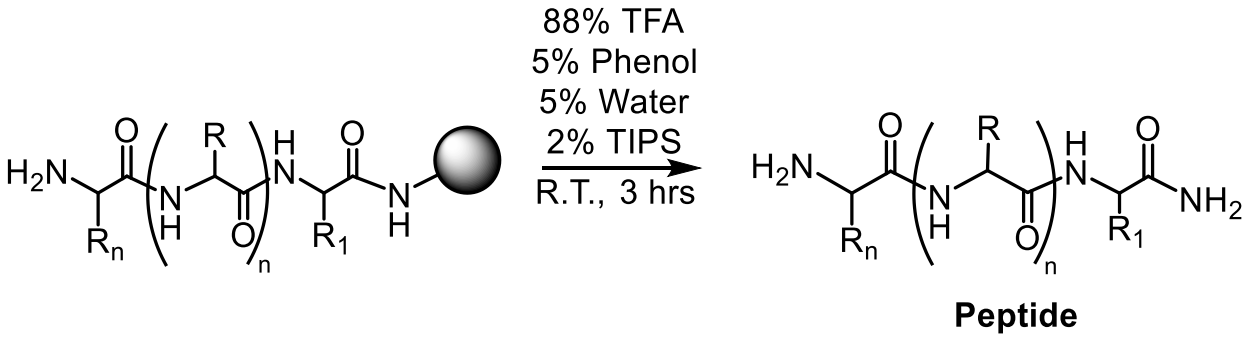
Peptide Cleavage from Resin Protocol

About

After a peptide has been synthesized on resin, and all desired on-resin modifications have been made, the peptide is ready to be cleaved off resin simultaneously with the removal of sidechain protecting groups. To perform the cleavage, trifluoroacetic acid (TFA) is used to remove the peptide from the resin linker and to remove acid labile protecting groups from the amino acid sidechains. The removal of the sidechain protecting groups leads to free electrophilic carbocations that can react with sensitive nucleophilic amino acid residues, altering the desired peptide. Therefore, a mixture of nucleophilic scavengers is often added to the TFA (termed a cleavage cocktail) to prevent side reactions. For more information on selecting a cleavage cocktail, see the Cleavage Cocktail Selection page on the PPMC website. The information below is the same for the various cleavage cocktails, and the only modification that would need to be made is the choice of a reagent table and the associated scavengers added to the reaction. This protocol will use the most commonly used cleavage cocktail within the Center, Reagent B, on a Rink amide resin.

Reaction Scheme



Glassware and Equipment

- 1 x Coarse Fritted Peptide Synthesis Reaction Vessel with Rubber Stopper
- 1 x White Rubber Septum Stopper, Sleeve Type
- 2 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 Analytical Balance

- 1 x Scoopula or Spatula
- 1 x Adjustable 1000-µL Micropipette
- 1-2 x 20-mL Scintillation Vials
- 2 x 50-mL Conical Centrifuge Tubes
- 2 x Glass Pasteur Pipette and Bulb
- 1 x Centrifuge
- 1 x Vortex Mixer
- 1 x Nitrogen Schlenk Line or Biotage V-10 Touch Evaporator

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 Acid (A38-212) in Dichloromethane
- Trifluoroacetic acid (60-017-61)
- Phenol (AA3321322)
- Triisopropylsilane (AC214922500)
- Water (From Milli-Q system or W5-4)
- Dithiothreitol (optional, BP172-5)
- Diethyl ether (E134-1)

Reagent Table

Table 1 – The reagent table for making one milliliter of Reagent B.[1] The highlighted cells represent the mass or volume of the given component that should be combined.

