The Use of the $N(\pi)$-Phenacyl Group for the Protection of the Histidine Side Chain in Peptide Synthesis [1]

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$N(\alpha)$-Benzyloxycarbonyl-$N(\tau)$-phenacyl-L-histidine (5) and $N(\alpha)$-benzyloxycarbonyl-$N(\pi)$-phenacyl-L-histidine (6) have been prepared. Under carboxy-activating conditions which cause gross racemisation of (5), (6) yields optically pure peptide. $N(\alpha)$-t-Butoxycarbonyl-$N(\pi)$-phenacyl-L-histidine (7) has also been prepared and the practicability of the $N(\pi)$-phenacyl protective group (which is unchanged by strong aqueous or anhydrous acids and essentially unchanged by aqueous alkali, but which is quantitatively cleaved on treatment with zinc–acetic acid or on photolysis) has been demonstrated in a synthesis of thyroliberin.

SERIOUS problems [2] including racemisation [3] attend peptide synthesis with unprotected histidine side chains, but in recent years many workers have nevertheless adopted this tactic as the lesser evil because of the manifold deficiencies of the protective groups which have been available to them. Thus forcing conditions are required for removal of N(im)-benzyl groups and furthermore the N(im)-benzyl derivatives so far described are liable to racemise on carboxy-activation.[4] So far as racemisation is concerned, N(im)-tolylsulphonyl and N(im)-dinitrophenyl appear to be safer [5,6] (although the literature on the subject is somewhat contradictory [7]) but both are susceptible to facile nucleophilic displacement and in our hands the consequent difficulties are often prohibitive. In fact, we find all acyl-, sulphonyl- and dinitrophenyl-type groups (with the possible exception of the piperidinocarbonyl group [8]) to be so labile to nucleophiles as to be of very limited value. Great chemical ingenuity [9] has been applied to the design of groups which do not have this propensity but which can still be easily removed, but this ingenuity has to some extent been self-defeating, resulting in complex chemistry and, in our experience, derivatives which are difficult to prepare and crystallise so that the problems created by these groups outweigh those they solve.

It is clear that the desiderata of a new N(im)-protecting group are (a) simplicity, (b) ease of preparation of the appropriate derivatives, (c) indifference to the presence of nucleophiles, (d) susceptibility to removal under mild conditions at the desired juncture, and (e) prevention of side reactions including racemisation. We have screened a wide range of 1-substituted imidazoles in a search for potential N(im)-protecting groups which might possess these characteristics.[10]

The properties of 1-phenacylimidazole (1), which are summarised in the Table, were promising. The carbonyl group is surprisingly unreactive towards primary amino-groups and N(im)-phenacylhistidine residues would not be expected to engage in side-reactions with amino-components during coupling. The reduction of the group on catalytic hydrogenation and the reactivity towards hydrazine would be a tactical limitation on the use of N(im)-phenacyl protection, but our criteria (a)—(e) seemed to be satisfied by the other properties of (1), and we were encouraged to investigate the possibility further.

The question of racemisation-inhibition, however, remained. Veber had suggested [3] that racemisation at histidine residues is associated with the basic and/or nucleophilic properties of $N(\pi)$ so that blockade of this position might be advantageous. In all the early work on N(im)-protection the location of the protecting group was unknown, and in most cases was not investigated. In only one case, that of N(im)-dinitrophenyl, has the position been proved: [11] chemical degradation involving opening of the heterocyclic ring established it as $N(\tau)$. In the N(im)-tolylsulphonyl and N(im)-acyl derivatives which have been described, steric considerations and Rapoport's n.m.r.[12] criterion all indicate that the single isomers which are obtained are $N(\tau)$-blocked.

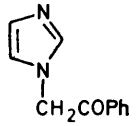
At the outset, therefore, syntheses of histidine derivatives with the proposed new protecting group at each of the two im-nitrogens were undertaken in order that comparative studies could be carried out. Treatment of $N(\alpha)$-benzyloxycarbonylhistidine methyl ester (2) (ob-

Experiments on the stability of 1-phenacylimidazole

Conditions	Result
CF_3CO_2H–20°–1 h	No reaction [a]
45% w/v HBr in AcOH–20°–1 h	No reaction [a]
6M-HCl–110°–18 h	No reaction [a]
1 equiv. 1M-NaOH–MeOH–20°–1 h	Slight discoloration but no significant reaction [a,b]
1 equiv. $PhCH_2NH_2$–DMSO–20°–7 d	No reaction [c]
NH_3–MeOH (sat.)–20°–2 d	No reaction [a]
10 equiv. N_2H_4–MeOH–20°–30 h	(1) consumed [d]
1 equiv. DCCI–DMF	No reaction [b]
5% Pd(C)–H_2–80% aq. ACOH–atmospheric pressure–1 h	CO group reduced giving 1-(1-imidazolyl)-2-phenylethanol in essentially quantitative yield
Na–NH_3–THF at 30°–0.5 h	(1) consumed [d]
Zn–aq. AcOH–20°–10 min	Quantitative conversion to imidazole and acetophenone
Medium-pressure Hg lamp–Pyrex vessel–EtOH–reflux–6 h	(1) consumed giving imidazole as the only heterocyclic product
Electrolysis–Hg cathode–1.3 V–0.5M–H_2SO_4–20°–3 h	(1) consumed giving imidazole as the major heterocyclic product but some 1-(1-imidazolyl)-2-phenylethanol also produced

