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ENZYMES ACTIVE IN ORGANIC MEDIA: SYNTHESIS OF OPTICALLY ACTIVE TRIFLUOROMETHYLATED COMPOUNDS VIA ASYMMETRIC ADDITION REACTIONS

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SUMMARY

Catalytic activity of the enzymes, lipasePL 266 and lipasePL 679(from *Alcaligenes sp*) and lipaseAL 865 (from *Achromobacter*) in organic media has been found. In their presence, (E)-ethyl 3-(trifluoromethyl)- and 2-(trifluoromethyl)-propenate are readily converted to chiral Michael adducts via addition of thiols or dialkylamines in organic media.

INTRODUCTION

Reports concerning enzymes active in an organic medium have appeared [1-8]. Synthetic methods which give a variety of versatile chiral fluorinated materials of high optical purity are now being studied in detail [9-18].

We wish to describe herein new catalytic reactions of the enzymes, lipasePL 266, lipasePL 679 and lipaseAL 865 in organic media, i.e. enzyme-assisted addition reactions. The catalytic activities of enzymes from *Alcaligenes sp*

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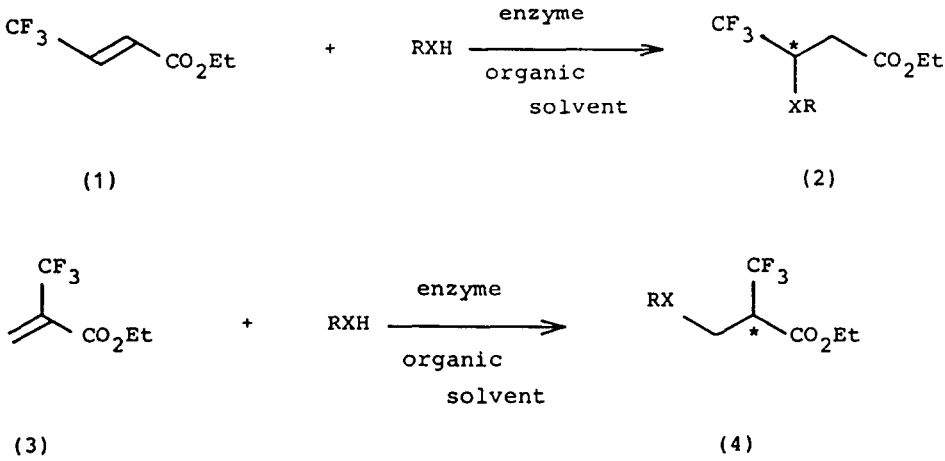
or *Achromobacter* (Meito Sangyo Co. Ltd.) in organic media are suitable for enzyme-assisted addition reactions to introduce a center of chirality into fluorocompounds. Without lipases, the chiral addition reaction did not proceed at all. Further, the activity of these enzymes was not sufficient to promote chiral addition reactions in water; the activities of these lipases in organic media were more than thirty times those in water.

RESULTS AND DISCUSSION

In the presence of lipases, (E)-ethyl 3-(trifluoromethyl)-propenate (1) and ethyl 2-(trifluoromethyl)propenate (3) are readily converted to chiral Michael-type adducts via addition of thiols or dialkylamines in various kinds of organic solvents (see Scheme I). The results in Table 1 show that non polar solvents are more suitable for these enzymatic reactions than are polar solvents. Detailed results are given in Tables 2 and 3. Though Michael addition was achieved using thiols and secondary amines as nucleophiles, alcohols, phenol and water did not react in this system.



Scheme I. X= S or $N(R)_2$

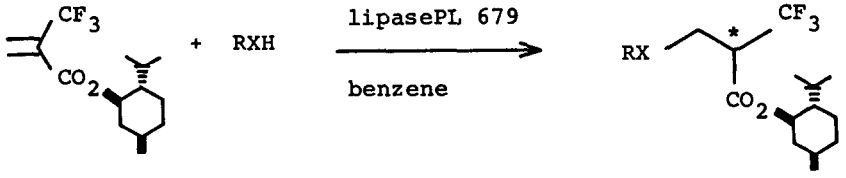
TABLE 1

Solvent effect in the addition reaction of ethyl 2-(trifluoromethyl)propenate (3) with thiophenol

