Microbiological Oxygenation of Bridgehead Azabicycloalkanes

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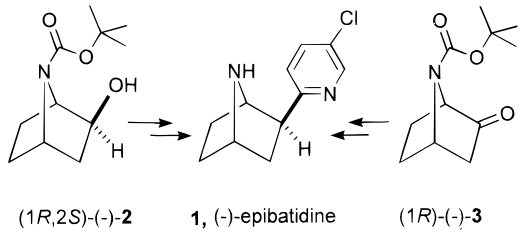
A series of N-substituted bridgehead azabicycloalkanes has been prepared and examined as substrates for microbiological oxygenation using the fungi *Beauveria bassiana, Rhizopus nigricans, Aspergillus ochraceus,* and *Rhizopus arrhizus*. Oxygenation using *B. bassiana* of *N*-tosyl-7-azabicyclo[2.2.1]heptane gave *N*-[*p*-(hydroxymethyl)benzenesulfonyl]-7-azabicyclo[2.2.1]heptane (56% yield), of *N*-(phenyloxycarbonyl)-7-azabicyclo[2.2.1]heptane gave the 2-*endo*-ol (56% yield, 51% ee), of N-BOC-7-azabicyclo[2.2.1]heptane gave the 2-*endo*-ol (10% yield), of *N*-Cbz-7-azabicyclo-[2.2.1]heptane gave the 2-*endo*-ol (28%), of *N*-(phenyloxycarbonyl)-8-azabicyclo[3.2.1]octane gave the 3-*endo*-ol, and of *N*-(phenyloxycarbonyl)-9-azabicyclo[3.3.1]nonane gave the 3-*exo*-ol (30%) and 3-one (16%). Oxygenation using *R. nigricans* of *N*-BOC-7-azabicyclo[2.2.1]heptane gave the 2-*endo*-ol (62% yield, 28% ee) and the 2-*exo*-ol (27% yield, 42% ee). Oxidation of the *N*-BOC-7-azabicyclo-[2.2.1]heptan-2-ols gives the 2-ketone, a synthetic intermediate useful for conversion to the natural product, epibatidine. Oxygenation of *N*-(phenyloxycarbonyl)-7-azabicyclo[2.2.1]heptane using *R. arrhizus* gives the 2-*endo*-ol (5% yield, 31% ee) and the 2-*exo*-ol (18% yield, 22% ee). Oxygenation of *N*-(phenyloxycarbonyl)-8-azabicyclo[3.2.1]octane using *A. ochraceous* gives the 3-*endo*-ol (36%) and the 3-one (4%).

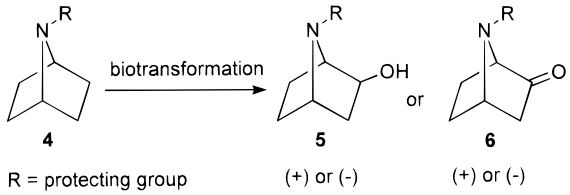
Introduction

(−)-Epibatidine (**1**) is a naturally occurring 7-azabicyclo-[2.2.1]heptane first isolated from the skin of the poisonous frog, *Epipedobates tricolor*.[2] (−)-Epibatidine is a very potent analgesic, but this desirable activity is accompanied by serious toxicity, which has heightened interest in the synthesis of analogs that may be free of side effects. This, together with the lack of abundance of the natural product, have led to intensive efforts aimed at the total synthesis of the compound. Numerous syntheses both of racemic and of the natural and unnatural enantiomers of epibatidine have been reported.[3] Fletcher and coworkers obtained (+)- and (−)-epibatidine through resolution and elaboration of (±)-alcohol **2**.[4] In an enantiospecific synthesis reported by Rapoport and coworkers, diastereomeric mixtures formed in the course of the synthesis did not require separation but instead converged on a single enantiomer, either (+)- or (−)-**3**, which in turn could be converted to the respective enantiomeric form of epibatidine.[5] Once both enantiomers of the natural product became available, pharmacological evaluations have shown that both have analgesic properties and are of approximately equal potency.[6]



(1*R*,2*S*)-(-)-**2** **1**, (-)-epibatidine (1*R*)-(-)-**3**

The use of microorganisms for the selective oxygenation of organic molecules is well documented; in particular, microbial transformations represent a powerful tool for oxygenation of unactivated hydrocarbon sites.[7] The list of substrates for biotransformation ranges from steroids and arenes to a wide array of cyclic, bicyclic, polycyclic, and acylic *N*-acylalkanes.[8−10] Our laboratories have considerable experience in performing microbiological oxygenation reactions including the oxygenation of a variety of *N*-acyl heterobicyclic alkanes.[11] However, the bridgehead azabicycloalkane series of 7-azabicyclo[2.2.1]-heptane,[12] 8-azabicyclo[3.2.1]octane, and 9-azabicyclo-[3.3.1]nonane has not been the subject of such oxygenation studies. We envisioned that selective oxygenation



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(1) Postdoctoral research fellow at Pharmacia & Upjohn, January 1, 1995−March 1, 1996. Present address: Department of Chemistry, Augustana College, Rock Island, IL 61201.

(2) Spande, T. F.; Garraffo, M. H.; Edwards, M. W.; Yeh, H. J. C.; Pannel, L.; Daly, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 3475.

(3) Early synthesis: Broka, C. A. *Tetrahedron Lett.* **1993**, *34*, 3251. A recent listing of syntheses may be found in: Zhang, C.; Trudell, M. L. *J. Org. Chem.* **1996**, *61*, 7189.

(4) Fletcher, S. R.; Baker, R.; Chambers, M. S.; Herbert, R. H.; Hobbs, S. C.; Thomas, S. R; Verrier, H. M.; Watt, A. P.; Ball, R. G. *J. Org. Chem.* **1994**, *59*, 1771.

(5) Hernández, A.; Marcos, M.; Rapoport, H. *J. Org. Chem.* **1995**, *60*, 2683.

(6) Bai, D.; Xu, R.; Chu, G.; Zhu, X. *J. Org. Chem.* **1996**, *61*, 4600.

(7) Johnson, R. A. In *Oxidation in Organic Chemistry*; Trahanovsky, W. S., Ed.; Academic Press: New York, 1978; Part C, p 131.

(8) Holland, H. L. *Organic Synthesis With Oxidative Enzymes*; VCH: New York, NY, 1992.

(9) Davies, H. G.; Green, R. H.; Kelley, D. R.; Roberts, S. M. *Biotransformations in Preparative Organic Chemistry: The Use of Isolated Enzymes and Whole Cell Systems*; Harcourt Brace Jovanovich: New York, NY, 1989.

(10) Vigne, B.; Archelas, A.; Furstoss, R. *Tetrahedron* **1991**, *47*, 1447 and references cited therein.

(11) Johnson, R. A.; Herr, M. E.; Murray, H. C.; Reineke, L. M.; Fonken, G. S. *J. Org. Chem.* **1968**, *33*, 3195.

(12) The chemistry of this ring system has been reviewed: Chen, Z.; Trudell, M. L. *Chem. Rev.* **1996**, *96*, 1179.

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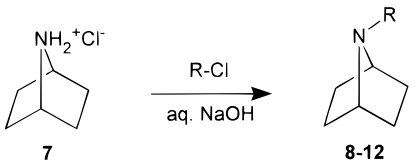
of *N*-acyl-7-azabicylo[2.2.1]heptanes **4** might lead to intermediates **5** or **6** useful for the synthesis of epibatidine and analogs thereof.

Our objective in this work, therefore, has been to obtain stereoselective oxygenation of the 7-azabicyclo[2.2.1]-heptane moiety and to study the outcome of these oxygenations as influenced by changes in both the N-substituent and the microorganism. We planned to extend our studies to the oxygenation of 8-azabicyclo-[3.2.1]octane and 9-azabicyclo[3.3.1]nonane substrates. For the microorganisms employed in these studies, the stereochemical information contained in the products should contribute to a better understanding of both the regio- and stereochemistry of substrate oxygenation. Described herein are the microbial hydroxylations of a series of *N*-acyl-7-azabicyclo[2.2.1]heptanes, of 8-(phenyloxycarbonyl)-8-azabicyclo[3.2.1]octane, and of 9-(phenyloxycarbonyl)-9-azabicyclo[3.3.1]nonane using the fungi *Beauveria bassiana*, *Rhizopus nigricans*, *Aspergillus ochraceus*, and *Rhizopus arrhizus* as the primary organisms.

