Organic Fluorine Compounds; Part XLVI[1]. γ-Fluoroglutamic Acid and Fluorofolic Acid

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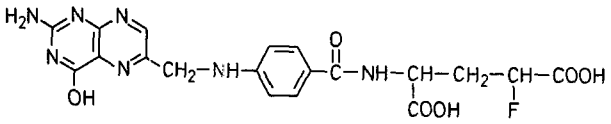
Recently, the synthesis of fluorofolic acid (**1**) was described[2] by Russian workers. We wish to report here briefly on this and related subjects, as the available reports on the Russian work contain no experimental details.



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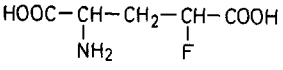

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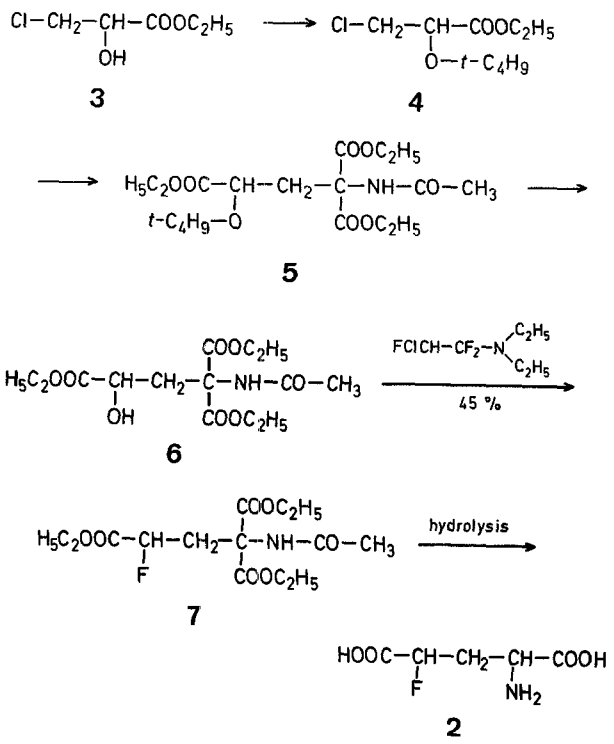
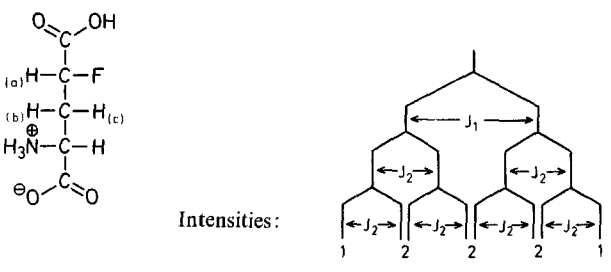
γ-Fluoroglutamic acid (**2**) was prepared following the procedure of Buchanan[2], with slight modifications. The method is based on the Michael reaction between diethyl fluoromalonate and ethyl α-acetamidoacrylate. A second known method[2,3,4] utilises the inverse Michael reaction, viz. between ethyl α-fluoroacrylate and diethyl acetamidomalonate. Alekseeva et al.[5] have reported that the reaction of tetraethyl 1-acetamidopropane-1,1,3,3-tetracarboxylate with perchloryl fluoride and the subsequent treatment of the reaction product with hydrochloric acid gives a good yield of γ-fluoroglutamic acid (**2**). We have developed a method for the preparation of **2** according to the following reaction scheme.



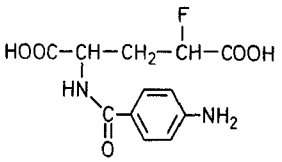
Ethyl 3-chloro-2-hydroxypropanoate (**3**) was etherified with isobutylene. The resultant O-*t*-butyl derivative **4** was condensed with diethyl acetamidomalonate and the ether group in **5** was hydrolyzed, giving **6**. The hydroxy group in **6** was replaced by fluorine using 2-chloro-1,1,2-trifluoroethyl-diethylamine[7] and the resultant crude **7** (45% yield) was hydrolyzed and decarboxylated to give **2**. This method does not afford higher overall yields than the previously reported ones; it has, however, some distinct advantages over the known methods, e.g., the applicability to the synthesis of analogs of **2** such as γ-chloroglutamic acid.

