Stereoselective Synthesis of Fluorinated Materials Catalyzed by an Antibody

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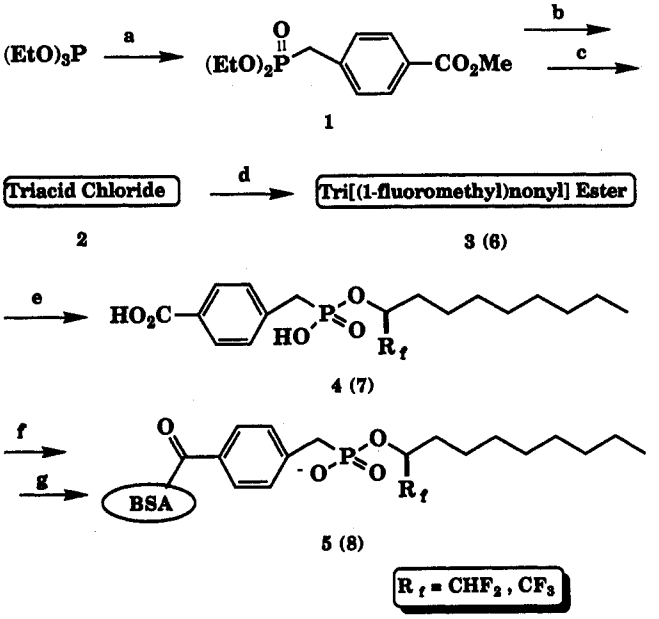
Abstract: A monoclonal antibody, elicited by a transition-state analogue for the hydrolysis of fluorinated esters, acted as an enzymelike catalyst for the preparation of chiral fluorinated compounds. The syntheses of (*R*)- or (*S*)-1-(fluoroalkyl)alkanols and an allylic alcohol possessing a trifluoromethyl group in high optical purity (>98% enantiomeric excess (ee)) by antibody-derived reagents are described. The role of molecular recognition by antibody reagents and its importance to the preparation of optically pure materials is described. Significantly, it has been found that a fluoromethyl group on the stereogenic center or an acyloyl group enhanced the optical purity of the resultant products.

Recent developments in the design of highly selective enzymelike catalysts are having an important impact on asymmetric organic synthesis.[2-7] Particularly, antibody reagents have been used as selective catalysts for acyl transfers, carbon–carbon bond cleavages, lactonizations, and Claisen rearrangements. The transition-state-analogue concept has been established as a valuable approach to the design of enzymelike catalysts for stereoselective chemical transformations. The synthesis of optically active fluorine-containing molecules with the appropriate functionalities may be prepared by enzymatic methods.[8] However, there are numerous disadvantages associated with these approaches; e.g., the resolution of fluorinated compounds, required for the study of differences in physical[9] and medicinal properties,[10] is not possible, efficient control of the conversion ratio is required in order to obtain both enantiomers, and highly enriched products must be prepared at low conversion so that unreacted substrates may be obtained in high optical purity. To obviate the above disadvantages, new methods are required. Accordingly, we have developed a new and effective method based upon antibody-catalyzed reactions for the enantio- and diastereoselective formation of organofluorine compounds.

Antibody-catalyzed enantioselective transformations of fluorinated materials facilitate the assembly of contiguous stereocenters with high relative as well as absolute stereocontrol.

Scheme I



[a] Arbuzov reaction: triethyl phosphite, methyl 4-(bromomethyl)-benzoate, 200 °C. [b] Acidic hydrolysis: concentrated HCl, 50 °C. [c] Key: $SOCl_2$. [d] Key: (1-fluoromethyl)nonanol, NaH, Et_2O. [e] 0.1 N NaOH aq. [f] Key: bovine serum albumin (BSA), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, dilute, HCl, pH 5.0. [g] Key: dialysis, NaCl buffer, pH 7.4.

Monoclonal Antibody

Enzymelike catalyst design (antibody reagent design) requires the preparation of haptens with structures that would mimic the site of catalytic activity. Antibody reagents in this paper were designed transition-state analogues.

The antigens required were prepared as shown in Scheme I. In the first step, haptens with fluoroalkyl groups attached to the stereogenic center were prepared from enantiomerically pure (*R*)- and/or (*S*)-1-(fluoroalkyl)alkanols. To form the desired antibody reagents, an immunogenic conjugate[11,12] was prepared by reaction of the phosphonate **4** with a carrier protein (bovine serum albumin). Lymphocytes from the spleen of BALC/c mice immunized with each type of the purified antigen (the BSA–phosphonate conjugate) were fused by standard protocols with use of mouse myeloma cells (P 3-X 63-Ag.8. U 1) as the fusion partner. Antibodies were screened by ELISA for cross-reactivity with the

(1) Research fellow on leave from Kashima Oil Co., Kioi-cho, Chiyoda-ku, Tokyo 102, Japan, 1987–1989.

