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Synthesis, Enzymatic Resolution and Absolute Configuration of Ethyl *trans*-3-(Trifluoromethyl)Pyroglutamate.

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Abstract: Ethyl *trans*-3-(trifluoromethyl)pyroglutamate **1** is synthesized in excellent yield; racemic **1** is enzymatically resolved with α-chymotrypsin-catalyzed hydrolysis affording both the enantiomerically pure enantiomers. The absolute configuration is established by X-ray analysis of the corresponding trichloroethylester. Copyright © 1996 Elsevier Science Ltd

Introduction.

The introduction of a fluorine atom or a fluorinated residue into a biologically-active molecule is known to have a marked effect on its physiological behaviour; fluoro-organic bioactive compounds in enantiomerically-pure form are therefore crucial to the detailed description of the recognition of these molecules by biological receptors.[1] Consequently, many attempts have been made to synthesize fluorine-containing aminoacids and describe their stereochemical characteristics.[2a]

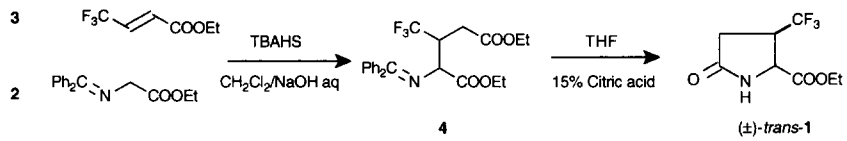
A recent study reports the synthesis of *cis* and *trans*-3-(trifluoromethyl)pyroglutamic esters,[3] which could be introduced into thyrotropin,[2b] a tripeptide regulating a wide variety of biological functions, with a view to studying structural requirements for biological activity. The synthesis was achieved *via* the addition of ethyl N-(diphenylmethylene)glycinate to ethyl 4,4,4-trifluorocrotonate; the product was recovered, after hydrolysis of the intermediate, as a pure *trans* isomer or as a mixture of *cis/trans* isomers, depending upon the experimental conditions. In the same work, the pure enantiomers of the *trans* form were obtained starting from a chiral imine and subsequently separating the resultant diastereoisomers.

In this paper we report a modified large-scale synthesis of the ethyl *trans*-3-(trifluoromethyl)pyroglutamate **1** together with its enzymatic resolution and absolute configuration, as determined by X-ray analysis of the trichloroethyl derivative.

Results and discussion.

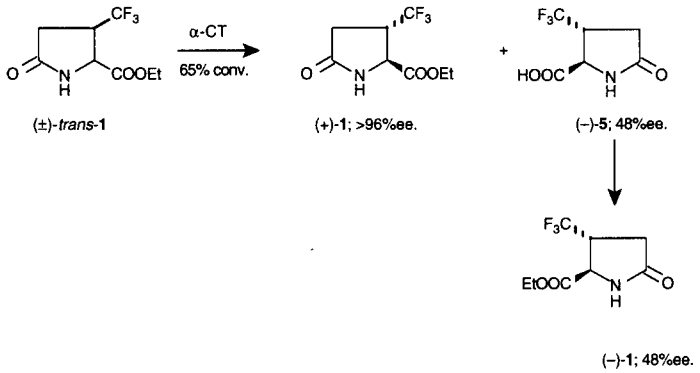
(±) Ethyl trans-*3-(trifluoromethyl)pyroglutamate* **1**: *synthesis and enzymatic resolution.* — Racemic **1** can be synthesized in gram-scale quantities and in high chemical yield with little modification of a previous report;[3] Michael addition of ethyl *N*-(diphenylmethylene)glycinate **2** to ethyl 4,4,4-trifluoro-*trans*-2-butenoate **3**, promoted by sodium hydroxide in the presence of a phase-transfer catalyst, stereoselectively affords the Michael

adduct **4**, in a single diastereoisomeric form (scheme 1); this compound, without purification, is deprotected with 15% citric acid and spontaneously cyclizes, affording **1** in 95% overall yield.



