Synthesis of Tricholomic Acid. V. 1) Synthesis of DL-Tricholomic Acid and Its three-Isomer from Diastereoisomers of β-Hydroxyglutamic Acid through α-Mono Esters 2) 

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Racemic tricholomic acid and its three-isomer (I) were prepared by a new route starting from the diastereoisomers of β-hydroxyglutamic acid (II) through α-mono esters (XI) and γ-benzyloxyamides (XIII).

In the preceding paper, 1) the synthesis of DL-tricholomic acid and its three-isomer (I) from β-hydroxyglutamic acid (II) via dialkyl β-chloroglutamate (III) and γ-hydroxamic acid (IV) was reported. Despite our efforts at further refinements of the reaction conditions, the result was not satisfactory from the view point of yield and stereospecificity. In this paper, first the discussion is given in regard to possible side reactions in the previous synthesis and then the synthesis of I by a new route is described.

![Chemical structure diagram]

Chart 1

Reaction of γ-methyl glutamate (VIII) with hydroxylamine under the same conditions as those previously employed in the transformation (III→IV) gave pyroglutamic acid (IX) in a high yield (86%), while the yield of the γ-hydroxamic acid (X) was less than 14%. This model reaction suggests that the formation of the pyrrolidine (VI) by an intramolecular cyclization would have predominated, thus leading to a principal side reaction in the previous synthesis. Moreover, III offers other possibilities to produce other by-products such as an α-hydroxamic acid (V) and a diketopiperazine (VII).

2) This work was presented at Meeting of Kinki Branch, Pharmaceutical Society of Japan, Osaka, June 1966 and published as a preliminary communication in Chem. Pharm. Bull. (Tokyo), 14, 1307 (1966).
3) Location: Jusonishino-cho, Higashiyodogawa-hu, Osaka.
In order to avoid the formation of these by-products a new synthetic route was designed, as shown in Chart 2.

\[
\begin{align*}
\text{HOOC-CH}_2\text{-CH-CH-COOR} & \xrightarrow{ZCl, \text{NaHCO}_3} \text{HOOC-CH}_2\text{-CH-CH-COOR} \\
\text{CH}_3 & \quad \text{CH}_3 \\
\text{Xa} : R = \text{CH}_3 & \quad \text{Xa} : R = \text{CH}_3 \\
b : R = \text{C}_2\text{H}_5 & \quad b : R = \text{C}_2\text{H}_5 \\
c : R = \text{CH}_3 & \quad c : R = \text{CH}_3 \\
\text{C}_6\text{H}_5\text{CH}_2\text{ONHOC-CH}_2\text{-CH-CH-COOR} & \xrightarrow{R'SO_2Cl, \text{Py}} \text{C}_6\text{H}_5\text{CH}_2\text{ONHOC-CH}_2\text{-CH-CH-COOR} \\
\text{R'SO}_2O & \quad \text{R'SO}_2O \\
\text{NHZ} & \quad \text{NHZ} \\
\text{XIVA} : R = \text{CH}_3, R' = \text{CH}_3 & \quad \text{XIVA} : R = \text{CH}_3 \\
b : R = \text{C}_2\text{H}_5, R' = \text{CH}_3 & \quad b : R = \text{C}_2\text{H}_5 \\
c : R = \text{CH}_3, R' = \text{CH}_3 & \quad c : R = \text{CH}_3 \\
d : R = \text{CH}_3, R' = \text{p-CH}_3\text{C}_6\text{H}_4 & \quad d : R = \text{CH}_3 \\
e : R = \text{C}_2\text{H}_5, R' = \text{p-CH}_3\text{C}_6\text{H}_4 & \quad e : \text{erythro}, t : \text{threo}, Z : \text{C}_6\text{H}_5\text{CH}_2\text{OCO}, \text{DCCI} : \text{C}_9\text{H}_{13}N = \text{C}=\text{NC}_4\text{H}_{11} \\
\text{H}_2/Pd \\
\text{HONHOC-CH}_2\text{-CH-CH-COOR} & \xrightarrow{\text{Et}_3\text{N}} \text{CH}_3\text{-CH-CH-COOR} \xrightarrow{1\text{n} \text{NaOH}} \text{CH}_3\text{-CH-CH-COOH} \\
\text{R'SO}_2O & \quad \text{R'SO}_2O \\
\text{NH}_2 & \quad \text{NH}_2 \\
\text{XVA} : R = \text{CH}_3, R' = \text{CH}_3 & \quad \text{XIVA} : R = \text{CH}_3 \\
b : R = \text{C}_2\text{H}_5, R' = \text{CH}_3 & \quad b : R = \text{C}_2\text{H}_5 \\
c : R = \text{CH}_3, R' = \text{CH}_3 & \quad c : R = \text{CH}_3 \\
d : R = \text{CH}_3, R' = \text{p-CH}_3\text{C}_6\text{H}_4 & \quad d : R = \text{CH}_3 \\
e : R = \text{C}_2\text{H}_5, R' = \text{p-CH}_3\text{C}_6\text{H}_4 & \quad \text{e: erythro, t: threo, Z: C}_6\text{H}_5\text{CH}_2\text{OCO, DCCI: C}_9\text{H}_{13}N=C=\text{NC}_4\text{H}_{11} \\
\text{Chart 2}
\end{align*}
\]

