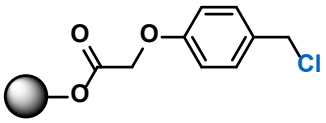
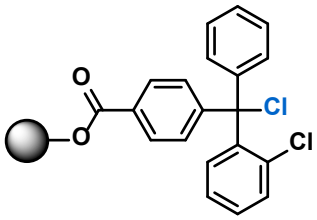
Cl-MPA ProTide and Cl-TCP(Cl) ProTide Resin Loading Procedure

This procedure details the simple and efficient method of loading Cl-MPA ProTide and Cl-TCP(Cl) ProTide resins with the Liberty Blue™ automated microwave peptide synthesizer.



Cl-MPA ProTide

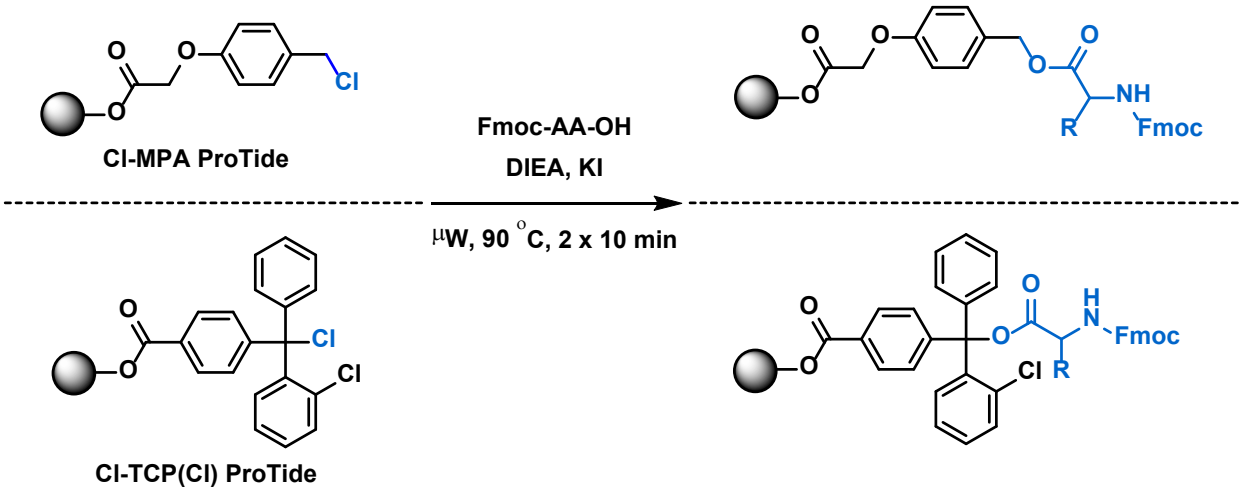


Cl-TCP(Cl) ProTide

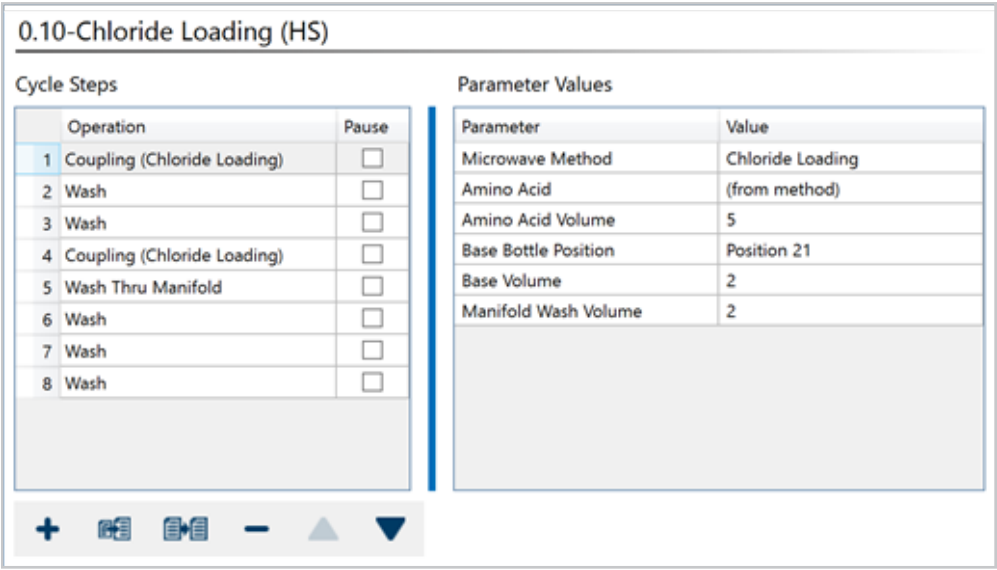
⚠ WARNING

Proper precautions must be taken to avoid contact with reagents or reagent vapors. Protective gear should be worn as outlined in the user's safety program for hazardous materials and the reagent manufacturer's safety data sheet. Refer to these guidelines for proper handling and disposal of the reagents. Dispose of all waste in accordance with all applicable local, state, and federal health and safety

