High Purity Peptides on a Wide Synthesis Scale: 5 µmol to 5 mmol

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Introduction

Recent advances in formulation and drug discovery have fueled a growing interest in peptide drug candidates. This has generated a challenge for the peptide chemists to develop new methods and provide relatively complex peptides efficiently and in high purity. We recently reported an optimized process for solid phase peptide synthesis that allows significant gains in product purity along with only a 4 minute standard cycle time and a 90% reduction in total waste produced.[1] The general applicability of the High Efficiency Solid Phase Peptide Synthesis (*HE*-SPPS) method was confirmed on a series of peptides at 100 µmol scale. New methods have been developed on the CEM Liberty Blue™ automated microwave peptide synthesizer to handle a wide range of synthesis scales from 5 µmol to 5 mmol using Fmoc-Rink amide SpheriTide® low loading (LL) or high loading (HL) resin. A small scale synthesis, such as 5 µmol, is highly desirable when expensive non-natural amino acids or labeled reagents are used. Synthesis at higher scales (1-5 mmol) allows the production of sufficient quantities for initiating animal studies.

Experimental

***HE*-SPPS Materials and Methods:** All peptides were synthesized on the CEM Liberty Blue automated microwave peptide synthesizer using Fmoc-Rink amide SpheriTide® LL resin (0.17 mmol/g substitution) or Fmoc-Rink amide SpheriTide® HL resin (1.05 mmol/g substitution). Post-deprotection washing with DMF was followed by coupling using a DIC/Oxyma activation method. The peptide resin was cleaved with TFA/TIS/H_2O/DODT (92.5/2.5/2.5/2.5) on the CEM Accent™ microwave cleavage system. The peptide was precipitated in cold ether and the crude material was analyzed without any purification.

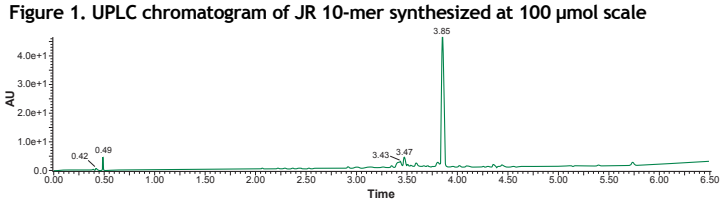
Analysis: Crude peptides were analyzed on a Waters UPLC ACQUITY H-Class with 3100 Single Quad MS using acetonitrile/water with 0.1% TFA as the solvent system on a C18 Column (1.7 µm, 2.1 x 100 mm).

Results and Discussion

(A) Standard Scale Synthesis at 100 µmol:

Well-known for its synthetic challenges, JR 10-mer (WFTTLISTIM-NH_2) was chosen as the test peptide. The final four residues involve difficult deprotection and coupling steps, and typical crude purities of 40-60% have been reported.[2] The peptide was synthesized at 100 µmol scale to establish a baseline purity level with standard default methods on the Liberty Blue using DIC/Oxyma coupling and a new deblocking cocktail, 10% piperazine in NMP/EtOH. Cycles involved a single 1 min/90 °C deprotection and a single 2 min/90 °C coupling for all amino acid residues using a 5-fold excess of reagents. The synthesis of JR 10-mer on Fmoc-Rink amide SpheriTide® LL resin at 100 µmol was completed in 51 min with a crude purity of 73% (Table 1, entry 4) (Figure 1).

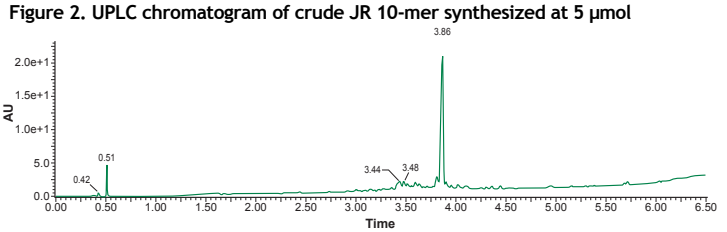
Figure 1. UPLC chromatogram of JR 10-mer synthesized at 100 µmol scale



(B) Small Scale Syntheses at 10 µmol and 5 µmol:

Initial attempts to synthesize the JR 10-mer peptide at 10 µmol using DIC/Oxyma activation with a standard 2 min/90 °C coupling and 1 min/90 °C deprotection resulted in lower crude purities. Optimization experiments were carried out with an aim to achieve the 100 µmol baseline purity levels. Thus, increasing the deprotection time to 5 min and the coupling to 10 min, while keeping the reaction temperature at 90 °C gave an improved crude purity of 66% (Table 1, entry 2). The purity level of crude JR 10-mer was further improved to 73% when the AA/DIC/Oxyma coupling reaction was performed in the presence of 0.1 equivalents DIEA[3] (Table 1, entry 3). Encouraged by the successful results at 10 µmol scale, the new AA/DIC/Oxyma-DIEA coupling procedure was applied to the synthesis at 5 µmol scale and JR 10-mer peptide was obtained in 70% crude purity (Table 1, entry 1) (Figure 2). Both synthesis scales at 10 µmol and 5 µmol used 5 equivalents of coupling reagents.

