Microbial Approach to the Practical Monofluorinated Chiral Synthons

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The synthetic approach to (+)- and (−)-2-fluoro-2-substituted malonic acid monoesters, based on the enantiotopic specificity of lipases and/or cellulases, which catalyze the stereospecific hydrolysis of the ester group in monofluorinated malonic acid diesters, is described. This microbial approach to the monofluorinated chiral synthons provides a new synthetic route for introduction of a center of chirality in fluorinated organic compounds.

The asymmetric synthetic opportunities provided by the catalytic activity of enzymes have increased in recent years.[1-4] Developments in microbial synthesis, especially, oxidative, reductive, carbon–carbon bond-forming reactions, and/or asymmetric hydrolysis, are remarkable. The importance of microbial behavior of halogen-containing compounds, which are hardly decomposed by microorganisms, has been recognized in living systems.[5,6] However, with the exception of the asymmetric reduction of halogen-containing carbonyl compounds by baker's yeast,[7,8] no reports concerning the use of microbially transformed halogenated compounds in synthetic reaction have appeared.

We recently outlined[9-11] the possibility of microbial transformation of fluorinated compounds under stereocontrol such as asymmetric induction with reductive and/or carbon–carbon bond-forming reactions and asymmetric hydrolysis. Recently considerable attention has been focused on the search for chiral synthetic tools for the preparation of fluorinated bioactive molecules.[12,13]

As part of our continuing effort to develop stereocontrolled syntheses of fluorinated compounds with high optical purity by use of microorganisms, we have found the microbial hydrolysis of 2-fluoro-2-substituted malonic acid diesters with several lipases or cellulases yields (+)- or (−)-2-fluoro-2-substituted malonic acid monoesters.

Results and Discussion

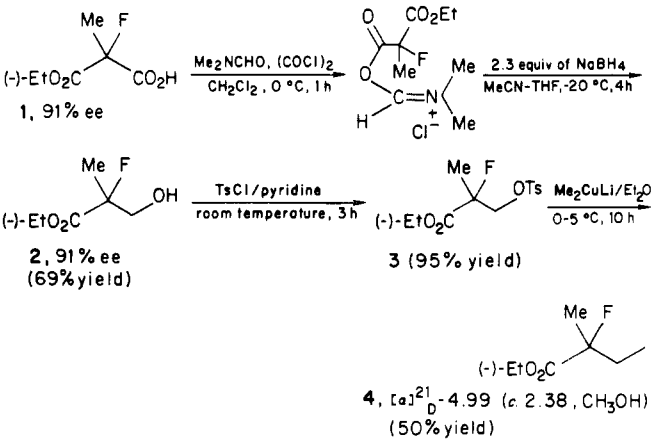
Asymmetric hydrolysis of prochiral compounds with enzymes of microbial or animal origin has been extensively studied.[14-25] However, it is difficult to transform halogenated compounds with microorganisms.

Nevertheless, Tonomura[26,27] and Goldman[28,29] have iso-

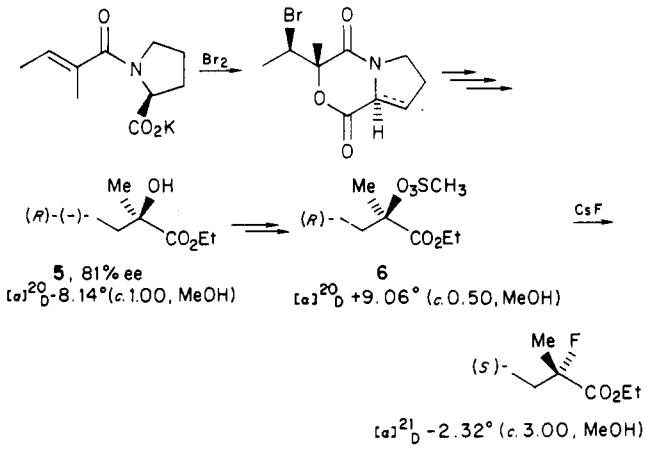
Table I. Asymmetric Hydrolysis with Pig Liver Esterase

R	yield, %	bp, °C (mmHg)	$[\alpha]_D$ (MeOH), deg	optical purity, % ee
Me	61	100–102 (3)	−4.15 (*c* 2.38)	16
Et	34	101–103 (2)	−4.88 (*c* 0.70)	24