Component	Mass (mg)	Density (g/mL)	Volume (µL)	Percentage
Trifluoroacetic acid (TFA)		1.490	930	0.88
Phenol	60	1.070	56.07	0.05
Triisopropylsilane (TIPS)		0.773	20	0.02
Water		1.000	50	0.05

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: 

Acetic Acid: 

Trifluoroacetic acid (TFA): 

Phenol: 

Triisopropylsilane (TIPS): 

Dithiothreitol (DTT): 

Diethyl ether 

CAUTION: TFA is an extremely corrosive liquid; great care must be taken when using this reagent. Phenol is toxic and mutagenic; this must be used in a fume hood with proper eye protection and lab coat.

Procedures

1. Begin by adding the resin into 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. 2 x DCM
    b. 2 x Methanol
    c. 3 x DCM
6. After draining the final DCM wash, swell the resin in 10% Acetic Acid in DCM by adding it to the reaction vessel and shaking for 10 minutes.[2] This prevents residual N,N'-Dimethylformamide (DMF) from reacting with the TFA when it is added.
7. While the resin is swelling, prepare the cleavage cocktail solution. This protocol details the use of Reagent B;[1] however, other cleavage cocktails can be explored.
    a. For a 0.10 mmol scale, at least 5 mL of cleavage solution should be made.
    b. For a 0.25 mmol scale, at least 10 mL of cleavage solution should be made.
8. The best process for making a cleavage cocktail involves adding the solid components to a 20 mL scintillation vial and then adding the liquid components, with TFA being added last.
    a. For Reagent B,[1] begin by weighing out the phenol ***in the fume hood*** in a 20-mL scintillation vial.
        i. 0.10 mmol: 300 mg phenol
        ii. 0.25 mmol: 600 mg phenol
    b. Once the phenol has been added, if necessary, weigh out dithiothreitol (DTT) in a small weight boat and add to the scintillation vial.
        i. DTT is useful if there is a sulfur moiety present on the peptide (Cys, Met).
    c. Next, add the TIPS by pipette to the scintillation vial.
        i. 0.10 mmol: 100 µL TIPS
        ii. 0.25 mmol: 200 µL TIPS

d. Next, pipette water to the scintillation vial.
    i. 0.10 mmol: 250 µL water
    ii. 0.25 mmol: 500 µL water
e. Finally, add the required amount of TFA to the scintillation vial ***in the fume hood***.
    i. 0.10 mmol: 4.65 mL TFA
    ii. 0.25 mmol: 9.30 mL TFA
f. Vortex the scintillation vial to ensure the solid components have been dissolved.

9. Once the cleavage cocktail has been made fresh and the resin has swelled for 10 minutes, drain the reaction vessel. Add the cleavage cocktail solution to the reaction vessel using a Pasteur pipette. Replace the red cap with the white rubber septum stopper and let the reaction vessel shake on the vortex mixer or on the stir plate for three hours.
    a. Be sure to check on the reaction over time, specifically after the first 15 minutes. The solution should remain a dark orange to yellow color. If the solution is a red or bright red color, then there is excess of carbocations in solution so extra TIPS should be added to scavenge those protecting groups.
10. After three hours, collect the cleavage cocktail and peptide solution in a 20-mL scintillation vial by connecting the reaction vessel to a clean 250-mL Erlenmeyer flask connected to a vacuum. Drain the reaction vessel by taking the white rubber septum stopper off, opening the stopcock on the reaction vessel, and turning on the vacuum.
    a. ***This must be done in the hood with proper personal protective equipment. It is advised that the hood sash be down as far as possible.***
11. Next, add 45-mL of chilled diethyl ether to a 50-mL conical centrifuge tube.
    a. It is often useful at this point to turn on the centrifuge and use the "fast temp" setting to lower the temperature to 4 °C.
12. Add the cleavage cocktail and peptide solution from the 250-mL Erlenmeyer flask dropwise to the diethyl ether using a Pasteur pipette.
    a. The peptide should precipitate as a white or light pink solid in the diethyl ether.
    b. Continue adding the solution until the conical centrifuge tube's volume reaches 50-mL. If there is still leftover cleavage cocktail and peptide solution, then a new 50-mL conical centrifuge tube should be filled with 45-mL of chilled diethyl ether and the process repeated.
13. Once all the cleavage cocktail and peptide solution from the Erlenmeyer flask has been precipitated, centrifuge the contents of the conical centrifuge tube at 4 °C and 4400 rpm for six minutes.
14. After the centrifugation, decant the diethyl ether from the conical centrifuge tube.

