Chemistry of 4-fluoroglutamic acid. Part 2. Separation of the diastereomers on a large scale. Preparation of *cis*- and *trans*-4-fluoro-5-pyrrolidone-2-carboxylic acids (4-fluoropyroglutamic acids)*

V. Tolman, V. Vlasáková

Institute of Nuclear Biology and Radiochemistry, Czechoslovak Academy of Sciences, Vídeňská 1083, 14220 Prague 4 (Czechoslovakia)

J. Němeček

Institute of Microbiology, Czechoslovak Academy of Sciences, Vídeňská 1083, 14220 Prague 4 (Czechoslovakia)

(Received January 4, 1992; May 10, 1992)

Abstract

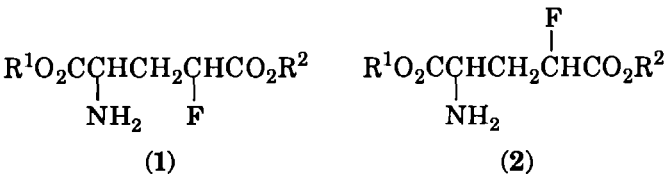
Separation of the 4-fluoroglutamic acid diastereomers has been achieved by crystallization of the hydrochlorides of their dialkyl esters **1b** and **2d**. Subsequent hydrolysis afforded the diastereomers **1a** and **2a** in 100% steric purity. Conversion of the acids into the 5-methyl esters followed by treatment with ammonia gives rise to the *trans* and *cis* isomers of 4-fluoro-5-pyrrolidone-2-carboxylic acid, **3** and **4**. NMR data are given.

Introduction

In the first part of this series [1], we described an optimized variant of the total synthesis of the 4-fluoroglutamic acid. This method, as well as other non-stereoselective syntheses of this compound [2–6], yields only a mixture of both racemic diastereomers, e.g. *erythro*-4-fluoroglutamic acid (**1a**) and its *threo* isomer **2a**. For the separation of this mixture, only one method has been reported [7] based on chromatography on a large Dowex 1 column. In our hands, this method proved to be unsuitable for any substantial scaling-up; moreover, both separated diastereomers were found to be only 95–97% sterically pure, the remainder being the other isomer [8, 9]. For these reasons – and also because of the need for highly pure **1a** and **2a** in multigram quantities – we have had to search for another separation method, based on the crystallization of suitable derivatives and thus applicable for work on any scale.

*Part of the lecture (by V.T.) at the 10th Winter Fluorine Conf., Jan. 28–Feb. 2, 1991, St. Petersburg, FL.

0022-1139/93/$6.00



(1) (2)

a : $R^1 = R^2 = H$
b : $R^1 = R^2 = Me$
c : $R^1 = H; R^2 = Me$

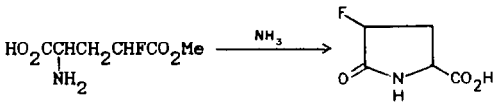
a : $R^1 = R^2 = H$
b : $R^1 = R^2 = Me$
c : $R^1 = H; R^2 = Me$
d : $R^1 = R^2 = Pr^i$

Theoretical and discussion

Although compounds **1a** and **2a** differ considerably in solubility, repeated recrystallization of the starting 1:1 mixture from water did not lead to an efficient separation but only to enriched preparations of **2a** still containing 10–20% of **1a**. In a search for simple derivatives of 4-fluoroglutamic acid, applicable to an efficient separation of the diastereomers by crystallization, we found the hydrochloride salts of 4-fluoroglutamic acid diesters to be the compounds of choice. When the mixture of **1a** and **2a** was esterified with HCl/methanol and the product recrystallized from methanol/ether, the hydrochloride salt of **1b** separated first and could be easily purified [10]. Attempts to purify the **2b** remaining in the mother liquors were unsuccessful, as they afforded — after numerous recrystallizations and with considerable loss of material — a product with steric purity never greater than 92%. A much more effective way to prepare pure **2a** consisted of the acid hydrolysis of the mother liquors back to the mixture of **1a** and **2a** in approx. 1:2 ratio, esterification with HCl/2-propanol and crystallization of the ester hydrochloride from acetone; after 2–3 such recrystallizations, the HCl salt of the *threo* ester **2d** was secured in high yield with high steric purity. Finally, acid hydrolysis of **1b**·HCl and **2d**·HCl yielded the acids **1a** and **2a**; most of the 4-fluoroglutamic acid remaining in the mother liquor was regenerated and re-used.

