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A New Approach for the Total Synthesis of L-γ-Carboxyglutamic Acid: Utility of Ruthenium Tetroxide Oxidation

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A new and convenient synthesis of optically pure L-γ-carboxyglutamic acid (L-Gla) (**1**) from L-proline as a chiral source was developed. Protection of *N-tert*-butyloxycarbonyl (Boc) prolinol (**4**) with a *tert*-butyldimethylsilyl group followed by oxidation with ruthenium tetroxide (RuO_4) afforded the corresponding lactam compound (**6**), which was carboxylated with lithium diisopropylamide and benzyloxycarbonylimidazole to afford the 4-benzyloxycarbonyl lactam derivative (**7**). Selective removal of the silyl group from **7**, followed by oxidation of the resulting alcohol (**8**) with pyridinium dichromate gave the carboxylic acid, which was converted into the ester derivatives (**10b—d**). Cleavage of the lactam bond of **10b** with excess benzyl alcohol in the presence of triethylamine gave γ,γ,α-tribenzyl *N*-Boc-L-γ-carboxyglutamate (**11**). Finally, **11** was hydrogenated over Pd on charcoal and deprotected with trifluoroacetic acid to produce L-γ-carboxyglutamic acid (**1**).

Keywords——L-γ-carboxyglutamic acid; ruthenium tetroxide oxidation; carboxylation; L-proline; L-prolinol; 1-benzyloxycarbonylimidazole; 4-benzyloxycarbonyl-1-*tert*-butyloxycarbonyl-L-pyroglutamic acid ester; catalytic hydrogenation; trifluoroacetic acid deprotection

In 1974, L-γ-carboxyglutamic acid (L-Gla) (**1**), a new type of tricarboxylic amino acid, was found in vitamin K-dependent blood clotting proteins such as prothrombin[1] and factor X.[2] Gla units are thought to be implicated in the formation of calcium-binding sites[3] on prothrombin. Since then, several synthetic approaches[4] to DL-Gla have been described. Recently, Danishefsky *et al.*[5] reported the first synthesis of optically active L-Gla using (+)-glutamic acid as a chiral source.

In this paper, we wish to describe a new and convenient synthesis of optically pure L-Gla (**1**) by an alternative route starting from L-proline (**2**). The key intermediate in our synthetic plan for L-Gla is the optically pure L-pyroglutamic acid derivative (**10**), because it possesses three protecting groups at the *C*- and *N*-terminal positions and the side-chain carboxylic acid moiety and can be easily ring-opened to yield glutamic acid derivatives. Our synthetic strategy for **10** includes the regioselective construction of a lactam carbonyl function at the 5-position in the pyrrolidine ring of the prolinol derivative (**5**), derived from L-proline, and direct introduction of a carboxyl moiety at the 4-position of the resulting lactam (**6**). Therefore, the following route was developed (Chart 1).

Synthesis

The synthetic approach to L-Gla (**1**) started with L-proline. The formation of the methyl ester of L-proline (**2**) followed by *N*-protection with *tert*-butyloxycarbonyl (Boc) group gave *N*-Boc-proline methyl ester[6] (**3**). Selective reduction of the ester group of **3** with lithium borohydride ($LiBH_4$) in tetrahydrofuran (THF) afforded the corresponding alcohol[6] (**4**) in 92% yield. Subsequent protection of Boc-prolinol (**4**) with *tert*-butyldimethylsilyl chloride in *N*,*N*-dimethylformamide (DMF) in the presence of a catalytic amount of 4-dimethylaminopyridine[7] (4-DMAP) afforded *N*-Boc-2-(*tert*-butyldimethylsilyl)oxymethylpyrrolidine (**5**) in

98% yield. An efficient conversion of **5** into the lactam (**6**) was subsequently achieved by ruthenium tetroxide (RuO_4) oxidation[8,9] which had been developed for the transformation of *N*-protected cyclic amines to the corresponding lactams. Namely, oxidation of **5** with RuO_4

