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AN ENZYME-ASSISTED POLYMERIZATION. EFFECT OF THE REACTION MEDIUM FOR THE CONTROLLED POLYMERIZATION OF FLUOROAMINO ACIDS

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SUMMARY

Chiral fluoroamino acids were polymerized with cellulase and/or modified cellulase. A number of chiral fluorinated polyamides of narrow molecular weight distribution were prepared. The degree of polymerization is controlled by the matching of enzyme-form and reaction medium.

INTRODUCTION

Enzymes for the hydrolysis of esters and/or amides have been studied in detail [1-5]. However, their catalytic ability for the synthesis of halogenated compounds remains unexplored from a practical point of view.

On consideration of the catalytic activity and/or capability of enzymes, it is expected that materials having two functional groups in the molecule, will be polymerized by enzymes [6] to give products having a narrow molecular weight distribution.

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This work describes the effect of the reaction medium [7-9] on an enzyme-assisted process for the controlled polymerization of optically pure fluoroamino acids [10-15].

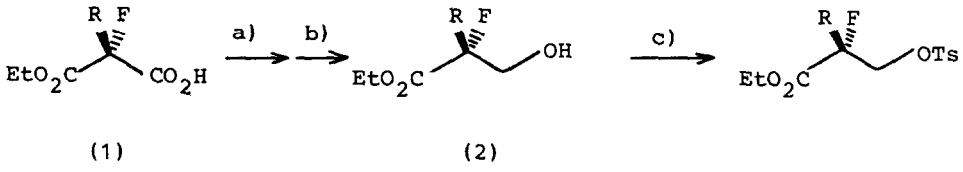
RESULTS AND DISCUSSION

Route to optically pure (R)- or (S)-α-fluoro-β-alanine and its derivatives

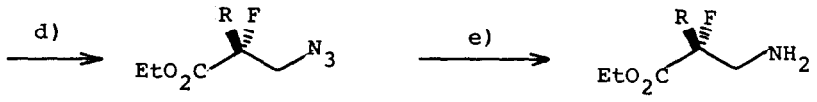
The previously reported optical resolution by asymmetric hydrolysis is a useful and important design feature for achieving the desired monofluorinated chiral materials [11-13].

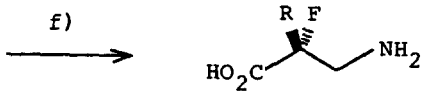
A brief outline of the synthetic strategies employed in preparing optically pure (R)- or (S)-α-fluoro-β-alanine and its derivatives is shown in Scheme I.

The synthetic intermediates are the optically pure hydroxyesters (2) which are prepared by the reduction of the corresponding optically pure 2-fluoro-2-substituted malonic acid monoethyl ester (1) with N,N-dimethylchloromethyleniminium chloride and sodium borohydride. Protection with tosyl chloride followed by treatment of the tosylesters with sodium azide gave the corresponding azide precursors which were converted to ethyl esters of α-fluoro-β-alanine [20-23] and its derivatives with sodium borohydride. The next step was an enzymatic hydrolysis to obtain the corresponding acids (3) that are often unstable under strongly basic conditions. This synthetic route to optically pure fluoroamino acids is a convenient stereocontrolled transformation. Reactions of α-alkyl derivatives which led to the desired α-fluoro-α-substituted-β-alanines were done in almost the same way as for (R)-α-fluoro-β-alanines.
The properties and nmr spectra of the amino acids synthesised are reported in Tables 1 and 2.

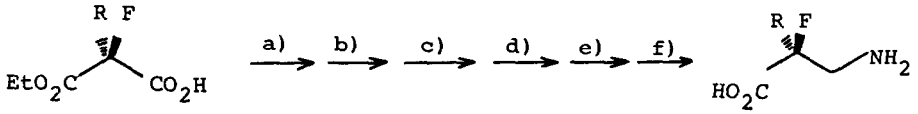


(1) (2)





(3)



a) $Me_2NCHO/(COCl)_2/CH_2Cl_2/0°C$ b) $NaBH_4/MeCN-THF/-20°C$

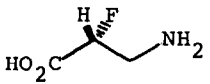
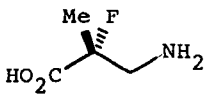
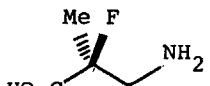
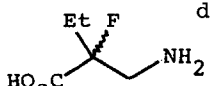
c) TsCl/pyridine d) NaN_3/i-PrOH/reflux e) $NaBH_4$/EtOH

f) lipase-MY.

