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SYNTHESIS OF AMINO ACID ESTERS BY PAPAIN

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Abstract: A wide range of N-Boc-amino acid esters were synthesized from N-Boc-amino acids and alcohol using papain as catalyst. Suitable biphasic reaction mixtures were found for most amino acids to achieve high yield of ester synthesis. With N-Boc-L-aspartic and glutamic acids only the α carboxyl group is esterified, without racemisation.

In enzymatically catalyzed peptide synthesis by serine or thiol proteases, N-protected amino acid alkylesters are often needed[1-2].

Lipases have been used for a long time for the esterification of acids [3]. However attempts to esterify amino acids failed[3c]. Esterases themselves have been mostly employed in transesterification reactions[4].

Some examples are known of esterification of amino acids by proteolytic enzymes. N-acetyl tryptophan ethyl ester[5] as well as N-acetyl-tyrosine[6] and N-benzoyl-phenylalanine[7] ethyl esters have been synthesized using chymotrypsin and biphasic aqueous reaction mixtures. In these conditions the equilibrium

$$\text{Amino acid} + \text{alcohol} \rightleftharpoons \text{Amino ester} + H_2O$$

is shifted to the right because the ester is continously removed in the organic phase.
It would be interesting to extend the range of amino acids being esterified enzymatically. Papain itself is a good candidate since it is commercially available, inexpensive and has a broad specificity.

In this work it was found that papain is, in fact, able to catalyze the esterification of a wide range of N-tbutyloxycarbonyl (N-Boc) protected amino acids in a biphasic system.

For each amino acid conditions were optimized to obtain almost quantitative yields of esterification, as seen in table 2. No protection of the side chain is necessary. In dicarboxylic amino acids only the α carboxyl group is esterified, without racemisation. Esterification of

N-Boc-D,L-aspartic acid with benzylalcohol, for example, gives only the L ester the optical rotation of which is identical to that of an authentic sample.

$$\underset{\text{D,L}}{\text{Boc-Asp-OH}} \xrightarrow[C_6H_5CH_2OH]{\text{papain}} \underset{\text{L}}{\text{Boc-Asp-OCH}_2C_6H_5} + \underset{\text{D}}{\text{Boc-Asp-OH}}$$

We checked that the D-amino acid is not esterified at all.
Acylase-catalyzed hydrolysis of the N-acyl group of a broad range of amino acid substrates has been used for the resolution of amino acids[8]. Also, resolutions of racemic aromatic acids are possible by stereospecific chymotryptic hydrolysis of L-amino acid esters[8]. The stereospecific esterification of N-Boc-amino acids by papain could be an other method of resolution.

Poor yields of esterification were found with the sterically hindered valine, isoleucine, threonine and we have not been able to find good conditions for the esterification of the basic amino acids lysine, arginine, histidine. However side chain protected Boc-Lys-OH (or Arg) have been reported to be esterified by papain in homogeneous media[9].

As in the esterification of N-acetyl-tyrosine by chymotrypsin[6] the reactions with papain were run in a concentrated citrate-phosphate buffer (1 M) at pH 4.2. The organic solvent (methylene chloride) effectively extracts the ester formed. Unlike what is claimed in the litterature[10] we have not been able to synthesize the esters in reasonable yields by using aqueous ethanol alone.

The papain-catalyzed synthesis of esters of N-Boc-amino acids in a biphasic system depends on the ratio β of the aqueous and organic phase volumes ($\beta = V_{org}/V_{aq}$). For each amino acid this ratio was optimized to obtain the best yield of esterification.

For the hydrophilic aspartic and glutamic acids a high proportion of organic solvent is necessary ($\beta = 17$).

For the hydrophobic N-Boc-phenylalanine, leucine, tryptophane, methionine a small amount of methylene chloride is needed ($\beta = 0.3$). These N-Boc-amino acids are soluble in the organic solvent; this might explain the difference observed in the conditions used for the esterification of N-benzoyl-phenylalanine by chymotrypsin where a high ratio β is more suitable[7].

For other amino acids like glycine, alanine, serine, tyrosine, variations in the value of β from 17 to 0.3 does not alter dramatically the yield of esterification. However the best conditions were found for a small ratio ($\beta = 1.6$). With N-Boc-tyrosine for example, a 63% yield is observed with $\beta = 17$ and a 90% yield with $\beta = 1.6$.

The esterification by papain is quite interesting in the case of aspartic and glutamic acids since the chemical methods always give the diesters. Furthermore for these two amino acids the amount of papain used in a standard procedure can be reduced by half without altering the yield of esterification. This is not true for alanine or phenylalanine, for example, where a lower yield of ester is observed when the amount of papain is reduced.

