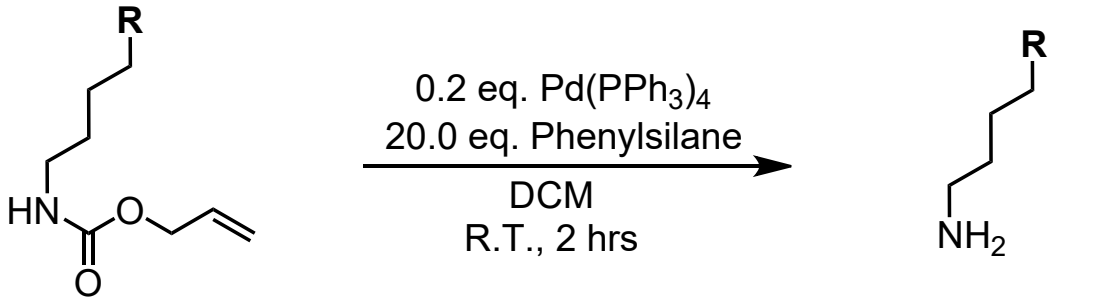
Alloc Protecting Group Removal Protocol

About

For orthogonal protection of amines, alcohols, and indoles, the allyloxycarbonyl (Alloc) group is a great choice. Other allyl-based groups include allyl protection for carboxylic acids, phenols, and imidazoles, as well as allyloxycarbonylaminomethyl (Allocam) for thiol protection. These protecting groups are completely orthogonal to the t-Bu and Fmoc protecting groups. This has allowed the protecting groups to be used in conjunction for the preparation of branched,[1] cyclic,[2,3] and protected peptides[4] through solid-phase peptide synthesis. This chemistry was first introduced by Lloyd-Willliams and coworkers.[5] The following procedure is based on those by Thieriet et. al.[6] Protection with the alloc group has been previously used with bundlemers for side-chain protection.[4] Within the PPMC, the alloc group is typically used for protection of lysine residues. These can be incorporated into a peptide and deprotected for further modification on the remaining free amine. Note that the N-terminus of the growing peptide must be protected (either still protected by Fmoc or capped through acetylation) to prevent subsequent reactions adding to the N-terminus of the original peptide in addition to those on the originally alloc protected residue.

Reaction Scheme



Alloc Protected Lysine

Deprotected Lysine (Free Amine)

Note: **R** represents the peptide backbone, connected to 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 Analytical Balance
- 1 x Scoopula or Spatula
- 1 x Adjustable 1000-µL Micropipette
- 1 x 8-mL Scintillation Vial

Materials

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

- Dichloromethane (AC610050040)
- Methanol (A412-4)
- Tetrakis(triphenylphosphine)palladium(0), $Pd(PPh_3)_4$ (AC202380010)
- Phenylsilane (AC291690050)

Reagent Tables

Table 1 – The reagent table for making the deprotection solution for a 0.10 mmol peptide synthesis. The highlighted cells represent the mass or volume of the given component that should be combined.

Component	Ratio	Moles (mmol)	Molecular Weight (g/mol)	Mass (g)	Density (g/mL)	Volume (mL)
Peptide Resin	1.0	0.10				
$Pd(PPh_3)_4$	0.2	0.02	1155.58	0.0231		
Phenylsilane	20.0	2.00	108.22	0.2164	0.877	0.247
Dichloromethane (DCM)			84.93		1.330	3.753

Table 2 – The reagent table for making the deprotection solution for a 0.25 mmol peptide synthesis. The highlighted cells represent the mass or volume of the given component that should be combined.

Component	Ratio	Moles (mmol)	Molecular Weight (g/mol)	Mass (g)	Density (g/mL)	Volume (mL)
Peptide Resin	1.0	0.25				
$Pd(PPh_3)_4$	0.2	0.05	1155.58	0.0578		
Phenylsilane	20.0	5.00	108.22	0.5411	0.877	0.617
Dichloromethane (DCM)			84.93		1.330	7.383

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:

$Pd(PPh_3)_4$:

Phenylsilane:

