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NEW STEREOSPECIFIC SYNTHESES AND X-RAY DIFFRACTION STRUCTURES OF (-)-D-ERYTHRO- AND (+)-L-THREO-4-FLUOROGLUTAMIC ACID

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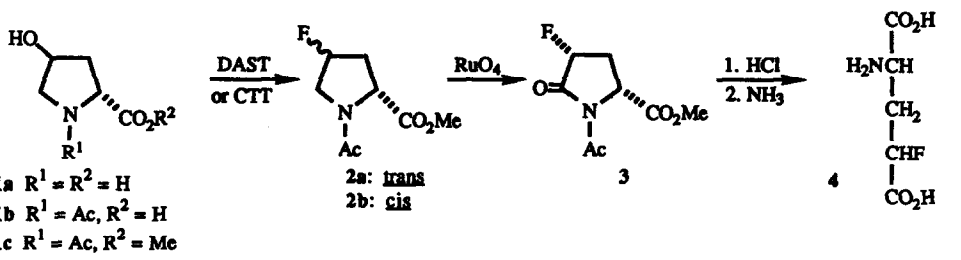
Abstract. Stereospecific syntheses of (+)-L-*threo* and (-)-D-*erythro*-4-fluoroglutamic acid are based on the hydrolysis of methyl 1-acetyl-4-fluoro-L-pyrrolidin-5-one-2-carboxylate, prepared from *trans*- and *cis*-4-hydroxyprolines, respectively.

After the discovery of the antimetabolic properties of fluorocitric acid[1] and 5-fluorouracil,[2] 4-fluoroglutamic acid of undefined stereochemistry was synthesized with the expectation, that, as a potential antimetabolite of glutamic acid, it might inhibit growth of bacteria and neoplastic tissues.[3-7] Its derivative, fluoromethotrexate, was found to possess antitumor properties.[8-10] Current interest focuses on the effects of 4-fluoroglutamic acid, especially its L-*threo* isomer, on important enzyme systems[11-19] and on the effects on central neuronal mechanisms.[20-25] The first syntheses of 4-fluoroglutamic acid gave racemic mixtures of *erythro* and *threo* forms.[3-7] These mixtures were later separated into DL-*erythro* and DL-*threo*, and L-*erythro* and L-*threo*-4-fluoroglutamic acids on ion exchangers,[26-28] by means of gas-liquid chromatography,[29,30] and by fractional crystallizations.[31] However, no stereoisomer was prepared synthetically. In this communication, straightforward stereospecific syntheses of (+)-L-*threo*-4-fluoroglutamic and of (-)-D-*erythro*-4-fluoroglutamic acid from commercially available materials are described.

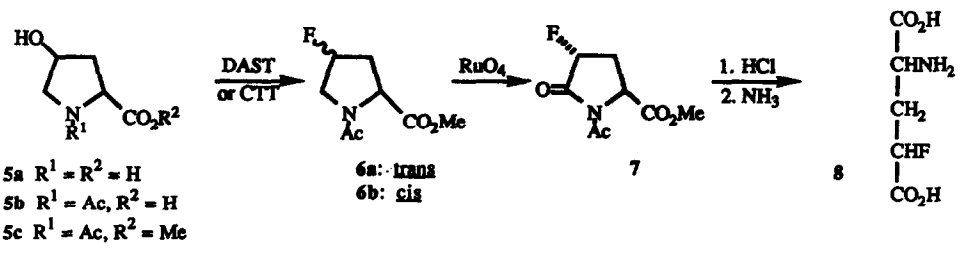


1a $R^1 = R^2 = H$
1b R^1 = Ac, $R^2 = H$
1c R^1 = Ac, R^2 = Me

2a: trans
2b: cis

3

4



5a $R^1 = R^2 = H$
5b R^1 = Ac, $R^2 = H$
5c R^1 = Ac, R^2 = Me

6a: trans
6b: cis

7

8

Acetylation and subsequent methylation of *trans*-4-hydroxy-L-proline (**1a**) according to the literature[32-34] gave compounds **1b** and **1c**. Treatment of **1c** with diethylaminosulfur trifluoride (DAST)[35] or with 2-chloro-1,1,2-trifluorotriethylamine(CTT)[36] gave, in a 50% yield, an oily, chromatographically inseparable mixture of 56-67% of *trans*- (**2a**) and 33-44% of *cis* (**2b**) methyl 1-acetyl-4-fluoro-L-prolinate. ^{19}F NMR ($CDCl_3$): -174.14 ppm (m). The assignment of *trans* to **2a** and *cis* to **2b** is based on comparison with chemical shifts of *trans* and *cis*-4-fluoro-L-proline described in the literature.[37] The ^1H NMR spectrum of a mixture of **2a** and **2b** is shown in Fig. 1.