The esterification of N-Boc-amino acids by papain under biphasic conditions can be compared with the formation of N-Boc-amino acid phenylhydrazides catalyzed by the same enzyme in water alone[11] (in this case precipitation of the product shifts the equilibrium towards synthesis). In general higher yields are observed for esterification. Glycine, serine, aspartic acid, asparagine phenylhydrazides, for example, are obtained in a somewhat low yield (48%, 55%, 34%, 33% respectively) whereas esterification of the same amino acids works well (82%, 75%, 82%, 80% yield respectively).

Our results show that papain is able to catalyze the esterification of a broad range of N-Boc-amino acids under suitable biphasic conditons. The esterification is stereospecific and could be used in the resolution of amino acids.

The fact that papain works well on N-t-butyloxycarbonyl (N-Boc) protected amino acids is an other advantage since this group can be removed easily. Also, esterification with benzylalcohol

Table 2. Physical properties of N-Boc-amino acid esters.

	X-OR	mp °C	$[\alpha_D]$,deg[a]	Yield[d] %	calc/found %C	%H	%N
1	Boc-Leu-OC_2H_5	-	- 37	75	60.20 60.62	9.72 9.81	5.40 5.13
2	Boc-Phe-OC_2H_5	-	- 5.7	76	65.50 65.39	7.90 7.88	4.77 4.67
3	Boc-Trp-OC_2H_5	154	- 9.0	78	65.04 64.78	7.27 7.23	8.42 8.41
4	Boc-Met-OC_2H_5	46	- 35.8	76	51.96 52.12	8.35 8.25	5.05 5.10
5	Boc-Cys(Bzl)-OC_2H_5	50	- 43.8	74	60.15 60.35	7.42 7.38	4.12 4.08
6	Boc-Val-OC_2H_5	-	- 21.6	27	58.79 58.57	9.38 9.32	5.71 5.81
7	Boc-Ile-OC_2H_5	-	- 15.6	22	60.20 60.28	9.71 9.68	5.40 5.42
8	Boc-Gly-OC_2H_5	-	-	82	53.18 53.57	8.43 8.47	6.89 6.87
9	Boc-Ala-OC_2H_5	-	- 42.5	88	55.28 55.34	8.81 8.94	6.45 6.35
10	Boc-Tyr-OC_2H_5	90	+ 2	90	62.12 62.37	7.49 7.58	4.53 4.42
11	Boc-Ser-OC_2H_5	60	- 21.7	75	51.49 51.42	8.21 8.18	6.00 5.95
12	Boc-Thr-OC_2H_5	51	- 24.5	40	53.42 53.29	8.56 8.53	5.66 5.57
13	Boc-Glu-OC_2H_5	-	- 28.9	90	53.35 52.27	7.68 7.84	5.08 5.02
14	Boc-Glu-$OCH_2C_6H_5$	94	- 29.5[b]	78	60.52 60.58	6.87 6.87	4.15 4.12
15	Boc-Gln-O-C_2H_5	101	- 25.5	85	52.54 52.45	8.08 8.05	10.21 10.41
16	Boc-Asp-OC_2H_5	107	- 21.7	83	50.56 50.56	7.33 7.36	5.36 5.37
17	Boc-Asp-$OCH_2C_6H_5$	97	- 22.5[c]	70	59.43 59.36	6.54 6.42	4.33 4.35
18	Boc-Asn-OC_2H_5	105	- 17.5	82	50.76 50.77	7.74 7.90	10.76 10.27

a) in methanol, c 1 g/100 ml; b) ref[12]; c) ref[13]; d) after purification on silicagel.

could be useful when a deprotection of the ester by hydrogenolysis is needed.

The most interesting examples are aspartic and glutamic acids since only the α carboxyl group is esterified. Advantage of this selective esterification could be taken for the enzymatic synthesis of peptides with serine or thiol proteases.

EXPERIMENTAL

Papain was purchased from Sigma chemical Co as a crude powder with a specific activity of 2.9 units per mg/solid. The powder was used without further purification.

Boc-amino acids as well as Boc-Asp-OBzl and Boc-Glu-OBzl were purchased from Novabiochem. Absolute ethanol was used.

Mass spectra and elemential analysis of N-Boc-amino acid esters prepared enzymatically were in agreement with the theory. Furthermore authentic samples of Boc-amino acid ethyl esters were synthesized by using a standard procedure[14].

Optical rotation were measured on a Perkin-Elmer 241 MC polarimeter.