(2) (a) Pollack, S. J.; Jacobs, J. W.; Schultz, P. G. *Science* **1986**, *234*, 1570. (b) Schultz, P. G. *Ibid.* **1988**, *240*, 426.

(3) Schultz, P. G. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1283 and references cited therein.

(4) (a) Tramontano, A.; Janda, K. D.; Lerner, R. A. *Science* **1986**, *234*, 1566. (b) Tramontano, A.; Janda, K. D.; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6736.

(5) Tramontano, A.; Ammann, A.; Lerner, R. A. *J. Am. Chem. Soc.* **1988**, *110*, 2282.

(6) (a) Jacobs, J. W.; Schultz, P. G.; Sugasawara, R.; Powell, M. *J. Am. Chem. Soc.* **1987**, *109*, 2174. (b) Jackson, D. Y.; Jacobs, J. W.; Sugasawara, R.; Reich, S. H.; Bartlett, P. A.; Schultz, P. G. *Ibid.* **1988**, *110*, 4841. (c) Cochran, A. G.; Sugasawara, R.; Schultz, P. G. *Ibid.* **1988**, *110*, 7888.

(7) Napper, A. D.; Benkovic, S. J.; Tramontano, A.; Lerner, R. A. *Science* **1987**, *237*, 1041. (b) Janda, K. D.; Schloeder, D.; Benkovic, S. J.; Lerner, R. A. *Ibid.* **1988**, *244*, 1188.

(8) (a) Kitazume, T.; Sato, T.; Kobayashi, T.; Lin, J. T. *J. Org. Chem.* **1986**, *51*, 1003. (b) Kitazume, T.; Nakayama, M. *Ibid.* **1986**, *51*, 2795. (c) Lin, J. T.; Yamazaki, T.; Kitazume, T. *Ibid.* **1987**, *52*, 3211. (d) Kitazume, T.; Kobayashi, T.; Yamamoto, T.; Yamazaki, T. *Ibid.* **1987**, *52*, 3218. (e) Kitazume, T.; Ikeya, T. *Ibid.* **1988**, *53*, 2350. (f) Yamazaki, T.; Yamamoto, T.; Kitazume, T. *Ibid.* **1989**, *54*, 83. (g) Kitazume, T.; Ikeya, T.; Murata, K. *J. Chem. Soc., Chem. Commun.* **1986**, 1331. (h) Kitazume, T.; Takeda, M.; Lin, J. T.; Yamazaki, T. *J. Fluorine Chem.* **1989**, *43*, 177.

(9) (a) Yashino, K.; Ozaki, M.; Taniguchi, H.; Ito, M.; Satoh, K.; Yamazaki, N.; Kitazume, T. *Jpn. J. Appl. Phys.* **1987**, *26*, L77. (b) Suzuki, Y.; Hagiwara, T.; Kawamura, I.; Okamura, N.; Kitazume, T.; Kakimoto, M.; Imai, Y.; Ouchi, H.; Takezoe, H.; Fukuda, A. *Liq. Cryst.* **1989**, *6*, 167. (c) Johno, M.; Itoh, K.; Lee, Y.; Ouchi, T.; Takezoe, H.; Fukuda, A.; Kitazume, T. *Jpn. J. Appl. Phys.* **1990**, *29*, L107.

(10) Filler, R.; Kobayashi, Y. *Biomedical Aspects of Fluorine Chemistry*; Kodansha and Elsevier Biomedical: Amsterdam, 1983.

(11) (a) Nishima, T.; Tsuji, A.; Fukushima, D. *Steroids* **1974**, *24*, 861. (b) Erlanger, B. *Methods Enzymol.* **1980**, *70*, 85.

(12) Niman, H. L.; Elder, J. H. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5624.

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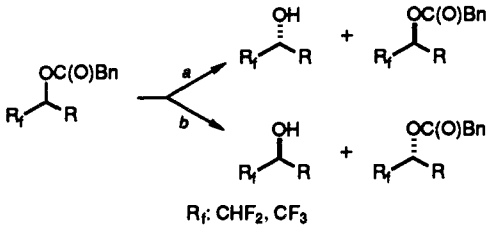
Table I. Antibody-Catalyzed Asymmetric Hydrolysis. Synthesis of Optically Pure 1,1-Difluoro-2-alkanols with Antibody



R_f: CHF_2, CF_3

substrate	antibody reagent[c]	hc (%)[d]	yield (%)	$[\alpha]^{21}_D$ (deg) (c, $CHCl_3$)	op[e] (% ee)	abs confign
n-$C_6H_{13}CH(OH)CHF_2$	A[a]	49	48	+19.6 (1.06)	98	R
			46	−20.3 (1.99)	>98	S[f]
n-$C_6H_{13}CH(OH)CHF_2$	B[b]	48.5	47	−20.1 (1.14)	99	S
			46	+19.8 (1.16)	>98	R[f]
n-$C_7H_{15}CH(OH)CHF_2$	A	49.5	46	+17.4 (1.17)	>97	R
n-$C_7H_{15}CH(OH)CHF_2$	B	47.5	48	−17.7 (1.19)	>98	S
n-$C_8H_{17}CH(OH)CHF_2$	A	49	47	+7.49 (1.15)	99	R
			48	−7.41 (1.07)	>98	S[f]
n-$C_8H_{17}CH(OH)CHF_2$	B	48.5	46	−7.50 (1.11)	>98	S
			48	+7.48 (1.14)	>98	R[f]