Scheme I. Determination of the Absolute Configuration of 2-Fluoro-2-methylmalonic Acid Monoester



Scheme II



(1) Jones, J. B. "Enzymic and Non-Enzymic Catalysis"; Dunnill, P., Wiseman, A., Blakeborough, N., Eds.; Ellis Horwood-Wiley: Chichester, New York, 1980.
(2) Whitesides, G. M.; Wong, C.-H. *Aldrichimica Acta* **1983**, *16*, 27 and references cited therein.
(3) Jones, J. B.; Sih, C. J.; Perlman, D. "Application of Biological Systems in Organic Chemistry"; Wiley: New York, 1976.
(4) Retey, J.; Robinson, J. A. "Stereospecificity in Organic Chemistry and Enzymology"; Verlag-Chemie: Basel, 1982.
(5) Motosugi, K.; Souda, K. *Kagaku (Kyoto)* **1982**, *37*, 544 and references cited therein.
(6) Filler, R.; Kobayashi, Y. "Biomedicinal Aspects of Fluorine Chemistry"; Kodansha and Elsevier Biomedical: 1983.
(7) Bucciarelli, M.; Forni, A.; Moretti, I.; Torre, G. *J. Chem. Soc., Chem. Commun.* **1978**, 456.
(8) Bucciarelli, M.; Forni, A.; Moretti, I.; Torre, G. *Synthesis* **1983**, 897.
(9) Kitazume, T.; Ishikawa, N. *Chem. Lett.* **1984**, 587.
(10) Kitazume, T.; Ishikawa, N. *Chem. Lett.* **1984**, 1811.
(11) Kitazume, T.; Ishikawa, N. *Chem. Lett.* **1983**, 237.
(12) Quistad, G. B.; Cerf, D. C.; Schooley, D. A.; Staal, G. B. *Nature (London)* **1981**, *289*, 176.
(13) Tsushima, T. *Kagaku to Seibutsu* **1982**, *20*, 770 and references cited therein.
(14) Huang, F. C.; Lee, L. F. H.; Mittal, R. S. D.; Ravikumar, P. R.; Chan, J. A.; Sih, C. J.; Capsi, E.; Eck, C. R. *J. Am. Chem. Soc.* **1975**, *97*, 4144.
(15) Chen, C. S.; Fujimoto, Y.; Sih, C. J. *J. Am. Chem. Soc.* **1981**, *103*, 3580.
(16) Schneider, M.; Engel, N.; Boensmann, H. *Angew. Chem., Int. Ed. Engl.* **1984**, 23 and references cited therein.
(17) Francis, C. J.; Jones, J. B. *J. Chem. Soc., Chem. Commun.* **1984**, 579.
(18) Oritani, T.; Kudo, S.; Yamashita, K. *Agric. Biol. Chem.* **1982**, *46*, 757.
(19) Ohta, H. *Yuki Gosei Kagaku Kyokaishi* **1983**, *41*, 1018 and references cited therein.
(20) Mori, K.; Akao, H. *Tetrahedron* **1980**, *36*, 91.
(21) Sugai, T.; Kuwahara, S.; Hoshino, C.; Matsuo, N.; Mori, K. *Agric. Biol. Chem.* **1982**, *46*, 2579.
(22) Ohno, M.; Kobayashi, S.; Iimori, T.; Wang, Y. F.; Izawa, T. *J. Am. Chem. Soc.* **1981**, *103*, 2405.
(23) Ito, Y.; Shibata, T.; Arita, M.; Sawai, H.; Ohno, M. *J. Am. Chem. Soc.* **1981**, *103*, 6739.
(24) Kotani, H.; Kuze, Y.; Uchida, S.; Miyabe, T.; Iimori, T.; Okano, K.; Kobayashi, S.; Ohno, M. *Agric. Biol. Chem.* **1983**, *47*, 1363.

