Decomposition of copper – amino acid complexes by oxalic acid dihydrate

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Abstract: A facile approach to the synthesis of some side-chain-protected amino acids via oxalic acid dihydrate as the copper sequestering reagent is presented. The copper in the amino acid complex reacted with oxalic acid dihydrate to form insoluble cupric oxalate, with the free amino acid released. Compared with conventional methods, this method is convenient, inexpensive, and environmentally friendly.

Key words: side-chain-protected amino acid, oxalic acid dihydrate, cupric oxalate, synthesis.

Résumé : On a mis au point une approche simple à la synthèse des divers acides aminés à chaîne latérale protégée impliquant le dihydrate de l'acide oxalique comme réactif de séquestration de du cuivre. Le cuivre du complexe avec l'acide aminé réagit avec le dihydrate de l'acide oxalique pour former l'oxalate cuivrique insoluble et la libération de l'acide aminé libre. Par comparaison avec les autres méthodes conventionnelles, cette méthode est pratique, peu coûteuse et écologique.

Mots-clés : acide aminé à chaîne latérale protégée, dihydrate de l'acide oxalique, oxalate cuivrique, synthèse.

[Traduit par la Rédaction]

Introduction

Since the emergence of glycylglycine synthesis from a diketopiperazine by Fischer and Fourneau[1] in 1901, peptide synthesis has undergone tremendous progress and it is now possible to routinely synthesize a protein consisting of over 200 amino acids. However, when the amino acids involved are trifunctional, such as lysine, ornithine, etc., it is necessary to protect their side-chain functional groups. This is accomplished by first reacting the primary amino acids with cupric ions to form the stable chelate complex, followed by a reaction with acylation reagents to facilitate selective protection of the side-chain functional group.

To recover the side-chain-protected amino acids, it is necessary to decompose the copper – amino acid complexes, for which several methods are available involving several important reagents including EDTA,[2,3] potassium cyanide,[4] 8-quinolinol,[5] hydrogen sulfide,[6,7] thioacetamide,[8] sodium borohydride,[9] and sodium sulfide.[10] EDTA is one of the most widely employed copper-sequestering reagents. However, the resultant copper–EDTA complex during the course of the reaction is water-soluble, which can lead to effluent problems with the water-soluble side-chain-protected amino acids. When copper is removed from the complexes using KCN, H_2S, or thioacetamide, there is a marked drawback in that these reagents are highly toxic and, thioacetamide in particular, is a potential cancer-causing chemical. When copper is removed from the complexes employing 8-quinolinol, there is a low yield of protected amino acids, with the cost greatly increased. Similarly, in the case of copper removal using sodium borohydride, issues such as heat generation during bulk production of protected amino acids also result. Despite the fact that recent research has confirmed the efficiency of sodium sulfide in the detachment of copper from the complexes, its serious hazards to the environment are noteworthy.

Given the limitations of the methods concerning the copper(II) deprotection discussed previously, it is difficult to realize the industrialization. Several studies have shown that oxalic acid can be preferable for its inexpensiveness and friendliness to the environment and can form complexes with copper.[11–13] In this paper, we report a highly convenient and efficient method in which oxalic acid dihydrate is employed to detach the copper(II) complexes, to prepare the side-chain amino-protected acetyl, Boc, Fmoc, and Z (benzyloxy carbonyl) ornithines,and lysines, respectively.

Results and discussion

The behavior of copper in oxalic acid solutions has been well-studied. The results have shown that copper ions with oxalate can easily form complexes; however, the complexation ability in the solution depends on pH (1–1.5).[13] In continuation of our efforts towards the decoppering reaction, we employed oxalic acid dihydrate to sequester copper from the copper – amino acid complexes. In general, cupric oxalate is insoluble in water or diluted hydrochloric acid. To accelerate

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Table 1. Preparation of the side-chain-protected amino acids.

Entry	Product[a]		Yield (%)	Purity (%)
1	H-Orn(Z)-OH	**a**	70	98.5
2	H-Orn(Fmoc)-OH	**b**	65	98.3
3	H-Orn(acetyl)-OH	**c**	78	97.9
4	H-Lys(Z)-OH	**d**	74	98.8
5	H-Lys(Fmoc)-OH	**e**	69	97.7
6	H-Lys(acetyl)-OH	**f**	65	98.0
7	H-Orn(Boc)-OH	**g**	59	97.1
8	H-Lys(Boc)-OH	**h**	63	96.9

[a]All compounds were characterized by ^{1}H NMR and elemental analysis.

the reaction, diluted hydrochloric acid was selected as the vehicle, with the concentrations of the acid investigated. It was observed that, with the acid maintained at a concentration of 0.01 mol/L, the method for the removal of copper ions by oxalic acid dihydrate was effective and could completely eradicate the sulfide problem during the course of the reaction (the pH of the solutions is ~1.1). The results in Table 1 show that the oxalic acid dihydrate-mediated detachment of copper ions from all the copper amino acid complexes resulted in protected amino acids of high purity with no racemization.