Papain catalyzed esterifications

The experiments were performed on 0.5 mmole of N-tbutyloxycarbonyl-amino acids. Papain (100 mg) was dissolved in a 1 M citrate-phosphate buffer pH 4.2 and activated with 18 mg of cysteine and 30 μl of a 1 M solution of EDTA (ethylenediamine tetraacetic acid tetrasodium salt, trihydrate). The amino acid was added. The amount of buffer, methylene chloride and alcohol are the following: Boc-Leu-OH, Boc-Phe-OH, Boc-Trp-OH, Boc-Met-OH, Boc-Cys-(Bzl)OH: 3/1/1; Boc-Val-OH, Boc-Ile-OH: 3/0.5/1; Boc-Gly-OH, Boc-Ala-OH, Boc-Tyr-OH, Boc-Ser-OH, Boc-Thr-OH: 3/5/1; Boc-Glu-OH, Boc-Asp-OH: 3/50/1.5; Boc-Gln-OH, Boc-Asn-OH: 1.5/50/1.5.

The reactions were run at 37° for 8 h.

The mixture was extracted with methylene chloride. The organic phase was washed with 20% sodium bicarbonate (except for aspartic and glutamic acids), water and dried over sodium sulfate. The solvent was evaporated and the Boc-amino acid esters were purified by chromatography on silica gel (Merk: 0.040-0.063 mm). The solvents used for elution were methylene chloride for esters 1, 2, 3, 4, 5, 6, 7, 8, 9; CH_2Cl_2/CH_3OH: 100/0.5 for esters 10, 11, 12,; $CH_2Cl_2/CH_3OH/ACOH$: 100/1/0.2 to 0.5 for esters 13, 15, 16, 18.

In the case of the benzyl esters 14 and 17 elution was first made with hexane to remove excess of benzylalcohol, then CH_2Cl_2. The products were finally eluted with CH_2Cl_2/ CH_3OH/ACOH: 100/1/0.2.

REFERENCES

[1] H.D. Jakubke, P. Kuhl, A. Könnecke, Angew Chem. Int. Edit. **24**, 85 (1985).
[2] J.S. Fruton, Adv. Enzymol. Relat. Areas Mol. Biol. **53**, 239 (1982).
[3] a) L. Velluz, Bull. Soc. Chim. Biol., **16**, 909 (1934);
b) G. Langrand, J. Baratti, G. Buono, C. Triantaphylides, Tetrahedron Lett., **27**, 29, (1986);
c) G. Kirchner, M. Scollar, A. Klibanov, J. Am. Chem. Soc., **107**, 7072 (1985).
[4] B. Cambou, A.M. Klibanov, J. Am. Chem. Soc., **106**, 2687 (1984).
[5] a) A.M. Klibanov, G.P. Samokhin, K. Martinek, I.V. Berezin, Biotechnol. Bioeng., **19**, 1351 (1977).
b) H. Kise, H. Shirato, Tetrahedron Lett., **26**, 6081 (1985).
[6] a) D. Tarquis, P. Monsan, G. Durand, Bull. Soc. Chim. Fr, II-76 (1980)
b) J.-L. Vidaluc,M. Baboulene, V. Speziale, A. Lattes, P. Monsan, Tetrahedron, **39**, 269 (1983).
[7] K. Martinek, A.N. Semenov, I.V. Berezin, Biochim. Biophys. Acta, **658**, 76 (1981).
[8] J.B. Jones, C.J. Sih, D. Perlman: Applications of Biochemical Systems in Organic Chemistry: Techniques of chemistry vol X, chapter IV; J. Wiley 1976.
[9] L. Pinheiro da Silva, J. Dovicchi, J. MUradian, W. Seidel, M. Tominaga, Braz, J. Med. Biol. Res., **14**, 373 (1981).
[10] I. Matsumoto, Jap. Patent 58 152 856(1983) Chem. Abstr. 100 86 122 j (1984).
[11] a) V. Cerovsky, K. Jost, Collect. Czech. Chem. Commun., **49**, 2557 (1984);
b) W. Kullman, J. Biol. Chem., **255**, 8234 (1980).
[12] E. Schröder, E. Klieger, Justus Liebigs Annln. Chem., **673**, 196 (1964).
[13] J. Halstrom, O. Schou, K. Kovacs, K. Brunfeldt, Hoppe-Seyler's, Z. Physiol. Chem., **351**, 1576 (1970).
[14] O. Yonemitsu, T. Hamada, Y. Kanaoka, Tetrahedron Lett., 1819 (1969).