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Decomposition of copper–amino acid complexes by sodium sulfide

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Abstract—Sodium sulfide very efficiently removes copper from protected amino acid–copper complexes. The copper in the amino acid complex was reduced to insoluble cuprous sulfide and the free amino acid was released in pure form. This method is very convenient and rapid, requiring only 5–10 min and 0.55–0.75 equiv of sodium sulfide.
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Since the synthesis of glycylglycine from a diketopiperazine by Emil Fisher[1] in 1901, peptide synthesis has undergone tremendous progress and now it is possible to routinely synthesize a protein consisting of up to 200 amino acids. Research involving over a hundred years of peptide synthesis,[2] including solid phase and chemical ligation methods, reveals the use of protection and deprotection techniques for efficient, ordered and specific protein synthesis. One of the simplest routes for simultaneous protection of α-amino and α-carboxyl functional groups of an amino acid is by copper complexation. When the amino acids involved are trifunctional, the protection of the third functional group on the side-chain is essential. Thus, protection of the ε-amino group in lysine, the γ-amino group of ornithine, the γ-carboxylic group of glutamic acid, the β-carboxylic group of aspartic acid, the phenolic group of tyrosine, the hydroxy groups of serine and threonine and the thiol group of cysteine assumes significance in peptide syntheses. The regioselective protection of the side-chain amino groups of lysine[3–7] and ornithine[6,8,9] is invariably carried out with Diboc, Fmoc-Nosu, benzyl chloroformate, benzoyl chloride, acetic anhydride, *N*-ethoxyphthalimide, and *p*-toluene sulfonyl chloride. The phenolic group of tyrosine[6,10] is protected as the corresponding benzyl ether, whereas the side-chain carboxyl groups of aspartic acid[11,12] and glutamic acid[11,12] are protected as benzyl esters, methyl esters and as other alkyl esters.

In copper complex-mediated peptide syntheses, the amino acid is first reacted with a copper(II) ion to give a stable square planar copper(II) complex. Subsequently, the copper–amino acid complex is reacted with the amino acid side-chain protecting reagent as the α-amino and α-carboxyl groups are bound to copper(II); the protecting group selectively reacts with the side-chain functional group of a given amino acid. In order to recover the side-chain protected amino acid, it is necessary to decompose the copper–amino acid complex, for which several methods are available. A few important examples include EDTA,[5,13] chelating ion exchange resins[7,11] potassium cyanide,[14] 8-quinolinol,[9] hydrogen sulfide,[3,4,8,10] hydrochloric acid,[10] thioacetamide[6] and sodium borohydride.[15] EDTA is one of the most widely employed copper sequestering reagents. However, the copper–EDTA complex formed during the course of the reaction is water soluble causing effluent problems with the water-soluble side-chain protected amino acids. Cleavage of copper from the complex using toxic potassium cyanide is hazardous, while using hydrogen sulfide needs prolonged heating in a highly acidic solution which leads to racemization of the amino acid, and a non-colloidal copper sulfide solution. The use of thioacetamide for the removal of copper requires a strongly acidic medium where migration of side-chain alkyl groups and the presence of colloidal copper sulfide restrict its wider use. When copper is removed from the complex employing 8-quinolinol, low yields of protected amino acids are obtained due to new copper(II)–quinolinate complex formation. Similarly, when copper is removed using a chelating ion exchange resin (chelex-100), regeneration of resin becomes laborious and the entire operation works out costly. Recent advances in the area of drug delivery have led to a

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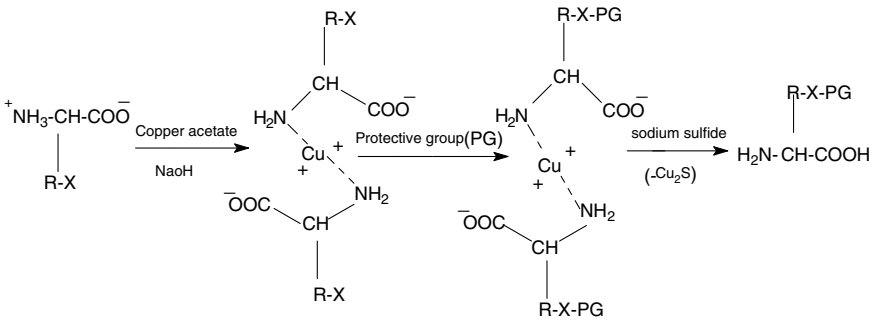

Scheme 1.

