Syntheses of New Phosphorus-Containing Azabicycloalkanes and Their Microbial Hydroxylation Using *Beauveria bassiana*

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Representative novel phosphorus-containing azabicyclic substrates have been synthesized and subsequently microbially hydroxylated in fair to good yields using the microorganism *Beauveria bassiana*. (7-Azabicyclo[2.2.1]hept-7-yl)phosphonic acid diethyl ester was hydroxylated at the unactivated methylene carbon to give (2-*endo*-hydroxy-7-azabicyclo[2.2.1]hept-7-yl)phosphonic acid diethyl ester in 43% yield and 64% ee, while *N*-(diphenylphosphinoyl)-7-azabicyclo[2.2.1]heptane was similarly hydroxylated to give 2-*endo*-hydroxy-7-(diphenylphosphinoyl)-7-azabicyclo[2.2.1]-heptane in 35% yield and 20% ee. (7-Azabicyclo[2.2.1]hept-7-yl)phosphonic acid diphenyl ester yielded two distinct hydroxylated products: monohydroxylated (2-*endo*-hydroxy-7-azabicyclo[2.2.1]-hept-7-yl)phosphonic acid diphenyl ester in 7% yield and 7% ee and dihydroxylated (2-*endo*-hydroxy-7-azabicyclo[2.2.1]hept-7-yl)phosphonic acid phenyl, *p*-hydroxyphenyl ester in 37% yield and 77% ee. HPLC studies indicated that the monohydroxylated metabolite is formed first during fermentation, and becomes a substrate for a second enzymatic hydroxylation at one of the aromatic rings with induced enantioselection, to give the dihydroxylated metabolite. All microbially hydroxylated metabolites were easily *N*-deprotected using TFA−CH_2Cl_2 (1:1). Thus, *N*-phosphinyl groups are good facilitators of hydroxylation reactions with *B. bassiana* and offer a new choice for an *N*-substituent when substrates are hydroxylated with this microorganism. By offering a new *N*-substituent, this work extends the general utility of *B. bassiana* as a preparatively useful unactivated methylene hydroxylator.

Introduction

Microbial hydroxylation of unactivated methylene carbons[1] still remains one of the most impressive examples of transformations not easily emulated with conventional organic chemistry.[2] Our laboratory has become increasingly interested in microbial hydroxylations of unactivated methylenes in certain azabicyclic substrates of the type illustrated in Figure 1. These 7-azabicyclo[2.2.1]-heptanes are of interest to us because they are easy to prepare and constitute intermediates in a synthesis of the natural product epibatidine.[3] The field of microbial hydroxylations is well-documented, with pioneering work done by Fonken and Johnson et al.,[1c,4] and later work done by the research groups of Holland,[1d,5] Furstoss [1h,6] and Roberts.[1b,7] More recent contributions from Griengl,[8]

1 R = COC_6H_5
2 R = $SO_2C_6H_5$
3 R = $P(O)(OEt)_2$
4 R = $P(O)(OC_6H_5)_2$
5 R = $P(O)(C_6H_5)_2$

Figure 1. 7-Azabicyclo[2.2.1]heptanes for microbial hydroxylation.

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Haufe,[9] and Turner[10] have helped to solidify the field of microbial hydroxylations and establish this biotransformation as a routine endeavor.

Our first report in this field explored the microbial hydroxylation of *N*-benzoyl substrate **1** and *N*-benzenesulfonyl substrate **2**[11] using the popular and well-studied fungus *Beauveria bassiana* (ATCC 7159).[12] Other *N*-substituents and related functionalities have included various amides,[4,6] lactams,[6f,i] *N*-carbamoyl,[6e−g,9] and *N*-acetyl[4e,7c] groups. Recently, Johnson's research group reported on the microbial hydroxylation of a variety of *N*-substituted azabicycloalkanes, utilizing *N*-BOC, *N*-Cbz, *N*-tosyl, and *N*-phenyloxycarbonyl functional groups.[4m] All of the above-mentioned functional groups are electron-rich centers which, according to the prevailing theory,[1c,4a,g−j] act as anchoring/directing groups while the substrate is at the active site of the enzyme system(s) performing the oxidation. Recent modifications to this theory include Haufe's refined distance model[9b] to allow greater prediction of the hydroxylation position, and the suggestion by Turner that the active site of the hydroxylating enzyme contains a defined aromatic binding pocket.[10] Given these literature precedents, we hypothesized that *N*-phosphinyl substituents, which are also electron-rich moieties, might also constitute acceptable anchoring groups for these microbial hydroxylations. Shown in Figure 1 are the specific phosphinyl substituents that we examined (**3**−**5**).

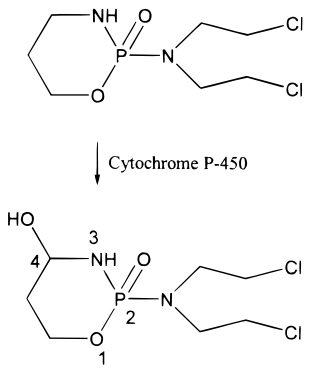

Figure 2. Metabolic activation step for cyclophosphamide.

There are several reasons why we envisaged these azabicyclic phosphoramidates and phosphinamides to be good substrates. First, the P═O bond of these substrates would mimic the carbonyl and sulfonyl functionalities of known anchoring groups and thus might be expected to function in the same manner while at the enzyme's active site. Roberts et al. postulated that the carbonyl/sulfonyl might be participating in noncovalent bonding with an amino acid residue at the active site of the oxidating enzyme.[7d] Second, these molecules are structurally similar to phosphates, which are ubiquitous in nature and living systems. Thus, we anticipated that these substrates would be biologically compatible with the fungal cells of *B. bassiana* without being toxic to them.