15. Add 45-mL of fresh chilled diethyl ether to any conical centrifuge tubes that have peptide.
16. Re-suspend the initial peptide pellet(s) using the hand vortex.
17. Centrifuge the contents of the conical centrifuge tube(s) again at 4 °C and 4400 rpm for six minutes.
18. After centrifugation, decant the diethyl ether again.
19. Perform the process of washing the precipitate with diethyl ether one to three more times. This includes:
    a. Add 45-mL of fresh chilled diethyl ether to the conical centrifuge tube(s).
    b. Resuspend the initial peptide pellet(s) using the hand vortex.
    c. Centrifuge the contents at 4 °C and 4400 rpm for six minutes.
    d. Decant the diethyl ether.
20. Once you have completed the desired amount of diethyl ether washes, there are two options for drying the peptide precipitate.
    a. Dry Under Nitrogen (Preferred):
        i. Any conical centrifuge tube(s) that contain peptide pellet should have Parafilm wrapped around the top.
        ii. Place a hole in the Parafilm for the inlet of Nitrogen.
        iii. Place a hypodermic needle into the Parafilm as an exit for Nitrogen.
        iv. Place the conical centrifuge tube(s) in a clamp and place the Nitrogen Schlenk line into the inlet hole on the Parafilm.
        v. Start the Nitrogen flow and allow the pellet to dry overnight.
        vi. Store the crude product in the freezer until ready for purification.
    b. Dry Using the Biotage V-10 Touch Evaporator (Faster):
        i. Redissolve the peptide pellet in methanol.
        ii. Add the peptide in methanol solution to a different 20-mL scintillation vial.
        iii. Place the vial on the Biotage V-10 Touch Evaporator and run the “Volatile” method to remove all solvents.
        iv. Store the crude product in the freezer until ready for purification.

Reaction Mechanisms

The reaction mechanism for the removal of the peptide from the resin by TFA is given in **Figure 1**. The following figures then detail the removal of the sidechain protecting groups. **Figure 2**, **Figure 3**, **Figure 4**, **Figure 5**, and **Figure 6** show the reaction mechanisms for the removal of the Boc, Pbf, tBu, OtBu, and Trt groups, respectively.