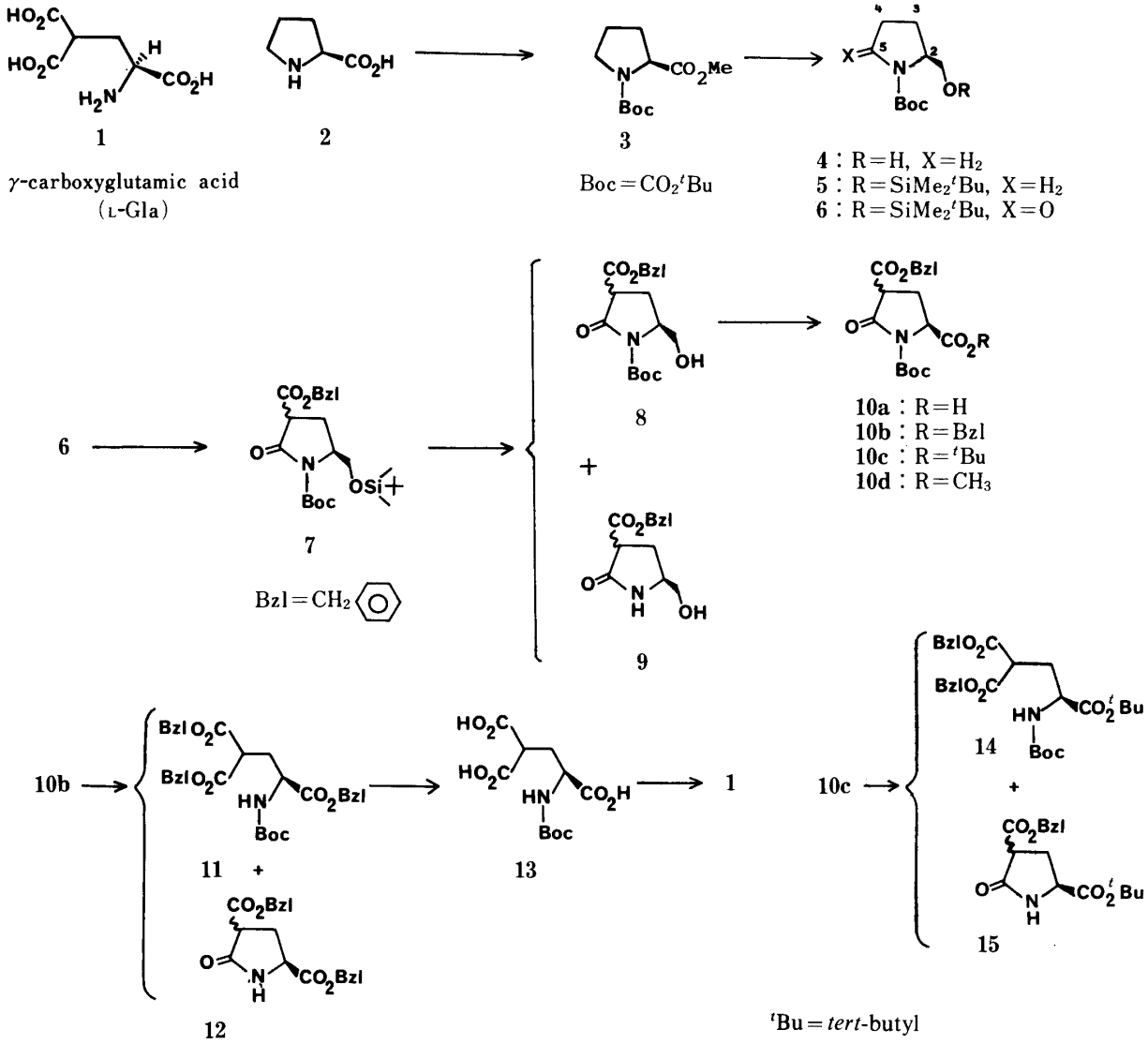
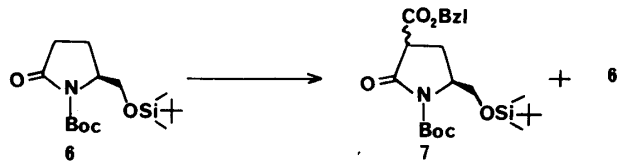


Chart 1

TABLE I. Preparation of (2*S*)-1-*tert*-Butyloxycarbonyl-4-benzyloxycarbonyl-2-(*tert*-butyldimethylsilyl)oxymethyl-5-pyrrolidone (7)



Carboxylating reagents (mole eq to **6**)	Yield (%) **7**	**6**
Z-Cl[a] (2.0)	25	45
Z-Im[b] (2.0)	65	20

a) Z-Cl: $C_6H_5CH_2O_2CCl$. *b*) Z-Im: $C_6H_5CH_2O_2C$-imidazole.

(catalytic amounts of RuO_2 hydrate-10% aqueous $NaIO_4$) in a two-phase system using ethyl acetate (AcOEt) gave the corresponding lactam derivative (**6**)[10] in 90% yield, regioselectively. The structure of **6** was established by the following data [carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum δ: 175.05 ppm (s, lactam carbonyl carbon). Mass spectrum (MS) *m/e*: 330 ($M^+ + 1$). Infrared (IR) spectrum: 1780 cm^{-1} (imide carbonyl)].