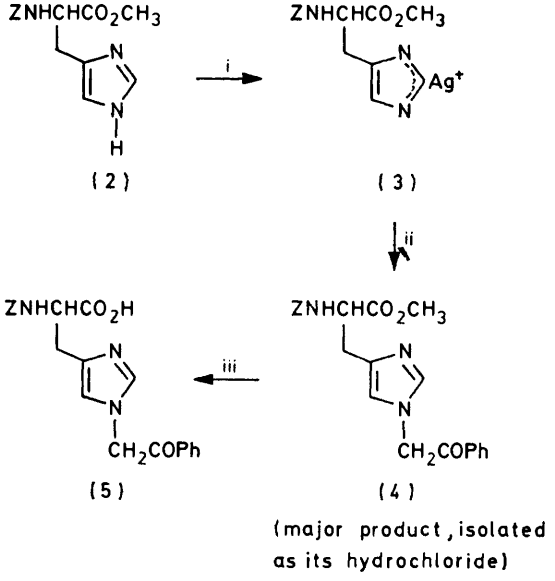
[a] 1-Phenacylimidazole recovered in 80—90% yield after neutralisation. [b] T.l.c. [c] N.m.r. [d] Complex mixture produced.

tained in crystalline form for the first time) with phenacyl bromide gave a complex mixture, as might have been expected from experience [13] with the $N(\alpha)$-benzoyl derivative, but treatment of the corresponding silver salt (3) with phenacyl bromide in dimethyl sulphoxide was



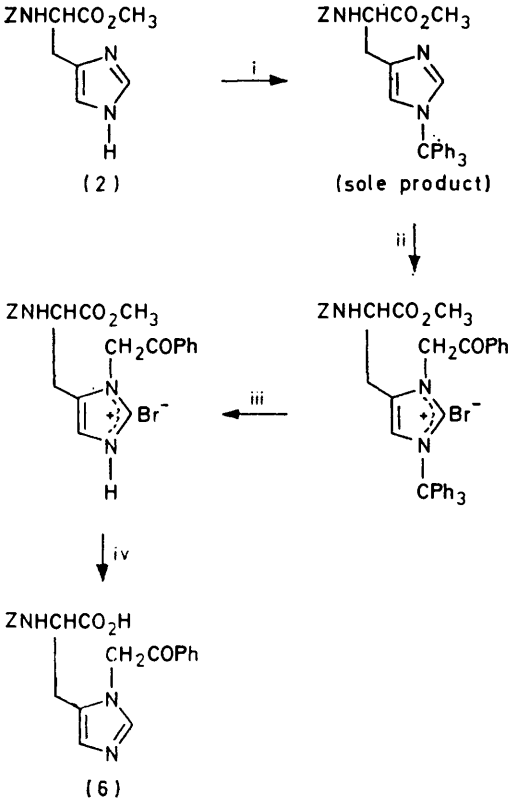
(1)

cleaner and afforded a mixture from which the major product (4) could be isolated easily as its hydrochloride which gave the acid (5) on saponification as outlined in Scheme 1. The isomeric acid (6) was obtained by more circuitous means as shown in Scheme 2. The strategy of selective $N(\tau)$-blockade by triphenylmethylation, highly selective for steric reasons, followed by $N(\pi)$-alkylation and finally removal of the $N(\tau)$-triphenylmethyl group is novel and apparently rather general: we have employed it for several related sequences with similar success. It is analogous in principle to that devised by Olofson and Kendall [14] and others [15] for position-controlled alkylation of 4(5)-substituted imidazoles (acylation, which is selective for the least hindered nitrogen, followed by alkylation and deacylation) but has the advantage that the alkylation step in our approach is performed under mild conditions whereas that in the published procedures requires rather vigorous conditions because the acylated intermediate is deactivated. The positions of the im-protecting group in the isomers (5) and (6) follow from the methods of synthesis but were also corroborated by Rapoport's cross-ring coupling constant criterion.[12]



SCHEME 1 Reagents: i, $AgNO_3$; ii, $PhCOCH_2Br$–Me_2SO; iii, aq. NaOH

Comparison of the racemisation-susceptibility of the isomers (5) and (6) under coupling conditions which were designed to exacerbate the danger produced a very clear-cut result (Scheme 3), confirming Veber's suggestion. It is obvious that a free $N(\pi)$-nitrogen is a very undesirable feature although it should be noted that our experiment provides no basis for deciding whether the free $N(\pi)$-



SCHEME 2 Reagents: i, Ph_3CCl–Et_3N; ii, $PhCOCH_2Br$–Et_2O–20°–3 days; iii, aq. AcOH–100°–10 min; iv, aq. NaOH

