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Application Note

Rapid Synthesis of Difficult Peptides Using a Novel Resin

Introduction

The properties of a resin are well known to have a major impact on the quality of a peptide made by SPPS. Recently, CEM scientists sought to improve the performance of peptide resins, building on the synthetic improvements realized with the Liberty Blue™ automated microwave peptide synthesizer that utilizes *HE*-SPPS[1] methodology. In this investigation, research focused on SpheriTide® resins, which are unique compared to traditional polystyrene based resins. SpheriTide resins are made using a specific synthetic process that does not involve radical polymerization. The result is a highly structured resin with linker sites that are spaced with a minimum distance between each other. The growing peptides are built off these specific sites which are spaced to avoid clustering, thereby allowing high purity syntheses even at loadings greater than 1 mmol/g. The result is hydrophilic, peptide- like backbone that prevents intramolecular peptide aggregation. The solid support creates a matrix that is an ideal environment for coupling and deprotection (the backbone is similar to DMF or NMP).

In order to test the new resin and further explore *HE*-SPPS methodology, two peptides were chosen. EGFRvIII and $^{1\text{-}42}$Beta Amyloid were selected based on known synthetic challenge. All peptides were first synthesized using polystyrene resin, then tested with either high loading or low loading Rink amide SpheriTide resin.

Results

The EGFRvIII peptide (LEEKKGNYVVTDHC) was synthesized to compare *HE*-SPPS and standard room temperature synthesis. Standard room temperature conditions with low loading Rink amide MBHA PS (loading = 0.38 mmol/g) produced only a trace amount of product. This is consistent with published reports where multiple deprotections were required after each coupling (up to eight in some cases) and >12 hour couplings were used to obtain crude purities of 40-70%.[2] The same resin under *HE*-SPPS conditions generated product with 72% crude purity in little over an hour total time. Using high-loading Rink amide MBHA PS resin (loading = 0.76 mmol/g) resulted in a substantial decline in crude purity, down to 49% (Table 1).

Table 1. EGFRvIII synthesis results*

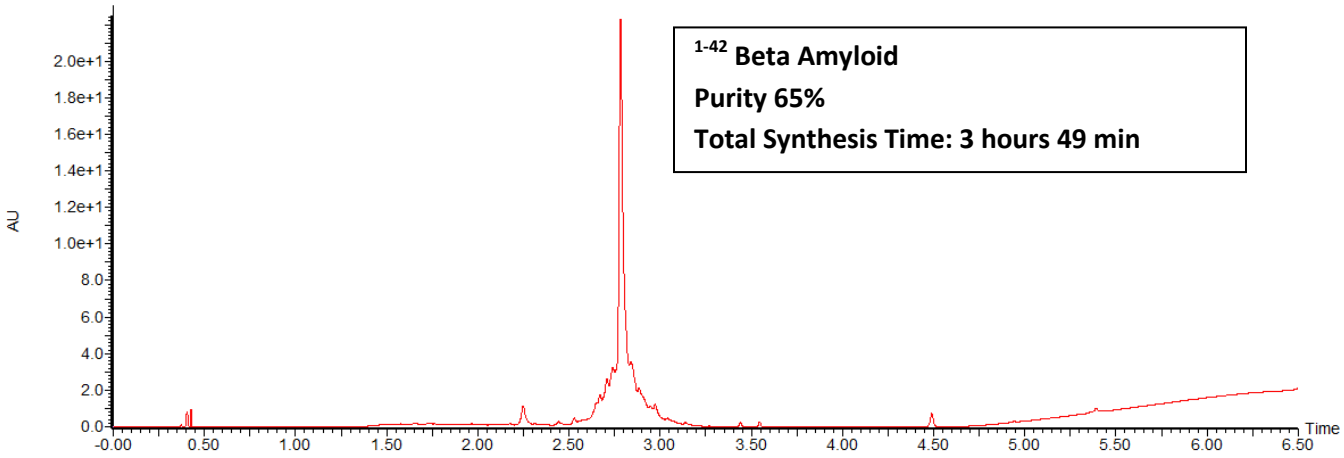
#	Method	Resin	Target	Time
1	Room Temperature SPPS†	0.38 meq Rink amide MBHA PS	< 10%	17h38min
2	*HE*-SPPS‡	0.38 meq Rink amide MBHA (LL)	72%	1h17min
3		0.76 meq Rink amide MBHA (HL)	49%	
4		1.05 meq Rink amide SpheriTide (HL)	67%§	

* All synthesis performed at 0.1 mmol scale. All peptides were cleaved using typical TFA cocktail on CEM Accent Peptide Cleavage System at 38 °C for 30 min followed by lyophilization and UPLC-MS analysis.
† Room temperature runs used 1 hr coupling and 5+10 min deprotection steps.
‡ *HE*-SPPS procedure as described in Reference 1.
§ Crude yield = 96%

Advantageously, the high loading Rink amide SpheriTide resin (loading = 1.05 mmol/g) achieved a comparable results to the low-loading MBHA resin, demonstrating that the unique

properties of the SpheriTide resin can allow high loading equivalencies to compete with conventional low loading resins in certain difficult sequences. The higher crude purity of the high loading resin could be attributed to (1) the hydrophilic nature of the resin which prevents intra-chain aggregation of the growing peptide and (2) the even distribution of initiation sites.

To further demonstrate the versatility of HE-SPPS and SpheriTide, highly complex $^{1\text{-}42}$Beta Amyloid (DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA), a notoriously difficult sequence was prepared. $^{1\text{-}42}$Beta Amyloid is well-known for being challenging both synthetically and analytically. Using Rink amide SpheriTide (LL) (loading = 0.17 mmol/g), the sequence was synthesized in 65% crude purity.



*** Typical *HE*-SPPS conditions[3] at 0.1 mmol scale: 1 min deprotection at 90 °C and 2 min coupling at 90 °C (Fmoc-Arg(Pbf)-OH coupling was 4 min at 90°C) . Peptide was cleaved using typical TFA cocktail on CEM Accent Peptide Cleavage System at 38 °C for 30 min followed by lyophilization and UPLC-MS analysis**

Conclusion

SpheriTide resin, when coupled with *HE*-SPPS techniques on the Liberty Blue microwave peptide synthesis system, results in high quality peptides in a short timeframe. Even challenging sequences can be prepared with high crude purities in high yields.

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. J. I. Finneman, M. J. Pozzo, *Novel Approach for Optimization of a 'Difficult' Peptide Synthesis by Utilizing Quantitative Reaction Monitoring Assays.* J. Pep. Sci. **8**, 511 (2012)