## *N*-2,2,2-Trichloroethoxycarbonyl-L-amino Acids

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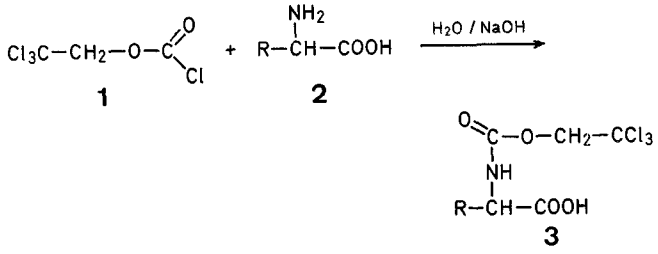
The 2,2,2-trichloroethoxycarbonylurethane (troc-urethane) was first recommended as a useful protecting group for amines by Windholz and Johnston[1]. Urethanes were prepared by reaction of 2,2,2-trichloroethyl carbonochloridate with the amine in pyridine or under Schotten-Baumann conditions. The trichloroethoxycarbonyl group was later removed by reduction with zinc dust. Sletzinger et al.[2] prepared this type of derivative in phosphate buffer (pH 8.5–9.0) as one step in a synthesis of cephalosporin.

The relatively mild removal of this protecting group and its moderate stability to acid[1] suggested the development of a method for the preparation of *N*-2,2,2-trichloroethoxycarbonyl derivatives of L-amino acids and their application in peptide synthesis. Chauvette et al.[3] prepared the *N*-troc derivative of D-α-phenylglycine in a synthesis of cephalexin and Yajima et al.[4] prepared ε-*N*-troc-L-lysine by reaction of the carbonochloridate with copper lysinate in the synthesis of the peptide, dog fish melanotrophin. These investigators also used this reagent for protection of terminal hydrazide groups. With the exception of these cases, the troc group has apparently not been applied in amino acid or peptide chemistry.

This report describes the preparation of *N*-2,2,2-trichloroethoxycarbonyl-L-amino acids **3** according to the following Scheme.




The derivatives **3** were prepared by alternate addition of sodium hydroxide solution and 2,2,2-trichloroethyl carbonochloridate (**1**) to a stirred aqueous solution of the amino acid **2** cooled in an ice-bath. The substitution of milder bases as sodium carbonate or sodium hydrogen carbonate generally gave lower yields. To maximize yields a large excess of the carbonochloridate **1** was used. Troc chloride (**1**) is less reactive to amines than benzyl carbonochloridate and the by-product bis[trichloroethyl] carbonate is always formed as was observed by Evans et al.[5] in reactions of **1** in base. It was necessary to remove this troublesome by-product from the basic reaction mixture before isolation of the troc-amino acid **3**.

Fifteen *N*-troc-L-amino acids **3** were prepared and all except troc-leucine, troc-isoleucine, and three troc-lysine derivatives were obtained crystalline. The last five compounds, obtained as oils or glasses, were crystallized as the *t*-butylamine salts. These derivatives have advantages over the commonly used dicyclohexylamine salts in that $^1$H-N.M.R. spectra are more easily interpreted and the original compounds are more easily regenerated from the salts. Physical constants and analytical data of the troc derivatives **3** are recorded in Tables 1 and 2. Troc derivatives of tryptophan or serine (unprotected hydroxy group) could not be prepared. Attempts to prepare α-troc-lysine from *N*-ε-benzylidenelysine by the Bezas and Zervas[6] method for the preparation of α-benzyloxycarbonyllysine resulted in only small yields of product contaminated with lysine. An alternate synthesis of α-troc-lysine by catalytic hydrogenolyses of α-troc-ε-benzyloxycarbonyllysine with 10% Pd—C and hydrogen also failed. The α-benzyloxycarbonyl-ε-troc-lysine was found to be a useful derivative for the preparation of α-benzyloxycarbonyllysine by zinc reduction.

