α-Benzyl-γ-L-glutamyltaurine as a Useful Intermediate for the Synthesis of a Brain-Peptide, γ-L-Glutamyltaurine[1]

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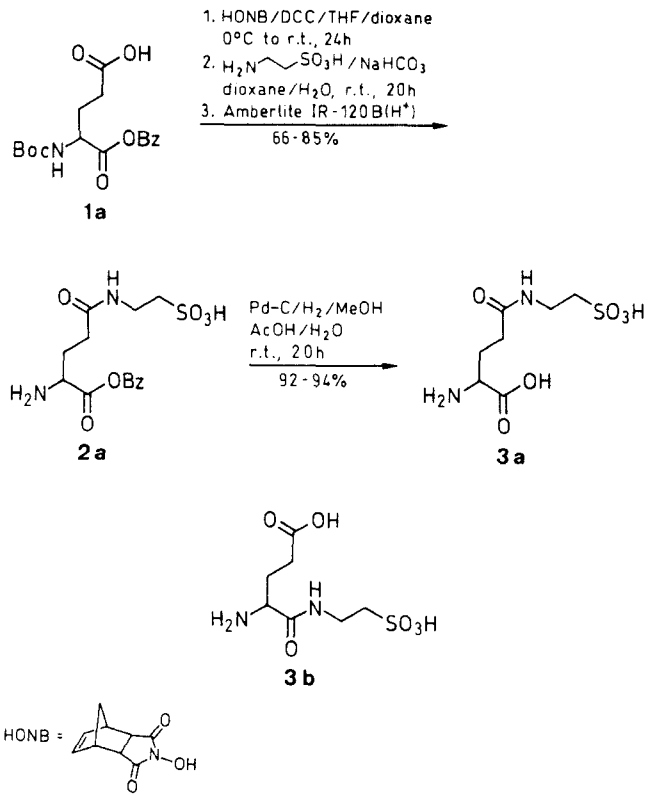
The synthesis of a neuropeptide, γ-L-glutamyltaurine (**3a**), was achieved via α-benzyl-γ-L-glutamyltaurine (**2a**) as a useful intermediate, which was prepared from α-benzyl *N-tert*-butoxycarbonyl-L-glutamate (**1a**) and taurine (2-aminoethanesulfonic acid) by active ester method. The preparation of its α-isomer **3b** was also described.

In our previous report,[1] we reported the isolation of pure glutamyltaurine (Glu-Tau) from bovine brains and the proof of its γ-linkage by the B/E linked scan SIMS (sputtered ion mass spectrometry) technique, which we have recently been developing.[2,3] The γ-Glu-Tau (glutaurine, **3a**) might be one candidate for a neurotransmitter, because glutaurine-like immunoreactivities have been shown to be localized in the specific neurons at the certain areas in the rat brain.[4,5,6] Although glutaurine **3a** was originally isolated in 1977 from parathyroid[7] and its periperal activities have been so far investigated by a Hungarian group,[7,8] its role in the central nervous system has not yet been evaluated well.

Therefore, we think that supplying a pure **3a** and its derivatives is still important.

Here we describe a simple synthetic method for **3a** by using α-benzyl-γ-L-glutamyltaurine (**2a**) as a useful intermediate. During our synthetic studies[2,4,9] on taurine containing oligopeptides, we have found out that a molecule, which forms a zwitter ion of sulfonic acid and amino groups, can be purified simply by crystallization; by contrast, a molecule with a sulfonic acid generally requires some additional purification procedures such as ion-exchange chromatography.[10-12] Since **2a** exists as a zwitter ion, **2a** became a useful intermediate which was also easily purified by crystallization.



