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Synthesis of α - and γ -(*N*-Carbobenzoxy- and *N*-*tert*-Butyloxycarbonyl-L-Glutamyl)cholines¹⁾

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In order to examine the lipotropic and hypotensive effects of glutamylcholines, α - and γ -(*Z*- and Boc-glutamyl)cholines (**4a**, **4'a**, **4b** and **4'b**) were synthesized by the removal of the *tert*-butyl and benzyl groups of the corresponding *tert*-butyl (**3a** and **3'a**) and benzyl (**3b** and **3'b**) choline esters which were obtained by the esterification of *tert*-butyl *Z*-glutamates (**1a** and **1'a**) and benzyl Boc-glutamate dicyclohexylamine salts (**1b** and **1'b**) with *N,N*-dimethylaminoethyl chloride, followed by methylation with methyl halide.

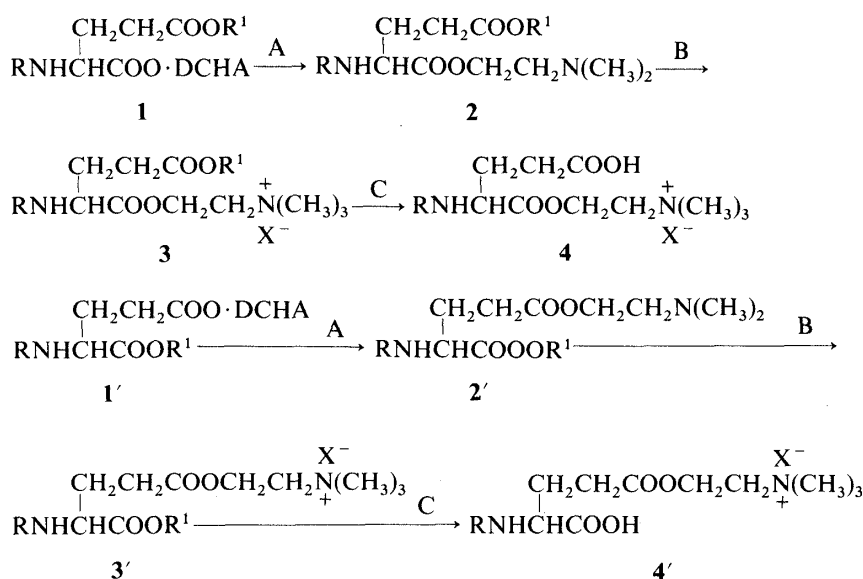
Keywords—glutamyl cholines synthesis; α -(*Z*-glutamyl)choline; γ -(*Z*-glutamyl)choline; α -(Boc-glutamyl)choline; γ -(Boc-glutamyl)choline; *tert*-butyl *Z*-glutamate; benzyl Boc-glutamate; *N,N*-dimethylaminoethyl chloride; dicyclohexylamine salt; hypotensive effect

In 1955, the lipotropic action and hypotensive effect of *N*-carbobenzoxy(*Z*)-glutamylcholine were reported by Nishizawa.²⁾ The choline ester used in the above examinations was a mixture of α (**4a**, X=Cl)- and γ (**4'a**, X=Cl)-(*Z*-glutamyl)cholines which had been prepared from *Z*-glutamic anhydride and choline chloride by Kotake *et al.*³⁾ and Matsukawa *et al.*⁴⁾ The latter authors presumed that the relative mobility of α -glutamylcholine on paper chromatography (PC) is higher than that of γ -glutamylcholine from the chemical behavior of the above mixture.

In order to compare the pharmacological activity of α -glutamylcholine with that of γ -glutamylcholine, we attempted to separate the above mixture by silica gel and aluminum oxide column chromatography, but without success, since a part of the choline esters was hydrolyzed easily to give glutamic acid and choline. In this paper, therefore, we describe the synthesis of α - and γ -(*Z*- and *tert*-butyloxycarbonyl(Boc)-glutamyl)cholines.

First, γ - and α -*tert*-butyl *Z*-glutamates (**1a** and **1'a**), and γ - and α -benzyl Boc-glutamates (**1b** and **1'b**), key intermediates for the synthesis of *Z*- and Boc-glutamylcholine, were prepared from glutamic acid by the methods described in the experimental section. An attempt to prepare **1a** and **1'a** by the reaction of *Z*-glutamic anhydride with *tert*-butyl alcohol in the presence of acids and bases⁵⁾ was unsuccessful.

In the previous paper,⁶⁾ the esterification of *Z*-amino acids with *N,N*-dimethylaminoethyl chloride in the presence of triethylamine afforded *N,N*-dimethylaminoethyl esters of *Z*-amino acids. As shown in Chart 1, the dicyclohexylamine (instead of the triethylamine) salts of γ - and α -*tert*-butyl *Z*-glutamates (**1a** and **1'a**) reacted with *N,N*-dimethylaminoethyl chloride to give γ -*tert*-butyl α -(*N,N*-dimethylamino)ethyl (**2a**) and α -*tert*-butyl γ -(*N,N*-dimethylamino)ethyl (**2'a**) *Z*-glutamates in 97% and 98% yields, respectively. These dimethylaminoethyl esters (**2a** and **2'a**) were colorless oils, and their proton magnetic resonance (¹H-NMR) spectra exhibited a 6H singlet due to two methyls of the dimethylamino group at δ 2.26. Methylation of these



a: R = carbobenzoxy (Z), R¹ = *tert*-butyl

b: R = *tert*-butyloxycarbonyl (Boc), R¹ = benzyl

X = Cl, Br, I, Tos DCHA = dicyclohexylammonium

A, (CH₃)₂NCH₂CH₂Cl; B, CH₃X;

C, HCl–AcOEt or TFA–CH₂Cl₂ (removal of *tert*-butyl group),

Pd–carbon/H₂ (removal of benzyl group).