Preparation of the \( \alpha \)-halesters (XI) was done in accordance with the method for the synthesis of glutamic acid \( \alpha \)-esters.4 Dialkyl \( \beta \)-hydroxyglutamates (XVII) were treated with tritylchloride and triethylamine to give N-tritylamino acid diesters (XVIII), which were partially hydrolyzed with \( 1\text{n} \) sodium hydroxide on an equivalent molar basis. The yield of the partial hydrolysis was high due to steric effect of the bulky trityl group, and the removal of the trityl group was effected easily by warming with 50% acetic acid.

\[
\begin{align*}
\text{ROOC-CH}_2\text{-CH-CH-COOR} & \xrightarrow{\text{TRICI, Et}_3\text{N}} \text{ROOC-CH}_2\text{-CH-CH-COOR} \\
\text{OH} & \quad \text{OH} \\
\text{NH}_2-HCl & \quad \text{NH}-\text{TRI} \\
\text{XVIIa} : R = \text{CH}_3 & \quad \text{XVIIa} : R = \text{CH}_3 \\
b : R = \text{C}_2\text{H}_5 & \quad b : R = \text{C}_2\text{H}_5 \\
c : R = \text{CH}_3 & \quad c : R = \text{CH}_3 \\
\text{HOOC-CH}_2\text{-CH-CH-COOR} & \xrightarrow{50\% \text{AcOH}} \text{NaOOOC-CH}_2\text{-CH-CH-COOR} \\
\text{OH} & \quad \text{OH} \\
\text{NH}_2 & \quad \text{NH} \\
\text{Xa} : R = \text{CH}_3 & \quad \text{Xa} : R = \text{CH}_3 \\
b : R = \text{C}_2\text{H}_5 & \quad b : R = \text{C}_2\text{H}_5 \\
c : R = \text{CH}_3 & \quad c : R = \text{CH}_3 \\
\text{TRI: (C}_6\text{H}_{13})_3\text{C} \\
\text{Chart 3}
\end{align*}
\]

The structure of XI was confirmed by elemental analysis, paper chromatography (PPC) \(^5\) and more exactly by nuclear magnetic resonance (NMR) studies described later.

These \(\alpha\)-mono esters were converted to the \(N\)-carbobenzyloxy compounds (XII) by the procedure used for the synthesis of \(N\)-carbobenzyloxytheonine. \(^6\) Condensation of XII and \(O\)-benzylhydroxylamine by the action of \(N,N'\)-dicyclohexylcarbodiimide (DCCI) \(^7\) gave \(\gamma\)-benzyloxyamides (XIII). Of the two diastereoisomers of XIII, the \(\text{erythro}\)-isomers were easily obtained as crystals, while the \(\text{threo}\)-isomer was obtained as an oily substance which was successfully crystallized by purification with silicagel.

