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Synthesis of α- and γ-(*N*-Carbobenzoxy- and *N*-*tert*-Butyloxycarbonyl-L-Glutamyl)cholines[1)]

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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.

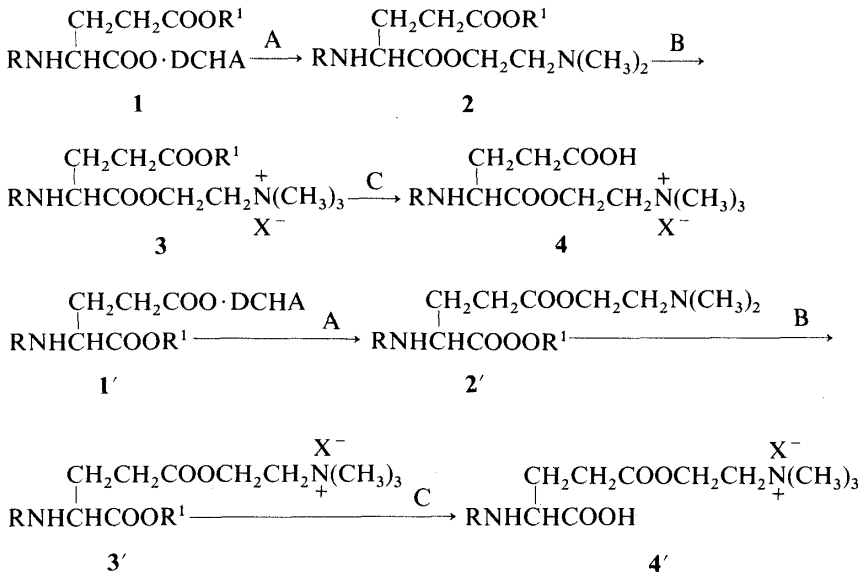
Keywords——glutamyl cholines synthesis; α-(Z-glutamyl)choline; γ(Z-glutamyl)choline; α-(Boc-glutamyl)choline; γ-(Boc-glutamyl)choline; *tert*-butyl Z-glutamate; benzyl Boc-glutamate; *N*,*N*-dimethylaminoethyl chloride; dicyclohexylamine salt; hypotensive effect

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 bases[5)] 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 (^{1}H-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), R^1 = *tert*-butyl
b: R = *tert*-butyloxycarbonyl (Boc), R^1 = benzyl
X = Cl, Br, I, Tos DCHA = dicyclohexylammonium
A, $(CH_3)_2NCH_2CH_2Cl$; B, CH_3X;
C, HCl–AcOEt or TFA–CH_2Cl_2 (removal of *tert*-butyl group),
Pd–carbon/H_2 (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. ^{1}H-NMR spectra of these *tert*-butyl choline esters (**3a** and **3'a**) exhibited a 9H singlet due to three methyls of the cholinyl 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 5 N 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 ^{1}H-NMR spectra of these choline esters (**4a** and **4'a**) exhibited a 9H singlet due to three methyls of the cholinyl 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 ^{1}H-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 ^{1}H-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}H-NMR spectra of α- and γ-(Boc-glutamyl)cholines (**4b** and **4'b**) exhibited a 9H singlet due to three methyls of the cholinyl 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}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 $(CH_3)_4Si$ 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 F_{254} Wako Pure Chemical Industry Ltd.) and detection was achieved by ultaviolet (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–H_2O (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→Z-glutamic acid[7]→Z-glutamic anhydride[13]→α-benzyl Z-glutmate[8]→α-benzyl γ-*tert*-butyl Z-glutamate[9]→**1a**.[10] Total yield: 14%. mp 139—142 °C. $[\alpha]_D^{27}$ +7.5°. (Lit.[10] mp 139—140 °C. $[\alpha]_D^{25}$ −7.3° (*c* = 1, MeOH)).

(B) Z-Glutamic acid[7]→(*S*)-3-Z-5-oxo-4-oxazolidinepropionic acid→γ-*tert*-butyl (*S*)-3-Z-5-oxo-4-oxazolidine-propionate→**1a**.[11] Total yield: 21%. mp 140—144 °C. $[\alpha]_D^{29}$ +6.6°. (Lit.[11] mp 138—140 °C. $[\alpha]_D^{25}$ +6.1° (*c* = 3.9, MeOH)). Free acid: ^{1}H-NMR δ: 1.42 (9H, s, $C(CH_3)_3$), 1.98—2.40 (2H, m, $-CH_2-$), 2.41 (2H, m, $COCH_2$), 4.40 (1H, m, COCH), 5.13 (2H, s, $C\underline{H}_2C_6H_5$), 7.37 (5H, s, C_6H_5).

