CHEMISTRY LETTERS, pp. 1255-1256, 1993.

Enzymatic Transamination to (2*S*)-5,5,5-Trifluoroleucine

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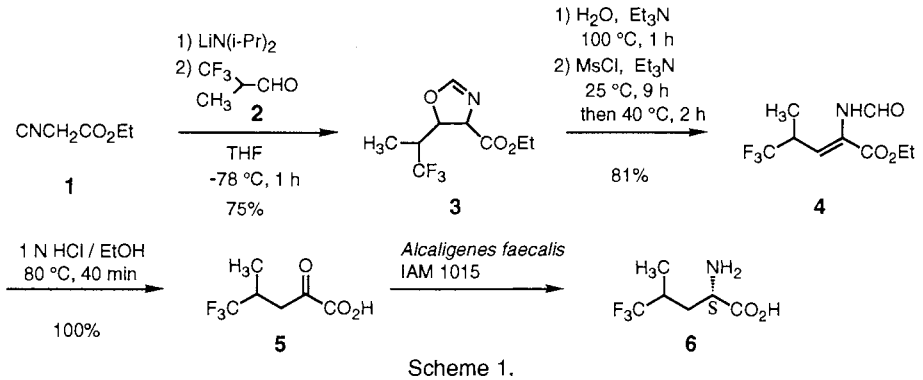
5,5,5-Trifluoro-4-methyl-2-oxopentanoic acid was conveniently synthesized and transformed into (2*S*)-5,5,5-trifluoroleucine with high optical purity by enzymatic transamination using *Alcaligenes faecalis* IAM 1015.

Fluorine containing amino acids[1] have been extensively studied in recent years because of their unique biological activities as potent enzyme inhibitors and also as key units of physiologically important peptides suc as renin,[2] neurokinin[3] and enkephalin analogs.[4] In accord with the requirement for optically active fluorinated amino acids, much effort has been directed toward the development of practical methods for enantioselective synthesis.[1,5] Although a few approaches[6] have been reported for the synthesis of chiral trifluoroleucine by optical resolution, it seems to remain limitations in the introduction of fluorine containing blocks or in the efficiency of the resolution.

Our previous research[7] on enzymatic synthesis resulted in the efficient production of fluorine-containing phenylalanine derivatives by transamination using *Alcaligenes faecalis*. In seeking to extend this finding to the aliphatic systems,[8] we chose 5,5,5-trifluoro-4-methyl-2-oxopentanoic acid (**5**) as a precursor of transamination. We first report herein a facile synthesis of **5** and its enzymatic transformation to (2*S*)-5,5,5-trifluoroleucine (**6**).

Preparation of **5** is outlined in Scheme 1. Condensation[9] of ethyl isocyanoacetate (**1**) with the known aldehyde **2**[10] afforded oxazoline **3** in 75% yield, which was converted to **4** by a hydrolysis-dehydration sequence. The ester **4** was directly obtained in 40% yield from **1** and **2** through isomerization in the presence of lithium diisopropylamide (in THF at -78 °C for 5 min then slowly warmed up to room temperature during 30 min and stirred for 1 h). Acid hydrolysis of **4** provided the desired keto acid **5**[11] quantitatively.



Scheme 1.

The enzymatic reactions were carried out using the resting cells of *Alcaligenes faecalis* IAM 1015 grown in a nutrient medium (50 ml, initial pH 7.0) in a shaking flask at 30 °C for 18 h. The cells were harvested by centrifugation and washed with a phosphate buffer (pH 7.0, 10 mM) to obtain wet cells for the transamination. The reaction with *L*-glutamic acid (100 mM) or *L*-aspartic acid (100 mM) was performed at 37 °C for 55 h in a volume of 1 ml of 100 mM phosphate buffer (pH 8.0) containing 46 mM of **5**, 1 mM of pyridoxal phosphate, and 7.1 mg of wet cells (corresponding to 1.2 mg of dry cells). The results are shown in Fig 1.

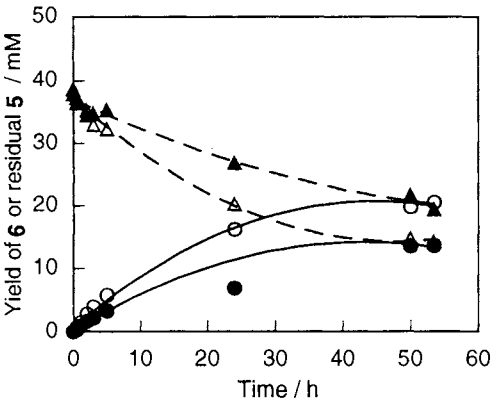


Fig. 1. Production of trifluoroleucine.