One final piece of suggestive evidence comes from cyclophosphamide,[13] an anti-cancer drug that has been in clinical use for decades (Figure 2). Cyclophosphamide becomes metabolically activated to the actual drug substance via methylene hydroxylation at C-4 by a cytochrome P450 enzyme system in the liver.[14] Once it enters tumor cells, the six-membered ring opens and the drug then alkylates the cell's DNA. Given the structural similarity between cyclophosphamide and our proposed phosphorus-containing substrates, it was reasonable to expect that these molecules would be taken up by the cultures and be compatible with their cellular physiology. For these reasons, we have pursued the synthesis of a few representative phosphorus-containing azabicyclic substrates and their subsequent microbial hydroxylation using *B. bassiana*. Herein, we describe the results of these experiments.

Results

Hassner has reported a synthesis of certain *N*-alkyl-7-azabicyclo[2.2.1]heptanes in four steps starting from monoprotected 1,4-cyclohexanedione.[15] However, we chose

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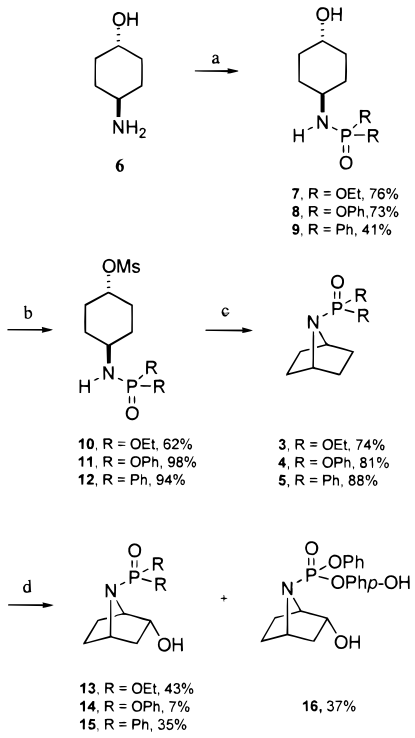

Figure 3. Synthesis and microbial hydroxylation of substrates **3**−**5**. Reagents and conditions: (a) $R_2P(O)Cl$, Et_3N, DMF, 0 °C; (b) MsCl, Et_3N, CH_2Cl_2, 0 °C; (c) KO^{t}Bu, 1:1 benzene−DMF; (d) stage II *B. bassiana* cultures, initial pH 5.0, 29 °C, 7 days.

to use our shorter route starting from commercially available *trans*-4-aminocyclohexanol hydrochloride.[11] After the hydrochloride salt was converted to its free base **6**, it was treated with the appropriate phosphorus chloride (diethylchlorophosphate, diphenylchlorophosphate, or diphenylphosphinic chloride) and triethylamine in DMF (Figure 3). The resulting alcohols **7**−**9** were converted to mesylates **10**−**12** using methanesulfonyl chloride and triethylamine in CH_2Cl_2. Cyclization occurred in good yields in the presence of potassium *tert*-butoxide to give the azabicyclic substrates **3**−**5**. Thus, these substrates are synthesized in only three steps and in good overall yields.

Substrates **3**−**5** were subjected to stage II liquid cultures of *B. bassiana* at 28−29 °C, 250 rpm, and 50% relative humidity for 168 h (7 days) of continuous biotransformation (Figure 3). Diethylphosphoramidate **3** was hydroxylated at the unactivated methylene to give metabolite **13** in 43% yield and 64% enantiomeric excess, with 92% conversion. Diphenylphosphinamide **5** was hydroxylated in 35% yield, 20% enantiomeric excess, and 88% conversion to provide metabolite **15**. Hydroxylation of diphenyl phosphoramidate **4** resulted in metabolite **14** in 7% yield and 7% enantiomeric excess and dihydroxylated metabolite **16** in 37% yield and 77% enantiomeric excess. Table 1 compares biotransformation data using two different growth media.

Discussion

Research concerning phosphoramidates and phosphinamides continues to accumulate in the literature, although papers on the former are more abundant.[16−21] Diethylchlorophosphate, diphenylchlorophosphate, and diphenylphosphinic chloride have all been used as phosphorylating agents[22] or *N*-blocking and coupling reagents in peptide synthesis.[23] Although it might appear that we are also using these phosphinyl moieties as *N*-protecting groups, our primary interest in them lies in their function as facilitators of unactivated methylene hydroxylation using *B. bassiana*.[24]

Table 1. Biotransformation Data for Substrates 3−5

		medium A[a]		medium B[a]				
substrate	metab	% conv[b]	% yield[c]	% conv[b]	% yield[c]	% ee[d]	$[\alpha]^{25}_D$	abs conf
3	**13**	97	36	92	43	64	+2.7	(1*S*)
4	**14**	100	11	100	7	7	+3.5	(1*S*)
4	**16**	100	34	100	37	77	+4.7	
5	**15**	99	3	88	35	20	−8.1	(1*R*)

[a] The compositions of medium A and medium B are provided in the Experimental Section. [b] Based on the amount of substrate recovered by column chromatography. [c] After isolation and purification by column chromatography. [d] Based on ^{1}H and ^{19}F NMR analyses of Mosher ester derivatives and confirmed by HPLC resolution of the same derivatives. These % ee values apply to metabolites isolated from both growth media.

For all three substrates examined, we indeed observed the desired hydroxylation, and in moderate to good yields (Table 1). Originally, only medium A was utilized in these biotransformations, but at the end of the fermentation the pH of the cultures was found to be very acidic (1.3−3.7) when using this medium. Although this acidity did not appear to be detrimental to the yields of metabolites **13**, **14**, and **16**, we suspected that it might have contributed to the very low yield (3%) of metabolite **15**. For this reason, medium B was added to our experimental protocol and all substrates were reexamined. Table 1 indicates that medium B (ending pH 5.1−7.5) is the preferred growth medium, with the isolated yields of metabolites **13** and **16** slightly higher, and the yield of metabolite **15** *much* higher (35%). Hydroxylation of the aromatic ring of substrate **4** was an unforeseen discovery, since our

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(24) We were unable to isolate hydroxylated metabolites when the free amine 7-azabicyclo[2.2.1]heptane was subjected to cultures of *B. bassiana* following our biotransformation protocol.