[a] Antibody made from (+)-hapten. [b] Antibody made from (−)-hapten. [c] Antibody reagent (A; K_{cat} 0.94 ± 0.2 min^{-1}; K_m 410 ± 90 μM) generated from (+)-hapten with (*R*)-(+)-1-(difluoromethyl)nonyl group. Antibody reagent (B; K_{cat} 0.91 ± 0.3 min^{-1}; K_m 380 ± 80 μM generated from (−)-hapten with (*S*)-(−)-1-(difluoromethyl)nonyl group. [d] The hydrolysis conversion was determined by ^{19}F NMR signal intensity. [e] The optical purity was determined by ^{19}F NMR after conversion of the compound to its diastereomeric ester by optically active MTPA. [f] The products were obtained from the recovered benzylates.

BSA–hapten conjugate, i.e., for the inhibition of binding to the BSA–hapten conjugate by free hapten. Antibodies were purified from ascites fluid by protein A Sepharose 4B affinity chromatography and were determined to be >94% homogeneous by sodium dodecyl sulfate poly(acrylamide) gel electrophoresis.[13]

Results and Discussion

It is ideally possible to obtain both enantiomers in 50% yield in 100% enantiomeric excess (ee) by enzymatic optical resolution. However, in the enzymatic resolution of fluorinated compounds, strict control of the conversion ratio is required to prepare both enantiomers.[14] To obviate the disadvantages of enzymatic resolution, we examined antibody-catalyzed asymmetric hydrolysis of racemic fluoromethylated esters (Table I).

Asymmetric hydrolysis of racemic 1-(difluoromethyl)nonyl phenylacetate antibody derived from a racemic hapten produced (*R*)-(+)-1,1-difluoro-2-decanol in an enantiomeric excess of 47% when the hydrolysis was carried to less than 45%. The rate of the antibody-catalyzed reaction relative to that of the uncatalyzed reaction was 914 times faster.[15,16] Since the optical purity of (*R*)-1,1-difluoro-2-decanol prepared from the antibody derived from the racemic hapten is insufficient for use as a practical asymmetric synthon, antibodies based on the chiral haptens were prepared. An antibody induced from a (+)-hapten (95% ee) catalyzed the stereospecific hydrolysis of racemic ester. (*R*)-(+)-1,1-Difluoro-2-decanol was isolated in 99% ee (49% hydrolysis conversion). The *S* enantiomer (>98% ee) was prepared from the recovered ester by a cellulase (*Trichoderma viride*) hydrolysis or by chemical methods (2 mol/L aqueous NaOH–acetone system). Further, an antibody induced from (−)-hapten (96% ee) catalyzed the same hydrolysis, forming the *S* enantiomer in >98% ee (48.5% hydrolysis conversion). The *R* enantiomer (>98% ee) was obtained from the recovered ester.

Table II

R_f	hc (%)	op[b] (% ee)
CH_3	17	racemic
CH_2F	34	27 (*R*)
CHF_2	49	99 (*R*)
CF_3	36	14 (*R*)
CF_2Cl	12	4 (*R*)

[a] Antibody generated from (+)-hapten–CHF_2 (>98% ee). [b] In the parentheses are shown their absolute configurations.

In the previous antibody-catalyzed system, it must be determined if contaminating protease such as acetyl cholinesterase is responsible for the hydrolysis conversion. This question could not be answered definitely even after purification by affinity chromatography and gel electrophoreses to remove protease contaminants. Therefore, we chose to explain the relationships that result in a molecular recognition by the antibody reagent. We examined the affinity of antibody induced by difluoromethylated hapten **4** for complex molecules containing various fluoroalkyl groups and different-length carbon chains attached to stereogenic center. The optical purity is independent of the length of the carbon chain attached to the stereogenic carbon. A moderate effect on optical purity was observed on changing the acyl group from benzyl to acetyl. The enantioselectivity was decreased from 99% ee (49% hydrolysis conversion) to 37% ee (24% hydrolysis conversion) when the product formed was (*R*)-(+)-1,1-difluoro-2-decanol (Table II). Generally, it was found that difluoromethyl groups attached to the stereogenic center improved the optical purity of the hydrolysis relative to other methyl or monofluoro- or trifluoromethyl groups.

From these results, 1-(trifluoromethyl)alkyl phenylacetates were predicted to be hydrolyzed by the antibody induced by the hapten containing a trifluoromethyl group.

