User Bulletin No. 40

Synergy™ Peptide Synthesizer

February 1994 (updated 07/2002)

SUBJECT: Simplified Cleavage for Synthesis on the Synergy™ System

This user bulletin contains procedures to simplify the operation of Synergy™ and the subsequent peptide work up. Firstly, a Synthesis Start-Up Check List is provided, which is designed to stay with the instrument and act as a quick reference guide when setting up a synthesis on Synergy™.

An alternative cleavage procedure is included. The procedure is different from section 3 of the User's Manual, in that the cleaved-resin is filtered off prior to centrifugation and precipitation of the peptide. This ensures that a greater yield of peptide will be recovered. Minor changes to the cleavage cocktail have also been made (i.e., the addition of water). Water has been shown to be important in the deprotection of the Pmc group Fmoc-Arg (Pmc)[1].

The peptide solubility guide provides helpful information to aid in the dissolution of the synthetic peptides after cleavage. The solubility of a synthetic peptide is sequence dependent and varies from peptide to peptide.

These three procedures are printed on laminated sheets, so you can keep them in the lab and refer to them as you work without the possibility of damage by chemicals.

1. King, D.S., Fields, C.G. & Fields, G.B. *Int. J. Peptide Protein Res.* **1990**, 36: 255-266.

Applied Biosystems

Synergy™ Synthesis Start-up Checklist

1. Check the external gas supply.

   Regulated pressure: 60-75 psi Gas tank pressure: ≥ 300 psi

2. Check levels of five reagent bottles.

Reagent	Bottle size (ml)	ml/cycle	mm/cycle	Cycles/bottle
HBTU	40	0.4	0.5	100
DIEA	40	0.4	0.5	100
Piperidine	200	2.0	1.0	100
THF	200	2.0	1.0	100
DMF	4000	60.0	3.0*	67

* DMF mm/cycle value applies to that part of the reagent bottle below the neck, where the bottle sides do not taper.

3. Check waste bottle, empty full waste bottle.

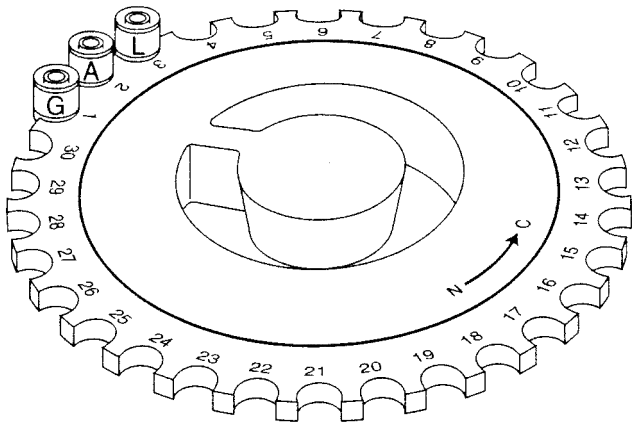
   Waste generated per cycle: approximately 65ml

4. Check printer power, paper supply, and cable connections.

5. Select and load PSC and AACs.

   AACs: Load C-terminal (Position 1) -> N-terminal

   PSCs: Install according to User Bulletin #39



6. Check the run file.

   Standard pre-programmed run file

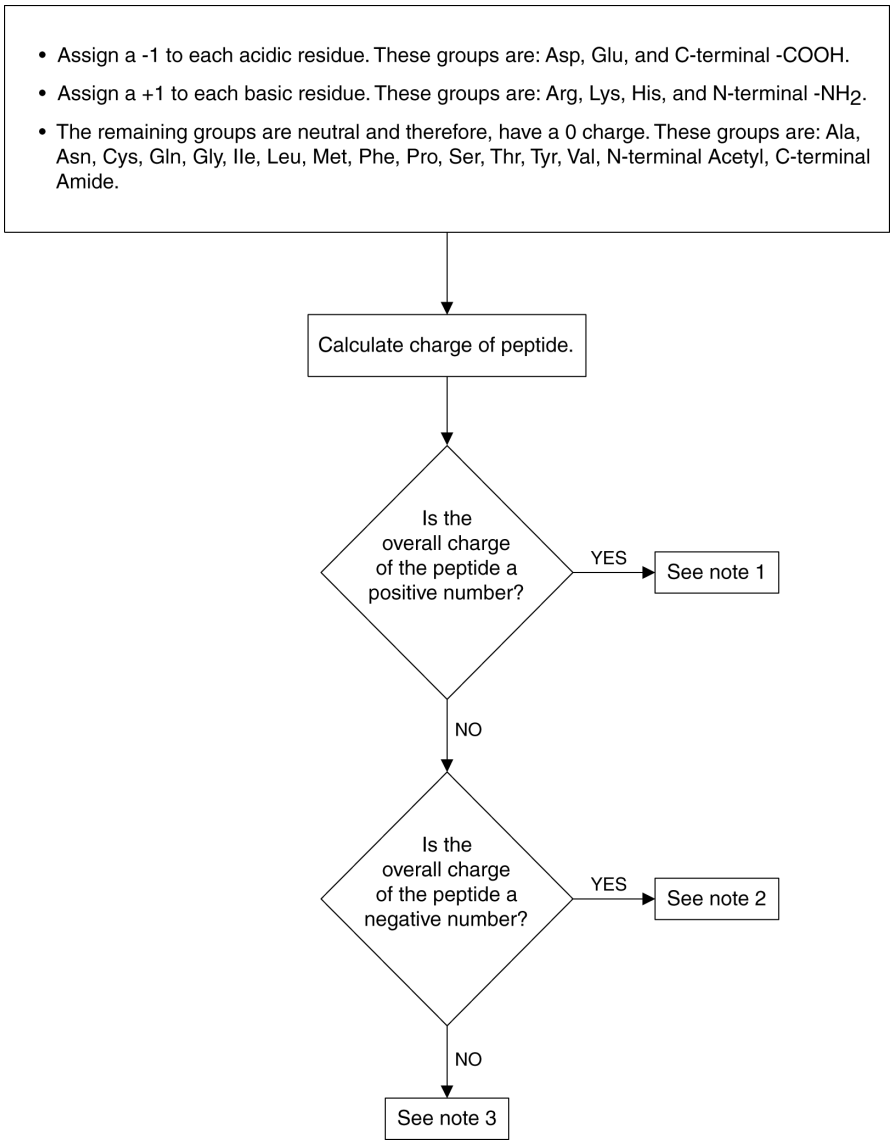
Line Name	Modules	Action
BEG	bf	initial resin solvation
L (cycle line) -1	jdacgfi	deprotection and coupling
END	de	Fmoc deprotection and dry peptide-resin