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Table II. Microbial Hydrolysis of Diethyl 2-Fluoro-2-methylmalonate

origin	method[h]	yield, %	$[\alpha]_D$ (MeOH), deg	optical purity,[g] % ee
		Triacylglycerol Lipase		
Candida cylindraceae[a]	A	87	−20.9 (*c* 2.81)	91
Candida cylindracea	B	60	−20.6 (*c* 1.95)	91
Porcine pancreas[b]	B	23	−12.0 (*c* 1.20)	61
Aspergillus Sp.[c]	B	80	−6.46 (*c* 1.11)	28
Chromobacterium viscosum[d]	C	67	0 (*c* 1.87)	0
Rhizopus delemar[e]	A		no reaction	
α-Chymotrypsin[b]	D	82	−16.0 (*c* 1.70)	70
		Cellulase		
Trichoderma viride[f]	A	60	+13.1 (*c* 2.24)	56
Trichoderma viride[c]	A	53	+9.97 (*c* 2.34)	44
Aspergillus nijer[c]	A	52	+4.39 (*c* 1.54)	19

[a] Meito Sangyo Co. Ltd. [b] Sigma Co. Ltd. [c] Amano Seiyaku Co. Ltd. [d] Toyo Jozo Co. Ltd. [e] Tanabe Seiyaku Co. Ltd. [f] Yakult Pharmaceutical Industry Co. Ltd. [g] The optical purifies were determined by GLC and/or ^{19}F NMR after conversion of the malonic acid monoesters to their diastereomeric amides by optically active α-methylbenzylamine. [h] Method: A, 3 g of lipase/10 mmol of substrate/100 mL of buffer solution (pH 7.3), 40–41 °C, 6 h; B, 2 g of lipase/10 mmol of substrate/50 mL of buffer solution (pH 7.3), 40–41 °C, 3 h; C, 3 mg of lipase/10 mmol of substrate/70 mL of buffer solution (pH 7.3), 40–41 °C, 23 h; D, 100 mg of α-chymotrypsin/6 mmol of substrate/60 mL of buffer solution (pH 7.8), 27 °C, 5 h.

lated an enzyme which is able to decompose a carbon–fluorine bond. However, no enzyme for microbial hydrolysis of fluorinated compounds has been reported.

We now report the asymmetric hydrolysis of 2-fluoro-2-methyl malonic acid diesters with pig liver esterase (PLE), giving the optically active (−)-2-fluoro-2-methyl malonic acid monoesters.

$$MeCF(CO_2R)_2 \xrightarrow[40\text{–}41\ ^\circ C/6\ h]{PLE} MeCF(CO_2H)CO_2R$$

Since the optical purity is insufficient for them as a practical chiral synthon (Table I), a wide variety of lipases or cellulases were examined to search for practical routes to monofluorinated chiral synthons with high optical purity.

The results shown in Table II clearly suggest the great advantage of the fluorine atom to obviate racemization under these conditions.

The asymmetric hydrolysis by esterase (*Candida cylindracea*) proceeded smoothly to afford the (−)-2-fluoro-2-methylmalonic acid monoesters and by cellulase (*Trichoderma viride*) to afford the enantiomeric (+)-2-fluoro-2-methylmalonic acid monoesters.

To improve the yield and optical activity of 2-fluoro-2-methylmalonic acid monoester, various reaction conditions were examined (Table III).

The microbial hydrolysis of 2-fluoro-2-substituted malonic acid diesters with both esterase and cellulase gave the optically active (+)- or (−)-2-fluoro-2-substituted malonic acid monoesters. However, in the case of 2-fluoromalonic acid diesters, only the (+)-2-fluoromalonic acid monoester was formed. The results shown in Table IV clearly demonstrate the effect of the nature of the substituent groups on the 2-position of 2-fluoromalonic acid diesters.

To achieve the desired structure to determine the absolute configuration, brief outlines of the conversion operators determining the synthetic strategies are shown in Schemes I and II.

In Scheme I, the synthetic intermediate is the optically pure hydroxy ester **2**. The optically active (−)-2-fluoro-2-methylmalonic acid monoester was selectively reduced with *N,N*-dimethylchloromethyleniminium chloride and sodium borohydride to give good yields of the optically pure (−)-ethyl 2-fluoro-3-hydroxy-2-methylpropionate, $[\alpha]_D$ (MeOH) −8.16° (*c* 1.81), 91% ee. Protection with tosyl chloride followed by treatment of the hydroxyester with methyl lithium-copper iodide gave (−)-ethyl 2-fluoro-2-methyl butyrate, $[\alpha]_D$ (MeOH) −4.99 (c 2.38), 90% ee.

