The Preparation of Methionine-Containing Peptides with Trifluoroacetyl Protection of Amino Groups and 2,2,2-Trichloroethyl Ester Protection of Carboxyl Groups*

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In a previous paper[1], a method for the preparation of 2,2,2-trichloroethyl esters was described. This paper presents the synthesis of a number of protected dipeptides, one tripeptide, and a tetrapeptide derivative containing methionine with *N*-trifluoroacetyl and 2,2,2-trichloroethyl as protecting groups.

Marinier et al.[2] employed amino acid trichloroethyl esters in peptide synthesis where the benzyloxycarbonyl group was used to protect the amino function. Peptides which contain methionine, however, present difficulties in removal of benzyloxycarbonyl groups by catalytic hydrogenolysis, hydrogen bromide in acetic acid, or sodium in liquid ammonia. In the first case, reaction is very slow because of catalyst poisoning and with the other two methods, methionine is partly destroyed. These problems are eliminated by the use of the *t*-butoxycarbonyl[3] derivative for amino protection, a procedure that is almost routine in solid phase syntheses.

Weygand and Frauendorfer[4] have prepared peptides with the amino group protected by trifluoroacetyl and the carboxyl group protected as the *t*-butyl ester and have shown that the trifluoroacetyl group may be readily removed with sodium borohydride in ethanol with only slight damage to the *t*-butyl ester and with no effect on a free carboxyl group. The ease of preparation of *N*-trifluoroacetyl amino acids and of amino acid trichloroethyl esters and the possibility of removal of the ester group by reduction with zinc dust suggested the combination of these two derivatives for peptide synthesis as an alternative procedure.

The syntheses described here follow the equations in which one or both amino acid reactants **1** and/or **2** is a methionine derivative. All amino acids are of the L-configuration.

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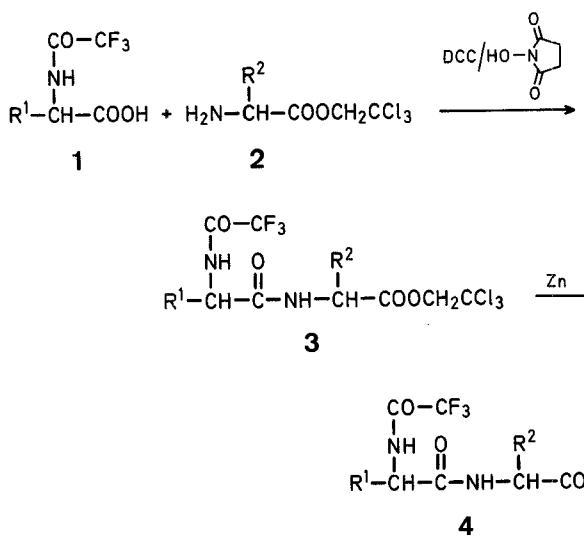

After zinc reduction, the cycle can be repeated by reaction of the *N*-trifluoroacetyl-peptide **4** with a second amino acid trichloroethyl ester. Finally, the free peptide can be obtained by borohydride reduction after removal of the last trichloroethyl ester group. The order cannot be reversed since borohydride attacks the ester but not the carboxyl group. In these reactions, the ester is applied as the tosylate salt followed by neutralization with the appropriate tertiary amine.

Since Weygand et al.[5] have observed serious racemization in the preparation of *N*-trifluoroacetyl-peptides with dicyclohexylcarbodiimide (DCC) at room temperature, but only slight racemization at −10 °C, the coupling reactions in this report were all performed at low temperature, and *N*-hydroxysuccinimide was used as an additive to further minimize racemization. The value of this reagent was demonstrated by Wünsch and Dries[6], Weygand[4], and by Izumiya and Muraoka[7].

The identity and purity of the *N*-trifluoroacetyl-peptide trichloroethyl esters **3** were established by ^{1}H-N.M.R. spectrometry in deuterated dimethyl sulfoxide when the integrated proton resonance intensities were compared for NH, CH_2—CCl_3, *t*-CH, and where possible for CH_3S and CH_3. Table 1 gives physical data for nine *N*-trifluoroacetyl dipeptide trichloroethyl esters and the tri- and tetramethionyl esters.

