Selectivity of Biohydroxylation with *Beauveria bassiana* of *trans*-2-Fluorocycloalkyl *N*-Phenylcarbamates

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Biohydroxylation with *Beauveria bassiana* of racemates and the pure enantiomers of *trans*-2-fluorocyclohexyl- **3** and *trans*-2-fluorocycloheptyl *N*-phenylcarbamates **6** were investigated and compared with results found for the corresponding nonfluorinated parent compounds. In all cases, mixtures of diastereomeric products hydroxylated in the 4-position were isolated, besides products of *p*-hydroxylation of the aromatic ring and succeeding compounds derived from these primary reaction products. The regioselectivity of hydroxylation by this fungus is not changed by a single fluorine substituent attached closely to the electron-rich anchoring group in the *trans*-2-position. There is a different influence on the diastereoselectivity of hydroxylation depending on the absolute configuration of the fluorinated substrates. While the transformation of the (*S*,*S*)-2-fluorocycloalkyl *N*-phenylcarbamates is not diastereoselective giving almost 1:1 mixtures of *cis*- and *trans*-4-hydroxyl compounds, the corresponding reactions of the (*R*,*R*)-isomers led preferentially to the products trans-hydroxylated in the 4-position. The transformation of the racemic fluorinated six-membered *N*-phenylcarbamate **3** led to products having a very small enantiomeric excess. The fluorine substituent slightly increased the enantioselectivity of transformation of the racemic seven-membered substrate **6** compared to the C_s-symmetric nonfluorinated carbamate. Thus, the fluorine substituent in the trans-2-position in these examples did not change the regioselectivity but rather influenced the stereochemistry of biotransformation, depending on the absolute configuration of the substrate and ring size.

Introduction

The selective hydroxylation of nonactivated hydrocarbon positions is continuing to attract the attention of organic chemists.[1] However, there are almost no chemical methods available[2] that can compete in selectivity with microbiological oxygenations involving mono-oxygenases[3] in whole-cell systems.[4] The fungus *Beauveria bassiana* is one of the most frequently used microorganisms for this purpose, and its biocatalytic reactions have been recently reviewed.[5] This fungus is known to hydroxylate, in addition to amides[4−6] and carbamates,[7−9] saturated N-containing heterocycles.[4a,10] Even saturated hydrocarbon positions of cycloalkanes, saturated steroids or terpenes, and side chains of aromatic hydrocarbons not having any electron-rich functional binding group may be hydroxylated.[5b] Moreover, it is well-known that this fungus can also *p*-hydroxylate aromatic rings of *N*-benzoylamines, *N*-phenylcarbamates,[11] or substituted heteroaromatics.[12] Thioethers have been shown to be

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† X-ray analyses.

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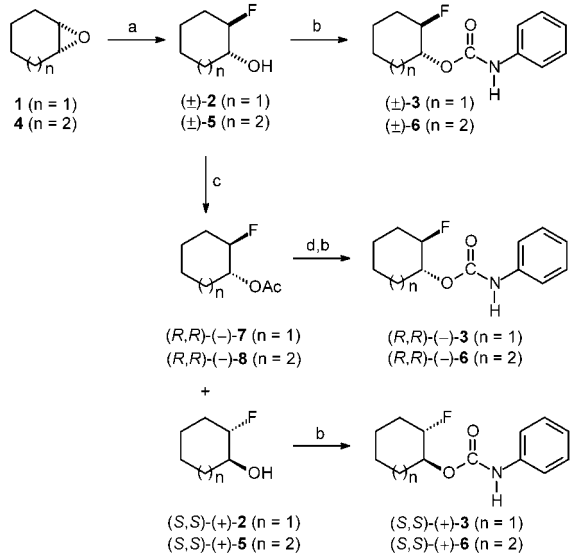
oxygenated to sulfoxides,[13] and epoxides were formed from olefins.[6a,10a] Even a Baeyer−Villiger oxidation of a ketone has been facilitated by this microorganism.[14] Recently, a new concept for anchoring/protecting groups has been introduced for hydroxylation reactions with *B. bassiana*.[15]

In 1997, we investigated the regio-, diastereo-, and enantioselectivity of hydroxylation by *B. bassiana* of cycloheptyl *N*-phenylcarbamate[7] and extended[8] the original distance models by Fonken et al.[6a] and Furstoss et al.[16] By using the racemic *trans*-2-fluorocycloheptyl *N*-phenylcarbamate, the first information regarding the influence of a single fluorine substituent attached neighbored to the anchoring group was obtained.[7] This is most interesting in connection with the enzyme-inhibiting abilities of several fluorinated compounds,[17] the well-known ability of the fluorine substituent to modify the electronic properties of molecules,[18] and the compound's ability to act as a hydrogen-bond acceptor.[19]

We now present our results on the biohydroxylation of the pure enantiomers of six- and seven-membered *trans*-2-fluorocycloalkyl *N*-phenylcarbamates.

Scheme 1[a]



[a] Reagents and conditions: (a) neat $Et_3N{\cdot}3HF$, 115 °C, 4 h for **1** and 155 °C, 4 h for **4**; (b) phenyl isocyanate, petroleum ether (110−140 °C), reflux, 4 h, recrystallization; (c) PCL, vinyl acetate, $(i\text{-Pr})_2O$, rt, 50% cv, (d), KOH, MeOH, rt.

Results and Discussion

The racemic fluorinated *N*-phenylcarbamates **3** and **6** were prepared in good yields from the corresponding epoxides **1** and **4** by ring opening with triethylamine trihydrofluoride ($Et_3N{\cdot}3HF$)[20] and subsequent treatment with the phenyl isocyanate of the fluorohydrins **2** and **5**. The corresponding enantiopure carbamates were available from the optically active fluorohydrins obtained by enzymatic deracemization using *Pseudomonas cepacia* lipase (PCL) and vinyl acetate in diisopropyl ether. The enantiomerically enriched fluorohydrins **2** and **5** were separated from the also-formed acetates **7** and **8**, which were hydrolyzed afterward.[21] Following treatment with phenyl isocyanate of both enantiomeric fluorohydrins, the enantiopure carbamates **3** and **6** were isolated after recrystallization from petroleum ether (Scheme 1). The optical purity of the carbamates was determined after reduction with $LiAlH_4$ in ether to the respective fluorohydrins **2** and **5** and subsequent gas-chromatographic analysis using a chiral β-cyclodextrin stationary phase.

All biotransformations of compounds **3** and **6** were done following a standard procedure using a 2 L fermenter with 200 mg of substrate per liter of a growing culture of *B. bassiana*. In a standard medium,[7] the culture with the substrate was aerated with 1.5 L air per minute at 30 °C for 72 h.

