Biohydroxylations of Cbz-protected alkyl substituted piperidines by *Beauveria bassiana* ATCC 7159

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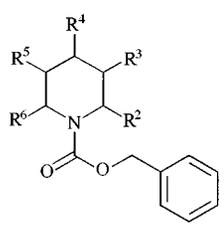
Received (in Cambridge) 24th July 1998, Accepted 2nd September 1998

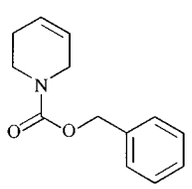
N-Benzyloxycarbonyl (Cbz) protected piperidines are hydroxylated with greater regioselectivity than the corresponding *N*-benzoyl analogues when incubated with the fungus *Beauveria bassiana* ATCC 7159. Cbz-protected piperidines **1**–**3**, **5**–**7**, have been biotransformed by growing cell suspensions of this fungus to yield predominantly 4-hydroxylated products in up to 48% yield. The regiospecificity of hydroxylation was only compromised significantly with *N*-Cbz-3-methylpiperidine **3** and *N*-Cbz-2-methylpiperidine **4** where hydroxylation occurred in both the 3 and 4 positions.

Introduction

The hydroxylation of non-activated carbon centres remains a challenge in organic synthesis. In most cases, a biocatalytic route is favoured, most notably in the industrial conversion of steroids by fungi.[1] Such biocatalytic methods are preferred for reasons of both regio- and stereoselectivity and the technique of fungal biohydroxylation has now been applied to the production of a wide range of hydroxylated derivatives of xenobiotic compounds. Most notable of these is the use of the entomopathogen *Beauveria bassiana* to selectively hydroxylate a wide range of organic compounds including N-containing heterocycles.[2] Pioneering work by the group of Fonken and Johnson at the Upjohn company established that the biohydroxylation of N-heterocycles was much improved by their conjugation to an aromatic moiety linked to the heterocycle *via* a bridge with an electron rich centre.[3] Detailed studies revealed that *Sporotrichum sulfurescens* ATCC 7159, now redesignated *Beauveria bassiana*, would selectively hydroxylate *N*-benzoylalkylpiperidines to give optically active hydroxypiperidines.[4] Hydroxypiperidines are constituents of many pharmacologically and biochemically important natural products including pseudoconhydrine[5] and esters of 1-methylpiperidin-3-ol[6] which have cholinergic or anticholinergic properties.

A particular feature of biohydroxylations reactions is the possible formation of a large number of reaction products given that the reaction is not generally directed by a particular functional group. For example, *N*-benzoylpiperidine studied by Johnson can potentially lead to six different hydroxylation products, and the number increases dramatically with substitution within the piperidine ring. It is therefore of great interest to understand how to direct regio- and stereoselectivity of hydroxylation to give the desired alcohol. Changes in selectivity can either be achieved by using a different biocatalyst or by temporarily changing the structural characteristics of the substrate using protecting groups.[7] Here we show that the selectivity of biohydroxylation by *Beauveria bassiana* can be modified by using benzyloxycarbonyl (Cbz) instead of benzoyl protected piperidines. The Cbz-group has the additional advantage that it is more amenable to facile removal than a benzoyl group.[8] A number of Cbz-protected alkylpiperidines **1**–**8** were therefore synthesised and incubated with *Beauveria bassiana* ATCC 7159 and the results of biotransformation compared with those obtained with the corresponding *N*-benzoyl analogues.





8

1 R = H

2 R^n = H, R^4 = Me

3 R^n = H, R^3 = Me

4 R^n = H, R^2 = Me

5 R^n = H, R^2, R^6 = Me

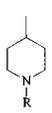
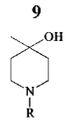
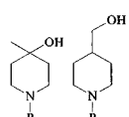
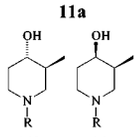
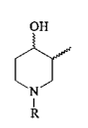
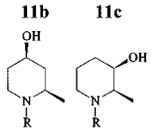
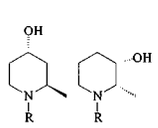
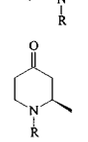
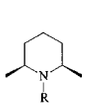
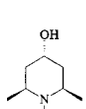
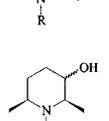
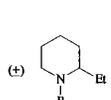
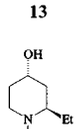
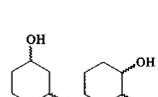
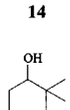
6 R^n = H, R^2 = Et

7 R^n = H, R^3, R^3 = Me

Results and discussion

Cbz-protected alkylpiperidines **1**–**8** were synthesised from the unprotected piperidines by treatment with benzyl chloroformate in the presence of potassium carbonate. The results of the biohydroxylations are summarised in Table 1, including a comparison with those obtained using the *N*-benzoyl analogues, as reported previously.[4] The unsubstituted Cbz-protected piperidine **1** is hydroxylated almost exclusively at the 4-position to give **9** (33% yield), in common with results obtained with *N*-benzoyl[4] and *N*-phenyl[9] derivatives. The structure was easily assigned from inspection of the ^{13}C NMR spectrum which clearly showed no loss of symmetry in the hydroxylated product. This selectivity was completely conserved when the 4-methylpiperidine derivative **2** was the substrate, with hydroxylation occurring at the tertiary carbon to give **10** (45% yield). This is not surprising as the radical mechanism by which hydroxylation by *Beauveria bassiana* is proposed to occur[10] would favour this product due to the stability of the tertiary radical intermediate. It is interesting to note that biohydroxylation of the *N*-benzoyl derivative[4] resulted in a 1 : 2 mixture of the 4-hydroxy compound and the 4-hydroxymethyl compound as a result of competing hydroxylation of the C-4 methyl group.