Trichloroethyl ester groups were removed by the procedure of Just and Grozinger[8] in which the combination zinc dust and 4/1 (vol/vol) of tetrahydrofuran/1 molar potassium dihydrogen phosphate was used. This was found to be preferable to the more commonly used zinc/acetic acid in that less zinc was solubilized and products were easier to purify. Table 2 gives physical data for the eleven *N*-trifluoroacetyl-peptides **4** obtained by zinc reduction of the compounds **3** of Table 1. Again, completion of reaction and purity was established by ^{1}H-N.M.R. spectrometry, absence of CH_2CCl_3 resonance and ratios of the other integrated resonances.

Specific rotations were measured with a Perkin-Elmer 241 polarimeter with a cell of 1 dm path length. ^{1}H-N.M.R. spectra were measured with a JEOL-PFT-100 spectrometer. L-Amino acids and

Table 1. *N*-Trifluoroacetyl Peptide 2,2,2-Trichloroethyl Esters **3** (*N*-F_3C—CO—A,B—OCH_2CCl_3)

Product No.	A	B	Yield [%]	m.p. [°C]	$[\alpha]_D$ ($[\alpha]_{436}$)[a] in $CHCl_3$	$[\alpha]_D$ ($[\alpha]_{436}$)[a] in DMF	Molecular formula[b]	^{1}H-N.M.R. (DMSO-d_6/TMS) δ [ppm]
3a	Ala	Met	90	133–135°	−22.0° (−44.6°)	−41.8° (−85.6°)	$C_{12}H_{16}Cl_3F_3N_2O_4S$ (447.7)	1.37 (d, 3H, CH_3); 1.84–2.20 (m, 5H, SCH_3+CH_2); 2.40–2.79 (m, SCH_2 + solvent); 4.16–4.74 (m, 2H, N—CH); 4.94 (q, 2H, CH_2CCl_3); 8.61 (d, 1H, NH); 9.57 (d, 1H, NH)
3b	Val	Met	93	84–85°	−19.9° (−39.0°)	−32.2° (−67.7°)	$C_{14}H_{20}Cl_3F_3N_2O_4S$ (475.7)	0.94 (t, 6H, CH_3); 1.74–2.35 (m, 6H, SCH_3 + CH_2 + CH); 2.35–2.80 (m, CH_2S + solvent); 4.22 (t, 1H, N—CH); 4.39–4.75 (m, 1H, N—CH); 4.97 (q, 2H, CH_2CCl_3); 8.74 (d, 1H, NH); 9.50 (d, 1H, NH)
3c[c]	Leu	Met	95	oil	−31.8° (−65.6°)	−39.6° (−80.0°)	$C_{15}H_{22}Cl_3F_3N_2O_4S$ (489.8)	0.90 (s, 6H, CH_3); 1.45–2.30 (m, 8H, SCH_3 + CH_2 + CH); 4.20–4.75 (m, 2H, N—CH); 4.96 (q, 2H, CH_2CCl_3); 8.68 (d, 1H, NH); 9.58 (d, 1H, NH)
3d	Phe	Met	86	106–107°	+ 4.82° (+15.6°)	−16.1° (−24.3°)	$C_{18}H_{20}Cl_3F_3N_2O_4S$ (523.8)	1.72–2.28 (m, 5H, SCH_3 + CH_2); 2.28–2.57 (m, 2H, CH_2); 2.58–3.20 (m, CH_2 + solvent); 4.42–4.78 (m, 2H, N—CH); 4.97 (q, 2H, CH_2CCl_3); 7.25 (s, $5H_{arom}$); 8.84 (d, 1H, NH); 9.66 (d, 1H, NH)
3e	Met	Ala	77	73°	+ 1.2° (+ 5.4°)	−20.0° (−40.4°)	$C_{12}H_{16}Cl_3F_3N_2O_4S$ (447.7)	1.40 (d, 3H, CH_3); 1.80–2.80 (m, SCH_3 + CH_2 + solvent); 4.25–4.70 (m, 2H, N—CH); 4.90 (q, 2H, CH_2CCl_3); 8.70 (d, 1H, NH); 9.40–9.70 (m, 1H, NH)
3f[c]	Met	Val	80	oil	+ 1.0° (+ 6.17°)	−11.0° (−20.1°)	$C_{14}H_{20}Cl_3F_3N_2O_4S$ (475.7)	0.90 (t, 6H, CH_3); 2.07 (s, 3H, SCH_3); 3.35 (m, 5H, CH_2 + CH); 4.20–4.70 (m, 2H, N—CH); 4.95 (q, 2H, CH_2CCl_3); 8.20–8.65 (m, 1H, NH); 9.55 (d, 1H, NH)
3g	Met	Leu	87	82°	— (—)	−25.6° (−49.4°)	$C_{15}H_{22}Cl_3F_3N_2O_4S$ (489.8)	0.91 (s, 6H, CH_3); 1.40–2.20 (m, 8H, SCH_3 + CH_2 + CH); 4.20–4.70 (m, 2H, N—CH); 4.94 (q, 2H, CH_2CCl_3); 8.65 (d, 1H, NH); 9.58 (d, 1H, NH)