The biotransformation of the racemic *trans*-2-fluorocyclohexyl *N*-phenylcarbamate (±)-**3** using a fresh culture of *B. bassiana* gave six products. Some amount of starting material was always recovered (Scheme 2).

The two products (−)-**9** and (+)-**10**, hydroxylated exclusively in the 4-position, were formed in a 1:1 ratio as determined by ^{19}F NMR spectroscopy of the crude reaction product. The two compounds were isolated in 6.9% (6% ee) and 7.1% (33% ee) yield, respectively. The

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Scheme 2

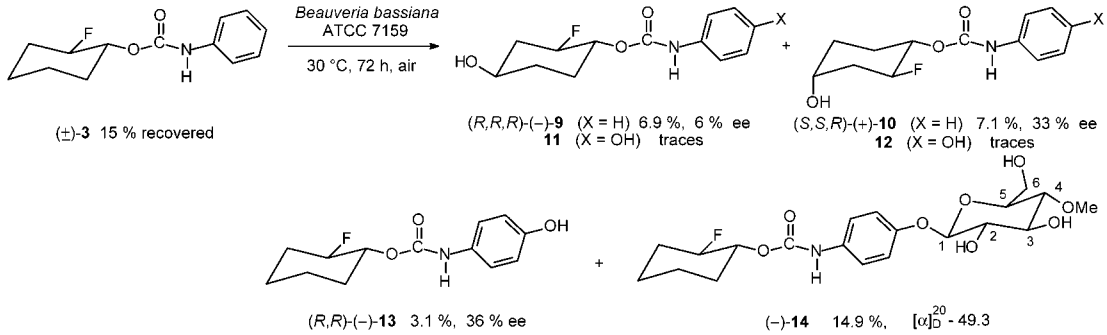

Table 1. Comparison of the Ratio (^{19}F NMR) of Products Hydroxylated in the Cyclohexane Ring and Isolated Yields of All Products Obtained by Biotransformation of *trans*-2-Fluorocyclohexyl *N*-Phenylcarbamate (±)-3 and the Pure Enantiomers (1*S*,2*S*)-(+)-3 or (1*R*,2*R*)-(−)-3

carbamate **3** (recovered (%))	product ratio (%)[a]		absolute configuration, yield [%], and enantiomeric excess (%)[b]				
	Σ **9** + **11**	Σ **10** + **12**	**9**	**10**	**11**	**12**	**13**
(*S*,*S*)-(+)-**3** [20]	49	51	(*S*,*S*,*S*)-(+)- [7.8]	(*S*,*S*,*R*)-(+)- [7.3]	n.d. [trace]	n.d. [trace]	(*S*,*S*)-(+)- [2.3]
(*R*,*R*)-(−)-**3** [13]	69	31	(*R*,*R*,*R*)-(−)- [12.3]	(*R*,*R*,*S*)-(−)- [3.4]	n.d. [trace]	n.d. [trace]	(*R*,*R*)-(−)- [5.8]
(±)-**3** [15]	50	50	(*R*,*R*,*R*)-(−)- [6.9] (6% ee)	(*S*,*S*,*R*)-(+)- [7.1] (33% ee)	n.d. trace	n.d. trace	(*R*,*R*)-(−)- [3.1] (36% ee)

[a] Determined from the crude product mixture by ^{19}F NMR. [b] The enantiomeric excesses (ee) of the transformation products of racemic **3** were determined by comparison of the measured optical rotations with those of the tranformation products of the pure enantiomers (cf. the Experimental Section).

structures of these products follow from their ^{1}H, ^{13}C, and ^{19}F NMR spectra and their mass spectra. These structures were further confirmed by X-ray analyses[22] of the enantiopure compounds obtained by biohydroxylation (vide infra) of (1*S*,2*S*)-(+)-2-fluorocyclohexyl *N*-phenylcarbamate (1*S*,2*S*)-(+)-**3** (cf. Figures 1 and 2 in the Supporting Information).

The enantiomeric excesses of all products were determined by comparison of the measured rotations with those of the products formed from the enantiopure carbamates (1*S*,2*S*)-(+)-**3** or (1*R*,2*R*)-(−)-**3**, respectively. Two succeeding products, **11** and **12**, which were derived most probably from the primary formed compounds (−)-**9** and (+)-**10** by para hydroxylation, were detected only in traces by ^{19}F NMR spectroscopy of the crude product mixture and by GC/MS coupling experiments of the silylated crude product mixture. The other two products, **13** (3.1% yield, 36% ee) and **14** (14.9% yield), were not hydroxylated in any alicyclic position at all, but were formed by para hydroxylation of the phenyl ring. The OH group was partially glycosylated afterward by the microorganism. However, this glycosylation was observed only in cases when a culture not older than 10 days was used. When we used a 5-month-old culture, the same products **9**−**13** were formed in very similar amounts as with the fresh culture, except that the glycoside **14** was absent. All of the isolated products that were hydroxylated in an alicyclic position bore the OH group at carbon 4. Furthermore, the isolated main hydroxylation products were optically active. The enantiomeric excesses were rather small in all cases, showing that there was a quite weak influence of the fluorine substituent. This fact would be expected if there is only a small geometric deviation from the C_s symmetry of the nonfluorinated parent carbamate. The diastereoselectivity of 9:1 in the nonfluorinated case[9b] changed to a 1:1 ratio of the respective cis/trans isomers **9** and **10** with regard to the relative configuration of the introduced hydroxyl group and the already existing carbamate function. Interestingly enough, studies on biotransformations of fluorinated steroids[23] and terpenes[24] using microorganisms other than *Beauveria bassiana*, when compared to the nonfluorinated parents, have demonstrated that a fluorine substituent frequently alters the regiochemistry of hydroxylation and occasionally also the enantioselectivity of hydroxylation.[25]

To check the influence of the fluorine in more detail, we investigated the biotransformation of both pure enantiomers. Treatment of (1*S*,2*S*)-(+)-**3** or (1*R*,2*R*)-(−)-**3** with a 5-month-old culture of *B. bassiana* gave the corresponding enantiopure products **9**−**13**. The ratio of diastereomeric products hydroxylated in the cyclohexane ring was determined by ^{19}F NMR spectroscopy of the crude product mixtures. The results are shown in Table 1, together with the isolated yields of products **9**−**13**.

From comparison of the results of the enantiopure compounds with the racemic **3**, one can conclude that the differences in transition-state energies and hence in the rates of hydroxylation of the enantiomers are not significantly different. Consequently, the enantiodifferentiation of the racemate is quite inefficient as indicated by the low enantiomeric excess in the products derived from the six-membered ring compound (±)-**3**.