Results and Discussion

***N*-Acyl-7-azabicyclo[2.2.1]heptanes.** A series of *N*-acyl-7-azabicyclo[2.2.1]heptane substrates was prepared by acylation of the hydrochloride salt **7**[13] under Schotten−Baumann conditions. The characterization of



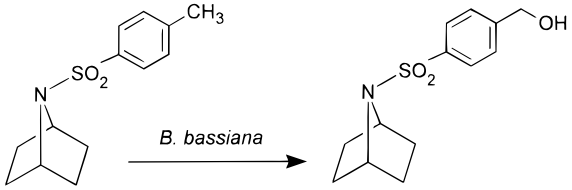
7 **8-12**

8, R = *p*-$(CH_3)C_6H_4SO_2$-, 68%
9, R = PhOC(O)-, 58%
10, R = $(CH_3)_3$COC(O)-, 68%
11, R = $PhCH_2OC(O)$-, 85%
12, R = PhC(O)-, 58%

derivatives **8**−**12** was straightforward, although we observe sets of signals for each of two rotamers in some NMR spectra as a consequence of restricted rotation about the N−CO bond. This phenomenon was described for the *N*-acetyl derivative of epibatidine.[2] We find that the number of chemically nonequivalent carbons in the ^{13}C NMR spectra, at room temperature, is dependent on the nature of the N-substituent. In the ^{13}C NMR spectrum of benzamide **12**, four distinct signals are observed for the bicycloalkane methylene carbons. In contrast, only two sharp signals are observed for the bicycloalkane methylene carbons in derivatives **8** and **11**, whereas two broader signals are observed for **9** and **10**. NMR evidence for restricted rotation has frequently been reported for other amides as well.[14]

Exploratory bioconversions were carried out in shake flasks on 10−20 mg of substrate in 100 mL of nutrient media. Large-scale conversions were carried out on 0.5−2.0 g of substrate in 10 L of media either in a 10 L fermentation tank or in multiple shake flasks. Microorganisms were selected from inspection of the considerable wealth of microbial transformations reported both from others and from these laboratories.[7] Initially, substrates **8**, **9**, and **11** were each bioconverted using *B. bassiana*, *R. nigricans*, and *A. ochraceous*. Workup of fermentation samples taken periodically followed by analysis of crude extracts by GCMS allowed us to detect possible oxygenated products and to estimate the extent of conversion of starting materials. The crude extracts were diluted to a concentration theoretically equivalent to that of prepared standards. Direct comparison of integration (GCMS) allowed for approximation of conversion to oxygenated products and overall recovery. From these results, we were able to choose conditions for scale up. Large-scale fermentations were monitored starting at the 3 day point and every 12−24 h thereafter. Large-scale fermentation times were 3−6.5 days in length.

We were surprised to find that in the bioconversion of **8** with *B. bassiana*, the product (**13**) was exclusively the result of hydroxylation of the *p*-phenylmethyl group in preference to oxygenation of the bicyclic ring carbons. The site of hydroxylation was easily determined from the ^1H NMR spectrum of the product **13**. The hydroxymethyl



8 **13** (54%)

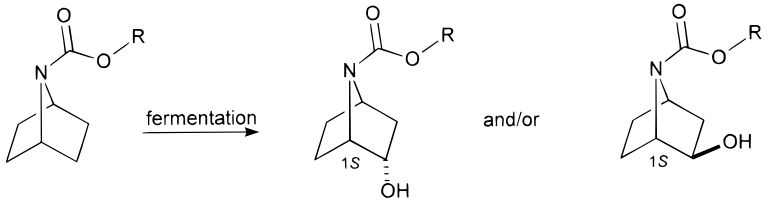
product **13** was also the only product formed, as detected by GCMS, in small-scale transformations of **8** with the microorganisms *R. nigricans* and *A. ochraceous*. In each case, the GCMS fragmentation pattern and retention time of the product were identical to that observed for **13**. Conversion of **8** to **13** using *R. nigricans* was slightly higher than was observed for *B. bassiana*, but only a trace of **13** was detected in a fermentation using *A. ochraceus*.

The other *N*-acyl-7-azabicyclo[2.2.1]heptane substrates (**9**−**12**) yielded products in which hydroxylation occurred only on the bicycloalkane moiety. We first examined bioconversion of phenyloxycarbonyl derivative **9**. Hydroxylation of **9** using *B. bassiana* gave a single product in an isolated yield of 46% that was characterized as the *endo*-alcohol **14** (see Table 1). Substrate **9** is achiral, but substitution of any of the methylene groups will introduce chirality into the molecule. Product **14** has $[\alpha]_D$ +5.2°, indicating that a degree of asymmetric induction occurred during the oxygenation process. *Note that the structures used to illustrate this discussion are drawn to represent the absolute configuration of the enantiomer obtained in excess from the oxygenation process.* In a small-scale bioconversion of **9** with *R. nigricans*, a 20% yield (determined by GCMS) of **14** was observed, while no product was detected in a fermentation using *A. ochraceus*.

The assignment of configuration to the hydroxyl group in **14** is based on NMR data accumulated at 50 °C; sharper signals were observed at this temperature, which allowed for a more detailed analysis of coupling constants. A ^{13}C DEPT spectrum exhibited three methylene carbon signals, six tertiary carbon signals, and two quaternary carbon signals, consistent with assignment of hydroxylation to a secondary carbon. ^1H NMR couplings observed for H-2 were consistent with the vicinal Karplus angles required by the *endo*-hydroxy stereo-

(13) Hassner, A.; Belostotskii, A. M. *Tetrahedron Lett.* **1995**, *36*, 1709.
(14) Iida, H.; Watanabe, Y.; Kibayashi, C. *J. Org. Chem.* **1985**, *50*, 1818.

Table 1. Results of the Oxygenation of *N*-Acyl-7-azabicyclo[2.2.1]heptanes Using Several Microorganisms



9, R = Ph
10, R = *t*-Bu
11, R = CH_2Ph

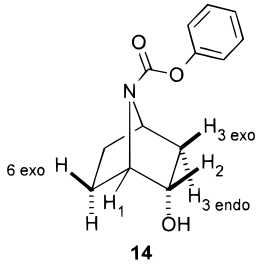
14, R = Ph
15, R = *t*-Bu
16, R = CH_2Ph

2, R = *t*-Bu
17, R = CH_2Ph

compd	microorganism	compd	yield (%)	abs config	ee[a] (%)	compd	yield (%)	abs config	ee[a] (%)
9	*B. bassiana*	**14**	46	(1*S*)	51				
9	*R. nigricans*	**14**	20	nd[b]	nd				
10	*B. bassiana*	**15**	10	(1*S*)	26	**2**	0		
10	*R. nigricans*	**15**	62	(1*S*)	28	**2**	27	(1*S*)	42
11	*B. bassiana*	**16**	28	(1*R*)	5	**17**	0		
11	*R. nigricans*	**16**	14	(1*S*)	36	**17**	8	(1*S*)	57
11	*R. arrhizus*	**16**	5	(1*S*)	31	**17**	18	(1*S*)	22

[a] Enantiomeric excess (ee) determined by ^1H and ^{19}F NMR of Mosher esters (see Experimental Section). [b] Not determined.

chemistry (see illustration of **14**). The observed couplings



14

H_2, δ 4.40, dddd

$J\ H_2\text{-}H_{3\ exo}$ = 9.7 Hz
$J\ H_2\text{-}H_1$ = 4.8 Hz
$J\ H_2\text{-}H_{3\ endo}$ = 3.4 Hz
$J\ H_2\text{-}H_{6\ exo}$ = 1.5 Hz

were confirmed by a COSY experiment, including the coupling between H-2 and H-6_{exo}. A correlation between C-2 (δ 70.6) and H-2 was also observed in a HETCOR experiment. Similar H−H couplings are reported for other *endo* N-substituted 7-azabicyclo[2.2.1]heptan-2-ols.[4]

The configuration of the hydroxyl group was confirmed by conversion of **14** to the *N*-BOC derivative **15**, which has been reported as the racemate[4] and also is obtained from bioconversions described below using *N*-BOC substrate **10**. Hydrolysis of the phenyloxycarbonyl group of **14** was achieved with aqueous base using 18-crown-6 as a phase-transfer catalyst. Consumption of starting material was complete after 18 h of reflux, and after reaction with BOC anhydride, an 85% yield of **15** was obtained. When we did the hydrolysis of **14** using *tert*-butyl ammonium iodide as the phase-transfer catalyst,[15] the reaction required 48 h and a lower yield of **15** was isolated.

The enantiomeric excess of **14** was measured in two ways. First, the Mosher esters **18a** and **18b** were prepared, and the ratio of diastereoisomers was quantitated by both ^1H and ^{19}F NMR. Second, the alcohol **15** derived above from **14** was oxidized *via* a Swern oxidation to ketone **3** allowing correlation of the optical rotation to that reported[5] for the optically pure **3**. Both approaches gave ee values of 51% that can be assigned to **14**. Comparison of the sign of the optical rotations of the ketones from the two sources allows assignment of 1*S* as the absolute configuration of the bioconversion product. This absolute configuration is opposite that found in natural (−)-epibatidine.[4]

We then explored the stereoselectivity of the oxygenation process as a consequence of changes in the N-substituent (see Table 1). In small-scale bioconversions of the BOC derivative **10**, major and minor hydroxylated products were detected with either *B. bassiana* and *R. nigricans* but not with *A. ochraceous*. The same two products were formed in either fermentation judging from the GCMS data. When the bioconversion of **10** with *B. bassiana* was scaled up in a 10 L tank, the minor product was detected in trace amounts and only the *endo* alcohol **15** was isolated. GCMS analysis prior to workup suggested a high yield of **15**, but we experienced difficulties in isolation of the pure product and obtained this compound in only 10% yield. The NMR data for the product are identical to that reported in the literature for **15**. Following oxidation to ketone **3**, correlation of optical rotation to the literature data[5] for enantiomerically pure **3** gave an ee of 26% and 1*S* absolute configuration. The 26% ee for **15** was confirmed by NMR analysis of Mosher esters **19a** and **19b**.