Table 1. (Continued)

Product No.	A	B	Yield [%]	m.p. [°C]	$[\alpha]_D$ ($[\alpha]_{436}$)[a] in $CHCl_3$	in DMF	Molecular formula[b]	^{1}H-N.M.R. (DMSO-d_6/TMS) δ [ppm]
3h	Met	Phe	80	92–93°	− 4.2° (− 3.4°)	−23.1° (−45.3°)	$C_{18}H_{20}Cl_3F_3N_2O_4S$ (523.8)	1.60–2.60 (m, 7H, SCH_3 + CH_2); 2.70–3.20 (m, CH_2 + solvent); 4.25–4.80 (m, 2H, N—CH); 4.90 (q, 2H, CH_2CCl_3); 7.25 (s, 5H_{arom}); 8.75 (dd, 1H, NH); 9.50 (dd, 1H, NH)
3i	Met	Met	94	67–68°	− 3.5° (− 3.9°)	−35.0° (−68.7°)	$C_{14}H_{20}Cl_3F_3N_2O_4S_2$ (507.8)	2.05 (m, 10H, SCH_3 + CH_2); 2.43–2.57 (SCH_2 + solvent); 4.41–4.68 (m, 2H, N—CH); 4.93 (q, 2H, CH_2CCl_3); 8.70 (d, 1H, NH); 9.61 (d, 1H, NH)
3j	Met	$(Met)_2$	93	148–150°	−11.7° (−21.2°)	−30.0° (−58.6°)	$C_{19}H_{29}Cl_3F_3N_3O_5S_3$ (639.0)	1.70–2.80 (m, SCH_3 + CH_2 + solvent); 4.10–4.65 (m, 3H, N—CH); 4.87 (q, 2H, CH_2CCl_3); 8.25 (d, 1H, NH); 8.45 (d, 1H, NH); 9.50 (d, 1H, NH)
3k	Met	$(Met)_3$	78	108–110° (softens at 106°)	−20.3° (−40.3°)	−28.0° (−54.6°)	$C_{24}H_{38}Cl_3F_3N_4O_6S_4$ (770.2)	1.70–2.60 (m, SCH_3 + CH_2 + solvent); 4.20–4.65 (m, 4H, N—CH); 4.90 (q, 2H, CH_2CCl_3); 8.00 (d, 1H, NH); 8.30 (d, 1H, NH); 8.50 (d, 1H, NH); 9.55 (d, 1H, NH)

[a] Specific rotations were measured at 4–5% concentration (g/100 ml).
[b] The microanalyses were in fair agreement with the calculated values (C ±0.42, H ±0.43, N ±0.32); exceptions: **3a** C +0.81, **3e** C −0.79, **3k** C +0.57.
[c] Not analyzed.