All the \(\gamma\)-benzyloxyamides (XIIIa–c) were then converted to the mesylates (XIVA–c) or the tosylates (XIVd, e) by the usual procedure, and their structures were confirmed by the NMR spectra (Fig. 1).

\[
\text{XIVA: } R - CH_3, \quad R' - CH_3, \quad e \\
\text{XIVb: } R = CH_2CH_2, \quad R' = CH_3, \quad e' e' f \\
\text{XIVc: } R = CH_3, \quad R' = CH_3, \quad e \\
\text{XIVd: } R = CH_3, \quad R' = p - CH_2C_6H_4, \quad e \\
\text{XIVE: } R = CH_2CH_2, \quad R' = p - CH_3C_6H_4, \quad e \\
\]

Fig. 1. Nuclear Magnetic Resonance Spectra of XIV in CDCl\(_3\) at 100 Mc

The catalytic hydrogenolysis of XIVe by the method of Plattner, et al. \(^7\) (palladium black in ethanol, in the presence of one mole equivalent of hydrobromic acid at room temperature), somewhat surprisingly, led to a diester (XIX), the structure of which was identified by the elemental analysis, infrared (IR) spectrum and color reactions. \(^8\) In the absence of hydrobromic acid, however, the catalytic hydrogenolysis (palladium black in aqueous methanol at 10–15 \(^\circ\)) gave XVe in a high yield. Each of five benzyloxyamides (XIVA–e) was subjected to this modified hydrogenolysis, and to cyclization with triethylamine, followed by hydrolysis

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{ONHOC-CH}_2-\text{CH}-\text{CH}-\text{COOC}_2\text{H}_4 & \quad \xrightarrow{H_2/Pd} \quad \text{C}_6\text{H}_5\text{OOC-CH}_2-\text{CH}-\text{CH}-\text{COOC}_2\text{H}_4 \\
\text{p-CH}_3\text{C}_6\text{H}_4\text{SO}_3\text{H} & \quad \xrightarrow{HBr-\text{EtOH}} \quad \text{p-CH}_3\text{C}_6\text{H}_4\text{SO}_4\text{H} \\
& \quad \text{NH}_3 \\
\end{align*}
\]

Chart 4

---

5) XIA, b, c are located between II and XVII on the chromatogram.
8) Ninhydrin reaction is positive, while FeCl\(_3\) reaction is negative.
of the α-ester without isolation of intermediates (XV, XVI) to give α-amino-3-oxo-5-isoxazolidineacetic acid (I).

Isolation and identification of I were carried out by the procedures described in the preceding paper and by ion-exchange chromatography on Dowex–50 and the racemic diastereomers (Ia, b) of α-amino-3-oxo-5-isoxazolidineacetic acid were obtained from all sulfonates (XIVa—e) in about 10% yields without any noticeable effect of the substituents and the configuration of XIV (Table I). It was of interest to note that the cyclization of

<table>
<thead>
<tr>
<th>XIV</th>
<th>Ia (theo)</th>
<th>Yield (%)</th>
<th>Ib (erythro)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (erythro, R: CH₃, R': CH₃)</td>
<td>12.5</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>b (erythro, R: C₆H₅, R': CH₃)</td>
<td>12.7</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>c (theo, R: CH₃, R': CH₃)</td>
<td>2.1</td>
<td>12.8</td>
<td></td>
</tr>
<tr>
<td>d (erythro, R: CH₃, R': p-CH₃C₆H₄)</td>
<td>12.1</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>e (erythro, R: C₆H₅, R': p-CH₃C₆H₄)</td>
<td>12.2</td>
<td>1.6</td>
<td></td>
</tr>
</tbody>
</table>

the hydroxamic acids (XV) had brought for the inversion of the configuration at β-asymmetric carbon yielding erythro-I from theo-XIV and theo-I from erythro-XIV. The overall yield in the last three steps was not improved by the kind of sulfonate under various reaction conditions.

NMR Spectra of the Mono Esters (XIIa, b, c)

The NMR spectra of XIIa, b, c provide a clear proof for their structures. For example, the ester signal of XIIa appeared as a singlet, while that of the corresponding dimethyl ester (XVIIa) as a doublet, and the chemical shift of the singlet was almost equal to that of the lower-field peak of the doublet as shown in Fig. 2. The same relationship was observed in the ethyl esters (XVIIb—XIIb) and the theo-esters (XVIIc—XIIc) (Table II). Since the singlet was assigned to the α-esters (XI), the higher field signal of the doublet observed with the diesters should be assigned to the γ-esters. Thus, the NMR spectra provided a useful criterion not only for the identification of α- and γ-esters of β-hydroxyglutamic acid but also for the purity test. Chemical shifts of various esters of α-amino-dicarboxylic acids are listed in Table II.

Fig. 2. Nuclear Magnetic Resonance Spectra of XIIa and XVIIa in D₂O at 60 Mc

9) Isolation and separation by ion-exchange chromatography on IR–120 and Dowex–1, and identification by PFC, paper electrophoresis (PE) and IR and NMR spectroscopies.

