Stereospecific syntheses of all four stereoisomers of 4-fluoroglutamic acid

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Abstract

(+)-L-*threo*-4-Fluoroglutamic acid [(+)-(2*S*, 4*S*)-fluoroglutamic acid] has been synthesized starting with the natural (−)-4-*trans*-hydroxy-L-proline. Its acetylation at nitrogen followed by esterification with diazomethane afforded methyl 1-acetyl-*trans*-4-hydroxy-L-prolinate which was converted to methyl 1-acetyl-*cis*-4-fluoro-L-prolinate by means of diethylaminosulfur trifluoride (DAST) or 2-chloro-1,1,2-trifluorotriethylamine. The mixture was oxidized by ruthenium tetroxide to methyl 1-acetyl-*cis*-4-fluoro-L-pyrrolidin-5-one-2-carboxylate, whose acid hydrolysis yielded the title compound. A similar sequence of reactions converted *cis*-4-hydroxy-D-proline to (−)-D-*erythro*-4-fluoroglutamic acid [(−)(2*R*, 4*S*)-fluoroglutamic acid]. (−)-D-*threo*-4-Fluoroglutamic acid [(−)-(2*R*, 4*R*)-fluoroglutamic acid] was prepared analogously from *trans*-4-hydroxy-D-proline, obtained from its diastereomer by inversion of configuration at carbon 4 of the pyrrolidine ring using the diethyl azodicarboxylate–triphenylphosphine procedure. *cis*-4-Hydroxy-L-proline, necessary for the synthesis of (+)-L-*erythro*-4-fluoroglutamic acid [(+)-(2*S*, 4*R*)-fluoroglutamic acid], was prepared from *trans*-4-hydroxy-L-proline by benzyloxycarbonylation at the nitrogen, oxidation of the 1-benzyloxycarbonyl-*trans*-4-hydroxy-L-proline to 1-benzyloxycarbonyl-4-oxo-L-proline, its reduction to 1-benzyloxycarbonyl-*cis*-4-hydroxy-L-proline and deprotection of the latter at the nitrogen. (−)-*cis*-4-Fluoro-L-proline and (+)-*trans*-4-fluoro-D-proline were isolated after the hydrolysis of incompletely oxidized methyl 1-acetyl-*cis*-4-fluoro-L-prolinate and methyl 1-acetyl-*trans*-4-fluoro-D-prolinate, respectively.

Introduction

The discovery of the antimetabolic properties of fluoroacetic acid [1] and of 5-fluorouracil [2] initiated interest in incorporating fluorine into amino acids, in the hope that the fluorinated amino acids might act as antimetabolites of amino acids, interfere with the synthesis of proteins and thus exhibit cancerostatic activity. Among the first monofluorinated aliphatic amino acids prepared was 4-fluoroglutamic (γ-fluoroglutamic) acid.

Its first preparation was based on the Michael addition of diethyl acetamidomalonate to ethyl 2-fluoroacrylate followed by acid hydrolysis [3]. The fluoroacrylate was synthesized from ethyl acrylate according to the method of Henne and Fox [4], or from diethyl fluorooxalacetate according to that of Gault *et al.* [5].

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Independently and practically simultaneously, Pattison *et al.* carried out the Michael addition to ethyl 2-acetamidoacrylate of diethyl fluoromalonate, prepared from diethyl malonate and perchloryl fluoride, which is now difficult to come across because of its potentially dangerous properties [6].

In the next synthesis, Tolman and Veres used the same fluorinating agent, perchloryl fluoride, for the fluorination of tetramethyl 2-acetamido-2,4-dicarboxyglutarate at position 4 [7]. The same procedure was used by Alexeeva *et al.*, except that the ethyl ester was employed [8].

Bergmann's original approach to 4-fluoroglutamic acid by the ammonolysis of 2-bromo-4-fluoroglutaric acid failed [9]. However, he succeeded in preparing the 4-fluoroglutamic acid via the acetamidomalonate synthesis using ethyl 3-chloro-2-t-butoxypropionate, deprotection of the hydroxy group and substitution of fluorine for the hydroxy group using 2-chloro-1,1,2-trifluorotriethylamine [10].

All the 4-fluoroglutamic acids prepared by hydrolyses of the esters as described above were racemic, and their configuration was not determined. Their melting points were within the range 188–192 °C [3, 6, 10] and 180–186 °C [7, 8].

Later, Unkeless and Goldman prepared D-4-fluoroglutamic acid (a mixture of *erythro* and *threo*) by the treatment of DL-4-fluoroglutamic acid with glutamic acid decarboxylase, converting the L enantiomers to α-amino-γ-fluorobutyric acid and leaving the D-amino acids behind [11]. They also succeeded in separating DL-*erythro*- and DL-*threo*-4-fluoroglutamic acids on ion-exchangers, and in isolating L-*erythro*- and L-*threo*-4-fluoroglutamic acids by enzymatic cleavage of the L-*N*-leucyl-4-fluoroglutamic acids by leucine aminopeptidase [12]. Unfortunately, neither the melting points nor the NMR spectra of any of the individual compounds were mentioned in their papers.

A better characterization of both L-*erythro*- and L-*threo*-4-fluoroglutamic acid was achieved by Bory *et al.* [13], who reported both proton and fluorine NMR spectra and specific rotations for both diastereomers. However, the melting points were again lacking.

Separation of the diastereomers of 4-fluoroglutamic acid was further accomplished by Tolman *et al.*, who separated the DL diastereomers by gas–liquid chromatography after appropriate derivatization [14]. The enantiomers of both 4-fluoroglutamic acids were prepared by a chromatographic technique using chiral columns [15]. Tolman *et al.* also isolated successfully all the four stereoisomers by physical methods [16]. They reported the melting points for DL-*erythro* and DL-*threo*-4-fluoroglutamic acid as being 172 °C (99% *erythro*) and 189 °C (91% *threo*), respectively. Judging from the melting points, the DL-4-fluoroglutamic acids synthesized thus far were probably *threo*. Certainly Bergmann's DL-4-fluoroglutamic acid [10] was *threo*, since the pattern of its ^{19}F NMR spectra is identical with that of the compound characterized as the *threo* isomer by Bory *et al.* [13], i.e. quintet 1:2:2:2:1, in contrast to the *erythro* isomer which shows a heptet 1:1:1:2:1:1:1.

4-Fluoroglutamic acid alone proved to be inefficient as a cancerostatic, but its derivative fluoromethotrexate showed promising cancerostatic activity

[17–19]. 4-Fluoroglutamic acid also became a focus of interest in the enzymologic studies [11, 12, 18–23] and in the studies of the effects on central neuronal mechanism [24, 25].

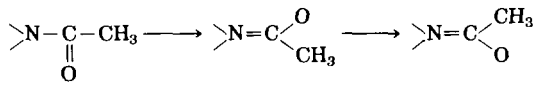
Since DL-fluoroglutamic acid available from the suppliers of chemicals is a mixture of diastereomers and of enantiomers and is very expensive, it was desirable to try to find a stereospecific synthesis which would yield pure stereoisomers. The syntheses of (+)-L-*threo*-, (−)-D-*erythro*-, (−)-D-*threo*- and (+)-L-*erythro*-4-fluoroglutamic acids starting from (−)-*trans*-4-hydroxy-L-proline and from (+)-*cis*-4-hydroxy-D-proline, respectively, will be described in this paper*.