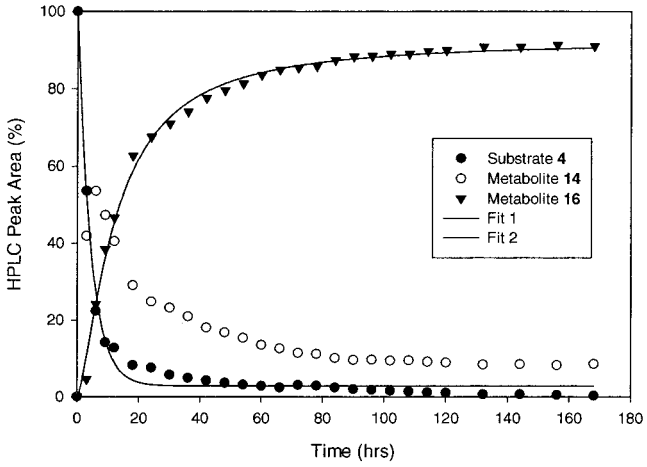

Figure 4. Time course of the biotransformation of substrate **4** (closed circles) to metabolite **14** (open circles) and metabolite **16** (closed triangles) by *B. bassiana*.

previous work with *N*-benzoyl and *N*-benzenesulfonyl substrates[11] did not display any aromatic ring hydroxylation.[6e,h,7f,9a] The observation that substrate **4** gave rise to metabolites **14** and **16** caused us to speculate that perhaps monohydroxylated product **14** is formed first during the fermentation, and dihydroxylated product **16** is formed second and accumulates at the expense of product **14**. To test this hypothesis, a biotransformation of substrate **4** was performed, during which 1 mL aliquots of culture were taken at predetermined time points.

Normal-phase HPLC analysis of organic extracts of the aliquots, summarized as a time course plot in Figure 4, clearly indicates that metabolite **14** was indeed formed first, with significant amounts of product formed within the first 3 h of fermentation. Metabolite **16** formed soon afterward, its accumulation occurring at the expense of metabolite **14**. The amount of substrate **4** decreases exponentially over the course of the experiment. This observation in which an enzymatic hydroxylation product itself becomes a substrate for a second enzymatic hydroxylation has previously been reported for *B. bassiana*. Furstoss et al. reported double hydroxylation of a cyclohexyl *N*-phenyl carbamate[6h] over a decade ago, and more examples of this are found in a review.[25] Furthermore, the hydroxyl group of metabolite **14** (7% ee by ^{19}F NMR analysis of the corresponding Mosher[26] esters) apparently has induced enantioselection in the second hydroxylation step to give metabolite **16** (77% ee, as determined using the same method). Although Furstoss et al. have reported glucosylation of phenol metabolites,[6e,h] we did not isolate any glucoside conjugate of the phenol of metabolite **16**.

In comparing our results with those of Johnson et al. in hydroxylations of 7-azabicyclo[2.2.1]heptane systems using *B. bassiana*, we find a similar range of percent yields and enantioselectivities overall, but also some important differences.[4m] Our diethyl phosphoramidate substrate **3** is structurally most similar to Johnson's *t*-BOC substrate, yet both our isolated yield (43% vs 10%) and ee (64% vs 26%) are higher. Diphenyl phosphoramidate **4** is similar to Johnson's *N*-phenyloxycarbonyl substrate. While the yield and % ee for monohydroxylated metabolite **14** are much lower than those for Johnson's *N*-phenyloxycarbonyl substrate, the yield for the dihydroxylated metabolite **16** is quite comparable (37% vs 46%), but the ee is somewhat higher (77% vs 51%). Moreover, Johnson et al. did not observe any aromatic ring *para*-hydroxylation. Diphenylphosphinamide **5** could be compared with our previously studied *N*-benzoyl-7-azabicyclo[2.2.1]heptane substrate **1**.[11] Compared to the metabolite derived from **1**, the yield of metabolite **15** is noticeably lower (35% vs 57%), but the enantioselectivities are quite close (20% vs 22%).

Table 2. Deprotection Data for Metabolites 13−16

metab	% yield[a]	time (h)	metab	% yield[a]	time (h)
13	91	6	**16**	97	10
14	95	10	**15**	99	3

[a] Determined indirectly using gas chromatography.

We have found that all four microbial metabolites **13**−**16** are readily *N*-deprotected to give the free secondary amine using TFA−CH_2Cl_2 (1:1) at room temperature (Table 2). These reaction conditions have been previously studied by Ramage et al.[27] Deprotection occurs in relatively high yield and in reasonable reaction times. The short reaction time for deprotection of diphenylphosphinamide metabolite **15** highlights the extreme acid-lability of the N−P bond of diphenylphosphinamides found by Ramage et al.[27] The ability to easily deprotect these *N*-phosphinyl metabolites after the biotransformation step further extends their potential synthetic utility as valuable intermediates.

In conclusion, we have demonstrated that *N*-phosphinyl substituents are good facilitators of these hydroxylations of unactivated carbons with *B. bassiana*. The extent of bioconversion of substrates, the fair to good isolated yields of hydroxylated metabolites, and the enantiomeric excess (in two cases) are all encouraging results, even though the yields and ee are not yet high enough to be synthetically useful. These *N*-phosphinyl substituents are easily cleaved under acidic conditions, a fact which extends their potential as intermediates. Finally, results with these *N*-phosphinyl substituents suggest that there may be other anchoring/activating groups yet to be discovered in the search for ways to effect hydroxylation of unactivated carbons with *B. bassiana*.

