Pergamon

Tetrahedron 57 (2001) 2833–2839

TETRAHEDRON

Studies on *Rhizopus arrhizus* mediated enantioselective reduction of arylalkanones

Neeta A. Salvi and Subrata Chattopadhyay*

Bio-Organic Division, Bhabha Atomic Research Centre, Mumbai 400 085, India

Received 7 September 2000; revised 8 January 2001; accepted 25 January 2001

Abstract—The effect of substitution on the biotransformation of various arylalkanones using *Rhizopus arrhizus* was investigated. The organism was found to be promising for the reduction of phenylalkanones and arylethanones with good to excellent enantioselectivity. The reduction followed Prelog's rule giving the (*S*)-carbinols in all the cases. The enantioselectivity of the reaction improved with increasing size of the groups flanking the carbonyl function and the electron withdrawing capacity of the substituents in the aromatic ring. However, the yield was dramatically affected with increased hydrophobicity of the substrates. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

The asymmetric reduction of ketones represents a pivotal transformation[1] in organic synthesis as the product carbinols are often useful target bioactive compounds or their precursors. For this reaction, the use of an enzymatic protocol offers an alternative to chemical methods and has aroused interest among organic chemists.[2a–c] At present, various redox enzymes are commercially available; however, their widespread use is restricted due to the prohibitive cost and the need to use costly cofactors in stoichiometric amounts. In this regard, the use of whole cell systems is emerging as an economical and eco-friendly alternative.[2a–c] Amongst various microorganisms, the use of baker's yeast in asymmetric reduction is very well documented.[3a,b] Although operationally simple, the microbial methods often suffer either from limited substrate specificity of most of the microbes and/ or moderate to good enantioselectivity except in few cases. Thus, there is a need to develop more efficient protocols for bioreduction. To this end, techniques have recently been introduced to improve the enantioselectivity of microbial reduction. These include use of the acetone powder of *Geotrichum candidum* for reduction of *para*-substituted acetophenones,[4a] application of purified enzymes from baker's yeast[4b] for reduction of alkanones and use of various yeast strains[4c] in the presence of selective enzyme inhibitors for reduction of α-keto esters, etc. However, these protocols involve external addition of the cofactor or tedious isolation of the enzymes. Exploration of other microorganisms is an alternative approach to address this issue.

During our recent studies on the biotransformation using *Rhizopus arrhizus*, we have established its versatility for stereoselective reduction of ketones.[5,6] In addition, its biomass was found to be effective for the removal of trace amounts of radioactive pollutants from nuclear waste.[7,8] It was therefore interesting to evaluate the potentials of *R. arrhizus* to bring about microbial reduction of arylalkanones, as chiral arylalkylcarbinols are useful intermediates for agrochemicals and pharmaceuticals.[9,10] Hence, we studied the microbial reduction of a wide range of substrates so as to understand the steric and electronic effects of substituents on the reactivity and the stereoselectivity of the organism. The chosen substrates can be sub-divided into three categories, viz. phenyl alkanones, substituted phenyl ethanones and fused arylethanones.

2. Results and discussion

A series of aromatic ketones were subjected to bioreduction using *R. arrhizus* in modified Czepak Dox medium.[11] In each case, the reaction was carried out for different incubation periods so as to optimize the yield vis-à-vis enantiomeric purity. The product alcohols were isolated and purified by preparative TLC. The optimized enantiomeric purities and yields for each of the three classes of substrates are listed in Tables 1–3. In general, the microbe showed moderate to excellent enantiopreference in the reduction. A total of 36 substrates were targeted for reduction. The organism could reduce 24 of these compounds to furnish the (*S*)-carbinols in all the cases. The degree of enantioselectivity was found to be governed by the steric demands of the substrates and the electronic effects of the substituents as revealed in the following analysis.

Keywords: microbial reactions; reduction; ketones; enantioselective.

* Corresponding author. Fax: +91-22-5505151;
e-mail: schatt@apsara.barc.ernet.in

PII: S0040-4020(01)00134-X

Table 1. Microbial reduction of 1-phenylalkanones using *R. arrhizus*

Entry No.	Substrate	Incub. period (days)	Product	Yield (%)[a]	Ee (%)[b]	Config.
1	**1a**	7	**4a**	33	69	*S*
2	**1b**	7	**4b**	53	80	*S*
3	**1c**	7	**4c**	42	92	*S*
4	**1d**	7	**4d**	45	92	*S*
5	**1e**	14	**4e**	15	92	*S*
6	**1f**	14	**4f**	15	92	*S*
7	**1g**	14	–	0	–	–
8	**1h**	14	–	0	–	–
9	**1i**	14	–	0	–	–
10	**1j**	14	–	0	–	–
11	**1k**	4	**4k**	26	34	*S*
12	**1l**	7	**4l**	15	74	*S*
13	**1m**	2	**4m**	60	82	*S*

[a] Isolated yields.
[b] Based on ^{1}H NMR analysis of the corresponding (*R*)-MTPA esters.

