The Differentiation of π- and τ-Derivatised Histidines

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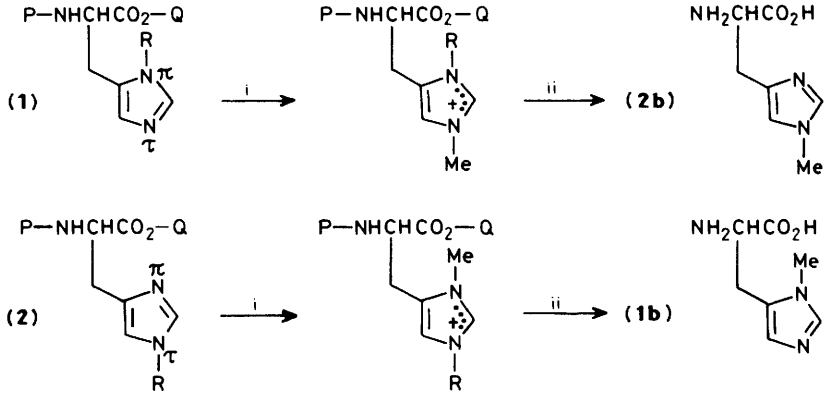
Two simple methods of differentiating π- and τ-derivatised histidines unambiguously are described. The first involves conversion into one of the two known *im*-methyl-L-histidines, which, to avoid all possible confusion arising from ambiguous nomenclature and previous work, have been correlated with *N*(α)-t-butoxycarbonyl-*N*(π)-benzyloxymethyl-L-histidine, whose structure has been established by *X*-ray crystallography. The second method, which is appropriate for *im*-substituents of type RCH_2–, involves the measurement of nuclear Overhauser effects. If the substituent is at the π-position, the CH_2 signal is enhanced if the low-field adjacent proton between the heterocyclic nitrogens is irradiated, but not if the high-field, more distant, ring proton is irradiated. If the substituent is at the τ-position, the CH_2 signal is enhanced whichever of the equidistant ring protons is irradiated.

The differentiation of π- and τ-derivatised histidine residues, (**1**) and (**2**), is a long standing problem [1] which has been much confused by the currency in biochemical and chemical circles of completely contrary conventions for the numerical designation of the heterocyclic nitrogens,[2] and by arbitrary structural representations which are in fact erroneous.[3] Various experimental approaches to the question can be found in the literature. I.r. spectroscopy [4] and mass spectrometry [5] have been used, but tenuous correlations are involved and general criteria based on these techniques cannot be defined. Chemical degradation [6] has provided a clear structural assignment in a few examples, but can only be achieved in a specific manner in special cases. An empirical rule based on the coupling constant between the protons on the imidazole ring has been proposed by Matthews and Rapoport.[1] According to this rule, compounds of the π-series should exhibit a cross-ring coupling constant between the two aromatic protons of the imidazole in the range 0.9—1.0 Hz whereas those with τ-orientation should give a value of 1.1—1.4 Hz. This seems a slender distinction, although we have yet to encounter an exception to the rule. Unfortunately, owing to overlapping of the signals in the aromatic region and/or line broadening, it is not always possible to measure the coupling constant with the necessary precision, even with advanced instrumentation. In practice the commonest way of making π–τ assignments has been simply to assume that in the reaction of histidine side-chains with equimolecular amounts of electrophilic reagents the main product will be that arising from attack at the least hindered, *i.e.* τ, position.[7] In our experience this is a valid assumption. Acylating agents, 2,4-dinitrophenyl fluoride, toluene-*p*-sulphonyl chloride, and very hindered alkylating agents generally give a greatly predominating product which has the τ-orientation in all the cases we have investigated. Alkylating agents which do not have overwhelming steric demands, however, give mixtures which may contain up to 30—40% of the π-substituted isomer. If an excess of alkylating agent is employed, under some circumstances the mixture of monoalkyl isomers and disubstituted product can contain comparable amounts of each of the monoalkyl isomers. In the extreme case of the unhindered highly reactive methylating agent methyl methanesulphonate, it has been reported that the second-order rate constant for first reaction at the π-nitrogen of *N*(α)-acetylhistidine methylamide is significantly greater than that for first reaction at the τ-nitrogen, and the generalisation that τ-substituted products are invariably predominant when histidine side-chains react with electrophiles has been contradicted.[8] The situation is further complicated by the fact that the yields of isolated products are often mediocre: the mere fact that one product can easily be isolated in a pure state does not necessarily allow the conclusion that it is the major component and thus the τ-derivative.