Chart 1

dimethylaminoethyl esters (**2a** and **2'a**) with methyl halide (X = Cl, Br, and I) and methyl tosylate (X = Tos) afforded γ -*tert*-butyl α -choline ester (**3a**: X = Cl, Br, I and Tos) and α -*tert*-butyl γ -choline ester (**3'a**: X = Cl, Br, I and Tos) in 83–100% and 74–100% yields, respectively. The iodide and tosylate of the above α - and γ -choline esters crystallized as colorless needles, but the other halides were semi-solids. ¹H-NMR spectra of these *tert*-butyl choline esters (**3a** and **3'a**) exhibited a 9H singlet due to three methyls of the choliny group at δ 3.20–3.45. Removal of the *tert*-butyl group from the *tert*-butyl choline esters (**3a** and **3'a**) was achieved by treatment with 50% trifluoroacetic acid–dichloromethane and 5N hydrogen chloride–AcOEt to give the desired α (**4a**, X = Cl, Br, I and Tos)- and γ (**4'a**: X = Cl, Br, I and Tos)-(Z-glutamyl)cholines. The ¹H-NMR spectra of these choline esters (**4a** and **4'a**) exhibited a 9H singlet due to three methyls of the choliny group at δ 3.18–3.30. The methylene protons of the benzyl group in α -(Z-glutamyl)cholines (**3a** and **4a**) appeared as an AB quartet at δ near 5.10 (J = 12 Hz), but those of the γ -(Z-glutamyl)cholines (**3'a** and **4'a**) appeared as a singlet at δ 5.10. The upper spot on thin layer chromatography (TLC) and PC of the reaction product which was obtained from Z-glutamic anhydride and choline chloride^{3,4)} was identical with that of α -(Z-glutamyl)choline chloride (**4a**, X = Cl) and the lower one was identical with that of γ -(Z-glutamyl)choline chloride (**4'a**, X = Cl).

Next, the synthesis of γ - and α -(Boc-glutamyl)cholines (**4b** and **4'b**) was performed in a similar manner. Esterification of the dicyclohexylamine salts of γ - and α -benzyl Boc-glutamate (**1b** and **1'b**) with *N,N*-dimethylaminoethyl chloride gave γ -benzyl α -(*N,N*-dimethylamino)ethyl (**2b**) and α -benzyl γ -(*N,N*-dimethylamino)ethyl (**2'b**) Boc-glutamates, respectively. The ¹H-NMR spectra of these esters (**2b** and **2'b**) exhibited a 6H singlet due to two methyls of the dimethylamino group at δ 2.30 and 2.28, respectively. Methylation of these dimethylaminoethyl esters (**2b** and **2'b**) with methyl halide (X = Cl, Br and I) gave α -(γ -benzyloxy-Boc-glutamyl)choline (**3b**, X = Cl, Br and I) and γ -(α -benzyloxy-Boc-glutamyl)choline (**3'b**, X = Cl, Br), whose ¹H-NMR spectra exhibited a 9H singlet due to three

methyls of the choline group at δ 3.42—3.49. α -(γ -Benzyloxy-Boc-glutamyl)choline iodide (**3b**, X=I) crystallized as colorless needles, but the other halides were semi-solids. Removal of the benzyl groups from the (benzyloxy-Boc-glutamyl)cholines (**3b** and **3'b**) was carried out by hydrogenolysis with palladium-carbon in isopropyl alcohol to give α (**4b**: X=Cl and Br)- and γ (**4'b**: X=Cl and Br)-(Boc-glutamyl)cholines. Hydrogenolysis of α -(γ -benzyloxy-Boc-glutamyl)choline iodide (**3b**: X=I) failed to give the starting material. $^1\text{H-NMR}$ spectra of α - and γ -(Boc-glutamyl)cholines (**4b** and **4'b**) exhibited a 9H singlet due to three methyls of the choliny group at δ 3.26—3.36.

It is interesting that α -(Z-glutamyl)cholines were more effective than γ -(Z-glutamyl)cholines in terms of their hypotensive action in spontaneously hypertensive rats (SHR). The details will be reported elsewhere.

Experimental

All melting points were taken on a microscopic hot stage apparatus (Yanagimoto) and are uncorrected. Optical rotation were measured in methanol solution ($c=1$) with a JASCO DIP-4 polarimeter. $^1\text{H-NMR}$ spectra were taken with a Varian XL 200 spectrometer in CDCl_3 unless otherwise noted. The chemical shifts were recorded in ppm on the δ scale from $(\text{CH}_3)_4\text{Si}$ as an internal standard. The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. TLC was performed on precoated silica gel plates 0.25 mm thick (Silicagel 70 F₂₅₄ Wako Pure Chemical Industry Ltd.) and detection was achieved by ultraviolet (UV) irradiation, by using the combination of hydrogen bromide and ninhydrin for Z- and Boc-derivatives and by using the Dragendorff reagent for choline derivatives. *Rf* values refer to the following solvent systems (v/v): *Rf*₁ CHCl_3 -MeOH-AcOH (95:5:3); *Rf*₂ *n*-BuOH-AcOH-H₂O (4:1:2).

Materials—L-Glutamic acid ("L" is omitted elsewhere) was purchased from Kyowa Hakko Kogyo Co., Ltd.

The key intermediates were prepared from glutamic acid through the following synthetic pathways.

