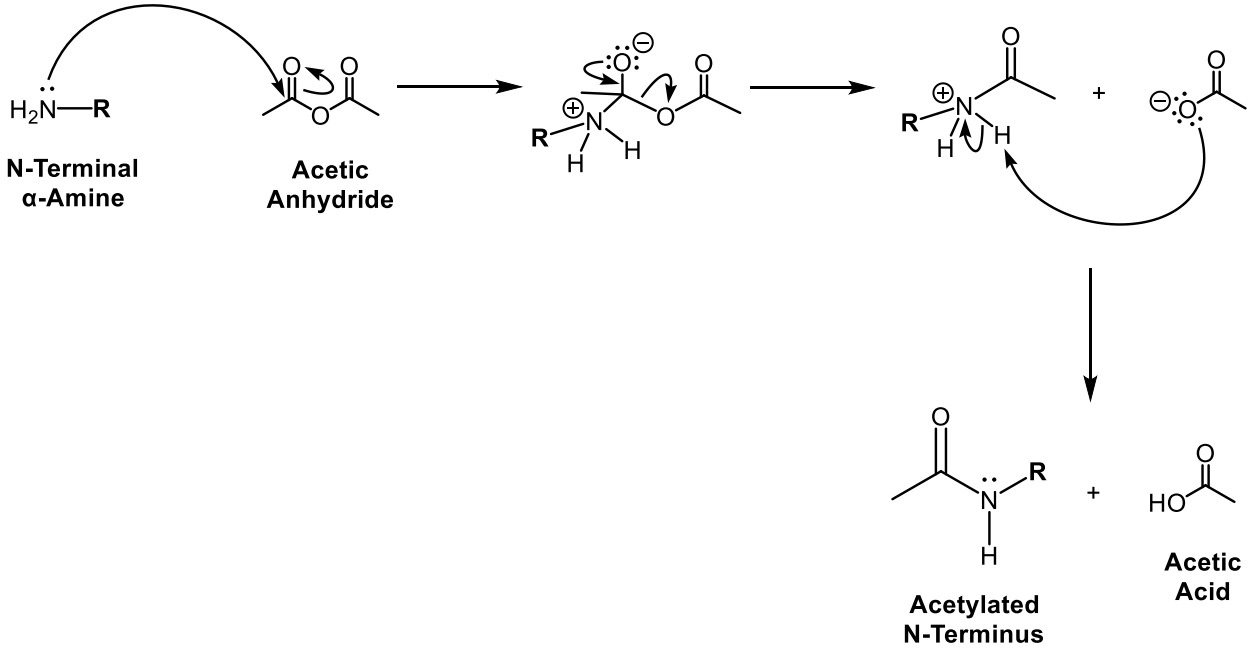


Figure 1 – The reaction mechanism for attack of a free amine with acetic anhydride, which is used in the capping/acetylation of the N-terminus of peptides on resin. **R** represents the rest of the peptide.[2]

References

(1) Amblard, M.; Fehrentz, J.-A.; Martinez, J.; Subra, G. Methods and Protocols of Modern Solid Phase Peptide Synthesis. *Mol. Biotechnol.* **2006**, *33* (3), 239–254. https://doi.org/10.1385/MB:33:3:239.

(2) Montalbetti, C. A. G. N.; Falque, V. Amide Bond Formation and Peptide Coupling. *Tetrahedron* **2005**, *61* (46), 10827–10852. https://doi.org/10.1016/j.tet.2005.08.031.