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π-Allyloxymethyl Protection of Histidine

S.J. HARDING and J.H. JONES*

The Dyson Perrins Laboratory, University of Oxford, Oxford, OX1 3BJ, UK

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Abstract: Experimental details are presented for the introduction and application of π-allyloxymethyl protection for histidine side-chains. Copyright © 1999 European Peptide Society and John Wiley & Sons, Ltd.

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INTRODUCTION

Histidine remains for a peptide synthesis a problem child. The introduction of τ-trityl protection [1] was an important advance, but the received wisdom which rapidly built up around it, that it somehow provided immunity from racemization, was unfounded [2,3]. In fact, from that point of view τ-trityl protection is no better than τ-benzyl, which has led to some instances of gross racemization in real experience as well as in model situations.

For complete security against racemization, π-blockade is indispensable. Given that mild final removal conditions are also very desirable, π-*t*-butoxymethyl protection [4] is in principle very attractive, but general convenient methods for making the key intermediates have eluded us; a protecting group which entails a lot of elaborate and expensive chemistry for its deployment is clearly a self-defeating nonsense. We therefore returned to the problem a few years ago, and have made an exhaustive investigation [5,6], in model systems, of the possibilities for π-protection with groups which are susceptible to mild acidolysis, or other gentle deprotective conditions.

Our efforts were largely unrewarded. In particular, we found the obvious strategem of locating a trityl group at the π-position to be beyond our ingenuity. Other acid-labile groups which could in principle be π-located, and which in other situations are roughly comparable to trityl in acid-sensitivity (ferrocenylmethyl, 4,4′-dimethoxybenzhydryl, 2,4,6-trimethoxybenzyl, 5*H*-dibenzo-suberenyl) either turned out to be insufficiently acid-labile when attached to an *im*-nitrogen, or were ruled out of serious contention by inordinately tricky reagent chemistry. But while we were so engaged, Guibé and his colleagues reported [7,8] on the use of π-allyl-*im*-protection. We had also explored this possibility in our models, but had been unable to achieve Pd^0-catalysed cleavage. In retrospect, this was the result of our ineptitude or inexperience with this process, because with their kind advice we have been able to confirm that π-allyl-*im*-protection is indeed feasible.

Of the allyl-based class of protecting groups, however, our most favoured candidate at present is π-allyloxymethyl. In our model systems, the on–off chemistry is all smooth, and applications in simple but demanding exercises seem very promising. We outlined [6] one of these at the 25th (1998) European Peptide Society Symposium in Budapest. This paper sets forth the corresponding experimental details.

EXPERIMENTAL

Microanalyses, NMR and MS measurements were performed by Dyson Perrins Laboratory Services.

* Correspondence to: Balliol College, Oxford, OX1 3BJ, UK. E-mail: john.jones@balliol.ox.ac.uk