Table 2. *N*-Trifluoroacetyl Peptides **4** (*N*-F_3C—CO—A,B—OH) from 2,2,2-Trichloroethyl Esters **3**

Product No.	Yield [%]	m.p. [°C]	$[\alpha]_D$ ($[\alpha]_{436}$) in CH_3CN	in DMF	Molecular formula[a]	^{1}H-N.M.R. (DMSO-d_6/TMS) δ [ppm]
4a	89	142–143°	−33.5° (−70.0°)	−28.7° (−58.9°)	$C_{10}H_{15}F_3N_2O_4S$ (316.3)	1.24 (d, 3H, CH_3); 1.56–2.24 (m, 5H, SCH_3 + CH_2); 2.24–2.80 (m, SCH_2 + solvent); 4.12–4.70 (m, 2H, N—CH); 8.26 (d, 1H, NH); 9.50 (d, 1H, NH)
4b	88	206–207°	— —	−22.4° (−46.8°)	$C_{12}H_{19}F_3N_2O_4S$ (344.4)	0.88 (t, 6H, CH_3); 1.70–2.30 (m, 6H, SCH_3 + CH_2 + CH); 2.50 (t, 2H, SCH_2); 4.00–4.58 (m, 2H, N—CH); 8.41 (d, 1H, NH); 9.40 (d, 1H, NH)
4c	79	205–207°	−36.9° (−75.2°)	−32.4° (−65.6°)	$C_{13}H_{21}F_3N_2O_4S$ (358.4)	0.90 (q, 6H, CH_3); 1.30–2.60 (m, 8H, SCH_3 + CH_2 + CH); 3.28 (m, 2H, SCH_2); 4.31 (m, 2H, N—CH); 8.36 (d, 1H, NH); 9.49 (d, 1H, NH)
4d	73	186–187°	− 8.1° (− 9.2°)	+ 2.1° (+15.6°)	$C_{16}H_{19}F_3N_2O_4S$ (392.4)	1.80–2.24 (m, 5H, SCH_3 + CH_2); 2.25–3.34 (m, CH_2 + solvent); 4.52 (m, 2H, N—CH); 7.30 (m, 5H_{arom}); 8.55 (d, 1H, NH); 9.60 (d, 1H, NH)
4e[b]	60	oil	− 5.39° (−10.9°)	− 8.62° (−17.2°)	$C_{10}H_{15}F_3N_2O_4S$ (316.3)	1.25 (d, 3H, CH_3); 1.50–2.65 (m, SCH_3 + CH_2 + solvent); 3.90–4.70 (m, 2H, N—CH); 8.35 (d, 1H, NH); 9.45 (d, 1H, NH)
4f[b]	84	oil	− 0.5° (+ 1.0°)	− 8.92° (−18.8°)	$C_{12}H_{19}F_3N_2O_4S$ (344.4)	0.90 (t, 6H, CH_3); 1.80–2.34 (m, 6H, SCH_3 + CH_2 + CH); 2.34–2.66 (m, 2H, SCH_2); 4.0–4.7 (m, 2H, N—CH); 8.00–8.40 (m, 1H, NH); 9.52 (d, 1H, NH)
4g	79	137°	— —	− 6.93° (−12.0°)	$C_{13}H_{21}F_3N_2O_4S$ (358.4)	0.90 (t, 6H, CH_3); 1.40–2.30 (m, 8H, SCH_3 + CH_2 + CH); 2.55 (t, 2H, SCH_2); 4.06–4.74 (m, 2H, N—CH); 8.36 (d, 1H, NH); 9.53 (d, 1H, NH)
4h	54	120–121°	− 9.58 (−15.3°)	−10.8° (−17.3°)	$C_{16}H_{19}F_3N_2O_4S$ (392.4)	1.54–3.34 (m, SCH_3 + CH_2 + solvent); 4.50 (d, 2H, N—CH); 7.28 (s, 5H_{arom}); 8.45 (t, 1H, NH); 9.32–9.66 (m, 1H, NH)
4i	72	157–158°	−19.1° (−35.6°)	−26.1° (−49.8°)	$C_{12}H_{19}F_3N_2O_4S_2$ (376.4)	1.86–2.06 (m, 10H, SCH_3 + SCH_2); 2.43–2.58 (m, CH_2 + solvent); 4.34 (m, 2H, N—CH); 8.41 (d, 1H, NH); 9.56 (d, 1H, NH)
4j	73	145–147° (dec)	−24.7° (−49.2°)	−16.5° (−30.6°)	$C_{17}H_{28}F_3N_3O_5S_3$ (507.6)	1.70–2.70 (m, SCH_3 + CH_2 + solvent); 4.35 (m, 3H, N—CH); 8.10 (d, 1H, NH); 8.30 (d, 1H, NH); 9.50 (t, 1H, NH)
4k	78	127° (softens at 110°)	— —	−19.8° (−38.2°)	$C_{22}H_{37}F_3N_3O_6S_4$ (638.8)	1.6–2.7 (m, SCH_3 + CH_2 + solvent); 4.2–4.6 (m, 4H, N—CH); 7.7–8.5 (m, 3H, NH); 9.55 (d, 1H, NH)

