Synthesis and Inhibitory Properties of α(Chlorofluoromethyl)-α-amino Acids, a Novel Class of Irreversible Inactivators of Decarboxylases

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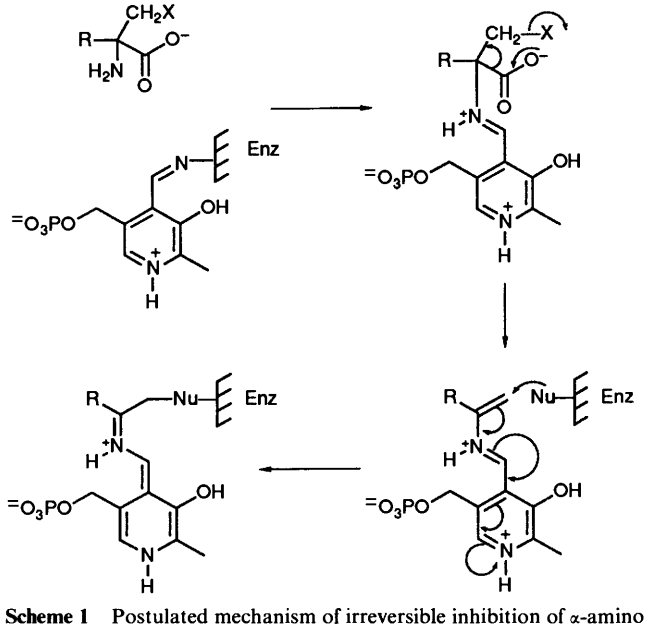
The synthesis of α-(chlorofluoromethyl)-ornithine **7**, -metatyrosine **8** and -glutamic acid **14** is described. Separation of diastereoisomers and relative configuration assignment by X-ray analysis are reported. Assignment of absolute configuration of the four enantiomers of α-(chlorofluoromethyl)-ornithine **7** is also described. The inhibitory properties against ornithine decarboxylase, aromatic amino acid decarboxylase and glutamate decarboxylase are reported in terms of diastereoselectivity (**8** and **14**) or enantioselectivity (**7**).

Over the past 10 years, a number of α-halogenomethyl-α-amino acids have been designed and synthesized as irreversible mechanism-based inhibitors of their corresponding pyridoxal phosphate (PLP)-dependent α-amino acid decarboxylases[1] (Scheme 1). α-(Difluoromethyl)ornithine (eflornithine), an inhibitor of ornithine decarboxylase (ODC) (EC 4.1.1.17), has proven to be of therapeutic relevance for the treatment of African sleeping sickness[2] and of *Pneumocystis carinii* pneumonia,[3] the most frequent opportunistic infection associated with acquired immune deficiency syndrome (AIDS).



Scheme 1 Postulated mechanism of irreversible inhibition of α-amino acid decarboxylases by α-halogenomethyl-α-amino acids

The synthesis of α-halogenomethyl-α-amino acids is well documented. The most convenient approach consists of a direct halogenomethylation of the anions derived from either malonate esters[4,5] or Schiff's base esters of α-amino acids.[6,7] The α-chlorofluoromethyl-α-amino acids, due to the asymmetry of the mixed halogenomethyl group, are obtained as mixtures of four isomers.

Here, we examine some of their actions on three decarboxylases chosen as models for our *in vitro* studies: ornithine decarboxylase,[8] which catalyses the first step in the biosynthesis of polyamines, aromatic amino acid decarboxylase (AADC),[9] and glutamic acid decarboxylase (GAD),[10] both of which are involved in the biosynthesis of neurotransmitters (dopamine and γ-aminobutyric acid, respectively).

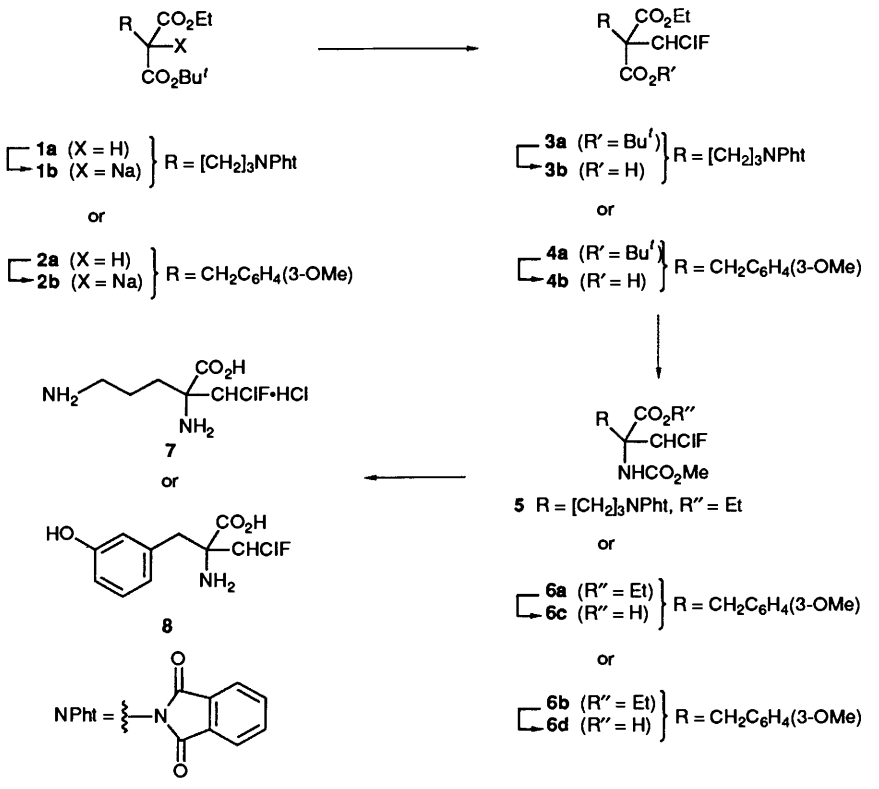
Several questions could be addressed in a comparison of α-chlorofluoromethyl-α-amino acids with other α-halogenomethyl-α-amino acids such as α-mono- or α-di-fluoromethyl α-amino acids: (i) how potent would the inhibitors be in terms of affinity and efficiency, (ii) how selective would the inhibition be in terms of diastereoisomer configuration, (iii) how selective would the inactivation be in terms of enantiomer configuration.

Results and Discussion

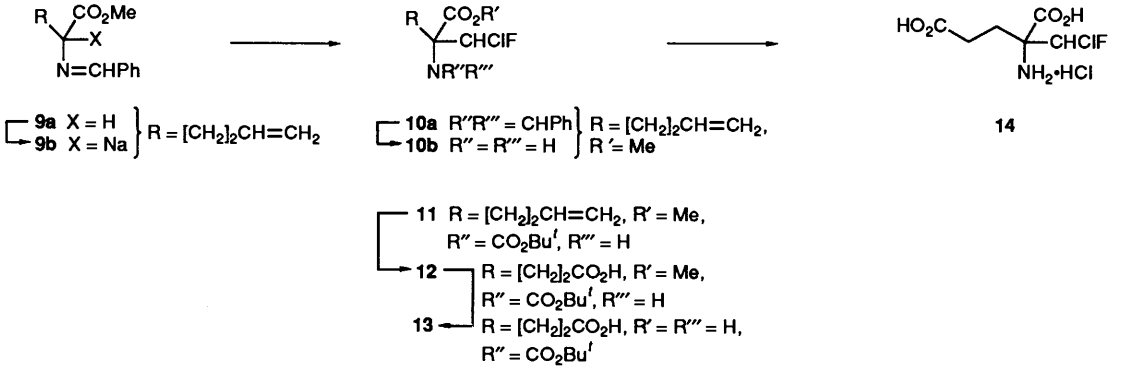
Chemistry.—Our general synthetic approach to α-chlorofluoromethyl-α-amino acids is outlined in Schemes 2 and 3.[7] This class of compounds can be generated by halogenomethylation of carbanions derived from malonates **1** (or **2**) (followed by rearrangement) or Schiff's base esters **9** and hydrolysis (Scheme 2 or 3 respectively).

(a) α-(*Chlorofluoromethyl*)*ornithine* **7**.—In view of the poor yield in the conversion of methyl ester **9a** (X = H) into α-chlorofluoromethyl imine **10a**, α-(chlorofluoromethyl)ornithine **7** was prepared *via* route 1 (Scheme 2). Alkylation, at room temperature, of the sodio derivative **1b** [sodium hydride (1 mol equiv.)] of the readily available diester **1a** with a large excess of dichlorofluoromethane† (Freon 21), at room temperature afforded *tert*-butyl ester **3a** in 30% yield. Conversion of the malonic acid monoester **3b** [obtained by treatment of diester **3a** with trifluoroacetic acid (TFA)] into compound **7** was achieved under the standard Curtius rearrangement sequence in 50% overall yield. Owing to the asymmetry of the chlorofluoromethyl substituent, carbamate **5** was obtained as a 1:1 mixture of diastereoisomers which were easily separated by chromatography. The relative configuration of each pair of enantiomers was determined by X-ray diffraction analysis of the fully protected intermediates **5a** (*RR,SS*) and **5b** (*RS,SR*) (Fig. 1).

† Available from Fluorochem. Ltd., Peakdale Road, Glossop, Derbyshire, SK13 9XE, England. Freon 21 was condensed at −20 °C in a graduated cylinder.



Scheme 2 Syntheses of α-(chlorofluoromethyl)ornithine and α-chlorofluoromethyl-*m*-tyrosine



Scheme 3 Synthesis of α-(chlorofluoromethyl)glutamic acid

Resolution and Absolute Configuration of the Enantiomers of Compound **7**.—Separation of the four isomers of α-(chlorofluoromethyl)ornithine **7** was achieved by reversed-phase high-performance liquid chromatography, with L-proline and copper as chiral mobile phase, as described by Wagner *et al.*[11] The configuration of each isomer was established through reduction of one anantiomer (from each diastereoisomeric pair) of α-(chlorofluoromethyl)ornithine to (*R*)- or (*S*)-α-(fluoromethyl)-ornithine [Bu_3SnH, cat. azoisobutyronitrile (AIBN), benzene or toluene; reflux; ref. 7] (Scheme 4) and correlation with authentic samples, by comparison of HPLC retention times.

(b) *α-Chlorofluoromethyl-*m*-tyrosine* **8**.—α-Chlorofluoromethyl-*m*-tyrosine **8** was prepared in 18% overall yield from the monosubstituted malonate **2a** in a similar way to the ornithine analogue **7** (Scheme 2). Chromatographic separation of the diastereoisomers **6a** and **6b** was performed at the methylcarbamate intermediate stage. An additional hydrolysis step under basic conditions [lithium hydroxide (2 mol equiv.), water–1,2-dimethoxyethane (DME)] was performed prior to the acidic treatment (47% HBr; 100 °C) during the final conversion of **6a** or **6b** into the product **8a** or **8b**, respectively. The relative configuration of one pair of enantiomers was determined by X-ray diffraction analysis of the acid **6c** (Fig. 1).

(c) *α(Chlorofluoromethyl)glutamic Acid* **14**.—α-(Chlorofluoromethyl)glutamic acid **14** was prepared *via* route 2 (Scheme 3), from the substituted Schiff's base ester **9a**. Sodium hydride (1 mol equiv.) in tetrahydrofuran (THF) at 45 °C converted the Schiff's base ester **9a** into the corresponding anion **9b**, which was alkylated at room temperature with Freon 21 ($CHCl_2F$) * to yield the Schiff's base ester **10a**. Hydrolysis to the free amino ester **10b** and derivatization of the free amine with di-*tert*-butyl dicarbonate afforded the carbamate intermediate **11**, which was separated into two diastereoisomeric pairs of enantiomers by medium-pressure liquid chromatography (MPLC). Subsequent oxidation of the double bond

* Available from Fluorochem. Ltd., Peakdale Road, Glossop, Derbyshire, SK13 9XE, England. Freon 21 was condensed at −20 °C in a graduated cylinder.