Table 2. Microbial reduction of 1-arylethanones using *R. arrhizus*

Entry No.	Substrate	Incub. period (days)	Product	Yield (%)[a]	Ee (%)[b]	Config.
1	**2a**	7	**5a**	51	68	*S*
2	**2b**	7	**5b**	72	91	*S*
3	**2c**	7	**5c**	62	94	*S*
4	**2d**	7	**5d**	59	96	*S*
5	**2e**	7	**5e**	58	10	*S*
6	**2f**	7	**5f**	63	12	*S*
7	**2g**	7	**5g**	10	67	*S*
8	**2h**	7	**5h**	55	46	*S*
9	**2i**	7	**5i**	72	72	*S*
10	**2j**	7	**5j**	59	85	*S*
11	**2k**	7	**5k**	42	93	*S*
12	**2l**	7	**5l**	15	92	*S*
13	**2m**	7	**5m**	14	93	*S*
14	**2n**	7	**5n**	15	94	*S*
15	**2o**	7	**5o**	15	99	*S*
16	**2p**	7	**5p**	68	90	*S*

[a] Isolated yields.
[b] Based on ^{1}H NMR analysis of the corresponding (*R*)-MTPA esters.

Table 3. Microbial reduction of 1-polyarylethanones using *R. arrhizus*

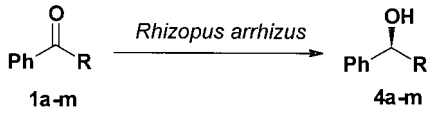
Entry No.	Substrate	Incub. period (days)	Product	Yield (%)[a]	Ee (%)[b]	Config.
1	**3a**	14	**6a**	18	74	*S*
2	**3b**	14	**6b**	20	89	*S*
3	**3c**	14	**6c**	3	68	*S*
4	**3d**	14	**6d**	0	–	–
5	**3e**	14	**6e**	0	–	–
6	**3f**	14	**6f**	0	–	–
7	**3g**	14	**6g**	0	–	–

[a] Isolated yields.
[b] Based on ^{1}H NMR analysis of the corresponding (*R*)-MTPA esters.

2.1. Reduction of 1-phenylalkanones

In order to explore the effect of size of the alkyl group on the reactivity and the enantioselectivity, various 1-phenylalkanones **1a–m** were subjected to reduction with *R. arrhizus* (Scheme 1). The result of the study is shown in Table 1. Within this class, increasing length of the alkyl chain (Table 1, entries 1–6) led to an increase in enantioselectivity giving the product alcohols in 69–92% enantiomeric excess (ee). However, the effect tapered off beyond the propyl group and no change in the ees was seen on changing R from propyl to hexyl groups. In terms of the rate of the reaction, it was found that compounds **1a–d** were easily reduced within 7 days of incubation. Thereafter the reaction was very slow and even after 14 days only **1e** and **1f** could be reduced in poor yields, while longer alkanones, **1g–1j**, could not be reduced. This might be attributed to the size of the alkyl side chain and/or their lower solubility in the reaction medium.

Reduction of α-phenylacetophenone (deoxybenzoin, **1k**) proceeded with poor enantioselectivity as expected due to the similar sizes of the groups flanking the carbonyl group. Surprisingly 2-benzoylpyridine (**1m**) was converted into the



1a-m 4a-m

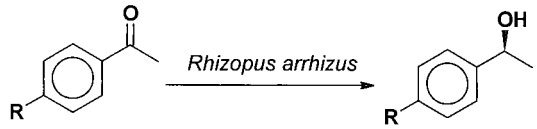
Substrate	R
1a	CH_3
1b	CH_3CH_2
1c	$CH_3(CH_2)_2$
1d	$CH_3(CH_2)_3$
1e	$CH_3(CH_2)_4$
1f	$CH_3(CH_2)_5$
1g	$CH_3(CH_2)_7$
1h	$CH_3(CH_2)_9$
1i	$CH_3(CH_2)_{10}$
1j	$CH_3(CH_2)_{11}$
1k	$PhCH_2$
1l	*cy*-Hexyl
1m	Pyridyl

Scheme 1.

corresponding alcohol with appreciable yield (60%) and enantioselectivity (82% ee) in spite of its apparent structural similarity with **1k**. The presence of a heteroatom or branching in the side chain was found to be detrimental for the reduction. This was revealed with substrates, viz. α-methoxy and α-cyano acetophenones as well as α-trifluromethyl and cyclopropylphenyl ketones, which did not undergo any bioconversion (data not shown). The ability of the organism to reduce **1l** (Table 1, entry 12), albeit in poor yield, in contrast to cyclopropylphenyl ketone could be attributed to the greater flexibility of the cyclohexyl group.