The diastereomers **1a** and **2a** may be readily cyclized to the corresponding lactams **3** and **4**, i.e. the 4-fluoro-5-pyrrolidone-2-carboxylic acids. In our previous work [4], we prepared these compounds through the thermal cyclization of diethyl 4-fluoroglutamate; they were also prepared by the pyrolysis (at 170 °C) of the separated **1a** and **2a** [7]. In this paper another access to **3** and **4** is described, based on the action of ammonia on the monoesters **1c** and **2c**, prepared from the acids using methanol with thionyl chloride [11]. No 4-fluoroglutamine was formed under these conditions, the

lactams **3** and **4** being the sole products. The *trans*-lactam **3** arises from the *erythro* acid **1a**, while the *cis* isomer **4** is formed from the *threo* acid **2a**.



(**1c**) ⟶ (**3**) (*trans*)
(**2c**) ⟶ (**4**) (*cis*)

The seric relevance of **3** and **4** had been formerly established by their correlation with *trans*- and *cis*-4-fluoroprolines [7]. The larger magnetic non-equivalence between the H-3 protons in **3** than in **4** (0.623 and 0.117 ppm, respectively) could be rationalized by a simultaneous shielding effect of both the F and COOH groups exerted on the *same* proton located on their side of the ring. The spatial proximity of H-2 and H-4 leads to an observable cross-peak in the NOESY (Nuclear Overhauser Effect Spectroscopy) [12] spectrum only in the case of the *cis*-lactam **3**.

Experimental

GC analysis was performed on a Varian gas chromatograph model 3700, equipped with a flame ionization detector, using a capillary column (20 m×0.2 mm i.d.) constructed from fused silica and coated with OV-275 (Chrompack). All samples were analyzed isothermally at 160 °C using nitrogen as a carrier gas (p_i=8 psig). Both the dimethyl and diisopropyl esters of 4-fluoroglutamic acid were analyzed for their *erythro/threo* composition as the *N*-trifluoroacetyl derivatives [13]. NMR spectra were recorded using a Varian VXR-400 spectrometer (399.95 MHz for ^{1}H, 100.577 MHz for ^{13}C and 376.289 MHz for ^{19}F nuclei). All spectra were measured in perdeuteriomethanol at 25 °C. Solvent resonance was used as a secondary reference for ^{1}H and ^{13}C NMR peaks (3.33 and 49.3 ppm, respectively); internal C_6F_6 was employed for ^{19}F NMR peaks. The correctness of the NMR parameters was checked by spectrum simulation. Apparent multiplicity is reported for proton signals (H, F coupling values (Hz) italicized); proton-caused multiplicity (determined by APT) is given for ^{13}C NMR signals (coupling values (Hz) to fluorine in parentheses).

*Separation of the 4-fluoroglutamic acid diastereomers (**1a**, **2a**)*

*(a) Preparation of the esters **1b** and **2b***

A mixture of 4-fluoroglutamic acid (59.4 g, 0.36 mol) (approx. 1:1 diastereomeric mixture [1]) and methanol (600 ml), half-saturated with hydrogen chloride was refluxed for 6 h, evaporated *in vacuo* and re-evaporated twice with methanol (100 ml) to remove most of the hydrogen chloride. The remainder was dissolved in methanol (100 ml), and dry ether (100 ml) was carefully added in portions to the filtered solution. The mixture was allowed