Table 1 Comparison of biohydroxylation of *N*-benzoyl and *N*-Cbz protected alkylpiperidines by *Beauveria bassiana* ATCC 7159

Substrate	Combined yield[a] Hydroxylated products (%) R = $PhCH_2OCO$	Major products R = $PhCH_2OCO$	Major products R = PhCO (data taken from ref. 4)
 **1**	33	 **9**	
 **2**	45	 **10**	
(±)  **3**	24	 **11a**  **11b** **11c**	 
(±)  **4**[b]	14	 **12a** **12b**	 
 **5**	5	 **13**	
(±)  **6**	45	 **14**	
 **7**	48	 **15**	–
 **8**	0	–	–

[a] Combined yields calculated prior to separation. [b] Preparation of substrates **4** and **8** where R = Cbz have been reported previously.[21,22]

The insertion of an O–CH_2 into the protecting group appears to have improved the regioselectivity of hydroxylation.

Three products (**11a–c**) were isolated from the transformation of *N*-benzyloxycarbonyl-3-methylpiperidine **3**, of which **11a** and **11c** were very difficult to separate by either chromatography on silica or preparative HPLC. The mixture of **11a** and **11c** was therefore subjected to acetylation conditions in an attempt to ease separation. Only one of the two products was acetylated (as shown by NMR and mass spectrometry) resulting in a mixture of **11a** and *O*-acetylated **11c**, which were then easily separated by preparative HPLC.

Product **11a** was found to be unaltered by the acetylation conditions. Mass spectrometry (atmospheric pressure chemical ionisation) showed an $(M + 1)^+$ peak at 250.1, confirming that **11a** is a monohydroxylated product. Since the characteristic C*H*OH signal was missing in the NMR spectrum, **11a** was assigned as the 3-methyl-3-hydroxy derivative.

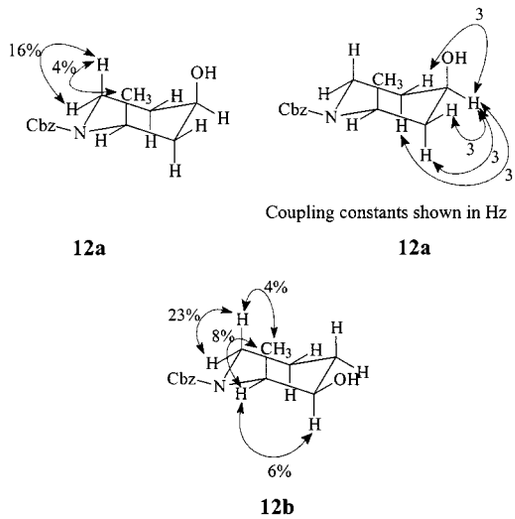
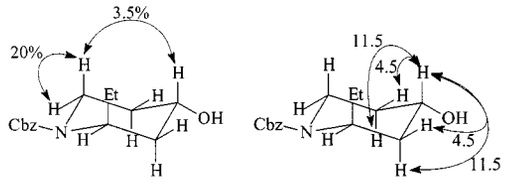


Fig. 1 NOE enhancements and coupling constants used in assigning hydroxylated products **12a** and **12b**.

The structure of **11c** was confirmed by NMR spectroscopy of the *O*-acetylated derivative. From the coupling pattern, it was possible to deduce that the hydroxy moiety was introduced in the axial position since the adjacent proton signal showed only three small couplings (*J* 3, 5 and 5 Hz). It was however not possible to establish the relative position of the methyl group from simple decoupling experiments since the C3-H signal is not easily identifiable. It is thought however that the methyl is most likely to lie in the equatorial position since a 3,4-diaxial conformation would be disfavoured and thus prone to ring-flip to give the 3,4-diequatorial conformation.

Product **11b** was identified by NMR spectroscopy as the isomer of **11c**, the 4-hydroxy-3-methyl derivative with the hydroxy group located equatorially. The signal due to the C-4 proton showed two large and one small couplings (*J* 3, 12, and 14 Hz) implying that the methyl group also adopts an equatorial position. The regioselectivity obtained in the biohydroxylation of **3** is the same as that observed for the *N*-benzoylpiperidine.[4] Evidently in this case, alignment of the piperidine ring in the enzyme active site is a more significant factor for hydroxylation regioselectivity than ease of radical formation which would favour formation of **11a**. When the fungus was challenged with the 2-methylpiperidine derivative **4** as substrate, equal amounts of products hydroxylated in the 3 (**12b**) and 4 (**12a**) positions but no 2-hydroxylated products were obtained. The structures of **12a** and **12b** were confirmed by NOE experiments, as shown in Fig. 1. The preference of 2-alkyl substituents for axial rather than equatorial positions is well documented in alkylpiperidine amides.[11,12] These conformational effects favour the 2,4-diaxial chair over the diequatorial conformation of **12a**.

