Monatshefte für Chemie 128, 207–210 (1997)

Monatshefte für Chemie
Chemical Monthly

Syntheses of Methylketones of Peptides with C-Terminal Optically Active Lysine: Removal of the Benzyloxycarbonyl Group

K. Midura-Nowaczek

Institute of Chemistry, University of Warsaw, Białystok Branch, PL-15443 Białystok, Poland

Summary. Several methods for the removal of the benzyloxycarbonyl group from Z-Ala-Phe-Lys(Z)-CH_3 have been tested. Only catalytic hydrogenation in acetic acid gives the desired products with satisfactory yield and without racemization at C-α of lysine.

Keywords. Tripeptide methylketone; Benzyloxycarbonyl group; Racemization; Catalytic hydrogenation.

Synthesen von Methylketonen von Peptiden mit C-terminalem optisch aktivem Lysin: Entfernung der Benzyloxycarbonylschutzgruppe

Zusammenfassung. Verschiedene Methoden zur Entfernung der Benzyloxycarbonylschutzgruppe von Z-Ala-Phe-Lys(Z)-CH_3 wurden untersucht. Nur durch katalytische Hydrierung Essigsäure können die gewünschten Produkte in zufriedenstellender Ausbeute und ohne Racemisierung am C-α des Lysins erhalten werden.

Introduction

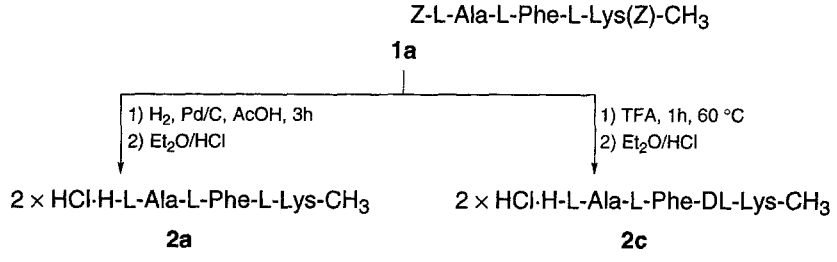
Synthetic methylketones of peptides are reversible inhibitors of serine and cysteine proteases [1–3]. They are easy to synthesize and they have a higher chemical stability than other reversible peptide inhibitors, *e.g.* aldehydes. This group of peptides are also useful as ligands in affinity chromatography [4,5]. *Fittkau et al.* have synthesized methylketones of peptides as inhibitors of thermitase and subtilisin [1,5]. *McMurray* and *Dyckes* obtained methylketones of peptides as inhibitors of trypsin by a modified *Dakin-West* reaction. Complete racemization at C-α of lysine was observed during conversion of *Boc*-Ala-Lys(Z)-OH and *Boc*-Lys(Z)-Ala-Lys(Z)-OH into the respective methylketones under *Dakin-West* conditions [6,7]. In search of reversible active centre directed inhibitors of plasmin, the synthesis of the protected tripeptide methylketone Z-Ala-Phe-Lys(Z)-CH_3 with an optically active C-terminal lysine has been reported. The problem of racemization at C-α of lysine during the removal of a *t*-butoxycarbonyl group using of ethyl ether/HCl has been investigated by ^1H NMR spectroscopy [8]. *Fittkau et al.* used HBr/AcOH or catalytic hydrogenation (Pd) in MeOH/HCl for this purpose [1,9]. According to their investigations, aminomethylketones and methylketones of peptides with an

unsubstituted aminogroup are stable only as salts under anhydrous conditions. Aminomethylketones and methylketones of dipeptides with a free amino group can form by-products *via* reaction between the N-terminal amine and the methylketone moiety [9]. *McMurray* and *Dyckes* employed *TFA* or catalytic reduction in methanol using palladium/carbon as the catalyst for the removal of the benzyloxycarbonyl group from *Boc-L*-Lys(*Z*)-*L*-Ala-*DL*-Lys(*Z*)-CH_3 [6,7]. According to the ^1H NMR spectrum, an application of the latter method to the final deprotection of *Z*-Ala-Phe-Lys(*Z*)-CH_3 did not give the expected result. ^1H NMR analysis (200 MHz) was used to rationalize this problem and develop a method for the removal of the benzyloxycarbonyl group from a tripeptide methlylketone with an optically active C-terminal lysine. The ketomethyl peak (integrating for three protons) of 2HCl·H-*L*-Ala-*L*-Phe-*L*-Lys-CH_3 appears as a singlet at 1.96 ppm (*DMSO*-d_6) or 1.98 ppm (D_2O). The corresponding peak of 2HCl·H-*L*-Ala-*L*-Phe-*D*-Lys-CH_3 appears as a singlet at 2.05 ppm (*DMSO*-d_6) or 2.04 ppm (D_2O). Therefore, an evaluation of final deprotection methods with respect to a racemization at C-α of lysine by ^1H NMR is possible.

