Effect of Acyl Chain Length and Branching on the Enantioselectivity of *Candida rugosa* Lipase in the Kinetic Resolution of 4-(2-Difluoromethoxyphenyl)-Substituted 1,4-Dihydropyridine 3,5-Diesters

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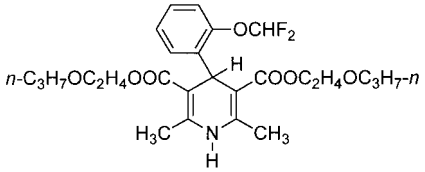
Since 2,6-dimethyl-4-aryl-1,4-dihydropyridine 3,5-diesters themselves are not hydrolyzed by commercially available hydrolases, derivatives with spacers containing a hydrolyzable group were prepared. Seven acyloxymethyl esters of 5-methyl- and 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)-phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate were synthesized and subjected to *Candida rugosa* lipase (CRL) catalyzed hydrolysis in wet diisopropyl ether. A methyl ester at the 5-position and a long or branched acyl chain at C3 gave the highest enantiomeric ratio (*E* values). The most stereoselective reaction ($E = 21$) was obtained with 3-[(isobutyryloxy)methyl] 5-methyl 4-(2-difluoromethoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, and this compound was used to prepare both enantiomers of 3-methyl 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate. The absolute configuration of the enzymatically produced carboxylic acid was established to be 4*R* by X-ray crystallographic analysis of its 1-(*R*)-phenylethyl amide.

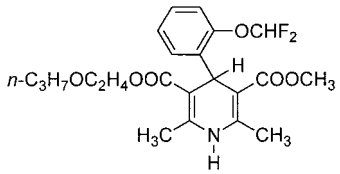
Introduction

The pharmacology of 1,4-dihydropyridine (1,4-DHP) derivatives is at the eve of a novel boom. After the synthesis, study, and development of a set of antihypertensive and antianginal drugs,[1,2] the interest is growing toward pharmacological activities that are not connected with their calcium antagonist properties, like neurotropic (antiamnestic, anticonvulsant, neuroregulatory), antidiabetic, and membrane protecting as well as anticancer, antibacterial, and antiviral activities.[3−6]

2,6-Dimethyl-3,5-bis[2-(propoxy)ethoxycarbonyl]-4-[2-(difluoromethoxy)phenyl]-1,4-dihydropyridine (cerebrocrast) is a novel, highly active preparation with antidiabetic, nootropic, neuromodulatory, cognition and memory enhancing, neuroprotective (for age-related neuronal damages, antihypoxic, antialcohol) properties; regulatory mode action; and neuropeptide effects.[5,6]



cerebrocrast



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Pharmaceutical evaluations of chiral 1,4-DHPs revealed that their stereoisomers usually have different biological activities. Sometimes the undesired enantiomer caused serious side effects, while in other cases enantiomers were reported to even have the opposite action profile (calcium antagonist−calcium agonist; hypotensive activity−hypertensive activity).[7] Ever since the differences in biological action and toxicity for a number of unsymmetrical dihydropyridines were reported for the

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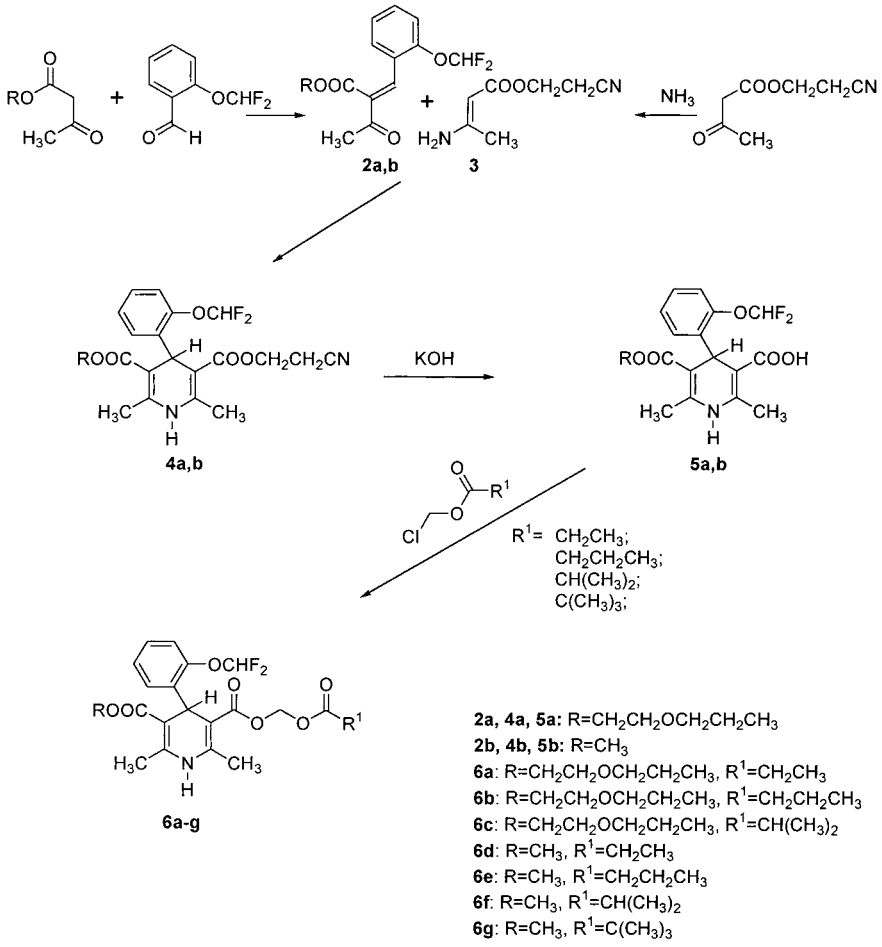
Scheme 1. Preparation of the Dihydropyridine Substrates 6



2a, 4a, 5a: R=$CH_2CH_2OCH_2CH_2CH_3$
2b, 4b, 5b: R=CH_3
6a: R=$CH_2CH_2OCH_2CH_2CH_3$, R^1=CH_2CH_3
6b: R=$CH_2CH_2OCH_2CH_2CH_3$, R^1=$CH_2CH_2CH_3$
6c: R=$CH_2CH_2OCH_2CH_2CH_3$, R^1=$CH(CH_3)_2$
6d: R=CH_3, R^1=CH_2CH_3
6e: R=CH_3, R^1=$CH_2CH_2CH_3$
6f: R=CH_3, R^1=$CH(CH_3)_2$
6g: R=CH_3, R^1=$C(CH_3)_3$

first time,[8] the demand for enantiopure dihydropyridines appeared, and in 1991, the first successful enantioselective chemoenzymatic transformation of a prochiral dihydropyridine-3,5-dicarboxylic diester was carried out.[9] The chemoenzymatic synthesis of some biological active DHPs, (e.g., Nicardipine, Nilvadipine) was recently achieved in several laboratories.[10,11]

Hydrolytic enzymes are not capable of hydrolyzing alkyl esters at the position 3 and 5 of 4-aryl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates, presumably because of steric and electronic factors.[9,12] To overcome this difficulty, spacers have been introduced at these positions. The spacer should contain a group that is easily hydrolyzed by enzymes (e.g., an ester group) in order to allow kinetic resolution or enzymatic asymmetrization. The ethoxycarbonylmethyl spacer has been used to asymmetrize a number of 4-aryl-1,4-DHPs by us[13] and others.[14,15] Moderate to excellent ee values were obtained, but this spacer group is not easy to remove. The acyloxymethyl moieties were introduced by the groups of Sih[16] and Achiwa[17] and have the advantage that, after enzymatic hydrolysis, a free carboxyl group is liberated due to the spontaneous loss of formaldehyde. This versatile spacer has also been applied for the kinetic resolution of sterically hindered tertiary alcohols.[18]

To investigate the differences in biological activities of both enantiomers of an asymmetric analogue of cerebrocrast, two chemoenzymatic approaches to the synthesis of both enantiomers of 3-methyl 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate (**1**) using various acyloxymethyl spacers are described in this paper. The relationship between the structure of the substrates and the rate and selectivity of the enzymatic transformation is studied.

Results and Discussion

The racemic substrates **6a**−**g** were synthesized as depicted in Scheme 1. These compounds have one of the desired ester groups at the 5-position and a variety of acyloxymethyl esters at the 3-position that are amenable

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to enzymatic hydrolysis. The first step is the reaction of the benzylidene acetoacetates **2a,b** and 2-cyanoethyl 3-aminocrotonate **3** by a modified Hantzsch cyclization.