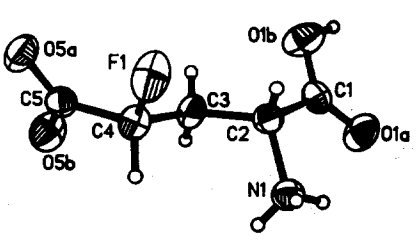
Oxidation of the mixture of **2a** and **2b** in ethyl acetate with ruthenium tetroxide, generated in situ from ruthenium dioxide and sodium periodate[38,39] afforded 46% of sterically almost uniform methyl 1-acetyl-*cis*-4-fluoro-L-pyrrolidin-5-one-2-carboxylate (**3**) containing 0-9% of the *trans*-isomer. Mp 105.5-106° (MeOH); ^{19}F NMR: -188.65 ppm (ddd). The finding that from a mixture of methyl 1-acetyl-*cis*- and *trans*-4-fluoro-L-prolinate **2a** and **2b** essentially only one isomer **3** was isolated, is somewhat surprising. It seems likely that epimerization at carbon 4 took place via the enolization of the carbonyl group. However, it is difficult to prove this hypothesis because the oxidation with ruthenium tetroxide was never complete and gave considerable amounts of byproducts. Hydrolysis by refluxing for 4 hours with concentrated hydrochloric acid followed by neutralization with 18% aqueous ammonia to pH 3 converted **3** to (+)-L-*threo*-4-fluoroglutamic acid [(+)-(2S,4S-fluoroglutamic acid] (**4**) in a 77% yield: Mp 196-196.5° dec, $[\alpha]_D^{25}$ + 13.35° ± 0.05° (*c* 1, 1M HCl); lit.[28] $[\alpha]_D^{20}$ + 14.6° (*c* 1, 1M HCl).

Analogous sequential treatment of *cis*-4-hydroxy-D-proline (**5a**) with acetic anhydride and with diazomethane gave **5b** and **5c**, respectively. Reaction of **5c** with DAST[35] or CTT[36] gave a mixture of 79-82% of methyl 1-acetyl-*trans* 4-fluoro-D-prolinate (**6a**) and 18-21% of its *cis* isomer **6b** in a 40% yield. Surprisingly, the product's composition did not change after several recrystallizations from hexane-benzene 20:1. Mp 100.5-101.5°, ^{19}F NMR ($CDCl_3$): -178.16 ppm (m).

Oxidation of the above mixture of **6a** and **6b** with ruthenium tetroxide gave a 57.7% yield of methyl 1-acetyl-*trans*-4-fluoro-D-pyrrolidin-5-one-2-carboxylate (**7**), mp 98.2-98.6° (MeOH); ^{19}F NMR ($CDCl_3$): -193.43 (ddd). Hydrolysis under the conditions described in the preparation of **4** converted **7** to (-)-D-*erythro*-4-fluoroglutamic acid (**8**) [(-)-(2R, 4S)-fluoroglutamic acid] in a 55% yield. Mp 180-180.5° till 182-183° dec (depending on the rate of heating). $[\alpha]_D^{25}$-36.1±0.015° (*c* 1, 1M HCl); lit.[28]+28° (*c* 1, 1M HCl) for 87% pure *erythro*-L-compound and +32° for pure compound.

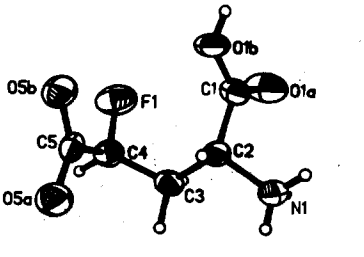
NMR spectra of both 4-fluoroglutamic acids **4** and **8** are listed in the footnote[40] and shown in Fig. 2.

The x-ray crystal structure of both L-*threo*-4-fluoroglutamic acid and D-*erythro*-4-fluoroglutamic were obtained and ORTEP plots for both molecules are shown in Figures 1 and 2, respectively.[41,42]



L-threo-4-Fluoroglutamic acid

FIGURE 1



D-erythro-4-Fluoroglutamic acid

FIGURE 2

Acknowledgement: Our sincere thanks are due to Drs. H. M. Bell, N. Castagnoli, H. C. Dorn and R. H. White for useful hints, and to Messrs. G. Iannaccone and W. R. Bebout for the NMR spectra. Elemental analyses were done by Galbraith Laboratories, Inc., Knoxville, TN.

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40. NMR data of 4-fluoroglutamic acids:
    4: ^{19}F NMR (in CF_3CO_2H): -191.71 ppm (q, 1:2:2:2:1, $J_{HF\ gem}$ 48.17 Hz, $J_{HF\ vic}$ 24.01 Hz). ^1H NMR (in CF_3CO_2H): 2.81 ppm (dt, 2, CH_2; $J_{HF\ vic}$ 25.10 Hz, J_{HH} 5.60 Hz): 4.51 ppm (m, 1, CHN); 5.38 ppm (dt, 1, CHF; $J_{HF\ gem}$ 48.03 Hz; J_{HH} 5.78 Hz); 7.67 ppm (broad s, 3, CNH_3). Lit.[28] -191.4 ppm (q, 1:2:2:2:1; J_{HF} 47.3 Hz, J_{HF} 24.4); 2.75 ppm (dt, J_{HF} 23.4 Hz, J_{HH} 5.4), 4.5 (m), 5.26 ppm (dt, J_{HF} 48 Hz, J_{HH} 5.4 Hz); 7.42 ppm (s). **8**: ^{19}F NMR (in CF_3CO_2H): –191.39 ppm (heptet 1:1:1:2:1:1:1); $J_{HF\ gem}$ 48.49 Hz, $J_{HF\ vic}$ 34.65 Hz, 13.93 Hz). ^1H NMR (in CF_3CO_2H): 2.68 ppm (t, 1, CH_2; $J_{HF\ vic}$ 14.33); 3.00 ppm (t, 1, CH_2, $J_{HF\ vic}$ 13.48) (two diastereotropic H in CH_2); 4.55 ppm (m, 1, CHN); 5.48 ppm (dd, 1, CHF; $J_{HF\ gem}$ 47.9 Hz, J_{HH} 8.3 Hz); 7.85 (m, 3, CNH_3). Lit.[28] -191.9