In Scheme II, (*R*)-(−)-2-hydroxy-2-methylbutyric acid, $[\alpha]_D$ (MeOH) −8.14° (*c* 1.00), 81% ee, prepared by Koga,[30] was reacted with methanesulfonyl chloride. Treatment of **4** with cesium fluoride in triethylene glycol for 1 h at 110 °C gave (*S*)-(−)-ethyl 2-fluoro-2-methylbutyrate with the desired absolute configuration, $[\alpha]_D$ (MeOH) −2.32° (*c* 3.00).

These results establish the absolute configuration of (−)-2-fluoro-2-methylmalonic acid monoethyl ester prepared from diethyl 2-fluoro-2-methylmalonate with the microbial hydrolysis.

The general rule based on the study of stereoselective hydrolysis of symmetrical diesters with pig liver esterase (PLE) by Tamm also predict formation of the *S* enantiomer.[31]

Mimic Effect of Fluorine Atom. It is of particular interest to compare fluorine with other halogens or alkyl groups, in order to confirm that fluorine mimics hydrogen. The results shown in Table V clearly suggest the mimic effect of fluorine in the microbial hydrolysis and the advantage of fluorine in obviating racemization under these conditions. Comparison of the fluorine atom with the methyl group clearly indicates that the difference in van der Waals radii is an important factor in this esterase system.

Microbial hydrolysis based on the mimic effect of fluorine atom appears to be the most convenient one-step process for preparing monofluorinated chiral synthons. The microbial approach to the new chiral synthons of monofluorinated compounds may open a new avenue for biologically active compounds containing fluorine.

Experimental Section

General Procedures. All microbial transformation were carried out in the Jarfermentor. All commercially available reagents were used without purification. Infrared spectra were obtained on a JASCO A-102 spectrometer and KBr pellets. The ^1H (internal Me_4Si) and ^{19}F (external CF_3CO_2H) NMR spectra were recorded on a Varian EM-390 spectrometer. Mass spectra were obtained on a Hitachi M-52 spectrometer at 20 eV. Specific rotations were recorded by using a JASCO DIP-140 digital po-

(25) Oritani, T.; Yamashita, K. *Yuki Gosei Kagaku Kyokaishi* **1983**, *41*, 1054.
(26) Kawasaki, H.; Tone, N.; Tonomura, K. *Agric. Biol. Chem.* **1981**, *45*, 29.
(27) Kawasaki, H.; Tone, N.; Tonomura, K. *Agric. Biol. Chem.* **1981**, *45*, 35.
(28) Goldman, P. *Science (Washington, D.C.)* **1969**, *164*, 1123.
(29) Goldman, P.; Milne, G. W.; Pignataro, M. T. *Arch. Biochem. Biophys.* **1967**, *118*, 178.
(30) Terashima, S.; Jew, S. S.; Koga, K. *Tetrahedron* **1979**, *35*, 2337, 2345.
(31) Mohr, P.; Waespe-Savcevic, N.; Tamm, C.; Gawronsca, K.; Gawronski, J. K. *Helv. Chim. Acta* **1983**, *66*, 2501.

Table III

entry	$MeCF(CO_2Et)_2$, mmol	lipase-MY, g	ratio[a]	buffer[b] solution, mL	reactn time, h	yield, %	optical purity, % ee
1	10	2.0	6.0	50	3	60	91
2	10	3.0	9.0	100	6	87	91
3	100	28.0	8.4	600	6	83	91
4	150	20.0	4.0	800	6	64	90
5	190	30.0	4.7	600	6	72	93

[a] Lipase/substrate (unit/mmol). [b] pH = 7.3.

Table IV. Asymmetric Hydrolysis

$$RCF(CO_2Et)_2 \xrightarrow[40\text{–}41\ ^\circ C]{\text{hydrolases}} RCF(CO_2Et)CO_2H$$

