

## Papain catalysed Esterification of *N*-Protected Amino Acids

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*N*-Protected amino acids and amino diacids were converted to their corresponding esters catalysed by immobilized papain with a high concentration of alcohols under mild conditions in a two-phase system.

The esterification of amino acids with alcohols is an important reaction. Although there are many chemical methods,<sup>1</sup> only a few simple processes are effective under mild conditions.<sup>2</sup> Recently the mono-esterification of *N*-protected diacids using chloroformate activation was reported<sup>3</sup> but the products contained about 10–34% of positional isomers and separation was difficult. We describe here a new enzymatic procedure for preparative esterification of *N*-protected amino acids and amino diacids using papain as catalyst.

Although  $\alpha$ -chymotrypsin can catalyse the formation of *N*-acetyl tryptophan ethyl ester,<sup>4</sup> its use is limited to aromatic amino acids. Our previous study<sup>5</sup> and others<sup>6</sup> have shown that papain can retain its activity in the presence of high concentrations of organic solvents. Recently Mitin reported that papain has high esterase activity at pH 8–9<sup>7</sup> and Wong was able to synthesise unusual amino acids by controlling the amidase activity of papain catalysed reactions,<sup>8</sup> using immobilized papain in the presence of high concentrations of organic solvent. Here we use immobilized papain as the catalyst and alcohols as nucleophiles in the synthesis of amino acid esters.

Papain is a thio-protease, and is known to be active at pH 5–6. The protease specificity of papain is broad and in general bulky aliphatic or aromatic groups are preferred as acyl donors. This requirement is easily met with the use of common amino protecting groups such as benzyloxycarbonyl (Cbz), *t*-butoxycarbonyl (Boc), and *p*-methoxybenzyloxycarbonyl (Moz). Papain catalysed hydrolysis of benzyl and *p*-nitrophenyl esters in aqueous solution is known. Here we have used methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, and

benzyl alcohol as nucleophiles to synthesise commonly encountered amino acid esters. Most of the primary alcohols react rapidly, but secondary alcohols react slowly. The enzyme was immobilized on XAD-8 resin.<sup>8</sup>

The reactions were carried out under thermodynamic control. The esters are more soluble in the reaction solutions than the substrates. Use of the minimum amount of water will shift the equilibrium of the enzymatic reaction in favour of the ester products. In a typical reaction, papain (0.5 g; *Carica papaya*, 3.5 m Anson-E/mg, from Merck) in McIlVine buffer (0.5 ml; pH 5.0), and adsorbent (2 g; Amberlite XAD-8, from Sigma, dried over KOH) were stirred for 30 min. After adsorption of the enzyme on XAD-8, the *N*-protected amino acid (2 mmol) in a mixture of the alcohol (2 ml) and ethyl acetate (15 ml) was added. The mixture was incubated at 37 °C with constant shaking for about 12 h until all the acyl amino acid had been consumed (t.l.c.). The resulting mixture was washed (NaHCO<sub>3</sub>) and concentrated to give the esters. In order to increase the concentration of nucleophile in the aqueous layer at the enzyme surface, acetone (3 ml) was added in reactions involving benzyl or *n*-butyl alcohol. For the diacids, the products were washed with water and isolated by preparative flash column chromatography. The esters were characterized by comparison (optical rotation and n.m.r. spectra) with chemically prepared samples.

The most interesting results were obtained for the diacid esters: cystine monoester, aspartyl  $\alpha$ -ester, and glutaryl  $\alpha$ -ester. The thio-protease papain can catalyse the formation of cystine monoesters without interference of the disulphide bond of the cystine. *N*-Protected aspartyl  $\alpha$ -benzyl ester is

**Table 1.** Papain catalysed synthesis of amino acid esters.

Acyl donor	Nucleophile	Product	% Yield	$[\alpha]_D^{25/0}$ (c 2, MeOH)	M.p., t/°C
Cbz-Phe-OH <sup>a</sup>	BzIOH	Cbz-Phe-OBzl	76	-12.0	63—65
Moz-Phe-OH	MeOH	Moz-Phe-OMe	70	-13.5	58—60
Boc-Ala-OH	BzIOH	Boc-Ala-OBzl	75	-40.5	Oil
Boc-Leu-OH	MeOH	Boc-Leu-OMe	80	+15.0	147—149
Cbz-Val-OH	MeOH	Cbz-Val-OMe	83	+17.0	54—56
Moz-Asp-OH	BzIOH	Moz-Asp- $\alpha$ -OBzl	55	-15.6 <sup>d</sup>	105—106 <sup>c</sup>
Moz-Glu-OH	BzIOH	Moz-Glu- $\alpha$ -OBzl	57	-13.0 <sup>d</sup>	166—167 <sup>c</sup>
Moz-Glu-OH	MeOH	Moz-Glu- $\alpha$ -OMe	71	-11.0 <sup>d</sup>	170—172 <sup>c</sup>
Moz-Tyr-OH	BzIOH	Moz-Tyr-OBzl	77	+3.2	77—79
Moz-Trp-OH	BzIOH	Moz-Trp-OBzl	74	-7.5	105—107
Boc-Cys-OH	BzIOH	Boc-Cys-OBzl	53	-66.0 <sup>d</sup>	72—74 <sup>c</sup>
Boc-Cys-OH		Boc-Cys-OH			
Moz-Ala-OH	MeOH	Moz-Ala-OMe	82	-24.0	Oil
Moz-Ala-OH	EtOH	Moz-Ala-OEt	81	-25.1	Oil
Moz-Ala-OH	Pr <sup>n</sup> OH	Moz-Ala-OPr <sup>n</sup>	82	-22.9	Oil
Moz-Ala-OH	Pr <sup>i</sup> OH	Moz-Ala-OPr <sup>i</sup>	17 <sup>b</sup>	-23.0	Oil
Moz-Ala-OH	Bu <sup>n</sup> OH	Moz-Ala-OBu	70	-21.0	44—47
Moz-Ala-OH	BzIOH	Moz-Ala-OBzl	76	-29.5	58—60

<sup>a</sup> Cbz = benzyloxycarbonyl; Boc = t-butyloxycarbonyl; Moz = *p*-methoxybenzyloxycarbonyl; Phe = phenylalanine; Cys = cysteine; Ala = alanine; Leu = leucine; Val = valine; Asp = aspartic acid; Glu = glutamic acid; Tyr = tyrosine; Trp = tryptophan. <sup>b</sup> Reaction for 96 h. <sup>c</sup> Dicyclohexylamine salt. <sup>d</sup> Optical rotation in dimethylformamide (c 2) solution.

commonly used in solid phase peptide synthesis for the conversion of the peptide C-terminal aspartic residue into asparagine.<sup>9</sup> *N*-Protected glutaryl  $\alpha$ -esters are useful starting materials for the synthesis of glutathione.

Table 1 shows the yield of acyl amino esters, the yields generally being high. The amino protecting groups do not affect the yields. Esterifications using benzyl or *n*-butyl alcohol need longer reaction times, owing to their poor solubility in the aqueous layer. All the primary alcohols tested can be used as nucleophiles, and give similar yields (70—80%). The only secondary alcohol used (Pr<sup>i</sup>OH) gave a low yield (15%). Use of aspartic and glutamic acid as an acyl donor led to exclusive esterification at the  $\alpha$ -position. The reaction of the cystine derivative gave 53% of the monoester and a small amount of diester (3—5% by h.p.l.c.). Finally, proline did not react in the enzyme catalysed reaction. As has been reported before, the rigid proline ring is excluded from the enzyme active site.

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