82. D(–)- and L(+)-γ-Carboxyglutamic Acid (Gla): Resolution of Synthetic Gla Derivatives

by **Walter Märki, Max Oppliger, Peter Thanei** and **Robert Schwyzer**

Institut für Molekularbiologie und Biophysik

Eidgenössische Technische Hochschule, CH-8093 Zürich

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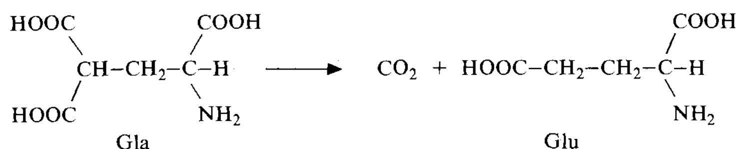
Summary

The chemical resolution of γ,γ'-di-*t*-butyl DL-*N*-benzyloxycarbonyl-γ-carboxyglutamate is described in detail (preliminary account see [1]). The D(–)-derivative was obtained as a crystalline quinine salt, and the L(+)-derivative as a crystalline salt with (–)-ephedrine in yields of 44 and 70%, respectively. Physical data are indicated for the enantiomers of γ,γ'-di-*t*-butyl *N*-benzyloxycarbonyl-γ-carboxyglutamate, γ,γ'-di-*t*-butyl γ-carboxyglutamate, and γ-carboxyglutamic acid. The absolute configurations and optical purities of the γ,γ'-di-*t*-butyl (+)- and (–)-*N*-benzyloxycarbonyl-γ-carboxyglutamates were determined by removal of the protecting groups and decarboxylation to optically active glutamic acid.

The new amino tricarboxylic acid, γ-carboxyglutamic acid (Gla), has been detected in the blood-clotting factors II (prothrombin), VII, IX, and X [1], in Protein C [2], and in calcium-binding proteins (osteocalcin) of mineralized vertebrate tissues [3]. Gla is synthesized by the vitamin K dependent, enzymatic carboxylation of L-glutamic acid residues contained in the precursors of these proteins [4] [5]. Isolation of Gla in sufficient quantities for the determination of gross physical properties, like specific rotation, has been precluded because of its instability under the usual hydrolytic conditions (decarboxylation of the malonic acid moiety):



Although six syntheses of DL-Gla and derivatives have been published in the past two years [5-8], only one preliminary report on the resolution of synthetic Gla derivatives [9] and one report on the asymmetric synthesis of L-Gla derivatives [10], both from this laboratory, have appeared.

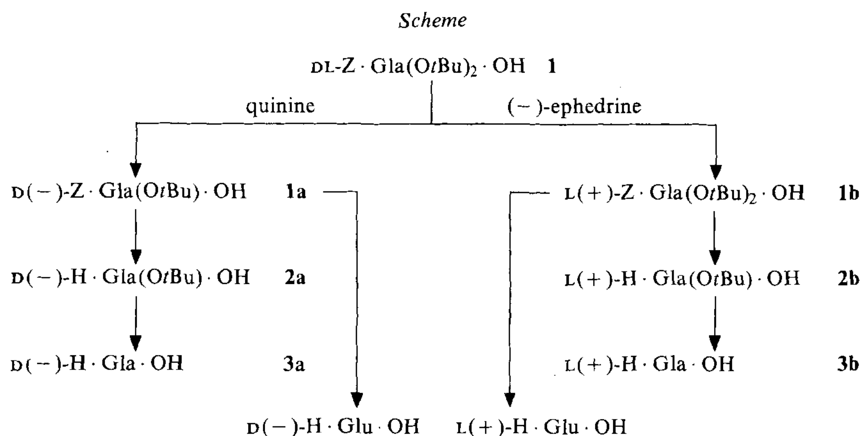
The purpose of this paper is to describe in detail the resolution of Z · Gla $(OtBu)_2$ · OH by quinine and ephedrine, the preparation, and the properties of the D(–)- and L(+)-enantiomers of Z · Gla $(OtBu)_2$ · OH, H · Gla $(OtBu)_2$ · OH, and H · Gla · OH (preliminary account [9]).