The ^{1}H-N.M.R. spectrum of **2** recorded by us is identical with that reported by Buchanan[3]. In the ^{19}F-N.M.R. spectrum, two apparent anomalies are found: (1) the splitting of the fluorine absorption is at the first sight expected to give a quartet but a quintet is found; (2) the relative intensities of the quartet would be 1:3:3:1, the observed intensities of the quintet are 1:2:2:2:1. These observations may be rationalized by the following scheme:



The fluorine absorption is split by $H_{(a)}$ into a doublet, the latter by $H_{(b)}$ into a quartet. The quartet should be split to give an octet under the influence of $H_{(c)}$; however, three pairs of lines coincide. The calculated coupling constants $J_2 = 23.48$ and $J_1 = 49.60$ cps agree with those found in the ^{1}H-N.M.R. spectrum, the chemical shift (relative to CCl_3F) is 178.35 ppm.

γ-Fluoroglutamic acid (**2**) was converted into the N-(4-aminobenzoyl) derivative (**8**), using the method of Anderson[8], by successive treatment of N-benzyloxycarbonyl-4-aminobenzoic acid with N-hydroxysuccinimide and γ-fluoroglutamic acid, followed by elimination of the N-benzyloxycarbonyl group. A previously reported synthesis[9] of acid **8** used a different reaction sequence. Condensation of acid **8** with 4-hydroxy-2,5,6-triaminopyrimidine (**9**) and 2,3-dibromopropanal yielded α-fluorofolic acid (**1**).



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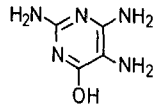

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Purification of compound **1** was accomplished by dissolving the crude product in hot aqueous alkali (pH 10–12), neutralizing the solution, filtering the cooled solution, and precipitating compound **1** by adjusting the filtrate to pH 3.1–3.2 (somewhat lower than the optimum pH for precipitation of unsubstituted folic acid). The U.V. spectrum of **1** was practically identical with that of folic acid (both in 0.1 *N* hydrochloric acid and in 0.1 *N* aqueous sodium hydroxide). The I.R. spectrum of **1** was very similar to that of folic acid, except for the fluorine absorption in the 1000–1200 cm^{-1} region in the case of **1**.

γ-Fluoroglutamic Acid (2):

Method A; from Ethyl α-Acetamidoacrylate and Diethyl Fluoromalonate[3]: A solution of ethyl α-acetamidoacrylate[10] (32.0 g) in absolute ethanol (50 ml) was added dropwise and with stirring to a mixture of ethanolic sodium ethoxide (prepared from 0.412 g of sodium and 100 ml of ethanol) and diethyl fluoromalonate[11] (37.8 g). The mixture was stirred at room temperature for 12 hr, then acidified with hydrochloric acid, filtered, and concentrated. The viscous residue (60 g) was refluxed with conc. hydrochloric acid (300 ml) for 12 hr, evaporated in vacuo, the residue taken up with water, the solution evaporated, and the residue again taken up with water. The syrup obtained on evaporation was dissolved in hot water and the solution treated with excess silver oxide. The filtered solution was evaporated to dryness and the residue triturated with ethanol. The product obtained upon evaporation of the solution was dried over phosphorus pentoxide in vacuo at 110° to give the anhydrous acid **2**; yield: 23.0 g (60%); m.p. 188–192°.

$C_5H_8FNO_4$	calc.	C 36.4	H 4.9	F 11.5	N 8.5
(165.1)	found	36.3	5.0	11.2	8.7

Method B; from β-Chloroactic Acid:

Ethyl 3-Chloro-2-hydroxypropanoate (Ethyl β-Chlorolactate, **3**): Crude β-chlorolactic acid[12] (450 g) was refluxed with absolute ethanol (900 ml) containing 4% hydrogen chloride for 5 hr. Ester **3** was isolated by distillation of the reaction mixture; yield: 330 g (60%); b.p. 104–109°/25 torr; m.p. 35–37.

$C_5H_9ClO_3$	calc.	C 39.4	H 5.9
(152.6)	found	39.7	5.4

I.R. (neat): ν_{max}, 3500 (OH), 1750 (C=O), 1200, 870, 750 (C—Cl) cm^{-1}.

Ethyl 2-t-Butoxy-3-chloropropanoate (**4**): In a hydrogenation bottle liquid isobutylene (200 ml) was poured into a solution of compound **3** (60 g) in dichloromethane (120 ml) at acetone/dry ice temperature. After a reaction time of 3 days, the mixture was washed with aqueous sodium hydrogen carbonate and water and the product isolated by distillation; yield: 50 g (62%); b.p. 104–106°/20 torr.

$C_9H_{17}ClO_3$	calc.	C 51.7	H 8.2	Cl 17.0
(208.7)	found	51.3	7.8	16.2

I.R. (neat): ν_{max}, 1700 (t-C_4H_9), 1100, 850, 740 (C—Cl) cm^{-1}.