Scheme 1

In order to obtain **1** in enantiomerically-pure form, we resorted to enzymatic catalysis, which is now increasingly employed in the resolution of racemates, because of its high chemo-, regio- and enantio-selectivity.[4] Several commercially available hydrolytic enzymes were tested for the resolution of **1**, the highest selectivity being observed in α-chymotrypsin-catalyzed hydrolysis (enantioselectivity ratio E=12).[5] This peptidase catalyzes the chemospecific hydrolysis of the ester function, and at 65% conversion pyroglutamic acid **5** and unreacted **1** are recovered in 48 and >96% ee. respectively (scheme 2).

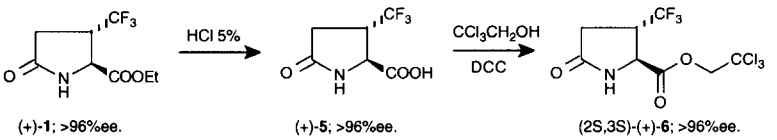


Scheme 2

In a typical resolution, racemic **1** is suspended in phosphate buffer (0.1 mol dm^3 and NaCl 0.1 mol dm^3; pH 7.5) at 37 °C and α-chymotrypsin is added (enzyme/substrate ratio 1/2.5). After 3 hours the suspension is extracted three times with methylene chloride, and the organic phase is dried ($MgSO_4$) and concentrated; column chromatography (SiO_2; ethyl acetate/*n*.hexane 1:1) of the resulting residue affords (+)-**1** in nearly enantiomerically-pure form (ee. >96%) and in 33% chemical yield (from starting material). The collapsed enzyme is separated from the aqueous phase by centrifugation, the supernatant is acidified with HCl 10% and evaporated to dryness *in vacuo*; the residue is dissolved in anhydrous ethanol and refluxed with thionyl chloride. After the usual work-up, the crude product is purified by column chromatography (SiO_2; ethyl acetate/*n*.hexane 1:1) affording (-)-**1** in 46% chemical yield and 48% ee; crystallization from hexane/ethanol 95:5 of this sample affords racemic **1** as a precipitate, while from mother liquors (-)-**1** is recovered in nearly enantiomerically-pure form (> 96% ee.; 17% chemical yield from starting material).

Determination of the absolute configuration.— A previous report[3] assigns the *trans* relative configuration to compound **1**, on the basis of the $J_{H_2\text{-}H_3}$ coupling constant; in order to confirm this attribution and to determine

its absolute configuration, we decided to convert (+)-**1** into a derivative suitable for X-ray diffraction. The enantiomerically-pure (+)-**1**, >96% ee., was hydrolysed in HCl 5% at 70 °C for 3 hours; the hydrochloric acid was removed in vacuum and the resulting solid crystallized from THF/pentane to give (+)-*trans*-3-(trifluoromethyl)pyroglutamic acid (**5**) as colourless crystals, ee. >96% in 95% yield, scheme 3.



Scheme 3

Attempts to obtain a crystalline and stable potassium salt from **5** were unsuccessful. The acid (+)-**5** was then converted into the trichloroethylester **6**: treatment of (+)-**5** with trichloroethanol and dicyclohexylcarbodiimide in anhydrous THF afforded a crude product which was purified by column chromatography, to afford (+) trichloroethyl *trans*-3-(trifluoromethyl)pyroglutamate, **6** (ee. >96%, 64% yield). We observed that in compounds **1**, **5**, and **6**, the $J_{H_2\text{-}H_3}$ coupling constants are very similar (3.2-3.5 Hz) and consistent with a *trans* relationship between the substituents.[6] Crystallization from methylene chloride afforded the enantiomerically pure form of (+)-**6** as colourless crystals whose absolute configuration was determined by X-ray diffraction analysis, figure 1. The results of the single-crystal X-ray structure analysis unambiguosly show the absolute configurations at the C2 and C3 centres to be *S*, for both atoms. The molecule exhibits eclipsed conformation around the C2-C3 bond. The penta-atomic ring displays an envelope conformation with the C3 atom displaced 0.214 (5) Å from the mean plane through the other four ring atoms, the latter being coplanar within the bounds of experimental accuracy. All bond lengths and angles lie within the normal range for related compounds. A strong N-H···O3 hydrogen bond determines helical arrangement of the molecules. Further contributions to the crystal packing arise from many short Van der Waals contacts, mainly involving fluorine and chlorine atoms.