Results

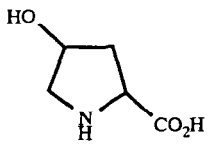
A chiral starting material, suitable for the stereospecific syntheses of 4-fluoroglutamic acids, was found in the natural and relatively cheap (−)-*trans*-4-hydroxy-L-proline (**1a**) (see Scheme 1). It was first converted to 1-acetyl-*trans*-4-hydroxy-L-proline (**2a**) by treatment with acetic anhydride in acetic acid [26]. The acetylated acid was esterified to its methyl ester **3a** by diazomethane [27]. Methyl 1-acetyl-*trans*-4-hydroxy-L-prolinate (**3a**) was then treated with diethylaminosulfur trifluoride (DAST) [28] or with 2-chloro-1,1,2-trifluorotriethylamine [29], which replaced the hydroxylic group by fluorine with predominant inversion of configuration, yielding mixtures consisting of 4.5% of methyl 1-acetyl-*trans*-4-fluoro-L-prolinate (**4a**) and 95.5% of its *cis*-diastereomer **4c****. The composition of the fluorination product

*When the present work was essentially finished, it was learned via a personal communication from Dr J. K. Coward of the University of Michigan that his group was working independently on the synthesis of (+)-L-*threo*-4-fluoroglutamic acid using practically the same reaction sequence. The synthesis of (+)-L-*threo*-4-fluoroglutamic acid was reported by the author at the 9th Winter Fluorine Conference in St. Petersburg, FL, Feb. 2, 1989, and that of (−)-D-*erythro*-4-fluoroglutamic acid at the 10th Winter Fluorine Conference in St. Petersburg, FL, Jan. 29, 1991. Syntheses of both 4-fluoroglutamic acids together with their X-ray diffraction data have been published as a communication [35].

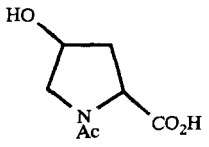
The ratio of the diastereomers of methyl 1-acetyl-4-fluoro-prolinates was determined from the ratio of signals at −174 ppm (*cis* isomers) and −178 ppm (*trans* isomers). The signals in the ^{1}H NMR spectra were rather complex and showed two sets of signals for the hydrogens at position 5 (geminal with fluorine), at position 2 (geminal with methoxy carbonyl) and in methyl groups in the ester and in acetyl. The ratios of the two sets were different in isomers **4a, **d** and **4b**, **c**. Originally, these ratios were erroneously ascribed to the ratios of the *cis* and *trans* diastereomers (see ref. 35). But when the ^{1}H NMR spectra of compounds **4a** and **4b** were taken at temperatures of 60, 80 and 100 °C, respectively, the two sets of above signals merged to single signals. This shows that the differences between the signals are most probably due to the generation of a new pair of *EZ* stereoisomers in the form of the amide groups having the double bond between nitrogen and the acetyl carbon, i.e.



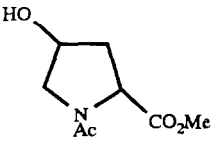
The ratio of the two sets in the *cis* compounds **4b**, **c** was approximately 6:4, and in the *trans* compounds **4a**, **d** about 8:2.



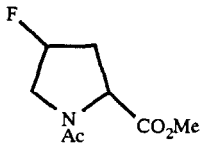
1 a-d



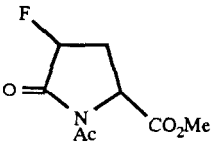
2 a-d



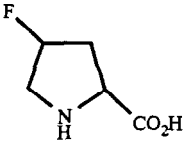
3 a-d



4 a-d



5 a-d



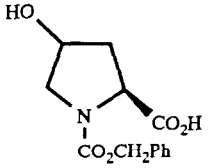
6 a-d

a: trans-L-; b: cis-D-; c: cis-L-; d: trans-D-

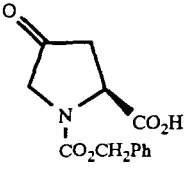
$HO_2CCHFCH_2CH(NH_2)CO_2H$

7 a: (+)-threo-L-; b: (-)-erythro-D-
c: (-)-threo-D-; d: (+)-erythro-L-

8



9



a: trans-L-
b: cis-L-

Scheme 1.

depended on the reaction conditions. The best stereospecificity was achieved using DAST and carrying out the reaction at ice-bath temperature [30]. A similar mixture of diastereomers containing 13–18% of **4b** and 82–87% of **4d** was obtained by treatment of compound **3b**, prepared from *cis*-4-hydroxy-D-proline (**1b**) by acetylation and treatment with diazomethane.

Oxidation of the mixture of the fluorinated esters **4a**, **4c** with ruthenium tetroxide, generated *in situ* from ruthenium dioxide and sodium periodate [31], afforded methyl 1-acetyl-*cis*-4-fluoro-L-pyrrolidin-5-one-2-carboxylate (methyl 1-acetyl-4-fluoro-L-pyroglutamate) (**5c**). The yields were not optimized and were rather low as the oxidation was incomplete with up to 30% of the starting material unaffected even after several days.

The results of the oxidation improved if much larger amounts of the ruthenium dioxide and especially sodium periodate were used than recommended in the literature [31]. It was also noticed that not every ruthenium

dioxide reacted as expected. According to the literature [32], the essential prerequisite for a successful conversion to ruthenium tetroxide is the use of ruthenium dioxide hydrate with at least 20% water. Anhydrous oxide or its hydrate with less than 20% water gave poor results. Alternative oxidants for the conversion of amides to imides, such as organic peroxy acids [33] or benzyltriethylammonium permanganate [34], failed entirely.

The *cis* configuration of compound **5c** was inferred from its hydrolysis with hydrochloric acid to (+)-L-*threo*-4-fluoroglutamic acid [(+)-(2*S*, 4*R*)-fluoroglutamic acid)] (**7a**) whose NMR spectra and specific rotation agree with those of the *threo* isomer [13].

The *trans* configuration of compound **5d**, prepared by the oxidation of **4d**, obtained in turn from **1b** by the sequence of reactions described for the synthesis of **5c**, was similarly proven by its hydrolysis to (−)-D-*erythro*-4-fluoroglutamic acid [(−)-(2*R*, 4*S*)-fluoroglutamic acid] (**7b**) in agreement with the literature data on its physical constants [13].

The X-ray diffraction structures of both 4-fluoroglutamic acids **7a** and **7b** have been published in a preliminary publication [35].

The starting materials for the syntheses of the antipodes **7c** and **7d** of the described 4-fluoroglutamic acids **7a** and **7b**, viz. *trans*-4-hydroxy-D-proline (**1d**) and *cis*-4-hydroxy-L-proline (**1c**), respectively, are either very expensive or not available and, hence, had to be prepared by inversion of configuration at carbon 4 of the pyrrolidine ring.

The most straightforward inversion of configuration can be achieved by the method using diethyl azodicarboxylate and triphenylphosphine [36]. In the presence of formic acid, methyl 1-acetyl-*cis*-4-hydroxy-D-prolinate (**3b**) was converted to the formate of methyl 1-acetyl-*trans*-4-hydroxy-D-prolinate **3d**. During the isolation of the product by chromatography on Florisil, the formate suffered hydrolysis and **3d** was obtained by elution with acetone, albeit in the low yield of 21–37%. The disadvantage of this method of inversion is the necessity for chromatography in the isolation of the product and the circumstance that the product is isolated only after all the less polar byproducts have been eluted first. Alternatives for better isolation of the product are being pursued.

Treatment of **3d** with DAST in the described manner afforded a mixture containing 86–88% of methyl-1-acetyl-*cis*-4-fluoro-D-proline (**4b**) and 12–14% of its *trans* isomer **4d**. Subsequent oxidation with ruthenium tetroxide gave methyl 1-acetyl-*cis*-4-fluoro-D-pyrrolidin-5-one-2-carboxylate (**5b**) which on hydrolysis yielded D-*threo*-4-fluoroglutamic acid (**7c**).