3,5-Bis(2-cyanoethyl) 1,4-dihydropyridinedicarboxylates have already been transformed by seaprose S (*Aspergillus melleus*) with good enantioselectivity.[14,15] Unfortunately, this enzyme is not commercially available. Furthermore, in our hands, enzymes from the same species or genus such as protease P6 (*Aspergillus melleus*) and acylase 30 000 (*Aspergillus sp.*) hardly showed any activity toward compounds **4a,b**. However, the 2-cyanoethyl ester group can be easily removed under basic conditions[19] to give the monoacids **5a,b** in good yields. The substrates **6a**−**g** were synthesized from the isolated or in situ generated monoacids by treatment with the corresponding acyloxymethyl chloride prepared according to reported methods.[20,21] The alkylation occurred smoothly in the case of **6g**, but in the other cases, the products **6a**−**f** were isolated from mixtures of byproducts in 10−70% yield. The low yield of the propionyloxymethyl derivatives **6a** and **6d** is due to their instability. The set of derivatives **6a**−**g** differing in the bulkiness of the acyloxymethyl ester and alkyl ester at positions 3 and 5 allowed us to establish the relationship between the structure of the substrate and the reactivity and enantioselectivity of the enzyme.

Screening of the transformation of **6c** and **6f** with lipase AH, lipase PS, *Candida antarctica B* lipase (CAL-B), *Burkholderia cepacia* lipase, and *Mucor miehei* lipase showed that these enzymes can catalyze the hydrolysis of the substrates in wet diisopropyl ether. However, the enantiopreference was rather moderate, and these enzymes were not investigated further. *Candida rugosa* lipase (CRL) was found to be the most active and enantioselective of all the tested enzymes, and this enzyme was used for all subsequent experiments (see Scheme 2).

According to the literature, CRL together with CAL-B are the most universal enzymes for different purposes and were already widely applied because of their unique stereoselectivity.[13,22,23]

The enantioselectivity of *Candida rugosa* lipase catalyzed kinetic resolution of racemic substrates **6a**−**g** in water-saturated diisopropyl ether (IPE) was investigated, and the obtained data are summarized in Table 1. From this table it is clear that increasing the steric bulk of the acyl group in compounds **6** decreases the reaction rates.[26] The pivaloyloxymethyl derivative **6g** is not reactive toward CRL. Only CAL-B and *Mucor miehei* lipase were able to hydrolyze **6g**: in 8 days of incubation with *Mucor miehei* lipase, the conversion to monoacid **5b** was 35% with 13% ee.

Scheme 2. Enzymatic Resolution of the Dihydropyridines 6

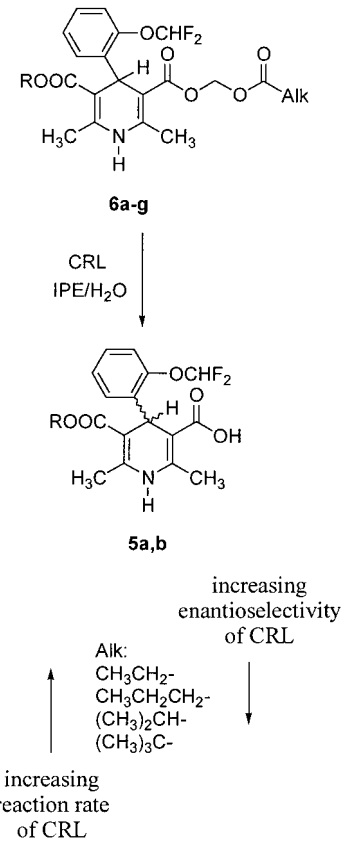

Table 1. Enantiomeric Ratio[24] (*E*) and Reaction Rate of the *Candida rugosa* Lipase Catalyzed Kinetic Resolution of Racemic 1,4-Dihydropyridine Substrates 6a−g in Water-Saturated Diisopropyl Ether[a]

compd	enantiomeric ratio (*E* value)[b]	time of 50% conversion, h
6a	1.7 ± 0.07	4
6b	3.0 ± 0.1	6
6c	11.0 ± 0.6	6
6d	4.0 ± 0.2	4.0
6e	6.0 ± 0.1	4.5
6f	21.0 ± 1.3	5
6g		no reaction

[a] For the details, see the Experimental Section. [b] The data were fitted using the computer program EIVFIT.[25]

Substrates having a methyl ester at C5 give higher *E* values than those having 2-propoxyethyl esters. Furthermore, the enantioselectivity of CRL increased together with the steric hindrance of acyloxymethyl ester group. The highest enantiomeric ratio ($E = 21$) was reached for the isobutyryloxymethyl ester **6f**. This rather high enantioselectivity is remarkable, since there are no less than six single bonds between the chiral center and the carbonyl group where the serine residue of the enzyme attacks. This means that there is much conformational flexibility in the substrate, which increases the chance that both enantiomers will fit in the enzyme active site (in different conformations). Increasing the size and branching of the acyloxymethyl group may limit the available space for the 4-aryl-1,4-DHP moiety inside the enzyme and therefore enhance the steric recognition. Furthermore, it should be noted that the reaction is

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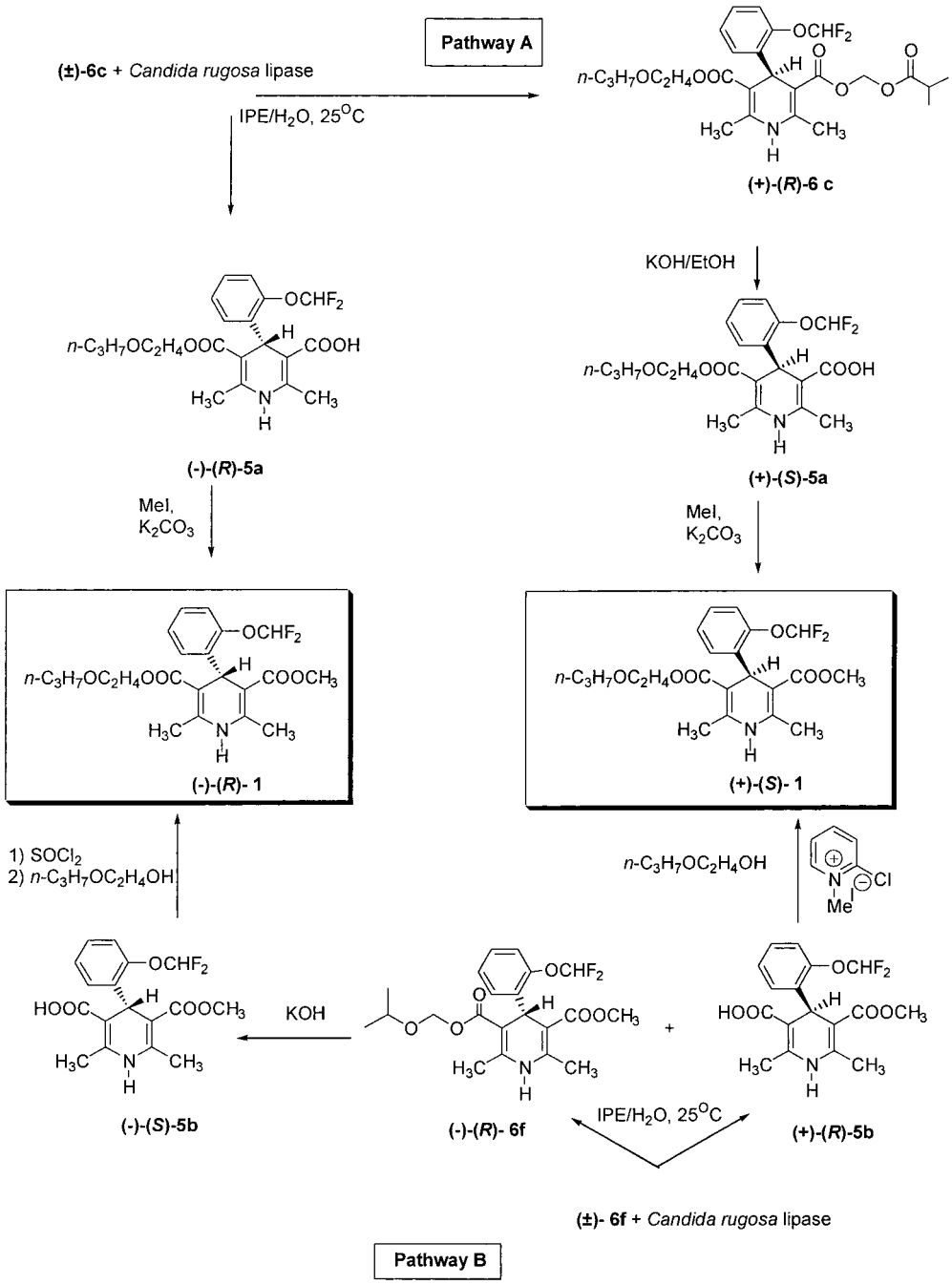
Scheme 3. Chemoenzymatic Routes to Both Enantiomers of the Cerebrocrast Analogue 1



performed in an organic solvent and it is well-appreciated that reactions in nonaqueous solvents have increased enantioselectivity.[27]

Our goal was the preparative synthesis of both enantiomers of **1**. Several synthetic roads can be envisaged for this purpose. The two isobutyryloxymethyl derivatives **6c** and **6f** have been chosen as the starting materials, as in these cases the highest enantioselectivity of CRL was observed. The CRL-catalyzed kinetic resolution was stopped halfway to give the optically active monoacid **5a,b** and the remaining optically active substrates **6c,f** (Scheme 3, Table 2). The remaining substrates **(+)-(*R*)-6c** and **(−)-(*R*)-6f** were hydrolyzed chemically to the monoacids **(+)-(*S*)-5a** and **(−)-(*S*)-5b**. The pathway A is more preferable from a synthetic point of view because 2-propoxyethanol (propylcellosolve) was already introduced to the dihydropyridine ring; however, pathway B gives higher ee values for the products.

