

Standard practices for Fmoc-based solid-phase peptide synthesis in the Nowick laboratory (Version 1.6.3)

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Contents	1
Contributions to this guide	2
General	2
Solid Supports	2
Loading 2-chlorotrityl chloride resin	3
Capping 2-chlorotrityl chloride resin	3
Loading rink amide resin	4
Capping rink amide resin	5
Checking resin loading	5
Solid-phase peptide synthesis	5
Manual (hand) coupling	6
Alloc deprotection	7
Dde deprotection	7
Automated synthesis – PS3 synthesizer	8
Automated synthesis – CEM synthesizer	8
Cleavage form solid support	9
Cleavage of side-chain protected linear peptide from 2-chlorotrityl chloride resin	9
Cleavage of linear peptide from Rink Amide resin	9
Preparing cyclic peptides	10
Cyclization	10
Global deprotection of acid labile protecting groups	10
Subsequent chemistries on "isolated" peptides	11
Removing TFA esters with MeOH reflux	11
Ether precipitation	11
Oxidation to form a disulfide bond	12
DMSO oxidation	12
ACM deprotection and disulfide bond formation	13
Purification of peptides	13
Reverse-phase HPLC purification	13
General operation of the semi-preparative RP-HPLCs	14
Checking fractions, and lyophilizing	14

Contributions to this guide

Latest revision: September 19th 2018

A.G.K. wrote the majority of protocols, made the majority of chemdraws, and compiled the first few word document versions of this guide. P.J.S. added the Removal of TFA esters by MeOH reflux and Cleavage of linear peptide from Rink Amide resin protocols, in addition to compiled the guide as LaTeX document. H.J.Y. added the removal of alloc protecting group and dde protecting group, corrected the Chemdraws, hand coupling section, in addition to compiled the guide as Word document.

The majority of these protocols exist due to countless other unmentioned sources passing their knowledge down from student to student, in addition to a number of academic sources, that may be added to this guide in the future.

General

Peptide synthesis is pretty robust and fool proof; however, there are a few things that can really mess up the reproducibility of these protocols. Probably chief amongst them is the quality of DMF. It is incredibly important the you use the "good" DMF. This means either getting it off of the solvent system, or opening a new bottle. This is particularly true for steps that fall under the **Solid-phase peptide synthesis** section of this guide.

"good DMF" Alfa aesar DMF (43465 N,N-Dimethylformamide, anhydrous, amine free, 99.9%)

Solid Supports

The first step in solid-phase peptide synthesis is choosing what functional group you want your C-terminus to be:

If you want your C-terminus to be a carboxylic acid use 2-chlorotrityl resin.

If you want your C-terminus to be an amide use Rink amide resin.

If you are making a macrocyclic peptide use 2-chlorotrityl resin.

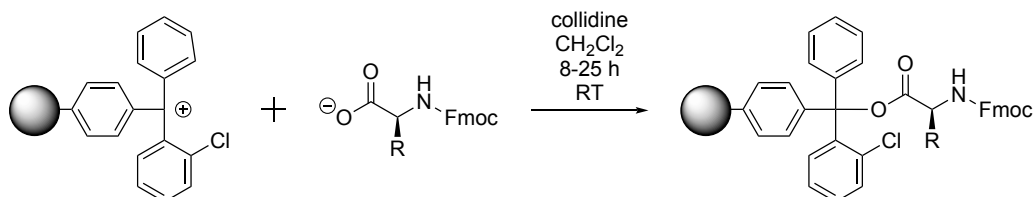
Once your choice of resin is made you will need to load your first amino acid onto the resin.

Loading 2-chlorotrityl chloride

The purpose of this step is to covalently link the first amino acid onto the resin.

Prep time: 30 min

Reaction time: 8–24 h



Scheme 1. Loading 2-chlorotrityl chloride resin. Resin is drawn as carbocation, whereas amino acid is drawn as carboxylate, to ease explanation of chemistry.

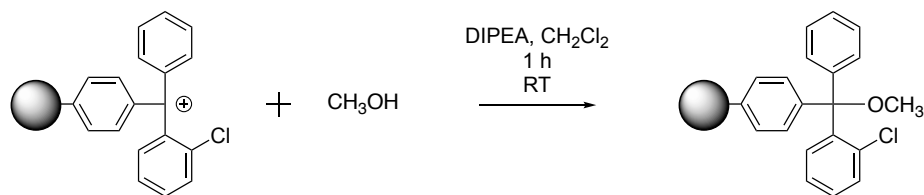
1. Weigh out appropriate amount of resin. Generally, we use 300 mg for a 0.1 mmol scale synthesis. Transfer the resin into a PolyPrep chromatography column (BioRad).
2. Swell resin for at least 30 min (longer is okay) at room temperature in dry CH₂Cl₂.
3. Weigh out an appropriate amount of the first amino acid and dissolve it in 8 mL CH₂Cl₂ w/ 0.3 ml 2,4,6-collidine. *When making a macrocyclic peptide our first amino acid is almost always Boc-Orn(Fmoc)-OH. Use ca. 100 mg of Boc-Orn(Fmoc)-OH*
4. Using a flow of nitrogen gas, push out all CH₂Cl₂ from the column that contains the swelled resin and add the amino acid/CH₂Cl₂/collidine mixture
5. Rock for at least 8 h (no longer than 24 h).
6. Move on to Capping 2-chlorotrityl Resin.