One major concern about employing this series of substrates with a whole cell biotransformation system was the potential lability of the Cbz-protecting group to other enzymes in the fungus. Microbial carbamate hydrolases have been reported[13] and degradation of alkyl substituted aromatics by benzylic hydroxylation has been well studied.[14] It had also been reported that the inclusion of inverted urethane-type linkers in hydroxylation substrates for this fungus led to no biotransformation whatsoever.[15] In only one case were these possibilities implicated by results. When *N*-benzyloxycarbonyl-*cis*-2,6-dimethylpiperidine **5** (270 mg) was incubated with *Beauveria bassiana* only 10 mg of hydroxylated product was recovered. ^1H and ^{13}C NMR indicated this compound to once again be the 4-hydroxy



14 **14**

Fig. 2 NOE enhancements and coupling constants used in assigning 4-hydroxylated product **14** of biotransformation of **6**.

derivative **13**. However, a large amount of benzoic acid (92 mg) was also recovered from the reaction mixture suggesting a degradative mechanism involving a benzylic hydroxylase as has been previously described.[14] This degradation pathway is not relevant in the *N*-benzoyl analogues which is reflected in the better yield reported.

Although no active cell-free extract, nor pure enzyme work has been successfully conducted using preparations from *Beauveria bassiana* ATCC 7159, the results of the numerous whole-cell studies have been used to construct a crude model for the active site of what is assumed to be the sole hydroxylating species in this fungus.[15,16] This model suggests an optimum distance from the electron-rich site of attachment to the site of hydroxylation of between 3.3 and 6.2 Å.[16] The results with *N*-benzyloxycarbonylalkylpiperidines suggest that the active site of the *Beauveria* hydroxylating system is very sensitive to relatively small changes in the nature of the protecting group. The hydroxylase is clearly now disposed to hydroxylation in the 4-position, except where there is a 2- or 3-methyl substituent, when the selectivity is compromised. The distance from the carbonyl group to the putative site of hydroxylation is, of course, unchanged from *N*-benzoyl to *N*-benzyloxycarbonyl substrate. The results presented herein would suggest that the more critical characteristic for selective hydroxylation is the distance from carbonyl to an aromatic binding pocket. In addition, greater flexibility of the *N*-benzyloxycarbonyl molecule is afforded by rotation around the benzylic centre. This would appear to allow improved accommodation by the active site and therefore greater regioselectivity for some substrates.

The change in regioselectivity of hydroxylation around the piperidine ring from the 4- to the 3-position with regioisomeric alkyl substitution is difficult to rationalise in terms of steric restrictions in the active site of the hydroxylase. For piperidines **1** and **2** access to the favoured 4-position by the hydroxylating species is unrestricted, but methyl substitution at the 2- and 3-position must move the 4-position sufficiently far from this species that hydroxylation also occurs at the 3-position. One would expect that an extension to the 2-alkyl chain would therefore result in even more pronounced selectivity for the 3-position. However, when *N*-benzyloxycarbonyl-2-ethylpiperidine **6** is the substrate, hydroxylation occurs almost exclusively at the 4-position to give the 4-hydroxypiperidine derivative **14** (Fig. 2) in good yield.

In Johnson's work,[4] the relative position of the hydroxy group in the hydroxylated products from the 2-alkylpiperidines was assigned by analogy to the products of the 2-ethyl transformation and it has been well reported that, in rigid systems, the hydroxy group is introduced in a *trans* orientation with respect to the amide functional group.[2a] By the use of NMR analysis we have established that the hydroxy group is also introduced into the equatorial position in most cases, with the exceptions of the 2-methyl-4-hydroxy product **12a** where the OH was in an axial position (this was shown by the lack of an NOE enhancement between the axial C2-H and the C4-H and also from the coupling constants of the C*H*OH signal, showing no axial–axial coupling) and the 3-methyl-4-hydroxy product (the acetylated derivative of which was isolated) **11c** where the C*H*OAc signal showed no diaxial couplings.

It is interesting to note that Johnson and co-workers observed some enantiodifferentiation by the *Beauveria* hydroxylating system when operating on *N*-benzoalkylpiperidines.[4] The optical purity of our products has not been determined but it was seen that in all cases, a 72 hour incubation of the substrate with the fungus resulted in complete substrate disappearance implying that no substrate enantiomer (where relevant) is preferred to any great extent. It is likely, on the basis of previous work,[7] that the enantioselectivity and resulting enantiomeric excess (if relevant) will depend on many factors, including the time course of the reaction. There could be preferential hydroxylation of one enantiomer of the substrate at a single position as well as preferential metabolism of one of the product molecules, both of these examples would effect the enantiomeric excess of the identified product. An investigation into these factors is currently underway in our laboratories and will be published at a later date. Since the yield of hydroxylated products was approximately 50% it is unlikely that a resolution process is operating.