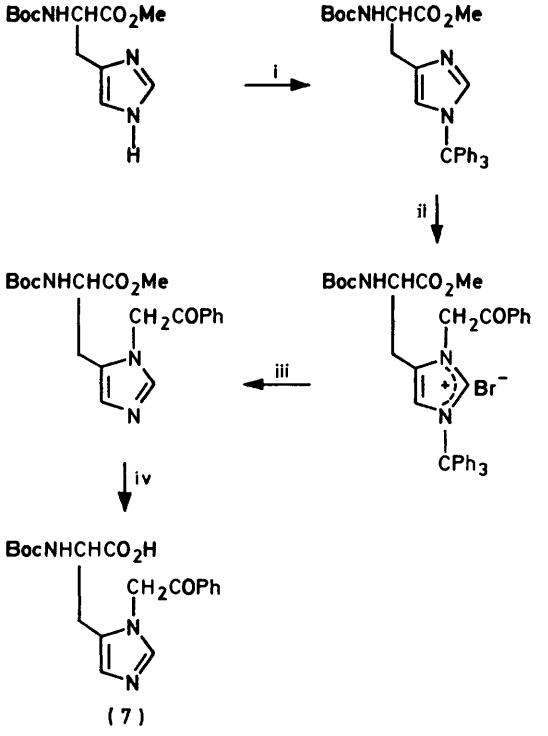
Z-His(τ-CH_2COPh)OH ⟶ Z-His(τ-CH_2COPh)ProNH_2 containing His which was 35% D

Z-His(π-CH_2COPh)OH ⟶ Z-His(π-CH_2COPh)ProNH_2 containing His which was <2% D

SCHEME 3 Conditions: 1 equiv. acid + 1 equiv. DCCI in DMF (20 ml $mmol^{-1}$) at 0 ° for 1 h; then equiv. ProNH_2,HCl + 1 equiv. Et_3N added; mixture left at 0 ° for 2 h then at 20° for 16 h

nitrogen leads to racemisation by simple intramolecular base action or by intramolecular nucleophilic attack to give an optically labile heterocyclic acylating agent. This question is the subject of work still in progress.

Satisfied by these preliminary investigations that the $N(\pi)$-phenacyl protection of histidine is in principle a suitable method, we have embarked upon an investigation of its practicability. The t-butoxycarbonyl derivative (7) has also been prepared (Scheme 4) and used in the simple synthesis of thyroliberin shown in Scheme 5: (6) has been used in an analogous synthesis. The intermediates proved to have convenient properties and the operations involved were uncomplicated by any noteworthy problems except that removal of last traces

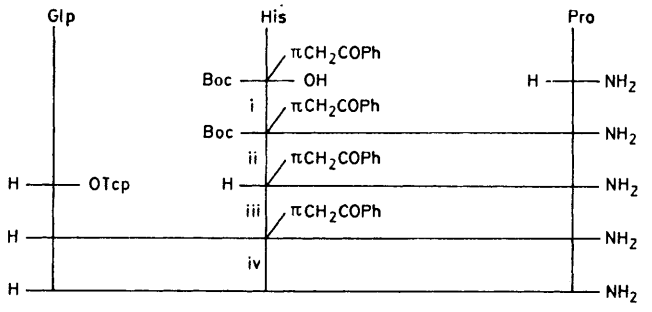


SCHEME 4 Reagents: i, Ph_3CCl–Et_3N; $PhCOCH_2Br$–Et_2O–20°–2 days; iii, AgOAc–aq. AcOH–20°–2 days; iv, aq. NaOH

of zinc after zinc–acetic acid final deprotection was a little troublesome at first. We were however able to find chromatographic conditions which dealt with this difficulty and in any case the alternative final deprotection by photolysis gave excellent results. Thyroliberin in a high state of purity and which was indistinguishable from authentic material was obtained after deprotection by either method.

The fact that im-phenacylhistidine derivatives have substantial u.v. extinction coefficients and also develop intensely and characteristically with Pauly's reagent facilitated the monitoring of analytical and preparative chromatography.

It would be premature to offer $N(\pi)$ phenacyl protection as the definitive solution to all the problems associated with histidine in peptide synthesis, but our experience with it so far is very encouraging.



SCHEME 5 Reagents: i, DCCI–HOBt–DMF; ii, CF_3CO_2H; iii, DMF; iv, Zn–aq. AcOH or *hν*

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. N.m.r. spectra were recorded with a Perkin-Elmer R32 spectrometer operating at 90 MHz with tetramethylsilane as internal standard. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter in a 1 dm cell. Phenacylimidazole was prepared by the method of Jones and Hysert.[13] All the compounds for which elemental analyses are reported were obtained in a chromatographically homogeneous state and had i.r. and n.m.r. spectra [10] consistent with their formulation as single isomers of the structures stated.

N(α)-*Benzyloxycarbonyl-*L*-histidine Methyl Ester* (2).—This was prepared by the method of Holly and Sondheimer [16] as modified by Seltzman [17] on a 100 mmolar scale, by treatment of L-histidine methyl ester dihydrochloride with benzyl chloroformate and triethylamine in chloroform, and was obtained initially as a pale yellow non-flowing oil (22.5 g, 75%). The oil was triturated with boiling ether (25 ml) and the triturant was decanted and allowed to evaporate slowly (2 days), giving a crystalline solid (2 mg) of m.p. 105—107°. The bulk of the oil was then crystallised from dichloromethane–light petroleum, using some of the crystalline material as seeds to give $N(\alpha)$-benzyloxycarbonyl-L-histidine methyl ester (19.5 g, 65%); m.p. 103—106° (lit.,[16] oil; lit.,[17] oil); $[\alpha]_D^{20}$ −16.3° (*c* 1.1 in MeOH) (Found: C, 59.2; H, 5.6; N, 13.6. Calc. for $C_{15}H_{17}N_3O_4$: C, 59.3; H, 5.7; N, 13.9%).

