



## Practical Synthesis of *L-erythro*- and *L-threo*-4-Fluoroglutamic Acids Using Aminoacylase

Yoshitsugu Kokuryo, Takuji Nakatani, Kobee Kobayashi, Yoshinori Tamura, Kenji Kawada,\* and Mitsuaki Ohtani

Shionogi Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan

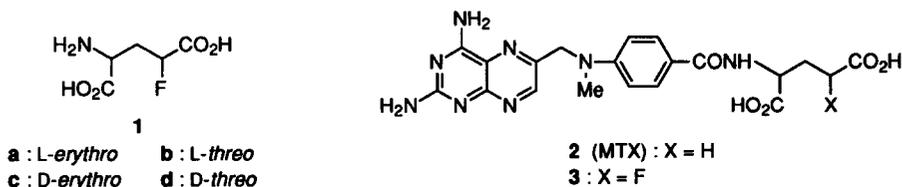
**Abstract:** Enantiomerically pure *L-erythro*- and *L-threo*-4-fluoroglutamic acids **1a** and **1b** were conveniently prepared. The key steps in this synthesis relied upon separation of diastereomers of *N*-chloroacetyl-4-fluoroglutamic acid 5-methyl ester **7** by recrystallization and enzymatic resolution of enantiomers of the resulting **7(a+c)** and **7(b+d)** by aminoacylase. Protection of the  $\gamma$ -carboxyl group as a methyl ester was found to be crucial for this enzymatic reaction.

Copyright © 1996 Elsevier Science Ltd

### INTRODUCTION

In the field of amino acids and peptides, increasing attention has been attracted to fluorine-containing amino acids due to their interesting biological activities.<sup>1</sup> Among them, 4-fluoroglutamic acid **1** derivatives have wide potential utility as enzyme substrates or inhibitors, agonists or antagonists of glutamate receptors, and parts of pharmacologically useful peptides. Specifically, **1** was employed for modification of the antitumor agent methotrexate **2** (MTX), leading to the less toxic analogue **3** with some favorable features for high-dose treatment of MTX-resistant cancers (Scheme 1).<sup>2</sup> This result was ascribed to the extreme electronegativity of fluorine<sup>3</sup> which caused acidity enhancement of the  $\gamma$ -carboxyl group and hence decreased its *in vivo* polyglutamate formation.<sup>4</sup> Considering the significant biological effect of chirality,<sup>5</sup> enantiomerically pure fluorinated MTX derivatives for the treatment of rheumatoid arthritis<sup>6</sup> as well as cancer are of particular interest.<sup>7</sup>

Scheme 1



Preparations of pure stereoisomers of **1** have been reported by either stereospecific synthesis starting from enantiomerically pure proline derivatives<sup>8</sup> or enzymatic hydrolysis of *L-N*-leucyl-4-fluoroglutamic acids by leucine aminopeptidase.<sup>9</sup> However, in order to obtain a large amount of this important compound in enantiomerically pure form, a more practical procedure was needed for large-scale, safe, and efficient production. Here, we describe a practical synthesis of (2*S*,4*R*)-*L*-*erythro*- and (2*S*,4*S*)-*L*-*threo*-4-fluoroglutamic acids **1a** and **1b** based on enzymatic resolution with aminoacylase.

