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Studies on the Synthesis and Resolution of γ-Carboxyglutamic Acid Derivatives[1,2]

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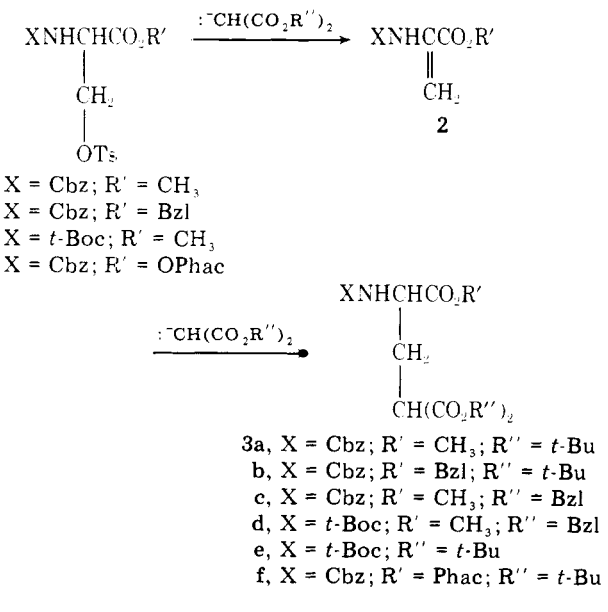
The synthesis and resolution of *N*-(benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamic acid (**5a**) is described. Resolution using quinine allowed separation of the D enantiomer from the racemic mixture in 15% yield from *N*-(benzyloxycarbonyl)-*O*-tosyl-*d,l*-serine methyl ester (**1a**). Liberation of the L enantiomer from the remaining oily quinine salt followed by purification of the L-tyrosine hydrazide salt of **5a** provided an overall yield of 13% of the L enantiomer from **1a**. The synthesis of *N*-(benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-D-γ-carboxyglutamyl-γ,γ-di-*tert*-butyl-D-γ-carboxyglutamic acid (**19**) is described.

Since the identification of γ-carboxyglutamic acid (Gla)[4] in 1974, the synthesis of Gla derivatives has been the target of several laboratories.[5-7] To date two synthetic approaches have yielded the desired amino acid. One route utilizes a procedure developed by Wheland[8] for the preparation of glutamic acid (Scheme I). As might be expected from similar syntheses of cysteine[9] and selenocysteine,[10] loss of leaving groups from the β carbon of alanine derivatives was accompanied by extensive racemization at the α-carbon atom.[5b,c] The second approach and the only reported asymmetric synthesis of Gla[5g] utilized a modified Strecker synthesis to afford γ,γ-di-*tert*-butyl-L(−)-*N*-phthaloyl-γ-carboxyglutamate. In spite of the high optical purity of the product obtained by this route, the primary source of optically pure Gla for further synthetic studies has been via Scheme I and classical resolution.[7] This report describes in detail our studies on the development of routes to optically pure Gla derivatives on a synthetically useful scale.

Results and Discussion

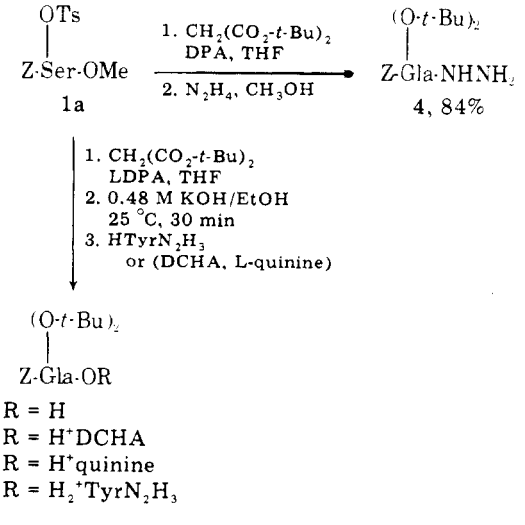
Synthesis. The preparation of the *O*-tosyl-L-serine derivatives (**1a–d**, Scheme I) was based on general procedures previously reported.[9a,b,10] The tosylation reaction was temperature dependent; reactions carried out below −5 °C were more successful. Although **1c** and **1d** were generated and used as in Scheme I, they were not easily purified without losses. The presence of pyridine particularly hampered purification.

Scheme I



Treatment of the appropriate *O*-tosyl-L-serine derivative with excess di-*tert*-butyl malonate using either sodium hydride or lithium diisopropylamide as the base provided the crude Gla derivatives. Of these esters, the *N*-(benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamic acid α-methyl ester (**3a**) and the *N*-(*tert*-butyloxycarbonyl)-γ,γ-dibenzyl-γ-methyl ester (**3d**) seemed to have properties appropriate for further study. In order to evaluate the stability of the amine triester, solutions of **3a** and **3d** were deblocked at the amino group with palladium and hydrogen and trifluoroacetic acid, respectively, followed by neutralization of the amine salt. The γ,γ-di-*tert*-butyl methyl ester resulting from **3a** exhibited a substantially longer lifetime (TLC, ninhydrin) than the dibenzyl methyl ester resulting from **3d**. Thus, **3a** was utilized in subsequent studies since the rate of apparent cyclization to the pyro-γ-carboxyglutamic acid derivative should be repressed relative to **3d**.

The triester, **3a**, was smoothly converted to the hydrazide, **4**. Hydrazinolysis could be performed on the crude reaction mixture resulting from the reaction of excess di-*tert*-butyl



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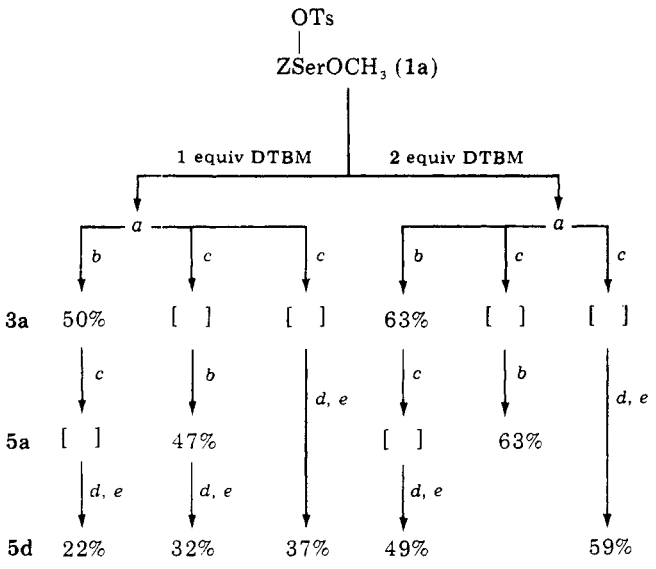
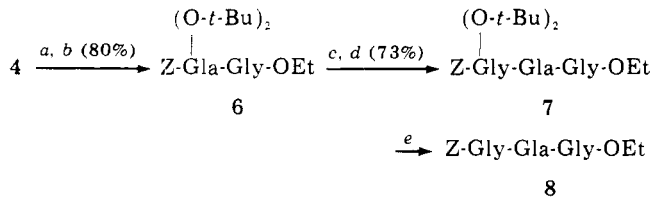
Scheme II. Process Efficiency Evaluation



T = tyrosine hydrazine, DTBM = di-*tert*-butyl malonate, [ ] = product not isolated

[a] Crude reaction mixture from which aliquots are drawn for subsequent procedures. [b] Silica gel chromatography. [c] 25 min, 0.48 M KOH/EtOH, room temperature. [d] $HTyrN_2H_3$, CH_3OH (min). [e] Anhydrous ether.

Scheme III



[a] 1 N BuONO, HCl. [b] HGlyOEt. [c] H_2, Pd/C. [d] ZGlyOCO-*i*-Bu. [e] 50:50 CH_2Cl_2/F_3CCO_2H.

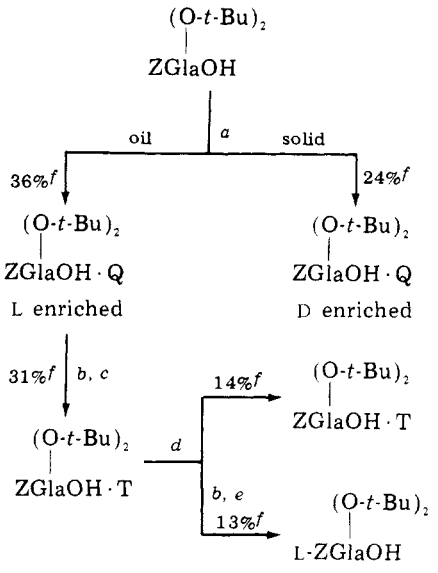
malonate with the *O*-tosyl-L-serine ester. This combination of steps avoided the necessity for chromatographic separation of **3a** from excess malonate and provided racemic **4** in good yield and purity. In a similar manner, the hydrolysis of the methyl ester of **3a** could be performed directly on the crude reaction mixture, and thus the acid **5a** could be obtained directly from **1a.** The acid **5a** was conveniently isolated as either the L-tyrosine hydrazide salt (**5d**) or the *N,N*-dicyclohexylamine salt (**5b**).

In order to secure large amounts of **5a** for chemical resolution, the process efficiency of the conversion of **1a** to **5d** was evaluated using 1 and 2 equiv of lithium di-*tert*-butyl malonate (Scheme II). The yields of **5d** indicate that 2 equiv of nucleophile was more advantageous when **3d** was not isolated and that acceptable yields of **5d** could be obtained directly from **1a** without isolation of the intermediate ester (**3a**) or acid (**5a**).