N(α)-*Benzyloxycarbonyl-*L*-histidine Methyl Ester Silver Salt* (3).—This was prepared by the method of Seltzman [17] on a 50-mmolar scale, by treatment of $N(\alpha)$-benzyloxycarbonyl-L-histidine methyl ester with ammoniacal silver nitrate in aqueous methanol, and was obtained as a microcrystalline *solid* which discoloured on standing in sunlight (19.5 g, 91%), m.p. 134—137° (decomp.) (Found: C, 43.6; H, 4.2; N, 10.0. Calc. for $C_{15}H_{16}AgN_3O_4$: C, 44.0; H, 3.4; N, 10.2%).

N(α)-*Benzyloxycarbonyl*-N(τ)-*phenacyl-*L*-histidine Methyl Ester* (4).—The preceding silver salt (4.1 g, 10 mmol) was dissolved in dry, distilled dimethyl sulphoxide (50 ml) and phenacyl bromide (2.0 g, 10 mmol) dissolved in chloroform (10 ml) was added dropwise over 40 min to the well stirred solution in the dark at room temperature. After 1 h, the mixture was diluted with chloroform (10 ml) and was filtered through a Celite pad. The filtrate, which was deep orange, was then washed with water (5 × 100 ml), dried, and saturated with hydrogen chloride gas. The solution became much lighter in colour and on the addition of ether (100 ml), a yellow solid (3.2 g), m.p. 185—205°, precipitated. This material was separated and reprecipitated twice from methanol–ether to give *methyl ester hydrochloride* (2.1 g, (46%), m.p. 205—210° (decomp.); $[\alpha]_D^{20}$ −20.4° (*c* 1 in MeOH) (Found: C, 60.4; H, 5.1, N, 9.0. $C_{23}H_{24}ClN_3O_5$ requires C, 60.3; H, 5.3; N, 9.2%). The free base was obtained by suspending the hydrochloride salt (1.1 g) in chloroform (40 ml) and shaking with saturated sodium hydrogencarbonate solution (40 ml) until all the solid had dissolved. The organic layer was then washed with water (20 ml), dried, and light petroleum was added to turbidity. *Methyl ester* crystallised readily as needles (0.9 g, 89%), m.p. 67—70°;

$[\alpha]_D^{20}$ +20.8 (*c* 1 in $CHCl_3$) (Found: C, 63.0; H, 5.9; N, 9.5. $C_{23}H_{23}N_3O_5 \cdot H_2O$ requires C, 62.9; H, 5.7; N, 9.6%).

N(α)-*Benzyloxycarbonyl*-N(τ)-*phenacyl*-L-*histidine* (5).—The preceding methyl ester hydrochloride (5.5 g, 12 mmol) was dissolved in methanol (25 ml) and 1.0M-sodium hydroxide solution (25 ml, 25 mmol) was added slowly with vigorous stirring at room temperature. The solution immediately turned orange. After 40 min, saponification appeared to be complete (t.l.c.). Methanol was evaporated and the residue was diluted with water (100 ml). The solution was washed with chloroform (2 × 50 ml) and the aqueous phase was acidified to pH 4 with 1.0M-hydrochloric acid. The pale yellow precipitate which formed was separated and recrystallised from methanol–water to give *protected acid* as a monohydrate (4.4 g, 86%), m.p. 119—123°; $[\alpha]_D^{20}$ +10.2° (*c* 1 in MeOH); δ [$(CD_3)_2SO$] 2.89br (2H, d, *J* 6 Hz, $CHCH_2$), 4.26 (1 H, m, $NHCHCH_2$), 5.01 (2 H, s, $OCH_2C_6H_5$), 5.65 (2 H, d, CH_2CO), 6.87br (1 H, s, H-5 of imidazole), 7.32 (5 H, s, C_6H_5 of benzyl), 7.35—7.75 (5 H, complex, H-2 of imidazole H-3, -4 and -5 and phenacyl, NHCH), and 8.01 (2 H, dd, *J* 8 and 2 Hz, H-2 and -6 of phenacyl) (Found: C, 62.2; H, 5.3; N, 9.7. $C_{22}H_{21}N_3O_5H_2O$ requires C, 62.1; H, 5.45; N, 9.9%). On irradiation of the signal at δ 2.89, the band at δ 6.87 simplified to a doublet enabling the cross-ring coupling constant of 1.3 Hz to be measured, corroborating [12] the substitution pattern of the imidazole ring. Deprotection and digestion with L-amino-acid oxidase [18] showed the acid to be >98% optically pure.

