Peptide Synthesis Protocol on SynPhase™ PA Lanterns

The hydrophilic surface of SynPhase PA (polyamide) Lanterns is based on a proprietary grafted dimethylacrylamide co-polymer, and is efficiently wetted in both polar (including aqueous) and non polar solvents. Two distinct loading levels in the PA range cater for different applications;

- a low loading version is optimized for peptide synthesis.
- a high loading version is ideal for small organic molecule synthesis, particularly in polar solvents when polystyrene (PS) is not suitable.

A general synthetic protocol for a complete amino acid coupling cycle using low loading Rink Amide PA Lanterns is described below. The procedure benefits from the convenient handling and washing techniques derived from the modular nature of SynPhase Lanterns, and is readily applied to multiple peptide synthesis as previously described for the SynPhase product range[1]. Furthermore, SynPhase PA Lanterns can be readily used in commercial peptide synthesizers[2]. The modular format of the Lanterns means that no weighing is required.

Materials and Methods

Peptides can be synthesized on SynPhase PA RAM D-series Lanterns (loading 8μmol/ Lantern, catalog code: SP-PA-D-RAM-008).

Fmoc protected amino acids should be used throughout.

Side chain protection can be afforded by: Trt for Cysteine, Histidine, Glutamine and Asparagine; Boc for Lysine and Tryptophan; *t*Bu for Tyrosine, Threonine, Serine, Aspartic acid and Glutamic acid; Pbf for Arginine.

The general coupling conditions for SynPhase PA Lanterns utilizes a solution of 80% distilled DMF and 20% AR grade DCM, with reagents at the following ratios and concentrations:

	AA	HOBt	DIC
Reagent ratio	1	1.2	1
Final conc. (mM)	120	144	120

This activated amino acid solution is prepared from equal volumes of an amino acid stock solution and activator solution. For each Lantern a minimum of 500μL of the activated amino acid solution is required.

AA:	Fmoc-amino acid-OH
AR:	analytical reagent
Boc:	*t*-butoxycarbonyl
DCM:	dichloromethane
DIC:	*N,N'*-diisopropylcarbodiimide
DMF:	dimethylformamide
EDT:	ethanedithiol
Fmoc:	9-fluorenylmethoxycarbonyl
HATU:	*N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethan-aminium hexafluorophosphate *N*-oxide
HOBt:	*N*-hydroxybenzotriazole (monohydrate)
Pbf:	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
pip:	piperidine
rt:	room temperature
*t*Bu:	*t*-butyl
TFA:	trifluoroacetic acid
TFFH:	tetramethylfluoroformamidinium hexafluorophosphate
Trt:	trityl

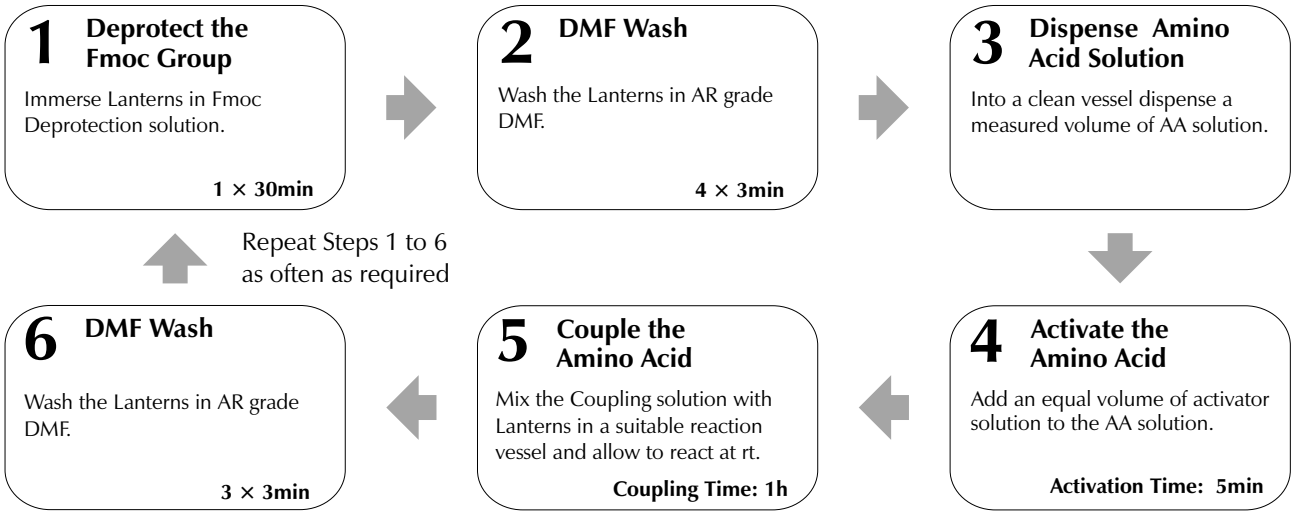
Reagent Preparation

Amino acid stock solution:
Prepare a 240mM amino AA solution (240mM in 60% distilled DMF and 40% AR grade DCM).

Activator solution:
Prepare a solution containing 288mM HOBt and 240mM of DIC in distilled DMF. An amount equal to the AA stock solution volume being used for each AA used in the coupling cycle is required. The activator solution should be prepared fresh and used immediately.

Fmoc Group Deprotection solution:
Prepare a solution of 20% pip in distilled DMF.

Coupling Scheme



SynPhase D-series Lanterns require typical working volumes of 500μL. Actual volumes depend upon the dimensions of the reaction vessel and the number of Lanterns used. The procedure may require slight optimization depending upon the nature of the peptide(s). For difficult couplings, two hour coupling times and/or the use of sonication may be beneficial. The use of HATU or TFFH is recommended for sterically demanding couplings.

Side Chain Deprotection

Simultaneous side chain deprotection and cleavage can be carried out using 2.5mL per Lantern of a solution of 82.5% TFA/5% thioanisole/5% anisole/5% water/2.5% EDT for 2hr. The TFA solution can be reduced under vacuum (or a gentle stream of N_2) and the cleaved peptide precipitated in 8mL cold diethyl ether/petroleum ether (bp 40-60°C) (1:2v/v), washed with 4mL cold diethylether/petroleum ether, and then air dried.

References

1 Maeji, N.J., Bray, A.M., Valerio, R.M. and Wang, W., Larger Scale Multipin™ Peptide Synthesis, *Peptide Res.*, 1995, **8**, 33-38.

2 See SynPhase Application Note SAN002, Automated Fmoc-Peptide Synthesis: Use of SynPhase™ PA Lanterns and PS-Resin in an ACT 496Ω Multiple Synthesizer.

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