Step One: Program Cycle



1. Navigate to the Cycle Editor (Edit > Cycles). Select the appropriate synthesis scale filter, and the "Amino Acid" and "High-Swelling" filters.
2. Highlight the "Chloride Loading (HS)" or "Trityl DCA Loading (HS)" cycle. If applicable, rename "Trityl DCA Loading (HS)" to "Chloride Loading (HS)".
3. Edit the "Chloride Loading (HS)" cycle to utilize the following Cycle Steps and Parameter Values (for the 0.1 mmol synthesis scale):



(If using software v1.5 or earlier, the Coupling Cycle Step will read “Coupling (Trityl Loading)” and the Microwave Method Parameter Value will read “DCA Trityl Loading”)

3.1. Wash Step Parameter Values:
- Volume: 4.0 mL
- Drain Time: 5.0 sec

3.2. Wash Thru Manifold Step Parameter Values:
- Volume: 4.0 mL
- Drain Time: 5.0 sec

(For the Parameter Values for other synthesis scales, contact peptide.support@cem.com.)

4. Select “Save” and close the Cycle Editor.

Step Two: Apply Cycle to a Liberty Method

1. Navigate to the Liberty Method Editor (Edit > Liberty Methods) and create a new Liberty Method.

2. Edit the Liberty Method, utilizing the following method options:
- C–Terminus: Acid
- Resin–Type: Chloride Loading (or “Trityl DCA Loading” if using software v1.5 or earlier)
- Resin Cycle: Chloride Loading No Swelling (or “Trityl DCA Loading No Swelling” if using software v1.5 or earlier)

3. In the Amino Acid Cycles grid, double-click the C-terminal amino acid and select the “Chloride Loading” cycle from the drop-down menu, if not already selected.

4. Select “Save” and close the Cycle Editor.

5. Load the Liberty Method into the resin indicator position.

6. Open the Usage Calculator (Calculators > Usage Calculator) and ensure sufficient reagent solutions are prepared. See “Step Three: Prepare Reagents” for important reagent preparation tips and considerations.

7. Press play to start the Liberty Method.

Step Three: Prepare Reagents

1. Weigh Cl-MPA ProTide or Cl-TCP(Cl) ProTide resin and transfer into a clean, dry reaction vessel. Secure the reaction vessel onto the attenuator and place into microwave cavity.

⚠ WARNING
The Cl-TCP(Cl) ProTide and Cl-MPA ProTide resins can hydrolyze, reducing yield.

NOTE
If using HT-12 or HT-24, load the resin into a clean, dry centrifuge tube and place directly onto the desired HT position. No solvent should be added.

2. Prepare a 1.0 M DIEA + 0.125 M KI solution and load onto Position 21.
   - **2.1.** Dissolve anhydrous KI (0.52 g) in DMF (20 mL).
   - **2.2.** Add DIEA (4.35 mL) to the KI solution.
   - **2.3.** Dilute the solution up to volume of 25 mL.

NOTE
For solution concentration for other synthesis scales, contact: peptide.support@cem.com

3. If employing Cl-TCP(Cl) ProTide resin, add 0.1 equivalents of DIEA to the HOBt or Oxyma solution.

WARNING
Cl-TCP(Cl) ProTide resin is hyper-acid sensitive. Addition of DIEA to the HOBt or Oxyma solution is necessary for prevention of premature cleavage, increasing synthesis yield.

4. Prepare any additional necessary reagents, load onto instrument, and begin the Liberty Method.

Optional: Protected Cleavage with Cl-TCP(Cl) ProTide

Resins with hyper-acid sensitive linkers like Cl-TCP(Cl) ProTide can be treated with 1% TFA to cleave the peptide from resin while the protecting groups stay intact.

NOTE
The Liberty Blue is not equipped to perform peptide cleavage; all cleavage procedures must be performed off-system.

The procedure below is for peptide cleavage at the 0.10 mmol scale. Adjust as necessary.

1. Transfer the peptidyl resin from the Liberty Blue reaction vessel to a filtered syringe vessel.
2. Rinse the resin thoroughly with DCM.
3. Add 1% TFA in DCM (double the resin bed volume) to the syringe vessel.
4. After 2 min at room temperature, collect the filtrate in a centrifuge tube containing a solution of 10% pyridine in MeOH (3 mL).
5. Repeat steps 3 and 4 four additional times.
6. Combine all filtrates, transferring quantitatively with MeOH and concentrate the solution (< 3 mL).
7. Transfer the solution to a clean 50-mL centrifuge tube with a small amount of MeOH (< 2 mL).
8. Dilute the solution to 35 mL with ice-cold deionized water to precipitate the protected peptide.
9. Centrifuge the peptide solution for 5 min at 3500 rpm, or until completion of peptidyl pellet formation. Upon decantation of the supernatant the peptide is ready for lyophilization and/or analysis.