R	origin of hydrolase	method[f]	bp, °C (mmHg)	yield, %	$[\alpha]_D$ (MeOH), deg	O.P.,[d] %
H	*Candida cylindracea*[a]	A	104–106 (0.9)	79	+11.4 (*c* 1.56)	82
	Trichoderma viride[b]	A		73	+5.34 (*c* 2.80)	38
Me	*Candida cylindracea*	A	88–89 (0.6)	87	−20.9 (*c* 2.81)	91
	Candida cylindracea	B		60	−20.6 (*c* 1.95)	91
	Trichoderma viride[b]	A		42	+13.1 (*c* 2.24)	56
Et	*Candida cylindracea*	A	94–95 (0.7)	87	−14.0 (*c* 1.86)	93
	Candida cylindracea	B		62	−13.8 (*c* 2.08)	93
	Trichoderma viride[b,c]	A	no reaction			
n-Pr	*Candida cylindracea*	B	108–111 (0.9)	30	−2.90 (*c* 1.51)	33
n-Bu	*Candida cylindracea*	A	93–97 (0.7)	78	−1.53 (*c* 2.26)	11
	Candida cylindracea	B		8[e]		
sec-Bu	*Candida cylindracea*	A		25[e]		
Bzl	*Candida cylindracea*	B		18[e]		

[a] Meito Sangyo Co. Ltd. [b] Yakult Pharmaceutical Industry Co. Ltd. [c] Meiji Seika Co. Ltd. [d] The optical purities were determined by GLC and/or ^{19}F NMR after conversion of the malonic acid half-esters to their diastereomeric amides by optically active α-methylbenzylamine. [e] Determined by means of ^{19}F NMR using $PhCF_3$ as the internal standard. [f] Method: A, 3 g of lipase/10 mmol of substrate/100 mL of buffer solution (pH 7.3), 6 h; B, 2 g of lipase/10 mmol of substrate/50 mL of buffer solution (pH 7.3), 3 h.

Table V. Mimic Effect of Fluorine Atom at the 2-Position

$$MeCX(CO_2Et)_2 \xrightarrow[40\text{–}41\ ^\circ C/3\ h]{\text{lipase-MY}^a} MeCX(CO_2H)CO_2Et$$

X	yield, %	optical purity, % ee	van der Waals radius, Å
H	83	*b*	1.2
F	60	91	1.35
Cl		no reaction	1.80
Br		no reaction	1.95
CH_3		no reaction	2.0

[a] Meito Sangyo Co. Ltd.; 2 g/10 mmol of substrate. [b] Cannot be determined because of racemization.

larimeter. Yields were those of the isolated products.

2-Fluoro-2-methylmalonic Acid Monoethyl Ester. A suspension of lipase-MY (*Candida cylindracea*, Meito Sangyo Co. Ltd., 30 g) in buffer solution (600 mL, pH 7.3) was prepared from 1/15 M aqueous Na_2HPO_4 solution (460.8 mL) and 1/15 M aqueous KH_2PO_4 solution (139.2 mL) and was stirred for 15 min at 40–41 °C in a Jarfermentor (M-100, Tokyo Rikakikai Co. Ltd.). Into the mixture was added diethyl 2-fluoro-2-methylmalonate (20 g, 104 mmol), and then the whole mixture was stirred at 40–41 °C. After 6 h of stirring, the flocculant (200 ppm solution prepared from p-713, Dai-ichi Kogyo Seiyaku, 100 mL) was added into the stirring mixture for a few minutes. After 1 h of standing, the mixture was acidified with 1 N HCl and then the precipitates were separated by filtration. The oily materials were extracted with diethyl ether. The ethereal extract was dried over anhydrous magnesium sulfate and the solvent was removed. Distillation gave the corresponding malonic acid monoester in 87% (14.8 g) yield, bp 88–89 °C (0.6 mmHg).

Determination of Optical Purity. A mixture of 1-methyl-2-chloropyridinium iodide (0.31 g, 1.2 mmol), (*S*)-(−)-2-fluoro-2-methylmalonic acid monomethyl ester (0.16 g, 1.0 mmol), $[\alpha]_D$ (MeOH) −20.6° (*c* 1.00), triethylamine (0.24 g, 2.4 mmol), and (*R*)-(+)-α-methylbenzylamine, $[\alpha]_D$ +37.6° (neat), in methylene chloride (10 mL) was heated at 40 °C. After 2 h of heating, the whole mixture was poured into water, and then oily materials were extracted with diethyl ether. The ethereal layer was washed with 1 N HCl solution, 5% aqueous $NaHSO_4$, saturated $Na_2S_2O_3$ solution, and then brine. After removing the solvent, the diastereomeric ratio was determined by GLC (carrier gas N_2, flow 20 mL/min, column 3 mm × 3 m packed by Silicone GEXE-60 on Chromosorb W) at 200 °C.

