THE USE OF SEQUESTERING AGENTS IN THE PREPARATION OF 
\( \varepsilon \)-ACYL-L-LYSINE AND \( \delta \)-ACYL-L-ORNITHINE DERIVATIVES*

By R. Ledger† and F. H. C. Stewart†

In peptide syntheses involving lysine it is frequently necessary to mask the 
\( \varepsilon \)-amino function of this amino acid with a suitable acyl protecting group. The 
preferential \( \varepsilon \)-acylation of lysine is usually accomplished by treatment of an aqueous 
solution of the amino acid cupric complex with the appropriate acid chloride, followed 
by removal of the copper as the insoluble sulphide using gaseous hydrogen sulphide. 
In this process the cupric ion forms a stable chelate complex with the \( \varepsilon \)-amino and 
carboxyl groups, thereby preventing acylation of the former. The \( \varepsilon \)-benzyloxy-
carbonyl,\(^1\) \( \varepsilon \)-p-nitrobenzyloxy carbonyl,\(^2\) \( \varepsilon \)-benzoyl,\(^3\) \( \varepsilon \)-p-toluenesulphonyl,\(^4\) and 
\( \varepsilon \)-formyl\(^5\) derivatives of L-lysine have been prepared by various modifications of this 
general method.

The use of hydrogen sulphide for decomposition of the \( \varepsilon \)-acyl cupric complexes 
is often unsatisfactory, particularly in larger scale preparations, owing to the low 
solubility of some \( \varepsilon \)-acyl-L-lysine derivatives in aqueous solution. Moreover, the 
procedure may involve prolonged boiling of the amino acid derivative with cupric 
sulphide, with the possibility of some racemization. For preparative purposes it has 
now been found that removal of the copper can be effected more conveniently by means 
of the sequestering agent ethylenediaminetetraacetic acid (EDTA), or, in certain 
circumstances, a chelating resin. In this connection it is noteworthy that Zahn and 
Pätzold\(^6\) have similarly used potassium cyanide to sequester cupric ions in the 
synthesis of an \( \varepsilon \)-peptide derivative of lysine. Both potassium cyanide and ethylene-
diamine were also examined as sequestering agents in the present work, but were not 
as generally satisfactory as EDTA.

For the preparation of \( \varepsilon \)-acyl-L-lysine derivatives which are sparingly soluble 
in cold water the cupric complex is dissolved in hot EDTA solution, whereupon the 
product crystallizes out on cooling, and the metal remains in solution as the EDTA 
complex (Method A). Alternatively, for preparing larger quantities of very slightly 
soluble \( \varepsilon \)-acyl compounds, the complex is dissolved in an acidic EDTA solution, 
which is then neutralized to precipitate the product (Method B). As would be expected 
the \( \delta \)-acyl derivatives of L-ornithine can also be prepared in the same way. The

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compounds which have been obtained using EDTA to decompose the cupric complexes are collected in Table 1, which includes two hitherto unreported \( p \)-bromobenzenesulphonyl derivatives.

In the case of acyl derivatives which are soluble in water, such as \( \varepsilon \)-formyl-\( L \)-lysine, a different procedure is adopted. An acidic solution of the cupric complex is treated with a chelating resin, and the \( \varepsilon \)-acylamino acid eluted with water (Method C). Citrulline\(^7\) (\( \delta \)-ureido-\( L \)-ornithine) has also been prepared by the resin method (Table 1).