N(α)-*Benzyloxycarbonyl*-N(τ)-*triphenylmethyl*-L-*histidine Methyl Ester*.—Triphenylmethyl chloride (2.78 g, 10 mmol) and triethylamine (1.4 ml, 10 mmol) were dissolved in dry benzene (20 ml) and *N*(α)-benzyloxycarbonyl-L-histidine methyl ester (3.03 g, 10 mmol) was added. The mixture was heated at reflux temperature for 1 h and the precipitated triethylammonium chloride was removed by filtration. The filtrate was washed with water (20 ml), dried, and the solvent was evaporated, leaving a yellow oil which did not solidify even after trituration with light petroleum. The residue was dissolved in ether (10 ml) and the solvent was evaporated quickly under high vacuum, leaving the N(τ)-*triphenylmethyl derivative* as a crisp foam (4.5 g, 82%), m.p. 58—63°; $[\alpha]_D^{20}$ +12.8° (*c* 1 in $CHCl_3$) (Found: C, 74.7; H, 5.8; N, 7.6. $C_{34}H_{31}N_3O_4$ requires C, 74.85; H, 5.7; N, 7.7%).

The procedure used by Hartter [19] for the corresponding t-butoxycarbonyl derivative gave in this case material which was contaminated with triphenylmethanol, but which was, however, adequate for the next stage.

N(α)-*Benzyloxycarbonyl*-N(τ)-*triphenylmethyl*-N(π)-*phenacyl*-L-*histidine Methyl Ester Imidazolium Bromide*.—Phenacyl bromide (3 g, 15 mmol) and the preceding *N*(τ)-triphenylmethyl derivative (8.2 g, 15 mmol) were dissolved in ether (50 ml) and stirred at room temperature for 3 days. The solvent was decanted from the yellow, oily precipitate which had formed and this was triturated with ether (4 × 100 ml) until it solidified (7 g). The washings and liquor were combined and concentrated to 20 ml and left at room temperature overnight. More product precipitated and this was solidified as before (3.5 g). The two crops were combined, but all attempts at recrystallisation failed and so this material was thoroughly dried to give the *imidazolium bromide* (10.4 g, 93%), m.p. 111—116°; $[\alpha]_D^{20}$ −3.2° (*c* 1 in $CHCl_3$); δ ($CDCl_3$) 3.21 (2 H, complex, $CHCH_2$), 3.62 (3 H, s, OCH_3), 4.59 (1 H, m, $NHCHCH_2$), 4.93 (2 H, s, $OCH_2C_6H_5$), 6.4—6.8 (3 H, complex, NHCH and CH_2CO), 6.92 (1 H, s, H-5 of imidazole), 7.1—7.6 [23 H, complex, C_6H_5 of benzyl, $C(C_6H_5)_3$, H-3, -4 and -5 of phenacyl], 8.09 (2 H, dd, *J* 8 and 2 Hz, H-2 and -6 of phenacyl), and 8.96 (1 H, s, H-2 of imidazole) (Found: C, 67.4; H, 5.2; Br, 10.5; N, 5.8 $C_{42}H_{38}BrN_3O_5$ requires C, 67.7; H, 5.1; Br, 10.7; N, 5.6%). This material was not hygroscopic and was stable at room temperature for long periods.

N(α)-*Benzyloxycarbonyl*-N(π)-*phenacyl*-L-*histidine Methyl Ester Hydrobromide*.—The preceding imidazolium bromide (8 g, 10.7 mmol) was dissolved in 50% acetic acid (50 ml) and heated to 100° for 10 min. On cooling, a crystalline precipitate of triphenylmethanol formed which was removed by filtration. The filtrate was evaporated under high vacuum to give a viscous oil which was dissolved in chloroform (30 ml) and this solution was added dropwise to a vigorously stirred, large volume of ether (500 ml). The powdery precipitate which formed was separated by filtration and washed with ether (250 ml). The filter cake was, however, never dried completely because the material was still exceedingly hygroscopic. The ether-wet solid was dissolved in chloroform (100 ml) and crystallisation was initiated by addition of a few drops of ether. The crystals, which were not hygroscopic, were removed by filtration; a second crop was obtained by the addition of more ether to the mother-liquor. This second crop was still hygroscopic and so was crystallised from chloroform–ether to give more stable material. The two non-hygroscopic crops were then combined and recrystallised from chloroform alone to give *methyl ester hydrobromide* (4.5 g, 84%), m.p. 123—127°; $[\alpha]_D^{20}$ −12.5° (*c* 1 in MeOH) (Found: C, 54.7; H, 4.9; Br, 15.3; N, 8.15. $C_{23}H_{24}BrN_3O_5$ requires C, 55.0; H, 4.8; Br, 15.9; N, 8.4%).