2.2. Reduction of 1-arylethanones

Next, the role of substituents in the aromatic moiety on the course of the biotransformation was studied (Scheme 2). For this, reduction of a series of 1-arylethanones was carried out with *R. arrhizus* and the results are presented in Table 2. In this case, various *para*-substituted phenylethanones (**2a**–**p**) were consistently converted to the corresponding (*S*)-alcohols. In general, the *para*-substitution in the phenyl ring improved the enantioselectivity of the reduction (except in two cases) as compared to that for unsubstituted substrate **1a**. Introduction of electron withdrawing substituents is expected to assist the reaction. Indeed, the *p*-halogen-substituted acetophenones **2a**–**d** were reduced with high enantioselectivity possibly due to the −I effect of the substituents. However, except for the fluoro compound **2a** (Table 2, entry 1), the effect of the differential electronegativities of the halogen substitutions were negligible on the % ees of the reaction (Table 2, entries



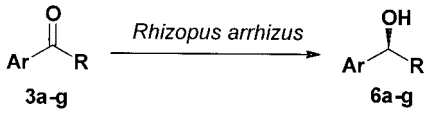
2a-p 5a-p

Substrate	R
2a	F
2b	Cl
2c	Br
2d	I
2e	OCH_3
2f	OCH_2CH_5
2g	OPh
2h	CN
2i	CH_3
2j	CH_3CH_2
2k	$(CH_3)_2CH$
2l	$(CH_3)_3C$
2m	$CH_3(CH_2)_3$
2n	*cy*-Hexyl
2o	Ph
2p	CF_3

Scheme 2.

2–4). The yields, however, showed a gradual decrease with increasing size of the halogens. In the case of substrates possessing *p*-alkoxy groups, there was a large drop in the % ee of the products (Table 2, entries 5, 6). In these cases, possibly the +M effect of the substituents outweighs their −I effect leading to the above result. This was vindicated in the case of the reduction of **2g** (Table 2, entry 7), where the substituent *p*-phenoxy group has a profound −I effect. As a consequence, its reduction showed a vastly improved enantioselectivity, although due to the solubility constraints of the substrate the yield was again poor. Considering the modest −I effect of the cyano substituent, reduction of **2h** gave the product with only 46% ee. Thus, it appears that electronic effect of the substituents in the aromatic ring has a definite role in the enantioselectivity of the reaction. Again, among the various components, the mesomeric effect was found to be more important than the inductive effect.

The minor role of the inductive effect was also manifested in the reduction of the *p*-alkyl-substituted substrates (**2i**–**n**). Reduction of these compounds showed a marked improvement in % ee, which increased with the bulk of the alkyl group. Here also, the enantioselectivity reached a plateau



3a-g 6a-g

Substrate	Ar	R
3a	1-Naphthyl	Me
3b	2-Naphthyl	Me
3c	2-Fluorenyl	Me
3d	9-Anthracenyl	Me
3e	9-Anthracenyl	CF_3
3f	9-Phenanthrenyl	Me
3g	1-Pyrenyl	Me

Scheme 3.

after **2j** (Table 2, entries 9–14) irrespective of the chain length and the extent of branching in the alkyl group. Presence of a phenyl group instead of an alkyl group at the *p*-position (compound **2o**) gave the product with excellent ee (99%) due to the combined electronic and size effects. The predominant steric effect on the enantioselectivity of the reduction was also obvious from the fact that substrate **2p** with a CF_3 substiuent gave the corresponding alcohol with much better % ee as compared to the fluoro-compound **2a**. However, in spite of increasing the enantioselectivity, the increased molecular sizes of the substituents, in general, led to poor yields in these transformations (Table 2, entries 9–16).

2.3. Reduction of polyarylethanones

The utility of the organism in asymmetric reduction was also probed using some polyarylethanones as the substrates (Scheme 3). The reduction of the bulkier substrates was, as expected, very slow. Some amount of conversion could be obtained with the naphthylethanones **3a** and **3b** only, but 14 days of incubation were required (Table 3). Bulkier substrates **3c**–**g** did not show practically any bioconversion due to their insolubility in the aqueous medium. In this case also, the same sense of enantiopreference was observed leading to the carbinols with (*S*)-configuration.