Capping 2-chlorotrityl chloride resin

The purpose of this step is to covalently link a small nucleophile (methanol) to the unreacted carbocations on the 2-chlorotrityl chloride resin.

Prep time: 10 mins

Reaction time: 1 hour



Scheme 2. Capping 2-chlorotrityl chloride resin. Resin is drawn as carbocation to ease explanation of chemistry.

1. Wash the loaded resin 3X with CH₂Cl₂.
2. Make the capping solution. The capping solution is CH₂Cl₂:MeOH :DIPEA (17:2:1). Make this fresh each time by adding 1 mL MeOH and 0.5 mL diisopropylethylamine (DIPEA, or DIEA) to 9 mL of CH₂Cl₂.
3. Dump the capping solution on to the loaded resin and rock for 1 hour at room temperature. It is not recommended to extend the reaction time, as exchange of the loaded amino acid with MeOH is a possibility.
4. After 1 h, push out the capping solution with nitrogen and wash the resin 2X with CH₂Cl₂ and 1X with DMF. *At this point you can analyze how efficient your resin was loaded. We typically skip this step, though, as loading 2-chlorotrityl resin is VERY reproducible if you do not stray from the protocol detailed above.*

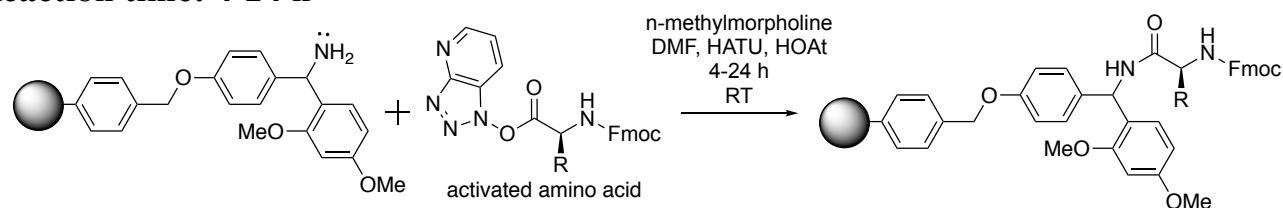
- Your loaded resin is now ready to go through repeated Fmoc-deprotections and amino acid couplings to build the rest of your peptide. These deprotections and couplings can be done manually (hand coupling) or on an automatic synthesizer. Regardless of your preferred method, the order of operation and chemistry is identical.

Loading rink amide resin

The purpose of this step is to covalently link the first amino acid to the resin

Prep time: 1.5 h

Reaction time: 4-24 h



Scheme 3. Loading Rink Amide resin. Note in this scheme the resin has already been Fmoc-protected

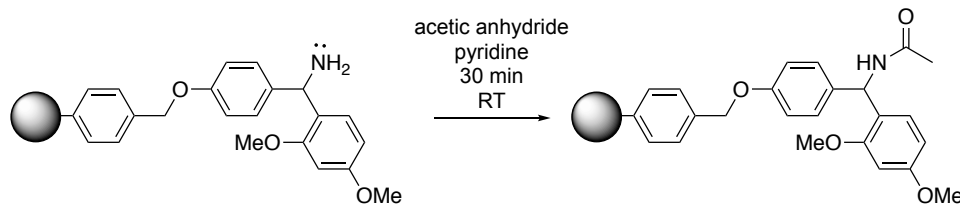
- Weigh out appropriate amount of resin. Generally we use 300 mg for a 0.1 mmol scale synthesis. Dump the resin into a PolyPrep chromatography column (BioRad).
- Swell resin for 1 hour in DMF. After 1 h has passed, use a flow of nitrogen gas to push out the DMF out of the column.
- Cleave Fmoc from the resin by adding 8 mL of 20% (v/v) piperidine in DMF. Allow the resin to rock in the 20% piperidine solution for 1 h. When there is 15 min left start preparing the amino acid you want to load the resin with.
- Preparing (or activating) the amino acid: weigh out 5 equivalents of the amino relative to achieving 100% loading of the resin. The amount of amino you want to weigh out is most easily figured out using Ryans Excel mass spec calculator. Also weigh out 4.5 equivalents of HATU and 4.5 equivalents of HOAt. Combine the amino acid, HATU, and HOAt in a glass scintillation vial and add 8 ml of 20% *N*-methylmorpholine (v/v) in DMF (alternatively, you can use 20% collidine (v/v) in DMF).
- After the Fmoc cleavage from the resin is complete use a flow of nitrogen to push out the 20% piperidine.
- Wash the resin 3X with DMF from the solvent system.
- Dump the amino acid/HATU/HOAt solution onto the resin and rock at room temperature for at least 4 h. Do not exceed 24 h.
- Next, use a flow of nitrogen to push out the amino acid solution.
- Wash the loaded 3X with DMF from the solvent system.
- Move on to Capping Rink Amide Resin