L-*erythro*-4-Fluoroglutamic acid (**7d**) can be prepared from methyl 1-acetyl-*trans*-4-fluoro-L-prolinate (**4a**) obtainable from methyl 1-acetyl-*cis*-4-hydroxy-L-prolinate (**3c**), which in turn was prepared from the *trans* isomer **3a** by inversion at carbon 4. The procedure used for the analogous conversion of **3b** to **3d** proved to be not very good. The alternative of converting the acetyl derivatives **2a** to **2c** by oxidation followed by stereoselective reduction has been described as being unsuccessful [37]. However, the oxidation–reduction sequence was found to work for *N*-benzyloxycarbonyl prolines

[37]. 1-Benzyloxycarbonyl-*trans*-4-hydroxy-L-proline (**8a**) was oxidized with chromium trioxide to 1-benzyloxycarbonyl-4-keto-L-proline (**9**), whose stereospecific reduction with sodium borohydride gave 1-benzyloxycarbonyl-*cis*-4-hydroxyproline (**8b**). Deprotection of **8b** afforded *cis*-4-hydroxy-L-proline (**1c**).

The routine acetylation and methylation of **1c** followed by treatment of the product **3c** with DAST yielded a mixture containing 88.5–100% of *trans*-**4a** and 0–11.5% of *cis*-**4c**. Oxidation of this mixture gave pure methyl 1-acetyl-*trans*-4-fluoro-L-pyrrolidin-5-one-2-carboxylate (**5a**) whose hydrolysis led to L-*erythro*-4-fluoroglutamic acid (**7d**).

The oxidations of methyl 1-acetyl-4-fluoroprolinates with ruthenium tetroxide were often incomplete, leaving part of the starting material intact. From the products of such oxidations of methyl 1-acetyl-*cis*-4-fluoro-L-prolinate (**4c**) contaminated with 4.5% of the *trans* isomer **4a**, (−)-*cis*-4-fluoro-L-proline (**6c**) and (−)-*trans*-4-fluoro-L-proline (**6a**) were isolated by an ion-exchange technique. Similarly, (+)-*trans*-4-fluoro-D-proline (**6d**) was obtained from incompletely oxidized methyl 1-acetyl-*trans*-4-fluoro-D-prolinate (**4d**).

Experimental

All the starting materials were of commercial grade and were used without further purification. Melting points were determined on a Thomas–Hoover Unimelt apparatus and are reported uncorrected. ^{1}H and ^{19}F NMR spectra were taken on a Bruker WP270 SY instrument at 270 MHz, and on a Bruker WP 200 SY instrument at 188.312 MHz, respectively, using solutions in deuteriochloroform, deuterated dimethyl sulfoxide, deuterium oxide and trifluoroacetic acid (when necessary), with tetramethylsilane (TMS), sodium 4,4-dimethyl-4-silapentanesulfonate (DSS), trifluoroacetic acid (TFA) and hexafluorobenzene (HFB) as internal standards. ^{19}F NMR shifts are negative east (upfield) from fluorotrichloromethane (F11). Specific rotations were measured on a Perkin-Elmer 241 polarimeter. Elemental analyses were done by Galbraith Laboratories, Inc., Knoxville, TN.

With the exception of (−)-D-*erythro*-4-fluoroglutamic acid, all the three isomers exhibited specific rotations which differed from those reported in the literature. However, all three isomers were found to be diastereomerically and enantiomerically pure by chiral chromatography.

*1-Acetyl-*trans*-4-hydroxy-L-proline (**2a**)*

The title compound was prepared from Aldrich's *trans*-4-hydroxy-L-proline, m.p. 273–275 °C (dec.), $[\alpha]_D^{25} - 75.3$ °C (c 20, H_2O), (**1a**) according to the literature [38], in 73.8% yield. Melting point, 133–134 °C (AcOEt), lit. values [26], 131–132 °C, [38], 133–134 °C; monohydrate, m.p., 75–78 °C, lit. value [38] 74–76 °C, $[\alpha]_D^{25} - 120.8°$ (c 3.2, H_2O), lit. values [38], $[\alpha]_D^{20} - 116.5°$ (c 3.2, H_2O), [26], $[\alpha]_D^{28.5} - 117.6°$ (c 3, H_2O), [27], $[\alpha]_D - 118°$ (c 1.5).

*1-Acetyl-*cis*-4-hydroxy-D-proline (**2b**)*

The title compound was prepared analogously from Aldrich's *cis*-4-hydroxy-D-proline, m.p. 251 °C (dec.), $[\alpha]_D$ +58° (c 2, H_2O) (**1b**), in a quantitative yield. Melting point 144–145 °C (AcOEt), lit. value [39], 145.5 °C.

*Methyl 1-acetyl-*trans*-4-hydroxy-L-prolinate (**3a**)*

Esterification of **2a** with diazomethane according to the literature [27] gave **3a** in 92.5% yield. Melting point 80–82.5 °C (dioxan/ether 1:4), lit. value [27], 78 °C.

*Methyl 1-acetyl-*cis*-4-hydroxy-D-prolinate (**3b**)*

Compound **2b** afforded similarly a quantitative yield of **3b**, m.p., 81–82 °C (EtOH Et_2O), lit. value [40], 79–80 °C for DL form; $[\alpha]_D^{25}$ +45.76° (c 1, $CHCl_3$).

*Methyl 1-acetyl-*cis*-4-fluoro-L-prolinate (**4c**) (nc)*

(a) Procedure using diethylaminosulfur trifluoride

In an apparatus consisting of a 50 ml round-bottomed flask, an adapter fitted with a separatory funnel and a calcium chloride tube, and a magnetic stirring bar, 1.87 g (0.01 mol) of methyl 1-acetyl-*trans*-4-hydroxy-L-prolinate (**3a**) was dissolved in 15 ml dichloromethane, the solution cooled with an ice–water bath and a solution of 1.89 g (0.0117 mol, 17% excess) of diethylaminosulfur trifluoride (DAST) added dropwise over a period of 40 min. After 15 h the mixture was poured onto 5 g of ice, the aqueous layer separated, the organic layer washed with a 5% solution of sodium bicarbonate to pH 7, dried with anhydrous magnesium sulfate and then evaporated at 44 °C at 15 mmHg pressure. The residue, an orange syrup (1.30 g, 68.7%), was a mixture consisting of 95.5% *cis* isomer **4c** and 4.5% *trans* isomer **4a**, as determined by the ratio of the signals in the ^{19}F NMR spectrum at −174.2 and −178.3 ppm, respectively. The pure compound **4c**, which gave a satisfactory elemental analysis, was obtained as a light yellow oil by chromatography over silica gel containing 10% water on elution with ethyl acetate after eluting the impurities with ether. The same results were obtained using a reverse technique, adding a solution of **3a** to a solution of DAST in dichloromethane.

Data for **4c**: Analysis: Found: C, 50.67; H, 6.87; F, 10.32; N, 7.33%. Calcd. for $C_8H_{12}FNO_3$ (189.19): C, 50.77; H, 6.40; F, 10.04, N, 7.40%. ^{19}F NMR ($CDCl_3$, HFB) δ: −174.14 (m) ppm. ^1H NMR ($CDCl_3$, TMS) δ: 2.05 (40%) and 2.13 (60%) (s, 3, $COCH_3$); 2.34 (m, 1 CH_2); 2.56 (m, 1, CH_2); 3.76 (60%) and 3.82 (40%) (s, 3, OCH_3, partly covers signal of another H); 3.91 (t, 1, CH_2N); 4.53 (40%) and 4.79 (60%) (d, 1, $CHCO_2H$); 5.26 (40%) and 5.34 (60%) (1, dt, CHF, $J_{HF_{gem}}$ = 52.4 Hz) ppm. At 100 °C in DMSO-d_6, the following signals were observed: 1.94 (CH_3CO); 3.65 (OCH_3); 4.61 ($CHCO_2H$); and 5.29 (CHF) ppm.

