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Efficient synthesis of (*R*)- and (*S*)-1-amino-2,2-difluorocyclopropanecarboxylic acid via lipase-catalyzed desymmetrization of prochiral precursors

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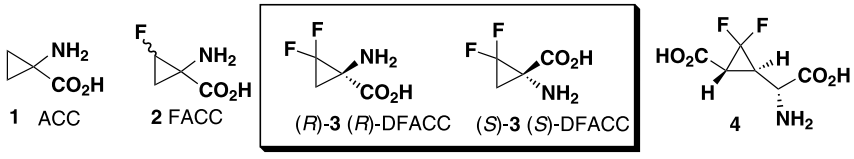
Abstract—The asymmetric syntheses of (+)-(*R*)-1-amino-2,2-difluorocyclopropane-1-carboxylic acid and its enantiomer have been accomplished. Key reactions in the synthetic design are lipase-catalyzed desymmetrization of a prochiral diol and a prochiral diacetate.

1. Introduction

1-Aminocyclopropane-1-carboxylic acid (ACC) **1** present in many plants, has several interesting biological activities.[1] For example, **1** is an intermediate in the biosynthesis of the ripening hormone ethylene,[2] a component of bacterial phytotoxines,[3] and an intermediate in the biosynthesis of azetidine-2-carboxylic acid.[4] Furthermore, **1** acts as a potent glycine agonist on the *N*-methyl-D-aspartate (NMDA) receptor ion channel.[5] Several peptides containing **1** as an amino acid residue also show diverse biological activities.[6]

In medicinal chemistry, the replacement of a carbon–hydrogen bond in a biologically important compound with a carbon–fluorine bond often has dramatic effects on biological activity.[7] The fluorinated analogues of ACC are attractive synthetic targets for a number of reasons. For example, such analogues are β-fluoroamino acids, a class of compounds known to be irreversible inhibitors of PLP-linked enzymes (such as is ethylene synthetase). In addition, the presence of fluorine would introduce chirality not present in the symmetrical parent compound. Examination of such analogues with respect to their activities at the NMDA receptor would provide another application. For the latter purpose, racemic mono-fluorinated ACC [1-amino-2-fluorocyclopropane-1-carboxylic acid (FACC)] **2** has been synthesized previously and found to have potencies comparable to the parent ACC at the NMDA receptor.[8]



Scheme 1.

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Based on the same principles, *gem*-difluorinated ACC (DFACC) **3** also represents an interesting synthetic target. The *gem*-difluorocyclopropane moiety previously has been introduced into the structures of several novel amino acids analogues that have been shown to possess important biological activities. For example, Taguchi et al. reported that one of the stereoisomers of 2-(2-carboxy-3,3-difluorocyclopropyl)glycines **4** is a potent agonist for metabotropic glutamate receptors.[9] In addition to the potential introduction of interesting biological properties, the presence of the *gem*-difluorocyclopropane moiety in a molecule can be expected to impart altered chemical and physical properties. Ito et al. synthesized a series of optically active *gem*-difluorocyclopropane compounds using lipase-catalyzed reactions and reported that they have interesting biological and physical properties.[10]

In a previous communication,[11] we reported the synthesis of (+)-(*R*)-1-amino-2,2-difluorocyclopropane-1-carboxylic acid (+)-(*R*)-**3**. Herein we describe the full details of this work and also report the synthesis of the (−)-(*S*)-**3** (Scheme 1).

2. Synthetic strategy

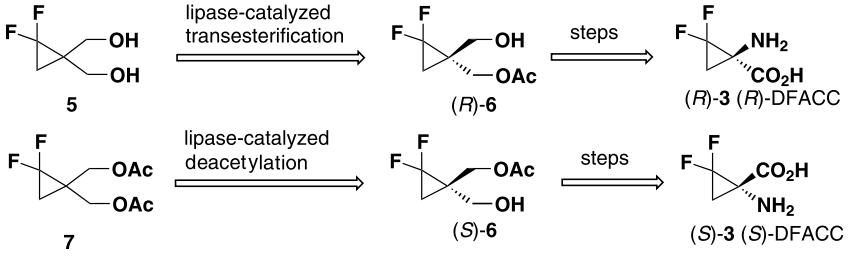
The desymmetrization of prochiral alcohols or acetates through lipase-catalyzed reactions is now a widely used methodology to obtain optically active compounds.[12] An important advantage is that the lipase-catalyzed reaction proceeds under very mild conditions that tolerate many functional groups. On the basis of these considerations, we based our synthesis of (+)-(*R*)-**3** and (−)-(*S*)-**3** on lipase-catalyzed reactions as depicted in Scheme 2. Chiral monoacetates (*R*)-**6** and (*S*)-**6**, products of the lipase-catalyzed asymmetric reactions of the prochiral diol **5** or the prochiral diacetate **7**, were the key intermediates in the synthetic design of (+)-(*R*)-**3** and (−)-(*S*)-**3**. According to the empirical rule, it was expected that the lipase-catalyzed asymmetric transesterification of **5** would give the (*R*)-monoacetate (*R*)-**6**, and the lipase-catalyzed asymmetric deacetylation of **7** would afford the (*S*)-monoacetate (*S*)-**6**.[13] We assumed that, with (*R*)-**6** and (*S*)-**6** in hand, subsequent functional groups transformations would provide ready access to the title compounds.

3. Results and discussion

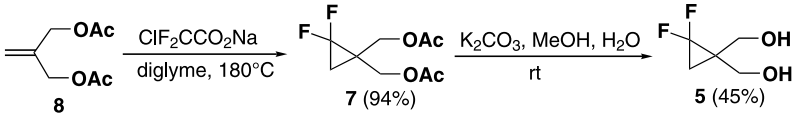
3.1. Synthesis of (*R*)-3 and determination of the absolute configuration

Addition of difluorocarbene, derived from sodium chlorodifluoroacetate in diglyme at 180°C,[14] to alkene **8** afforded the key prochiral diacetate **7** containing the *gem*-difluorocyclopropane moiety. Alkaline hydrolysis of **7** provided the pro-chiral diol **5** (Scheme 3).