A large-scale bioconversion of BOC-derivative **10** using *R. nigricans* afforded both *endo* alcohol **15** and *exo* alcohol **2**. The isomers were separable by SiO_2 chromatography and were isolated in an excellent total yield of 89%. Enantiomerically pure **2** has been reported,[4] and comparison of NMR and optical rotation data allowed assignment of the absolute configuration and ee as shown in Table 1. Following oxidation of **15** to ketone **3**, correlation of the optical rotation with literature data[5] gave an ee of 28% that again was confirmed by analysis of Mosher esters **19a** and **19b** derived from **15**.

We next examined the *N*-(benzyloxycarbonyl) (Cbz) derivative **11** as a potential substrate. Hydroxylated products were detected in exploratory bioconversions of **11** using *B. bassiana* and *R. nigricans* but not with *A. ochraceus*. A large-scale bioconversion using *B. bassiana* gave an alcohol to which we have assigned structure **16** on the basis of an ^1H NMR pattern similar to those observed for **14** and **15**. To confirm the structure, the Cbz group was removed by transfer hydrogenation followed by derivatization of the crude amine with *tert*-butyl dicarbonate to give **15**. ^1H and ^{13}C NMR data obtained

(15) Nelsen, S. F.; Hollinsed, W. C.; Kessel, C. R.; Calabrese, J. C. *J. Am. Chem. Soc.* **1978**, *100*, 7876.

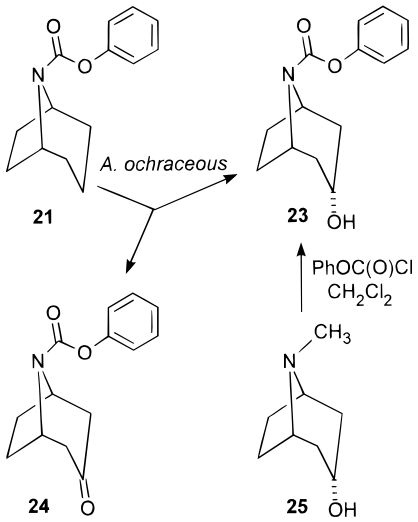
on this derivative correlated with NMR data acquired on **15** obtained in prior fermentations. A minor product, identical to **17** (described below) in GCMS retention time and fragmentation pattern, was detected in the exploratory bioconversion of **11** using *B. bassiana*. This minor product was formed to a much lesser extent in the large-scale fermentation, and only the *endo* isomer **16** was isolated. A large-scale bioconversion of **11** using *R. nigricans* produced both *endo* alcohol **16** and *exo* alcohol **17**. The enantiomeric excess of *endo* alcohol **16** was determined by analysis of Mosher esters **20a** and **20b**, while characterization of *exo* alcohol **17** was carried out by comparison of NMR and optical rotation data to that obtained for **17** as described below.

An effort was then made to identify other organisms capable of biotransformation with this class of substrates. A limited screen of fungi (ca. 50 microorganisms) was carried out on substrate **11**, and several were identified as producing **16** and **17** with *R. arrhizus* being the most productive. A large-scale bioconversion of **11** using *R. arrhizus* yielded *endo* alcohol **16** and *exo* alcohol **17** and was the only experiment in which the *exo* alcohol predominated (see Table 1). After chromatographic separation, the structure and absolute configuration of *exo* alcohol **17** were determined by conversion to the *N*-BOC derivative **2** and comparison of NMR and optical rotation data to that reported for enantiomerically pure **2**.[4]

Finally, in the case of benzoyl derivative **12**, hydroxylated products were detected (GCMS) on an exploratory scale in bioconversions using *B. bassiana* and *R. nigricans*, but these products were not isolated and characterized due to low conversion (5−10%) of starting material.

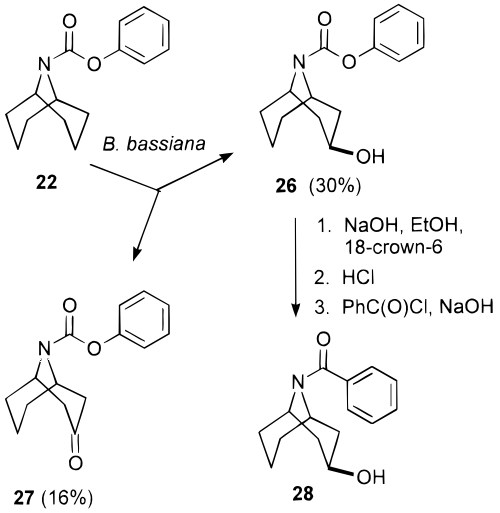
8-(Phenyloxycarbonyl)-8-azabicyclo[3.2.1]-octane and 9-(Phenyloxycarbonyl)-9-azabicyclo-[3.3.1]nonane. We have extended our biotransformation studies to the 8-azabicyclo[3.2.1]octane and 9-azabicyclo-[3.3.1]nonane systems. Phenyloxycarbonyl derivatives **21** and **22** were chosen as model substrates and were readily prepared by reaction of the *N*-methyl precursors, tropane and deoxopsuedopelletierine,[15] with phenyl chloroformate.

Bioconversion of **21** using *A. ochraceous* gave an alcohol (**23**, 36%) and a ketone (**24**, 4%). The ^{13}C NMR spectrum



of the alcohol **23** exhibited seven signals for saturated carbon atoms in the room-temperature spectrum (see Experimental Section). The presence of seven signals at first suggests that the seven carbons of the bicyclic ring system are nonequivalent and that the hydroxyl group is in an asymmetric location on the molecule. Inspection of the spectrum, however, shows three closely paired sets of signals, raising the possibility that equivalent carbon atoms are giving two signals each because of unequal magnetic fields generated by slow rotation of the acyl group about the carbonyl−nitrogen bond. Indeed, signals for C-1 and C-5, C-2 and C-4, and for C-6 and C-7 coalesced into broad singlets upon acquisition of the ^{13}C NMR spectrum at 50 °C. Consequently, the hydroxyl group in **23** must be symmetrically placed on the molecule, and the C-3 position is the only logical choice for this substitution.

The melting point for our sample of **23** (151−153 °C) was identical to the literature value (151−153 °C) for the 3-*endo* alcohol,[16] but a mp for the epimeric alcohol has not been reported. We confirmed the configuration of the hydroxyl group in **23** by repeating the literature conversion of tropine **25** to the Cbz derivative **23** as reported.[16] ^{1}H and ^{13}C NMR and melting point data obtained on the authentic sample correlated with data obtained on the **23** we obtained from biotransformation.



Bioconversion of **22** using *A. ochraceus* also gave an alcohol (**26**, 30%) and a ketone (**27**, 16%). Assignment of the oxygen substituent to the C-3 position in both compounds could be made on the basis of analysis of the ^{1}H and ^{13}C NMR spectral data (both molecules give spectra having only five nonaromatic carbon signals, indicating symmetrical placement of the substituents). The configuration of the hydroxyl group in **26** was determined by conversion of carbamate **26** to benzamide **28**.[17] The melting point of **28** (153−154 °C) compares well with the reported melting point (152−153 °C) for the *exo* alcohol and differs from the reported melting point (116−117 °C) for the *endo* alcohol.[17]

We were surprised that hydroxylation of **21** and **22** had given *endo* and *exo* alcohols, respectively, since the structures of the two substrates are quite similar. One clear difference between the experiments is that they used different microorganisms for the bioconversions of the two compounds. We, therefore, carried out a small-scale bioconversion of **21** using *B. bassiana* and examined it by GCMS. The analytical results showed that alcohol

(16) Leete, E. *Phytochemistry* **1972**, *11*, 1713.
(17) Alder, K.; Dortmann, H. A. *Chem. Ber.* **1953**, *86*, 1544.

23 was produced by this microorganism just as it was with *A. ochraceous*; the difference, therefore, does not lie in the use of different microorganisms in the two experiments. Clearly, there are subtle structural details of enzymic active sites that we do not yet appreciate.

Summary

The best chemical yield observed in this study of the oxygenation of *N*-acyl-7-azabicyclo[2.2.1]heptanes (Table 1) is the oxygenation of **10** using *R. nigricans* to give a combined 89% yield of 2-*endo*- and 2-*exo*-alcohols (**15** and **2**). Oxidation of both **15** and **2** will generate ketone **3** in good yield. Since ketone **3** has been carried on to epibatidine as described by Fletcher and co-workers,[4] our results provide a link between the synthesis of 7-benzyl-7-azabicyclo[2.2.1]heptane described by Hassner and Belostotskii[13] and the conversion of ketone **3** into epibatidine and thereby constitute a formal synthesis of the unnatural enantiomer of this compound.