10) This procedure was useful for the separation of diastereomers of I.
TABLE II. Chemical Shifts of Various Esters of 
\(\alpha\)-Aminodicarboxylic Acids in D\(_2\)O

<table>
<thead>
<tr>
<th>Substance</th>
<th>(\alpha)-Ester</th>
<th>(\gamma)-Ester ((\beta)-Ester)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOOC-(\text{CH}_2)-(\text{CH})-(\text{CH})-COOCH(_3)</td>
<td>6.08</td>
<td>—</td>
</tr>
<tr>
<td>(\text{OH}) (\text{NH}_2)</td>
<td>6.06</td>
<td>6.20</td>
</tr>
<tr>
<td>CH(_2)OOC-(\text{CH}_2)-(\text{CH})-(\text{CH})-COOCH(_3)</td>
<td>((c))</td>
<td>8.62(^b)</td>
</tr>
<tr>
<td>(\text{OH}) (\text{NH}_2).HCl</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>(\text{OH})</td>
<td>8.63(^b)</td>
<td>8.70(^b)</td>
</tr>
<tr>
<td>C(_2)H(_2)OOC-(\text{CH}_2)-(\text{CH})-(\text{CH})-COOCH(_3)</td>
<td>((t))</td>
<td>6.06</td>
</tr>
<tr>
<td>(\text{OH}) (\text{NH}_2).HCl</td>
<td>6.03</td>
<td>6.17</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>6.25</td>
</tr>
<tr>
<td>CH(_2)OOC-(\text{CH}_2)-(\text{CH})-(\text{CH})-COOCH(_3)</td>
<td>((t))</td>
<td>6.11</td>
</tr>
<tr>
<td>(\text{OH}) (\text{NH}_2).HCl</td>
<td>—</td>
<td>6.20</td>
</tr>
<tr>
<td>CH(_2)OOC-(\text{CH}_2)-(\text{CH})-(\text{COOH})</td>
<td>—</td>
<td>6.12</td>
</tr>
</tbody>
</table>

\(^a\) \(s\) and \(t\) designate erythro and threo, respectively. \(^b\) Chemical shift of methyl proton.

Experimental\(^{11}\)

Reaction of \(\gamma\)-Methyl L-GLutamate (VIII) with Hydroxylamine—A solution of 110 mg of hydroxylamine hydrochloride and 190 mg of NaOH in 2 ml of H\(_2\)O was added dropwise under stirring at \(-5^\circ\) to a solution of 300 mg of VIII in 5 ml of EtOH. The mixture was stirred at \(-5^\circ\)—room temperature for 3 hr. The solution was diluted with H\(_2\)O and passed through a column of Amberlite IR-120 (H\(^+\)-form). The effluent and washings were combined and evaporated to give crystals, 168 mg (86\%), mp 161\(-162^\circ\) (EtOH-H\(_2\)O). IR spectrum of the crystal was identical with that of an authentic IX, mp 162\(-163^\circ\).\(^{13}\) Ninhydrin positive part of 5\% NH\(_4\)OH eluate was evaporated to a white solid (30 mg), which was identified as a mixture of X and L-glutamic acid by PPC.

General Preparation of N-Trityl \(\beta\)-Hydroxyglutamic Acid Dialkyl Ester (XVIII, see Table III)—To a suspension of 0.05 mole of XVII in 120 ml of dry CH\(_2\)Cl\(_2\) was added 11 g (0.11 mole) of triethylamine and 0.05 mole of tritylchloride, and the mixture was stirred for 5 hr at room temperature. Chloroform was added and the solution was washed with H\(_2\)O, dried over Na\(_2\)SO\(_4\), and evaporated \(in\) \(\text{vacuo}\) to dryness. The residue was recrystallized from MeOH to yield XVIII.

General Preparation of N-Trityl \(\beta\)-Hydroxyglutamic Acid \(\alpha\)-Ester (XI, see Table III)—To a hot solution of 0.02 mole of XI in 60 ml of MeOH was added one drop of phenolphthalein solution and 20 ml of 1\% NaOH. The red colored solution was refluxed for several minutes until the color had faded and evaporated \(in\) \(\text{vacuo}\). The residue was treated with 40 ml of 50\% AcOH for 20 min at about 50\(^\circ\) and white precipitates that separated were filtered, washed with H\(_2\)O. The filtrate and the washing were combined and concentrated to a crystalline residue, which was recrystallized from H\(_2\)O-EtOH to yield XI.