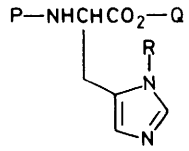
Scheme. The differentiation of π- and τ-derivatised histidines by conversion into *im*-methylhistidines. Conditions: i, methylation; ii, deprotection and hydrolysis. Compounds (**2b**) and (**1b**) are distinguished by amino acid analysis

Table 1. The differentiation of π- and τ-derivatised histidines by conversion into *im*-methylhistidines

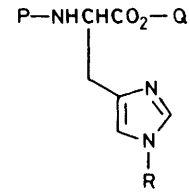
Structure assigned	Proportions of (**1b**) and (**2b**) produced on methylation and deprotection and/or hydrolysis	
	(**1b**) (%)	(**2b**) (%)
ZHis(πBum)OMe[a] (**1c**)	3	97
ZHis(τBum)OMe (**2d**)	100	0
ZHis(πMem)OMe (**1d**)	0	100
ZHis(τMem)OMe (**2e**)	100	0
ZHis(πSem)OMe (**1e**)	<1	>99
ZHis(τSem)OMe (**2f**)	>99	<1

[a] This experiment also confirms the orientation of the corresponding carboxylic acid which is derived from compound (**1c**): the acid is the key intermediate in the use[12] of π-t-butoxymethyl protection for histidine side-chains.

	P	Q	R
(1) **a**;	Boc	H	Bom
b;	H	H	Me
c;	Z	Me	Bum
d;	Z	Me	Mem
e;	Z	Me	Sem
f;	Z	Me	Phen
g;	Z	Me	Tom



(2) **a**;	Boc	Me	Boc
b;	H	H	Me
c;	Z	Me	Trt
d;	Z	Me	Bum
e;	Z	Me	Mem
f;	Z	Me	Sem
g;	Z	Me	Phen
h;	Boc	H	Bzl
i;	Z	Me	Ppc
j;	Z	Me	Tos



The following abbreviations are used for protecting groups: Z, $PhCH_2OCO$–; Boc, Me_3COCO–; Bom, $PhCH_2OCH_2$–; Bum, Me_3COCH_2–; Tos, 4-$MeC_6H_4SO_2$–; Ppc, piperidinocarbonyl; Trt, Ph_3C–; Mem, $MeOCH_2CH_2OCH_2$–; Sem, $Me_3SiCH_2CH_2OCH_2$–; Phen, phenacyl; Tom, 2,4,6-trimethylbenzyloxymethyl.

For many years the response to the problem in the field of peptide synthesis was to ignore it. Various imidazole-protecting groups were employed without regard to their location. We have shown, however, that location at the π-position is a fundamental *desideratum* for histidine side-chain protection, particularly with regard to the suppression of racemisation.[9] We have therefore sought to disentangle some of the confusions over the question and to establish a more secure and general basis for π–τ assignments not based on previous work, which we now report.