A highly stereocontrolled hydrolysis of 1-(trifluoromethyl)alkyl phenylacetates was effected by the antibodies generated from (+) hapten **7** in 96% ee or (−) hapten **7** in 95% ee in Scheme I (Table III).

Stereoselective transformation of trifluoromethylated alkanols containing various functionalized groups is also possible. Several

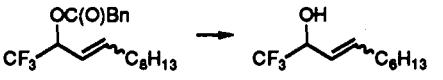
(13) Laemmli, V. *Nature (London)* **1970**, *227*, 680.
(14) Kitazume, T.; Asai, M.; Tsukamoto, T.; Lin, J. T.; Yamazaki, T. *J. Fluorine Chem.*, in press.
(15) Kinetic constants were determined by the method of initial rates data. Kinetic parameters for the hydrolysis of esters from the Lineweaver–Burk plots were determined. The value of K_{cat} and the Michaelis constant, K_m, were found to be 0.94 ± 0.2 min^{-1} and 410 ± 90 μM, respectively.
(16) The antibody (400 μM, Lowry assay, with a molecular weight of 150 000 for immunoglobulin G) was incubated at 25 °C in 100 mM phosphate buffer at pH 7.3. The racemic ester (20 mM) was hydrolyzed for 25 h at 25–27 °C in this buffer solution. The antiody was removed by Centricon filtration; alcohol was isolated from the diethyl ether extraction followed by column chromatography on silica.

Table III. Synthesis of Optically Pure 1,1,1-Trifluoro-2-alkanols with Antibody

substrate	antibody reagent[a]	hc[b] (%)	yield (%)	$[\alpha]^{21}_D$ (deg) (*c*, $CHCl_3$)	op[c] (% ee)	abs confign
n-$C_6H_{13}CH(OH)CF_3$	A	49	43	+28.3 (1.05)	98.5	*R*
			44	−27.5 (1.49)	>97	*S*[d]
n-$C_6H_{13}CH(OH)CF_3$	B	48.5	48	−28.6 (1.14)	>98	*S*
			43	+28.1 (1.16)	>98	*R*[d]
n-$C_8H_{17}CH(OH)CF_3$	A	48.5	46	+24.4 (1.24)	99	*R*
			45	−24.1 (1.27)	98	*S*[d]
n-$C_8H_{17}CH(OH)CF_3$	B	48	44	−24.7 (1.14)	99	*S*
			45	+24.3 (1.26)	>98	*R*[d]

[a] Antibody reagent (A; K_{cat} 0.89 ± 0.3 min^{-1}, K_m 430 ± 50 μM) generated from (+)-hapten with (*R*)-(+)-1-(trifluoromethyl)nonyl group. Antibody reagent (B; K_{cat} 0.86 ± 0.3 min^{-1}, K_m 390 ± 70 μM) generated from (−)-hapten with (*S*)-(−)-1-(trifluoromethyl)nonyl group. [b] The hydrolysis conversion was determined by ^{19}F NMR signal intensity. [c] The optical purity was determined by ^{19}F NMR after conversion of the compound to its diastereomeric ester by optically active MTPA. [d] The products were obtained from the recovered benzylates.

Table IV



hapten abs confign	K_{cat} (min^{-1})	K_m (μM)	hc[a] (%)	yield (%)	$[\alpha]^{21}_D$ (deg) (*c*, $CHCl_3$)	op[b] (% ee)	abs confign
(*R*)-(*E*)-(+)	0.87 ± 0.2	390 ± 80	23.5	21	+2.54 (1.09)	99	(*R*)-(*E*)
(*S*)-(*E*)-(−)	0.94 ± 0.2	410 ± 70	24	22	−2.57 (1.17)	98.5	(*S*)-(*E*)
(*R*)-(*Z*)-(+)	0.91 ± 0.2	370 ± 90	23	20	+21.3 (1.15)	98.5	(*R*)-(*Z*)
(*S*)-(*Z*)-(−)	0.89 ± 0.3	440 ± 50	23.5	19	−20.9 (1.03)	>98	(*S*)-(*Z*)

[a] The hydrolysis conversion was determined by ^{19}F NMR signal intensity. [b] The optical purity was determined by ^{19}F NMR after conversion of the compound to its diastereomeric ester by optically active MTPA.

haptens containing a trifluoromethyl group such as the *E* and *Z* allylic alcohols (see Scheme II) have been prepared. Obviously, the antibody induced by the *E* hapten acted on the *E* allylic alcohol with a selectivity of >99% (hydrolysis conversion >48.5%), and the antibody from the *Z* hapten acted on the *Z* isomer with a selectivity of >98.5% (hydrolysis conversion 49%). The antibody reagents demonstrate an improvement in enantioselectivity of 82% ee (27% hydrolysis conversion of benzylate derivative of *E* allylic alcohol) to 99% ee (48.5% hydrolysis conversion of racemate), giving (*R*)-(*E*)-(+) isomer in comparison with the product formed by lipase-MY (*Candida cyclindracea*). These antibody-catalyzed hydrolyses were effective in separating four isomers directly from the racemic ester. (*R*)-(*E*)-(+)-1-Hexyl-3-hydroxy-4,4,4-trifluoro-1-butene[8h] (99% ee) was separated from the racemic ester by hydrolysis (conversion 23.5%) by use of the antibody generated from (*R*)-(*E*) hapten (>98% ee). The *E* isomer, (*S*)-(*E*)-(−)-1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butene (98.5% ee), was separated from the recovered ester by use of the antibody generated from (*S*)-(*E*)-(−) hapten. Furthermore, (*R*)-(*Z*)-(+) and (*S*)-(*Z*)-(−) isomers could be obtained in the same system.