We first examined the lipase-catalyzed transesterification of pro-chiral diol **5** with vinyl acetate as the acyl donor. Three commercially available lipases [porcine pancreatic lipase (PPL), Novozym 435 from *Candida antarctica*, and Amano PS from *Pseudomonas cepacia*] were examined with respect to their abilitites to selectively esterify **5** in organic solvents. Since the hydrophobicity of organic solvents can influence the enantioselectivity of lipase-catalyzed reaction,[15] a variety of organic solvents were examined. These results are shown in Table 1. The PPL-catalyzed reaction proceeded very slowly and gave the monoacetate with poor enantioselectivity (runs 1–5). Although Novozym 435 gave a rapid reaction, the enantiomeric excesses were unsatisfactory (runs 6 and 7). Excellent enantioselectivity (91.3% ee) with a high chemical yield was obtained when **5** was treated with lipase PS in benzene: di-*i*-propyl ether = 20:1 (run 9). The selectivity was improved further with an increase in the hydrophobicity of the solvent (runs 8 and 9). Although the absolute configuration of the monoacetate was not determined at this stage, we assumed that it had the (*R*)-configuration according to the empirical rule developed by Kazlauskas.[13] Using reaction conditions similar to run 9, we carried out acetylation of **5** on a preparative scale (1.2 g, 8.7 mmol) and obtained (*R*)-**6** with 91% ee in 94% isolated yield.

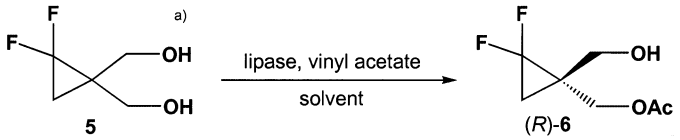


Scheme 2.



Scheme 3.

Table 1. Lipase-catalyzed acetylation of the diol **5**



Run	Lipase[b] (mg)	Solvent (ml)	Vinyl acetate (equiv.)	Reaction time (h)	Ee (%)[c]	Yield (%)	
						(*R*)-**6**	Diacetate
1	PPL (10)	*i*-Pr_2O (2)	1	145	–	22.2	0.6
2	PPL (100)	PhH (2), *i*-Pr_2O (0.1)	1	146	23.5	91.0	6.1
3	PPL (100)	THF (2)	10	96	–	62.2	4.1
4	PPL (100)	AcOEt (2)	10	48	35.5	76.0	5.9
5	PPL (100)	CH_3CN (2)	10	96	–	68.1	8.3
6	Novozym435 (10)	PhH (1), *i*-Pr_2O (1)	1	1	45.8	90.8	2.6
7	Novozym435 (10)	*i*-Pr_2O (2)	1.5	1	37.5	89.6	10.1
8	PS (10)	PhH (1), *i*-Pr_2O (1)	10	1.5	88.6	95.1	1.7
9	**PS (10)**	**PhH (2), *i*-Pr_2O (0.1)**	**10**	**1.5**	**91.3**	**96.5**	**1.2**
10	PS (10)	CH_3CN (2)	10	0.5	–	73.9	3.7

[a] 10 mg of **5** was used.
[b] PPL: porcine pancreatic lipase (Amano), Novozym 435:*Candida antarctica* (Novo), PS: *Pseudomonas cepacia* (Amano).
[c] Determined by HPLC analysis (DAICEL CHIRALCEL OB-H, hexane/Pr*i*OH = 20/1) of the corresponding benzoyl ester of **6**.

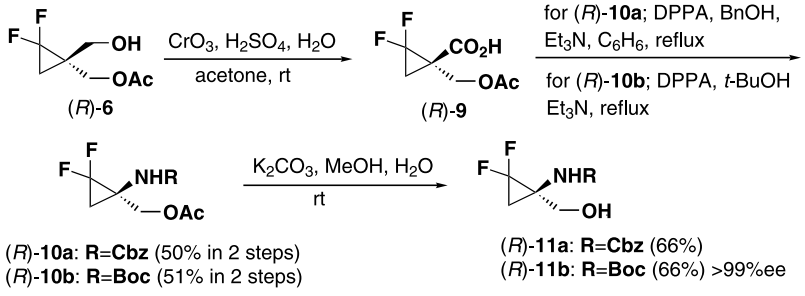
With the chiral mono-alcohol (*R*)-**6** in hand, we proceeded with the reaction sequence designed to produce the target amino acid (*R*)-**3**. First, the hydroxyl group of (*R*)-**6** was oxidized to the carboxylic acid (*R*)-**9** with Jones reagent, and (*R*)-**9** was converted to carbamates (*R*)-**10** using Shioiri's method [treatment of (*R*)-**9** with diphenylphosphoryl azide (DPPA), benzyl alcohol or *t*-butanol, and triethylamine].[16] Removal of the acetyl group of (*R*)-**10** afforded the *N*-protected aminoalcohols (*R*)-**11**. Both (*R*)-**10** and (*R*)-**11** were purified by crystallization. By this procedure we prepared (*R*)-**11b** in over 99% ee (Scheme 4).

At this stage, we prepared ester **12** from (*R*)-**11a** and (*S*)-(+)-*O*-acetylmandelic acid in order to determine the absolute configuration. The X-ray crystallographic analysis of **12** showed that **11a**, as predicted has the (*R*)-configuration. The ORTEP plot of **12** is shown in Scheme 5.

