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STEREOCONTROLLED SYNTHESIS OF 4,4,4-TRIFLUOROTHREONINE

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Abstract. Stereoisomers of unnatural 4,4,4-trifluorothreonine are obtained through enzymatic resolution, and the absolute configuration of these materials is determined. 4,4,4-Trifluorothreonine thus prepared was evaluated for antifungal or antitumor activity.

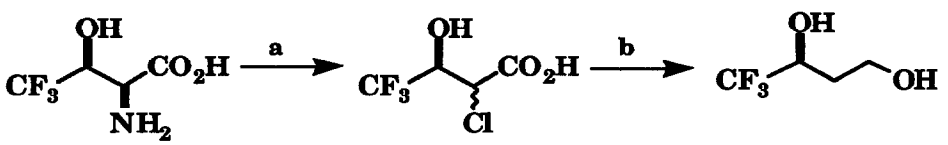
Research work on the biological utility of fluoro analogues of amino acids and their derivatives, which are receiving considerable attention as antifungal, antitumor and chemotherapeutic agents, has been extensive in recent years.[1-5] Obviously, the fundamental requirement in the design of these compounds is to obtain amino materials with high optical purity. In this field, fluorinated threonines (F_n-Thr; n=1,2 or 3) are interesting not only because of their potential pharmaceutical utility but their versatility as chiral building blocks with three distinguishable functionalities. In the literature, F_3-Thr has been reported in racemic as well as in optically active form, however, stereocontrolled synthesis of all isomers of F_3-Thr have not reported.[6,7] Herein, we would like to report the stereocontrolled synthesis of F_3-Thr and their antifungal, antitumor activities.

Stereoisomers of 4,4,4-trifluorothreonine were prepared via the synthetic strategy shown in Scheme 1. The substrates for the enzymatic resolution were prepared from the condensation of imine with trifluoroacetaldehyde followed by diacetylation. It was possible to separate the erythro (***anti***) and threo (***syn***) diastereoisomers by column chromatography on silica gel. The asymmetric hydrolysis of ***syn***-(**3**) with lipase MY (*Candida cylindracea* : Meito Sangyo Co. Ltd.) produces the optically active ***syn***-2*R*,3*R*-(**4a**) alcohol. The ee of ***syn***-2*R*,3*R*-(**4a**) obtained at 37% conversion was 86% ee. The optically pure ***syn***-2*R*,3*R*-(**4a**) [>97%

ee, $[\alpha]_D^{23}$ +18.7 (c 1.13, MeOH)] was obtained from the hydrolysis of the acetate derived from *syn*-2*R*,3*R*-(**4a**) with 86% ee.[8] The desired *syn*-(2*R*,3*R*)-4,4,4-trifluorothreonine, (+)-(**5a**) [>97% ee, $[\alpha]_D^{23}$ +12.5 (c 1, H_2O); mp 209-211°C], was obtained from the acidic hydrolysis of *syn*-2*R*,3*R*-(**4a**) (5h reflux in 1.2N HCl). F_3-Thr was purified by ion exchange chromatography on DOWEX 50W and recrystallized from acetone. The *syn*-2*S*,3*S*-(**4b**) [>93% ee, $[\alpha]_D^{23}$ -17.9 (c 1.07, MeOH)] was prepared from the recovered acetate by hydrolysis using a cellulase (*Trichoderma viride*), and then *syn*-2*S*,3*S*-(**4b**) was also converted to the *syn*-2*S*,3*S*-(-)-(**5b**), [>93%ee, $[\alpha]_D^{23}$ -12.1 (c 1, H_2O); mp 210-213°C]. When the hydrolysis of *anti* isomer was carried to less than 25% with lipase-MY *anti*-2*S*,3*R*-(**4c**) [95% ee, $[\alpha]_D^{23}$ +26.9 (c 1.15, MeOH)] was obtained. Furthermore, *anti*-2*R*,3*S*-(**4d**) [89% ee, $[\alpha]_D^{23}$ -25.4 (c 1.43, MeOH)] was prepared from the corresponding *anti*-2*S*,3*R*-(**4c**) acetate derivative which was recovered from the hydrolysis conversion (74%) of the *anti* isomer with lipase-MY. *Anti*-2*S*,3*R*-(-)-(**5c**) [>95% ee, $[\alpha]_D^{23}$ -11.9 (c 1, H_2O); mp 190-193°C], and *anti*-2*R*,3*S*-(-)-(**5d**) [>93% ee, $[\alpha]_D^{23}$ +11.5 (c 1, H_2O); mp 191-193°C] were also prepared in the same manner.

