

# Novabiochem<sup>®</sup> Peptide resin loading protocols



# Peptide resin loading protocols

## 1. Merrifield resins

Merrifield resin can be loaded with Boc-amino acids as described in Method 1, or can be purchased pre-loaded with the C-terminal amino acid.

### Method 1: Attachment of Boc-amino acids to Merrifield resin

1. Dissolve Boc-amino acid in EtOH (2 ml/mmol) and add water (0.5 ml/mmol). Adjust pH to 7 with 2M aq. Cs<sub>2</sub>CO<sub>3</sub>. Evaporate solution to dryness. Add dioxane and evaporate to dryness. Repeat evaporation with dioxane.
2. Pre-swell Merrifield resin in DCM for 1 h and then wash with DMF. Add Cs salt (1.2 eq.) in DMF to the resin and heat at 50 °C o/n. The reaction may be catalyzed by the addition of KI (0.1 eq.). At the end of this time, wash the resin with 3x DMF, 3x DMF/water (1:1), 3x DMF, 3x DCM, 3x MeOH. Dry *in vacuo* over KOH.

## 2. Hydroxymethyl-functionalized resins

One of the simplest methods for esterification to hydroxymethyl-functionalized linkers is to use the symmetrical anhydride of the protected amino acid in the presence of a catalytic amount of pdimethylaminopyridine (DMAP) (see Method 2). However, due to the basic character of this material, enantiomerization and dipeptide formation can be expected; the amount depending on the quantity of DMAP used, the length of the reaction and the nature of the amino acid. Cysteine and histidine are particularly prone to enantiomerization and should not be loaded by this method. For these residues, the use of 2-CITrt resin is recommended; esterification of the C terminal residue is free from enantiomerization and dipeptide formation [1] because attachment does not involve activation of the incoming protected amino acid (Method 3).

### Method 2: Attachment to hydroxymethyl resins using symmetrical anhydride

1. Place the resin (1 g) in a clean, dry flask, and add sufficient DMF to just cover and allow to swell for 30 min. Add extra DMF if necessary just to cover the resin.
2. Dissolve the Fmoc amino acid (10 eq. relative to resin loading) in dry DCM. One or two drops of DMF may be needed to aid complete dissolution.
3. Add a solution of diisopropylcarbodiimide (5 eq. relative to resin loading) in dry DCM to the amino acid solution.
4. Stir the mixture for 20 min at 0 °C, keeping the reaction mixture free of moisture with a calcium chloride drying tube.
5. Remove the DCM by evaporation under reduced pressure using a rotary evaporator.
6. Dissolve the residue in the minimum of DMF and add the solution to the resin prepared in step 1.
7. Dissolve DMAP (0.1 eq. relative to resin loading) in DMF and add this solution to the resin/amino acid mixture. Stopper the flask and allow the mixture to stand at rt for 1 h with occasional swirling.
8. Remove a small sample of resin (20 mg) and wash, dry and estimate the level of first residue attachment using the procedure described in Method 6. If the value obtained is less than 70% the first residue attachment procedure should be repeated.

**Note:** This method is not suitable for His or Cys.

The MSNT method [2, 3] is the method of choice in difficult circumstances, such as loading of HMBA resins or when attaching enantiomerization prone amino acid derivatives [4, 5]. When peptide acids containing Pro as the C-terminal residue are desired, the use of trityl-based resins is recommended. Once the resin is loaded the substitution of the resin can be easily determined using Method 6.

### Method 3: Attachment to hydroxymethyl resins using MSNT/Melm

1. Place the resin in a dry reaction vessel. Swell and wash with DCM, add sufficient DCM to cover resin and flush vessel with nitrogen.
2. Weigh the appropriate Fmoc amino acid (5 eq.) into a dry round bottom flask. Add dry DCM to dissolve the amino acid derivative (approximately 3 ml/mmol); one or two drops of THF can be added to aid dissolution.
3. Add Melm (3.75 eq.) followed by MSNT (5eq.). Flush flask with nitrogen and seal. Stir the mixture until the MSNT has dissolved.
4. Using a syringe, transfer the amino acid solution to the vessel containing the resin.
5. Allow the mixture to stand at rt for 1 h, with gentle agitation.
6. Wash with DCM (5 times) and DMF (5 times).
7. Remove a small sample of resin (20 mg) and wash, dry and estimate the level of first residue attachment using the procedure described in Method 6.
8. If the value obtained is less than 70% the first residue attachment procedure should be repeated.

## 3. Loading amino-functionalized resins

Attachment of amino acid derivatives and other carboxylic acids to linkers containing primary amino groups can normally be effected using standard methods of amide bond formation. Hydroxylamine, Weinreb amide, and resins functionalized with secondary amines are much more difficult to load; for these the use of HOAt/DIPCDI or HATU/DIPEA activation is required.