Asymmetric Hydrolysis with Pig Liver Esterase. A suspension of pig liver esterase (180 μL, 3.2 M $(NH_4)_2SO_4$ solution, Type I, Sigma) and diethyl 2-fluoro-2-methylmalonate (1.9 g, 10

Table VI. NMR Data for 2-Alkyl-2-fluoromalonic Acid Monoesters

substrate	^{19}F NMR	^1H NMR
$CHF(CO_2H)CO_2Et$	115.8 (d, J_{FH} = 40.3 Hz)	1.34 (CH_3, t, $J_{CH_3CH_2}$ = 7.1 Hz), 4.34 (CH_2, d), 5.40 (CH, d), 10.20 (CO_2H, s)
$MeCF(CO_2H)CO_2Me$	78.0 (q, J_{FCH_3} = 20.9 Hz)	1.80 (CH_3, d), 3.87 (CH_3, s), 11.43 (CO_2H, s)
$MeCF(CO_2H)CO_2Et$	77.8 (q, J_{FCH_3} = 21.4 Hz)	1.32 (CH_3, t, $J_{CH_3CH_2}$ = 7.1 Hz), 1.77 (CH_3, d), 4.27 (CH_2, q), 10.90 (CO_2H, s)
$EtCF(CO_2H)CO_2Me$	89.4 (t, J_{FCH_2} = 21.0 Hz)	0.95 (CH_3, t, $J_{CH_3CH_2}$ = 7.1 Hz), 2.10 (CH_2, dq), 3.86 (CH_3, s), 11.46 (CO_2H, s)
$EtCF(CO_2H)CO_2Et$	89.4 (t, J_{FCH_2} = 21.5 Hz)	1.07 (CH_3, t, $J_{CH_3CH_2}$ = 7.1 Hz), 1.40 (CH_3, t, $J_{CH_3CH_2}$ = 6.8 Hz), 2.22 (CH_2, dq), 4.37 (CH_2, q), 11.0 (CO_2H, s)
$PrCF(CO_2H)CO_2Et$	87.4 (t, J_{FCH_2} = 22.0 Hz)	0.98 (CH_3, t, $J_{CH_3CH_2}$ = 7.0 Hz), 1.10–1.74 (m, 2 × H), 1.29 (CH_3, m, $J_{CH_3CH_2}$ = 7.2 Hz), 1.98 (CH_2CF, dm), 4.68 (CH_2, q), 11.0 (CO_2H, s)
$BuCF(CO_2H)CO_2Et$	87.4 (t, J_{FCH_2} = 21.2 Hz)	0.96 (CH_3, t, $J_{CH_3CH_2}$ = 5.6 Hz), 1.10–1.72 (m, 4 × H), 1.35 (CH_3, t, $J_{CH_3CH_2}$ = 6.5 Hz), 2.10 (CH_2CF, dm), 4.27 (CH_2, q), 12.90 (CO_2H, s)
sec-$BuCF(CO_2H)CO_2Et$	97.3 (t, J_{FCH_2} = 28.0 Hz)	0.96 (CH_3, t, $J_{CH_3CH_2}$ = 5.6 Hz), 1.02 (CH_3, t, $J_{CH_3CH_2}$ = 7.1 Hz), 1.00 -1.88 (m), 1.36 (CH_3, t, $J_{CH_3CH_2}$ = 7.1 Hz), 2.40 (d, m, 1 × H), 4.37 (CH_2, q), 10.34 (CO_2H, s)
$PhCH_2CF(CO_2H)CO_2Et$	84.5 (t, J_{FCH_2} = 23.4 Hz)	1.23 (CH_3, t, $J_{CH_3CH_2}$ = 7.2 Hz), 3.35 (d, 2 × H), 3.49 (q, 4 × H), 7.50 (m, 5 × H), 10.60 (CO_2H, s)

mmol) in buffer solution (25 mL, pH 7.3) was stirred at 40–41 °C. After 6 h of stirring, the whole solution was acidified to pH 2 by 1 N HCl solution, and then oily materials were extracted with ethyl acetate (30 mL × 3). Workup gave the corresponding monoester in a yield of 34% (0.55 g), $[\alpha]_D$ (MeOH) −4.88° (*c* 0.7), 24% ee.