Several simple peptide derivatives of racemic Gla were prepared in order to examine the facility of coupling at the amino and carboxy ends of the molecule. The synthesis of *N*-(benzyloxycarbonyl)glycyl-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamylglycine ethyl ester (**7**) is shown in Scheme III. Smooth and essentially quantitative removal of the γ-*tert*-butyl ester groups of **7** could be achieved by treatment with a 50:50 (v/v) solution of TFA in methylene chloride (Scheme IV).

Resolution. With the availability of **5a** and the knowledge that the combination of the *N*-benzyloxycarbonyl and *tert*-butyl ester groups could be removed from Gla derivatives

Scheme IV. Resolution of Enriched L Enantiomer via the L-Tyrosine Hydrazide Salt



T = $TyrN_2H_3$, Q = L-quinine

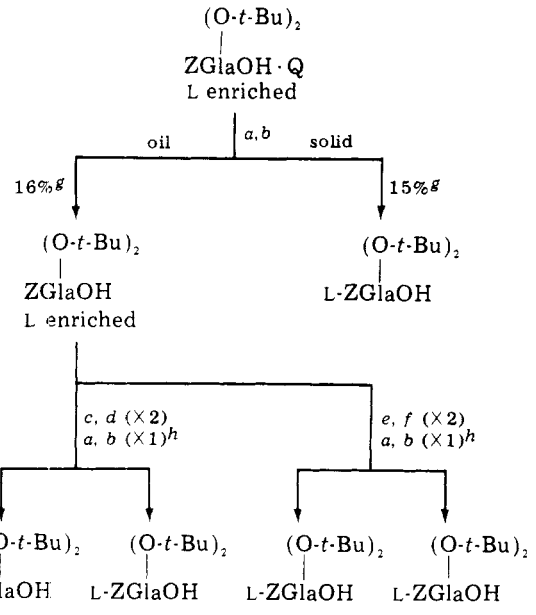
[a] 1 equiv of Q. [b] 20% citric acid. [c] 1 equiv of T, CH_3OH. [d] CH_3OH recrystallization. [e] CCl_4 recrystallization (×1). [f] Based on *d,l*-**1a**.

without problem, the resolution of Gla was considered. Enzymatic resolution of *N*-acetyl-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamic acid, from acetylation of γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamic acid (**6a**) in our hands as others[7a] experienced, was unsuccessful. Likewise, the separation of diastereoisomers from dipeptide derivatives such as **14, 15, 16,** and **17** did not appear to be a promising route to synthetic quantities of optically pure Gla derivatives.

Attempts to prepare the crystalline D-benzoyltartaric acid or the D-camphor sulfonic acid salts of γ,γ-di-*tert*-butyl-γ-carboxyglutamic acid α-methyl ester were unsuccessful; the brucine salt of the acid **5a** was also prepared, but remained as an oil. The L-tyrosine hydrazide salt (**5d**) could, however, be readily obtained and could be purified by recrystallization from methanol. Although salt **5d** could be resolved by numerous recrystallizations from methanol, only a low yield (5% overall from **1a**) of the L enantiomer of **5a** could be secured. In addition, the crystallization process was slow and the **5a** obtained had an optical purity of only 80–90%. Finally, the recovered tyrosine hydrazide salt of the D enantiomer of **5a,** a low-melting solid, could not be further resolved by recrystallization.

The timely report of Schwyzer et al.[7a] using quinine to obtain the D-enantiomer of **5c** combined with our studies using L-tyrosine hydrazide provided a solution to several of these problems. As mentioned above, racemic **5a** could be conveniently isolated from crude reaction mixtures as the L-tyrosine hydrazide salt (55–65% from **1a**). Since samples of **5d** enriched in the L enantiomer of **5a** were more easily resolved, quinine was used to separate the D enantiomer from the racemate (Scheme IV). The initial partition of the quinine salt of **5a** gave 36% of the solid D-quinine D-acid salt and 53% of the D-quinine L-acid as an oil. Purification of the former provided a 15% yield (from *d,l*-**5a**) of the D enantiomer of **5a.** Liberation of the acid **5a** from the oily D-quinine L-acid salt using 20% citric acid followed by treatment of the acid with L-tyrosine hydrazide provided 75% of the solid salt of the L enantiomer. Recrystallization from methanol provided the pure L enantiomer of **5a.** The overall yield of **5a** from **1a** via this scheme involving the quinine and tyrosine hydrazide salts was 15% for the D enantiomer and 13% for the L enantiomer.

Scheme V



% ee: 91 15 16 84

T = L-HTryN$_2$H$_3$, E = L-ephedrine, Q = L-quinine

[a] 20% citric acid. [b] CCl_4/pentane recrystallization. [c] 1 equiv of T. [d] CH_3OH recrystallization. [e] 1 equiv of E. [f] Ethyl acetate/pentane recrystallization. [g] % based on starting serine tosylate. [h] Ratios are the same in each case; volumes are proportional to the amount of solid obtained from each salt.

Another route to the L enantiomer of **5a** was reported by Schwyzer et al.[7a] and was used successfully in our laboratory. Recrystallization of the crude L enantiomer fraction of **5a** (liberated from the oily D-quinine L-acid fraction with 20% citric acid) from carbon tetrachloride or carbon tetrachloride/pentane provided a 15% overall yield of the optically pure L enantiomer of **5a** and 16% of recovered mother liquor enriched in the L enantiomer (Scheme V). This route to the L-enantiomer of **5a** is the method of choice for the rapid preparation of synthetically useful quantities of this substance.

Attempts to further enrich the mother liquors by formation and separation of the quinine salt were unsuccessful; however, both the tyrosine hydrazide and ephedrine[7b] salts could be obtained and purified. Recrystallization of the salts was pursued until no more solid could be obtained from either filtrate. Each filtrate was then reconverted to the acid **5a**, and the rotation of the oil was determined; virtually no difference between the amount of rotation of the acid remaining in the filtrate from either salt was observed. The crystalline salt fractions from ephedrine and tyrosine hydrazide were collected and likewise converted into **5a**. Rotations indicated that although the percent yield of solid salt was higher with ephedrine, the enantiomeric excess of the acid obtained from the tyrosine hydrazide salt was considerably greater.

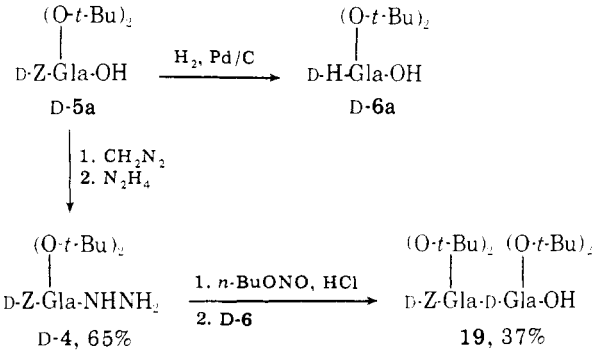
Determination of Optical Purity. The criteria that were developed for the determination of the optical purity of **5a** are given in Table I. Conversion of **5a** or *N*-(benzyloxycarbonyl)-γ-*tert*-butyl-L-glutamic acid to the optically pure glutamic acid hydrochloride by hydrolysis with hydrochloric acid was accompanied by a 1–2% loss of enantiomeric excess. The value of 20.3 ± 0.5° (*c* 1, H_2O) for the specific rotation of glutamic acid hydrochloride was used as the standard value.

The specific rotations for the enantiomers of **5a** were found to be most reliable when methanol was used as the solvent. Consistent with the previous reports,[2b,7b] we have found an inversion of the Cotton effect of both glutamic and γ-carboxyglutamic acid derivatives on changing solvent from methanol to chloroform. The problems of reproducibility encountered with chloroform as a solvent could have been due to the presence of either water or alcohol in the chloroform, although alumina-filtered chloroform was not more reliable.

Table I. Optical Purity Criteria for Z-Gla(O-*t*-Bu)$_2$-OH

	mp, °C	$[\alpha]^{22}_D$, deg
1. quinine salt	139.5–140	−71.9 (*c* 1, $CHCl_3$)
tyrosine hydrazide salt	149–150	−21.6 (*c* 1, CH_3OH)
2. free acid (D)	87–88	+11.4 (*c* 1.1, CH_3OH)
(L)	87–89	−11.2 (*c* 1.1, CH_3OH)
3. glutamic acid hydrochloride (98%)		±20.0 (*c* 1, H_2O)

Synthesis Using Chiral 5a. The preparation of *N*-(benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-γ-carboxyglutamyl-γ,γ-di-*tert*-butyl-D-γ-carboxyglutamic acid (**19**) was accomplished using optically pure **5a**. Treatment of **5a** (liberated from the quinine salt) with diazomethane generated the ester (**3a**), which was not isolated but directly converted to the hydrazide D-**4**. Conversion of D-4 to the azide and acylation



with γ,γ-di-*tert*-butyl-D-γ-carboxyglutamic acid (D-**6a**) provided the optically pure dipeptide derivative **19**.