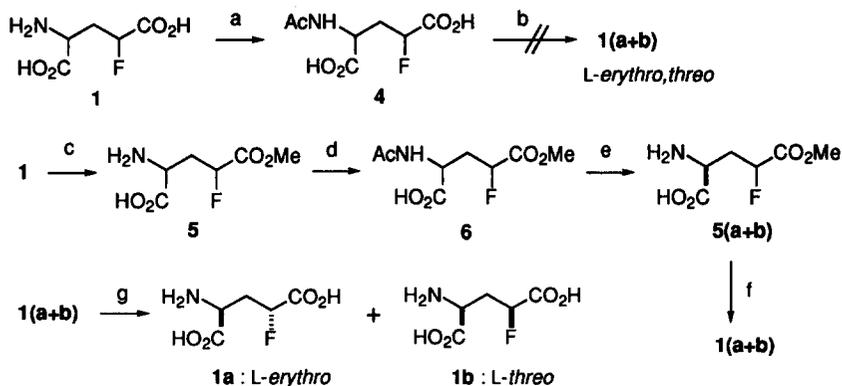
## RESULTS AND DISCUSSION

Among a variety of reported synthetic approaches for preparing racemic **1**,<sup>10</sup> we adopted a convenient method involving Michael addition of diethyl acetamidomalonate to ethyl 2-fluoroacrylate and hydrolysis of the resulting adduct.<sup>11</sup> Owing to a recent improvement in obtaining 2-fluoroacrylate,<sup>12</sup> this method seemed to be suitable for large-scale synthesis. With a large amount of racemic diastereomers of **1** in hand, we next sought to develop a convenient resolution procedure.

In order to prepare enantiomerically pure amino acids, numerous methods have been exploited using various specific enzymes such as acylases, amidases, esterases, and transaminases.<sup>13</sup> Preparation of enantiomerically pure **1** using leucine aminopeptidase has been reported,<sup>9</sup> but this enzyme lacked stereospecificity toward the *erythro* isomer (87% e.e.). In addition, this procedure was unsuitable for large-scale production since the separation of *erythro* and *threo* diastereomers was based on ion-exchange chromatography on a Dowex 1 column. Therefore, we decided to utilize widely applied aminoacylase (acylase I, EC 3.5.1.14.) for the resolution of enantiomers<sup>2b</sup> and search for a convenient method based on crystallization for the separation of diastereomers.

First, we studied deacylation of *N*-acetyl-4-fluoroglutamic acid **4**<sup>14</sup> using aminoacylase from porcine kidney or *Aspergillus* as shown in Scheme 2. Aminoacylase has been known to catalyze the hydrolysis of *N*-acyl-*L*-glutamic acid.<sup>15</sup> Moreover, in the case of *N*-acetyl-3-fluoroglutamic acid, this enzyme was reported to afford enantiomerically pure *L* isomer of 3-fluoroglutamic acid.<sup>16</sup> However, as for the *N*-acetylated derivative **4**, all attempts to obtain deacylated fluoroglutamic acid **1(a+b)** were unsuccessful. Considering minimal steric disturbance by fluorine substitution<sup>3</sup> as well as a broad range of substrate acceptance by aminoacylase,<sup>15</sup> the absence of reactivity with **4** was probably due to the high acidity of the  $\gamma$ -carboxylic acid rather than sterical problems. Consequently, we decided to protect the  $\gamma$ -carboxylic acid of **4** as an ester.

Selective methylation of the  $\gamma$ -carboxyl group of **1** by thionyl chloride in methanol<sup>17</sup> and acetylation of ester **5** provided *N*-acetyl-4-fluoroglutamic acid 5-methyl ester **6**. Enzymatic reaction by porcine kidney aminoacylase under the reaction conditions of pH 7.0 for 17 h afforded the desired deacylated *L*-*erythro*- and *L*-*threo*-ester **5(a+b)**. Purification of the ester by ion-exchange column chromatography (Dowex 50W-X8) using 1 N aqueous ammonia led the subsequent hydrolysis to provide 4-fluoroglutamic acid **1(a+b)** in 18% yield. This product proved to consist of only *L* isomers according to HPLC.<sup>9b</sup> Recrystallization of the diastereomeric mixture of *L* isomers **1(a+b)** provided the *L*-*threo* isomer **1b**, and the *L*-*erythro* isomer **1a** could be isolated from the mother liquor. However, the diastereomeric purity of products was in the range of 88 to 97% and that of **1a** was rather lower. Much more efficient resolution and recrystallization were necessary.