Two further substrates have been tested with the fungus which do not correlate with the substrate used by Fonken but add to the overall series and the investigation of the tolerance of the active site to changes in the substrate. It was found on incubation of the 3,3-dimethylpiperidine derivative **7** with the fungus that the 4-hydroxylated product **15** was produced. It was also noted that the rate of this hydroxylation was enhanced and that no substrate remained after two days, whereas other reactions required three days for complete substrate disappearance. This is likely to be a consequence of the reaction being directed by the two methyl groups.

Somewhat unexpectedly, when the fungus was challenged with the unsaturated piperidine derivative, *N*-benzyloxycarbonyl-1,2,3,6-tetrahydropyridine **8**, no biohydroxylation was noted but unreacted starting material was recovered. This result was surprising since both allylic hydroxylation and epoxidations[17] are known to be catalysed by P-450 monoxygenases.

Conclusion

This study has established that the regioselectivity of hydroxylation by *Beauveria bassiana* ATCC 7159, an organism much used for biotransformations of this type, may be significantly altered by changes to the linker attaching the putative hydroxylation site to the aromatic recognition group. It appears that regioselectivity is not only determined by distance of hydroxylation site to the carbonyl group, which is the same for both *N*-benzoyl and *N*-Cbz protected piperidines. The distance to the aromatic sidechain is also important, suggesting that the active site of the enzyme contains a defined aromatic binding pocket.

Experimental

Apparatus and chemicals

All chemicals were purchased from Aldrich Chemical Company, Poole, Dorset UK unless specified otherwise. ^{1}H and ^{13}C NMR analysis was performed on a Bruker AC250 spectrometer (at 250 MHz and 63 MHz respectively) and on a WH-360 spectrometer (at 360 MHz and 90 MHz). Spectra were recorded in deuterochloroform. Chemical shifts are quoted in parts per million (ppm) and these chemical shifts were referenced internally (^{1}H, C*H*Cl$_3$, 7.62 ppm; ^{13}*C*HCl$_3$, 77.0 ppm). Coupling constants (*J*) are quoted in Hz. Carbon multiplicity was established by DEPT (distortionless enhancement by polarisation transfer). Infra-red spectra were recorded on a Perkin-Elmer FT-IR spectrometer (Paragon 1000) as thin films. Mass spectra were run using electron impact (EI) on a Finnigan 4600 instrument for nominal mass and a Kratos MS50TC instrument for high

resolution spectra. Thin layer chromatography was performed on glass sheets coated with silica gel Merck 60F-254 (0.24 mm, Art. 5715). Components were detected by UV (254 nm) and visualised by treating the plate with ammonium molybdate solution and heating. Wet flash chromatography was carried out on silica gel (Merck 9385, Kieselgel 60). Gas chromatography was carried out using a Hewlett-Packard 6890 series GC system (employing a HP-5 5% phenyl methyl siloxane capillary column (30.0 m × 320 μm × 0.25 μm)). Oven temperature gradient from 200 °C (2 min) ramp to 250 °C (20 °C min^{-1}; 10 min); inlet temperature 200 °C and detector temperature 300 °C. The HPLC system used was a Waters 486 controller using Millenium software using Phenomenex ODS-2 5 μ column (25 cm × 4.6 mm) using CH_3CN–0.1% NH_4OAc solution as eluent.

General method for the preparation of Cbz-protected alkylpiperidines

The substituted piperidine (0.010 mol) and benzyl chloroformate (0.012 mol) were added to a stirred solution of potassium carbonate (0.030 mol) in tetrahydrofuran (50 cm^3) under an atmosphere of argon. After stirring for 1 h, water (10 cm^3) was added and the solution was stirred for 1 h. Water (150 cm^3) was then added and the mixture extracted with ethyl acetate (3 × 150 cm^3). The combined organic extracts were washed with saturated sodium carbonate solution (160 cm^3) and brine (2 × 120 cm^3) and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed *in vacuo* and the residue purified by wet flash chromatography using petroleum ether–ethyl acetate mixture as eluent.

***N*-Benzyloxycarbonylpiperidine 1.**[18] Carbamate (**1**) was isolated as a colourless oil (2.04 g, 93%); ν_{max} 1701 cm^{-1}; δ_H 1.46–1.63 (6H, m, C(3)H_2, C(4)H_2 and C(5)H_2), 3.41–3.46 (4H, m, C(2)H_2 and C(6)H_2), 5.12 (2H, s, CH_2Ph), 7.25–7.38 (5H, m, aromatic); δ_C 24.1 (t, C3 and C5), 25.4 (t, C4), 44.6 (t, C2 and C6), 66.6 (t, CH_2Ph), 127.6 (d, aromatic), 127.6 (d, aromatic), 128.2 (d, aromatic), 136.8 (s, aromatic), 155.1 (s, C=O); *m*/*z* (EI) required 219.12593, found 219.12558, 219 (14%, M^+), 128 (12, M − CH_2Ph), 91 (100, $PhCH_2$), 84 (10, M − CO_2CH_2Ph).