N(α)-*Benzyloxycarbonyl*-N(π)-*phenacyl*-L-*histidine* (6).—The preceding methyl ester hydrobromide (4.0 g, 8 mmol) was dissolved in methanol (30 ml) and 1.0M-sodium hydroxide solution (17 ml, 17 mmol) was added. After stirring for 1 h at room temperature, the mixture was diluted with water (75 ml) and methanol was evaporated. 1M-Hydrochloric acid was added to adjust the pH to 3.8 and the aqueous layer was extracted with ethyl acetate (2 × 10 ml). This resulted in the removal of all the coloured impurities from the aqueous layer and caused only a small loss of product. The aqueous layer was then partially evaporated under vacuum to remove the last traces of organic solvents and sodium chloride (solid) was added in portions. On storage at 0° for 24 h, *protected acid* (6) crystallised out and was separated by filtration and thoroughly dried. This material was chromatographically homogeneous and was therefore used without further purification (2.6 g, 81%), m.p. 205—208° (decomp.); $[\alpha]_D^{20}$ +10.9° (*c* 0.5 in pyridine); $[\alpha]_D^{20}$ +2.8° (*c* 0.6 in MeOH); δ [$(CD_3)_2SO$] 2.86 (2 H, complex, $CHCH_2$), 4.21 (1 H, m, $NHCHCH_2$), 4.99 (2 H, s, $OCH_2C_6H_5$), 5.73 (2 H, s, CH_2CO), 6.81 (1 H, s, H-4 of imidazole), 7.32 (5 H, s, C_6H_5 of benzyl), 7.4—7.8 (5 H, complex, NHCH, H-2 of imidazole, H-3, -4, and -5 of phenacyl), and 7.97 (2 H, dd, *J* 8 and 2 Hz, H-2 and -6 of phenacyl). On irradiating the band at δ 2.86, the signal at δ 6.81 remained a singlet at room temperature. However, at 140°, the singlet was resolved into a broad doublet enabling the cross-ring coupling constant of 0.9 Hz, to be measured, corroborating [12] the substitution pattern of the imidazole ring. The entire spectrum was recorded again on cooling to check that no decomposition had occurred at the higher temperature (Found: C, 64.8; H, 5.5; N, 10.2. $C_{22}H_{21}N_3O_5$ requires C, 64.85; H, 5.2; N, 10.3%). Deprotection and digestion

with L-amino-acid oxidase [18] showed the acid to be >98% optically pure.

Comparison of the Optical Lability of N(α)-*Benzyloxycarbonyl*-N(τ)-*phenacyl*-L-*histidine and* N(α)-*Benzyloxycarbonyl*-N(π)-*phenacyl*-L-*histidine*.—The protected histidine derivative (5) or (6) (0.1 mmol) was dissolved in freshly distilled dimethylformamide (1.0 ml) and the solution was cooled to −5°. *NN'*-Dicyclohexylcarbodi-imide (21 mg, 0.1 mmol) as a precooled solution in dimethylformamide (1.0 ml) was added and the mixture was stirred at 0° for 1 h, after which L-prolineamide hydrochloride (15 mg, 0.1 mmol) and triethylamine (14 μl, 0.1 mmol) were added. After a further 2 h at 0° the cooling bath was removed and the mixture was left at room temperature for 16 h, and then filtered. The filtrate was evaporated and the residue was dissolved in chloroform (2 ml). The solution was filtered and applied to a silica p.l.c. plate (200 × 200 × 1 mm) which was eluted consecutively with 5, 7, and 10% methanol in chloroform. This gave three bands well separated from each other consisting of *N*-acylurea (near the solvent front), dipeptide (the middle band), and other components (at lowest R_F). The band containing dipeptide was scraped off, allowing a wide margin on either side to ensure that no fractionation of diastereoisomers occurred, and the silica was extracted with methanol. [In the *N*(τ)-phenacyl series each of the LL and DL diastereoisomeric peptide derivatives has been isolated and fully characterised,[10] and it was possible to prove that they are not separated from each other under the conditions used in this experiment.] The optical purity of the histidine residue in the dipeptide material thus isolated was determined with L-amino-acid oxidase [18] after deprotection and hydrolysis: the histidine in the dipeptide obtained from the *N*(τ)-phenacyl acid (5) was found to be 35 ± 2% D whereas that from the *N*(π)-phenacyl acid (6) was <2% D, both results being corrected for racemisation of the histidine during hydrolysis of the peptide.

N(α)-*t*-*Butoxycarbonyl*-N(τ)-*triphenylmethyl*-L-*histidine Methyl Ester*.—This was prepared by the method of Hartter [19] and obtained as a foam contaminated with triphenylmethanol (as in the published procedure) which was used in the following preparation without further purification.

N(α)-*t*-*Butoxycarbonyl*-N(τ)-*triphenylmethyl*-N(π)-*phenacyl*-L-*histidine Methyl Ester Imidazolium Bromide*.—The preceding derivative (6.00 g, 12 mmol) was dissolved in ether (10 ml), and phenacyl bromide 2.39 g, 12 mmol) was added to the stirred solution. After 48 h at room temperature, the precipitate was separated and washed with ether (4 × 20 ml) to yield the *imidazolium bromide* (8.10 g, 95%), m.p. 177—179°; $[\alpha]_D^{20}$ −9.2° (*c* 1.0 in MeOH) (Found: C, 65.8; H, 5.7; Br, 11.3; N, 5.9. $C_{39}H_{40}BrN_3O_5$ requires C, 65.9; H, 5.7; Br, 11.3; N, 5.9%).

N(α)-*t*-*Butoxycarbonyl*-N(π)-*phenacyl*-L-*histidine Methyl Ester*.—The bromide salt (7.10 g, 10 mmol) was dissolved in 80% acetic acid (20 ml), and silver acetate (1.67 g, 10 mmol) was added to the stirred solution. The precipitated silver bromide was then filtered off, and the solution was left to stir at room temperature. After 48 h the solvent was removed and saturated sodium hydrogencarbonate (100 ml) was added to the residue. The mixture was then extracted with ether (5 × 50 ml), the organic extract was dried briefly and then allowed to stand at room temperature for 48 h, when the *methyl ester* crystallised from the solution (3.25 g, 84%), m.p. 139—141°; $[\alpha]_D^{20}$ −9.3° (*c* 1.0 in MeOH) (Found: C, 62.2; H, 6.4; N, 10.8. $C_{20}H_{25}N_3O_5$ requires C, 62.0; H, 6.5; N, 10.9%).