The *N-tert*-butoxycarbonyl (Boc) glutamic acid α- and γ-benzyl ester, **1a** and **1b**, were used for the starting material for syntheses of γ-L-glutamyltaurine (**3a**) and its α-L-isomer **3b**, respectively. The *N*-hydroxy-5-norbornene-2,3-dicarboximide (*N*-hydroxybicyclo[2.2.1]hept-5-ene-2,3-dicarboximide, HONB) was first coupled with the protected glutamic acid, **1a** or **1b**, by dicyclohexylcarbodiimide (DCC) to form active ester, then the ester was coupled with taurine sodium salt in a mixed solvent of dioxane and water. After removal of the organic solvent in vacuo, sodium cation and the Boc protecting-group were both removed at the same time by treatment with Amberlite IR-120B (H^+ form). Evaporation of the water in vacuo gave a solid, which was washed by ethanol to remove ethanol-soluble HONB. The residue was purified by recrystallization from aqueous ethanol to give pure intermediate, **2a** or **2b**.

Hydrogenation of the benzyl ester over 10% Pd-C in aqueous methanol containing acetic acid gave quantitatively the corresponding glutamyltaurine **3a** or **3b** so that purification required only recrystallization from aqueous ethanol.

All reagents and solvents were of commercial quality: **1a** was from BACHEM Feinchemikalien AG, Switzerland; **1b**, HONB, and DCC were from Peptide Institute, Inc., Japan; Taurine and $NaHCO_3$ were from Kishida Chemical Co. Japan. Melting points, taken with Yamato MP-21 apparatus, are uncorrected. Microanalyses, optical rotations at the Na-D line at 24°C, and IR spectra were obtained with Perkin-Elmer Model 240B elemental analyser, JASCO DIP-140 polarimeter, and a Hitachi Model 260-30 spectrophotometer, respectively. ^1H NMR spectra in 0.1 N DCl/D_2O were obtained by using *t*-BuOH (1.23 ppm) as an internal standard with a Bruker AM-400 spectrometer.

α-Benzyl-γ-L-glutamyltaurine (2a):

To an ice cooled solution of the *N*-Boc-L-glutamic acid α-benzyl ester **1a** (16.9 g, 50 mmol) and HONB (9.50 g, 53 mmol) in a mixed solvent of THF (100 mL) and dioxane (100 mL), DCC (10.9 g, 53 mmol) was added and the mixture was stirred at the same temperature for 40 min, then at r.t. for 24 h. The resulting *N,N'*-dicyclohexylurea was filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in dioxane (100 mL), and then an aqueous solution of taurine sodium salt, prepared from taurine (6.26 g, 50 mmol) and $NaHCO_3$ (4.62 g, 55 mmol) in H_2O (100 mL), was added at r.t. After stirring of the mixture for 20 h followed by evaporation of the organic solvent in vacuo, the remaining aqueous solution was applied to a column packed with Amberlite IR-120B (H^+ form; 60 mL). After H_2O elution (500 mL),

the combined eluent was concentrated to give a solid, which was recrystallized from H_2O/EtOH to give pure **2a**; yield: 14.7 g (85%); mp 213–214°C (dec); $[\alpha]_D$ + 6.4° (*c* = 1.0, H_2O).

$C_{14}H_{20}N_2O_6S$	calc.	C 48.83	H 5.85	N 8.13
(344.4)	found	48.99	6.16	7.91

MS (SIMS): *m/z* = 345 (M + 1).

IR (KBr): ν = 3325, 3100–2900, 1740, 1650, 1550, 1195, 1040 cm^{-1}.

^1H NMR: δ = 2.13–2.27 (m, 2H), 2.32–2.47 (m, 2H), 2.98 (ddd, 1H, *J* = 7, 7, 14 Hz), 3.01 (ddd, 1H, *J* = 7, 7, 14 Hz), 3.46 (ddd, 1H, *J* = 7, 7, 14 Hz), 3.50 (ddd, 1H, *J* = 7, 7, 14 Hz), 4.20 (t, 1H, *J* = 6.5 Hz), 5.28, 5.31 (ABq, 2H, *J* = 12 Hz), 7.40–7.48 (m, 5H).