General Preparation of N-Carboxbenzoxyl-\(\beta\)-hydroxyglutamic Acid \(\alpha\)-Alkyl Ester (XII, see Table III)—To a solution of 0.01 mole of XI and 2 g of NaHCO\(_3\) in 30 ml of H\(_2\)O was added 2 ml of carbobenzoxychloride at 0\(^\circ\), and the mixture was stirred for 4 hr at 0\(^\circ\)—room temperature. The solution was washed with ether,

11) Melting points are uncorrected. Procedures and instrumentation used were the same as previously reported,\(^{10}\) unless otherwise indicated.

12) M. Bergmann and L. Zervas, \(Z.\) \(\text{Physiol. Chem.},\) \(221,\) 51 (1933).
### Table III. R'OC-CH₂-CH-CH-COOR

<table>
<thead>
<tr>
<th>R</th>
<th>R'</th>
<th>X</th>
<th>Configuration</th>
<th>mp (°C)</th>
<th>Yield (%)</th>
<th>IR (cm⁻¹)</th>
<th>Formula</th>
<th>Calcd. C</th>
<th>Calcd. H</th>
<th>Calcd. N</th>
<th>Found C</th>
<th>Found H</th>
<th>Found N</th>
</tr>
</thead>
<tbody>
<tr>
<td>XVIIaCH₃CH₃TRI⁺</td>
<td>erythro</td>
<td>140.5–141</td>
<td>94</td>
<td>1730, 1747</td>
<td>C₁₂H₁₅O₂N</td>
<td>72.03</td>
<td>6.28</td>
<td>3.23</td>
<td>71.97</td>
<td>6.14</td>
<td>3.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XVIIbC₆H₅C₆H₅TRI⁺</td>
<td>erythro</td>
<td>98–99</td>
<td>84.5</td>
<td>1730, 1743</td>
<td>C₁₂H₁₅O₂N</td>
<td>72.36</td>
<td>6.77</td>
<td>3.04</td>
<td>72.58</td>
<td>6.57</td>
<td>3.25</td>
<td></td>
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</tr>
<tr>
<td>XVIIcC₆H₅CH₃TRI⁺</td>
<td>threo</td>
<td>146.5</td>
<td>89</td>
<td>1726, 1745</td>
<td>C₁₂H₁₅O₂N</td>
<td>72.03</td>
<td>6.28</td>
<td>3.23</td>
<td>71.98</td>
<td>6.25</td>
<td>3.27</td>
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<tr>
<td>XIαC₆H₅HHErythro</td>
<td>181 (d.ᵃ)</td>
<td>93.5</td>
<td>1748</td>
<td>C₁₆H₁₅O₂N</td>
<td>40.68</td>
<td>6.26</td>
<td>7.91</td>
<td>40.87</td>
<td>6.35</td>
<td>7.78</td>
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<tr>
<td>XIbC₆H₅HHErythro</td>
<td>165.5–166 (d.)</td>
<td>96</td>
<td>1743</td>
<td>C₁₆H₁₅O₂N</td>
<td>43.97</td>
<td>6.85</td>
<td>7.32</td>
<td>44.06</td>
<td>6.76</td>
<td>7.34</td>
<td></td>
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<tr>
<td>XIcC₆H₅HHTheiro</td>
<td>163 (d.)</td>
<td>72</td>
<td>1745</td>
<td>C₁₆H₁₅O₂N</td>
<td>40.68</td>
<td>6.26</td>
<td>7.91</td>
<td>40.58</td>
<td>6.35</td>
<td>7.93</td>
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<tr>
<td>XIIαC₆H₅HZ⁺</td>
<td>erythro</td>
<td>120.5</td>
<td>90</td>
<td>1726</td>
<td>C₁₆H₁₅O₂N</td>
<td>54.01</td>
<td>5.51</td>
<td>4.50</td>
<td>54.23</td>
<td>5.47</td>
<td>4.50</td>
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<tr>
<td>XIIbC₆H₅HHZ⁺</td>
<td>erythro</td>
<td>108</td>
<td>90</td>
<td>1723</td>
<td>C₁₆H₁₅O₂N</td>
<td>55.58</td>
<td>5.89</td>
<td>4.31</td>
<td>55.61</td>
<td>5.78</td>
<td>4.13</td>
<td></td>
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</tr>
</tbody>
</table>

ᵃ) TRI: (C₆H₅)₂C; ²: C₆H₅CH₆CO, d.: decomp.
ᵇ) A crude product contaminated with β-hydroxyglutamic acid was isolated, however, the pure diester was obtained by chromatography on IRC-50 (6.5% acetate).