The yield of esterification of the monoacid **(+)-(*R*)-5b** with 2-propoxyethanol (propylcellosolve) using 2-chloromethylpyridinium iodide (Mukaiyama reagent)[28] was 22%, together with unconverted starting material. This forced us to look for a better method of esterification, which was found in treatment of **(−)-(*S*)-5b** with 4 equiv of $SOCl_2$ and a subsequent reaction with propylcellosolve to give **(−)-(*R*)-6** in 45% yield. Using less $SOCl_2$ (1−2 equiv) leads to decarboxylation instead of esterification.

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Table 2. Synthesis of Both Enantiomers of 3-Methyl 5-(2-Propoxyethyl) 4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylates, (+)-(*S*)-1 and (−)-(*R*)-1

pathway	compound	ee, %	$[\alpha]^{20}_D$ (solvent)
A	**(+)-(*R*)-6c**	62	+16.0 (MeOH)
A	**(−)-(*R*)-5a**	88[a]	−32.6 (MeOH)
A	**(+)-(*S*)-5a**	62	+29.8 (MeOH)
A	**(+)-(*S*)-1**	62	+13.0 ($CHCl_3$)
A	**(−)-(*R*)-1**	88	−17.0 ($CHCl_3$)
B	**(−)-(*R*)-6f**	79	−15.0 ($CHCl_3$)
B	**(+)-(*R*)-5b**	77	+42.7 ($CHCl_3$)
			−12.7 (MeOH)
B	**(−)-(*S*)-5b**	79	−46.9 ($CHCl_3$)
			+16.6 (MeOH)
B	**(+)-(*S*)-1**	71[b]	+16.8 ($CHCl_3$)
B	**(−)-(*R*)-1**	89[b]	−18.7 ($CHCl_3$)

[a] Determined after crystallization. [b] Obtained from another experiment, where the degree of conversion of the enzymatic reaction was higher than 50%.

Determination of optical yields of the products of the enzymatic reactions has been performed in several ways. The most convenient analysis using HPLC on chiral stationary phases was applied for compound **5a**, since the separation of enantiomers (R_s) was ∼1.2. In other cases (**5b, 6c, 6f, 1**) the optimal conditions of separation using different chiral HPLC columns were not found, and R_s was at best ∼0.6−0.8, which was not sufficient for the determination of the ee of the enzymatic products. So, the data obtained by this method were only used to determine the approximate ee. The determination of ee of **5b** has been done via coupling to (*R*)-(+)-α-methylbenzylamine;[29] analysis of the obtained diastereomers on a reversed phase column gave R_s >1.2. Enantiomeric excesses of the intermediates **6c,f** and target compound **1** derived from **6c** were assumed to be the same as for **5a** and **5b**. The enantiomeric excess of **1** obtained from **5b** has been determined by ^{1}H NMR using a chiral shift reagent.

Initial attempts to synthesize derivatives suitable for the determination of the absolute configuration by X-ray diffraction analysis failed. A number of esters of **5b** and optically active or heavy atom containing alcohols were synthesized, but none of them led to enantiopure crystalline material. Even when the racemic mixtures were crystalline, the enantiopure materials were found to be oils, until the derivative of **(±)-5b** and (*R*)-(+)-α-methylbenzylamine was synthesized. The epimers of **7** (Scheme 4) were separated on reversed-phase silica gel, and both diastereomers appeared to be crystalline. The crystals of the first eluted amide **(+)-(*R,S*)-7** were submitted to X-ray analysis. An ORTEP plot of **(+)-(*R,S*)-7** is given in the Supporting Information. Besides the indicated intramolecular hydrogen bond, the structure also contains an intermolecular hydrogen bond, donated by N1−H to O32, creating an infinite one-dimensional chain of hydrogen-bonded molecules, running in the *a*-direction. The asymmetric unit contains one molecule of cocrystallized chloroform, involved in a short C−H···O interaction with O11. Further details of intra- and intermolecular geometry are given in the Supporting Information.

The absolute configuration was initially chosen to be in accordance with the known configuration of C24. The anomalous signal of the cocrystallized chloroform proved to be strong enough for the ab initio determination of the absolute configuration of an enantiopure sample.[30] The Flack *x*-parameter[31] amounted to −0.04(8); the *x* parameter for the inverted structure was 1.03(8), indicating a correct assignment of absolute structure.

The acid **(−)-(*S*)-5b**, derived from the remaining substrate **(−)-(*R*)-6f**, was converted to the corresponding amide **7** and appeared to have the same retention time as the **(+)-(*R,S*)**-isomer that was subjected to X-ray analysis. In the same way, the amide derived from the enzymatic product **(+)-(*R*)-5b** coeluted with **(−)-(*R,R*)-7**. So, in the hydrolysis of **6f**, CRL preferentially reacts with the *S*-isomer. The same enantiopreference of CRL was observed for the hydrolysis of **6c** and other derivatives.

Conclusions

The chemoenzymatic synthesis of both enantiomers of 3-methyl 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)-phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate has been performed in moderate to good optical yields, depending on the strategy of the synthesis. The reaction of *Candida rugosa* lipase was dependent on the structure of the substrates: increasing the steric bulk of the hydrolyzed acyloxymethyl ester leads to lower reaction rates until the substrate becomes unreactive toward this enzyme at all. At the same time, the enantioselectivity of CRL increases until an enantiomeric ratio (*E*) of 21 is reached for the isobutyryloxymethyl ester. This fine-tuning of the structure of the acyl moiety on the spacer provides a useful method to perform kinetic resolution of hindered or unreactive carboxylic acids. Using this strategy, the CRL-catalyzed hydrolysis of the isobutyryloxymethyl derivative **6f** yielded a practical method for the synthesis of both enantiomers of a chiral 1,4-dihydropyridine. The absolute configuration of the enzymatic product was assigned by X-ray analysis of its (*R*)-(+)-1-phenylethylamine derivative.

Experimental Section

Materials and Methods. All reagents were purchased from Aldrich, Acros, or Merck and used without further purification. HPLC grade solvents were from Labscan (Dublin, Ireland). Flash column chromatography was performed on Merck silica gel 60 (230−400 mesh or 70−230 mesh) and Baker bond phase C18 for flash from J. T. Baker (Deventer, The Netherlands). Preparative TLC was performed on 20 × 20 cm silica gel TLC−PET F_{254} foils (Fluka). *Candida rugosa* lipase [lipase (EC 3.1.1.3) type VII from *Candida rugosa*] was purchased from Sigma. Lipase AH and lipase PS were supplied by Amano Pharmaceutical Co., Ltd. (Japan). Immobilized *Candida antarctica* lipase B (Novozym 435) was a gift from Novo Nordisk A/S (Bagsvaerd, Denmark). Lipase from *Burkholderia cepacia* (CHIRAZYME L-1, c.-f., lyo.) and *Mucor miehei* lipase (CHIRAZYME L-9, c.-f, lyo.) were gifts from Boehringer-Mannheim (Mannheim, Germany). Enzymatic reactions were carried out in a orbital shaker at 25 °C (250 rpm). ^{1}H NMR spectra were recorded at 90, 200, or 400 MHz. ^{13}C spectra were recorded at 50 MHz. Melting points are uncorrected. The conversions and *E*-values of the enzymatic reactions were analyzed by HPLC on a 4.6 × 250 mm column packed with 5 μm Spherisorb, ODS-2 (Phase Separations) with the solvent system acetonitrile/water/acetic acid (60:40:0.1) as the mobile phase at a flow rate of 1.0 mL/min. Alternatively, a 4.6 × 250 mm Alltima

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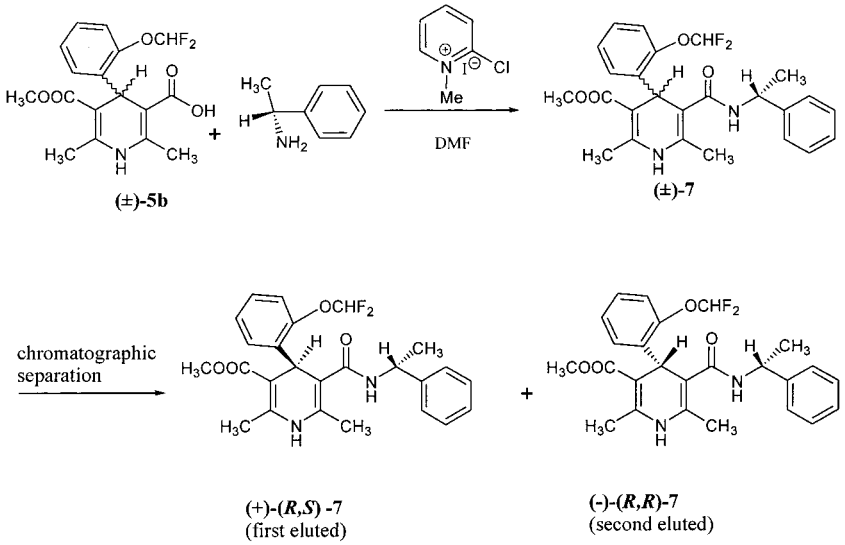
Scheme 4. Determination of the Stereochemistry of the Enzymatic Reaction



C18 5U (Alltech) column was used with the eluent methanol/water/acetic acid (68:38:0.01) equipped with a detector set at 254 nm. Determination of enantiomeric excesses of **5a** was performed by direct analysis on the chiral column Chirex 3011, 4.6 × 250 mm, 5 μm (Phenomenex) with detection at 254 nm. The eluent was methanol/dichloromethane (1:2) at a flow rate of 1.0 mL/min.