7. Check run control menu settings.

   Standard Settings: Run Begin: YES, Run End: YES, Print: YES

8. Push the “START” soft key in the Run Control Menu.

Peptide Solubility Guide



In order to determine the best solvent for your peptide, test a small sample (approx. 0.5mg) of your peptide for solubility using the guide above.

Note 1 If your peptide has a basic nature, the peptide will probably dissolve in water. If not try 10% and increasing strengths of acetic acid. If the peptide still does not dissolve, add TFA (<50µl) to solubilize the peptide and dilute to 1ml with water.

Note 2 Peptides with an acidic nature may also dissolve in water or acetic acid. If these fail to dissolve, add NH_4OH (<50µl) and dilute to 1ml with water.

Note 3 Peptides which are neutral may require the addition of organic solvents e.g. methanol, isopropanol or acetonitrile. The addition of denaturants such as urea or guanidinium-HCl may also be required.

If your peptides are slow to dissolve, sonicate the solution for 10mins.

Synergy™ Fmoc Cleavage Procedure

The following procedure uses a universal cleavage cocktail, which is suitable for all peptide sequences.

1. Prepare fresh cleavage mixture as follows:

WARNING	***CHEMICAL HAZARD. Trifluoroacetic acid (TFA) is an extremely dangerous and corrosive liquid. Always wear gloves, a lab coat and eye protection when handling it. All procedures must be conducted in a properly operating fume hood.***

To make 2mls, mix the following reagents IN THIS ORDER:

100µl Thioanisole

50µl Water

50µl Ethanedithiol

1.8mls Trifluoroacetic acid (TFA)

Chill this mixture in an ice/salt bath to -5°C to -10°C

Note *Always use good quality reagents with >99% purity.*

2. To a 5ml sample vial, add the resin and a micro stir bar. Cool the sample vial in an ice bath.
3. Add enough of the cold cleavage mixture to make a slurry, using the chart below as a guide:

Weight of Resin (mg.)	Amount of Cleavage Mixture (µl)
0 - 10	100
10 - 25	200
25 - 50	400
50 - 100	700
100 - 200	1200

4. Screw cap on vial, place on a magnetic stirrer and stir the mixture at 0°C for 10 - 15 mins. Allow the reaction to continue to stir at room temperature for a further 1hr 45 mins (Total time = 2hrs). Increase this time by 30 mins for each additional arginine, to a maximum total time of 3hrs.
5. Filter off the resin using a sintered funnel (medium porosity) or a Pasteur pipette plugged with glass wool. Wash the resin with neat TFA (approximately 0.5ml). Retain the filter for step 7.
6. Add approximately 8mls of cold ether (either diethyl or methyl t-butyl ether, MTBE) to one 10ml empty, capped centrifuge tube.
7. Add approximately 0.5ml of the peptide-TFA filtrate (from step 5), drop by drop, to the ether-containing tube. The peptide should now precipitate, if it does not, follow

the Ether Extraction Process below. Centrifuge the capped tube, containing the peptide, for 5 mins at room temperature.

8. Decant off the supernatant ether and re-suspend the pellet in fresh ether. Add more peptide-TFA solution and repeat steps 7 & 8 until all the peptide is precipitated.
9. Wash the final peptide pellet in fresh ether at least 4 times, as in step 8.
10. Decant the final ether wash and place the peptide in a fume hood to dry.
11. The final peptide pellet may then be dissolved in an aqueous solution and lyophilized. For cysteine-containing peptides, add approximately 1mM of dithiothreitol (DTT) as a stabilizer, prior to lyophilization.

Ether Extraction Process

WARNING ***CHEMICAL AND FIRE HAZARD. Ether is volatile and highly flammable. Perform this procedure in a working fume hood.***

1. Transfer the ether-TFA-peptide mixture to a 50ml capped centrifuge tube, adding any remaining peptide-TFA filtrate. Add to this tube approximately 10mls of 30% aqueous acetic acid.
2. Shake the capped tube for 10 mins at room temperature to extract the peptide into the aqueous layer (bottom).
3. Spin briefly to separate the layers and remove the top ether layer into a clean, 50 ml capped centrifuge tube. Save the aqueous layer containing the peptide.
4. Add another 10ml of acetic acid to the ether. Shake, spin and remove the top ether layer.
5. Repeat steps 3 and 4 once more.
6. Combine the aqueous layers containing the peptide, and remove residual ether by rotary evaporation.
7. Lyophilize the aqueous phase. For cysteine-containing peptides, add approximately 1mM of dithiothreitol (DTT) as a stabilizer, prior to lyophilization.

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