Experimental Section

General Methods. The microorganism used was *B. bassiana*, ATCC 7159 (Manassas, VA).[12] Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded using NaCl film or KBr disk techniques. Optical rotations were determined using a JASCO P-1020 polarimeter. ^{1}H NMR spectra were recorded at 360 MHz, with chemical shifts referenced to $CDCl_3$ (δ 7.27), $(CD_3)_2SO$ (δ 2.50), or TMS (δ 0.00) as an internal standard. ^{13}C NMR spectra were recorded at 90 MHz, with chemical shifts referenced to either $CDCl_3$ (δ 77.23) or $(CD_3)_2SO$ (δ 39.51). ^{19}F NMR spectra were recorded at 282 MHz, with chemical shifts referenced to $CFCl_3$ (δ −98.07) as an external standard. ^{31}P NMR spectra were recorded at 360 MHz, with chemical shifts referenced to H_3PO_4 (δ 0.00) as an external standard. All mass spectra were obtained at an ionizing voltage of 70 eV, in EI or FAB

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ionization modes, at The University of Iowa Mass Spectrometry Facility. Analytical HPLC was performed on a normal-phase column with UV detection at 254 nm. Elemental analyses were performed at Galbraith Laboratories, Inc., Knoxville, TN. Methylene chloride and triethylamine were each distilled from calcium hydride before use. Column chromatography was carried out using silica gel 60 (230−400 mesh). All reactions were monitored by TLC on Merck 60 F_{254} precoated silica gel plates (250 μm thickness), and spots were visualized with UV light or with a series of two dip solutions: ninhydrin, followed by *p*-anisaldehyde (heating after each dip). Commercially available (Aldrich) *trans*-4-aminocyclohexanol hydrochloride was converted to the free base form by exhaustively extracting a solution of the HCl salt in 4 N aqueous NaOH with ethyl acetate and subsequently removing the solvent. All other solvents and materials were of reagent grade and used without further purification.

HPLC Analysis of Fermentation Extracts. Aliquots (1 mL) of culture medium were taken at predetermined time points. Each aliquot was shaken with 1 mL of CH_2Cl_2 and then centrifuged. The organic layer was transferred to a clean vial, concentrated to the absence of solvent, and redissolved in 100 μL of mobile phase. A 20 μL sample of the resulting solution was injected for analysis. Chromatographic parameters: normal-phase silica analytical column (250 mm × 4 mm, 5 μm packing); 7:3 *n*-heptane−2-propanol mobile phase; UV detection at 254 nm; 1.0 mL/min flow rate.

GC Analysis for Determination of Yield of *N*-Deprotection. A linear standard curve was generated using pure 2-*endo*-hydroxy-7-azanorbornane product. After TLC analysis had indicated a completed deprotection reaction, an aliquot of the reaction mixture was injected for analysis. The area under the product peak was translated into a percent yield using the standard curve and the corresponding equation for a straight line. Chromatographic parameters: Alltech Heliflex AT-1 capillary column (30 m × 0.53 mm i.d.); helium carrier gas; flame ionization detector at 220 °C; injector temperature 200 °C; initial column temperature at 100 °C, increasing 30 °C/min to 250 °C, and then hold; 1.0 μL injection volume.

(*trans*-1,4-Cyclohexanolyl)phosphoramidic Acid Diethyl Ester (7). A solution containing *trans*-4-aminocyclohexanol free base **6** (1.84 g, 15.97 mmol) at 0 °C in DMF (50 mL) was treated with triethylamine (1.93 g, 19.10 mmol) under argon. The reaction mixture was treated 10 min later with diethylchlorophosphate (2.62 g, 15.21 mmol) under argon. The reaction mixture was stirred for 3.5 h and then filtered through filter paper to remove white solids which had developed. The DMF was removed by distillation, leaving a residue which was purified by flash chromatography on a silica gel column. Elution with 9:1 chloroform−methanol afforded **7** as a colorless oil: yield 3.06 g (76%); R_f 0.30 (9:1 chloroform−methanol); ^{1}H NMR ($CDCl_3$) δ 4.06 (4 H, qd, J = 1.4, 7.1 Hz), 3.60 (1 H, m), 2.98 (1 H, br s), 2.45 (1 H, br s), 1.99 (4 H, t, J = 15.0 Hz), 1.87 (1 H, br s), 1.33 (6 H, td, J = 7.1, 0.7 Hz), 1.43−1.15 (4 H, m); ^{13}C NMR ($CDCl_3$) δ 69.3, 62.3 (d, J_{CP} = 5.0 Hz), 50.1, 34.1, 33.5 (d, J_{CP} = 5.1 Hz), 16.3 (d, J_{CP} = 7.2 Hz); ^{31}P NMR ($CDCl_3$) δ 8.84; IR (thin film) ν_{max} 3253 (br), 1453, 1235, 1040, 977 cm^{-1}; EIMS *m*/*z* (relative intensity) 251 (M^+, 2.5), 192 (100), 136 (71); HRMS calcd for $C_{10}H_{22}NO_4P$ 251.1286, found 251.1292.

(*trans*-1,4-Cyclohexanolyl)phosphoramidic Acid Diphenyl Ester (8). A solution containing *trans*-4-aminocyclohexanol free base **6** (1.26 g, 10.97 mmol) at 0 °C in DMF (30 mL) was treated with triethylamine (1.33 g, 13.18 mmol) under argon. The reaction mixture was treated 5 min later with diphenylchlorophosphate (2.80 g, 10.43 mmol) under argon. The reaction mixture was stirred for 4 h, diluted with water, and extracted with ethyl acetate. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was concentrated under diminished pressure to afford a residue, which was purified by flash chromatography on a silica gel column. Elution with 4:1 ethyl acetate−petroleum ether and then ethyl acetate afforded **8** as a white solid: yield 2.82 g (74%); R_f 0.36 (95:5 chloroform−methanol); mp 136−137 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 7.39 (4 H, t, J = 7.8 Hz), 7.23 (4 H, d, J = 8.6 Hz), 7.18 (2 H, d, J = 7.3 Hz), 5.72 (1 H, dd, J = 9.4, 13.2 Hz), 4.49 (1H, d, J = 4.1 Hz), 3.30 (1 H, br s), 2.97 (1 H, br s), 1.72 (2 H, d, J = 8.8 Hz), 1.65 (2 H, d, J = 8.8 Hz), 1.15 (4 H, m); ^{13}C NMR ($(CD_3)_2SO$) δ 150.8 (d, J_{CP} = 6.7 Hz), 129.7, 124.7, 120.2 (d, J_{CP} = 5.3 Hz), 68.0, 50.4, 34.0, 32.7 (d, J_{CP} = 4.4 Hz); ^{31}P NMR ($CDCl_3$) δ −1.29; IR (KBr) ν_{max} 3476, 3235, 1193, 947 cm^{-1}; EIMS *m*/*z* (relative intensity) 347 (M^+, 13), 329 (21), 289 (100), 250 (67), 77 (78); HRMS calcd for $C_{18}H_{22}NO_4P$ 347.1286, found 347.1279.