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. 2 x DCM
    b. 2 x Methanol
    c. 3 x DCM
6. Next, begin the preparation of the deprotection solution by weighing and adding 0.2 equivalents of $Pd(PPh_3)_4$ for every equivalent of peptide to an 8-mL scintillation vial.
    a. 0.10 mmol: 23.1 mg $Pd(PPh_3)_4$
    b. 0.25 mmol: 57.8 mg $Pd(PPh_3)_4$
7. In the same 8-mL vial, pipette 20 equivalents of phenylsilane for every equivalent of peptide.
    a. 0.10 mmol: 247 µL phenylsilane
    b. 0.25 mmol: 617 µL phenylsilane
8. Add DCM to the 8-mL vial. For a 0.10 mmol scale, the total volume should be 4-mL. For a 0.25 mmol scale, the total volume should be 8-mL.
    a. 0.10 mmol: 3.753 mL DCM
    b. 0.25 mmol: 7.383 mL DCM
9. Shake the 8-mL scintillation vial till everything is mixed and dissolved.
10. Add the vial's contents to the peptide synthesis 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 two hours.
11. After two hours, 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.

12. Once the deprotection solution has been removed from the reaction vessel, perform the following washes (allowing it to shake for at least one minute per wash).
    a. 1 x DCM
    b. 1 x Methanol
    c. 1 x DCM
13. After completing those washes, repeat steps 6-10, once more, to perform a second deprotection.
14. After the second deprotection, drain the reaction vessel as before. Perform the following washes with one to two minutes of shaking before performing the next rinse:
    a. 3 x DCM
    b. 1 x Methanol
    c. 3 x DCM
    d. 1 x Methanol
    e. 3 x DCM
15. The alloc group deprotection is now completed. Further modifications can be performed utilizing the free amine, or the peptide can be cleaved from resin.

Reaction Mechanisms

Before acting as a catalyst for the alloc deprotection, the tetrakis(triphenylphosphine)palladium(0), or $Pd(PPh_3)_4$, undergoes equilibrium dissociations of the ligand, triphenylphosphine (PPh_3), as seen in **Figure 1**. The equilibrium is largely in favor of the tricoordinated species, while further dissociation is less likely to occur without some sort of driving force.[7]

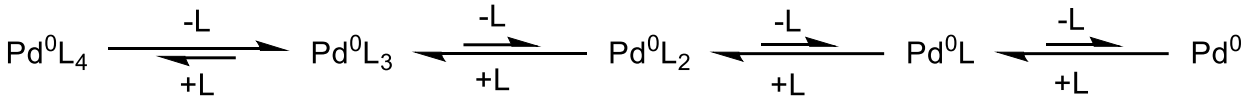


Figure 1 – The ligand dissociation of the palladium(0) compound. If using tetrakis(triphenylphosphine)palladium(0), the ligand, L, is represented by PPh_3 (triphenylphosphine).[7]

The catalytic behavior of the palladium(0) is described by the Tsuji-Trost allylation reaction. This process is depicted in **Figure 2**. It involves the Pd^0L_2 form that coordinates with the allyl group. The first step involves an equilibrium coordination of the allyl group with the palladium. In this specific protocol, the allyl functional group on the alloc group on the peptide, is coordinated to the palladium. The second step involves formation of a π-allyl complex. The actual mechanism for this step is given in **Figure 3**. The third and final step involves nucleophilic attack on the π-allyl complex and regeneration of the palladium. The mechanism for this nucleophilic attack is shown in **Figure 4**. In this protocol, the nucleophile is phenylsilane. The nature of the nucleophile determines the reaction mechanism. For soft nucleophiles, categorized as those whose conjugate acid has a pKa less than 25, direct attack at the allylic carbon site occurs. Meanwhile, hard nucleophiles tend to coordinate to the palladium. Then, reductive elimination occurs through internal delivery of the nucleophile to the carbon. It is essential that a good nucleophile be present to prevent side reactions that lead to allylamines.[7] Phenylsilane has been shown to be an efficient at preventing these side reactions and is known to act as a hard nucleophile with its ability to act as a hydride donor.