Next, we attempted to prepare the 4-benzyloxycarbonylprolinol derivative (**7**) from **6**, as a synthetic intermediate for Gla having a carboxylic acid side-chain at the 4-position. In order to introduce a carboxyl moiety to the 4-position, direct carboxylation *via* the lactam enolate of **6** was investigated.

Initially, treatment of **6** with lithium diisopropylamide (LDA) in THF at -78 °C for 1 h followed by reaction with benzyloxycarbonyl chloride (Z-Cl) afforded **6** and the 4-benzyloxycarbonylprolinol derivative (**7**) in 45% and 25% yields, respectively. However, when benzyloxycarbonylimidazole[11] (Z-Im) was used instead of Z-Cl, the yield of **7** was found to increase to 65%. The results are summarized in Table I. In the ^{13}C-NMR spectrum of **7**, the C_2 and C_4 carbon signals were split into two peaks: C_2 (δ: 57.23 and 57.37 ppm), C_4 (δ: 48.97 and 49.95 ppm). This splitting can be explained by the existence of two diastereoisomers, which could not be separated by normal chromatographic methods. The silyl group of **7** was selectively removed with *p*-toluenesulfonic acid (*p*-TsOH) in MeOH at room temperature for 3 h to give *N*-Boc-4-benzyloxycarbonyl-2-hydroxymethyl-5-pyrrolidone (**8**) and 4-benzyloxycarbonyl-2-hydroxymethyl-5-pyrrolidone (**9**) in 78% and 8% yields, respectively.

According to the procedure of Ohfune and Tomita,[10] the prolinol derivative (**8**) was easily converted into the proline derivative (**10**) without racemization. Thus, oxidation of **8** with pyridinium dichromate (PDC) in DMF at 40 °C for 15 h followed by esterification with alcohol and dicyclohexylcarbodiimide (DCC) in the presence of 4-DMAP afforded the corresponding ester derivatives (**10b—d**) in 70%—78% yields from **8**. Solvolysis of **10b** by heating with excess benzyl alcohol in the presence of triethylamine (TEA) at 105 °C for 24 h gave the tribenzyl ester (**11**) and the *N*-deprotected compound (**12**) in 69% and 10% yields, respectively. The three protective benzyl groups of **11** were removed by hydrogenolysis in the presence of 10% Pd on charcoal to give the *N*-Boc-γ-carboxylglutamic acid (**13**) in 85% yield.

Finally, deprotection of the Boc group of **13** by treatment with trifluoroacetic acid (TFA) in dichloromethane at 0—5 °C for 30 min gave the desired L-Gla (**1**), mp 156—158 °C; $[\alpha]_D^{25}$ +34.1° (c=1.0, 6N HCl). [lit.[5] mp 154—155 °C (dec); $[\alpha]_D$ +33.9° (c=1.2, 6N HCl)]. Furthermore, **1** was decarboxylated by refluxing with 6N hydrochloric acid to afford L-glutamic acid, and its specific rotation was in good agreement with that of authentic L-glutamic acid. The result indicates that racemization does not occur during the course of the reaction described above. In addition, in order to apply this methodology to the synthesis of peptides containing L-Gla units, we examined the solvolysis of **10c** having different protective groups at the α-carboxyl and the side-chain carboxyl groups. Solvolysis of **10c** by a procedure similar to that used for **10b** with benzyl alcohol in the presence of TEA afforded **14** and **15** in 63% and 9% yields, respectively.

Thus, we have developed a new and convenient synthesis of optically pure L-Gla from L-proline as a chiral source. This methodology for L-Gla should be applicable to the synthesis of peptides containing the L-Gla unit.

Experimental

All melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO IRA-2 or Hitachi 270-30 spectrometer. MS were measured on a JEOL JMS D-300 spectrometer. NMR spectra were obtained at 23 °C using tetramethylsilane as an internal standard with a JEOL JNM-MH-100 or a JEOL JNM-FX-100 spectrometer. Optical rotations were measured with a JASCO DIP-4 spectrometer. All organic extracts were dried over anhydrous sodium sulfate. Column chromatography was

performed on Merck silica gel (70-230 and 230-400 mesh).

Materials——L-Proline was obtained from Sigma Chemical Co. RuO_2 hydrate was obtained from Aldrich Chemical Co. *n*-Butyllithium (1.6 M in hexane solution) was obtained from Kanto Chemical Co., Inc. 1-Benzyloxycarbonylimidazole was prepared from imidazole and carbobenzyloxy chloride by the reported procedure.[11] THF was freshly distilled under nitrogen from lithium aluminum hydride. Diisopropylamine was distilled under nitrogen from calcium hydride and stored over 5A molecular sieves prior to use.