The best enantioselectivity observed is in the oxygenation of **9** using *B. bassiana* to give the 2-*endo*-alcohol **14** with 51% ee and in 46% isolated yield. The predominant enantiomer of **14** is shown to have the 1*S* absolute configuration, which is opposite to that required by natural (−)-epibatidine. While these results demonstrate the potential of microbiological oxygenations to deliver enantioselective reactions, in this case they are neither of the correct absolute configuration nor of high enough enantioselectivity to be of use in the synthesis of either natural (−)-epibatidine or its unnatural enantiomer.

In previous work with *B. bassiana* we have drawn attention to a spatial arrangement in the products that can be summarized as a trans orientation of the newly introduced hydroxyl group(s) to an existing functional group (usually an amide group) in the substrate.[7] Such an orientation is seen in the present results in the hydroxylation of the 7-azabicyclo[2.2.1]heptane (products **14**−**16**)[18] and 8-azabicyclo[3.2.1]octane (**23**) systems. The surprising result in this work is in the hydroxylation of *N*-(phenyloxycarbonyl)-9-azabicyclo[3.3.1]nonane (**22**), which produces the *cis*-3-alcohol **26**.

Finally, oxygenation of the *p*-toluenesulfonamide **8** by *B. bassiana* on the methyl group of the toluene rather than on the bicyclic hydrocarbon ring system suggests that the tosyl group finds easier entry into the active site cavity of the hydroxylase than does the bicyclic system. The bioconversion of other *p*-toluenesulfonamide derivatives of saturated cyclic hydrocarbons using *B. bassiana* has resulted in oxygenation of the saturated portion of the substrate.[19]

Experimental Section

7-(*p*-Toluenesulfonyl)-7-azabicyclo[2.2.1]heptane (8). A 250 mL separatory funnel was charged with crude salt **7** (6.20 g) and shaken vigorously with 100 mL of 2 N NaOH. *p*-Toluenesulfonyl chloride (3.8 g, 20 mmol) was added, and the funnel was vigorously shaken for 10−15 min. The aqueous mixture was then extracted with CH_2Cl_2 (4 × 100 mL). The organic phases were dried (Na_2SO_4) and concentrated to give 4.6 g of crude product, which was recrystallized from 3:2 hexane:EtOAc to give 3.3 g (0.0131 mol, 68% based on the 7-benzyl-7-azabicyclo[2.2.1]heptane precursor[13] of **7**) of **8** as a white solid: mp 120−121 °C; ^{1}H NMR ($CDCl_3$) δ 1.38 (dm, J = 7.2 Hz, 4 H), 1.78 (dm, J = 7.2 Hz, 4 H), 2.42 (s, 3 H), 4.18 (m, 2 H), 7.28 (d, J = 8.0 Hz, 2 H), 7.80 (d, J = 8.2 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 21.4, 30.0, 59.1, 127.4, 129.3, 137.7, 143.2; IR (mull) 604 (s), 674 (s), 1055 (s), 1088 (s), 1152 (s), 1199 (m), 1334 (s), 1608 (m), 2922 (s), 2956 (s) cm^{-1}; GCMS m/z 251 (M^+). Anal. Calcd for $C_{13}H_{17}NO_2S$: C, 62.12; H, 6.82; N, 5.58. Found: C, 62.05; H, 6.82; N, 5.56.

7-(Phenyloxycarbonyl)-7-azabicyclo[2.2.1]heptane (9). A 250 mL separatory funnel was charged with crude salt **7** (6.20 g) and shaken vigorously with 2 N NaOH (100 mL). Phenyl chloroformate (3.1 g, 20 mmol) was added, and the funnel was vigorously shaken for 10−15 min. The aqueous mixture was then extracted with CH_2Cl_2 (4 × 50 mL). The organic phases were dried (Na_2SO_4) and concentrated to give a solid crude product that was recrystallized from hexane to give 2.5 g (0.0115 mol, 58% based on the precursor of **7**) of **9** as a white solid: mp 70−71 °C; ^{1}H NMR ($CDCl_3$) δ 1.51 (d, J = 6.8 Hz, 4 H), 1.90 (d, J = 6.8 Hz, 4 H), 4.44 (s, 2 H), 7.13 (m, 2 H), 7.19 (m, 1 H), 7.36 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 30.2, 56.9, 122.0, 125.5, 129.6, 151.7, 153.8; IR (mull) 1042 (m), 1165 (s), 1196 (s), 1391 (s), 1718 (s), 2922 (s), 2956 (s) cm^{-1}; GCMS m/z 217 (M^+). Anal. Calcd for $C_{13}H_{15}NO_2$: C, 71.87; H, 6.96, 6.45. Found: C, 71.77; H, 6.95; N, 6.40.

7-[(1,1-Dimethylethoxy)carbonyl]-7-azabicyclo[2.2.1]-heptane (10). A 250 mL flask was charged with crude salt **7** (5.0 g), di-*tert*-butyl dicarbonate (7.0 g, 0.032 mol), dioxane (100 mL), and 2 N NaOH (50 mL) and stirred at rt for 24 h. The solvents were removed by rotary evaporation. H_2O (100 mL) was added, and the product was extracted with CH_2Cl_2 (3 × 75 mL). The CH_2Cl_2 phases were dried (Na_2SO_4) and concentrated to give an oily residue. Chromatography on SiO_2 (5% EtOAc:hexane, R_f 0.4) gave 2.2 g (0.0111 mol, 68%) of **10** as a clear oil: ^{1}H NMR ($CDCl_3$) δ 1.37 (d, J = 7.2 Hz, 4 H), 1.43 (s, 9 H), 1.74 (m, 4 H), 4.20 (bs, 2 H); ^{13}C NMR ($CDCl_3$) δ 28.66, 29.95, 56.50, 79.58. 156.17; IR (mull) 1090 (m), 1150 (s), 1185 (m), 1318 (m), 1369 (s), 1390 (m), 1702 (s), 2954 (m), 2975 (m) cm^{-1}; HRMS calcd 197.1416, obsd 197.1418. Anal. Calcd for $C_{11}H_{19}NO_2$: C, 66.97; H, 9.71; N, 7.10. Found: C, 65.67; H, 9.61; N, 6.89.

7-(Benzyloxycarbonyl)-7-azabicyclo[2.2.1]heptane (11). A 250 mL separatory funnel containing crude salt **7** (5.0 g) and 2 N NaOH (100 mL) was shaken for several minutes. Benzyl chloroformate (3.4 g, 20.0 mmol) was added, and the funnel was shaken vigorously for 10 min. The mixture was extracted with CH_2Cl_2 (4 × 50 mL), and the combined CH_2Cl_2 phases were dried (Na_2SO_4) and concentrated to give a crude oil. Chromatography on SiO_2 (5% EtOAc−hexane, R_f 0.25) gave 3.25 g (0.0140 mol, 85% based on the precursor to **7**) of **11** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.42 (d, J = 7.3 Hz, 4 H), 1.78 (m, 4 H), 4.31 (bs, 2 H), 5.12 (s, 2 H), 7.34 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 30.06, 56.57, 67.00, 128.17, 128.28, 128.8, 137.31, 155.96; IR 698 (m), 1093 (m), 1153 (s), 1260 (m), 1304 (m), 1313 (s), 1349 (m), 1394 (m), 1707 (s), 2953 (s) cm^{-1}; HRMS calcd 231.1259, obsd 231.1261. Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.09; H, 7.35; N, 6.06.

7-Benzoyl-7-azabicyclo[2.2.1]heptane (12). A 250 mL separatory funnel containing crude salt **7** (6.0 g) and 2 N NaOH (100 mL) was shaken for several minutes. Phenyl chloroformate (3.4 g, 20.0 mmol) was added, and the funnel was shaken vigorously for 10 min. The mixture was extracted with CH_2Cl_2 (4 × 50 mL), and the combined CH_2Cl_2 phases were dried (Na_2SO_4) and concentrated to give a crude solid. Chromatography on SiO_2 (5% CH_3CN−CH_2Cl_2, R_f 0.35) gave 2.32 g (0.0115 mol, 58% based on the precursor of **7**) of **12** as a white solid: mp 75−76 °C; ^{1}H NMR ($CDCl_3$) δ 1.49 (d, J = 8.4 Hz, 4 H), 1.75−1.98 (m, 4 H), 4.12 (bs, 1 H), 4.75 (bs, 1 H), 7.42 (m, 3 H), 7.54 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 29.1, 30.9, 54.1, 59.1, 128.1, 128.6, 130.7, 136.7, 169.0; IR (mull) 1621 (s), 1615 (s), 1455 (s), 1445 (s), 1461 (m), 1576 (m), 2922 (s),

(18) Epimeric alcohols **15**/**2** and **16**/**17** may represent direct hydroxylation products or, alternately, could arise through a sequence of enzymic conversions: (a) hydroxylation, (b) dehydrogenation, and (c) reduction. We have no direct evidence to choose between the two possibilities but note that the intermediate ketone of the latter mechanism was not detected.

(19) (a) Fonken, G. S.; Herr, M. E.; Murray, H. C.; Reineke, L. M. *J. Org. Chem.* **1968**, *32*, 3182. (b) Johnson, R. A.; Herr, M. E.; Murray, H. C.; Fonken, G. S. *J. Org. Chem.* **1968**, *32*, 3187.