Experimental Section[11]

***N*-(Benzyloxycarbonyl)-L-serine Phenacyl Ester (20).** To a solution of 2.4 g of *N*-(benzyloxycarbonyl)-L-serine[12] and 1.39 mL of triethylamine in dimethylformamide was added 1.9 g of phenacyl bromide in 2 mL of dimethylformamide. After being stirred for 17 h, the reaction mixture was filtered and the solvent was removed in vacuo, leaving a yellow oil which solidified on standing. The solid was dissolved in ethyl acetate, and the organic solution was washed sequentially with saturated sodium bicarbonate, water, and saturated brine. After the mixture was dried over anhydrous magnesium sulfate, the solvent was removed in vacuo to leave 2.93 g of crude solid (mp 108.5–110 °C). Recrystallization from ether/ethyl acetate solvent gave 1.90 g of ester (53%): mp 113.5–114.5 °C; $[\alpha]^{25}_D$ −29.3° (*c* 1.0, methanol).

Anal. Calcd for $C_{19}H_{19}NO_6$: C, 63.86; H, 5.36; N, 3.92. Found: C, 63.84; H, 5.38; N, 3.94.

***N*-(Benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamic Acid α-Methyl Ester (3a).** To a slurry of 59 mg of sodium hydride in 5 mL of dimethylformamide (freshly distilled from calcium hydride) at 0 °C was added 741 mg of di-*tert*-butyl malonate[13] in 3 mL of dimethylformamide, and the solution was stirred for 24 h at room temperature.[14] The reaction mixture was added dropwise over 15 min to a solution of 900 mg of **1a**[9a] in 3 mL of dimethylformamide. After the mixture was stirred for 20 h under a nitrogen atmosphere,[15] the solvent was removed in vacuo and the resulting oil was partitioned between 20% citric acid and ethyl acetate. The collected organic extracts were washed with saturated sodium bicarbonate and saturated brine and finally dried over magnesium sulfate. Removal of the solvent in vacuo left 0.79 g of oil which was chromatographed on silica gel (eluted with a linear gradient from methylene chloride to 5% ethyl acetate in methylene chloride). Pooled product-containing fractions gave 0.47 g of oil (84%) which was homogeneous by TLC (system A):

$[\alpha]^{24}_D$ 0.38° (c 7.7, methanol); NMR ($DCCl_3$) δ 1.43, 1.45 (2s, 18, 2t-Bu), 1.95–2.6 (broad, 2, β-CH_2), 3.31 (dd, J_1 = 6.4 Hz, J_2 = 7.5 Hz, γ-CH), 3.71 (s, 3, OCH_3), 4.44 (m, 1, α-CH), 5.10 (s, 2, CH_2Bzl), 5.61 (d, J = 8.4 Hz, 1, NH), 7.33 (s, 5, ArH).

Anal. Calcd for $C_{23}H_{33}NO_8$: C, 61.18; H, 7.37; N, 3.10. Found: C, 61.29, H. 7.42; N, 3.14.

***N*-(*tert*-Butoxycarbonyl)-*O*-tosyl-*d,l*-serine Methyl Ester (1c).** Following the procedure of Weinstein et al.,[16] 20 g of *d,l*-serine methyl ester hydrochloride was converted to 22.6 g of crude *N*-(*tert*-butoxycarbonyl)serine methyl ester, a viscous oil (70%) which was not further purified. The crude oil was dissolved in pyridine and cooled to −15 °C, and 25.1 g of freshly recrystallized *p*-toluenesulfonyl chloride was added over 20 min. After being stirred for 6 h at −15 °C, the reaction mixture was poured onto cracked ice and the aqueous mixture was extracted with ethyl acetate. The collected organic extracts were washed with 20% citric acid, saturated sodium bicarbonate, and saturated brine. (An additional washing with ice-cold 1.0 N hydrochloric acid was required to remove all of the pyridine followed by washes with saturated sodium bicarbonate and saturated brine.) After the mixture was dried over magnesium sulfate, removal of solvent left 30.3 g of solid. Recrystallization from absolute ethanol gave 23.6 g (61%) of crystalline tosylate, mp 95–97 °C.

Anal. Calcd for $C_{16}H_{23}NO_7 \cdot (C_2H_5OH)_{1/2}$: C, 51.50; H, 6.61; N, 3.53. Found: C, 52.09; H, 6.45; N, 3.85.

***N*-(*tert*-Butoxycarbonyl)-γ,γ-dibenzyl-*d,l*-γ-carboxyglutamic Acid α-Methyl Ester (3d).** The sodium salt of 0.70 g of dibenzyl malonate was prepared with 50 mg of sodium hydride in 5 mL of dimethylformamide (freshly distilled from calcium hydride) as described above. The solution was added at room temperature to a solution of 0.46 g of the *l* enantiomer of **1c** (prepared as described above for **1c**, mp 95–97 °C) in 3 mL of dimethylformamide over 10 min. The reaction was then stirred for 20 h, the solvent was removed, and the mixture was worked up in the usual fashion. After the mixture was dried over magnesium sulfate, the ethyl acetate was evaporated to leave 0.97 g of oil. Chromatography of the oil on silica gel (eluting with a linear gradient from methylene chloride to 5% ethyl acetate in methylene chloride) gave 0.33 g (60%) of **3d**: mp 82–84 °C; $[\alpha]^{24}_D$ +1.4° (c, 5, methanol): NMR ($CDCl_3$) δ 1.37 (s, 9, *t*-Bu), 1.95–2.75 (m, 2, β-CH_2), 3.5–3.75 (m, 4, γ-CH and OCH_3), 4.40 (m, 1, α-CH), 5.07 (s, 2, CH_2Bzl), 5.10 (s, 2, CH_2Bzl), 5.40 (d, J = 8, NH), 7.25 (brd s, 10, ArH).

Anal. Calcd for $C_{26}H_{31}NO_8$: C, 64.31; H, 6.44; N, 2.89. Found: C, 64.24; H, 62.46; N, 2.89.

***N*-(Benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamic Acid Dicyclohexylamine Salt (5b).** A solution of 2.41 g (5.34 mmol) of **3a** was added dropwise at room temperature to a 0.48 N solution of potassium hydroxide in absolute ethanol. After 30 min, the reaction mixture was cooled to 0 °C and brought to pH 7 with ice-cold 1.0 N hydrochloric acid. After the solvent was removed, the resulting oil was dissolved in ethyl acetate and the solution was washed with 20% citric acid, water, and saturated sodium chloride. After being dried over magnesium sulfate, solvent was removed and the resulting oil was dissolved in 10 mL of anhydrous ether and treated with 1.05 mL (5.33 mmol) of dicyclohexylamine. Filtration after standing overnight at room temperature gave 1.51 g of solid (46% based on starting tosylate), mp 132.4–134 °C. Recrystallization from a carbon tetrachloride/petroleum ether mixture (1:2) raised the melting point to 140–142.5 °C.

Anal. Calcd for $C_{34}H_{54}N_2O_8$: C, 65.99; H, 8.80; N, 4.53. Found: C, 65.80; H, 8.26; N, 4.49.

***N*-(Benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamic Acid α-Phenacyl Ester (3f). A. From 5a.** To a solution of 173 mg of **5a** and 55 μL of triethylamine in 1 mL of dimethylformamide was added 78.5 mg of phenacyl bromide in one portion, followed by a 1-mL rinse with solvent. After being stirred for 22 h at room temperature, solvent was removed and the mixture was worked up in the usual fashion to give 182 mg of solid. Recrystallization from an ethyl acetate and petroleum ether solvent pair gave 112 mg of crystalline solid (51%), mp 107.5–109 °C.

Anal. Calcd for $C_{30}H_{37}NO_9$: C, 64.85; H, 6.71; N, 2.52. Found: C, 64.79; H, 6.74; N, 2.52.

B. From 2o. To a solution of 9.33 g of *N*-(benzyloxycarbonyl)-L-serine phenacyl ester in 40 mL of pyridine cooled to −15 °C was added in one portion 4.94 g of *p*-toluenesulfonyl chloride. After being stirred at −15 °C for 5 h, the reaction mixture was warmed to 0 °C, stirred for an additional 9 h, and poured on 300 g of cracked ice. The aqueous mixture was extracted three times with ethyl acetate, and the collected organic washes were washed twice with ice-cold 1 N hydrochloric acid, twice with saturated sodium bicarbonate, and twice with saturated brine. After the mixture was dried over magnesium sulfate, the solvent was removed in vacuo to leave a yellow oil. Trituration with petroleum ether yielded 10.4 g (77%) of solid, mp 72–78 °C. The tosylate was not further purified, but was used directly as follows.

To a solution of the crude tosylate at 0 °C in 10 mL of dimethylformamide was added a solution of the sodium salt of 8.78 g of di-*tert*-butyl malonate, prepared as described with 0.97 g of sodium hydride in 50 mL of dimethylformamide. After 20 h, the reaction mixture was worked up in the usual fashion to give 12.6 g of oil which solidified on very long standing. A 5.82-g portion of this material was chromatographed on silica gel using an elution gradient of 100% chloroform to 5% methanol in chloroform. Three major fractions were obtained, each of which had at least three components on TLC. Recrystallization of one fraction (585 mg) from ethyl acetate gave 420 mg of solid, mp 104–108 °C. The NMR spectrum and TLC mobility were identical with those of an authentic sample of **3f** prepared as described above.