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Allyl Chloromethyl Ether

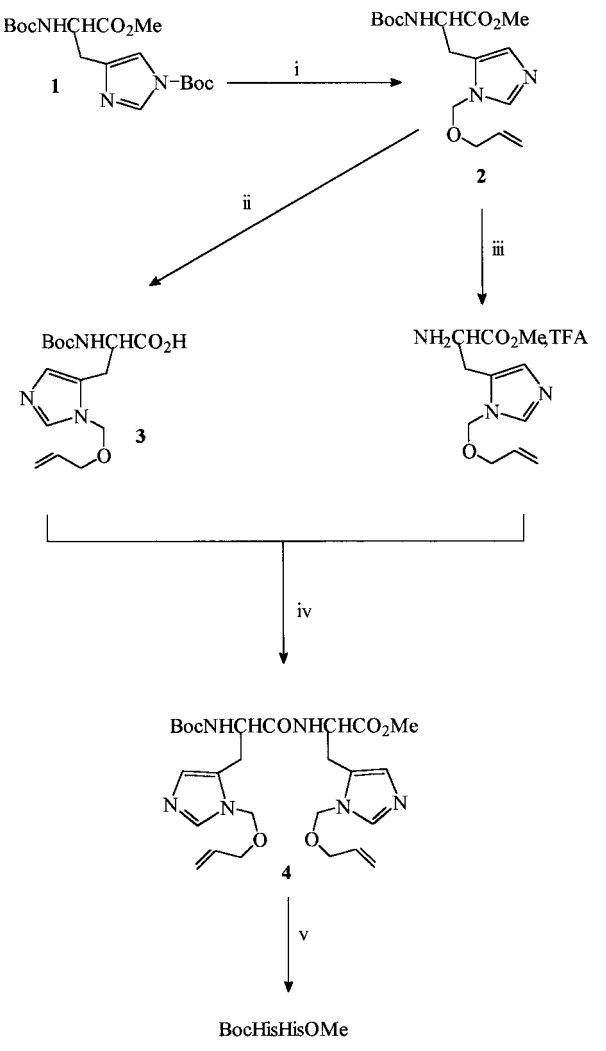
The procedure was based on that of du Preez *et al.* [9]. Freshly distilled thionyl chloride (10.43 g) was added dropwise to a stirred mixture of distilled allyl alcohol (8.48 g) and dry paraformaldehyde (4.38 g) which had been cooled to −25°C. When the mixture had clarified, the top layer was separated, dried ($CaCl_2$) and filtered. The filtrate was distilled *in vacuo* to give *allyl chloromethyl ether* (8.32 g, 53%) as a noxious colourless liquid of b.p. 42–44°C (60mmHg). H^1-NMR ($CDCl_3$): δ 4.21 (2H, d, $J = 5.9$ Hz, allyl CH_2O); 5.26–5.38 (2H, m, CH_2=CH); 5.52 (2H, s, OCH_2Cl); 5.88 (1H, m, CH_2=C*H*). ^{13}C-NMR ($CDCl_3$): δ 70.40 (CH_2=*C*H); 81.90 (allyl CH_2O); 119.10 (CH_2=*CH*); 132.50 (OCH_2Cl).

N(α)-*t*-Butoxycarbonyl-*N*(π)-allyloxymethyl-L-histidine Methyl Ester Hydrochloride

Allyl chloromethyl ether (1.05 g) was added to a solution of *N*(α)-*t*-butoxycarbonyl-*N*(τ)-*t*-butoxycarbonyl-L-histidine methyl ester (**1**) (Scheme 1) (3.63 g) in dichloromethane (30 ml). The solution was stirred at room temperature for 24 h. The solvent was evaporated. Methanol (20 ml) was added and evaporated. Ether (50 ml) was added. The resulting white precipitate was separated and recrystallized from methanol–ether to give *N(α)-t-butoxycarbonyl-N(π)-allyloxymethyl-L-histidine methyl ester hydrochloride* (2.64 g, 71%) as a white powder of m.p. 129–130°C. NMR ($CDCl_3$): δ 1.39 (9H, s, Bu^t); 3.27 (2H, m, His CH_2); 3.76 (3H, s, OCH_3); 4.15 (2H, m, allyl CH_2O); 4.58 (1H, m, α-CH); 5.26 (2H, m, CH_2=CH); 5.50 (1H, brd, NH); 5.76 (2H, s, NCH_2O); 5.81 (1H, m, CH_2=C*H*); 7.24 (1H, s, *im*-H4); 9.78 (1H, s, *im*-H2). *m*/*z* (APCI$^+$): 340 (100%, MH$^+$). Calculated for $C_{16}H_{27}N_3O_5$: M = 340. $[\alpha]_D^{24}$ + 13.0° (*c* 0.1, MeOH). Found: C 50.89%, H 6.90%, N 11.10%. Calculated for $C_{16}H_{27}ClN_3O_5$: C 51.13%, H 6.92%, N 11.19%.