$^1$H-N.M.R. spectral data of compounds **3** are given in Table 1. The $\underline{CH_2}$—$CCl_3$ resonance appeared as a sharp singlet at $\delta = 4.7$–$4.8$ ppm (in DMSO-$d_6$/TMS), except for *N*-troc-L-proline in which case the $\underline{CH_2}$—$CCl_3$ absorption appeared as a doublet centered at $\delta = 4.75$ ppm. At 90 °C the doublet coalesced to a singlet suggesting two structures for this derivative which interconvert rapidly at higher temperatures.

The following protected dipeptides were prepared by the dicyclohexylcarbodiimide procedure with added *N*-hydroxysuccinimide: *N*-Troc-Phe-Met-$OC_2H_5$, *N*-Troc-Leu-Met-$OC_2H_5$, *N*-Troc-Met-Met-$OC_2H_5$, and *N*-Troc-Met-Phe-$OC_4H_9$-*t*. The first three were obtained crystalline and the last as an oil. By the zinc dust reduction method of Just and Grozinger[7] (zinc dust/tetrahydrofuran/1 molar $KH_2PO_4$:5/1 v/v), the troc group was removed from *N*-Troc-Phe-Met-$OC_2H_5$ to yield $H_2$N-Phe-Met-$OC_2H_5$ isolated as the crystalline hydrochloride, and from *N*-Troc-Met-Phe-$OC_4H_9$-*t* to yield the crystalline hydrochloride of $H_2$N-Met-Phe-$OC_4H_9$-*t*. Of the frequently described solvent combinations such as acetic acid, formic acid, refluxing methanol and dimethylformamide + acetic acid, the Just and Grozinger procedure seemed the most satisfactory. Less zinc was solubilized and removal of zinc salts by hydrogen sulfide was usually unnecessary. However, yields in these zinc reductions are variable apparently independent of the solvent used, and the methods of activating zinc dust are not always completely satisfactory.

Specific rotations were measured with a Perkin-Elmer Model 21 polarimeter with a cell of 1.0 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 Co. or Pierce. Zinc dust was obtained from Mallinckrodt and activated by the procedure of Tsuda et al.[8] L-Methionine ethyl ester was prepared by the method of Brenner and Huber[9] and L-phenylalanine *t*-butyl ester hydrochloride was prepared by the method of Roeske[10]. Melting points are uncorrected capillary melting points.

**N-Troc-L-methionine; Typical Procedure:**

A solution of L-methionine (7.00 g; 0.0469 mol) in 1 normal sodium hydroxide solution (55 ml) is stirred in an ice-bath and trichloroethyl carbonochloridate (**1**; 20.0 g, 0.094 mol) and 1 normal sodium hydroxide solution (12.5 ml) are added alternately in small portions over 7 h with con-

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**Table 1.** *N*-(2,2,2-Trichloroethoxycarbonyl)-L-amino Acids **3**

