Facile Enzymatic Synthesis of *N*-Acyl-enkephalin Amides

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A novel three step protease catalysed synthesis of enkephalin precursors from simple readily available substrates is described.

In recent years a wide range of endogenous bioactive peptides have been isolated, characterized and synthesized.[1] Although most peptides are currently prepared by solution or solid phase chemical methods,[2] enzyme catalysed syntheses[3] have become increasingly important, and preparative scale methodologies have been developed for a number of oligopeptides such as angiotensin, caerulin, cholecystokinin, dynorphin,[4] enkephalins and other opioids.

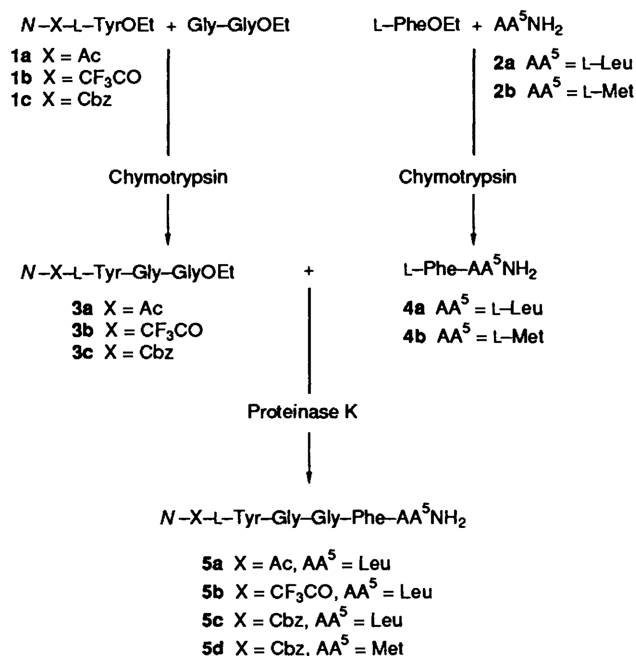
Since their discovery in 1975,[5] the enkephalins have attracted much interest as a class of potent analgesics.[6] A number of linear and convergent protease-catalysed syntheses of endogenous opioids have been reported.[7] However, these have all employed multistep methodologies with complex protection/deprotection schemes, leading to poor overall yields and limited scope for their preparative scale application.† In this communication we describe an efficient, highly simplified enzymic synthesis of a range of Leu- and Met-enkephalin derivatives in organic media.

Following our observation that the specificity of α-chymotrypsin towards *N*-unprotected amino acid esters is greatly increased in low-water media,[8] L-PheOEt was used as acyl donor for the synthesis of the *C*-terminal dipeptide fragments of Leu- and Met-enkephalin (Scheme 1, Table 1). Compounds **4a** and **4b** were prepared in 89–90% yields using **2a** and **2b** as amino components respectively. A range of *N*-acylated *N*-terminal tripeptides **3a–c** were also synthesized in 88–89% yields from **1a–c** by this method. Finally, the direct coupling of the two fragments gave the final products **5a–c** in overall yields of 45–59%. This compares very favourably with the yields obtained by conventional solution phase chemical syntheses.[9]

This novel methodology has several distinct advantages over reported syntheses: (i) The use of an *N*-unprotected substrate as

† Chymotrypsin, carboxypeptidase Y, papain, and thermolysin have been used in syntheses typically involving 7 to 13 steps, with yields ranging from 6 to 17%.

‡ For full details of the BLDSC deposition scheme see 'Instructions for Authors,' *J. Chem. Soc., Perkin Trans. 1*, 1992, Issue 1.