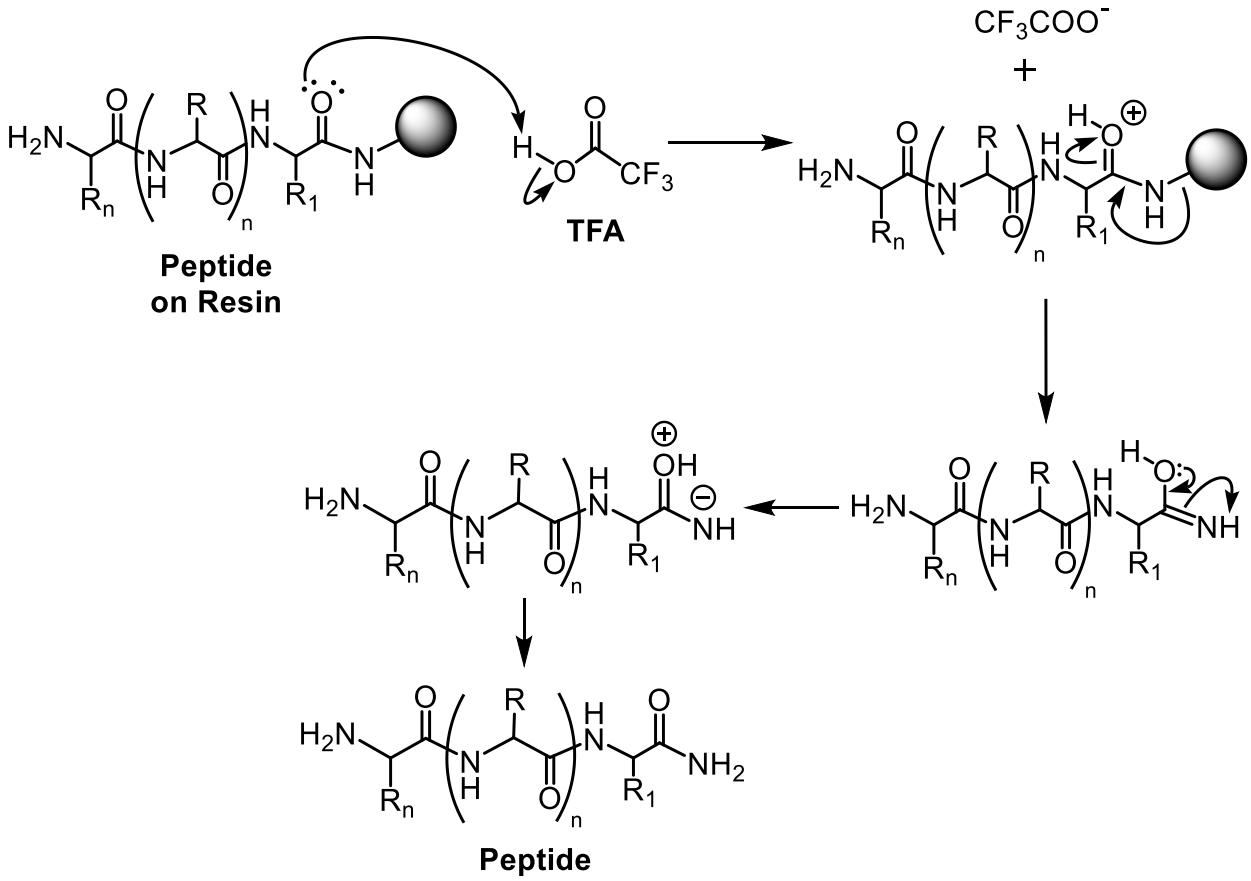


Figure 1 – The reaction mechanism for the cleavage of peptide from Rink amide resin with TFA. The full linker structure to the polystyrene bead is not depicted for simplicity, as well as the reaction of the positively charged linker/polystyrene resin with a nucleophile scavenger present in the cleavage process.[3]

