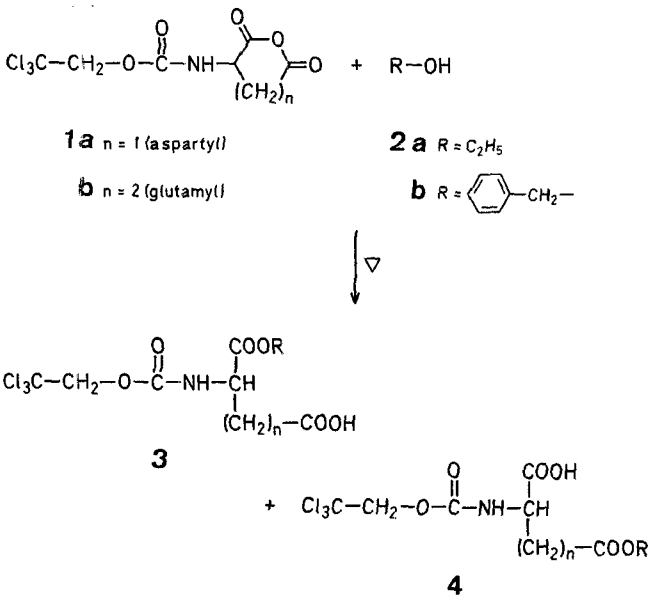

1a n = 1 (aspartyl) 2a $R = C_2H_5$

b n = 2 (glutamyl) b $R = C_6H_5CH_2-$

3

4

## The Synthesis of *N*-2,2,2-Trichloroethoxycarbonyl-L-glutamyl- and -L-aspartyl α-Ethyl and α-Benzyl Monoesters

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The preparation of *N*-2,2,2-trichloroethoxycarbonyl(Troc)-L-glutamyl and -L-aspartyl monoesters has been studied to obtain useful intermediates for the preparation of glutamyl and aspartyl peptides. The Troc protecting group is removable under mild conditions (zinc dust reduction)[1]. The procedures described yield predominately the α-monoethyl or α-monobenzyl esters and hence the products are useful primarily for the preparation of γ-glutamyl or β-aspartyl peptides. The γ-glutamyl or β-aspartyl esters could be obtained pure in only very small yields.

*N*-Troc-glutamic acid[2] on reaction with acetic anhydride yields *N*-Troc-glutamic anhydride **(1b)** as an oil which has not been crystallized, *N*-Troc-aspartic acid[2] with acetic anhydride yields the crystalline *N*-Troc-aspartic anhydride **(1a)**. These anhydrides on heating with ethanol or benzyl alcohol yield mixtures of the corresponding monoesters (α and γ) of Troc-glutamic acid or mixtures of the monoesters (α and β) of Troc-aspartic acid as shown below.

The crude reaction products **3**+**4** are obtained as oils which are converted to the crystalline *t*-butylamine salts in approximately 75–85% yields based on amino acid. The isomer distribution calculated from specific rotations of the mixed salts showed that approximately 70–75% esterification occurred at the α position and 25–30% at the more distant β or γ position. This agrees with similar syntheses involving anhydride opening of *N*-benzyloxycarbonylglutamic anhydride[3] or of *N*-trifluoroacetylglutamic anhydride[4] with ethanol in which cases the α ester predominates. Fractional crystallization of the *t*-butylamine salts from ethanol or ethanol/ether mixtures yields the pure α-monoethyl or α-monobenzyl ester amine salts as the less soluble isomers and very small yields of the corresponding β- or γ-ester amine salts from the mother liquors. The tedious isolation of the β- or γ-ester salts as pure compounds is of no synthetic value, but is necessary to be able to assess the optical purity of the α-esters by rotational methods. Data are presented in the Table. The α-glutamyl ester salts had negative specific rotations in ethanol and the γ-derivatives had positive rotations. With the aspartyl ester salts, both isomers had positive rotations but the α-derivatives were less positive than the β-esters.

