aapptec

Technical Support
Information Bulletin 1073

Wang Resin

Wang resin is the standard peptide synthesis resin used with Fmoc-chemistry. The resin is acid labile and finished peptides can be easily cleaved by treatment with 50 % (v/v) TFA/DCM. These relatively mild cleavage conditions have made this resin popular also in solid phase organic synthesis.

The first amino acid is attached to Wang resin using an activating agent such as dicyclohexylcarbodiimide (DCC) and a catalytic amount of 4-dimethylamino-pyridine (DMAP). These conditions can lead to partial epimerization of the amino acid, so HOBt is normally added to reduce racemization. Even with HOBt added, racemization can be a problem if the first amino acid is Cys or His. If DMF is used as the solvent in the coupling reaction, it should be degassed under vacuum or sparged with nitrogen first to remove any dimethylamine that may be contaminating it. The dimethylamine can remove the Fmoc group of the attached amino acid and lead to C-terminal oligomeric impurities. After the first amino acid is attached, the resin should be end-capped with acetic anhydride to block any unreacted active sites on the resin.

Fmoc-amino acid fluorides can also be used to attach the first amino acid. This method is useful where racemization may be a problem.

Attachment of the First Amino Acid

A. DIC/HOBt Method

1. In a round bottom flask suspend the resin in 9:1 v/v CH_2Cl_2/DMF (approximately 15 mL per gram of resin).

2. In a separate flask dissolve 1.5 to 2.5 equivalents (relative to the resin) of the Fmoc-amino acid in a minimum amount of DMF. Add the same equivalency of HOBt. Stir the mixture until the HOBt dissolves. If the HOBt doesn't dissolve completely, add just enough DMF to bring it into solution. Add this solution to the resin.

3. In a separate flask dissolve 0.1 equivalent (relative to the resin) of DMAP in a minimum amount of DMF.

4. Add 1.0 equivalent (relative to the amino acid) of DIC to the resin mixture then add the DMAP solution. Fit a drying tube onto the flask.

5. Agitate the mixture with a mechanical shaker for 2 to 3 hours at room temperature. Add 2 equivalents (relative to the resin) of acetic anhydride and

pyridine to the reaction flask and mix an additional 30 minutes at room temperature to end cap any unreacted hydroxyl groups on the resin.

6. Filter the resin in a fine porosity sintered glass funnel and wash it 3 times with DMF, then 3 times with DCM, and finally 3 times with methanol. In each wash use enough solvent to slurry the resin. After the final methanol wash, dry the resin in vacuo to a constant weight. The substitution of the resin can be estimated from the weight gain of the resin. For an accurate measurement of the resin substitution, the amount of Fmoc released from a weighed sample of resin can be measured spectrophotometrically.

Cleavage of the Peptide From the Resin

A. TFA Proceedure[1]

1. Place the resin in a round bottom flask and add 20% piperidine in DMF until the resin is just covered. Let the mixture stand for 30 minutes to remove the N-terminal Fmoc group.

2. Transfer the resin to a sintered glass funnel with fine porosity and apply vacuum. Wash the resin 3 times with DMF. Slurry the resin in DCM three times to remove the DMF.

3. Slurry the resin in 50%TFA in DCM (v/v) containing scavengers as required by the amino acid composition of the peptide. Swirl the mixture occasionally during the reaction time. The reaction time will depend on the amino acid composition of the peptide. If there are no Arg(Mtr) or Arg(Pmc) groups, cleavage will take 1.5 to 2 hours. If the peptide contains Arg(Pmc), allow 2 to 4 hours for cleavage. If the peptide contains Arg(Mtr), over 6 hours may be required for complete removal of the Mtr group.

4. Filter the resin in a fine sintered glass funnel. Wash the resin 3 times with small portions of TFA.

5. Combine the filtrates and add 8-10 times the volume of cold ether. If necessary, keep the mixture at 4°C overnight to precipitate the peptide. Filter the peptide using a fine sintered glass funnel. Wash the crude peptide further with cold ether.

B. TMSBr Procedure[2]

If the peptide contains Arg(Mtr), this procedure will cleave the peptide from the resin and remove the Mtr group more rapidly than the TFA procedure.

1. Place the resin in a round bottom flask and add 20% piperidine in DMF until the resin is just covered. Let the mixture stand for 30 minutes to remove the N-terminal Fmoc group.

2. Transfer the resin to a sintered glass funnel with fine porosity and apply vacuum. Wash the resin 3 times with DMF. Slurry the resin in DCM three times to remove the DMF.

---

[1] Based on results reported in Jubilut, GN; Cilli, EM; Crusca, E; Silva, EH; Okada, Y; Nakaie, CR. *Chem Pharm Bull (Tokyo)* **2007**, *55*, 468-470.

[2] Based on procedures in Guo,S.; et al. *Chem. Pharm. Bull*. **1988**, *36*, 4989; Yajima, H.; et al. *Tetrahedron* **1988**, *44*, 805-819.

3. For 100 mg of peptide-resin, mix 250 uL of ethanedithiol, 50 uL of m-cresol, 590 uL of thioanisole and 3.75 mL of TFA. Cool the mixture in an ice bath then add 660 ul of TMSBr. Cool the cleavage mixture to 0 ºC, then add 100 mg of the peptide resin. Allow the mixture to stand 15 minutes under nitrogen.

4. Filter the resin in a fine sintered glass funnel. Wash the resin 3 times with small portions of TFA.

5. Combine the filtrates and add 8-10 times the volume of cold ether. If necessary, keep the mixture at 4 °C overnight to precipitate the peptide. Filter the peptide using a fine sintered glass funnel. Dissolve the precipitated peptide in 20% aqueous acetic acid and lyophilize.