Unsuccessful attempts to obtain optically active Gla derivatives. During our previous experiments [8], we had noticed that the condensation of L-Z · Ala(J) · OMe with di-*t*-butyl malonate proceeded *via* methyl benzyloxycarbonyl-dehydroalaninate and led to a completely racemized product, Z · Gla$(OtBu)_2$ · OMe. However, with dimethyl malonate, the resulting Z · Gla$(OMe)_2$ · OMe was slightly, but definitely optically active (we did not hydrolyse this material to Gla as was erroneously implied by *Weinstein et al.* [7]). We therefore decided to try the condensation of di-*t*-butyl malonate also with L-Z · Ala(Br) · OMe and L-Z · Ala(Cl) · OMe. The latter compounds were synthesized *via* a crystalline modification of L-Z · Ser · OMe (see exper. part). No optically active product was obtained, indicating a deleterious effect of the two *t*-butylester groups relative to the two smaller methylester groups on the direct S_N2 condensation into the halogenated alanine side chain. Thus, it proved to be impossible to retain the α-carbon atom configuration introduced by the use of L-serine as starting material (asymmetric induction, however, proved to be successful [10]).

All our attempts to cleave DL-*N*-acetyl- or -chloroacetyl-Gla$(OtBu)_2$ · OH with kidney acylase were of no avail. We also did not succeed in preparing suitable diastereomeric salts with L-tyrosine hydrazide, brucine (partial success only with Ac · Gla$(OtBu)_2$ · OH, see exper. part and *Table 1*), quinidine, cinchonidine, and cinchonine.

*Resolution of γ,γ'-di-*t*-butyl* DL-*N-benzyloxycarbonyl-γ-carboxyglutamate* (**1**) *(Scheme).* The diastereomeric salt of the *laevo* enantiomer **1a**[1]) crystallized from an equimolar solution of **1** and quinine in ethyl acetate. After removal of the quinine

Scheme



from the mother liquors with an anion exchange resin, the *dextro* enantiomer **1b**[1]) was purified by repeated crystallizations from carbon tetrachloride/pentane. A similar treatment of the crystalline diastereoisomeric salt yielded **1a**. Acid hydrolysis and decarboxylation of the two enantiomers resulted in electrophoretically pure glutamic acid (Glu). A comparison of the specific rotations of the so produced (not recrystallized!) Glu with that produced by the hydrolysis of authentical

[1]) Sign of the optical rotation of the particular Z · Gla$(OtBu)_2$ · OH enantiomer in chloroform (opposite sign in methanol).

L-Z · Glu(O*t*Bu) · OH *(Table 1)* proved that **1a** has the D- and **1b** the L-configuration. The optical purity of both compounds was found to be greater than 98%.

The *dextro* enantiomer **1b** was obtained directly as a crystalline diastereomeric salt with (–)-ephedrine from ethyl acetate/pentane mixtures. Removal of the alcaloid produced pure, crystalline **1b** in 51% yield. Treatment of the mother liquors in the same manner yielded another 11% **1b.**

In the hydrophilic solvent methanol **1b** (L) exhibits negative optical rotation, very much like L-Z · Glu(O*t*Bu) · OH *(Table 2)*. In chloroform, however, its rotation is almost exactly reversed; the Glu derivative behaves similarly. This may be the consequence of different preferred conformations in the two solvents. The actual existence of preferred side-chain conformations of Gla is indicated by the 360 MHz ^{1}H-NMR. spectrum of Gla in D_2O at pH 7.9 (see below and Fig. 1)[2]).

Table 1. *Determination of the absolute configuration and optical purity of γ-carboxyglutamic acid derivatives*

Educt	Hydrolysis, 3h, 110°C	Product (electrophoresis)	$[\alpha]^{21}_{546}$°	$[\alpha]^{21}_{D}$°	c, H_2O	% Optical purity	Absolute configuration
L-Glu	6N HCl	Glu	+25.2±0.2	+21.4	1.5	=100	L
L-Z · Glu(O*t*Bu)	6N HCl	Glu	+24.6±0.2	+20.6	1.5	=100	L
L-Z · Glu(O*t*Bu)	conc. HCl	Glu	+23.2±0.4	+19.3	2	=100	L
(+)-Z · Gla(O*t*Bu)$_2$[a])	6N HCl	Glu	+24.2±0.4	+20.4	1	99	L
(+)-Z · Gla(O*t*Bu)$_2$[a])	conc. HCl	Glu	+21.7±0.6	+15.9	0.4	94–96	L
(–)-Z · Gla(O*t*Bu)$_2$[b])	6N HCl	Glu	–24.0±0.4	–20.5	1	98–99	D
(–)-Z · Gla(O*t*Bu)$_2$[b])	conc. HCl	Glu	–23.6±0.5	–21.3	0.4	100	D
(+)-Ac · Gla(O*t*Bu)$_2$[c])	6N HCl, 16 h, 108°	Glu	+10.5±0.2	+ 8.8	2	50–55	L>D

[a]) *Dextro* in chloroform, *laevo* in methanol.
[b]) *Laevo* in chloroform, *dextro* in methanol.
[c]) *Dextro* in chloroform.