N(α)-*t*-*Butoxycarbonyl*-N(π)-*phenacyl*-L-*histidine* (7).—The methyl ester (3.00 g, 7.8 mmol) was dissolved in methanol (3 ml), 1.0M-sodium hydroxide (7.8 ml) was added, and the mixture was left to stir at room temperature. After 1 h water was added (25 ml), methanol was evaporated, and the pH of the solution was adjusted to 3.8 with 1.0M-hydrochloric acid. Sodium chloride was added to saturate the aqueous phase which was then extracted exhaustively with chloroform (6 × 50 ml). Drying, evaporation, and trituration with ether gave a yellow solid, which was reprecipitated from methanol–ether to give the *protected acid* as a hemihydrate (2.57 g, 85%), m.p. 181—183°; $[\alpha]_D^{20}$ +9.4° (*c* 1.0 in MeOH) (Found: C, 59.5; H, 6.2; N, 10.8. $C_{19}H_{23}N_3O_5 \cdot 0.5\ H_2O$ requires C, 59.7; H, 6.3; N, 11.0%). Deprotection and digestion with L-amino-acid oxidase [18] showed the optical purity of the acid to be >98%.

N(π)-*Phenacylthyroliberin*.—*Route A*. *N*(α)-t-Butoxycarbonyl-*N*(π)-phenacyl-L-histidine hemihydrate (50 mg, 0.13 mmol) was dissolved in dimethylformamide (2 ml) and the solution was cooled to 0°. To this were added L-prolineamide hydrochloride (20 mg, 0.13 mmol), triethylamine (18 μl, 0.13 mmol), 1-hydroxybenzotriazole (20 mg, 0.13 mmol), and *NN'*-dicyclohexylcarbodi-imide (27 mg, 0.13 mmol), in that order, and the mixture was allowed to attain room temperature. After 15 h the precipitated *NN'*-dicyclohexylurea was filtered off, the solvent was evaporated, and the residue was dissolved in chloroform (10 ml). The organic phase was washed with saturated sodium hydrogencarbonate (2 × 10 ml), dried, and evaporated to yield an oil which was dissolved in 20% methanol–chloroform (1 ml) and was applied to a size B Merck pre-packed silica gel 60 column. The column was eluted with 20% methanol–chloroform: pooling of the appropriate fractions and evaporation gave the *t-butoxycarbonyldipeptide* (49 mg, 80%), m.p. 229—232°; $[\alpha]_D^{20}$ −30.1° (*c* 1.0 in MeOH) (Found: C, 59.2; H, 6.5; N, 14.3. $C_{24}H_{30}N_5O_5 \cdot H_2O$ requires C, 59.2; H, 6.2; N, 14.4%).

The protected dipeptide (50 mg, 0.11 mmol) was dissolved in trifluoroacetic acid (5 ml) at room temperature. After 0.5 h trifluoroacetic acid was evaporated and the residue was triturated with ether. The crude *N*(α)-deprotected dipeptide bistrifluoroacetate salt was dissolved in dimethylformamide (2 ml), and L-pyroglutamic acid trichlorophenyl ester [20] (36 mg, 0.11 mmol) and triethylamine (31 μl, 0.22 mmol) were added. The mixture was left at room temperature for 16 h, the solvent was evaporated, and the residue was triturated with ether. The resulting solid was dissolved in chloroform–methanol–40% acetic acid (3 : 2 : 1) (1 ml) and applied to a size B Merck prepacked silica gel 60 column. Elution with the same solvent mixture and pooling of the appropriate fractions followed by evaporation gave crude protected tripeptide which was dissolved in 0.2M-acetic acid (2 ml) and applied to a Sephadex G 10 column (2.5 × 100 cm). The column was eluted with 0.2M-acetic acid: pooling of the appropriate fractions and evaporation gave N(π)-*phenacylthyroliberin* (40 mg, 75%), m.p. 139—146°; $[\alpha]_D^{20}$ −44.8° (*c* 1.0 in MeOH) (Found: C, 53,6; H, 6.0; N, 15.4. $C_{24}H_{28}N_6O_5 \cdot 3H_2O$ requires C, 53.9; H, 6.4; N, 15.7%).

Route B. *N*(α)-Benzyloxycarbonyl-*N*(π)-phenacyl-L-histidine (122 mg, 0.3 mmol), 1-hydroxybenzotriazole (62 mg, 0.4 mmol), and L-prolineamide hydrochloride (60 mg, 0.4 mmol) were dissolved in dimethylformamide (3 ml).

