Journal of Peptide Science, Vol. 3, 391–394 (1997)

SHORT COMMUNICATION

The Regiospecific *N*-Derivatization of Histidine Side Chains: Reinvestigation of a Supposed N^{τ} to N^{π} Migration

JOHN H. JONES and VICTORIA L. WALKER

The Dyson Perrins Laboratory, South Parks Road, Oxford, UK

Received 29 March 1997
Accepted 6 May 1997

Abstract: A report claiming that AcHisOMe reacts regiospecifically with 4-fluoronitrobenzene to give AcHis[τPh(NO_2)]OMe, which on treatment with H_2/Pd(C) undergoes a partial τ–π shift to give some AcHis[πPh(NH_2)]OMe, cannot be substantiated. 4(5)-Methylimidazole, a model for AcHisOMe, gives on reaction with 4-fluoronitrobenzene a 4 : 1 mixture of the regioisomers 1-(4-nitrophenyl),4-methylimidazole, corresponding to τ-substitution, and 1-(4-nitrophenyl),5-methylimidazole, corresponding to π-substitution, each of which has been isolated and fully characterized, including proof of orientation. In both cases, treatment with H_2/Pd(C) gives a single product, without any change of orientation in either case. © 1997 European Peptide Society and John Wiley & Sons, Ltd.

J. Pep. Sci. 3: 341–394

No. of Figures: 2. No. of Tables: 0. No. of References: 9

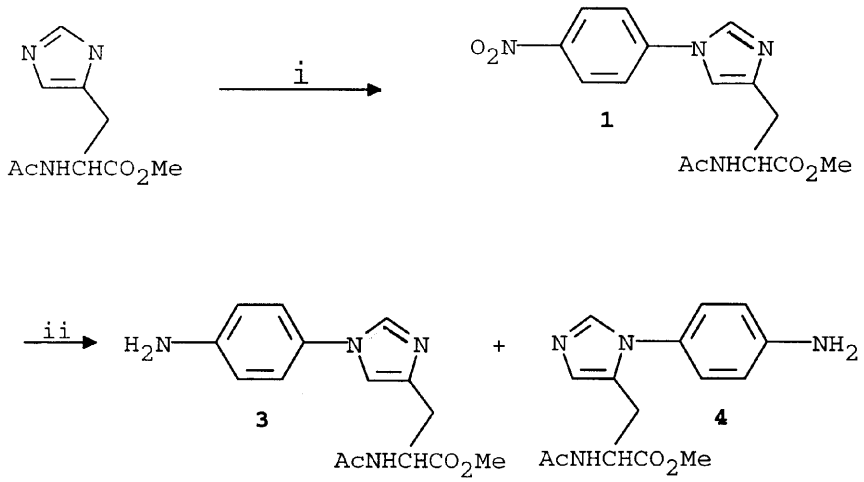
Keywords: histidine side chains; regiospecific *im*-derivatization.

The regiospecific *N*-derivatization of histidine side chains is not easily achieved, and rather circuitous routes must usually be followed [1–7]. N^{τ}-Derivatives are in general more accessible than N^{π}-derivatives, which are of particular interest for side-chain protection in peptide synthesis, a topic which has had our attention for some years. We were therefore very interested in a paper [8] by Bambal and Hanzlik with the sub-title 'Observation of an unusual Pd-catalyzed N^{τ}- to N^{π}-aryl substituent migration', which described the transformations shown in Scheme 1.

Address for correspondence: Dr John Jones, Balliol College, Oxford, OX1 3BJ, UK.