Table 2. *Physical data of glutamic acid* (Glu), *γ-carboxyglutamic acid* (Gla), *and derivatives*

Compound	$[\alpha]^{20}_{546}$ (c, solvent)		$[\alpha]^{20}_{D}$ (c, solvent)	
D-Gla, NH_3	–44.6±0.5°	(1, 6N HCl)	–37.5±0.5°	(1, 6N HCl)
L-Gla	+41.7±0.5°	(1, 6N HCl)	+35.3±0.5°	(1, 6N HCl)
L-Glu	+36°	(5, 5N HCl)	+31.4°	(1, 6N HCl)
D-Gla(O*t*Bu)$_2$	– 6.8±0.3°	(1, methanol)	– 5.7±0.3°	(1, methanol)
L-Gla(O*t*Bu)$_2$	+ 6.6±0.3°	(1, methanol)	+ 5.6±0.3°	(1, methanol)
L-Glu(O*t*Bu)	–	–	+10.1°	(1, water)
D-Z · Gla(O*t*Bu)$_2$	–13.7±0.3°	(1.1, chloroform)	–11.3±0.3°	(1.1, chloroform)
L-Z · Gla(O*t*Bu)$_2$	+14.7±0.3°	(1.1, chloroform)	+12.3±0.3°	(1.1, chloroform)
	–13.8±0.3°	(1.1, methanol)	–10.9±0.3°	(1.1, methanol)
L-Z · Glu(O*t*Bu)	+13.3±0.3°	(1.1, chloroform)	+11.5±0.3°	(1.1, chloroform)
	–16.0±1°	(2, methanol)	– 9.4°	(1, ethanol)

[2]) The solution conformation of Gla and its derivatives and peptides is presently being investigated on collaboration with our Biophysics Group (Prof. Dr. *K. Wüthrich*).

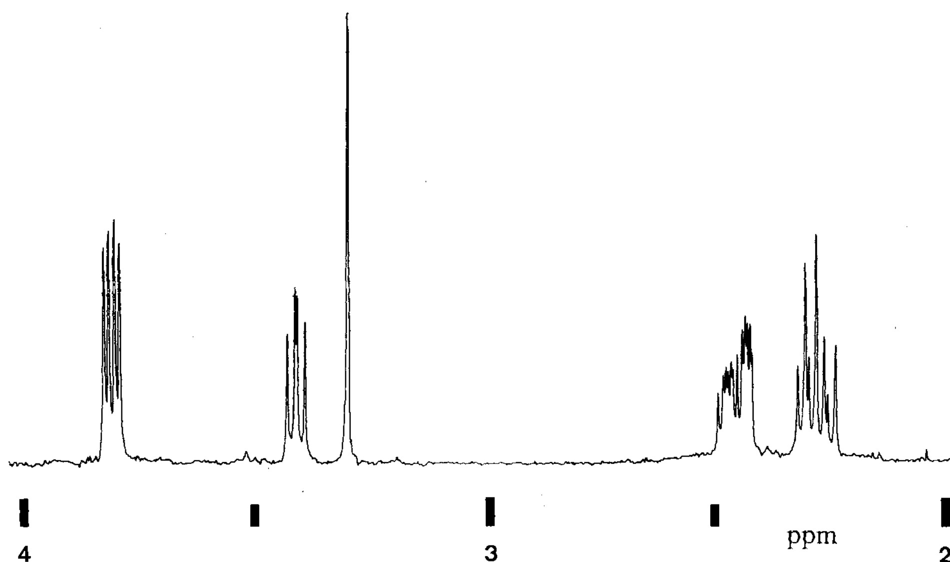


Fig. 1. *360 MHz ^{1}H-NMR. spectrum of* DL-*γ-carboxyglutamic acid* in D_2O at an apparent pH = 7.92 and with tetramethylammonium chloride as internal standard (3.3 ppm). D- and L-Gla (**3a, 3b**) gave identical spectra. The α-proton appears at about 3.8 ppm, the γ-proton (partly exchanged against a deuteron) at about 3.4 ppm. The singlet at 3.30 is tetramethylammonium, and the signals between 2.5 and 2.2 ppm arise from the β-protons.