Dicyclohexylamine Salt of α-*tert*-Butyl Z-Glutamate (**1'a**): (A) Glutamic acid→γ-benzyl glutamate[12]→γ-benzyl Z-glutamate[12]→γ-benzyl α-*tert*-butyl Z-glutamate[10]→**1'a**.[10] Total yield: 25%. mp 147—150 °C. $[\alpha]_D^{25}$ −13.7°. (Lit.[10] mp 148—149 °C. $[\alpha]_D^{25}$ −14.1° (*c* = 1, MeOH)).

(B) Z-Glutamic acid[7]→Z-pyroglutamic acid[13]→*tert*-butyl Z-pyroglutamate[10]→**1'a**.[10] Total yield: 22%. mp 147—150 °C. $[\alpha]_D^{31}$ −13.6°. (Lit.[10] mp 149—150 °C. $[\alpha]_D^{25}$ −13.8° (*c* = 1, MeOH)). Free acid: ^{1}H-NMR δ: 1.43 (9H, s, $C(CH_3)_3$), 2.02—2.40 (2H, m, $-CH_2-$), 2.40 (2H, m, $COCH_2$), 4.28 (1H, m, –COCH, 5.12 (2H, s, $C\underline{H}_2C_6H_5$), 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→Boc-glutamic acid[14]→Boc-glutamic anhydride[9]→**1'b**.[9] Total yield: 32%. mp 163—168 °C. $[\alpha]_D^{26}$ −16.5°. (Lit.[9] mp 172 °C. $[\alpha]_D^{25}$ −19.2° (*c* = 0.7, MeOH)). Free acid: ^{1}H-NMR δ: 1.43 (9H, s, $C(CH_3)_3$), 1.87—2.31 (2H, m, $-CH_2-$), 2.41 (2H, m, $COCH_2$), 4.39 (1H, m, COCH), 5.19 (2H, s, $C\underline{H}_2C_6H_5$), 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. $[\alpha]_D^{28}$ −17.2°. MS *m/z*: 408 (M^+). Rf_2 0.39. ^{1}H-NMR δ: 1.40 (9H, s, $C(CH_3)_3$), 1.90—2.20 (2H, m, $-CH_2-$), 2.26 (6H, s, $N(CH_3)_2$), 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, $C\underline{H}_2C_6H_5$), 7.35 (5H, s, C_6H_5). *Anal.* Calcd for $C_{21}H_{32}N_2O_6$: 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. $[\alpha]_D^{28}$ −20.4°. MS *m/z*: 408 (M^+). Rf_2 0.37. ^{1}H-NMR δ: 1.43 (9H, s, $C(CH_3)_3$), 1.84—2.40 (2H, m, $-CH_2-$), 2.26 (6H, s, $N(CH_3)_2$), 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, $C\underline{H}_2C_6H_5$), 7.34 (5H, s, C_6H_5). *Anal.* Calcd for $C_{21}H_{32}N_2O_6$: 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°. MS *m/z*: 408 (M^+). Rf_2 0.40. ^{1}H-NMR δ: 1.42 (9H, s, $C(CH_3)_3$), 1.90—2.40 (2H, m, $-CH_2-$), 2.30 (6H, s, $N(CH_3)_2$), 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, $C\underline{H}_2C_6H_5$), 7.36 (5H, s, C_6H_5), *Anal.* Calcd for $C_{21}H_{32}N_2O_6$: 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°. MS *m/z*: 408 (M^+). Rf_2 0.37. ^{1}H-NMR δ: 1.39 (9H, s, $C(CH_3)_3$), 1.90—2.30 (2H, m, $-CH_2-$), 2.28 (6H, s, $N(CH_3)_2$), 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, $C\underline{H}_2C_6H_5$), 7.32 (5H, s, C_6H_5). *Anal.* Calcd for $C_{21}H_{32}N_2O_6$: 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=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=Cl) (12 g, 87%) as a semi-solid. $[\alpha]_D^{26}$ −15.2°. MS *m/z*: 408 ($M^+ - CH_3$). Rf_2 0.33. ^{1}H-NMR δ: 1.40 (9H, s, $C(CH_3)_3$), 2.00—2.30 (2H, br.m, $-CH_2-$), 2.27 (2H, m, $COCH_2$), 3.32 (9H, s, $N(CH_3)_3$), 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, $C\underline{H}_2C_6H_5$), 7.37 (5H, s, C_6H_5).