Consequently, four stereoisomers were separated in high optical purity from the racemic ester by using the corresponding antibody reagent (Table IV).

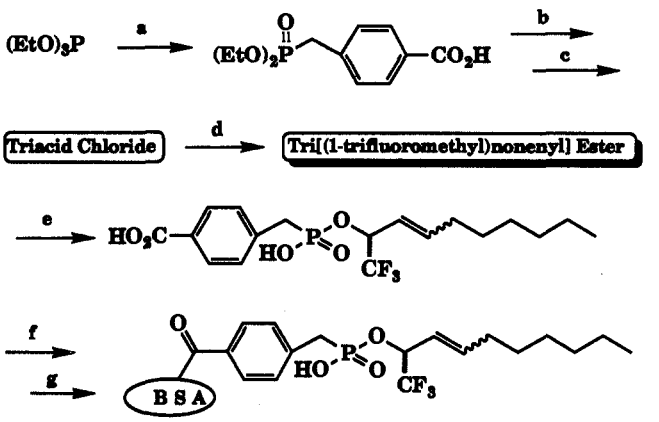
We believe that the procedures reported provide selective syntheses of optically pure fluoromethylated alkanols. These results clearly imply that antibody-catalyzed asymmetric resolution has significant advantages relative to other enzymatic methods that have been applied to organofluorine chemistry.

Experimental Section

General Procedures. All asymmetric hydrolyses were carried out in the "CULSTIR" flask. Infrared spectra were obtained by using a Jasco A-102 spectrometer and KBr pellets. Nuclear magnetic resonance (NMR) spectra were recorded at 200 MHz for ^1H NMR (internal Me_4Si) and at 56.5 MHz for ^{19}F NMR (external CF_3CO_2H) and ^{31}P NMR (external 85% H_3PO_4) in $CDCl_3$. Specific rotations were recorded by using a Jasco DIP-140 digital polarimeter. Yields were those of isolated products.

Synthesis of Antigen 5. (a) Phosphonate 4. A mixture of triethyl phosphite (10 g) and methyl 4-(bromomethyl)benzoate (4.6 g, 20 mmol) was heated at 200 °C for 5 h. Distillation under reduced pressure gave the compound **1** in 57% yield. Compound **1** was converted into the free triacid via usual acidic hydrolysis (1-h reflux in concentrated HCl) followed by removal of solvent and then reaction with freshly distilled thionyl chloride to form the corresponding triacid chloride **2**. Subsequent condensation with (*R*)-(+)-1-(difluoromethyl)nonanol (40 mmol, $[\alpha]^{21}_D$ +7.45 (*c* 1.14, $CHCl_3$), >98% ee) and triacid chloride (10 mmol) in pyridine (5 mL)–CH_2Cl_2 (50 mL) system afforded the corresponding tris[1-(difluoromethyl)nonyl] ester **3**. The ester was then hydrolyzed with 0.1 M aqueous NaOH at 50 °C and purified by reversed-phase high-pressure liquid chromatography (Bondapak C_{18}, 5–30% CH_3CN gradient in 0.1 M aqueous triethylammonium bicarbonate buffer, pH 7.4) to give phosphonate **4** in 41% yield: $[\alpha]^{21}_D$ +44.1° (*c* 1.14, MeOH), 98% ee; ^{19}F NMR ($CDCl_3$) δ 48.1 (ddd, $J_{F,F}$ = 260, $J_{F,H}$ = 53, $J_{F,H}$ = 11 Hz), 52.7 (ddd, $J_{F,H}$ = 52.5, $J_{F,H}$ = 11 Hz); ^1H NMR ($CDCl_3$) δ 1.1–3.1 (17 H, m), 3.38–3.73 (4 H, m), 5.53 (CHF_2, dt, $J_{H,H}$ = 5.1 Hz), 7.13–7.67 (ArH), 10.5 (CO_2H); ^{31}P NMR (relative to 85% H_3PO_4) 31.4; high-resolution mass for $C_{18}H_{27}PO_5F_2$, calcd 392.379, found 392.409.