Scheme I

TABLE 1

Physical properties of α-fluoro-α-substituted-β-alanines(3)

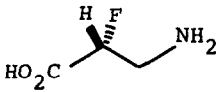
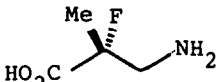
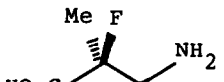
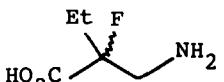
Product[a]	Over all Yield (%)	Molecular formula	Mp (°C)	$[\alpha]_D$/MeOH	O.P.[b] %e.e.	Elemental analysis (%) C found(calcd)	H found(calcd)	N found(calcd)
	34	$C_3H_6NO_2F$ (107.1)	260-261 (261)[c]	+11.8(c 1.08)	99			
	41	$C_4H_8NO_2F$ (121.1)	275-277	-18.7(c 1.25)	99	39.56(39.67)	6.84(6.66)	11.74(11.57)
	43	$C_5H_8NO_2F$ (121.1)	274-277	+18.4(c 1.87)	97	39.74(39.67)	6.45(6.66)	11.34(11.57)
 [d]	39	$C_5H_{10}NO_2F$ (135.1)	292-295	-12.7(c 1.29)	96	44.35(44.44)	7.34(7.46)	10.59(10.37)

[a] Products were purified by column chromatgraphy on silica gel. [b] Optical purity

[c] Mukherjee,K.L.,Heidelberger,C. J. Biol. Chem., 235.(1960)433 [d] The absolute configuration is not determined.

TABLE 2

^{1}H and ^{19}F NMR spectra of α-fluoro-α-substituted-β-alanines (3)

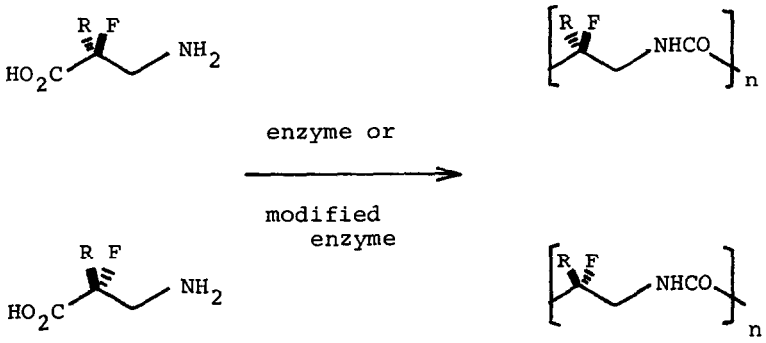
Product	^{1}H-N.M.R. ($CDCl_3$) δ[ppm]	^{19}F-N.M.R. ($CDCl_3/CF_3CO_2H$) δ[ppm]
	3.48(d.d.d,1H,$J_{H_A-H_B}$ = 2.1, $J_{H_A-H_{vic}}$ = 3.0,J_{H_A-F}= 21 Hz);3.61(d.d.d, 1H,$J_{H_B-H_{vic}}$ = 8.2, J_{H_B-F}= 15 Hz);5.27(d.d.d,1H,J_{H-F}= 46 Hz); 7.4(br,NH_2);10.6(br,CO_2H)	+78.4(d.d.d)
	1.19(d,3H,J_{CH_3-F}= 6.3 Hz);3.50(d.d,1H,$J_{H_A-H_B}$ = 2.6, J_{H_A-F}= 23 Hz); 3.63(d.d,1H,J_{H_B-F}= 15 Hz);7.2(br,NH_2);10.7(br,CO_2H)	+84.5(q.d.d)
	1.20(d,3H,J_{CH_3-F}= 6.4 Hz);3.50(d.d,1H,$J_{H_A-H_B}$ = 2.6, J_{H_A-F}= 23 Hz); 3.64(d.d,1H,J_{H_B-F}= 15.2 Hz);7.3(br,NH_2);10.7(br,CO_2H)	+84.4(q.d.d)
	1.23(t,3H,$J_{CH_3-CH_2}$= 7.4 Hz);3.30(d.q,2H,J_{CH_2-F}= 18 Hz);3.49(d.d,1H, $J_{H_A-H_B}$= 2.4, J_{H_A-F}= 24 Hz);3.64(d.d,1H,J_{H_B-F}= 16.2 Hz); 7.4(br,NH_2);11.2(br,CO_2H)	+87.2(t.d.d)

Enzyme-assisted polymerization

This process of polymerization gives polyamides of narrow molecular weight distribution. In particular, the degree of polymerization can be controlled by the matching of enzyme-form and reaction medium. Tables 3 and 4 summarize the results.