Results and Discussion

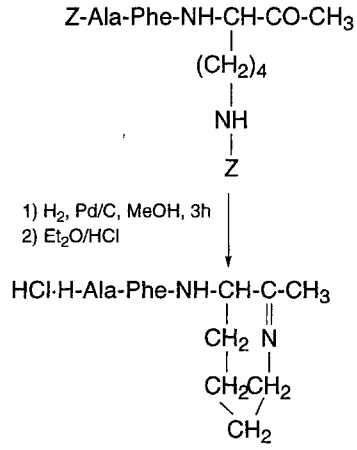
In search of an efficient method for the removal of the benzyloxycarbonyl group from a tripeptide methylketone, catalytic hydrogenation using 5% palladium/carbon as a catalyst in methanol, methanol/acetic acid, methanol/HCl, and acetic acid has been tested. A cleavage of the benzyloxycarbonyl group with trifluoracetic acid was also examined. According to the ^1H NMR spectra, the best method for the removal of the benzyloxycarbonyl group from *Z*-Ala-Phe-Lys(*Z*)-CH_3 seems to be catalytic hydrogenation in glacial acetic acid.

It affords the desired products (**2a,b**) in high yield and without recemization at C-α of lysine. The ketomethyl group is also stable in the case of reaction with *TFA*, but a partially racemized product (**2c**) was obtained (Scheme 1). Therefore, this method can only be used for the removal of the *Z* group from tripeptide methylketones with racemic C-terminal lysine, for example *Dakin-West* reaction products.



Scheme 1

Catalytic hydrogenation of *Z*-Ala-Phe-Lys(*Z*)-CH_3 in methanol (3 h) with the use of Pd/C as a catalyst, followed by precipitation with ethyl ether/HCl, gives predominatly a by-product resulting from a reaction between the ketomethyl and ε amino groups (Scheme 2). A structure for the by-product is proposed on the basis of the ^1H NMR spectrum of the crude product. No signals of ketomethyl and



Scheme 2

Lys-ε-NH_3^+ protons were observed. On the other hand, the spectrum shows a singlet at $\delta = 2.2$ (substrate **1a**) or 2.4 (substrate **1b**) ppm attributable to $-N=C-CH_3$ protons. Signals of α-CH, ε-CH_2, and α-NH protons from the lysine residue were shifted downfield with respect to **2a,b**.

Catalytic hydrogenation in methanol with addition of HCl or acetic acid results in a decrease of the ketomethyl signal. Partial racemization was also observed in the case of methanol/HCl.

2a and **2b** are stable under anhydrous condition. Their stability in aqueous solution was examined by ^1H NMR spectroscopy. Compound **2a** was dissolved in D_2O and kept for one week at room temperature. Then, its ^1H NMR spectrum was recorded and compared with the spectrum recorded just after dissolution **2** of **a** in D_2O. The obtained results suggest that hydrochlorides of tripeptide methylketones with optically active C-terminal lysine are also stable in water solution.

Experimental

Z-L-Ala-*L*-Phe-*L*-Lys(*Z*)-CH_3 (**1a**) and *Z-L*-Ala-*L*-Phe-*D*-Lys(*Z*)-CH_3 (**1b**) were obtained as described earlier [8]. Melting points were measured on a Boëtius apparatus. NMR spectra were recorded on a Bruker AC 200F spectrometer. Elemental analyses were performed on an Analyser CHNSO model 2400 (Perkin-Elmer). Specific rotations were measured using a Polamat A apparatus (Carl Zeiss, Jena). TLC was performed on silica gel plates (Kieselgel 60 F_{254}, Merck) using the following solvent systems: A: *n*-butanol/pyridine/acetic acid/water (15:15:12:10); B: pyridine/acetic acid/water (1:10:89); C: *n*-butanol/acetic acid/25% ammonia solution {5,5:3:1,5). The chromatograms were stained by 1) spraying with ninhydrine 2) exposing the plate to chlorine vapour for 15 min and spraying with tolidine reagent after 20 min.

(L-Alanyl-L-phenylalanyl-L-lysyl) methane dihydrochloride (2HCl·H-L-Ala-L-Phe-L-Lys-CH_3, **2a***)*

100 mg (0.16 mmol) of **1a** were dissolved in 3 ml of glacial acetic acid, and 5% Pd/C (30 mg) was added. After catalytic hydrogenation (3 h), the mixture was filtered. Ethyl ether saturated with HCl (2*N*,0.1 ml) was added to the filtrate. The final product was quickly precipitated by addition of 20 ml of ethyl ether. The obtained white solid was washed several times with dry ethyl ether and dried over NaOH and P_2O_5.