N(α)-*t*-Butoxycarbonyl-*N*(π)-allyloxymethyl-L-histidine Methyl Ester (2)

The preceding hydrochloride (1.33 g) was dissolved in dichloromethane (50 ml). The solution was washed with aqueous $NaHCO_3$ (50 ml), dried and the solvent was evaporated to give *N(α)-t-butoxycarbonyl-N(π)-allyloxymethyl-L-histidine methyl ester* (**2**) (Scheme 1) as a colourless oil (1.20 g, 99%). NMR ($CDCl_3$): δ 1.40 (9H, s, Bu^t); 3.14 (2H, m, His CH_2); 3.72 (3H, s, OCH_3); 3.91 (2H, d, $J = 5.9$ Hz, allyl CH_2O); 4.55 (1H, m, α-CH); 5.27 (5H, m, CH_2=CH, NCH_2O and NH); 5.83 (1H, m, CH_2=C*H*); 6.84 (1H, s, *im*-H4); 7.48 (1H, s, *im*-H2). Irradiation at δ 3.14 resulted in a 1.2% nOe at δ 6.84, a 3.2% nOe at δ 5.27 and a 6.6% nOe at δ 4.55. Irradiation at δ 3.91 resulted in a 1% nOe at δ 7.48, a 3.2% nOe at δ 5.83, a 5.4% nOe at δ 5.27, a 1.2% nOe at δ 4.55 and a 1% nOe at δ 3.14. Irradiation at δ 6.84 resulted in a 1.9% nOe at δ 5.27, a 2.7% nOe at δ 4.55 and a 3% nOe at δ 3.14. Irradiation at δ 7.48 resulted in a 1% nOe at δ 6.84, a 1.2% nOe at δ 5.83, a 6% nOe at δ 5.27 and a 2.4% nOe at δ 3.91.



Scheme 1 Conditions: (i) CH_2=$CHCH_2OCH_2Cl/CH_2Cl_2$, then treatment of the HCl salt with $NaHCO_3$, 70% overall; (ii) aq. NaOH, 77%; (iii) TFA, quant., used immediately; (iv) DCCI/HOBt/MeCN, 95%; (v) $Pd(PPh_3)_4$/*N*,*N*′-dimethylbarbituric acid/CH_2Cl_2, reflux, 94%.

N(α)-*t*-Butoxycarbonyl-*N*(π)-allyloxymethyl-L-histidine Monohydrate (3)

The preceding methyl ester (677 mg) was dissolved in methanol (8 ml). Aqueous NaOH (1 M, 2.3 ml) was added. The solution was kept at room temperature for 15 min. The methanol was evaporated. Dilute hydrochloric acid (1 M, 2.3 ml) was added. The mixture was extracted with chloroform (2 × 50 ml), the chloroform was evaporated to give *N(α)-t-butoxycarbonyl-N(π)-allyloxymethyl-L-histidine monohydrate* (**3**) (Scheme 1) (502 mg, 77%) as a white powder of m.p. 113–115°C. NMR ($CDCl_3$): δ 1.42 (9H, s, Bu^t); 3.25 (2H, m, His CH_2); 3.94 (2H, d, $J = 5.3$ Hz, allyl CH_2O); 4.37 (1H, m, α-CH); 5.24 (2H, m, C*H*$_2$=CH, 5.38 (2H, m, NCH_2O); 5.69 (1H, brd, NH); 5.78 (1H, m, C*H*$_2$=CH), 6.98 (1H, s, *im*-H4); 8.04 (1H, s, *im*-H2); 10.09 (1H, brs, CO_2H). $[\alpha]_D^{28}$ +5.6° (*c* 1.0, MeOH). *m/z* ($APCI^+$): 327 (15%, MH^+). Calculated for $C_{15}H_{23}N_3O_5$: M = 326. Found: C 52.14%, H 7.19%, N 12.12%. Calculated for $C_{15}H_{23}N_3O_5.H_2O$: C 52.48%, H 7.29%, N 12.24%.