2953 (s) cm^{-1}. Anal. Calcd for $C_{13}H_{15}NO$: C, 77.58; H, 7.513; N, 6.96. Found: C, 77.27; H, 7.38; N, 7.04.

Biotransformation Process. The cultures used in these experiments were *B. bassiana* (ATCC 7159, UC-1365), *R. arrhizus* (ATCC 11145, UC-4041), *R. nigricans* (UC-4285), and *A. ochraceus* (NRRL 405, UC-4115). Fermentation media was prepared by mixing cerelose (25 g/L) and Pharmamedia (Archer Daniels Midland Co., 25 g/L) with tap water and adjusting the pH to 7.2 with NH_4OH. Fermentation tanks containing 10 L of sterilized media (autoclaved at 180 °C for 90 min) were innoculated with 100 mL of the appropriate seed culture (pregrown in a 500 mL flask for 3 days on two rotary shakers at 250 rpm). Surfactant (SAG 471, Union Carbide) (0.4 g/L) was added to the tank, the air flow adjusted to 12 L/min, and agitation was set at 250 rpm. The culture was allowed to grow for 24 h at 28 °C, after which time the substrate was added as a 0.1 g/mL acetone or DMF solution. The fermentation or transformation was continued additional days until the transformation was judged optimum by chromatographic analysis. The contents of the tank were transferred to a 25 L vat equipped with a drain and air-driven propeller agitator. CH_2Cl_2 (8 L) was added, and the mixture was agitated for 1−2 h. The contents were suction-filtered in 2 L portions through a layer of Celite on a polypropylene pad, changing the Celite with each filtration. The CH_2Cl_2 was separated, dried (Na_2SO_4), and concentrated by rotary evaporation to give the crude residue, purified by column chromatography as detailed below. Similarly, shake flask fermentations were carried out in 500 mL Erlenmeyer flasks equipped with air-permeable microbe filters using 100 mL sterilized media prepared as described above. The flasks were placed on automated shakers (250 revolutions per min) at 28 °C for the duration of the fermentations. No surfactant was added. For extraction, 100 mL of CH_2Cl_2 was added to each flask, and the flasks were shaken for 1 h. To remove the biomass, collective filtration of the flask contents through Celite was carried out. The organic phase was separated, dried, and concentrated. Fermentations were monitored by removal and workup of a sample aliquot (ca. 25 mL) followed by GCMS and TLC analysis of the crude residue.

Preparation of 7-[(*p*-Hydroxymethyl)benzenesulfonyl]-7-azabicyclo[2.2.1]heptane (13) by Bioconversion of 7-(*p*-Toluenesulfonyl)-7-azabicyclo[2.2.1]heptane (8) Using *B. bassiana*. Bioconversion of **8** (1.00 g, 3.98 mmol) using *B. bassiana* in a 10 L tank was carried out for 5 days. Following workup (see general procedure above), the crude residue was twice chromatographed on SiO_2 [(1) 5% CH_3CN−CH_2Cl_2, R_f 0.25; (2) 40% acetone−hexane, R_f 0.40] to give 0.572 g (0.00205 mol, 54%) of **13** as a white solid: mp 121−122 °C; ^{1}H NMR ($CDCl_3$) δ 1.37 (d, J = 7.2 Hz, 4 H), 1.75 (d, J = 7.2 Hz, 4 H), 2.58 (s, 1 H), 4.16 (s, 2 H), 4.75 (s, 2 H), 7.44 (d, J = 8.3 Hz, 2 H), 7.82 (d, J = 8.3 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 30.5, 59.7, 64.5, 127.2, 128.0, 139.8, 146.6; IR (mull) 677 (s), 1054 (s), 1091 (s), 1150 (s), 1320 (s), 1412 (s), 1458 (s), 2925 (s), 2955 (s), 3493 (s) cm^{-1}; GCMS m/z 267 (M^+), 238, 171, 107, 77, 68, 41. Anal. Calcd for $C_{13}H_{17}NO_3S$: C, 58.41; H, 6.41; N, 5.24. Found: C, 58.55; H, 6.47; N, 5.22.

Preparation of 7-(Phenyloxycarbonyl)-7-azabicyclo-[2.2.1]heptan-2-*endo*-ol (14) by Bioconversion of 7-(Phenyloxycarbonyl)-7-azabicylo[2.2.1]heptane (9) Using *B. bassiana*. Bioconversion of **9** (1.00 g, 4.60 mmol) using *B. bassiana* in a 10 L tank was carried out for 5 days. Following workup (see general procedure above), the crude residue was chromatographed three times on SiO_2 [(1) 20% acetone−hexane, R_f 0.20; (2) 95:4.5:0.5 $CHCl_3$:MeOH:Et_3N, R_f 0.25; (3) 30% acetone−hexane, R_f 0.40] to give 0.495 g (0.00212 mol, 46%) of **14** as a white solid: mp 103−104 °C; ^{1}H NMR (50 °C, $CDCl_3$) δ 1.13 (dd, J = 12.7, 3.4 Hz, 1 H), 1.61 (ddd, J = 11.4, 9.0, 3.9 Hz, 1 H), 1.75 (dddd, J = 12.3, 3.6, 3.1, 1.5 Hz, 1 H), 1.89 (m, 1 H), 1.9 (s, 1 H), 2.25 (m, 1 H), 2.30 (m, 1 H), 4.31 (dd, J = 4.5, 3.8 Hz, 1 H), 4.33 (dd, J = 4.5, 3.8 Hz, 1 H), 4.40 (dddd, J = 9.7, 4.8, 3.4, 1.5 Hz, 1 H), 7.08 (m, 2H), 7.16 (m, 1H), 7.36 (m, 2H); ^{13}C NMR (50 °C) ($CDCl_3$) δ 20.8, 29.9, 39.3, 57.7, 60.2, 70.6, 121.4, 125.1, 129.1, 152.2, 153.4; $[\alpha]^{25}_D$ + 5.2° (c = 1.10, $CHCl_3$); GCMS m/z 234 (M^+ + H), 140, 94, 79, 41; IR (mull) 1042 (s), 1201 (s), 1380 (s), 1706 (s), 1720 (s), 2924 (s), 2952 (s), 3461 (s) cm^{-1}. Anal. Calcd for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.01. Found: C, 67.02; H, 6.56; N, 5.97.

Alcohol **14** was converted to Mosher esters **18a** and **18b**; the esters were analyzed by ^{19}F NMR (51.9% ee) and ^{1}H NMR (50.0% ee) spectroscopy.

Preparation of Mosher Esters 18a and 18b. Mosher esters **18a** and **18b** were prepared by the general procedure described below for **20a** and **20b**. The mixture of diastereoisomers was purified by chromatography over SiO_2 (10% EtOAc−hexane, R_f 0.15) to give a viscous oil: ^{1}H NMR ($CDCl_3$) δ **18a** 3.54 (OCH_3), **18b** 3.58 (OCH_3); ^{19}F NMR ($CDCl_3$ vs internal $CFCl_3$ standard) **18a** −71.93 (s, CF_3), **18b** −71.85 (s, CF_3).

Preparation of [(1,1-Dimethylethoxy)carbonyl]-7-azabicyclo[2.2.1]heptan-2-*endo*-ol (15) by Bioconversion of [(1,1-Dimethylethoxy)carbonyl]-7-azabicyclo[2.2.1]-heptane (10) Using *B. bassiana*. Bioconversion of **10** (1.00 g, 5.06 mmol) using *B. bassiana* in a 10 L tank was carried out for 4 days. Following workup (see general procedure above), the crude residue was twice chromatographed on SiO_2 [(1) 5% MeCN−CH_2Cl_2, R_f 0.20; (2) 10% acetone−hexane, R_f 0.1] to give 0.111 g (0.00052 mol, 10%) of **15** as a viscous oil: ^{1}H NMR ($CDCl_3$) δ 1.05 (dd, J = 12.7, 3.5 Hz, 1 H), 1.41 (s, 9 H), 1.45−1.75 (m, 3 H), 2.65 (bs, 1 H), 4.09 (m, 2 H), 4.30 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 20.66, 28.26, 29.79, 39.07, 57.38, 60.00, 70.64, 79.73, 155.69; GCMS m/z 213 (M^+), 157, 113, 69, 57, 41; $[\alpha]^{25}_D$ +1.2° (c = 1.00, $CHCl_3$).

Alcohol **15** was converted to Mosher esters **19a** and **19b** followed by ^{19}F NMR (25.2% ee) and ^{1}H NMR (26.7% ee) analysis. Swern oxidation of a sample of **15** (0.082 g) gave ketone **3**, $[\alpha]_D$ +19.0° (c = 1.00, $CHCl_3$).

Preparation of Mosher Esters 19a and 19b. Mosher esters **19a** and **19b** were prepared by the general procedure described below for **20a** and **20b**. The mixture of diastereoisomers was purified by chromatography over SiO_2 (20% EtOAc−hexane, R_f 0.45) to give a viscous oil: ^{1}H NMR ($CDCl_3$) δ **19a** 3.5 (OCH_3), **19b** 3.55 (OCH_3); ^{19}F NMR ($CDCl_3$ vs internal $CFCl_3$ standard) **19a** −71.99 (s, CF_3), **19b** −71.90 (s, CF_3).