Methyl L-1-*tert*-Butyloxycarbonylprolinate (3)——This was prepared according to the reported procedure.[9]

L-1-*tert*-Butyloxycarbonylprolinol (4)——$LiBH_4$ (1.6 g, 73 mmol) was added portionwise to a stirred solution of **3** (12.0 g, 49 mmol) in THF (100 ml) at below 5 °C. After being stirred at room temperature for 15 h, the reaction mixture was cooled and then H_2O and 2 N HCl were added carefully. The whole was extracted with AcOEt. The extracts were washed with brine, dried, and concentrated *in vacuo* to leave an oil, which was purified by distillation to give **4** (9.1 g, 92%) as a colorless oil. bp 115 °C (1 mmHg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3425, 1680. MS *m/e*: 201 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.50 (9H, s, $C(CH_3)_3$), 1.55—2.24 (4H, m, C_3-H and C_4-H), 3.56 (2H, m, C_5-H), 3.73 (2H, d, J=7 Hz, CH_2O), 4.15 (1H, m, C_2-H), 4.65 (1H, br s, OH). $[\alpha]_D^{11}$ −64.8° (c=1.20, MeOH). [lit.[6] $[\alpha]_D^{20}$ −47.2° (c=1.0, MeOH)].

(2*S*)-1-*tert*-Butyloxycarbonyl-2-(*tert*-butyldimethylsilyl)oxymethylpyrrolidine (5)——*tert*-Butyldimethylsilyl chloride (5.8 g, 38 mmol) was added portionwise to a mixture of **4** (6.5 g, 32 mmol), TEA (3.8 g, 37 mmol) and 4-DMAP (0.2 g, 1.6 mmol) in DMF (50 ml). After being stirred at room temperature for 3 h, the reaction mixture was poured into ice-water and extracted with AcOEt. The extract was washed with 5% HCl and brine, dried and then concentrated *in vacuo*. The residue was purified by column chromatography on SiO_2 with AcOEt–hexane (1 : 1, v/v) as an eluent to give **5** (9.9 g, 98%) as a colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1684. MS *m/e*: 316 (M^+ + 1). ^{1}H-NMR ($CDCl_3$) δ: 0.05 (6H, s, $Si(CH_3)_2$), 0.92 (9H, s, $SiC(CH_3)_3$), 1.51 (9H, s, $CO_2C(CH_3)_3$), 1.68—2.16 (4H, m, C_3-H and C_4-H), 3.28—3.52 (2H, m, CH_2O), 3.60—3.92 (3H, m, C_2-H and C_5-H). *Anal.* Calcd for $C_{16}H_{33}NO_3Si$: C, 60.91; H, 10.54; N, 4.44. Found: C, 61.14; H, 10.39; N, 4.28. $[\alpha]_D^{11}$ −68.5° (c=1.20, MeOH).

(2*S*)-1-*tert*-Butyloxycarbonyl-2-(*tert*-butyldimethylsilyl)oxymethyl-5-pyrrolidone (6)——A solution of **5** (9.0 g, 28 mmol) in AcOEt (60 ml) was added to a mixture of RuO_2 hydrate (120 mg) and 10% aqueous $NaIO_4$ (150 ml). The mixture was vigorously stirred with a mechanical stirrer at room temperature for 1 h in a sealed flask. The organic layer was withdrawn and the aqueous layer was extracted with three 30-ml portions of AcOEt. The AcOEt solution was treated with isopropyl alcohol (2 ml) to destroy the RuO_4 oxidant. Black-colored RuO_2 which precipitated from the solution was filtered off and the filtrate was washed with H_2O, dried, and concentrated *in vacuo* to leave a brown oil, which was purified by column chromatography on SiO_2 with AcOEt–hexane (1 : 3, v/v) as an eluent to give **6** (8.5 g, 90%) as a colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1780, 1640. MS *m/e*: 330 (M^+ + 1). ^{1}H-NMR ($CDCl_3$) δ: 0.06 (6H, s, $Si(CH_3)_2$), 0.92 (9H, s, $SiC(CH_3)_3$), 1.59 (9H, s, $CO_2C(CH_3)_3$), 1.92—2.88 (4H, m, C_3-H and C_4-H), 3.75 and 4.01 (total 2H, AB part of ABX pattern, J=11, 3 Hz and J=11, 4 Hz, CH_2O), 4.16—4.33 (1H, m, C_2-H). ^{13}C-NMR ($CDCl_3$) δ: −5.57 (q, $Si(CH_3)_3$), 18.21 (s, $SiCMe_2{}^tBu$), 21.14 (t, C_3), 25.73 and 25.87 (each q, $SiC(\underline{C}H_3)_3$), 28.12 (q, $CO_2C(\underline{C}H_3)_3$), 32.37 (t, C_4), 58.98 (d, C_2), 64.45 (t, CH_2O), 82.71 (s, $CO_2\underline{C}Me_3$), 150.34 (s, $\underline{C}O_2CMe_3$), 175.05 (s, C_5). *Anal.* Calcd for $C_{16}H_{31}NO_4Si$: C, 58.32; H, 9.48; N, 4.25. Found: C, 58.54; H, 9.30; N, 4.18. $[\alpha]_D^{15}$ −72.5° (c=1.20, $CHCl_3$) [lit.[10] $[\alpha]_D^{25}$ −61° (c=1.1, $CHCl_3$)].