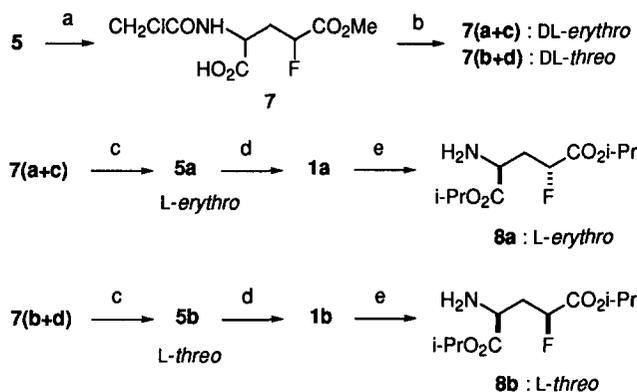
Scheme 2<sup>a</sup>

<sup>a</sup> (a)  $\text{Ac}_2\text{O}$ , aq. NaOH (87%); (b) aminoacylase; (c)  $\text{SOCl}_2$ , MeOH (79%); (d)  $\text{Ac}_2\text{O}$ ,  $\text{Et}_3\text{N}$ , DMF; (e) aminoacylase, pH 7.0, 17 h; (f) ion-exchange resin using 1 N  $\text{NH}_3$  (18% for d, e, f); (g) recrystallization.

Chloroacetyl amino acids have been reported to have higher enzymatic reactivities than acetyl derivatives and be stable against spontaneous hydrolysis.<sup>15b</sup> Therefore, *N*-chloroacetyl-4-fluoroglutamic acid 5-methyl ester **7** was prepared from **5** by using chloroacetyl chloride in acetonitrile as shown in Scheme 3. This led to the *erythro* isomer **7(a+c)** being secured in over 99% diastereomeric purity through recrystallization from 2-butanone, and **7(b+d)** enriched by the *threo* isomer being obtained from the mother liquor. Pure *threo* isomer could be prepared at a later stage by recrystallization of free amino acid **1b** or diisopropyl ester **8b**. In particular, as described below, diisopropyl ester **8b** was a suitable derivative for purification of the *threo* isomer by large-scale recrystallization. With the achievement of efficient separation of diastereomers, we next examined the enzymatic reaction using chloroacetyl derivative **7** as a potential substrate.

Reactivity of **7** with porcine kidney aminoacylase at various pH values was measured by colorimetric determination of free amino acid with ninhydrin.<sup>18</sup> We observed that the optimal pH value for the reaction was around 6.6, and chloroacetyl derivative **7** was deacylated two times faster than acetyl derivative **6**. Next, progress of the reaction was monitored at pH 7.0 and the conversion yield was found to reach a maximum level after a few hours. After termination of the reaction, no promotion of the deacylation was observed by addition of aminoacylase. On the other hand, further deacylation proceeded by adding more substrate. These results suggested that termination of the reaction was not due to deactivation of aminoacylase but to decomposition of the substrate. Namely, the ester moiety of **7** was assumed to be easily hydrolyzed even under enzymatic reaction conditions because of the inductive effect of fluorine to produce unreactive free fluorocarboxylic acid.

In order to avoid hydrolysis of the methyl ester, the carboxylic acid was converted to a more bulky ester, such as the ethyl and isopropyl derivatives. However, the enzymatic reactivity of ethyl and isopropyl esters was markedly low compared with methyl ester **7**.

Scheme 3<sup>a</sup>

<sup>a</sup> (a)  $\text{CH}_2\text{ClCOCl}$ ,  $\text{CH}_3\text{CN}$ ; (b) recrystallization (**7(a+c)**: 47% for a, b); (c) aminoacylase, pH 6.0, 3 h; (d) ion-exchange resin using 1 N  $\text{NH}_3$  (86% for c, d); (e)  $\text{SOCl}_2$ , *i*-PrOH (95%).

As a result, we decided to employ methyl ester **7** as a substrate and sought to establish optimal reaction conditions for preventing hydrolysis of the  $\alpha$ -fluoro methyl ester. Although substrate **7** was rather unstable even under pH 7.0, good results were obtained by conducting the reaction at pH 6.0 for 3 h using 2 N aqueous ammonia to adjust the pH. Under these reaction conditions, enzymatic deacylation of fluorinated methyl ester **7(a+c)** followed by purification with ion-exchange column chromatography afforded enantiomerically pure L-isomer **1a** in 86% isolated yield. The relative structure of both *L-erythro*-4-fluoroglutamic acid **1a** and *L-threo*-4-fluoroglutamic acid **1b** were confirmed by X-ray crystallographic analysis.<sup>19</sup>