Preparation of 15 and [(1,1-Dimethylethoxy)carbonyl]-7-azabicyclo[2.2.1]heptan-2-*exo*-ol (2) by Bioconversion of [(1,1-Dimethylethoxy)carbonyl]-7-azabicyclo-[2.2.1]heptane (10) Using *R. nigricans*. Bioconversion of **10** (0.250 g, 1.27 mmol) using *R. nigricans* in 10 shake flasks was carried out for 3.5 days. Following workup, the residue was first chromatographed on SiO_2 (10% acetone−CH_2Cl_2, R_f 0.30) to give a mixture of *endo* and *exo* isomers that were then separated on SiO_2 (40% EtOAc−hexane, R_f *endo* 0.20, R_f *exo* 0.10) to give 0.169 g (0.793 mmol, 62%) of *endo* isomer **15** and 0.075 g (0.352 mmol, 27%) of *exo* isomer **2**, both as viscous oils: ^{1}H and ^{13}C NMR ($CDCl_3$) for **15** are as given above. *Exo* isomer **2**: ^{1}H NMR ($CDCl_3$) δ 1.20−1.29 (m, 2 H), 1.44 (s, 9 H), 1.57−1.73 (m, 3 H), 1.82 (dd, J = 13.1, 6.8 Hz, 1 H), 1.95 (bs, 1 H), 3.85 (dm, J = 5.7 Hz, 1 H), 4.10 (d, J = 5.1 Hz, 1 H), 4.22 (t, J = 4.6 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 23.97, 28.15, 28.30, 42.52, 55.42, 63.19, 74.41, 79.90, 156.68; $[\alpha]^{25}_D$ +8.8° (c = 0.639, CH_2Cl_2); GCMS m/z 213 (M^+), 157, 113, 69, 68, 57, 41.

Alcohol **15** was converted to Mosher esters **19a** and **19b**; ^{19}F NMR analysis: 29.3% ee. Swern oxidation of **15** (0.050 g) gave ketone **3**, $[\alpha]_D$ +20.7° (c = 1.00, $CHCl_3$).

Preparation of 7-(Benzyloxycarbonyl)-7-azabicyclo-[2.2.1]heptan-2-*endo*-ol (16) by Bioconversion of 7-(Benzyloxycarbonyl)-7-azabicyclo[2.2.1]heptane (11) Using *B. bassiana*. Bioconversion of **11** (1.30 g, 5.62 mmol) using *B. bassiana* in a 10 L tank was carried out for 6.5 days. Following workup (see general procedure above), the residue was chromatographed three times on SiO_2 [(1) 20% EtOAc−hexane, R_f 0.30; (2) 15% acetone−hexane, R_f 0.10; (3) 12% acetone−CH_2Cl_2, R_f 0.30) to give 0.388 g (0.00157 mol, 28%) of **16** as a viscous oil: ^{1}H NMR ($CDCl_3$) δ 1.07 (dd, J = 12.7, 3.4 Hz, 1 H), 1.49−1.66 (m, 2 H), 1.77 (m, 1 H), 2.14−2.22 (m, 2 H), 2.50 (m, 1 H), 4.22 (m, 2 H, bridgehead H), 4.30 (m, 1 H, carbinol H), 5.09 (s, 2 H, benzylic H), 7.33 (s, 5 H, aromatic); ^{13}C NMR ($CDCl_3$) δ 20.78, 29.91, 39.18, 57.40, 59.99, 66.90, 70.66, 127.84, 128.07, 128.52, 136.64, 155.57; IR (mull) 3434 (m), 2908 (m),

1706 (s), 1682 (s), 1455 (m), 1406 (s), 1352 (s), 1302 (s), 1099 (s), 1086 (s) cm^{-1}; $[\alpha]^{25}_D$ −0.3° (c = 1.00, $CHCl_3$); HRMS calcd (M^+ + H) 248.1287, obsd 248.1289. Anal. Calcd for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66. Found: C, 66.79; H, 6.83; N, 5.45.

Alcohol **16** was converted to Mosher esters **20a** and **20b** as described below, and the esters were submitted to ^{19}F (4.1% ee) and ^1H (5.9% ee) NMR analysis.

General Procedure for Preparation of Mosher Esters. Preparation of 20a,b from 16. To a 1 mL reaction vial equipped with a septum and spin vane were added $CHCl_3$ (0.5 mL), Et_3N (13 μL,0.1 mmol), **16** (0.008 g, 0.032 mmol), and a single crystal of DMAP. (*R*)-(−)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.0164 g, 0.064 mmol) was then added via μL syringe (either neat or as a $CDCl_3$ solution), and the mixture was stirred overnight at rt. The contents of the reaction were eluted through a short column of SiO_2 (20% EtOAc−hexane) to give a purified mixture of diastereomers **20a** and **20b** as a viscous oil: ^1H NMR ($CDCl_3$) δ **20a** 3.51 (OCH_3), **20b** 3.54 (OCH_3); ^{19}F NMR ($CDCl_3$ vs internal $CFCl_3$ standard) **20a** −71.96 (s, CF_3), **20b** −71.89 (s, CF_3).

Preparation of 7-(Benzyloxycarbonyl)-7-azabicyclo-[2.2.1]heptan-*endo*-2-ol (16) and 7-(Benzyloxycarbonyl)-7-azabicyclo[2.2.1]heptan-2-(*exo*)-ol (17) by Bioconversion of 7-(Benzyloxycarbonyl)-7-azabicyclo[2.2.1]heptane (11) Using *R. nigricans*. Bioconversion of **11** (0.125 g, 0.540 mmol) using *R. nigricans* in 10 shake flasks was carried out for 4.5 days. Following workup (see general procedure above) the crude extract residue was chromatographed on SiO_2 (40% EtOAc−hexane, R_f *endo*-**16** 0.30, R_f *exo*-**17** 0.20). The *endo* isomer **16** was purified further by chromatography on SiO_2 (10% MeCN−CH_2Cl_2, R_f 0.25) to give 0.020 g (0.81 mmol, 15%): ^1H, ^{13}C NMR as given previously; $[\alpha]^{25}_D$ +1.5° (c = 1.00, $CHCl_3$). *Endo* alcohol **16** was converted to Mosher esters **20a** and **20b** as described above followed by ^{19}F (36.5% ee) and ^1H (35.1% ee) NMR analysis.

The *exo* isomer **17** was further chromatographed on SiO_2 (10% MeCN−CH_2Cl_2, R_f 0.20) to give 0.012 g (0.48 mmol, 8%) as a viscous oil: ^1H NMR ($CDCl_3$) δ 1.22−1.30 (m, 2 H), 1.58−1−77 (m, 3 H), 1.83 (dd, J = 13.1, 6.8 Hz, 1 H), 2.2 (b, 1 H), 3.88 (d, J = 5.9 Hz, 1 H), 4.20 (d, J = 5.2 Hz, 1 H), 4.34 (t, J = 4.5 Hz, 1 H), 5.11 (s, 2 H), 7.25−7.35 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 24.03, 28.33, 42.26, 55.37, 63.22, 66.96, 74.21, 127.91, 128.04, 128.50, 136.66, 156.52; HRMS calcd for $C_{14}H_{17}NO_3$ (M^+ + H) 248.1287, obsd 248.1289; $[\alpha]^{25}_D$ +11.6° (c = 1.00, $CHCl_3$).

Preparation of 7-(Benzyloxycarbonyl)-7-azabicyclo-[2.2.1]heptan-*endo*-2-ol (16) and 7-(Benzyloxycarbonyl)-7-azabicyclo[2.2.1]heptan-2-*exo*-ol (17) by Bioconversion of 7-(Benzyloxycarbonyl)-7-azabicyclo[2.2.1]heptane (11) using *R. arrhizus*. Bioconversion of **11** (0.500 g, 2.16 mmol) using *R. arrhizus* was carried out in 32 shake flasks for 3.5 days. Two isomeric products were separated by chromatography on SiO_2 (15% MeCN−CH_2Cl_2, R_f *endo*-**16** 0.20, R_f *exo*-**17** 0.15). Isomer **16** was further chromatographed on SiO_2 (50% EtOAc−hexane, R_f 0.30) to give 0.028 g (5%): $[\alpha]^{25}_D$ +1.0° (c = 1.00, $CHCl_3$). The *endo* isomer **16** was converted to Mosher esters **20a** and **20b** as described above followed by ^{19}F (32.4% ee) and ^1H (30.2% ee) NMR analysis.

The isomer **17** was chromatographed twice on SiO_2 [(1) 15% acetone−CH_2Cl_2, R_f 0.30; (2) 50% EtOAc−hexane, R_f 0.20] to give 0.098 g (0.39 mmol, 18%) of pure material; $[\alpha]^{25}_D$ +4.4° (c = 1.00, $CHCl_3$).