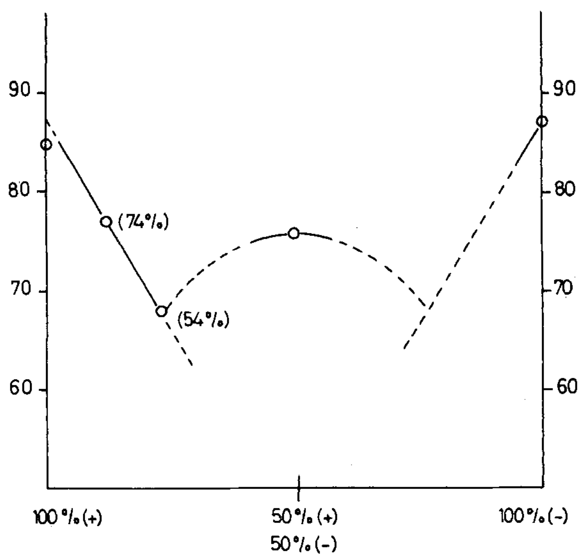


Fig. 2. *Melting points of* L(+)- *and* D(−)-*Z · Gla(OtBu)$_2$ · OH mixtures.* Ordinate: m.p. in degrees centrigade. Abscissa: mixture composition in mol percent.

DL-Z · Gla(O*t*Bu)$_2$ · OH appears to crystallize as a racemic compound. This is suggested by the melting points of the enantiomers and their mixtures *(Fig. 2)*.

D(−) and L(+) γ,γ'-di-t-butyl γ-carboxyglutamates, **2a** *and* **2b** *(Scheme).* Catalytic hydrogenation of **1a** and **1b** yielded pure, crystalline **2a** and **2b**, respectively. The sign of the optical rotation of **2b** (L) in methanol was the same as that of the corresponding L-Glu derivative, H · Glu(O*t*Bu) · OH, in water *(Table 2)*. The absolute configurations of **2a** and **2b** were inferred from this result and from their genealogy.

D(−)- and L(+)-γ-carboxyglutamic acid, **3a** *and* **3b** *(Scheme).* To obtain these compounds, **2a** and **2b** were treated with cold, concentrated hydrochloric acid in order to remove the *t*-butyl groups. **3a** (D) was first characterized as its monoammonium salt, **3b** (L) as its monohydrochloride dihydrate. Trifluoroacetic acid treatment of **2b**, azeotropic removal of the reagent with toluene, and crystallization from water/ethanol produced 'free' L-Gla, **3b**.

The total yield of L-Gla is in the range of 12%, 35%, and 45% with respect to serine, di-*t*-butyl malonate, and DL-Z · Gla(O*t*Bu)$_2$ · OH, respectively. It is better than that hitherto obtained for a derivative with the asymmetric synthesis [10].

When subjected to the usual analytical procedure for acid hydrolysis of peptides and proteins, Gla is quantitatively converted to Glu. In a test mixture of amino acids, authentical synthetic Gla emerges at 23 min, *i.e.* after cysteic acid and before aspartic acid *(Fig. 3)*.

Its electrophoretic mobility is greater than that of Asp, and its electrophoretic isoelectric point is around pI=2.4 *(Fig. 4)*. Preliminary titration experiments

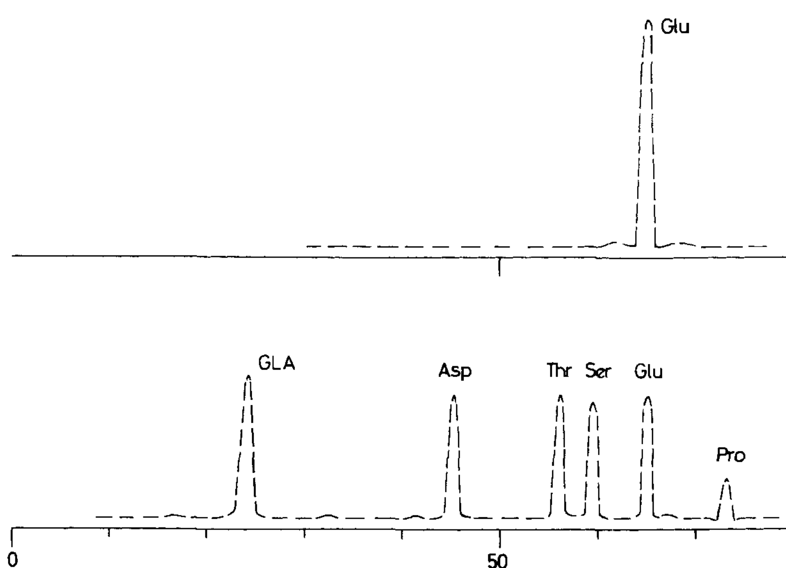


Fig. 3. *Behaviour of Gla under the conditions of amino acid analysis.* Upper panel: glutamic acid from Gla by acid hydrolysis. Lower panel: Gla added to a standard mixture of amino acids. Abscissa: elution time (min).