(2*S*)-1-*tert*-Butyloxycarbonyl-4-benzyloxycarbonyl-2-(*tert*-butyldimethylsilyl)oxymethyl-5-pyrrolidone (7)——Under a nitrogen atmosphere, a mixture of diisopropylamine (3.6 g, 36 mmol) and *n*-butyllithium (24 ml, 36 mmol) in THF (15 ml) was stirred at −78 °C (dry ice-acetone bath), then a solution of **6** (6.0 g, 18 mmol) in THF (15 ml) was added and the reaction mixture was stirred for 30 min. A solution of carbobenzyloxy chloride (36 mmol) in THF (10 ml) was added dropwise and the reaction mixture was stirred at −78 °C for 1 h and then at room temperature for 1 h. The mixture was quenched by addition of aqueous NH_4Cl and then extracted with AcOEt. The extract was washed with brine, dried, and concentrated *in vacuo* to leave a brown oil, which was purified by flash column chromatography on SiO_2 with AcOEt–hexane (1 : 4, v/v) as an eluent. From the earlier part of the eluate, **6** (2.7 g, 45%) was obtained. All spectral data were identical with those of the starting material **6**. $[\alpha]_D^{15}$ −72.3° (c=1.0, $CHCl_3$). From the later part, **7** (2.1 g, 25%) was obtained as a pale yellowish oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1790, 1734. MS *m/e*: 407 (M^+ + 1 − CMe_3), 363 (M^+ + 1 − Boc). ^{1}H-NMR ($CDCl_3$) δ: 0.04 (6H, s, $Si(CH_3)_2$), 0.89 (9H, s, $SiC(CH_3)_3$), 1.54 (9H, s, $CO_2C(CH_3)_3$), 2.10—2.66 (2H, m, C_3-H), 3.58—4.10 (3H, m, OCH_2 and C_4-H), 4.16—4.32 (1H, m, C_2-H), 5.19 (2H, s, CH_2Ar), 7.33 (5H, s, aromatic H). ^{13}C-NMR ($CDCl_3$) δ: −5.62 (q, $Si(CH_3)_2$), 18.21 (s, SiC), 25.68 (t, C_3), 25.88 (q, $SiC(\underline{C}H_3)_3$), 28.08 (q, $CO_2C(\underline{C}H_3)_3$), 48.97 and 49.95 (d, C_4), 57.23 and 57.37 (d, C_2), 64.30 (t, CH_2OSi), 67.33 (t, $CO_2\underline{C}H_2$), 83.35 (s, $CO_2\underline{C}(CH_3)_3$), 128.22, 128.37, 128.71 and 135.74 (aromatic C), 150.00 (s, $\underline{C}O_2C(CH_3)_3$), 169.14 (s, C_5 or $\underline{C}O_2CH_2Ar$), 169.43 (s, C_5 or $\underline{C}O_2CH_2Ar$). *Anal.* Calcd for $C_{24}H_{37}NO_6Si$: C, 62.17; H, 8.04; N, 3.02. Found: C, 62.48; H, 8.25; N, 3.26. $[\alpha]_D^{12}$ −42.5° (c=1.28, $CHCl_3$). By using carbobenzyloxyimidazole in place of carbobenzyloxy chloride in the procedure described above, **6** (1.2 g, 20%) and **7** (5.5 g, 65%) were obtained, respectively.