Conversion of 7-(Phenyloxycarbonyl)-7-azabicyclo-[2.2.1]heptan-*endo*-2-ol (14) to 7-[(1,1-Dimethylethoxy)-carbonyl]-7-azabicyclo[2.2.1]heptan-*endo*-2-ol (15). A 15 mL flask equipped with a magnetic stir bar and cold water condenser was charged with **14** (from bioconversion of **9** using *B. bassiana*, 0.210 g, 0.901 mmol), EtOH (3 mL), H_2O (1.2 mL), NaOH (0.2 g, 5 mmol), and 18-crown-6 ether (0.026 g, 0.1 mmol) and refluxed for 18 h. The reaction was monitored by TLC for consumption of starting material (30% acetone−hexane, R_f 0.4), and when complete, the pH was carefully adjusted to 1−2 with concd HCl and the mixture refluxed for an additional 1 h and cooled to rt. The solvents were removed by rotary evaporation, and the residue was diluted with dioxane (4 mL), H_2O (2 mL), and NaOH to pH 10−11. $(Boc)_2O$ (0.295 g, 1.4 mmol) was added, and the mixture was stirred 6 h at rt. The solvents were evaporated, and the residue was diluted with 15 mL of CH_2Cl_2 and washed with 10 mL of H_2O. The aqueous phase was back-extracted with CH_2Cl_2 (10 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated. The residue was chromatographed on SiO_2 (10% acetone−hexane, R_f 0.15) to give 0.164 g (0.77 mmol, 85%) of **15** as a viscous oil (^1H, ^{13}C NMR purity >95%).

Swern oxidation of a sample of **15** (0.162 g, derived from **14**) gave ketone **3**, $[\alpha]_D$ +37.7° (c = 1.00, $CHCl_3$).

Conversion of 7-(Benzyloxycarbonyl)-7-azabicyclo-[2.2.1]heptan-*endo*-2-ol (16) to 7-[(1,1-Dimethylethoxy)-carbonyl]-7-azabicyclo[2.2.1]heptan-*endo*-2-ol (15). A 15 mL flask equipped with a magnetic stir bar was charged with 10% Pd−C (0.10 g) under a stream of N_2 followed by addition of MeOH (5 mL), **16** (from bioconversion of **11** using *B. bassiana*, 0.050 g, 0.202 mmol), and ammonium formate (0.063 g, 1.0 mmol). The mixture was stirred for 3 h at rt and then filtered through a layer of Celite. The MeOH was removed by evaporation, and the residue was diluted with dioxane (2 mL) and H_2O (1 mL) followed by addition of NaOH (0.10 g) and $(Boc)_2O$ (0.088 g, 0.4 mmol). The mixture was vigorously stirred overnight at rt. The solvents were removed by evaporation, and the residue was diluted with 10 mL of CH_2Cl_2 and washed with an equal volume of H_2O. The aqueous phase was back-extracted with 10 mL of CH_2Cl_2, and the combined CH_2Cl_2 phases were dried (Na_2SO_4) and concentrated. The residue was chromatographed on SiO_2 (10% acetone−hexane, R_f 0.15) to give 0.021 g (0.098 mmol, 48%) of **15**: $[\alpha]^{25}_D$ −0.3° (c = 1.00, $CHCl_3$). Alcohol **15** was converted to Mosher esters **19a** and **19b** as described above followed by ^{19}F (6.7% ee) and ^1H (5.5% ee) NMR analysis.

Conversion of 7-(Benzyloxycarbonyl)-7-azabicyclo-[2.2.1]heptan-*exo*-2-ol (17) to 7-[1,1-Dimethylethoxy)carbonyl]-7-azabicyclo[2.2.1]heptan-*exo*-2-ol (2). Following the procedure described for conversion of **16** to **15**, **17** (from bioconversion of **11** using *R. arrhizus*, 0.030 g, 0.12 mmol), 10% Pd−C (0.030 g), MeOH (5 mL), ammonium formate (0.038 g, 0.60 mmol), NaOH (0.08 g), and $(Boc)_2O$ (0.050 g) were used for conversion to **2**. After workup, chromatography on SiO_2 (40% EtOAc−hexane, R_f 0.25) gave 0.016 g (0.075 mmol, 63%) of **2**: $[\alpha]^{25}_D$ +4.5° (c = 0.639, CH_2Cl_2).

Conversion of 7-[(1,1-Dimethylethoxy)carbonyl]-7-azabicyclo[2.2.1]heptan-*endo*-2-ol (15) to 7-[(1,1-Dimethylethoxy)carbonyl]-7-azabicyclo[2.2.1]heptan-2-one (3). To a 25 mL two-neck flask equipped with a magnetic stir bar, N_2 source, and low-temperature thermometer was added CH_2Cl_2 (7 mL) followed by oxalyl chloride (0.045 g, 0.35 mmol). The solution was cooled to −78 °C via dry ice/2-propanol bath. DMSO (0.055 g in 1 mL of CH_2Cl_2, 0.70 mmol) was added via syringe, maintaining the temperature at <−60 °C, and the solution was stirred for an additional 10 min at −70 °C. A 1 mL CH_2Cl_2 solution of **15** (0.050 g, 0.23 mmol) was added dropwise at −70 °C, after which the solution was stirred for an additional 20 min. The reaction was quenched with Et_3N (0.12 g, 3.9 mmol), and the mixture was allowed to warm to rt. The mixture was washed with H_2O (10 mL), and the aqueous phase was back-extracted with an equal volume of CH_2Cl_2. The combined CH_2Cl_2 phases were dried (Na_2SO_4) and concentrated. Chromatography on SiO_2 (5% acetone−hexane, R_f 0.15) gave 0.038 g (0.18 mmol, 77%) of **3** as a clear oil: ^1H NMR ($CDCl_3$) δ 1.44 (s, 9 H), 1.51−1.67 (m, 2 H), 1.96−2.02 (m, 3 H), 2.46 (dd, J = 17.4, 5.3 Hz, 1 H), 4.23 (d, J = 4.8 Hz, 1 H), 5.46 (1, J = 4.5 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 24.43, 27.56, 28.20, 45.24, 56.05, 63.94, 80.84, 140.34, 155.06; GCMS m/z 211 (M^+), 127, 83, 68, 57, 41.

Preparation of 8-(Phenyloxycarbonyl)-8-azabicyclo-[3.2.1]octane (21). A 25 mL flask equipped with a septum was charged with tropane (2.5 g, 20 mmol) and CH_2Cl_2 (15 mL). The solution was cooled to 5 °C with an ice bath, and a 5 mL CH_2Cl_2 solution of phenyl chloroformate (3.10 g, 19.8 mmol) was added dropwise via syringe over 5 min. The ice bath was removed, and the solution was stirred overnight at rt. The solution was washed with 0.1 N NaOH (15 mL) and 1 N HCl (15 mL), and the aqueous phases were each back-extracted with CH_2Cl_2 (10 mL). The combined CH_2Cl_2 phases

were dried (Na_2SO_4) and concentrated to give a crude solid residue that was recrystallized from hexane to give 2.77 g (12 mmol, 60%) of **21** as a white solid: mp 70−71 °C; ^{1}H NMR ($CDCl_3$) δ 1.4−2.1 (m, 10 H), 4.34 (m, 1 H), 4.42 (m, 1 H), 7.15 (m, 3 H), 7.35 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 16.7, 27.6, 28.3, 30.6, 31.6, 54.2, 54.8, 121.7, 125.0, 129.2, 151.4, 151.6; GCMS *m*/*z* 231 (M^+), 158, 138, 110, 95, 77, 67, 55; IR (mull) 2926 (s), 1710 (s), 1410 (s), 1206 (s), 1175 (m), 1034 (m), 1325 (m) cm^{-1}. Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.76; H, 7.14; N, 5.98.

Preparation of 9-(Phenyloxycarbonyl)-9-azabicyclo-[3.3.1]nonane (22). 9-Methyl-9-azabicyclo[3.3.1]nonane was prepared by Wolff−Kishner reduction of pseudopelletierine as described in the literature.[15] Demethylation was carried out as described above for the preparation of **21** and as reported in the literature, using 9-methyl-9-azabicyclo[3.3.1]nonane (2.20 g, 15.8 mmol), CH_2Cl_2 (20 mL), and a 10 mL CH_2Cl_2 solution of phenyl chloroformate (3.1 g, 20 mmol). Following workup, the crude residue was recrystallized from hexane to yield 1.1 g (4.5 mmol, 28%) of **22** as a white solid: mp 99−100 °C (lit.[15] mp 100−102 °C); ^{1}H NMR ($CDCl_3$) δ 1.75 (m, 6 H), 1.90−2.21 (m, 6 H), 4.38 (s, 1 H), 4.47 (s, 1 H), 7.14 (m, 3 H), 7.36 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 20.5, 29.7, 30.3, 46.8, 47.9, 122.1, 125.3, 129.5, 152.1, 153.3.