| Product | Yield [%] | m.p. [°C] | Specific Rotations[a] | | | | Molecular formula[b] | $^1$H-N.M.R. (DMSO-$d_6$/TMS, 20 °C) $\delta$ [ppm] |
|---|---|---|---|---|---|---|---|---|
| | | | ethanol | | DMF | | | |
| | | | $[\alpha]_D^{25}$ | $[\alpha]_{436}^{25}$ | $[\alpha]_D^{25}$ | $[\alpha]_{436}^{25}$ | | |
| troc-Gly | 67 | 123–125° | — | — | — | — | $C_5H_6Cl_3NO_4$ (250.5) | 3.70 (d, 2H); 4.80 (s, 2H); 7.95 (t, 1H) |
| troc-Ala | 73 | 77° | −18.4° | −37.20° | −22.0° | −45.64° | $C_6H_8Cl_3NO_4$ (264.5) | 1.27 (d, 3H); 3.8–4.3 (m, 1H); 4.72 (s, 2H); 7.92 (d, 1H) |
| troc-Val | 72 | 80.5–81° | − 3.2° | − 5.5° | − 8.9° | −18.0° | $C_8H_{12}Cl_3NO_4$ (292.6) | 0.90 (d, 6H); 1.9–2.4 (m, 1H); 3.7–4.2 (m, 1H); 4.80 (s, 2H); 7.9 (d, 1H) |
| troc-Leu | 66 | oil[c] | −19.3° | −38.07° | −25.0° | −49.98° | $C_9H_{14}Cl_3NO_4$ (306.6) | 0.90 (t, 6H); 1.3–2.0 (m, 3H); 3.8–4.3 (m, 1H); 4.79 (s, 2H); 7.85 (d, 1H) |
| troc-Ileu | 59 | oil[c] | + 1.3° | + 3.6° | − 6.3° | −12.6° | $C_9H_{14}Cl_3NO_4$ (306.6) | 0.7–1.0 (m, 6H); 1.1–2.0 (m, 3H); 3.90 (q, 1H); 4.80 (s, 2H); 7.85 (d, 1H) |
| troc-Phe | 66 | 129–130° | + 4.5° | +13.3° | −28.95° | −57.38° | $C_{12}H_{12}Cl_3NO_4$ (340.6) | 2.7–3.3 (m, 2H); 4.1–4.4 (m, 1H); 4.70 (s, 2H); 7.20 (s, 5H); 7.90 (d, 1H) |
| troc-Pro | 84 | 64–65° | −44.27° | −89.73° | −41.90° | −85.58° | $C_8H_{10}Cl_3NO_4$ (290.5) | 1.7–2.6 (m, 4H); 3.20–3.70 (dd, 2H); 4.10–4.50 (dt, 1H); 4.65–4.90 (d, 2H)[f] |
| troc-Met | 76 | 80–81° | −12.3° | −24.2° | −23.8° | −48.68° | $C_8H_{12}Cl_3NO_4S$ (324.6) | 2.10 (s, 5H); 3.9–4.3 (m, 1H); 4.80 (s, 2H); 8.00 (d, 1H) |
| troc-Glut | 95 | 74° | −12.4° | −23.84° | −22.81° | −46.00° | $C_8H_{10}Cl_3NO_6$ (322.5) | 1.7–2.5 (m, 2H); 3.8–4.2 (m, 1H); 4.80 (s, 2H); 7.90 (d, 1H) |
| troc-Glut($NH_2$) | 82 | 112.5–113° | − 7.4° | −13.4° | −17.0° | −36.41° | $C_8H_{11}Cl_3N_2O_5$ (321.5) | 1.5–2.4 (m, 4H); 3.8–4.3 (m, 1H); 4.78 (s, 2H); 7.15 (d, 2H); 7.95 (d, 1H) |
| troc-Asp($NH_2$) | 73 | 154–155° (dec) | − 0.31° | − 2.5° | −12.1° | −26.53° | $C_7H_9Cl_3N_2O_5$ (307.5) | 2.4–2.8 (m, 2H); 4.3–4.5 (m, 1H); 4.78 (s, 2H); 7.08 (d, 2H); 7.80 (d, 1H) |
| troc-Asp | 89 | 146–147° (dec) | − 3.4° | − 9.16° | −31.97° | −68.40° | $C_7H_8Cl_3NO_6$ (308.5) | 2.3–2.9 (m, 2H); 4.2–4.5 (m, 1H); 4.75 (s, 2H); 7.85 (d, 1H) |
| α-troc-ε-Cbz-Lys[d] | 90 | oil[c] | − 5.4° | −10.0° | −13.4° | −26.61° | $C_{17}H_{21}Cl_3N_2O_6$ (455.7) | 1.2–2.0 (m, 2H); 2.4–3.2 (m, 2H); 3.8–4.2 (m, 1H); 4.76 (s, 2H); 4.97 (s, 2H); 7.35 (s, 5H); 7.7–8.3 (m, 2H) |
| α-Cbz-ε-troc-Lys[e] | 90 | oil[c] | − 2.4° | − 4.1° | − 6.6° | −12.5° | $C_{17}H_{21}Cl_3N_2O_6$ (455.7) | 1.1–2.0 (m, 2H); 2.3–3.2 (m, 2H); 3.8–4.2 (m, 1H); 4.75 (s, 2H); 5.0 (s, 2H); 7.32 (s, 5H); 7.4–7.8 (m, 2H) |
| α,ε-di-troc-Lys | 42 | oil[c] | − 5.7° | −11.2° | − 9.7° | −19.4° | $C_{12}H_{16}Cl_6N_2O_6$ (497.0) | 1.0–2.0 (m, 2H); 2.3–3.6 (m, 2H); 3.8–4.2 (m, 1H); 4.74 (s, 4H); 7.60 (m, 1H); 7.95 (d, 1H) |