Our absolute reference point is the structure of *N*(α)-t-butoxycarbonyl-*N*(π)-benzyloxymethyl-L-histidine (**1a**), which rests on *X*-ray crystallography.[10] This also establishes the structure (**2a**) of the bis-t-butoxycarbonyl-L-histidine methyl ester[11] from which it can be prepared in good yield. Treatment of compound (**1a**) with excess of methyl iodide followed by hydrolysis and amino acid analysis gave the commercially available amino acid described as '3-methyl-L-histidine' which is thus confirmed as *N*(τ)-methyl-L-histidine (**2b**). It follows that the commercially available isomer '1-methyl-L-histidine' is *N*(π)-methyl-L-histidine (**1b**). Treatment of *N*(α)-benzyloxycarbonyl-N(*im*)-triphenylmethyl-L-histidine methyl ester with excess of methyl iodide followed by hydrolysis gave (**1b**) predominantly, confirming the structure as (**2c**). Compounds (**2a**) and (**2c**) are thus subsidiary reference structures which are clearly established: preparation from them gives a firm basis for proposing a structure, although it involves the assumption that no premature loss of *im*-protection has taken place *en route*. In our experience so far this does not happen but it seems desirable to obtain confirmation independently where possible. This can be done by conversion into (**1b**) or (**2b**) in cases where the existing imidazole substituent is cleavable after methylation of the unencumbered nitrogen atom. The isomer pairs (**1c**) and (**2d**), (**1d**) and (**2e**), and (**1e**) and (**2f**), for example, which had been prepared as part of a programme involving the investigation and development of novel protecting groups for histidine side-chains, were readily distinguished by this means (Table 1).

The availability of a fair number of compounds of firmly established structure enabled the demonstration of another criterion, namely that in π-substituted structures of type (**1**) in which R = $R'CH_2$, a nuclear Overhauser enhancement (n.O.e.) of the signal from the substituent methylene group is observed on irradiation of the proton between the two heterocyclic nitrogens (which is generally well downfield and easily identified) but not on irradiation of the other more distant proton; an n.O.e. of the methylene signal is observed in the τ-substituted isomer on irradiation of either ring proton as they are equidistant. A typical example is shown in the Figure; data for five pairs of isomers are summarised in Table 2.

We now outline the application of these principles to some cases of interest. A sample of *N*(α)-t-butoxycarbonyl-*N*(*im*)-benzyl-L-histidine of commercial origin proved to be a pure single isomer: the n.O.e. criterion indicated it to have the τ-benzyl structure (**2h**), consistent with the known tendency of this intermediate to lead to gross racemisation on coupling. Secondly, we had in hand a specimen of *N*(α)-benzyloxycarbonyl-*N*(*im*)-piperidinocarbonyl-L-histidine methyl ester of indeterminate structure. This specimen, which, remarkably, had been kept, with no detectable deterioration, without special precautions, in an ordinary cupboard for a decade, had been isolated in poor yield after treatment of *N*(α)-benzyloxycarbonyl-L-histidine methyl ester with piperidine-1-carbonyl chloride. It was a single pure isomer by various criteria and had a sharp m.p., but its m.p. differed substantially from that previously reported[13] for a compound prepared in this way. There was therefore doubt as to the location of the piperidinocarbonyl group: this was resolved by methylation and hydrolysis which gave only (**1b**), thus confirming the structure of our compound to be (**2i**). Our third case is *N*(α)-benzyloxycarbonyl-*N*(*im*)-(2,4,6-trimethylbenzyloxy)methyl-L-histidine methyl ester, which was prepared in the course of development of an acid-labile side-chain-protecting group for histidine. It was prepared by alkylation of the triphenylmethyl derivative (**2c**) and we therefore hoped that it would be π-substituted but this could not be safely assumed as impure unstable (2,4,6-trimethylbenzyloxy)methyl chloride had been used. Hydrogen chloride, which might have caused premature loss of the triphenylmethyl group, had almost certainly been present. In the event both the n.O.e. and methylation criteria established the structure (**1g**) unambiguously.