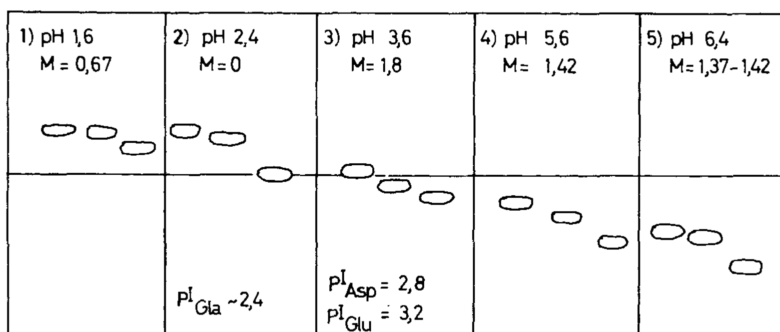


Fig. 4. *Electrophoretic behaviour of Gla on cellulose thin-layer plates* (400 V, 5 mA, 50 min). The spots are, from left to right on each plate: Glu/Asp/Gla.

indicate a pK(1) of about 1.7±0.2 and an over-all association constant for Ca^{2+} about equal to that of Glu. We shall report separately on these and similar properties [11].

The 360 MHz ^{1}H-NMR. spectrum of *Fig. 1* indicates the expected slow exchange of the γ-proton against solvent deuterons and the non-equivalence of the two β-protons under the conditions of the experiment: both the α- and γ-protons are detected as double doublets. An exact NMR. analysis is under way.

We wish to thank the *Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung* for financial acid. This work is part of the doctoral thesis of *W. M.* Abbreviations of amino acids and derivatives are according to *E. Wünsch* 'Synthese von Peptiden', Bd. 15, *Houben-Weyl*, 'Methoden der organischen Chemie', *E. Müller*, Herausgeber, Georg Thieme Verlag, Stuttgart 1974.

Experimental Part

General procedures and materials. See *Märki & Schwyzer* [8]. Additional solvents (volume parts) for thin-layer chromatography (TLC.) are: I: 2-propanol/water/pyridine 7:6:6; CM: chloroform/methanol 1:1; EMA: acetic acid/methanol/acetone 7:2:1.

Diastereomeric salts of Gla derivatives with optically active bases were decomposed as follows: The sulfonic acid resin Amberlyst 15 (*Rohm and Haas Co.*, Philadelphia) in its acid cycle was washed repeatedly with methanol. The alcaloid salts were dissolved in methanol and chromatographed over columns containing 10 ml of the resin per mmol salt. The columns were washed with 5 times their volume of methanol. This eluate was evaporated. The viscous residue consisted of the desired amino acid derivative.

The absolute configuration and the optical purity of the Gla derivatives were determined as follows: 0.1 mmol of the product was suspended in 1 ml of 6N HCl containing minute amounts of phenol as antioxidant and the ampoule sealed in a high vacuum. Hydrolysis was carried out at 110° for 3 h and the solution evaporated over P_2O_5 and KOH at about 0.001 Torr. The residue was not further purified and its optical rotation measured. Part of the solution was subjected to thin-layer electrophoresis to make sure that it contained pure glutamic acid. The specific rotations were calculated on the basis of the weights of the non-hydrolysed educts. The optical purity in per cent is defined as: ($[\alpha]_D$ found · 100)/ ($[\alpha]_D$ calc.) wherein the calculated value is derived either from the literature or from measurements with the pure compounds, or both.

The amino acid analyses were carried out with a *Beckman* Model 121 Amino Acid Analyser using a column of *Beckman* AA 15 resin, 0.6×52 cm.

*γ,γ'-Di-*t*-butyl* DL-N-*benzyloxycarbonyl-γ-carboxyglutamate* (**1**). This compound was prepared in crystalline form according to [8]. It was further characterized by its cyclohexylamine salt: colourless needles from ethyl acetate, m.p. 119-121°, $[\alpha]_D^{20} = 0°$ ($c = 1.1$, $CHCl_3$).