Capping Rink amide resin

The purpose of this step is to cap unreacted amines on rink amide so that the next amino acids you couple are not attached to the resin

Prep time: 5 min

Reaction time: 30 min



Scheme 4. Capping unreacted sites on Rink amide resin

1. Prepare the capping solution by combining acetic anhydride and pyridine in a 3:2 ratio of acetic anhydride:pyridine. Make this fresh each time. Crudely by using a Pasteur pipette to combine 3 "squirts" of acetic anhydride with 2 "squirts" of pyridine in a scintillation vial.
2. Dump the capping solution on the resin and rock for 30 min at room temperature.
3. After the resin is done capping push out the capping solution with nitrogen and wash the resin 4X with DMF.
4. Your loaded resin is now ready to go through repeated Fmoc-deprotections and amino acid couplings to build the rest of your peptide. These deprotections and couplings can be done on an automatic synthesizer or manually (hand coupling).

Checking resin loading

The purpose of this step is to determine the mmol of amino acid that are on your resin. The procedure is exactly same regardless of the resin you are using.

Prep time: 20 mins

Reaction time: 5 mins

1. Take a small portion (1–2 mg) of resin and transfer it to a new polyprep column. Dry the resin by blowing nitrogen through it.
2. While the resin drying find a 3-mL quartz cuvette and add exactly 3 mL of 20% piperidine in DMF to it. Blank the UV/Vis with the 20% piperidine at 290 nm.
3. Weigh as close to 1 mg of dried resin as you can and add it to 3 mL 20% piperidine in DMF in the quartz cuvette.
4. Allow the resin to sit in the 20% piperidine for at least 5 min. Longer reaction times are fine.
5. Take a UV/Vis reading at 290 nm against the 20% piperidine blank.
6. Use Ryan's Excel mass spec calculator to determine your loading percentage (present in our group website under LINKS). This will dictate the mmol of each amino acid you want to use when synthesizing your peptide. Good loading percentages fall between 50-70%.

Solid-phase peptide synthesis

The purpose of this step is to sequentially add amino acids to the resin to build a peptide chain. There are two main steps in coupling an amino to peptide chain. The first step is deprotecting Fmoc from the amino on the resin to expose an amine. The second step is coupling an activated amino acid to the exposed amine. These steps are done exactly the same on 2-chlorotriyl chloride and rink amide resin.

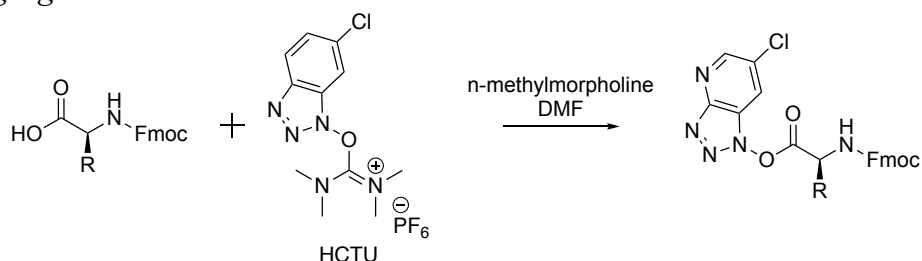
Manual (hand) coupling

You will need stock solutions of 20% piperidine in DMF in addition to 20% NMM (or collidine) in DMF for this.