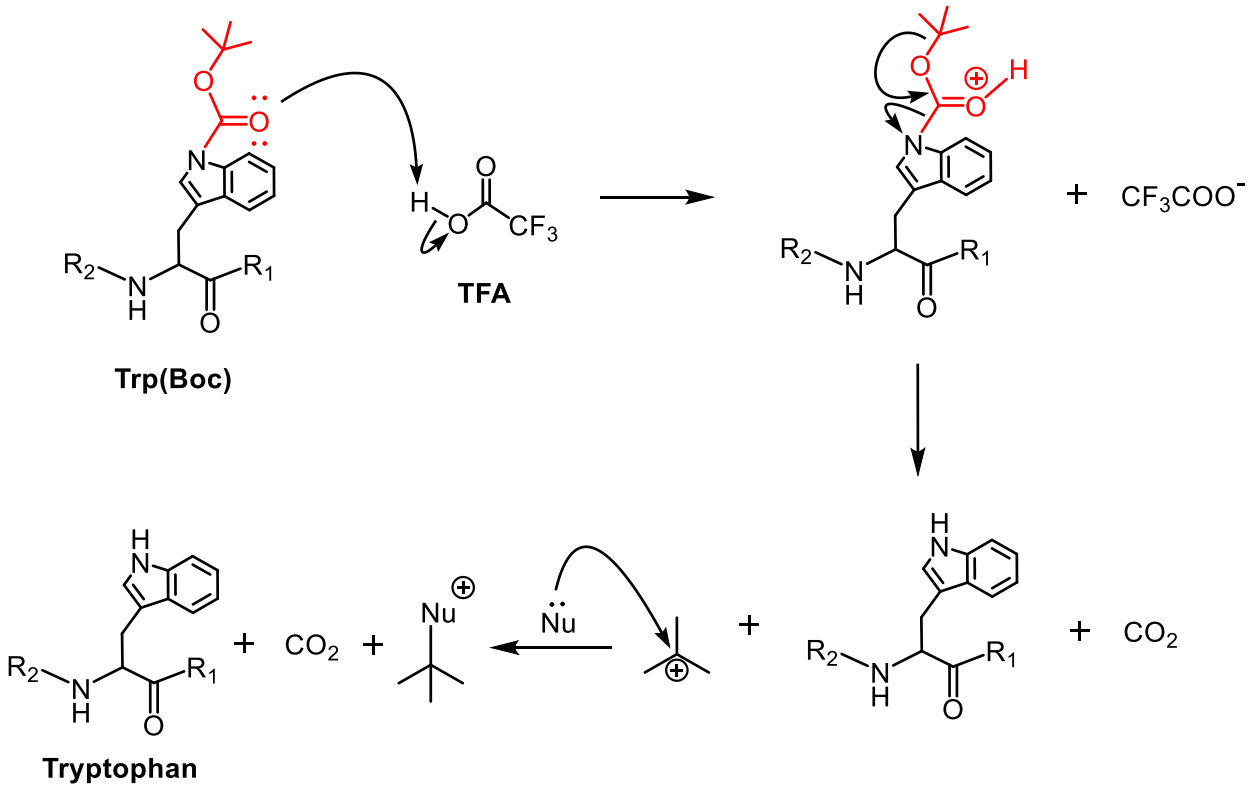


Figure 2 – The reaction mechanisms for the removal of the Boc (tert-butoxycarbonyl) protecting group from a protected Tryptophan residue by TFA. The Boc group is depicted in red. R_1 and R_2 are peptide chains, and Nu is a nucleophile scavenger present in the cleavage process.[3]