*Quinine salt of γ,γ'-di-*t*-butyl* D(–)-N-*benzyloxycarbonyl-γ-carboxyglutamate.* 2.66 g (6.08 mmol) of DL-Z · Gla(O*t*Bu)$_2$ · OH (**1**) and 1.97 g (6.08 mmol) of quinine (m.p. 175°; $[\alpha]_{546}^{20} = -154 \pm 3°$, $c = 1.5$, $CHCl_3$) were dissolved in 50 ml of hot ethyl acetate and kept at RT. for 12 h. Crystallization was completed by cooling to 4° for 15 h. The colourless needles were gathered by filtration and washed with ice-cold ethyl acetate: 1.82 g (2.4 mmol, 40% of the theoretical yield), m.p. 132-133°; $[\alpha]_D^{20} = -76.8°$ and $[\alpha]_{546}^{20} = -94.1°$ (both $c = 1$, $CHCl_3$).

The filtrate and washings were evaporated to dryness, the residue dissolved in CCl_4 and treated with pentane. Another 204 mg of the salt (4.4% yield) were obtained.

*γ,γ'-Di-*t*-butyl* D(–)-N-*benzyloxycarbonyl-γ-carboxyglutamate* (**1a**). The quinine was removed according to the general procedure. The viscous residue was crystallized from CCl_4/pentane in the form of dense aggregates of needles. 1.63 g (2.1 mmol) of the quinine salt yielded 781 mg (1.79 mmol, 83%) **1a**; m.p. 86-88°; Rf 0.68 CM, 0.37 CME. - NMR.: 10.05 (*broad signal*, 1H); 7.4 (*s*, 5H); 5.6-5.3 (*broad signal*, 1H); 5.15 (*s*, 2H); 4.7-4.3 (*m*, 1H); 3.35 (*t*, 1H); 2.6-2.2 (*many signals*, 2H); 1.45 (2*s*, 18H).

$C_{22}H_{31}NO_8$ (437.5) Calc. C 60.40 H 7.14 N 3.20% Found C 60.32 H 7.19 N 3.28%

*γ,γ'-Di-*t*-butyl* L(+)-N-*benzyloxycarbonyl-γ-carboxyglutamate* (**1b**). The filtrate and washings of the crystalline quinine salt were evaporated to dryness. The quinine was removed from the residue according to the general procedure. Attempts to further resolve the mixture of L- and DL-acids *via* the quinidine salts were only partly successful, because the L-Z · Gla(O*t*Bu)$_2$ · OH salt was only obtained as a gel (m.p. 83-93°, $[\alpha]_D^{20} = +100 \pm 0.5°$ and $[\alpha]_{546}^{20} = 123 \pm 0.5°$, both $c = 1$, $CHCl_3$).

Therefore the acid mixture was simply recrystallized. The L-isomer appeared from CCl_4/pentane mixtures at 4° as dense aggregates of needles and from diisopropyl ether/pentane at 4° usually as colourless needles, m.p. 87-89°, in seldom cases as hard, thick crystals. The TLC. and NMR. results were identical with those of the D-isomer, **1a**. The (–)-ephedrine salt of **1b** (below) yielded an identical product in 62% over-all yield from **1**.

$C_{22}H_{31}NO_8$ (437.5) Calc. C 60.40 H 7.14 N 3.20% Found C 60.23 H 7.11 N 3.13%

The cyclohexylamine salt was obtained as colourless needles from ethyl acetate, m.p. 122-126°, $[\alpha]_D^{20} = +11.7°$, $[\alpha]_{546}^{20} = +14.7°$ (both $c = 1.1$, $CHCl_3$).

(–)-*Ephedrine salt of γ,γ'-di-*t*-butyl* L(+)-N-*benzyloxycarbonyl-γ-carboxyglutamate.* 437 mg (6.08 mmol) **1** were dissolved in 0.3 ml ethyl acetate and combined with a solution of 83 mg (0.5 mmol) (–)-ephedrine in 0.2 ml ethyl acetate. The clear mixture was diluted with much pentane and kept at 4° for 2 days. The solid precipitate was recrystallized from ethyl acetate/pentane: 173 mg (58%) large, colourless needles, m.p. 122-124°. $[\alpha]_{546}^{20} = -7.7°$, $[\alpha]_D^{20} = -6.7°$ (both $c = 1.2$, $CHCl_3$); $[\alpha]_{546}^{20} = -17.7°$, $[\alpha]_D^{20} = -14.5°$ (both $C = 1.0$, MeOH).

$C_{32}H_{46}N_2O_9$ (602.7)	Calc.	C 63.77	H 7.69	N 4.65	C 62.03	H 7.73	N 4.52%
with 2.73% EtOAc	Found	C 62.03	H 7.71	N 4.60%			

The product was decomposed to **1b** with Amberlyst 15, yield 85%. Treatment of the mother liquors with (–)-ephedrine resulted in another 13% of pure salt, or 11% of **1b**. Total yield of **1b** from **1** = 62%.