***N*-(Benzyloxycarbonyl)-γ,γ-dibenzyl-*d,l*-γ-carboxyglutamic Acid Methyl Ester (3c).** Preparation of the sodium salt of 3.57 g of dibenzyl malonate was accomplished using 0.30 g of sodium hydride in 50 mL of dimethylformamide as described above. This solution was added dropwise over 2–3 min to a solution of 2.55 g of tosylate **1a** in 10 mL of dimethylformamide at room temperature. After the mixture was stirred for 20 h, 0.72 mL of glacial acetic acid was added, the solvent was removed, and the resulting oil was worked up in the usual manner to yield 5.40 g of yellow oil. The oil was chromatographed on silica gel (eluting with a linear gradient from methylene chloride to 10% methanol in methylene chloride) to yield 2.35 g of **3c** as a clear colorless oil (72%): $[\alpha]^{24}_D$ +1.28° (c 5.4, methanol); NMR ($CDCl_3$) δ 2.06–2.80 (m, 2, β-CH_2), 3.66 and 3.64 (dd, J_1 = 6 Hz, J_2 = 8 Hz, and s, 4, respectively, γ-CH and OCH_3), 4.51 (m, 1, α-CH), 5.06, 5.08, 5.12 (3 overlapping singlets, 6, CH_2Bzl), 5.65 (d, J = 8.5, 1, NH), 7.26 (s, 15, ArH).

Anal. Calcd for $C_{29}H_{29}NO_8$: C, 67.04; H, 5.63; N, 2.70. Found: C, 66.80; H, 5.70; N, 2.64.

***N*-(Benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamic Acid Hydrazide (4).** To a stirred solution of diisopropylamine (5.82 mL, 41.6 mmol) in 5 mL of dry tetrahydrofuran (distilled from lithium aluminum hydride under nitrogen) under a nitrogen atmosphere was added 17.35 mL of a 2.4 N solution of *n*-butyllithium in hexane. The solution was stirred for 5 min at room temperature and then cooled to 0 °C. A solution of 8.98 g (41.6 mmol) of di-*tert*-butyl malonate in 10 mL of dry tetrahydrofuran was added dropwise, and the mixture was stirred for 20 min after the addition was complete. This solution was added to a stirred solution of tosylate **1a** (8.06 g, 19.8 mmol) in 30 mL of dry tetrahydrofuran (distilled from lithium aluminum hydride under nitrogen) at 0 °C under a nitrogen atmosphere.

The reaction mixture was stirred at room temperature for 20 h and condensed in vacuo. The residue was taken up in an ethyl acetate/20% citric acid solution, the layers were separated, and the aqueous layer was washed with ethyl acetate. The combined organic layers were washed with 20% citric acid solution and saturated sodium chloride solution, dried over magnesium sulfate, and condensed to an oil in vacuo. The residue was dissolved in 15 mL of methanol and treated with 5 mL of hydrazine hydrate. After being stirred for 3 h at room temperature, the reaction mixture was evaporated to dryness to produce an oily residue which solidified on trituration with hexane. Recrystallization from aqueous methanol produced 7.61 g of **4** (84%): mp 111.1–112 °C; NMR ($CDCl_3$) δ 1.45 (s, 9, *t*-Bu), 1.47 (s, 9, *t*-Bu), 2.23 (m, 2, β-CH_2) 3.34 (t, J = 7 Hz, 1, γ-CH), 3.87 (brd, 2, NH_2), 4.27 (m, 1, α-CH) 5.11 (s, 2, $ArCH_2$), 5.85 (d, J = 7 Hz, 1, NH), 7.35 (s, 5, ArH), 8.03 (m, 1, NH).

Anal. Calcd for $C_{22}H_{33}N_3O_7$: C, 59.44; H, 7.16; N, 9.11. Found: C, 59.21; H, 7.18; N, 9.02.

***d,l*-γ,γ-Di-*tert*-butyl-γ-carboxyglutamic Acid (6a).** A solution of the lithium salt of 12.7 g of di-*tert*-butyl malonate in tetrahydrofuran was prepared as described and carefully added to a solution of 19.3 g of **1b**[10] in 25 mL of tetrahydrofuran at 0 °C. The reaction mixture was allowed to stir for 24 h, during which time it warmed to room temperature. Following the addition of 4.7 mL of glacial acetic acid, solvent was removed in vacuo from the solution and the resulting oil was partitioned between 20% citric acid and ether. The combined organic extracts were washed with saturated sodium bicarbonate, water, and saturated brine and dried over magnesium sulfate. Removal of the solvent provided an oil which was dissolved in 250 mL of anhydrous methanol and hydrogenated in the presence of 0.9 g of 10% palladium on charcoal catalyst. When TLC indicated completion of the reaction, the catalyst was removed by filtration and the solvent was evaporated in vacuo to leave 12.9 g of crude waxy solid. Recrystallization from methanol gave 8.1 g of a white solid; mp 142–143.5

°C (66% based on starting tosylate **1b**).

The melting point was found to vary considerably with the drying procedure: desiccator, P_2O_5, 24 h, mp 138–139 °C; pistol, P_2O_5, 22 °C, 24 h, mp 143–143.5 °C; pistol, P_2O_5, 55 °C, 24 h, mp 148.5–150 °C. The difference is due to complexed methanol as indicated by the analytical data.

Anal. Calcd for $C_{14}H_{25}NO_6$: C, 55.43; H, 8.31; N, 4.62. Found: C, 55.24; H, 8.35; N, 4.56.

γ,γ-Di-*tert*-butyl-*d,l*-γ-carboxyglutamic Acid Methyl Ester Oxalate Salt (9). Hydrogen gas was bubbled for 2 h through a solution of 343 mg of **3a** containing 0.25 g of 10% palladium on charcoal in 12 mL of glacial acetic acid. The mixture was filtered through Celite, and 68.1 mg of anhydrous oxalic acid was added. Removal of the solvent in vacuo followed by trituration with ether gave 210 mg (68%) of a white solid, mp 133.5–135 °C.

Anal. Calcd for $C_{17}H_{29}NO_{10}(CH_3CO_2H)$: C, 48.81; H, 7.12; N, 3.00. Found: C, 48.45; H, 6.89; N, 3.20.

***d,l*-γ-Carboxyglutamic Acid α-Methyl Ester Hydrochloride (10).** Hydrogen was bubbled for 6 h through a solution of 0.54 g of **3c** containing 0.5 g of 10% palladium on charcoal in 15 mL of glacial acetic acid. After the mixture was filtered through Celite, 0.1 mL of concentrated hydrochloric acid was added and the solution was condensed to one-tenth its volume. Precipitation from the solution with an excess of anhydrous ether, filtration, and drying for 24 h in vacuo over phosphorus pentoxide provided 107 mg (43%) of **10** as a white solid: mp 122.5–123 °C; NMR (D_2O, chemical shift downfield from acetone) δ 2.05 (t, J = 7.5 Hz, 1, α-CH), 1.62 (s, 3, OCH_3), 0.28 (dd, J = 7 Hz, 2, β-CH_2).

Anal. Calcd for $C_7H_{12}NO_6Cl(H_2O)$: C, 32.39; H, 5.43; N, 5.40. Found: C, 32.01; H, 5.15; N, 5.41.

***N*-(*tert*-Butoxycarbonyl)-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamic Acid Hydrazide (11).** The sodium salt of 0.75 g of di-*tert*-butyl malonate was prepared with 59 mg of sodium hydride in 5 mL of dimethylformamide as described above. The reaction mixture was added to a solution of 640 mg of L-tosylate **1c** in 3 mL of dimethylformamide, and the reaction was stirred for 20 h. After 0.15 mL of glacial acetic acid was added, the solvent was removed and the residue was worked up in the usual fashion to give 0.72 g of oil. Chromatography on silica gel (linear gradient from methylene chlorine to 2% ethyl acetate in methylene chloride) gave 0.29 g (41%) of crude ester **3e.**

To a solution of 172.1 mg (0.412 mmol) of **3e** in 1 mL of methanol was added 30 drops (1.5 mmol) of hydrazine hydrate. After 3 h at room temperature, the reaction mixture was condensed in vacuo to 145.8 mg (85%) of hydrazide **11.** An analytical sample was obtained by recrystallization from aqueous methanol: mp 82–83 °C; NMR ($CDCl_3$) δ 1.40 (s, 24, O-*t*-Bu), 2.20 (m, 2, β-CH_2), 3.28 (t, $J \approx 12$ Hz, 1, γ-CH), 4.18 (m, α-CH_2), 5.65 ($J \approx 15$ Hz, 1, NH).

Anal. Calcd for $C_{19}H_{35}N_3O_7$: C, 54.68; H, 8.39; N, 10.07. Found: C, 54.67; H, 8.43; N, 10.02.

***N*-(*tert*-Butoxycarbonyl)-*d,l*-γ-carboxyglutamic Acid α-Methyl Ester (12).** A mixture of 518.8 mg (1.07 mmol) of crude ester **3d,** prepared as described above, and 52.2 mg of 10% palladium on charcoal catalyst in 10 mL of methanol was stirred under 1 atm of hydrogen for 2 h. After the catalyst was separated and the solvent was removed, the resulting solid was dried in vacuo. Recrystallization from an ether/hexane solvent gave 226.1 mg (70%) of **12,** mp 125–126.5 °C.

Anal. Calcd for $C_{12}H_{19}NO_8$: C, 47.21; H, 6.23; N, 4.59. Found: C, 47.16; H, 6.25; N, 4.56.