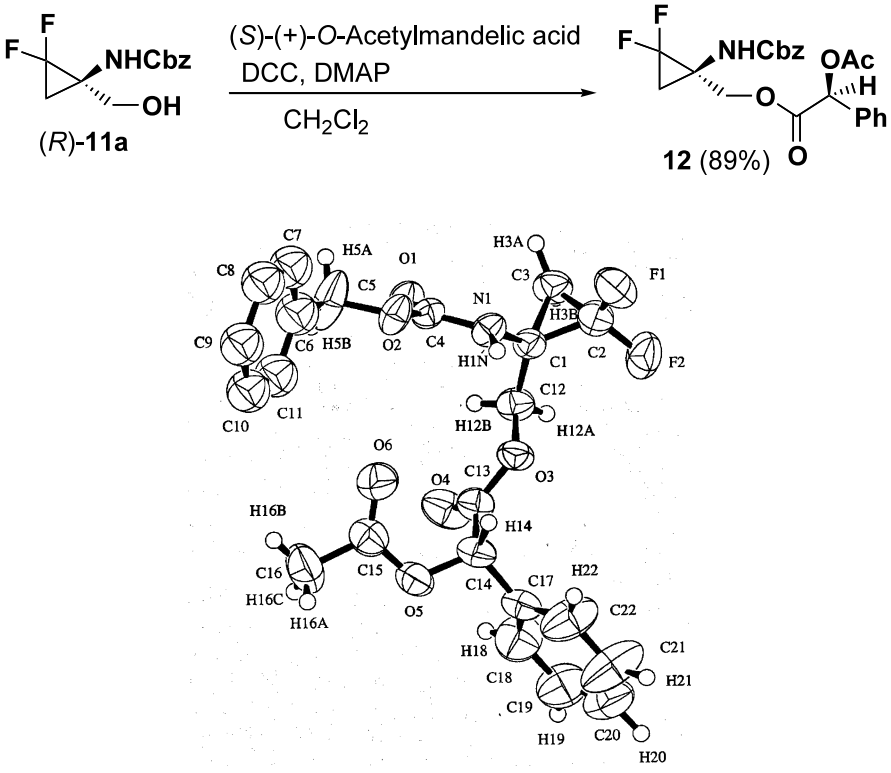
N-Protected aminoalcohols (*R*)-**11** were oxidized with Jones' reagent into *N*-protected DFACC (*R*)-**13**. In the case of *N*-CbzDFACC (*R*)-**13a**, all attempts (H_2–Pd/C–AcOEt, TMSI–CH_3CN or aq. KOH–MeOH) to remove the Cbz group from (*R*)-**13a** were unsuccessful. The structure of (*R*)-**13a** was confirmed by the conversion of (*R*)-**13a** into the methyl ester (*R*)-**14**. Fortunately, acid hydrolysis of *N*-BocDFACC (*R*)-**13b** afforded the desired (+)-(*R*)-DFACC as a hydrochloride (*R*)-**3**·HCl (Scheme 6).

3.2. Synthesis of (*S*)-3

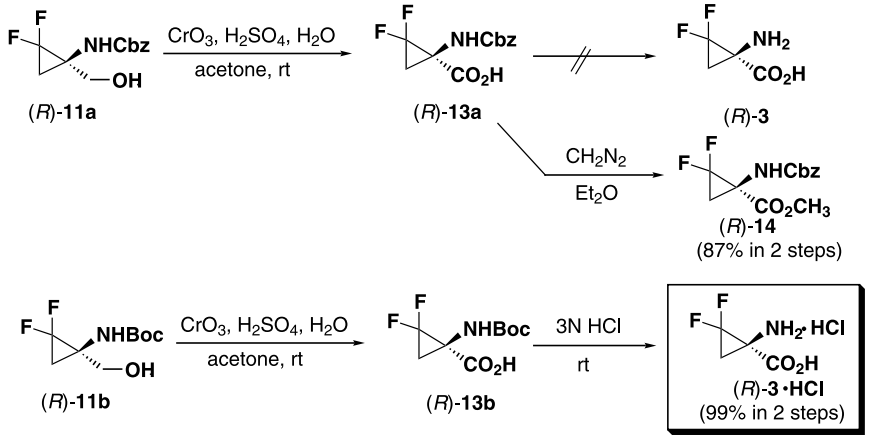
We next examined the lipase-catalyzed deacetylation of the prochiral diacetate **7**. The results are summarized in Table 2. We first tried enantioselective deacetylation of **7** using Amano PS in organic solvents (runs 1–6). However, the reactions were very slow in all the trials. We next investigated the hydrolysis of **7** using several types of lipase in the presence of organic solvents (runs 7–10). Although PS-catalyzed hydrolysis proceeded smoothly, a considerable amount of diol was obtained (run 7). Two kinds of PPL-catalyzed hydrolyses also gave no satisfactory results (runs 8 and 9). We finally



Scheme 4.



Scheme 5. ORTEP diagram of **12** with 50% thermal ellipsoids. The minor disordered contribution of phenyl group of benzyloxycarbonyl group is omitted for clarity.



Scheme 6.

achieved highly enantioselective and smooth hydrolysis of **7** by using Amano PS in a mixed solvent consisting of acetone and phosphate buffer (run 10). We then carried out preparative scale (1.17 g, 5.3 mmol) hydrolysis of **7** under reaction conditions similar to run 10 and obtained (*S*)-**6** with 93.5% ee in 68% isolated yield.

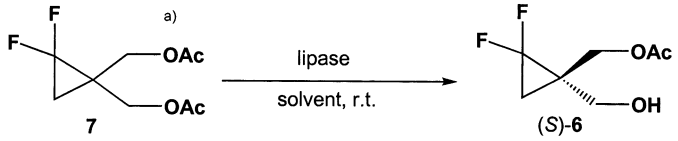
Recently, Ito et al. reported the synthesis of the chiral monoacetate of 1,1-difluoro-2,3-(bishydroxymethyl)-cyclopropane through the lipase-catalyzed hydrolysis of the diacetate, along with synthetic applications of the product.[10] Their results along with our own demonstrate that such diacetates containing the *gem*-difluoromethylcyclopropane moiety are good substrates for lipase-catalyzed reactions.