(22) Details of the X-ray crystal structure analyses are available in the Supporting Information.

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Scheme 3

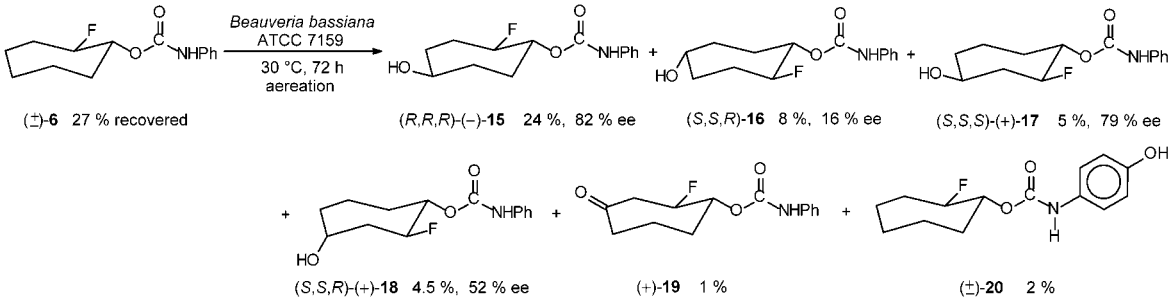

Table 2. Comparison of the Ratio (^{19}F NMR) of Products Hydroxylated in the Cycloheptane Ring and Absolute Configuration of Products of Biotransformation of *trans*-2-Fluorocycloheptyl *N*-Phenylcarbamate (±)-6[7] and the Pure Enantiomers (1*R*,2*R*)-(−)-6 or (1*S*,2*S*)-(+)-6

	absolute configuration, ratio, yield [%], and enantiomeric excess of products (%)[a]			
carbamates	**15**	**16**	**17**	**18**
(*R*,*R*)-(−)-**6**	(*R*,*R*,*R*)-(−)- 78	(*R*,*R*,*S*)-(−)- 15	(*R*,*R*,*R*)-(−)- 1	(*R*,*R*,*S*)-(−)- 6
(*S*,*S*)-(+)-**6**	(*S*,*S*,*S*)-(+)- 23	(*S*,*S*,*R*)-(+)- 34	(*S*,*S*,*S*)-(+)- 27	(*S*,*S*,*R*)-(+)- 16
(±)-**6**	(*R*,*R*,*R*)-(−)- 54, [24%] (82% ee)	n.d.[b] 24, [8%] (16% ee)	(*S*,*S*,*S*)-(+)- 14, [5%] (79% ee)	(*S*,*S*,*R*)-(+)- 8, [4.5%] (52% ee)

[a] The enantiomeric excesses (ee) of the transformation products of racemic **6** were determined by comparison of the measured optical rotations with those of the tranformation products of the pure enantiomers (cf. the Experimental Section). [b] Should be the (*S*,*S*,*R*)-enantiomer by comparison of the amounts of the respective **16** obtained by transformation of the pure enantiomers of **6**.

With the seven-membered compounds **6**, more pronounced effects have been observed. The products, isolated yields, and enantiomeric excesses of biotransformation of racemic *trans*-2-fluorocycloheptyl *N*-phenylcarbamate (±)-**6** have already been reported (Scheme 3).[7] Two *trans*-4-hydroxylated compounds (with regard to the haptophoric carbamate function), (−)-**15** (24% yield, 82% ee) and (+)-**17** (4.5% yield, 79% ee), were isolated. One of those was a 1,4-fluorohydrin, while the other was a 1,3-fluorohydrin, but both of them were hydroxylated in a formal 4-position in relation to the anchoring group. Moreover, two cis-configurated products, **16** (8% yield, 16% ee), which should be the (*S*,*S*,*R*)-enantiomer, and (+)-**18** (5% yield, 52% ee), were isolated having the same regiochemistry as the trans compounds. Furthermore, the 4-keto derivative (+)-**19** (1%, absolute configuration has not been determined) and the phenol **20** (2%), showing no rotation, were isolated. All compounds that were hydroxylated in the alicyclic ring were optically active and posessed higher enantiomeric excesses than the corresponding six-membered analogues.

We next determined the product ratio in the crude reaction mixture of treatment of both enantiomers of **6**. After transformation of (1*R*,2*R*)-(−)-**6** and (1*S*,2*S*)-(+)-**6** under standard conditions, all products isolated in our former study[7] were identified in the crude product mixture by ^{19}F NMR. Relative amounts and absolute configurations of the products are given in Table 2 together with the ratios, yields, and enantiomeric excesses of the products isolated in our earlier study.[7] In contrast to the transformation of the six-membered compounds **3**, no products hydroxylated both in an alicyclic and aromatic position were found in the product mixtures of the transformation of **6** under the same conditions.

Comparing the results of biotransformation of the enantiomers of the six- and the seven-membered ring *trans*-2-fluorocycloalkyl *N*-phenylcarbamates **3** and **6**, respectively, one can see that the two (*S*,*S*)-configurated compounds were formally hydroxylated nondiastereoselectively. From (*S*,*S*)-(+)-**3** a 49:51 ratio of trans (Σ **9** + **11**) and cis (Σ **10** + **12**) and from (*S*,*S*)-(+)-**6** a 50:50 ratio of trans (Σ **15** + **17**) and cis compounds (Σ **16** + **18**) was observed. In contrast, (*R*,*R*)-(−)-**3** gave a 69:31 ratio of trans and cis compounds, and for the seven-membered ring (*R*,*R*)-(−)-**6** this ratio is even 79:21. However, for the products of compounds **6** this is a formal ratio, because two different positions of the seven-membered ring have been hydroxylated in the two trans and the two cis compounds. Another comparison of transformation products of the seven-membered ring enantiomers is interesting, namely the regioselectivity of hydroxylation (positions 4 or 5, i.e., formation of 1,4-fluorohydrins **15** and **16** compared to 1,3-fluorohydrins **17** and **18**, respectively). While from (*R*,*R*)-(−)-**6**, 93% of the isomeric 2-fluoro-5-hydroxycycloheptyl *N*-phenylcarbamates **15** and **16** were formed, the transformation of (*S*,*S*)-(+)-**8** gave only 57% of the corresponding carbamates **15** and **16**. Furthermore, for the products of transformation of (±)-**3** only 6% ee and 33% ee were found for the trans or the cis products **9** and **10**, respectively. The enantiomeric excesses for the corresponding trans products **15** (82% ee) and **17** (79% ee) or the cis products **16** (16% ee) and **18** (52% ee) were larger, showing a stronger influence of the fluorine substituent in the transformation of the seven-membered ring compounds.