*γ,γ'-Di-*t*-butyl* D(–)-*γ-carboxyglutamate*, **2a**. 219 mg (0.5 mmol) **1a** were catalytically hydrogenated according to [8] in order to remove the benzyloxycarbonyl group. The crude solid product was washed with diethyl ether, yield 144 mg (0.48 mmol or 95%). It was pure without recrystallization from water, m.p. 165.5-167.5 (dec.); Rf 0.46 BEWI.

$C_{14}H_{25}NO_6$ (303.36) Calc. C 55.43 H 8.31 N 4.62% Found C 55.42 H 8.43 N 4.76%

*γ,γ'-Di-*t*-butyl* L(+)-*γ-carboxyglutamate*, **2b**. This isomer was prepared in exactly the same manner as its antipode, **2a**. Yield 96% (precipitated from methanol with diethyl ether), pure solid, m.p. 166-167° (dec.). Crystallization from water with a small amount of methanol resulted in colourless needles, yield 70%; m.p. 170-171° (dec.).

$C_{14}H_{25}NO_6$ (303.36) Calc. C 55.43 H 8.31 N 4.62% Found C 55.23 H 8.30 N 4.55%

D(–) *γ-Carboxyglutamic acid monoammonium salt* (**3a**). 94 mg (0.31 mmol) of D-H · Gla(O*t*Bu)$_2$ · OH (**2a**) were dissolved in 1 ml of cold, concentrated hydrochloric acid and kept at 0° for 15 min. The solution was evaporated at 0.001 Torr over P_2O_5 and KOH. The hygroscopic residue was quickly dissolved in 1 ml of glacial acetic acid, treated with a slight excess of ammonium acetate (35 mg = 0.42 mmol), and lyophilized. The residue was repeatedly triturated with ethanol: yield 53 mg (0.25 mmol, 82%) colourless solid; m.p. 157–159° (decomposition with evolution of CO_2). At pH 6.4 and 40 V/cm, the compound migrates about 50 mm towards the anode in 50 min. This corresponds to about 1.4 times the distance travelled by aspartic acid under the same conditions. The electrophoretic and TLC. aspects are those of a pure compound, Rf 0.23 1. – ^{1}H-NMR. confirmed the expected structure and the analytical purity (see *Fig. 1*).

$C_6H_{12}N_2O_6$ (208.1) Calc. C 34.62 H 5.81 N 13.46% Found C 34.63 H 6.00 N 13.66%

L(+)-*γ-Carboxyglutamic acid* (**3b**). This isomer was obtained by dissolving **2b** in trifluoroacetic acid/water, 9:1 (*v/v*), evaporating the solution after 90 min at 20°, azeotropic removal of the reagent with toluene, triturating with ether, and recrystallizing from water/ethanol, 1:1 (*v/v*). Colourless, very small crystals, yield 75%, m.p. 167–167.5° (dec.).

$C_6H_9NO_6$ (191.14) Calc. C 37.70 H 4.75 N 7.32% Found C 37.59 H 4.85 N 7.29%

Methyl L-*serinate hydrochloride*. Preparation according to [8]. Yield 97%; m.p. 162–166°, $[\alpha]_D^{25} = +1.8°$ (*c* = 0.8, dimethylformamide).

$C_4H_{10}ClNO_3$ (155.6)	Calc.	C 30.88	H 6.48	Cl 22.79	N 9.00%
	Found	C 30.89	H 6.56	Cl 22.56	N 9.07%

Methyl L-N-*benzyloxycarbonyl-serinate*. Preparation according to [8]. Yield 96%; colourless needles, m.p. 35–37°; $[\alpha]_D^{25} = -15°$ (*c* = 0.8, methanol); Rf 0.35 CME.

$C_{12}H_{15}NO_5$ (253.23) Calc. C 56.91 H 5.97 N 5.53% Found C 56.83 H 5.99 N 5.51%

Methyl L-*β-bromoalaninate*. A solution of 6.6 g (16.2 mmol) methyl L-*N*-benzyloxycarbonyl-*O*-tosyl-serinate [8] and 1.75 g (20 mmol) lithium bromide in 50 ml dry acetone was stirred in the dark at 20° for 72 h. The precipitated lithium toluenesulfonate was removed by filtration and the filtrate evaporated. The residue was recrystallized from diethylether/pentane and from pentane. Yield 3.2 g (10.1 mmol, 63%); m.p. 63–65°; $[\alpha]_D^{20} = -21.2°$ (*c* = 1, dimethylformamide); Rf 0.2 HE1, 0.45 HE2. – NMR. 7.35 (*s*, 5H); 5.8–5.5 (*broad signal*, 1H); 5.15 (*s*, 2H); 4.9–4.6 (*m*, 1H); 3.8–3.7 (*m*, 5H: *β*-CH_2 + OCH_3).