Finally, both free amino acids **1a** and **1b** were converted to isopropyl esters **8a** and **8b**, respectively, in preparation for the synthesis of enantiomerically pure MTX analogue **3**.<sup>2</sup> Large-scale purification of the *threo* diastereomer was achieved effectively by recrystallization of hydrochloride of **8b** from 2-butanone.<sup>20</sup>

In conclusion, we were able to develop a practical way of preparing enantiomerically pure *L-erythro*-4-fluoroglutamic acid **1a** and *L-threo*-4-fluoroglutamic acid **1b** through separation of diastereomers of **7** by recrystallization and subsequent enzymatic resolution of enantiomers of the resulting **7(a+c)** and **7(b+d)** by aminoacylase. With substantial supplies of both enantiomerically pure glutamates **8a** and **8b** in hand, we next plan to prepare various fluorinated MTX derivatives in an attempt to develop useful antifolates with reduced toxicity.

## EXPERIMENTAL SECTION

**General.** Unless otherwise stated, all reactions were carried out under nitrogen atmosphere with anhydrous solvents that had been dried over type 4A molecular sieves. Melting points are uncorrected. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were determined at 200 and 50.3 MHz, respectively. Sodium 4,4-dimethyl-4-silapentane-1-sulfonate was used as an internal standard in deuterium oxide. Coupling values to fluorine of <sup>13</sup>C NMR signals are given in parentheses. High resolution-liquid secondary ion mass spectra (HR-LSIMS) were determined using *m*-nitrobenzyl alcohol as a matrix. Cation- and anion-exchange chromatography was performed using Dowex 50W-X8 resin (Muromachi, 100-200 mesh) and AG1X4 resin (Bio-Rad, 200-400

mesh, formate form), respectively. TLC was performed on Merck pre-coated cellulose plate with 6:5:3 butanol-water-acetic acid and compounds were visualized with 1% (w/w) ninhydrin in methanol. Diastereomeric and enantiomeric purity was determined by HPLC using Cosmosil 5C18 column (Nakalai, 15 × 0.4 cm) with *N,N*-di-*n*-propyl-L-alanine (8 mM) and cupric acetate (4 mM) (detected by post-column derivatization with *ortho*-phthalaldehyde)<sup>21</sup> or Crownpack CR(+) column (Daicel, 15 × 0.4 cm) with aqueous perchloric acid (pH 1.6). Aminoacylase enzymes from porcine kidney (1430 units/mg) and *Aspergillus* were from Sigma and Amano, respectively.

**(2*RS*,4*RS*)-DL-erythro,threo-N-Acetyl-4-fluoroglutamic acid 5-Methyl Ester 6.** To a solution of 4.75 g (27.0 mmol) of **5**<sup>17</sup> in 80 mL of *N,N*-dimethyl formamide were added 9.41 mL (67.5 mmol) of triethylamine and 3.06 mL (32.4 mmol) of acetic anhydride. The mixture was stirred for 2 h at 25 °C and then concentrated. The residue was evaporated *in vacuo* at 60 °C to afford 5.88 g of **6** as a colorless oil, which was used for the enzymatic reaction without further purification. IR (nujol) 3700-2500 (br), 1772, 1644, 1535 cm<sup>-1</sup>. <sup>1</sup>H NMR (D<sub>2</sub>O) δ 2.28-2.64 (m, 2H), 2.85 and 3.01 (each s, 3H), 3.81 and 3.82 (each s, 3H), 4.51-4.65 (m, 1H), 5.19 and 5.30 (each ddd, *J* = 3.6, 8.8, 47.9 Hz, and 5.1, 5.2, 47.2 Hz, 1H). HR-LSIMS *m/z* 222.0784 (M + H)<sup>+</sup> (calcd for C<sub>8</sub>H<sub>13</sub>FNO<sub>5</sub> *m/z* 222.0778).