Enantiomeric excesses of **(−)-(*R*)-1** and **(+)-(*S*)-1** were determined by ^{1}H NMR (400 MHz) using Eu(hfc)$_3$ in $CDCl_3$ solution. The ee was calculated from the splitting of the resonance of one of the 2,6-CH_3 groups into two singlets.

2-[2-(Difluoromethoxy)benzylidene]methyl Acetoacetate (2b). 2-(Difluoromethoxy)benzaldehyde (26.15 g, 0.15 mol) and methyl acetoacetate (21.37 g, 0.18 mol) were added to a mixture of 2-propanol (10 mL), piperidine (1 mL), and acetic acid (1 mL). After being stirred at room temperature for 6 h, the solvent was removed in vacuo. The residue was twice crystallized from diethyl ether−hexane mixture to give 21.80 g (53.2%) of **2b** as white crystals: mp 51−53 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.44 (3H, s), 3.77 (3H, s), 6.53 (1H, t, J_{H-F} = 73.2 Hz), 7.20−7.45 (4H, m), 7.82 (1H, s).

Anal. Calcd for $C_{13}H_{12}F_2O_4$: C, 57.78; H, 4.47. Found: C, 57.87; H, 4.28.

2-Cyanoethyl 3-aminocrotonate (3)[32] was prepared by a modified method: To 10.00 g (0.06 mol) of 2-cyanoethyl acetoacetate were added 15 mL (0.20 mol) of 25% aqueous ammonia and 0.3 mL of acetic acid with stirring. The reaction mixture was maintained at room temperature for 2 h, after which the white precipitate was filtered off. Crystallization from methanol gave 6.50 g (71%) of **3** as white crystals: mp 88 °C; ^{1}H NMR (DMSO-d_6, 90 MHz) δ 1.85 (3H, s), 2.60 (2H, t, J = 6.5 Hz), 4.00 (2H, t, J = 6.5 Hz), 4.45 (1H, s), 7.20 (2H, br s).

Anal. Calcd for $C_7H_{10}N_2O_2$: C, 54.53; H, 6.53; N, 18.17. Found: C, 54.35; H, 6.51; N, 17.95.

3-(2-Cyanoethyl) 5-(2-Propoxyethyl) 4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate (4a). 2-(Difluoromethoxy)benzaldehyde (17.21 g, 0.10 mol) and propoxyethyl acetoacetate (18.82 g, 0.10 mol) were added to a mixture of 2-propanol (150 mL), a catalytic amount of piperidine, and acetic acid (0.5 mL). After being stirred at reflux for 5 h, the solvent was removed in vacuo. The residue was dissolved in ether and washed with water twice, dried over $MgSO_4$, and concentrated under reduced pressure to give 18.2 g (0.53 mol) of crude (*E*,*Z*)-2-[2-(difluoromethoxy)benzylidene]propoxyethyl acetoacetate (**2a**), which was used without further purification: ^{1}H NMR ($CDCl_3$, 90 MHz) δ (major peaks) 1.46 (2H, sextet, J = 7.0 Hz), 2.25 and 2.38 (3H, s), 3.30 (2H, t, J = 7.0 Hz), 3.40−3.70 (2H, m), 4.15−4.35 (2H, m), 6.48 and 6.50 (1H, t, J = 72.5 Hz), 6.95−7.50 (4H, m), 7.80 and 7.82 (1H, s).

Crude **2a** (18.2 g, 0.53 mol) and 8.15 g (0.53 mol) of 2-cyanoethyl 3-aminocrotonate **3** were refluxed in 50 mL of isopropyl alcohol for 6 h. After cooling until −5 °C overnight, the precipitate containing 90% of the main product was filtered off. The precipitate was flash chromatographed with chloroform/hexane/acetone (9:7:1) to give 17.5 g (85%) of **4a** as pale yellow crystals: mp 123−124 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.86 (3H, t, J = 7.0 Hz), 1.53 (2H, sextet, J = 7.0 Hz), 2.27 (3H, s), 2.28 (3H, s), 2.63 (2H, t, J = 6.5 Hz), 3.32 (CH_2, t, J = 7.0 Hz), 3.55 (2H, t, J = 5.1 Hz), 4.12 (2H, t, J = 5.3 Hz), 4.19 (2H, t, J = 6.5 Hz), 5.23 (1H, s), 6.03 (1H, br s), 6.58 (1H, dd, J_{H-F} = 73.8, 76.8 Hz), 6.96−7.18 (3H, m), 7.37 (1H, dd, J = 2.0, 7.0 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 10.50 (CH_3), 17.85 (CH_2), 19.55 (CH_3), 19.73 (CH_3), 22.78 (CH_2), 35.55 (CH), 58.18 (CH_2), 63.06 (CH_2), 68.56 (CH_2), 72.72 (CH_2), 101.57 (C), 103.10 (C), 116.91 (CN), 117.30 (CH, t, J = 264.6 Hz, $OCHF_2$), 118.22 (CH), 125.18 (CH), 127.81 (CH), 131.71 (CH), 138.25 (C), 144.53 (C), 146.16 (C), 149.29 (C), 166.78 (C), 167.46 (C); IR (Nujol) 2260 cm^{-1} (CN); MS *m*/*z* 478 (M^+); HRMS calcd for $C_{24}H_{28}F_2N_2O_6$ 478.1915, found: 478.1910.

Anal. Calcd for $C_{24}H_{28}F_2N_2O_6$: C, 60.24; H, 5.89; N, 5.85. Found C, 59.88; H, 5.90; N, 5.78;

3-(2-Cyanoethyl) 5-Methyl 4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate (4b). A solution of 5.00 g (19 mmol) of **2b** and 2.86 g (19 mmol) of 2-cyanoethyl 3-aminocrotonate was refluxed in 20 mL of ethanol for 6 h. After cooling until −5 °C, the precipitate was filtered off. Crystallization from ethanol gave 5.20 g (69%) of **4b** as pale yellow crystals: mp 124−125 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.29 (3H, s), 2.30 (3H, s), 2.64 (2H, t, J = 6.4 Hz), 3.59 (3H, s), 4.20 (2H, t, J = 6.4 Hz), 5.25 (1H, s,), 5.86 (1H, br s), 6.52 (1H, dd, J_{H-F} = 73.7, 76.6 Hz), 6.96−7.19 (3H, m), 7.36 (1H, dd, J = 2.0, 7.0 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 17.81 (CH_2), 19.49 (CH_3), 19.80 (CH_3), 35.11 (CH), 50.92 (CH_3), 58.19 (CH_2), 101.71 (C), 103.32 (C), 116.91 (CN), 117.23 (CH, t, J = 279.7 Hz, $OCHF_2$), 117.89 (CH), 125.23 (CH), 127.83 (CH), 131.47 (CH), 138.24 (C), 144.16 (C), 146.10 (C), 149.02 (C), 166.64 (C), 167.85 (C); IR (Nujol) 2260 cm^{-1} (CN); MS *m*/*z* 406 (M^+); HRMS calcd for $C_{20}H_{20}F_2N_2O_5$ 406.1440, found 406.1333.

Anal. Calcd for $C_{20}H_{20}F_2N_2O_5$: C, 59.11; H, 4.96; N, 6.89. Found: C, 59.00; H, 5.05; N, 6.72.