$C_{12}H_{14}BrNO_4$ (316.4)	Calc.	C 45.59	H 4.46	Br 25.28	N 4.43%
	Found	C 45.85	H 4.57	Br 25.05	N 4.42%

Methyl L-*β-chloroalaninate*. 497 mg (1.22 mmol) methyl L-*N*-benzyloxycarbonyl-*O*-tosyl-serinate [8] were reacted with 206 mg (4.9 mmol) lithium chloride in 6 ml dry acetone according to the procedure for the bromo derivative. Yield 295 mg (1.1 mmol, 89%); colourless crystals, m.p. 54–55°; $[\alpha]_D^{20} = -21.8°$ (*c* = 0.8, dimethylformamide); Rf 0.47 HE2. – NMR. practically identical with that of the bromo compound, except 4.0–3.9 (*d*, J_{AD} = 3 Hz, 2H, *β*-CH_2) and 3.8 (*s*, 3H, OCH_3).

$C_{12}H_{14}ClNO_4$ (271.7)	Calc.	C 53.04	H 5.19	Cl 13.05	N 5.16%
	Found	C 52.94	H 5.15	Cl 12.92	N 4.99%

γ,γ'-Di-t-butyl DL-N-*chloroacetyl-γ-carboxyglutamate*. 310 mg (1 mmol) *γ,γ'*-di-*t*-butyl DL-*γ*-carboxyglutamate were dissolved in 6 ml aqueous, saturated sodium hydrogencarbonate solution, cooled to 0° and treated with 200 µl chloroacetylchloride. The pH was kept at about 8 by gradual addition of a total of 2 ml 2N NaOH. After stirring for 15 h the product was isolated by the usual procedure with ethyl acetate. Column chromatography on silica gel and elution with chloroform/methanol 19:1 (*v/v*) yielded 350 mg (0.92 mmol, 92%) of a viscous oil that was crystallized from carbon tetrachloride/pentane. Yield 85%, m.p. 115–118°; Rf 0.3 CME, 0.66 BEW1. – NMR. 8.1 (*broad signal*); 7.6–7.4 (*d*, 1H); 4.9–4.4 (*m*, 1H); 4.1 (*s*, 2H); 3.4 (*d*×*d*, 1H); 2.6–2.2 (*m*, 2H); 1.5 (2*s*, 18H).

γ,γ'-Di-t-butyl DL-N-*acetyl-γ-carboxyglutamate*. Preparation from *γ,γ'*-di-*t*-butyl DL-*γ*-carboxyglutamate and *p*-nitrophenyl acetate in dimethylformamide/pyridine. Yield 86%, prisms from $CHCl_3/CCl_4$, m.p. 125–127°; Rf 0.57 BEW1, 0.72 EMA. – NMR. as expected.

*Partial resolution of γ,γ'-di-*t*-butyl* DL-*N-acetyl-γ-carboxyglutamate.* 520 mg (1.5 mmol) γ,γ'-di-*t*-butyl DL-*N*-acetyl-γ-carboxyglutamate and 595 mg (1.5 mmol) brucine (m.p. 178°, $[\alpha]^{20}_{546} = -136 \pm 5°$, $c = 2.5$, chloroform) were dissolved in 2-propanol and the solution treated dropwise with diisopropylether until turbid. After 5-6 days at 4°, the solid precipitate was isolated: 300 mg (27%); m.p. 88-120°; $[\alpha]^{20}_{546} = -12.7 \pm 0.5°$, $[\alpha]^{20}_{D} = -8.8°$ (both $c = 1$, chloroform). The compound was dissolved in 2-propanol and treated with chloroform and dilute hydrochloric acid to pH about 2 of the aqueous phase. The chloroform was subjected to the usual isolation procedure and the residue recrystallized from carbon tetrachloride. Yield 115 mg (0.33 mmol, 44%) of a colourless, crystalline solid, m.p. 125-127°; $[\alpha]^{20}_{546} = +21°$, $[\alpha]^{20}_{D} = +17.7°$ (both $c = 1.1$, chloroform). Optical purity somewhat more than 50%, indicating that at least 75% of the product has the L-configuration *(Table 1)*.

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