**(2*S*,4*R*:2*R*,4*S*)-DL-erythro-N-Chloroacetyl-4-fluoroglutamic acid 5-Methyl Ester 7(a+c) and (2*S*,4*S*:2*R*,4*R*)-DL-threo-N-Chloroacetyl-4-fluoroglutamic acid 5-Methyl Ester 7(b+d).** To a suspension of 48 g (268 mmol) of **5**<sup>17</sup> in 640 mL of acetonitrile was added 27.6 mL (347 mmol) of chloroacetyl chloride. The mixture was refluxed for 2 h and then concentrated. The residue was diluted with 200 mL of acetone and filtered. The filtrate was concentrated and the residue was recrystallized from 2-butanone to afford 31.9 g (47%) of **7(a+c)** as colorless crystals (*erythro*, >99%). Evaporation of the mother liquor gave 36.6 g of **7(b+d)** as a colorless oil (*threolexythro*, 94:6). **7(a+c)**: mp 129-130 °C. IR (KBr) 3600-2400 (br), 1768, 1650, 1540 cm<sup>-1</sup>. <sup>1</sup>H NMR (D<sub>2</sub>O) δ 2.05-2.48 (m, 2H), 3.40 (s, 3H), 3.83 (s, 2H), 4.42 (m, 1H), 4.98 (ddd, *J* = 5.4, 5.6, 47.0 Hz, 1H). <sup>13</sup>C NMR (D<sub>2</sub>O) δ 33.01 (d, *J* = 20.6 Hz), 42.41, 49.22 (d, *J* = 4.0 Hz), 53.59, 86.75 (d, *J* = 182.1 Hz), 170.16, 172.14 (d, *J* = 24.6 Hz), 174.33. Anal. Calcd for C<sub>9</sub>H<sub>11</sub>ClFNO<sub>5</sub>: C, 37.59; H, 4.34; Cl, 13.87; F, 7.43; N, 5.48. Found: C, 37.53; H, 4.45; Cl, 13.60; F, 7.41; N, 5.59. **7(b+d)**: <sup>1</sup>H NMR (D<sub>2</sub>O) δ 2.01-2.37 (m, 2H), 3.48 (s, 3H), 3.92 (s, 2H), 4.35 (m, 1H), 4.86 (ddd, *J* = 3.0, 8.2, 47.6 Hz, 1H). <sup>13</sup>C NMR (D<sub>2</sub>O) δ 33.26 (d, *J* = 20.6 Hz), 41.76, 49.58 (d, *J* = 2.0 Hz), 53.64, 86.73 (d, *J* = 181.1 Hz), 170.33, 172.39, 174.19. HR-LSIMS *m/z* 256.0388 (M + H)<sup>+</sup> (calcd for C<sub>9</sub>H<sub>12</sub><sup>35</sup>ClFNO<sub>5</sub> *m/z* 256.0388).

**(2*S*,4*R*)-L-erythro-4-Fluoroglutamic acid 1a.** A suspension of 26.5 g (100 mmol) of **7(a+c)** in 1.7 L of distilled water was brought to pH 6.0 with 2 N aqueous ammonia. To this solution was added 200 mg of porcine kidney aminoacylase (1430 units/mg) and then incubated for 3 h at 28 °C under shaking. The mixture was adjusted to pH 6.0 during the reaction. After the mixture was brought to pH 3.0 by adding 42.5 mL of acetic acid, the crude mixture was separated by 680 mL of Dowex 50W-X8 column using 3.4 L of water as eluent first until the eluent became neutral and then 1 N aqueous ammonia to elute the hydrolyzed amino acid. The ninhydrin positive fractions were collected and concentrated below 30 °C *in vacuo* to obtain the ammonium salt of **1a**. The salt was subjected 340 mL of AG1X8 column using 3.4 L of water as the first eluent and then 1 N aqueous formic acid. The latter fractions were concentrated and dried *in vacuo* to give 9.4 g (86%) of **1a** as colorless crystals, which were identical in all respects with published data.<sup>8,9</sup>

**(2*S*,4*S*)-L-threo-4-Fluoroglutamic acid 1b.** Compound **1b** was obtained from **7(b+d)**, using a procedure similar to that described above, as colorless crystals, which were identical in all respects with published data.<sup>8,9</sup>