Thus, the study demonstrated the applicability of the simple microbial whole cell system of *R. arrhizus* for the biotransformation of a broad assortment of structurally different arylalkanones. The reactivity and the enantioselectivity were governed mainly by the steric factors of the groups flanking the reaction site. Increasing the size of the two flanking groups by incorporating hydrophobic groups (increasing the alkyl chain length or fusing aryl group) led to a drop in conversion and an improvement in the enantioselectivity. The polarity effect of the substituents in the case of arylethanones was more drastic for +M groups. Presence of electron withdrawing substituents in the phenyl ring improved the % ees of the reduced product. However, with +I groups like alkyl, the steric factor was more crucial.

For the determination of % ees, the carbinols were converted[12] into the corresponding MTPA esters with (*R*)-MTPA. The % ees were then assayed by the ^{1}H NMR analyses of the respective esters. The configurations of the carbinols **1a**–**d**, **1m**, **2a**, **2c**, **2e**, **2i**, **2j**, **2p**, **3a** and **3b** were determined by comparison of their $[\alpha]_D$ values with those reported. [13a–f] Incidentally, in the ^{1}H NMR spectra, the methoxy resonances of the MTPA esters of the designated compounds appeared as two singlets at δ 3.44–3.48 (minor) and 3.54–3.57 (major). Hence the downfield NMR signal could be attributed to those generated from the (*S*)-carbinols. A similar observation was also reported earlier by Brown et al.[13d] Since the MTPA esters of the rest of the carbinols also showed the OMe resonances at δ 3.54–3.57 as the major peak, they should also have (*S*)-configuration. The formation of (*S*)-alcohols could be rationalized by Prelog's rule.[14a,b]

3. Experimental

All the substrates (Aldrich, Fluka and Lancanster) were used as received. (*R*)-(+)-α-Methoxy-α-trifluoromethylphenylacetic acid (MTPA) was supplied by Aldrich. The fungus *R. arrhizus* was obtained from the National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, India. The IR spectra as thin films were scanned with a Nicolet FT-IR model Impact 410 spectrophotometer. The ^{1}H NMR spectra in $CDCl_3$ were recorded with a Bruker Ac-200 (200 MHz) spectrometer. The optical rotations were recorded with a Jasco DIP 360 digital polarimeter. The fungus from PDA slant was cultivated on 150 ml sterilized modified Czepak Dox medium in 500 mL Erlenmeyer flasks at room temperature on a rotary shaker (150 rpm).

3.1. Preparation of microbial culture

The modified Czepak Dox medium was prepared by mixing $FeSO_4$ (10 mg), K_2HPO_4 (50 mg), $MgSO_4$ (250 mg), KCl (0.5 g), KH_2PO_4 (0.95 g), $NaNO_3$ (2.0 g), yeast extract (0.5 g), cornsteep liquor (5 g) and glucose (8 g) in distilled H_2O (1 L). The medium was adjusted to pH 4.5–4.8 and sterilized. The fungus from PDA slant was incubated in the above medium (150 mL) and allowed to grow under static conditions at 25°C for 72 h.

3.2. Microbial reduction of substrates

The substrates (100 mg) in EtOH (1 mL) were added to the culture and shaken for the periods shown in the Tables. Substrate and organism controls were also run simultaneously in each case. At the end of fermentation, the mycelial mass was filtered from the culture medium. The filtrate was extracted with chloroform (3×50 mL), washed with water (2×20 mL) and dried. Removal of solvent gave an oily residue designated as the filtrate extract. The mycelial mass was washed with hot acetone (3×50 mL) and the extract concentrated in vacuo to give an oily residue.

This was taken into water and extracted with EtOAc (3×50 mL), the organic extract washed with H_2O and brine and dried. Removal of solvent in vacuo gave the mycelial extract as oil. Controls were also extracted in a similar way. The transformed products and unchanged substrates were isolated and purified by preparative TLC (silica gel G, EtOAc/Pet. Ether). All the products were characterized by IR and ^{1}H NMR specroscopy. The major amounts of the product alcohols were obtained from the filtrate extracts while the mycelial extracts provided the unchanged substrates.

3.2.1. 4a.[13a–c] Colourless liquid; $[\alpha]_D^{23}=-43.7$ (*c* 0.90, $CHCl_3$); IR: 3350, 1060, 749, 732 cm^{-1}; ^{1}H NMR: δ 1.46 (d, *J*=6 Hz, 3H), 2.43 (bs, D_2O exchangeable, 1H), 4.86 (q, *J*=6 Hz, 1H), 7.0–7.5 (m, 5H); MS *m/z* (rel. int. %): 122 (M^+, 15.1), 107 (38.9), 104 (100), 78 (95.1), 63 (21.4), 51 (54.8).