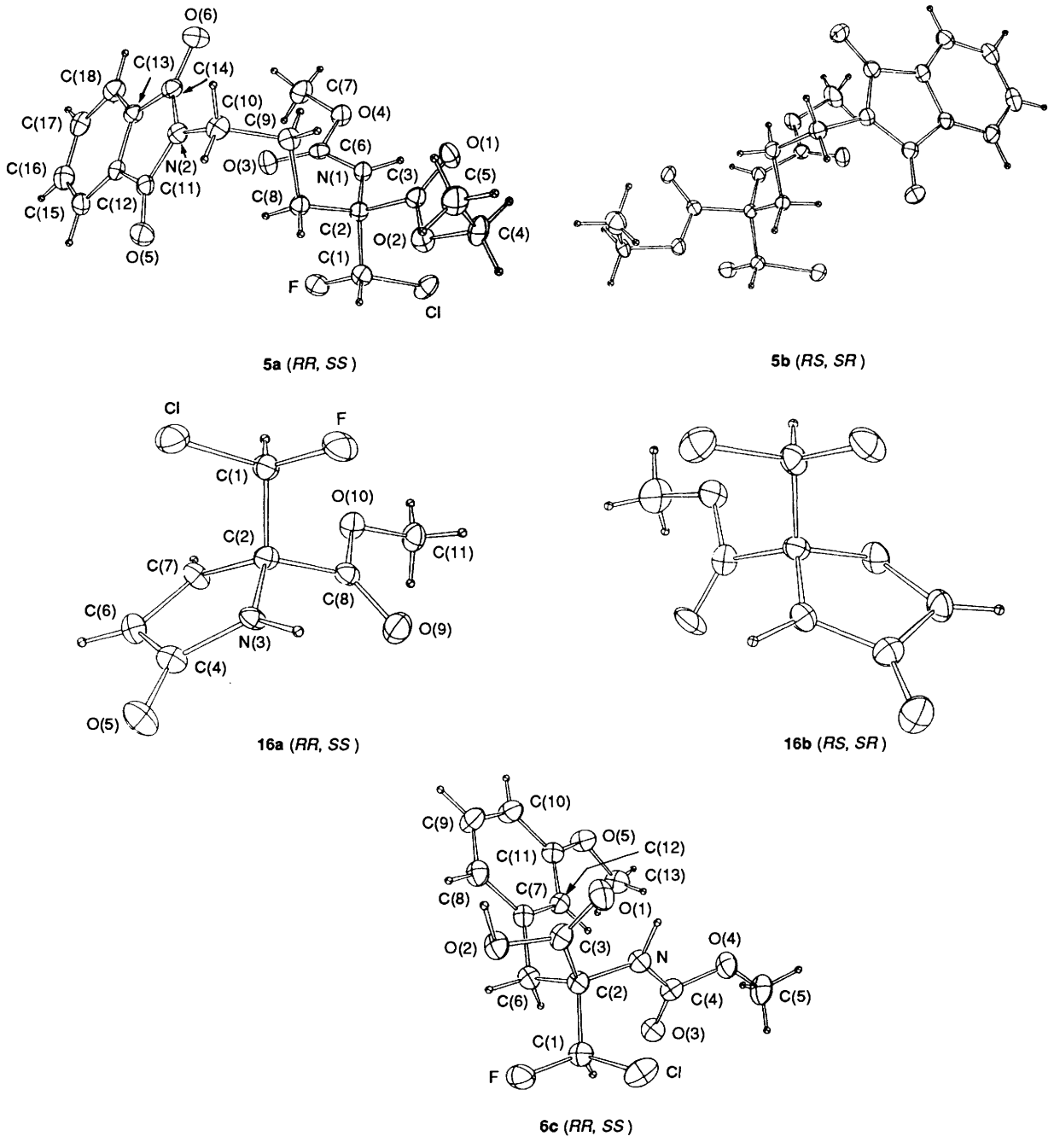
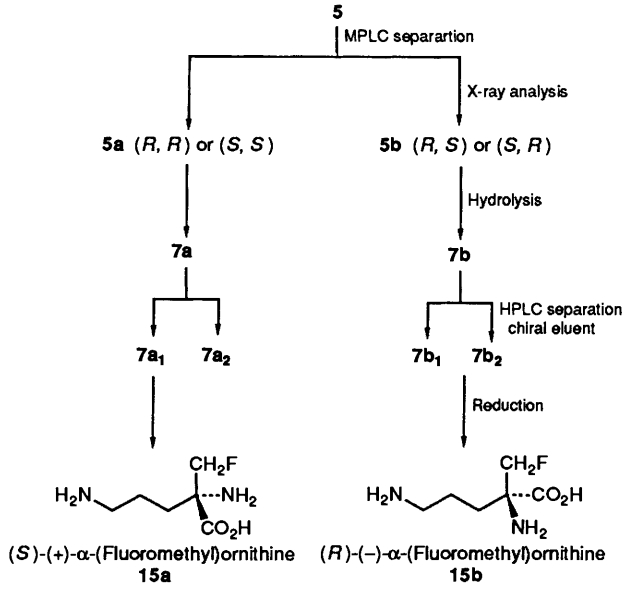


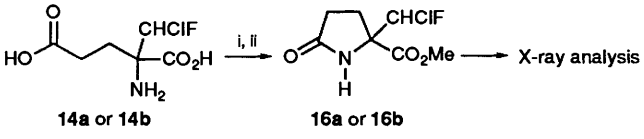
Fig. 1 ORTEP plot of all molecules analysed by X-ray diffraction, including the atomic numbering scheme. Thermal vibrational ellipsoids are scaled to enclose 50% of the electron density. An arbitrary, small radius value has been used for all hydrogen atoms.

($KMnO_4$, water; 0 °C), followed by hydrolysis of the ester (LiOH, water–DME; room temp.) and removal of the amine protecting group (HCl–Et_2O; room temp), yielded the expected α-(chlorofluoromethyl)glutamic acid **14a** or **14b** (*RR,SS* or *RS,SR* respectively). The relative configuration of each pair of enantiomers of compound **14** was determined by X-ray diffraction analysis of the lactam esters **16** (Fig. 1), formed by spontaneous cyclization of the α-amino acids **14a** and **14b** in water at room temperature followed by esterification [diazomethane, (Et_2O; room temp.) (Scheme 5).

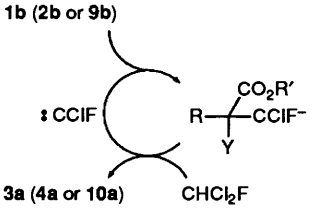
In summary, three types of α-chlorofluoromethyl-α-amino acids bearing either a neutral, basic, or acidic side chain were prepared from either monosubstituted malonate diesters or monosubstituted Schiff's base esters. Alkylation of the carbanions derived from differently substituted Schiff's base esters or malonates with dichlorofluoromethane gave access to this new family of putative irreversible inhibitors of pyridoxal phosphate-dependent decarboxylases in fair-to-good yields. The halogenomethylation reaction proceeds without diastereoselectivity. It can be rationalized by a chain mechanism (Scheme 6) involving α-chlorofluorocarbene due to the propensity of halogenoforms to form carbenes under those reaction conditions.[12] A similar mechanism of alkylation had been proposed in the preparation of α-difluoromethyl-α-amino acids from Schiff's base esters carbanions and chlorodifluoromethane (Freon 22).[6] The presence of two asymmetric centres confers to this category of α-alkylated α-amino acids new properties in terms of selectivity and efficacy of inactivation of their corresponding decarboxylases. The easy separation at an intermediate stage of these α-chlorofluoromethyl-α-amino acids in pairs of enantiomers (MPLC) and the determination of their configuration (by X-ray analysis of intermediates or final



Scheme 4 Separation and determination of absolute configuration of each enantiomer of α-(chlorofluoromethyl)ornithine



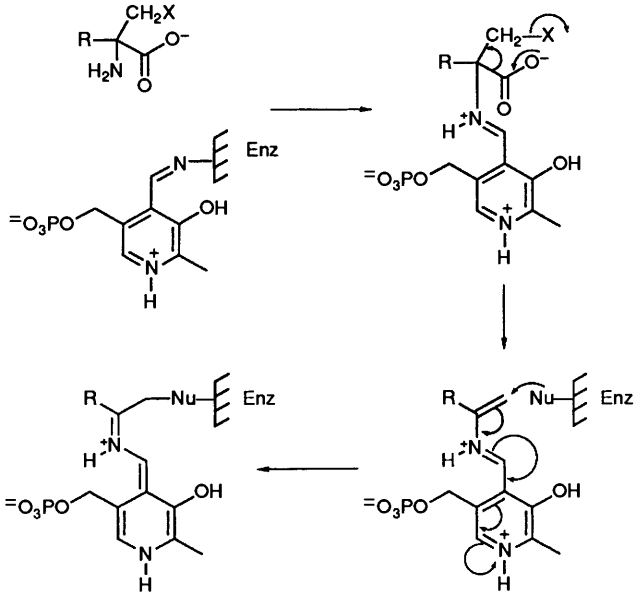
Scheme 5 Syntheses of α-(chlorofluoromethyl)pyroglutamates **16a** and **16b**. *Reagents:* i, water; ii, CH_2N_2.



Scheme 6 Postulated mechanism of halogenomethylation

products) has permitted the comparison of kinetic parameters for the *in vitro* inactivation (diastereoselectivity) of their corresponding decarboxylases. Owing to the larger chemical-shift effects observed for fluorine resonances in comparison with proton resonances, ^{19}F NMR spectroscopy can be applied to the determination of the stereoisomeric composition of mixtures or to the assignment of relative configuration of pairs of enantiomers of α-chlorofluoromethyl-α-amino acids. By correlation with the results from the X-ray analyses, it appears that the ^{19}F signal at low field corresponds to the *RR* or *SS* enantiomers whereas the ^{19}F signal at high field corresponds to the *RS* or *SR* enantiomers (Table 1). These results are valid for a whole range of such α-dihalogenomethyl-α-amino acids and can thus be used for the determination of relative configurations or enantiomeric excesses for any other such compounds.

Enzyme Inactivation.—Mammalian ODC. Determination of the absolute configuration of each isomer (Scheme 4) allows us to understand both the diastereo- and the enantio-selectivity of inhibition of ODC by such α-amino acids analogues. The four stereoisomers of α-(chlorofluoromethyl)ornithine produced a time-dependent loss of rat liver ODC activity which followed pseudo-first-order kinetics for approximately two half-lives. Loss of activity was related to the concentration of inhibitor. By plotting the time of half-inactivation as a function of the reciprocal of the inhibitor concentration, according to the method of Kitz and Wilson,[13] straight lines were obtained. These lines intercepted the positive *y*-axis, demonstrating saturation effects which involve the enzyme active-site in the inhibitory process (not shown). Kinetic constants K_i (apparent dissociation constant) and $\tau_{\frac{1}{2}}$ (time of half-inactivation extrapolated to infinite concentration of inhibitor) could be extrapolated from Kitz and Wilson plots (see Table 2). While the $\tau_{\frac{1}{2}}$-values of the four stereoisomers are rather similar, the K_i-values are quite different, showing an important steric effect in the recognition step. However, when the complex EI is formed, inactivation occurs irrespective of stereochemistry. Such a result was somewhat unexpected since ODC is believed to decarboxylate only the (*S*)-enantiomer of ornithine.[14] However, the most efficient isomer, **7b**$_2$ (*RS* enantiomer), is of the opposite α-configuration to that of the natural substrate (*S*-ornithine) whereas the weakest, **7b**$_1$ (*SR* enantiomer), is of the same α-configuration as *S*-ornithine. A comparable finding was made for the *R*-(−)-enantiomer of α-(fluoromethyl)ornithine, which is approximately three times more potent than its antipode.[15] When compared with the (−)-enantiomer of α-(difluoromethyl)-ornithine (DFMO) of unknown absolute configuration, (*R,S*)-α-(chlorofluoromethyl)ornithine (**7b**$_2$) is roughly four times less potent in terms of apparent dissociation constant (K_i) and equipotent in terms of $\tau_{\frac{1}{2}}$. Direct substitution of one of the halogens by a nucleophilic residue of the enzyme active site is unlikely and therefore a decarboxylation step, with concomitant elimination of halide, has to occur prior to ODC inactivation. As in α-chlorofluoromethylated-α-amino acids, either the chloride ion or the fluoride ion can leave,* and so both chloro- or fluoro-vinyl-PLP adduct species can be generated (Scheme 7).