4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-5-[(2-propoxyethoxy)carbonyl]-1,4-dihydro-3-pyridinecarboxylic Acid (5a). Crushed KOH (0.73 g, 13 mmol) was added to a solution of 4.78 g (10 mmol) of **4a** in 40 mL of ethanol. The reaction mixture was stirred at 40 °C for 3 h and then 6 h at

(32) Grohe, K.; Heitzer, H. *Liebigs Ann. Chem.* **1973**, 1025−1035.

room temperature. Then the reaction mixture was evaporated and the residue was dissolved in water and washed twice with 30 mL of chloroform. The ice-cooled aqueous layer was acidified with HCl to pH 4.0−5.0. The precipitated product was filtered off, washed with water, and dried over $MgSO_4$ to give 2.90 g (86%) of **4a** as a pale yellow powder: mp 138−140 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 0.82 (3H, t, J = 7.0 Hz), 1.46 (2H, sextet, J = 7.0 Hz), 2.22 (6H, s), 3.30 (2H, t, J = 7.0 Hz), 3.45−3.51 (2H, m), 3.95−4.06 (2H, m), 5.12 (1H, s), 6.93 (1H, t, J_{H-F} =76.6 Hz), 6.92−7.30 (4H, m), 8.75 (1H, br s), 11.63 (1H, br s); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 10.46 (CH_3), 18.28 (2 × CH_3), 22.39 (CH_2), 34.85 (CH), 62.35 (CH_2), 67.95 (CH_2), 71.81 (CH_2), 100.70 (C), 101.80 (C), 117.10 (CH, t, J = 254.6 Hz, $OCHF_2$), 117.66 (CH), 125.00 (CH), 127.39 (CH), 131.17 (CH), 139.17 (C), 145.04 (C), 145.94 (C), 148.43 (C), 167.04 (C), 168.74 (C).

Anal. Calcd for $C_{21}H_{25}F_2NO_6$: C, 59.29; H, 5.92; N, 3.29. Found C, 59.38; H, 6.00; N, 3.85.

4-[2-(Difluoromethoxy)phenyl]-5-(methoxycarbonyl)-2,6-dimethyl-1,4-dihydro-3-pyridinecarboxylic Acid (5b). This compound was prepared via the same method used for compound **5a**, but beginning with 2.20 g (5.0 mmol) of **4b**, after addition of 0.30 g (5.3 mmol) of KOH, the reaction mixture was stirred for 6 h at room temperature, and the washing step with chloroform was omitted. Compound **5b** was obtained in 86% (1.65 g), as a pale yellow powder: mp 153−154 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 2.21 (3H, s), 2.24 (3H, s), 3.49 (3H, s), 5.14 (1H, s), 6.97 (1H, t, J_{H-F} = 76.0 Hz), 6.99−7.28 (4H, m) 8.76 (1H, br s), 11.61 (1H, br s); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 18.12 (CH_3), 18.21 (CH_3), 34.23 (CH), 50.42 (CH_3), 100.96 (C), 101.85 (C), 117.07 (CH, t, J = 254.6 Hz, $OCHF_2$), 117.43 (CH), 125.14 (CH), 127.42 (CH), 130.79 (CH), 139.30 (C), 145.14 (C), 145.63 (C), 148.05 (C), 167.51 (C), 168.68 (C).

Anal. Calcd for $C_{17}H_{17}NF_2O_5$: C, 57.79; H, 4.85; N, 3.96. Found C, 57.65, H, 4.82, N, 3.85.

3-[(Propionyloxy)methyl] 5-(2-Propoxyethyl) 4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate (6a). To a solution of 478 mg (2.0 mmol) of **4a** in 5 mL of ethanol was added a solution of 135 mg (2.4 mmol) of KOH in 1 mL of ethanol at room temperature. The resulting mixture was stirred for 1 h, after which the reaction mixture was evaporated until the solvent was completely removed. The residue was diluted with 2 mL of dry DMF, after which 139 mg (2.6 mmol) of propionyloxymethyl chloride was added. The reaction mixture was stirred overnight and then diluted with $CHCl_3$ and washed successively twice with water and twice with brine, dried over $MgSO_4$, and evaporated. The remaining residue was flash chromatographed on silica gel with petroleum ether (bp 40−60 °C)/ethyl acetate (7:3) to give 683 mg (67%) of 90% pure **6a** as a yellow oil, which can be purified on a preparative TLC silica gel plate with petroleum ether (bp 40−60 °C)/acetone (5:2): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.89 (3H, t, J = 7.5 Hz), 1.05 (3H, t, J = 7.5 Hz), 1.60 (2H, sextet, J = 7.5 Hz), 2.15−2.30 (2H, m), 2.24 (3H, s), 2.27 (3H, s), 3.36 (2H, t, J = 7.4 Hz), 3.53−3.63 (2H, m), 4.11−4.17 (2H, m), 5.22 (1H, s), 5.69 (2H, s), 6.16 (1H, s), 6.53 (1H, t, J_{H-F} =74.7 Hz), 6.91−7.16 (3H, m), 7.35 (1H, dd, J = 2.1, 7.4 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 8.65 (CH_3), 10.50 (CH_3), 19.38 (CH_3), 19.88 (CH_3), 22.80 (CH_2), 27.23 (CH_2), 36.09 (CH), 62.98 (CH_2), 68.47 (CH_2), 72.88 (CH_2), 78.41 (CH_2), 100.93 (C), 103.12 (C), 116.90 (CH, t, J = 254.7 Hz, $OCHF_2$), 117.71 (CH), 124.70 (CH), 127.65 (CH), 132.15 (CH), 137.56 (C), 144.12 (C), 147.06 (C), 149.85 (C), 166.10 (C), 167.46 (C), 173.23 (C); MS m/z 511 (M^+); HRMS calcd for $C_{25}H_{31}F_2NO_8$ 511.2018, found 511.2022.

3-[(Isobutyryloxy)methyl] 5-(2-Propoxyethyl) 4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate (6c). This compound was prepared via the same method used for compound **6a**. Beginning with 957 mg (2 mmol) of **4a**, 135 mg (2.4 mmol) of KOH, and 355 mg (2.6 mmol) of isobutyryloxymethyl chloride, **6c** was obtained in 72% (762 mg): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.89 (3H, t, J = 7.4 Hz), 1.01 (3H, d, J = 6.8 Hz), 1.05 (3H, d, J = 6.8 Hz), 1.56 (2H, sextet, J = 7.4 Hz), 2.25 (3H, s), 2.29 (3H, s), 2.42 (1H, septet, J = 6.8 Hz), 3.36 (CH_2, t, J = 6.7 Hz), 3.50−3.64 (2H, m), 4.04−4.20 (2H, m), 5.22 (1H, s), 5.68 (2H, s), 6.01 (1H, br s), 6.55 (1H, dd, J_{H-F} = 74.1, 76.2 Hz), 6.93−7.15 (3H, m), 7.33 (1H, J = 2.1, 7.3 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 10.50 (CH_3), 18.57 (2 × CH_3), 19.34 (CH_3), 19.91 (CH_3), 22.79 (CH_2), 33.63 (CH), 36.00 (CH), 62.97 (CH_2), 68.45 (CH_2), 72.89 (CH_2), 78.51 (CH_2), 101.01 (C), 103.14 (C), 117.00 (CH, t, J = 250.1 Hz, $OCHF_2$), 117.62 (CH), 124.75 (CH), 127.65 (CH), 132.08 (CH), 137.65 (C), 144.06 (C), 146.98 (C), 149.84 (C), 166.05 (C), 167.50 (C), 172.85 (C); MS m/z 525 (M^+); HRMS calcd for $C_{26}H_{33}F_2NO_8$ 525.2174, found 525.2175.

3-[(Isobutyryloxy)methyl] 5-Methyl 4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate (6f). To a solution of 3.00 g (8.5 mmol) of **5b** in 6 mL of dry DMF was added 1.41 g (10.2 mmol) of K_2CO_3 at room temperature, and the reaction mixture was stirred for 2 h, after which 1.50 g (11.0 mmol) of isobutyryloxymethyl chloride was added. The mixture was stirred overnight, diluted with $CHCl_3$, washed with water (three times) and brine, dried over $MgSO_4$, and evaporated. The remaining residue was flash chromatographed on silica gel with petroleum ether (bp 40−60 °C)/ethyl acetate (2:1) followed by crystallization from ethanol to give 2.67 g (71%) of **6f** as white crystals: mp 112−113 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.04 (3H, d, J = 6.7 Hz), 1.06 (3H, d, J = 6.7 Hz), 2.27 (3H, s), 2.31 (3H, s), 2.44 (1H, septet, J = 6.7 Hz), 3.58 (1H, s), 5.23 (1H, s), 5.69 (2H, s), 5.81 (1H, br s), 6.53 (1H, dd, J_{H-F} = 74.2, 76.1 Hz), 6.93−7.16 (3H, m), 7.32 (1H, dd, J = 2.1, 7.3 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 18.57 (2 × CH_3), 19.28 (CH_3), 19.93 (CH_3), 35.65 (CH), 35.73 (CH), 50.90 (CH_3), 78.50 (CH_2), 101.11 (C), 103.31 (C), 116.84 (CH, t, J = 256.7 Hz, $OCHF_2$), 117.57 (CH), 124.82 (CH), 127.70 (CH), 131.78 (CH), 137.69 (C), 143.98 (C), 147.01 (C), 149.70 (C), 166.05 (C), 167.99 (C), 175.88 (C); MS m/z 453 (M^+); HRMS calcd for $C_{22}H_{25}NO_7F_2$ (M^+) m/z 453.1599, found 453.1597.