Conclusion

This study has shown that the fungus *B. bassiana* selectively monohydroxylates fluorinated cycloalkyl *N*-phenylcarbamates. Comparing these results with those of the nonfluorinated cyclohexyl-[9b] or cycloheptyl *N*-phenylcarbamates,[7] one can conclude that in case of the six-membered and the seven-membered substrates the regiochemistry of hydroxylation with respect to the anchoring group was not changed by fluorine substitution in the trans 2-position. The trans diastereoselectivity of

hydroxylation, which was 88:12 for the nonfluorinated compound,[9b] decreased to 69:31 for (*R*,*R*)-(−)-**3** and decreased to 49:51 for (*S*,*S*)-(+)-**3**. For the products of transformation of the nonfluorinated seven-membered ring carbamate a 92:8 ratio of trans to cis 4-hydroxylated compounds was found, considering that the ketone isolated in 12% yield was formed exclusively from *trans*-4-hydroxycycloheptyl *N*-phenylcarbamate.[7] From (*R*,*R*)-(−)-**6**, the *trans*-5-hydroxy derivative **15** was formed with a 84:16 selectivity over the cis isomer **16** and the *trans*-4-hydroxy derivative **17** was formed with a 16:84 selectivity compared to the cis isomer **18**. The reverse diastereoselectivity was observed for (*S*,*S*)-(+)-**6**. While the 5-hydroxy compounds **15** and **16** were formed in a 40:60 ratio, the 4-hydroxy isomers **17** and **18** were formed in a 63:37 ratio (cf. Table 2). Thus, the fluorine substituent in the trans 2-position to the electron-rich anchoring group in these examples did not change the regioselectivities, but influenced the stereochemistry of biotransformation differently, depending on the absolute configuration of the substrates.

Experimental Section

General Methods. ^{1}H NMR (300.1 MHz), ^{13}C NMR (75.5 MHz), and ^{19}F NMR spectra (282.3 MHz) were recorded from ca. 20% solutions in $CDCl_3$. Chemical shifts are reported as δ values (ppm) relative to TMS (^{1}H), $CDCl_3$ (^{13}C) or $CFCl_3$ (^{19}F), respectively, as internal standards. The multiplicity of ^{13}C signals was determined by the DEPT operation. Mass spectra (electron impact ionization, 70 eV) were recorded by GC/MS coupling. The enantiomeric excesses of the fluorohydrins **2** and **5** and the corresponding acetates **7** and **8** or the carbamates **3** and **6**, respectively, after hydrolysis were determined by chiral GC with FID using a β-cyclodextrin column, 30 m × 0.25 mm, 0.25 μm, , isotherm 96 °C for **2** and 111 °C for **5** , N_2 as carrier gas. The product ratio of microbial transformations were determined by ^{19}F NMR spectroscopy of the crude product mixtures. The products were separated by column chromatography (silica gel, 70−230 mesh, diethyl ether/pentane 1:1). Optical rotations were determined at Na_D line, λ = 589 nm. Microanalyses were carried out by the Mikroanalytisches Laboratorium, Organische Chemie, University of Münster.

The (±)-*trans*-2-fluorocycloalkanols have been prepared from the corresponding epoxides by ring opening with triethylamine trishydrogen fluoride ($Et_3N{\cdot}3HF$)[20] and cleaved by lipase-catalyzed deracemization as described previously.[21]

Synthesis of *N*-Phenylcarbamates 3 and 6 from Fluorohydrins. The racemic or enantiopure fluorohydrins **2** or **5** (4 mmol) were dissolved in petroleum ether (110−140 °C, 40 mL), and phenyl isocyanate (571 mg, 4.8 mmol) was added (syringe). The mixture was refluxed for 4 h and subsequently concentrated to ∼20 mL. This solution was stored in a refrigerator for 12−24 h, and the precipitate was separated and recrystallized from petroleum ether. To get the optically active carbamates with >98% ee, the carbamates **3** and **6** had to be recrystallized three to five times, depending on the ee of the fluorohydrin that was used in the preparation.

Determination of the Enantiopurity of the Carbamates 3 or 6. The enantiomeric excess has been determined using the fluorohydrins obtained by reduction of the carbamates **3** or **6**, respectively. Analogously to a known protocol,[26] the respective carbamate (0.15 mmol) was refluxed with $LiAlH_4$ (23 mg, 0.6 mmol) in diethyl ether (5 mL) for 5 h. After careful addition of water (5 mL), the phases were separated and the aqueous phase was extracted with diethyl ether (5 mL). The combined organic phase was dried ($MgSO_4$), the solvent was evaporated, and the residue was analyzed by chiral GC (β-cyclodextrin column, 30 m × 0.25 mm, 0.25 μm, isotherm, 91 or 107 °C, respectively, for the six- or the seven-membered ring fluorohydrins, N_2 as carrier gas).

(±)-*trans*-2-Fluorocyclohexyl *N*-Phenylcarbamate 3. Yield: 0.80 g (84%). Mp: 118 °C (petroleum ether). ^{1}H NMR: δ 1.21−1.85 (m, 6H), 2.04−2.23 (m, 2H), 4.44 (dddd, $^2J_{H,F}$ = 50.3 Hz, $^3J_{Ha,Ha}$ = 10.3 Hz, $^3J_{Ha,Ha}$ = 8.4 Hz, $^3J_{Ha,He}$ = 4.8 Hz, 1H), 4.79−4.90 (m, 1H), 6.77 (br s, 1H), 7.05 (t, $^3J_{H,H}$ = 7.3 Hz, 1H), 7.27 (t, $^3J_{H,H}$ = 7.9 Hz, 2H), 7.38 (br d, $^3J_{H,H}$ = 7.6 Hz, 2H). ^{13}C NMR: δ 22.8 (dt, $^3J_{C,F}$ = 10.2 Hz), 23.0 (t), 29.7 (dt, $^3J_{C,F}$ = 7.6 Hz), 30.4 (dt, $^2J_{C,F}$ = 17.8 Hz), 75.1 (dd, $^2J_{C,F}$ = 20.4 Hz), 92.1 (dd, $^1J_{C,F}$ = 178.0 Hz), 118.7 (2d), 123.4 (d), 129.0 (2d), 137.8 (s), 152.8 (s). ^{19}F NMR: δ −181.7 (d, $^2J_{F,H}$ = 47.7 Hz). GC−MS *m*/*z*: 237 (46), 150 (5), 138 (4), 137 (23), 132 (18), 119 (58), 118 (7), 94 (8), 93 (100), 91 (22), 81 (56), 77 (17), 72 (9), 64 (14), 59 (20), 57 (53), 55 (21), 51 (10), 41 (35).