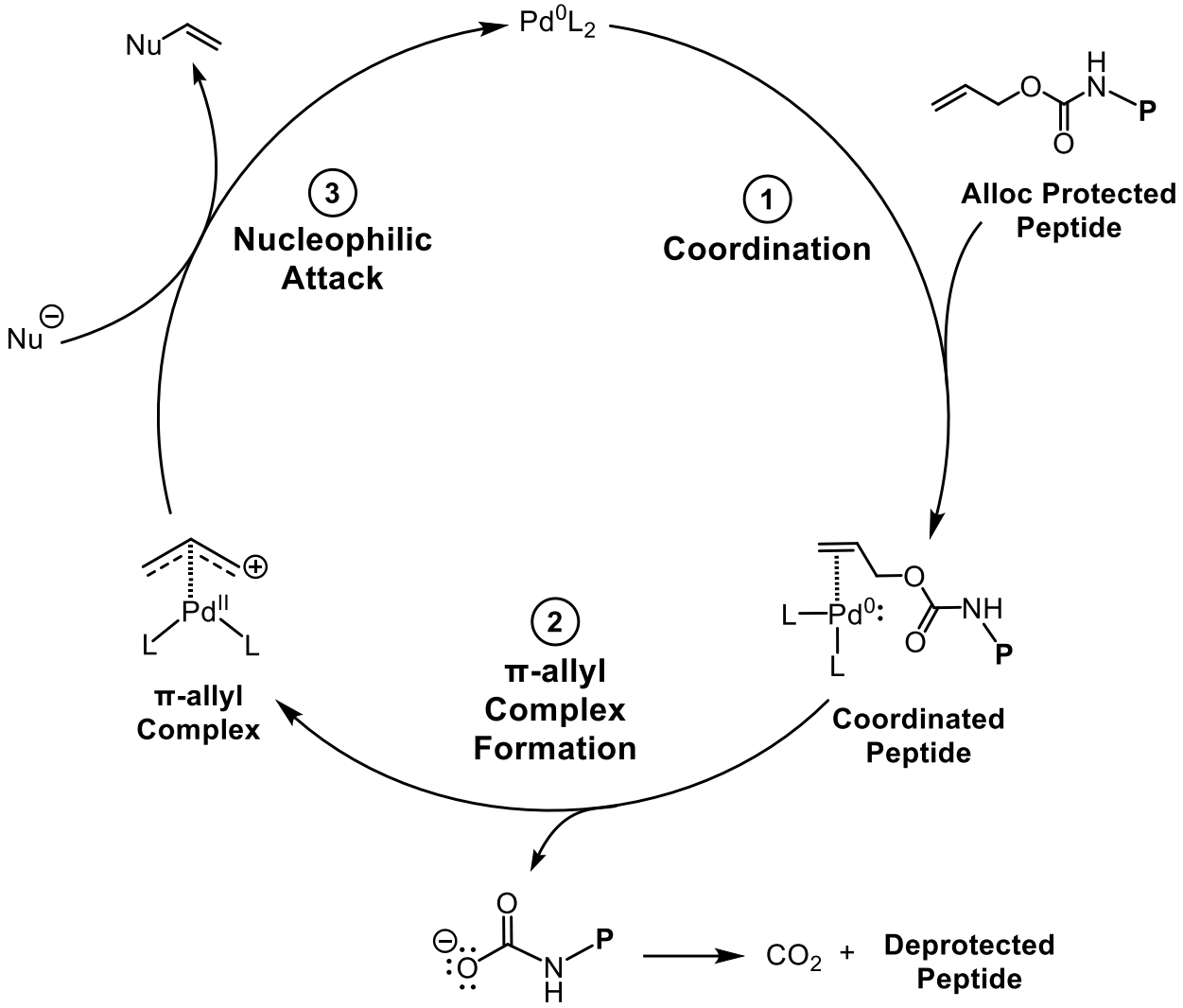


Figure 2 – An overview of Tsuji-Trost allylation.[7] The ligand associated with the palladium, L, is PPh_3. The **P** group represents the rest of the peptide connected to the alloc protected amine functional group.