Organic solvent[a]	Yield (%)	$[\alpha]_D$/MeOH	Optical purity % e.e.
hexane	74	+1.69 (c 1.24)	39
benzene	86	+2.90 (c 1.38)	67
$CF_2ClCFCl_2$	79	+2.47 (c 1.47)	57
CH_3COCH_3	3		0
tetrahydrofuran	18		0

[a] lipasePL 679 (*Alcaligenes sp* No 679, 1.0 g), thiophenol (20 mmol) and ethyl 2-(trifluoromethyl)propenate (10 mmol) in organic solvent (50 mL) were used in the above reaction.

To obtain materials with improved optical purity, we have attempted to use the chiral esters of (1R,2S,5R)-(-)- or (1R,2R,5R)-(+)-menthol as the Michael acceptor. The results shown in Schemes II and III show that those enzyme-assisted Michael addition reactions in organic media exhibited high degrees of diastereoselectivities, which were confirmed by HPLC analyses, and the enantioselectivity was concluded as being > 98 % ee in each case.



[α]D/MeOH -76.0 (c 1.52)

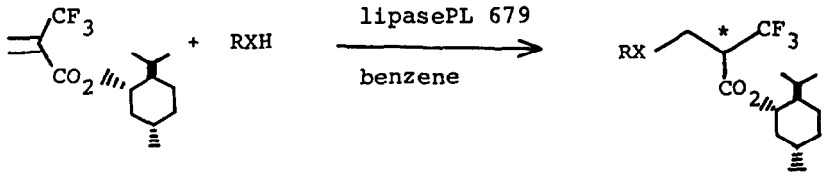
(5a)

RX:

(6) PhS ; Yield. 94 %
$[\alpha]_D$/MeOH -47.7 (c 2.10)

(7) Et_2N; Yield. 73 %
$[\alpha]_D$/MeOH -36.2 (c 1.44)

(continued)



$[\alpha]_D$/MeOH +76.1 (c 1.57)

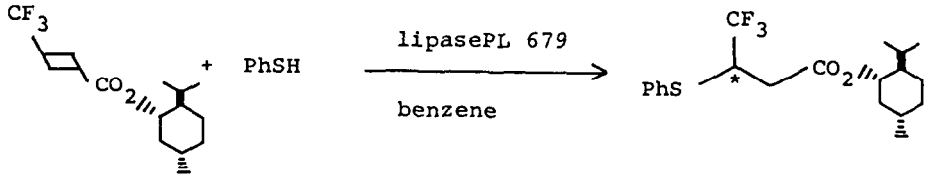
(5b)

RX:

(8) PhS ; Yield. 79 %
$[\alpha]_D$/MeOH +47.5 (c 1.79)

(9) Et_2N; Yield. 83 %
$[\alpha]_D$/MeOH +35.7 (c 1.04)

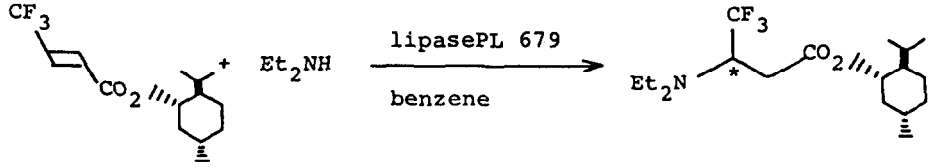
Scheme II



$[\alpha]_D$/MeOH -64.2 (c 1.43)

(5c)

(10) $[\alpha]_D$/MeOH -26.7
(c 1.41)



$[\alpha]_D$/MeOH -64.2 (c 1.43)

(5d)

(11) $[\alpha]_D$/MeOH -23.4
(c 1.06)

Scheme III

TABLE 2

Asymmetric Michael addition reaction of ethyl 3-(trifluoromethyl)propenate (1)

