

Large-Scale Syntheses on the *Symphony*[®] and *Prelude*[™] with No-Foam Beads

PTI has just developed No-Foam beads for large-scale syntheses on the *Symphony*[®] and *Prelude*[™]. When chemically inert No-Foam beads are added to a reaction vessel, it is possible to run higher scales on each instrument. No-Foam beads suppress foaming and improve mixing at large scales. This application note reviews large-scale synthesis protocols on the *Symphony*[®] and *Prelude*[™] using No-Foam beads.

Guidelines for Calculating Synthesis Scales

Different reaction vessels have different capacities, which need to be taken into account along with peptide size and resin loading when planning a synthesis. Longer peptides would require smaller scales, while higher resin loadings would allow larger scales. As a rough guideline, use the factors in Table 1 and Equation 1 to calculate the maximum scale for a synthesis.

Table 1: Maximum total mass for each reaction vessel type, where the total mass equals the sum of the resin mass and final peptide mass. The final peptide mass takes into consideration the growth of the resin as the peptide chain elongates.

Maximum Total Mass (g)	Reaction Vessel
~1.1	<i>Symphony</i> [®] Plastic RV
~1.5	<i>Symphony</i> [®] Large Glass RV
~1.7	<i>Prelude</i> [™] 40 mL Plastic RV

$$Scale (mmol) = \frac{MaximumTotalMass (g)}{\left(\frac{1}{Loading (mmol / g)} + \frac{MW (g / mol)}{1000 (mmol / mol)} \right)}$$

Equation 1: Input the resin loading (in mmol/g), molecular weight (MW) of the final peptide (in g/mol), and maximum total mass for the reaction vessel being used (in g) from Table 1 to obtain the maximum suggested scale (in mmol) for the synthesis.

For example, to calculate the maximum scale for synthesizing ⁶⁵⁻⁷⁴ACP using 0.41 mmol/g substituted Fmoc-Gly-Wang resin in the *Symphony*[®] plastic RV, the result would be:

$$\frac{1.1g}{\left(\frac{1}{0.41mmol / g} + \frac{1063g / mol}{1000mmol / mol} \right)} = 0.300mmol$$

These guidelines only provide a rough estimate because additional factors such as peptide and resin fluffiness can only be taken into account after the synthesis has been started, and may require the reaction to be scaled back.

Symphony[®] Large-Scale Protocol

With No-Foam beads, ⁶⁵⁻⁷⁴ACP (H-VQAAIDYING-OH) was synthesized on Fmoc-Gly-Wang resin (0.41 mmol/g) at the 300 μmol scale (~0.7g) in the disposable plastic reaction vessel and the 400 μmol scale (~1 g) in the large glass reaction vessel on the *Symphony*[®]. 9 g of No-Foam beads were added to each reaction vessel. The synthesis and cleavage protocols are shown in Tables 2 & 3 below.

Table 2: *Symphony*[®] Large-Scale Synthesis Protocol. Vol refers to the number of 1.25 mL aliquots.

Step	Solvent/Operation	Vol	Mix Time	Drain	Rep
1	DMF	8	0:00:30	ON	3
2	20% Piperidine/DMF	8	0:02:30	ON	2
3	DMF	8	0:00:30	ON	6
4	Reagent (AA)	4	0:00:00	OFF	1
5	HBTU/NMM/DMF	4	0:10:00	ON	1
6	DMF	8	0:00:30	ON	1
7	Reagent (AA)	4	0:00:00	OFF	1
8	HBTU/NMM/DMF	4	0:10:00	ON	1
9	DMF	8	0:00:30	ON	3

Table 3: *Symphony*[®] Large-Scale Cleavage Protocol. Vol refers to the number of 1.25 mL aliquots.

Step	Solvent/Operation	Vol	Mix Time	Drain	Rep
1	DMF	8	0:00:30	ON	3
2	20% Piperidine/DMF	8	0:02:30	ON	2
3	DMF	8	0:00:30	ON	6
4	Methylene Chloride	8	0:00:30	ON	6
5	Dry	1	0:10:00	ON	1
6	TFA	6	2:00:00	ON	1
7	TFA	2	0:00:30	ON	1
8	Methylene Chloride	8	0:00:30	ON	3
9	Dry	1	0:02:00	ON	1

Peptides were analyzed on a Varian Microsorb C18 column (4.6 x 50 mm, 5 μ m, 300 Å). 50 μ L of a 3 mg/mL aqueous peptide solution was injected into a Varian Pro-Star HPLC and separated in a 5-95% gradient of aqueous ACN containing 0.1% TFA over 7 minutes. HPLC results are shown in Figures 1 & 2.