The structures of the new compounds are established by microanalysis, $^1$H-N.M.R. spectra and decisively by alternate syntheses from known compounds and by fractional alkaline extraction. In the $^1$H-N.M.R. spectra, integration of proton resonances (Table) of *t*-butyl, benzyl or ethyl, trichloroethyl, and tertiary C—H protons gave ratios consistent with the assigned structures. *t*-Butyl protons appeared as 9 proton singlets at $\delta = 1.17$–$1.24$ ppm in benzyl esters and as multiplets (12H) overlapping the $CH_3$ resonances in ethyl esters. The $CH_2$ of ethyl appeared as a quartet centered at $\delta = 3.8$–$4.25$ ppm. The $Cl_3C-CH_2-$ appeared as 2H sharp singlets at $\delta = 4.70$–$4.75$ ppm, benzylic protons as 2H singlets at $\delta = 5.00$–$5.08$ ppm and aromatic protons as 5H singlets at $\delta = 7.30$–$7.35$ ppm. The known β-benzyl-L-aspartate[5] on reaction with trichloroethyl carbonochloridate yielded a product whose *t*-butylamine salt was identical with *N*-Troc-β-benzyl aspartate salt **(4ab)**. Similarly, γ-benzyl-L-glutamate[5] was converted to the *N*-Troc-derivative whose *t*-butylamine salt was identical with *N*-Troc-γ-benzyl glutamate amine salt **(4bb)**.

Identification of the isomeric ethyl esters was established by fractional alkaline extraction according to the procedure of Le Quesne and Young[6]. In the fractional alkaline extraction of a mixture of two isomeric organic acids from an organic solvent, the acid with the more highly ionized carboxyl will be extracted first. Hence, with *N*-protected glutamyl or aspartyl monoester mixtures, the isomer with a free α-carboxyl (β- or γ-ester) should be concentrated in the first fractions and the isomer with free β- or γ-carboxyl (α-ester) should be concentrated in later fractions. This procedure was applied to mixtures of *N*-Troc-glutamyl monoethyl esters **(3ba**+**4ba)**. The ester isolated from the first fractions when converted to the *t*-butylamine salt was identical with the γ-ethyl ester **(4ba)** and the corresponding α-ester **(3ba)** was isolated from the last fractions. Similarly, *N*-Troc β-ethyl aspartate **(4aa)** was extracted first from a mixture of α- and β-mono esters.

When *t*-butylamine was removed from the pure salts, the free monoester acids were usually obtained as non-crystallizing oils. The *N*-Troc-α-glutamyl benzyl ester **(3bb)** could be crystallized as the free acid after removal of *t*-butylamine from the salt.

Specific rotations were measured with a Perkin-Elmer Model 21 polarimeter with a cell of 1 decimeter path length. $^1$H-N.M.R. spectra were obtained with a JEOL-PFT-100 spectrometer. Organic reagents and L-amino acids were purchased from Aldrich Chemical Company. Melting points are uncorrected capillary melting points.

**N-Troc-L-glutamic Anhydride (1b):**
The method of preparation is an adaptation of the procedure of Le Quesne and Young[6]. *N*-Troc-L-glutamic acid[2] (11.3 g, 0.035 mol) is covered with acetic anhydride (100 ml; protected from moisture) and kept at room temperature for 2 days. The solution is then heated in a water bath at 50–55 °C for 2 h, concentrated in vacuo (oil pump) at room temperature for 20 h, and finally at 50–55 °C for 4 h to give a pale straw colored viscous oil which could not be crystallized; yield: 10.0 g (93%); $[\alpha]_D^{25}$: −44.6°; $[\alpha]_{436}^{25}$: −92.24° (*c* 6.3, DMF). The starting material has $[\alpha]_D^{25}$: −22.8°; $[\alpha]_{436}^{25}$: −46.00 in the same solvent.

**N-Troc-L-aspartic Anhydride (1a):**
This compound is prepared from *N*-Troc-aspartic acid[2] (8.80 g, 0.029 mol) by the same procedure as in the previous example to yield the crystalline anhydride; yield: 6.87 g (83%); m.p. 124–125 °C; $[\alpha]_D^{25}$: −28.33°; $[\alpha]_{436}^{25}$: −61.93° (*c* 5.7, DMF).

| | | | | | |
|---|---|---|---|---|---|
| $C_7H_6Cl_3NO_5$ | calc. | C 28.94 | H 2.08 | N 4.82 | Cl 36.62 |
| (290.5) | found | 28.5 | 2.2 | 4.7 | 36.3 |

**N-Troc-α-benzyl-L-glutamate *t*-Butylamine Salt (3bb); Typical Procedure:**
*N*-Troc-glutamic anhydride (**1b**; 7.64 g, 0.0251 mol) and benzyl alcohol (26 g, 0.24 mol) are heated together in a hot water bath at 70–75 °C for 2 h and the solution then concentrated in vacuo (oil pump) at 80–85 °C to remove most of the excess benzyl alcohol. The product, a pale amber oil, is dissolved in ether (125 ml), cooled to 0 °C, and treated with *t*-butylamine (5 ml, 0.048 mol). The resulting crystalline mixture is refrigerated overnight, filtered, and washed with ether to give the mixed *t*-butylamine salts; yield: 10.7 g (87%); $[\alpha]_D^{22}$: −7.6°; $[\alpha]_{436}^{22}$: −14.8° (*c* 5, ethanol). Recrystallation of the salt from ethanol (60 ml) gives a fraction; yield: 5.94 g (49%); $[\alpha]_D^{22}$: −15.2° (ethanol). A second recrystallization from ethanol gives pure **3bb** salt; yield: 5.38 g (Table).