Prep time: variable

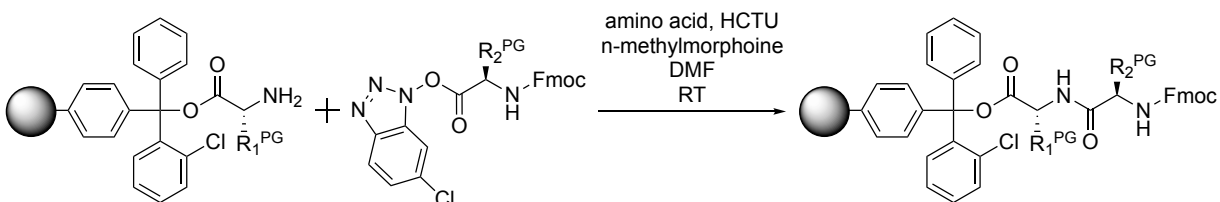
Reaction time: variable

1. Transfer your loaded resin to a hand coupling vessel.
2. Deprotect Fmoc from the amino acid on your resin by adding 5 mL of 20% piperidine. Mix for 5 min by bubbling in nitrogen gas. We typically add a second 5 mL of piperidine, but it's probably not necessary.
3. Wash 3X with DMF from the solvent system.
4. Prepare activated amino acid by weighing out an appropriate amount of amino acid and coupling agent (HCTU) and dissolving them in 20% *N*-methylmorpholine in DMF or 20% collidine in DMF. *We almost always use HCTU as the coupling agent, unless you are coupling from an *N*-methyl amino acid other than *N*-methyl glycine. In that case use HATU and HOAt as the coupling agents.*



Scheme 5. Activating an amino acid, shown using HCTU, although we commonly use HATU or HBTU as well.

5. Dump solution of amino acid/coupling agent onto the resin and mix for *ca.* 20 min. Longer coupling times (1–12 hours) are OK and are recommended as your peptide increases in length.



Scheme 6. Coupling an amino acid, shown using HCTU onto 2-chlorotrityl chloride. This is general to all resin/activating choices.

6. Repeat steps 2–5 until you have added each amino acid in your sequence.
7. After coupling your last amino acid cleave the final Fmoc group by treating your peptide on resin with 20% piperidine in DMF.
8. Wash 3X with DMF

*At this point it is advisable to analyze the success of the synthesis by analytical HPLC and mass spectrometry. We call this "checking the linear." To do this, use the tip of a thin spatula or the tip of a Pasteur pipette to transfer a very small amount of resin to a small glass vial. Treat the resin with roughly 400 μ L of trifluoroacetic acid (TFA) to globally deprotect the peptide. If you have trityl (Trt) protected side chains (C, H, Q, N) in your peptide add a single drop of triisopropylsilane (TIPS) to the reaction. This will make the

HPLC and mass spec data easier to interpret, as you will not observe Trt-protected side chains. Place the vial in the sonicator for 20 min. After 20 min, filter the reaction through a Pasteur pipette plugged with a small amount of cotton into a new HPLC vial. This separates the peptide from the resin. Rotovap deprotected peptide and dilute the peptide with 5 drops of water and take an HPLC and mass spec.

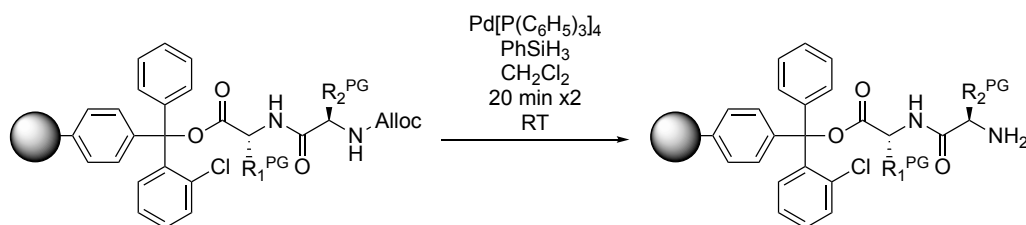
9. If you are synthesizing a macrocyclic peptide proceed to Cleavage of Protected Peptide from 2-chlorotrityl Resin. If you are synthesizing a linear peptide on either 2-chlorotrityl resin or rink amide resin proceed to Cleavage and Deprotection of Linear Peptide.

Alloc deprotection

The purpose of this step is to remove alloc protecting groups during peptide synthesis.

Prep time: 5 min

Reaction time: 40 min



Scheme 7. Alloc deprotection on 2-chlorotrityl resin.

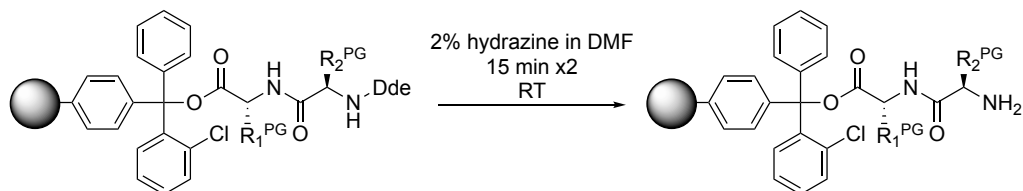
1. Wash the resin in a polyprep column 3X with CH_2Cl_2 .
2. Based on loading, weigh out 0.1 equiv. of tetrakis(triphenylphosphine)palladium(0) and dissolve in CH_2Cl_2 (6 mL).
3. Add 20 equiv. of phenylsilane (density 0.878 g/cm^3) to the resulting solution.
4. Transfer the solution to resin in a poly-prep column.
5. Put it on the rocker for 20 minutes.
6. Repeat steps 1–5.
7. Wash the resin in a poly-prep column 3X with CH_2Cl_2 .
8. Check alloc deprotection by cleaving small portion of resin with 20% HFIP in CH_2Cl_2 (15 min).
9. Rotovap the cleaved solution and re-dissolve in MeCN.
10. Using the ESI-MS, search for the alloc deprotected mass.