RXH	Solvent	Lipase PL or AL	Yield (%)	$[\alpha]_D$/MeOH	Optical purity [a] % e.e.
PhSH	hexane	PL 679	47	-0.94 (c 1.25)	33
	benzene	PL 679	53	-2.16 (c 1.14)	78
	benzene	PL 266	46	-1.49 (c 1.78)	54
	benzene	AL 865	38	-1.37 (c 1.26)	50
	$CF_2ClCFCl_2$	PL 679	52	-1.41 (c 1.04)	51
	$CF_2ClCFCl_2$	PL 266	41	-1.71 (c 1.05)	61
	$CF_2ClCFCl_2$	AL 865	64	-1.51 (c 1.41)	54
BuSH	benzene	PL 679	56	-2.81 (c 1.08)	61
	benzene	AL 865	67	-3.09 (c 1.07)	67
Et_2NH	hexane	PL 679	47	-2.14 (c 1.24)	44
	benzene	PL 679	53	-3.16 (c 1.27)	65
	benzene	PL 266	51	-2.52 (c 1.63)	51
	benzene	AL 865	62	-3.75 (c 1.34)	77
	$CF_2ClCFCl_2$	PL 679	54	-1.99 (c 1.45)	41
	$CF_2ClCFCl_2$	PL 266	50	-2.58 (c 1.37)	50
	$CF_2ClCFCl_2$	AL 865	65	-2.89 (c 1.46)	56
Bu_2NH	benzene	PL 679	55	-2.47 (c 1.57)	49
	benzene	AL 865	49	-1.96 (c 1.49)	38

[a] The optical purities were determined by ^{19}F nmr signal intensities by commercially available (+)-tris[di(perfluoro-2-propoxypropionyl)metanate]europium(III).

TABLE 3

Asymmetric Michael addition reaction of ethyl 2-(trifluoromethyl)propenate (3)

RXH	Solvent	Lipase PL or AL	Yield (%)	$[\alpha]_D$/MeOH	Optical purity[a] % e.e.
PhSH	hexane	PL 679	74	+1.69 (c 1.24)	39
	benzene	PL 679	86	+2.90 (c 1.38)	67
	benzene	PL 266	79	+2.47 (c 1.47)	57
	benzene	AL 865	76	+3.21 (c 1.68)	74
	$CF_2ClCFCl_2$	PL 679	83	+2.77 (c 1.16)	64
	$CF_2ClCFCl_2$	PL 266	58	+2.10 (c 1.57)	49
BuSH	benzene	PL 679	76	+3.34 (c 1.08)	59
	$CF_2ClCFCl_2$	AL 865	81	+3.85 (c 1.41)	68
Et_2NH	hexane	PL 679	76	+2.09 (c 1.87)	21
	benzene	PL 679	82	+6.46 (c 2.57)	65
	benzene	PL 266	76	+6.44 (c 1.34)	64
	benzene	AL 865	69	+5.99 (c 1.42)	62
	$CF_2ClCFCl_2$	PL 679	69	+5.69 (c 1.35)	57
	$CF_2ClCFCl_2$	PL 266	59	+5.09 (c 1.68)	51
Bu_2NH	benzene	PL 679	68	+5.21 (c 1.21)	67
	$CF_2ClCFCl_2$	PL 266	74	+4.96 (c 1.36)	64

[a] The optical purities were determined by ^{19}F nmr signal intensities by commercially available (+)-tris[di(perfluoro-2-propoxypropionyl)metanate]europium(III).

EXPERIMENTAL

(-)-Ethyl 3-phenylthio-4,4,4-trifluorobutanate (2a)(nc)

A suspension of lipasePL 679 (*Alcaligenes sp* No 679, 1 g), ethyl 3-(trifluoromethyl)propenate (1)(1.8 g, 10 mmol)[13] and thiophenol (1.65 g, 15 mmol) in benzene (50 ml) was stirred at 40-41°C. After 24 h of stirring, the solvent was removed. The resulting crude products were chromatographed on silica gel (10:1, hexane/ethyl acetate) to give optically active compound (2a) 1.48 g (53 %) as an oil, after evaporation of the solvent.
^{19}F NMR ($CDCl_3$): δ -6.4 (d, J_{CF_3-CH}= 8.5 Hz) ppm.
^{1}H NMR ($CDCl_3$): δ 1.35(CH_3, t, $J_{CH_3-CH_2}$= 7.0 Hz), 3.30-3.45(3xH, m), 4.31(CH_2, q), 7.2(Ar-H).
Analysis. Found : C, 51.67 ; H, 4.57 %
Calcd for $C_{12}H_{13}SO_2F_3$: C, 51.79 ; H, 4.71 %