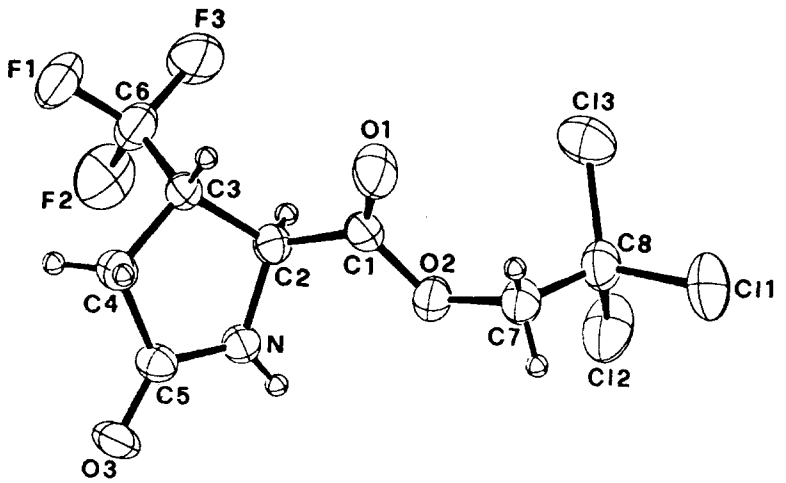


Fig. 1 — ORTEP drawing of the molecular structure of (2*S*,3*S*)-(+) trichloroethyl *trans*-3-(trifluoromethyl)pyroglutamate **6**. Thermal ellipsoids enclose 50% probability.

The chemical correlation between (+)-**1**, (+)-**5** and (2S,3S)-(+)-**6**, not involving the C(2) and C(3) stereogenic centres, allow the same (2S,3S) absolute configurations to be assigned to all the dextrorotatory stereoisomers, and in particular to (+) ethyl *trans*-3-(trifluoromethyl)pyroglutamate **1**.

Conclusion.

(±) Ethyl *trans*-3-(trifluoromethyl)pyroglutamate **1** can be stereoselectively synthesized in gram-scale quantity and excellent chemical yield by means of the addition of ethyl *N*-(diphenylmethylene)glycinate **2** to ethyl 4,4,4-trifluoro-*trans*-2-butenoate (**3**) in phase-transfer catalysis and subsequent hydrolysis of the Michael adduct. α-Chymotrypsin-catalyzed hydrolysis allows the resolution of racemic **1**, providing both enantiomerically-pure enantiomers; the relative and absolute configuration of (+)-**1** is unambiguously stated by single-crystal X-ray diffraction analysis of the derived trichloroethyl ester (+)-**6**.

Experimental.

^{1}H-NMR spectra were recorded in $CDCl_3$ solution on a Bruker AMX 400 WB spectrometer. Chemical shifts are reported in δ values from TMS as internal standard (s singlet, d doublet, m multiplet, t triplet, b broad signal). Coupling constants (*J*) are given in Hz. Optical rotations were measured at 20 °C on a Perkin-Elmer 241 polarimeter and are in 10^{-1} deg cm^2 g^{-1}. GLC analyses were performed on a Hewlett-Packard 5890 A gas chromatograph, carrier helium gas; conversions were evaluated on a DB-1 column (30 m x 0.53 mm I.D. and 5 μm film phase) from J&W Scientific, while the enantiomeric purities (ee's) were evaluated on a chiral B-DEX 120 column (30 mx 0.53 mm I.D. and 0.25 μm film phase) from Supelchem. Accuracy was within ±2%. Mass spectra were determined on a Hewlett-Packard 5970 mass selective detector. Chromatographic purification of the compounds was performed on silica gel (ϕ 0.05-0.20 mm). The enzyme α-chymotrypsin (from bovine pancreas, 74.6 U/mg) was purchased from Fluka. Elemental analyses were performed with a Carlo Erba Elemental Analyzer.