(*S*,*S*)-(+)-3. Yield: 0.58 g (61%). Mp: 119 °C (petroleum ether). $[\alpha]^{22}_D$: +58.9 (*c* 1.0, $CHCl_3$), >98% ee. Anal. Calcd for $C_{13}H_{16}NO_2F$ (237.3): C, 65.81; H, 6.80; N, 5.90. Found: C, 65.78; H, 6.77; N, 6.09.

(*R*,*R*)-(−)-3. Yield: 0.63 g (66%). Mp: 118−119 °C (petroleum ether). $[\alpha]^{22}_D$: −57.8 (*c* 1.0, $CHCl_3$), >98% ee. Anal. Calcd for $C_{13}H_{16}NO_2F$ (237.3): C, 65.81; H, 6.80; N, 5.90. Found: C, 66.00; H, 6.60; N, 6.09.

(±)-*trans*-2-Fluorocycloheptyl *N*-Phenylcarbamate 6. Yield: 0.89 g (89%). Mp: 94 °C (petroleum ether). ^{1}H NMR: δ 1.38−2.05 (m, 10H), 4.62 (dm, $^2J_{H,F}$ = 48.4 Hz, 1H), 4.90−5.06 (m, 1H), 6.56 (br s, 1H), 7.04 (tt, $^3J_{H,H}$ = 6.9 Hz, $^4J_{H,H}$ = 1.3 Hz, 1H), 7.28 (t, $^3J_{H,H}$ = 8.0 Hz, 2H), 7.36 (dd, $^3J_{H,H}$ = 8.6 Hz, $^4J_{H,H}$ = 1.0 Hz, 2H). ^{13}C NMR: δ 21.8 (dt, $^3J_{C,F}$ = 7.6 Hz), 23.1 (t), 28.3 (t), 29.8 (dt, $^3J_{C,F}$ = 7.6 Hz), 30.9 (dt, $^2J_{C,F}$ = 20.3 Hz), 78.7 (dd, $^2J_{C,F}$ = 22.9 Hz), 95.8 (dd, $^1J_{C,F}$ = 172.9 Hz), 118.8 (2d, C-10), 123.5 (d), 129.1 (2d, C-11), 137.9 (s), 152.9 (s). ^{19}F NMR: δ −171.7 (m). GC−MS *m*/*z*: 252 (6), 251 (37), 231 (4), 138 (6), 137 (45), 124 (13), 119 (26), 95 (53), 93 (100), 91 (11), 77 (15), 73 (19), 67 (13), 57 (18), 55 (28), 43 (7), 41 (23).

(*S*,*S*)-(+)-6. Yield: 0.62 g (62%). Mp: 93−94 °C (petroleum ether). $[\alpha]^{22}_D$ +44.1 (*c* 1.0, $CHCl_3$), >98% ee. Anal. Calcd for $C_{14}H_{18}NO_2F$ (251.3): C, 66.91; H, 7.22; N, 5.57. Found: C, 66.84; H, 7.20; N, 5.91.

(*R*,*R*)-(−)-6. Yield: 0.59 g (59%). Mp: 94−95 °C (petroleum ether). $[\alpha]^{22}_D$: −44.2 (*c* 0.5, $CHCl_3$), >98% ee. Anal. Calcd for $C_{14}H_{18}NO_2F$ (251.3): C, 66.91; H, 7.22; N, 5.57. Found: C, 66.72; H, 7.16; N, 5.86.

Biotransformation with *B. bassiana*.[7] All media and equipment were sterilized in an autoclave at 121 °C and 2.3 bar for 20 min prior to use. The fungus *B. bassiana* ATCC 7159 was mobilized on potato dextrose agar at 30 °C in Petri dishes for 70 h. Subsequently, the vegetative cells of the light yellow culture were incubated in 75 mL of a culture medium made from 20 g/L corn steep liquor, pH 5.0 and 10 g/L glucose. This mixture was shaken (180 rpm) under nitrogen for 48 h. This culture broth was used to inoculate the main culture (1.425 L). The mixture was stirred (200 rpm) in a 2 L autoclave while being aerated with 1.5 L of air per min at 30 °C for 24 h. Then 300 mg of the respective carbamate **3** or **6** in DMF (3 mL) was added and fermented under the mentioned conditions for 72 h. Subsequently, the mycelium was separated by centrifugation. The mycelium was stirred with CH_2Cl_2 (300 mL) for 48 h and separated by filtration. The aqueous layer was continuously extracted with CH_2Cl_2 (200 mL) for 7 days. The combined organic layer was dried over $MgSO_4$, and the solvent was removed in a vacuum. The crude product mixture was analyzed by ^{19}F NMR spectroscopy and gas chromatography prior to separation by column chromatography (silica gel, cyclohexane/ethyl acetate, gradient). The partially resolved products were purified by HPLC, Nucleosil 50/7, $CHCl_3$/methanol, 50:1.

Biotransformation of (±)-*trans*-2-Fluorocyclohexyl *N*-Phenylcarbamate (±)-3. From the transformation of carbamate (±)-**3** (450 mg, 1.90 mmol) in addition to the transformation products **9**−**14** was recovered 69 mg (15%) of (+)-**3**. $[\alpha]^{20}_D$: +3.8 (c, 1.2, $CHCl_3$); 9% ee (calculated from the value of optical rotation of the pure enantiomer). The relative

(26) *Organikum*, 18th ed.; Deutscher Verlag der Wissenschaften: Berlin, 1990; p 430.

amounts of compounds **9**−**13** found in the crude product mixture are given in Table 1.