Finally, to extend the range of *im*-substituents of proven location, we examined the *im*-tolylsulphonyl derivative of *N*(α)-benzyloxycarbonyl-L-histidine methyl ester. Only one of the two possible isomers can be detected in this instance, and the cross-ring coupling constant of 1.26 Hz was consistent with τ-orientation, but further proof seemed desirable as the examples on which the Matthews and Rapoport rule[1] rests do not include any sulphonyl derivatives. Because of the nature of

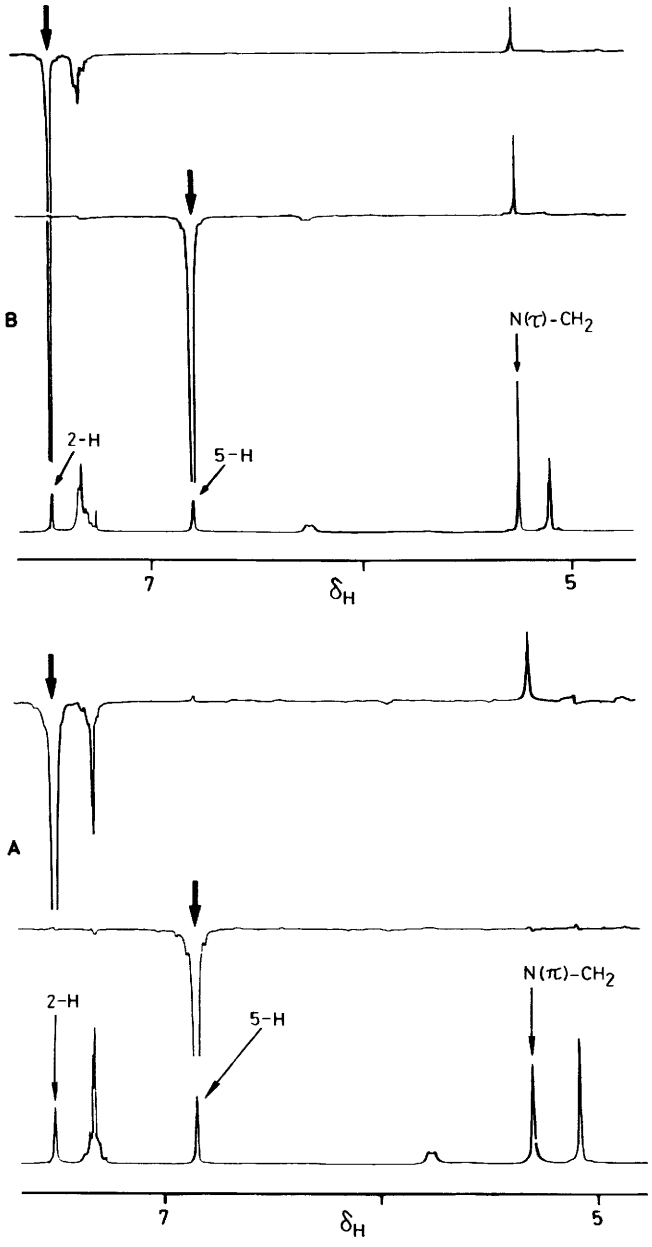


Figure. An example of the use of nuclear Overhauser effects for the differentiation of π- and τ-derivatised histidines. Effects shown by (A) the π-substituted structure (**1d**) and (B) the τ-substituted isomer (**2e**)

the *im*-substituent the n.O.e. approach was not applicable and methylation of the deactivated system with methyl iodide proved impossible.

Even trimethyloxonium fluoroborate followed by hydrolysis gave a poor conversion into *N*(π)-methylhistidine, but not a trace of the isomeric amino acid was produced; these results thus established the structure (**2j**).

Experimental

M.p.s were determined with a Kofler hot-stage apparatus. Mass spectra were determined on a V.G. Micromass ZAB 1F mass spectrometer. N.m.r. spectra were recorded with a Bruker WH 300 spectrometer operating at 300 Mz. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter in a 1 dm cell. All the compounds discussed in this paper were obtained in a chromatographically homogeneous state and had full n.m.r. spectra consistent with their formulation as single isomers of the structures stated.