***N*-(Benzyloxycarbonyl)glycyl-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamic Acid α-Methyl Ester (13).** Hydrogen gas was bubbled for 45 min through a solution of 1.30 g of **3a** in 40 mL of glacial acetic acid containing 500 mg of 10% palladium on charcoal. After being filtered through Celite, the solution was condensed to one-third its volume and 5 mL of saturated hydrochloric acid in anhydrous ether was added. Evaporation in vacuo yielded an oil from which traces of acid were removed by evaporation with dry tetrahydrofuran, ethyl acetate, and anhydrous ether. The resulting hydrochloride salt **10** was used directly in the following reaction.

Under a nitrogen atmosphere a solution of 605 mg of *N*-(benzyloxycarbonyl)glycine and 322 μL of *N*-methylmorpholine in 1.5 mL of tetrahydrofuran (freshly distilled from calcium hydride) was cooled to −7 °C and 394 μL of isobutyl chloroformate in 500 μL of tetrahydrofuran was added. After the mixture was stirred for 30 min, a filtered solution of 1.16 g of the hydrochloride **10** and 394 mg of *N*-methylmorpholine in 9 mL of tetrahydrofuran was added and the reaction mixture was stirred overnight at 0 °C. After the solvent was removed, the resulting oil was partitioned between ethyl acetate and 20% citric acid. The collected organic washes were extracted with saturated sodium bicarbonate and saturated brine and dried over magnesium sulfate. Removal of the solvent gave 1.28 g of oil which solidified on standing. Recrystallization from anhydrous ether/petroleum ether solvent gave 0.92 g (63%) of **13:** mp 91.5–93 °C; NMR ($CDCl_3$) δ 1.45 (s, 18, O-*t*-Bu), 2.39 (m, 2, β-CH_2), 3.30 (t, J = 6 Hz, 1, γ-CH), 3.70 (s, 3, OCH_3), 3.91 (brd d, $GlyCH_2$), 4.66 (m, 1, α-CH), 5.12 (s, 2, $BzlCH_2$), 5.94 (brd t, 1, GlyNH), 7.12 (brd d, 1, GlaNH), 7.34 (s, 5, ArH).

Anal. Calcd for $C_{25}H_{36}N_2O_9$: C, 59.04; H, 7.14; N, 5.51. Found: C, 59.09; H, 7.17; N, 5.53.

***N*-(Benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamylglycine Ethyl Ester (6).** To a solution of 0.86 g of **4** (1.87 mmol) in 4 mL of dry tetrahydrofuran (distilled from lithium aluminum hydride under nitrogen) at −23 °C under a nitrogen atmosphere was added dropwise 6.94 mmol (1.99 mL) of a 3.49 M solution of hydrochloric acid in tetrahydrofuran followed by 0.5 mL of *n*-butyl nitrite. After the mixture was stirred for 10 min, the pH was adjusted to 10 with triethylamine. The reaction mixture was diluted with 10 mL of tetrahydrofuran, and the temperature was raised to 0 °C. A solution of 900 mg (8.7 mmol) of ethyl glycinate in 5 mL of dry tetrahydrofuran was added, and the reaction was stirred at 0 °C for 3 h. (The reaction may be monitored by TLC using 2% methanol in chloroform.) The reaction mixture was condensed in vacuo, and the residue was taken up in saturated sodium bicarbonate/ethyl acetate. The aqueous layer was washed with ethyl acetate, and the organic layers were combined. After being washed with 20% citric acid and saturated sodium chloride and dried with magnesium sulfate, the organic layer was condensed to an oil which was crystallized from aqueous methanol to produce 811.1 mg (80%) of a white solid: mp 97.5–99 °C; NMR ($CDCl_3$) δ 1.24 (t, J = 7 Hz, 3, CH_3), 1.45 (s, 18, *t*-Bu), 2.2 (brd, 2, $GlaCH_2$), 3.44 (t, J = 7 Hz, 1, Gla-γ-CH), 3.99 (d, J = 5 Hz, 2, $GlyCH_2$), 4.18 (q, J = 7 Hz, 2, $GlyOCH_2$), 4.35 (brd, 1, Gla-α-CH), 5.09 (s, 2, $ArCH_2$), 6.05 (d, J = 8 Hz, 1, NH), 7.32 (s, 5, ArH).

Anal. Calcd for $C_{26}H_{38}N_2(H_2O)$: C, 57.77; H, 7.41; N, 5.19. Found: C, 57.57; H, 7.08; N, 5.15.

***N*-(Benzyloxycarbonyl)glycyl-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamylglycine Ethyl Ester (7).** A solution of 4.83 g (8.95 mmol) of **6** in 150 mL of absolute ethanol was stirred with 450 mg of 10% palladium on carbon catalyst under hydrogen at atmospheric pressure for 2 h, filtered through Supercell, and condensed in vacuo to an oil. The product was used immediately without purification.

To a stirred solution of 3.27 g (15.6 mmol) of *N*-(benzyloxycarbonyl)glycine in 10 mL of dry tetrahydrofuran (distilled from lithium aluminum hydride under nitrogen) at −5 °C was added 1.75 mL (15.6 mmol) of *N*-methylmorpholine followed by 2.05 mL (15.6 mmol) of isobutyl chloroformate. After the mixture was stirred for 15–20 min, a solution of 8.95 mmol of dipeptide-free base (prepared as above) in 10 mL of dry tetrahydrofuran was added and the reaction was stirred overnight at 5 °C. The reaction mixture was condensed, and the residue was taken up in ethyl acetate/water. The aqueous layer was washed with ethyl acetate, and the combined organic layers were washed with saturated sodium bicarbonate, water, 20% citric acid, and saturated sodium chloride. Drying with magnesium sulfate and condensation in vacuo produced an oil (6.9 g) which was recrystallized from ether/petroleum ether to yield 3.7 g (73%) of **7:** mp 103–106 °C; NMR ($CDCl_3$) δ 1.24 (t, J = 7 Hz, 3, $GlyOCH_2$), 1.46 (s, 18, *t*-Bu), 2.25 (brd, 2, Gla-β-CH_2), 3.42 (t, J = 7 Hz, 1, Gla-γ-CH), 3.90 (d, J = 5 Hz, 2, $GlyCH_2$), 3.98 (d, J = 5 Hz, 2, $GlyCH_2$), 4.19 (q, J = 7 Hz, 3, CH_3), 4.6 (brd, 1, Gla-α-CH), 5.12 (s, 2, $ArCH_2$), 5.85 (t, J = 5 Hz, 1 NH), 7.35 (s, 5, ArH).

Anal. Calcd for $C_{28}H_{41}N_3O_{10}$: C, 58.03; H, 7.08; N, 7.25. Found: C, 58.01; H, 7.08; N, 7.30.

***N*-(Benzyloxycarbonyl)glycine-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamyl Hydrazide (18).** To a solution of 0.65 g (12.7 mmol) of dipeptide ester **13** in 3.5 mL of methanol was added 0.65 mL of hydrazine hydrate, and the solution was stirred for 3 h at room temperature. Removal of the solvent provided an oil which was taken up in ethyl acetate, and the resulting solution was washed with saturated brine and dried over magnesium sulfate. Removal of solvent gave 0.59 g of solid which was recrystallized from methanol and water to yield 0.42 g (65%) of hydrazide, mp 80.5–82 °C.

Anal. Calcd for $C_{24}H_{36}N_4O_8 \cdot (H_2O)_{1/2}$: C, 53.81; H, 7.34; N, 10.47. Found: C, 53.67; H, 7.45; N, 10.43.

***N*-(Benzyloxycarbonyl)glycyl-*d,l*-γ-carboxyglutamylglycine Ethyl Ester (8).** To an ice-cold, stirred solution of 1.02 g (1.76 mmol) of **7** in 30 mL of methylene chloride was added dropwise 20 mL of 99% trifluoroacetic acid. The mixture was stirred for 70 min at 0 °C and then for 15 min at room temperature. Removal of solvent yielded a hydroscopic oil. An analytical sample of **7** as the half-hydrate was obtained in low yield by crystallization of the oil from ethyl acetate/

hexane. The white solid obtained decomposed over the range 76 to 94 °C.

Anal. Calcd for $C_{20}H_{25}N_3O_{10}\cdot 0.5H_2O$: C, 50.42; H, 5.46; N, 8.82. Found: C, 50.50; H, 5.50; N, 8.84.

***N*-(Benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamyl-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamylglycine Ethyl Ester (14).** *N*-(Benzyloxycarbonyl)-di-*tert*-butyl-*d,l*-γ-carboxyglutamyl azide was prepared from 5.87 g (13.0 mmol) of hydrazide **4** and coupled in situ with γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamylglycine ethyl ester, prepared from 5.62 g (11.1 mmol) of the fully protected peptide **6**, in a manner analogous to that described for the preparation of **6**. Workup provided 8.84 g of a crude oil which was chromatographed over a 5.5 × 22 cm column of silica gel G using 1% methanol in chloroform as solvent. The main fraction yielded an oil which was crystallized from aqueous methanol to give **14** as 1.53 g (17%) of a mixture of diastereoisomers, melting at 108–118 °C.

Anal. Calcd for $C_{40}H_{61}N_3O_{14}$: C, 59.48; H, 7.56; N, 5.20. Found: C, 59.39; H, 7.65; N, 5.20.