to stand overnight at laboratory temperature and then for 2 d at 5 °C, the crystals filtered, washed with a small volume of a 1:3 mixture of methanol/ether, then with ether and dried. Yield, 20.2 g; m.p., 139–140 °C (fraction A; *erythro/threo*, 93:7). Evaporation of the mother liquor and double recrystallization of the residue from methanol/ether gave 2.5 g of a second crop (fraction B, 97:3). The fractions were combined and recrystallized from methanol (80 ml) and ether (80 ml); after work-up of the mother liquors the pure **1b**·HCl amounted to 19.0 g (m.p., 144 °C).

After isolation of **1b**·HCl, the combined mother liquors were evaporated and the oily residue hydrolyzed with 6 N HCl (700 ml) for 3.5 h at 70 °C. After evaporation, the greater part of the hydrogen chloride was removed by evaporating twice with a little water, then taking up the residue into water (90 ml) and adjusting the pH to *c.* 3 by adding pyridine. Careful dilution with ethanol (600 ml in total), followed by refrigeration at 5 °C for 2 d gave 30.0 g of 4-fluoroglutamic acid, enriched by the *threo* form (**1a**: **2a**, *c.* 1:3). The mother liquor was evaporated, dissolved in water (100 ml) and poured down a column of Dowex 50×8 (200 ml) in a H^+ cycle. After washing out the Cl^- ions, the second crop (7.9 g) of 4-fluoroglutamic acid was eluted using 2 M pyridine.

A mixture of the regenerated acid (37.9 g) and dry 2-propanol (200 ml) was refluxed for 3.5 h under a slow stream of hydrogen chloride. After evaporation, the semi-solid mass was evaporated once with chloroform (100 ml), then acetone (300 ml) was added and the whole refluxed for 2 h, filtered hot and allowed to stand overnight. The voluminous crystals of **2d**·HCl were washed with acetone and dried; yield, 28.6 g (fraction C; *erythro/threo*, 2.5:97.5). Work-up of the mother liquors gave 4.5 g of the second portion (fraction D, 2:98). A suspension of fractions C + D in acetone (150 ml) was refluxed with stirring for 15 min, the insoluble **2d**·HCl was filtered hot and subjected again to the same operation. (This procedure is more effective for the preparative scale than repeated crystallizations from acetone, due to the very slight solubility of pure **2d**·HCl in this solvent.) Together with the material secured by the work-up of the hot extracts, 25.0 g of **2d**·HCl was obtained (m.p., 145–146 °C (acetone)).

The combined mother liquors were again hydrolyzed with 6 N HCl and the regenerated 4-fluoroglutamic acid (13.5 g) was subjected to the same cycle of operations, giving further portions of **1b**·HCl (4.0 g) and **2d**·HCl (7.1 g) of the same purity as in the first cycle.

The final yields of the separated ester hydrochlorides were analyzed as follows.

Compound **1b**·HCl: 23.0 g (55.8%); m.p., 144 °C. Analysis: Found: C, 36.35; H, 5.70; Cl, 15.37, N, 6.13%. $C_7H_{13}ClFNO_4$ requires: C, 36.61; H, 5.70; Cl, 15.44; N, 6.10%. GC purity >99% of the *erythro* form. ^{1}H NMR δ: 2.444 (dddd, 1H (H-3u, 15.5, 10.3, 5.9, *15.4*)); 2.630 (dddd, 1H (H-3d, 15.5, 7.0, 2.8, *35.1*)); 3.844 (s, 3H ($COOCH_3$)); 3.890 (s, 3H ($COOCH_3$)); 4.345 (dd, 1H (H-2, 7.0, 5.9)); 5.344 (ddd, 1H (H-4, 10.3, 2.8, *48.4*)) ppm. ^{13}C NMR δ: 34.25 (t (C-3, 20.7)); 51.22 (d (C-2, 2.5)); 53.60 (q (OCH_3));

54.29 (q (OCH_3)); 87.65 (d (C-4, 182.9)); 170.24 (s (C-1)); 170.25 (s (C-5, 22.8)) ppm. ^{19}F NMR δ: −191.77 ppm.