3.2.2. 4b.[13a–e] Colourless liquid; $[\alpha]_D^{24}=-44.4$ (*c* 0.63, $CHCl_3$); IR: 3380, 1060, 758, 697 cm^{-1}; ^{1}H NMR: δ 0.8 (t, *J*=6.2 Hz, 3H), 1.6–1.7 (m, 2H), 2.23 (bs, D_2O exchangeable, 1H), 4.46 (t, *J*=6 Hz, 1H), 7.0–7.5 (m, 5H); MS *m/z* (rel. int. %): 136 (M^+, 6.3), 118 (88.1), 117 (100), 107 (47.4), 91 (52.5), 79 (51.7), 77 (48), 65 (18.1), 63 (22.4), 51 (30.8).

3.2.3. 4c.[13a,b,e] White solid; $[\alpha]_D^{24}=-44.9$ (*c* 0.45, $CHCl_3$); IR: 3360, 1050, 755, 698 cm^{-1}; ^{1}H NMR: δ 0.9 (t, *J*=6.2 Hz, 3H), 1.5–2.3 (m, 4H), 2.45 (bs, D_2O exchangeable, 1H), 4.86 (t, *J*=6 Hz, 1H), 7.0–7.5 (m, 5H); MS *m/z* (rel. int. %): 150 (M^+, 11.8), 132 (87.1), 117 (100), 115 (89.7), 107 (83), 91 (84.8), 79 (77.2), 77 (78), 65 (35.3), 63 (34.4), 51 (56.3).

3.2.4. 4d.[13a] White solid; $[\alpha]_D^{24}=-39.3$ (*c* 0.57, $CHCl_3$); IR: 3380, 1060, 750, 699 cm^{-1}; ^{1}H NMR: δ 0.9 (t, *J*=7 Hz, 3H), 1.5–1.7 (m, 6H), 2.03 (bs, D_2O exchangeable, 1H), 4.43 (t, *J*=6 Hz, 1H), 6.9–7.3 (m, 5H); MS *m/z* (rel. int. %): 146 (85.1), 128 (14.4), 117 (100), 115 (93), 107 (57), 104 (79), 91 (83.4), 79 (42.6), 77 (47.1), 65 (26.3), 63 (24.2), 51 (31.8).

3.2.5. 4e. White solid; $[\alpha]_D^{24}=-35.0$ (*c* 0.88, $CHCl_3$); IR: 3360, 1060, 755, 735 cm^{-1}; ^{1}H NMR: δ 0.93 (t, *J*=7 Hz, 3H), 1.4–1.6 (m, 8H), 2.0 (bs, D_2O exchangeable, 1H), 4.91 (t, *J*=6 Hz, 1H), 7.1–7.4 (m, 5H); MS *m/z* (rel. int. %): 160 (81.1), 131 (16.8), 126 (16.8), 117 (100), 115 (95.2), 107 (76.7), 104 (95.9), 91 (81.8). Anal. Calcd for $C_{12}H_{18}O$: C 80.85, H 10.18; Found: C 81.10, H 10.10.

3.2.6. 4f. White solid; $[\alpha]_D^{24}=-29.2$ (*c* 0.89, $CHCl_3$); IR: 3350, 1060, 758, 705 cm^{-1}; ^{1}H NMR: δ 0.9 (t, *J*=6.2 Hz, 3H), 1.4–1.6 (m, 10H), 1.95 (bs, D_2O exchangeable, 1H), 4.76 (t, *J*=6 Hz, 1H), 7.2–7.4 (m, 5H); MS *m/z* (rel. int. %): 192 (M^+, 6.8), 174 (41.2), 145 (6.4), 131 (24.8), 125 (25.1), 117 (100), 115 (51), 107 (52.8), 104 (99.8), 91 (46.2). Anal. Calcd for $C_{13}H_{20}O$: C 81.20, H 10.48; Found: C 81.44, H 10.60.

3.2.7. 4k.[13b] White solid; $[\alpha]_D^{24}=+0.08$ (*c* 2.56, $CHCl_3$); IR: 3330, 1070, 758, 702 cm^{-1}; ^{1}H NMR: δ 2.3 (bs, D_2O exchangeable, 12H), 3.33 (d, *J*=6 Hz, 2H), 5.23 (t, *J*=6 Hz, 1H), 7.3–8.0 (m, 10H); MS *m/z* (rel. int. %): 122 (93.2), 105 (100), 77 (78.8), 51 (41.2).