### Table IV. C₆H₅CHONHOC-CH₂-CH-CH-COOR

<table>
<thead>
<tr>
<th>R</th>
<th>R'</th>
<th>Configuration</th>
<th>mp (°C)</th>
<th>Yield (%)</th>
<th>r-Value (100 Mc, CDCl₃)</th>
<th>Formula</th>
<th>Calcd. C</th>
<th>Calcd. H</th>
<th>Calcd. N</th>
<th>Found C</th>
<th>Found H</th>
<th>Found N</th>
</tr>
</thead>
<tbody>
<tr>
<td>XIIIaCH₃H</td>
<td>erythro</td>
<td>87–89</td>
<td>91.5</td>
<td>—</td>
<td>C₁₆H₁₅O₂N</td>
<td>60.57</td>
<td>5.81</td>
<td>6.73</td>
<td>60.45</td>
<td>6.06</td>
<td>7.04</td>
<td></td>
</tr>
<tr>
<td>XIXbC₆H₅H</td>
<td>erythro</td>
<td>106</td>
<td>83</td>
<td>—</td>
<td>C₁₆H₁₅O₂N</td>
<td>61.38</td>
<td>6.09</td>
<td>6.51</td>
<td>61.57</td>
<td>6.35</td>
<td>6.18</td>
<td></td>
</tr>
<tr>
<td>XIIIC⁺</td>
<td>C₆H₅H</td>
<td>threo</td>
<td>110–112</td>
<td>36</td>
<td>5.04, 5.23, 6.45, —</td>
<td>C₁₆H₁₅O₂N</td>
<td>60.57</td>
<td>5.81</td>
<td>6.73</td>
<td>60.80</td>
<td>5.75</td>
<td>6.77</td>
</tr>
<tr>
<td>XIVA⁺</td>
<td>C₆H₅Ms⁻</td>
<td>erythro</td>
<td>139–140</td>
<td>85</td>
<td>5.00, 5.25, 6.33, 7.07</td>
<td>C₁₆H₁₅O₂NS</td>
<td>53.43</td>
<td>5.30</td>
<td>5.69</td>
<td>53.23</td>
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<td>5.57</td>
</tr>
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<td>XIVb⁺</td>
<td>C₆H₅Ms⁻</td>
<td>erythro</td>
<td>106–110</td>
<td>83</td>
<td>4.99, 5.23, 8.78—a</td>
<td>C₁₆H₁₅O₂NS</td>
<td>54.32</td>
<td>5.52</td>
<td>5.51</td>
<td>54.07</td>
<td>5.52</td>
<td>5.50</td>
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<tr>
<td>XIVc⁺</td>
<td>C₆H₅Ms⁻</td>
<td>threo</td>
<td>108</td>
<td>84</td>
<td>5.02, 5.21, 6.43, 7.10</td>
<td>C₁₆H₁₅O₂NS</td>
<td>53.43</td>
<td>5.30</td>
<td>5.69</td>
<td>53.38</td>
<td>5.26</td>
<td>5.58</td>
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<tr>
<td>XIVd⁺</td>
<td>C₆H₅Ts⁻</td>
<td>erythro</td>
<td>120–121.5</td>
<td>85</td>
<td>5.04, 5.30, 6.41, 7.71-a</td>
<td>C₁₆H₁₅O₂NS</td>
<td>58.93</td>
<td>5.50</td>
<td>4.91</td>
<td>59.05</td>
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<td>4.74</td>
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<tr>
<td>XIVE⁺</td>
<td>C₆H₅Ts⁻</td>
<td>erythro</td>
<td>94</td>
<td>—</td>
<td>5.03, 5.30, 8.82—a</td>
<td>C₁₆H₁₅O₂NS</td>
<td>59.57</td>
<td>5.52</td>
<td>4.79</td>
<td>59.43</td>
<td>5.31</td>
<td>4.58</td>
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</table>

ᵃ) Oily raw product was purified by chromatography on silica gel (benzene-EtOH; 8:2) and recrystallized from benzene.
ᵇ) Ms.: CH₃SO⁺, Ts. = p-CH₃C₆H₄SO₃⁻.
ᶜ) Chemical shift of methyl protons; triplet
ᵈ) Chemical shift of methyl protons; singlet
acidified to pH 3 with 6N HCl and extracted with ether. The extract, dried over Na₂SO₄, was condensed to a white crystalline residue, which was recrystallized from CHCl₃-CCl₄-ether to yield XII.