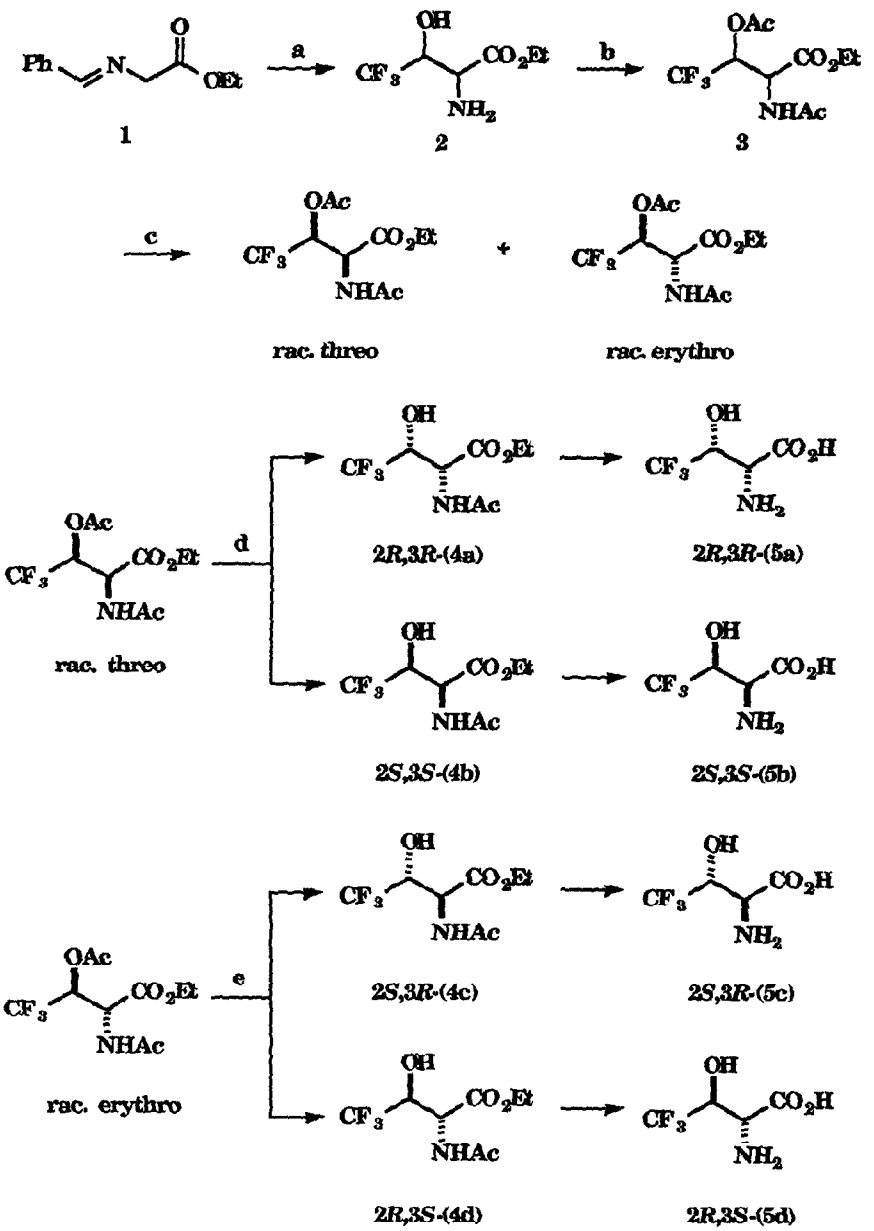
We investigated the absolute configuration of optically pure F_3-Thr as shown in Scheme 2. *Syn*-(-)-(**5b**) [>93%ee, $[\alpha]_D^{23}$ -12.1 (c 1, H_2O); mp 210-213°C], was transformed to (S)-(-)-3-hydroxy-4,4,4-trifluorobutanol **6** with known absolute configuration, $[\alpha]_D^{23}$ -5.71 (c 1.04, MeOH), >91%ee [lit.[9] :$[\alpha]_D^{23}$ -6.14 (c 0.94, MeOH), >96%ee. These results establish that absolute configuration of F_3-Thr, *syn*-(-)-(**5b**) is 2*S*,3*S*-enantiomer.

Then, F_3-Thr stereoisomers were examined for their growth inhibitory action towards tumor cell lines. Table 1 compares the results for F_3-Thr using the established antimetabolite, 5-fluorouracil (5-FU), as a reference. Unfortunately, a comparison of IC_{50} values revealed that only the (2S,3S) isomer, the same stereostructure as naturally occurring L-threonine, possessed activity, which showed less effective inhibition of cell growth than 5-FU.



syn-(-)-(**5 b**) (S)-(-)-(**6**)

a) $NaNO_2$, KCl, 1N H_2SO_4, 0°C ⟶ r.t, 6h b) $LiAlH_4$, THF, r.t.



a) LDA, THF, -78°C, CF_3CHO derived from $CF_3CH(OEt)OH$ and c.H_2SO_4; yield. 64% b) AcCl, pyr., Et_2O; yield. 85% c) column chromatography
d) lipase MY e) cellulase

Table 1 **Effective F_3-Thr on the growth of L1210 cell *in vitro*** [10]

F_3-Thr	IC_{50} (μg/mL)
(2S,3S)	27.0
(2S,3R)	>100
(2R,3R)	87
(2R,3S)	>100
5-FU	0.36

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8. Asymmetric hydrolysis: A suspension of lipase MY (Meito Sangyo Co. Ltd., 6.0 g), and **syn**-(**3**) (5.7 g) in a buffer solution (pH 7.3, 120 ml) was stirred at 40-41°C. After 96h of stirring, the mixture was acidified with 1N HCl and oily materials was extracted with ethyl acetate. After determining the hydrolysis conversion (37%) by 19F NMR signal intensities, the products were separated by column chromatography on silica gel, producing *syn*-2*R*,3*R*-(**4a**) (86% ee, 1.6 g) and **syn** (**3**)(3.2 g). Further, *syn*-2*R*,3*R*-(**4a**)(86% ee) was converted to the acetate by AcC and then enzymatic resolution with lipase MY was carried out. After determining the hydrolysis conversion (41%), the mixture was worked up similarly, giving *syn*-2*R*,3*R*-(**4a**)(97% ee).
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10. Tumor cell lines (1×10^4 cells/well) was incubated in the presence or absence of compound for 72 h. Then, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] was added for $OD^{570-700}$ measurements. IC_{50} (μg/mL) was given as the concentration at 50% inhibition of cell growth.
% Inhibition = {1-($OD^{570-700}$ of sample well)/($OD^{570-700}$ of control well)}.