Scheme 7 Postulated mechanism of inhibition of α-amino acid decarboxylases by α-chlorofluoromethyl- or α-difluoromethyl-α-amino acids

Mammalian AADC. This is inactivated irreversibly by both pairs of enantiomers of α-chlorofluoromethyl-*m*-tyrosine, **8a** and **8b**. However, kinetics of inactivation were not first-order as observed for α-mono- and α-di-fluoromethyl-3,4-dihydroxyphenylalanine.[17,18] Times of half-inactivation were extrapolated from the slopes of time-courses of inactivation (Table 3).

* Preliminary experiments indicate no significant difference in the elimination rate between chloride ion or fluoride ion for α-(chlorofluoromethyl)-*m*-tyrosine (reactions performed in buffer, pH 7.4, in the presence of PLP, and monitored by ^{19}F NMR spectroscopy). Full details will be published elsewhere.

Table 1 ^{19}F and ^{1}H NMR data obtained for the various diastereoisomeric pairs of enantiomers

	RR and *SS*			*RS* and *SR*		
α-Chlorofluoro derivative	$^{19}F^{a}$	$^{1}H^{b}$	$^{2}J(F-H)^{c}$	$^{19}F^{a}$	$^{1}H^{b}$	$^{2}J(F-H)^{c}$
Glutamic acid **14**	−57.9	6.81 **14a**	46.5	−66.6	6.78 **14b**	48.0
Ornithine **7**	−59.8	6.75 **7a**	46.5	−68.5	6.71 **7b**	48.1
(3-Hydroxyphenyl)alanine **8**	−63.0	6.88 **8a**	46.3	−71.0	6.82 **8b**	47.9

[a] Chemical shift in ppm (TFA as external reference). [b] Chemical shift in ppm (TSP as internal reference). [c] Coupling constant in Hz.

Table 2 Mammalian ornithine decarboxylase

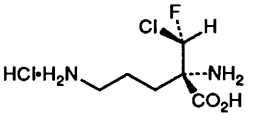
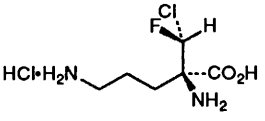
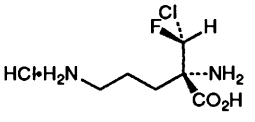
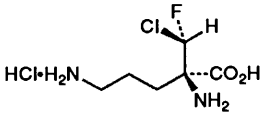
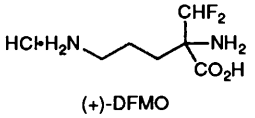
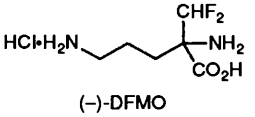
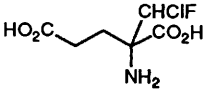
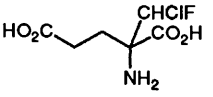
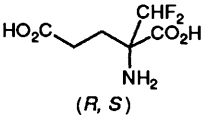
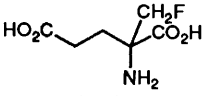
Structure	K_i (10^{-6} mol dm^{-3})	$\tau_{\frac{1}{2}}$ (min)
 **7a$_1$** (*S, S*)	490	3.7
 **7a$_2$** (*R, R*)	2200	1.5
 **7b$_1$** (*S, R*)	440	1.6
 **7b$_2$** (*R, S*)	49	2.7
 (+)-DFMO	120	2.3
 (−)-DFMO	11	2.7

Table 3 Bacterial glutamic acid decarboxylase

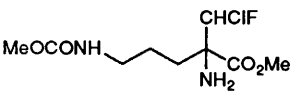
Structure	K_i (mmol dm^{-3})	$\tau_{\frac{1}{2}}$ (min)
 **14a** (*RR, SS*)	2.1	7.9
 **14b** (*RS, SR*)	0.6	1.3
 (*R, S*)	0.001	10
mammalian enzyme	>1	($\tau_{\frac{1}{2}}$ O 180 min at 3 mmol dm^{-3})
 (*R, S*) (ref. 17*b*)		

The (*RR,SS*) pair of enantiomers **8a** is more active than the *RS,SR*) pair and is 10 times more potent than α-difluoromethyl-*m*-tyrosine. Based on the inhibition constants, these α-dihalogenomethyl-*m*-tyrosines are, however, substantially less potent than both (*S*)-α-fluoromethyl-3,4-dihydroxyphenylalanine [(*R*)-α-fluoromethyl-DOPA being completely inactive],[17a] and (*R,S*)-α-difluoromethyl-DOPA.[18]

Mammalian GAD. This is not inhibited by α-(chlorofluoromethyl)glutamate. This was not unexpected since α-(difluoromethyl)glutamate itself does not inactivate mammalian GAD either, contrary to the situation with α-(fluoromethyl)glutamic acid which was found[17b] to inhibit rat brain GAD with a K_i higher than 10^{-3} mol dm^{-3}. However, when α-(chlorofluoromethyl)glutamic acids were tested on bacterial GAD, irreversible time- and concentration-dependent inactivation occurred.

Whereas the times of half-inactivation extrapolated to infinite concentration of inhibitor ($\tau_{\frac{1}{2}}$) for the pairs of enantiomers of α-(chlorofluoromethyl)glutamic acid and for α-(difluoromethyl)-glutamic acid are in the same order, the K_is are approximately 3 orders of magnitude greater (Table 4). These data demonstrate that only the affinity of α-(chlorofluoromethyl)glutamic acids for the active site of bacterial GAD is affected, the large size difference between a chlorine and fluorine atom being the most likely explanation (Van der Waal's radii of 1.80 and 1.35 Å, respectively).

Whereas for mammalian GAD only (fluoromethyl)glutamic acid seems to be active,[17b] for mammalian AADC, α-(difluoromethyl)* and both pairs of enantiomers of α-chlorofluoromethyl-*m*-tyrosine are active, with some diastereoselectivity shown even for the latter compounds. Despite the fact that only (*S*)-α-fluoromethyl-DOPA is a potent inhibitor of AADC, we cannot reach any conclusions on enantioselectivity with the data in hand.



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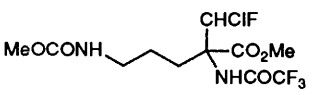

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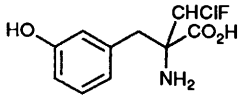
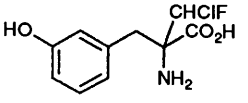
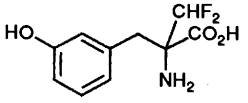
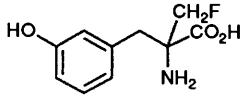
* Unpublished results.

Table 4 Mammalian aromatic amino acid decarboxylase (AADC)

Structure	K_i (10^{-6} mmol dm^{-3})	τ_i (min) [concn.]
 **8a** (*RR, SS*)	No saturation kinetics	18 [100 μmol dm^{-3}]
 **8b** (*RS, SR*)	No saturation kinetics	27 [500 μmol dm^{-3}]
 (*R, S*)	No saturation kinetics	2 [100 μmol dm^{-3}]
 (*S*)	0.04 (ref. 17*a*)	4 [0.1 μmol dm^{-3}]

Conclusions.—In conclusion, α-chlorofluoromethyl-α-amino acids are potent irreversible inhibitors of ornithine decarboxylase and of aromatic amino acid decarboxylase. Their efficacy is generally weaker when compared with the corresponding α-difluoromethyl-α-amino acid, suggesting either a steric effect (during the recognition process) and (or) the generation of less reactive alkylating species (during the inactivation process).

The most important finding is, however, that all four enantiomers of α-(chlorofluoromethyl)ornithine **7** are active, demonstrating lack of enantiospecificity and moderate stereoselectivity of ODC. This observation had already been made by Danzin *et al.*[15] for the two enantiomers of DFMO or (monofluoromethyl)ornithine (MFMO). Direct substitution of a halogen by a nucleophilic residue of the enzyme-active site being most unlikely, a decarboxylation step should occur prior to inactivation of ODC. That the four enantiomers of α-(chlorofluoromethyl)ornithine are inhibitors of ODC suggests some disturbance in the action of the classical catalytic basic residue involved in the decarboxylation reaction. Such non-stereospecific decarboxylation may be explained by a misplacement of the PLP-inhibitor complex in the enzyme active-site induced by the α-substituent.

Experimental

M.p.s were determined on a Büchi or a Mettler FP5 melting point apparatus and are uncorrected, as are boiling points. Microanalyses were conducted on a Perkin-Elmer 240 CHN analyser. IR spectra were taken on a Perkin-Elmer IR-577 spectrophotometer. UV spectra were recorded on a Beckman DU-7 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian Associates T-60 (60 MHz), a EM-390 (90 MHz), a Brücker AM-360 (360 MHz) or a Brücker AC200F (200 MHz) spectrometer and are reported in parts per million from internal tetramethylsilane, 3-(trimethylsilyl)[$^2H_4$]propionic acid sodium salt (TSP) or 3-(trimethylsilyl)propanesulfonic acid sodium salt on the δ-scale. ^{19}F NMR spectra were recorded on a Brücker AM-360 (338.8 MHz) spectrometer and are reported in parts per million on the φ-scale. Trifluoroacetic acid (TFA) or hexafluorobenzene (C_6F_6) was used as the standard. *J*-Values are given in Hz. Mass spectrometry was performed on a Finnigan TSQ/U6/triple-stage quadrupole.