(1*R*,2*R*,4*R*)-(−)-2-Fluoro-4-hydroxycyclohexyl *N*-Phenylcarbamate (−)-9. Yield: 33 mg (6.9%). Mp: 162−164 °C (cyclohexane/ethyl acetate). $[\alpha]^{22}_D$: −2.8 (*c* 0.5, $CHCl_3$), 6% ee. ^{1}H NMR: δ 1.31−2.48 (m, 6H), 3.67−3.83 (m, 1H), 4.49 (dddd, $^2J_{H,F}$ = 49.8 Hz, $^3J_{Ha,Ha}$ = 10.7 Hz, $^3J_{Ha,Ha}$ = 8.4 Hz, $^3J_{Ha,He}$ = 4.8 Hz, 1H), 4.79−4.93 (m, 1H, 1-CH), 6.57 (br. s, 1H), 7.05 (tt, $^3J_{H,H}$ = 7.2 Hz, $^4J_{H,H}$ = 1.4 Hz, 1H), 7.29 (t, $^3J_{H,H}$ = 8.0 Hz, 2H), 7.36 (dd, $^3J_{H,H}$ = 7.9 Hz, $^4J_{H,H}$ = 1.2 Hz, 2H). ^{13}C NMR: δ 25.0 (dt, $^3J_{C,F}$ = 5.1 Hz), 32.0 (t), 39.0 (dt, $^2J_{C,F}$ = 15.3 Hz), 67.3 (dt, $^3J_{C,F}$ = 10.1 Hz), 74.4 (dd, $^2J_{C,F}$ = 20.3 Hz), 89.9 (dd, $^1J_{C,F}$ = 180.6 Hz), 118.8 (2d), 123.7 (d), 129.1 (2d), 137.6 (s), 152.7 (s). ^{19}F NMR: δ −183.2 (m). GC−MS *m*/*z*: 254 (4), 253 (29), 138 (4), 137 (16), 120 (6), 119 (22), 99 (56), 93 (100), 79 (17), 77 (17), 73 (18), 65 (15), 55 (10), 43 (9), 41 (30). Anal. Calcd for $C_{13}H_{16}NO_3F$ (253.3): C, 61.65; H, 6.37; N, 5.53. Found: C, 61.28; H, 6.38; N, 5.72.

(1*S*,2*S*,4*R*)-(+)-2-Fluoro-4-hydroxycyclohexyl *N*-Phenylcarbamate (+)-10. Yield: 34 mg (7.1%). Mp: 123 °C ($CHCl_3$). $[\alpha]^{22}_D$: +11.3 (c, 2.8, $CHCl_3$), 33% ee. ^{1}H NMR: δ 1.50−2.21 (m, 6H), 4.09−4.20 (m, 1H), 4.82 (dddd, $^2J_{H,F}$ = 33.6 Hz , $^3J_{Ha,Ha}$ = 8.6 Hz, $^3J_{Ha,Ha}$ = 7.4 Hz, $^3J_{Ha,He}$ = 4.3 Hz, 1H), 4.81−4.98 (m, 1H, 1-CH), 6.69 (br s, 1H), 7.05 (tt, $^3J_{H,H}$ = 7.3 Hz, $^4J_{H,H}$ = 1.4 Hz, 1H), 7.29 (tt, $^3J_{H,H}$ = 7.9 Hz, $^4J_{H,H}$ = 1.9 Hz, 2H), 7.37 (dd, $^3J_{H,H}$ = 8.5 Hz, $^4J_{H,H}$ = 1.2 Hz, 2H). ^{13}C NMR: δ 24.2 (dt, $^3J_{C,F}$ = 3.9 Hz), 30.0 (t), 36.9 (dt, $^2J_{C,F}$ = 18.5 Hz), 66.1 (dt, $^3J_{C,F}$ = 8.7 Hz), 73.1 (dd, $^2J_{C,F}$ = 21.6 Hz), 89.5 (dd, $^1J_{C,F}$ = 174.9 Hz), 118.7 (2d), 123.6 (d), 129.1 (2d), 137.7 (s), 152.7 (s). ^{19}F NMR: δ −189.0 (m). GC−MS *m*/*z*: 254 (6), 253 (38), 138 (4), 137 (20), 120 (7), 119 (46), 117 (2), 99 (26), 93 (100), 91 (5), 77 (16), 69 (14), 65 (8), 57 (7), 43 (6), 41 (24). High-resolution MS: calcd for $C_{13}H_{16}NO_3F$ 253.1114, found 253.1102.

(1*R*,2*R*)-(−)-2-Fluorocyclohexyl *N*-(*p*-Hydroxyphenyl)-carbamate (−)-13. Yield: 15 mg (3.1%). $[\alpha]^{23}_D$: −16.7 (c, 1.7, $CHCl_3$). ^{1}H NMR: δ 1.18−1.82 (m, 6H), 2.02−2.20 (m, 2H), 4.42 (dddd, $^2J_{H,F}$ = 50.5 Hz, $^3J_{Ha,Ha}$ = 10.2 Hz, $^3J_{Ha,Ha}$ = 8.4 Hz, $^3J_{Ha,He}$ = 4.8 Hz, 1H), 4.72−4.88 (m, 1H), 6.50 (br s, 1H), 6.73 (dt, $^3J_{H,H}$ = 9.5 Hz, $^4J_{H,H}$ = 2.8 Hz, 2H), 7.17 (br d, $^3J_{H,H}$ = 8.8 Hz, 2H). ^{13}C NMR: δ 22.9 (dt, $^3J_{C,F}$ = 7.5 Hz), 23.1 (t), 29.8 (dt, $^3J_{C,F}$ = 5.1 Hz), 30.5 (dt, $^2J_{C,F}$ = 17.8 Hz), 75.2 (dd, $^2J_{C,F}$ = 17.8 Hz), 92.2 (dd, $^1J_{C,F}$ = 178.0 Hz), 115.8 (2d), 121.6 (2d), 130.5 (s), 152.5 (s) 153.7 (s). ^{19}F NMR: δ −181.7 (d, $^2J_{F,H}$ = 50.5 Hz). GC−MS *m*/*z*: 255 (0.5), 254 (10), 253 (77), 154 (4), 153 (63), 140 (12), 135 (22), 110 (8), 109 (100), 108 (24), 107 (6), 82 (7), 81 (71), 79 (10), 65 (4), 59 (16), 55 (21), 53 (15), 52 (9), 51 (4), 41 (38). High-resolution MS: calcd for $C_{13}H_{16}NO_3F + NH_4^+$ 271.1458, found 271.1440.