Enzymatic immobilization and modification have been recognized as important techniques for modifying the catalytic activities and/or capabilities of enzymes [16-18]. Furthermore, an enzyme modified by reaction with a fluorine-containing molecule is much more soluble in organic solvents than water [19]. Since it seems that the main-driving force of enzyme-assisted polymerization is interactions between the polyamide and the binding pocket of the enzyme, one might expect that the enzyme-polyamide binding in organic solvents becomes tighter to produce a long chain polymer because the polyamide will be much more soluble in the organic medium than water.

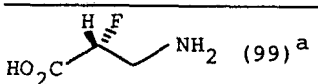
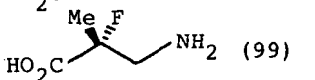
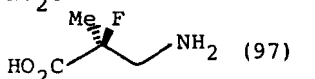
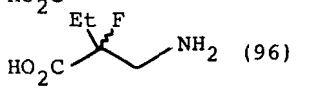
The results presented in Tables 3 and 4 offer possibilities for the biosynthetic polymerization of amino acids possessing fluorine that may offer control of molecular weight size and distribution.



Scheme II

TABLE 3

Polyamides by enzyme-assisted polymerization in water

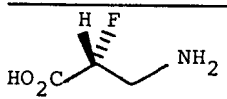
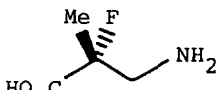
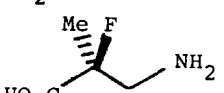
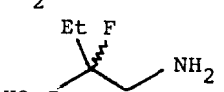
Monomer(3)	Yield (%)[b]	$[\alpha]_D$/benzene	polymer		dispersity
			M_n	M_w	$D=M_w/M_n$
 (99)[a]	74	+6.7 (c 0.58)	8600	9600	1.12
 (99)	61	-8.4 (c 0.97)	6400	8100	1.27
 (97)	71	+7.6 (c 1.04)	7500	10200	1.36
 (96)	69	-5.1 (c 0.89)	5800	7200	1.24

[a] Optical purity of monomer

[b] The samples were subjected to gel permeation chromatography (GPC) in tetrahydrofuran at 50°C. The analysis was done with Shimadzu LC-5A high performance liquid chromatography using a Shodex GPC KF-803 column equipped a reflactive index detector Shodex RI. The flow rate was 1.5 ml/min.

TABLE 4

Polyamides by modified enzyme[a] - assisted polymerization in organic solvent

Monomer(3)	solvent	Yield (%)	$[\alpha]_D$/benzene	Polymer M_n	Polymer M_w	dispersity $D=M_w/M_n$
	benzene	43	+8.2 (c 1.04)	13200	14400	1.09
	hexane	32	+7.8 (c 0.95)	11400	13500	1.18
	benzene	51	-9.2 (c 1.23)	9100	10100	1.11
	hexane	47	-9.4 (c 1.07)	9800	12600	1.29
	benzene	62	+8.4 (c 1.14)	11600	13400	1.16
	hexane	38	+8.1 (c 0.84)	9700	11700	1.21
	benzene	54	-6.5 (c 0.85)	8900	10200	1.45
	hexane	29	-6.7 (c 1.18)	9500	12700	1.34

[a] To attempt the enzymatic modification with fluorine-containing molecules, 2-trifluoromethyl propenoic acid chloride was allowed to react with cellulase (*Trichoderma viride*, Yakult Pharmaceutical Industry Co. Ltd.) in an N,N-dimethylformamide-water system at room temperature. After 1 day of stirring, the solvent was removed under dynamic vacuum under 30°C [19].

