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Novel Chemoenzymatic Synthesis of Peptide C-Terminal Amides from Ester Precursors

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Abstract: A novel two step chemoenzymatic procedure for the preparation of peptide C-terminal amides has been developed.

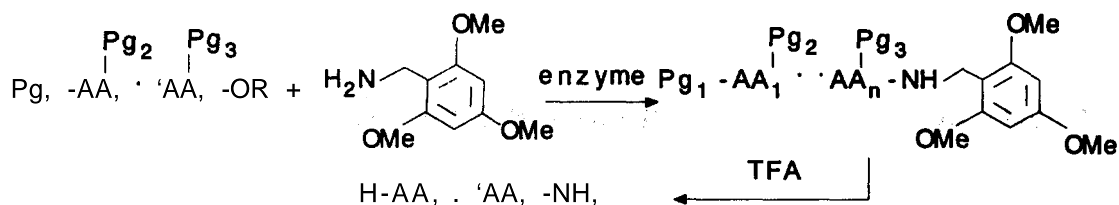
Recent advances in multiple peptide synthesist make possible the simultaneous preparation and analysis of very large peptide libraries. It is due to this progress, that in the nearest future we may see a dramatic in&ease in the number of peptide-based pharmaceuticals, such as neuropeptide hormones.2 The fact that almost half of ail known neuropeptides are C-terminal amides demonstrates the importance of the carboxamido group for full biological activity of these compounds.[3]

The preparation of substantial amounts of these peptides can be better achieved by solution-, solid-phase peptide synthesis. or enzymatic techniques. Each of these methods has its own advantages and limitations. in solution-phase peptide synthesis, the preparation of peptide C-terminal amides often suffers due to the poor solubility of amide intermediates in commonly used organic solvents. This can be overcome by use of C-terminal esters and ammonoiysis with ammonia at the conclusion of the synthesis. However, this reaction is often slow, low yielding, prone to racemization and lacks selectivity when other esters (eg. benzyl) are present in the molecule.[4] Solid phase synthesis allows the preparation of peptide amides but the high cost of the required resins restricts the adaptability of this technique especially on a large scale. Amidation can also be achieved by enzyme-catalyzed hydroxylation of C-terminal Gly residues, transpeptidation or aminolysis.[6] These reactions normally employ peptide precursors, the sequences of which are either one amino acid shorter7 or contain an alternative C-terminal amino acid to the desired final product.*

Here we report a novel two step chemoenzymatic method for the preparation of C-terminal amides from the peptides of desired length.[9] First, regioselective enzyme-mediated coupling of the corresponding peptide ester to DmbNH2 or $TmbNH_2$[10] is carried out in aqueous-organic solvent mixtures or in organic solvents using papain or subtiiisin. Second, the resulting product is treated with trifluoroacetic acid to give a fully deprotected peptide C-terminal amide (see Figure).

The resulting peptida di- and tri-methoxylwnzylamides were isolated in good yield and high purity after a simple work-up procedure.[11] In addition, papain-catalyzed reactions were fast and complete in less than 2 h. It is worth mentioning that different esters, such as methyl, 2-chloroethyl and plienacyl were successfully employed.

To demonstrate the versatility of this technique, ZGlu(^{t}Bu)Lys(Boc)NHTmb, a peptide which, in addition to the benzylamide moiety, contains three of the most widely used protecting groups was prepared on a large scale (1.1 g). This compound was treated with TFA/thioanisole to give the desired fully deprotected C-terminal amide ($HGluLysNH_2$) in quantitative yield.[12]



Peptide ester	Enzyme	T i e	Yield **(%)**
ZLeuLys(Z)OMe	**Papain**	20 min	76
BocLeuGlu(Bn)OPac	Papain	65 min	69
BocMetLeuOMe	Papain	60 min	63
ZGlu(^{t}Bu)Lys(Boc)OMe	Papain	2h	87
ZPheOCH$_2$CH$_2$Cl^a	Subtilisin	24h	64

a. Reaction was carried out in *tert*-**amyl** alcohol with DmbNH2 (3 eq.)

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9. In a typical experiment protected **peptide** ester (80 mg) and **TmbNH$_2$** hydrochloride (3eq.) are suspended in 6-8 **mL** 0.2 M carbonate buffer **pH 9** containing 40% **MeCN. 2-Mercaptoethanol (25 µL)** is added followed by papain (S-10 mg) and the reaction stirred at RT **(20-120** min). Water is added to complete precipitation of **theproduct,** which is filtered, washed with water, and dried.
10. Nonstandard abbreviations: Dmb. **2,4-dimethoxybenzyl; Tmb, 2,4,6-trimethoxybenzyl;** TFA, **trifluoroacetic** acid; Pg, **protecting** group; **AA,** amino acid,
11. All compounds were characterized by TLC, HPLC, ^{1}H NMR and MS.
12. **ZGlu(^{t}Bu)Lys(Boc)NHTmb** (1 **mmol)** was stirred in TFA (21 **mL), thioanisole (5.9 mL)** and H20 (2.1 **mL)** overnight at RT. Solvent was evaporated and residue taken up in H20, washed with Et20 and **lyophilized.** In addition to standard analyses (Ref. 10) **HGluLysNH$_2$** was characterized by FAB MS **and high resolution** MS.

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