(*S*)-(−)-Ethyl 3-Hydroxy-2-fluoro-2-methylbutyrate. After a mixture of *N*,*N*-dimethylformamide (3.4 mL) and oxalyl chloride (8 mL) in methylene chloride (40 mL) was stirred for 1 h at 0 °C, the solvent was removed under dynamic vacuum. Into the reaction vessel were added acetonitrile (30 mL) and tetrahydrofuran (100 mL) with a syringe under an atmosphere of nitrogen, and then (*S*)-(−)-2-fluoro-2-methylmalonic acid monoethyl ester (6.56 g, 40 mmol, 91% ee) was added at −30 °C. After 1 h of stirring at −30 °C, a solution of sodium borohydride (3.5 g, 93 mmol) in *N*,*N*-dimethylformamide (20 mL) was added slowly at −78 °C, cooling with the dry ice–acetone bath. After adding the above solution, the reaction mixture was stirred for 4 h at −20 °C, and the mixture was quenched with 3 N HCl (50 mL). Oily materials were extracted with ethyl acetate, and the organic layer was washed with 1 N HCl, 5% aqueous $NaHCO_3$, water, and brine. On removal of the solvent, distillation gave (*S*)-(−)-ethyl 3-hydroxy-2-fluoro-2-methylbutyrate (4.11 g, 27.4 mmol) in a yield of 69%, $[\alpha]_D$ (MeOH) −8.16° (*c* 1.81), bp 84–85 °C (8 mmHg), 91% ee: ^{19}F NMR ($CDCl_3$) δ 84.0 (ddq, J_{F-H_A} = 18 Hz, J_{F_A} = 21 Hz, J_{F-CH_3} = 23 Hz); ^1H NMR ($CDCl_3$) δ 1.33 (CH_3, t; $J_{CH_3-CH_2}$ = 7.1 Hz), 1.50 (CH_3, d), 2.67 (OH), 3.77 (H_A, d), 3.88 (H_B, d), 4.27 (CH_2, q).

(*S*)-(−)-Tosylate of 2. A mixture of (*S*)-(−)-ethyl 3-hydroxy-2-fluoromethylbutyrate (2.0 g, 13 mmol) and tosyl chloride (3.0 g, 16 mmol) in pyridine (20 mL) was stirred at room temperature. After 3 h of stirring, the mixture was poured into water and then oily materials were extracted with ethyl acetate. Tosylate was purified by column chromatography on silica gel using the *n*-hexane–diethyl ether (5:1) as an eluent, in 95% yield: $[\alpha]_D$ (MeOH) −1.79° (*c* 1.34); ^{19}F NMR ($CDCl_3$) δ 82.3 (ddq, J_{FCH_3} = 19.5 Hz, J_{FH_A} = 18.3 Hz, J_{FH_B} = 14.7 Hz); ^1H NMR ($CDCl_3$) δ 1.29 (CH_3, t; $J_{CH_3CH_2}$ = 7.4 Hz), 1.52 (CH_3, d), 2.46 (CH_3, s), 4.13 (H_B, d), 4.15 (H_A, d), 4.18 (CH_2, q), 7.30–7.75 (Ar H).

(*S*)-(−)-Ethyl 2-Fluoro-2-methylbutyrate. Into a solution of Me_2CuLi which was prepared from copper iodide (7.6 g, 40 mmol) and methyllithium (1.3 N, 80 mmol) in freshly dried diethyl ether (20 mL) at −20 °C was added (*S*)-(−)-tosylate (3)(5.8 g, 20 mmol) in diethyl ether (10 mL) slowly at 0–5 °C. After 10 h of stirring at 0–5 °C, the reaction mixture was worked up in the usual manner, giving the product in a 50% yield: $[\alpha]_D$ (MeOH) −4.99° (*c* 2.38); ^{19}F NMR ($CDCl_3$) δ 78.2 (ddq; J_{FH_A} = 20.7 Hz, J_{FCH_3} = 16.9 Hz, J_{FCH_3PW} = 17.9 Hz); ^1H NMR ($CDCl_3$) δ 0.94 (CH_3, t, $J_{CH_3CH_2}$ = 7.1 Hz), 1.32 (CH_3, t, $J_{CH_3CH_2}$ = 6.8 Hz), 1.49 (CH_3, d), 1.60–2.20 (m, 2 × H), 4.17 (CH_2, 2 × H).