**N-Troc-γ-benzyl-L-glutamate *t*-Butylamine Salt (4bb):**
The mother liquor from the first recrystallization of the α-ester, after four recrystallizations from ethanol gives pure **4bb** salt; yield: 0.8 g (Table).

**N-Troc-L-glutamyl α-Benzyl Ester: from 3bb:**
*t*-Butylamine salt **3bb** (2.318 g, 0.00477 mol) is suspended in ethyl acetate (125 ml), 2 normal hydrochloric acid (50 ml) is added, and the two liquids are stirred together until both phases are free of solid. The organic phase is separated, washed with saturated sodium chloride solution (2 × 20 ml), dried with sodium sulfate, and concentrated in vacuo to an oil which crystallizes very slowly. Recrystallization from ethyl acetate/hexane gives the product ester; yield: 1.87 g (95%); m.p. 71.5–72.0 °C; $[\alpha]_D^{25}$: −21.9°; $[\alpha]_{365}^{25}$: −70.77° (*c* 3.5, ethanol).

| | | | | | |
|---|---|---|---|---|---|
| $C_{15}H_{16}Cl_3NO_6$ | calc. | C 43.66 | H 3.91 | N 3.40 | Cl 25.78 |
| (412.7) | found | 43.5 | 3.8 | 3.5 | 25.6 |

**N-Troc-α- and -γ-ethyl-L-glutamate *t*-Butylamine Salts (3ba and 4ba):**
These are prepared as in the preceding examples with ethanol (50 mol excess) substituted for benzyl alcohol. Ethanol/ether (1:2) is employed in the fractional crystallization. As in the previous case, the α-ester salt separates first and the γ-ester salt is obtained from the mother liquor.

**N-Troc-L-aspartyl Benzyl and Ethyl Ester *t*-Butylamine Salts (3ab, 4ab, 3aa, 4aa):**
These compounds are prepared under the same conditions and with the same molar proportions as for the corresponding glutamyl esters. Ethanol is used for crystallizing the major products, α-esters, and from the mother liquors, the γ-esters are obtained by crystallization from ethanol/ether mixtures.

**N-Troc-β-benzyl L-Aspartate *t*-Butylamine Salt (4ab) from β-Benzyl Aspartate:**
Dibenzyl-L aspartate *p*-toluenesulfonate is converted by copper-catalyzed hydrolysis to β-benzyl aspartate by the procedure of Prestidge et al[5]. β-Benzyl L-aspartate (2.5 g, 0.0112 mol) in saturated borax solution (200 ml) is stirred continuously at 3–7 °C while trichloroethyl carbonochloridate (5.1 g, 0.024 mol) is added in small quantities over a 7 h period. The product is isolated as described previously[2] as an oil which is converted to the *N*-Troc-β-benzyl aspartate *t*-butylamine salt; yield: 1.4 g (27%); with $[\alpha]_D^{25}$: +14.8°; $[\alpha]_{365}^{25}$: +47.4°; and m.p. 148–149 °C; agreeing with **4ab**.