α-(γ-*tert*-Butyloxy-Z-glutamyl)choline Bromide (**3a**, X=Br): Yield: 98%. Semi-solid. $[\alpha]_D^{25}$ −14.3°. MS *m/z*: 408 ($M^+ - CH_3$). Rf_2 0.33 ^{1}H-NMR δ; 1.41 (9H, s, $C(CH_3)_3$), 2.00—2.30 (2H, m, $-CH_2-$), 2.37 (2H, m, $COCH_2$), 3.37 (9H, s, $N(CH_3)_3$), 4.09 (2H, m, NCH_2), 4.35 (1H, m, COCH), 4.61 (2H, m, OCH_2), 5.09 (2H, ABq, $J=12$ Hz, $C\underline{H}_2C_6H_5$), 7.37 (5H, s, C_6H_5).

α-(γ-*tert*-Butyloxy-Z-glutamyl)choline Iodide (**3a**, X=I): Yield: 97%. Colorless needles. mp 74—79 °C (from CH_2Cl_2 + hexane). $[\alpha]_D^{26}$ −12.5°. MS *m/z*: 408 ($M^+ - CH_3$). Rf_2 0.33. *Anal.* Calcd for $C_{22}H_{35}IN_2O_6$: C, 48.01; H, 6.41; N, 5.09. Found: C, 48.00; H, 6.49; N, 5.04. ^{1}H-NMR δ: 1.43 (9H, s, $C(CH_3)_3$), 2.00—2.30 (2H, m, $-CH_2-$), 2.40 (2H, m, $COCH_2$), 3.41 (9H, s, $N(CH_3)_3$), 4.12 (2H, m, NCH_2), 4.36 (1H, m, COCH), 4.65 (2H, m, OCH_2), 5.12 (2H, ABq, $J=12$ Hz, $C\underline{H}_2C_6H_5$), 7.38 (5H, s, C_6H_5).

α-(γ-*tert*-Butyloxy-Z-glutamyl)choline Tosylate (**3a**, X=Tos): Yield: 83%. Colorless needles. mp 102—105 °C (from CH_2Cl_2 + hexane). $[\alpha]_D^{26}$ −12.6°. MS *m/z*: 408 ($M^+ - CH_3$). Rf_2 0.33. *Anal.* Calcd for $C_{29}H_{42}N_2O_9S$: C, 58.57; H, 7.11; N, 4.71. Found: C, 58.72; H, 6.94; N, 4.79. ^{1}H-NMR δ: 1.41 (9H, s, $C(CH_3)_3$), 2.00—2.30 (2H, m, $-CH_2-$), 2.30 (2H, m; $COCH_2$), 2.33 (3H, s, $C_6H_4C\underline{H}_3$), 3.20 (9H, s, $N(CH_3)_3$), 3.92 (2H, m, NCH_2), 4.34 (1H, m, COCH), 4.64 (2H, m, OCH_2), 5.07 (2H, ABq, $J=12$ Hz, $C\underline{H}_2C_6H_5$), 7.15 (2H, d, $J=8$ Hz, ArMe, *ortho* H), 7.35 (5H, s, C_6H_5), 7.75 (2H, d, $J=8$ Hz, $ArSO_3$, *ortho* H).

α-(γ-Benzyloxy-Boc-glutamyl)choline Chloride (**3b**, X=Cl): Yield: 99%. Semi-solid. $[\alpha]_D^{25}$ −16.1°. MS *m/z*: 408 ($M^+ - CH_3$). Rf_2 0.34. ^{1}H-NMR δ: 1.40 (9H, s, $C(CH_3)_3$), 1.98—2.30 (2H, m, $-CH_2-$), 2.51 (2H, m, $COCH_2$) 3.46 (9H, s, $N(CH_3)_3$), 4.12 (2H, m, NCH_2), 4.30 (1H, m, COCH), 4.62 (2H, m, OCH_2), 5.09 (2H, s, $C\underline{H}_2C_6H_5$), 7.35 (5H, s, C_6H_5).

α-(γ-Benzyloxy-Boc-glutamyl)choline Bromide (**3b**, X=Br): Yield: 93%. Semi-solid. $[\alpha]_D^{26}$ −12.6°. MS *m/z*: 408 ($M^+ - CH_3$). Rf_2 0.34. ^{1}H-NMR δ: 1.40 (9H, s, $C(CH_3)_3$), 1.98—2.30 (2H, m, $-CH_2-$), 2.48 (2H, m, $COCH_2$), 3.46 (9H, s, $N(CH_3)_3$), 4.13 (2H, m, NCH_2), 4.27 (1H, m, COCH), 4.62 (2H, m, OCH_2), 5.08 (2H, s, $C\underline{H}_2C_6H_5$), 7.33 (5H, s, C_6H_5).