Proofs of Structure by Conversion into im-*Methylhistidines.*—Using the short column only of a Jeol JLC 5AH amino-acid analyser, conditions were optimised (45 °C; pH 4.28; 0.35M-sodium citrate buffer) for the separation of '1-methyl-L-histidine,' '3-methyl-L-histidine,' and L-histidine and their determination when mutually admixed. The histidine derivative whose orientation was under investigation (*ca.* 10 mg) was dissolved in methyl iodide (5 ml). In cases of insufficient solubility, enough dimethylformamide (DMF) to dissolve the derivative was first added. After 24 h at room temperature removal of methyl iodide and DMF (if any) gave a residue which was heated with 'constant boiling' hydrochloric acid under reflux for 24 h. If methoxyethoxymethyl groups were present, *i.e.* with (**1d**) and (**2e**), the residue was gently warmed for 5 h with anhydrous zinc bromide (5 mol equiv.) in methylene dichloride (10 ml) before the hydrochloric acid treatment. The hydrochloric acid treatment alone sufficed to remove all blocking groups in the other cases. The hydrochloric acid was evaporated off and the residue was redissolved to give a solution in 0.01M-hydrochloric acid for amino acid analysis as detailed above.

Nuclear Overhauser Effect Measurements.—Equilibrium n.O.e. measurements were performed by the difference method at 300 MHz. Samples were approximately 50 mM solutions in $CDCl_3$, D_2O, or $(CD_3)_2SO$, and were degassed by passage of a stream of nitrogen or by alternate freezing and pumping. The n.O.e. was generated by pre-irradiation of each of the imidazole protons for 5 s in separate experiments; in order to achieve sufficient selectivity between these and the nearby phenyl resonances it proved necessary to use r.f. field strengths of 5—15 Hz. Under these conditions saturation was not complete, but was sufficient to give readily detectable effects. Acquisition of the perturbed and unperturbed (with irradiation off-resonance) signals was interleaved during the course of the accumulation. Percentage n.O.e.s are reported relative to the intensity of the same line in the unperturbed spectrum.

N(α)-*t-Butoxycarbonyl-N*(π)-*benzyloxymethyl*-L-*histidine Hydrate* (**1a**)-H_2O.—This was the material used for *X*-ray crystallography.[10] Methylation followed by hydrolysis as described above gave only material identical with Sigma '3-methyl-L-histidine.'

τ-*Methyl*-L-*histidine* (**2b**).—This was obtained from Sigma as '3-methyl-L-histidine,' lot number 88C-0040.

π-*Methyl*-L-*histidine* (**1b**).—This was obtained from Sigma as '1-methyl-L-histidine,' lot number 79C-0355.

Compounds (**1c**), (**2c** *and* **d**).—These compounds were prepared in connection with the development of π-t-butoxymethyl protection for histidine side-chains. A preliminary communication has appeared[12] and full details will be submitted for publication shortly.

N(α)-*Benzyloxycarbonyl-N*(π)-(*2-methoxyethoxy*)*methyl*-L-*histidine Methyl Ester* (**1d**).—To a stirred solution of *N*(α)-benzyloxycarbonyl-*N*(τ)-t-butoxycarbonyl-L-histidine methyl ester (prepared by analogy with bis-t-butoxycarbonyl-L-histidine methyl ester[11] and obtained as an oil; 20.17 g, 50 mmol) in methylene dichloride cooled to 0 °C was added dropwise a

Table 2. The differentiation of π- and τ-derivatised histidines by nuclear Overhauser effects[a]