Glu for NH_2 donor: Yield of **6** (○); Residual **5** (△)
Asp for NH_2 donor: Yield of **6** (●); Residual **5** (▲)

In contrast to the transamination[7] of fluorinated phenylpyruvic acids, *L*-glutamic acid was more effective than *L*-aspartic acid as a donor of amino group in the reaction rate and selectivity. While the reaction using *L*-aspartic acid produced *L*-alanine as a side product, no *L*-alanine was observed in the reaction mixture with *L*-glutamic acid. A larger scale production using *L*-glutamic acid gave **6** in 39% yield as a 1 : 1 mixture of (2*S*, 4*S*) and (2*S*, 4*R*) diastereomers,[12] which was isolated after purification by ion exchange chromatography.

References

1) J. T. Welch and S. Eswarakrishnan, "Fluorine in Bioorganic Chemistry," John Wiley & Sons, New York (1991); "Biochemical Aspects of Fluorine Chemistry," ed by R. Filler and Y. Kobayashi, Kodansha Ltd., Tokyo and Elsevier Biomedical, Amsterdam (1982).
2) K. Akahane, T. Kamijo, K. Iizuka, T. Taguchi, Y. Kobayashi, Y. Kiso, and H. Umeyama, *Chem. Pharm. Bull.*, **36**, 3447 (1988).
3) T. Sakurada, H. Kuwahara, S. Sakurada, K. Kisara, M. Ohba, and E. Munekata, *Neuropeptides*, **9**, 197 (1987).
4) S. Salvadori, C. Bianchi, L. H. Lazarus, V. Scaranari, M. Attila, and R. Tomatis, *J. Med. Chem.*, **35**, 4651 (1992); J. Watanabe, S. Tokuyama, M. Takahashi, H. Kaneto, M. Maeda, K. Kawasaki, T. Taguchi, Y. Kobayashi, Y. Yamamoto, and K. Shimokawa, *J. Pharmacobio-Dyn.*, **14**, 101 (1991).
5) T. Kitazume, J. T. Lin, and T. Yamazaki, *Tetrahedron Asymm.*, **2**, 235 (1991); I. Ojima, K. Kato, K. Nakanishi, T. Fuchikami, and M. Fujita, *J. Org. Chem.*, **54**, 4511 (1989).
6) a) K. Weinges and E. Kromm, *Liebigs Ann. Chem.*, **1985**, 90; b) T. Taguchi, A. Kawara, S. Watanabe, Y. Oki, H. Fukushima, Y. Kobayashi, M. Okada, K. Ohta, and Y. Iitaka, *Tetrahedron Lett.*, **42**, 5117 (1986); c) T. Tsushima, K. Kawada, S. Ishihara, N. Uchida, O. Shiratori, J. Higaki, and M. Hirata, *Tetrahedron*, **44**, 5375 (1988).
7) K. Uchida and H. Tanaka, *Yuki Gosei Kagaku Kyokaishi*, **46**, 977 (1988).
8) Difluoroaspartic acid was only reported on the transamination. P.A. Briley, R. Eisenthal, R. Harrison, and G. D. Smith, *Biochem. J.*, **161**, 383 (1977).
9) D. Hoppe and U. Schollkopf, *Liebigs Ann. Chem.*, **763**, 1 (1972).
10) I. Ojima, K. Hirai, M. Fujita, and T. Fuchikami, *J. Organomet. Chem.*, **279**, 203 (1985).
11) ^{1}H NMR ($CDCl_3$): δ 1.17 (d, 6.2 Hz, 3 H), 2.70-3.30 (m, 3H), 8.80 (br s, 1 H). ^{19}F NMR ($CDCl_3$): δ -74.1 (d, 8.3 Hz).
12) $[\alpha]_D^{20}$ +12° (c=1.0, 4 N HCl) [lit. $[\alpha]_D^{21.5}$ +13.2 (c=1.00, 4 N HCl)].[6c] ^{1}H NMR specra were identical to the reported data.[6a] Optical purity (> 99%) was determined by chiral HPLC analysis. Daicel Chiralpak WH, 0.25 mM $CuSO_4$, 1 ml/min, 60 °C, UV 254 nm and fluorecence (o-phthalaldehyde, Ex 364 nm, Em 455 nm), (2*R*): t_R = 10.83, (2*S*): t_R= 12.14 min.

(Received March 25, 1993)