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Microbial Oxygenation of Cycloheptyl-*N*-phenylcarbamate and (±)-*trans*-2-Fluorocycloheptyl-*N*-phenylcarbamate with *Beauveria bassiana*

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Abstract: The title biotransformation of **1** gave *trans*-4-hydroxycyclohept-1-yl-carbamate (**3**, 10%, 62% ee), its consecutive ketone **5** (12%) and the racemic *cis*-isomer **4** (1.8%). Similarly, the racemic *trans*-2-fluoro analogue **2** yielded mainly four optically active alcohols **7** (24%,, 82% ee), **8** (8%, 16% ee), **9** (4.5%, 52% ee) and **10** (5%, 79% ee) by partial kinetic resolution. The fluorine substituent near the anchoring electron rich group caused a remarkable drop in regio- and diastereoselectivity, but increased the enantioselectivity. Obviously, there is not simply an isosteric substitution of a hydrogen in this position, but the electronic effect by fluorine strongly influences the binding mode. Probably **1** and the two enantiomers of **2** can be attached in different geometry to the active site of the enzyme.
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INTRODUCTION

The selective oxygenation of hydrocarbon positions is a major topic in organic synthesis.[1] Much of the relevant work is aimed to introduce a hydroxyl group to a specific position remote from an already existing hetero substituent in the molecule. Frequently, a hydroxylation mediated by monooxygenases in whole cell systems is the method of choice.[2] The generally high regio-, diastereo-, and enantioselectivity of such enzymes is usually not achieved with acceptable overall yields by convenient chemical approaches.[1] In particular, the fungus *Beauveria bassiana*[3] is a versatile microbiological oxidant for this type of oxygenation. Transformations of relatively simple substrates bearing amide, sulfonate, lactam, carbamate or azide functions[4] are well documented over the past 30 years.[5] *B. bassiana* is known to prefer *trans*-hydroxylation[5a,d,f,g] with respect to an existing electron rich substituent in the substrate.[4d] This part is responsible for the initial binding to the active site of the monooxygenase and its nature has been shown to effect the regio- and diastereoselectivity of the hydroxylation.[5b] Anchoring substituents like amido,[5a,b,d] and carbamato[4a] in contrast to sulfonato[5a] govern enantioselective hydroxylation in a specific distance[4d] from these groups.

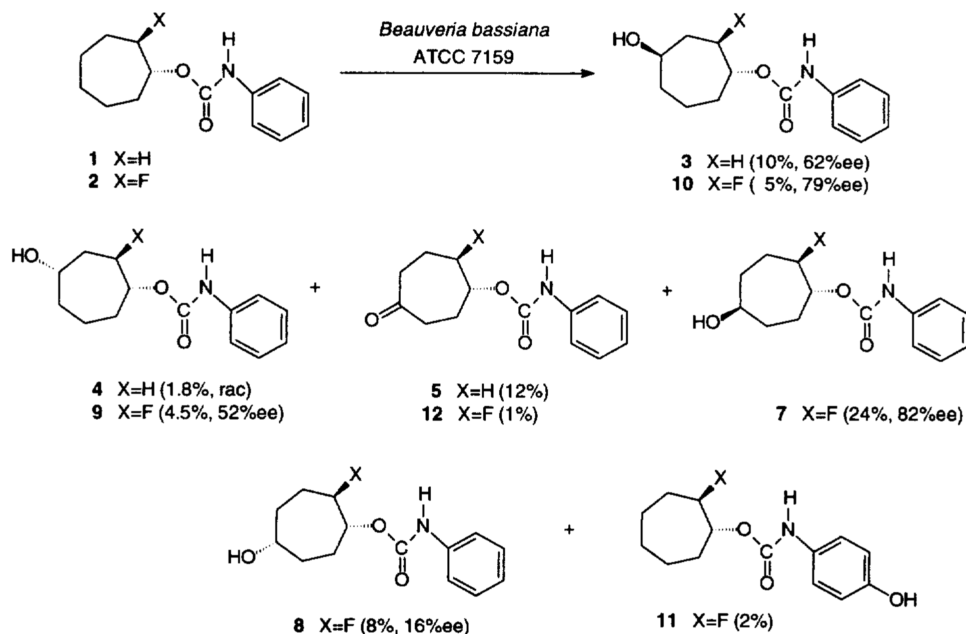
We were interested to study the hydroxylation of a prochiral cycloalkyl-*N*-phenylcarbamate, which should allow in principle the regio-, diastereo-, and enantioselective oxygenation by *B. bassiana*. Therefore, we have chosen the *N*-phenylcarbamate **1**, containing a seven-membered ring. Moreover, we investigated the influence of a fluorine substituent near to the electron rich group binding to the enzyme's active site on the selectivity of the oxygenation. Fluorine is the second smallest substituent and can mimic a hydrogen atom in its steric requirement.[6] Thus, a comparative study on microbial oxygenation of **1** and **2** would allow to consider both, the ability of this fungus for asymmetric hydroxylation of prochiral **1** as well as kinetic resolution of racemic **2** by oxygenation. Since fluorine is the most electronegative of all elements, the electronic properties of neighboured groups can be modified significantly.[7] In particular, the negatively charged fluorine can act as a hydrogen bond acceptor.[6a,8] Therefore, the 2-position is suggested to be most

interesting for the replacement of hydrogen by fluorine and (±)-*trans*-2-fluorocycloheptyl-*N*-phenylcarbamate (**2**) has been chosen. Interestingly enough, studies on other monooxygenase mediated hydroxylations of fluorinated steroids[9] and terpenes[10] compared to the parent compounds have demonstrated that fluorine substituents in other positions frequently alters the regiochemistry. However, there is less known on changes of the stereochemistry.

On the other hand, it is well known that organofluorine chemistry is a rapidly developing field of research.[6,7] Presently, the synthetic access to enantiomerically enriched fluorinated compounds is limited.[11] Biotransformations, especially enzymatic resolutions of fluorinated esters by lipases or esterases,[12] and microbial reductions of fluoro ketones[13] have proven to be versatile methods for the synthesis of optically active substances.[14] The majority of the published results on microbial transformations of fluorinated materials include the above mentioned analogues of natural products[9,10] and fluoroaromatics.[15] These studies, however, are not focused on synthesis of optically active compounds. To the best of our knowledge, there are no reports on biooxygenation of fluorinated compounds by *B. bassiana*.