***N*-Benzyloxycarbonyl-4-methylpiperidine 2.** Carbamate (**2**) was isolated as a colourless oil (2.08 g, 89%); ν_{max} 1701 cm^{-1}; δ_H (250 MHz) 0.93 (3H, d, *J* 6.5, CH_3), 1.03–1.18 (1H, m, C(4)H), 1.46–1.61 (4H, m, C(3)H_2 and C(5)H_2), 2.75 (2H, ddd, *J* 2.5, 13 and 13, C(2)Hax and C(6)Hax), 4.13 (2H, br d, *J* 13, C(2)Heq and C(6)Heq), 5.11 (2H, s, CH_2Ph), 7.24–7.36 (5H, m, aromatic); δ_C 63 MHz) 21.7 (q, CH_3), 30.8 (d, C4), 33.8 (t, C3 and C5), 44.1 (t, C2 and C6), 66.8 (d, CH_2Ph), 127.7 (d, aromatic), 127.8 (d, aromatic), 128.3 (d, aromatic), 136.9 (s, aromatic), 155.2 (s, C=O); *m*/*z* (EI) required 233.14158, found 233.14262, 233 (81%, M^+), 218 (4, M − CH_3), 142 (78, M − CH_2Ph), 126 (70, M − OCH_2Ph), 98 (73, M − CO_2CH_2Ph), 91 (62, CH_2Ph), 77 (13, Ph).

***N*-Benzyloxycarbonyl-3-methylpiperidine 3.** Carbamate (**3**) was isolated as a colourless oil (2.14 g, 92%); ν_{max} 1701 cm^{-1}; δ_H (250 MHz) 0.87 (3H, d, *J* 6.5, CH_3), 1.01–1.13 (1H, m, C(3)H), 1.39–1.81 (4H, m, C(4)H_2 and C(5)H_2), 2.42 (1H, dd, *J* 11 and 13, C(2)Hax), 2.71–2.81 (1H, m, C(6)Hax), 4.00–4.08 (2H, m, C(2)Heq and C(6)Heq), 5.12 (2H, s, CH_2Ph), 7.25–7.37 (5H, m, aromatic); δ_C (63 MHz) 18.7 (q, CH_3), 25.7 (d, C3), 30.8 (t, C4 or C5), 32.7 (t, C4 or C5), 44.2 (t, C2 or C6), 51.1 (t, C2 or C6), 66.7 (t, CH_2Ph), 127.6 (d, aromatic), 127.7 (d, aromatic), 128.3 (d, aromatic), 136.9 (s, aromatic), 155.1 (s, C=O); *m*/*z* (EI) required 233.14158, found 233.14109, 233 (35%, M^+), 142 (46, M − CH_2Ph), 126 (26, M − OCH_2Ph), 98 (27, M − CO_2CH_2Ph), 91 (100, CH_2Ph).

***N*-Benzyloxycarbonyl-*cis*-2,6-dimethylpiperidine 5.**[19] Carbamate (**5**) was isolated as a colourless oil (2.19 g, 89%); ν_{max} 1693 cm^{-1}; δ_H (250 MHz) 1.20 (6H, d, *J* 7, 2 × CH_3), 1.41–1.77 (6H, m, C(3)H_2, C(4)H_2 and C(5)H_2), 4.32–4.38 (2H, br m, C(2)H and C(6)H), 5.13 (2H, s, CH_2Ph), 7.25–7.36 (5H, m, aromatic); δ_C 13.5 (t, C4), 20.7 (q, C7 and C8), 29.8 (t, C3 and C5), 45.9 (d, C2 and C6), 66.6 (t, CH_2Ph), 127.5 (d, aromatic), 127.6 (d, aromatic), 128.3 (d, aromatic), 137.0 (s, CH_2Ph), 155.5 (s, C=O); *m*/*z* (EI) required 247.157229, found 247.15832, 247 (4%, M^+), 32 (22, M − CH_3), 156 (13, M − CH_2Ph), 91 (100, $PhCH_2$).

***N*-Benzyloxycarbonyl-2-ethylpiperidine 6.** Carbamate (**6**) was isolated as a colourless oil (2.28 g, 92%); ν_{max} 1693 cm^{-1}; δ_H (250 MHz) 0.83 (3H, t, *J* 7.5, C(8)H_3), 1.34–1.80 (8H, m, C(3)H_2, C(4)H_2, C(5)H_2 and C(7)H_2), 2.75–2.86 (1H, m, C(6)Hax), 4.02–4.07 (1H, br m, C(2)Heq or C(6)Heq), 4.16–4.23 (1H, br m, C(2)Heq or C(6)Heq), 5.17 (2H, s, CH_2Ph), 7.25–7.37 (5H, m, aromatic); δ_C (63 MHz) 10.6 (q, C8), 18.8 (t, C7), 22.4 (t, C3, C4 or C5), 25.5 (t, C3, C4 or C5), 27.9 (t, C3, C4 or C5), 38.9 (t, C6), 52.2 (d, C2), 66.7 (t, CH_2Ph), 127.6 (d, aromatic), 127.6 (d, aromatic), 128.3 (d, aromatic), 137.0 (s, aromatic), 155.6 (s, C=O); *m*/*z* (EI) required 247.15723, found 247.15706, 247 (6%, M^+), 218 (87%, M − CH_2CH_3), 156 (9, M − CH_2Ph), 140 (5, M − OCH_2Ph), 112 (6, M − CO_2CH_2Ph), 91 (67, CH_2Ph).