General Preparation of N-Carbobenzyloxy-β-hydroxyglutamic Acid α-Alkyl Ester γ-Benzoylamide (XIII, see Table IV)—A solution of 0.03 mole of DCCI in 25 ml of tetrahydrofuran was slowly added to a stirred solution of 0.03 mole of XII and 0.033 mole of O-benzylhydroxylamine in 100 ml of tetrahydrofuran. The mixture was stirred for 6 hr at room temperature and the precipitated dicyclohexylurea was removed by filtration. Evaporation of the filtrate under reduced pressure yielded a crystalline residue. The residue was recrystallized from ether to yield XIII.

General Preparation of N-Carbobenzyloxy-β-mesyloxy- (or β-tosyloxy-) glutamic Acid α-Alkyl Ester γ-Benzoylamide (XIV, see Table IV)—To a solution of 0.01 mole of XIII in 20 ml of dry pyridine was added 0.01 mole of mesyl chloride (or tosyl chloride) and the solution was allowed to stand at 5° overnight. The solution was treated with 200 g of ice-water and extracted with three 100 ml portions of CH₂Cl₂. The extract was washed with H₂O, dried over Na₂SO₄, and condensed to a heavy syrup under reduced pressure. The residue was crystallized from MeOH to yield XIV.

Catalytic Hydrogenolysis of XVe by the Conventional Procedure—To a solution of 552 mg of XVe in 20 ml of EtOH was added 0.18 ml of 47% HBr and 30 mg of palladium black, and the reaction mixture was subjected to catalytic reduction at room temperature for 16 hr according to the conventional procedure. The reaction mixture was filtered and the filtrate was condensed to 8 ml under reduced pressure. One half of the concentrate was left standing in a refrigerator overnight to yield 100 mg of XIX, mp 133—134°. Anal. Calcd. for C₄₂H₃₄O₊₂NS: C, 51.46; H, 6.21; O, 29.99; N, 3.75. Found: C, 51.32; H, 5.65; O, 29.86; N, 4.23. The other half of the concentrate was subjected to cyclization, hydrolysis and isolation according to the procedure described in the next experiment.

General Preparation of α-Amino-3-oxo-5-isoxazolidineacetic Acid (I, see Table I)—To a solution of 2 g of XIV in 160 ml of MeOH-H₂O (5:1) was added 150 mg of Pd-black, and the reaction mixture was subjected to catalytic reduction at 15° for 3 hr. After the reaction had completed, the catalyst was removed by filtration and the solution was concentrated under reduced pressure. The aqueous solution thus obtained was treated with 10 ml of Et₂N at 0° and stirred for 3 hr, during which the reaction temperature raised to 18°. The solution was stirred for further 2 hr at room temperature after addition of 10 ml of 1N NaOH. Excess Et₂N was removed by evaporation under reduced pressure at low temperature and the resulting solution was passed through a column (3 x 15 cm) of ion-exchanger (Amberlite IR-120, H-type). After being washed with H₂O, the column was eluted with 0.5N NH₄OH. The eluate was concentrated under reduced pressure keeping the temperature below 50°. The residue (ca. 200 mg) was chromatographed on a column (4 x 120 cm) of Dowex-1 ion-exchanger (200—400 mesh) with 0.5N AcOH as solvent in an automated fraction collector. Each fraction (15 ml) was examined by ninhydrin reaction. Ninhydrin positive by-products were collected in fractions 10 to 16, and a diastereomeric mixture of I in fractions 18 to 26. The fractions 18 to 26 were combined and condensed to a crystalline residue under reduced pressure.

Diastereoisomers of I in the mixture were separated by a column (4.5 x 130 cm) of Dowex-50 ion-exchanger (200—400 mesh) with the eluent of 0.1 mole pyridine—acetate buffer (pH 3.1). The identity of each diastereoisomer with authentic sample was established by the mixed melting point determination, IR-spectroscopy, PPC, PE and the automatic amino acid analysis.