

Tetrazole-Peptide Conjugate Synthesis

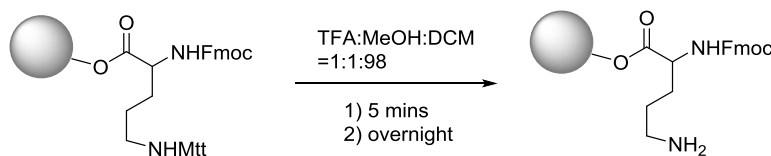
Example of GAAA(tet)K

Day 1:

Amount: 30 mg Fmoc-Lysine (Mtt) -Wang Resin weighed into a 3 mL syringe cylinder

Note: this amount of resin will give raw final product of 10-15 mg.

1) Use 1% TFA/ 1%MeOH/ 98% DCM (~ 2 mL) to immerse the beads for 5mins; then use the same amount of the solution to immerse the beads overnight

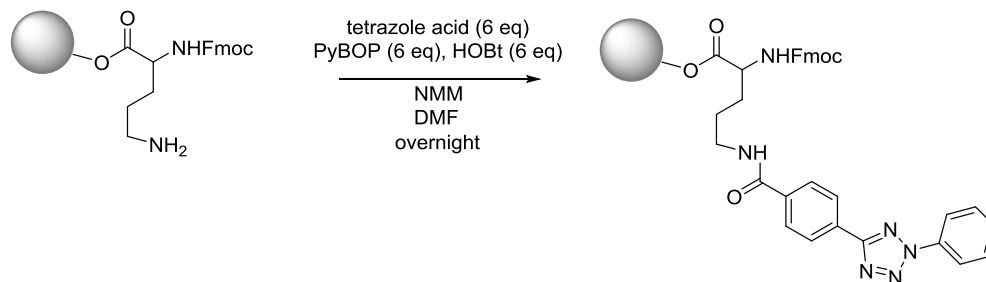


Note: the 1% TFA solution are made and stored in the bottle, but one should make fresh solution once every 2 weeks. The old solution would result in incomplete deprotection indicated by less red color after tetrazole coupling step.

Day 2:

2) Wash the beads with the following solvent (each time 2 mL) 2*DCM; then 2* MeOH; then again 2*DCM; then 2* 1% DIEA in DMF; then 4* DMF

3) make the tetrazole coupling cocktail: 6 equiv. tetrazole acid (28.8 mg)/ 6 equiv. PyBOP (56.2 mg)/ 6 equiv. HOBT (20.43 mg)/ 6*1.4 equiv. NMM (30 uL) in 1.2 ml DMF; immerse the beads overnight.



Note: All the coupling cocktail should be prepared at least 10 minutes before use (the solution should turn yellow).

Day 3:

4) Wash with fresh DMF 8 times (each time 1.5 ml)

5) Follow the general procedure of solid phase peptide synthesis for peptide elongation:

Deprotection with 20% PIP (2 mL, shake for 10 mins) > Wash (2mL each time, 6-8 times) > Coupling (shake for 20 mins) > Wash (2mL each time, 6-8 times) > Repeat.

Note: Remember to use fresh & uncontaminated DMF and wipe the needle for every sucking solvent/solution.

6) Solutions for 3 times of Alanine coupling can be made at once:

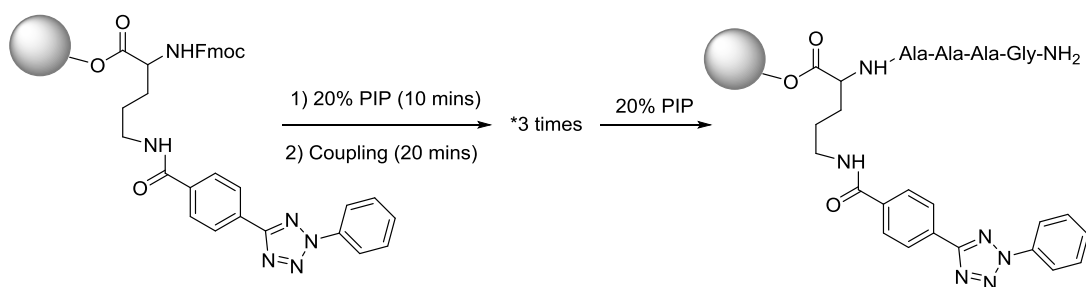
Fmoc-Ala-OH: 50.4 mg; PyBOP: 84.3 mg; HOBt: 30.6 mg; 45 uL NMM in 2.1 mL DMF

Each time take 0.7 mL, immerse and shake for 20 minutes.

7) Solutions for Glycine coupling:

Fmoc-Gly-OH: 17 mg; PyBOP: 28.1 mg; HOBt: 10.3 mg; 15 uL NMM in 0.7 mL DMF

8) After the last step of deprotection to remove the Fmoc on Glycine, wash the beads with DMF 6 times (* 2 mL) then DCM 6 times (* 2mL).



9) The TFA cleavage solution is made by adding 50 uL Et₃SiH and 50 uL water in 2 mL TFA. Carefully suck 1~1.5 mL TFA solution into the syringe. Try to avoid any splashing or leaking. Wear suitable PPE if necessary. Eject the product solution (should be of pink color) into a pre-weighed vial under gentle flow of air. Perform a second cleavage with ~0.5 mL TFA solution. Combine all the product solution and dry with airflow.