**Table 1**

\( \varepsilon \)-ACYL-\( L \)-LYSINES AND \( \delta \)-ACYL-\( L \)-ORNITHINES

Rotations at concentration 2.0 in 2N HCl unless otherwise indicated.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Method</th>
<th>Overall Yield (%)</th>
<th>Yield from Complex (%)</th>
<th>Melting Point</th>
<th>([\alpha]_D)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \varepsilon )-Benzyloxy carbonyl-( L )-lysine</td>
<td>B</td>
<td>85</td>
<td>95</td>
<td>278–280(^\circ)</td>
<td>17.3</td>
<td>1</td>
</tr>
<tr>
<td>( \varepsilon )-p-Toluenesulphonyl-( L )-lysine</td>
<td>A</td>
<td>78</td>
<td>97</td>
<td>238 dec.</td>
<td>13.4</td>
<td>4</td>
</tr>
<tr>
<td>( \varepsilon )-Benzyloxyl-( L )-lysine</td>
<td>A</td>
<td>72</td>
<td>96</td>
<td>253–255</td>
<td>24.5</td>
<td>3</td>
</tr>
<tr>
<td>( \varepsilon )-p-Nitrobenzyloxy carbonyl-( L )-lysine</td>
<td>A</td>
<td>66</td>
<td>90</td>
<td>230–240</td>
<td>15.5</td>
<td>2</td>
</tr>
<tr>
<td>( \varepsilon )-p-Bromobenzenesulphonyl-( L )-lysine*</td>
<td>A</td>
<td>84</td>
<td>96</td>
<td>264–265</td>
<td>14.8</td>
<td>—</td>
</tr>
<tr>
<td>( \varepsilon )-Formyl-( L )-lysine</td>
<td>C</td>
<td>59</td>
<td>97</td>
<td>228 dec.</td>
<td>15.6(^\dagger)</td>
<td>5</td>
</tr>
<tr>
<td>( \delta )-Benzyloxy carbonyl-( L )-ornithine</td>
<td>B</td>
<td>86</td>
<td>94</td>
<td>254–255</td>
<td>17.8</td>
<td>8</td>
</tr>
<tr>
<td>( \delta )-p-Bromobenzenesulphonyl-( L )-ornithine(^\ddagger)</td>
<td>A</td>
<td>88</td>
<td>92</td>
<td>254–256</td>
<td>17.9(^\S)</td>
<td>—</td>
</tr>
<tr>
<td>Citrulline</td>
<td>C</td>
<td>73</td>
<td>96</td>
<td>228–230</td>
<td>19.5</td>
<td>7</td>
</tr>
</tbody>
</table>

* Found: C, 39.8; H, 4.8; Br, 21.7. Calc. for \( C_{12}H_{17}BrN_2O_4S \): C, 39.5; H, 4.7; Br, 21.9%.

\( ^{\dagger} \) Concentration 1.3 in saturated NaHCO\(_3\).

\( ^{\ddagger} \) Found: C, 37.6; H, 4.3; N, 8.2. Calc. for \( C_{11}H_{15}BrN_2O_4S \): C, 37.6; H, 4.3; N, 8.0%.

\( ^{\S} \) Concentration 1.0.

In all cases the yields obtained by both processes have exceeded 90\% based on the amount of copper complex, and the products are optically pure. The use of sequestering agents should be particularly useful for the introduction of \( \varepsilon \)-substituents which would be attacked chemically under the rather vigorous conditions of the hydrogen sulphide procedure.

**Experimental**

The microanalyses were carried out by the Australian Microanalytical Service, Melbourne. Melting points are uncorrected.

The copper complexes of known \( \varepsilon \)-acyl-\( L \)-lysine and \( \delta \)-acyl-\( L \)-ornithines were prepared as described in the literature, and the new \( p \)-bromobenzenesulphonyl derivatives by the method of Roeske et al.\(^4\) Three experimental procedures were used for the decomposition of the complexes, according to the solubility of the product.

\(^7\) Kurtz, A. C., *J. Biol. Chem.*, 1937, **122**, 477.

\(^8\) Barras, B. C., and Elmore, D. T., *J. Chem. Soc.*, 1957, **3134**.
Method A.—The cupric complex (0.005 mole) was dissolved in boiling EDTA solution (0.1N; 100 ml). On cooling the acylamino acid crystallized out; it was collected, washed with water and ethanol, and dried.

Method B.—The complex (0.005 mole) was dissolved in 2N HCl (50 ml), and EDTA solution (0.1N; 100 ml) added. The solution was then neutralized with 2N NaOH, and cooled. The precipitated product was treated as in Method A.

Method C.—A solution of the complex (0.0005 mole) in 0.5N HCl (2 ml) was applied to a column of Chelex 100 chelating resin (1 by 10 cm; H+ form), and the product eluted with water. The eluate was neutralized with 2N lithium hydroxide, and evaporated to small volume (c. 2 ml) in vacuo. Addition of ethanol (10 ml) precipitated the product, which was collected after storage overnight at 4°.

Method A is suitable for most small scale preparations. On a larger scale with more sparingly soluble acyl derivatives prohibitive volumes of solution would be necessary, and in these cases Method B is used. Method C is only required for water-soluble products. Details of the various derivatives prepared are given in Table 1.