(2*S*)-1-*tert*-Butyloxycarbonyl-4-benzyloxycarbonyl-2-hydroxymethyl-5-pyrrolidone (8) and (2*S*)-4-Benzyloxycarbonyl-2-hydroxymethyl-5-pyrrolidone (9)——*p*-Toluenesulfonic acid (2.1 g, 10 mmol) was added to a solution of **7** (5.0 g, 10 mmol) in MeOH (20 ml) at room temperature, and the mixture was stirred for 2 h. The reaction mixture was made alkaline by adding sat. aq. $NaHCO_3$ under cooling and then concentrated *in vacuo*. After addition of H_2O

(20 ml), the whole was extracted with $CHCl_3$. The extract was washed with brine, dried, and concentrated *in vacuo* to leave an oil, which was purified by column chromatography on SiO_2 with AcOEt–hexane (1 : 1, v/v) as an eluent. From the earlier part of the eluate, **8** (2.9 g, 78%) was obtained. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1788, 1734. MS *m/e*: 350 ($M^+ + 1$). ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s, $C(CH_3)_3$), 2.00—2.60 (2H, m, C_3-H), 3.20—3.23 (1H, m, OH), 3.44—4.04 (3H, m, C_4-H and CH_2O), 4.08—4.18 (1H, m, C_2-H), 5.15 (2H, s, CH_2Ar), 7.06 (5H, s, aromatic H). ^{13}C-NMR ($CDCl_3$) δ: 23.97 and 25.34 (each t, C_3), 28.03 (q, $C(\underline{C}H_3)_3$), 49.02 and 49.66 (each d, C_4), 57.62 and 58.25 (each d, C_2), 63.82 and 64.45 (each t, CH_2OH), 67.48 and 67.82 (each t, $CO_2\underline{C}H_2$), 83.79 and 84.23 (each s, $CO_2\underline{C}(CH_3)_3$), 128.32, 128.46, 128.71, 135.35 and 135.59 (aromatic C), 150.09 and 150.68 (each s, $\underline{C}O_2C(CH_3)_3$), 168.95, 169.34, 169.48 and 169.92 (each s, C_5 and $\underline{C}O_2CH_2Ar$). *Anal.* Calcd for $C_{18}H_{23}NO_6$: C, 61.88; H, 6.64; N, 4.01. Found: C, 62.05; H, 6.39; N, 4.17. $[\alpha]_D^{15}$ −29.5° (c = 1.24, $CHCl_3$). From the later part, **9** (0.3 g, 8%) was obtained. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1780, 1736. MS *m/e*: 249 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 2.04—2.58 (2H, m, C_3-H), 3,30—3.78 (4H, m, CH_2OH and C_4-H), 4.00—4.18 (1H, m, C_2-H), 5.11 (2H, s, CH_2Ar), 7.26 (5H, s, aromatic H), 7.44 (1H, br s, NH). $[\alpha]_D^{25}$ −20.4° (c = 1.10, $CHCl_3$).

(2*S*)-1-*tert*-Butyloxycarbonyl-4-benzyloxycarbonyl-5-oxoproline Benzyl Ester (10b)——Pyridinium dichromate (11.2 g, 29 mmol) was added to a solution of **8** (3.0 g, 8.5 mmol) in DMF (15 ml) and the mixture was stirred at 40 °C for 15 h. After addition of H_2O (60 ml), the whole was extracted with $CHCl_3$. The extract was washed with 5% AcOH and brine, dried, and concentrated *in vacuo* to leave a brown oil (2.7 g), which was directly used in the esterification without purification. Benzyl alcohol (0.92 g, 8.5 mmol), DCC (1.8 g, 8.7 mmol) and 4-DMAP (0.1 g, 0.8 mmol) were added to an ice-cooled solution of the crude product obtained above in CH_2Cl_2 (20 ml). The mixture was stirred at room temperature for 3 h. The resulting *N,N*-dicyclohexylurea was filtered and the filtrate was washed with 5% AcOH and brine, dried, and concentrated *in vacuo* to leave a brown oil, which was purified by column chromatography on SiO_2 with AcOEt–hexane (1 : 2, v/v) as an eluent, to give **10b** (2.7 g, 70%) as a colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1794, 1735. MS *m/e*: 397 ($M^+ + 1 - CMe_3$), 353 ($M^+ + 1 - Boc$). ^{1}H-NMR ($CDCl_3$) δ: 1.41 (9H, s, $C(CH_3)_3$), 2.00—2.80 (2H, m, C_3-H), 3.44—3.72 (1H, m, C_4-H), 4.52—4.68 (1H, m, C_2-H), 5.04 and 5.11 (total 4H, each s, 2 × CH_2Ar), 7.19 and 7.24 (total 10H, each s, aromatic H). $[\alpha]_D^{25}$ −8.7° (c = 1.0, $CHCl_3$). By using ^{t}BuOH and MeOH in place of benzyl alcohol in the procedure described above, **10c** (74%) and **10d** (78%) were obtained, respectively.