RESULTS AND DISCUSSION

Cycloheptyl-N-phenylcarbamate. The *N*-phenylcarbamate **1** is readily available (86% yield) by addition of cycloheptanol to phenylisocyanate. Oxygenation of **1** (200 mg/L) with *B. bassiana* gave the optically active alcohol **3** (10%), its racemic epimer **4** (1.8%), and ketone **5** (12%) (Scheme 1). Moreover, 24% of **1** was recovered.



Scheme 1

The *trans*-configuration of carbamoyl and hydroxyl substituents in **3** was determined by X-ray crystal diffraction (Figure 1)[16].

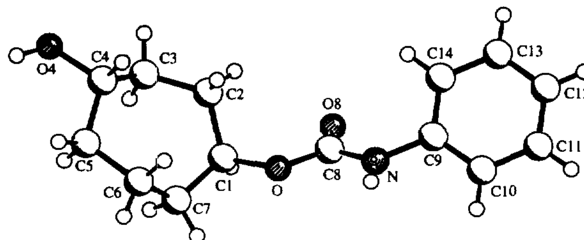


Figure 1: X-Ray structure[16] of *trans-4*-hydroxycycloheptyl-*N*-phenylcarbamate (**3**)

Alcohol **3** exhibited an enantiomeric excess of 62%, determined by Mosher's method.[17] The minor *cis*-alcohol **4** could not be obtained in a pure form (contamined by about 40% of **3**). The structure was deduced by comparison of their ^{1}H and ^{13}C NMR spectra to be epimeric with **2**. The signals of C(4) (bearing the hydroxyl group) appear at δ 71.9 ppm for **3** and at δ 71.5 ppm for **4** and correlate to the respective multiplets at δ 3.89 ppm in the ^{1}H NMR spectra. Only a minor substituent effect has been observed on the chemical shift of C(1) (bearing the carbamoyl group) at δ 75.24 ppm for **3** and at δ 74.98 ppm for **4**. This is quite similar to the value of δ 75.15 ppm for **1**, which supports the hydroxylation position at C(4).

The ^{13}C signal of the carbonyl carbon of **5** appears at δ 213.38 ppm and it's position relative to C(1) has been proved by X-ray crystal diffraction (Figure 2). The ketone **5** had an optical rotation of $[\alpha]_D$ +6.1°. In order to prove that ketone **5** was derived from alcohol **3** and/or **4** by a dehydrogenase reaction,[18] a sample of **3** (62% ee) was oxidised with Jones' reagent.[19] The resulting ketone exhibited a smaller optical rotation of $[\alpha]_D$ +4.5°. This result indicates that **5** formed in the biotransformation of **1** is obviously derived mainly, if not exclusively, from *trans*-alcohol **3** by a highly enantioselective dehydrogenase reaction.

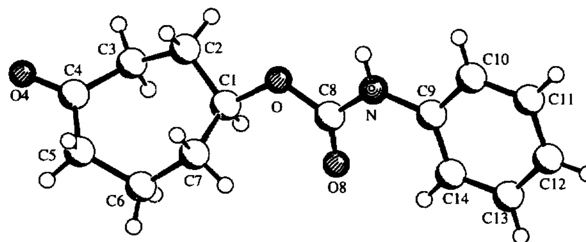


Figure 2: X-Ray structure[16] of 4-oxocycloheptyl-*N*-phenylcarbamate (**5**)

In conclusion, the highly asymmetric microbial oxygenation of prochiral **1** occurred regioselectively at the 4-position relative to the carbamate moiety choosing mainly one of the diastereotopic faces of one of the enantiotopic methylene groups. The remarkable discrimination (1:12) of both diastereotopic pseudo-equatorial hydrogens at C(4) and C(5) relative to the carbamate moiety in **1** by *B. bassiana* will be discussed in more detail in the following publication in this issue.

trans-2-Fluorocycloheptyl-N-phenylcarbamate. *trans*-2-Fluorocycloheptyl-*N*-phenylcarbamate (**2**) was prepared (56% overall yield) from the fluorohydrin **6** by reaction with phenylisocyanate. **6** was obtained from 8-oxabicyclo[5.1.0]octane by ring opening[20] using triethylamine trishydrofluoride[21] ($Et_3N{\cdot}3HF$).

Biotransformation of the racemic fluorinated carbamate **2** (200 mg/L) with *B. bassiana* gave mainly four isomeric alcohols **7** (24%, 82% ee), **8** (8%, 16% ee), **9** (4.5%, 52% ee) and **10** (5%, 79% ee) resulting from hydroxylation of the carbocyclic system in 4 or 5 position, respectively. The enantiomeric excess was determined, after chromatographic separation, ^{19}F NMR spectroscopically by means of Mosher's method.[17] Additionally, 2% of the *p*-hydroxylated product **11** and 1% of the ketone **12** were identified and 27% of **2** was recovered (Scheme 1). The ability of *B. bassiana* to *p*-hydroxylate other carbamates has been previously reported by Furstoss and co-workers.[4a-c] The separation of the alcohols was achieved by HPLC using a preparative, chiral reversed phase column. Fluorohydrin **8** could, however, not been isolated in a pure form and was contaminated with about 34% of **7**. Moreover, a lack of about 30% in product yields of the reaction of **2** and even 50% in the transformation of **1** suggests the formation of hydrophilic degradation products and probably the formation of glycoside conjugates[4,22] of the *p*-hydroxylated product **11**.

The structure of the isolated products **7-12** was mainly established by their ^{1}H, ^{13}C and ^{19}F NMR data. The stereochemistry of the major product **7** was confirmed also by X-ray single crystal diffraction (Figure 3).