Solvents and reagents were dried prior to use when deemed necessary. Reactions were routinely followed by ^{1}H NMR analysis of aliquots or by TLC analysis. Analytical TLC was performed with Merck precoated silica gel 60F-254 plates that were 0.25 mm thick. Bulb-to-bulb distillation was accomplished in a Büchi GKR-50 Kugelrohrapparat at the oven temperature and pressure indicated. Reactions described as run under nitrogen employed a mercury bubbler arranged so that the system could alternatively be evacuated, filled with inert gas, and left under a positive pressure.

tert-*Butyl Ethyl* 2-(3-*Phthalimidopropyl*)*malonate* **1a**.—A mixture of *tert*-butyl ethyl malonate (5.65 g, 30 mmol) and sodium hydride (1.6 g of a 45% dispersion in oil, 30 mmol) in anhydrous THF (40 cm^3) was stirred at room temperature under nitrogen until completion of the metallation (4 h). A solution of 1-iodo-3-phthalimidopropane (9.45 g, 30 mmol) in anhydrous THF (20 cm^3) was then added and the mixture was stirred at room temperature for 15 h, was hydrolysed, and extracted with diethyl ether (2 × 50 cm^3). The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. Filtration, and removal of the solvent under reduced pressure, yielded an oil. Chromatography on silica gel with ethyl acetate–cyclohexane as eluent (1 : 1; MPLC) afforded the *title diester* **1a** as a solid (5.58 g, 50%), m.p. < 50 °C (from diethyl ether–pentane) (Found: C, 63.9; H, 6.7; N, 3.7. $C_{20}H_{25}NO_6$ requires C, 63.97; H, 6.72; N, 3.73%); ν_{max}(KBr)/cm^{-1} 1773 (CO), 1747 (CO), 1732 (CO) and 1715 (CO); δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.23 (3 H, t, *J* 7, *Me*CH_2O), 1.43 (9 H, s, Bu^t), 1.70–2.00 (4 H, m), 3.27 (1 H, t, *J* 7, CH), 3.68 (2 H, t, *J* 7, NCH_2), 4.14 (2 H, q, *J* 7, MeCH_2O) and 7.71 (4 H, m); R_f [silica gel; ethyl acetate–cyclohexane (1 : 1)] 0.47; *m/z* (DI, NH_3) 393 (MNH_4^+, 100%), 337 (27), 320 (24), 293 (40), 276 (63) and 258 (25).

tert-*Butyl Ethyl* 2-*Chlorofluoromethyl*-2-(3-*phthalimidopropyl*)*malonate* **3a**.—A mixture of *tert*-butyl ethyl 2-(3-phthalimidopropyl)malonate **1a** (3.89 g, 10.4 mmol) and sodium hydride (0.55 g of a 45% dispersion in oil, 10.4 mmol) in anhydrous THF (50 cm^3) was stirred under nitrogen at room temperature until completion of the metallation (2 h). The mixture was then cooled to −20 °C, and Freon 21 ($CHCl_2F$) (15 cm^3) was added through a refrigerated addition funnel. The mixture was stirred at room temperature for 15 h, then was hydrolysed, and extracted with diethyl ether (2 × 50 cm^3). The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. Filtration, and removal of the solvent under reduced pressure, yielded an oil. Chromatography on silica gel with ethyl acetate–cyclohexane as eluent (2:8; MPLC) afforded the *title diester* **3a** as crystals (1.37 g, 30%), m.p. 96–97 °C (from diethyl ether–pentane) (Found: C, 57.0; H, 5.5; N, 3.1. $C_{21}H_{25}ClFNO_6$ requires C, 57.08; H, 5.70; N, 3.17%); ν_{max}(KBr)/cm^{-1} 1773 (CO), 1755 (CO), 1735 (CO), 1715 (CO) and 1036 (CX); δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.23 (3 H, t, *J* 7, *Me*CH_2O), 1.43 (9 H, s, Bu^t), 1.65–2.30 (4 H, m), 3.67 (2 H, t, *J* 7), 4.19 (2 H, q, *J* 7, MeCH_2O), 6.60 (1 H, d, J_{HF} 48, CHClF) and 7.73 (4 H, m); R_f [silica gel; ethyl acetate–cyclohexane (1 : 1)] 0.55.

Ethyl 2-*Chlorofluoromethyl*-2-*methoxycarbonylamino*-5-*phthalimidopentanoate* **5**.—A mixture of diester **3a** (1.46 g, 3.3 mmol) and TFA (10 cm^3) was stirred at 0 °C for 0.5 h. The solvent was removed under reduced pressure to yield crude ethyl 2-chlorofluoromethyl-2-(3-phthalimidopropyl)malonate **3b**, which was used in the next step without purification; δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.33 (3 H, t, *J*, *Me*CH_2O), 1.60–2.52 (4 H,

m), 3.77 (2 H, t, *J* 7), 4.32 (2 H, q, *J* 7, MeCH_2O), 6.63 (1 H, d, J_{HF} 48, CHClF), 7.80 (4 H, m) and 10.2 (1 H, br s).

The crude monoester was dissolved in thionyl dichloride (10 cm^3) and the mixture was heated at reflux for 2 h. The solvent was removed under reduced pressure to yield the crude acyl chloride, which was used in the next step without purification; δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.34 (3 H, t, *J* 7, *Me*CH_2O), 1.60–2.65 (4 H, m), 3.76 (2 H, t, *J* 7), 4.31 (2 H, q, *J* 7, MeCH_2O), 6.70 (1 H, d, J_{HF} 48, CHClF) and 7.82 (4 H, m).

To a solution of crude acyl chloride in acetone (5 cm^3) at 0 °C was added a solution of sodium azide (0.26 g, 4 mmol) in water (0.5 cm^3). The mixture was stirred at 0 °C for 0.75 h. Water (5 cm^3) was then added and the mixture was extracted with diethyl ether (2 × 25 cm^3). The combined organic layers were dried over anhydrous magnesium sulfate. Filtration, and removal of the solvent under reduced pressure, yielded crude acyl azide as an oil (1.30 g, 95% from the *tert*-butyl ethyl diester), δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.27 (3 H, t, *J* 7, *Me*CH_2O), 1.57–2.51 (4 H, m), 3.69 (2 H, t, *J* 7), 4.27 (2 H, q, *J* 7, MeCH_2O), 6.64 (1 H, d, J_{HF} 48, CHClF) and 7.74 (4 H, m).

A solution of crude acyl azide (1.30 g, 3.3 mmol) in anhydrous methanol (30 cm^3) was heated at reflux for 5 h. The solvent was removed under reduced pressure. Chromatography on silica gel with ethyl acetate–cyclohexane as eluent (2:8; MPLC) allowed the separation of the two pairs of enantiomers of the expected carbamate as pure crystalline materials.

1st Eluted pair of enantiomers **5a** (0.58 g, 42%), m.p. 115 °C (from ethyl acetate–pentane) (Found: C, 52.4; H, 5.0; N, 6.7. $C_{18}H_{20}ClFN_2O_6$ requires C, 52.12; H, 4.86; N 6.75%); ν_{max}(KBr)/cm^{-1} 3411 (NH), 1770 (CO), 1752 (CO), 1732 (CO), 1711 (CO) and 1075 (CX); δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.35 (3 H, t, *J* 7, MeCH_2O) 1.40–2.85 (4 H, m), 3.60 (s) and 3.70 (t, *J* 7) (together 5 H), 4.30 (2 H, q, *J* 7, MeCH_2O), 5.95 (1 H, br s), 6.55 (1 H, d, J_{HF} 48, CHClF) and 7.75 (4 H, m); R_f [silica gel; ethyl acetate–cyclohexane (1:1)] 0.45; *m*/*z* (DI, NH_3) 432 (MNH_4^+, 100%), 434 (MNH_4^+ + 2, 37), 415 (MH^+, 38), 417 (MH^+ + 2, 24), 400 (20) and 357 (20).

2nd Pair of enantiomers **5b** (0.50 g, 36%), m.p. 121 °C (from ethyl acetate–pentane) (Found: C, 51.9; H, 5.0; N, 6.6%); ν_{max}(KBr)/cm^{-1} 3395 (NH), 1769 (CO), 1732 (br, CO), 1711 (CO) and 1075 (CX); δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.28 (3 H, t, *J* 7, *Me*CH_2O), 145–2.90 (4 H, m), 3.55 (s) and 3.65 (t, *J* 7) (together 5 H), 4.25 (2 H, q, *J* 7, MeCH_2O), 5.85 (1 H, br s), 6.50 (1 H, d, J_{HF} 49, CHClF) and 7.78 (4 H, m); R_f [silica gel; ethyl acetate–cyclohexane (1:1)] 0.41; *m*/*z* (DI, NH_3) data identical with those of **5a**.

2,5-*Diamino*-2-(*chlorofluoromethyl*)*pentanoic Acid Hydrochloride* **7**.—The same procedure was used to hydrolyse each pair of enantiomers of carbamates **5a** and **5b**. A mixture of ethyl 2-chlorofluoromethyl-2-methoxycarbonylamino-5-phthalimidopentanoate (0.207 g, 0.5 mmol), acetic acid (1 cm^3) and 6 mol dm^{-3} hydrochloric acid (5 cm^3) was heated at 100 °C for 4 h. The solvent was removed under reduced pressure. The residue was taken up in conc. hydrochloric acid (10 cm^3) and the mixture was heated at 100 °C for 16 h. The solvent was removed under reduced pressure. The residue was triturated with cold water and the solid material was filtered off, and rinsed with cold water. The filtrate was concentrated under reduced pressure, and the same operation was repeated. The filtrate was treated with active charcoal. Filtration, and removal of the solvent under reduced pressure, left a sticky oil, which was dissolved in ethanol (5 cm^3). Propylene oxide (3 mol equiv.) was added and the mixture was left at room temperature for 15 h. Crystals were formed; filtration, and drying in high vacuum, yielded the expected amino acid.

1st Pair of enantiomers of α-(*chlorofluoromethyl*)*ornithine*·HCl **7a**. The hydrolysis of carbamate **5a** (0.5 mmol) yielded α-(*chlorofluoromethyl*)*ornithine*·*HCl* **7a** (0.075 g, 64%) (Found: C, 30.3; H, 5.4; N, 11.9. $C_{16}H_{13}Cl_2FN_2O_2$ requires C, 30.65; H, 5.57; N, 11.92%); m.p. 170 °C; ν_{max}(KBr)/cm^{-1} 3400br (NH), 3000br (OH), 1640s (CO), 1510 (NH) and 1050 (CX); δ_H(200 MHz; D_2O; TSP) 1.40–2.10 (4 H, m), 3.04 (2 H, t, *J* 7, C$H_2$$NH_2$) and 6.75 (1 H, d, J_{HF} 46.5, CHClF); R_f [silica gel; chloroform–17% ammonium hydroxide (7:3)] 0.59 (ninhydrin); *m*/*z* (FAB, xenon) 199 (MH^+, 100%), 201 (MH^+ + 2, 34), 182 (25), 148 (12) and 136 (20).

2nd Pair of enantiomers of α-(*chlorofluoromethyl*)*ornithine*·HCl **7b**. The hydrolysis of carbamate **5b** (0.5 mmol) yielded α-(chlorofluoromethyl)ornithine·HCl **7b** (0.080 g, 70%) (Found: C, 30.6; H, 5.6; N, 11.4%); m.p. 160 °C; ν_{max}(KBr)/cm^{-1} 3400br (NH), 3000br (OH), 1660s (CO) and 1040 (CX); δ_H(200 MHz; D_2O; TSP) 1.55–2.25 (4 H, m), 3.05 (2 H, t, *J* 7, C$H_2$$NH_2$) and 6.71 (1 H, d, J_{HF} 48.1, CHClF); R_f [silica gel; chloroform–17% ammonium hydroxide (7:3)] 0.57 (ninhydrin); *m*/*z* (FAB, xenon) data were identical with those of **7a**.

Resolution of pairs of enantiomers of α-(*chlorofluoromethyl*)*ornithine* **7**. Each pair of enantiomers (**7a** or **7b**) was resolved through the chiral-eluent HPLC procedure as described in ref. 11 into optically pure stereoisomers. Pair **7a** yielded enantiomers $\mathbf{7a_1}$ and $\mathbf{7a_2}$, whereas pair **7b** yielded enantiomers $\mathbf{7b_1}$ and $\mathbf{7b_2}$.

Assignment of the Absolute Configuration of Pure Enantiomers of α-(*chlorofluoromethyl*)*ornithine* **7**.—*Reduction of* α-(*chlorofluoromethyl*)*ornithine to* α-(*fluoromethyl*)*ornithine by using tributyltin hydride*. The reduction of enantiomerically pure α-(chlorofluoromethyl)ornithine $\mathbf{7a_1}$ or $\mathbf{7b_2}$ to enantiomerically pure α-(fluoromethyl)ornithine has been performed. The following procedure was used for either $\mathbf{7a_1}$ or $\mathbf{7b_2}$.

