Solid-phase peptide synthesis: The greener, the better

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Introduction

In the last years, the role of peptides in the drug discovery arena has been enormously increased as demonstrated by the number of peptide based drugs authorized by medicinal agencies.[1] The concept of solid-phase peptide synthesis (SPPS) pioneered by Merrifield in the 60's [2] and the later development of the Fmoc/tBu strategy are the cornerstones that have made peptides the subject of study in many research areas. Thus, the demand for synthetic peptides is increasing, and considerable efforts have been channeled into optimizing the synthetic process itself, i.e. the coupling reagents, protecting groups, automation of the process, etc. However, little attention has been paid for making the process greener. The "solid-phase" itself could be considered green in some aspects. For instance, the synthesis of a peptide comprising 20 amino acids involves 41 chemical steps in only one reactor, with a clear minimization of mechanical losses and mass transfer. However, SPPS involves the use of large excesses of reagents, which are removed by simple filtration and exhaustive washing steps between each reaction, and therefore the consumption of solvents is the main impediment to be considered an full eco-process. DMF, the main solvent used in SPPS, is a highly reprotoxic solvent and is classified as a Substance of Very High Concern (SVHC).[3] However, it is considered to be difficult to replace because of its properties.

The work presented here is focused to the replacement of DMF by γ -Valerolactone (GVL), a biomass derived solvent, in all steps of SPPS: coupling, Fmoc removal, and washes; and its compatibility with micro-waves conditions. Furthermore, the substitution of diethyl ether (DEE) by cyclopentyl methyl ether (CPME) in the precipitation step, after global deprotection is also described.

Results and Discussion

Initially, comprehensive review and evaluation of physical properties of selected green solvents were conducted in order to decide which was the best choice. Based on some properties, such as capacity to solubilize Fmoc-amino acids, coupling reagents and additives, aside from Fmoc removal efficiency [4], GVL has proved to be the best green solvent of choice for peptide chain elongation whereas, CPME has proved its ability as a green precipitating ether. It is worth mentioning that CPME has limited ability to solubilize Fmoc amino acids, excluding it from the elongation protocol.

For the study, four different model peptides were synthesised based in their synthetic difficulty and different length: (i) the decamer acyl carrier protein (⁶⁵⁻⁷⁴ ACP, H-VQAAIDYING-OH), (ii) the decamer Jung-Redemann (JR, H-WFTTLISTIM-NH₂), (iii) the ABC 20-mer (H-VYWTSPFMKLIHEQCNRADG-NH₂), and (iv) the 28-mer thymosin (H-SDAAVDTSEITTKDLKEKKEVVEEAEN-NH₂). The syntheses were carried out under microwave conditions using a CEM Liberty Blue: 0.1 mmol scale, 5-fold excess of reagents, DIC/Oxyma as coupling reagent and 20% Piperidine for Fmoc removal. The same cycle was used for all syntheses: coupling time 165 s, deprotection time 95 s, both at 90°C using GVL to solubilize all the reagents and for washing steps.

For comparison of the synthetic efficiency, two syntheses of each peptide were made: one with GVL as a green solvent and another one with DMF as a standard solvent. The HPLC analysis of the obtained crude peptides shown similar purities for both solvents[5], Fig 1 shows the example of JR peptide, where the GVL synthesis even lead higher purity than DMF.

Figure 1: HPLC chromatograms for JR decamer peptide synthesized using GVL vs. DMF

Furthermore, the quality of the crude peptides obtained after precipitation by addition of three different chilled ethers namely DEE, TBME (t-butylmethylether) and CPME were compared. Thus, three identical aliquots of each peptidyl-resin were treated with the standard cleavage solution (TFA/H₂O/TIS 95:2.5:2.5), and then the corresponding ether was added for provoking precipitation. No significant differences were found in the purity of the peptides as shown by HPLC as shown in Figure 2. Additionally the percentage of peptide recovery was not affected by the ether used.[6]

Figure 2: Comparison of 65-74ACP decamer peptide (A) and ABC 20mer (B) precipitated with CPME vs. DEE and TBME; D. peptide precipitated with CPME (green), DEE (blue) and TBME (red)

Conclusion

We have developed a very efficient green methodology for SPPS. It involves the total substitution of the hazardous DMF by GVL during the synthetic process and the substitution of DEE by CPME in the precipitation step. GVL showed full compatibility with automation and use of MW during the process. The protocols are applicable to all both PS and PEG-based solid supports.

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