Compound	Solvent	Chemical shifts, δ_H		Enhancements of the N(*im*)–CH_2 signal, %	
		Ring protons	N(*im*)–CH_2	On irradiation of the low-field ring proton	On irradiation of the high-field proton
BocHis(πBom)OH (**1a**)	$CDCl_3$	7.94, 6.97	5.41	0.9	None
HHis(τMe)OH (**2b**)	D_2O	7.38, 6.77	3.47	1.2	3.00
HHis(πMe)OH (**1b**)	D_2O	7.52, 6.75	3.46	0.3	None
ZHis(πBum)OMe[b] (**1c**)	$CDCl_3$	7.48, 6.80	5.22	1.1	None
ZHis(τBum)OMe (**2d**)	$CDCl_3$	7.52, 6.80	5.23	1.9	1.5
ZHis(πMem)OMe (**1d**)	$CDCl_3$	7.51, 6.85	5.28	2.2	None
ZHis(τMem)OMe (**2e**)	$CDCl_3$	7.48, 6.81	5.25	2.0	2.0
ZHis(πSem)OMe (**1e**)	$CDCl_3$	7.48, 6.83	5.19	1.6	None
ZHis(τSem)OMe (**2f**)	$CDCl_3$	7.48, 6.79	5.18	1.4	1.5
ZHis(τPhen)OMe (**2g**)	$CDCl_3$	7.55, 6.69	5.33	Not determined	1.4
ZHis(πPhen)OMe (**1f**)	$CDCl_3$	7.43, 6.88	5.35	Not determined	None

[a] Other nuclear Overhauser effects, which are not reported, eno also observed. [b] This experiment also confirms the orientation of the corresponding carboxylic acid which is derived from compound (**1c**): the acid is the key intermediate in the use[12] of π-t-butoxymethyl protection for histidine side chains.

solution of (2-methoxyethoxy)methyl chloride (5.68 ml, 50 mmol) in CH_2Cl_2 (10 ml). After 1 h the ice-bath was removed and the mixture was stirred at room temperature for 3 h. The solution was diluted with CH_2Cl_2 (600 ml) and extracted with 6% aqueous citric acid (5 × 100 ml). The combined aqueous layers were neutralised with solid $NaHCO_3$ and extracted three times with CH_2Cl_2 (200 ml). The extracts were dried (Na_2SO_4) and evaporated to give crude *N*(α)-benzyloxycarbonyl-*N*(π)-(2-methoxyethoxy)methyl-L-histidine methyl ester (**1d**) as an oil (15.85 g, 81%). Pure material of $[\alpha]_D^{20}$ +5.2° (*c* 1.06 in $CHCl_3$) was obtained by chromatography on a silica gel column with chloroform–methanol (9:1 v/v) as eluant [Found: M^+ and $(M + H)^+$, 391 and 392. Calc. for $C_{19}H_{25}N_3O_6$: *M*, 391].

N(α)-*Benzyloxycarbonyl*-*N*(τ)-(*2-methoxyethoxy*)*methyl*-L-*histidine Methyl Ester* (**2e**).—*N*(α)-Benzyloxycarbonyl-L-histidine methyl ester[9] (3.03 g, 10 mmol) and triethylamine (1.39 ml, 10 mmol) were dissolved in CH_2Cl_2 (15 ml). The solution was cooled in an ice-bath and a solution of (2-methoxyethoxy)-methyl chloride (Fluka, 1.25 ml, 11 mmol) in CH_2Cl_2 (5 ml) was added dropwise. The mixture was stirred for 2 h at room temperature, diluted with CH_2Cl_2 (50 ml), and extracted with 6% aqueous citric acid (5 × 40 ml). The aqueous layers were combined, basified (pH 7.5) with solid Na_2CO_3, and extracted with CH_2Cl_2 (3 × 80 ml). The extracts were dried (Na_2SO_4) and concentrated to give an oil, which was applied to a silica gel column (3 × 70 cm). Elution with chloroform–methanol (9:1 v/v) gave *N*(α)-benzyloxycarbonyl-*N*(τ)-(2-methoxyethoxy)-methyl-L-histidine methyl ester (**2e**) (1.27 g, 32%) as an oil, $[\alpha]_D^{20}$ +19.9° (*c* 1.36 in $CHCl_3$) [Found: M^+ and $(M + H)^+$, 391 and 392. Calc. for $C_{19}H_{25}N_3O_6$: *M*, 391].