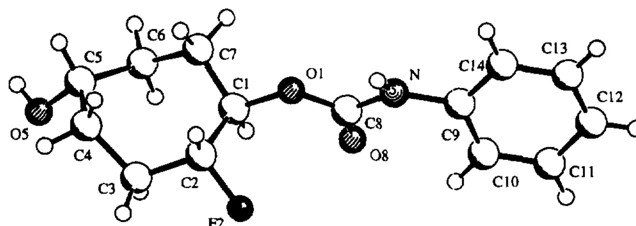


Figure 3: X-Ray structure[16] of *t*-2-fluoro-*t*-5-hydroxycycloheptyl-*r*-*N*-phenylcarbamate (**7**)

The fluorine atom and the carbamate moiety in **7** occupy equatorial positions, while the pseudo equatorial hydroxyl group at C(5) is *trans*-configurated relative to the carbamate moiety. The structural assignment of the epimeric alcohol **8** was deduced mainly from its ^{13}C and ^{19}F NMR data, which were found to be similar to those of **7**. The absence of any significant γ-*anti* substituent effect of an hydroxyl group on the chemical shift of C(1) at δ 78.2 ppm in **7** and at δ 77.9 ppm in **8** compared to δ 78.7 ppm in **2**, indicates that the hydroxyl group should be bound to C(5), and not to C(6) in **8**. Moreover, the lack of ^{13}C^{19}F couplings of the carbons bearing the hydroxyl groups at δ 70.5 ppm in **7** and δ 69.5 ppm in **8** prove the assignment of the hydroxylation to C(5), and not to C(3) conclusively also for **8**. The expected doublets for C(4) due to $^{3}J_{C,F}$ coupling in both **7** and **8** appear at δ 24.8 ppm ($^{3}J_{C,F}$ = 7.6 Hz) and δ 24.3 ppm ($^{3}J_{C,F}$ = 7.6 Hz). The hydroxyl groups at C(5) in **7** and **8** cause only minor shielding effects on the ^{19}F NMR signals, which were observed at δ -171.9 ppm and δ -172.1 ppm for **7** and **8**, compared to δ -171.3 ppm for the substrate **2**. In contrast, the ^{19}F signals of alcohols **9** (δ -173.5 ppm) and **10** (δ -176.3 ppm) are significantly deshielded by the hydroxyl group at C(4). The ^{13}C signals of the hydroxylated carbons for these compounds appear at δ 68.1 ppm (**9**) and 66.3 ppm (**10**), and have coupling constants of $^{3}J_{C,F}$ = 7.6 Hz or $^{3}J_{C,F}$ = 10.2 Hz, respectively. The relative configuration of alcohols **9** and **10** were assigned by two independent nuclear Overhauser measurements.[23] First, the saturation of the CHF and C*H*OH signals in compound **10** showed a significant enhancement for these protons, while compound **9** did not give any observable NOE. These results confirm that the fluorine substituent and the hydroxyl group in **10** are located on the same side of the cycloheptyl ring in a chair conformation (*cis*-configuration) and hence the microbial hydroxylation occurred at C(4) in *trans*-position to the carbamate moiety.

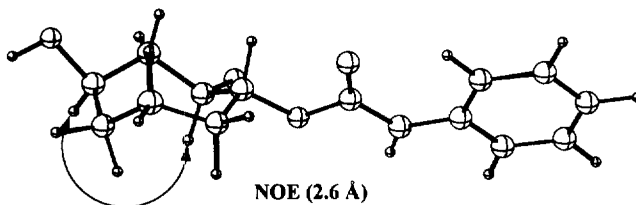


Figure 4: Calculated chair conformer of **10** (AM1)

In a second experiment, the spectrum of **9** showed a significant NOE of the proton belonging to the C*H*OH group and the proton neighboured to the carbamoyl function. The close proximity of these two protons can only be explained by a boat-conformation of the seven membered ring. The hydroxyl function occupies a pseudo equatorial position relative to the carbamoyl moiety and, hence, these functional groups are in *cis*-configuration. A similar NOE could not be observed for **10**.

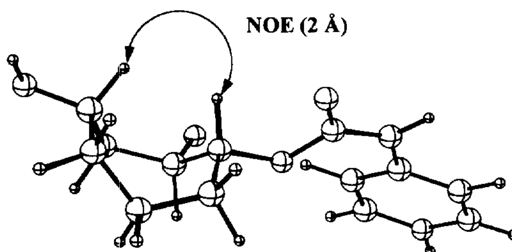


Figure 5: Calculated boat conformer of **9** (AM1)

These results support the previous assignment of the configuration of alcohols **9** and **10**. In order to establish the measured NOEs, we optimized the structures **9** and **10** on the basis of their geometrical parameters at AM1 level (Figures 4, 5).[24] From this analysis the larger $^3J_{C,F}$ value of C(4) i.e. the carbon bearing hydroxyl group in **10** (compared to that observed for **9**) can be rationalized on the basis of a pseudo *anti*-C(4)-F arrangement (dihedral angle 215°) of a twist-chair conformation. Similar consideration for **9** suggests a pseudo *gauche*-orientation of C(4)-F in a boat conformation (dihedral angle 117°). The vicinal fluorine-carbon spin-spin coupling constant ($^3J_{C,F}$) has a Karplus-type dihedral angle dependence and in general $^3J_{C,F\,anti}$ is larger than $^3J_{C,F\,gauche}$ for any given system.[25] This further supports for the structural assignments given above.

The ketone **12** exhibiting an optical rotation of $[\alpha]_D$ +3.1° was isolated as a consecutive product of one or two of the optically active alcohols. The ^{13}C signal of the carbonyl group C(5) appears at δ 211.5 ppm and lacked vicinal $^{13}C^{19}F$ coupling. For the neighbouring γ-C(4) fragment a $^3J_{C,F}$ coupling constant of 7.4 Hz was found, while the corresponding coupling constant to the other γ-C(7) fragment was nearly zero and could not be detected, which is not unusual for these types of molecules.[25] Thus, we conclude that **12** was derived from **7** and/or **8** by a dehydrogenase.[18,5g] However, **12** was obtained in much lower quantity compared to the unfluorinated parent **5**, and a corresponding ketone derived from **9** and/or **10** was not observed. Perhaps the fluorinated products do inhibit the dehydrogenase activity of the fungus.