***N*-(Benzyloxycarbonyl)glycyl-*d,l*-γ,γ-di-*tert*-butyl-γ-carboxyglutamyl-*d,l*-γ,γ-di-*tert*-butyl-γ-carboxyglutamylglycine Ethyl Ester (15).** Hydrogen gas was passed through a solution of 440 mg of **6** in 10 mL of methanol containing 50 mg of 10% palladium on charcoal catalyst until TLC of the reaction mixture indicated no starting material. After the reaction mixture was filtered and the solvent was removed by evaporation, the resultant oil was dissolved in 1 mL of tetrahydrofuran (freshly distilled from calcium hydride) and added to the solution of the acyl azide prepared as indicated below.

A solution of 420 mg of **18** in 2 mL of freshly distilled tetrahydrofuran was cooled to −23 °C, and 0.87 mL of a saturated solution of hydrogen chloride in tetrahydrofuran was added dropwise. After 10 min 0.23 mL of isobutyl nitrite was added in one portion, and after 10 min the solution was adjusted to pH 10 by addition of triethylamine. The temperature was adjusted to 0 °C, and the solution of the reduced dipeptide prepared above was added in one portion. The reaction mixture was stirred for 24 h at 4 °C, the solvent was then removed, and the resulting oil was partitioned between ethyl acetate and 20% citric acid. The ethyl acetate solution was then washed with saturated sodium bicarbonate, water, and saturated brine and dried over magnesium sulfate. Recrystallization of the product yielded 290 mg of solid and 250 mg of oil in the mother liquor. The oil was chromatographed on silica gel (gradient elution from methylene chloride to 5% methanol in methylene chloride) to yield 145 mg (19%) of **15** as a mixture of diastereoisomers: NMR ($CDCl_3$) δ 1.24 (brd t, 3, CH_3), 1.48 (s, 36, O-*t*-Bu), 2.35 (m, 4, γ-CH_2), 3.42 (m, 2, β-CH), 3.6–4.4 (m, 7, GlyCH_2, α-CH, CH_2CH_3), 4.52 (m, 1, α-CH), 5.16 (s, 2, BzlCH_2) 5.96 (m, 1, NH), 7.5–8.4 (m, s overlap, 8, ArH + NH).

Anal. Calcd for $C_{42}H_{64}N_4O_{15}$: C, 58.32; H, 7.47; N, 6.48. Found: C, 58.40; H, 7.48; N, 6.58.

***N*-(Benzyloxycarbonyl)-L-leucyl-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamic Acid Methyl Ester (16).** Hydrogen gas was bubbled through a solution of 1.54 g of **3a** in 25 mL of anhydrous methanol containing 0.14 g of 10% palladium on charcoal catalyst. Removal of the catalyst and solvent provided 1.2 g of oil which was used as described below without further purification.

To a solution of 1.14 g of *N*-(benzyloxycarbonyl)-L-leucine (**19**) (obtained from the dicyclohexylamine salt as an oil) and 0.35 g of *N*-methylmorpholine in 2 mL of tetrahydrofuran (distilled from lithium aluminum hydride) at −10 °C was added 0.47 g of isobutyl chloroformate. After the mixture was stirred for 30 min, a solution of the crude free base of the triester (1.2 g) in 2 mL of tetrahydrofuran was added dropwise over 5 min and the reaction was stirred with slow warming to room temperature for 15 h. After the solvent was removed, the residue was partitioned between ethyl acetate and 20% citric acid and the combined organic extracts were washed with saturated brine and dried over magnesium sulfate. After the solvent was removed in vacuo, the resulting oil (2.00 g) was chromatographed on silica gel (eluting with a concentration gradient from methylene chloride to 5% acetone in methylene chloride) to yield 1.27 g of an oil, presumably the diastereomers of **16**. Attempted separation of the diastereomers by column chromatography was unsuccessful: NMR ($CDCl_3$) δ 0.93 (6, brd d, *J* = 4 Hz, LeuCH_3), 1.3–1.8 (s + brd s, 20, O-*t*-Bu + Leu-β-CH_2), 3.22 (t, *J* = 7 Hz, 1, Gla-γ-CH), 3.71 (s + m, 4, OCH_3 + Leu-β-CH), 4.35 (m, 1, Leu-γ-CH), 4.66 (m, 1, Gla-α-CH), 5.15 (brd s, 2, BzlCH_2), 5.34 (t, 1, NH), 7.35 (s + m, 6, ArH + NH).

Anal. Calcd for $C_{37}H_{44}N_2O_9$: C, 61.68; H, 7.85; N, 4.96. Found: C, 61.57; H, 7.90; N, 5.00.

***N*-(Benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-*d,l*-γ-carboxyglutamyl-L-proline Benzyl Ester (17).** To a solution of hydrazide **4** in 4.6 mL of tetrahydrofuran (freshly distilled from lithium aluminum hydride) at −23 °C was added 2.32 mL of anhydrous saturated hydrogen chloride in tetrahydrofuran. After the mixture was stirred for 10 min, 0.6 mL of isobutyl nitrite was added in one portion and stirring was continued for 10 min. The solution was brought to pH 10 by addition of triethylamine, and the temperature was raised to 0 °C. A solution of L-proline benzyl ester (obtained from the tosylate salt by partitioning between ether and 33% sodium hydroxide saturated with potassium chloride) in 4 mL of dry tetrahydrofuran was added to the solution over 5 min, and the reaction was stirred for 24 h at 4 °C. After being warmed to room temperature, the mixture was poured into saturated sodium bicarbonate and the aqueous mixture was extracted with ethyl acetate. The collected organic layers were washed with 20% citric acid and saturated brine and then dried over magnesium sulfate. Removal of the solvent in vacuo left 1.73 g of oil which was chromatographed on silica gel (eluted with a linear gradient from methylene chloride to 5% acetone in methylene chloride) to provide 0.83 g (60%) of a clear oil, presumably a mixture of the diastereoisomers of **17**. The mixture could not be separated by elution chromatography.

Anal. Calcd for $C_{34}H_{44}N_2O_8$: C, 65.47; H, 6.95; N, 4.49. Found: C, 65.31; H, 7.13; N, 4.50.

Determination of Optical Purity of Gla Derivatives. Sample Preparation. A degassed solution of a weighed amount of protected amino acid derivative in 6 N hydrochloric acid was heated at 110 °C in a vacuum-sealed tube. At the end of 3 h the hydrochloric acid was removed in vacuo and the resulting solid was dissolved in 2–3 mL of distilled, deionized water to give a nearly opaque dispersion which could not be used for polarimeter measurements. Extraction of the solution with three 2-mL portions of ether yielded a clear aqueous layer which gave a white powder on lyophilization. The powder was redissolved three times in 2 mL of water and lyophilized to insure the complete removal of hydrochloric acid and finally was dried in vacuo with phosphorus pentoxide.

The optical rotations of the glutamic acid hydrochloride samples were obtained from weighed amounts of solid (2.0 ± 0.1 mg in 2 mL of distilled, deionized water).[17] Enantiomeric excess was determined by comparison with standard values obtained in the following fashion.

Preparation of Authentic Samples of D- and L-Glutamic Acid Hydrochloride. A solution of 2.4 g of L-glutamic acid (enantiomeric excess determined by comparison with published values as 98.1 ± 0.3%)[18] in 25 mL of 6 N hydrochloric acid was warmed on a steam bath for 5 min to dissolve all solid. After the mixture was cooled overnight at 0 °C, the crystals were filtered and vacuum dried over phosphorus pentoxide to leave 2.66 g (90%) of crystalline solid: mp 202–203.5 °C; $[\alpha]^{21}_D$ 21.4° (*c* 1.00, H_2O), $[\alpha]^{22}_D$ +23.2° (*c* 6.25, H_2O), $[\alpha]^{22}_{578}$ +24.2° (*c* 6.25, H_2O), $[\alpha]^{22}_{546}$ +27.7° (*c* 6.25, H_2O). Lyophilization of the filtrate gave an additional 256 mg of solid: mp 196 °C; $[\alpha]^{21}_D$ +20.6° (*c* 1.0, H_2O).

A similar procedure starting with D-glutamic acid (enantiomeric excess 98%) yielded the corresponding hydrochloride: $[\alpha]^{22}_D$ −20.6° (*c* 1.00, H_2O), $[\alpha]^{22}_D$ −23.5° (*c* 6.25, H_2O).

The average rotation and the precision to be expected from the determination of rotation for 20.0 ± 0.1 mg samples in 2 mL of distilled, deionized water were determined to be $[\alpha]^{23}_D$ +20.3 ± 0.36° and $[\alpha]^{23}_{578}$ +21.3° ± 0.35° and $[\alpha]^{23}_{546}$ +24.3 ± 0.41°, or an expected deviation then is nearly 2% out of 20.7°. Compared to the values reported by Schwyzer and co-workers,[7b] this sample of L-glutamic acid hydrochloride has an enantiomeric excess of 98.5%, a value consistent with the determined value for the starting L-glutamic acid.