The (*S*)-monoacetate (*S*)-**6** was converted into the desired (*S*)-DFACC hydrochloride (*S*)-**3**·HCl using the same sequence of reactions that was used for the synthesis of (*R*)-DFACC hydrochloride (*R*)-**3**·HCl (Scheme 7).

4. Conclusion

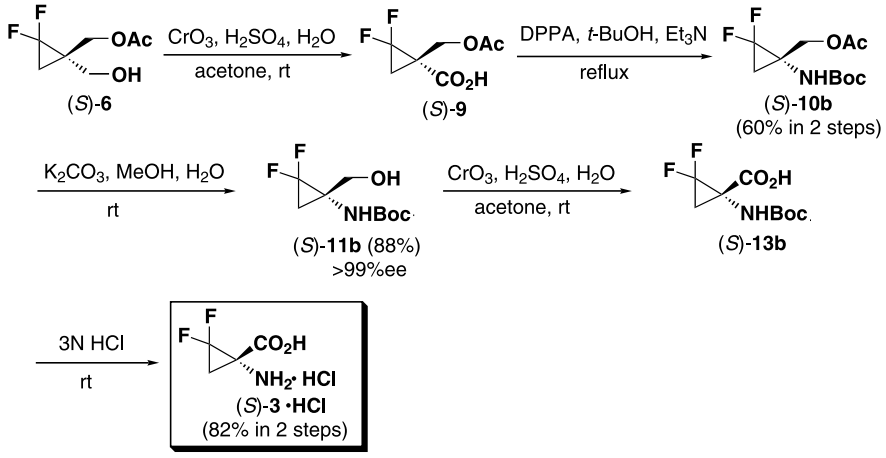
We have synthesized the enantiomers of 1-amino-2,2-

Table 2. Lipase-catalyzed deacetylation of the diacetate **7**



Run	Lipase[b] (mg)	Solvent (ml)	Acyl acceptor (ml)	Reaction time (h)	Ee (%)[c]	Yield (%)	
						(*S*)-**6**	Diol
1	PS (10)	Benzene (2)	*n*-BuOH (0.002)	3	–	0	0
2	PS (10)	Benzene (2)	H_2O (0.004)	42	–	3.1	0.3
3	PS[d] (50)	Benzene (2)	H_2O (0.004)	6	–	1.24	0.01
4	PS[d] (50)	*i*-Pr_2O (2)	H_2O (0.0012)	16	–	8.6	0.1
5	PS[d] (50)	THF (2)	H_2O (0.0012)	1.5	–	0.4	0
6	PS (10)	Benzene (2)	MeOH (0.2)	3.5	–	0	0
7	PS (10)	*i*-Pr_2O (1)	H_2O (1)	6	–	81.0	14.4
8	PPL (Amano)(50)	*i*-Pr_2O (1)	H_2O (1)	2	–	0.6	0
9	PPL (Sigma)(50)	Acetone (1)	H_2O[e] (2)	47	–	45.7	1.4
10	**PS (50)**	**Acetone (0.7)**	**H_2O[e] (1.4)**	**9.5**	**91.7**	**86.2**	**3.4**

[a] 10 mg of diacetate was used.
[b] PS: *Pseudomonas cepacia* (Amano), PPL: porcine pancreatic lipase (Amano or Sigma).
[c] Determined by HPLC analysis (DAICEL CHIRALCEL OB-H, hexane/PriOH = 20/1) of the corresponding benzoyl ester of **6**.
[d] Immobilized lipase was used. This lipase preparation contains 5% of lipase PS and 95% of Celite.
[e] 0.07 M phosphate buffer (pH 7) was used.



Scheme 7.

difluorocyclopropane-1-carboxylic acid [DFACC] (**3**). The (*R*)-isomer [(*R*)-DFACC] (*R*)-**3** was obtained from the (*R*)-monoacetate (*R*)-**6**, prepared by lipase-catalyzed transesterification of the prochiral diol **5**. The (*S*)-isomer [(*S*)-DFACC] (*S*)-**3** was obtained from the (*S*)-monoacetate (*S*)-**6**, available from the lipase-catalyzed deacetylation of the prochiral diacetate **7**. Results of the biological evaluation of (*R*)-DFACC (*R*)-**3** and (S)-DFACC (*S*)-**3**, currently underway, will be reported elsewhere.

5. Experimental

5.1. General procedures

Amano PPL and Amano PS were supplied by Amano Enzyme, Inc. Novozym 435 and Sigma PPL were supplied by Novo Nordisk and Sigma, respectively. The infrared spectra (IR) were measured using a Jasco IR-8300 FT-IR spectrophotometer or a Perkin–Elmer 1600 series FT-IR spectrophotometer. The ^1H, ^{19}F and ^{13}C NMR spectra were obtained using a JEOL GX270, JEOL JNM-400, Varian Gemini 300 or Varian UNITY plus 500 instrument with tetramethylsilane (for ^1H) and chlorotrifluoromethane (for ^{19}F) as the internal standards. The mass spectra (MS) and high-resolution mass spectra (HR-MS) were measured with a JEOL JMS D-200 spectrometer using electron ionization (EI) method. The fast atom bombardment mass spectra (FAB-MS) were measured with a JEOL JMS AX-500 using *m*-nitrobenzylalcohol as a matrix. Melting points were measured with a Yanaco MP-S3 melting-point apparatus. Optical rotations were measured with a

Perkin–Elmer 241 polarimeter. Gas chromatograms were recorded on a Shimadzu GC-14B with an OV 101 bonded capillary column (Gasukuro Industry), 17 m×0.25 mm (90°C), or a CP Cyclodextrin-β-236-M-19 capillary column (Chrompack), 25 m×0.25 mm (120°C). HPLC analyses were carried out on a Hitachi L-6250 intelligent pump with a Hitachi L-4000 UV detector using a chiral column CHIRALCEL OB-H (Daicel), 250 mm×4.6 mm (flow 0.45 mL/min; solvent *n*-hexane:*i*-propanol = 20:1). Column chromatography was performed on silica gel (Wakogel C300 or Merck Kieselgel 60). Jones reagent was prepared as follows; Chromium trioxide (26.7 g) was resolved in diluted sulfuric acid [sulfuric acid (23 mL) and water (40 mL)] and the resulting mixture was diluted with water to 100 mL.