Scheme II



[a] Arbuzov reaction: triethyl phosphite, methyl 4-(bromomethyl)-benzoate, 200 °C. [b] Acidic hydrolysis: concentrated HCl, 100 °C. [c] Key: $SOCl_2$. [d] Key: (*E*)- or (*Z*)-1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butene, NaH, Et_2O. [e] Key: 0.1 N NaOH aq. [f] Key: bovine serum albumin (BSA), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, dilute HCl, pH 5.0. [g] Key: dialysis, NaCl buffer, pH 7.4.

(b) Antigen 5. A mixture of compound **4** (3.92 g, 10 mmol) and bovine serum albumin (BSA) (2 g) in water (30 mL) was stirred for 24 h at room temperature. The precipitate was purified by chromatography on Sephadex G-50.

Synthesis of Antigen 8. (a) Phosphonate 7. In the previous reaction, (*R*)-(+)-1-(trifluoromethyl)nonanol (40 mmol, $[\alpha]^{21}_D$ +24.0 (*c* 1.44, MeOH), >98% ee) and triacid chloride (10 mmol) in pyridine (5 mL)–

CH_2Cl_2 (50 mL) were used to afford the corresponding tirs[1-(trifluoromethyl)nonyl] ester **6**. The ester was then hydrolyzed with 0.1 M aqueous NaOH at 50 °C and purified by reversed-phase high-pressure liquid chromatography (Bondapak C_{18}, 5–30% CH_3CN gradient in 0.1 M aqueous triethylammonium bicarbonate buffer, pH 7.4) to give phosphonate **7** in 46% yield: $[\alpha]^{21}_D$ +37.4° (*c* 1.27, MeOH), >98% ee; ^{19}F NMR ($CDCl_3$) δ −4.1 (d, $J_{F,H}$ = 7.5 Hz); ^1H NMR ($CDCl_3$) δ 1.11–3.21 (17 H, m), 3.35–3.76 (4 H, m), 7.13–7.67 (ArH), 10.7 (CO_2H); ^{31}P NMR (relative to 85% H_3PO_4) 31.8; high-resolution mass for $C_{18}H_{26}PO_5F_3$, calcd 410.369, found 410.513.

(b) Antigen 8. A mixture of compound **7** (4.10 g, 10 mmol) and bovine serum albumin (BSA) (2 g) in water (30 mL) was stirred for 24 h at room temperature. The precipitate was purified by chromatography on Sephadex G-50.

Synthesis of Antigen 11. (a) Phosphonate 10. In the previous reaction, (*R*)-(*E*)-(+)-1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butene (40 mmol, $[\alpha]^{23}_D$ +2.56 (*c* 1.16, MeOH), >98% ee) and triacid chloride (10 mmol) in pyridine (5 mL)–CH_2Cl_2 (50 mL) were combined to afford the corresponding tris(1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butenyl) ester 9. After the usual workup, **9** was purified by reversed-phase HPLC (Bondapak C_{18}, 5–30% CH_3CN gradient in 0.1 M aqueous triethylammonium bicarbonate buffer, pH 7.4) to give phosphonate **10** in 68% yield: $[\alpha]^{23}_D$ +11.7° (c 1.31, MeOH), >98% ee; ^{19}F NMR ($CDCl_3$) δ 1.44 (d, $J_{F,H}$ = 6.5 Hz); ^1H NMR ($CDCl_3$) δ 0.89–2.43 (13 H, m), 4.32 (dq, $J_{H,Hvic}$ = 6.0 Hz), 5.51 (dd, $J_{H,Htrans}$ = 15.7 Hz), 6.03 (dt, $J_{H,Hvic}$ = 6.5 Hz), 7.13–7.67 (ArH), 10.7 (CO_2H); ^{31}P NMR (relative to 85% H_3PO_4) 31.7; high-resolution mass for $C_{18}H_{24}PO_5F_3$, calcd 408.353, found 408.463.

(b) Antigen 11. A mixture of compound **10** (4.08 g, 10 mmol) and bovine serum albumin (BSA) (2 g) in water (30 mL) was stirred for 24 h at room temperature. The precipitate was purified by chromatography on Sephadex G-50.

Synthesis of Antigen 14. (a) Phosphonate 13. In the previous reaction, (*S*)-(*E*)-(−)-1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butene (40 mmol, $[\alpha]^{23}_D$ −2.60 (*c* 1.44, MeOH), 99% ee) and triacid chloride (10 mmol) in pyridine (5 mL)–CH_2Cl_2 (50 mL) were used to afford the corresponding tris(1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butenyl) ester **12**. After the usual workup, the ester was purified by reversed-phase HPLC (Bondapak C_{18}, 5–30% CH_3CN gradient in 0.1 M aqueous triethylammonium bicarbonate buffer, pH 7.4) to give phosphonate **13** in 57% yield: $[\alpha]^{23}_D$ −11.9° (*c* 1.25, MeOH), 99% ee; high-resolution mass for $C_{18}H_{24}PO_5F_3$, calcd 408.353, found 407.975.