Diethyl α-Acetamido-α-ethoxycarbonyl-γ-hydroxyglutarate (**6**): To a solution prepared from sodium (2.0 g), diethyl acetamidomalonate (16 g), and absolute ethanol (150 ml), ester **4** (16 g) was added dropwise with stirring. The reaction was complete within 12 hr at room temperature. Sodium chloride was removed by centrifugation and washed with ethanol. The combined liquid phases were concentrated in vacuo yielding crude, oily diethyl *α-acetamido-γ-t-butoxy-α-ethoxycarbonylglutarate* (**5**); yield: 15 g [I.R. (neat): ν_{max}, 3400 (NH), 1750 (ester C=O), 1650 (amide C=O), 1460, 1350 (t-C_4H_9), 1030, 870 cm^{-1}]. The crude compound **5** (15 g) was dissolved in glacial acetic acid and a small amount of *p*-toluenesulfonic acid was added. The mixture was refluxed for 30 min, then poured into water, and extracted with dichloromethane. The extract on evaporation gave **6**; yield: 12 g (92%); m.p. 50–52°.

$C_{14}H_{23}NO_8$	calc.	C 50.5	H 6.9	
(333.3)	found	50.1	7.0	

I.R. (neat): ν_{max}, 3500, 3300, 1750 (ester C=O), 1600 (amide C=O) cm^{-1}.

γ-Fluoroglutamic Acid (**2**): A mixture of compound **6** (22 g), dichloromethane (50 ml), and 2-chloro-1,1,2-trifluoroethyl-diethylamine (22 g) was stirred for 60 hr in a well-stoppered flask at room temperature, then washed with 10% potassium carbonate solution and water, and distilled in vacuo. At 80–82°/8 torr, N,N-diethylchlorofluoroacetamide distilled. The viscous oily residue was diethyl α-acetamido-α-ethoxycarbonyl-γ-fluoroglutarate (**7**); yield: 10 g (45%); the compound was not purified further; it was refluxed with conc. hydrochloric acid (40 ml) for 12 hr and the resultant solution evaporated in vacuo. The residue was twice taken up in water and brought to dryness, then dissolved in hot water and treated with silver oxide. The filtrate was brought to a volume of ~20 ml and kept at 0°; it had pH 2.8. The *monohydrate of γ-fluoroglutamic acid* crystallized; yield: 2.9 g colorless crystals; m.p. 183–185° (dec.). The I.R. and ^{1}H-N.M.R. data were identical with those of the anhydrous acid. In the R_f values, a slight difference was observed (propanol/water 7:3, ninhydrin): 0.35 for the hydrate, 0.40 for the dried product.

Pteroyl-γ-fluoroglutamic Acid (α-Fluorofolic Acid, 1):

4-Benzyloxycarbonylaminobenzoic Acid: To a solution of sodium hydroxide (8 g) and 4-aminobenzoic acid (27.4 g) in water (300 ml) at 0–5°, benzyloxycarbonyl chloride (34 g) was added dropwise and with stirring; the pH of the mixture was kept at 8–9 by the occasional addition of 2 *N* aqueous sodium hydroxide. After 2 hr, the mixture was acidified to pH 2–3 with conc. hydrochloric acid. The precipitated product was isolated by filtration and recrystallized from butanol; yield: 40 g (82%); m.p. 224–226°.

$C_{15}H_{13}NO_4$	calc.	C 66.4	H 4.8	N 5.2
(271.3)	found	66.7	4.9	5.0

N-(4-Benzyloxycarbonylaminobenzoyloxy)-succinimide: Dicyclohexylcarbodiimide (30.2 g) was added with cooling to a solution of 4-benzyloxycarbonylaminobenzoic acid (40 g) and N-hydroxysuccinimide (17 g) in dioxane (400 ml). The mixture was stirred for 2 hr and then allowed to stand at 0°. Dicyclohexylurea was removed by filtration and washed with dioxane. The filtrate was concentrated in vacuo, the residual yellow oil was triturated with ether, the ether evaporated, and the product recrystallized from 1,2-dimethoxyethane; yield: 35 g (65%); m.p. 235–236°.

$C_{19}H_{16}N_2O_6$	calc.	C 62.0	H 4.6	N 7.3
(368.3)	found	62.0	4.3	7.4

I.R. (KBr): ν_{max}, 3350 (N—H), 1750 (ester C=O), 1600 (amide C=O) cm^{-1}.

γ-Fluoro-N-(4-benzyloxycarbonylaminobenzoyl)-glutamic Acid: A solution of the foregoing compound (5.54 g) in 1,2-dimethoxyethane (150 ml) was added to a stirred solution of γ-fluoroglutamic acid (**2**; 2.47 g) and sodium hydrogen carbonate (5.54 g) in water (50 ml) at room temperature. After 18 hr, the mixture was acidified to pH 2 with conc. hydrochloric acid and chilled for 1 hr; yield: 4.18 g (70%); m.p. 140–142°.