**(2S,4R)-L-erythro-4-Fluoroglutamic acid 1,5-Diisopropyl Ester Hydrochloride 8a-HCl.** To a suspension of 8.3 g (50.3 mmol) of **1a** in 350 mL of isopropanol was added 89.7 g (754 mmol) of thionyl chloride. The mixture was refluxed for 7 h and then concentrated. The crystalline residue was recrystallized from 2-butanone to afford 12.2 g (85%) of **8a-HCl** as colorless crystals. mp 149-150 °C.  $[\alpha]_D^{24} +21.8$  (c 0.5, CHCl<sub>3</sub>). IR (CHCl<sub>3</sub>) 3300-2300 (br), 1745, 1376, 1239, 1227 cm<sup>-1</sup>. <sup>1</sup>H NMR (D<sub>2</sub>O) δ 1.28 (d, *J* = 6.2 Hz, 6H), 1.30 (d, *J* = 5.8 Hz, 3H), 1.31 (d, *J* = 6.0 Hz, 3H), 2.31-2.82 (m, 2H), 4.43 (m, 1H), 5.06-5.15 (m, 1H), 5.11 (q, *J* = 6.2 Hz, 1H), 5.45 (ddd, *J* = 3.4, 9.4, 48.8 Hz, 1H), 8.92 (br s, 3H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 22.01, 22.12, 33.22 (d, *J* = 19.8 Hz), 50.72, 70.64, 71.85, 86.27 (d, *J* = 61.1 Hz), 168.37, 168.81. Anal. Calcd for C<sub>11</sub>H<sub>21</sub>ClFNO<sub>4</sub>: C, 46.24; H, 7.41; Cl, 12.41; F, 6.65; N, 4.90. Found: C, 45.88; H, 7.44; Cl, 12.16; F, 6.54; N, 5.13.

**(2S,4S)-L-threo-4-Fluoroglutamic acid 1,5-Diisopropyl Ester Hydrochloride 8b-HCl.** Compound **8b-HCl** was obtained from **1b** using a procedure similar to that described above, with all spectral data in accord with published values of DL-*threo*-**8-HCl**.<sup>20</sup>  $[\alpha]_D^{24} +2.0$  (c 0.5, CHCl<sub>3</sub>).

#### REFERENCES AND NOTES

1. (a) Kollonitsch, J. In *Biomedical Aspects of Fluorine Chemistry*; Filler, R., Kobayashi, Y., Eds.; Kodansha: Tokyo, 1982, pp. 93-122. (b) Tsushima, T.; Kawada, K. In *Fluorine Chemistry in Pharmaceutical Sciences, Basic and Experimental*; Kobayashi, Y., Kumadaki, I., Taguchi, T., Eds.; Hirokawa Publishing: Tokyo, 1993, pp. 55-72 (in Japanese). (c) Symposium on *Fluoro-Amino Acids and Peptides in Medicinal Chemistry*; 210th ACS Meeting, Chicago, August, 1995.
2. (a) Tsushima, T.; Kawada, K.; Shiratori, O.; Uchida, N. *Heterocycles* **1985**, *23*, 45. (b) Tsushima, T.; Kawada, K.; Ishihara, S.; Uchida, N.; Shiratori, O.; Higaki, J.; Hirata, M. *Tetrahedron* **1988**, *44*, 5375.
3. For a recent review on fluorine chemistry, see: (a) *Chemistry of Organic Fluorine Compounds II, A Critical Review*; Hudlicky, M.; Pavlath, A. E. Eds.; American Chemical Society: Washington, D.C., 1995. For recent results in this field, see: (b) *Fluoroorganic Chemistry: Synthetic Challenges and Biomedical Rewards (Tetrahedron Symposium-in-Print)*; *Tetrahedron* **1996**, *52*, 1-330.
4. (a) Galivan, J.; Ingless, J.; McGuire, J. J.; Nimec, Z.; Coward, J. K. *Proc. Natl. Acad. Sci., U.S.A.* **1985**, *82*, 2598. (b) Coward, J. K.; McGuire, J. J.; Galivan, J. In *Selective Fluorination in Organic and Bioorganic Chemistry*; Welch, J. T. Ed.; American Chemical Society: Washington, D.C., 1991; pp. 196-204.
5. (a) *Drug Stereochemistry*; Wainer, I. W., Drayer, D. E. Eds.; Marcel Dekker: New York, 1988. (b) Stinson, S. C. *Chem. Eng. News* **1995**, October 9, 44. (c) Cannarsa, M. J. *Chem. Ind. (London)* **1996**, May 6, 374.
6. For general reviews, see: (a) Bannwarth, B.; Labat, L.; Moride, Y.; Schaefferbeke, T. *Drugs* **1994**, *47*, 25. (b) DeGraw, J. I.; Colwell, W. T.; Pipe, J. R.; Sirotak, F. M.; Smith, R. L. *Curr. Med. Chem.* **1995**, *2*, 630.
7. MTX analogue containing D-glutamic acid was reported to be a poor inhibitor of cell growth, see: (a) Cramer, S. M.; Schornagel, J. H.; Kalghatgi, K. K.; Bertino, J. R.; Horvath, C. *Cancer Res.* **1984**, *44*, 1843. The *in vitro* activity of L-*threo*-**3** has been reported recently, see: (b) Hart, B. P.; Haile, W. H.; Licato, N. J.; Bolanowska, W. E.; McGuire, J. J.; Coward, J. K. *J. Med. Chem.* **1996**, *39*, 56.
8. (a) Hudlicky, M.; Merola, J. S. *Tetrahedron Lett.* **1990**, *31*, 7403. (b) Avent, A. G.; Bowler, A. N.; Doyle, P. M.; Marchand, C. M.; Young, D. W. *Tetrahedron Lett.* **1992**, *33*, 1509. (c) Hudlicky, M. J. *Fluorine*