Compound **2d**·HCl: 32.1 g (62.4%); m.p., 145–146 °C (acetone). Analysis: Found: C, 45.93; H, 7.63; Cl, 12.73; N, 4.83%. $C_{11}H_{21}ClFNO_4$ requires: C, 46.23; H, 7.41; Cl, 12.41; N, 4.90%. GC purity >99.5% of the *threo* form. ^1H NMR δ: 1.19 (d, 6H (CH_3-a, 6.2)); 1.32 (d, 3H (CH_3-b, 6.3)); 1.34 (d, 3H (CH_3-b, 6.3)); 2.57 (dddd, 1H (H-3u, 4.9, 9.4 15.5, *16.7*)); 2.66 (dddd, 1H (H-3d, 3.4, 8.0, 15.5, *32.3*)); 4.00 (hep, 2H (CH-4, 6.2)); 4.17 (s, 2H (NH_2)); 4.24 (dd, 1H (H-2, 4.9, 8.0)); 5.15 (hep, 2H (CH-b, 6.3)); 5.38 (ddd, 1H (H-4, 3.4, 9.4, *48.3*)) ppm. ^{13}C NMR δ: 21.09 (q (CH_3)); 21.14 (q (CH_3)); 24.54 (q ($2CH_3$)); 32.49 (t (C-3, 21.1)); 49.41 (d (C-2, 2.3)); 63.70 (d (CH)); 71.36 (d (CH)); 85.1 (d (C-4, 183.4)); 167.42 (s (C-1)); 168.72 (s (C-5, 23.1)) ppm. ^{19}F NMR δ: −190.55 ppm.

From the final mother liquors, further 4.2 g of 4-fluoroglutamic acid was regenerated.

(b) Preparation of erythro*-4-fluoroglutamic acid (**1a**)*

A solution of **1b**·HCl (4.58 g, 20 mmol; GC > 99% pure) in 5 N hydrochloric acid (35 ml) was heated at 70 °C for 3 h, evaporated *in vacuo*, excess hydrogen chloride removed by threefold evaporation with water (25 ml) and the remainder dried in a desiccator over P_2O_5 and NaOH. The semi-solid mass was dissolved in water (6 ml), the pH adjusted to 3.5 with pyridine and the acid crystallized by slow addition of ethanol (40 ml). After refrigeration overnight, the product was collected, thoroughly washed with ethanol, then with ether and dried. Yield, 3.16 g (96%); m.p., 171–173 °C (lit [7] does not report the m.p.). Analysis: Found: C, 36.09; H, 4.89; N, 8.50%. $C_5H_8FNO_4$ requires: C, 36.37; H, 4.89; N, 8.48%. GC purity corresponded to 100% of the *erythro* form.

(c) Preparation of threo*-4-fluoroglutamic acid (**2a**)*

Compound **2d**·HCl (5.72 g, 20 mmol; GC > 99.2% pure) was treated in the same way as **1b**·HCl. Yield, 2.77 g (84%); m.p. 194–195 °C (lit. [7] does not report the m.p.). Analysis: Found: C, 36.12; H, 4.77; N, 8.54%. $C_5H_8FNO_4$ requires: C, 36.37; H, 4.89; N, 8.48%. GC purity corresponded to 100% of the *threo* form.

