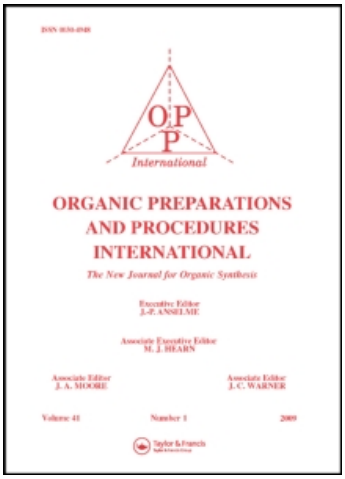

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SYNTHESIS OF γ-L-GLUTAMYL-TAURINE (GLUTAURINE)†

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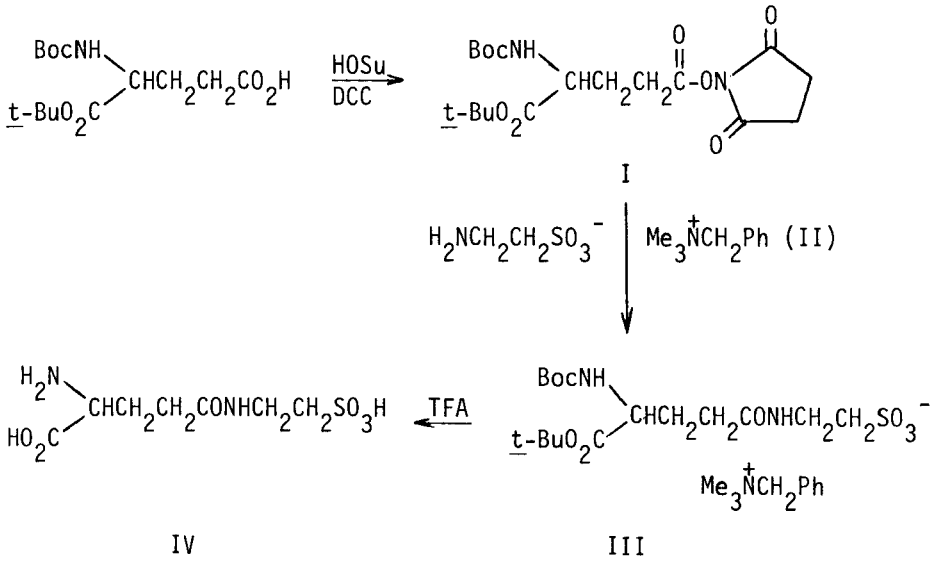
Glutaurine (γ-L-glutamyl-taurine)[1] was originally isolated from mammalian parathyroids.[1-3] Other analogs of glutamic acid or aspartic acid with taurine, cholamine, homotaurine and cystamine have also been reported.[4] Glutaurine has been reported to influence the metabolism of Vitamin A[5] and to antagonize the prednisolone inhibition of thymus culture growth.[6] Glutaurine is reported to have other pharmacologically and therapeutic properties such as radiation protection, promotion of wound healing, protection against stress and infections.[3]

The synthesis of glutaurine was recently described *via* three different pathways[7] using benzyl side chain protection. These procedures required prolonged catalytic hydrogenolysis (presumably to circumvent catalyst poisoning by the S-containing intermediate) or purification by ion-exchange chromatography. An alternate synthesis was devised using intermediates with side-chain protecting groups which do not require deprotection by catalytic hydrogenation.

Our synthesis of glutaurine was carried out using t-butyl side-chain protection by the procedure outlined in Scheme 1. Boc-L-Glu(OH)-OtBu was converted to the N-hydroxysuccinimide ester, I, by the procedure of Anderson[8] and coupled with taurine trimethylbenzylammonium salt, II, (prepared from taurine and Triton B). Intermediate III was fully deprotected in one step using trifluoroacetic acid and the final product, IV, was obtained as white prisms. Glutaurine was obtained directly without chromatographic purification in 71.2% yield and characterized by IR, NMR, specific rotation and elemental analysis. Peptide analysis of IV (pH 3.20; 0.2M Na^+; containing isopropanol) gave a homogeneous peak at t = 9 min (under these conditions aspartic acid emerges at t = 22 min). This new procedure was found to be somewhat more convenient than those previously reported.[7]



Scheme 1

EXPERIMENTAL

Materials and Methods

N-t-Butyloxycarbonyl-L-glutamic acid-α-t-butyl ester and taurine were purchased from Chemical Dynamics Corp. Dimethylformamide (reagent grade, Matheson, Coleman and Bell) was distilled from ninhydrin at reduced pressure and stored over molecular sieve. Dicyclohexylcarbodiimide (Aldrich Chemical Co.) was purified by distillation at reduced pressure. Triethylamine (Pierce Chemical Co.) was of Sequanal grade purity. All other solvents were of reagent grade purity and used without further purification.