Dicyclohexylamine Salt of γ -*tert*-Butyl Z-Glutamate (**1a**): (A) Glutamic acid $\rightarrow$ Z-glutamic acid⁷⁾ $\rightarrow$ Z-glutamic anhydride¹³⁾ $\rightarrow$ α -benzyl Z-glutamate⁸⁾ $\rightarrow$ α -benzyl γ -*tert*-butyl Z-glutamate⁹⁾ $\rightarrow$ **1a**.¹⁰⁾ Total yield: 14%. mp 139—142°C. $[\alpha]_{\text{D}}^{27} + 7.5^\circ$. (Lit.¹⁰⁾ mp 139—140°C. $[\alpha]_{\text{D}}^{25} - 7.3^\circ$ ($c=1$, MeOH)).

(B) Z-Glutamic acid⁷⁾ $\rightarrow$ (S)-3-Z-5-oxo-4-oxazolidinepropionic acid $\rightarrow$ γ -*tert*-butyl (S)-3-Z-5-oxo-4-oxazolidinepropionate $\rightarrow$ **1a**.¹¹⁾ Total yield: 21%. mp 140—144°C. $[\alpha]_{\text{D}}^{29} + 6.6^\circ$. (Lit.¹¹⁾ mp 138—140°C. $[\alpha]_{\text{D}}^{25} + 6.1^\circ$ ($c=3.9$, MeOH)). Free acid: $^1\text{H-NMR}$ δ : 1.42 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.98—2.40 (2H, m, $-\text{CH}_2-$), 2.41 (2H, m, COCH_2), 4.40 (1H, m, COCH), 5.13 (2H, s, $\text{CH}_2\text{C}_6\text{H}_5$), 7.37 (5H, s, C_6H_5).

Dicyclohexylamine Salt of α -*tert*-Butyl Z-Glutamate (**1'a**): (A) Glutamic acid $\rightarrow$ γ -benzyl glutamate¹²⁾ $\rightarrow$ γ -benzyl Z-glutamate¹²⁾ $\rightarrow$ γ -benzyl α -*tert*-butyl Z-glutamate¹⁰⁾ $\rightarrow$ **1'a**.¹⁰⁾ Total yield: 25%. mp 147—150°C. $[\alpha]_{\text{D}}^{25} - 13.7^\circ$. (Lit.¹⁰⁾ mp 148—149°C. $[\alpha]_{\text{D}}^{25} - 14.1^\circ$ ($c=1$, MeOH)).

(B) Z-Glutamic acid⁷⁾ $\rightarrow$ Z-pyroglutamic acid¹³⁾ $\rightarrow$ *tert*-butyl Z-pyroglutamate¹⁰⁾ $\rightarrow$ **1'a**.¹⁰⁾ Total yield: 22%. mp 147—150°C. $[\alpha]_{\text{D}}^{31} - 13.6^\circ$. (Lit.¹⁰⁾ mp 149—150°C. $[\alpha]_{\text{D}}^{25} - 13.8^\circ$ ($c=1$, MeOH)). Free acid: $^1\text{H-NMR}$ δ : 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 2.02—2.40 (2H, m, $-\text{CH}_2-$), 2.40 (2H, m, COCH_2), 4.28 (1H, m, $-\text{COCH}$), 5.12 (2H, s, $\text{CH}_2\text{C}_6\text{H}_5$), 7.37 (5H, s, C_6H_5).

Dicyclohexylamine salt of γ -benzyl Boc-glutamate (**1b**) was purchased from Wako Pure Chemical Industry Ltd.

Dicyclohexylamine Salt of α -Benzyl Boc-Glutamate (**1'b**): Glutamic acid $\rightarrow$ Boc-glutamic acid¹⁴⁾ $\rightarrow$ Boc-glutamic anhydride⁹⁾ $\rightarrow$ **1'b**.⁹⁾ Total yield: 32%. mp 163—168°C. $[\alpha]_{\text{D}}^{26} - 16.5^\circ$. (Lit.⁹⁾ mp 172°C. $[\alpha]_{\text{D}}^{25} - 19.2^\circ$ ($c=0.7$, MeOH)). Free acid: $^1\text{H-NMR}$ δ : 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.87—2.31 (2H, m, $-\text{CH}_2-$), 2.41 (2H, m, COCH_2), 4.39 (1H, m, COCH), 5.19 (2H, s, $\text{CH}_2\text{C}_6\text{H}_5$), 7.37 (5H, s, C_6H_5).

General Procedure for Preparation of γ (or α)-Substituted α (or γ)-(N,N-Dimethylamino)ethyl Z- and Boc-Glutamate (2a**, **2'a**, **2b** and **2'b**): A Typical Example**— γ -*tert*-Butyl α -(N,N-Dimethylamino)ethyl Z-Glutamate (**2a**): A solution of N,N-dimethylaminoethyl chloride (7.8 g, 72 mmol) in benzene (30 ml) was added to a solution of **1a** (18.8 g, 36 mmol) in AcOEt (240 ml), and the mixture was refluxed at 90°C for 16 h. After removal of dicyclohexylammonium chloride by filtration, the filtrate was washed successively with 0.1 N HCl, 10% Na_2CO_3 and brine, then dried over MgSO_4 . The solvent was removed *in vacuo* to give **2a** (14.3 g, 97%) as a colorless oil. $[\alpha]_{\text{D}}^{28} - 17.2^\circ$. MS *m/z*: 408 (M^+). *Rf*₂ 0.39. $^1\text{H-NMR}$ δ : 1.40 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.90—2.20 (2H, m, $-\text{CH}_2-$), 2.26 (6H, s, $\text{N}(\text{CH}_3)_2$), 2.32 (2H, m, COCH_2), 2.56 (2H, t, $J=6$ Hz, NCH_2), 4.24 (2H, t, $J=6$ Hz, OCH_2), 4.40 (1H, m, COCH), 5.10 (2H, s, $\text{CH}_2\text{C}_6\text{H}_5$), 7.35 (5H, s, C_6H_5). Anal. Calcd for $\text{C}_{21}\text{H}_{32}\text{N}_2\text{O}_6$: C, 61.74; H, 7.90; N, 6.86. Found: C, 61.63; H, 7.72; N, 6.51.