2-*Amino*-2-*chlorofluoromethyl*-5-(*methoxycarbonylamino*)*pentanoate* $\mathbf{17a_1}$ *or* $\mathbf{17_2}$.—2,5-Diamino-2-chlorofluoromethylpentanoic acid hydrochloride $\mathbf{7a_1}$ (0.050 g) was suspended in THF (3 cm^3) and phosgene were condensed (≈1 cm^3). The mixture was stirred overnight at room temperature and a clear solution was obtained. The mixture was evaporated to dryness and the residue was dissolved in methanol (3 cm^3). The solution was saturated with dry hydrogen chloride and was stirred 24 h at room temperature. The solvent was then evaporated off. The residue was dissolved in water. Sodium hydrogen carbonate was added to neutralize the hydrochloride. The solution was then extracted with ethyl acetate (3 ×). The combined organic layers were dried over anhydrous sodium sulfate. Filtration, and removal of the solvent under reduced pressure, afforded an oil. The expected carbamate was purified by preparative TLC [silica gel; ethyl acetate–hexane (1:1)]. Carbamate $\mathbf{17a_1}$ was isolated (0.028 g, 48%), carbamate $\mathbf{17b_2}$ was similarly isolated (0.017 g, 50%) after reaction of enantiomer $\mathbf{7b_2}$ (0.028 g).

Methyl 2-*Chlorofluoromethyl*-5-*methoxycarbonylamino*-2-(*trifluoroacetylamido*)*pentanoate* $\mathbf{18a_1}$ *or* $\mathbf{18b_2}$.—To a solution of methyl 2-amino-2-chlorofluoromethyl-5-(methoxycarbonylamino)pentanoate $\mathbf{17a_1}$ (0.028 g) in anhydrous dichloromethane (5 cm^3) was added trifluoroacetic anhydride (0.2 cm^3) at room temperature. The mixture was stirred for 15 h at room temperature. The solvent and excess of anhydride were then removed under reduced pressure and the expected amide $\mathbf{18a_1}$ was isolated (0.029 g, 76%); similarly, amide $\mathbf{18b_2}$ was isolated (0.020 g, 87%) from amine $\mathbf{17b_2}$ (0.017 g); $\mathbf{18a_1}$ φ($CDCl_3$; C_6F_6) 17.938 (d, J_{HF} 48.9, 1 F) and 85.781 (s, 3 F); $\mathbf{17b_2}$ φ($CDCl_3$; C_6F_6) 19.372 (d, J_{HF} 48.7, 1 F) and 85.870 (s, 3 F).

α-(*Fluoromethyl*)*ornithine* **15a** *or* **15b**.—The crude trifluoroacetamide $\mathbf{18a_1}$ (or $\mathbf{18b_2}$) was dissolved in anhydrous toluene. Tributyltin hydride (0.1 cm^3) and AIBN (one crystal) were

added. The mixture was refluxed under nitrogen for 3 h. The excess of tributyltin hydride was consumed with tetrachloromethane. The mixture was evaporated to dryness and the completion of the reduction was checked by ^{19}F NMR analysis. The crude reduction product was then dissolved in 6 mol dm^{-3} aq. hydrochloric acid and heated at 100 °C for 24 h. The resulting mixture was washed twice with diethyl ether, and the aqueous layer was evaporated to dryness to leave the expected α-(fluoromethyl)ornithine bishydrochloride. Isomer **15a** (0.011 g) was isolated from amide **18a**$_1$; similarly, isomer **15b** (0.015 g) was isolated from amide **17b**$_2$ (0.020 g).

15a φ(D_2O; TFA) −151.86 (t, J_{HF} 45.6 Hz); **15b** φ(D_2O; TFA) −152.17.

Compound **15a** was found to be identical with an authentic sample of (*S*)-(+)-(fluoromethyl)ornithine (HPLC; ref. 11), and compound **15b** was found to be identical with an authentic sample of (*R*)-(−)(fluoromethyl)ornithine (HPLC; ref. 11).

The absolute configuration can thus be assigned to each isomer of α-(chlorofluoromethyl)ornithine, isomers **7a** and **7b** being, respectively, racemic mixtures of *RR* and *SS* enantiomers or *RS* and *SR* enantiomers.

(*S,S*)-α-(Chlorofluoromethyl)ornithine **7a**$_1$ showed $[\alpha]^{25}$ + 0.90 (*c* 0.98, water, pH 1.75);* (*R,R*)-α-(chlorofluoromethyl)-ornithine **7a**$_2$ showed $[\alpha]^{25}$ −1.5 (*c* 1.02, water, pH 1.80); (*S,R*)-α-(chlorofluoromethyl)ornithine **7b**$_1$ showed $[\alpha]^{25}$ +9.4 (*c* 1.00, water, pH 1.82); (*R,S*)-α-(chlorofluoromethyl)ornithine **7b**$_2$ showed $[\alpha]^{25}$ 9.9 (*c* 1.04, water, pH 1.95).

tert-*Butyl Ethyl* 2-(3-*Methoxybenzyl*)*malonate* **2a**.—A mixture of *tert*-butyl ethyl malonate (23 g, 120 mmol) and sodium hydride (6.4 g of a 45% dispersion in oil, 120 mmol) in anhydrous THF (200 cm^3) under nitrogen was stirred at room temperature for 3 h. A solution of 3-methoxybenzyl bromide (24.59 g, 120 mmol) in anhydrous THF (50 cm^3) was then added, and the mixture was stirred at room temperature for 15 h. The mixture was then hydrolysed by addition of water (100 cm^3) and was extracted with diethyl ether (3 × 100 cm^3). The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. Filtration, and removal of the solvent under reduced pressure, yielded an oily residue (34.7 g), which was purified by fractional distillation under reduced pressure. tert-*Butyl ethyl* 2-(3-*methoxybenzyl*)*malonate* **2a** was isolated as a yellowish oil (13 g, 35%), b.p. 146–148 °C/0.1 mbar† (Found: C, 66.5; H, 7.75. $C_{17}H_{24}O_5$ requires C, 66.20; H, 7.85%); δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.22 (3 H, t, *J* 7, *Me*CH_2O), 1.43 (9 H, s, Bu^t), 3.00–3.30 (2 H, m, PhCH_2), 3.38–3.67 (1 H, m, 2-H), 3.72 (3 H, s, MeO), 4.10 (2 H, q, *J* 7, MeCH_2O), 6.50–6.83 (3 H, m) and 7.10 (1 H, m); R_f [silica gel; ethyl acetate–cyclohexane (1:1)] 0.61; *m/z* (DI, NH_3) 326 (MNH_4^+, 100%), 309 (MH^+, 10), 270 (64), 253 (65) and 226 (6).

tert-*Butyl Ethyl* 2-*Chlorofluoromethyl*-2-(3-*methoxybenzyl*)-*malonate* **4a**.—A mixture of *tert*-butyl ethyl 2-(3-methoxybenzyl)malonate **2a** (10.66 g, 34.6 mmol) and sodium hydride (1.85 g of a 45% dispersion in oil, 34.6 mmol) in anhydrous THF (70 cm^3) under nitrogen was stirred at 50 °C until the metallation was complete (2 h). The mixture was cooled to −20 °C and Freon 21 (40 cm^3) was added through a refrigerated addition funnel. The temperature was allowed to rise to ambient and the mixture was stirred for 16 h. The solution was hydrolysed by addition of water (50 cm^3) and was extracted with diethyl ether (3 × 80 cm^3). The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. Filtration, and removal of the solvent under reduced pressure, yielded an oil (11.65 g). Chromatography on silica gel with ethyl acetate–cyclohexane as eluent (5:95; MPLC) yielded the *title diester* **4a** as an oil (6.27 g, 48%) (Found: C, 57.95; H, 6.35. $C_{18}H_{24}ClFO_5$ requires C, 57.70; H, 6.45%); δ_H(90 MHz; $CDCl_3$; Me_4Si) 1.23 (3 H, t, *J* 7.5, *Me*CH_2O) 1.43 (9 H, s, Bu^t), 3.40 (2 H, br s), 3.75 (3 H, s, MeO), 4.20 (2 H, q, *J* 7.5, MeCH_2O), 6.38 (1 H, d, J_{HF} 48, CHClF), 6.70–6.86 (3 H, m) and 7.13 (1 H, m); R_f [silica gel; ethyl acetate–cyclohexane (1:1)] 0.61.

Ethyl 3-*Chloro*-3-*fluoro*-2-*methoxycarbonylamino*-2-(3-*methoxybenzyl*)*propanoate* **6a**/**b**.—A mixture of diester **4a** (5.57 g, 15.5 mmol) and TFA (20 cm^3) was stirred at 0 °C for 1 h. The solvent was removed under reduced pressure to yield ethyl hydrogen 2-chlorofluoromethyl-2-(3-methoxybenzyl)malonate **4b** (used in the next step without purification); δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.27 (3 H, t, *J* 7, *Me*CH_2O), 3.42 (2 H, br s), 3.73 (3 H, s, MeO), 4.27 (2 H, q, *J* 7, MeCH_2O), 6.37 and 6.40 (1 H, 2 d, J_{HF} 48, CHFCl), 6.63–6.80 (3 H, m), 7.13 (1 H, m) and 10.60 (1 H, br s, CO_2H).

A mixture of the crude monoester and thionyl dichloride (35 cm^3) was heated at reflux for 2 h. The solvent was removed under reduced pressure to yield the expected acyl chloride as an oil (used in the next step without purification), δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.28 (3 H, t, *J* 7, *Me*CH_2O), 3.50 (2 H, br s), 3.75 (3 H, s, MeO), 4.30 (2 H, q, *J* 7, MeCH_2O), 6.27 and 6.35 (1 H, 2 d, J_{HF} 48, CHClF), 6.67–6.92 (3 H, m) and 7.18 (1 H, m).

To a solution of the crude acyl chloride in acetone (30 cm^3) at 0 °C was added a solution of sodium azide (1.12 g, 17.7 mmol) in water (3 cm^3). The mixture was stirred at 0 °C for 0.75 h. Water (30 cm^3) was added and the mixture was extracted with diethyl ether (2 × 50 cm^3). The combined organic layers were dried over anhydrous magnesium sulfate. Filtration, and removal of the solvent under reduced pressure, yielded the expected acyl azide as an oil (4.6 g, 87% overall yield from **4**), δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.13 and 1.16 (3 H, 2 t, *J* 7, *Me*CH_2O), 3.30 (2 H, br s), 3.65 (3 H, s, MeO), 4.10 and 4.13 (2 H, 2 q, *J* 7, MeCH_2O), 6.28 (1 H, d, J_{HF} 48, CHClF), 6.57–6.80 (3 H, m) and 7.07 (1 H, m).

A mixture of crude acyl azide (4.6 g, 13.4 mmol) and anhydrous methanol (30 cm^3) was heated at reflux for 12 h. The solvent was removed under reduced pressure. Chromatography on silica gel with ethyl acetate–cyclohexane as eluent (5:95; MPLC) allowed the separation of the diastereoisomeric pair of esters **6**.

1st Eluted pair of enantiomers **6a** (2.49 g, 53%) (Found: C, 51.7; H, 5.45; N, 3.9. $C_{15}H_{19}ClFNO_5$ requires C, 51.80; H, 5.50; N, 4.05%); δ_H(90 MHz; $CDCl_3$; Me_4Si) 1.37 (3 H, t, *J* 7.5, *Me*CH_2O), 3.38 (1 H, dd, J_{HH} 13.5, J_{HF} 3), 3.70 and 3.75 (6 H, 2 s, 2 × MeO), 3.78 (1 H, d, J_{HH} 13.5), 4.33 (2 H, q, J_{HH} 7, MeCH_2O), 5.87 (1 H, br s, NH), 6.60–6.90 (3 H, m), 6.93 (1 H, d, J_{HF} 49, CHClF) and 7.20 (1 H, m); TLC R_f 0.58 [silica gel; ethyl acetate–cyclohexane (1:1)].