Dde deprotection

The purpose of this step is to remove Dde protecting groups during peptide synthesis.

Prep time: 5 min

Reaction time: 30 min



Scheme 8. Dde deprotection on 2-chlorotrityl resin.

1. Wash the resin in a poly-prep column 3X with DMF.

2. Add 5 mL of 2% hydrazine in DMF
3. Put it on the rocker for 15 min.
4. Repeat steps 1–3.
5. Wash the resin in a poly-prep column 3X with DMF.
6. Check Dde deprotection by cleaving small portion of resin with 20% HFIP in CH₂Cl₂ (15 min).
7. Rotovap the cleaved solution and re-dissolve in MeCN.
8. Using the ESI-MS, search for Dde deprotected mass.
*2% hydrazine in DMF will deprotect Fmoc protecting group, so plan your synthesis before hand.

Automated synthesis – PS3 synthesizer

To use the PS3 synthesizer you must first weigh out 4 equivalents of each amino acid you wish to couple along with 4 equivalents of coupling agent (HCTU or HATU/HOAt) and add the amino acid and coupling agent to amino acid vials. You will also need to book time on the google calendar in advance. Consult with a Nowick lab group member who is in charge of the machine.

Prep time: 1–3 h

Reaction time: variable, typically less than 24 h

1. Transfer your resin to the appropriate reaction vessel, and attach it to the synthesizer. Save the poly-prep column as you can reuse this column when your synthesis is complete.
2. Check the solvent levels and waste levels on the instrument. If the waste is more than halfway full attach a new waste container.
3. Program your sequence into the PS3. See list of programs taped to wall in the synthesizer room for your options. Generally, use **Program 12** for each amino acid coupling. You must end with **Program 5** in order to remove the final Fmoc group.
4. When your peptide finishes synthesizing remove the reaction vessel from the synthesizer and transfer the resin to the poly-prep column. If you are making a macrocyclic peptide proceed to Cleavage of Protected Peptide from 2-chlorotrityl chloride Resin. If you are synthesizing a linear peptide on either 2-chlorotrityl resin or rink amide resin proceed to Global Deprotection of Acid Labile Protecting Groups.

*At this point it is advisable to analyze the success of the synthesis by analytical HPLC and mass spectrometry. We call this "checking the linear." To do this, use the tip of a thin spatula or the tip of a Pasteur pipette to transfer a very small amount of resin to a small glass vial. Treat the resin with a 400 µL of trifluoroacetic acid (TFA) to globally deprotect the peptide. If you have trityl (Trt) protected cysteines in your peptide add a single drop of triisopropylsilane (TIPS) to the reaction. This will make the HPLC and mass spec data easier to interpret, as you will not observe Trt-protected cysteines. Place the vial in the sonicator for 20 min. After 20 min, filter the reaction through a Pasteur pipette plugged with a small amount of cotton into a new HPLC vial. This separates the peptide from the resin. Rotovap deprotected peptide and dilute the peptide with 5 drops of water and take an HPLC and mass spec.

Automated synthesis – CEM synthesizer

The CEM is not equipped to do "difficult" couplings such as coupling to N-methyl valine, etc. If you have one of these difficult couplings in your sequence, hand couple past it, or use the PS3.

1. Coordinate with Nowick lab member who is in charge of this machine.

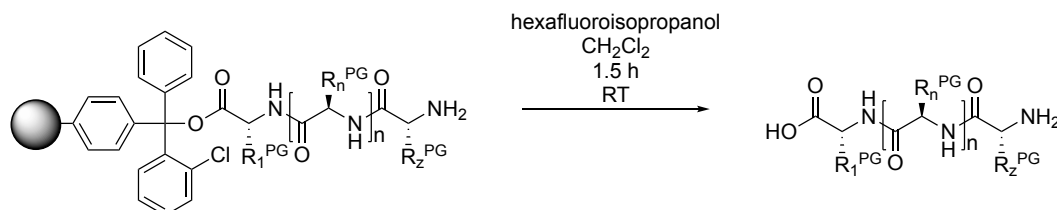
Cleavage form solid support

Cleavage of side chain protected linear peptide from 2-chlorotrityl chloride resin

The purpose of the step is to cleave the peptide from 2-chlorotrityl resin to expose a free carboxy terminus and maintain the protecting groups on the amino acid side chains

Prep time: 10 min

Reaction time: 1.5 h



Scheme 9. Cleaving a side chain protected peptide form 2-chlorotrityl chloride resin with HFIP.