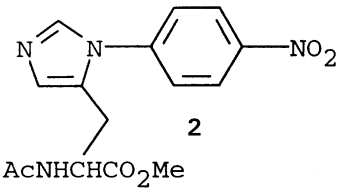
CCC 1075-2617/97/050391–04 $17.50

Of the nitroarylation stage (i), it was said 'As expected ... electrophilic attack ... occurred exclusively at the less-hindered N^{τ} imidazole nitrogen giving rise to a single regioisomer ....' Of the reduction stage (ii), it was said that 'Catalytic hydrogenation ... over Pd/charcoal gave a 3 : 1 mixture of two products ...' which were separated and identified as **3** and **4** respectively. Evidence was presented that 'rearrangement occurred at some intermediate stage of the hydrogenation process', but neither the reaction nor its mechanism were further defined. It seemed possible that this remarkable and completely unprecedented observation could be the basis for the development of novel chemistry for N^{π}-derivatization, and we have therefore reinvestigated reactions (i) and (ii). Pure **1** could not be isolated following the literature protocol. Scrutiny of the



Scheme 1 Conditions: (i) 4-fluoronitrobenzene/K_2CO_3/DMF/110 °C/13 h; (ii) H_2/5% Pd(C)/EtOH/2.5 h/20 °C.

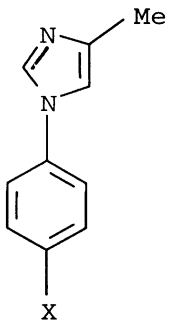
product by NMR revealed it to be a mixture even after repeated reprecipitation, although comparison with the NMR data of Bambal and Hanzlik did show that **1** was the major component. Signals consistent with the presence of traces of the isomer **2** were also



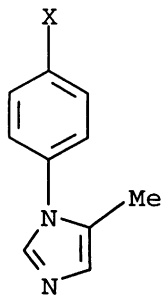
observed. Hydrogenation of this mixture as described by the same authors gave another mixture, from which no crystalline or pure substances could be isolated. Again, comparison with the published NMR data showed that the major component was the N^{τ}-derivative **3** described by Bambal and Hanzlik, but we were not able to detect any of the signals unique to the N^{π}-isomer **4** which were reported by them. In view of the difficulty of interpreting the spectra of our complex mixtures fully, we decided to investigate the two reactions in the simplest possible model system, namely using 4(5)-methylimidazole as the substrate.

Treatment of 4(5)-methylimidazole with 4-fluoronitrobenzene under the conditions used by Bambal and Hanzlik for the derivatization of N^{α}-acetylhistidine methyl ester showed that this reaction is certainly not regiospecific. The two regioisomers **5** and **6**, each of which was isolated in a pure state by fractional crystallization and very thoroughly characterized (including nOe proof of orientation), were formed in a combined yield of 77%, in approximate proportions of 4 : 1 respectively.

In our experience, this is typical of the isomer ratios produced by the generality of electrophiles on reaction with 4(5)-methylimidazole or histidine side chains. The only reagents that do react regiospecifically are those (a) that involve steric hindrance sufficient to impose specificity (e.g. triphenylmethyl chloride [1]); (b) that react in a freely reversible manner (e.g. acetic anhydride [4]), so that thermodynamic control operates; or (c) that bridge N^{α} and N^{π} (e.g. carbonyl diimidazole [5]). Of course the fact that a reaction provides convenient access to a single regioisomer does not necessarily mean that it is regiospecific. The reaction of Boc_2O with histidine side chains, for example, is not regiospecific [4], but a good yield of pure BocHis(τ-Boc)OMe can be isolated easily, nevertheless, after reaction of Boc_2O with HisOMe [2]. Many earlier papers–including one of our own [9]–are misleading on this point.



5 X=NO_2
7 X=NH_2



6 X=NO_2
8 X=NH_2

Thus although 2,4-dinitrophenylation of histidine side chains with 2,4-dinitrofluorobenzene has invariably led to τ-Dnp-derivatives as the sole isolated products [9], the inference of regiospecificity is false.

Hydrogenation of **5** under the conditions of Bambal and Hanzlik gave **7** only, and hydrogenation of **6** gave **8** only: in both cases the hydrogenation products were demonstrated to be homogeneous before any purification or manipulations that might have led to fractionation or loss of minor products; and they were fully characterized (including nOe proof of orientation). We conclude that the rearrangement reported by Bambal and Hanzlik does not in fact take place, the hydrogenation reported by them presumably having been performed on a mixture of the regioisomers **1** and **2**.