In summary, regioselective microbial hydroxylation of a formal 4-position relative to the carbamate moiety is always observed in prochiral **1** and racemic **2**. In addition to the expected *trans*-hydroxylation[5a,d,g,f]

we also found some *cis*-hydroxylation in particular with the fluorinated compound **2**. Thus, fluorine substitution does not appear to favour *trans*-hydroxylation as much as the parent substrate **1**. The *trans:cis* product ratio in the fluorinated compounds **7**, **8**, **9**, and **10** is only one half of that in the non-fluorinated species **3**, **4**, and **5**. On the other hand the fluorine substituent enhanced the enantioselectivity of hydroxylation, particularly for **7** (82% ee) and **10** (79% ee). Moreover, the fluorinated analogue **9** of the parent racemic *cis*-alcohol **4** shows an enantiomeric excess of 52%, and the alcohol **8** is also optically active. These results indicate that there is an enantiomeric discrimination during the microbial hydroxylation of the racemic *trans*-2-fluorocycloheptyl-*N*-phenylcarbamate (**2**) with *B. bassiana*. However, the absolute configuration of the products could not be determined yet. Hence, it could not be decided which product originates from which enantiomer of the educt. Further studies with the pure enantiomers of **2** are scheduled to get information on the geometry of the enzyme's active site. This enzyme has yet to be purified from fungal sources and characterised, but is thought to be a *cytochrome* P-450[26] depend monooxygenase.

The mode of action of P-450 enzymes involves the radical hydrogen abstraction/oxygen rebound process established by Groves et al.[27] or a concerted mechanism recently published by Newcomb et al.[28] Taking the first approach into account the microbial oxidation proceeds by a hydrogen atom abstraction to form a hydroxy iron(IV) species of the enzyme and a carbon radical followed by a radical rebound of an OH-group from the iron species. In the case that the H-abstraction is enantioselective concerning the two enantiotopic methylene groups in 4-position of **1** only one enantiomer of one diastereomer can be formed on condition that the formed carbon centred radical imprisoned in a radical cage is not able to rearrange. If the radical, on the other hand, is able to invert, the alcohol of opposite configuration should be formed, however, exhibiting the same enantioselectivity like the other diastereomer.[27a,29] In this way by abstraction of one of the *trans*-hydrogens from the prochiral **1**, mainly compound **3** is formed. However, also the disfavoured enantiotopic methylene group of **1** seems to be attacked causing the only moderate 62% ee of **3**. For the formation of the minor alcohol **4** no enantioselectivity could be detected at all.

Considering the second approach to the mechanism of hydroxylation, different orientations to the enzymes active site could be responsible for different „side-on“ approaches to the particular C-H bonds at C(4) and C(5), since fluorine is supposed to be able to alter the intrinsic nature of the anchoring group of **1** or the enantiomers of **2**. This will be discussed in more detail in the succeeding publication presenting an modified enzyme substrate model for the enzyme's action.

EXPERIMENTAL

Biotransformation Conditions:[30] The bioconversion medium was comprised of a mixture of corn steep liquor (20 g/L, Sigma) and glucose (10 g/L, Merck) in water. During the transformation the medium was adjusted to pH 5 with aqueous sodium hydroxide. All media were sterilized (123°C, 2.3 bar) by an autoclave prior to use. Flasks containing 150 ml of the medium were inoculated with loops of vegetative cells of *Beauveria bassiana*, which were grown on potato-dextrose agar (Difco) Petri dishes. The precultures were shaken (250 rpm) for 48 h at 30°C. Transformations were conducted in 4 L three-necked flasks containing 3 L of medium, inoculated by the addition of 150 mL of a preculture. Cultures were then shaken on a rotatory shaker (100 rpm) with aeration (1 L/min) at 30°C. After 24 h of growth, the educt (600 mg) dissolved in

dimethylformamide (5 mL) was added to each culture. A conversion time of 72 hours was used. The mycelium of the culture broth was filtered off and washed twice with 500 mL dichloromethane. The culture broth (3 L) was extracted three times with 1 L portions of dichloromethane. The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude residue was analysed by TLC. Biooxygenation products and recovered substrate were isolated by silica gel chromatography. HPLC was used for the final separations. Yields of products are given in % of converted substrate.

Analytical Methods: Melting Points (uncorrected): Reichert melting point apparatus. *Optical Rotation*: Perkin Elmer polarimeter 241 (20°C, λ_{Na}= 589 nm). *^1H, ^{13}C NMR*: Bruker WM 300, Varian 600 MHz Unity Plus, solvent ($CDCl_3$) as internal standard. Assignments of signals were made on the basis of ^1H,^1H-, ^1H,^{13}C-correlation (HMQC), and ^1H-TOCSY experiments. The multiplicity of the ^{13}C signals is given for proton decoupled spectra. *^{19}F NMR*: Bruker WM 300, with $CDCl_3$ as solvent and α,α,α trifluorotoluene (^{19}F, δ -69 ppm from $CFCl_3$) as internal standard. *MS*: Finnigan MAT 8230 GC-MS, capillary columns: HP5, HP1, EI (70eV), CI (NH_3) and HRMS (CI). *GC*: Hewlett Packard Series II with integrator HP 3396A, quarz capillary column: 25 m, 0.33 mm x 0.25 µm HP1 and 25m, 0.20 mm x 0.11 µm HP5, carrier gas N_2, FID. *HPLC*: Knauer system containing pump 64, differential refractometer, retriver™ (Fa. Isco); column: nucleosil 50-7 (Knauer) with eluent cyclohexane/ethylacetate 20:1; chiral column: varioprep VP 250/21 (Macherey-Nagel) with eluent chloroform/methanol 500:1. *TLC*: Silica gel 60 F_{254} aluminium plates (Merck), Detection: a) UV (254nm), b) spraying reagent (5% $(NH_4)_6Mo_7O_{24}x4H_2O$ in ethanol) and developing on a heat plate (500°C). *Elemental Analysis*: Microanalytical Laboratory, Institute of Organic Chemistry, University of Münster.