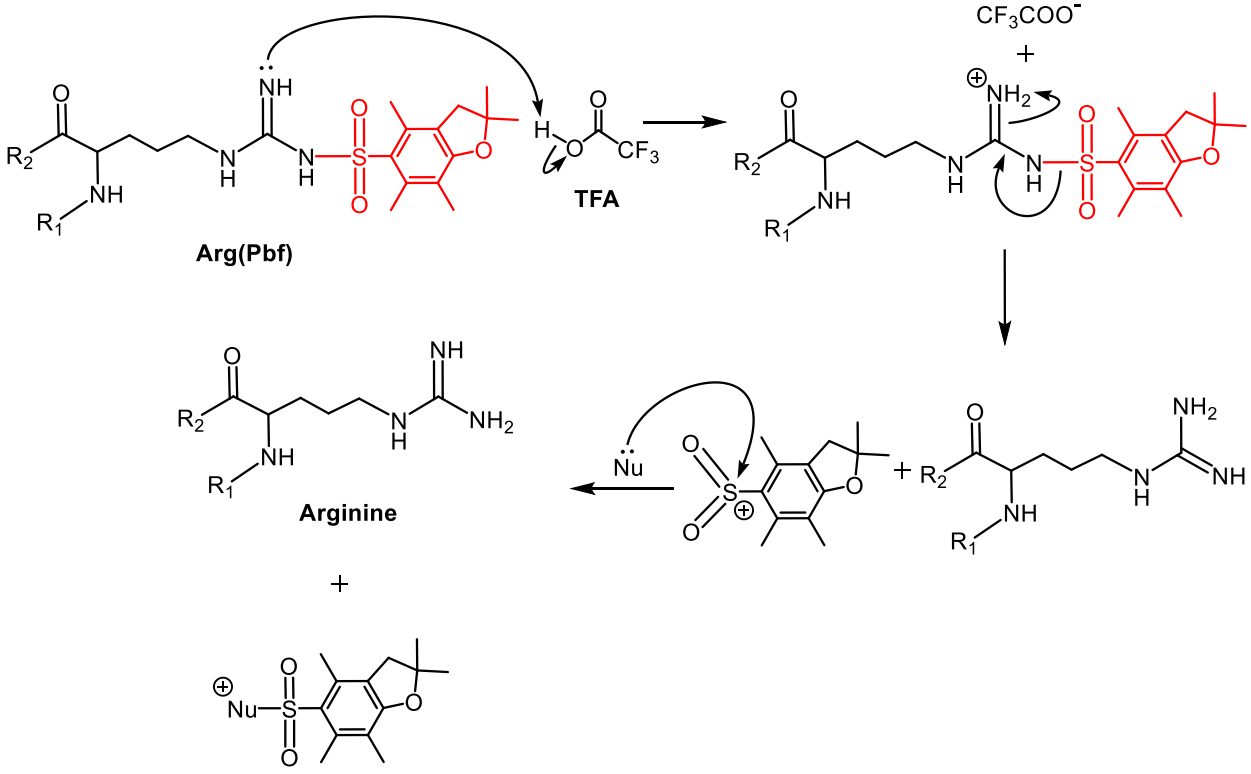


Figure 3 – The reaction mechanism for the removal of the Pbf (2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl) protecting group from a protected Arginine residue by TFA. The Pbf group is depicted in red. R_1 and R_2 are peptide chains, and Nu is a nucleophile scavenger present in the cleavage process.[3]

