Tetrahedron Letters No. 22, pp 2261 - 2264, 1972. Pergamon Press. Printed in Great Britain.

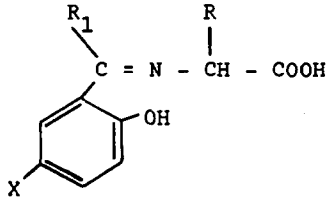
THE USE OF KETIMINE DERIVATIVES OF AMINO ACIDS IN PEPTIDE SYNTHESIS

A. Hope and B. Halpern

Department of Chemistry, Wollongong University College, Wollongong, N.S.W., 2500, Australia.

(Received in UK 18 April 1972; accepted for publication 27 April 1972)

In order to synthesize complex peptides, it is necessary to find protecting groups which intermittently protect α-amino functions and which can be removed selectively in the presence of other blocked trifunctional amino acid residues. Combinations of Cbz, t-Boc and Nps protecting groups*, which have graded acid labilities, are most commonly employed, but the removal of the Nps group by hydrogen chloride in aprotic solvents can lead to partial fission of t-Boc groups[1,2] and side reactions with Try and Cys[3]. We report that the ketimine condensates (I) of o-hydroxy substituted aromatic ketones with amino acids can be utilized in peptide synthesis and the protecting group removed under conditions which leave the t-Boc group and acid sensitive amino acids intact.



R_1 = Ph or Alkyl

X = Cl or Me

(I)

The ketimines (Table I)are stable, optically pure, yellow solids which can be condensed with amino acid esters by the DCC method to form sterically pure

* Abbreviations used are those recommended by IUPAC-IUB (J. Biol. Chem., 241, 2491 (1966).

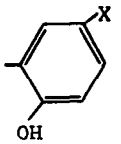
N-protected peptide derivatives in good yield. In a typical experiment, a mixture of L-Try (613 mg, 3 mmole), tetramethylammonium hydroxide (3 mmole, 7.5 ml of 0.4 mmole/ml methanol), 5-chloro-2-hydroxybenzophenone (699 mg, 3 mmole) and a few sticks of molecular sieve (3A) was left at 25°C for one day. After removal of the solvent (< 30°C), the residue was triturated with water (40 ml), filtered to remove unreacted ketone and acidified to pH 4 with citric acid. Extraction and crystallisation from ethyl acetate-ether (1:1) yielded 978 mg (72%) N-(α-phenyl-5-chloro-2-hydroxybenzylidene)-L-tryptophane, m.p. 160-2°C, $(\alpha)_D^{22}$ -364° (c, 0.5, MeOH), found: C, 68.52; H, 4.63; N, 7.13; $C_{24}H_{19}N_2O_3Cl$ requires: C, 68.82; H, 4.57; N, 6.69 %. The steric purity (< 1% D) of the derivative was confirmed by hydrolysis with 80% acetic acid at 80°C, followed by g.l.c. analysis of the recovered Try, as the N-TFA-Try-(+) sec butyl ester[4]. The L-Try ketimine (419 mg, 1 mmole) was coupled with L-Try-OEt (233 mg, 1 mmole) using DCC (206 mg, 1 mmole) in dichloromethane (5 ml). After evaporation of the solvent followed by column chromatography (silica, benzene-chloroform) N-(α-phenyl-5-chloro-2-hydroxybenzylidene) L-Try-L-TryOEt (594 mg, 91% yield) was obtained; m.p. 82-3°C, $(\alpha)_D^{22}$ -172° (c, 0.5, MeOH); found: C, 70.09; H, 5.45; N, 8.59; $C_{37}H_{33}N_4O_4Cl$ requires: C, 70.19; H, 5.25; N, 8.85 %. In a similar fashion a large number of protected di- and tri-peptides derived from the amino acid condensates in Table I have been prepared and characterized. The protecting group can be removed by 80% acetic acid at 80°C in 20 min. or at 25°C in 10 hrs. Under the same conditions it takes > 110 days to cleave the t-Boc group from t-Boc-Gly-OEt[5]. The arylidene function is also smoothly hydrolysed by alcoholic hydrochloric acid solution (1N, 25°) in 15 min or p-toluenesulphonic acid monohydrate in ether (1 equivalent, 25°C) in 4 hrs. Finally we have shown that peptide bond formation and subsequent cleavage of the protecting group proceed without racemization, by synthesizing sterically pure cyclo-L-Try-L-Ala and cyclo-L-Leu-L-Leu (t.l.c: $CHCl_3$ - MeOH - CH_3COOH (14:2:1))[6] and TFA-L-Val-L-ValOMe (g.l.c., 1% XE 60 on Chrom W)[7] from the corresponding crude ketimine peptide ester reaction mixtures.

References

1. K. Poduška, Coll. Czech. Chem. Comm., 31, 2955 (1966).
2. W. Kessler and B. Iselin, Helv. Chim. Acta, 49, 130 (1966).
3. J.C. Anderson, A.M. Barton, P.M. Hardy, G.W. Kenner, J.K. MacLeod, J. Preston, R.C. Sheppard and J.S. Morley, Acta Chim. Acad. Sci. Hung., 44, 187 (1965).
4. G. Pollock, V. Oyama and R. Johnson, J. Gas Chrom., 3, 174 (1965).
5. P. Sieber and B. Iselin, Helv. Chim. Acta, 51, 622 (1968).
6. J.W. Westley, V.A. Close, D.E. Nitecki and B. Halpern, Anal. Chem., 40, 1888 (1968).
7. F. Weygand, A. Prox, L. Schmidhammer and W. Konig, Angew. Chem. (Int. Ed.), 2, 183 (1963).

TABLE I

KETIMINE DERIVATIVES OF AMINO ACIDS[a]

L-Amino Acids[b]	Ph - CO  X =	% Yield	m.p. (°C)	$[\alpha]_D^{22}$ (c, 0.5, methanol)
Gly	Cl	55	156	-
Ala	Cl	71	182	-24
Val	Cl	53	146	-52
Ser	Cl	62	152	-32
Thr	Cl	58	93	-40
Try	Cl	72	161	-364
O-Bz-Tyr	Cl	47	74	-174
Im-Bz-His	Cl	73	132	- 84
Leu	Me	55	128	- 88
Ile	Me	52	136	- 68
Phe	Me	75	79	-240
S-Bz-Cys	Me	42	59	-136

(a) All compounds gave satisfactory elemental analysis.

(b) The condensates of Met, ε-Cbz-Lys, γ-Bz-Glu, γ-Bz-Asp, t-Boc-Orn and ω-NO_2-Arg are oily or amorphous materials, which have been used without further purification in peptide synthesis.