α -*tert*-Butyl γ -(N,N-Dimethylamino)ethyl Z-Glutamate (**2'a**): Yield: 98%. Colorless oil. $[\alpha]_{\text{D}}^{28} - 20.4^\circ$. MS *m/z*: 408 (M^+). *Rf*₂ 0.37. $^1\text{H-NMR}$ δ : 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.84—2.40 (2H, m, $-\text{CH}_2-$), 2.26 (6H, s, $\text{N}(\text{CH}_3)_2$), 2.42 (2H, m, COCH_2), 2.55 (2H, t, $J=6$ Hz, NCH_2), 4.16 (2H, t, $J=6$ Hz, OCH_2), 4.26 (1H, m, CHCO), 5.10 (2H, s, $\text{CH}_2\text{C}_6\text{H}_5$), 7.34 (5H, s, C_6H_5). Anal. Calcd for $\text{C}_{21}\text{H}_{32}\text{N}_2\text{O}_6$: C, 61.74; H, 7.90; N, 6.86. Found: C, 61.38; H, 8.16; N,

6.84.

γ -Benzyl α -(*N,N*-Dimethylamino)ethyl Boc-Glutamate (**2b**): Yield: 73%. Colorless needles. mp 46–48 °C (from hexane). $[\alpha]_D^{26} -16.0^\circ$. MS m/z : 408 (M^+). R_f 0.40. $^1\text{H-NMR}$ δ : 1.42 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.90–2.40 (2H, m, $-\text{CH}_2-$), 2.30 (6H, s, $\text{N}(\text{CH}_3)_2$), 2.48 (2H, m, COCH_2), 2.64 (2H, t, $J=6$ Hz, NCH_2), 4.26 (2H, t, $J=6$ Hz, OCH_2), 4.36 (1H, m, COCH), 5.12 (2H, s, $\text{CH}_2\text{C}_6\text{H}_5$), 7.36 (5H, s, C_6H_5). Anal. Calcd for $\text{C}_{21}\text{H}_{32}\text{N}_2\text{O}_6$: C, 61.74; H, 7.90; N, 6.86. Found: C, 61.53; H, 7.48; N, 6.88.

α -Benzyl γ -(*N,N*-Dimethylamino)ethyl Boc-Glutamate (**2'b**): Yield: 97%. Colorless oil. $[\alpha]_D^{25} -13.9^\circ$. MS m/z : 408 (M^+). R_f 0.37. $^1\text{H-NMR}$ δ : 1.39 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.90–2.30 (2H, m, $-\text{CH}_2-$), 2.28 (6H, s, $\text{N}(\text{CH}_3)_2$), 2.32 (2H, m, COCH_2), 2.54 (2H, t, $J=6$ Hz, NCH_2), 4.14 (2H, t, $J=6$ Hz, OCH_2), 4.32 (1H, m, COCH), 5.14 (2H, s, $\text{CH}_2\text{C}_6\text{H}_5$), 7.32 (5H, s, C_6H_5). Anal. Calcd for $\text{C}_{21}\text{H}_{32}\text{N}_2\text{O}_6$: C, 61.74; H, 7.90; N, 6.86. Found: C, 61.43; H, 8.11; N, 6.74.

General Procedure for Preparation of α (or γ)-(γ (or α)-Substituted *Z*- and Boc-Glutamyl)cholines (3a,3'a**, **3b** and **3'b**): A Typical Example**— α -(γ -*tert*-Butyloxy-*Z*-glutamyl)choline Chloride (**3a**, $X=\text{Cl}$): A solution of methyl chloride (15 g, 0.3 mol) in benzene (75 ml) was added to a solution of **2a** (12.3 g, 0.03 mol) in AcOEt (15 ml) in a bomb tube. The reaction mixture was allowed to stand at room temperature for 7 d (overnight in the case of the other methyl halides). The solvent was evaporated off *in vacuo*, and the residue was washed with CH_2Cl_2 –EtOH (1 : 1) twice to give **3a** ($X=\text{Cl}$) (12 g, 87%) as a semi-solid. $[\alpha]_D^{26} -15.2^\circ$. MS m/z : 408 ($M^+ - \text{CH}_3$). R_f 0.33. $^1\text{H-NMR}$ δ : 1.40 (9H, s, $\text{C}(\text{CH}_3)_3$), 2.00–2.30 (2H, br.m, $-\text{CH}_2-$), 2.27 (2H, m, COCH_2), 3.32 (9H, s, $\text{N}(\text{CH}_3)_3$), 3.97 (2H, br.t, NCH_2), 4.35 (1H, m, COCH), 4.53 (2H, m, OCH_2), 5.09 (2H, ABq, $J=12$ Hz, $\text{CH}_2\text{C}_6\text{H}_5$), 7.37 (5H, s, C_6H_5).

α -(γ -*tert*-Butyloxy-*Z*-glutamyl)choline Bromide (**3a**, $X=\text{Br}$): Yield: 98%. Semi-solid. $[\alpha]_D^{25} -14.3^\circ$. MS m/z : 408 ($M^+ - \text{CH}_3$). R_f 0.33. $^1\text{H-NMR}$ δ : 1.41 (9H, s, $\text{C}(\text{CH}_3)_3$), 2.00–2.30 (2H, m, $-\text{CH}_2-$), 2.37 (2H, m, COCH_2), 3.37 (9H, s, $\text{N}(\text{CH}_3)_3$), 4.09 (2H, m, NCH_2), 4.35 (1H, m, COCH), 4.61 (2H, m, OCH_2), 5.09 (2H, ABq, $J=12$ Hz, $\text{CH}_2\text{C}_6\text{H}_5$), 7.37 (5H, s, C_6H_5).