(*R*)-(+)-Mesylate 6. A mixture solution of (*R*)-(−)-**5** (20 mmol), ethanol (3.8 mL), benzene (4.4 mL), and concentrated H_2SO_4 (4 drops) was refluxed. After 4 h, the reaction mixture was poured into water, and then oily materials were extracted with diethyl ether. After removing the solvent, crude hydroxy ester was obtained. Into the solution of crude hydroxy ester in pyridine (5 mL) was added methanesulfonyl chloride (1.8 g, 16 mmol), and then the whole solution was stirred for 1 day at room temperature. The reaction mixture was worked up in the usual manner. The mesylate was purified by column chromatography on silica gel using a mixture of *n*-hexane–diethyl ether (2:1) as an eluent, in 51% yield: $[\alpha]^{21}_D$ +9.06° (*c* 0.50, MeOH); ^1H NMR ($CDCl_3$) δ 0.97 (CH_3, t, $J_{CH_3CH_2}$ = 7.5 Hz), 1.30 (CH_3, t, $J_{CH_3CH_2}$ = 7.1 Hz), 1.70 (CH_3, s), 1.90 (CH_2, q), 3.03 (CH_3, s), 4.23 (CH_2, q); IR (C=O) 1740 cm^{-1}.

(*R*)-(+)-Mesylate 6 to (*S*)-(−)-2-Fluoro-2-methylbutyrate. To a solution of cesium fluoride (1.82 g, 12 mmol) and triethylene glycol (5 mL) heated at 110 °C was added (*R*)-(+)-mesylate **6** (10 mmol). The product was collected by the trap to trap distillation under dynamic vacuum: $[\alpha]^{21}_D$ −2.32° (*c* 3.00, MeOH).

Syntheses of Tetrahydrofuro[2,3-*b*]benzofurans: A Synthesis of (±)-Aflatoxin B_2

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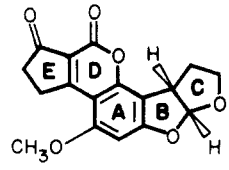
Aldehyde *O*-aryl oximes, on treatment with hydrogen chloride in tetrahydrofuran, are converted to 2-hydroxy-2,3-dihydrobenzofurans and their corresponding 4-chlorobutanol ketals. The major reaction pathway, a 3,4-oxaza Cope rearrangement, is accompanied by Beckmann fragmentation, the relative amount of which is sensitive to the stereochemistry of the oxime and the specific acid conditions. With appropriately substituted oximes, the 2,3,3a,8a-tetrahydrofuro[2,3-*b*]benzofuran ring system is efficiently prepared, as is demonstrated for the 4-hydroxy-6-methoxy derivative and its regioisomer. The former compound provides a total synthesis of aflatoxin B_2.

The aflatoxin are extremely toxic and carcinogenic fungal metabolites that frequently occur as contaminants in a large variety of foods. Their profound biological activity, wide distribution, and unusual structures have generated considerable synthetic activity and resulted in several total syntheses of the racemic mycotoxins, particularly aflatoxin B_2 (**1**).[1]



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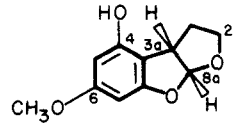

2

In general, the previous syntheses can be considered to consist of three stages: (1) preparation of an appropriately substituted ring A moiety with functionally differentiated phenolic groups, (2) elaboration of the ring A moiety, frequently a coumarin, into the tricyclic ABC ring system, and (3) annulation of the 2-pyran and its fused ring onto the tetrahydrofuro[2,3-*b*]benzfuran (**2**), to add rings D and E. The latter stage 3 (**2**→**1**) has been effectively accomplished.[2] Routes to furobenzofuran **2**, however, have been

(1) Recently reviewed by: Schuda, P. F. *Top. Curr. Chem.* **1980**, *91*, 75.

(2) (a) Roberts, J. C.; Sheppard, A. H.; Knight, J. A.; Roffey, P. *J. Chem. Soc. C* **1968**, 22. (b) Büchi, G.; Weinreb, S. M. *J. Am. Chem. Soc.* **1971**, *93*, 746.

0022-3263/86/1951-1006$01.50/0