5.2. [1-(Acetoxymethyl)-2,2-difluorocyclopropyl]methyl acetate 7

To a solution of 2-(acetoxymethyl)prop-2-enyl acetate **8** (2.00 g, 11.6 mmol) in diglyme (5.0 mL) was added a solution of sodium chlorodifluoroacetate (10.6 g, 69.8 mmol) in diglyme (20 mL) at 180°C under an inert atmosphere. The reaction mixture was stirred under the same conditions for 30 min. The mixture then was poured onto ice-water and extracted with *n*-hexane (50 mL×3). The extract was dried over anhydrous magnesium sulfate and the solvent was evaporated. The residue was purified using column chromatography (*n*-hexane:ethyl acetate = 5:1) to afford the pure **7** (2.43 g, 94%) as a colorless oil. IR (neat) cm^{-1}: 1742, 1654, 1636, 1560, 1542, 1508, 1475, 1375, 1227, 1035; ^1H NMR ($CDCl_3$) δ: 1.44 (2H, t, J = 8.8 Hz), 2.07 (6H, s), 4.08 (2H, d, J = 12.5 Hz), 4.28 (2H, dt, J = 12.0, 2.2 Hz); ^{19}F NMR ($CDCl_3$) δ: −137.94 (2F, t, J_{FH} = 8.8 Hz); FAB-MS (m/z) 223 (M^++H), 202 (M^+−HF); FAB-HRMS calcd for $C_9H_{13}F_2O_4$ (M^++H), 223.0782; found 223.0785.

5.3. [2,2-Difluoro-1-(hydroxymethyl)cyclopropyl]methan-1-ol 5

To a solution of **7** (2.40 g, 10.9 mmol) in water (20 mL) and methanol (20 mL) was added potassium carbonate (1.47 g, 10.9 mmol) at 0°C and the reaction mixture was stirred for 1 h under the same condition. The methanol then was removed by rotary evaporation and the remaining aqueous solution was extracted with ethyl acetate (50 mL×3). The organic extract was washed with brine, dried over anhydrous magnesium sulfate and evaporated. The residue was recrystallized from *n*-hexane/ether to give **5** (678 mg, 45%) as colorless needles. Mp 54°C (hexane/ether); IR (neat) cm^{-1}: 3313, 2960, 2363, 1463, 1398, 1367, 1306, 1270, 1238, 1187, 1148, 1023, 970, 961, 945, 904, 744; ^1H NMR ($CDCl_3$) δ: 1.31 (2H, t, J = 8.9 Hz), 2.15 (2H, br), 3.85 (4H, brt); ^{19}F NMR ($CDCl_3$) δ: −138.14 (2F, t, J_{FH} = 8.9 Hz); FAB-MS (m/z) 139 (M^++H); FAB-HRMS calcd for $C_5H_9F_2O_4$ (M^++H), 139.0571; found 139.0547.

5.4. Lipase-catalyzed transesterification of 5

In a typical experiment (run 9), Amano PS (10 mg) was placed in a vial to which was added **5** (10 mg), vinyl acetate (10 equiv.), benzene (2 mL) and diisopropyl ether (0.1 mL). The resulting suspension was then magnetically stirred at 35°C and the reaction course was followed by GC analysis (OV 101). The reaction was quenched by filtration and the filtrate was concentrated under reduced pressure. The residue was chromatographed on a silica gel column using hexane-ethyl acetate (1:1) as an eluent to give (*R*)-**6** as colorless oil.

We also conducted transesterification of **5** on a preparative scale. Using the same procedure as for the small scale transesterification, **5** (1.20 g, 8.70 mmol) was converted into (*R*)-**6** (1.47 g, 94%) with 91% ee.

5.5. (*R*)-(−)-[2,2-Difluoro-1-(hydroxymethyl)cyclopropyl]methyl acetate (*R*)-6

$[\alpha]_D^{29.5}$ −3.2 (*c* 1.38, $CHCl_3$); IR (neat) cm^{-1}: 3346, 1734, 1700, 1476, 1378, 1309, 1245, 1198, 1035, 905, 668; ^1H NMR ($CDCl_3$) δ: 1.32 ~ 1.38 (2H, m), 2.08 (3H, s), 2.43 (1H, br), 3.65 (2H, t-like), 4.20 (1H, dd, J = 12.2, 2.0 Hz), 4.31 (1H, dt, J = 12.2, 2.0 Hz); ^{19}F NMR ($CDCl_3$) δ: −136.56 ~ −137.28 (1F, d-like), −139.14 ~ −139.41 (1F, d-like); ^{13}C NMR ($CDCl_3$) δ: 18.87 (t, J_{CF} = 11.78 Hz), 20.69 (s), 31.48 (t, J_{CF} = 11.25 Hz), 60.14 (d, J_{CF} = 7.43 Hz), 61.43 (d, J_{CF} = 7.43 Hz),114.30 (t, J_{CF} = 316.58 Hz), 171.56 (s); FAB-MS (m/z) 181 (M^++H); FAB-HRMS calcd for $C_7H_{11}F_2O_3$ (M^++H), 181.0676; found 181.0680.