***N*-Benzyloxycarbonyl-3,3-dimethylpiperidine 7.** Carbamate (**7**) was isolated as a colourless oil (2.20 g, 89%); ν_{max} 1701 cm^{-1}; δ_H 0.89 (6H, s, C(7)H_3 and C(8)H_3), 1.32–1.37 (2H, m, C(4)H_2 or C(5)H_2), 1.55–1.60 (2H, m, C(4)H_2 or C(5)H_2), 3.13 (2H, s, C(2)H_2), 3.40 (2H, dd, *J* 5.5 and 5.5, C(6)H_2), 5.12 (2H, s, CH_2Ph), 7.25–7.36 (5H, m, aromatic); δ_C 25.8 (q, C7 and C8), 30.8 (s, C3), 37.6 (t, C4), 44.3 (t, C2 or C6), 55.3 (t, C2 or C6), 66.7 (t, CH_2Ph), 127.6 (d, aromatic), 127.7 (d, aromatic), 128.3 (d, aromatic), 137.0 (s, aromatic), 155.5 (s, C=O); *m*/*z* (EI) required 247.15723, found 247.15813, 247 (36%, M^+), 156 (42, M − CH_2Ph), 140 (20, M − OCH_2Ph), 112 (17, M − CO_2-CH_2Ph), 91 (63, $PhCH_2$).

Maintenance and growth of microorganism

Beauveria bassiana ATCC 7159 was obtained from the American Type Culture Collection, USA. The organism was maintained on malt extract agar plates at room temperature and these were subcultured at regular intervals. A loop of fungus was used to inoculate 60 cm^3 of sterile medium containing 7.5 g dm^{-3} corn steep solids and 10 g dm^{-3} glucose in distilled water adjusted to pH 4.85 in a 250 cm^3 Erlenmeyer flask. After three days growth on an orbital shaker at 200 rpm at 25 °C, a culture was used to inoculate 600 cm^3 of the same medium in a 2 dm^3 Erlenmeyer flask. This culture was again grown for three days.

General biotransformation procedure

A solution of 66 mg substrate in 1 cm^3 ethanol was prepared and this was added to the three day old culture of *Beauveria bassiana* ATCC 7159. After a further three days incubation as above, the fungal cells were removed from the broth by centrifugation and the supernatant extracted into ethyl acetate. Purification of metabolites was routinely carried out using flash silica chromatography with petroleum ether–ethyl acetate gradients as eluent.

Biohydroxylation of *N*-benzyloxycarbonylpiperidine **1** produced hydroxylated product (**9**) which was isolated as a colourless oil (100 mg, 33%). The product **9** was identical to previously reported material as judged by NMR spectroscopy.[20]

Biohydroxylation of *N*-benzyloxycarbonyl-4-methylpiperidine 2. Hydroxylated product (**10**) was isolated as a pale yellow solid (130 mg, 45%); mp 76 °C; δ_H (250 MHz) 1.24 (3H, s, CH_3), 1.45–1.56 (5H, m, C(3)H_2, C(5)H_2 and C(4)OH), 3.34–3.23

(2H, dt, *J* 7 and 13, C(2)Hax and C(6)Hax), 3.80 (2H, br d, *J* 13, C(2)Heq and C(6)Heq), 5.11 (2H, s, CH_2Ph), 7.25–7.36 (5H, m, aromatic); δ_C (63 MHz) 30.0 (q, CH_3), 38.2 (t, C3 and C5), 40.2 (t, C2 and C6), 66.9 (t, CH_2Ph), 67.7 (s, C4), 127.7 (d, aromatic), 127.8 (d, aromatic), 128.3 (d, aromatic), 136.7 (s, aromatic), 155.1 (s, C=O); *m*/*z* (EI) required 249.13649, found 249.13637, 249 (44%, M^+), 158 (14, M − $PhCH_2$), 142 (16, M − $PhCH_2$), 91 (100, $PhCH_2$).

Biohydroxylation of *N*-benzyloxycarbonyl-3-methylpiperidine 3. Three products **11a**–**c** were isolated from this biohydroxylation, two of which (**11a** and **11c**) were found to be very closely related and thus difficult to separate. These two products were separated by subjecting the mixture to acetylation conditions (10 mol equiv. acetic anhydride, 5 mol equiv. pyridine and catalytic dimethylaminopyridine) under which conditions one of the products was acetylated **11c** and one was left untouched **11a**. The products were then separated by preparative HPLC.