[a] Specific rotations were measured at 3.5–4.0% concentration.
[b] Crystalline compounds gave satisfactory microanalyses: C ±0.58, H ±0.28, N ±0.39.
[c] Characterized as *t*-butylamine salt, see Table 2.
[d] Prepared by reaction of trichloroethyl carbonochloridate with ε-benzyloxycarbonyllysine.
[e] Prepared by reaction of trichloroethyl carbonochloridate with α-benzyloxycarbonyllysine.
[f] At 90 °C, for proline, the $CH_2$ at 3.20–3.70 became a triplet, the CH at 4.10–4.50 became a doublet of doublets and the $CH_2$ doublet at 4.65–4.90 coalesced to a singlet at 4.75.

tinuous stirring at 0 °C. The air of the flask is displaced by argon. The mixture is then stirred overnight at room temperature and extracted with ethyl acetate (4 × 75 ml) to remove bis[trichloroethyl] carbonate. The alkaline phase is then chilled and acidified (hydrochloric acid) with precipitation of an oil. The oily suspension is extracted with ethyl acetate (3 × 75 ml) and after drying with sodium sulfate, the ethyl acetate fraction is concentrated in vacuo to a colorless oil which is crystallized from ether/hexane (1:3) to give *N*-troc-L-methionine; yield: 11.11 g (76%).

The ethyl acetate extracts of the original basic reaction mixture on concentration yield the by-product, bis[trichloroethyl] carbonate identified by mass spectra, $^1$H-N.M.R., and $^{13}$C-N.M.R. in agreement with the data of Evans et al.[5]. Ethyl acetate is chosen as an extracting agent at this stage, since ether or dichloromethane yield severe emulsions. This is found to be true for the other compounds except for the three lysine derivatives when the opposite is true. Ether is then preferable.

**N-Troc-Phe-Met-$OC_2H_5$; Typical Procedure:**

A solution of troc-phenylalanine (6.75 g, 0.0198 mol) and L-methionine methyl ester hydrochloride (4.255 g, 0.0199 mol) in dichloromethane (200 ml) and dimethylformamide (70 ml) is stirred at −10 °C and the hydrochloride neutralized by the addition of diisopropylethylamine (4 ml). Air in the flask is displaced by argon and dicyclohexylcarbodiimide (4.45 g, 0.0211 mol) is added. Approximately 20 min later, *N*-hydroxysuccinimide (2.19 g, 0.019 mol) is added and the suspension is stirred at −10 °C for 6 h, stored at −20 °C for 16 h, and at 0 °C for 72 h. The mixture is then concentrated in vacuo to a thick slurry whereupon ethyl acetate (300 ml) is added followed by 1 normal hydrochloric acid (10 ml) and acetic acid (2 ml) to destroy excess dicyclohexylcarbodiimide. The mixture is stirred for 30 min and filtered. The ethyl acetate filtrate is extracted successively with 1 normal hydrochloric acid (5 × 40 ml), 5% sodium hydrogen carbonate solution (5 × 40 ml), and saturated sodium chloride solution (25 ml). Concentration of the ethyl acetate extract in vacuo gives the crystalline product which is recrystallized from 1:2 ethyl acetate/hexane or 2:1 ethanol/water; yield: 9.7 g (>95%); m.p. 88–89 °C; $[\alpha]_D^{25}$: −16.7°, $[\alpha]_{436}^{25}$: −29.63°, $[\alpha]_{365}^{25}$: −37.57° (*c* 4.7, DMF).