α -(γ -*tert*-Butyloxy-*Z*-glutamyl)choline Iodide (**3a**, $X=\text{I}$): Yield: 97%. Colorless needles. mp 74–79 °C (from CH_2Cl_2 + hexane). $[\alpha]_D^{26} -12.5^\circ$. MS m/z : 408 ($M^+ - \text{CH}_3$). R_f 0.33. Anal. Calcd for $\text{C}_{22}\text{H}_{35}\text{IN}_2\text{O}_6$: C, 48.01; H, 6.41; N, 5.09. Found: C, 48.00; H, 6.49; N, 5.04. $^1\text{H-NMR}$ δ : 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 2.00–2.30 (2H, m, $-\text{CH}_2-$), 2.40 (2H, m, COCH_2), 3.41 (9H, s, $\text{N}(\text{CH}_3)_3$), 4.12 (2H, m, NCH_2), 4.36 (1H, m, COCH), 4.65 (2H, m, OCH_2), 5.12 (2H, ABq, $J=12$ Hz, $\text{CH}_2\text{C}_6\text{H}_5$), 7.38 (5H, s, C_6H_5).

α -(γ -*tert*-Butyloxy-*Z*-glutamyl)choline Tosylate (**3a**, $X=\text{Tos}$): Yield: 83%. Colorless needles. mp 102–105 °C (from CH_2Cl_2 + hexane). $[\alpha]_D^{26} -12.6^\circ$. MS m/z : 408 ($M^+ - \text{CH}_3$). R_f 0.33. Anal. Calcd for $\text{C}_{29}\text{H}_{42}\text{N}_2\text{O}_9\text{S}$: C, 58.57; H, 7.11; N, 4.71. Found: C, 58.72; H, 6.94; N, 4.79. $^1\text{H-NMR}$ δ : 1.41 (9H, s, $\text{C}(\text{CH}_3)_3$), 2.00–2.30 (2H, m, $-\text{CH}_2-$), 2.30 (2H, m, COCH_2), 2.33 (3H, s, $\text{C}_6\text{H}_4\text{CH}_3$), 3.20 (9H, s, $\text{N}(\text{CH}_3)_3$), 3.92 (2H, m, NCH_2), 4.34 (1H, m, COCH), 4.64 (2H, m, OCH_2), 5.07 (2H, ABq, $J=12$ Hz, $\text{CH}_2\text{C}_6\text{H}_5$), 7.15 (2H, d, $J=8$ Hz, ArMe, *ortho* H), 7.35 (5H, s, C_6H_5), 7.75 (2H, d, $J=8$ Hz, ArSO₃, *ortho* H).

α -(γ -Benzoyloxy-Boc-glutamyl)choline Chloride (**3b**, $X=\text{Cl}$): Yield: 99%. Semi-solid. $[\alpha]_D^{25} -16.1^\circ$. MS m/z : 408 ($M^+ - \text{CH}_3$). R_f 0.34. $^1\text{H-NMR}$ δ : 1.40 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.98–2.30 (2H, m, $-\text{CH}_2-$), 2.51 (2H, m, COCH_2), 3.46 (9H, s, $\text{N}(\text{CH}_3)_3$), 4.12 (2H, m, NCH_2), 4.30 (1H, m, COCH), 4.62 (2H, m, OCH_2), 5.09 (2H, s, $\text{CH}_2\text{C}_6\text{H}_5$), 7.35 (5H, s, C_6H_5).

α -(γ -Benzoyloxy-Boc-glutamyl)choline Bromide (**3b**, $X=\text{Br}$): Yield: 93%. Semi-solid. $[\alpha]_D^{26} -12.6^\circ$. MS m/z : 408 ($M^+ - \text{CH}_3$). R_f 0.34. $^1\text{H-NMR}$ δ : 1.40 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.98–2.30 (2H, m, $-\text{CH}_2-$), 2.48 (2H, m, COCH_2), 3.46 (9H, s, $\text{N}(\text{CH}_3)_3$), 4.13 (2H, m, NCH_2), 4.27 (1H, m, COCH), 4.62 (2H, m, OCH_2), 5.08 (2H, s, $\text{CH}_2\text{C}_6\text{H}_5$), 7.33 (5H, s, C_6H_5).

α -(γ -Benzoyloxy-Boc-glutamyl)choline Iodide (**3b**, $X=\text{I}$): Yield: 86%. Colorless needles. mp 95–97 °C (from CH_2Cl_2 + hexane). $[\alpha]_D^{26} -13.3^\circ$. MS m/z : 408 ($M^+ - \text{CH}_3$). R_f 0.34. Anal. Calcd for $\text{C}_{22}\text{H}_{35}\text{IN}_2\text{O}_6$: C, 46.84; H, 6.55; N, 5.20. Found: C, 46.53; H, 6.32; N, 5.10. $^1\text{H-NMR}$ δ : 1.40 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.98–2.30 (2H, m, $-\text{CH}_2-$), 2.51 (2H, m, COCH_2), 3.49 (9H, s, $\text{N}(\text{CH}_3)_3$), 4.12 (2H, m, NCH_2), 4.29 (1H, m, COCH), 4.63 (2H, m, OCH_2), 5.10 (2H, s, $\text{CH}_2\text{C}_6\text{H}_5$), 7.35 (5H, s, C_6H_5).