*Chem.* **1993**, *60*, 193.

9. (a) Unkeless, J. C.; Goldman, P. *Mol. Pharmacol.* **1971**, *7*, 293. (b) Bory, S.; Dubois, J.; Gaudry, M.; Marquet, A.; Lacombe, L.; Weinstein, S. *J. Chem. Soc., Perkin Trans. 1* **1984**, 475.
10. Tolman V. *J. Fluorine Chem.* **1993**, *60*, 179, and references cited therein.
11. Several kilograms of the adduct were purchased from Daikin Industries, Ltd., see: ref. 12.
12. (a) Ohmori, A.; Takaki, S.; Kitahara, T. European Patent 136668 B1, 1987. (b) *Idem.* Japan Patent Koho 1-50214, 1989.
13. For a general review, see: (a) Williams, R. M. In *Synthesis of Optically Active  $\alpha$ -Amino Acids*; Pergamon Press: New York, 1989; pp. 257-279. For reviews on enzymatic preparation of fluorine-containing amino acids, see: (b) Matsumura, Y.; Urushihara, M. In *Fluorine-containing Amino Acids: Synthesis and Properties*; Kukhar, V. P., Soloshonok, V. A., Eds.; John Wiley & Sons: New York, 1995; pp 243-265. (c) Miyazawa, T. *Ibid.* pp. 267-294.
14. Burde, N. L.; Alekseeva, L. V.; Lundin, B. N. *Zh. Obshch. Khim.* **1968**, *38*, 439.
15. (a) Gentzen, I.; Löffler, H.-G.; Schneider, F. *Z. Naturforsch.* **1980**, *35c*, 544. (b) Chenault, H. K.; Dahmer, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 6354.
16. Vidal-Cros, A.; Gaudry, M.; Marquet, A. *J. Org. Chem.* **1985**, *50*, 3163.
17. Tolman, V.; Veres, K. *Collect. Czech. Chem. Commun.* **1967**, *32*, 4460.
18. Rosen, H. *Arch. Biochem. Biophys.* **1957**, *67*, 10.
19. Data of X-ray crystallographic analysis of both **1a** and **1b** can be obtained from Mr. H. Nakai of these Laboratories. The X-ray crystal structures of **1b** and **1c** were also reported by Hudlicky and Merola, see: ref. 8a.
20. Tolman *et al.* reported that DL-*threo*-**8**-HCl was secured in high diastereomeric purity by recrystallization from acetone, see: Tolman, V.; Vlasakova, V.; Nemecek, J. *Fluorine Chem.* **1993**, *60*, 185.
21. (a) Benson, J. R.; Hare, P. E. *Proc. Natl. Acad. Sci., U.S.A.* **1975**, *72*, 619. (b) Gil-Av, E.; Tishbee, A.; Hare, P. E. *J. Am. Chem. Soc.* **1980**, *102*, 5115.

(Received in Japan 1 October 1996)