(-)-Ethyl 3-butylthio-4,4,4-trifluorobutanate (2b)(nc)

A suspension of lipaseAL 865 (*Achromobacter* No 865, 1 g), ethyl 3-(trifluoromethyl)propenate (1.8 g, 10 mmol) and butanethiol (1.8 g, 20 mmol) in benzene(50 ml) was stirred at 40-41°C. After 24 h of stirring, work up gave the corresponding product 1.72 g (67 %).
^{19}F NMR ($CDCl_3$): δ -6.3(d, J_{CF_3-CH}= 8.0 Hz) ppm.
^{1}H NMR ($CDCl_3$): δ 1.33-1.42(6xH), 2.10-3.50(9xH), 4.33(CH, q)
Analysis. Found : C, 46.87 ; H, 6.37 %
Calcd for $C_{10}H_{17}SO_2F_3$: C, 46.50 ; H, 6.63 %

(-)-Ethyl 3-diethylamino-4,4,4-trifluorobutanate (2c)(nc)

A suspension of lipasePL 266 (*Alcaligenes sp*,No 266, 1.0 g), ethyl 3-(trifluoromethyl)propenate (1.8 g, 10 mmol) and diethylamine (1.5 g, 20 mmol) in benzene (50 ml) was stirred at 40-41°C. After 24 h of stirring, work up gave the corresponding product 1.23 g (51 %).

^{19}F NMR ($CDCl_3$): δ -8.0 (d, J_{CF_3-CH}= 8.5 Hz) ppm.
^{1}H NMR ($CDCl_3$): δ 1.14(CH_3, t, $J_{CH_3-CH_2}$ = 6.5 Hz), 1.33 (CH_3, t, $J_{CH_3-CH_2}$ = 7.0 Hz), 2.50-2.83(CH, m), 2.60(CH_2, q), 3.83(CH_2, m), 4.10(CH_2, q).
Analysis. Found : C, 49.82 ; H, 7.75 ; N, 5.68 %
Calcd for $C_{10}H_{18}NO_2F_3$: C, 49.79 ; H, 7.52 ; N, 5.81 %

(-)-Ethyl 3-dibutylamino-4,4,4-trifluorobutanate (2d)(nc)

In the above system, lipasePL 679 (*Alcaligenes sp*, No 679, 1.0 g), dibutylamine (2.6 g, 20 mmol) and ethyl 3-(trifluoromethyl)propenate (1.8 g, 10 mmol) in benzene (50 ml) were used, and then worked up similarly, to give the product 1.46 g (49 %).
^{19}F NMR ($CDCl_3$) : δ -11.9 (d, J_{CF_3-CH}= 8.5 Hz) ppm
^{1}H NMR ($CDCl_3$) : δ 1.10-1.16(6xH), 1.20-3.80(18xH), 4.30(CH, q)
Analysis. Found : C, 56.34 ; H, 8.53 ; N, 5.06 %
Calcd for $C_{14}H_{26}NO_2F_3$: C, 56.55 ; H, 8.81 ; N, 4.71 %

(+)-Ethyl 3-phenylthio-2-(trifluoromethyl)propanate (4a)(nc)

A suspension of lipasePL 679 (*Alcaligenes sp*, No 679,1.0 g) ethyl 2-(trifluoromethyl)propenate (3)(1.8 g, 10 mmol)[13] and thiophenol (2.2 g, 20 mmol) in benzene (50 ml) was stirred at 40-41°C. After 24 h of stirring, the solvent was removed. The resulting crude products were chromatographed on silica gel (5:1, hexane/ethyl acetate) to give optically active compound in a yield of 86 % (2.39 g).
^{19}F NMR ($CDCl_3$): δ -11.7 (d, J_{CF_3-CH}= 7.5 Hz) ppm.
^{1}H NMR ($CDCl_3$): δ 1.35(CH_3, t, $J_{CH_3-CH_2}$ = 7.1 Hz), 3.41-3.71(CH, m), 4.04(CH_2), 4.28(CH_2, q), 7.20-7.51(Ar-H).
Analysis. Found : C, 51.44 ; H, 4.53 %
Calcd for $C_{12}H_{13}SO_2F_3$: C, 51.79 ; H, 4.71 %