α-(γ-Benzyloxy-Boc-glutamyl)choline Iodide (**3b**, X=I): Yield: 86%. Colorless needles. mp 95—97 °C (from CH_2Cl_2 + hexane). $[\alpha]_D^{26}$ −13.3°. MS *m/z*: 408 ($M^+ - CH_3$). Rf_2 0.34. *Anal.* Calcd for $C_{22}H_{35}IN_2O_6$: C, 46.84; H, 6.55; N, 5.20. Found: C, 46.53; H, 6.32; N, 5.10. ^{1}H-NMR δ: 1.40 (9H, s, $C(CH_3)_3$), 1.98—2.30 (2H, m, $-CH_2-$), 2.51 (2H, m, $COCH_2$), 3.49 (9H, s, $N(CH_3)_3$), 4.12 (2H, m, NCH_2), 4.29 (1H, m, COCH), 4.63 (2H, m, OCH_2), 5.10 (2H, s, $C\underline{H}_2C_6H_5$), 7.35 (5H, s, C_6H_5).

γ-(α-*tert*-Butyloxy-Z-glutamyl)choline Chloride (**3'a**, X=Cl): Yield: 99%. Semi-solid. $[\alpha]_D^{26}$ −19.8°. MS *m/z*: 408 ($M^+ - CH_3$). Rf_2 0.32. ^{1}H-NMR δ: 1.43 (9H, s, $C(CH_3)_3$), 1.84—2.40 (2H,.m, $-CH_2-$), 2.45 (2H, m, $COCH_2$), 3.43 (9H, s, $N(CH_3)_3$), 4.00 (2H, m, NCH_2), 4.20 (1H, m, COCH), 4.55 (2H, m, OCH_2), 5.10 (2H, s, $C\underline{H}_2C_6H_5$), 7.38 (5H, s, C_6H_5).

γ-(α-*tert*-Butyloxy-Z-glutamyl)choline Bromide (**3'a**, X=Br): Yield: 96%. Semi-solid. $[\alpha]_D^{25}$ −18.6°. MS *m/z*: 408 ($M^+ - CH_3$). Rf_2 0.32. ^{1}H-NMR δ: 1.46 (9H, s, $C(CH_3)_3$), 1.85—2.40 (2H, m, $-CH_2-$), 2.50 (2H, m, $COCH_2$), 3.45 (9H, s, $N(CH_3)_3$), 4.10 (2H, m, NCH_2), 4.21 (1H, m, COCH), 4.60 (2H, m, OCH_2), 5.10 (2H, s, $C\underline{H}_2C_6H_5$), 7.37 (5H, s, C_6H_5).