(b) Procedure using 2-chloro-1,1,2-trifluorotriethylamine

In the same apparatus as described in the preceding paragraph, a solution consisting of 6.00 g (0.0316 mol) of 2-chloro-1,1,2-trifluorotriethylamine [29] in 20 ml dichloromethane was added portionwise to a magnetically-stirred solution of 5.20 g (0.0278 mol) of methyl 1-acetyl-*trans*-4-hydroxy-L-prolinate (**3a**) in 45 ml dichloromethane. The slightly exothermic reaction was accompanied by a change of color to a yellow tinge. After 16.5 h at room temperature, the mixture was shaken with 5 ml water, the organic layer washed with 7 ml of a saturated solution of sodium bicarbonate to pH 7, dried over anhydrous magnesium sulfate and then evaporated at 41 °C at 20 mmHg pressure. The yellow oily residue was distilled to give 3.85 g (73%) of the coproduct *N,N*-diethylchlorofluoroacetamide [b.p., 46–50 °C/0.05 mmHg] and 4.11 g (78%) of a mixture of *trans* and *cis* methyl 1-acetyl-4-fluoro-L-prolinate (**4a** and **4c**) [b.p. 105–110 °C/0.025 mmHg]. The light yellow product contained 14% of the coproduct, *N,N*-diethylchlorofluoroacetamide. It was used without further purification, as the coproduct can be easily separated in the final stage of the synthesis of 4-fluoroglutamic acid.

*Methyl 1-acetyl-*trans*-4-fluoro-D-prolinate (**4d**) (nc)*

Method a

A solution consisting of 5.25 g (0.0326 mol) of *N,N*-diethylaminosulfur trifluoride (DAST) in 15 ml dichloromethane was added over a period of 15 min to a stirred solution consisting of 5.37 g (0.0288 mol) of methyl 1-acetyl-*cis*-4-hydroxy-D-prolinate (**3b**) in 25 ml dichloromethane cooled in a dry-ice/acetone bath. After 16 h while the bath warmed to room temperature, the mixture was washed with 5 ml water, 15 ml of a 5% solution of sodium bicarbonate, 2 ml water, dried with magnesium sulfate and then evaporated at 40 °C at 8 mmHg pressure. The partly crystalline residue was triturated with 2 ml benzene and the mixture filtered by suction to give 1.68 g of yellow crystals. Recrystallization from a mixture of 15% benzene and 85% hexane gave a product melting at 100–101.5 °C. From the mother liquors, additional amounts of the crystalline product were obtained to yield ultimately 2.45 g (49.8%) of methyl 1-acetyl-*trans*-4-fluoro-D-prolinate (**4d**). Although the reaction of **3b** was never undertaken with ice cooling rather than dry-ice cooling, it is most probable that the same results would have been obtained, as this was the case with the preparation of all the rest of the stereoisomers **4a**, **b**, **c**.

Method b

A solution consisting of 3.50 g (0.0185 mol) of 2-chloro-1,1,2-trifluorotriethylamine in 5 ml dichloromethane was added all at once to a stirred solution consisting of 2.64 g (0.014 mol) of methyl 1-acetyl-*cis*-4-hydroxy-D-prolinate (**3b**) in 15 ml dichloromethane at room temperature. After 15.5 h, the mixture was treated with 5 ml water, neutralized with sodium bicarbonate, washed with 3 ml water, dried with magnesium sulfate and then evaporated

at 40 °C at 9 mmHg pressure. The yellow oil, a mixture of the product **4b** and **4d** and the coproduct *N,N*-diethylchlorofluoroacetamide, was distilled to give 2.20 g of partly crystalline **4d** [b.p., 103–109 °C/0.04 mmHg] containing 26% of the coproduct. Crystallization from 5% benzene in hexane gave **4d**, m.p., 100.5–101.5 °C. Total yield of **4d** was 1.59 g (60%).

Data for **4d**: Analysis: Found: C, 50.75; H, 6.41; F, 9.92; N, 7.28%. Calcd. for $C_8H_{12}FNO_3$ (189.19): C, 50.77; H, 6.40; F, 10.04; N, 7.40%. ^{19}F NMR ($CDCl_3$, HFB) δ: −178.16 (m) ppm. ^1H NMR ($CDCl_3$, TMS) δ: 1.88 (20%) and 2.00 (80%) (s, 3, $COCH_3$); 1.95–2.14 (m, 1, CH_2); 2.44–2.59 (m, 1, CH_2); 3.68 (80%) and 3.74 (20%) (s, 3, OCH_3, partly covers signals of H in CH_2N); 3.62–3.80 (m, 2, CH_2N, partly covered by the signal of OCH_3); 4.23 (20%) and 4.49 (80%) (t, 1, $CHCO_2H$); 5.15 (20%) and 5.25 (80%) (1, dt, $J_{HF_{gem}} = 51.88$ Hz) ppm. At 100 °C in DMSO-d_6, the following signals were observed: 1.95 (CH_3CO); 3.66 (OCH_3); 4.52 ($CHCO_2H$; and 5.33 (CHF) ppm.

*Methyl 1-acetyl-*cis*-4-fluoro-L-pyrrolidin-5-one-2-carboxylate (**5c**) (nc)*

Method a

A solution consisting of 1.68 g (0.0083 mol) of methyl 1-acetyl-4-fluoro-L-prolinate (**4a, c**) [from procedure a above using diethylaminosulfur trifluoride] in 30 ml ethyl acetate was added portionwise over 25 min to a stirred yellow solution of ruthenium tetroxide, prepared in a 125 ml Erlenmeyer flask by adding 2.0 g (*c.* 0.0132 mol) of ruthenium dioxide dihydrate to a solution of 9 g (0.021 mol) of sodium periodate in 81 ml water. Addition of each portion of the solution of the methyl ester to the solution of the oxidant produced instantaneous blackening of the mixture which resumed its original orange color within seconds. After stirring the reaction mixture overnight at room temperature, the lower aqueous layer was separated, extracted with three 20 ml portions of ethyl acetate, and the combined organic layers were stirred overnight with 2 ml isopropyl alcohol to reduce the excess of oxidant. After the suction filtration removing the black ruthenium dioxide, the filtrate was washed with 25 ml water, the organic layer separated, treated with activated charcoal, dried with anhydrous magnesium sulfate and evaporated at 30–35 °C/20 mmHg. The yellow oil (1.00 g) crystallized after cooling and seeding, giving 0.78 g (46%) of light yellow crystals of methyl 1-acetyl-*cis*-4-fluoro-L-pyrrolidin-5-one-2-carboxylate (**5c**), m.p., 104–105 °C. Crystallization from a small amount of methanol gave 0.37 g (22%) of an analytically pure product, m.p., 105–106 °C. The rest of the crude product was the recovered methyl 1-acetyl-4-fluoro-L-prolinate (**4a, c**).

Data for **5c**: Analysis: Found: C, 47.24; H, 4.95; F, 9.42; N, 6.81%. Calcd. for $C_8H_{10}FNO_4$ (203.17): C, 47.29; H, 4.96; F, 9.35; N, 6.90%. ^{19}F NMR ($CDCl_3$, HFB) δ: −188.68 (ddd, $J_{HF_{gem}} = 51.44$ Hz, $J_{HF_{vic}} = 23.43$ Hz) ppm. ^1H NMR ($CDCl_3$, TMS) δ: 2.56 (s, 3, CH_3CO); 2.26 (m, 1, CH_2; $J_{HF_{vic}} = 24.10$ Hz, $J_{HH_{gem}} = 14.48$ Hz, $J_{HH_{vic}} = 5.56$ Hz); 2.80 (m, 1, CH_2; $J_{HF_{vic}} = 20.20$ Hz, $J_{HH_{gem}} = 14.48$ Hz, $J_{HH_{vic}} = 8.32$ Hz); 3.79 (s, 3, OCH_3); 4.64 (dd, 1, CHN; $J_{HH} = 8.46$, 5.68 Hz); 5.14 (dt, 1, CHF; $J_{HF_{gem}} = 51.28$ Hz, $J_{HH} = 7.58$, 5.6 Hz) ppm.