(+)-Ethyl 3-butylthio-2-(trifluoromethyl)propanate (4b)(nc)

In the above system, lipaseAL 865 (*Achromobacter*, No 865, 1 g), butanethiol (1.8 g, 20 mmol) and ethyl 2-(trifluoromethyl) propenate (1.8 g, 10 mmol) in benzene (50 ml) were used, and then worked up similarly. The product 1.96 g (76 %) was obtained.

^{19}F NMR ($CDCl_3$) : δ -10.9 (d, J_{CF-CH}= 8.4 Hz) ppm

^{1}H NMR ($CDCl_3$) : δ 1.30-1.45(6xH), 2.14-3.65(9xH), 4.30(CH, q)

Analysis. Found : C, 46.85 ; H, 6.97 %

Calcd for $C_{10}H_{17}SO_2F_3$: C, 46.50 ; H, 6.63 %

(+)-Ethyl 3-diethylamino-2-(trifluoromethyl)propanate (4c)(nc)

In the above system, lipasePL 266 (*Alcaligenes sp* ,1.0 g), diethylamine (1.5 g, 20 mmol) and ethyl 2-(trifluoromethyl)-propenate (1.8 g, 10 mmol) in benzene (50 ml) were used, and then worked up similarly. The product 1.83 g (76 %) was obtained.

^{19}F NMR ($CDCl_3$): δ -12.5 (d, J_{CF_3-CH}= 8.5 Hz) ppm.

^{1}H NMR ($CDCl_3$): δ 1.13(CH_3, t, $J_{CH_3-CH_2}$ = 6.7 Hz), 1.33(CH_3, t, $J_{CH_3-CH_2}$= 7.1 Hz), 2.61(CH_2, q), 3.29-3.64(CH, m), 4.05 (CH_2, m), 4.26(CH_2, q).

Analysis. Found : C, 49.55 ; H, 7.64 ; N, 5.97 %

Calcd for $C_{10}H_{18}NO_2F_3$: C, 49.79 ; H, 7.52 ; N, 5.81 %

(-)-Ethyl 3-dibutylamino-2-(trifluoromethyl)propanate (4d)(nc)

In the above system, lipasePL 679 (*Alcaligenes sp*,No 679, 1.0 g), dibutylamine (1.8 g, 20 mmol) and ethyl 2-(trifluoromethyl)propenate (1.8 g, 10 mmol) in benzene (50 ml) were used, and then worked up similarly. The product 2.01 g (68 %) was obtained.

^{19}F NMR ($CDCl_3$) : δ -10.6 (d, J_{CF_3-CH}= 8.5 Hz) ppm

^{1}H NMR ($CDCl_3$) : δ 1.11-1.37(6xH), 1.23-3.97(18xH), 4.34(CH, q)

Analysis. Found : C, 56.19 ; H, 9.04 ; N, 4.50 %

Calcd for $C_{14}H_{26}NO_2F_3$: C, 56.55 ; H, 8.81 ; N, 4.71 %

(-)-Menthyl 2-(trifluoromethyl)propenate (5a)[10]

Into a mixture solution of (1R,2S,5R)-(-)-menthyl (10 mmol) and pyridine (2 ml) in dichloromethane (30 ml), 2-(trifluoromethyl)propenoic acid chloride (11 mmol) in dichloromethane (5 ml) was added at room temperature. After 12h of stirring, the reaction mixture was poured into water, and then oily materials was separated. Removal of the solvent, distillation gave the compound (5a) in a yield of 87%.
bp 72°C/0.48 mmHg.
^{19}F NMR ($CDCl_3$): δ -12.0 (CF_3) ppm.
^{1}H NMR ($CDCl_3$): δ 0.33-2.17(18xH), 4.76(1xH,m), 6.33(1xH), 6.66(1xH).