Scheme 1

Table 1 Protease-catalysed oligopeptide synthesis

Product[a]	Scale (mmol)	Time (h)	Yield (%)	M.p.[b] (°C)	$[\alpha]^{20c}$	FAB-MS (M + H), obsvd.[d]	(M + H), calcd.
3a	15.6	60	89	194–196	+31.4	366.171 42	366.166 49
3b	12.5	60	88	174–177	+22.3	420.149 75	420.138 22
3c	18.7	60	89	169–171	+8.1	458.201 46	458.192 71
4a	30.0	40	90	125–127	−22.3	278.187 23	278.186 84
4b	20.3	60	89	148–150	−13.2	296.141 96	296.143 26
5a	1.55	60	66	154–156	+9.5	597.300 35	597.303 64
5b	1.44	60	51	197–199	+10.6	651.275 25	651.275 37
5c	1.36	60	60	185–187	−13.2	689.333 42	689.329 85
5d	3.24	80[e]	54	174–176	−12.2	707.292 76	707.286 27

[a] Preparations: equimolar quantities of acyl and amino components (free bases) were dissolved to a final concentration of 250 mmol dm^{-3} in 9:1 acetonitrile–ethanol containing 4% water (**3a–4b**) or in 94:3:3 acetonitrile–ethanol–formamide to a final concentration of 50 mmol dm^{-3} (**5a–5d**). α-Chymotrypsin (**3a–4b**) or proteinase K (**5a–5d**) adsorbed on Celite, as previously described,[10] was added to a final concentration of 100 or 200 mg cm^{-3} respectively, and the mixture was shaken at 37 °C. The reaction mixture was then filtered, treated with 2.0 g of Dowex 50X4 (**5a–5d** only), rotary evaporated and purified by reverse phase low-pressure chromatography on Sorbsil RP18, C200 using a methanol-water gradient. Satisfactory elemental analyses (±0.6% for C,H,N) were obtained for **3c**, **4a**, **4b**, **5c** and **5d**. In addition all products were fully characterized by 400 MHz ^1H NMR, 100 MHz ^{13}C-NMR, COSY and NOESY spectroscopy. Spectra agreed well with literature data.[10] Experimental details and physical data for compounds **3–5** are available from the British Library as a supplementary publication (SUP. PUBL. NO. 56875).‡ [b] Lyophilized products. [c] *c* = 1.0, MeOH for **3a–5b**; *c* = 1.0, 9:1 MeOH–$HCONH_2$ for **5c**, **5d**. [d] 8 keV Xe, Glycerol matrix. [e] After 40 h of incubation the enzyme was filtered off, a new batch of proteinase K was added and the incubation continued for a further 40 h.

the acyl donor and an amino acid ester as the acyl acceptor* in the synthesis of corresponding *C*- and *N*-terminal oligopeptide fragments allows direct coupling, without the need for deprotection and activation; (ii) acyl donors and acceptors were used in a 1:1 molar ratio, thus minimizing work-up procedures† that are normally required in conventional syntheses employing an excess of acyl or amino component;‡ (iii) the use of organic media allowed the synthesis of di- and tri-peptide components at high concentrations.§ This work demonstrates the considerable simplification that can be achieved compared to conventional protocols utilizing enzymes. Combined with the well-documented advantages of protease-catalysed peptide synthesis in organic solvents,[10] such as high solubility of reactants, greatly reduced hydrolysis and dispensation with side chain protection, this methodology provides an attractive approach to the preparative scale synthesis of bioactive peptides.

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* Gly-GlyOEt, which was used in the present synthesis, is a good substrate for chymotrypsin, and is readily hydrolysed under different reaction conditions, *e.g.* in the absence of ethanol or at a higher water concentration.

† Note that the preparative yields of **3** and **4** are reported. Conversions greater than 95% were achieved in reactions I and II, allowing 'straight through' enzymic coupling of the di- and tri-peptides without their prior isolation.

‡ Enzymatic and solution phase chemical syntheses have typically employed 10–100% excess of amino component and 10–50% excess of acyl component respectively.

§ Substrate concentrations up to 1.0 mol dm^{-3} could be used without adversely affecting kinetics or yields.

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