The copper complexes of the following amino acids were prepared in accordance with the methods in literature: N^{ε}-Boc–lysine,[14] N^{δ}-Boc–ornithine,[15] N^{ε}-*Z*–lysine, N^{δ}-*Z*–ornithine,[8] N^{ε}-Fmoc–lysine, N^{δ}-Fmoc–ornithine,[16] N^{δ}-acetyl–ornithine, and N^{ε}-acetyl–lysine.[17]

The copper – amino acid complexes were treated with oxalic acid dihydrate in diluted hydrochloric acid for 1–2 h at 80 °C to afford side-chain-protected amino acids **a**–**j** in yields of 55%–80% (Scheme1 and Table 1). The isolated products were characterized by ^{1}H NMR and elemental analyses.

Conclusion

In conclusion, we have confirmed that oxalic acid dihydrate is a useful reagent in removing copper from amino acid copper complexes during the preparation of several side-chain-protected amino acids. This method may also be applicable to bulk production of side-chain-protected amino acids.

Experimental

Melting points were measured in open capillaries and are uncorrected. Optical rotations were measured on a PerkinElmer model 341 automatic polarimeter. Elemental analysis was performed on a PerkinElmer CHNS analyzer. ^{1}H NMR spectra were recorded in CD_3SOCD_3 on a Bruker Avance 500 spectrometer; chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS), serving as an internal standard. Solvents and reagents were purchased from respective suppliers and used without further purification.

General procedure for the preparation of side-chain-protected amino acids a–h

To dissolve the copper – amino acid complex (10.0 mmol) in a capped flask at ambient temperature, diluted hydrochloric acid (30 mL, 0.01 mol/L) was added, followed by the addition of oxalic acid dihydrate (10.3 mmol), and the resulting mixture was stirred at 80 °C (the pH of the solutions was ~1.1 at this time). At the end of stirring for 1–3 h, the consequent cupric oxalate precipitate was filtered. The filtrate was neutralized with dilute NaOH. The resulting precipitate was isolated by filtration and then rinsed with distilled water (50 mL × 4) to obtain the side-chain-protected amino acids **a**–**f**. The water-soluble products (**g**–**h**) were isolated by the removal of the solvent and extraction of the residue with acetone.

H-Orn (*Z*)-OH (compound a)

White crystals (70%), mp 248–250 °C, $[\alpha]_D^{20}$ +17.6° (lit.[18] mp 228–230 °C, $[\alpha]_D^{20}$ +17.5°, *c* 0.01, water/acetone = 1:1, and equal volumes of hydrochloric acid). ^{1}H NMR δ: 7.35–7.45 (m, 5H, Ar-H), 5.08 (s, 2H, Ar–CH_2), 3.37–3.44 (m, 1H, α-H), 3.19–3.23 (m, 2H, δ-H), 1.81–1.86 (m, 2H, γ-H), 1.65–1.70 (m, 2H, β-H). Anal. calcd for $C_{13}H_{18}N_2O_4$: C 58.63, H 6.81, N 10.52; found: C 58.57, H 6.84, N 10.48.

H-Orn (Fmoc)-OH (compound b)

White crystals (65%), mp 151–153 °C (lit.[19] mp 152–154 °C). ^{1}H NMR δ: 3.41–3.46 (m, 1H, α-H), 3.17–3.23 (m, 2H, δ-H), 1.78–1.84 (m, 2H, γ-H), 1.61–1.67 (m, 2H, β-H), 4.53–4.69 (m, 2H, $COOCH_2$), 4.31–4.37 (m, 1H, Fmoc-9H), 7.29–7.80 (m, 8H, Ar-H). Anal. calcd for $C_{20}H_{22}N_2O_4$: C 67.76, H 6.26, N 7.91; found: C 67.83, H 6.31, N 7.87.

H-Orn (Acetyl)-OH (compound c)

White crystals (78%), mp 246–249 °C (lit.[20] mp 248–250 °C). ^{1}H NMR δ: 3.38~3.45 (m, 1H, α-H), 3.18–3.24 (m, 2H, δ-H), 1.81–1.87 (m, 2H, γ-H), 1.58–1.65 (m, 2H, β-H), 2.01 (s, 3H, CH_3). Anal. calcd for $C_7H_{14}N_2O_3$: C 48.27, H 8.01, N 16.08; found: C 48.20, H 8.09, N 16.01.