1. Prepare the cleavage solution. The cleavage solution is 20% hexafluoroisopropanol (HFIP) in CH₂Cl₂. Prepare this fresh each time by adding 3.5 mL HFIP to 11.5 mL CH₂Cl₂ in a small graduated cylinder. Alternatively, 1 squirt of HFIP into 4 squirts of CH₂Cl₂ with a Pasteur pipette.
2. Add about half of the cleavage solution to the resin in the polyprep column. If you have washed the resin with CH₂Cl₂ before you add the cleavage solution, it will most likely turn red. Rock for 1 h at room temperature, and then drain into a clean round bottom flask.
3. Add the other half of the cleavage solution to the resin and rock for an additional 30 min. After the 30 min is complete drain into the same round bottom flask.
4. Use the rotovap to evaporate the HFIP and CH₂Cl₂. At this point you may put your peptide on the highvac for extended period of time or proceed to the next step.
5. If you are making a macrocyclic peptide proceed to Macrolactamization if you are making a linear peptide proceed to Global Deprotection of Acid Labile Protecting Groups.

Cleavage of linear peptide from Rink Amide resin

The purpose of the step is to cleave the peptide from Rink Amide resin. This will cleave the peptide from the resin, as well as deprotect the side chain protecting groups.

Prep time: 1 h

Reaction time: 1.5 h

1. Wash the completed peptide on resin 3X with CH₂Cl₂, and transfer it into a poly prep column.
2. Dry resin under a stream on N₂ gas for *ca.* 1 h.
3. Add 10 mL of an 18:1:1 TFA:H₂O:TIPS (prepared by adding 9 mL TFA and 0.5 mL of both H₂O and TIPS) to the resin. Let rock for 1–1.5 hours.
4. Collect solution in a 250-mL round bottom flask.
5. Remove TFA with the use of a rotovap.

Preparing cyclic peptides

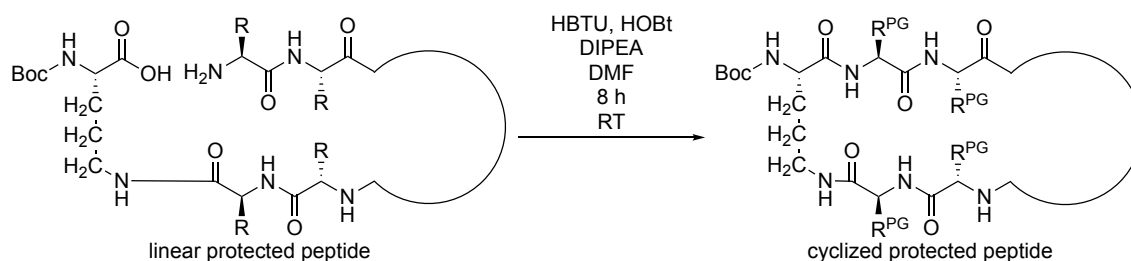
These steps pick up from cleaving the linear-protected peptide from 2-chlorotrityl chloride resin.

Cyclization

The purpose of this step is to covalently link the N- and C-termini of the peptide to create a macrolactam (aka a cyclic peptide, macrocycle, etc.).

Prep time: 5–30 min

Reaction time: 1 day to whenever you get to it



Scheme 10. Cyclizing a peptide with HBTU/HOBt and DIPEA in DMF. NMM can be used in place of DIPEA (PJS and NLT thinks its cleaner).

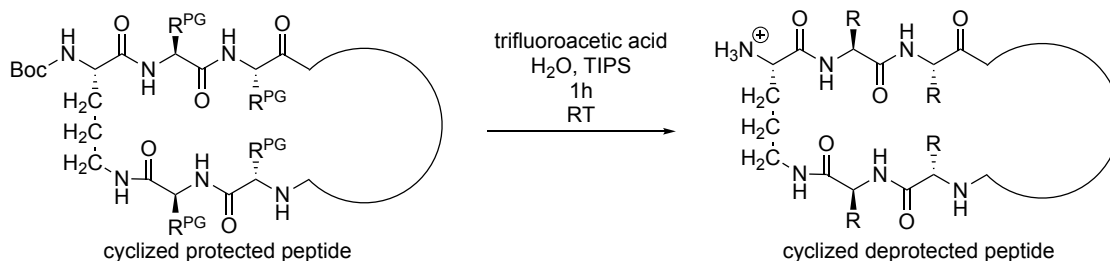
1. Add DMF from the solvent system to the round bottom flask that contains the linear peptide. *ca.* 125 mL
2. Add 6 equiv. HBTU (typically 250 mg), 6 equiv. HOBT (typically 100 mg), and 300 μ L of diisopropylethylamine (DIPEA) and let stir at RT under N_2 for at least 8 h. Alternatively, N-methylmorpholine (NMM) can be used in place of DIPEA.
3. Use the rotovap to evaporate the DMF. You will likely have to heat the DMF to 55-70 $^{\circ}$ C to achieve a moderate rate of evaporation.
4. After the DMF has evaporated attach the round bottom flask to the highvac to further dry the peptide. The more DMF you get rid of, the better. Once your peptide is dry, proceed to Global Deprotection of Acid Labile Protecting Groups.