Product **11a** was isolated as a colourless oil (10 mg, 3%); δ_H (360 MHz) 1.21 (3H, s, CH_3), 1.49–1.79 (5H, m, C(3)OH, $C(4)H_2$ and $C(5)H_2$), 2.96–3.03 (2H, br m, C(2)Hax and C(6)Hax), 3.68 (1H, br s, C(2)Heq), 3.84 (1H, ddd, *J* 4.5, 4.5 and 13, C(6)Heq), 5.13 (2H, s, CH_2Ph), 7.28–7.35 (5H, m, aromatic); *m*/*z* (EI) required 249.13649, found 249.13725, 249 (5%, M^+), 114 (26, M − $PhCH_2OCO$), 91 (100, $PhCH_2$).

Hydroxylated product **11b** was isolated as a colourless oil (10 mg, 3%); δ_H (360 MHz) 0.99 (3H, d, *J* 6.5, CH_3), 1.43–1.59 (3H, br m, three of C(3)H, C(4)OH and $C(5)H_2$), 1.91 (1H, br dd, *J* 3.5 and 13, C(3)H, C(4)OH or C(5)H), 2.53 (1H, br s, C(2)Hax or C(6)Hax), 2.90 (1H, ddd, *J* 3, 12 and 14, C(4)H), 3.30 (1H, br m, C(2)Hax or C(6)Hax), 4.05–4.13 (2H, br m, C(2)Heq and C(6)Heq), 5.11–5.12 (2H, d, *J* 2, CH_2Ph), 7.28–7.36 (5H, m, aromatic); *m*/*z* (EI) required 249.13649, found 249.13616, 249 (8%, M^+), 158 (11, M − $PhCH_2$), 142 (M − $PhCH_2O$), 91 ($PhCH_2$).

The *O*-acetylated product of **11c** was isolated as a colourless oil (5 mg); δ_H (360 MHz) 0.88 (3H, d, *J* 12.5, CH_3), 1.67–1.93 (3H, br m, C(3)H and $C(5)H_2$), 2.07 (3H, s, CH_3CO_2), 3.00–3.15 (1H, br m, C(2)Hax or C(6)Hax), 3.25–3.28 (1H, br m, C(2)Hax or C(6)Hax), 3.70 (1H, br s, C(2)Heq or C(6)Heq), 3.75 (1H, ddd, *J* 4.5, 4.5 and 13.5, C(2)Heq or C(6)Heq), 5.01 (1H, ddd, *J* 3, 3 and 5, C(4)Heq), 5.12 (2H, d, *J* 2, CH_2Ph), 7.28–7.39 (5H, m, aromatic); *m*/*z* (EI) required 291.14706, found 291.14768; 291 (3%, M^+), 200 (11, M − $PhCH_2$), 91 (100, $PhCH_2$).

Biohydroxylation of *N*-Benzyloxycarbonyl-2-methylpiperidine 4. Hydroxylated product **12a** was isolated as a colourless oil (20 mg, 7%); δ_H (250 MHz) 1.35 (3H, d, *J* 7, CH_3), 1.46–1.91 (5H, m, $C(3)H_2$, C(4)OH and $C(5)H_2$), 3.34 (1H, ddd, *J* 5, 12.5 and 13.5, C(6)Hax), 3.90 (1H, ddd, *J* 3.5, 4 and 12.5, C(6)Heq), 4.16 (1H, q, *J* 3, C*H*OH), 4.31–4.42 (1H, m, C(2)Heq), 5.12 (2H, s, CH_2Ph), 7.25–7.36 (5H, m, aromatic); δ_C (90 MHz) 19.1 (q, CH_3), 32.2 (t, C3 or C5), 33.5 (t, C3 or C5), 36.4 (t, C6), 45.7 (d, C2), 64.7 (d, CHOH), 66.8 (t, CH_2Ph), 127.7 (d, aromatic), 127.8 (d, aromatic), 128.4 (d, aromatic), 136.9 (s, aromatic), 155.2 (s, C=O); *m*/*z* (EI) required 249.13649, found 249.136337, (FAB) 234 (4%, M − CH_3), 158 (15, M − $PhCH_2$), 142 (31, M − $PhCH_2O$), 114 (2, M − $PhCH_2CO_2$), 91 (100, $PhCH_2$).

Hydroxylated product **12b** was isolated as a colourless oil (20 mg, 7%); δ_H (360 MHz) 1.12 (3H, d, *J* 7, CH_3), 1.43–1.78 (5H, m, C(3)OH, $C(4)H_2$ and $C(5)H_2$), 2.79 (1H, ddd, *J* 3, 13 and 13.5, C(6)Hax), 3.74–3.80 (1H, m, C*H*OH), 3.95 (1H, br d, *J* 12.5, C(6)Heq), 4.51 (1H, q, *J* 6.5, C(2)H), 5.12 (2H, s, CH_2Ph), 7.25–7.38 (5H, m, aromatic); δ_C (90 MHz) 9.2 (q, CH_3), 24.0 (t, C5 or C4), 27.1 (t, C5 or C4), 37.6 (t, C6), 51.0 (d, C2), 67.0 (t, CH_2Ph), 69.0 (d, CHOH), 127.7 (d, aromatic), 127.9 (d, aromatic), 128.4 (d, aromatic), 136.7 (s, aromatic), 155.3 (s, C=O); *m*/*z* (EI) required 249.13649, found 249.13637, (APCI †) 250 (100%, M + 1), 217 (3, M − $OHCH_3$), 158 (7, M − $PhCH_2$).