$C_{19}H_{25}Cl_3N_2O_5S$ (499.8) calc. C 45.66 H 5.04 N 5.60 Cl 21.28
found 46.1 5.17 5.71 20.7

$^1$H-N.M.R. (DMSO-$d_6$/TMS): $\delta$ = 1.13 (t, 3H); 1.7–2.1 (m, 5H); 4.10 (q, 2H); 4.3–4.5 (m, 2H); 4.67 (s, 2H); 7.30 (s, 5H); 7.85 (d, 1H); 8.40 ppm (d, 1H).

**Table 2.** *t*-Butylamine Salts[a] of Oily *N*-2,2,2-Trichloroethoxycarbonyl-L-amino Acids

| Troc-L-amino Acid | m.p. [°C] | Molecular formula[b] |
|---|---|---|
| troc-Leu[c] | 168–169° (dec) | $C_{13}H_{25}Cl_3N_2O_4$ (379.7) |
| troc-Ileu | 178–179° (dec) | $C_{13}H_{25}Cl_3N_2O_4$ (379.7) |
| α-troc-ε-Cbz-Lys | 131° (dec) | $C_{21}H_{32}Cl_3N_3O_6$ (528.9) |
| α-Cbz-ε-troc-Lys | 120° (dec) | $C_{21}H_{32}Cl_3N_3O_6$ (528.9) |
| α,ε-di-troc-Lys | 137° (dec) | $C_{16}H_{27}Cl_6N_3O_6$ (570.1) |

[a] The salts had N.M.R. spectra very similar to those of the oily acids with the addition of $NH_3^{\oplus}$ bands and the *t*-butyl hydrogen singlet at $\delta = 1.25$–$1.3$ ppm.

[b] Satisfactory microanalyses obtained: C ±0.4, H ±0.14, N ±0.10, Cl ±0.6.

[c] In a typical preparation, a solution of *t*-butylamine (1.0 ml) in ether (6 ml) is added to troc-leucine (1.40 g) in ether (15 ml). After refrigeration overnight, the amine salt crystallizes; yield: 1.42 g (82%).

**N-Troc-Leu-Met-$OC_2H_5$:**
Prepared as described above and recrystallized from 1:3 ethyl acetate/hexane or 2:1 ethanol/water; yield: 89%; m.p. 100–100.5 °C; $[\alpha]_D^{25}$: −23.0°, $[\alpha]_{436}^{25}$: −44.11°, $[\alpha]_{365}^{25}$: −64.86° (*c* 4.326, DMF).

| | | | | | |
|---|---|---|---|---|---|
| $C_{16}H_{27}Cl_3N_2O_5S$ | calc. | C 41.25 | H 5.85 | N 6.01 | Cl 22.83 |
| (465.8) | found | 41.4 | 5.92 | 6.04 | 22.5 |

$^1$H-N.M.R. (DMSO-$d_6$/TMS): $\delta = 0.90$ (m, 6H); 1.15 (t, 3H); 3.9–4.5 (m, 4H); 4.80 (s, 2H); 7.80 (d, 1H); 8.25 ppm (d, 1H).

**N-Troc-Met-Met-$OC_2H_5$:**
Prepared as described above and recrystallized; yield: 98%; m.p. 60 °C; $[\alpha]_D^{25}$: −19.3°, $[\alpha]_{436}^{25}$: −35.95°, $[\alpha]_{365}^{25}$: −50.67 (*c* 4.79, DMF).

| | | | | |
|---|---|---|---|---|
| $C_{15}H_{25}Cl_3N_2O_5S_2$ | calc. | C 37.23 | H 5.21 | N 5.79 |
| (483.9) | found | 37.6 | 5.43 | 5.60 |

$^1$H-N.M.R. (DMSO-$d_6$/TMS): $\delta = 1.17$ (t, 3H); 3.9–4.5 (m, 4H); 4.75 (s, 2H); 7.85 (d, 1H); 8.30 ppm (d, 1H).

**N-Troc-Met-Phe-$OC_4H_9$-*t*:**
Prepared from *N*-troc-L-methionine and L-phenylalanine *t*-butyl ester as described above; yield: 97%; oil; $[\alpha]_D^{25}$: +13.9°, $[\alpha]_{436}^{25}$: +33.35° (*c* 7.604, ethyl acetate); $[\alpha]_D^{25}$: −10.7°, $[\alpha]_{436}^{25}$: −18.1° (*c* 5.067, DMF).