Starting Materials: 8-Oxabicyclo[5.1.0]octane, phenylisocyanate, and cycloheptanol were purchased from Aldrich. Triethylamine trishydrofluoride was a gift of Hoechst AG, Frankfurt am Main, but is commercially available from several suppliers such as Fluka, Aldrich or Acros.

General Method for the Preparation of Mosher's Esters: Mosher esters of **3**, **4**, **7**, **8**, **9**, and **10** were synthesised as reported by Neises and Steglich.[31] The enantiomeric excess has been deduced from the measured (^{19}F NMR of the CF_3 groups) diastereomeric ratio in the crude ester mixture.

Preparation of trans-2-Fluorocycloheptanol (**6**). Caution ! Although triethylamine trishydrofluoride is less corrosive than anhydrous HF itself, any contact with the skin must be avoided. The experiments should be done under an efficient working hood and personal safety protection equipment should be worn.

A heterogeneous mixture of 8-oxabicyclo[5.1.0]octane (5 g, 0.044 mol) and $Et_3N{\cdot}3HF$ (7.1 g, 0.044 mol) was stirred for 3 h at 155°C. After this period the solution was poured into ice-water, neutralized with dilute ammonia, and extracted three times with 25 mL portions of dichloromethane. The combined organic layers were washed with brine and dried over $MgSO_4$. After evaporation of the solvent, distillation of the residue afforded a colorless liquid of **6** (4.36 g, 75%); bp 90-92°C (25 mm); FT-IR (neat): ν 3574, 3386, 2932, 2863, 1457, 1387, 1346, 1056, 1034, 996 cm^{-1}; ^1H NMR: δ 1.34-2.01 (m, 10 H), 2.54 (m, 1 H, OH), 3.72 (m, 1 H, CHOH), 4.21-4.44 (dddd, $^2J_{H,F}$ = 49.2 Hz, $^3J_{Ha,Ha}$ = 9.1 Hz, $^3J_{Ha,Ha}$ = 7.9 Hz, $^3J_{He,Ha}$ = 3.9 Hz, 1 H, CHF); ^{13}C NMR: δ 21.1 (d, $^3J_{C,F}$ = 10.2 Hz, C-4), 22.3 (s, C-6), 27.1 (s, C-5), 30.3 (d, $^2J_{C,F}$ = 20.3 Hz, C-3), 30.8 (d, $^3J_{C,F}$ = 7.6 Hz, C-7), 75.8 (d, $^2J_{C,F}$ = 20.4 Hz, C-1), 99.5 (d, $^1J_{C,F}$ = 167.8 Hz, C-2); ^{19}F NMR: δ -171.3 (m); EI GC/MS: 132 (M^+, 0.8), 114 (12), 112 (4), 94 (30), 68 (76), 57 (100). Anal. calcd. for $C_7H_{13}FO$ (132.2) C 63.61, H 9.91 found C 63.36, H 10.20%.

General Procedure for the Preparation of N-Phenylcarbamates: A mixture of 1.43 g (12 mmol) phenylisocyanate and 10 mmol of the respective alcohol in 50 ml dry petroleum ether (130-150°C) is heated at 120°C for 3 h and remains at room temperature overnight. The solvent is removed under vacuum and the residue is purified by chromatography (silica gel, cyclohexane/ethyl acetate (9:1).

Cycloheptyl-N-phenylcarbamate (**1**). Yield: 8.6 g (85%); mp 82-84°C; FT-IR (CCl_4): ν 3443, 3344, 3061, 3031, 3931, 2860, 1736, 1521, 1441, 1207 cm^{-1}; ^1H NMR: δ 1.31-1.69 (m, 10 H), 1.86-1.95 (m, 2 H), 4.86 (septet, 1H, CHOCO), 6.47 (s, 1 H, NH), 6.9 (t, J_{para} = 6.8 Hz, 1 H), 7.21 (t, J_{meta} = 7.9 Hz, 2 H), 7.3 (d, J_{ortho} = 7.6 Hz, 2 H); ^{13}C NMR: δ 22. 8 (s, C-3, C-6), 28.2 (s, C-4, C-5), 34.0 (s, C-2, C-7), 75.15 (s, C-1), 118.5 (s, C-10, C-14), 123.1 (s, C-12), 129.0 (s, C-11, C-13), 138.1 (s, C-9), 153.2 (s, C-8); EI GC/MS: 233 (M^+, 20), 138 (8), 137 (52), 132 (12), 120 (6), 119 (24), 97 (32), 93 (58), 91 (13), 55 (100). Anal. calcd. for $C_{14}H_{19}O_2N$ (233.3) C 72.07, H 8.20, N 6.00 found C 72.08, H 8.05, N 6.02%.

trans-4-Hydroxycycloheptyl-N-phenylcarbamate (**3**). Yield: 97 mg (10%); mp 109-112°C; $[\alpha]_D$ +4.9° (c 0.105, ethyl acetate); FT-IR (CCl_4): ν 3544, 3201, 2926, 1683, 1593, 1442, 1253, 1212, 1008 cm^{-1}; ^1H NMR: δ 1.43-2.17 (m, 11 H, 1 OH), 3.89 (m, 1 H, CHOH), 4.95 (m, 1 H, CHOCO), 6.97 (t, J_{para} = 7.3 Hz, 1 H), 7.21 (t, J_{meta} = 8.0 Hz, 2 H), 7.29 (d, J_{ortho} = 7.6 Hz, 2 H); ^{13}C NMR: δ 18.0 (s, C-6), 28.0 (s, C-2 or C-3), 31.7 (s, C-2 or. C-3), 34.0 (s, C-7 or C-5), 37.6 (s, C-7 or C-5), 71.9 (s, C-4), 75.24 (s, C-1), 118.6 (s, C-10, C-14), 123.3 (s, C-12), 129.0 (s, C-11, C-13), 138.1 (s, C-9); 153.1 (s, C-8); EI GC/MS: 249 (M^+, 10), 138 (7), 137 (42), 120 (11), 119 (44), 113 (11), 95 (88), 93 (100), 91 (38), 57 (24), 55 (38); HRMS: calcd. for $C_{14}H_{18}NO_3$ + NH_4^+ 267.1708, found 267.1683.

cis-4-Hydroxycycloheptyl-N-phenylcarbamate (**4**). Yield: 17.5 mg (1.8%); ^{13}C NMR: δ 18.7 (s, C-6), 27.6 (s, C-2 or C-3), 31.1 (s, C-2 or C-3), 34.2 (s, C-5 or C-7), 37.8 (s, C-5 or C-7), 71.5 (s, C-4), 74.98 (s, C-1), 118.6 (s, C-10, C-14), 123.3 (s, C-12), 129.0 (s, C-11, C-13), 138.1 (s, C-9); 153.1 (s, C-8).

