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Short Communication

Highly efficient synthesis of endomorphin-2 under thermodynamic control catalyzed by organic solvent stable proteases with in situ product removal

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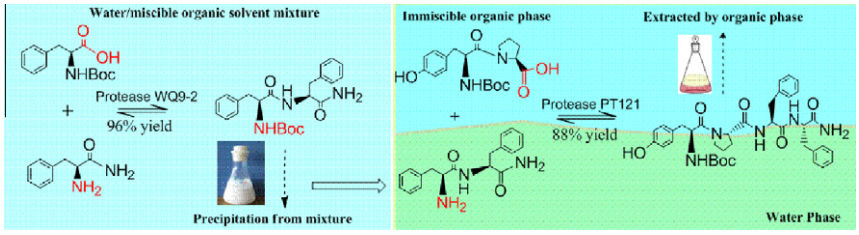
GRAPHICAL ABSTRACT



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ABSTRACT

An efficient enzymatic synthesis of endomorphin-2 (EM-2) was achieved using organic solvent stable proteases in nonaqeous media, based on thermodynamic control and an in situ product removal methodology. The high stability of biocatalysts in organic solvents enabled the aleatoric modulation of the nonaqueous reaction media to shift thermodynamic equilibrium toward synthesis. Peptide Boc-Phe-Phe-NH_2 was synthesized with a high yield of 96% by the solvent stable protease WQ9-2 in monophase medium with an economical molar ratio of the substrate of 1:1. The tetrapeptide Boc-Tyr-Pro-Phe-Phe-NH_2 was synthesized with a yield of 88% by another organic solvent tolerant protease PT121 from Boc-Tyr-Pro-OH and Phe-Phe-NH_2 in an organic–aqueous biphasic system. The reaction–separation coupling in both enzymatic processes provides "driving forces" for the synthetic reactions and gives a high yield and high productivity without purification of the intermediate, thereby making the synthesis more amenable to scale-up.

1. Introduction

Over the past ten years, scientific and technological advances have established biocatalysis as a practical and environmentally friendly alternative to traditional metallo- and organocatalysis in chemical synthesis, both in the laboratory and on an industrial scale (Bornscheuer et al., 2012; Fernández-Lucas et al., 2012). Proteases are effective biocatalysts for the synthesis of peptides under nonaqueous conditions and give a more selective process, in turn preventing the formation of by-products (Bordusa, 2002; Gupta and Khare, 2006). The stereospecificity of proteases guarantees the formation of stereochemically uniform products and only semi-permanent protection of the carboxy and amino in Cα backbone would be required.

Endomorphin-2 (EM-2, Tyr-Pro-Phe-Phe-NH_2) has recently drawn much attention owing to the distinct medicinal values of this compound. It can relieve severe pain through activation of μ-opioid receptors, being more effective than endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2) and has shown great promise as analgesics of comparable potency to morphine (Liu and Wang, 2012). In addition, EM-2 can effectively protect neurons against biological effects

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