γ -(α -*tert*-Butyloxy-*Z*-glutamyl)choline Chloride (**3'a**, $X=\text{Cl}$): Yield: 99%. Semi-solid. $[\alpha]_D^{26} -19.8^\circ$. MS m/z : 408 ($M^+ - \text{CH}_3$). R_f 0.32. $^1\text{H-NMR}$ δ : 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.84–2.40 (2H, m, $-\text{CH}_2-$), 2.45 (2H, m, COCH_2), 3.43 (9H, s, $\text{N}(\text{CH}_3)_3$), 4.00 (2H, m, NCH_2), 4.20 (1H, m, COCH), 4.55 (2H, m, OCH_2), 5.10 (2H, s, $\text{CH}_2\text{C}_6\text{H}_5$), 7.38 (5H, s, C_6H_5).

γ -(α -*tert*-Butyloxy-*Z*-glutamyl)choline Bromide (**3'a**, $X=\text{Br}$): Yield: 96%. Semi-solid. $[\alpha]_D^{25} -18.6^\circ$. MS m/z : 408 ($M^+ - \text{CH}_3$). R_f 0.32. $^1\text{H-NMR}$ δ : 1.46 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.85–2.40 (2H, m, $-\text{CH}_2-$), 2.50 (2H, m, COCH_2), 3.45 (9H, s, $\text{N}(\text{CH}_3)_3$), 4.10 (2H, m, NCH_2), 4.21 (1H, m, COCH), 4.60 (2H, m, OCH_2), 5.10 (2H, s, $\text{CH}_2\text{C}_6\text{H}_5$), 7.37 (5H, s, C_6H_5).

γ -(α -*tert*-Butyloxy-*Z*-glutamyl)choline Iodide (**3'a**, $X=\text{I}$): Yield: 74%. Colorless needles. mp 75–81 °C (from CH_2Cl_2 + hexane). $[\alpha]_D^{27} -16.6^\circ$. MS m/z : 408 ($M^+ - \text{CH}_3$). R_f 0.32. Anal. Calcd for $\text{C}_{22}\text{H}_{35}\text{IN}_2\text{O}_6$: C, 48.01; H, 6.41; N, 5.09. Found: C, 47.84; H, 6.25; N, 5.11. $^1\text{H-NMR}$ δ : 1.44 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.80–2.40 (2H, m, $-\text{CH}_2-$), 2.48 (2H, m, COCH_2), 3.42 (9H, s, $\text{N}(\text{CH}_3)_3$), 4.04 (2H, m, NCH_2), 4.24 (1H, m, COCH), 4.55 (2H, m, OCH_2), 5.06 (2H, s, $\text{CH}_2\text{C}_6\text{H}_5$), 7.34 (5H, s, C_6H_5).

γ -(α -*tert*-Butyloxy-Z-glutamyl)choline Tosylate (**3'a**, X = Tos): Yield: 85%. Semi-solid. $[\alpha]_D^{24} - 15.2^\circ$. MS m/z : 408 ($M^+ - CH_3$). R_f 0.32. 1H -NMR δ : 1.44 (9H, s, $C(CH_3)_3$), 1.80–2.40 (2H, m, $-CH_2-$), 2.32 (3H, s, $CH_3C_6H_4$), 2.43 (2H, m, $COCH_2$), 3.28 (9H, s, $N(CH_3)_3$), 3.83 (2H, m, NCH_2), 4.18 (1H, m, OCH_2), 4.42 (2H, m, OCH_2), 5.08 (2H, s, $CH_2C_6H_5$), 7.13 (2H, d, $J = 8$ Hz, ArMe, *ortho* H), 7.34 (5H, s, C_6H_5), 7.75 (2H, d, $J = 8$ Hz, $ArSO_3$, *ortho* H).

γ -(α -Benzyloxy-Boc-glutamyl)choline Chloride (**3'b**, X = Cl): Yield: 98%. Semi-solid. $[\alpha]_D^{25} - 19.3^\circ$. MS m/z : 408 ($M^+ - CH_3$). R_f 0.31. 1H -NMR δ : 1.40 (9H, s, $C(CH_3)_3$), 1.80–2.30 (2H, m, $-CH_2-$), 2.46 (2H, m, $COCH_2$), 3.42 (9H, s, $N(CH_3)_3$), 4.03 (2H, m, NCH_2), 4.31 (1H, m, $COCH$), 4.55 (2H, m, OCH_2), 5.12 (2H, s, $CH_2C_6H_5$), 7.36 (5H, s, C_6H_5).

γ -(α -Benzyloxy-Boc-glutamyl)choline Bromide (**3'b**, X = Br): Yield: 90%. Semi-solid. $[\alpha]_D^{18} - 16.5^\circ$. MS m/z : 408 ($M^+ - CH_3$). R_f 0.31. 1H -NMR δ : 1.40 (9H, s, $C(CH_3)_3$), 1.80–2.30 (2H, m, $-CH_2-$), 2.48 (2H, m, $COCH_2$), 3.48 (9H, s, $N(CH_3)_3$), 4.09 (2H, m, NCH_2), 4.34 (1H, m, $COCH$), 4.58 (2H, m, OCH_2), 5.16 (2H, s, $CH_2C_6H_5$), 7.36 (5H, s, C_6H_5).

General Procedure for Preparation of Z-Glutamylcholines (4a and 4'a): A Typical Example— α -(Z-Glutamyl)choline Chloride (**4a**, X = Cl). Method A: A solution of **3a** (X = Cl) (5.0 g, 11 mmol) in AcOEt (15 ml) was added to 5 N HCl–AcOEt (98 ml) under ice cooling and the mixture was stirred for 2 h. The hydrogen chloride was removed *in vacuo* at $5^\circ C$ and the residue was triturated with CH_2Cl_2 –AcOEt (1 : 1) (40 ml) four times. The residue was dried over P_2O_5 *in vacuo* to give **4a** (X = Cl) (4.1 g, 93%) as a semi-solid.