(±) Ethyl trans-*3-(trifluoromethyl)pyroglutamate* **1**.[3]— 10 g of *N*-(diphenylmethylene)glycine ethyl ester (37.4 mmol), 1.5 g of tetrabutylammonium hydrogen sulphate (TBAHS), 60 ml of 10% sodium hydroxyde and 60 ml of dichloromethane are stirred for 15 min. at 0 °C. Thereafter, 6.3 g of ethyl 4,4,4-trifluoro-*trans*-2-butenoate (37.4 mmol, 5.6 ml) are added and the whole two-phase system is vigorously stirred at 0 °C for 2 h. The reaction mixture is diluted with water/dichloromethane (500 ml/ 250 ml) and the aqueous layer is extracted three times with 150 ml of dichloromethane. The combined organic layers are washed with water (150 ml) and saturated sodium chloride (150 ml). After drying over magnesium sulphate, the solvent is evaporated *in vacuo* at 30 °C. The resulting, slightly-yellow oil (ca. 15 g) is dissolved in 100 ml of tetrahydrofuran (THF) and stirred for 7 days with 70 ml of 15% citric acid at room temperature. The THF is evaporated *in vacuo* (30 °C). The resulting solid is crystallized from *n*-hexane/ethanol (9:1) to give 8.0 g (95%) of ethyl *trans*-3-(trifluoromethyl)pyroglutamate in colourless needles (mp.: 96-97 °C). *Anal.* Calcd for $C_8H_{10}NO_3F_3$: C, 42.67; H, 4.48; N, 6.22. Found: C, 42.56; H, 4.45; N, 6.12. ^{1}H-NMR, ($CDCl_3$)δ_H 1.35 (3H, t, *J* 7.1), 2.57 (1H, dd, *J* 17.8, 5.3), 2.70 (1H, dd, *J* 17.8, 10.0), 3.44 (1H, m), 4.31 (2H, q, *J* 7.1), 4.34 (1H, d, *J* 3.2), 6.74 (1H, b); MS *m/z* 225 (M^+).

Enzymatic resolution.— Racemic **1** (600 mg; 2.66 mmol) is added to 0.1 mol dm^{-3} potassium phosphate buffer [100 mL containing NaCl (0.1 mol dm^{-3})], pH 7.5 at 37 °C and treated with α-chymotrypsin (240 mg) with vigorous mechanical stirring. Hydrolysis is stopped after 3 h, and the aqueous phase extracted with

dicholorometane (5 x 80 ml); the combined organic layers are dried over magnesium sulphate and concentrated under reduced pressure; the crude residue is purified by column chromatography (silica gel, ethyl acetate/*n*.hexane 1:1 eluant) affording 196 mg of **1** (33% yield) as a solid, m.p. 50-51 °C, $[\alpha]_D$ +20.3 (c. 1.1, $CHCl_3$), ee. > 96%. *Anal.* Calcd for $C_8H_{10}NO_3F_3$: C, 42.67; H, 4.48; N, 6.22. Found: C, 42.59; H, 4.41; N, 6.13. Spectroscopic data are identical to those reported for the racemic form. The aqueous phase is centrifuged (10 min, 3000 rpm) and the enzyme filtered off; the pH of the solution is adjusted to pH 1 (HCl 10%), and the solvent removed under reduced pressure. The crude residue is desiccated overnight (anhydrous calcium chloride) and then refluxed with anhydrous ethanol (10 ml) and thionyl chloride (2 ml) for two hours. The suspension is concentrated *in vacuo*, diluted with methylene chloride (30 ml), washed with satd. $NaHCO_3$, H_2O and dried over magnesium sulphate. The solvent is removed and the residue purified by column chromatography (silica gel, ethyl acetate/*n*.hexane 1:1 eluant) affording 274 mg of **1** (46% yield) as a solid (m.p. 75-81 °C) showing $[\alpha]_D$ -9.8 (c. 1.0, $CHCl_3$), ee. 48%. This sample is crystallized from *n*.hexane/ethanol 95:5 affording 151 mg of racemic **1** (mp. 96-97 °C); from the mother liquor, 103 mg of (-)-**1**, (m.p. 50-51 °C), $[\alpha]_D$ -19.94 (c. 1.0, $CHCl_3$), ee. >96%. are recovered. *Anal.* Calcd for $C_8H_{10}NO_3F_3$: C, 42.67; H, 4.48; N, 6.22. Found: C, 42.59; H, 4.41; N, 6.13. Spectroscopic data are identical to those reported for the racemic form.