ppm (heptet; 1:1:1:2:1:1:1); $J_{HF\ gem}$ 49.6 Hz, $J_{HF\ vic}$, 33.4, and 16.6 Hz); 2.63 ppm (m, 2H, CH_2), 4.54 ppm (m, 1, CH), 5.35 (td, J_{HF} 48, J_{HH} 9.2, 3, 1H, CHF); 7.44 (s, 3H, NH_3).

41. Crystals of D-*erythro*-4-fluoroglutamic acid suitable for x-ray diffraction were obtained by slow evaporation of an aqueous solution. D-*erythro*-4-fluoroglutamic acid crystallizes in the monoclinic system, space group $P2_1$, two molecules per unit cell with cell constants: a=5.217(2) Å, b=10.316(4) Å, c=6.099(3) Å, β=97.72(4)°, V=325.2(2) $Å^3$, d_{calcd}=1.686 gcm^{-3}. 712 observed, independent reflections ($F > 3.0\sigma(F)$) were collected on a Nicolet R3/mV diffractometer in the range $3.5 < 20 < 55$ with Mo K_a radiation (λ=0.71073 Å). The structure was solved using direct methods and refined with full matrix least squares refinement. All non-hydrogen atoms were located and refined anisotropically. All hydrogen atoms were located and refined as riding on the attached non-hydrogen atom. The nitrogen atom was clearly identified by examination of the thermal parameters as well as by the number of hydrogen atoms attached. The structure refined to an R value of 3.91%, (wR=4.51%). In like fashion, the structure of L-*threo*-4-fluoroglutamic acid was obtained: orthorhombic space group $P2_12_12_1$ with a=6.391(2)(Å); b=6.723(2)(Å); c=15.147(5)(Å); z=4. For 810 observed, independent reflections, R=3.76% (wR=4.98%).[41]

42. *The atomic coordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK. Any request should be accompanied by the full literature citation for this communication.*

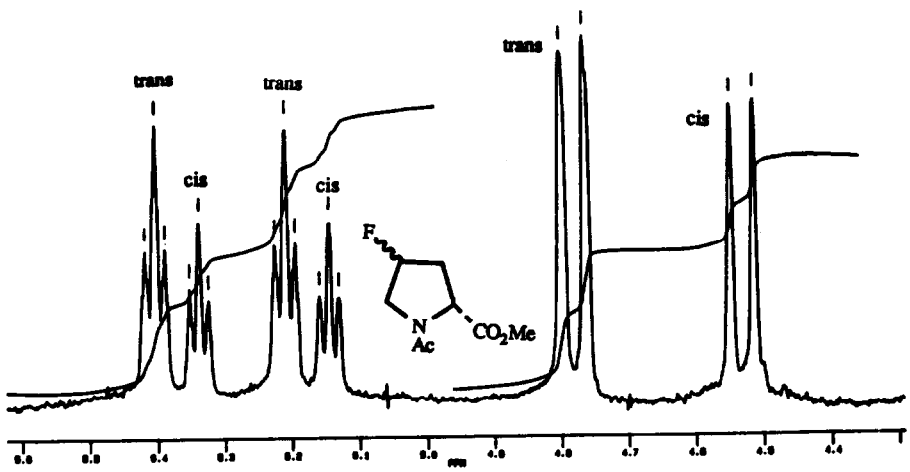

Fig. 1

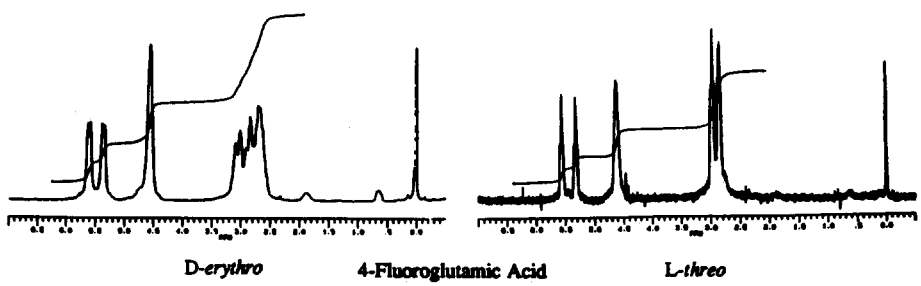

Fig. 2

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