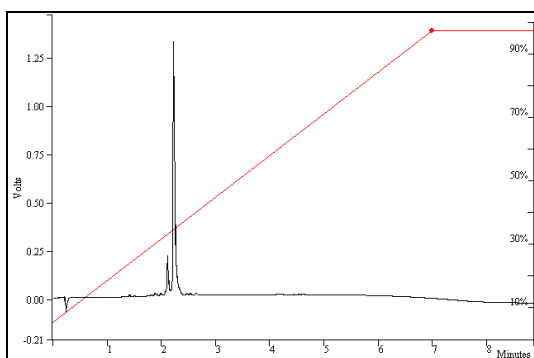


Figure 1: HPLC of ⁶⁵⁻⁷⁴ACP synthesized at the 300 μ mol scale (~0.7 g of resin) in a disposable plastic RV on the *Symphony*®.

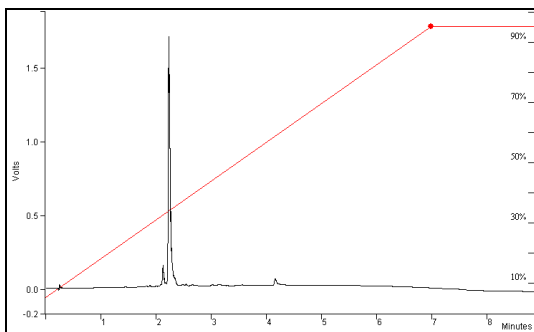


Figure 2: HPLC of ⁶⁵⁻⁷⁴ACP synthesized at the 400 μ mol scale (~1 g of resin) in a large glass RV on the *Symphony*®. **Prelude™ Large-Scale Protocol**

With No-Foam beads, G-LHRH (H-GHWSYGLRPG-NH₂) was synthesized on Fmoc-Rink MBHA resin (0.47 mmol/g) at the 500 μ mol scale (~1 g) in the 40 mL plastic reaction vessel on the *Prelude*™. 5 g of No-Foam beads were added to the reaction vessel. The synthesis and cleavage protocols are shown in Tables 4 & 5 below.

Table 4: *Prelude*™ Large-Scale Synthesis Protocol. Volumes are given in milliliters.

Step	Solvent/Operation	Vol	Mix Time	Drain	Rep
1	DMF	15	0:00:30	ON	3
2	20% Piperidine/DMF	15	0:02:30	ON	2
3	DMF	15	0:00:30	ON	6
4	Reagent (AA)	6	0:00:00	OFF	1
5	HBTU/NMM/DMF	6	0:10:00	ON	1
6	DMF	15	0:00:30	ON	1
7	Reagent (AA)	6	0:00:00	OFF	1
8	HBTU/NMM/DMF	6	0:10:00	ON	1
9	DMF	15	0:00:30	ON	3

Table 5: *Prelude*™ Large-Scale Cleavage Protocol. Volumes are given in milliliters.

Step	Solvent/Operation	Vol	Mix Time	Drain	Rep
1	DMF	15	0:00:30	ON	3
2	20% Piperidine/DMF	15	0:02:30	ON	2
3	DMF	15	0:00:30	ON	6
4	Methylene Chloride	15	0:00:30	ON	6
5	Dry	0	0:10:00	ON	1
6	Cleave and Collect	15	0:00:00	OFF	1
7	Cleave Mix	0	2:00:00	ON	1
8	Collect	10	0:00:30	ON	1
9	Methylene Chloride	15	0:00:30	ON	3
10	Dry	0	0:02:00	ON	1

The peptide was analyzed on a Varian Pro-Star HPLC as described earlier. HPLC results are shown in Figure 3.

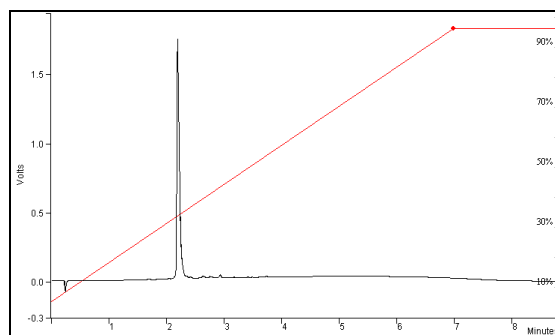


Figure 3: HPLC of G-LHRH synthesized at the 500 μ mol scale (~1 g of resin) in a 40 mL plastic RV on the *Prelude*[™].

To order No-Foam beads, call PTI Customer Service at 1-800-477-6834.

Cat. No.	Description	Quantity
XXX-XXX-XX	No-Foam Beads	X g