resurgence of research interest in the large-scale protection of peptide pharmaceuticals. Therefore, economically viable methods are desirable for the manufacture of amino acid derivatives. Detachment of copper from the complex using sodium borohydride is efficient; however, heat generation during bulk production of protected amino acids and on a few occasions the reduction of protected esters to alcohols are problems. In view of the limitations of the above copper(II) deprotection methods, there is a need to develop efficient copper deprotection routes to prepare protected amino acids. In this letter, we report a highly convenient and efficient method for the preparation of side-chain amino protected acetyl, phthalyl, tosyl, Boc, Fmoc, Z (benzyloxy carbonyl) and benzoyl ornithines and lysines. The γ/β-benzyl esters of glutamic acid/aspartic acid and benzyl ethers of tyrosine involve a copper(II) amino acid complex as the intermediate. A simple and cost effective reagent, sodium sulfide, is used to carry out the detachment of copper from the complex. The method involved in the preparation of the side-chain protected amino acids is shown in Scheme 1.

The copper complexes of the following amino acids were prepared by literature methods: N^{ε}-Boc-lysine,[7] N^{δ}-Boc-ornithine,[10] N^{ε}-Z-lysine and N^{δ}-Z-ornithine,[6] N^{ε}-tosyl-lysine,[4] N^{δ}-tosyl-ornithine,[16] N^{ε}-Fmoc-lysine and N^{δ}-Fmoc-ornithine,[17] *O*-benzyl tyrosine,[9] N^{δ}-acetyl-ornithine and N^{ε}-acetyl-lysine,[3] N^{δ}-phthalyl-ornithine and N^{ε}-phthalyl-lysine,[18] N^{δ}-benzoyl-ornithine and N^{ε}-benzoyl-lysine,[19] γ- and β-esters of aspartic acid and the γ-ester of glutamic acid.[12]

The procedure is illustrated by a typical example. To a stirred suspension of N^{ε}-Z-lysine–copper complex (1 g, 2.9 mmol) in water (30 ml) was added sodium sulfide (0.17 g, 2.18 mmol). After stirring for 10 min, the cuprous sulfide precipitate formed was filtered. The clear, colorless filtrate was neutralized with dilute HCl. On cooling, N^{ε}-Z-lysine precipitated and was filtered and washed with water to yield 0.75 g (93%). Other water insoluble products (Table 1, entries 2–7 and 9–17) were isolated in a similar manner. The water-soluble products (Table 1, entries 1 and 8) were isolated by removing the solvent and by extracting the residue with methanol.

Generally, the oxide salts of copper(I) are less soluble in water and precipitate more readily than copper(II) salts. Similarly, the sulfide of copper(I) easily precipitates out, either in acidic or basic medium. Therefore, sodium sulfide removed the copper from all types of amino acid complexes more readily than other methods. We employed sodium sulfide to sequester copper from the complex as cuprous sulfide.[20] We found that an aqueous solution of sodium sulfide is environment friendly, and evolves no hydrogen sulfide during the course of reaction. From Table 1 is clear that the sodium sulfide-mediated detachment of copper from all the copper amino acid complexes resulted in protected amino acids of high purity and in higher yields without any racemization.[21] We noted that in all the cases, cuprous sulfide precipitates out rapidly at room temperature (25–30 °C) without giving any colloidal solution at neutral pH. Thus, the supernatant liquid is colorless and the protected amino acid is obtained completely free of cuprous sulfide contamination. Removal of copper

Table 1. Preparation of side-chain protected lysine, ornithine, tyrosine, glutamic acid and aspartic acid from their complexes using sodium sulfide

Entry	Product[a]	Yield[b] (%)
1	H–Lys(Boc)–OH	93
2	H–Lys(Fmoc)–OH	96
3	H–Lys(Z)–OH	93
4	H–Lys(Ts)–OH	94
5	H–Lys(Benzoyl)–OH	82
6	H–Lys(Phthalyl)–OH	88
7	H–Lys(Acetyl)–OH	79
8	H–Orn(Boc)–OH	93
9	H–Orn(Fmoc)–OH	98
10	H–Orn(Z)–OH	96
11	H–Orn(Ts)–OH	94
12	H–Orn(Benzoyl)–OH	89
13	H–Orn(Phthalyl)–OH	83
14	H–Orn(Acetyl)–OH	79
15	H–Tyr(Bzl)–OH	93
16	H–Glu(OBz)–OH	84
17	H–Asp(OBz)–OH	87

[a] The HPLC and ^{1}H NMR data of the isolated products were identical to those of authentic samples.

[b] Isolated yields. Yields were calculated from the respective copper complexes.

requires neither acidic nor basic media. A significant difference between earlier detachment methods and the present sodium sulfide method is that the latter requires only 0.55–0.75 equiv of sodium sulfide for excellent yields of products.

In conclusion, we have found that sodium sulfide is a useful reagent for removing copper from amino acid copper complexes during the preparation of several side-chain protected amino acids. The method may also be employed for bulk production of side-chain protected amino acids.

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