$C_{20}H_{19}FN_2O_7$	calc.	C 57.4	H 4.5	F 4.5	N 6.7
(418.4)	found	56.8	4.8	4.2	6.9

I.R. (KBr): ν_{max}, 3300 (N—H), 1750 (C=O), 1050 (C—F) cm^{-1}.

N-(4-Aminobenzoyl)-γ-fluoroglutamic acid (**8**)[9]: A 33% solution of hydrogen bromide in glacial acetic acid was added to the foregoing compound (4 g) and the mixture allowed to stand at room temperature with occasional shaking until the evolution of carbon dioxide ceased ($1^1/_2$ hr). Dry ether (300 ml) was then added and the reaction flask kept in the refrigerator for 4 hr. The solid hydrobromide which separated was isolated by filtration, washed with dry ether, and dried in vacuo. It was then dissolved in aqueous sodium hydroxide (2 equiv) and the solution acidified to pH 2 with conc. hydrochloric acid. Compound **8** precipitated; yield: 2.2 g (80%); m.p. 105–107°.

$C_{12}H_{13}FN_2O_5$	calc.	C 50.7	H 4.6	F 6.7
(284.2)	found	51.1	4.8	6.0

I.R. (KBr): ν_{max}, 3400 (NH_2), 3280, 1730 (C=O), 1130 (C—F) cm^{-1}.

α-Fluorofolic Acid (**1**): 4-Hydroxy-2,5,6-triaminopyridine sulfate (2.55 g) was dissolved in a solution of barium chloride (0.80 g) in water (25 ml). The mixture was stirred and heated at 60° for 3 hr, and then filtered to remove barium sulfate. To the filtrate (pH 1–2), N-(4-aminobenzoyl)-γ-fluoroglutamic acid (**8**; 2.84 g) was added with stirring. The solution was maintained at pH 3.6 throughout the reaction. To this solution was added, slowly ($1^1/_2$ hr) and with stirring, a solution of 2,3-dibromopropanal (2.35 g) in ethanol (50 ml) and the mixture was stirred for a further 2 hr. The precipitated product was collected by filtration, washed with ethanol, and dried; yield of crude product: 0.80 g (17%).

Crude **1** (2.50 g) was dissolved in hot aqueous sodium hydroxide (1000 ml) at pH 10–12 and the solution brought to pH 7. The mixture was cooled and filtered, the filtrate adjusted to pH 3, and the precipitate collected by filtration. It was again dissolved in aqueous sodium hydroxide (100 ml) at pH 10–12 and the solution diluted with hot water (250 ml); then, the pH was adjusted to 3.1–3.2 by the addition of acetic acid. Upon cooling, pure **1** crystallized; yield: 0.825 g (5.6%); m.p. 265° (charring).

$C_{19}H_{18}FN_7O_6 \cdot H_2O$	calc.	C 47.8	H 4.2	F 4.0	N 20.5
(467.4)	found	47.9	4.4	3.9	20.3

U.V. Spectra of α-fluorofolic acid and (for comparison) of folic acid:

	0.1 *N* Hydrochloric Acid
Folic Acid	250[s] (10900), 298 (16000), 337[s] (6800)
α-Fluorofolic Acid	250[s] (18700), 298 (22000), 337[s] (9000), 400 (2600)
	0.1 *N* Sodium Hydroxide
Folic Acid	220 (15900), 258 (21000), 285 (20800), 365 (7500)
α-Fluorofolic Acid	220 (18400), 258 (28500), 280 (23200), 365 (8100), 445[s] (1500)

γ-Chloroglutamic Acid:
Thionyl chloride (6.5 g) was added during 2 hr, with vigorous stirring, to a solution of diethyl α-acetamido-α-ethoxycarbonyl-γ-hydroxyglutarate (**6**; 6 g) in pyridine (10 ml). The mixture was refluxed for 30 min and then evaporated. The crude viscous residue (8 g) was refluxed with conc. hydrochloric acid (10 ml) for 10 hr and the resultant solution evaporated in vacuo. The residue was taken up in water and the solution evaporated; this procedure was repeated twice. The residue of the last evaporation was triturated with ethanol. Evaporation of the ethanolic solution gave γ-chloroglutamic acid; yield: 1.2 g (32%); m.p. 192–194°.

$C_5H_8ClNO_4$	calc.	C 33.1	H 4.4	Cl 19.5	N 7.8
(181.6)	found	33.2	5.1	19.7	7.8

I.R. (KBr): ν_{max}, 3450, 3150, 2600, 1650 (C=O), 750 (C—Cl) cm^{-1}.

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