Method B: **3a** (X = Cl) (4.6 g, 10 mmol) was treated with trifluoroacetic acid– CH_2Cl_2 (1 : 1) (15 ml) in the same manner as in method A to give **4a** (X = Cl) (3.9 g, 97%) as a semi-solid. $[\alpha]_D^{24} - 16.1^\circ$. MS m/z : 353 ($M^+ + 1 - CH_3$). R_f 0.27. 1H -NMR ($CDCl_3 + CD_3OD$) δ : 2.00–2.30 (2H, m, $-CH_2-$), 2.47 (2H, m, $COCH_2$), 3.18 (9H, s, $N(CH_3)_3$), 3.77 (2H, m, NCH_2), 4.27 (1H, m, $COCH$), 4.56 (2H, m, OCH), 5.09 (2H, ABq, $J = 12$ Hz, $CH_2C_6H_5$), 7.33 (5H, s, C_6H_5). $PtCl_6$ salt: pale yellow needles. mp 119 – $121^\circ C$ (from EtOH). Anal. Calcd for $C_{36}H_{54}N_4O_{12} \cdot PtCl_6$: C, 37.84; H, 4.76; N, 4.90. Found: C, 37.98; H, 4.84; N, 4.67.

α -(Z-Glutamyl)choline Bromide (**4a**, X = Br): Yield: 90% (method A), 97% (method B). Semi-solid. $[\alpha]_D^{25} - 12.7^\circ$. MS m/z : 353 ($M^+ + 1 - CH_3$). R_f 0.27. 1H -NMR ($CDCl_3 + CD_3OD$) δ : 2.00–2.30 (2H, m, $-CH_2-$), 2.46 (2H, m, $COCH_2$), 3.19 (9H, s, $N(CH_3)_3$), 3.72 (2H, m, NCH_2), 4.27 (1H, m, $COCH$), 4.58 (2H, m, OCH_2), 5.10 (2H, ABq, $J = 12$ Hz, $CH_2C_6H_5$), 7.37 (5H, s, C_6H_5).

α -(Z-Glutamyl)choline Iodide (**4a**, X = I): Yield: 82% (method A), 85% (method B). Semi-solid. $[\alpha]_D^{25} - 12.1^\circ$. MS m/z : 353 ($M^+ + 1 - CH_3$). R_f 0.27. 1H -NMR ($CDCl_3 + CD_3OD$) δ : 2.00–2.26 (2H, m, $-CH_2-$), 2.47 (2H, m, $COCH_2$), 3.24 (9H, s, $N(CH_3)_3$), 3.85 (2H, m, NCH_2), 4.32 (1H, m, $COCH$), 4.56 (2H, m, OCH_2), 5.10 (2H, ABq, $J = 12$ Hz, $CH_2C_6H_5$), 7.38 (5H, s, C_6H_5).

α -(Z-Glutamyl)choline Tosylate (**4a**, X = Tos): Yield: 59% (method A), 93% (method B). Semi-solid. MS m/z : 353 ($M^+ + 1 - CH_3$). R_f 0.27. $[\alpha]_D^{25} - 13.1^\circ$. 1H -NMR, ($CDCl_3 + CD_3OD$) δ : 2.00–2.30 (2H, m, $-CH_2-$), 2.37 (3H, s, $CH_3C_6H_4$), 2.47 (2H, m, $COCH_2$), 3.22 (9H, s, $N(CH_3)_3$), 3.81 (2H, m, NCH_2), 4.32 (1H, m, $COCH$), 4.55 (2H, m, OCH_2), 5.10 (2H, ABq, $J = 12$ Hz, $CH_2C_6H_5$), 7.20 (2H, d, $J = 8$ Hz, ArMe, *ortho* H), 7.34 (5H, s, C_6H_5), 7.76 (2H, d, $J = 8$ Hz, $ArSO_3$, *ortho* H).

γ -(Z-Glutamyl)choline Chloride (**4'a**, X = Cl): Yield: 69% (method A), 96% (method B). Semi-solid. $[\alpha]_D^{24} - 10.5^\circ$. MS m/z : 353 ($M^+ + 1 - CH_3$). R_f 0.24. 1H -NMR ($CDCl_3 + CD_3OD$) δ : 1.95–2.40 (2H, m, $-CH_2-$), 2.49 (2H, m, $COCH_2$), 3.18 (9H, s, $N(CH_3)_3$), 3.27 (2H, m, NCH_2), 4.29 (2H, m, $COCH$), 4.53 (2H, m, OCH_2), 5.09 (2H, s, $CH_2C_6H_5$), 7.36 (5H, s, C_6H_5). $PtCl_6$ salt: pale yellow needles. mp 163 – $165^\circ C$ (from EtOH). Anal. Calcd for $C_{36}H_{54}N_4O_{12} \cdot PtCl_6$: C, 37.84; H, 4.74; N, 4.90. Found: C, 38.08; H, 5.01; N, 4.82.

γ -(Z-Glutamyl)choline Bromide (**4'a**, X = Br): Yield: 79% (method A), 91% (method B). Semi-solid. $[\alpha]_D^{24} - 9.0^\circ$. MS m/z : 353 ($M^+ + 1 - CH_3$). R_f 0.24. 1H -NMR ($CDCl_3 + CD_3OD$) δ : 1.94–2.40 (2H, m, $-CH_2-$), 2.54 (2H, m, $COCH_2$), 3.20 (9H, s, $N(CH_3)_3$), 3.70 (2H, m, NCH_2), 4.28 (1H, m, $COCH$), 4.58 (2H, m, OCH_2), 5.14 (2H, s, $CH_2C_6H_5$), 7.38 (5H, s, C_6H_5).