***N*-(Diphenylphosphinoyl)-*trans*-1,4-aminocyclohexanol (9).** A solution containing *trans*-4-aminocyclohexanol free base **6** (1.16 g, 10.09 mmol) at 0 °C in DMF (30 mL) was treated with triethylamine (1.23 g, 12.12 mmol) under argon. The reaction mixture was treated 10 min later with diphenylphosphinic chloride (2.27 g, 9.60 mmol). The reaction mixture was stirred for 4 h, diluted with water, and extracted with ethyl acetate. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was concentrated under diminshed pressure to afford a residue which was purified by flash chromatography on a silica gel column. Elution with 9:1 chloroform−methanol afforded **9** as a white solid: yield 1.29 g (41%); R_f 0.29 (9:1 chloroform−methanol); mp 215−216 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 7.79 (2 H, dd, J = 1.5, 11.5 Hz), 7.77 (2 H, dd, J = 1.8, 11.7 Hz), 7.47 (6 H, m), 5.15 (1 H, t, J = 9.5 Hz), 4.44 (1 H, d, J = 4.5 Hz), 3.30 (1 H, m), 2.69 (1 H, m), 1.85 (2 H, d, J = 11.0 Hz), 1.74 (2 H, d, J = 11.0 Hz), 1.33 (2 H, qd, J = 2.9, 13.2 Hz), 1.02 (2 H, qd, J = 2.9, 13.2 Hz); ^{13}C NMR ($(CD_3)_2SO$) δ 134.5 (d, J_{CP} = 126.0 Hz), 131.7 (d, J_{CP} = 9.4 Hz), 131.3 (d, J_{CP} = 2.2 Hz), 128.4 (d, J_{CP} = 11.5 Hz), 68.1, 49.6, 34.4, 33.4 (d, J_{CP} = 5.1 Hz); ^{31}P NMR ($CDCl_3$) δ 25.62; IR (KBr) ν_{max} 3350 (br), 3256, 1438, 1179, 1104 cm^{-1}; EIMS *m*/*z* (relative intensity) 315 (M^+, 15), 257 (60), 201 (100); HRMS calcd for $C_{18}H_{22}NO_2P$ 315.1388, found 315.1386.

General Procedure for Preparation of Mesylates 10−12. A solution containing alcohol (**7**−**9**) in CH_2Cl_2 at 0 °C was treated under argon with triethylamine (3.0 equiv). The reaction mixture was treated 10 min later with methanesulfonyl chloride (2.0 equiv) under argon. The reaction mixture was stirred at 0 °C for 3 h and then added to 2 volumes of water. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was concentrated under diminished pressure to afford a residue which was purified by flash chromatography on a silica gel column.

(*trans*-1,4-Cyclohexanol methanesulfonyl)phosphoramidic Acid Diethyl Ester (10). Elution with 95:5 methylene chloride−methanol afforded **10** as white, needlelike crystals: yield 2.47 g (62%); R_f 0.42 (9:1 chloroform−methanol); mp 70−71 °C; ^{1}H NMR ($CDCl_3$) δ 4.62 (1 H, m), 4.05 (4 H, qd, J = 1.5, 7.1 Hz), 3.04 (1 H, br s), 3.01 (3 H, s), 2.71 (1 H, br s), 2.10 (4 H, m), 1.66 (2 H, qd, J = 2.7, 12.7 Hz), 1.33 (8 H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 79.9, 62.0 (d, J_{CP} = 5.0 Hz), 48.7, 38.5, 32.1 (d, J_{CP} = 5.0 Hz), 30.8, 16.1 (d, J_{CP} = 7.3 Hz); ^{31}P NMR ($CDCl_3$) δ 8.60; IR (KBr) ν_{max} 3219, 1455, 1350, 1223, 1032 cm^{-1}; EIMS *m*/*z* (relative intensity) 329 (M^+, 1.1), 233 (36), 192 (100). Anal. Calcd for $C_{11}H_{24}NO_6PS$: C, 40.12; H, 7.34; N, 4.25. Found: C, 40.06; H, 7.49; N, 4.21.

(*trans*-1,4-Cyclohexanol methanesulfonyl)phosphoramidic Acid Diphenyl Ester (11). Gradient elution with 1:1 ethyl acetate−petroleum ether, ending with ethyl acetate, afforded **11** as a white, crystalline solid: yield 3.40 g (98%); R_f 0.56 (9:1 chloroform−methanol); mp 116−117 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 7.40 (4 H, t, J = 7.8 Hz), 7.25 (4 H, d, J = 8.5 Hz), 7.19 (2 H, t, J = 7.3 Hz), 5.85 (1 H, dd, J = 9.4, 12.8 Hz), 4.50 (1 H, m), 3.15 (3 H, s), 3.13 (1 H, br s), 1.97 (2 H, d, J = 9.3 Hz), 1.74 (2 H, d, J = 10.1 Hz), 1.53 (2 H, qd, J = 3.1, 12.3 Hz), 1.31 (2 H, qd, J = 2.4, 12.3 Hz); ^{13}C NMR ($(CD_3)_2SO$) δ 150.7 (d, J_{CP} = 6.5 Hz), 129.8, 124.8, 120.2 (d, J_{CP} = 4.3 Hz), 79.9, 48.9, 37.7, 31.6 (d, J_{CP} = 5.7 Hz), 30.5; ^{31}P NMR ($CDCl_3$) δ −1.42; IR (KBr) ν_{max} 3226, 1347, 1180 cm^{-1}; EIMS *m*/*z* (relative intensity) 425 (M^+, 0.5), 329 (59), 275 (79), 250 (100). Anal. Calcd for $C_{19}H_{24}NO_6PS$: C, 53.64; H, 5.67; N, 3.29. Found: C, 53.09; H, 5.79; N, 3.09.