N(α)-*Benzyloxycarbonyl*-N(π)-(2-*trimethylsilylethoxy*)-*methyl*-L-*histidine Methyl Ester* (**1e**).—To a stirred solution of *N*(α)-benzyloxycarbonyl-*N*(τ)-t-butoxycarbonyl-L-histidine methyl ester (20.17 g, 50 mmol) in ethyl acetate (70 ml) cooled to 0 °C was added dropwise a solution of 2-(trimethylsilyl)ethoxy-methyl chloride (Fluka 8.85 ml, 50 mmol) in ethyl acetate (10 ml). After 2 h the solution was allowed to attain room temperature and was then stirred for another 2 h. The mixture was diluted with ethyl acetate (600 ml) and extracted with 6% aqueous citric acid (5 × 100 ml). The combined aqueous extracts were neutralised with solid $NaHCO_3$ and extracted four times with CH_2Cl_2 (200 ml). The combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give *N*(α)-benzyloxycarbonyl-*N*(π)-(2-trimethylsilylethoxy)-methyl-L-histidine methyl ester (**1e**) (13.66 g, 63%) as an oil, $[\alpha]_D^{20}$ +14.7° (*c* 1.07 in $CHCl_3$) [Found: M^+ and $(M + H)^+$, 433 and 434. Calc. for $C_{21}H_{31}N_3O_5Si$: *M*, 433].

N[α)-*Benzyloxycarbonyl*-N(τ)-(2-*trimethylsilylethoxy*)-*methyl*-L-*histidine Methyl Ester* (**2f**).—*N*(α)-Benzyloxy-carbonyl-L-histidine methyl ester (3.03 g, 10 mmol) and triethylamine (1.39 ml, 10 mmol) were dissolved in ethyl acetate (10 ml). The solution was cooled in an ice-bath and a solution of 2-(trimethylsilyl)ethoxymethyl chloride (1.95 ml, 11 mmol) in ethyl acetate (6 ml) was added dropwise. The mixture was stirred for 1 h at 0 °C and for 6 h at room temperature, diluted with ethyl acetate (80 ml), and extracted with 6% aqueous citric acid (5 × 50 ml). The aqueous layers were combined, basified (pH 7.5) with solid Na_2CO_3, and extracted with CH_2Cl_2 (3 × 100 ml). The extracts were dried (Na_2SO_4) and the solvent was removed under reduced pressure. The remaining oil was purified by chromatography on a silica gel column (3 × 70 cm) with chloroform–methanol (9:1 v/v) as eluant. *N*(α)-Benzyl-oxycarbonyl-*N*(τ)-(2-trimethylsilylethoxy)methyl-L-histidine methyl ester (**2f**) (1.17 g, 27%) was obtained as an oil, $[\alpha]_D^{20}$ +19.3° (*c* 1.53 in $CHCl_3$) [Found: M^+ and $(M + H)^+$, 433 and 434. Calc. for $C_{21}H_{31}N_3O_5Si$: *M*, 433].

N(α)-*Benzyloxycarbonyl*-*N*(τ)-*phenacyl*-L-*histidine Methyl Ester* (**2g**).—This was as described previously.[9]

N(α)-*Benzyloxycarbonyl*-N(π)-*phenacyl*-L-*histidine Methyl Ester* (**1f**).—This was obtained from the corresponding hydrobromide[9] in the usual way.

The Structure of N(α)-*t-Butoxycarbonyl*-N(τ)-*benzyl*-L-*histidine* (**2h**).—This was obtained from Sigma, lot number 91C-0320. Nuclear Overhauser effects were determined in $CDCl_3$: irradiation of the low-field imidazole ring proton (δ 7.81) gave a 1.7% enhancement of the N–CH_2 signal (δ 5.06), and irradiation of the high-field imidazole ring proton (δ 6.69) gave a 1.2% enhancement.