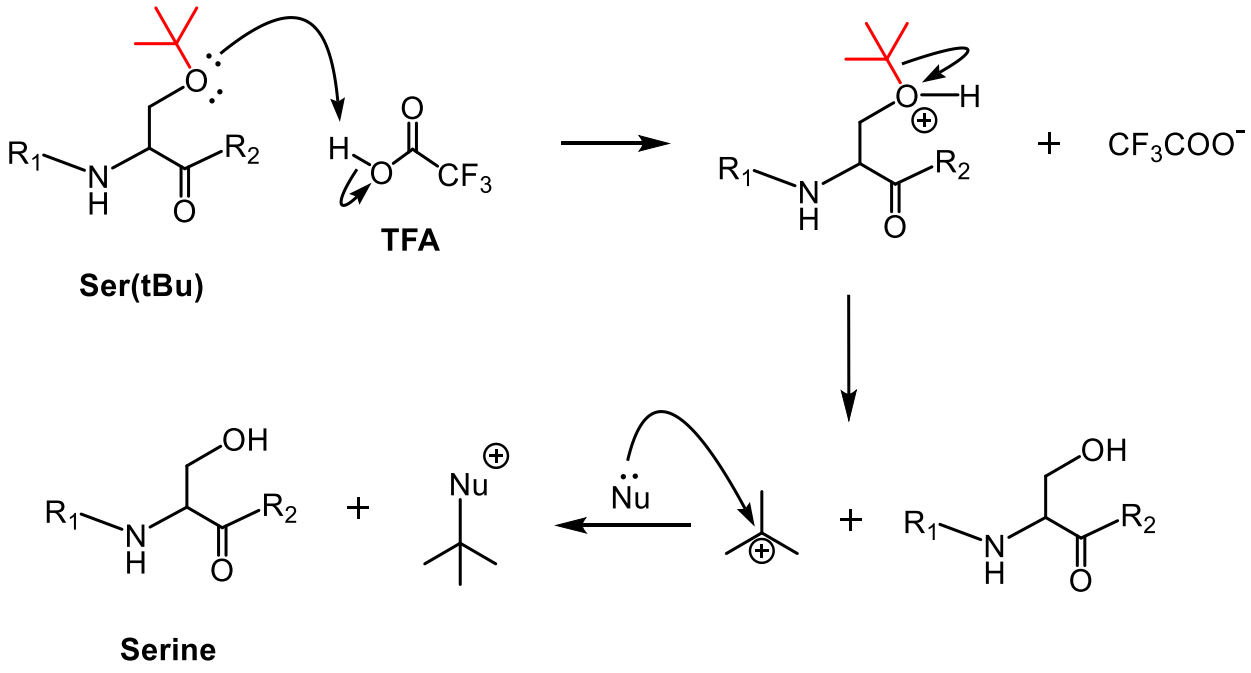


Figure 4 – The reaction mechanism for the removal of the tBu (tert-butyl) protecting group from a protected Serine residue by TFA. The tBu group is depicted in red. R_1 and R_2 are peptide chains, and Nu is a nucleophile scavenger present in the cleavage process.[3]

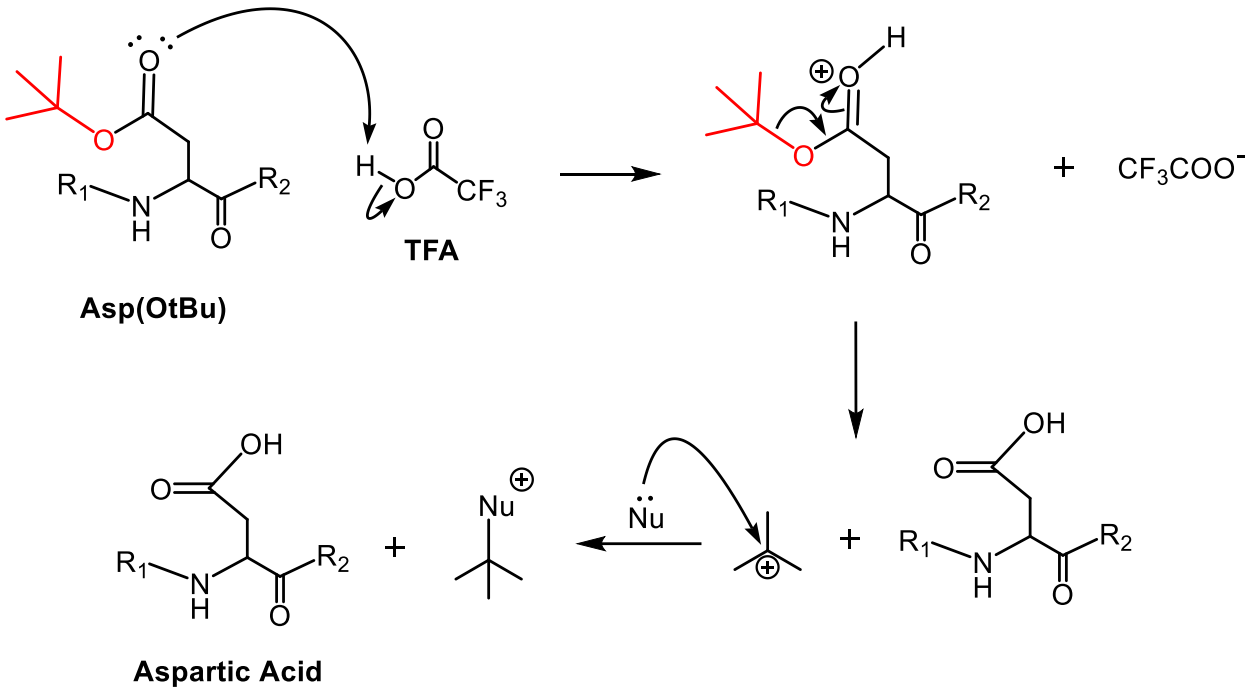


Figure 5 – The reaction mechanism for the removal of the OtBu (*O*-tert-butyl) protecting group from a protected Aspartic Acid residue by TFA. The OtBu group is depicted in red. R_1 and R_2 are peptide chains, and Nu is a nucleophile scavenger present in the cleavage process.[3]