***N*-(Diphenylphosphinoyl)-*trans*-1,4-aminocyclohexanol Methanesulfonate (12).** Elution with 95:5 chloroform−methanol afforded **12** as a white solid: yield 1.33 g (94%); R_f

0.47 (10% MeOH/$CHCl_3$); mp 175−176 °C; ^{1}H NMR ($CDCl_3$) δ 7.89 (2 H, dd, J = 1.3, 11.9 Hz), 7.87 (2 H, dd, J = 1.5, 12.0 Hz), 7.48 (6 H, m), 4.59 (1 H, m), 3.05 (1 H, m), 2.97 (3 H, s), 2.74 (1 H, dd, J = 6.0, 10.0 Hz), 2.20 (2 H, d, J = 12.7 Hz), 2.11 (2 H, d, J = 12.7 Hz), 1.57 (2 H, qd, J = 3.3, 12.8 Hz), 1.39 (2 H, qd, J = 3.1, 12.7 Hz); ^{13}C NMR ($CDCl_3$) δ 132.9 (d, J = 139.5 Hz), 132.2 (d, J_{CP} = 9.5 Hz), 128.8 (d, J_{CP} = 12.3 Hz), 79.9, 49.1, 38.9, 33.8 (d, J_{CP} = 5.1 Hz), 31.5; ^{31}P NMR ($CDCl_3$) δ 24.25; IR (KBr) ν_{max} 3137, 1438, 1351, 1198, 1112 cm^{-1}; EIMS m/z (relative intensity) 394 (M + H, 100)$^+$, 298 (79), 201 (52); HRMS (M + H)$^+$ calcd for $C_{19}H_{25}NO_4PS$ 394.1242, found 394.1246.

General Procedure for Preparation of Bicyclic Substrates 3−5. A solution containing mesylate (**10**−**12**) in 1:1 DMF−benzene at 0 °C was treated under argon with potassium *tert*-butoxide (3.0 equiv). The reaction mixture was stirred for 3 h and then added to 8 volumes of water and 1 volume of additional benzene. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was concentrated under diminished pressure to afford a residue, which was purified by flash chromatography on a silica gel column.

(7-Azabicyclo[2.2.1]hept-7-yl)phosphonic Acid Diethyl Ester (3). Elution with 9:1 ethyl acetate−petroleum ether afforded **3** as a colorless oil: yield 1.24 g (74%); R_f 0.71 (9:1 chloroform−methanol); ^{1}H NMR ($CDCl_3$) δ 4.05 (6 H, m), 1.75 (4 H, d, J = 6.8 Hz), 1.39 (4 H, d, J = 6.8 Hz), 1.30 (6 H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 62.6 (d, J_{CP} = 5.8 Hz), 57.8 (d, J_{CP} = 2.2 Hz), 31.0 (d, J_{CP} = 5.9 Hz), 16.4 (d, J_{CP} = 6.8 Hz); ^{31}P NMR ($CDCl_3$) δ 6.46; IR (thin film) ν_{max} 1275, 1256, 1028 cm^{-1}; EIMS m/z (relative intensity) 233 (M$^+$, 28), 204 (100), 148 (90); HRMS calcd for $C_{10}H_{20}NO_3P$ 233.1181, found 233.1171.

(7-Azabicyclo[2.2.1]hept-7-yl)phosphonic Acid Diphenyl Ester (4). Elution with 4:6 ethyl acetate−petroleum ether afforded **4** as a white, crystalline solid: yield 2.06 g (81%); R_f 0.73 (9:1 chloroform−methanol); mp 75−76 °C; ^{1}H NMR ($CDCl_3$) δ 7.31 (4 H, t, J = 7.5 Hz), 7.25 (4 H, d, J = 7.6 Hz), 7.14 (2 H, t, J = 7.0 Hz), 4.25 (2 H, s), 1.72 (4 H, d, J = 6.5 Hz), 1.41 (4 H, d, J = 6.3 Hz); ^{13}C NMR ($CDCl_3$) δ 151.4 (d, J_{CP} = 8.0 Hz), 129.7, 124.8, 120.3 (d, J_{CP} = 5.1 Hz), 58.5 (d, J_{CP} = 2.1 Hz), 31.0 (d, J_{CP} = 5.8 Hz); ^{31}P NMR ($CDCl_3$) δ −4.75; IR (KBr) ν_{max} 3050, 1279, 1222, 1095 cm^{-1}; EIMS m/z (relative intensity) 329 (M$^+$, 35), 300 (100), 77 (46). Anal. Calcd for $C_{18}H_{20}NO_3P$: C, 65.65; H, 6.12; N, 4.25. Found: C, 65.64; H, 6.26; N, 4.15.

***N*-(Diphenylphosphinoyl)-7-azabicyclo[2.2.1]heptane (5).** Elution with 1:1 ethyl acetate−petroleum ether and then 7:3 ethyl acetate−petroleum ether afforded **5** as a white, crystalline solid: yield 882 mg (88%); R_f 0.58 (9:1 chloroform−methanol); mp 184−185 °C; ^{1}H NMR ($CDCl_3$) δ 7.92 (2 H, dd, J = 1.3, 11.6 Hz), 7.90 (2 H, dd, J = 1.4, 11.6 Hz), 7.44 (6 H, m), 3.82 (2 H, m), 1.92 (4 H, d, J = 6.5 Hz), 1.42 (4 H, d, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$) δ 133.6 (d, J = 131.5 Hz), 132.4 (d, J_{CP} = 8.7 Hz), 131.6 (d, J_{CP} = 2.9 Hz), 128.6 (d, J = 12.4 Hz), 58.5, 31.5 (d, J_{CP} = 6.5 Hz); ^{31}P NMR ($CDCl_3$) δ 22.63; IR (KBr) ν_{max} 1438, 1215, 1060 cm^{-1}; EIMS m/z (relative intensity) 297 (M$^+$, 59), 268 (63), 201 (100); HRMS calcd for $C_{18}H_{20}NOP$ 297.1283, found 297.1292.