4-Oxocycloheptyl-N-phenylcarbamate (**5**). Yield: 116 mg (12%); mp 96-99°C; $[\alpha]_D$ +6.1° (c 0.18, CH_3OH); FT-IR (CCl_4): ν 3300, 2935, 2863, 1731, 1701, 1606, 1540, 1444, 1510, 1222, 1049, 1025 cm^{-1}; ^1H NMR: δ 1.68-1.75 (m, 1 H), 1.85-2.05 (m, 5 H), 2.43-2.55 (m, 3 H), 2.62-2.71 (m, 1 H), 5.02 (septet, 1 H, CHOCO), 6.61 (br s, 1 H, NH), 7.06 (t, J_{para} = 7.2 Hz, 1 H), 7.30 (t, J_{meta} = 8.0 Hz, 2 H), 7.37 (d, J_{ortho} = 7.9 Hz, 2 H); ^{13}C NMR: δ 19.0 (s, C-6), 29.0 (s), 35.0 (s), 37.75 (s), 43.5 (s, C-5), 74. 1 (s, C-1), 118.7 (s, C-10, C-14), 123.6 (s, C-12), 129.1 (s, C-11, C-13), 137.8 (s, C-9), 152.7 (s, C-8), 213.4 (s, C-4); EI GC/MS: 247 (M^+, 20), 138 (4), 137 (24), 120 (10), 119 (100), 110 (14), 93 (61), 91 (56), 55 (82); HRMS: calcd. for $C_{14}H_{17}NO_3$ + NH_4^+ 265.1552, found 265.1545.

4-Oxocycloheptyl-N-phenylcarbamate (**5**) *from Oxidation of Alcohol* **3**. A sample of **3** (7 mg, 0.028 mmol) in an acetone solution (5 mL) was oxidized with Jones reagent.[19] The crude reaction product was purified by HPLC to give crystalline product of **12**. Yield: 4 mg (57%); mp 97-99°C; $[\alpha]_D$ +4.5° (c 4, $CHCl_3$). The ^1H NMR spectrum is identical with that of the biooxygenation product **5**.

trans-2-Fluorocycloheptyl-N-phenylcarbamate (**2**). Yield: 7.12 g (81%); mp 79-81°C (colorless crystals); FT-IR (CCl_4): ν 3321, 3146, 2863, 1700, 1602, 1540, 1444, 1223, 1064 cm^{-1}; ^1H NMR: δ 1.42-1.81 (m, 7 H), 1.82-2.05 (m, 3 H), 4.63 (dm, $^2J_{C,F}$ = 47.6 Hz, 1 H, CHF), 5.00 (m, 1 H, CHOCO), 6.60 (s, 1 H, NH), 7.05 (t, J_{para} = 7.9 Hz, 1 H), 7.29 (m, J_{meta} = 7.8 Hz, 2 H), 7.38 (d, J_{ortho} = 8.3 Hz, 2 H); ^{13}C NMR: δ 21.6 (d, $^3J_{C,F}$ = 7.4 Hz, C-4), 23.0 (s, C-6), 28.2 (s, C-5), 29.7 (d, $^3J_{C,F}$ = 10.2 Hz, C-7), 30.8 (d, $^2J_{C,F}$ = 20.3 Hz, C-

3), 78.7 (d, $^2J_{C,F}$ = 22. 9 Hz, C-1), 95.8 (d, $^1J_{C,F}$ = 172.9 Hz, C-2), 118.7 (s, C-10, C-14), 123.4 (s, C-12), 129.0 (s, C-11, C-13), 137.8 (s, C-9), 152.9 (s, C-8); ^{19}F NMR: δ -171.3 (m); EI GC/MS: 251 (M^+, 36), 231 (11), 138 (5), 137 (40), 119 (100), 95 (57), 93 (98), 91 (48), 57 (64), 55 (56). Anal. calcd. for $C_{14}H_{18}FNO_2$ (251.3) C 66.91, H 7.22, N 5.57 found C 67.09, H 7.47, N 5.69%.

t-2-Fluoro-t-5-hydroxycycloheptyl-r-N-phenylcarbamate (**7**). Yield: 223 mg (24%); mp 76-78°C; $[\alpha]_D$ -22° (c 0.12, $CHCl_3$); FT-IR (CCl_4): ν 3399, 3316, 2923, 2849, 1704, 1601, 1544, 1445, 1321, 1227 cm^{-1}; ^{1}H NMR: δ 1.51-1.63 (m, 2 H), 1.75-1.88 (m, 3 H, OH), 1.99-2.13 (m, 3 H), 3.83 (m, 1 H, CHOH), 4.51-4.71 (dm, $^2J_{H,F}$ = 47.9 Hz, 1 H, CHF); 5.00 (m, 1 H, CHOCO), 6.70 (s, br, NH), 7.05 (t, J_{para} = 7.27 Hz, 1H), 7.29 (t, J_{meta} = 7.9 Hz, 2H), 7.37 (d, J_{ortho} = 7.9 Hz, 2H); ^{13}C NMR: δ 24.8 (d, $^3J_{C,F}$ = 7.6 Hz, C-4), 25.6 (d, $^2J_{C,F}$ = 22.9 Hz, C-3), 30.3 (d, $^3J_{C,F}$ = 7.6 Hz, C-7), 31.9 (s, C-6), 70.6 (s, C-5), 78.2 (d, $^2J_{C,F}$ = 25.4 Hz, C-1), 95.0 (d, $^1J_{C,F}$ = 175.5 Hz, C-2), 118.8 (s, C-10, C-14), 123.6 (s, C-12), 129.1 (s, C-11, C-13), 137.8 (s, C-9), 152.8 (s, C-8); ^{19}F NMR: δ -171.6 (m); EI GC/MS: 267 (M^+, 7), 249 (0.2), 247 (8), 138 (8), 137 (15), 119 (61), 113 (23), 93 (100), 91 (30), 55 (33); HRMS: calcd for $C_{14}H_{18}FNO_3 + NH_4^+$ 285.1614, found 285.1630.