10c: (74%). Colorless needles, mp 102—103 °C (AcOEt–isopropyl ether). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1796, 1740. MS *m/e*: 363 ($M^+ + 1 - CMe_3$), 319 ($M^+ + 1 - Boc$). ^{1}H-NMR ($CDCl_3$) δ: 1.41 and 1.44 (total 18H, each s, 2 × $C(CH_3)_3$), 1.88—2.61 (2H, m, C_3-H), 3.29—3.61 (1H, m, C_4-H), 4.17—4.33 (1H, m, C_2-H), 4.91 and 4.93 (total 2H, each s, CH_2Ar), 6.94 and 6.96 (total 5H, each s, aromatic H). *Anal.* Calcd for $C_{22}H_{29}NO_7$: C, 62.99; H, 6.97; N, 3.34. Found: C, 62.85; H, 7.00; N, 3.23. $[\alpha]_D^{25}$ −8.5° (c = 1.0, $CHCl_3$).

10d: (78%). A colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1784, 1730. MS *m/e*: 321 ($M^+ + 1 - CMe_3$), 277 ($M^+ + 1 - Boc$). ^{1}H-NMR ($CDCl_3$) δ: 1.57 (9H, s, $C(CH_3)_3$), 2.09—2.85 (2H, m, C_3-H), 3.65—3.85 (1H, m, C_4-H), 3.67 (3H, s, CO_2CH_3), 4.35—4.68 (1H, m, C_2-H), 5.16 and 5.19 (total 2H, each s, CH_2Ar), 7.27 and 7.30 (total 5H, each s, aromatic H). *Anal.* Calcd for $C_{19}H_{23}NO_7$: C, 60.47; H, 6.14; N, 3.71. Found: C, 60.26; H, 6.41; N, 3.59. $[\alpha]_D^{25}$ −9.8° (c = 1.0, $CHCl_3$).

Solvolysis of 10b and (2*S*)-1-*tert*-Butyloxycarbonyl-4-benzyloxycarbonyl-5-oxoproline *tert*-Butyl Ester (10c) with Benzyl Alcohol——General Procedure: A mixture of 1-*tert*-butyloxycarbonyl-4-benzyloxycarbonyl-5-oxoproline ester, benzyl alcohol and TEA was heated at 105 °C for 24 h. The excess benzyl alcohol was removed by distillation, and the residue was purified by column chromatography on SiO_2 with AcOEt–hexane as an eluent.

i) γ,γ,α-Tribenzyl *N*-*tert*-Butyloxycarbonyl-L-γ-carboxyglutamate (**11**) and (2*S*)-4-Benzyloxycarbonyl-5-oxoproline Benzyl Ester (**12**): In the general procedure, **10b** (2.5 g, 5.5 mmol), benzyl alcohol (50 ml), and TEA (0.4 ml) were used. The crude product, obtained after work-up as described above, was purified by column chromatography on SiO_2 with AcOEt–hexane (1 : 2, v/v) as an eluent. From the earlier part of the eluate, **11** (2.1 g, 69%) was obtained. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 1730. MS *m/e*: 561 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.40 and 1.42 (9H, each s, $C(CH_3)_3$), 1.94—2.66 (2H, m, β-H), 3.58 (1H, t, J = 7 Hz, γ-H), 4.26—4.56 (1H, m, α-H), 5.04 (1H, m, NH), 5.08 and 5.11 (total 6H, each s, 3 × CH_2Ar), 7.26 and 7.31 (total 15H, each s, 3 × aromatic H). *Anal.* Calcd for $C_{32}H_{35}NO_8$: C, 68.43; H, 6.28; N, 2.49. Found: C, 68.56; H, 6.42; N, 2.48. $[\alpha]_D^{25}$ +7.5° (c = 1.0, $CHCl_3$). From the later part, **12** (0.1 g, 10%) was obtained. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1740, 1722. MS *m/e*: 353 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 2.14—2.94 (2H, m, C_3-H), 3.30—3.60 (1H, m, C_4-H), 4.10—4.41 (1H, m, C_2-H), 5.10, 5.13 and 5.17 (total 4H, each s, 2 × CH_2Ar), 7.32 (11H, s, NH and aromatic H).