Method b

The oxidation of methyl 1-acetyl-4-fluoro-L-prolinate (**4a, c**) with ruthenium tetroxide was also achieved using the less pure ester containing 14% of *N,N*-diethylchlorofluoroacetamide as the impurity [from procedure b above using 2-chloro-1,1,2-trifluorotriethylamine]. The procedure was carried out using 3.77 g of **4a, c** (0.0172 mol of the pure ester **4a, c**) dissolved in 75 ml ethyl acetate, and oxidizing the mixture with a solution of 2.80 g (0.0165 mol) of ruthenium dioxide dihydrate in a solution of 22 g (0.103 mol) of sodium periodate in 200 ml water. After 23.5 h at room temperature, the reaction mixture was worked-up as described in procedure a. The ethyl acetate extracts (three times with 50 ml portions of ethyl acetate) left, after evaporations at 50 °C/0.1 mmHg, 2.01 g of a yellow solid which on recrystallization from a few ml methanol afforded 0.71 g (20.4%) of methyl 1-acetyl-*cis*-4-fluoro-L-pyrrolidin-5-one-2-carboxylate (**5c**), m.p. 93–96 °C. Evaporation of the mother liquors at 40 °C/20 mmHg gave 0.98 g of a yellow oil which was a mixture of **5c** and unreacted **4c**. It was used for the preparation of (+)-L-*threo*-4-fluoroglutamic acid (**7a**) and of *cis*-4-fluoro-L-proline (**6c**) in procedure b (*vide infra*).

The aqueous layer after the extraction of the reaction mixture with ethyl acetate was evaporated to dryness at 50 °C/20 mmHg, and the white solid was twice boiled with 100 ml portions of ethyl acetate. The combined extracts gave upon evaporation at 50 °C/0.07 mmHg 1.43 g of a yellow oil containing, according to ^{19}F NMR spectroscopy, 68.5% (0.98 g) of the recovered methyl 1-acetyl-4-fluoro-L-prolinate, 13.5% of *N,N*-diethylchlorofluoroacetamide and 18% of a compound with a signal at −192.5 ppm, probably 1-acetyl-*cis*-4-fluoro-L-pyrrolidin-5-one-2-carboxylic acid) (*N*-acetylpyroglutamate).

*Methyl 1-acetyl-*trans*-4-fluoro-D-pyrrolidin-5-one-2-carboxylate (**5d**) (nc)*

To a vigorously stirred solution consisting of 11.3 g (0.0528 mol) of sodium periodate in 102 ml water was added all at once 1.5 g of ruthenium dioxide hydrate (Aesar, 58% Ru). The black suspension turned to a bright yellow solution, to which a solution of 0.85 g (0.00418 mol) of methyl 1-acetyl-*trans*-4-fluoro-D-prolinate (79% of *trans*-**4d** and 21% of *cis*-**4b**) in 50 ml ethyl acetate was added dropwise over a period of 20 min.

After 31 h at room temperature, the orange ethyl acetate layer was separated, the aqueous layer extracted with four 20 ml portions of ethyl acetate, the combined organic layers stirred with 2.5 ml isopropyl alcohol overnight, the black ruthenium dioxide filtered off, the filtrate decolorized by activated charcoal, dried with magnesium sulfate and evaporated at 40 °C/12 mmHg, to give 0.62 g (68%) of **5d**, lanolin-like smelling crystals, m.p. 75–80 °C, containing, according to ^{19}F NMR spectroscopy, 87% of **5d** and 13% of unreacted **4d**. Recrystallization from methanol gave 0.37 g (40.6%) of pure **5d**, m.p. 98.2–98.6 °C; $[\alpha]_D^{25}$ $-63.5 \pm 0.6°$ (c 1, $CHCl_3$).

Data for **5d**: Analysis: Found: C, 47.53; H, 4.89; F, 9.38; N, 6.85%. Calcd. for $C_8H_{10}FNO_4$ (203.17): C, 47.29; H, 4.96; F, 9.35; N, 6.90%. ^{19}F NMR ($CDCl_3$, HFB) δ: −193.46 (ddd, $J_{HF_{gem}}$ = 51.14 Hz, $J_{HF_{vic}}$ = 22.37 Hz) ppm. ^1H NMR ($CDCl_3$, TMS) δ: 2.59 (s, 3, CH_3CO); 2.37–2.69 (m, 2, CH_2); 3.80 (s, 3, OCH_3); 4.80 (dd, 1, CHN); 5.29 (ddd, 1, CHF, $J_{HF_{gem}}$ = 51.55 Hz, J_{HH} = 9.53 Hz) ppm.

*(+)-D-*threo*-4-Fluoroglutamic acid (**7a**)*

Method a

A solution consisting of 0.71 g (0.0035 mol) of methyl 1-acetyl-*cis*-4-fluoro-L-pyrrolidin-5-one-2-carboxylate (**5c**) in 3 ml concentrated hydrochloric acid was stirred and refluxed for 4 h. The resulting light yellow solution was evaporated at 50 °C/11 mmHg to yield a light brown oil (0.91 g) which solidified to give light yellow rosette-like crystals of the hydrochloride of 4-fluoroglutamic acid, m.p. of crude material, 173–176 °C (dec.). The salt (0.78 g) was dissolved in a minimum amount (2 ml) of warm distilled water, 17% aqueous ammonia was added to reach pH 3 and the solution cooled in ice water. 4-Fluoroglutamic acid was collected in two crops, 0.33 g [m.p., 196–197.5 °C (dec.)] and 0.04 g [m.p., 191–192 °C (dec.)]. After evaporation of the filtrate at 45 °C/20 mmHg, an additional 0.01 g of product [m.p., 186–187 °C (dec.)] was obtained after adding ethanol. Altogether 0.38 g (65.8%) of (+)-L-*threo*-4-fluoroglutamic acid (**7a**) was obtained.

Method b

The residue obtained after evaporation of the mother liquors in the procedure b of the preparation of **5c** (0.98 g) was combined with the residue obtained on evaporation of the ethyl acetate extract of the solids from the aqueous layer after oxidation of methyl 1-acetyl-*cis*-4-fluoro-L-prolinate (**5c**) (1.43 g). The resulting oil was dissolved in 12 ml concentrated hydrochloric acid and the dark orange solution stirred and refluxed for 4 h. The dark brown solution was decolorized with activated charcoal and the light yellow filtrate was evaporated at 60 °C/11–13 mmHg to yield a light yellow-greenish syrup (2.33 g) which was a mixture of L-*threo*-4-fluoroglutamic acid (**7a**) and 4-fluoro-*cis*-L-proline (**6c**).

The syrup was dissolved in distilled water, the solution neutralized with 1 N sodium hydroxide to pH 7, diluted to 100 ml and treated with 65 g of the strongly basic anion-exchanger AG1-X8 (BioRad) in an acetate cycle. Elution with three 125 ml portions of distilled water gave *cis*-4-fluoro-L-proline (0.94 g of **6c** in the form of the copper salt) (33.3%). Elution of the ion-exchanger with three 125 ml portions of 1.5 N formic acid afforded 0.32 g (0.0019 mol) of L-*threo*-4-fluoroglutamic acid (**7a**). The overall yield of **7a** based on **4a**, **c** was 0.70 g (24.6%). Recrystallization from water gave the amino acid **7a**, m.p., 194–195 °C (dec.).