To determine the ee of (*R*)-**6** by HPLC, a sample of (*R*)-**6** was benzoylated: several drops of benzoyl chloride were added to 1 mL of a dry pyridine solution cooled to 0°C containing one drop of (*R*)-**6**. The mixture was stirred overnight at room temperature, was then poured into cold 6 M HCl, and the resulting mixture was extracted with ether. The organic layer was washed successively with water, a saturated solution of sodium hydrogen carbonate, and brine, then dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was chromatographed on a silica gel column using hexane–ethyl acetate (5:1) as an eluent to give benzoylated (*R*)-**6**. An aliquot of the combined fractions containing (*R*)-**6** was analyzed by HPLC (CHIRALCEL OB-H) to determine the ee of (*R*)-**6**.

5.6. (*R*)-(+)-{2,2-Difluoro-1-[(phenylmethoxy)carbonylamino]cyclopropyl}methyl acetate (*R*)-10a

To a solution of (*R*)-**6** (1.00 g, 5.56 mmol) in acetone (13.9 mL) was added Jones reagent (5.5 mL) at room temperature and the reaction mixture was stirred for 2 h. *i*-Propanol (5 mL) was added and the solution was diluted with ether (60 mL). The resulting mixture was filtered through Celite®. The filtrate was dried over anhydrous magnesium sulfate and evaporated to afford the crude carboxylic acid [(*R*)-**9**] which was dissolved in benzene (21.5 mL). Triethylamine (0.775 mL, 5.56

mmol) and diphenylphosphoryl azide (1.19 mL, 5.56 mmol) were added to the benzene solution of crude (*R*)-**9** and the resulting mixture was heated under reflux for 30 min under a nitrogen atmosphere. Benzyl alcohol (5.0 mL) was added to the solution and the reaction mixture was heated under reflux for 24 h under a nitrogen atmosphere. Then, 5% aqueous citric acid was added and the resulting mixture was extracted with benzene (30 mL×3). The extract was dried over anhydrous magnesium sulfate and evaporated to afford crude (*R*)-**10a**. The crude product was purified by recrystallization (*n*-hexane/ethyl acetate) to give pure (*R*)-**10a** (642 mg, 50%) as colorless crystals.

Mp 90∼93°C (*n*-hexane/ethyl acetate); $[\alpha]_D^{29}$ +30.8 (*c* 0.50, $CHCl_3$); IR (neat) cm^{-1}: 3332, 3019, 1741, 1706, 1529, 1457, 1367, 1250, 1041, 1028; ^1H NMR ($CDCl_3$) δ: 1.75 (2H, br), 2.07 (3H, s), 4.07 (1H, dd, $J=12.4$, 2.2 Hz), 4.54 (1H, d, $J=10.3$ Hz), 5.12 (2H, br), 5.28 (1H, br), 7.36 (5H, br); ^{19}F NMR ($CDCl_3$) δ: −139.85 (2F, q-like); FAB-MS (*m*/*z*) 300 (M^++H); FAB-HRMS calcd for $C_{14}H_{16}F_2NO_4$ (M^++H), 300.1047; found 300.1050.

5.7. (*R*)-(+)-{2,2-Difluoro-1-[(*t*-butoxy)carbonylamino]-cyclopropyl}methyl acetate (*R*)-10b

To a solution of (*R*)-**6** (1.00 g, 5.56 mmol) in acetone (7.0 mL) was added Jones reagent (2.8 mL) at room temperature and the mixture was stirred for 2 h. *i*-Propanol (2 mL) was added and the solution was diluted with ether (60 mL). The resulting mixture was filtered through Celite®. The filtrate was dried over anhydrous magnesium sulfate and evaporated to afford the crude carboxylic acid [(*R*)-**9**] which was dissolved in *t*-butanol (15.0 mL). Triethylamine (0.390 mL, 2.80 mmol) and diphenylphosphoryl azide (765 mg, 2.80 mmol) were added to the solution of crude (*R*)-**9** and the resulting mixture was heated under reflux for 24 h under a nitrogen atmosphere. The solvent was evaporated and the crude product was purified by recrystallization (*n*-hexane) to give pure (*R*)-**10b** (375 mg, 51%) as colorless crystals. Mp 67°C (*n*-hexane); $[\alpha]_D^{27}$ +29.75° (*c* 0.97, $CHCl_3$); IR (neat) cm^{-1}: 3336, 1741, 1690, 1513, 1457, 1254, 1219, 1040; ^1H NMR ($CDCl_3$) δ: 1.46 (9H, s), 1.68–1.69 (2H, m), 2.10 (3H, s), 4.60 (2H, br) 5.40 (1H, br); ^{19}F NMR ($CDCl_3$) δ: −138.82∼−140.90 (2F, m); MS (*m*/*z*) 266 (M^++H); HRMS calcd for $C_{11}H_{18}F_2NO_4$ (M^++H), 266.1240; found 266.1163.

5.8. Benzyl (*R*)-(+)-*N*-[2,2-difluoro-1-(hydroxymethyl)-cyclopropyl]carbamate (*R*)-11a

To a stirred solution of **10a** (299 mg, 1.00 mmol) in methanol (5 mL) was added 0.20 M aqueous solution of potassium carbonate (5.0 mL, 1.0 mmol) and the resulting mixture was stirred at room temperature for 30 min. The reaction mixture was extracted with chloroform (30 mL×3) and the combined extract was dried over anhydrous magnesium sulfate. The solvent was evaporated and the crude product was purified by recrystallization (*n*-hexane/ethyl acetate) to afford pure (*R*)-**11a** (149 mg, 66%) as colorless crystals.

Mp 68∼71°C (*n*-hexane/ethyl acetate); $[\alpha]_D^{28}$ +33.7 (*c* 0.26, $CHCl_3$); IR (neat) cm^{-1}: 3323, 1695, 1526, 1476, 1282, 1253; ^1H NMR ($CDCl_3$) δ: 1.62∼1.82 (2H, br), 2.95 (1H, br), 3.82 (2H, br), 5.14 (2H, s), 5.43 (1H, br), 7.36 (5H, br); ^{19}F NMR ($CDCl_3$) δ: −139.16 (2F, t, $J=10.0$ Hz); MS (*m*/*z*) 257 (M^+); HRMS calcd for $C_{12}H_{13}F_2NO_3$ (M^+), 257.0863; found 257.0818.