3.2.8. 4l.[13c] White solid; $[\alpha]_D^{24}=-28.1$ (*c* 0.26, $CHCl_3$); IR: 3330, 1070, 758, 702 cm^{-1}; ^{1}H NMR: δ 1.5–2.3 (m, partially D_2O exchangeable, 12H), 4.26 (d, *J*=7 Hz, 1H), 7.0–7.3 (m, 5H); MS *m/z* (rel. int. %): 190 (M^+, 8.8), 172 (100), 157 (6.3), 143 (22.4), 141 (17.8), 129 (78.2), 127 (77.4), 117 (47.4), 115 (81.7), 107 (95), 104 (83.1), 91 (95.3).

3.2.9. 4m.[13h] White solid; $[\alpha]_D^{24}=+106.7$ (*c* 1.68, $CHCl_3$); IR: 3300, 1060, 697, 609 cm^{-1}; ^{1}H NMR: δ 2.2 (bs, D_2O exchangeable, 1H), 5.76 (s, 1H), 7.0–7.8 (m, 8H), 8.6 (d, *J*=8 Hz, 1H); MS *m/z* (rel. int. %): 185 (M^+, 67.2), 167 (22.4), 155 (23.8), 139 (78.1), 108 (84.5), 83 (8.1), 79 (100), 77 (77.8), 63 (5.7), 51 (43.4).

3.2.10. 5a.[13c] Colourless liquid; $[\alpha]_D^{24}=-38.7$ (*c* 4.15, $CHCl_3$); IR: 3360, 1060, 845 cm^{-1}; ^{1}H NMR: δ 1.66 (d, *J*=6 Hz, 3H), 2.58 (bs, D_2O exchangeable, 1H), 5.06 (q, *J*=6 Hz, 1H), 7.3–7.7 (m, 4H); MS *m/z* (rel. int. %): 122 (100), 101 (26.7), 96 (36.8), 83 (5.7), 75 (16.2), 63 (7.2), 57 (5.3).

3.2.11. 5b.[13a,e] Colourless liquid; $[\alpha]_D^{27}=-48.4$ (*c* 7.29, $CHCl_3$); IR: 3360, 1050, 898, 828 cm^{-1}; ^{1}H NMR: δ 1.43 (d, *J*=6 Hz, 3H), 2.96 (bs, D_2O exchangeable, 1H), 4.83 (q, *J*=6 Hz, 1H), 7.2–7.5 (m, 4H); MS *m/z* (rel. int. %): 156 (M^+, 15.6), 141 (52.7), 138 (100), 121 (7.8), 113 (22.4), 103 (96.4), 87 (6.8), 77 (97.8), 63 (24.4), 51 (47.8).

3.2.12. 5c.[13c,e] Pale yellow liquid; $[\alpha]_D^{26}=-37.0$ (*c* 5.0, $CHCl_3$); IR: 3340, 1060, 898, 828 cm^{-1}; ^{1}H NMR: δ 1.43 (d, *J*=6 Hz, 3H), 2.87 (bs, D_2O exchangeable, 1H), 4.78 (q, *J*=6 Hz, 1H), 7.0–7.7 (m, 4H); MS *m/z* (rel. int. %): 200 (M^+-1, 6.1), 184 (100), 182 (53.4), 157 (12.4), 121 (9.3), 107 (55.7), 77 (56.8), 63 (21.7), 51 (21.1).

3.2.13. 5d. Pinkish solid; $[\alpha]_D^{25}=-32.7$ (*c* 5.99, $CHCl_3$); IR: 3335, 1060, 898, 879 cm^{-1}; ^{1}H NMR: δ 1.33 (d, *J*=6 Hz, 3H), 2.43 (bs, D_2O exchangeable, 1H), 4.76 (q, *J*=6 Hz, 1H), 6.8–7.8 (m, 4H); MS *m/z* (rel. int. %): 248 (M^+-1, 8.7), 233 (14.8), 230 (100), 127 (33.4), 121 (6.3), 103 (77.2), 77 (99.2), 63 (16.8), 51 (40.8). Anal. Calcd for C_8H_9OI: C 38.73, H 3.66; Found: C 38.70, H 3.60.

3.2.14. 5e.[13c,d] Colourless liquid; $[\alpha]_D^{27}=-1.1$ (*c* 4.7, $CHCl_3$); IR: 3380, 1060, 832 cm^{-1}; ^{1}H NMR: δ 1.40 (d, *J*=6 Hz, 3H), 2.26 (bs, D_2O exchangeable, 1H), 3.72 (s, 3H), 4.83 (q, *J*=6 Hz, 1H), 6.7–7.1 (m, 4H);. MS *m/z* (rel. int. %): 134 (100), 119 (82.4), 103 (81.4), 91 (99.6), 77 (17.8), 65 (68.6), 51 (21.6).