Method c

A solution consisting of 0.33 g (0.00163 mol) of methyl 1-acetyl-4-fluoro-*cis*-L-pyrrolidin-5-one-2-carboxylate (**5c**) in 1 ml concentrated hydro-

chloric acid was refluxed for 4 h. The contents of the flask were diluted with 5 ml water and the milky solution extracted with 5 ml ether. The aqueous layer was separated, filtered and evaporated at 50 °C/15 mmHg to give 0.33 g of an almost colorless oil. The oil was diluted with 10 ml distilled water, the chloride ions removed by treatment with freshly prepared silver oxide and the solution, after the removal of silver chloride, evaporated at 35–40 °C/20 mmHg to give a glassy residue (0.18 g, still wet, yield less than 67%). The glassy residue was stirred with 0.5 ml distilled water and stored overnight in a refrigerator. Crystals that deposited at the bottom of the test tube were redissolved in water and allowed to cool in the refrigerator. The white crystals were filtered with suction and washed with a drop of water. The product **7a** melted at 191–192 °C (dec.) [197–198.5 °C (dec.) after recrystallization from 0.5 ml water].

The melting points of the 4-fluoroglutamic acid varied depending on the rate of heating. In some cases, melting points as high as 200–201 °C were found when the samples were inserted into the apparatus at 150–160 °C.

Data for **7a**: Analysis: Found: C, 36.34; H, 4.75; F, 11.14; N, 8.41%. Calcd. for $C_5H_8FNO_4$ (165.04): C, 36.37; H, 4.87; F, 11.51; N, 8.48%. $[\alpha]_D^{25}$ +13.35° (c 1, 1 N HCl) (diastereomerically and enantiomerically pure by chiral GC [41]), lit. values [13], $[\alpha]_D^{20}$ +14.6°, [41], $[\alpha]_D^{20}$ +15.03° (1 N HCl). ^{19}F NMR (in CF_3CO_2H) δ: −191.33 (quintet 1:2:2:2:1, $J_{HF_{gem}}$ = 48.57 Hz, $J_{HF_{vic}}$ = 24.24 Hz) ppm. ^{1}H NMR (in CF_3CO_2H) δ: 2.77 (dt, 2H, CH_2, $J_{HF_{vic}}$ = 24.75 Hz, J_{HH} = 5.7 Hz); 4.55 (m, 1H, $CH\overset{+}{N}H_3$); 5.34 (dt, 1H, CHF, $J_{HF_{gem}}$ = 48.1 Hz, J_{HH} = 5.8 Hz); 6.96 (m, $\overset{+}{N}H_3$) ppm. Lit. values [13]: 2.75 (dt, J = 23.4 Hz, 5.4 Hz); 4.5 (m); 5.26 (dt, J = 48 Hz, 5.4 Hz); 7.42 (s) ppm.

*(−)-*D-erythro-*4-Fluoroglutamic acid (7b)*

Method a

The mixture of **4d** and **5d** containing 1.25 g (0.002 mol) **5d** was refluxed with 7 ml concentrated hydrochloric acid for 4 h. The light brown liquid was cooled, extracted with two 5 ml portions of dichloromethane, the aqueous layer decolorized with activated charcoal and evaporated at 53 °C/8 mmHg. The crystalline residue [1.53 g, containing, according to ^{19}F NMR spectroscopy, 75% of the hydrochloride of D-*erythro*-4-fluoroglutamic acid (**7b**) and 22% of the hydrochloride of *trans*-4-fluoro-D-proline (**6d**)] was dissolved in about 3 ml warm water and neutralized with 18% aqueous ammonium hydroxide to pH 3. Since no product crystallized even in a refrigerator, 20 ml ethanol was added; this precipitated a semisolid material. After decanting the supernatant, the residue was dissolved in 2 ml boiling water and the solution allowed to evaporate. From the semicrystalline residue, 0.17 g (0.00103 mol) of crude crystalline D-*erythro*-4-fluoroglutamic acid (**7b**), m.p., 175–181 °C, was obtained by suction filtration. The filtrate was neutralized with 1 N sodium hydroxide to pH 7 and treated with 30 ml of a suspension of the ion-exchanger AG1-X8 in an acetate cycle. Elution with water afforded *trans*-4-fluoro-D-proline (**6d**) and 0.39 (0.0026 mol) of crystalline D-*erythro*-4-

fluoroglutamic acid (**7b**). The yield of crude crystalline **7b** was 54.9%. After recrystallization from water, 0.33 g (32.3%) of pure **7b** was obtained, m.p., 180–180.5 °C or 180.5–182 °C (dec.) depending on the rate of heating. The mixed melting point with L-*threo*-4-fluoroglutamic acid (**7a**) [m.p., 196–196.5 °C (dec.)] was 180–180.5 °C.

Method b

A solution consisting of 0.87 g (0.0043 mol) of **5d** in 3.75 ml concentrated hydrochloric acid was refluxed for 4 h. The light green solution was evaporated at 40 °C/11 mmHg leaving 1.20 g of a viscous syrup that solidified after scratching to the rosette-like crystals, the hydrochloride of **7b**. The solid was dissolved in 2.5 ml warm water and neutralized by 2.5 ml of 18% aqueous ammonium hydroxide (pH 3) to give 0.36 g of crystalline **7b**. An additional 0.02 g of **7b** was obtained by evaporation of the mother liquor, and 0.16 g of **7b** was isolated by adding 7 ml ethanol to the filtrate. The combined crude products were recrystallized from a minimum amount of water to yield 0.32 g (45.3%) of pure **7b**, m.p., 175.5–179 °C. An additional recrystallization afforded analytically pure (−)-D-*erythro*-4-fluoroglutamic acid (**7b**), m.p., 181–182 °C (dec.); $[\alpha]_D^{25}$ −36.1±0.015 ° (c 1, 1 N HCl), lit. values [41], $[\alpha]_D^{20}$ −36.41° (1 N HCl), [13], $[\alpha]_D^{20}$ +32° for the L-*erythro* compound.

Data for **7b**: Analysis: Found: C, 36.37; H, 4.88; F, 11.31; N, 8.47% . Calcd. for $C_5H_8FNO_4$ (165.04): C, 36.37; H, 4.87; F, 11.51; N, 8.48%. ^{19}F NMR (in CF_3CO_2H) δ: −191.39 (heptet 1:1:1:2:1:1:1, $J_{HF_{gem}}$ = 47.91 Hz, $J_{HF_{vic}}$ = 20.67, 13.95 Hz) ppm. ^1H NMR (in CF_3CO_2H) δ: 2.76 (tm, 1, CH_2, $J_{HF_{vic}}$ = 16.81 Hz); 3.00 (tm, 1, CH_2, $J_{HF_{vic}}$ = 17.07 Hz) (two diastereotopic H in CH_2); 4.55 (m, 1, CHN); 5.48 (ddd, 1, CHF, $J_{HF_{gem}}$ = 48.17 Hz, J_{HH} = 8.3 Hz); 7.85 (m, 3, $\overset{+}{N}H_3$) ppm. Lit. values [13]: −191.9 (sextet (?) 1:1:1:2:1:1:1, J_{HF} = 49.6, 33.4, 16.6 Hz); 2.63 (m, 2H, CH_2); 4.54 (m, 1, CH); 5.35 (td, 1H, CHF, J_{HF} = 48, $J_{HH_{gem}}$ = 9.2, $J_{HH_{vic}}$ = 3.1 Hz); 7.44 (s, 3H) ppm.