5.9. *t*-Butyl (*R*)-(+)-*N*-[2,2-difluoro-1-(hydroxymethyl)-cyclopropyl]carbamate (*R*)-11b

To a stirred solution of **10b** (300 mg, 1.13 mmol) in methanol (5 mL) was added a 0.20 M aqueous solution of potassium carbonate (5.65 mL, 1.13 mmol) and the resulting mixture was stirred at room temperature for 30 min. The reaction mixture was extracted with chloroform (30 mL×3) and the combined extract was dried over anhydrous magnesium sulfate. The solvent then was evaporated to afford crude (*R*)-**11b**. The crude product was purified by recrystallization (*n*-hexane) to afford pure (*R*)-**11b** (165 mg, 66%) as colorless crystals. According to the GC analysis (CP Cyclodextrin-β-236-M-19) of this compound, the enantiomeric excess was over 99% ee. Mp 89∼90°C (*n*-hexane); $[\alpha]_D^{29}$ +33.2 (*c* 0.83, $CHCl_3$); IR (neat) cm^{-1}: 3366, 1691, 1676, 1521, 1283, 1021; ^1H NMR ($CDCl_3$) δ: 1.46 (9H, s), 1.70∼1.81 (2H, m), 3.35 (1H, br), 3.73∼3.81 (2H, m), 5.21 (1H, br); ^{19}F NMR ($CDCl_3$) δ: −138.99 (2F, t-like); MS (*m*/*z*) 223 (M^+), 123 (M^++H−*t*-BuOCO); HRMS calcd for $C_4H_7F_2NO$ (M^++H−*t*-BuOCO): 123.0496; found 123.0461.

5.10. (+)-{2,2-Difluoro-1-(*R*)-[(2-phenylacetyloxy)amino]-cyclopropyl}methyl-2-(*S*)-acetyloxy-2-phenylacetate 12

To a stirred solution of (*R*)-**11a** (20.0 mg, 0.08 mmol) in dichloromethane (2 mL) were added dicyclohexylcarbodiimide (19.0 mg), *N*,*N*-dimethylaminopyridine (1.0 mg. 0.01 mmol), and (*S*)-*O*-acetylmandelic acid (18.0 mg, 0.093 mmol). The resulting mixture was stirred at room temperature for 1 h, and then diluted with ethyl acetate (10 mL). The mixture was filtered through Celite® and the filtrate was washed with water. The organic layer was dried over anhydrous magnesium sulfate, the solvent was evaporated, and the crude product was purified by recrystallization (*n*-hexane/ethyl acetate) to afford pure **12** (30 mg, 89%) as colorless crystals. Mp 99∼101°C (*n*-hexane/ethyl acetate); $[\alpha]_D^{28}$ +81.7 (*c* 0.09, $CHCl_3$); IR (neat) cm^{-1}: 3332, 3019, 1741, 1706, 1529, 1457, 1367, 1250, 1041, 1028; ^1H NMR ($CDCl_3$) δ: 1.68–1.71 (2H, m), 2.14 (3H, s), 4.09 (1H, d, $J=12.0$ Hz), 4.75 (1H, d, $J=12.0$ Hz), 5.05∼5.17 (2H, dd-like), 5.26 (1H, br), 5.86 (1H, s), 7.31∼7.48 (10H, m); ^{19}F NMR ($CDCl_3$) δ: −138.41∼−141.15 (2F, q-like); MS (*m*/*z*) 434 (M^++H), 433 (M^+); HRMS calcd for $C_{22}H_{21}F_2NO_6$ (M^+), 433.1337; found 433.1303.

6. X-Ray crystallographic data for 12

A colorless prismatic crystal of **12** was mounted on a glass fiber. All measurements were made on a Rigaku

AFC7R diffractometer with graphite-monochromated Mo–Kα radiation ($\lambda = 0.71069$ Å) and a rotating anode generator. The structure was solved by direct methods (Sir92[17]), and the non-hydrogen atoms were refined anisotropically except for those of the disordered phenyl group of the benzyloxycarbonyl group. $C_{22}H_{21}F_2NO_6$, $M = 433.40$, monoclinic, space group $P2_1$ (no. 4), $a = 10.129(3)$, $b = 9.069(2)$, $c = 12.606(2)$ Å, $\beta = 109.500(14)°$, $V = 1091.5(4)$ Å^3, $Z = 2$, $F(000) = 444$, $\mu = 0.107$ mm^{-1}, $D_{calcd} = 1.319$ g cm^{-3}. Final goodness of fit = 1.422, $R_1 = 0.0572$, $wR_2 = 0.1703$. Crystallographic data (excluding structure factors) for the structure of **12** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 137783. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: data_request@ccdc.cam.ac.uk].

6.1. (*R*)-(+)-Benzyl 2-[2,2-difluoro-1-(methoxycarbonyl)-cyclopropylamino]acetate (*R*)-14