3.2.15. 5f. Pale yellow solid; $[\alpha]_D^{24}=-0.2$ (*c* 6.30, $CHCl_3$); IR: 3400, 1060, 898, 836 cm^{-1}; ^{1}H NMR: δ 1.43–1.46 (merged d and t, 6H), 2.0 (bs, D_2O exchangeable, 1H), 4.03 (q, *J*=7 Hz, 2H), 4.83 (q, *J*=6 Hz, 1H), 6.8–7.3 (m, 4H); MS *m/z* (rel. int. %): 148 (79.8), 120 (100), 105 (5.3), 103 (6.1), 91 (79.8), 77 (22.4), 65 (56.3), 63 (26), 51 (18.7). Anal. Calcd for $C_{10}H_{14}O_2$: C 72.26, H 8.49; Found: C 72.50, H 8.37.

3.2.16. 5g. Pale yellow liquid; $[\alpha]_D^{23}=-40.0$ (*c* 0.81, $CHCl_3$); IR: 3380, 1060, 898, 845 cm^{-1}; ^1H NMR: δ 1.53 (d, *J*=6 Hz, 3H), 2.16 (bs, D_2O exchangeable, 1H), 5.03 (q, *J*=6 Hz, 1H), 7.1–7.6 (m, 9H); MS *m/z* (rel. int. %): 196 (100), 181 (9.8), 177 (8.7), 167 (76.7), 153 (56.1), 141 (24.2), 139 (11.2), 128 (10.4), 120 (25.1), 115 (27.8), 102 (30.6), 98 (6.4), 94 (5.7), 91 (79.3), 77 (98.6), 65 (81.3), 51 (87). Anal. Calcd for $C_{14}H_{14}O_2$: C 78.48, H 6.59; Found: C 78.64, H 6.47.

3.2.17. 5h.[13g] Pale yellow liquid; $[\alpha]_D^{25}=-40.2$ (*c* 2.23, $CHCl_3$); IR: 3410, 1070, 898, 845 cm^{-1}; ^1H NMR: δ 1.25 (d, *J*=6 Hz, 3H), 1.82 (bs, D_2O exchangeable, 1H), 4.72 (q, *J*=6 Hz, 1H), 7.0–7.3 (m, 4H). MS *m/z* (rel. int. %): 147 (M^+, 8.1), 132 (81), 104 (100), 77 (42), 63 (14.7), 51 (27.8).

3.2.18. 5i.[13a] Colourless liquid; $[\alpha]_D^{22}=-45.3$ (*c* 5.81, $CHCl_3$); IR: 3390, 1060, 878, 819 cm^{-1}; ^1H NMR: δ 1.45 (d, *J*=6 Hz, 3H), 2.34 (s, 3H), 4.5 (bs, D_2O exchangeable, 1H), 4.93 (q, *J*=6 Hz, 1H), 7.0–7.6 (m, 4H); MS *m/z* (rel. int. %): 136 (M^+, 17.1), 121 (47.4), 118 (82.4), 117 (100), 115 (51.4), 103 (14.8), 93 (49.8), 91 (94.5), 79 (12.4), 77 (38.6), 65 (32.3), 63 (21.9), 51 (18).

3.2.19. 5j. Colourless liquid; $[\alpha]_D^{23}=-44.9$ (*c* 5.98, $CHCl_3$); IR: 3360, 1060, 898, 836 cm^{-1}; ^1H NMR: δ 1.1–1.6 (merged d and t, 6H), 2.63 (q, *J*=6 Hz, 2H), 2.8 (bs, D_2O exchangeable, 1H), 4.86 (q, *J*=6 Hz, 1H), 7.0–7.5 (m, 4H); MS *m/z* (rel. int. %): 150 (M^+, 10.2), 135 (22.4), 132 (64.4), 121 (6.8), 117 (100), 115 (47.8), 105 (12.4), 103 (12.1), 91 (42.6), 79 (54.6), 65 (16.8), 63 (16.1), 51 (9.8). Anal. Calcd for $C_{10}H_{14}O$: C 79.96, H 9.39; Found: C 80.14, H 9.22.

3.2.20. 5k. Colourless liquid; $[\alpha]_D^{23}=-45.8$ (*c* 3.34, $CHCl_3$); IR: 3370, 1060, 898, 8368 cm^{-1}; ^1H NMR: δ 1.2 (d, *J*=7 Hz, 6H) 1.45 (d, *J*=6 Hz, 3H), 1.63 (bs, D_2O exchangeable, 1H), 2.74–2.81 (m, 1H), 4.67 (q, *J*=6 Hz, 1H), 7.1–7.7 (m, 4H); MS *m/z* (rel. int. %): 149 (8.8), 146 (22.2), 131 (100), 129 (15.4), 115 (21.6), 103 (14.7), 91 (58.4), 77 (24.8), 65 (12.8), 63 (12.2), 51 (11.1). Anal. Calcd for $C_{11}H_{16}O$: C 80.44, H 9.82; Found: C 80.26, H 9.88.