2nd Eluted pair of enantiomers **6b** (2.13 g, 46%) (Found: C, 51.9; H, 5.40; N, 3.9%); δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.27 (3 H, t, *J* 7, *Me*CH_2O), 3.27 (1 H, d, J_{HH} 14), 3.67 and 3.71 (6 H, 2 s, 2 × MeO), 3.78 (1 H, d, J_{HH} 14), 4.27 (2 H, q, J_{HH} 7, MeCH_2O), 5.75 (1 H, br s, NH), 6.53–6.87 (3 H, m), 6.73 (1 H, d, J_{HF} 49, CHClF) and 7.15 (1 H, m); R_f 0.57 [silica gel; ethyl acetate–cyclohexane (1:1)].

3-*Chloro*-3-*fluoro*-2-*methoxycarbonylamino*-2-(3-*methoxybenzyl*)*propanoic Acid* **6c**.—To a solution of the ethyl ester **6a** (1.74 g, 5 mmol) in DME (15 cm^3) was added a solution of lithium hydroxide·H_2O (0.42 g, 10 mmol) in water (15 cm^3). The mixture was stirred at room temperature for 15 h. The solution was washed with diethyl ether (2 × 20 cm^3), acidified to pH 2–3, and extracted with diethyl ether (3 × 30 cm^3). The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. Filtration and removal of the

* New IUPAC recommendations state that values for $[\alpha]_D$ should be expressed in units of 10^{-1} deg cm^2 g^{-1}.

† 1 mbar = 10^2 Pa.

solvent under reduced pressure, yielded the expected *acid* **6c** (1.08 g, 67%), m.p. 167 °C (from diethyl ether–pentane) (Found: C, 49.25; H, 4.75; N, 4.35. $C_{13}H_{15}ClFNO_5$ requires C, 48.85; H, 4.75; N, 4.40%); ν_{max}(KBr)/cm^{-1} 3332s (NH), 3100br (OH), 1755 (CO), 1699 (CO) and 1053 (CX); δ_H(60 MHz; $CDCl_3$ + CD_3OD; Me_4Si) 3.35 (1 H, dd, J_{HH} 14, J_{HF} 3), 3.61 and 3.68 (6 H, 2 s, 2 × MeO), 3.71 (1 H, d, J_{HH} 14), 5.83 (1 H, br s, NH), 6.83 (1 H, d, J_{HF} 48, CHClF), 6.55–6.80 (3 H, m) and 7.10 (1 H, m); *m/z* (DI, NH_3) 257 (MNH_4^+, 100%), 240 (MH^+, 38) and 220 (10).

3-*Chloro*-3-*fluoro*-2-*methoxycarbonylamino*-2-(3-*methoxybenzyl*)*propanoic Acid* **6d**.—The same procedure was used as for the hydrolysis of ester **6a** to acid **6c**. Starting from compound **6b** (1.77 g, 5.1 mmol) of the expected acid **6d** (1.42 g, 87%) was obtained as an oil; δ_H(60 MHz, $CDCl_3$ + CD_3OD, Me_4Si), 3.58 (s) and 3.65 (s) (6 H, 2 × MeO), 5.85 (1 H, br s, NH), 6.58–6.90 (3 H, m), 6.70 (1 H, d, J_{HF} 49, CHClF) and 7.15 (1 H, m).

2-*Amino*-3-*chloro*-3-*fluoro*-2-(3-*hydroxyphenyl*)*propanoic Acid* **8**.—Each pair of enantiomers (**6c** or **6d**) was treated by the following procedure.

First pair of enantiomers **8a**. A mixture of the acid **6c** (0.64 g, 2 mmol) and 47% aq. hydrobromic acid (20 cm^3) was heated at 100 °C for 15 h. The solvent was removed under reduced pressure. The residue was taken up in water and treated with active charcoal. Filtration, and removal of the solvent under reduced pressure, left a sticky oil. Half of the oil was dissolved in propan-2-ol (or absolute ethanol) (10 cm^3). Propylene oxide (5 mol equiv.) was added and the mixture was left at room temperature for 50 h. Crystals (0.21 g, 84%) of the expected amino acid **8a** were formed, isolated by filtration, and dried in high vacuum.

1st Pair of enantiomers **8a**, m.p. 171 °C (Found: C, 48.6; H, 4.85; N, 5.3. $C_{10}H_{11}ClFNO_3$ requires C, 48.50; H, 4.50; N, 5.65%); ν_{max}(KBr)/cm^{-1} 3400vbr (NH), 3000vbr (OH), 1640 (CO) and 1060 (CX); λ_{max}(water)/nm 278, 273 and 214 (ε 6160); δ_H(250 MHz; D_2O + DCl; TSP) 3.05 (1 H, d, *J* 14.2, PhC*H*), 3.37 (1 H, d, *J* 14.2, PhC*H*), 6.77 (1 H, Ar m, 2-H), 6.84 (d) and 6.87 (dd) (2 H, Ar 4- and 6-H), 6.88 (1 H, d, J_{HF} 46.3, CHClF) and 7.28 (1 H, t, *J* 7, Ar 5-H); R_f [silica gel; butan-1-ol–acetic acid–water (6:2:2)] 0.54; *m/z* (FAB, xenon) 248 (MH^+, 100%), 250 (MH^+ + 2, 38) and 202 (17).

2nd Pair of enantiomers **8b**. Starting from compound **6d** (1.18 g, 3.7 mmol) and, after treatment of half of the amino acid hydrobromide with propylene oxide, isolated the second pair of enantiomers **8b** (0.275 g, 60%), m.p. 166 °C (Found: C, 48.25; H, 4.8; N, 5.4%); ν_{max}(KBr)/cm^{-1} 3200vbr (NH, OH), 1640 (CO), 1610 (CO) and 1070 (CX); λ_{max}(water)/nm 278, 273 and 214 (ε 6300); δ_H(250 MHz; D_2O + DCl; TSP) 3.18 (1 H, dd, *J* 14.2, J^2 1.5, ArC*H*), 3.50 (1 H, d, *J* 14.2, ArC*H*), 6.76 (1 H, m, Ar 2-H), 6.83 (d) and 6.87 (dd) (2 H, Ar 4- and 6-H), 6.82 (1 H, d, J_{HF} 47.9, CHClF) and 7.29 (1 H, t, *J* 7, Ar 5-H); R_f [silica gel; butan-1-ol–acetic acid–water (6:2:2)] 0.54; *m/z* (FAB, xenon) data were identical with those of compound **8a**.

Methyl 2-*Benzylideneamino*(*chlorofluoromethyl*)*hex*-5-*enoate* **10a**.—A mixture of ester **9a**[16] (16 g, 69 mmol) and sodium hydride (3.68 g of a 45% dispersion in oil, 69 mmol) in anhydrous THF (140 cm^3) was heated at 45 °C, under nitrogen, until completion of the metallation (2 h). The mixture was cooled to −20 °C. Freon 21 (20 cm^3) was added through a refrigerated addition funnel. The mixture was stirred at room temperature for 15 h. Hydrolysis, and removal of the solvent under reduced pressure, left an oily residue, which was triturated and extracted with diethyl ether (3 × 50 cm^3). The combined organic layers were dried over anhydrous magnesium sulfate. Filtration and removal of the solvent under reduced pressure, yielded an orange-red oil. Distillation under high vacuum yielded a 1:1 mixture (11.1 g) of methyl 2-benzylideneamino-2-(chlorofluoromethyl)hex-5-enoate **10a** and starting material **9a** (b.p. 175 °C/0.2 mbar).

Methyl 2-*Amino*-2-(*chlorofluoromethyl*)*hex*-5-*enoate* **10b**.—A solution of the crude mixture from the preceding step (**10a**; 11.1 g) in 1 mol dm^{-3} hydrochloric acid (60 cm^3) was stirred at room temperature for 2 h. The aqueous acidic solution was washed with diethyl ether (2 × 50 cm^3) and evaporated to dryness under reduced pressure. The residue was dissolved in water (10 cm^3). Sodium hydrogen carbonate was added until saturation and the basic aqueous layer was extracted with diethyl ether (3 × 50 cm^3). The combined organic layers were dried over anhydrous magnesium sulfate. Filtration, and removal of the solvent under reduced pressure, gave a 3:2 mixture of methyl 2-amino-2-(chlorofluoromethyl)hex-5-enoate and methyl 2-aminohex-5-enoate. Chromatography on silica gel with ethyl acetate–cyclohexane (2:8) as eluent yielded the expected methyl 2-amino-2-(chlorofluoromethyl)hex-5-enoate **10b** as an oil (2 g, 14% overall yield from **9a**); δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.65–2.50 (6 H, m), 3.73 and 3.76 (3 H, 2 s), 4.80–5.20 (2 H, m), 5.45–6.08 (1 H, m) and 6.27 (1 H, d, J_{HF} 50, CHClF).

Methyl 2-(tert-*Butoxycarbonylamino*)-2-(*chlorofluoromethyl*)-*hex*-5-*enoate* **11**.—A mixture of methyl 2-amino-2-(chlorofluoromethyl)hex-5-enoate **10b** (2 g, 9.5 mmol) and di-*tert*-butyl dicarbonate (2.29 g, 10.5 mmol) in anhydrous THF (30 cm^3) was heated at reflux under nitrogen for 120 h. The solvent was removed under reduced pressure. Chromatography on silica gel with ethyl acetate–cyclohexane (5:95) as eluent allowed separation of the two pairs of enantiomers (87% combined yield).

1st Eluted pair of enantiomers **11a**. R_f [silica gel; ethyl acetate–cyclohexane (2:8)] 0.61; δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.50 (9 H, s), 1.75–2.80 (4 H, m), 3.82 (3 H, s), 4.70–5.15 (2 H, m), 5.37–6.00 (m) and 5.67 (br s) (together 2 H) and 6.62 (1 H, d, J_{HF} 49, CHClF).

2nd Eluted pair of enantiomers **11b**. R_f [silica gel; ethyl acetate–cyclohexane (2:8)] 0.54; δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.43 (9 H, s), 1.80–2.73 (4 H, m), 3.80 (3 H, s), 4.78–5.23 (2 H, m), 5.42–6.10 (m) and 5.56 (br s) (together 2 H) and 6.61 (1 H, d, J_{HF} 49, CHClF).

1-*Methyl Hydrogen* 2-(tert-*Butoxycarbonylamino*)-2-(*chlorofluoromethyl*)*pentanedioate* **12**.—The same procedure was used to oxidize both pairs of enantiomers of carbamate **11**.

1st Pair of enantiomers **12a**. To a solution of potassium permanganate (2.26 g, 14.4 mmol) in water (100 cm^3) at 0 °C was added a solution of methyl 2-(*tert*-butoxycarbonylamino)-2-(chlorofluoromethyl)hex-5-enoate **11a** (1.5 g, 4.8 mmol) in acetic acid (20 cm^3). The mixture was stirred at 0 °C for 2 h, and then at room temperature for 15 h. The excess of potassium permanganate was destroyed by addition of 10% aq. sodium hydrogen sulfite in water. The mixture was then saturated with sodium chloride and extracted with diethyl ether (3 × 100 cm^3). The combined organic layers were dried over anhydrous magnesium sulfate. Filtration, and removal of the solvent under reduced pressure, yielded the expected acid **12a** as a sticky oil (1.24 g, 79%); δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.43 (9 H, s), 2.03–2.90 (4 H, m), 3.83 (3 H, s), 5.76 (1 H, br s), 6.67 (1 H, d, J_{HF} 49, CHClF) and 9.17 (1 H, br s).