$^1$H-N.M.R. (DMSO-$d_6$/TMS): $\delta = 1.27$ (s, 9H); 1.98 (s, 3H); 4.0–4.5 (m, 2H); 4.76 (s, 2H); 7.20 (s, 5H); 7.81 (d, 1H); 8.17 ppm (d, 1H).

**Zinc Dust Reduction of N-Troc-Phe-Met-$OC_2H_5$:**
*N*-Troc-Phe-Met-$OC_2H_5$ (4.40 g, 0.0088 mol) is dissolved in tetrahydrofuran (120 ml) and the air displaced with argon. Activated zinc dust (14 g) is added with vigorous magnetic stirring and immediately after 1 molar $KH_2PO_4$ (24 ml) is added and stirring continued for 7.5 h. The slurry is filtered by gravity and the zinc washed with fresh tetrahydrofuran (100 ml) and ethanol (100 ml). Water (20 ml) and 1 normal hydrochloric acid (10 ml) is added and the solution is concentrated in vacuo to ~50 ml. The acidic suspension is extracted with ether (3 × 75 ml) to remove starting material and the aqueous phase made alkaline with sodium hydrogen carbonate solution. This is again extracted with ethyl acetate (3 × 75 ml). The organic phase is treated with 1 normal hydrochloric acid (8 ml) to form the hydrochloride and concentrated in vacuo to 50 ml. Water is removed by 3 repeated additions of 75 ml portions of ethanol and vacuum concentration until the product is obtained as a foam. Crystallization is accomplished by the addition of ether to give *$H_2N$-Phe-Met-$OC_2H_5$·HCl*; yield: 2.60 g (82%); m.p. 86–87 °C; $[\alpha]_D^{25}$: −16.8°, $[\alpha]_{436}^{25}$: −28.6°, $[\alpha]_{365}^{25}$: −34.31° (*c* 3.433, DMF).

| | | | | | |
|---|---|---|---|---|---|
| $C_{16}H_{25}ClN_2O_3S$ | calc. | C 53.25 | H 6.98 | N 7.76 | Cl 9.82 |
| (360.9) | found | 53.6 | 7.04 | 7.60 | 9.6 |

$^1$H-N.M.R. (DMSO-$d_6$/TMS): $\delta = 1.15$ (t, 3H); 1.7–2.2 (m, 5H); 2.8–3.4 (m, 4H); 3.9–4.6 (m, 4H); 7.25 (s, 5H); 8.4 (s, 3H); 9.2 ppm (d, 1H).

**$H_2N$-Met-Phe-$OC_4H_9$-*t*·HCl:**
This compound is obtained by zinc reduction of *N*-troc-Met-Phe-$OC_4H_9$-*t* in a manner similar to the previous experiment. Reduction of *N*-troc-Met-Phe-$OC_4H_9$-*t* (4.47 g, 8.66 mmol) gives *$H_2N$-Met-Phe-$OC_4H_9$*-t as a non-crystallizing oil; yield: 2.08 g (70%).

This compound is converted to the hydrochloride which is recrystallized from ether; m.p. 151–153 °C (dec); $[\alpha]_D^{25}$: +16.1°, $[\alpha]_{436}^{25}$: +39.27° (*c* 3.8, DMF).

| | | | | | |
|---|---|---|---|---|---|
| $C_{18}H_{29}ClN_2O_3S$ | calc. | C 55.59 | H 7.52 | N 7.20 | |
| (389.0) | found | 56.0 | 7.63 | 7.14 | |

$^1$H-N.M.R. (DMSO-$d_6$/TMS): $\delta = 1.30$ (s, 9H); 1.8–2.2 (m, 5H); 3.0 (d, 2H); 3.8–4.0 (m, 1H); 4.3–4.6 (m, 1H); 7.25 (s, 5H); 8.45 (s, 3H); 9.10 ppm (d, 1H).

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