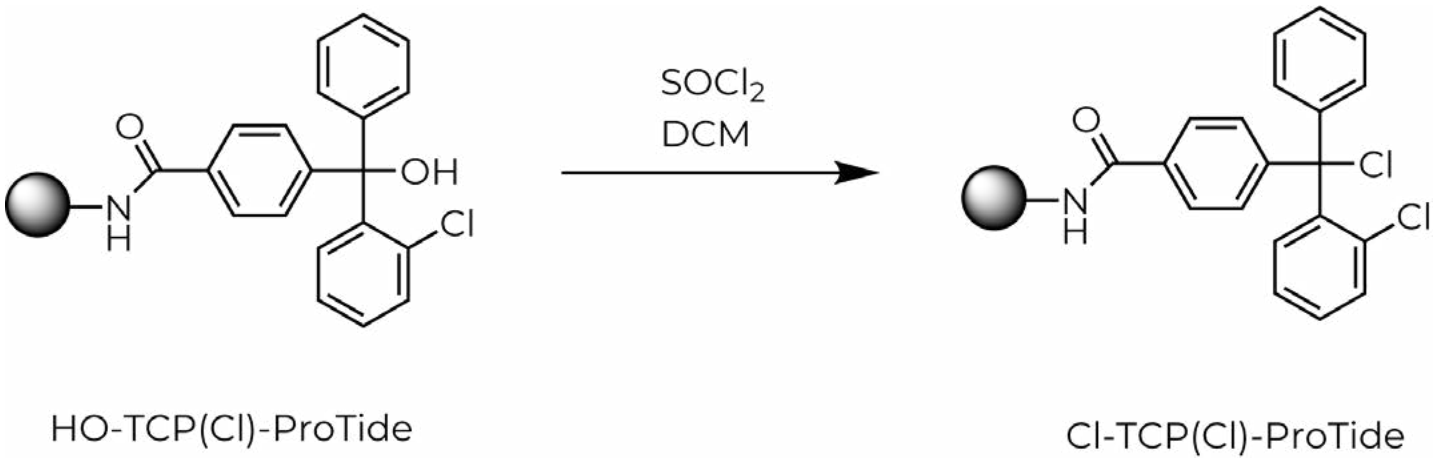
Chlorination Procedure for Cl-TCP(Cl) ProTide



HO-TCP(Cl)-ProTide

Cl-TCP(Cl)-ProTide

Introduction

The HO-TCP(Cl)-ProTide resin can be converted to the loading ready Cl-TCP(Cl)-ProTide through a simple chlorination reaction using $SOCl_2$. The procedure for both the chlorination and a resin loading test are provided here.

NOTE
Storage instructions are included for Cl-TCP(Cl)-ProTide, which can break down when not stored properly.

Reagents

Chlorination

- HO-TCP(Cl)-ProTide Resin (CEM P/N: R008)
- Thionyl Chloride, low iron ($SOCl_2$)
- Dichloromethane (DCM)
- Desiccant (for storage of product)

Resin Loading Test (ACP Synthesis)

- Piperidine
- Resin
- Oxyma Pure (CEM P/N: S001)
- 1,3-Diisopropylcarbodiimide (DIC)
- Amino Acids (CEM P/N: A001, A003, A004, A006, A008, A010, A019, A020)
- N,N-Dimethylformamide (DMF)
- N,N-Diisopropylethylamine (DIEA)
- Potassium Iodide (KI)
- Cleavage Cocktail: TFA/TIS/H_2O /DODT (92.5/2.5/2.5/2.5)
- Diethyl Ether
- Acetic Acid (AcOH)

Equipment

Chlorination (Scale to Reaction Size)

- 50 mL glass bottle (or larger as needed)
- Glass collection flask
- Vacuum pump
- Nitrogen line
- Fritted Funnel
- Rolling mixer

Resin Loading Test

- Liberty Blue 2.0 peptide synthesizer
- Razor cleavage system (recommended)
- Lyophilizer
- Centrifuge

Chlorination Procedure for Cl-TCP(Cl) ProTide[1]

1. A resealable bottle of at least 50 mL volume dried in a 180 °C oven overnight, then cool with blasting N_2 (g).
2. Add DCM (40 mL) and $SOCl_2$ (5 mL) to cooled bottle and mix well.
3. Add Resin (5 g) and seal, shake and vent the bottle (5x).
4. Roll suspension in mixer overnight, then the next morning add an additional 2 mL $SOCl_2$.
5. After an additional 6 hours, pour the contents of the glass bottle into fritted funnel and wash with 6 x (2 x resin volume) DCM.
6. Dry collected resin overnight by blowing N_2 until a free-flowing powder.
7. Store resin with desiccant packs in freezer.

Resin Loading Test

Peptide Synthesis

ACP (10 amino acid peptide amide, VQAAIDYING, 1063.2 amu) to be synthesized on the Liberty Blue 2.0 using previously published procedures[2] with slight modifications.[3]

Scale:	0.1 mmol
Activator Base:	1.0 M Oxyma Pure + 0.1 M DIEA
Activator:	1.0 M DIC in DMF
Position 21:	1 M DIEA + 0.125 M KI in DMF (freshly prepared)
Amino acids:	0.2 M in DMF
Deprotect:	20% Piperidine + 0.1M Oxyma Pure in DMF
Deprotection:	65 sec, 90 °C
Coupling:	125 sec, 90 °C

Resin Cleavage

1. The peptide resin should be washed three times with DCM immediately after synthesis.
2. Cleavage is then performed with 10 mL of freshly prepared TFA/TIS/H20 /DODT (92.5/2.5/2.5/2.5) solution for 30 min at 38 °C (on Razor) or for 3 hours at RT.
3. Filter the resin, saving the TFA solution and wash the resin with an additional 5mL TFA. Evaporate the TFA solution using a N_2 (g) stream until concentrated to approx. 5 mL.
4. Add ice cold diethyl ether to the TFA solution for precipitation in a 50 mL centrifuge tube and let sit for 30 min.
5. Collect the peptide precipitate via centrifugation and wash with cold ether two more times, careful not to lose any peptide.
6. Lyophilize the precipitated sample in a 10% AcOH solution.

Resin Loading Determination

The loading is determined by the recovered yield of peptide:

$$\textbf{Loading}_{\textit{pre-loaded resin}} = \frac{\textbf{1000}}{\textbf{MW}_{\textit{peptide}}} \textbf{ x } \frac{\textbf{Wt}_{\textit{recovered peptide}}}{\textbf{Wt}_{\textit{resin}}}$$

References

[1] For chlorination of differing resin amounts, scale glassware sizes and reagent amounts accordingly.

[2] Collins, J.M.; Porter, K.A.; Singh, S. K.; Vanier, G.S. *Org. Lett.* **2014**, *16*, 940 – 943.

[3] For more information on loading first amino acid onto Cl-TCP(Cl) resin, please see: Universal Loading Procedure for Chloride Resins, 2019. CEM Corporation Website; Technical Notes. https://cem.com/en/protide-chloride-loading-procedure (accessed July 12, 2022).

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