ii) *N*-(*tert*-Butyloxycarbonyl)-γ,γ-dibenzyl-L-γ-carboxyglutamic Acid α-*tert*-Butyl Ester (**14**) and (2*S*)-4-Benzyloxycarbonyl-5-oxoproline *tert*-Butyl Ester (**15**): In the general procedure, **10c** (0.5 g, 1.2 mmol), benzyl alcohol (20 ml), and TEA (0.1 ml) were used. The crude product, obtained after work-up as described above, was purified by column chromatography on SiO_2 with AcOEt–hexane (1 : 3, v/v) as an eluent. From the earlier part of the eluate, **14** (0.39 g, 63%) was obtained as a colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 1732. MS *m/e*: 527 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.41 and 1.44 (total 18H, each s, 2 × $C(CH_3)_3$), 1.92—2.68 (4H, m, β-H), 3.60 (1H, t, J = 7 Hz, γ-H), 4.12—4.44 (1H, m, α-H), 5.04 (1H, m, NH), 5.12 and 5.16 (total 4H, each s, 2 × CH_2Ar), 7.30 (total 10H, s, aromatic H). *Anal.* Calcd for $C_{29}H_{37}NO_8$: C, 66.02; H, 7.06; N, 2.66. Found: C, 65.87; H, 6.84; N, 2.79. $[\alpha]_D^{18}$ +8.3° (c = 1.0, $CHCl_3$). From the later part, **15** (0.04 g, 9%) was obtained. mp 124—125 °C. (Colorless needles from AcOEt–isopropyl ether). IR

$\nu_{max}^{CHCl_3}$ cm^{-1}: 3460, 1750, 1725. MS *m/e*: 319 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.54 (9H, s, $C(CH_3)_3$), 2.44—3.04 (2H, m, C_3-H), 3.52—3.84 (1H, m, C_4-H), 4.24—4.56 (1H, m, C_2-H), 5.50 (2H, s, CH_2Ar), 6.94 (1H, br s, NH), 7.82 (5H, s, aromatic H).

***N-tert*-Butyloxycarbonyl-L-γ-carboxyglutamic Acid (13)**——A solution of **11** (1.5 g, 2.7 mmol) in EtOH (20 ml) was hydrogenated over 10% Pd–C (0.2 g) at room temperature. The catalyst was removed by filtration. The filtrate was concentrated *in vacuo* to give **13** as a semi-solid. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3425, 1705. ^{1}H-NMR (acetone-d_6) δ: 1.46 (9H, s, $C(CH_3)_3$), 2.04—2.62 (2H, m, β-H), 3.50—3.85 (1H, m, γ-H), 4.14—4.46 (1H, m, α-H), 6.10 (1H, br d, J=10 Hz, NH), 9.46 (3H, br s, 3×CO_2H). *Anal.* Calcd for $C_{11}H_{17}NO_8$: C, 45.36; H, 5.88; N, 4.81. Found: C, 45.28; H, 5.71; N, 4.92. $[\alpha]_D^{25}$ −20.3° (*c*=1.0, MeOH).

L-γ-Carboxyglutamic Acid (1)——TFA (3 ml) was added to an ice-cooled solution of **13** (0.8 g, 1.4 mmol) in CH_2Cl_2 (5 ml) and the mixture was stirred at room temperature for 30 min. The reaction mixture was diluted with benzene (10 ml) and concentrated *in vacuo*. The residue was triturated with ether. The resulting powder was collected by filtration to give **1** (0.3 g, 90%). Recrystallization from H_2O–EtOH (1 : 1, v/v) afforded colorless prisms. mp 156—158 °C. $[\alpha]_D^{25}$ +34.1° (*c*=1.0, 6 N HCl). [lit.[5] mp 154—155 °C (dec). $[\alpha]_D$ +33.9° (*c*=1.2, 6 N HCl)]. IR ν_{max}^{KBr} cm^{-1}: 3000, 1690. ^{1}H-NMR (D_2O) δ: 2.26—2.51 (2H, m, β-H), 3.40 (1H, t, J=7 Hz, γ-H), 3.85—3.94 (1H, m, α-H). *Anal.* Calcd for $C_6H_9NO_6$: C, 37.70; H, 4.75; N, 7.33. Found: C, 37.52; H, 4.84; N, 7.26.

Decarboxylation of γ-Carboxyglutamic Acid——A solution of **1** (0.3 g, 1.5 mmol) in 6 N hydrochloric acid (10 ml) was refluxed for 5 h. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in H_2O (10 ml). The resulting solution was treated with powdered K_2CO_3 (0.75 mmol) under cooling. This mixture was allowed to stand at 5 °C for 20 h to give a colorless powder (0.2 g, 85%). mp 195—196 °C. $[\alpha]_D^{25}$ +30.9° (*c*=1.0, 5 N HCl). [lit.[12] $[\alpha]_D^{25}$ +32.2° (*c*=1.0, 5 N HCl)]. Its IR and NMR spectra were identical with those of authentic L-glutamic acid.

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