#### Method 4: Attachment of carboxylic acids to amino resins

1. Pre-swell the resin with DCM (polystyrene-based resins) or DMF (PEG-PS or PEGA resin) for 1 h. Wash resin thoroughly with DMF.
2. If the resin is Fmoc protected, treat with 20% piperidine in DMF for 20 min, then wash resin thoroughly with DMF.

##### Rink Amide, Sieber Amide, MBHA

3. Dissolve amino acid derivative or carboxylic acid (5 eq.) and HOBt in DMF. Add DIPCDI (5 eq.) and leave to stand for 10 min. Add mixture to resin. Leave for 1-6 h.

##### N-Alkylamino resins

3. Dissolve amino acid derivative or carboxylic acid (5 eq.) and HATU in DMF. Add DIPEA (10 eq.) and add immediately to resin. Leave for 6 h.
4. Remove a small quantity of resin and test this for the presence of unreacted amines using the TNBS test (primary amine resins) or chloranil test (secondary amine resins) as described in Methods 3-8 and 3-9. If the result is positive, wash the resin with DMF and repeat coupling. Continue this procedure until a negative result is obtained.

#### Method 5: Loading of trityl resins

NOTE: it is important to dry all solvents and glassware before use.

##### Attachment of carboxylic acids

1. Dissolve the carboxylic acid (0.6-1.2 eq. relative to the resin for 2-chlorotrityl resin and 2 eq. for NovaSyn® TGT chloride resin) and DIPEA (4 eq. relative to carboxylic acid) in dry DCM (approx. 10 ml per gram of resin) containing, if necessary, a small amount of dry DMF (just enough to facilitate dissolution of the acid). For pseudoproline dipeptides add 3ml of NMP/gram of resin.
2. Add this to the resin and stir for 30-120 min. For pseudoproline dipeptides leave to react o/n. At the end of this time, wash the resin with 3x DCM/MeOH/DIPEA (17:2:1), 3x DCM; 2x DMF, 2x DCM. Dry *in vacuo* over KOH.

Fmoc-amino acids are best dried before use by repeated evaporation from dioxane; determine loading using Method 6.

## 4. Trityl-based resins

In contrast to benzyl alcohol-based supports, attachment of amino acids to trityl-based resins, such as 2-chlorotrityl or NovaSyn® TGT resins, is free from enantiomerization [1], making them ideal for the immobilization of sensitive residues such as Cys and His. The resin also protects Cys from enantiomerization during chain extension. They are particularly useful in the synthesis of C-terminal prolyl peptides as the bulk of the trityl linker helps to prevent diketopiperazine formation [6 - 8].

When loading 2-chlorotrityl chloride resin, it is important to ensure that all amino-acid derivatives, glassware and solvent are thoroughly dried before use.

NovaSyn® TGT alcohol resins must be converted to the chloride form before attachment of the amino acid.

#### Method 4: Chloridation of NovaSyn® TGT alcohol resin

NOTE: it is important to dry all solvents and glassware before use.

1. Place NovaSyn® TGT alcohol resin in a sintered glass funnel and wash the resin consecutively with DMF (2x), dry DCM (3x) and dry toluene (3x).
2. Drain off excess toluene from the resin and transfer damp material to a round bottom flask equipped with a reflux condenser.
3. Add sufficient toluene to cover resin, then add freshly distilled AcCl (1 ml/g of resin). Heat at 60 - 70 °C for 3 h.
4. Slurry mixture to a sintered glass funnel. Wash resin with dry toluene (3x) and dry DCM (3x).
5. Drain excess solvent from resin and use immediately.

## 5. Fmoc loading test

For estimating the loading of resins derivatized with Fmoc-amino acids, the simplest approach involves cleaving the Fmoc group with DBU and measuring the solution concentration of the liberated dibenzofluvene by U.V. spectroscopy.

#### Method 6: Estimation of level of first residue attachment [9]

1. Take 3 x 10 mm matched silica UV cells.
2. Weigh dry Fmoc amino acid-resin (approx. 5 μmole with respect to Fmoc) into a 10 ml graduated flask. Add 2 ml of 2% DBU in DMF. Agitate gently for 30 min. Dilute solution to 10 ml with MeCN. Take 2 ml of this solution and dilute to 25 ml in a graduated flask.
3. Prepare a reference solution as in step 2, but without addition of the resin.
4. Fill two cuvettes with 3 ml of test solution and one cuvette with 3 ml of reference solution.

NOTE: Do not cross-contaminate the solutions. Allow the resin to settle to the bottom of the cells.

5. Place the cells in a spectrophotometer and record optical density at 304 nm.

6. Obtain an estimate of first residue attachment from equation below

Fmoc loading: mmole/g =  $(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{ref}}) \times 16.4 / \text{mg of resin}$ .

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