EXPERIMENTAL PART

NMR spectra were recorded on a Varian Gemini 200 instrument operating at 200 MHz, and nOes were determined on a Bruker AM500 instrument operating at 500 MHz; positive ion mass spectra were recorded on a Hewlett-Packard 1050 instrument with atmospheric pressure chemical ionisation.

The Reaction of 4-Fluoronitrobenzene with 4(5)-Methylimidazole

The protocol employed by Bambal and Hanzlik [8] for the reaction of N^{α}-acetylhistidine methyl ester with 4-fluoronitrobenzene was used. Excess potassium carbonate (25 g) was added to a solution of 4-fluoronitrobenzene (14.1 g, 0.1 mol) and 4(5)-methylimidazole (8.2 g, 0.1 mol) in dimethylformamide (40 ml); the mixture was heated at 110 °C for 13 h and then partitioned between water and chloroform. The chloroform layer was separated and dried ($MgSO_4$). The chloroform was removed *in vacuo*, giving an orange-yellow powder (15.72 g, 77%), which was shown by NMR to be a clean mixture of the isomers **5** and **6** (see below) in proportions of approximately 4 : 1 respectively.

4-Methyl,1-(4-nitrophenyl)imidazole, Compound 5

This was isolated in a homogeneous state by fractional crystallization of the above mixture from chloroform and light petroleum, when **5** separated first, and was obtained as a yellow powder of m.p. 153–154 °C. (Found: C, 59.4; H, 4.1; N, 20.5; *m/e* 204, 62%. $C_{10}H_9N_3O_2$ requires C, 59.1; H, 4.4; N, 20.7%, $M+1=204$.) ^{1}H-NMR ($CDCl_3$): 2.31 (3H, s, CH_3); 7.10 (1H, s, H5 of the imidazole ring); 7.53 (2H, d, $J=8.7$ Hz, H3 and H5 of the benzene ring); 7.89 (1H, s, H2 of the imidazole ring); 8.36 (2H, d, $J=8.7$ Hz, H2 and H6 of the benzene ring); irradiation at the 2.31 signal gave an nOe of 7% at the 7.10 signal, but no effect elsewhere.

5-Methyl,1-(4-nitrophenyl)imidazole, Compound 6

This was also isolated in a homogeneous state by fractional crystallization of the above mixture from chloroform and light petroleum, when **6** separated second, and was obtained as yellow needles of m.p. 115–117 °C. (Found: C, 59.25; H, 4.3; N, 20.9; *m/e* 204, 100%. $C_{10}H_9N_3O_2$ requires C, 59.1; H, 4.4; N, 20.7%; $M+1=204$.) ^{1}H-NMR ($CDCl_3$): 2.26 (3H, s, CH_3); 6.98 (1H, s, H4 of the imidazole ring); 7.50 (2H, d, $J=9.0$ Hz, H3 and H5 of the benzene ring); 7.63 (1H, s, H2 of the imidazole ring); 8.40 (2h, d, $J=9.0$ Hz, H2 and H6 of the benzene ring); irradiation at the 2.26 signal gave nOes of 5% and 6.7% respectively at the 6.98 and 7.50 signals.

4-Methyl,1-(4-aminophenyl)imidazole, Compound 7

The protocol employed by Bambal and Hanzlik [8] for the catalytic hydrogenation of the product from the reaction of N^{α}-acetylhistidine methyl ester with 4-fluoronitrobenzene was used. 4-Methyl,1-(4-nitrophenyl)imidazole, **5** (0.203 g, 1 mmol), was dissolved in ethanol (5 ml) and hydrogenated at 35 p.s.i. for 2.5 h over 5% palladium on charcoal (50% moist). Filtration and evaporation gave **7** as a pale brown solid (0.161, g, 85%) which was homogeneous by ^{1}H-NMR ($CDCl_3$): 2.30 (3H, s, CH_3); 3.79 (2H, bs, NH_2); 6.73 (2H, d, $J=8.6$ Hz, H3 and H5 of the benzene ring); 6.90 (1H, s, H5 of the imidazole ring); 7.14 (2H, d, $J=8.6$ Hz, H2 and H6 of the benzene ring); 7.63 (1H, s, H2 of the imidazole ring); irradiation at the 2.30 signal gave an nOe of 5% at the 6.90 signal, but no effect elsewhere; irradiation at the 6.90 signal gave nOes of 4.1% and 2.45% respectively at the 2.30 and 7.14 signals, but no effect elsewhere. Recrystallization from chloroform-light petroleum gave slightly discoloured crystals of m.p. 123–124 °C (Found: C, 69.3; H, 6.5; N, 24.2; *m/e* 174, 100%. $C_{10}H_{11}N_3$ requires C, 69.4; H, 6.4; N, 24.2%; $M+1=174$).