γ-Benzyl-α-L-glutamyltaurine · 0.33 H_2O (2b):

The γ-benzyl isomer of **2a** was similarly prepared from the corresponding γ-benzyl ester **1b**. Yield of **2b** as 0.33 hydrate was 66%; mp 211–212°C (dec); $[\alpha]_D$ + 37.0° (c = 1.0, H_2O).

$C_{14}H_{20}N_2O_6S \cdot 0.33H_2O$	calc.	C 47.99	H 5.95	N 7.99
(350.4)	found	48.07	6.01	7.99

MS (SIMS): *m/z* = 345 (M + 1).

IR (KBr): ν = 3300, 3100–2900, 1720, 1660, 1550, 1030 cm^{-1}.

^1H NMR: δ = 2.16 (ddd, 1H, *J* = 7, 7, 16 Hz), 2.20 (ddd, 1H, *J* = 7, 7, 16 Hz), 2.59 (t, 2H, *J* = 7 Hz), 3.04 (t, 2H, *J* = 7 Hz), 3.53 (ddd, 1H, *J* = 7, 7, 14 Hz), 3.60 (ddd, 1H, *J* = 7, 7, 14 Hz), 4.01 (t, 1H, *J* = 7 Hz), 5.17 (s, 2H), 7.38–7.47 (m, 5H).

γ-L-Glutamyltaurine (3a):

Hydrogenation of **2a** (3.44 g, 10 mmol) over a 10% Pd-C catalyst (0.3 g) in a mixed solvent of AcOH/MeOH/H_2O (20 mL/30 mL/10 mL) at r.t. for 20 h, followed by removal of the catalyst and evaporation of the solvent, gave a solid, which was recrystallized from H_2O/EtOH to give pure **3a**; yield: 3.25 g (92%); mp 222–223°C (dec); $[\alpha]_D$ + 20.1° (*c* = 1.0, H_2O) [Lit.[2] mp 224–225°C (dec); $[\alpha]_D$ + 20.3° (*c* = 1.0, H_2O)].

$C_7H_{14}N_2O_6S$	calc.	C 33.07	H 5.55	N 11.02
(254.3)	found	32.95	5.62	11.31

MS (SIMS): *m/z* = 255 (M + 1).

IR (KBr): ν = 3300, 3200–2500, 1750, 1650, 1230, 1030 cm^{-1}.

^1H NMR: δ = 2.19 (dddd, 1H, *J* = 7.5, 7.5, 7.5, 15 Hz), 2.26 (dddd, 1H, *J* = 7.5, 7.5, 7.5, 15 Hz), 2.48 (ddd, 1H, *J* = 7.5, 7.5, 15 Hz), 2.52 (ddd, 1H, *J* = 7.5, 7.5, 15 Hz), 3.08 (t, 2H, *J* = 6.5 Hz), 3.57 (t, 2H, *J* = 6.5 Hz), 4.11 (t, 1H, *J* = 7.5 Hz).

α-L-Glutamyltaurine (3b):

The α-isomer of **3a** was similarly prepared from the corresponding γ-benzyl ester **2b**. Yield of **3b** as 0.5 hydrate was 94%; mp 162–164°C (dec); $[\alpha]_D$ + 43.3° (*c* = 1.0, H_2O) [Lit.[2] mp 162–164°C (dec); $[\alpha]_D$ + 40.6° (*c* = 1.0, H_2O)].

$C_7H_{14}N_2O_6S \cdot 0.5H_2O$	calc.	C 31.94	H 5.74	N 10.64
(263.3)	found	31.83	6.01	10.34

MS (SIMS): *m/z* = 255 (M + 1).

IR (KBr): ν = 3450, 3300–2900, 1720, 1680, 1200, 1040 cm^{-1}.

^1H NMR: δ = 2.14 (ddd, 1H, *J* = 7, 7, 14 Hz), 2.19 (ddd, 1H, *J* = 7, 7, 14 Hz), 2.56 (t, 2H, *J* = 7 Hz), 3.11 (t, 2H, *J* = 6.5 Hz), 3.63 (t, 2H, *J* = 6.5 Hz), 4.03 (t, 1H, *J* = 7 Hz).

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