To a stirred solution of **11a** (50.0 mg, 0.19 mmol) in acetone (5 mL) was added Jones reagent (0.25 mL) and the mixture was stirred at room temperature for 1 h. *i*-Propanol (5 mL) was added and the solution was diluted with ether (90 mL). The resulting mixture was filtered through Celite®, the filtrate was dried over anhydrous magnesium sulfate and evaporated to give crude (*R*)-**13a**. The crude carboxylic acid (*R*)-**13a** was dissolved in ether (8 mL) and an ether solution of diazomethane [prepared from *N*-methyl-*N*-nitrosourea (98 mg), ether (4 mL), and 50% sodium hydroxide (4 mL)] was added. The reaction mixture was stored at ambient room temperature for 10 h. Acetic acid (0.5 mL) was added to the mixture to decompose excess diazomethane. The solution was washed with saturated aqueous sodium bicarbonate and brine, and the organic layer was dried over anhydrous magnesium sulfate and evaporated. The residue was purified using preparative thin layer chromatography (*n*-hexane:ethyl acetate = 3:1) to afford the pure (*R*)-**14** (47.0 mg, 87%) as a colorless oil. $[\alpha]_D^{27}$ +47.1 (*c* 0.37, $CHCl_3$); IR (neat) cm^{-1}: 3327, 1761, 1750, 1696, 1684; ^{1}H NMR ($CDCl_3$) δ: 1.93 (2H, br), 3.76 (3H, s), 5.12 (2H, s), 5.53 (1H, br), 7.34 (5H, br); ^{19}F NMR ($CDCl_3$) δ: −134.29 (1F, dq-like), −136.99 (1F, dq-like); MS (*m*/*z*) 285 (M^+); HRMS calcd for $C_{13}H_{13}F_2NO_4$ (M^+), 258.0813; found 258.0829.

6.2. (*R*)-(+)-1-Amino-2,2-difluorocyclopropanecarboxylic acid hydrochloride (*R*)-3·HCl

To a stirred solution of **11b** (50.0 mg, 0.22 mmol) in acetone (5 mL) was added Jones reagent (0.28 mL) and the mixture was stirred at room temperature for 1 h. *i*-Propanol (2 mL) was added and the solution was diluted with ether (90 mL). The resulting mixture was filtered through Celite®. The filtrate was dried over anhydrous magnesium sulfate and evaporated to afford crude carboxylic acid (*R*)-**13b**. The crude carboxylic acid (*R*)-**13b** was dissolved in 3N hydrochloric acid (0.74 mL, 2.22 mmol) and the reaction mixture was stirred at room temperature for 12 h. The mixture was concentrated in vacuo and the residue was purified by chromatography on DOWEX 50W-X8 column chromatography (3N hydrochloric acid as an eluent) to afford (*R*)-**3** hydrochloride (39.0 mg, 99%) as colorless crystals. Mp 170°C (decomp.); $[\alpha]_D^{27}$ +5.10° (*c* 1.05, H_2O); IR (KBr) cm^{-1}: 2899, 1722, 1427, 1186; ^{1}H NMR (DMSO-d_6) δ: 2.54–2.59 (2H, m); ^{19}F NMR (DMSO-d_6) δ: −133.77 (1F, dt, $J = 157.2$, 11.6 Hz), −136.17 (1F, ddd, $J = 157.2$, 11.6, 6.5 Hz); ^{13}C NMR ($CDCl_3$) δ: 20.53 (t, $J_{CF} = 9.2$ Hz), 79.05 (t, $J_{CF} = 33.4$ Hz), 109.26 (t, $J_{CF} = 286.5$ Hz), 164.6 (s); FAB-MS (*m*/*z*) 138 (M^++H); FAB-HRMS calcd. for $C_4H_6F_2NO_2$ (M^++H), 138.0367; found 138.0363.

6.3. Lipase-catalyzed deacetylation of 7

In a typical experiment (run 10), Amano PS (50 mg) was placed in a vial to which was added **7** (10 mg), acetone (0.4 mL) and 0.07 M phosphate buffer (pH 7) (1.4 mL). The resulting suspension was magnetically stirred at room temperature and the course of the reaction was followed by GC analysis (OV101). Based on this analysis, the mixture was stirred for 9.5 h and the reaction was saturated with sodium chloride and quenched by filtration through Celite®. After extraction with ethyl acetate, the organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was chromatographed on a silica gel column using hexane–ethyl acetate (1:1) as an eluent to give (*S*)-**6**. The ee of (*S*)-**6** was determined by HPLC analysis as described in the case of (*R*)-**6**. Preparative deacetylation was carried out by this procedure using the following conditions: Amano PS (5.0 g), **7** (1.17 g, 5.3 mmol), 0.07 M phosphate buffer (pH 7, 140 mL), acetone (70 mL), reaction time (16 h).

6.4. (*S*)-(+)-[2,2-Difluoro-1-(hydroxymethyl)cyclopropyl]methyl acetate (*S*)-6

$[\alpha]_D^{29}$ +3.3 (*c* 1.11, $CHCl_3$). The spectral data for this sample were identical with those of (*R*)-**6**.

6.5. (*S*)-(−)-{2,2-Difluoro-1-[(*t*-butoxy)carbonylamino]-cyclopropyl}methyl acetate (*S*)-10b

Using the same procedure described for the preparation of (*R*)-**10b**, (*S*)-**10b** was synthesized from (*S*)-**6** in 60% yield. $[\alpha]_D^{29}$ −29.8 (*c* 1.16, $CHCl_3$). The spectral data for this sample were identical with those of (*R*)-**10b**.

6.6. *t*-Butyl (*S*)-(−)-*N*-[2,2-difluoro-1-(hydroxymethyl)-cyclopropyl]carbamate (*S*)-11b

Using the same procedure described for the preparation of (*R*)-**11b**, (*S*)-**11b** was synthesized from (*S*)-**10b** in 88% yield. Mp 89 ~ 90°C (*n*-hexane); $[\alpha]_D^{28}$ −32.4 (*c* 0.68, $CHCl_3$). The spectral data for this sample were identical with those of (*R*)-**11b**. According to the GC analysis as described for (*R*)-**11b** (CP Cyclodextrin-β-236-M-19) the enantiomeric excess of this compound was over 99% ee.

6.7. (*S*)-(−)-1-Amino-2,2-difluorocyclopropanecarboxylic acid hydrochloride (*S*)-3·HCl

Using the same procedure as described for the preparation of (*R*)-**3**·HCl, (*S*)-**3**·HCl was synthesized from (*S*)-**11b** in 82% yield. Mp 170°C (decomp.); $[\alpha]_D^{27}$ −5.7 (*c* 0.77, H_2O). The spectral data for this sample were identical with those of (*R*)-**3**·HCl.

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