Accelerated room-temperature SPPS protocol
v. 2013.03.07

Synthesis

1. Calculate:
   a) amount of the C-terminal Fmoc-amino acid loaded on resin (in mmol)
   b) weight of each Fmoc-amino acid to be used in coupling steps (in g; need 5-fold molar excess per mmol resin)
   c) volume of HCTU solution needed for each coupling step (in mL; need 15 mL of HCTU solution per mmol resin per coupling)
   d) total volume of HCTU solution needed for the whole synthesis (in mL)
   e) volume of DIPEA needed for each coupling step (in mL; need 10-fold molar excess per mmol resin)
2. Place the resin into the reaction vessel, cover with DMF and shake for 30 min.
3. Prepare solutions:
   a) Fmoc-deprotecting solution (20% piperidine in DMF)
   b) HCTU solution for the whole synthesis (139 mg HCTU per mL of DMF)
   c) Fmoc-amino acid/HCTU solutions (dissolve each Fmoc-amino acid in the volume of HCTU solution needed for each step)
4. Open the stopcock to drain the solvent from the reaction vessel. Cover the resin with DMF, shake for 15 sec, drain. Repeat 2 more times.
5. Fmoc deprotection: cover the resin with piperidine solution, shake for 1 min, then drain. Repeat one more time.
6. Wash: cover the resin with DMF, shake for 15 sec, drain. Repeat 2 more times.
7. Coupling: add the necessary Fmoc-amino acid/HCTU solution, shake for 15 sec, add DIPEA, shake for 3 min, drain.
8. Wash: cover the resin with DMF, shake for 15 sec, drain. Repeat 2 more times.
9. Repeat steps 5 through 8 for each Fmoc-amino acid.
10. Final Fmoc deprotection.
11. After the final Fmoc deprotection, suspend the resin in DMF and transfer this suspension into a plastic vessel for MW synthesis. Wash 3 times with DMF (5 resin volumes), then 4 times with dichloromethane (5 resin volumes).

12. Remove all protecting groups and cleave the peptide off the resin according to the MW SPPS procedure (TFA deprotection). The TFA solution normally consists of 2% TIPS, 2% thioanisole, and 2% anisole in 10 mL TFA.

Work-up

1. While the TFA cleavage is underway, put ~30 mL of diethyl ether (label the tube) into either the freezer of the 4 °C fridge or in the -20 °C freezer.

2. After 30 minutes, when the TFA cleavage is finished, suction the cleavage solution into a 50 mL falcon tube and wash 3 times with DCM.

3. Add the diethyl ether until the crude peptide has fully precipitated out of solution. If no, or only a little, solid peptide appears, place in the -20 °C freezer until it does.

4. Centrifuge the precipitated peptide for 10 minutes at 7200 rpm to form a pellet. Decant the supernatant.

5. Resuspend the pellet in water. If needed, add a little methanol or HFIP to help dissolution.

6. After dissolved, place in -80 °C freezer to freeze the solution completely solid. Afterwards, put on lyophilizer (in jar filled with cold aluminum beads just removed from -80 °C freezer).

7. After 1-2 days of lyophilization, the peptide will be ready for HPLC. Calculate percent yield.

Specialized modifications

N-acetylation

1. After step 10 in the synthesis protocol, couple 0.5 mL of acetic anhydride in the same conditions (same HCTU concentration, DIEA coupling, etc.) as done for a regular amino acid.

N-myristoylation

1. After step 11 in the synthesis protocol, add 10x myristic anhydride (4° fridge) in 9 mL DMF, 1 mL pyridine. Note the myristic anhydride will not dissolve completely. Place in Discover microwave and select “Myristoylation” method. Afterwards, add some DCM to the plastic vessel so any crashed out myristic anhydride does not clog the waste collection

pipe. Drain the vessel, then wash again with DMF, then DCM, and finally proceed with TFA cleavage.