Preparation of 8-(Phenyloxycarbonyl)-8-azabicyclo-[3.2.1]octan-*endo*-3-ol (23) and 8-(Phenyloxycarbonyl)-8-azabicyclo[3.2.1]octan-3-one (24) by Bioconversion of 8-(Phenyloxycarbonyl)-8-azabicyclo[3.2.1]octane (21) Using *A. ochraceus*. Bioconversion of **21** (0.800 g, 3.46 mmol) using *A. ochraceous* was carried out in 80 shake flasks for 3 days. Following workup (see general procedure above), the residue was chromatographed on SiO_2 (5% MeCN−CH_2Cl_2, R_f alcohol **23** 0.10, R_f ketone **24** 0.45) to give 0.31 g (1.25 mmol, 36%) of **23** as a pale yellow solid (^{1}H NMR purity 95%). A portion of alcohol **23** (0.150 g) was further chromatographed on SiO_2 (90:9:1 EtOAc:MeOH:Et_3N, R_f 0.65) to give 0.136 g of **23**: mp 151−153 °C (lit.[16] mp 151−153 °C); ^{1}H NMR ($CDCl_3$) δ 1.72−1.82 (m, 2 H), 1.91−2.27 (m, 6 H), 4.00 (t, J = 4.9 Hz, 3 H), 4.33 (bs, 1 H), 4.42 (bs, 1 H), 7.15−7.39 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 27.72, 28.45, 38.16, 39.08, 52.91, 53.49, 64.85, 121.59, 125.03, 129.13, 151.19, 151.53; IR (mull) 3475 (s), 2924 (s), 1694 (s), 1426 (s), 1361 (m), 1206 (s), 1194 (s), 1043 (s) cm^{-1}. Anal. Calcd for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66. Found: C, 67.95; H, 6.90; N, 5.72.

The ketone **24** was then twice chromatographed on SiO_2 [(1) 15% EtOAc−hexane, R_f 0.10; (2) 5% MeCN−CH_2Cl_2, R_f 0.45] to give 0.031 g (0.12 mmol, 4%) of a white solid: mp 122−123 °C; ^{1}H NMR ($CDCl_3$) δ 1.78 (m, 2 H), 2.21 (m, 2 H), 2.47 (m, 2 H), 2.79 (m, 2 H), 4.74 (m, 2 H), 7.15 (dd, J = 5.4, 1.1 Hz, 2 H), 7.21 (m, 1 H), 7.39 (t, J = 7.4 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 28.66, 29.47, 48.63, 49.38, 53.44, 121.56, 125.56, 129.39, 150.96, 151.83, 207.45; IR (mull) 2924 (s), 1721 (s), 1707 (s), 1402 (s), 1342 (s), 1321 (m), 1282 (m), 1058 (s), 1000 (m) cm^{-1}; HRMS found 245.1063, $C_{14}H_{15}NO_3$ requires 245.1052, 152, 109, 94, 81, 67 *m*/*z*. Anal. Calcd for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.17; H, 5.71. Found: C, 68.54; H, 6.24; 5.71.

Conversion of Tropine (25) to 8-(Phenyloxycarbonyl)-8-azabicyclo[3.2.1]octan-*endo*-3-ol (23). Preparation of **23** was carried out according to the literature procedure[17] with the following modifications. A 25 mL flask was charged with tropine (**25**, 0.50 g, 3.5 mmol) and CH_2Cl_2 (15 mL). Phenyl chloroformate (1.2 g, 7.6 mmol) was added, and the solution was stirred for 24 h at rt. The reaction mixture was evaporated to dryness, diluted with 2 N HCl (20 mL), and extracted with Et_2O (3 × 75 mL). The combined Et_2O phases were washed with saturated $NaHCO_3$ (25 mL), dried (Na_2SO_4), and concentrated. The residue was chromatographed on SiO_2 (10% MeCN−CH_2Cl_2, R_f 0.20) to give 0.52 g (2.1 mmol, 60%) of **23** as a white solid: mp 153−154 °C (lit.[16] mp 151−153 °C); ^{1}H, ^{13}C NMR data are in agreement with that given for **23** above.

Preparation of 9-(Phenyloxycarbonyl)-9-azabicyclo-[3.3.1]nonan-3-one (27) and 9-(Phenyloxycarbonyl)-9-azabicyclo[3.3.1]nonan-*exo*-3-ol (26) by Bioconversion of 9-(Phenyloxycarbonyl)-9-azabicyclo[3.3.1]nonane (22) Using *B. bassiana*. Bioconversion of **22** (0.90 g, 3.7 mmol) using *B. bassiana* was carried out in a 10 L tank for 3.5 days. Following workup (see general procedure above), the crude residue was chromatographed on SiO_2 (5% MeCN−CH_2Cl_2, R_f **27** = 0.35). After elution of the ketone **27**, the alcohol was eluted with EtOAc (R_f **26** = 0.10). The ketone product was then twice chromatographed on SiO_2 [(1) 20% EtOAc−hexane, R_f = 0.15; (2) 5% MeCN−CH_2Cl_2, R_f = 0.35] to give 0.147 g (0.567 mmol, 15%) of **27** as a pale yellow solid: mp 125 °C; ^{1}H NMR ($CDCl_3$) δ 1.61−1.70 (m, 4 H), 1.87−1.93 (m, 2 H), 2.46 (dd, J = 16.3, 8.2 Hz, 2 H, −$COCH_2$−), 2.75 (dd, J = 16.6, 7.0 Hz, 2 H, −$COCH_2$−), 4.83 (bs, 1 H, bridgehead H), 4.92 (bs, 1 H, bridgehead H), 7.14 (m, 2 H), 7.24 (m, 1 H), 7.38 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 16.18, 30.72, 30.79, 45.61, 45.24, 48.16, 48.95, 121.64, 125.33, 129.39, 151.20, 152.84, 208.55; IR (mull) 2954 (s), 1717 (s), 1702 (s), 1411 (s), 1367 (s), 1346 (s), 1341 (s), 1207 (s), 1048 (s) cm^{-1}; HRMS calcd 259.1208, obsd 259.1209. Anal. Calcd for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.61; N, 5.40. Found: C, 69.42; H, 6.50; N, 5.45.

The alcohol product was then twice chromatographed on SiO_2 [(1) 20% MeCN−CH_2Cl_2, R_f = 0.30; (2) 30% acetone−hexane, R_f = 0.40] to give 0.290 g (1.11 mmol, 30%) of **26** as a white solid: mp 116−118 °C; ^{1}H NMR ($CDCl_3$) δ 1.60−1.85 (m, 7 H), 1.80−1.98 (m, 2 H), 2.04−2.15 (6 line m, 2 H), 4.50 (m, 1 H), 4.63 (m, 2 H), 7.10 (m, 2 H), 7.16 (m, 1 H), 7.35 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 20.76, 29.22, 29.81, 39.98, 40.43, 47.71, 48.58, 65.22, 122.09, 125.53, 129.61, 151.83, 152.95; HRMS calcd 261.1365, found 261.1371; IR (mull) 3454 (m), 2954 (s), 1693 (s), 1424 (s), 1203 (s), 1349 (w), 1114 (m), 1061 (s) cm^{-1}. Anal. Calcd for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33; N, 5.36. Found: C, 68.88; H, 7.43; N, 5.36.

Conversion of 9-(Phenyloxycarbonyl)-9-azabicyclo-[3.3.1]nonan-*exo*-3-ol (26) to 9-Benzoyl-9-azabicyclo[3.3.1]-nonan-*exo*-3-ol (28). A 10 mL flask equipped with a stir bar and cold H_2O condenser was charged with **26** (0.090 g, 0.344 mmol), EtOH (4 mL), H_2O (1.5 mL), 18-crown-6 ether (0.015 g, 0.057 mmol), and NaOH (0.3 g, 7.5 mmol). The solution was refluxed for 2 h, after which time consumption of **26** was noted by TLC (R_f **26** = 0.35, 50% EtOAc−hexane). After the solution was cooled to 50 °C, the pH was adjusted to 1−2 with concd HCl and refluxing was continued for an additional 30 min. The reaction mixture was cooled to rt, and the solvents were removed by rotary evaporation. The residue was diluted with H_2O (4 mL), and the pH was adjusted to 10−11 with NaOH, followed by addition of benzoyl chloride (0.141 g, 1.0 mmol). The mixture was stirred vigorously for 2 h at rt, diluted with H_2O (5 mL), and extracted with CH_2Cl_2 (3 × 10 mL). The combined CH_2Cl_2 phases were dried (Na_2SO_4) and concentrated to give a crude viscous residue. The residue was chromatographed on SiO_2 (75% EtOAc−hexane, R_f = 0.15) to give 0.023 g (0.093 mmol, 27%) of **28** as a white solid, mp 145−149 °C. Recrystallization from acetone−hexane gave crystals: mp 153−154 °C (lit.[17] mp 152−153 °C); ^{1}H NMR ($CDCl_3$) δ 1.50−2.15 (4 multiplets, 11 H), 3.95 (bs, 1 H), 4.63 (7 line m, 1 H), 4.97 (bs, 1 H), 7.39 (s, 5 H); ^{13}C NMR ($CDCl_3$) δ 20.53, 28.94, 30.06, 39.66, 40.45, 44.73, 51.06, 64.93, 126.30, 128.57, 129.53, 136.32, 169.24; GCMS *m*/*z* 245 (M^+), 228, 200, 140, 122, 105, 96, 77, 55, 27.

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