(−)-*trans*-2-Fluorocyclohexyl *N*-[*p*-(4′-Methyl-β-D-glucopyranosyl)]phenylcarbamate (−)-14. Yield: 121 mg (14.9%). Mp: 186−188 °C (cyclohexane/ethyl acetate). $[\alpha]^{22}_D$: −49.3 (*c* 0.5, methanol). ^{1}H NMR: δ 1.26−2.25 (m, 8H), 3.23 (dd, $^3J_{Ha,Ha}$ = 9.8 Hz, $^3J_{Ha,Ha}$ = 9.5 Hz), 3.38−3.94 (m, 6H), 3.63 (s, 3H), 4.50 (dddd, $^2J_{H,F}$ = 50.3 Hz, $^3J_{Ha,Ha}$ = 10.0 Hz, $^3J_{Ha,Ha}$ = 8.6 Hz, $^3J_{Ha,He}$ = 4.8 Hz, 1H), 4.69−4.89 (m, 1H), 7.08 (dt, $^3J_{H,H}$ = 9.1 Hz, $^4J_{H,H}$ = 2.7 Hz, 2H), 7.37 (br d, $^3J_{H,H}$ = 8.8 Hz, 2H). ^{13}C NMR: δ 24.1 (dt, $^3J_{C,F}$ = 10.2 Hz), 24.4 (t, C-5), 31.0 (dt, $^3J_{C,F}$ = 5.1 Hz), 31.8 (dt, $^2J_{C,F}$ = 17.8 Hz), 61.1 (q), 62.4 (t), 75.3, 77.4, 78.3, 80.9 (4d), 72.2 (dd, $^2J_{C,F}$ = 20.3 Hz), 93.6 (dd, $^1J_{C,F}$ = 175.5 Hz), 103.0 (d), 118.6 (2d), 121.8 (2d), 135.0 (s), 155.3 (s) 155.9 (s). ^{19}F NMR: δ −180.9 (d, $^2J_{F,H}$ = 53.4 Hz). ESI-MS, nanospray, *m*/*z*: 474 (40), 428 (100), 310 (18), 133 (13).

To determine the structure of (−)-**14**, this compound was acetylated and the structure of the product was determined.

(−)-*trans*-2-Fluorocyclohexyl *N*-[*p*-(4′-Methyl-β-D-glucopyranoacetyl)]phenylcarbamate. Compound (−)-**14** (22 mg, 0.05 mmol) was dissolved in acetic anhydride (1 mL, 10 mmol) and pyridine (2 mL, 25 mmol) and stored at rt for 12 h. Pyridine and acetic anhydride were removed in vacuo, and the residue was dissolved in CH_2Cl_2. The solution was washed with 2 N HCl (5 × 2 mL) and dried ($MgSO_4$) overnight. The solvent was removed, and the residue was filtered through a short silica gel column using cyclohexane/ethyl acetate 4:1 as an eluent. Yield: 27 mg (97%). $[\alpha]^{20}_D$: −22.9 (*c* 1.2, $CHCl_3$). ^{1}H NMR: δ 1.15−2.18 (m, 8H), 2.03 (s, 3H), 2.06 (s, 3H), 2.07 (s, 3H), 3.42 (t, $^3J_{Ha,Ha}$ = 7.1 Hz, 1H), 3.42 (s, 3H), 3.56−3.69 (m, 1H), 4.24 (dd, $J_{H,H}$ = 11.9 Hz, $J_{H,H}$ = 5.5 Hz, 1H), 4.39 (dd, $J_{H,H}$ = 11.9 Hz, $J_{H,H}$ = 2.4 Hz, 1H), 4.41 (dddd, $^2J_{H,F}$ = 52.6 Hz, $^3J_{Ha,Ha}$ = 10.3 Hz, $^3J_{Ha,Ha}$ = 8.3 Hz, $^3J_{Ha,He}$ = 4.8 Hz, 1H), 4.72−4.86 (m, 1H), 4.94 (d, $^3J_{Ha,Ha}$ = 7.6 Hz, 1H), 5.10 (dd, $^3J_{Ha,Ha}$ = 9.5 Hz, $^3J_{Ha,Ha}$ = 7.6 Hz, 1H), 5.21 (t, $^3J_{Ha,Ha}$ = 9.2 Hz, 1H), 6.59 (br s, 1H), 6.91 (dt, $^3J_{H,H}$ = 9.1 Hz, $^4J_{H,H}$ = 2.7 Hz, 2H), 7.26 (br d, $^3J_{H,H}$ = 9.1 Hz, 2H). ^{13}C NMR: δ 20.7, 20.8, 20.9 (3q), 22.9 (dt, $^3J_{C,F}$ = 10.1 Hz), 23.1 (t), 29.8 (dt, $^3J_{C,F}$ = 5.8 Hz), 30.5 (dt, $^2J_{C,F}$ = 18.4 Hz), 60.3 (q), 62.8 (t), 71.8 (d), 73.1 (d), 74.9 (d), 77.7 (d), 75.3 (d), 92.2 (dd, $^1J_{C,F}$ = 178.1 Hz), 99.6 (d), 117.9 (2d), 118.9 (2d), 132.3 (s), 153.0 (s) 153.2 (s), 169.7, 170.0, 170.6, (3s). ^{19}F NMR: δ −181.8 (d, $^2J_{F,H}$ = 53.4 Hz). ESI-MS, nanospray, *m*/*z*: 554 (12), 437 (25), 436 (100), 394 (8), 352 (5), 301 (13), 232 (13).

***cis*- and *trans*-2-Fluoro-4-hydroxycyclohexyl *N*-(*p*-Hydroxyphenyl)carbamates 11 and 12.** In the proton-decoupled ^{19}F NMR spectra of the crude product mixtures of biotransformations of **3** two more signals, δ = −182.7 and δ = −188.7 ppm, were detected (both <1% of the total integral of all fluorinated compounds) very close to the signals of products **9** (−183.2) or **10** (−189.0), respectively. These signals were assigned to compounds **11** and **12**, respectively. The structures of these compounds are supported by the fact that in the mass spectra of a silylated sample of the crude product mixture (after treatment with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide) the molecular ions of the disilylated compounds **11** and **12** were identified. Compound **11**. GC−MS *m*/*z*: 415 (8), 414 (10), 413 (27), 282 (5), 225 (20), 207 (100), 192 (21), 181 (13), 180 (6), 134 (4), 111 (4), 99 (10), 97 (6), 79 (11), 75 (16), 73 (43), 69 (7), 45 (9). Compound **12**. GC−MS *m*/*z*: 413 (27), 304 (2), 303 (8), 253 (21), 225 (18), 207 (34), 192 (75), 189 (27), 169 (39), 129 (8), 107 (10), 99 (57), 97 (72), 79 (83), 73 (100), 69 (36), 67 (16), 55 (14), 41 (19).

Transformation of (1*S*,2*S*)-(+)-2-Fluorocyclohexyl *N*-Phenylcarbamate (+)-3. From the transformation of carbamate (+)-**1** (450 mg, 1.90 mmol) in addition to the transformation products **9**−**13** was recovered 91 mg (20%) of (+)-**3**. The spectroscopic data of the isolated products agree with those determined for the products obtained by biotransformation of racemic **3**.

(1*S*,2*S*,4*S*)-(+)-2-Fluoro-4-hydroxycyclohexyl *N*-Phenylcarbamate (+)-9. Yield: 37 mg, (7.8%). Mp: 163−164 °C (cyclohexane/ethyl acetate). $[\alpha]^{20}_D$: +51.0 (*c* 1.0, $CHCl_3$). High-resolution MS: calcd for $C_{13}H_{16}NO_3F + NH_4^+$ 271.1458, found 271.1461.