2nd Pair of enantiomers **12b**. The expected acid **12b** (1.10 g, 99%) was isolated after treatment of carbamate **11b** (1.05 g) by the procedure described above; δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.43 (9 H, s), 2.23–2.70 (4 H, m), 3.81 (3 H, s), 5.77 (1 H, br s), 6.65 (1 H, d, J_{HF} 49, CHClF) and 9.20 (1 H, br s).

2-(tert-*Butoxycarbonylamino*)-2-(*chlorofluoromethyl*)*pentanedioic Acid* **13**.—The same procedure was used to hydrolyse both pairs of enantiomers of acid **12**.

Table 5

Structure	**5a**	**5b**	**16a**	**16b**	**6c**
Formula	$C_{18}H_{20}ClFN_2O_6$	$C_{18}H_{20}ClFN_2O_6$	$C_7H_9ClFNO_3$	$C_7H_9ClFNO_3$	$C_{13}H_{15}ClFNO_5$
Molecular weight	414.8	414.8	209.59	209.59	319.72
Crystal system	Triclinic	Triclinc	Monoclinic	Monoclinic	Monoclinic
a/Å	11.295 (5)	11.164 (5)	9.414 (3)	8.937 (3)	10.223 (3)
b/Å	11.685 (5)	11.252 (5)	7.117 (3)	10.447 (4)	9.075 (3)
c/Å	7.600 (3)	8.139 (3)	12.939 (5)	10.151 (4)	15.391 (4)
α/°	105.23 (3)	105.37 (3)			
β/°	96.69 (3)	105.64 (3)	95.37 (2)	106.14 (2)	93.57 (2)
γ/°	90.41 (3)	85.96 (3)			
V/Å^3	961	949	863	910	1425
Z	2	2	4	4	4
D_c/g cm^{-3}	1.393	1.409	1.59	1.51	1.49
Space group	$P\bar{1}$	$P\bar{1}$	$P2_1/c$	$P2_1/c$	$P2_1/c$
Wavelength (Å)	Cu-Kα = 1.5405	Cu-Kα = 1.5405	Cu-Kα = 1.5405	Cu-Kα = 1.5405	Cu-Kα = 1.5405
Absorption					
Linear coefficient μ/cm^{-1}	21.8 (μR = 0.3)	22.05	39.72	37.65	27.13
Crystal size (mm)	sphere/diameter 0.25 ± 0.01	0.20 × 0.21 × 0.23	0.10 × 0.20 × 0.25	0.20 × 0.20 × 0.35	0.13 × 0.15 × 0.07
Temperature/°C	−100	−100	−100	−100	−100
Philips PW 1100/16					
4-Circle diffractometer					
Scan mode	$\theta/2\theta$ flying step scan	$\theta/2\theta$ flying step scan	$\theta/2\theta$ flying step scan	$\theta/2\theta$ flying step scan	$\theta/2\theta$ flying step scan
Scan speed/° s^{-1}	0.024	0.020	0.024	0.024	0.024
Scan width/°	1.0 + 0.143 tanθ	1.0 + 0.143 tanθ	1.0 + 0.143 tanθ	1.0 + 0.143 tanθ	1.0 + 0.143 tanθ
Step width/°	0.05	0.05	0.05	0.05	0.05
θ Range measured/°	$4 \leqslant \theta \leqslant 57$	$4 \leqslant \theta \leqslant 57$	$5 \leqslant \theta \leqslant 57$	$5 \leqslant \theta \leqslant 57$	$4 \leqslant \theta \leqslant 57$
Octants	$\pm h, \pm k, +l$	$\pm h, \pm k, +l$	$+h, +k, \pm l$	$+h, +k, \pm l$	$+h, +k, \pm l$
Number of reflections	2547	2515	1340	1327	2170
Data $I \geqslant 3\sigma(I)$	2448	2371	1107	1087	1497
R(F)	0.039	0.037	0.055	0.056	0.033
$R_w(F)$	0.087	0.073	0.118	0.124	0.05

Table 6 Positional parameters and their esds (in parentheses) for compound **5a**

Atom	x	y	z
Cl	0.245 59(5)	0.095 23(4)	0.951 16(7)
F	0.386 5(1)	−0.052 4(1)	1.049 5(2)
O(1)	0.024 8(1)	0.063 1(1)	1.246 4(2)
O(2)	0.187 6(1)	0.172 6(1)	1.394 4(2)
O(3)	0.255 0(1)	−0.279 5(1)	1.085 9(2)
O(4)	0.076 9(1)	−0.288 6(1)	0.912 9(2)
O(5)	0.516 2(1)	−0.206 9(1)	1.527 5(2)
O(6)	0.159 2(1)	−0.414 8(1)	1.409 1(2)
N(1)	0.148 7(1)	−0.113 4(1)	1.079 5(2)
N(2)	0.325 1(2)	−0.287 1(1)	1.495 5(2)
C(l)	0.312 9(2)	0.033 9(2)	1.126 9(3)
C(2)	0.219 3(2)	−0.019 9(2)	1.214 7(3)
C(3)	0.131 4(2)	0.076 2(2)	1.285 3(3)
C(4)	0.114 9(2)	0.269 7(2)	1.482 9(3)
C(5)	0.110 1(2)	0.266 9(2)	1.678 0(3)
C(6)	0.169 2(2)	−0.230 9(2)	1.032 4(3)
C(7)	0.087 1(2)	−0.415 2(2)	0.850 7(3)
C(8)	0.284 9(2)	−0.059 6(2)	1.377 1(3)
C(9)	0.201 2(2)	−0.114 7(2)	1.479 6(3)
C(10)	0.269 7(2)	−0.183 2(2)	1.602 7(3)
C(11)	0.443 9(2)	−0.288 5(2)	1.466 1(3)
C(12)	0.461 1(2)	−0.408 7(2)	1.347 1(3)
C(13)	0.352 9(2)	−0.472 4(2)	1.308 1(3)
C(14)	0.264 8(2)	−0.393 5(2)	1.404 6(3)
C(15)	0.563 1(2)	−0.458 5(2)	1.276 7(3)
C(16)	0.550 8(2)	−0.574 5(2)	1.166 8(3)
C(17)	0.442 5(2)	−0.637 7(2)	1.130 0(3)
C(18)	0.341 2(2)	−0.487 7(2)	1.200 0(3)

Table 7 Positional parameters and their esds (in parentheses) for compound **5b**

Atom	x	y	z
Cl	0.633 28(4)	0.022 92(4)	0.949 16(6)
F	0.824 6(1)	−0.114 7(1)	0.953 8(1)
O(1)	1.002 7(1)	−0.056 3(1)	0.697 4(2)
O(2)	0.837 7(1)	−0.183 2(1)	0.569 6(2)
O(3)	0.743 6(1)	0.270 5(1)	0.910 8(2)
O(4)	0.943 9(1)	0.286 6(1)	0.065 3(2)
O(5)	0.816 1(1)	0.420 2(1)	0.572 2(2)
O(6)	0.449 6(1)	0.241 4(1)	0.523 1(2)
N(1)	0.879 5(1)	0.107 0(1)	0.886 1(2)
N(2)	0.640 5(1)	0.305 2(1)	0.518 6(2)
C(l)	0.736 3(2)	−0.065 6(2)	0.833 7(2)
C(2)	0.802 5(2)	0.010 1(2)	0.752 9(2)
C(3)	0.894 9(2)	−0.080 0(2)	0.670 2(2)
C(4)	0.910 8(2)	−0.274 2(2)	0.473 0(3)
C(5)	0.895 8(2)	−0.254 8(2)	0.295 1(3)
C(6)	0.844 4(2)	0.224 6(2)	0.948 1(2)
C(7)	0.920 2(2)	0.413 0(2)	1.146 3(3)
C(8)	0.706 5(2)	0.052 8(2)	0.604 2(2)
C(9)	0.765 1(5)	0.114 0(2)	0.499 7(2)
C(10)	0.676 2(2)	0.194 3(2)	0.400 2(2)
C(11)	0.715 9(2)	0.408 4(2)	0.596 8(2)
C(12)	0.646 8(2)	0.497 7(2)	0.709 0(2)
C(13)	0.533 9(2)	0.444 0(2)	0.690 1(2)
C(14)	0.530 0(2)	0.318 5(2)	0.570 1(2)
C(15)	0.678 5(2)	0.614 5(2)	0.815 0(3)
C(16)	0.593 3(2)	0.677 0(2)	0.903 0(3)
C(17)	0.479 1(2)	0.624 6(2)	0.883 6(3)
C(18)	0.448 4(2)	0.505 6(3)	0.775 9(3)

1st Pair of enantiomers **13a**. To a solution of the methyl ester **12a** (1.23 g, 3.7 mmol) in DME (9 cm^3) was added a solution of lithium hydroxide·H_2O (0.366 g, 8 mmol) in water (9 cm^3) at room temperature. The mixture was stirred at room temperature for 15 h, and was then washed with diethyl ether (2 × 15 cm^3). The aqueous layer was acidified to pH 3 and extracted with diethyl ether (3 × 30 cm^3). The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. Filtration, and removal of the solvent under reduced pressure, yielded the expected *dicarboxylic acid* **13a** (0.79 g, 68%), m.p. 108–109 °C (from diethyl ether–pentane) (Found: C, 42.3; H, 5.4; N, 4.5. $C_{11}H_{17}ClFNO_6$ requires C, 42.11; H, 5.46; N, 4.46%); ν_{max}(KBr)/cm^{-1} 3303s (NH), 3000br (OH), 1768 (CO), 1756 (CO), 1697 (CO), 1642 (CO) and 1053 (CX); δ_H(60

Table 8 Positional parameters and their esds (in parentheses) for compound **6c**

Atom	*x*	*y*	*z*
Cl	0.453 81(8)	0.035 15(8)	0.888 31(4)
F	0.589 8(2)	−0.199 4(2)	0.907 81(9)
C(1)	0.486 8(3)	−0.146 1(3)	0.856 0(2)
C(2)	0.366 6(2)	−0.247 9(3)	0.860 4(1)
C(3)	0.319 5(3)	−0.251 7(3)	0.953 6(2)
O(1)	0.217 8(2)	−0.199 0(2)	0.972 6(1)
O(2)	0.403 1(2)	−0.320 3(2)	1.008 7(1)
N	0.257 8(2)	−0.186 5(2)	0.807 1(1)
C(4)	0.261 0(2)	−0.144 6(3)	0.723 7(2)
O(3)	0.350 8(2)	−0.168 3(2)	0.676 8(1)
O(4)	0.152 9(2)	−0.071 1(2)	0.699 3(1)
C(5)	0.140 7(3)	−0.016 7(4)	0.611 5(2)
C(6)	0.409 8(2)	−0.402 6(3)	0.830 6(2)
C(7)	0.303 6(3)	−0.517 0(3)	0.824 1(2)
C(8)	0.285 6(3)	−0.613 6(3)	0.891 7(2)
C(9)	0.191 7(3)	−0.723 1(3)	0.882 0(2)
C(10)	0.115 4(3)	−0.737 7(3)	0.806 0(2)
C(11)	0.131 9(3)	−0.641 0(3)	0.737 9(2)
C(12)	0.225 9(3)	−0.531 1(3)	0.746 6(2)
O(5)	0.052 2(2)	−0.662 7(2)	0.664 6(1)
C(13)	0.079 2(3)	−0.578 2(4)	0.590 2(2)

Table 9 Positional parameters and their esds (in parentheses) for compound **16a**

Atom	*x*	*y*	*z*
Cl	0.349 23(9)	0.152 8(1)	0.967 80(7)
F	0.184 9(2)	−0.035 2(2)	1.074 2(2)
C(1)	0.193 6(4)	0.141 8(4)	1.034 3(3)
C(2)	0.199 8(3)	0.283 1(4)	1.122 3(2)
N(3)	0.331 2(3)	0.273 1(3)	1.189 8(2)
C(4)	0.421 9(3)	0.420 9(4)	1.180 6(3)
O(5)	0.540 0(2)	0.431 5(3)	1.227 6(2)
C(6)	0.349 6(4)	0.562 5(4)	1.108 2(3)
C(7)	0.197 7(3)	0.489 0(5)	1.081 0(3)
C(8)	0.071 3(4)	0.249 2(4)	1.183 6(3)
O(9)	0.075 0(3)	0.219 0(4)	1.276 2(2)
O(10)	−0.048 6(2)	0.259 2(3)	1.120 3(2)
C(11)	−0.181 2(4)	0.241 8(5)	1.168 9(3)

MHz; $CDCl_3$; Me_4Si) 1.43 (9 H, s, Bu^t), 2.30–2.87 (4 H, m, CH_2CH_2), 5.85 (1 H, br s), 6.67 (1 H, d, J_{HF} 48, CHClF) and 9.97 (2 H, br s); R_f [silica gel; chloroform–methanol–17% ammonium hydroxide (2:2:1)] 0.47.