General Biotransformation Procedure. Details describing the maintenance of cultures and biotransformation protocol are provided elsewhere.[11] Medium A: 20 g of D-glucose, 5 g of yeast extract (Difco), 5 g of soybean meal (Victoria Feed Co.), 5 g of NaCl, and 5 g of K_2HPO_4 per liter of water, pH adjusted to 5.0 prior to autoclaving. Medium B: 20 g of corn steep liquor (Sigma), 10 g of D-glucose per liter of water, pH adjusted to 5.0 prior to autoclaving. Media were sterilized for 15 min (125 mL DeLong flasks) or 20 min (1 L DeLong flasks) and allowed to cool to room temperature prior to inoculation.

(2-*endo*-Hydroxy-7-azabicyclo[2.2.1]hept-7-yl)phosphonic Acid Diethyl Ester (13). A solution containing (7-azabicyclo[2.2.1]hept-7-yl)phosphonic acid diethyl ester **3** (404.0 mg, 1.73 mmol) in ethanol (2 mL) was equally distributed among four stage II preparative scale liquid cultures of *B. bassiana* (prepared as described above). All flasks were then placed on an orbital shaker at 250 rpm, 29−30 °C, and 50% relative humidity for biotransformation. After 168 h, the cells were removed from the cultures by vacuum filtration and the filtrate was extracted with methylene chloride. The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was concentrated under diminished pressure to afford a residue which was purified by flash chromatography on a silica gel column. Elution with 9:1 chloroform−methanol afforded **13** as a colorless oil: yield 186.3 mg (43%); R_f 0.24 (95:5 chloroform−methanol); $[\alpha]^{25}_D$ +2.7° (c = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.33 (1H, m), 4.04 (4 H, quint, J = 7.3 Hz), 3.91 (2 H, td, J = 1.9, 4.8 Hz), 3.52 (1 H, br s), 2.19 (2 H, m), 1.79 (1 H, m), 1.67−1.49 (2 H, series of m), 1.30 (6 H, t, J = 7.1 Hz), 1.04 (1 H, dt, J = 3.8, 12.3 Hz); ^{13}C NMR ($CDCl_3$) δ 71.7 (d, J_{CP} = 10.1 Hz), 62.7 (d, J_{CP} = 6.5 Hz), 61.4, 58.9, 39.7 (d, J_{CP} = 5.8 Hz), 31.1 (d, J_{CP} = 4.4 Hz), 21.9 (d, J_{CP} = 4.4 Hz), 16.3 (d, J_{CP} = 6.5 Hz); ^{31}P NMR ($CDCl_3$) δ 6.10; IR (thin film) ν_{max} 3364 (br), 1236, 1024 cm^{-1}; EIMS m/z (relative intensity) 249 (M$^+$, 20), 205 (86), 112 (100); HRMS calcd for $C_{10}H_{20}NO_4P$ 249.1130, found 249.1148.

(2-*endo*-Hydroxy-7-azabicyclo[2.2.1]hept-7-yl)phosphonic Acid Diphenyl Ester (14) and (2-*endo*-Hydroxy-7-azabicyclo[2.2.1]hept-7-yl)phosphonic Acid Phenyl, *p*-Hydroxyphenyl Ester (16). A solution containing (7-azabicyclo[2.2.1]hept-7-yl)phosphonic acid diphenyl ester **4** (1.00 g, 3.04 mmol) in 1:1 ethanol−DMF (5 mL) was equally distributed among ten stage II preparative scale liquid cultures of *B. bassiana* (prepared as described above). All flasks were then placed on an orbital shaker at 250 rpm, 29−30 °C, and 50% relative humidity for biotransformation. After 168 h, the cells were removed from the cultures by vacuum filtration and the filtrate was extracted with methylene chloride. The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was concentrated to afford a residue which was purified by flash chromatography on a silica gel column. Elution with 9:1 chloroform−methanol afforded **14** as a pale yellow oil: yield 78 mg (7%); R_f 0.43 (9:1 chloroform−methanol); $[\alpha]^{25}_D$ +3.5° (c = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.31 (4 H, t, J = 7.8 Hz), 7.22 (4 H, d, J = 7.6 Hz), 7.15 (2 H, t, J = 7.2 Hz), 4.24 (1 H, m), 4.14 (1 H, td, J = 2.0, 4.7 Hz), 4.09, (1 H, td, J = 1.6, 4.5 Hz), 2.34 (1 H, s), 2.20 (1 H, m), 2.10 (1 H, m), 1.73 (1 H, m), 1.55 (2 H, m), 1.03 (1 H, dt, J = 3.8, 12.5 Hz); ^{13}C NMR ($CDCl_3$) δ 151.3 (d, J_{CP} = 6.5 Hz), 129.9, 125.1, 120.3 (dd, J_{CP} = 3.6, 5.1 Hz), 72.0 (d, J_{CP} = 9.3 Hz), 61.9 (d, J_{CP} = 2.1 Hz), 59.7 (d, J_{CP} = 2.2 Hz), 40.1 (d, J_{CP} = 5.1 Hz), 31.3 (d, J_{CP} = 3.7 Hz), 22.0 (d, J_{CP} = 3.6 Hz); ^{31}P NMR ($CDCl_3$) δ −5.07; IR (thin film) ν_{max} 3404 (br), 1591, 1489, 1190, 937 cm^{-1}; EIMS m/z (relative intensity) 345 (M$^+$, 17), 300 (27), 112 (100), 77 (75); HRMS calcd for $C_{18}H_{20}NO_4P$ 345.1130, found 345.1131.