**Table.** *N*-(2,2,2-Trichloroethoxycarbonyl)-L-glutamyl- and -L-aspartyl Monoester *t*-Butylamine Salts

| Product No. | *t*-Butylamine Salts R | n | Yield [%] | m.p. [°C] | $[\alpha]_D^{25}$ | $[\alpha]_{365}^{25}$ | Molecular Formula[a] | $^1$H-N.M.R. (DMSO-$d_6$/TMS, 20 °C) δ [ppm] |
|---|---|---|---|---|---|---|---|---|
| **3bb** | $C_6H_5CH_2$ | 2 | 44 | 162–164° | −16.1° | −49.21° | $C_{19}H_{27}Cl_3N_2O_6$ (485.8) | 1.18 (s, 9H); 4.75 (s, 2H); 5.08 (s, 2H); 7.35 (s, 5H) |
| **4bb** | $C_6H_5CH_2$ | 2 | 7.5 | 153–154° | +13.2° | +47.2° | $C_{19}H_{27}Cl_3N_2O_6$ (485.8) | 1.24 (s, 9H); 4.74 (s, 2H); 5.04 (s, 2H); 7.35 (s, 5H) |
| **3ba** | $C_2H_5$ | 2 | 47 | 155–156° | −15.4° | −44.43° | $C_{14}H_{25}Cl_3N_2O_6$ (423.7) | 1.0–1.3 (m, 12H); 3.8–4.25 (q, 2H); 4.75 (s, 2H); 8.65–8.80 (d, 1H) |
| **4ba** | $C_2H_5$ | 2 | 1 | 151–152° | +12.5° | +42.79° | $C_{14}H_{25}Cl_3N_2O_6$ (423.7) | 1.05–1.20 (m, 12H); 3.65 (m, 1H); 3.95 (q, 2H); 4.72 (s, 2H); 6.75 (d, 1H) |
| **3ab** | $C_6H_5CH_2$ | 1 | 64 | 144–145° | +3.3° | +6.30° | $C_{18}H_{25}Cl_3N_2O_6$ (471.8) | 1.17 (s, 9H); 4.4 (m, 1H); 4.77 (s, 2H); 5.08 (s, 2H); 7.30 (s, 5H); 7.95 (d, 1H) |
| **4ab** | $C_6H_5CH_2$ | 1 | 4 | 148–149° | +15.1° | +48.42° | $C_{18}H_{25}Cl_3N_2O_6$ (471.8) | 1.20 (s, 9H); 3.85–4.15 (m, 1H); 4.70 (s, 2H); 5.00 (s, 2H); 6.95 (d, 1H); 7.30 (s, 5H) |
| **3aa** | $C_2H_5$ | 1 | 61 | 140–141° | +2.6° | +4.90° | $C_{13}H_{23}Cl_3N_2O_6$ (409.7) | 1.18 (m, 12H); 4.05 (q, 2H); 4.75 (s, 2H); 7.9 (d, 1H) |
| **4a** | $C_2H_5$ | 1 | 3 | 158–159° | +16.6° | +52.65° | $C_{13}H_{23}Cl_3N_2O_6$ (409.7) | 1.0–1.3 (m, 12H); 2.5 (m, 1H); 4.0 (q, 2H); 4.75 (s, 2H); 6.9 (d, 1H) |

[a] Satisfactory microanalyses obtained: C ±0.22, H ±0.18, N ±0.19, Cl ±0.20.

The low yield is occasioned by severe emulsions and by the fact that reactions of trichlorethyl carbonochloridate with amino acids give good yields with sodium or potassium hydroxide but poor yields with hydrogen carbonates or borax as alkaline media.

**N-Troc-γ-benzyl L-Glutamate t-Butylamine Salt (4bb) from γ-Benzyl L-Glutamate:**

Dibenzyl glutamate p-toluenesulfonate was converted to γ-benzylglutamate as in the previous example and reaction with trichloroethyl carbonochloridate yielded N-Troc-γ-benzyl L-glutamate isolated as the t-butylamine salt **4bb** with $[\alpha]_D^{25}$: +13.0°; $[\alpha]_{365}^{25}$: +45.5° (c 3.4, ethanol).

**Fractional Alkaline Extraction of Mixed N-Troc-α- and -γ-ethyl Glutamates:**

A mixture of the N-Troc-monoethyl glutamates (4.90, 0.0205 mol) is dissolved in ethyl acetate (175 ml) and extracted serially with water (6 × 60 ml) each containing potassium hydrogen carbonate (120 mg), followed by a seventh extraction with water (100 ml) containing potassium hydrogen carbonate (250 mg), and a final extraction with water (100 ml) containing potassium hydrogen carbonate (1.00 g). The extractions are performed by vigorous stirring of the ethyl acetate solution with the aqueous potassium hydrogen carbonate solution for 15–20 min followed by separation in a separatory funnel. Each alkaline fraction is acidified and further extracted into ethyl acetate. Each ethyl acetate fraction after drying with sodium sulfate is concentrated in vacuo to an oil. The first four fractions are combined and converted to the t-butylamine salt; yield: 1.27 g; $[\alpha]_D^{25}$: +11.4°; $[\alpha]_{365}^{25}$: +40.3° (ethanol). Recrystallization from 1:4 ethanol/ether (22 ml) yields 0.97 g; $[\alpha]_D^{25}$: +12.5°; $[\alpha]_{365}^{25}$: +42.16° (c 3, ethanol) in agreement with **4ba.** The last fraction when similarly treated yields 0.69 g; $[\alpha]_D^{25}$: −14.4°; $[\alpha]_{365}$: −40.75 (ethanol) in fair agreement with **3ba.**

Fractional alkaline extraction of the mixed N-Troc-α- and -β-ethyl aspartates follows the same procedure.

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