Peptide analyses were performed on the Beckman Model 121M Amino Acid Analyzer. Tlc (100 μg load) was carried out on silica gel G plates (Analtech, Inc.) and developed with chlorine-tolidine. Melting points were determined on a Reichert hot stage apparatus and are uncorrected. Infrared and NMR spectra were measured and found to be compatible for all new products synthesized. Optical rotations were measured in a jacketed 1-dm cell on a Perkin Elmer Model 141 Polarimeter.

N-t-Butyloxycarbonyl-L-glutamic acid-α-t-butyl ester-γ-N-hydroxysuccinimide ester (I).- A solution of N-t-butyloxycarbonyl-L-glutamic acid-α-t-butyl ester (3.11 g, 10.3 mmol) in methylene chloride:dimethylformamide (50 mL:4 mL) was cooled to 0° and treated with N-hydroxysuccinimide (1.30 g, 11.3 mmol) and dicyclohexylcarbodiimide (2.33 g, 11.3 mmol). Stirring proceeded at 0° for 30 min and 25° for 17 h. The reaction mixture was filtered and the filtrate evaporated to dryness, taken up in methylene chloride (50 mL) and extracted with 10% $NaHCO_3$ (2 x 25 mL), saturated NaCl (2 x 25 mL), 1M citric acid (2 x 25 mL), saturated NaCl (2 x 25 mL), dried ($MgSO_4$) and evaporated. Crystallization from ethyl acetate-petroleum ether provided a white crystalline product: Yield, 3.26 g (79.1%); mp. 136.5-138.5°; $[\alpha]_D^{25}$ -15.14° (C 2.4, EtOH); R_f 0.85 (BuOH-AcOH-EtOAc-H_2O; 1:1:1:1).

Anal. Calcd. for $C_{18}H_{28}N_2O_8$: C, 53.99; H, 7.05; N, 7.00.
Found: C, 54.20; H, 7.04; N, 7.10.

Taurine trimethylbenzylammonium salt (II).- A mixture of taurine (1.25 g, 10.0 mmol) and Triton B (40% in methanol, 4.18 mL, 10.0 mmol) was evaporated to dryness and the residue taken up in DMF (2 mL) and evaporated to dryness again. This procedure was repeated two more times and the residual oil was taken up in 25.0 mL of DMF (final concentration: 0.40 mmol/mL) and used directly in the coupling reaction.

N-t-Butyloxycarbonyl-L-glutamyl-α-t-butyl ester-γ-taurine-trimethylbenzylammonium salt (III).- A mixture of Boc-Glu(OSu)-OtBu (I, 1.901 g, 4.75 mmol) and taurine trimethylbenzylammonium salt (II, 11.9 mL, 4.75 mmol) was stirred at 25° for 18 h. Triethylamine was added to maintain ph ∿8 (moist litmus paper) and the reaction mixture evaporated to dryness. The residue was taken up in DMF (10 mL) and evaporated to dryness again. This procedure was repeated two more times and the final residue was triturated with ether to afford a white amorphous product; R_f 0.44 (BuOH-AcOH-EtOAc-H_2O; 1:1:1:1) which was used directly in the deprotection step.

Glutaurine(γ-L-Glutamyl-taurine) (IV).- Intermediate III was treated with trifluoroacetic acid (35 mL) at 25° for 1.5 h. and the reaction mixture evaporated to dryness. The residue was evaporated from H_2O (4 x 10 mL), and crystallized from H_2O-isopropanol: Yield, 0.862 g (71.2% overall yield from I and II). Recrystallization from H_2O-isopropanol gave white prisms; mp. 223.5-224° dec; $[\alpha]_D^{25}$ + 23.94° (C 0.94, 1N HCl); R_f 0.21 (BuOH-AcOH-EtOAc-H_2O; 1:1:1:1); R_f 0.19 (BuOH-AcOH-Pyr-H_2O; 15:3:10:12). ^{1}H-NMR (D_2O) δ 2.74 (m,2H), 2.96 (m,2H), 3.55 (t,2H),

4.05 (t,2H), 4.56 (t,1H). IR (KBr) 3310, 1750, 1230, 1165 cm^{-1}. Lit.[7]; mp. 222-223°, $[\alpha]_D^{25}$ + 20.3° (C 3.6, H_2O). Peptide analysis in water using (conc., 514.2 nm/mL) [20 μL (10.3 nmole) applied] at 0.5 O.D. scale showed a single ninhydrin positive peak at retention time 9.0 min. Eluant: Citrate buffer, pH 3.20 (0.2M Na^+) containing i-PrOH.

Anal. Calcd. for $C_7H_{14}N_2O_6S$: C, 33.07; H, 5.55; N, 11.02; S, 12.61.
Found; C, 32.72; H, 5.56; N, 10.83; S, 12.34.

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† Abbreviations follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature [Biochem. J., 126, 773-780 (1972)]. Additional abbreviations: AcOH, acetic acid; BuOH, n-butanol; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; EtOAc, ethyl acetate; HOSu, N-hydroxysuccinimide; Pyr, pyridine, Tau, taurine (2-amino-ethanesulfonic acid); TFA, trifluoroacetic acid.

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