PCAS BioMatrix Inc.
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Analytical & Quality Control Dept.

General SPPS procedures for ChemMatrix® resins

ChemMatrix® resins are designed to be used with the same procedures as for polystyrene. The only difference is that swellings are higher in most solvents (including TFA). All the standard solvents used in SPPS such as DMF, NMP, DCM and MeOH give good swellings with ChemMatrix® resins (*see Chart no 1*) . Therefore, all kind of peptide synthesizers could be used by taking care of the final volume filled by the resin in the vessel. Many examples [1-4] with the automatic synthesizers (ABI-433A, CEM–Liberty...) using modified FastMoc® method gave very complexes peptides in high purities.

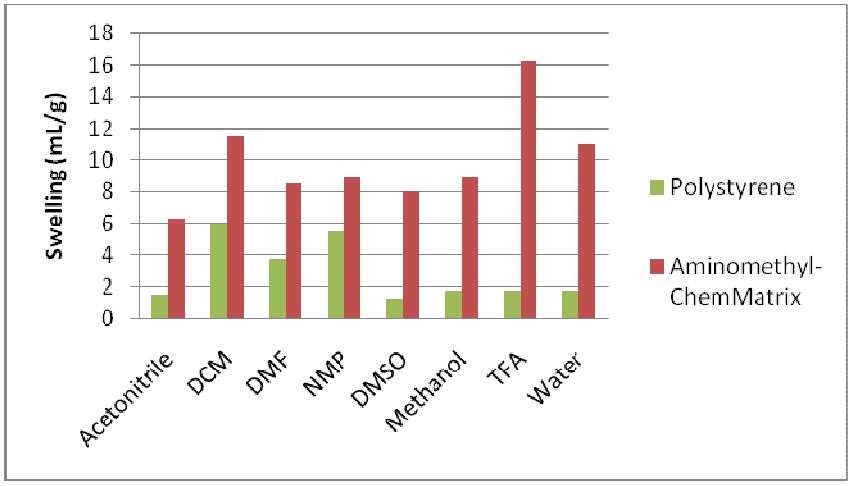

Chart no 1: Swellings of ChemMatrix® in solvents used in SPPS *versus* polystyrene.

Note that the following procedures can be improved by changing the coupling agents with more powerful ones: HBTU/HOBt or HCTU/6-Cl HOBt for PyBOP/HOBt or HATU/HOAt. N,N-diisopropylethylamine could be replaced by milder bases such as N-methylmorpholine to lower racemization in some cases.

The cleavage step relies on the use of TFA. Depending on the peptide sequence, all kind of cleavage cocktails could be used. If Arg(Pbf) is present in the sequence, the cleavage should be left 3 hours instead of one.

References:

1- Garcia-Martin, F. *et al.*, *J. Comb. Chem.*, **2006**, 8, 213-220.
2- Garcia-Martin, F. *et al.*, *Biopolymers (Peptide Science)*, **2006**, 84, 566-575.
3- Frutos, J. *et al.*, *Int. J. Pept. Res. Therapeutics*, **2007**, 13, 221-227.
4- De la Torre, B. *et al.*, *Int. J. Pept. Res. Therapeutics*, **2007**, 13, 265-270.

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Coupling step *(representative example)*

- Swell the resin with DMF (10mL/g of resin);
- Dissolve the Fmoc-amino acid (3 eq.) and HCTU (3 eq.) in a minimum of DMF followed by the addition of NMM (*N*-Methylmorpholine) (4-5 eq.);
- Add this solution to the swelled resin;
- Stir 30 minutes at room temperature;
- Filter and wash with DMF (3-4 times);
- Add a solution of 20% piperidine in DMF (10-12mL/g) to the resin and stir 30 minutes;
- Filter and wash with DMF (3-4 times);
- Begin the coupling of a new amino acid.

Cleavage step *(representative example)*

- Once the synthesis is over, wash the resin with methanol and then with Et_2O;
- Dry the resin for several hours under vacuum at room temperature;
- Add 20mL/g of a solution of TFA / H_2O / Et_3SiH (95/2,5/2,5) and stir one hour (3 hours if Arg(Pbf) are present);
- Filter and rinse the resin twice with TFA;
- Evaporate all the solvents to a minimum;
- Under stirring, add cold Et_2O (10-15mL/mL of residual volume of solvent with the peptide);
- Centrifugate the suspension and remove the supernatant ether;
- Repeat the last step twice;
- Dry the peptide under vacuum overnight (freeze drying could be also performed with a suitable solvent).

Trityl alcohol-ChemMatrix®: Chlorination and coupling procedures

- In a vessel for SPPS, 7,5g of resin is added then swelled with 100mL of dry DCM;
- Add 2 mL of SOCl2 (to get a final 2% SOCl2 in DCM);
- The vessel is shaked overnight;
- Drain the resin and rinse it with DCM (5x75mL) then with NMM 2%/DCM (3x75mL);
- Add a solution of the desired Fmoc-amino acid-OH (3eq.) with NMM (4 eq.) in DCM;
- The vessel is shaked overnight;
- Add 2,5 mL of a solution of 25% NMM / MeOH and shake for an hour;
- Drain the resin and rinse it with DCM, DMF, MeOH and ether (3x75mL each).
- Dry the resin overnight at room temperature (never heat it)