The Preparation and Structure of N(α)-*Benzyloxycarbonyl*-N(τ)-*piperidinocarbonyl*-L-*histidine Methyl Ester* (**2i**).—Piper-

idine-1-carbonyl chloride (1.33 g, 10 mmol) was added to a solution of triethylamine (1.01 g, 10 mmol) in dry benzene (15 ml) containing $N(\alpha)$-benzyloxycarbonyl-L-histidine methyl ester (3.03 g, 10 mmol). The mixture was heated under reflux for 2 h, cooled, and filtered, and the filtrate was washed successively with water and saturated aqueous sodium hydrogen carbonate, and dried ($MgSO_4$). Removal of the benzene under reduced pressure, trituration of the residue with light petroleum (b.p. 40—60 °C), and recrystallisation from ether gave the *piperidinocarbonyl derivative* (**2i**) as crystals (1.6 g, 39%), m.p. 77—80 °C; $[\alpha]_D^{20}$ −2.3° (*c* 1.04 in MeOH) (Found: C, 60.85; H, 6.3; N, 13.5. $C_{21}H_{26}N_4O_5$ requires C, 61.2; H, 6.4; N, 13.5%) {lit.,[13] m.p. 104 °C; $[\alpha]_D^{20}$ −2.5° (*c* 1 in MeOH)} for a derivative of uninvestigated orientation. Methylation and hydrolysis as described above gave only $N(\pi)$-methylhistidine, thus confirming the orientation shown in structure (**2i**).

The Structure of N(α)-*Benzyloxycarbonyl*-N(π)-(2,4,6-*trimethylbenzyloxy*)*methyl*-L-*histidine* (**1g**).—The preparation of this compound will be described shortly together with other work on the development of acid-labile protecting groups for histidine side-chains. Methylation and hydrolysis as described above gave only $N(\tau)$-methylhistidine. Nuclear Overhauser effects were determined in $(CD_3)_2SO$: irradiation of the low-field imidazole ring proton (δ 7.75) gave a 0.9% enhancement of the N–CH_2 signal (δ 5.40), whereas irradiation of the high-field imidazole ring proton (δ 6.73) gave no detectable effect on the N–CH_2 signal.

The Preparation and Structure of N(α)-*Benzyloxycarbonyl*-N(τ)-(4-*tolylsulphonyl*)-L-*histidine Methyl Ester* (**2j**).—$N(\alpha)$-Benzyloxycarbonyl-L-histidine methyl ester (0.92 g, 3.04 mmol) was dissolved in dry pyridine (7 ml). A solution of toluene-4-sulphonyl chloride (0.58 g, 3.04 mmol) dissolved in dry pyridine (10 ml) was added at 0 °C and the mixture was set aside at room temperature for 24 h. Most of the pyridine was evaporated off and the residue was dissolved in water (20 ml). Extraction with chloroform, and drying ($MgSO_4$) and evaporation of the extract, gave a chromatographically homogenous yellow solid (0.98 g, 70%) which was recrystallised from chloroform–light petroleum (b.p. 40—60 °C) to give N(α)-*benzyloxycarbonyl*-N(τ)-(4-*tolylsulphonyl*)-L-*histidine methyl ester* (**2j**), m.p. 79—80 °C; $[\alpha]_D^{20}$ + 28.0° (*c* 1 in $CHCl_3$) (Found: C, 57.8; H, 5.0; N, 9.2. $C_{22}H_{23}N_3O_6S$ requires C, 58.1; H, 5.1; N, 9.05%). No detectable *im*-methylation took place with methyl iodide under the conditions described above. The ester (10 mg) was treated with trimethyloxonium fluoroborate (200 mg) in dry methylene dichloride (5 ml) for 24 h at room temperature. A substantial amount of $N(\pi)$-methylhistidine, but no $N(\tau)$-methylhistidine, was detected on hydrolysis and amino acid analysis of the reaction mixture as before, thus confirming the orientation shown in structure (**2j**).

Acknowledgements

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