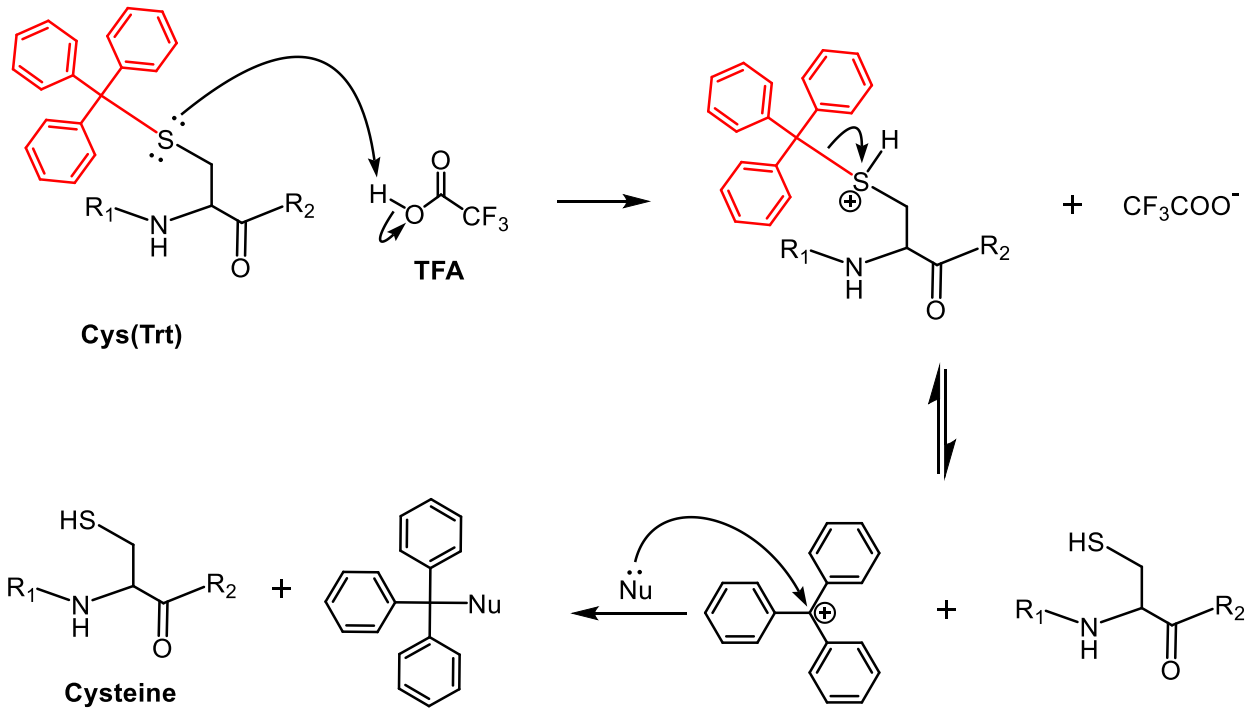


Figure 6 – The reaction mechanism for the removal of the Trt (triphenylmethyl) protecting group from a protected Cysteine residue by TFA. The Trt group is depicted in red. R_1 and R_2 are peptide chains, and Nu is a nucleophile scavenger present in the cleavage process.[3]

References

(1) Sole, N. A.; Barany, G. Optimization of Solid-Phase Synthesis of [Ala8]-Dynorphin A. *J. Org. Chem.* **1992**, *57* (20), 5399–5403. https://doi.org/10.1021/jo00046a022.

(2) Chan, W.; White, P. *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*; Oxford University Press, **1999**. https://doi.org/10.1093/oso/9780199637256.001.0001.

(3) Pires, D. A. T.; Bemquerer, M. P.; do Nascimento, C. J. Some Mechanistic Aspects on Fmoc Solid Phase Peptide Synthesis. *Int. J. Pept. Res. Ther.* **2014**, *20* (1), 53–69. https://doi.org/10.1007/s10989-013-9366-8.