EXPERIMENTAL

Ethyl 2-fluoro-3-azidopropionate

A mixture of the tosylate of (R)-(+)-ethyl 3-hydroxy-2-fluoropropionate (5.8 g, 20 mmol) ($[\alpha]_D$ +2.90 (c 1.26, MeOH); >99 %e.e.) and saturated aqueous sodium azide (55 mmol) in isopropanol (100 ml) was refluxed for 15 h, and the mixture was poured into water. Oily materials were extracted with methylene dichloride, and the organic layer was dried over magnesium sulfate. On removal of the solvent, azide was obtained in a yield of 86 %.

^{19}F-N.M.R. ($CDCl_3$/external CF_3CO_2H) : δ = +114.6(d.d.d, $J_{F-H_{gem}}$ = 46, $J_{F-H_{vic}}$ = 21.4, 20.0 Hz) ppm.

^{1}H-N.M.R. ($CDCl_3$) : δ = 1.27(t,CH_3, $J_{CH_3-CH_2}$ = 6.7 Hz); 4.21 (q,CH_2); 4.30(m,1H); 4.32(m,1H); 4.95(d.d.d,CHF).

α-Fluoro-β-alanine ethylester

A mixture of the above azide (10 mmol) and sodium borohydride (33 mmol) in ethanol (30 ml) was refluxed for 5h, and the mixture was quenched with saturated aqueous NH_4Cl solution. Oily materials were extracted with diethyl ether, and the organic layer was washed with 1N HCl, 5 % aqueous $NaHCO_3$, water and brine. On removal of the solvent, crude products were separated by column chromatography on silica gel using hexane-diethyl ether (10:1) as eluent to give the product in 81 % yield.

^{19}F-N.M.R. ($CDCl_3$/external CF_3CO_2H) : δ = +113.1(d.d.d, $J_{F-H_{gem}}$ = 45 ; $J_{F-H_{vic}}$ = 21.3, 20.1 Hz) ppm.

^{1}H-N.M.R. ($CDCl_3$) : δ = 1.26(t,CH_3, $J_{CH_3-CH_2}$ = 7.0 Hz); 4.16 (q,CH_2); 4.29(m,1H); 4.31(m,1H); 4.86(d.d.d,CHF); 8.26(br,NH_2).

Analysis : Found : C, 44.73 ; H, 7.19 %.

Cacld for $C_5H_{10}NO_2F$: C, 44.44, H, 7.46 %.

M.S.:m/e = 135.129 (M^+ requires 135.138).

(R)-(+)-α-Fluoro-β-alanine

A suspension of lipase-MY (*Candida cylindracea*, Meito Sangyo Co. Ltd., 3 g) in buffer solution (60 ml, pH 7.3) was prepared from 1/15 M aqueous Na_2HPO_4 solution (46.1 ml) and 1/15 M aqueous KH_2PO_4 solution (13.9 ml) and was stirred for 15 min at 40-41°C in 'CULSTIR' flask for suspension culture with double arms and jacket (100 ml, Sibata Scientific Technology Ltd.) Into the mixture was added α-fluoro-β-alanine ethyl ester (2.7 g, 20 mmol), and then the whole mixture was stirred at 40-41°C. After 5h of stirring, the mixture was acidified with 1N HCl and then the oily materials were extracted with ethyl acetate. The organic layer was dried over anhydrous magnesium sulfate and then the solvent removed. the products were separated by column chromatography on silica gel using hexane-diethyl ether (5:1) as eluent.
Mp. 260-261°C (lit. [23] mp. 260°C).

Other α-fluoro-α-substituted-β-alanines (3) were prepared in the same manner and the results are listed in Table 1.

Polymerization

(A) A suspension of cellulase (*Trichoderma viride*, Yakult Pharmaceutical Industry Co. Ltd., 5 g) in buffer solution (100 ml, pH 8.0) was prepared from 1/30 M aq. Na_2HPO_4 and KH_2PO_4 solution, was stirred for 15 min at 40-41°C. Into the mixture, the corresponding chiral fluoroamino acid (20 mmol) was added, and then the whole was stirred at 40-41°C. After 5 days of stirring at that temperature, the mixture was extracted with ethyl acetate. The product was purified by column chromatography on silica gel using hexane-ethyl acetate (5:1) as eluent.

(B) A suspension of modified enzyme [19] [cellulase-$C(O)(CF_3)C=CH_2$, 10 g] and the corresponding fluoroamino acid (20 mmol) in benzene (100 ml) was stirred at 40-41°C. After 5 days of stirring at 40-41°C, the mixture was worked up as usual.

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