5-Methyl,1-(4-aminophenyl)imidazole, Compound 8

5-Methyl,1-(4-nitrophenyl)imidazole was hydrogenated in the same way on the same scale and **8** was obtained (0.176 g, 93%) as an oil. (Found, *m/e* 174, 100%. $C_{10}H_{11}N_3$ requires $M+1=174$), which was homogeneous by ^{1}H-NMR ($CDCl_3$): 2.12 (3H, s, CH_3); 3.85 (2H, bs, NH_2); 6.74 (2H, d, $J=8.7$ Hz, H3 and H5 of the benzene ring); 6.88 (1H, s, H5 of the imidazole ring); 7.06 (2H, d, $J=8.7$ Hz, H2 and H6 of the benzene ring); 7.55 (1H, s, H2 of the imidazole ring); irradiation at the 2.12 signal gave an nOe of 5% at the 7.06 signal, and a weak effect at the 6.88 signal, but no effect elsewhere; irradiation at the 6.88 signal gave an nOe of 2.4% at the 2.12 signal, but no effect elsewhere; irradiation at the 7.55 signal gave an nOe of 2.3% at the 7.06 signal, but no effect elsewhere.

REFERENCES

1. A. R. Fletcher, J. H. Jones, W. I. Ramage and A. V. Stachulski (1979). The use of the *N*(π)-phenacyl group for the protection of the histidine side chain in peptide synthesis. *J. Chem. Soc. Perkin Trans. 1*, 2261–2267.
2. T. Brown, J. H. Jones and J. D. Richards (1982). Further studies on the protection of histidine side chains in peptide synthesis: the use of the π-benzyloxymethyl group. *J. Chem. Soc. Perkin Trans. 1*, 1553–1561.
3. J. C. Hodges (1987). Regiospecific synthesis of 3-substituted histidines. *Synthesis*, 20–24.
4. J. H. Jones, D. L. Rathbone and P. B. Wyatt (1987). The regiospecific alkylation of histidine side chains. *Synthesis*, 1110–1113.
5. C. J. Chivikas and J. C. Hodges (1987). Phenacyl-directed alkylation of imidazoles: a new regiospecific synthesis of 3-substituted histidines. *J. Org. Chem. 52*, 3591–3594.
6. J. H. Jones and P. B. Wyatt (1990). Syntheses of thyroliberin analogues in which the *C*-terminal amide and the τ-position of the histidine side-chain are bridged. Regiospecific τ-*im*-alkylation and 2-hydroxyphenyl ester cyclopeptide synthesis exemplified. *J. Chem. Res (S)*, 212–213; *J. Chem. Res (M)*, 1701–1711.
7. R. Jain and L. A. Cohen (1996). Regiospecific alkylation of histidine and histamine at N-1(τ). *Tetrahedron 52*, 5363–5370.
8. R. Bambal and R. P. Hanzlik (1994). Synthesis of N^{ε}-(*p*-bromophenyl)-L-lysine and N^{τ}-(*p*-bromophenyl)-L-histidine as models for adducts of bromobenzene 3,4-oxide to protein. Observation of an unusual Pd-catalysed N^{τ}- to N^{π}-aryl substituent migration. *J. Org. Chem. 59*, 729–732.
9. J. R. Bell and J. H. Jones (1974). The position of the dinitrophenyl group in *im*-dinitrophenylhistidine derivatives. *J. Chem. Soc. Perkin Trans. 1*, 2336–2339.