t-2-Fluoro-c-4-hydroxycycloheptyl-r-N-phenylcarbamate (**8**). Alcohol **8** was contaminated with 34% of 7. Yield: 74 mg (8%); ^{1}H NMR: δ 1.52-2.17 (m, 8 H, OH), 3.97 (m, 1 H, CHOH), 4.52-4.75 (dm, $^2J_{H,F}$ = 47.9 Hz, 1 H, CHF), 4.96 (m, 1H, CHOCO), 6.70 (s, br, 1 H, NH), 7.06 (t, J_{para} = 7.1 Hz, 1 H), 7.30 (t, J_{meta} = 8.0 Hz, 2 H), 7.39 (d, J_{ortho} = 7.6 Hz, 2 H); ^{13}C NMR: δ 24.3 (d, $^3J_{C,F}$ = 7.6 Hz, C-4), 25.1 (d, $^2J_{C,F}$ = 20.3 Hz, C-3), 29.9 (d, $^3J_{C,F}$ = 7.6 Hz, C-7), 31.6 (s, C-6), 69.5 (s, C-5), 77.9 (d, $^2J_{C,F}$ = 25.4 Hz, C-1), 95.2 (d, $^1J_{C,F}$ = 172.9 Hz, C-2), 118. 8 (s, C-10, C-14), 123.6 (s, C-12), 129.0 (s, C-13, C-11), 137.7 (s, C-9), 152.8 (s, C-9); ^{19}F NMR: δ -171.4 (m); EI GC/MS: 267 (M^+, 6), 247 (10), 137 (18), 120 (17), 119 (74), 113 (32), 93 (100), 91 (38), 55 (36).

t-2-Fluoro-c-4-hydroxycycloheptyl-r-N-phenylcarbamate (**9**). Yield: 46 mg (5%); $[\alpha]_D$ +8.3° (c = 0.12, $CHCl_3$); FT-IR (CCl_4): ν 3404, 3316, 2937, 2869, 1708, 1603, 1542, 1445, 1232 cm^{-1}; ^{1}H NMR: δ 1.22-2.2 (m, 8 H); 3,6 (s, 1 H, OH); 4.09 (m, 1 H, CHOH); 4.81-4.98 (dm, $^2J_{H,F}$ = 31.2 Hz, 1 H, CHF); 5 (m, 1 H); 6.98 (s, NH); 7.04 (t, J_{para} = 7.2 Hz, 1 H); 7.28 (t, J_{meta} = 7.5 Hz, 2 H); 7.37 (d, J_{ortho} = 7.9 Hz, 2 H); ^{13}C NMR: δ 19. 8 (s, C-6), 30.3 (d, $^3J_{C,F}$ = 7.4 Hz, C-7), 38.0 (s, C-5), 39.4 (d, $^2J_{C,F}$ = 22.9 Hz, C-3), 66.3 (d, $^3J_{C,F}$ = 7.6 Hz, C-4), 77.9 (d, $^2J_{C,F}$ = 22.9 Hz, C-1), 91.3 (d, $^1J_{C,F}$ = 170.4 Hz, C-2), 118.8 (s, C-14, C-10), 123.5 (s, C-12), 129.0 (s, C-13, C-11), 137.8 (s, C-9); 153.0 (s, C-8); ^{19}F NMR: δ -176.3 (m); EI GC/MS: 267 (M^+, 18), 247 (7), 137 (22), 119 (100), 113 (10), 93 (78), 91 (30), 55 (24).

t-2-Fluoro-t-4-hydroxycycloheptyl-r-N-phenylcarbamate (**10**). Yield: 42 mg (4.5%); mp 89-93°C; $[\alpha]_D$ +26° (c = 0.5, $CHCl_3$); FT-IR (CCl_4): ν 3412, 3352, 2934, 2849, 1721, 1618, 1558, 1448, 1218 cm^{-1}; ^{1}H NMR (CD_3OD): δ 1.28-1.34 (m, 1 H), 1.47-1.68 (m, 2 H), 1.75-1.80 (m, 1 H), 1.89-2.07 (m, 3 H), 2.1-2.25 (m, 1 H), 3.88 (m, 1 H, COH), 4.65 (dm, $^2J_{H,F}$ = 47.7 Hz), 5.01 (m, 1 H, CHOCR), 7.0 (t, J_{para} = 7.5 Hz), 7.57 (t, J_{meta} = 7.3 Hz), 7.41 (d, J_{ortho} = 7.7 Hz); ^{13}C NMR: δ 18.0 (s, C-6); 30.2 (d, $^3J_{C,F}$ = 7.6 Hz, C-7), 37.4 (s, C-5), 39.4 (d, $^2J_{C,F}$ = 20.3 Hz, C-3), 68.1 (d, $^3J_{C,F}$ = 10.2 Hz, C-4), 77.5 (d, $^2J_{C,F}$ = 22.7 Hz, C-1), 92.1 (d, $^1J_{C,F}$ = 172.9 Hz, C-1), 118.8 (s, C-10, C-14), 123.6 (s, C-12), 129.1 (s, C-11, C-13), 137.8 (s, C-9); 152.7 (s, C-8); ^{19}F NMR: δ -173.3 (m); EI GC/MS: 267 (M^+, 16), 247 (16), 137 (16), 119 (57), 113 (38), 93 (100), 91 (25), 55 (29).