Methyl 1-acetyl-trans-*4-hydroxy-*D-*prolinate (**3d**)*

According to the literature [36], to a solution of 1.00 g of 91% pure (0.0049 mol) methyl 1-acetyl-*cis*-4-hydroxy-D-prolinate (**3b**) in 58 ml anhydrous tetrahydrofuran was added successively 2.62 g (0.01 mol) of triphenylphosphine, 0.50 g (0.01 mol) of 91% formic acid, and finally a solution of 1.74 g (0.01 mol) of diethyl azodicarboxylate in 10 ml tetrahydrofuran which was dropped in over a period of 3 min. A slight warming of the reaction mixture was noted. After 24 h at room temperature, the yellow solution was evaporated to dryness at 75 °C/15 mmHg. The crystalline residue (6.10 g) was chromatographed over 120 g of Florisil. After successive elutions with 1260 ml benzene, 1050 ml ether and 400 ml ethyl acetate which led to the recovery of 4.48 g (92.2%) of the byproducts, elution with 450 ml acetone afforded 0.78 g (78%) of **3d** as a yellow oil which crystallized. Recrystallization from 2 ml ethyl acetate gave 0.21 g (21%) **3d**, m.p. 81–82 °C; $[\alpha]_D^{32}$ +92.0° (c 1, $CHCl_3$). The mixed melting point with the *cis* isomer

was 65–70 °C. In another experiment, the yield of crystalline product was 37.9%. Lit. value [27]: 78 °C for the L enantiomer.

*Methyl 1-acetyl-*cis*-4-fluoro-D-prolinate (**4b**) (nc)*

A solution of 2.58 g (0.0138 mol) of methyl 1-acetyl-*trans*-4-hydroxy-D-prolinate (**3d**) in 20 ml dichloromethane was added over a period of 50 min to a stirred solution of 2.60 g (0.016 mol) of DAST in 7 ml dichloromethane, cooled in an ice–water bath. After 15 h, the mixture was worked-up as described in the preparation of **4a**. Evaporation of the solution gave 2.05 g of a syrup containing 73.5% of a mixture of 88% *cis* (**4b**) and 12% *trans* methyl 1-acetyl-4-fluoro-D-prolinate (**4d**). Yield of the pure *cis* isomer **4b** was 50.8%.

*Methyl 1-acetyl-*cis*-4-fluoro-D-pyrrolidin-5-one-2-carboxylate (**5b**) (nc)*

A solution consisting of 2.05 g of crude **4b, d** containing 1.51 g of a mixture of 88% *cis* (**4b**) and 12% *trans* methyl 1-acetyl-4-fluoro-D-prolinate (**4d**) was added over a period of 15 min to a well-stirred yellow solution of ruthenium tetroxide, prepared by adding 2.8 g of ruthenium dioxide dihydrate to a solution of 38.5 g of sodium periodate in 310 ml water. After 45 h stirring at room temperature, the reaction mixture was worked-up as described for the preparation of **5c** and **5d**. The orange oil (0.95 g) did not crystallize. It was a mixture of 10% unreacted **4b, d** and 0.80 g (49.4%) of a mixture consisting of 81.5% *cis* (**5b**) and 18.5% *trans* methyl 1-acetyl-4-fluoro-D-pyrrolidin-5-one-2-carboxylate (**5d**). The yield of the pure *cis* isomer **5b** was 39.9% (44.7% based on reacted **4b, d**).

*(−)-D-*threo*-4-Fluoroglutamic acid (**7c**)*

Refluxing 0.94 g (0.0046 mol) of a mixture containing 10% of unreacted **4b**, 73% *cis* (**5b**) and 17% *trans* methyl 1-acetyl-4-fluoro-D-pyrrolidin-5-one-2-carboxylate (**5d**) with 7 ml concentrated hydrochloric acid for 4 h, evaporation of the solution at 60 °C/40 mmHg and removal of chloride ions with silver carbonate gave 0.38 g (51%) of crude **7c**. Recrystallization from water gave 0.12 g (16.1%) of pure **7c**, m.p. 196.5–197.5 °C (dec.) or 198.5–199.5 °C (dec.) depending on the rate of heating. The mixed melting point with D-*erythro*-4-fluoroglutamic acid (**7b**) was 186–188 °C (dec.) whereas with L-*threo*-4-fluoroglutamic acid (**7a**) no depression was observed; $[\alpha]_D^{26}$ $-13.1° \pm 2.4\%$, lit. value [41], $[\alpha]_D^{20}$ $-14.98°$ (1 N HCl); the compound **7c** was diastereomerically and enantiomerically pure [41].

Data for **7c**: ^{19}F NMR (in CF_3CO_2H) δ: -191.2 ppm. ^1H NMR (in CF_3CO_2H, DDS) δ: 2.86 (1H); 2.98 (1H) (two diastereotropic hydrogens in $-CH_2-$); 4.62 (1H, $CHNH_3$); 5.33 (1H, CHF); and 7.62 (m, 3H, $\overset{+}{N}H_3$) ppm.

*1-Benzyloxycarbonyl-*trans*-4-hydroxy-L-proline (**8a**)*

This was prepared according to the literature [37] in a 91.7% yield as an oil that did not crystallize even in the refrigerator (lit. value [37] m.p., 106–107 °C).

*1-Benzyloxycarbonyl-4-oxo-L-proline (**9**)*

This was prepared according to the literature [37] in a 53% yield, m.p., 99–100 °C (ether/hexane), lit. value [37], 101–102 °C.

*1-Benzyloxycarbonyl-*cis*-4-hydroxy-L-proline (**8b**)*

This was prepared according to the literature [37] in a 50% yield, m.p., 109–111.5 °C (AcOEt), lit. value [37], 110–111 °C.

cis*-4-Hydroxy-L-proline (**1c**)*

A solution consisting of 4.42 g (0.0167 mol) of 1-benzyloxycarbonyl-*cis*-4-hydroxy-L-proline (**8b**) in 7.3 ml of 36% hydrogen bromide in acetic acid was allowed to stand at room temperature for 2 h. Addition of 50 ml ether precipitated the hydrobromide of *cis*-4-hydroxy-L-proline. After 2.5 h stirring, the ether was decanted, the semisolid residue washed with 20 ml ether, the ether decanted, the semisolid residue dissolved in 8 ml methanol and the dark orange solution evaporated at 43 °C/16 mmHg to yield yellow crystals. The hydrobromide was treated with silver carbonate, the silver bromide removed by suction filtration and the filtrate evaporated at 40 °C/15 mmHg to give 2.05 g (92.3%) of crude *cis*-4-hydroxy-L-proline (**1c**). Crystallization from 70% aqueous ethanol yielded 63.8% of the pure compound **1c**, m.p., 254–256 °C; $[\alpha]_D^{25}$ −58.9° (c 2, H_2O). Lit. values [27]: m.p., 248 °C; $[\alpha]_D$ −58° (c 2, H_2O).

*Methyl 1-acetyl-*trans*-4-fluoro-L-prolinate (**4a**) (nc)*

cis-4-Hydroxy-L-proline (**1c**) (2.05 g, 0.0156 mol) was converted to methyl 1-acetyl-*cis*-4-hydroxy-L-prolinate (**3c**) by acetylation [38] followed by methylation [27]. The crude product (3.33 g, 0.0167 mol) dissolved in 30 ml dichloromethane was added dropwise over a period of 80 min into a stirred solution consisting of 3.75 g (0.0233 mol) of diethylaminosulfur trifluoride (DAST) in 12 ml dichloromethane cooled in an ice bath. After 4 h, the mixture was treated with 10 g of ice, the aqueous layer washed with a solution of sodium bicarbonate, then with water, dried with magnesium sulfate and evaporated at 43 °C/13 mmHg leaving 2.52 g of a dark brown oil that crystallized in the refrigerator. The crystals were spread over a porous plate and scraped off yielding 1.30 g of **4a** containing, according to ^{19}F NMR spectroscopy, 88.5% of methyl 1-acetyl-*trans*-4-fluoro-L-proline. Recrystallization from a mixture of 20% benzene and 80% hexane gave 0.88 g of a product, m.p., 91–95 °C. The overall yield based on *cis*-4-hydroxy-L-proline was 24.2%. ^{19}F NMR ($CDCl_3$, HFB) δ: −178.29 (m) ppm.