Further elution with 9:1 chloroform−methanol afforded **16** as a pale yellow oil: yield 403 mg (37%); R_f 0.30 (9:1 chloroform−methanol); $[\alpha]^{25}_D$ +4.7° (c = 1.0, MeOH); ^{1}H NMR ($(CD_3)_2SO$) δ 7.39 (2 H, t, J = 7.8 Hz), 7.22 (3 H, dd, J = 1.0, 5.2 Hz), 7.02 (2 H, dd, J = 1.0, 3.5 Hz), 6.73 (2 H, d, J = 9.0 Hz), 5.03 (1 H, d, J = 4.1 Hz), 4.01 (1 H, br s), 3.94 (1 H, m), 3.87 (1 H, br s), 2.09 (1 H, m), 1.87 (1 H, m), 1.60−1.30 (4 H, series of m), 0.89 (1 H, dt, J = 3.8, 12.1 Hz); ^{13}C NMR ($(CD_3)_2SO$) δ 154.3, 150.8 (d, J_{CP} = 7.2 Hz), 142.9 (d, J_{CP} = 8.0 Hz), 129.8, 124.7, 120.4 (dt, J_{CP} = 4.4, 82.8 Hz), 115.8, 79.2, 70.5 (d, J_{CP} = 9.1 Hz), 61.2, 58.7, 30.7 (d, J_{CP} = 3.6 Hz), 21.5 (d, J_{CP} = 3.7 Hz); ^{31}P NMR ($CDCl_3$) δ −4.34; IR (cast film) ν_{max} 3266 (br), 1186, 940 cm^{-1}; EIMS m/z (relative intensity) 361 (M$^+$, 20), 161 (89), 112 (100); HRMS calcd for $C_{18}H_{20}NO_5P$ 361.1079, found 361.1088.

2-*endo*-Hydroxy-7-(diphenylphosphinoyl)-7-azabicyclo[2.2.1]heptane (15). A solution containing 7-(diphenylphosphinoyl)-7-azabicyclo[2.2.1]heptane **5** (103 mg, 0.35 mmol) in 5:7 ethanol−DMF (1.2 mL) was added to one stage II preparative scale liquid culture of *B. bassiana* (prepared as described above) inside a sterile laminar flow hood. The flask was placed on an orbital shaker at 250 rpm, 29−30 °C, and 50% relative humidity for biotransformation. After 168 h, the cells were removed from the culture by vacuum filtration and the filtrate was extracted with methylene chloride. The combined organic layer was washed with brine and dried over Na_2SO_4. The

solvent was concentrated under diminished pressure to afford a residue which was purified by flash chromatography on a silica gel column. Elution with 9:1 chloroform−methanol afforded **15** as a white solid: yield 38 mg (35%); R_f 0.36 (9:1 chloroform−methanol); mp 160−161 °C; $[\alpha]^{25}_D$ −8.1° (c = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.88 (2 H, dd, J = 1.2, 11.8 Hz), 7.86 (2 H, dd, J = 1.4, 11.7 Hz), 7.44 (6 H, m), 4.60 (1 H, dd, J = 4.0, 9.3 Hz), 3.72 (1 H, td, J = 1.9, 5.1 Hz), 3.66 (1 H, m), 2.36 (2 H, m), 2.25 (1 H, m), 1.96 (1 H, m), 1.78 (1 H, m), 1.58 (1 H, m), 1.09 (1 H, dt, J = 2.4, 12.4 Hz); ^{13}C NMR ($CDCl_3$) δ 132.9 (d, J_{CP} = 132.1 Hz), 132.4 (d, J_{CP} = 16.0 Hz), 132.4, 131.9 (d, J_{CP} = 3.0 Hz), 128.7 (d, J = 12.3 Hz), 72.5 (d, J_{CP} = 7.0 Hz), 62.0, 59.6, 40.5 (d, J_{CP} = 4.4 Hz), 31.8 (d, J_{CP} = 6.5 Hz), 22.3 (d, J_{CP} = 6.6 Hz); ^{31}P NMR ($CDCl_3$) δ 22.87; IR (KBr) ν_{max} 3318 (br), 1439, 1171, 1121 cm^{-1}; EIMS m/z (relative intensity) 313 (M^+, 3.4), 201 (44), 112 (100); HRMS calcd for $C_{18}H_{20}NO_2P$ 313.1232, found 313.1225.

2-*endo*-((*R*)-α-Methoxy-α-trifluoromethylphenylacetic ester)-7-azabicyclo[2.2.1]hept-7-yl)phosphonic Acid Diethyl Ester: R_f 0.42 (95:5 chloroform−methanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.55, 3.51; ^{19}F NMR ($CDCl_3$, 282 MHz) δ −71.83, −71.91.

2-*endo*-((*R*)-α-Methoxy-α-trifluoromethylphenylacetic ester)-7-azabicyclo[2.2.1]hept-7-yl)phosphonic Acid Diphenyl Ester: R_f 0.59 (95:5 chloroform−methanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.54, 3.50; ^{19}F NMR ($CDCl_3$, 282 MHz) δ −71.78, −71.87.

2-*endo*-((*R*)-α-Methoxy-α-trifluoromethylphenylacetic ester-7-azabicyclo[2.2.1]hept-7-yl)phosphonic Acid Phenyl, *p*-((*R*)-α-Methoxy-α-trifluoromethylphenylacetic ester)phenyl Ester: R_f 0.61 (95:5 chloroform−methanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.68, 3.54, 3.50; ^{19}F NMR ($CDCl_3$, 282 MHz) δ −71.76, −71.87−71.95.

2-*endo*-((*R*)-α-Methoxy-α-trifluoromethylphenylacetic ester)-7-(diphenylphosphinoyl)-7-azabicyclo[2.2.1]-heptane: R_f 0.33 (95:5 chloroform−methanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.52, 3.49; ^{19}F NMR ($CDCl_3$, 282 MHz) δ −71.80, −71.95.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **3**−**5** and **7**−**16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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