(1*S*,2*S*,4*R*)-(+)-2-Fluoro-4-hydroxycyclohexyl *N*-Phenylcarbamate (+)-10. Yield: 35 mg (7.3%). Mp: 122 °C ($CHCl_3$). $[\alpha]^{22}_D$: +34.4 (*c* 1.2, $CHCl_3$). High-resolution MS: calcd for $C_{13}H_{16}NO_3F + NH_4^+$ 271.1458, found 271.1441.

(1*S*,2*S*)-(+)-2-Fluorocyclohexyl *N*-(*p*-Hydroxyphenyl)-carbamate (+)-13. Yield: 11 mg (2.3%). $[\alpha]^{22}_D$: +46.2 (*c* 0.6, $CHCl_3$). High-resolution MS: calcd for $C_{13}H_{16}NO_3F + NH_4$: 271.1458, found 271.1409.

Transformation of (1*R*,2*R*)-(−)-2-Fluorocyclohexyl *N*-Phenylcarbamate (−)-3. From the transformation of carbamate (−)-**3** (450 mg, 1.90 mmol) in addition to the transformation products **2**−**6** was recovered 59 mg (13%) of (−)-**3**. The spectroscopic data of the isolated products agree with those determined for the products obtained by biotransformation of racemic **3**.

(1*R*,2*R*,4*R*)-(−)-2-Fluoro-4-hydroxycyclohexyl *N*-Phenylcarbamate (−)-9. Yield: 59 mg (12.3%). Mp: 159−161 °C (cyclohexane/ethyl acetate). $[\alpha]^{20}_D$: −49.6 (*c* 1.0, $CHCl_3$). High-resolution MS: calcd for $C_{13}H_{16}NO_3F + H^+$ 254.1192, found 254.1177.

(1*R*,2*R*,4*S*)-(−)-2-Fluoro-4-hydroxycyclohexyl *N*-Phenylcarbamate (−)-10. Yield: 16 mg (3.4%). Mp: 122 °C ($CHCl_3$). $[\alpha]^{22}_D$: −33.2° (*c* 0.5, $CHCl_3$). High-resolution MS: calcd for $C_{13}H_{16}NO_3F$ 253.1142, found 253.1144.

(1*R*,2*R*)-(−)-2-Fluorocyclohexyl *N*-(*p*-Hydroxyphenyl)-carbamate (−)-13. Yield: 28 mg (5.8%). $[\alpha]^{22}_D$: −49.9° (*c* 0.9,

$CHCl_3$). High-resolution MS: calcd for $C_{13}H_{16}NO_3F + NH_4^+$ 271.1458, found 271.1424.

Biotransformation of (±)-*trans*-2-Fluorocycloheptyl *N*-Phenylcarbamate (±)-6. From the transformation of carbamates (±)-**6**, (+)-**6**, and (−)-**6** (450 mg, 1.79 mmol) with *B. bassiana* under standard conditions, in addition to some amount of starting material, the transformation products **15**−**18** were detected in the ^{19}F NMR spectra of the crude product mixtures. The relative amounts of compounds **15**−**18** determined by integration of the signals in the ^{19}F NMR spectra are given in Table 2. The structures of the products have already been determined.[7]

X-ray crystal structure analysis of (*S*,*S*,*S*)-(+)-9: formula $C_{13}H_{16}FNO_3$, $M = 253.27$, colorless crystal 0.20 × 0.20 × 0.20 mm, $a = 9.162(1)$ Å, $b = 11.137(1)$ Å, $c = 12.587(1)$ Å, $\beta = 99.20(1)°$, $V = 1267.8(2)$ Å^3, $\rho_{calcd} = 1.327$ g cm^{-3}, $\mu = 8.71$ cm^{-1}, empirical absorption correction via ψ scan data ($0.845 \le T \le 0.845$), $Z = 4$, monoclinic, space group $P2_1$(No. 4), $\lambda = 1.541\,78$ Å, $T = 223$ K, $\omega/2\theta$ scans, 2880 reflections collected ($+h, +k, \pm l$), $[(\sin\theta)/\lambda] = 0.62$ Å^{-1}, 2712 independent ($R_{int} = 0.032$) and 2519 observed reflections [$I \ge 2\sigma(I)$], 334 refined parameters, $R = 0.034$, $wR^2 = 0.093$, maximum residual electron density 0.18 (−0.17) e Å^{-3}, Flack parameter 0.0(2), two almost identical independent molecules in the asymmetric unit, connected by hydrogen bonds, hydrogen atoms at nitrogen atoms from difference Fourier calculations, others calculated, all refined as riding atoms.

X-ray crystal structure analysis of (*S*,*S*,*R*)-(+)-10: formula $C_{13}H_{16}FNO_3$, $M = 253.27$, colorless crystal 0.30 × 0.25 × 0.15 mm, $a = 8.783(2)$ Å, $b = 12.746(3)$ Å, $c = 22.848(6)$ Å, $\beta = 93.54(2)°$, $V = 2552.9(11)$ Å^3, $\rho_{calcd} = 1.318$ g cm^{-3}, $\mu = 8.66$ cm^{-1}, empirical absorption correction via ψ scan data ($0.781 \le T \le 0.881$), $Z = 8$, monoclinic, space group $P2_1/n$ (No. 14), $\lambda = 1.541\,78$ Å, $T = 223$ K, $\omega/2\theta$ scans, 5310 reflections collected ($\pm h, +k, +l$), $[(\sin\theta)/\lambda] = 0.62$ Å^{-1}, 5182 independent ($R_{int} = 0.151$) and 3941 observed reflections [$I \ge 2\sigma(I)$], 354 refined parameters, $R = 0.047$, $wR^2 = 0.131$, max. residual electron density 0.22 (−0.23) e Å^{-3}, fluorine atoms disordered between the C2/C6 (C22/C26) positions with the ratios of 0.85(1):0.15 (0.53(1):0.47), two almost identical independent molecules in the asymmetric unit, connected by hydrogen bonds, hydrogen atoms at nitrogen atoms from difference Fourier calculations, others calculated, all refined as riding atoms.

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Supporting Information Available: ^1H, ^{13}C, and ^{19}F NMR spectral data including assignments of the signals and the EI mass spectral data of the carbamates **3** and **6** and their transformation products, and details of the X-ray crystal structure analyses of (*S*,*S*,*S*)-(+)-**9** and (*S*,*S*,*R*)-(+)-**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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