[a] The microanalyses were in good agreement with the calculated values (C ±0.38, H ±0.14, N ±0.18).
[b] Not analyzed.

organic reagents were purchased from Aldrich Chemical Co. Zinc dust was obtained from Mallinckrodt. Amino acid 2,2,2-trichloroethyl esters **2** were prepared as the tosylate salts by azeotropic distillation as described earlier[1]. *N*-Trifluoroacetyl-L-amino acids **1** were prepared by the procedure of Weygand and Geiger[9] and had the physical properties described by Fones and Lee[10].

N-Trifluoroacetyl-L-phenylalanyl-L-methionine 2,2,2-Trichloroethyl Ester (3d); Typical Procedure:

To a cold solution of L-methionine trichloroethyl ester tosylate (10.69 g, 0.024 mol) in dichloromethane (300 ml) and dimethylformamide (50 ml) is added diisopropylethylamine[11] (4.2 ml, 0.024 mol) and *N*-trifluoroacetyl-L-phenylalanine (6.00 g, 0.023 mol). Air above the reaction mixture is displaced by argon. The reaction mixture is stirred and cooled in a salt/ice bath and dicyclohexylcarbodiimide (5.50 g, 0.027 mol) is added. After 20 min[12], *N*-hydroxysuccinimide (3.10 g, 0.027 mol) is added and the mixture is stirred for 7 h at −10 °C, stored at −20 °C for 48 h, and at 0 °C for 24 h. Excess dicyclohexylcarbodiimide is destroyed by the addition of 1 normal hydrochloric acid (10 ml) and acetic acid (3 ml). The mixture is filtered and concentrated in vacuo to a slurry which is dissolved in ethyl acetate (350 ml) and again filtered to remove dicyclohexylurea. The ethyl acetate solution is extracted successively with 1 normal hydrochloric acid (5 × 35 ml), 4% aqueous sodium hydrogen carbonate (5 × 35 ml), and finally with saturated salt solution (30 ml). After drying with sodium sulfate, the solution is concentrated in vacuo to a waxy solid. Crystallization from ethyl acetate/*n*-heptane (1:4) gives the product; yield: 11.5 g (96%). Aqueous ethanol can also be used for recrystallization.

$C_{18}H_{20}Cl_3F_3N_2O_4S$	calc.	C 41.28	H 3.85	N 5.35
(523.8)	found	41.60	3.99	5.45

N-Trifluoroacetyl-L-valyl-L-methionine (4b); Typical Procedure for Zinc Reduction[8]:

A solution of **3b** (2.033 g, 0.0043 mol) in tetrahydrofuran (20 ml) is stirred magnetically and the air displaced by argon[13]. Zinc dust (4 g) is added and immediately after 1 molar potassium dihydrogen phosphate solution (4 ml) is added. The slurry is stirred vigorously for 45 min and filtered. The zinc is washed with fresh tetrahydrofuran (5 × 25 ml). The combined filtrates are concentrated in vacuo to ~50 ml. 1 Normal hydrochloric acid (10 ml) and water (20 ml) are added and the suspension is concentrated in vacuo to 25 ml. The suspension of crystalline material is extracted with ethyl acetate (125 ml). The desired product is removed from the ethyl acetate solution by extraction into 4% sodium hydrogen carbonate solution (3 × 25 ml). The alkaline extracts are acidified with normal hydrochloric acid and reextracted with ethyl acetate (3 × 60 ml). Evaporation of the organic solvent leaves a crystalline product which is recrystallized from ethanol to give the product as prisms; yield: 1.30 g (88%).

$C_{12}H_{19}F_3N_2O_4S$	calc.	C 41.86	H 5.56	N 8.14
(344.4)	found	42.10	5.70	8.20

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* Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

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