Dimethyl threo*-4-fluoroglutamate hydrochloride (**2b**·HCl)*

A mixture of acid **2a** (1.15 g, 7 mmol) and methanol (7 ml) saturated with hydrogen chloride was refluxed for 3 h, evaporated and dried in a desiccator. Crystallization from methanol/ethyl acetate/ether gave 1.35 g (84.3%) of **2b**·HCl, m.p., 121–124 °C. Analysis: Found: C, 36.39; H, 5.70; Cl, 15.42; N, 6.18%. $C_7H_{13}ClFNO_4$ requires: C, 36.61; H, 5.70; Cl, 15.44; N, 6.10%. ^1H NMR δ: 2.550 (dddd, 1H (H-3u, 15.6, 7.3, 4.3, *28.5*)); 2.581 (dddd, 1H (H-3d, 15.6, 8.3, 5.4, *20.1*)); 3.851 (s, 3H ($COOCH_3$)); 3.891 (s, 3H, ($COOCH_3$)); 4.323 (dd, 1H (H-2, 7.3, 5.4)); 5.284 (ddd, 1H (H-4, 8.3, 4.3, *48.0*)) ppm. ^{13}C NMR δ: 34.12 (t (C-3, 21.0)); 51.00 (d (C-2, 1.9));

53.63 (q (OCH_3)); 54.38 (q (OCH_3)); 87.21 (d (C-4, 183.3)); 170.17 (s (C-1)); 170.29 (s (C-5, 22.9)) ppm. ^{19}F NMR δ: −191.85 ppm.

*4-Fluoroglutamic acid 5-methyl esters (**1c**) and (**2c**)*

*(a) Preparation of **1c***

To dry methanol (6.7 ml) cooled to −15 °C, thionyl chloride (1.65 g [1.01 ml], 13.9 mmol) was slowly added. After 10 min stirring at this temperature, **1a** (1.65 g, 10 mmol) was added and the solution was stirred for 30 min without cooling. Dry ether (60 ml) was then added, the resulting suspension refrigerated overnight, filtered, washed with ether and dried. Yield, 2.04 g (94.4%) of **1c**·HCl; m.p., 163–165 °C; after recrystallization from methanol/ether, m.p., 164–165 °C. Analysis: Found: C, 33.44; H, 5.17; Cl, 16.37; N, 6.20%. $C_6H_{11}ClFNO_4$ requires: C, 33.42; H, 5.14; Cl, 16.45; N, 6.50%.

Compound **1c**·HCl (645 mg, 3 mmol), dissolved in methanol (13 ml), was neutralized with triethylamine (305 mg [0.420 ml], 3 mmol), refrigerated overnight, filtered, washed with methanol until free from Cl^- anions, then with ether and dried. Yield, 509 mg (95%) of **1c**; m.p., 160.5–161.5 °C (water). Analysis: Found: C, 39.95; H, 5.60; N, 7.98%. $C_6H_{10}FNO_4$ requires: C, 40.22; H, 5.59; N, 7.82%.

*(b) Preparation of **2c***

Compound **2a** (1.65 g, 10 mmol) was esterified in the same way as for **1a**, except that the reaction time was shortened to 20 min. After adding ether (60 ml) and cooling at 5 °C for 3 h, the supernatant was decanted and dry ether (40 ml) was added to the crystals, the mixture refrigerated overnight and the filtered crystals washed with ether. Yield, 1.95 g (90.5%) of **2c**·HCl; m.p., 142–144 °C; after recrystallization from methanol/ether, m.p. 146–148 °C. Analysis: Found: C, 33.21; H, 5.10; Cl, 16.38; N, 6.19%. $C_6H_{11}ClFNO_4$ requires: C, 33.42; H, 5.14; Cl, 16.45; N, 6.50%.

Free **2c** was liberated from the salt in the same way as for **1c**; yield, 482 mg (90%); m.p., 143.5–144 °C. Analysis: Found: C, 40.01; H, 5.49; N, 7.63%. $C_6H_{10}FNO_4$ requires: C, 40.22; H, 5.59; N, 7.82%.