Biohydroxylation of *N*-benzyloxycarbonyl-*cis*-2,6-dimethylpiperidine 5. Hydroxylated product **13** was isolated as a colourless oil (10 mg, 5%); δ_H (360 MHz) 1.25 (6H, d, *J* 7, 2 × CH_3), 1.42–1.60 and 1.86–1.90 (5H, m, $C(3)H_2$, C(4)OH and $C(5)H_2$), 4.20 (1H, tt, *J* 11 and 4, C*H*OH), 4.49–4.57 (2H, m, C(2)Heq and C(6)Heq), 5.14 (2H, s, CH_2Ph), 7.23–7.38 (5H, m, aromatic); δ_C (63 MHz, DEPT) 21.7 (q, 2 × CH_3), 39.4 (t, C3 and C5), 47.2 (d, C2 and C6), 61.4 (d, CHOH), 66.9 (t, CH_2Ph), 127.7 (d, aromatic), 127.8 (d, aromatic), 128.4 (d, aromatic); *m*/*z* (EI) required 263.15214, found 263.15203, (FAB) 264 (M + 1).

Biohydroxylation of *N*-benzyloxycarbonyl-2-ethylpiperidine 6. Hydroxylated product **14** was isolated as a colourless oil (130 mg, 45%); δ_H (360 MHz) 0.85 (3H, t, *J* 7.5, $C(8)H_3$), 1.23–1.66 (5H, m, C(3)H, C(4)OH, C(5)H and $C(7)H_2$), 1.92 (2H, dd, *J* 2.5 and 12.5, C(3)H and C(5), 2.85 (1H, ddd, *J* 2.5, 12.5 and 12.5, C(6)Hax), 3.91 (1H, tt, *J* 4.5 and 11.5, C*H*OH), 4.16 (1H, br d, *J* 12.5, C(6)Heq), 4.33 (1H, br m, C(2)Heq), 5.12 (2H, d, *J* 2, CH_2Ph), 7.25–7.37 (5H, m, aromatic); δ_C (90 MHz) 10.7 (q, C8), 23.8 (t, C7), 35.0 (C3 and C5), 37.6 (t, C6), 52.8 (d, C2), 65.0 (t, CHOH), 67.0 (t, CH_2Ph), 127.6 (d, aromatic), 127.8 (d, aromatic), 128.3 (d, aromatic), 136.8 (s, aromatic), 155.4 (s, C=O); *m*/*z* (EI) required 263.15214, found 263.15253, (APCi) 264 (12%, M + 1), 220 (M − CH_3CH_2), 172 (7, M − $PhCH_2$), 156 (23, M − $PhCH_2O$), 91 (100, $PhCH_2$).

Biohydroxylation of *N*-benzyloxycarbonyl-3,3-dimethylpiperidine 7. Hydroxylated product **15** was isolated as a colourless oil (134 mg, 48%); δ_H (250 MHz; 345 K) 0.89 (3H, s, $C(7)H_3$ or $C(8)H_3$), 0.95 (3H, s, $C(7)H_3$ or $C(8)H_3$), 1.51–1.64 (2H, m, C(5)H and C(4)OH), 1.71–1.82 (1H, m, C(5)H), 2.85 (1H, d, *J* 13.5, C(2)Hax), 3.12–3.23 (1H, m, C(6)Hax), 3.40 (1H, dd, *J* 8.5 and 4.0, C*H*OH), 3.56 (1H, dd, *J* 1.5 and 13.5, C(2)Heq), 3.81–3.91 (1H, m, C(6)Heq), 5.13 (2H, s, CH_2Ph), 7.24–7.36 (5H, m, aromatic); δ_H (63 MHz) 18.4 (q, C7 or C8), 24.2 (q, C7 or C8), 29.5 (s, C3), 35.8 (t, C5), 41.5 (t, C2 or C6), 52.6 (t, C2 or C6), 66.9 (t, CH_2Ph), 74.7 (d, CHOH), 127.7 (d, aromatic), 127.8 (d, aromatic), 128.3 (d, aromatic), 136.7 (s, aromatic), 155.4 (s, C=O); *m*/*z* required 263.15214, found 263.15254, 263 (10%, M^+), 172 (5, M − $PhCH_2$), 128 (3, CO_2CH_2Ph), 91 (100, CH_2Ph), 77 (4, Ph).

Acknowledgements

We are grateful to the BBSRC and Zylepsis Ltd. for funding (to S. J. A. and G. G. respectively). We would also like to acknowledge the assistance of Mr J. Millar and Dr D. Reed with NMR studies.

† APCI = Atmospheric pressure chemical ionisation.

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Paper 8/05800H