γ -(Z-Glutamyl)choline Iodide (**4'a**, X = I): Yield: 68% (method A), 91% (method B). Semi-solid. $[\alpha]_D^{25} - 12.1^\circ$. MS m/z : 353 ($M^+ + 1 - CH_3$). R_f 0.24. 1H -NMR ($CDCl_3 + CD_3OD$) δ : 1.90–2.40 (2H, m, $-CH_2-$), 2.53 (2H, m, $COCH_2$), 3.30 (9H, s, $N(CH_3)_3$), 3.85 (2H, m, NCH_2), 4.35 (1H, m, $COCH$), 4.56 (2H, m, OCH_2), 5.12 (2H, s, $CH_2C_6H_5$), 7.39 (5H, s, C_6H_5).

γ -(Z-Glutamyl)choline Tosylate (**4'a**, X = Tos): Yield: 51% (method A), 91% (method B). Semi-solid. $[\alpha]_D^{25} - 10.2^\circ$. MS m/z : 353 ($M^+ + 1 - CH_3$). R_f 0.24. 1H -NMR ($CDCl_3 + CD_3OD$) δ : 1.95–2.40 (2H, m, $-CH_2-$), 2.37 (3H, s, $CH_3C_6H_4$), 2.51 (2H, m, $COCH_2$), 3.26 (9H, s, $N(CH_3)_3$), 3.79 (2H, m, NCH_2), 4.34 (1H, m, $COCH$), 4.54 (2H, m, OCH_2), 5.11 (2H, s, $CH_2C_6H_5$), 7.22 (2H, d, $J = 8$ Hz, ArMe, *ortho* H), 7.38 (5H, s, C_6H_5), 7.96 (2H, d, $J = 8$ Hz, $ArSO_3$, *ortho* H).

General Procedure for Preparation of Boc-Glutamylcholines (4b and 4'b): A Typical Example— α -(Boc-Glutamyl)choline Chloride (**4b**, X = Cl): A mixture of **3b** (X = Cl) (1 g, 2 mmol) and 5% Pd–carbon (0.7 g) and isopropyl alcohol (25 ml) was stirred under a hydrogen atmosphere at room temperature for 3 h. The catalyst was filtered off and the filtrate was evaporated *in vacuo*. The residue was washed with CH_2Cl_2 –Et₂O (1 : 2) twice to give **4b** (X = Cl) (0.54 g, 69%) as a semi-solid. $[\alpha]_D^{26} - 20.9^\circ$. MS m/z : 319 ($M^+ + 1 - CH_3$). R_f 0.26. 1H -NMR ($CDCl_3 + CD_3OD$) δ : 1.40 (9H, s, $C(CH_3)_3$), 2.00–2.20 (2H, m, $-CH_2-$), 2.43 (2H, m, $COCH_2$), 3.29 (9H, s,

N(CH₃)₃), 3.86 (2H, m, NCH₂), 4.21 (1H, m, COCH), 4.55 (2H, m, OCH₂). PtCl₆ salt: pale yellow needles. mp 164—168 °C (from EtOH). *Anal.* Calcd for C₃₀H₅₈N₄O₁₂·PtCl₆: C, 33.53; H, 5.44; N, 5.21. Found: C, 33.10; H, 5.55; N, 5.14.

α -(Boc-Glutamyl)choline Bromide (**4b**, X=Br): Yield: 78%. Semi-solid. $[\alpha]_D^{18}$ -17.9°. MS *m/z*: 319 ($M^+ + 1 - CH_3$). *Rf*₂ 0.26 ¹H-NMR (CDCl₃ + CD₃OD) δ : 1.42 (9H, s, C(CH₃)₃), 2.00—2.20 (2H, m, -CH₂-), 2.47 (2H, m, COCH₂), 3.37 (9H, s, N(CH₃)₃), 3.95 (2H, m, NCH₂), 4.25 (1H, m, COCH), 4.61 (2H, m, OCH₂).

γ -(Boc-Glutamyl)choline Chloride (**4'b**, X=Cl): Yield: 67%. Semi-solid. $[\alpha]_D^{26}$ -4.3°. MS *m/z*: 319 ($M^+ + 1 - CH_3$). *Rf*₂ 0.22. ¹H-NMR (CDCl₃ + CD₃OD) δ : 1.37 (9H, s, C(CH₃)₃), 1.95—2.20 (2H, m, -CH₂-), 2.45 (2H, m, COCH₂), 3.26 (9H, s, N(CH₃)₃), 3.79 (2H, m, NCH₂), 4.14 (1H, m, COCH), 4.49 (2H, m, OCH₂). PtCl₆ salt: pale yellow needles. mp 162—165 °C (from EtOH). *Anal.* Calcd for C₃₀H₅₈N₄O₁₂·PtCl₆: C, 33.53; H, 5.44; N, 5.21. Found: C, 33.64; H, 5.52; N, 4.93.

γ -(Boc-Glutamyl)choline Bromide (**4'b**, X=Br): Yield: 80%. Semi-solid. $[\alpha]_D^{18}$ -4.0°. MS *m/z*: 319 ($M^+ + 1 - CH_3$). *Rf*₂ 0.22. ¹H-NMR (CDCl₃ + CD₃OD) δ : 1.42 (9H, s, C(CH₃)₃), 1.95—2.30 (2H, m, -CH₂-), 2.50 (2H, m, COCH₂), 3.36 (9H, s, N(CH₃)₃), 3.90 (2H, m, NCH₂), 4.19 (1H, m, COCH), 4.56 (2H, m, OCH₂).

Reaction of Z-Glutamic Anhydride with *tert*-Butyl Alcohol: Z-Glutamic anhydride (2 g) was allowed to react with *tert*-butyl alcohol (8 ml) in the presence of catalyst (ZnCl₂ or BF₃·Et₂O, and 4-dimethylaminopyridine) at room temperature or by heating at 90 °C for 3 h. No spots of α - and γ -*tert*-butyl Z-glutamates were detected in these reaction mixture by the TLC.

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References and Notes

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