N(α)-*t*-Butoxycarbonyl-*N*(π)-allyloxymethyl-L-histidine-*N*(π)-allyloxymethyl-L-histidine Methyl Ester (4)

N(α)-*t*-Butoxycarbonyl-*N*(π)-allyloxycarbonyl-L-histidine methyl ester hydrochloride (503 mg) was dissolved in trifluoroacetic acid (2 ml). The solution was kept at room temperature for 30 min. The trifluoroacetic acid was evaporated. The resulting oil was kept in a desiccator over KOH for 72 h. The oil was taken up in acetonitrile (2 ml). **3** (433 mg) was added, followed by 1-hydroxybenzotriazole (224 mg). The solution was stirred at 0°C. Triethylamine (0.41 ml) was added, followed by dicyclohexylcarbodiimide (274 mg). The solution was stirred at 0°C for 1 h, then at room temperature for 20 h. The mixture was filtered, and the filtrate was evaporated. The residue was taken up in dichloro-methane (5 ml) and washed with aqueous $NaHCO_3$ (5 ml). The solution was dried and evaporated to give the crude dipeptide (693 mg, 95%) as a pale orange oil. A portion of this oil was subjected to centrifugally accelerated (Chromatotron) preparative layer chromatography, eluting with 5:1 chloroform–methanol. Evaporation of the solvent gave *N(α)-t-Butoxycarbonyl-N(π)-allyloxy-methyl-L-histidine-N(π)-allyloxymethyl-L-histidine methyl ester* (**4**) (Scheme 1) (156 mg) as a pale orange meringue. NMR ($CDCl_3$): δ 1.35 (9H, s, Bu^t); 2.85–3.20 (4H, m, His CH_2); 3.66 (3H, s, OCH_3); 3.87 (2H, d, $J = 5.7$ Hz, allyl CH_2O); 3.90 (2H, d, $J = 5.7$ Hz, allyl CH_2O); 4.37 (1H, m, α-CH); 4.78 (1H, m, α-CH); 5.15–5.30 (8H, m, C*H*$_2$=CH and NCH_2O); 5.65 (1H, d, NH); 5.80 (2H, m, C*H*$_2$=CH), 6.71 (1H, s, *im*-H4); 6.78 (1H, s, *im*-H2); 7.45 (1H, s, *im*-H2); 7.46 (1H, s, *im*-H2); 7.58 (1H brd, NH). $[\alpha]_D^{25}$ +2.2° (*c* 3.9, $CHCl_3$). *m/z* ($APCI^+$): 547 (100%, MH^+). Calculated for $C_{26}H_{38}N_6O_7$: M = 546.

N(α)-*t*-Butoxycarbonyl-L-histidyl-L-histidine Methyl Ester (5)

N(α)-*t*-Butoxycarbonyl-*N*(π)-allyloxymethyl-L-histidine-*N*(π)-allyloxymethyl-L-histidine methyl ester (130 mg) was dissolved in dichloromethane (3 ml). *N*,*N*′-dimethylbarbituric acid (371 mg) was added, followed by tetrakis(triphenylphosphine)-palladium(0) (10 mg). The solution was refluxed for 3 h. The solution was extracted with water (10 ml). The aqueous layer was evaporated. The residue was taken up in methanol (5 ml) and stirred with a large excess (0.5 g) of Amberlyst A-21 resin for 18 h. The methanol was evaporated. The residue was precipitated from chloroform–ether to give *N(α)-t-butoxycarbonyl-L-histidine-L-histidine methyl ester* (**5**) (Scheme 1) (91 mg, 94%) as a white powder of m.p. 120–130°C. NMR ($CDCl_3$): δ 1.44 (9H, s, Bu^t); 3.00–3.30 (4H, m, His CH_2); 3.47 (3H, s, OCH_3); 4.41 (1H, m, α-CH); 4.73 (1H, m, α-CH); 5.74 (1H, brd, NH); 6.74 (1H, s, *im*-H4); 6.84 (1H, s, *im*-H4); 7.51 (1H, s, *im*-H2); 7.52 (1H, s, *im*-H2); 7.69 (1H brd, NH). $[\alpha]_D^{25}$ −0.3° (*c* 2.7, MeOH). *m/z* ($APCI^+$): 429 (49%, MNa^+); 407 (100%, MH^+). Calculated for $C_{18}H_{26}N_6O_5$: M = 406.

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