The solution was cooled to −5° and *NN'*-dicyclohexylcarbodi-imide (82 mg, 0.4 mmol) as a precooled solution in dimethylformamide (1 ml) containing triethylamine (55 μl, 0.4 mmol) were added. After 1 h at 0°, the mixture was allowed to attain room temperature and was stirred for a further 16 h. A few drops of 50% acetic acid were added to the mixture to destroy the excess of di-imide. After a further 1 h, the urea was removed by filtration and the filtrate was evaporated, leaving an oily residue which was dissolved in chloroform (40 ml). The organic phase was washed with saturated sodium hydrogencarbonate solution (20 ml) and the aqueous phase was re-extracted with more chloroform (50 ml). The combined organic layers were dried and evaporated to give crude protected dipeptide as an oil. Chromatographic purification as at the dipeptide stage of Route A and reprecipitation from chloroform–light petroleum gave the *benzyloxycarbonyldipeptide*, m.p. 120—127°; $[\alpha]_D^{20}$ −57.6° (*c* 1.0 in $CHCl_3$) (Found: C, 62.7; H, 5.9; N, 13.3. $C_{27}H_{29}N_5O_5 \cdot H_2O$ requires C, 62.2; H, 6.0; N, 13.4%). The crude protected dipeptide was dissolved in hydrogen bromide in acetic acid (2 ml, 45% w/v). After 1 h ether (400 ml) was added and the pale orange solid which was formed was separated by decantation and then dissolved in the minimum volume of dimethylformamide (*ca.* 2 ml), together with L-pyroglutamic acid trichlorophenyl ester [20] (124 mg, 0.4 mmol). The solution was cooled to 0° and sufficient triethylamine (125 μl, 0.9 mmol) was added to bring the ' pH ' of the vapour above the mixture to 8 (moist indicator paper). The mixture was allowed to reach room temperature and after 16 h, the solvent was evaporated, leaving an oily residue which solidified readily on trituration with ether (2 × 50 ml). Chromatographic purification by the methods described above for Route A gave analytically and chromatographically pure $N(\pi)$-phenacylthyroliberin (96 mg, 62% overall) which was identical in all respects to that obtained by Route A.

Thyroliberin.—(*A*) *Deprotection with zinc–acetic acid.* $N(\pi)$-Phenacylthyroliberin (40 mg, 0.083 mmol) was dissolved in 50% aqueous acetic acid (3 ml) and zinc dust (100 mg, 1.5 mmol) was added in portions with vigorous shaking over 5 min. The excess of zinc was removed by filtration and the filtrate was evaporated to give an oily residue which was dissolved in water (5 ml). This solution was saturated with hydrogen sulphide gas and the precipitated zinc sulphide was removed by filtration through a Millipore membrane. The filtrate was evaporated to dryness and the residue was dissolved in 0.01M-trimethylammonium acetate buffer (pH 5.0; 2 ml). Ethylenediaminetetracetic acid disodium salt solution (0.27M; pH 5.0; 0.5 ml) was added and the solution was applied to a column (0.9 × 30 cm) of Whatman CM-32 cation exchange resin which was eluted with two column volumes of 0.01M-trimethylammonium acetate to remove the sodium acetate and then with an ionic strength gradient of the same buffer of 0.01—0.5M. The hormone emerged at an ionic strength of *ca.* 0.1M. The fractions which contained Pauly positive material were combined and evaporated. More water was added and evaporated to assist removal of the buffer salts, and the residue was then dissolved in water and lyophilised, giving, after high-vacuum drying over potassium hydroxide (pellets) and concentrated sulphuric acid, chromatographically pure thyroliberin (36 mg, 88%) which was indistinguishable from authentic material; amino acid analysis Glu 0.98; His 0.95; Pro 1.07 (Found: C, 43.8; H, 6.1; N, 15.8. Calc. for $C_{16}H_{22}N_6O_4 \cdot 1.5AcOH \cdot 4H_2O$: C, 43.5; H, 6.9; N, 16.0%). Measurement of the specific rotation using concentration values corrected for the water present in the product gave a value $[\alpha]_D^{20}$ −68° (*c* 0.4 in 1M-AcOH) for thyroliberin sesquiacetate: the correct value for the optical rotation has been the subject of careful investigation by Syrier and Beyerman [21] who reported $[\alpha]_D^{20}$ −68° (c *ca.* 1 in 1M-AcOH) for thyroliberin sesquiacetate of proven chemical and optical purity.

(*B*) *Deprotection by photolysis.* $N(\pi)$-Phenacylthyroliberin (50 mg, 0.104 mmol) was dissolved in ethanol (10 ml) and the solution was irradiated under reflux for 6 h in a Pyrex vessel using a Hanovia medium pressure mercury lamp. Removal of the ethanol and trituration with ether (3 × 5 ml) gave essentially pure thyroliberin which was purified by gel chromatography on a column (150 ml) of Sephadex G 10 eluted with 0.2M-acetic acid at a rate of 50 ml h^{-1}, fractions being collected every 6 min. Pauly positive material was present in fractions 22—27. Pooling of fractions 23—26 and lyophilisation gave chromatographically pure thyroliberin (33 mg, 75%) which was indistinguishable from authentic material and that obtained by the alternative deprotection procedure A (Found: C, 47.3; H, 6.5; N, 18.1%. Calc. for $C_{16}H_{22}N_6O_4 \cdot AcOH \cdot 2H_2O$: C, 47.2; H, 6.6; N, 18.3%). Determination of the specific rotation using concentration values corrected for the water present in the product and the deficiency of acetic acid gave a value of $[\alpha]_D^{20}$ −67° (*c* 0.35 in 1M-AcOH) for thyroliberin sesquiacetate.

We are grateful to Dr. M. Szelke for helpful discussions and information about his unpublished investigation of im-phenacyl protection.

[8/1983 *Received*, 14*th November*, 1978]

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