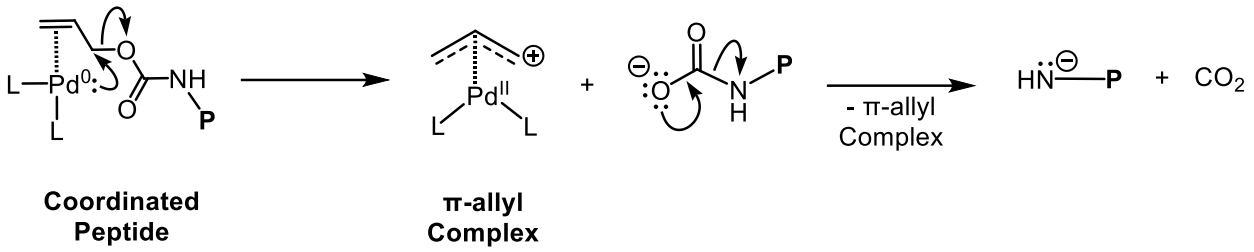


Figure 3 – The reaction mechanism for the second step of the Tsuji-Trost reaction that involves the production of a π-allyl complex. In this step, the Pd^0L_2 fragment displaces the leaving group, in this case the peptide, which leads to the π-allyl complex.[7] L is the ligand PPh_3 associated with the palladium. **P** is the rest of the peptide attached to the alloc protected amine functional group.

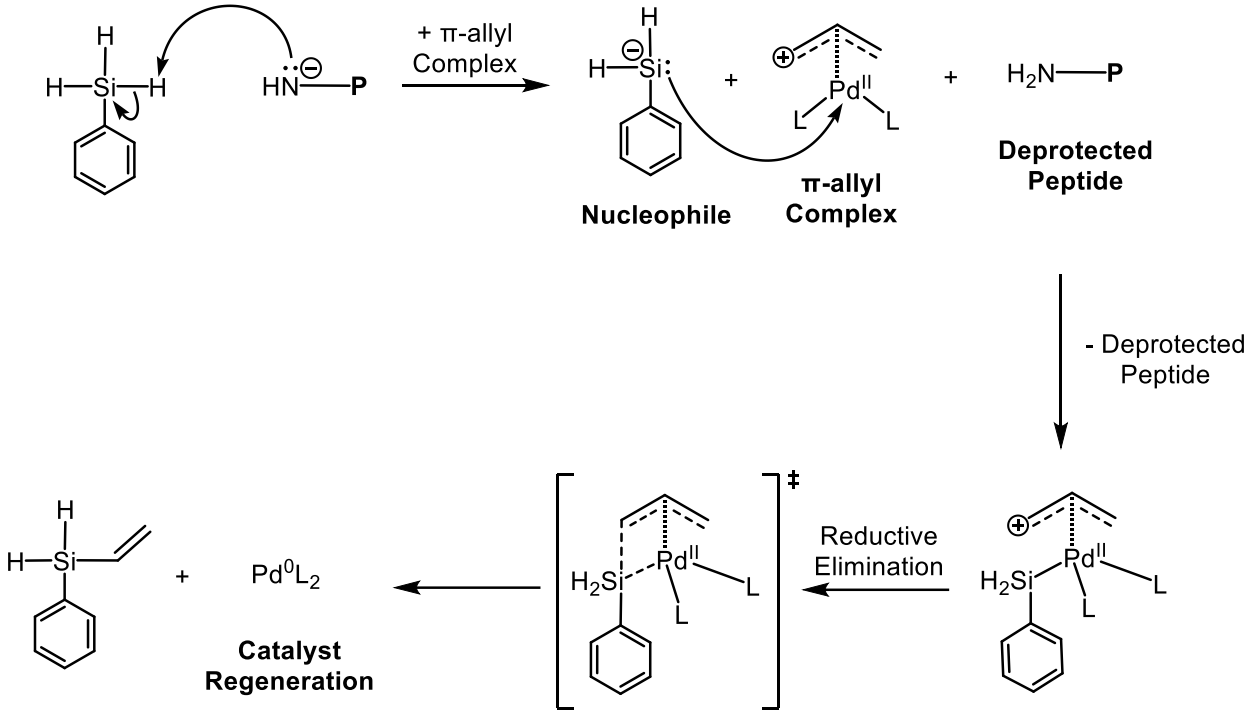


Figure 4 – The reaction mechanism for the third and final step of the Tsuji-Trost reaction.[7] Depicted first is the nucleophilic attack of the nitrogen atom from the peptide fragment of a hydrogen on phenylsilane, with the **P** group representing the rest of the peptide. This leads to the completely deprotected peptide. The final portion of the mechanism involves nucleophilic attack by the phenylsilane on the palladium. Depending on the nature of the nucleophile, this reaction mechanism is different. Here, phenylsilane is depicted as acting like a hard nucleophile. After the attack of the phenylsilane, reductive elimination occurs, regenerating the catalyst. L is the ligand PPh_3 associated with the palladium.

References

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