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Sodium borohydride efficiently removes copper from amino acid–copper complexes

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Abstract—Sodium borohydride very efficiently removed copper from amino acid–copper complexes. The copper in the amino acid–copper complex was reduced to insoluble copper(I) oxide and the free amino acid was released in pure form. This method is rapid, nontoxic and inexpensive compared to the currently used methods.

In peptide chemistry the copper complex method is used frequently to prepare sidechain protected derivatives of various amino acids such as lysine,[1–5] ornithine,[4,6,7] tyrosine,[4,8], aspartic acid,[9,10] glutamic acid,[9,10] etc. In this method, the amino acid is reacted with a copper(II) ion to obtain a stable complex. In the next stage, the copper–amino acid complex is reacted with the acylating agent. Since the alpha amino group and the carboxylic group are bound to the copper ion, the acylating agent selectively reacts with the side chain functional group. Finally, copper is removed from the complex to recover the sidechain protected amino acid. In the cases of aspartic acid and glutamic acid, the copper complex method is used to selectively hydrolyze the alpha ester from a diester.[9,10]

Although there are several methods to remove copper from the amino acid–copper complex, these methods are far from satisfactory. Some of the methods reported in the literature to remove copper are based on the use of EDTA,[3,7,11] hydrogen sulfide,[1,2,6,8] thioacetamide,[4] potassium cyanide,[12] metal ion exchange resins,[5,9] 8-quinolinol,[7] hydrochloric acid,[8] hydrobromic acid,[13] etc.

The EDTA method is the most widely used. Apart from being expensive, the copper–EDTA complex formed during the reaction is water soluble which causes effluent problems. Further, if the product is water soluble, it is difficult to separate the amino acid from the copper–EDTA complex. Often, the product obtained from the EDTA method is slightly greenish due to incomplete removal of copper, and requires repeated EDTA treatment. In the hydrogen sulfide method, copper precipitates out as copper sulfide. The toxicity and unpleasant odour of hydrogen sulfide makes this method unsuitable. The thioacetamide method is based on hydrogen sulfide,[4] and shares the same limitations: also, thioacetamide is a suspected carcinogen.[14] The toxicity of the reagent and the effluent generated makes the cyanide method unfavorable. Furthermore, metal ion-exchange resins and 8-quinolinol are expensive. The use of hydrochloric acid or hydrobromic acid is also unsuitable since the protecting groups such as *t*-Boc, amide, etc., are not stable in acid medium. Thus, there is a need for the development of newer, safer, and efficient methods for the removal of copper from amino acid–copper complexes.

In general, copper salts are water-soluble in the oxidized state and are insoluble in the reduced state. Several analytical methods for estimating reducing substances are based on the precipitation of the reduced copper (e.g., Fehling method for sugars). Hence, we tested a number of reducing agents for their ability to remove copper from amino acid complexes by reducing it to an insoluble salt. We have found that an aqueous solution of sodium borohydride is an excellent and a nontoxic agent for removing copper from the amino acid complexes. We have applied this method for preparing derivatives of several amino acids. In all the cases, products were obtained in high yield and in high purity and without any racemization (Tables 1 and 2).

Keywords: Sodium borohydride; Copper–amino acid complex; Copper removal.
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Table 1. Preparation of sidechain protected lysine, ornithine, and tyrosine from their copper complex using sodium borohydride

Entry	Product[a]	Yield[b] (%)
1	H-Lys(Boc)-OH	90
2	H-Lys(Fmoc)-OH	89
3	H-Lys(*Z*)-OH	88
4	H-Lys(Tos)-OH	91
5	H-Orn(Boc)-OH	87
6	H-Orn(Fmoc)-OH	79
7	H-Orn(*Z*)-OH	88
8	H-Orn(Tos)-OH	91
9	H-Tyr(Bzl)-OH	91

[a] The HPLC and ^{1}H NMR data of the isolated products were identical to those of authentic samples.
[b] Isolated yields. Yields are calculated from the respective copper complexes and are given in %.

Table 2. Reaction of sodium borohydride with copper complexes of Asp(OMe)-OH and Glu(OMe)-OH

Entry	Starting material	Product[a]	Solvent	Yield[b] (%)
1	$[H\text{-}Glu(OMe)OH]_2Cu$	H-Glu(OMe)-OH	MeOH	96
2	$[H\text{-}Glu(OMe)OH]_2Cu$	H-Glu-5-OH	Water	73
3	$[H\text{-}Asp(OMe)OH]_2Cu$	H-Asp(OMe)-OH	MeOH	89
4	$[H\text{-}Asp(OMe)OH]_2Cu$	H-Asp-4-OH	Water	85

[a] The HPLC and ^{1}H NMR data of the isolated products were identical to those of authentic samples.
[b] Isolated yields. Yields are calculated from the respective copper complexes and are given in %.

The procedure is illustrated by a typical example. To a stirred suspension of N^{ε}-*Z*-lysine–copper complex (1 g, 4.7 mmol) in water (30 mL), was added sodium borohydride (0.18 g, 4.7 mmol). After stirring for 15 min, the copper(I) oxide 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 (0.6 g, 88%).

Other water insoluble products (entries 2,4,6,7,8 and 9, Table 1) were isolated in a similar manner. The water-soluble products (entries 1 and 5, Table 1) were isolated by removing the solvent and by extracting the residue with acetone.

The required copper complexes of the following amino acids were prepared by reported literature methods: N^{ε}-Boc-lysine (Scott et al.[5]), N^{δ}-Boc-ornithine (Wunsch et al.[8]), N^{ε}-*Z*-lysine and N^{δ}-*Z*-ornithine (Neuberger and Sanger[1]), N^{ε}-Tosyl-lysine (Roeske et al.[2]), N^{δ}-Tosyl-ornithine (Erlanger et al.[15]), N^{ε}-Fmoc-lysine and N^{δ}-Fmoc-ornithine (Younghee and Richard[16]), *O*-benzyl tyrosine (Wiejak et al.[7]), and the β-ester of aspartic acid and the γ -ester of glutamic acid (Prestidge et al.[10]).

Sodium borohydride also removed copper efficiently from the copper complexes of the β-ester of aspartic acid and the γ-ester of glutamic acid. However, the reaction went further and the esters underwent reduction to give the corresponding alcohols (Table 2). Interestingly, when the reaction was carried out in methanol, instead of water as solvent, no reduction was observed and the esters were obtained in good yields (Table 2).

In all the cases, the reaction was rapid and complete in 5–10 min at ambient temperature and also at 0 °C. The reaction requires alkaline pH. In neutral and in acidic pH the reaction did not take place. For maximum yields, 1–0.75 equiv of sodium borohydride is required, lower yields were obtained with less equivalents. In the case of lysine and ornithine derivatives, water was found to be the best solvent, when methanol was used as solvent, the reaction was incomplete even after 1 h. All the products were analyzed for their enantiomeric purity by chiral HPLC assays.[17] No racemization was found to occur during any of the reactions.

In conclusion, we have found that sodium borohydride is a useful reagent for removing copper from amino acid–copper complexes during the preparation of several sidechain protected amino acids. The method is rapid, safe, and convenient compared to existing methods.

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