Figure 2. UPLC chromatogram of crude JR 10-mer synthesized at 5 µmol



(C) Large Scale Syntheses at 1 mmol and 5 mmol:

1 mmol synthesis of JR 10-mer peptide was performed on Fmoc-Rink amide SpheriTide® HL resin (1.05 mmol/g substitution). All amino acid cycles used a single 2.5 min/90 °C deprotection and a single 10 min/90 °C coupling with DIC/Oxyma in the presence of 0.1 equivalents DIEA.[3] Synthesis at 1.0 mmol was completed on the Liberty Blue system with a crude purity of 73% (Table 1, entry 5). Furthermore, the synthesis of JR 10-mer peptide at 5.0 mmol scale was also accomplished on Fmoc-Rink amide SpheriTide® HL resin (1.05 mmol/g substitution) with the new DIC/Oxyma-DIEA method[3] using only 3 equivalent coupling reagents. The microwave methods involved 4 min/90 °C single deprotection and 15 min/90 °C single coupling; the peptide was synthesized with a crude purity of 66% (Table 1, entry 6) (Figure 3). Use of a high-loading (HL) resin is desirable for large scale synthesis, as higher loading means less resin required to synthesize the same amount of peptide. Traditionally, higher loading resins result in a decline in purity and/or yield, as the high peptide density interferes with the synthetic process. SpheriTide resins, however, offer a unique hydrophilic environment resulting in fast coupling rates even at very high substitution levels.

Figure 3. UPLC chromatogram of crude JR 10-mer synthesized at 5 mmol

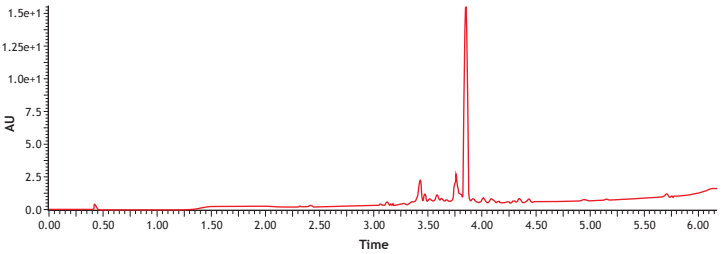


Table 1: Synthesis of JR 10-mer peptide: WFTTLISTIM-NH_2

Entry	Synthesis Scale [a]	SpheriTide® Resin	Deprotection Method	Coupling Reagent	Coupling Method	Crude Purity (%)
1	5 µmol	LL	5 min/90 °C	DIC/Oxyma 0.1 equiv. DIEA	10 min/90 °C	70
2	10 µmol	LL	5 min/90 °C	DIC/Oxyma	10 min/90 °C	66
3	10 µmol	LL	5 min/90 °C	DIC/Oxyma 0.1 equiv. DIEA	10 min/90 °C	73
4	100 µmol	LL	1 min/90 °C	DIC/Oxyma	2 min/90 °C	73
5	1 mmol	HL	2.5 min/90 °C	DIC/Oxyma 0.1 equiv. DIEA	10 min/90 °C	73
6	5 mmol	HL	4 min/90 °C	DIC/Oxyma 0.1 equiv. DIEA	15 min/90 °C	66

[a] Liberty Blue Reaction Vessel:
(i) 5 µmol to 100 µmol: standard 35 mL vessel;
(ii) 1 mmol to 5 mmol: 125 mL vessel

Conclusion

The combination of new *HE*-SPPS chemistry,[3] hydrophilic properties of SpheriTide® resin and ultrafast automation allows the synthesis of high purity peptides on the Liberty Blue in shortest possible synthesis time. Additional advantages of this unique combination include the use of only 5 equiv. coupling reagents for scales of 5 µmol to 1 mmol and 3 equivalents for 5 mmol scale, resulting in substantial savings in reagent cost and a 90% reduction in solvent usage and chemical waste.

References

1. J. M. Collins, K. A. Porter, S. K. Singh, G. S. Vanier; High-Efficiency Solid Phase Peptide Synthesis (*HE*-SPPS) *Org. Lett.* **16**, 940 (2014).

2 (a) T. Redemann, G. Jung; *Peptides-24th European Peptide Symposium*, 749 (1996); (b) L. A. Carpino, E. Krause; Synthesis of 'difficult' peptide sequences: application of a depsipeptide technique to the Jung-Redemann 10- and 26-mers and the amyloid peptide Aß(1-42) *Tetrahedron Lett.* **45**, 7519 (2004).

3. J. M. Collins, S. K. Singh, K. A. Porter; An Improved Coupling Method for Peptide Synthesis at Elevated Temperature, Presented at APS 2015, poster P382.