Global deprotection of acid labile protecting groups

The purpose of this step is to remove the acid labile protecting groups to expose the amino acid side chains. Common acid labile protecting groups we use include: trityl (Trt), *O*-*tert*-butyl (*OtBu*), *tert*-butyloxycarbonyl (Boc), and pentamethyldihydrobenzofuran-5-sulfonyl (*Pbf*). This step will not remove acetamidomethyl (*Acm*) from cysteine, alloc protecting group, or *dde* protecting group.

Prep time: 5 min

Reaction time: 1–1.5 h



Scheme 11. Global deprotection of a cyclized peptide. Note this is identical to cleaving a peptide

from Rink Amide resin.

1. Remove the round bottom flask from the highvac.
2. Prepare the deprotection solution (aka the deprotection cocktail) by combining 9 ml TFA, 0.5 mL TIPS, and 0.5 mL H₂O to the dried cyclized peptide.
3. Dump the deprotection solution into the round bottom flask that contains your peptide and stir for 1 h at room temperature under N₂ gas. *Optional: if peptide contains Trt-protected Cys, you may add 250 mg DTT to the TFA/TIPS/H₂O cocktail to avoid oxidation of the thiols. We have not really had a problem with omitting the DTT, though. Just make sure you check your mass spec for M+16 species.*
4. After 1 h, remove the stir bar and use the rotovap to evaporate as much TFA as possible. *Note: if you plan to do an ether precipitation leave a small puddle (1–2 mL) of TFA at the bottom of the flask.*
5. At this point you want to purify your peptide. There are two common means of purification we do in the lab; The first is reverse-phase HPLC (RP-HPLC), which will get your peptide 80–99% pure. If you ARE NOT taking your peptide through further chemistry, then RP-HPLC purification is the best to use at this point. Proceed to Purification Using Reverse-Phase HPLC The second is ether precipitation, which will get your peptide much less pure than RP-HPLC, but will remove A LOT of the protecting groups and coupling agents. Use ether precipitation if you ARE taking your peptide through further chemistry. Proceed to Ether Precipitation

Subsequent chemistries on "isolated" peptides

This section details a number of reactions you might be interested in doing on a purified peptide, such as disulfide bond formation, or removing TFA esters.

Removing TFA esters with MeOH reflux

*The purpose of this step is to remove TFA esters of serine or threonine that may form during global deprotection. TFA esters are seen in mass spect after global deprotection with M+96 species. You **cannot** do this if your peptide contains Q, N, E, D, or a C-terminal amide/acid, as you will trans-esterify with the methanol.*

Prep time: 5–10 min

Reaction time: 0.5–1 h

1. After removing the TFA from a global deprotection, dissolve your peptide in *ca.* 50 mL of methanol.
2. Equip the round bottom flask with a reflux condenser attached to a 2 gallon water re-circulator. Reflux for 30 min to 1 h.
3. Remove methanol with a rotovap, proceed to purification.

Ether precipitation

The purpose of this step is to "clean up" your peptide for further chemistry. Right now, the most common chemistry that we do after global deprotection is formation of disulfide bonds, but ether precipitation can be used for any post-deprotection chemistry.

Prep time: 5 min

Reaction time: 20 min

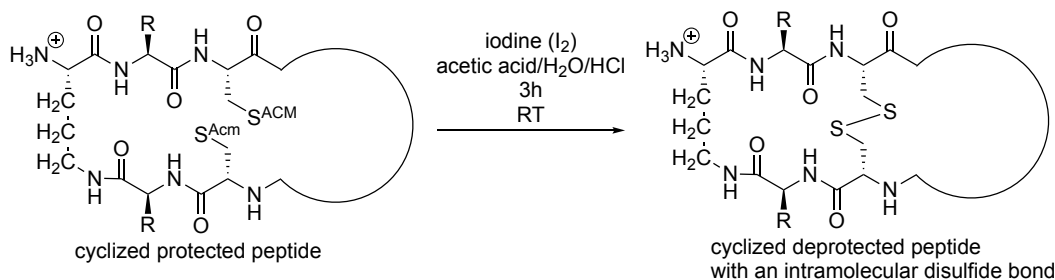
1. Obtain *ca.* 75 mL of ether from the solvent system and cool for 15 min in an ice bath.
2. After evaporation of the TFA from the global deprotection, leave a small puddle of TFA that contains your peptide.