Preparation of trans- *and* cis-*4-fluoro-5-pyrrolidone-2-carboxylic acids (**3**) and (**4**)*

(a) Preparation of trans-(*3*)

Compound **1c**·HCl (108 mg, 0.5 mmol) was dissolved in 25% aqueous ammonia (2 ml) and allowed to stand overnight at laboratory temperature. After evaporation, the residue was taken into a minimum amount of water, placed on a column of Dowex 50×8 (100–200 mesh; 0.9×3.5 cm) in a H^+ cycle and the product eluted with water. On evaporation of the eluate and drying, 59 mg (80%) of **3** resulted; m.p., 198–202.5 °C; after recrystallization from methanol/ether/hexane, m.p., 204–207 °C. This compound was identical with the 'form A' (m.p., 198–200 °C) described in the literature [4]. ^1H NMR δ: 2.232 (dddd, 1H (H-3u, 14.0, 6.4, 6.1, *26.8*)); 2.955 (dddd,

1H (H-3d, 14.0, 8.0, 7.9, *15.2*)); 4.237 (ddd, 1H (H-2, 8.0, 6.4, *2.8*)); 5.099 (ddd, 1H (H-4, 7.9, 6.1, *52.8*)) ppm. ^{13}C NMR δ: 33.21 (t (C-3, 20.7)); 53.22 (d (C-2, 3.1)); 89.32 (d (C-4, 182.8)); 174.24 (s (COOH)); 174.58 (s (C-5, 20.4)) ppm. ^{19}F NMR δ: −188.75 ppm.

(b) Preparation of cis-*(4)*

Compound **2c**·HCl (108 mg, 0.5 mmol) was treated in the same way as described in the preceding paragraph, yielding 58 mg (79%) of **4**; m.p., 175–178.5 °C; after recrystallization from methanol/ether/hexane, m.p., 180–181 °C. This product was identical with the 'form B', (m.p., 179–180 °C) described earlier [4]. ^{1}H NMR δ: 2.560 (dddd, 1H (H-3u, 14.1, 8.8, 6.6, *26.9*)); 2.677 (dddd, 1H (H-3d, 14.1, 7.7, 3.2, *14.1*)); 4.342 (dd, 1H (H-4, 8.8, 3.2)); 5.171 (ddd, 1H (H-4, 7.7, 6.6, *53.9*)) ppm. ^{13}C NMR δ: 33.36 (t (C-3, 21.3)); 54.09 (d (C-2, 4.1)); 89.16 (d (C-4, 182.1)); 174.81 (s (COOH)); 175.11 (s (C-5, 19.8)) ppm. ^{19}F NMR δ: −191.53 ppm.

References

1 V. Tolman, preceding paper.
2 M. Hudlický, *Collect. Czech. Chem. Commun., 26* (1961) 1414.
3 R. L. Buchanan, F. H. Dean and F. L. M. Pattison, *Can. J. Chem., 40* (1962) 1571.
4 V. Tolman and K. Vereš, *Collect. Czech. Chem. Commun., 32* (1967) 4460.
5 L. V. Alekseeva, B. N. Lundin and N. L. Burde, *Zh. Obshch. Khim., 37* (1967) 1754 [*Chem. Abs., 68* (1968) 22 221d].
6 E. D. Bergmann and L. Chun-Hsu, *Synthesis*, (1973) 44.
7 J. C. Unkeless and P. Goldmann, *Mol. Pharmacol., 7* (1971) 293.
8 J. Balada, student project, Charles University, Prague, Czechoslovakia, 1973.
9 V. Tolman and K. Živný, unpublished results.
10 V. Tolman, V. Vlasáková and K. Živný, *11th Int. Symp. Fluorine Chem.*, Aug. 5–9, 1985, Berlin, Germany.
11 R. A. Boissonas, S. Guttman, P.-A. Jaquenoud and J.-P Waller, *Helv. Chim. Acta, 38* (1955) 1491.
12 J. Jeener, B. H. Meier, P. Bachmann and R. R. Ernst, *J. Chem. Phys., 71* (1979) 4546.
13 A. Darbre and A. Islam, *Biochem. J., 106* (1968) 923.