H-Lys (Z)-OH (compound d)

White crystals (74%), mp 245–248 °C (lit.[8] mp 250 °C). ^{1}H NMR δ: 7.31–7.68 (m, 5H, Ar-H), 5.11 (s, 2H, Ar-CH_2), 3.13–3.22 (m, 2H, ε-H), 3.41–3.46 (m, 1H, α-H), 1.77–1.82 (m, 2H, β-H), 1.38–1.45 (m, 2H, γ-H), 1.57–1.64 (m, 2H, δ-H). Anal. calcd for $C_{14}H_{20}N_2O_4$: C 59.99, H 7.19, N 9.99; found: C 59.90, H 7.24, N 10.00.

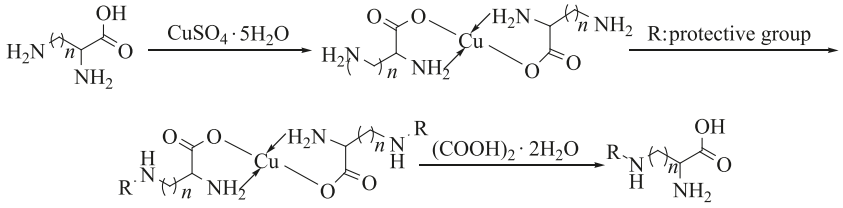
H-Lys (Fmoc)-OH (compound e)

White crystals (69%), mp 209–210 °C (lit.[19] mp 210–212 °C). ^{1}H NMR δ: 3.41–3.47 (m, 1H, α-H), 3.17–3.24 (m, 2H, ε-H), 1.32–1.39 (m, 2H, γ-H), 1.51–1.59 (m, 2H, δ-H), 1.79–1.85 (m, 2H, β-H), 4.74 (d, J = 3.21 Hz, 2H, OCH_2), 4.42 (t, J = 3.21 Hz, 1H, Fmoc-9H), 7.26~7.80 (m, 8H, Ar-H). Anal. calcd for $C_{21}H_{24}N_2O_4$: C 68.46, H 6.57, N 7.60; found: C 68.38, H 6.49, N 7.67.

H-Lys (Acetyl)-OH (compound f)

White crystals (65%), mp 245–249 °C (dec). ^{1}H NMR δ: 3.40–3.46 (m, 1H, α-H), 3.18–3.22 (m, 2H, ε-H), 1.30–1.38 (m, 2H, γ-H), 1.53–1.61 (m, 2H, δ-H); 1.73–1.85 (m, 2H, β-H), 2.00 (s, 3H, CH_3). Anal. calcd for $C_8H_{16}N_2O_3$: C 51.05, H 8.57, N 14.88; found: C 51.00, H 8.64, N 14.77.

Scheme 1.



a, H-Orn(Z)-OH: R=Z, *n*=3
b, H-Orn(Fmoc)-OH: R=Fmoc, *n*=3
c, H-Orn(Acetyl)-OH: R=Acetyl, *n*=3
g, H-Orn(Boc)-OH: R=Boc, *n*=3
d, H-Lys(Z)-OH: R=Z, *n*=4
e, H-Lys(Fmoc)-OH: R=Fmoc, *n*=4
f, H-Lys(Acetyl)-OH: R=Acetyl, *n*=4
h, H-Lys(Boc)-OH: R=Boc, *n*=4

H-Orn (Boc)-OH (compound g)

White crystals (59%), mp 210–213 °C, $[\alpha]_D^{20}$ +15.5° (lit.[21] mp 209.75 °C, $[\alpha]_D^{20}$ +15.2°,[17] *c* 1.0, acetic acid). ^{1}H NMR δ: 3.35–3.46 (m, 1H, α-H), 3.19–3.25 (m, 2H, δ-H), 1.81–1.92 (m, 2H, γ-H), 1.58–1.65 (m, 2H, β-H), 1.44 (s, 9H, 3 × CH_3). Anal. calcd for $C_{10}H_{20}N_2O_4$: C 51.71, H 8.68, N 12.06; found: C 51.66, H 8.60, N 12.11.

H-Lys (Boc)-OH (compound h)

White crystals (63%), mp 247–249 °C, $[\alpha]_D^{20}$ +17.1° (*c* 1, acetic acid). ^{1}H NMR δ: 3.37–3.44 (m, 1H, α-H), 3.14–3.21 (m, 2H, ε-H), 1.32–1.38 (m, 2H, γ-H), 1.50–1.58 (m, 2H, δ-H), 1.78–1.84 (m, 2H, β-H), 1.39 (s, 9H, 3 × CH_3). Anal. calcd for $C_{11}H_{22}N_2O_4$: C 53.64, H 9.00, N 11.37; found: C 53.70, H 8.95, N 11.33.

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