- Change the column on the semi-prep HPLC back to the C18 column and do another purification according to the procedure detailed in Purification Using Reverse-Phase HPLC.

ACM deprotection and disulfide formation

Prep time: 1 h

Reaction time: 3 h



Scheme 13. Formation of disulfide bond coupled to Acm deprotection.

- Prepare 0.1 M iodine (I_2) by dissolving 380 mg of I_2 in 15 mL glacial acetic acid (100% acetic acid). Sonicate for at least 30 min. All of the I_2 may not go into solution.
- Prepare 250 mL 50% aq. acetic by adding 125 mL of glacial acetic acid to 125 mL H_2O .
- Prepare 25 mL 1 M HCl by adding 2 mL 12 M HCl (conc. HCl) to 23 mL H_2O .
- Dissolve the peptide in 250 mL 50% aq. acetic acid, and 25 mL 1 M HCl.

Obtain an analytical HPLC trace before adding the I_2 .

- Add 5 mL of 0.1 M I_2 , and stir under N_2 for at least 3 h. Monitor your reaction every hour by analytical HPLC.
- After 3 h, take a mass spec to ensure that your reaction has gone to completion.
- Quench the unreacted I_2 by adding 1 M sodium thiosulfate drop-wise until the reaction turns clear.
- Use the rotovap to remove ALL of the acetic acid/ H_2O /HCl.
- Re-dissolve whatever is on the side of your flask in 2 mL 100% TFA and perform an ether precipitation according to the protocol above. The ether precipitation removes most of the I_2 .
- Dissolve the peptide pellets in an appropriate amount of MeCN/ H_2O and proceed to Purification Using Reverse-Phase HPLC.

Purification of peptides

Reverse-phase HPLC purification

The purpose of this section is to describe the steps involved with purifying a peptide using RP-HPLC. It is not meant to be a definitive guide on how to purify YOUR peptide. You will likely need to experiment to figure out what gradient works best for you.

- Dissolve the deprotected peptide in the round bottom flask in a small amount of MeCN (2 mL) and transfer to a 15-mL conical tube. Wash the round bottom flask with *ca.* 3 mL H_2O to collect the residual peptide in the round bottom flask and transfer to the 15-mL conical. The volume transferred to the 15-mL conical should not exceed 10 mL.
- To remove particulates, centrifuge 15-mL conical at 10,000 rpm for 5 min.
- Pass through a 0.2- μ m syringe filter into a new 15-mL falcon tube. Your peptide is now ready to inject on to the preparative RP-HPLC column. *Before you inject, it is advisable to obtain an*

analytical HPLC trace to gauge what percentage of MeCN your peptide will come out.

General operation of the semi-preparative RP-HPLCs

We have two Rainin prep HPLCs in the lab. The general procedure for using each instrument is the same. This is just a rough procedure. Purifying peptides by RP-HPLC will take lots of practice. Adopt the following to suit your needs

1. Make sure that the solvent bottles are full.
2. Turn on lamp and flow (10 mL/min) on HPLC. The absorbance should be set to 214 nm.
3. Wash column at 95% MeCN over 5 min.
4. Go to 20% MeCN over 5 min at 10mL/min.
5. Inject peptide and allow the peptide to load onto the column for 10 min.
6. Go to 30% MeCN over 20 min at 15 mL/min.
7. Go to 50% MeCN over 60 min at 15 mL/min. *Peptide is likely to come out when going from 30% to 50%.*
8. Collect fractions. Typically count 20 sec for each fraction.
9. After your peptide comes out, wash the column with 95% MeCN for at least 15 min. Then return the MeCN percentage to 50%.
10. The column is stored at 50% MeCN.
11. Turn off flow and UV lamp.

Checking fractions, and lyophilizing

1. To assess the purity of your fractions check them on the analytical HPLC. If there are impurities, an additional preparative HPLC is necessary.
2. Combine the pure fractions and take a "combined HPLC" and a "combined mass spec". It is recommended to save this data now, as opposed to having to recollect it for second-year report, orals, or publication.
3. Evaporate the MeCN/H₂O using a rotovap (if your peptide bumps add a liberal amount of isopropanol) *may not be advisable if your planning on doing serious NMR, as some students have noticed IPA sticking around.*
4. Re-dissolve the peptide film on the side of the flask in H₂O and transfer to a clean polypropylene 15-mL conical.
5. Freeze your peptide in dry ice and then transfer the peptide to a lyophilization vessel. Detach the lid of the 15-mL conical, and use a kimwipe and rubber band to cover the top of the 15-mL conical.
6. Attach the lyophilization vessel to the lyophilizer. 12–48 hours later you will have a dry powder of your peptide as a TFA salt.