Optical Purity of L-Glutamic Acid Obtained by Hydrolysis of *N*-(Benzyloxycarbonyl)-γ-*tert*-butyl-L-glutamic Acid. A 150-mg sample of *N*-(benzyloxycarbonyl)-γ-*tert*-butyl-L-glutamic acid[19] hydrolyzed and treated according to the previously described procedure provided 79 mg of glutamic acid hydrochloride, $[\alpha]^{21}_D$ +20.0° (*c* 1.00, H_2O). The calculated enantiomeric excess according to the above described standards is either 98.5% if the standard is optically pure or 97.1% if the enantiomeric excess of the standard is 98.5%.

Partial Resolution of 4c Using the L-Tyrosine Hydrazide Salt (5d). The lithium salt of 8.2 g (38 mmol) of di-*tert*-butyl malonate was prepared in tetrahydrofuran as described above using 10.5 mL (74.4 mmol) of diisopropylamine and 34.6 mL of *n*-butyllithium in hexane (75.1 mmol). The solution of salt was added to a solution of 15 g (36.8 mmol) of **1a** in 20 mL of tetrahydrofuran at 0 °C. The reaction mixture was allowed to stir for 24 h, during which time it warmed to room temperature. After addition of 4.3 mL of glacial acetic acid and removal of the solvent in vacuo, the mixture was worked up in the usual fashion to give 14.2 g of oil. A 4.6-g portion of the oil in 10 mL of hot absolute methanol was treated with 1.0 g of L-tyrosine hydrazide.[23] On cooling, 1.24 g of salt **5d** was obtained, mp 134–146 °C.

Several recrystallizations of the salt from methanol ultimately

yielded 140 mg of **5d** (2% based on **2**), $[\alpha]^{22}_D$ +19.5° (*c* 2.0, methanol). The free acid **5a** obtained by partitioning **4a** between ether and 20% citric acid was a clear, nearly colorless oil, $[\alpha]^{24}_D$ −10.2° (*c* 2.3, methanol). Hydrolysis of 46 mg of **5a** followed by removal of HCl gave 15.5 mg of residue. Dissolution of the solid in 2 mL of water gave an opaque mixture which became clear after three ether extractions. The aqueous solution was lyophilized, and the resulting solid was dried in vacuo over phosphorus pentoxide. The rotation of a sample, $[\alpha]^{23}_D$ +21.16° (*c* 0.7, 1 N hydrochloric acid), compared to that of a sample of L-glutamic acid similarly treated [$[\alpha]^{23}_D$ +27.6° (*c* 0.75, 1 N HCl)] indicated an optical purity of **4c** of no better than 77%, although the optical purity in subsequent runs varied from 77 to 87%.

Resolution of 4c Using a Combination of the Quinine and Tyrosine Hydrazide Salts. Isolation of the D Enantiomer. The lithium salt of 167 g of di-*tert*-butyl malonate in 242 mL of tetrahydrofuran was prepared as described above using 108 mL of diisopropylamine, 604 mL of tetrahydrofuran, and 322 mL of 2.4 M *n*-butyllithium in hexane. After being warmed to room temperature and stirred for an additional hour, the reaction mixture was added over 5 h to a solution of 157 g of *d,l*-**1a** (prepared in the same manner as **1a**, mp 103–105 °C) in 242 mL of tetrahydrofuran cooled to 0 °C. The reaction was allowed to stir for an additional 24 h, during which time it warmed to room temperature. After the dropwise addition of 44 mL of glacial acetic acid and removal of the solvent in vacuo, the mixture was worked up in the usual fashion to yield 240 g of oil. The oil was dissolved in 3 L of 0.48 N potassium hydroxide in ethanol and stirred for 25 min at room temperature. After adjustment to pH 7 with ice-cold 1.0 N hydrochloric acid, the ethanol was removed in vacuo. After workup as before, 225 g of crude oil was isolated.

The oil was dissolved in 150 mL of hot anhydrous methanol, 48.2 g of L-tyrosine hydrazide was added, and the mixture was warmed until all solid dissolved. After the mixture was cooled to room temperature, 1000 mL of anhydrous ether was added followed 15 min later by a second 1000 mL. The mixture was filtered after standing at −15 °C for 1 h, and the solid was air-dried, leaving 158 g of **5d:** mp 125–130 °C; $[\alpha]^{22}_D$ +22.6° (*c* 2.0, methanol). The free acid **5a** was recovered by partitioning the crude salt between ether and a solution of 48 g of citric acid in 150 mL of water. The ether extracts were washed with saturated brine, dried, and condensed in vacuo to 111 g of oil. Mixing of a solution of the oil in 50 mL of hot ethyl acetate with a dispersion of 80.8 g of L-quinine in 700 mL of hot ethyl acetate resulted in solution of all solids. Filtration after slow cooling and standing for 2 days gave 42.4 g of quinine salt, mp 130–135 °C. A second crop of 30.7 g was obtained from the filtrate for a total yield of 73.1 g (37% of the *d,l* free acid). The remaining material in the final filtrate (110.4 g) was a brown viscous oil (representing 56.4% of the *d,l* free acid). The conversion to the first set of separated quinine salts proceeded with 93% overall yield.

Following the procedure of Schwyzer et al.,[7] the quinine salt was recrystallized several times from ethyl acetate. Careful cooling procedures yielded a total of 46 g of salt (15% from **2** or 23% of the *d,l* acid **5a**). The salt was contained in four fractions of various levels of purity, including 28.2 g of highly purified salt: mp 139.5–140 °C; $[\alpha]^{22}_D$ −68.6° (*c* 1.0, methanol).

Anal. Calcd: C, 66.21; H, 7.28; N, 5.52. Found: C, 65.98; H, 7.28; N, 5.46.

The salt **5d** could be converted into **5a** by partitioning the purified salt between 20% citric acid and ether: mp 87–88 °C; $[\alpha]^{22}_D$ +11.4° (*c* 1.1, methanol). Hydrolysis of 150 mg of **5a** as previously described above yielded 61.4 mg of glutamic acid hydrochloride, $[\alpha]^{20}_D$ −20.3° (*c* 1.0, H_2O). The isolated free acid **5a** is therefore *N*-(benzyloxycarbonyl)-D-γ,γ-di-*tert*-butyl-γ-carboxyglutamic acid with an enantiomeric excess of 98.1 ± 1.5%.

Isolation of the L Enantiomer of 4c via 4a. The oily L-enriched quinine salt obtained on the first separation was partitioned between 20% citric acid and ether. The combined organic extracts were washed with saturated brine and dried over magnesium sulfate, and the solvent was removed in vacuo to leave 58.6 g of oil (representing 55.4% of the *d,l* free acid). The oil was dissolved in 140 mL of hot anhydrous methanol, 25.3 g of L-tyrosine hydrazide was added, and the solution was heated until all of the solid dissolved. Careful cooling overnight gave on first separation 56.7 g of solid: mp 134–137 °C; $[\alpha]^{24}_D$ +22.3° (*c* 2.0, methanol). Further crystallization and collection of fractions gave a total of 64 g of salt (27.2% from **1a**, representing 41.9% of *d,l*-**5a**) distributed among several fractions of differing purity, including 21 g of highly purified salt (9% from **1a**, 13.7% from *d,l*-**4c**): mp 149–150 °C; $[\alpha]^{22}_D$ −21.6° (*c* 1.0, methanol).

Anal. Calcd for $C_{31}H_{44}N_4O_{10}$: C, 58.84; H, 7.01; N, 8.86. Found: C, 58.72; H, 7.04; N, 8.84.

The free acid **5a** was obtained from the most highly purified fraction as previously described: mp 87–89 °C; $[\alpha]^{21}_D$ −12.4° (*c* 1.1, methanol).

Anal. Calcd for $C_{22}H_{31}NO_8$: C, 60.40; H, 7.14; N, 3.20. Found: C, 60.29; H, 7.17; N, 3.19.

Hydrolysis of 150 mg of this material as described above yielded 67.2 mg of glutamic acid hydrochloride, $[\alpha]^{20}_D$ +20.4° (*c* 1.0, water), indicating on comparison with the appropriate standards an enantiomeric excess of 98.6%. The absolute configuration of the material obtained from the tyrosine hydrazide salt is established as L.

Isolation of the L Enantiomer of 5a by Crystallization. The low-melting solid obtained from the filtrates of the recrystallization of the quinine salt obtained from 144.0 g of **5a** was partitioned between 20% citric acid and ether, and the combined organic fractions were washed with water and saturated brine. After the mixture was dried over magnesium sulfate, the solvent was removed in vacuo to leave 91.5 g of viscous oil (representing 66% of the *d,l*-**5a**). Several recrystallizations of this material from a carbon tetrachloride and petroleum ether solvent pair yielded essentially two fractions: 38 g (15% from *d,l*-**1a**) of highly purified *N*-(benzyloxycarbonyl)-L-γ,γ-di-*tert*-butyl-γ-carboxyglutamic acid [mp 85–86 °C, $[\alpha]^{20}_D$ −12.1° (*c* 1.1 methanol)], and 40 g of oil (16% from *d,l*-**1a**) from which no more L-**5a** could be crystallized.

Comparison of the Effectiveness of L-Tyrosine Hydrazide, Quinine, and (−)-Ephedrine in the Purification of the L Enantiomer of 5a. The rotation of 40 g of oil obtained from the mother liquor when purification of the L enantiomer of **5a** was accomplished by recrystallization of an L enriched sample of **5a**, $[\alpha]^{21}_D$ −2.7° (*c* 1.1, methanol), indicates an excess of 22% of the L enantiomer. In order to compare the separation effectiveness of L-tyrosine hydrazide and quinine with that of (−)-ephedrine, the oil was divided into three 15-g portions and transformed into salt with the appropriate bases as indicated below.