(b) Antigen 14. A mixture of compound **13** (4.08 g, 10 mmol) and bovine serum albumin (BSA) (2 g) in water (30 mL) was stirred for 24 h at room temperature. The precipitate was purified by chromatography on Sephadex G-50.

Synthesis of Antigen 17. (a) Phosphonate 16. In the above reaction, (*R*)-(*Z*)-(+)-1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butene (40 mmol, $[\alpha]^{23}_D$ +21.3 (*c* 1.09, MeOH), >98% ee) and triacid chloride (10 mmol) in pyridine (5 mL)–CH_2Cl_2 (50 mL) were combined to afford the corresponding tris(1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butenyl) ester **15**. After the usual workup, the ester was purified by reversed-phase HPLC (Bondapak C_{18}, 5–30% CH_3CN gradient in 0.1 M aqueous triethylammonium bicarbonate buffer, pH 7.4) to give phosphonate **16** in 65% yield: $[\alpha]^{23}_D$ +18.7° (*c* 1.27, MeOH), >98% ee; ^{19}F NMR ($CDCl_3$) δ 1.54 (d, $J_{F,H}$ = 6.4 Hz); ^1H NMR ($CDCl_3$) δ 0.94–2.41 (13 H, m), 4.65 (dq, $J_{H,Hvic}$ = 8.7 Hz), 5.43 (dd, $J_{H,Hcis}$ = 11.4 Hz), 5.88 (dt, $J_{H,Hvic}$ = 7.8 Hz), 7.15–7.66 (ArH), 10.4 (CO_2H); ^{31}P NMR (relative to 85% H_3PO_4) 31.9; high-resolution mass for $C_{18}H_{24}PO_5F_3$, calcd 408.353, found 408.743.

(b) Antigen 17. A mixture of compound **16** (4.08 g, 10 mmol) and bovine serum albumin (BSA) (2 g) in water (30 mL) was stirred for 24 h at room temperature. The precipitate was purified by chromatography on Sephadex G-50.

Synthesis of Antigen 20. (a) Phosphonate 19. In the previous reaction, (*S*)-(*Z*)-(−)-1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butene (40 mmol, $[\alpha]^{23}_D$ −21.0 (*c* 1.05, MeOH), >98% ee) and triacid chloride (10 mmol) in pyridine (5 mL)–CH_2Cl_2 (50 mL) were used to afford the corresponding tris(1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butenyl) ester **15**. After usual workup, the ester was purified by reversed-phase HPLC (Bondapak C_{18}, 5–30% CH_3CN gradient in 0.1 M aqueous triethylammonium bicarbonate buffer, pH 7.4) to give phosphonate 20 in 54% yield: $[\alpha]^{23}_D$ −18.5° (*c* 1.34, MeOH), >98% ee; high-resolution mass for $C_{18}H_{24}PO_5F_3$, calcd 408.353, found 408.006.

(b) Antigen 20. A mixture of compound 19 (4.08 g, 10 mmol) and bovine serum albumin (BSA) (2 g) in water (30 mL) was stirred for 24 h at room temperature. The precipitate was purified by chromatography on Sephadex G-50.

Asymmetric Hydrolysis with Antibody. (a) Synthesis of *R* Enantiomer. A mixture of the antibody (400 μM) made from (+) hapten **4** with 95% ee in a buffer solution (60 mL, pH 7.3) prepared from a $^1/_{15}$ M aqueous Na_2HPO_4 solution (46.1 mL) and $^1/_{15}$ M aqueous KH_2PO_4 solution (13.9 mL) and the phenylacetate derivative of 1,1-difluoro-2-decanol (20 mmol) was stirred at 25–27 °C. After being stirred for 15 h, the mixture was acidified with 1 N HCl and then the oily materials were extracted with diethyl ether. The ethereal extract was dried over anhydrous magnesium sulfate and the solvent removed. After the hydrolysis ratio (49%) was determined by ^{19}F NMR signal intensities with $C_6H_5CF_3$ as an internal standard, the 1,1-difluoro-2-decanol and benzyl ester were separated by column chromatography on silica gel by use of a mixture of *n*-hexane–diethyl ether (5:1). Final purification by distillation gave (*R*)-(+)-1,1-difluoro-2-decanol in 99% ee; bp 110–112 °C (16 mmHg); $[\alpha]^{21}_D$ +7.47 (*c* 1.15, $CHCl_3$), 99% ee; ^{19}F NMR ($CDCl_3$) δ 47.2 (ddd, $J_{Fa,Fb}$ = 275, $J_{F,H}$ = 54.6, $J_{F,H}$ = 11.1 Hz), 52.2 (ddd, $J_{F,H}$ = 54.5, $J_{F,H}$ = 11 Hz); ^1H NMR ($CDCl_3$) δ 0.70–1.00 (3 H, t, $J_{H,H}$ = 7.5 Hz), 1.10–1.70 (14 H, m), 2.07 (OH), 3.40–3.90 (1 H, m), 5.50 (CHF, dt, $J_{H,H}$ = 4.5 Hz); IR (cm^{-1}) 3375 (OH); high-resolution mass for $C_{10}H_{20}OF_2$, calcd 194.265, found 194.144.