trans-2-Fluorocycloheptyl-N-(p-hydroxyphenyl)carbamate (**11**). Yield: 19 mg (2%); ^{1}H NMR: δ 1.49-1.99 (m, 10 H), 4.63 (dm, $^2J_{C,F}$ = 47.6 Hz, 1 H, CHF), 5 (m, 1 H, CHOCO), 6.72 (d, J = 9.0 Hz, 2H), 7.23 (d, J = 8.8 Hz, 2H); ^{13}C NMR: δ 21.7 (d, $^3J_{C,F}$ = 7.6 Hz, C-4), 23.1 (s, C-6), 28.3 (s, C-5), 29.8 (d, $^3J_{C,F}$ = 7.6 Hz, C-7), 30.9 (d, $^2J_{C,F}$ = 20.3 Hz, C-3), 78.7 (d, $^2J_{C,F}$ = 22.9 Hz, C-1), 95.8 (d, $^1J_{C,F}$ = 172.9 Hz, C-2), 115.7 (s, C-11, C-13), 121.1 (s, C-14, C-10), 131.0 (s, C-9), 152.0 (s, C-9), 153.4 (s, C-12); ^{19}F NMR: δ -171.4 (m); EI GC/MS: 267 (M^+, 48), 247 (6), 154 (10), 153 (84), 136 (12), 135 (90), 109 (100), 107 (31), 95 (43), 55 (44); HRMS: calcd. for $C_{14}H_{18}FNO_3 + NH_4^+$ 285.1614, found 285.1631.

trans-2-Fluoro-4-oxocycloheptyl-N-phenylcarbamate (**12**). Yield: 9 mg (1%); mp 124-126°C; $[\alpha]_D$ +3.1° (c = 0.9, $CHCl_3$); ^{1}H NMR: δ 1.8-2.25 (m, 4 H), 2.34-2.79 (m, 4 H), 4.73 (dm, $^2J_{H,F}$ = 48.6 Hz, 1 H, CHF), 5.14 (m, 1 H, CHOCR), 6.75 (s, 1 H, NH), 7.06 (t, J_{para} = 7.2 Hz, 1 H), 7.29 (t, J_{meta} = 7.9 Hz, 2 H), 7.36 (d, J_{ortho} = 7.9 Hz, 2 H); ^{13}C NMR: δ 23.5 (s, C-7), 25.4 (d, $^2J_{C,F}$ = 20.3 Hz, C-3), 36.0 (d, $^3J_{C,F}$ = 7.4 Hz, C-4), 37.1 (s, C-6), 73.9 (d, $^2J_{C,F}$ = 27.8 Hz, C-1), 91.2 (d, $^1J_{C,F}$ = 175.5 Hz, C-2), 118.8 (s, C-10, C-14), 123.9 (s, C-12), 129.1 (s, C-11, C-13), 137.4 (s, C-9), 152.1 (s, C-8), 211.5 (s, C-5); ^{19}F NMR: δ -182.2 (m); EI GC/MS: 265 (M^+, 20), 245 (4), 137 (14), 120 (16), 119 (100), 93 (44), 91 (38), 55 (20); HRMS: calcd. for $C_{14}H_{16}FNO_3 + NH_4^+$ 283.1457, found 283.1456.

X-*Ray Analyses:*[16] Crystallographic data for **3**: formula $C_{14}H_{19}NO_3$, M = 249.30, 0.4 x 0.2 x 0.1 mm, a = 9.185(3), b = 10.331(4), c = 13.983(2) Å, V = 1326.9(7) Å^3, ρ_{calc} = 1.248 g cm^{-3}, μ = 7.10 cm^{-1}, Z = 4, orthorhombic, space group $P2_12_12_1$ (No. 19), λ = 1.54178 Å, T = 293 K, ω/2θ scans, 1887 reflections collected (-h, +k, ±l), [(sinθ)/λ] = 0.53 Å^{-1}, 1664 independent and 1194 observed reflections [$I \geq 2\,\sigma(I)$], 169 refined parameters, R = 0.048, wR^2 = 0.107, max. residual electron density 0.18 (-0.16) e Å^{-3}, Flack parameter 0.2(6), hydrogens calculated and refined as riding atoms.
Crystallographic data for **5**: formula $C_{14}H_{17}NO_3$, M = 247.29, 0.2 x 0.25 x 0.1 mm, a = 6.481(1), b = 13.241(1), c = 7.957(1) Å, β = 111.27(1)°, V = 636.3(1) Å^3, ρ_{calc} = 1.291 g cm^{-3}, μ = 7.40 cm^{-1}, Z = 2, monoclinic, space group $P2_1$ (No. 4), λ = 1.54178 Å, T = 223 K, ω/2θ scans, 1468 reflections collected (+h, +k, ±l), [(sinθ)/λ] = 0.62 Å^{-1}, 1352 independent and 1288 observed reflections [$I \geq 2\,\sigma(I)$], 167 refined parameters, R = 0.034, wR^2 = 0.093, max. residual electron density 0.18 (-0.17) e Å^{-3}, Flack parameter -0.1(3), hydrogens calculated and refined as riding atoms.
Crystallographic data for **7**: formula $C_{14}H_{18}NO_3F$, M = 267.29, 0.30 x 0.30 x 0.25 mm, a = 18.497(5), b = 13.433(3), c = 10.938(3) Å, V = 2717.8(12) Å^3, ρ_{calc} = 1.307 g cm^{-3}, μ = 8.40 cm^{-1}, Z = 8, orthorhombic, space group *Pbca* (No. 61), λ = 1.54178 Å, T = 223 K, ω/2θ scans, 2964 reflections collected (-h, +k, -l), [(sin θ)/λ] = 0.56 Å^{-1}, 2021 independent and 1158 observed reflections [$I \geq 2\,\sigma(I)$], 174 refined parameters, R = 0.071, wR^2 = 0.155, max. residual electron density 0.27 (-0.29) e Å^{-3}, hydrogens calculated and refined as riding atoms.
All data sets were collected with an Enraf Nonius CAD4 diffractometer. Programs used: data reduction MolEN,[32] structure solution SHELXS-86,[33] structure refinement SHELXL-93,[34] graphics SCHAKAL-92.[35]

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