(i) Quinine. As might have been expected, quinine was essentially ineffective at separating a mixture with this level of enantiomeric excess.

(ii) Tyrosine Hydrazide. The tyrosine hydrazide salt of a second aliquot (21.8 g) was recrystallized from methanol until no further solid could be obtained from the filtrate. Three fractions of crystals (a total of 10.5 g) and 7.83 g of oil were obtained from the filtrate. The filtrate was converted as before to 5.4 g of free acid, $[\alpha]^{22}_D$ +1.0° (*c* 1.0, methanol). The collected 10.5 g of salt was recrystallized as before from methanol, giving three fractions of crystals (a total of 6.4 g) and 2.69 g of filtrate. The filtrate was converted to 1.77 g of free acid, $[\alpha]^{23}_D$ +0.15° (*c* 1.0, methanol). The pooled salt fractions were converted to 4.59 g of oil, $[\alpha]^{23}_D$ −8.0° (*c* 1.0, methanol). The oil was dissolved in 11.1 mL of warm carbon tetrachloride, and 35.6 mL of pentane was added. Slow cooling followed by filtration gave 2.86 g of solid **5a**, $[\alpha]^{23}_D$ −10.9° (*c* 1.0, methanol). The filtrate yielded 1.20 g of oil as well, $[\alpha]^{23}_D$ −1.8° (*c* 1.0, methanol).

(iii) Ephedrine. The ephedrine salt of a third aliquot (20.7 g) was recrystallized from ethyl acetate/petroleum ether until no further salt could be obtained from the filtrate, giving four fractions of crystals (a total of 11.9 g) and 6.5 g of oil from the filtrate. The oil was converted as above to 4.69 g of **5a** as an oil, $[\alpha]^{22}_D$ +1.6° (*c* 1.0, methanol). The collected 11.9 g of solid was recrystallized as before from ethyl acetate/petroleum ether, giving three fractions of salt (a total of 8.8 g) and 2.25 g of oil from the filtrate. The filtrate was converted to 1.54 g of free acid as an oil, $[\alpha]^{23}_D$ −2.0° (*c* 1.0, methanol). The pooled salt fractions were converted to 5.24 g of oil, $[\alpha]^{23}_D$ −5.2° (*c* 1.0, methanol). The oil was dissolved in 12.5 mL of warm carbon tetrachloride, and 41 mL of pentane was added. Slow cooling followed by filtration gave 1.57 g of solid **5a**, $[\alpha]^{23}_D$ −10.1° (*c* 1.0, methanol). The filtrate yielded 3.00 g of oil as well, $[\alpha]^{23}_D$ −1.9° (*c* 1.0, methanol).

***N*-(Benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-D-γ-carboxyglutamic Acid Hydrazide (D-4).** The optically pure free acid **5a** was obtained from 2.24 g of the quinine salt in the usual manner and dissolved in 3 mL of anhydrous ether. An ether solution of diazomethane[21] was added until the yellow color persisted. After 10 min additional, the reaction mixture was quenched with acetic acid in ether and extracted with saturated brine. The dried ether layer was evaporated to yield 1.24 g (100%) of D-**3a** as a clear oil, $[\alpha]^{21}_D$ 1.25° (*c* 1.2, $CHCl_3$). The NMR spectrum and TLC mobility were identical with those of a sample of *d,l*-**3a**.

To a solution of 1.62 g of **3a** in 7.9 mL of methanol was added 0.52 mL of hydrazine hydrate, and the reaction mixture was stirred at room temperature for 4.5 h. Removal of the solvent and drying over P_2O_5 and 96% sulfuric acid provided 1.62 g of glassy solid. Crystallization from ether provided 1.05 g (65%) of optically pure **4**: mp 63.5–65 °C; $[\alpha]^{24}_D$ −6.2° (*c* 1.0, $CHCl_3$).

Anal. Calcd for $C_{22}H_{33}N_3O_7$: C, 58.52; H, 7.37; N, 9.31. Found: C,

58.46; H, 7.38; N, 9.29.

γ,γ-Di-*tert*-butyl-D-γ-carboxyglutamic Acid (D-6a). The free acid **5a** from 2.24 g of quinine salt was obtained by partitioning the salt between 20% citric acid and ether. The ether layer was washed with water and saturated brine and dried over anhydrous magnesium sulfate. The 1.25 g of oil obtained on removal of the solvent in vacuo was dissolved in 20 mL of anhydrous methanol and hydrogenated at 1 atm in the presence of 200 mg of 10% palladium on charcoal. After 1 h, the flow of hydrogen was discontinued, the catalyst was removed by filtration through a Celite bed, and the methanol was evaporated in vacuo to leave 808 mg of white solid (98% based on starting quinine salt): mp 163–164 °C; $[\alpha]^{26}_D$ −5.7° (*c* 1.0, CH_3OH) [literature values:[7b] mp 165–167.5 °C, $[\alpha]^{20}_D$ −5.7 ° (*c* 1, CH_3OH)].

***N*-(Benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-D-γ-carboxyglutamyl-γ,γ-di-*tert*-butyl-D-γ-carboxyglutamic Acid (19).** A solution containing 1.0 g of the optically pure hydrazide D-**4** in 4 mL of dimethylformamide (freshly distilled from calcium hydride) was cooled to −23 °C. The solution was treated with 0.58 mL of *n*-butyl nitrite followed by the dropwise addition of 1.0 mL of a saturated solution of hydrogen chloride in tetrahydrofuran. After the mixture was stirred for 10 min, the pH was adjusted to 9–10 with triethylamine and the temperature was raised to 0 °C. A slurry of 0.70 g of optically pure D-**6a** in 2 mL of dimethylformamide was added, and the mixture was stirred for 4 h at 0 °C. Workup in the usual manner provided 1.42 g of oil, which when chromatographed on silica gel using a chloroform/methanol gradient (100% chloroform to 10% methanol in chloroform) provided 0.59 g (37%) of **19** as an oil: $[\alpha]^{23}_D$ 11.6° (*c* 1.0, CH_3OH); NMR ($CDCl_3$) δ 1.45 (s, 36, O-*t*-Bu), 2.28 (m, 4, β-CH_2), 3.43 (m, 2, γ-CH), 4.40 (m, 2, α-CH), 5.13 (brd s, 2, $ArCH_2$), 6.05 (m, 1, NH), 7.36 (brd s, 6, NH + ArH).

Anal. Calcd for $C_{36}H_{54}N_2O_{13}$: C, 59.82; H, 7.53; N, 3.89. Found: C, 59.90; H, 7.57; N, 3.96.

Registry No.—**1a,** 21142-81-4; **1b,** 69941-99-7; L-**1c,** 56926-94-4; DL-**1c,** 69961-11-1; **1d,** 69980-66-1; **3a,** 56877-40-8; D-**3a,** 66513-60-8; **3b,** 59479-77-5; **3c,** 56991-25-4; **3d,** 56926-90-0; **3e,** 56926-89-7; **3f,** 69942-00-3; **4,** 56926-92-2; D-**4,** 66513-61-9; D-**5a,** 60686-52-4; L-**5a,** 60686-50-2; L-**5a** ephedrine salt, 62965-13-3; **5b,** 59524-08-2; D-**5c,** 62965-11-1; L-**5c,** 66438-58-2; D-**5d,** 69942-01-4; L-**5d,** 69942-02-5; **6,** 56926-93-3; **6a,** 56877-44-2; D-**6a,** 60686-53-5; **7,** 66438-55-9; **8,** 66438-56-0; **9,** 69942-03-6; **10,** 69942-04-7; **11,** 56991-24-3; **12,** 69942-05-8; **13,** 56926-91-1; **14,** 69942-06-9; **15,** 69942-07-0; **16,** 69942-08-1; **17,** 69942-09-2; **18,** 69942-10-5; **19,** 66438-60-6; **20,** 69942-11-6; *N*-(benzyloxycarbonyl)-L-serine, 1145-80-8; *N*-(*tert*-butoxycarbonyl)serine, methyl ester, 69942-12-7; *N*-(benzyloxycarbonyl)glycine, 1138-80-3; ethyl glycinate, 459-73-4; γ,γ-di-*tert*-butyl-DL-γ-carboxyglutamylglycine ethyl ester, 69942-13-8; *N*-(benzyloxycarbonyl)-di-*tert*-butyl-DL-γ-carboxyglutamylazide, 69942-14-9; γ,γ-di-*tert*-butyl-DL-γ-carboxyglutamic acid α-methyl ester, 59479-80-0; *N*-benzyloxcarbonyl-L-leucine, 2018-66-8; L-proline benzyl ester, 41324-66-7; L-tyrosine hydrazide, 7662-51-3.

References and Notes

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(19) Sample obtained from Fluka Chemical Co.: $[\alpha]^{23}_D$ −13.3° (*c* 2.00, CH_3OH), $[\alpha]^{23}_{546}$ −15.7° (*c* 2.00, CH_3OH) [lit. $[\alpha]^{20}_{546}$ −16° (*c* 2.0, CH_3OH)[7b,20] and $[\alpha]^{20}_D$ −13.3° (*c* 2.3, methanol)[5b]]. The calculated enantiomeric excesses based on the two reported values are 98.1 and 100%, respectively.

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