2nd Pair of enantiomers **13b**. The expected *dicarboxylic acid* (0.70 g, 68%) was isolated after hydrolysis of acid **12b** (1.10 g, 3.3 mmol), m.p. 119–120 °C (from diethyl ether–pentane) (Found: C, 41.95; H, 5.4; N, 4.55%); ν_{max}(KBr)/cm^{-1} 3315s (NH), 2990vbr (OH), 1753 (CO), 1679 (CO), 1629 (CO) and 1054 (CX); δ_H(60 MHz; $CDCl_3$; Me_4Si) 1.43 (9 H, s, Bu^t), 2.33–2.80 (4 H, m, CH_2CH_2), 5.90 (1 H, br s), 6.65 (1 H, d, J_{HF} 49, CHClF) and 10.50 (2 H, br s); R_f [silica gel; chloroform–methanol–17% ammonium hydroxide (2:2:1)] 0.48.

2-Amino-2-(chlorofluoromethyl)pentanedioic Acid·HCl **14**.—The same procedure was used to deprotect both pairs of enantiomers of diacid **13**.

1st Pair of enantiomers **14a**. To a solution of 2-(*tert*-butoxycarbonylamino)-2-(chlorofluoromethyl)pentanedioic acid **13a** (0.100 g, 0.32 mmol) in diethyl ether (1 cm^3) was added a saturated solution of hydrochloric acid in diethyl ether (5 cm^3) at room temperature. The mixture was kept for 4 days at room temperature during which time crystals formed. The expected amino acid **14a** (0.030 g, 37%) was isolated by filtration and drying in high vacuum, m.p. 196 °C (Found: C, 29.5; H, 4.1; N, 5.6. $C_6H_{10}Cl_2FNO_4$ requires C, 28.32; H, 4.03; N, 5.60%); ν_{max}(KBr)/cm^{-1} 3400w (NH), 3000vbr (OH), 1755 (CO), 1720 (CO) and 1050 (CX); δ_H(90 MHz; D_2O; TMPS) 2.20 (2 H, m), 2.52 (2 H, m), 6.81 (1 H, d, J_{HF} 46.5, CHClF); R_f [silica gel; butan-1-ol–acetic acid–water (6:2:2)] 0.29; *m/z* (FAB, xenon) 214 (MH^+, 100%) and 216 (MH^+ + 2, 34).

2nd Pair of enantiomers **14b**. The expected α-amino acid **14b** (0.033 g, 41%) was isolated after deprotection of acid **13b** (0.100 g, 0.32 mmol), m.p. 227 °C (Found: C, 29.0; H, 4.2; N, 5.4%); ν_{max}(KBr)/cm^{-1} 3400w (NH), 3000vbr (OH), 1755 (CO), 1720 (CO) and 1060 (CX); δ_H(90 MHz; D_2O; TMPS) 2.33 (2 H, m), 2.58 (2 H, m) and 6.78 (1 H, d, J_{HF} 48, CHClF); R_f [silica gel; butano-1-ol–acetic acid–water (6:2:2)] 0.29; *m/z* (FAB, xenon) data were identical with those of isomer **14a**.

Single-crystals Formation for X-Ray Diffraction Analysis.—Carbamates **5a** *and* **5b**. Suitable single crystals for X-ray diffraction experiments were obtained by slow recrystallization from dichloromethane–pentane at room temperature. Relative configuration of **5a** is *RR,SS*; that of **5b** is *RS,SR* (Fig. 1).

Pyroglutamates **16a** *and* **16b**. The methyl α-(chlorofluoromethyl)pyroglutamates were obtained by spontaneous cyclization of each pair of enantiomers **14a** or **14b** in water (D_2O) at room temperature (several days followed by NMR spectroscopy). The pyroglutamic acids were isolated after extraction of the aqueous solution with diethyl ether and usual work-up. Esterification was performed with diazomethane in diethyl ether (Scheme 5). Suitable single crystals for X-ray diffraction experiments were obtained by slow recrystallization from dichloromethane–pentane at room temperature (Fig. 1).

Methyl α-(chlorofluoromethyl)pyroglutamate **16b** *from diacid* **14b**. M.p. 155–156 °C; δ_H(360 MHz; $CDCl_3$; Me_4Si) 2.35–2.60 (4 H, m), 3.84 (3 H, s), 6.14 (1 H, br s) and 6.44 (1 H, d, J_{HF} 49.5); φ(340 MHz; $CDCl_3$; C_6F_6) 11.59 (d, J_{FH} 49.7).

α-(Chlorofluoromethyl)pyroglutamate **16a** *from diacid* **14a**. M.p. 128–129 °C; δ_H(360 MHz; $CDCl_3$; Me_4Si) 2.30–2.65 (4 H, m), 3.85 (3 H, s), 6.13 (1 H, br s) and 6.45 (1 H, d, J_{HF} 49; φ(340 MHz; $CDCl_3$; C_6F_6) 20.93 (d, J_{FH} 48.8). Relative configuration of **16a** is *RR,SS*; that of **16b** is *RS,SR* (Fig. 1).

Carbamate **6c**. Suitable single crystals for X-ray diffraction experiments were obtained by slow evaporation of an ethanolic solution at room temperature. Relative configuration of **6c** is *RR,SS* (Fig. 1).

X-Ray Experimental Section.—For all compounds the method used was the same. One single crystal was cut out from a cluster of crystals and mounted on a rotation-free goniometer head; for compound **5a**, a single crystal was carved to a nearly spherical shape. The crystal system for each compound was found from a systematic search in reciprocal space by using a Philips PW1100/16 automatic diffractometer. Quantitative data were obtained at −100 °C achieved by using a locally built gas-flow device. All experimental parameters used are given in Table 5. The resulting data-sets were transferred to a VAX computer, and for all subsequent calculations the Enraf–Nonius SDP/PDP package[19] was used with the exception of a local data-reduction program. Three standard reflections measured every hour during the entire data-collection periods showed no significant trend. The raw step-scan data were converted into intensities by using the Lehmann–Larsen method[20] and were then corrected for Lorentz and polarization factors.

The structures were solved by using MULTAN.[21] After refinements of the heavy atoms, difference-Fourier maps revealed maxima of residual electronic density close to the positions expected for hydrogen atoms; they were introduced in structure-factor calculations by their computed co-ordinates (C–H 0.95 Å) and isotropic temperature factors such as $B(H) = 1 + B_{eqv}$ (C) Å^2 but were not refined. At this stage empirical absorption

Table 10 Positional parameters and their esds (in parentheses) for compound **16b**

Atom	*x*	*y*	*z*
Cl	0.368 7(1)	0.050 4(1)	0.332 5(1)
F	0.115 1(3)	0.067 0(2)	0.398 3(2)
C(1)	0.223 3(4)	−0.025 2(4)	0.396 1(4)
C(2)	0.145 0(4)	−0.134 1(3)	0.301 7(4)
N(3)	0.064 5(3)	−0.087 9(3)	0.167 0(3)
C(4)	−0.092 1(4)	−0.075 3(4)	0.144 3(4)
O(5)	−0.180 2(3)	−0.029 4(3)	0.042 0(3)
C(6)	−0.134 5(4)	−0.129 3(4)	0.267 3(4)
C(7)	0.012 5(4)	−0.195 2(4)	0.354 4(3)
C(8)	0.266 2(4)	−0.236 0(4)	0.296 4(3)
O(9)	0.268 2(3)	−0.297 8(3)	0.197 6(3)
O(10)	0.364 5(3)	−0.248 2(2)	0.420 8(2)
C(11)	0.488 9(5)	−0.340 2(4)	0.436 6(5)

corrections were applied by using the method of Walker and Stuart[22] since face indexation was not possible under the cold gas stream; for compound **5a**, the values of the absorption factors come from ref. 23. Full least-squares refinements; $\sigma^2(F^2) = \sigma^2$ counts $+(pI)^2$. A final difference map revealed no significant maxima. The scattering factor coefficients and anomalous dispersion coefficients came, respectively, from ref. 24*a* and 24*b*. Fractional atomic co-ordinates are given in Tables 6–10.*

Biochemicals.—DL-[1-^{14}C]Ornithine (58 Ci mol^{-1}), L-3,4-dihydroxyphenyl-[1-^{14}C]alanine (5 Ci mol^{-1}) and DL-[1-^{14}C]-glutamate (50 Ci mol^{-1}) were purchased from Amersham International (Amersham, UK). Bacterial glutamate decarboxylase (type II from *Escherichia coli*), L-ornithine, L-3,4-dihydroxyphenylalanine and L-glutamate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other biochemical products were of the purest grade commercially available.

Enzyme Preparations and Assays of Time-dependent Inhibition.—ODC was obtained from the liver of rats which had been injected with thioacetamide (150 mg/kg body wt.) 18 h before sacrifice, and was purified ~10-fold by acidic treatment at pH 4.6 as described by Ono *et al.*[25] The specific activity of the preparation was 0.2 nmol CO_2 min^{-1} (mg of protein)$^{-1}$. Assay and determination of kinetic constants were performed essentially as previously described.[26] AADC was prepared and partially purified from hog kidney according to a published procedure.[27] The specific activity of the enzyme was 7.5 μmol CO_2 min^{-1} (mg of protein)$^{-1}$. Assay and determination of times for half-inactivation were performed essentially as previously described.[27] Mammalian GAD was prepared from rat brain.[28] Assay conditions have been published.[28] Specific activity of the preparation was 10 nmol CO_2 min^{-1} (mg of protein)$^{-1}$ Bacterial GAD, from Sigma Chemical Co., was used without further purification. Specific activity of this enzyme was 5 μmol CO_2 min^{-1} (mg of protein)$^{-1}$ when measured as described by Jung *et al.*[29] Determination of kinetic constants of inactivation of mammalian and bacterial GAD was performed as published by Danzin *et al.*[30]

* *Supplementary material* (see section 5.6.3 of Instructions to Authors, in the January issue): for compounds **5a**, **5b**, **6c**, **16a** and **16b** respectively: tables S2, S8, S14, S20, S26: temperature factors for anisotropic atoms; tables S3, S9, S15, S21, S27: hydrogen-atom positional parameters; tables S4, S10, S16, S22, S28: complete set of bond distances; tables S5, S11, S17, S23, S29: complete set of bond angles; tables S6, S12, S18, S24, S30: observed and calculated structure factors amplitudes (*10) for all observed reflections (68 pages).

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