(b) Synthesis of *S* Enantiomer. A suspension of cellulase (*Trichoderma viride*, Yakult Pharmaceutical Industry Co. Ltd., 2 g) in buffer solution (60 mL, pH 7.3) was stirred for 15 min at 40–41 °C in the "CULSTIR" flask (200 mL). Into the mixture was added the recovered (*S*)-phenylacetate derivative of 1,1-difluoro-2-decanol, and then the whole mixture was stirred at 40–41 °C. After being stirred for 5 h, the mixture was acidified with 1 N HCl and then the oily materials were extracted with diethyl ether. The ethereal extract was dried over anhydrous magnesium sulfate, and then the solvent was removed. The distillation gave the *S* enantiomer in a 48% yield: $[\alpha]^{21}_D$ −7.41 (*c* 1.07, $CHCl_3$), >98% ee.

(c) A mixture of the antibody (400 μM) made from (−) hapten **4** with 96% ee and the phenylacetate derivative of 1,1-difluoro-2-decanol (20 mmol) in buffer solution (60 mL) was used and worked up as usual. After the hydrolysis ratio (48.5 %) was determined by ^{19}F NMR signal intensities with $C_6H_5CF_3$ as an internal standard, the products were worked up as usual. Final purification was distillation, giving (*S*)-(−)-1,1-difluoro-2-decanol with >98% ee in a 46% yield ($[\alpha]^{21}_D$ −7.50 (*c* 1.11, $CHCl_3$). The *R* enantiomer with >98% ee was obtained from the recovered benzylate in a 48% yield ($[\alpha]^{21}_D$ +7.48 (*c* 1.14, $CHCl_3$)).

(d) A mixture of the antibody (400 μM) made from (+) hapten **8** with >98% ee and the benzylate derivative of 1,1,1-trifluoro-2-decanol (20 mmol) in buffer solution (60 mL) was used and worked up as usual. After the hydrolysis ratio (48.5%) was determined by ^{19}F NMR signal intensities with $C_6H_5CF_3$ as an internal standard, the products were worked up as usual. Final purification was distillation, giving (*R*)-(+)-1,1,1-trifluoro-2-decanol with 99% ee in a 46% yield ($[\alpha]^{21}_D$ +24.4 (*c* 1.24, MeOH)). The *S* enantiomer with 98% ee was obtained from the recovered benzylate in a 45% yield ($[\alpha]^{21}_D$ −24.1 (*c* 1.27, MeOH)).

(e) A mixture of the antibody (400 μM) made from (+) hapten **11** with >98% ee and the benzylate derivative of 1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butene (20 mmol) in buffer solution (60 mL) was used and worked up as usual. After the hydrolysis ratio (23.5%) was determined by ^{19}F NMR signal intensities with $C_6H_5CF_3$ as an internal standard, the products were worked up as usual. Final purification was distillation, giving (*R*)-(*E*)-(+)-1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butene with 99% ee in a 21% yield ($[\alpha]^{21}_D$ +2.54 (*c* 1.09, MeOH)).

(f) A mixture of the antibody (400 μM) made from (−) hapten **14** with >98% ee and the benzylate derivative of 1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butene (20 mmol) in buffer solution (60 mL) was used and worked up as usual. After the hydrolysis ratio (24%) was determined by ^{19}F NMR signal intensities with $C_6H_5CF_3$ as an internal standard, the products were worked up as usual. Final purification was distillation, giving (*S*)-(*E*)-(−)-1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butene with 98.5% ee in a 22% yield ($[\alpha]^{21}_D$ −2.57 (*c* 1.17, MeOH)).

(g) A mixture of the antibody (400 μM) made from (+) hapten **16** with >98% ee and the benzylate derivative of 1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butene (20 mmol) in buffer solution (60 mL) was used and worked up as usual. After the hydrolysis ratio (23%) was determined by ^{19}F NMR signal intensities with $C_6H_5CF_3$ as an internal standard, the products were worked up as usual. Final purification was distillation, giving (*R*)-(*Z*)-(+)-1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butene with 98.5% ee in a 20% yield ($[\alpha]^{21}_D$ +21.3 (*c* 1.15, MeOH)).

(h) A mixture of the antibody (400 μM) made from (−) hapten **20** with >98% ee and the benzylate derivative of 1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butene (20 mmol) in buffer solution (60 mL) was used and worked up as usual. After the hydrolysis ratio (23.5%) was determined by ^{19}F NMR signal intensities with $C_6H_5CF_3$ as an internal standard, the products were worked up as usual. Final purification was distillation, giving (*S*)-(*Z*)-(−)-1-hexyl-3-hydroxy-4,4,4-trifluoro-1-butene with >98% ee in a 19% yield ($[\alpha]^{21}_D$ −20.9 (*c* 1.03, MeOH)).