(-)-Menthyl 3-(trifluoromethyl)propenate (5c)[10]

In the above reaction, 3-(trifluoromethyl)propenoic acid chloride (11 mmol) was used and then worked up similarly. Distillation gave the compound (5c) in a yield of 79%.
bp 74-76°C/ 0.6 mmHg.
^{19}F NMR ($CDCl_3$): δ -12.0 (CF_3) ppm.
^{1}H NMR ($CDCl_3$): δ 0.31-2.21(18xH), 4.77(1xH,m), 6.35(1xH), 6.58(1xH).

(-)-Menthyl 3-phenylthio-2-(trifluoromethyl)propanate (6)(nc)

A suspension of lipasePL 679 (*Alcaligenes sp* , 1.0 g), l-menthyl 2-(trifluoromethyl)propenate (5)(10 mmol), and thiophenol (2.2 g, 20 mmol) in benzene (30 ml) was stirred at 40-41°C. After 5 h of stirring, the solvent was removed. The resulting crude products were chromatographed on silica gel (10:1, hexane/ethyl acetate) to give optically pure compound(6) 3.65 g (94 %).
$[\alpha]_D$/MeOH -47.7 (c 2.10).
^{19}F NMR (CCl_4): δ -10.2(d, J_{CF_3-CH}= 6.6 Hz) ppm.
^{1}H NMR (CCl_4): δ 0.60-2.13(18xH), 3.13-3.40(3xH), 4.73(1xH, d.d.d), 7.33(Ar-H).
Analysis. Found : C, 61.72 ; H, 6.86 %
Calcd for $C_{20}H_{27}SO_2F_3$: C, 61.83 ; H, 7.01 %

(+)-Menthyl 3-phenylthio-2-(trifluoromethyl)propanate (8)(nc)

In the above reaction, lipasePL 679 (1.0 g), d-menthyl 2-(trifluoromethyl)propenate (5b)(10 mmol), and thiophenol (2.2 g, 20 mmol) in benzene (30 ml) were used, and then worked up similarly. The product (8)(79 %) was obtained.
$[\alpha]_D$/MeOH +47.5 (c 1.79).
Analysis. Found : C, 61.78 ; H, 6.96 %
Calcd for $C_{20}H_{27}SO_2F_3$: C, 61.83 ; H, 7.01 %

(-)-Menthyl 3-phenylthio-3-(trifluoromethyl)propanate (10)(nc)

A suspension of lipase679 (1.0 g), l-menthyl 3-(trifluoromethyl)propenate (5c)(10 mmol), and thiophenol (2.2 g, 20 mmol) in benzene (30 ml) was stirred at 40-41°C. After 5 h of stirring, the solvent was removed. The resulting crude products were chromatographed on silica gel to give optically pure compound (10) 2.86 g(74 %).
$[\alpha]_D$/MeOH -26.7 (c 1.41).
^{19}F NMR ($CDCl_3$): δ -8.7 (d, J_{CF_3-CH}= 8.0 Hz) ppm.
^1H NMR ($CDCl_3$): δ 0.64-2.41(18xH), 3.21-3.42(3xH), 4.21(1H), 7.13-7.45(Ar-H).
Analysis. Found : C, 62.04 ; H, 7.31 %
Calcd for $C_{20}H_{27}SO_2F_3$: C, 61.83 ; H, 7.01 %

(-)-Menthyl 3-diethylamino-3-(trifluoromethyl)propanate (11)(nc)

In the above system, lipasePL679 (1.0 g), d-menthyl 3-(trifluoromethyl)propenate (5d)(10 mmol), and diethylamine (20 mmol) in benzene (50 ml) were used, and then worked up similarly. The product (11) 2.75 g (78 %) was obtained.
$[\alpha]_D$/MeOH -23.4 (c 1.06).
^{19}F NMR ($CDCl_3$): δ -8.9 (d, J_{CF_3-CH}= 7.5 Hz) ppm.
^1H NMR ($CDCl_3$): δ 0.50-2.54(28xH), 3.07-3.41(3xH, 4.56(1H)
Analysis. Found : C, 61.35 ; H, 9.36 ; N, 3.67 %
Calcd for $C_{18}H_{32}NO_2F_3$: C, 61.52 ; H, 9.18 ; N, 3.99 %

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