(+)-trans-*3-(trifluoromethyl)pyroglutamic acid* **5**.— A suspension of (+)-**1**, $[\alpha]_D$ +20.3, (190 mg, 0.84 mmol) in HCl 5% is stirred for 3 h at 70 °C; the resulting solution is concentrated and the residue crystallized from anhydrous THF/pentane: 158 mg of **5** (95% yield) are recovered as colourless crystals having $[\alpha]_D$ +25.3 (c. 1.2, CH_3OH), ee. >96%, m.p. 120-122 °C. The enantiomeric excess of (+)-**5** is determined by GLC after its conversion to the corresponding methyl ester with diazomethane in THF. *Anal.* Calcd for $C_6H_6NO_3F_3$: C, 36.56; H, 3.07; N, 7.11. Found: C, 36.38; H, 3.06; N, 7.07. ^{1}H-NMR (DMSO-D): δ_H 2.24 (1H, dd, *J* 17.6, 4.2), 2.65 (1H, dd, *J* 17.6, 10.3), 3.51 (1H, m), 4.15 (1H, d, *J* 3.3), 8.33 (1H, b), 13.82 (1H, b); MS *m/z* 197 (M^+).

(4S,5S)-(+) Trichloroethyl trans-*3-(trifluoromethyl)pyroglutamate* **6**.—A few crystals of 4-(N,N-dimethylamino)-pyridine (5 mg, 0.041 mmol) are added to a solution of (+)-**5**, $[\alpha]_D$ +25.3 (80 mg, 0.41 mmol), trichloroethanol (73 mg, 0.49 mmol) and dicyclohexylcarbodiimide (100 mg, 0.49 mmol) in anhydrous THF (5 ml), and the solution is magnetically stirred for 24 h at room temperature. The reaction mixture is filtered (dicyclohexylurea), and the solution concentrated; the crude residue is purified by column chromatography (silica gel, *n*-hexane/ethyl acetate 60/40 as eluant) affording 85 mg (64% yield) of **6** as a solid (m.p. 118-120 °C) having $[\alpha]_D$ +8.5 (c. 1.0, $CHCl_3$), ee. >96%. The enantiomeric excess of (+)-**6** is determined after its conversion to the methyl ester by transesterification with methanol. *Anal.* Calcd for $C_8H_7NO_3Cl_3F_3$: C, 29.25; H, 2.15; N, 4.26. Found: C, 29.53; H, 2.18; N, 4.20. ^{1}H-NMR ($CDCl_3$): δ_H 2.61 (1H, dd, *J* 17.9, 5.0), 2.75 (1H, dd, *J* 17.9, 10.0), 3.50 (1H, m), 4.51 (1H, d, *J* 3.5), 4.83 (1H, d, *J* 11.8), 4.94 (1H, d, *J* 11.8), 6.33 (1H, b); MS *m/z* 331 (M^+).

X-ray structure analysis.— X-ray data were collected at room temperature on a Siemens PARA-M18X rotating-anode diffractometer using graphite-monochromated Mo Kα radiation (52 KW, 100 mA). A colourless crystal (0.35x0.15x0.15 mm) was used. Cell constants were determined by least-squares refinement of 31 accurately centered reflections. Intensities were corrected for Lorentz-polarization effects, and an empirical absorption correction, based on the Ψ scan, was applied. The structure was solved by direct methods using a SHELX-86 program,[7] and refined on F^2 with SHELXL-93.[8] All non-H atoms were refined anisotropically, while H-atoms, located in a ΔF map, were refined isotropically. The absolute configuration was determined by

refinement on Flack *x* parameter,[9] whose final value was 0.08 (8). Tables of atomic coordinates and complete bond distances and angles have been deposited with the Cambridge Crystallographic Centre.

Crystal data:: $C_8H_7Cl_3F_3NO_3$, Mr328.50, monoclinic, space group $P2_1$, a = 9.484(2), b = 6.105(1), c = 10.743(2) Å, β = 102.69(1)°, V = 606.8(2) $Å^3$, Z = 2, D_{calc} = 1.798 Mg/m^3, μ 0.792 mm^{-1}, F (000) = 328, $2\vartheta_{max}$ = 52°; final R = 0.0362 and wR2 = 0.0844 for 2057 reflections with I > 2σ(I).

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