Anal. Calcd for $C_{22}H_{25}NO_7F_2$: C, 58.28; H, 5.56; N, 3.09. Found: C, 58.12; H, 5.53; N, 3.03.

3-[(Butyryloxy)methyl] 5-(2-Propoxyethyl) 4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate (6b). This compound was prepared via the same method used for compound **6f**, but beginning with 213 mg (0.5 mmol) of **5a**, 35 mg (0.25 mmol) of K_2CO_3, and 889 mg (0.65 mmol) of butyryloxymethyl chloride in 0.5 mL of dry DMF. Flash chromatography on silica gel with petroleum ether (bp 40−60 °C)/ethyl acetate (7:3) gave 137 mg (52%) of **6b** as a yellow oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.86 (3H, t, J = 7.6 Hz), 0.88 (3H, t, J = 7.6 Hz), 1.53 (2H, sextet, J = 7.6 Hz), 1.55 (2H, sextet, J = 7.6 Hz), 2.18 (2H, t, J = 7.6 Hz), 2.27 (3H, s), 2.29 (3H, s), 3.35 (2H, t, J = 7.6 Hz), 3.50−3.62 (2H, m), 4.05−4.19 (2H, m), 5.21 (1H, s), 5.69 (2H, ABq), 5.92 (1H, s), 6.53 (1H, dd, J_{H-F} = 74.3, 75.9 Hz), 6.92−7.15 (3H, m), 7.33 (1H, dd, J = 2.1, 7.4 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 10.50 (CH_3), 13.51 (CH_3), 18.03 (CH_2), 19.45 (CH_3), 19.98 (CH_3), 22.80 (CH_2), 35.77 (CH_2), 36.11 (CH), 62.98 (CH_2), 68.46 (CH_2), 72.88 (CH_2), 78.39 (CH_2), 101.02 (C), 103.19 (C), 116.97 (CH, t, J = 250.1 Hz, $OCHF_2$), 117.73 (CH), 124.72 (CH), 127.67 (CH), 132.14 (CH), 137.57 (C), 144.03 (C), 146.91 (C), 149.93 (C), 166.07 (C), 167.42 (C), 172.39 (C); MS m/z 525 (M^+); HRMS calcd for $C_{26}H_{33}F_2NO_8$ 525.2174, found 525.2182.

3-[(Propionyloxy)methyl] 5-Methyl 4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate (6d). This compound was prepared via the same method used for compound **6f**, but beginning with 1.06 g (3.0 mmol) of **5b**, 0.62 g (4.5 mmol) of K_2CO_3, and 0.55 g (4.5 mmol) of propionyloxymethyl chloride in 2 mL of dry DMF. Flash chromatography on silica gel with petroleum ether (bp 40−60 °C)/chloroform/isopropyl alcohol (10:10:1) followed by crystallization from hexanes−ethyl acetate and recrystallization from methanol gave 0.13 g (10%) of **6d** as a pale yellow powder: mp 115−116 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.04 (3H, t, J = 7.4 Hz), 2.22 (2H, q, J = 7.4 Hz), 2.25 (3H, s), 2.29 (3H, s), 3.58 (3H, s), 5.21 (1H, s), 5.63 (2H, s), 5.94 (1H, br s), 6.54 (1H, t, J_{H-F} = 75.0 Hz), 6.88−7.32 (4H, m); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 8.66 (CH_3), 19.34 (CH_3), 19.42 (CH_3), 27.24 (CH_2), 35.78 (CH), 50.91 (CH_3), 78.40 (CH_2), 101.06 (C), 103.31 (C), 116.84 (CH, t, J = 255.4 Hz, $OCHF_2$), 117.59 (CH), 124.79 (CH), 127.7 (CH), 131.85 (CH), 137.66 (C), 143.98 (C), 147.07

(C), 149.74 (C), 166.10 (C), 167.97 (C), 173.29 (C); MS *m*/*z* 439 (M^+); HRMS calcd for $C_{21}H_{23}F_2NO_7$ 439.1443, found 439.1437.

3-[(Butyryloxy)methyl] 5-Methyl 4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate (6e). This compound was prepared via the same method used for compound **6f**, but beginning with 71 mg (0.20 mmol) of **5b**, 33 mg (0.24 mmol) of K_2CO_3, and 35 mg (0.26 mmol) of butyryloxymethyl chloride in 0.5 mL of dry DMF. Purification by flash chromatography on silica gel with petroleum ether (bp 40−60 °C)/ethyl acetate (7:3) followed by crystallization from ethanol gave 64 mg (70%) of **6e**, a pale yellow powder: mp 98−99 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.84 (3H, t, J = 7.4 Hz), 1.49 (2H, sextet, J = 7.4 Hz), 2.13 (2H, t, J = 7.4 Hz), 2.17 (3H, s), 2.20 (3H, s), 3.52 (1H, s), 5.23 (1H, s), 5.63 (2H, s), 5.99 (1H, s), 6.46 (1H, dd, J_{H-F} = 74.4, 75.7 Hz), 6.84−7.20 (3H, m), 7.26 (1H, dd, J = 2.1, 7.3 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.50 (CH_3), 18.03 (CH_2), 19.28 (CH_3), 19.91 (CH_3), 35.72 (CH), 35.77 (CH_2), 50.90 (CH_3), 78.36 (CH_2), 101.04 (C), 103.30 (C), 116.84 (CH, t, J = 255.4 Hz, $OCHF_2$), 117.60 (CH), 124.82 (CH), 127.70 (CH), 131.80 (CH), 137.71 (C), 144.04 (C), 147.13 (C), 149.00 (C), 166.10 (C), 167.99 (C), 172.47 (C); MS: *m*/*z* 453 (M^+); HRMS calcd for $C_{22}H_{25}NO_7F_2$ 453.1599, found 453.1596.

3-Methyl 5-[(Pivaloyloxy)methyl] 4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate (6g). This compound was prepared via the same method used for compound **6f**, but beginning with 0.35 g (1 mmol) of **5b**, 0.20 g (1.5 mmol) of K_2CO_3, and 0.30 mL (2 mmol) of pivaloyloxymethyl chloride in 3 mL of dry DMF. The mixture was poured into water, and the precipitate was filtered off and crystallized from methanol to give 0.32 g (69%) of **6f** as a white powder: mp 153−155 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.05 (9H, s), 2.25 (3H, s), 2.29 (3H, s), 3.58 (1H, s), 5.22 (1H, s), 5.69 (2H, ABq), 5.92 (1H, br s, NH), 6.52 (1H, dd, J_{H-F} = 74.0, 76.3 Hz), 6.92−7.16 (3H, m), 7.31 (1H, dd, J = 2.1, 7.4 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 19.33 (CH_3), 19.98 (CH_3), 26.71 (3 × CH_3), 35.81 (CH), 38.60 (C), 50.87 (CH_3), 78.67 (CH_2), 101.23 (C), 103.31(C), 116.87 (CH, t, J = 256.0 Hz, $OCHF_2$), 117.60 (CH), 124.84 (CH), 127.69 (CH), 131.78 (CH), 137.79 (C), 143.89 (C), 146.74 (C), 149.76 (C), 165.93 (C), 167.97 (C), 177.22 (C); MS: *m*/*z* 467 (M^+); HRMS calcd for $C_{23}H_{27}F_2NO_7$ 467.1756, found 467.1748.

Anal. Calcd for $C_{23}H_{27}F_2NO_7$: C, 59.09; H, 5.82; N, 2.99. Found: C, 58.95; H, 5.79; N, 3.11.

General Procedure of *Candida rugosa* Lipase Catalyzed Kinetic Resolution of Racemic (±)-6c and (±)-6f. To a solution of 0.80 mmol of **(±)-6c,f** in 80 mL of water-saturated diisopropyl ether was added 200 mg of *Candida rugosa* lipase, and the resulting mixture was shaken for 6 h at 25 °C and monitored by HPLC. When the conversion reached 50%, the enzyme was removed by filtration and washed additionally with chloroform. The filtrate was concentrated under reduced pressure. The residue was flash chromatographed on silica gel with chloroform/petroleum ether (bp 40−60 °C)/isopropyl alcohol (10:20:1 ⇒ 5:5:1) to give the monoacids **(−)-(*R*)-5a** and **(+)-(*R*)-5b** and the remaining substrates **(+)-(*R*)-6c** and **(−)-(*R*)-6f**, respectively.

(4*R*)-4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-5-[(2-propoxyethoxy)carbonyl]-1,4-dihydro-3-pyridinecarboxylic Acid ((−)-(*R*)-5a): yield 139 mg (41%) as a white precipitate from ether; mp 62−65 °C; 88% ee (determined after crystallization), $[\alpha]^{20}_D$ −32.6 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.90 (3H, t, J = 7.4 Hz), 1.57 (2H, sextet, J = 7.4 Hz), 2.26 (3H, s), 2.29 (3H, s), 3.38 (CH_2, t, J = 7.4 Hz), 3.51−3.63 (CH_2, m), 4.08−4.24 (CH_2, m), 5.21 (1H, s), 5.85 (1H, br s), 6.39 (1H, dd, J_{H-F} = 72.8, 77.8 Hz), 6.98−7.15 (3H, m), 7.35 (1H, dd, J = 2.0, 7.6 Hz). The mass spectral data were identical to those described for **(±)-5a** and **(+)-(*S*)-5a**; HRMS calcd for $C_{21}H_{25}F_2N_1O_6$ 425.1650, found 425.1647.