Yield: 47 mg (65%); m.p.: 178–182 °C; $[\alpha]_D^{20} = +1.4$ ($C = 1$, AcOH); R_f: 0.71 (A), 0.61 (B), 0.56 (C); ^{1}H NMR (*DMSO*-d_6): $\delta = 8.90–8.78$ (d, 1H, Phe-α-NH), 8.70–8.62 (d, 1H, Lys-α-NH), 8.28–8.10 (s, 3H, Ala-NH_3^+), 8.1–7.9 (s, 3H, Lys-ε-NH_3^+), 7.4–7.1 (m, 5H, Phe-C_6H_5), 4.7–4.5 (m, 1H, Phe-α-CH), 4.15–4.05 (m, 1H, Lys-α-CH), 3.80–3.65 (m, 1H, Ala-α-CH), 3.20–2.80 (m, 2H, Phe-CH_2), 2.8–2.6 (m, 2H, Lys-ε-CH_2), 1.96 (s, 3H, $COCH_3$), 1.75–0.95 (m, 9H, Lys-β-CH_2, γ-CH_2, δCH_2, Ala-CH_3) ppm.

(L-Alanyl-L-phenylalanyl-D-lysyl) methane dihydrochloride (2HCl·H-L-Ala-L-Phe-D-Lys-CH_3, **2b**)

2b was obtained as described for the preparation of **2a** using **1b** as substrate.
Yield: 42 mg (57.8%); m.p.: 185–188 °C; $[\alpha]_D^{20} = +15.1$ ($C = 1$, AcOH); R_f identical to **2a**; ^{1}H NMR (*DMSO*-d_6) $\delta = 8.89–8.86$ (d, 1H, Phe-α-NH), 8.70–8.63 (d, 1H, Lys-α-NH), 8.3–8.1 (s, 3H, Ala-NH_3^+), 8.1–7.9 (s, 3H, Lys-ε-NH_3^+), 7.40–7.15 (m, 5H, Phe-C_6H_5), 4.7–4.5 (m, 1H, Phe-α-CH), 4.15–4.00 (m, 1H, Lys-α-CH), 3.80–3.65 (m, 1H, Ala-α-CH), 3.2–2.8 (m, 2H, Phe-CH_2), 2.8–2.6 (m, 2H, Lys-ε-CH_2), 2.05 (s, 3H, $COCH_3$), 1.75–0.95 (m, 9H, Lys-β-CH_2), γ, CH_2, δ-CH_2, Ala-CH_3) ppm.

(L-Alanyl-L-phenylalanyl-DL-lysyl) methane dihydrochloride (2HCl·H-L-Ala-L-Phe-DL-Lys-CH_3, **2c**)

50 mg (0.08 mmol) of **1a** were dissolved in 3 ml of *TFA* and kept at 60 °C for 1 h. Then the mixture was cooled to room temperature, and 1 ml of 2 *N* HCl/ethyl ether was added. The final product was precipitated and washed with dry ethyl ether. The obtained white solid was dried over NaOH and P_2O_5.
Yield: 32 mg (88.2%); m.p.: 128–32 °C; $[\alpha]_D^{20} = +5.6$ ($C = 1$, AcOH); ratio **2b:2a** 35:65 based on the ^{1}H NMR spectrum); $C_{19}H_{32}N_4O_3Cl_2 \cdot H_2O$ (453.4) calcd: C 50.33, H 7.56, N 12.35; found: C 50.2 ± 0.6, H 7.4 ± 0.3, N 12.5 ± 0.3.

Acknowledgements

Thanks are due to Dr. *L. Siergiejczyk* for assistance with the recording of some NMR spectra.

References

[1] Fittkau S, Jahreis G (1984) J Prakt Chem **326**: 48
[2] Fittkau S, Smalla K, Pauli D (1984) Biomed Biochim Acta **43**: 887
[3] Brömme D, Bartels B, Kirschke H, Fittkau S (1989) J Enzyme Inhib **3**: 13
[4] Peters K, Fittkau S (1990) Biomed Biochim Acta **49**: 173
[5] Peters K, Fittkau S (1993) J Chrom **648**: 91
[6] McMurray JS, Dyckes DF (1985) J Org Chem **50**: 1112
[7] McMurray JS, Dyckes DF (1986) Biochemistry **25**: 2298
[8] Midura-Nowaczek K (1996) J Prakt Chem **338** 380
[9] Fittkau S, Jahreis G, Peters K, Balaspiri L (1986) J Prakt Chem **328**: 529

Received July 12, 1996. Accepted September 16, 1996

Verleger: Springer-Verlag KG, Sachsenplatz 4–6, A-1201 Wien. – Herausgeber: Österreichische Akademie der Wissenschaften, Dr.-Ignaz-Seipel-Platz 2, A-1010 Wien und Gesellschaft Österreichischer Chemiker, Eschenbachgasse 9, A-1010 Wien. – Redaktion: Währinger Straße 38, A-1090 Wien. – Satz und Umbruch: Thomson Press Ltd., New Delhi, India. – Offsetdruck: Eugen Ketterl Gesellschaft m.b.H., Schopenhauerstraße 45, A-1180 Wien. – Verlagsort: Wien. – Herstellungsort: Wien. – Printed in Austria.