Hydrolysis of dipeptide *n*-heptyl esters with Newlase F

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Newlase F, a crude enzyme containing lipase and protease, can be used in the synthesis of peptide conjugates; the hydrolysis conditions were optimized to increase lipase activity and suppress protease activity and in addition, N-terminal protecting groups and peptide bonds were not affected.

Keywords: Newlase F, hydrolysis, esters, lipase, protease

Peptides conjugates, such as phospho-peptides,[1] are of great importance in biological studies, for example of protein phosphorylation/dephosphorylation.[2] However, the chemical synthesis of these compounds is severely complicated by their pronounced acid and base lability as well as their multifunctionality. Traditional peptide synthesis will lead to many side reactions such as β-elimination. Recently, it has been shown that enzymatic protecting group techniques[3] can provide a variety of protecting groups which can be removed selectively and orthogonally under the mildest and preferably neutral conditions. C-terminal heptyl esters can be selectively demasked through cleavage of the heptyl group by lipase from *Rhizopus niveus* (lipase N). But lipase N is expensive and not stable enough to use.

Newlase F is a crude enzyme which contains lipase and acid protease from *Rhizopus niveus*. In comparison with lipase N, Newlase F is cheaper, more stable and more available. In this paper, Newlase F was used to remove the C-terminal protecting groups from dipeptide esters. The hydrolysis reaction was monitored by HPLC with dipeptide heptyl esters as substrates.

When the pH was around 7.0 and temperature was around 30°C, the lipase in Newlase F had the highest catalytic activity. When the enzyme content reached 5.0 mg/ml, the hydrolysis was very fast and the hydrolysis reached equilibrium in one hour. Low levels of acetonitrile can promote the hydrolysis, while high levels of acetonitrile inhibit it. On the other hand, protease activity was inhibited distinctly with increase of acetonitrile concentration. Adding water-miscible organic solvents to the hydrolysis can improve the catalytic lipase activity of Newlase F. HPLC analyses indicated that the N-terminal protecting groups (Boc, Z) and peptide bonds with hydrophilic amino acid residues on either side were not affected by the crude enzyme Newlase F.

In mild conditions, Newlase F has been used in the synthesis of phospho-dipeptide Boc-Ala-Ser(OP(OPr^i)$_2$)-OH. Newlase F can remove the protecting group heptyl group from Boc-Ala-Ser(OP(OPr^i)$_2$)-OHept without any effect on the peptide bond and side chain phosphoryl group (Fig. 3).

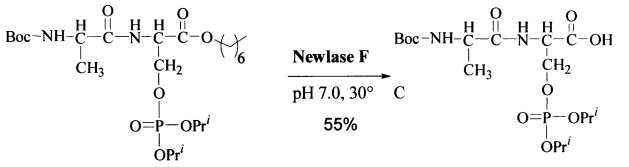

Fig. 3 Use of Newlase F in the synthesis of a phospho-dipeptide.

Caution: Newlase F may cause sensitisation when inhaled.

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Techniques used: HPLC, ^{31}P NMR, ^{1}H NMR, UV, ESI-MS.

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