(+)-3-[(Isobutyryloxy)methyl] 5-(2-Propoxyethyl) (4*S*)-4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate ((+)-(*R*)-6c): yield 194 mg (46%) as a colorless viscous oil; 62% ee; $[\alpha]^{20}_D$ +16.0 (*c* 1.0, MeOH); the ^{1}H NMR and mass spectral data were identical to those described for its racemic precursor **(±)-5c**; HRMS calcd for $C_{26}H_{33}F_2N_1O_8$ 525.2174, found 525.2171.

(+)-(4*R*)-4-[2-(Difluoromethoxy)phenyl]-5-(methoxycarbonyl)-2,6-dimethyl-1,4-dihydro-3-pyridinecarboxylic Acid ((+)-(*R*)-5b): yield 127 mg (45%) as a colorless viscous oil, which was triturated from the mixture of chloroform−hexane to give 90 mg (32%) of a white solid; mp 82−84 °C; 77% ee; $[\alpha]^{20}_D$ +42.7 (*c* 1.0, $CHCl_3$); $[\alpha]^{20}_D$ −12.7 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.28 (3H, s), 2.29 (3H, s), 3.58 (3H, s), 5.21 (1H, s,), 5.71 (1H, br s), 6.39 (1H, dd, J_{H-F} = 73.1, 77.4 Hz), 6.95−7.17 (3H, m), 7.33 (1H, dd, J = 2.0, 7.2 Hz); mass spectral data were identical to those described for its racemic precursor **(±)-5b**; HRMS calcd for $C_{17}H_{17}NO_5F_2$ (M^+) *m*/*z* 353.1075, found 353.1074.

(−)-3-[(Isobutyryloxy)methyl] 5-Methyl (4*R*)-4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate ((−)-(*R*)-6f): yield 164 mg (45%) as a colorless viscous oil; 79% ee; $[\alpha]^{20}_D$ −15.0 (*c* 1.0, $CHCl_3$); the ^{1}H NMR and mass spectral data were identical to those described for its racemic precursor **(±)-6f**; HRMS calcd for $C_{22}H_{25}NO_7F_2$ (M^+) *m*/*z* 453.1599, found 453.1592.

(+)-(4*S*)-4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-5-[(2-propoxyethoxy)carbonyl]-1,4-dihydro-3-pyridinecarboxylic Acid ((+)-(*S*)-5a). To a stirred solution of 158 mg (0.30 mmol) of **(+)-(*R*)-6c** in 3 mL of ethanol was added a solution of 19 mg (0.33 mmol) of KOH in 0.5 mL of ethanol. After being stirred at room temperature for 1.5 h, the reaction mixture was evaporated, diluted with water, acidified with diluted HCl until pH 5.0, and extracted with chloroform three times. The organic layer was washed with water and evaporated. The residue was chromatographed on silica gel with petroleum ether (bp 40−60 °C)/dichloromethane/isopropyl alcohol (5:5:1) to give 87 mg (68%) of **(+)-(*S*)-5a** as a white precipitate from ether: mp 86−87 °C; 62% ee; $[\alpha]^{20}_D$ +29.8 (*c* 1.0, MeOH). The ^{1}H NMR ($CDCl_3$, 200 MHz) and mass spectral data were identical to those described for **(±)-5a** and **(−)-(*R*)-5a**; HRMS calcd for $C_{21}H_{25}F_2N_1O_6$ 425.1650, found 425.1645.

(−)-(4*S*)-4-[2-(Difluoromethoxy)phenyl]-5-(methoxycarbonyl)-2,6-dimethyl-1,4-dihydro-3-pyridinecarboxylic Acid ((−)-(*S*)-5b). This compound was prepared via the same method used for compound **(+)-(*S*)-5a**, without purification by flash chromatography. After pH adjustment to 5, the precipitated product was filtered off and thoroughly washed with water to give 84 mg (79%) of **(−)-(*S*)-5b** as a white precipitate: mp 87−89 °C; 79% ee; $[\alpha]^{20}_D$ −46.9 (*c* 1.0, $CHCl_3$); the ^{1}H NMR ($CDCl_3$, 200 MHz) and mass spectral data were identical to those described for **(±)-5b** and **(+)-(*R*)-5b**; HRMS calcd for $C_{17}H_{17}NO_5F_2$ (M^+) *m*/*z* 353.1075, found 353.1069.

General Procedure for the Determination of the Enantiomeric Ratio (*E* Value) of the *Candida rugosa* Lipase Mediated Kinetic Resolution of Racemic Substrates 6a−f. To a solution of 0.04 mmol of **(±)-6a−d** in 4 mL of water-saturated diisopropyl ether was added 10 mg of *Candida rugosa* lipase, and the resulting mixture was shaken at 25 °C. During the investigated period, several samples of 50 μL each were taken. The solvent was removed by passing a gentle stream of nitrogen. The samples taken from the reactions of **6a−c** were analyzed for the conversion and enantiomeric excess on the chiral column Chirex 3011. The rate of the reactions of **6d−f** were analyzed on a reversed-phase HPLC column, and enantiomeric excesses were determined after conversion to diastereomers of amide **7**: the sample was diluted with 50 μL of DMF, treated with an excess of (*R*)-(+)-α-methylbenzylamine, and kept for 0.5 h at room temperature, after which a small excess of chloromethylpyridinium iodide was added. The reaction mixture was kept for another 2 h, evaporated, and diluted with mobile phase, and the pH of the sample was adjusted by adding acetic acid. The ee values of the reactions of **6e,f** were analyzed on a HPLC column packed with Spherisorb, ODS-2. The ee of the reaction of **6d** was analyzed on an Alltima HPLC column.

General Procedure for Preparation of (−)-(*R*)-1 and (+)-(*S*)-1 from the Monoacids (−)-(*R*)-5a and (+)-(*S*)-5a. To a stirred solution of 64 mg (0.15 mmol) of **(−)-(*R*)-5** or **(+)-(*S*)-5** in 0.4 mL of dry DMF was added 21 mg (0.15 mmol) of K_2CO_3 at room temperature, and the resulting mixture was stirred for 2 h, after which 0.028 mL (0.45 mmol) of MeI was added. The reaction mixture was stirred for another 2 h, diluted with water and extracted with chloroform. The organic layer was washed with water and brine (twice) and dried over $MgSO_4$. After removal of the solvent in vacuo, the residue was chromatographed on a silica gel coated preparative TLC plate with petroleum ether (bp 40−60 °C)/chloroform/isopropyl alcohol (10:1:1) to give the following.

(−)-3-Methyl 5-(2-Propoxyethyl) (4*R*)-4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate ((−)-(*R*)-1): yield 42 mg (63%) as a yellow oil; 88% ee; $[\alpha]^{20}_D$ −17.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.88 (3H, t, J = 7.4 Hz), 1.54 (2H, sextet, J = 7.4 Hz), 2.27 (3H, s), 2.28 (3H, s), 3.35 (CH_2, t, J = 7.4 Hz), 3.55 (2H, t, J = 4.8 Hz), 3.58 (3H, s), 4.13 (2H, t, J = 5.1 Hz), 5.26 (1H, s), 5.74 (1H, br s), 6.88 (1H, t, J_{H-F} = 75.3 Hz), 6.98−7.16 (3H, m), 7.35 (1H, J = 2.2, 7.2 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 10.45 (CH_3), 19.43 (CH_3), 19.56 (CH_3), 22.76 (CH_2), 35.71 (CH), 50.76 (CH_3), 62.89 (CH_2), 68.47 (CH_2), 72.79 (CH_2), 102.58 (C), 102.70 (C), 116.97 (CH, t, J = 255.0 Hz, $OCHF_2$), 118.04 (CH), 124.96 (CH), 127.57 (CH), 131.69 (CH), 138.31 (C), 144.37 (C), 144.68 (C), 149.43 (C), 167.50 (C), 168.05 (C); MS *m*/*z* 439 (M^+); HRMS calcd for $C_{22}H_{27}NO_6F_2$ (M^+) *m*/*z* 439.1806, found 439.1800.

(+)-3-Methyl 5-(2-Propoxyethyl) (4*S*)-4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate ((+)-(*S*)-1): yield 45 mg (68%) as a yellow oil; 62% ee; $[\alpha]^{20}_D$ +13.0 (*c* 1.0, $CHCl_3$); the ^{1}H NMR ($CDCl_3$, 200 MHz) and mass spectral data were identical to those described for **(−)-(*R*)-1**; HRMS calcd for $C_{22}H_{27}NO_6F_2$ (M^+) *m*/*z* 439.1806, found 439.1801.