*Methyl 1-acetyl-*trans*-4-fluoro-L-pyrrolidin-5-one-2-carboxylate (**5a**) (nc)*

A solution consisting of 0.85 g (0.0045 mol) of the 87% pure methyl 1-acetyl-*trans*-4-fluoro-L-prolinate (**4a**) in 35 ml ethyl acetate was added dropwise over a period of 5 min to a solution of ruthenium tetroxide, prepared by adding 1.15 g of ruthenium dioxide dihydrate to a solution of 16 g of sodium periodate in 145 ml water. After stirring the yellow mixture vigorously for 62.5 h, the organic layer was separated, the aqueous layer extracted

with four 30 ml portions of ethyl acetate, the combined organic solutions stirred for 3 h with 8 ml isopropyl alcohol, the precipitated ruthenium dioxide filtered with suction, the filtrate treated with magnesium sulfate and activated charcoal, filtered with suction, and the light yellow filtrate evaporated at 45 °C/12 mmHg giving 0.57 g (62.5%) of an oil that crystallized after seeding. Recrystallization from 2 ml methanol gave pure product **5a**, m.p. 98–99 °C; $[\alpha]_D^{25}$ +37.5° (c 2, MeOH). ^{19}F NMR ($CDCl_3$, HFB) δ: −193.43 (ddd, $J_{HF_{gem}}$ = 51.7 Hz, $J_{HF_{vic}}$ = 22.5 Hz) ppm.

*(+)-L-*erythro*-4-Fluoroglutamic acid (7d)*

A solution consisting of 0.52 g (0.00256 mol) of methyl 1-acetyl-*trans*-4-fluoro-L-pyrrolidin-5-one-2-carboxylate (**5a**) in 4 ml concentrated hydrochloric acid was refluxed for 4 h. The brown liquid was evaporated to dryness at 45 °C/12 mmHg, the residue dissolved in 10 ml water, the solution treated with silver carbonate, and the filtrate after removal of silver chloride evaporated at 40 °C/12 mmHg to give 0.33 g (78%) of a glassy residue. The solid was stirred with 1 ml warm distilled water and 1 ml ethanol was added producing a semisolid precipitate. On evaporation on a watch glass, the material crystallized to give 0.068 g (16.1%) of (+)-L-*erythro*-4-fluoroglutamic acid (**7d**), m.p., 173–177.5 °C. Two recrystallizations from minimum amounts of water gave diastereomerically and enantiomerically pure product [41], m.p., 180.5–182.5 °C or 182–183 °C (dec.) depending on the rate of heating; $[\alpha]_D^{25}$ +33.17° ± 1.2% (c 1, 1 N HCl), lit. values [13], +32° (c 1, 1 N HCl), [41], $[\alpha]_D^{20}$ +36.24° (1 N HCl).

Data for **7d**: ^{19}F NMR (CF_3CO_2H) δ: −191.64 (heptet 1:1:1:2:1:1:1 $J_{HF_{gem}}$ = 47.83 Hz, $J_{HF_{vic}}$ = 13.98, 33.85 Hz) ppm. ^1H NMR (CF_3CO_2H, DSS) δ: 2.74 (m, 1, CH_2); 2.98 (dm, 1, CH_2, $J_{HF_{vic}}$ = 34.82 Hz) (two diastereotopic H in CH_2); 4.64 (m, 1, CHN); 5.53 (dd, 1, CHF, $J_{HF_{gem}}$ = 48.91 Hz) ppm.

*(−)-*cis*-4-Fluoro-L-proline (6c)*

Hydrolysis of crude products arising from the oxidation of **4a, c** with ruthenium tetroxide yielded mixtures of the hydrochlorides of *cis*-4-fluoro-L-proline (**6c**), small amounts of *trans*-4-fluoro-L-proline (**6a**) and (+)-*threo*-L-4-fluoroglutamic acid (**7a**). Treatment of these mixtures, either as such or after neutralization with sodium hydroxide to pH 7, with anion-exchanger AGl-X8 (BioRad) in an acetate or formate cycle, followed by elution with water, afforded (−)-*cis*-4-fluoro-L-proline (**6c**), a small amount of (−)-*trans*-4-fluoro-L-proline (**6a**) and, by elution with 1.5 N formic acid, (+)-L-*threo*-4-fluoroglutamic acid (**7a**). The identity and configuration of **6c** were proven by its melting point, by ^{19}F and ^1H NMR spectroscopy, and by specific rotation. All the parameters correspond to the structure assigned by Gerig and McLeod [42]. Melting point, 266–267 °C (dec.) (90% ethanol); $[\alpha]_D^{25}$ −56.0 (c 1, H_2O). ^{19}F NMR (D_2O, TFA internal) δ: −176.59 (m) ppm. ^1H NMR δ: 2.73 (qd, 1, $J_{HF_{vic}}$ = 13.7 Hz); 2.97 (q, 1); 3.84 (dd, 1, $J_{HF_{vic}}$ = 36.7 Hz); 4.13 (t, 1, $J_{HF_{vic}}$ = 12.7 Hz); 4.94 (m, 1); 5.52 (d, 1, $J_{HF_{gem}}$ = 50.2 Hz) ppm. Lit. values [42]: m.p., 271 °C; $[\alpha]_D^{20}$ −54.9° (c 1, H_2O); ^{19}F NMR (D_2O,

HFB external) δ: −171.266 ppm; ^{1}H NMR δ: 2.93; 2.97; 3.88; 4.18; 4.68; 5.81 ppm. *trans*-4-fluoro-L-proline (**6a**) was not isolated but was present in the mixture with **6c** up to 7% and was identified by ^{19}F NMR (H_2O, TFA) δ: −176. 78 (m) ppm. Lit. value [42]: ^{19}F NMR (D_2O, HFB external) δ: −173.28 (m) ppm.

(+)-trans-*4-Fluoro-D-proline (6d)*

Hydrolysis of the product of the incomplete oxidation of methyl *trans*-4-fluoro-D-prolinate (**4d**), containing, according to NMR spectroscopy, 75% methyl *trans*-4-fluoro-D-pyrrolidin-5-one-2-carboxylate (**5d**) and 22% of the unreacted methyl *trans*-4-fluoro-D-prolinate (**4d**), by refluxing for 4 h with concentrated hydrochloric acid afforded a mixture of the hydrochlorides of **6d** and **7b**. Neutralization with sodium hydroxide to pH 7 followed by treatment with ion-exchanger AG1-X8 in an acetate cycle gave, on elution with water, a 43.6% yield of *trans*-4-fluoro-D-proline (**6d**), m.p. 258 °C (dec.). The mixed melting point with *cis*-4-fluoro-L-proline (**6c**) (m.p., 255–257 °C) was 242–245 °C. ^{19}F NMR (H_2O, TFA) δ: −178.18 (m) ppm. Lit. values [42]: m.p., 264 °C (dec.). ^{19}F NMR (D_2O, HFB external) δ: −173.281 (m) ppm.

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Note added in proof

After this paper, containing the correct interpretation of the ^{1}H NMR spectra of compounds **4a** and **4c**, had been submitted to the editor of *J. Fluorine Chem.* on December 27, 1991 (received on January 8, 1992), a paper entitled "Stereospecific Synthesis of 4-Fluoroglutamic Acids" by A. G. Avent, A. N. Bowler, P. M. Doyle, C. M. Marchand and D. W. Young was published in *Tetrahedron Lett., 33* (1992) 1509 (received on November 21, 1991). The paper interprets the spectra of compounds **4a** and **4b** based on the variable temperature ^{1}H NMR measurements as is described in footnote ** on p. 195 above.