γ-(α-*tert*-Butyloxy-Z-glutamyl)choline Iodide (**3'a**, X=I): Yield: 74%. Colorless needles. mp 75—81 °C (from CH_2Cl_2 + hexane). $[\alpha]_D^{27}$ −16.6°. MS *m/z*: 408 ($M^+ - CH_3$). Rf_2 0.32 *Anal.* Calcd for $C_{22}H_{35}IN_2O_6$: C, 48.01; H, 6.41; N, 5.09. Found: C, 47.84; H, 6.25; N, 5.11. ^{1}H-NMR δ: 1.44 (9H, s, $C(CH_3)_3$), 1.80—2.40 (2H, m, $-CH_2-$), 2.48 (2H, m, $COCH_2$), 3.42 (9H, s, $N(CH_3)_3$), 4.04 (2H, m, NCH_2), 4.24 (1H, m, COCH), 4.55 (2H, m, OCH_2), 5.06 (2H, s, $C\underline{H}_2C_6H_5$), 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°. MS *m/z*: 408 ($M^+ - CH_3$). Rf_2 0.32. ^{1}H-NMR δ: 1.44 (9H, s, $C(CH_3)_3$), 1.80—2.40 (2H, m, $-CH_2-$), 2.32 (3H, s, $C\underline{H}_3C_6H_4$), 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, $C\underline{H}_2C_6H_5$), 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°. MS *m/z*: 408 ($M^+ - CH_3$). Rf_2 0.31. ^{1}H-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, $C\underline{H}_2C_6H_5$), 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°. MS *m/z*: 408 ($M^+ - CH_3$). Rf_2 0.31. ^{1}H-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, $C\underline{H}_2C_6H_5$), 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 m mol) 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 °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 m mol) 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°. MS *m/z*: 353 ($M^+ + 1 - CH_3$). Rf_2 0.27. ^{1}H-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, $C\underline{H}_2C_6H_5$), 7.33 (5H, s, C_6H_5). $PtCl_6$ salt: pale yellow needles. mp 119—121 °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°. MS *m/z*: 353 ($M^+ + 1 - CH_3$). Rf_2 0.27. ^{1}H-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, $C\underline{H}_2C_6H_5$), 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°. MS *m/z*: 353 ($M^+ + 1 - CH_3$). Rf_2 0.27. ^{1}H-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, $C\underline{H}_2C_6H_5$), 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 ($M^+ + 1 - CH_3$). Rf_2 0.27. $[\alpha]_D^{25}$ −13.1°. ^{1}H-NMR, ($CDCl_3 + CD_3OD$) δ: 2.00—2.30 (2H, m, $-CH_2-$), 2.37 (3H, s, $C\underline{H}_3C_6H_4$), 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, $C\underline{H}_2C_6H_5$), 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°. MS *m/z*: 353 ($M^+ + 1 - CH_3$). Rf_2 0.24. ^{1}H-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, $C\underline{H}_2C_6H_5$), 7.36 (5H, s, C_6H_5). $PtCl_6$ salt: pale yellow needles. mp 163—165 °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°. MS *m/z*: 353 ($M^+ + 1 - CH_3$). Rf_2 0.24. ^{1}H-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, $C\underline{H}_2C_6H_5$), 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°. MS *m/z*: 353 ($M^+ + 1 - CH_3$). Rf_2 0.24. ^{1}H-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, $C\underline{H}_2C_6H_5$), 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°. MS *m/z*: 353 ($M^+ + 1 - CH_3$). Rf_2 0.24. ^{1}H-NMR ($CDCl_3 + CD_3OD$) δ: 1.95—2.40 (2H, m, $-CH_2-$), 2.37 (3H, s, $C\underline{H}_3C_6H_4$), 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, $C\underline{H}_2C_6H_5$), 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–Et_2O (1 : 2) twice to give **4b** (X=Cl) (0.54 g, 69%) as a semi-solid. $[\alpha]_D^{26}$ −20.9°. MS *m/z*: 319 ($M^+ + 1 - CH_3$). Rf_2 0.26. ^{1}H-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,

$N(CH_3)_3$), 3.86 (2H, m, NCH_2), 4.21 (1H, m, COCH), 4.55 (2H, m, OCH_2). $PtCl_6$ salt: pale yellow needles. mp 164—168 °C (from EtOH). *Anal.* Calcd for $C_{30}H_{58}N_4O_{12} \cdot PtCl_6$: 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 ^{1}H-NMR ($CDCl_3 + CD_3OD$) δ: 1.42 (9H, s, $C(CH_3)_3$), 2.00—2.20 (2H, m, $-CH_2-$), 2.47 (2H, m, $COCH_2$), 3.37 (9H, s, $N(CH_3)_3$), 3.95 (2H, m, NCH_2), 4.25 (1H, m, COCH), 4.61 (2H, m, OCH_2).

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. ^{1}H-NMR ($CDCl_3 + CD_3OD$) δ: 1.37 (9H, s, $C(CH_3)_3$), 1.95—2.20 (2H, m, $-CH_2-$), 2.45 (2H, m, $COCH_2$), 3.26 (9H, s, $N(CH_3)_3$), 3.79 (2H, m, NCH_2), 4.14 (1H, m, COCH), 4.49 (2H, m, OCH_2). $PtCl_6$ salt: pale yellow needles. mp 162—165 °C (from EtOH). *Anal.* Calcd for $C_{30}H_{58}N_4O_{12} \cdot PtCl_6$: 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. ^{1}H-NMR ($CDCl_3 + CD_3OD$) δ: 1.42 (9H, s, $C(CH_3)_3$), 1.95—2.30 (2H, m, $-CH_2-$), 2.50 (2H, m, $COCH_2$), 3.36 (9H, s, $N(CH_3)_3$), 3.90 (2H, m, NCH_2), 4.19 (1H, m, COCH), 4.56 (2H, m, OCH_2).

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 ($ZnCl_2$ or BF_3–Et_2O, 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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