(+)-(*S*)-1 from (+)-(*R*)-5b. To a stirred solution of 91 mg (0.26 mmol) of **(+)-(*R*)-5b** in 0.2 mL of dry DMF was added 0.107 mL (0.77 mmol) of triethylamine at room temperature. After the solution was stirred for 30 min, 0.053 mL (0.46 mmol) of 2-propoxyethanol and 64 mg (0.28 mmol) of 2-chloromethylpyridinium iodide were added, and the reaction mixture was stirred overnight. The reaction mixture was diluted with water and extracted with dichloromethane two times. The organic layer washed with water twice, dried over $MgSO_4$, and evaporated. The residue was flash chromatographed on silica gel with petroleum ether (bp 40−60 °C)/dichloromethane/isopropyl alcohol (5:5:1) to give 53 mg (58%) of unreacted **(+)-(*R*)-5b** and 25 mg (22%) of **(+)-(*S*)-1** as a yellow oil: 71% ee; $[\alpha]^{20}_D$ +16.6 (*c* 1.0, $CHCl_3$). The ^{1}H NMR and mass spectral data were identical to those described for **(−)-(*R*)-1** above. Please note: **(+)-(*R*)-5b** was obtained from another CRL-catalyzed hydrolysis of **6f** where the degree of conversion was higher than 50%.

(−)-(*R*)-1 from (−)-(*S*)-5b. To a stirred solution of 77 mg (0.22 mmol) of **(−)-(*R*)-5b** in 0.2 mL of dry DMF at 0 °C was added 0.064 mL (0.88 mmol) of $SOCl_2$, after which the reaction mixture was allowed to come to room temperature. After the solution was stirred for 2 h, 0.05 mL (0.44 mmol) of 2-propoxyethanol was added, and the reaction mixture was stirred for another 1 h. The reaction mixture was diluted with water and extracted with chloroform. The organic layer was washed with water (twice) and brine, dried over $MgSO_4$, and evaporated. The residue was flash chromatographed on silica gel with ethyl acetate/petroleum ether (bp 40−60 °C) (2:3) and purified again on a TLC silica plate with petroleum ether (bp 40−60 °C)/chloroform/isopropyl alcohol (10:1:1) to give 43 mg (45%) of **(−)-(*R*)-1** as a yellow oil: 89% ee; $[\alpha]^{20}_D$ −18.7 (*c* 1.0, $CHCl_3$). The ^{1}H NMR ($CDCl_3$, 200 MHz) and mass spectral data were identical to those described for **(−)-(*R*)-1**. HRMS calcd for $C_{22}H_{27}F_2NO_6$ 439.1806, found 439.1800. Please note: the starting material **(−)-(*R*)-5b** was obtained from another CRL-catalyzed hydrolysis of **6f** where the degree of conversion was higher than 50%.

Synthesis and Chromatographic Separation of Epimers of Methyl 4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-5-({[(1*R*)-1-phenylethyl]amino}carbonyl)-1,4-dihydro-3-pyridinecarboxylate (*R*,*S*)-(+)-7 and (*R*,*R*)-(−)-7. To a solution of 141 mg (0.4 mmol) of **(±)-5b** in 0.2 mL of dry DMF was added 0.309 mL (2.4 mmol) of (*R*)-(+)-α-methylbenzylamine at room temperature, and the reaction mixture was stirred for 1 h, after which 180 mg (0.8 mmol) of 2-chloromethylpyridinium iodide was added. The reaction mixture was stirred overnight, diluted with chloroform, washed with water (three times) and brine, dried over $MgSO_4$, and evaporated. The remaining residue was flash chromatographed on silica gel with chloroform/petroleum ether (bp 40−60 °C)/isopropyl alcohol (9:7:1) to give 84 mg (46%) of **7** as a colorless viscous oil. The mixture of two diastereomers was separated by flash chromatography on Baker bond phase C18 with acetonitrile/water/acetic acid (60:40:0.1) to give the following.

Methyl (4*S*)-4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-5-({[(1*R*)-1-phenylethyl]amino}carbonyl)-1,4-dihydro-3-pyridinecarboxylate ((*R*,*S*)-(+)-7): yield 29 mg (16%) as colorless needles from a mixture of EtOH, $CHCl_3$, and hexane; mp 101−103 °C; $[\alpha]^{20}_D$ +63.2 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.29 (3H, d, J = 7.0 Hz), 2.19 (3H, s), 2.22 (3H, s), 3.51 (3H, s), 4.96 (1H, quintet, J = 7.0 Hz), 5.03 (1H, s), 5.65 (1H, br s), 6.51 (1H, dd, J_{H-F} = 69.8, 79.6 Hz), 6.51 (1H, br d, J = 7.0 Hz), 6.00−7.37 (9H, m); ^{13}C NMR (50 MHz, $CDCl_3$ + CD_3OD) δ 17.29 (CH_3), 18.38 (CH_3), 21.51 (CH_3), 33.69 (CH), 48.66 (CH), 50.31 (CH_3), 100.07 (C), 105.65 (C), 116.8 (CH, dd, J = 254.4, 259.4 Hz, $OCHF_2$), 117.79 (CH), 125.46 (two CH from the phenyl), 126.16 (CH), 126.49 (CH), 127.67 (CH), 128.04 (two CH from the phenyl), 130.52 (CH), 138.85 (C), 139.73 (C), 143.40 (C), 146.53 (C), 147.03 (C), 167.67 (C), 168.34 (C). MS *m*/*z* 456 (M^+); HRMS calcd for $C_{25}H_{26}F_2N_2O_4$ 456.1861, found 456.1860. This isomer was the first to elute from the C18 column.

Methyl (4*R*)-4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-5-({[(1*R*)-1-phenylethyl]amino}carbonyl)-1,4-dihydro-3-pyridinecarboxylate ((*R*,*R*)-(−)-7): yield 29 mg (16%) as colorless needles from a mixture of $CHCl_3$ and hexane; mp 142−145 °C; $[\alpha]^{20}_D$ −112.2 (*c* 1.0, MeOH) or $[\alpha]^{20}_D$ −146.0 (*c* 1.0, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.40 (3H, d, J = 7.0 Hz), 2.16 (3H, s), 2.20 (3H, s), 3.53 (3H, s), 4.96 (1H, quintet), 5.06 (1H), 5.82 (1H, br s), 6.42 (1H, dd, J_{H-F} = 70.2, 79.2 Hz), 6.52 (1H, br d, J = 7.0 Hz), 6.88−7.33 (9H, m); ^{13}C NMR ($CDCl_3$, 50 MHz) 19.36 (CH_3), 19.92 (CH_3), 22.17 (CH_3), 33.69 (CH), 49.07 (CH), 50.94 (CH_3), 101.87 (C), 105.74 (C), 116.8 (CH, dd, J = 254.1; 260.2 Hz, $OCHF_2$), 118.05 (CH), 125.92 (two CH from the phenyl), 126.68 (two CH from the phenyl), 128.15 (CH), 128.30 (two CH from the phenyl), 131.04 (CH), 139.11 (C), 140.98 (C), 144.04 (C), 145.74 (C), 146.51 (C), 166.56 (C), 168.00 (C); MS *m*/*z* 456 (M^+); HRMS calcd for $C_{25}H_{26}F_2N_2O_4$ 456.1861, found 456.1863. This isomer was the second to elute from the C18 column.

Crystal data for compound (*R*,*S*)-(+)-7: $C_{25}H_{26}F_2N_2O_4 \cdot CHCl_3$, M_r = 575.85, colorless crystal (0.05 × 0.10 × 0.30 mm), orthorhombic, space group $P2_12_12_1$ (no. 19) with a = 7.8831(10) Å, b = 14.619(2) Å, c = 23.101(3) Å, V = 2662.2(6) Å^3, Z = 4, D_x = 1.437 g cm^{-3}, 17 412 reflections measured, 6034 independent, R_{int} = 0.0651 (1.5° < θ < 27.5°, T = 150 K, Mo Kα radiation, λ = 0.71073 Å) on a Nonius KappaCCD diffractometer on rotating anode. The structure was solved by automated direct methods (SHELXS86) and refined on F^2 (SHELXL-97) for 344 parameters. Refinement converged at a final *wR*2 value of 0.1415, *R*1 = 0.0578 (for 3626 reflections with $I > 2\sigma(I)$), S = 1.061. The N−H hydrogen atom coordinates were included as parameters in the refinement; all other hydrogen atoms were included on calculated positions, riding on their carrier atoms. A final difference Fourier showed no residual density outside −0.61 and 0.48 e Å^{-3}.

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra of compounds **1**, **4a,b**, **5b**, **6a**−**g**, **(+)-(*R,R*)-7**, **(−)-(*R,S*)-7** and a copy of the ^{1}H NMR spectrum of compound **5a**; a Crystallographic Information File of compound **(+)-(*R,S*)-7**; an ORTEP(PLATON) plot of **(+)-(*R,S*)-7**, drawn at 50% probability level; and a drawing of the crystal lattice structure of this compound. This material is available free of charge via the Internet at http://pubs.acs.org.

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