3.2.21. 5l. White solid; $[\alpha]_D^{24}=-38.8$ (*c* 1.54, $CHCl_3$); IR: 3355, 1060, 610 cm^{-1}; ^1H NMR: δ 1.26 (s, 9H), 1.44 (d, *J*=6 Hz, 3H), 1.56 (bs, D_2O exchangeable, 1H), 4.88 (q, *J*=6 Hz, 1H), 7.2–7.4 (m, 4H); MS *m/z* (rel. int. %): 163 (11.42), 160 (24), 145 (100), 128 (14.8), 117 (48.2), 115 (19.3), 105 (22.2), 103 (9.8), 91 (26.4), 77 (22.1), 65 (8.8), 63 (9.1), 51 (14.8). Anal. Calcd for $C_{12}H_{18}O$: C 80.85, H 10.18; Found: C 80.76, H 10.10.

3.2.22. 5m. Colourless liquid; $[\alpha]_D^{23}=-38.6$ (*c* 0.39, $CHCl_3$); IR: 3360, 1070, 941, 608 cm^{-1}; ^1H NMR: δ 1.3–2.0 (m, 10H), 2.63 (t, *J*=7 Hz, 2H), 2.7 (bs, D_2O exchangeable, 1H), 4.86 (q, *J*=6 Hz, 1H), 7.2–7.6 (m, 4H); MS *m/z* (rel. int. %): 160 (22.1), 117 (100), 115 (24.8), 91 (27.3), 77 (11.8), 65 (7.8), 51 (8.1). Anal. Calcd for $C_{12}H_{18}O$: C 80.85, H 10.18; Found: C 80.68, H 10.32.

3.2.23. 5n. White solid; $[\alpha]_D^{23}=-37.4$ (*c* 0.29, $CHCl_3$); IR: 3350, 1060, 900, 608 cm^{-1}; ^1H NMR: δ 1.3–1.5 (m, 13H), 1.7–1.9 (m, partially D_2O exchangeable, 2H), 4.83 (q, *J*=6 Hz, 1H), 7.0–7.3 (m, 4H); MS *m/z* (rel. int. %): 204 (M^+, 6.2), 189 (31.2), 186 (83.1), 143 (92.8), 130 (100), 128 (91.8), 117 (92.1), 115 (77.3), 104 (30.9), 91 (51.9), 83 (21.4), 77 (31.2), 67 (8.8), 65 (14.7), 63 (12.1), 51 (12.4). Anal. Calcd for $C_{14}H_{20}O$: C 82.30, H 9.87; Found: C 82.16, H 10.08.

3.2.24. 5o. White solid; $[\alpha]_D^{28}=-43.7$ (*c* 0.75, $CHCl_3$); IR: 3320, 1060, 750 cm^{-1}; ^1H NMR: δ 1.8 (d, *J*=6 Hz, 3H), 2.30 (bs, D_2O exchangeable, 1H), 5.23 (q, *J*=6 Hz, 1H), 7.4–8.0 (m, 9H); MS *m/z* (rel. int. %): 198 (M^+, 7.5), 183 (12.6), 180 (100), 178 (32.1), 165 (22.4), 152 (29.8), 115 (11.8), 102 (9.9), 89 (12.1), 77 (23.4), 63 (11.1), 51 (11.7). Anal. Calcd for $C_{14}H_{14}O$: C 84.81, H 7.12; Found: C 84.69, H 7.31.

3.2.25. 5p.[13c] Colourless liquid; $[\alpha]_D^{22}=-31.5$ (*c* 5.49, $CHCl_3$); IR: 3350, 1070, 898, 845 cm^{-1}; ^1H NMR: δ 1.56 (d, *J*=6 Hz, 3H), 5.03 (q, *J*=6 Hz, 1H), 7.3–7.8 (m, 4H); MS *m/z* (rel. int. %): 175 (62.4), 172 (17.4), 151 (8.4), 147 (15.4), 145 (17.7), 127 (100), 103 (18.1), 95 (7.1), 77 (21), 69 (22.8), 63 (8.1), 51 (17).

3.2.26. 6a.[13b,c,e,g] Pinkish solid; $[\alpha]_D^{27}=-53.6$ (*c* 1.80, $CHCl_3$); IR: 3370, 1060, 898, 810 cm^{-1}; ^1H NMR: δ 1.56 (d, *J*=6 Hz, 3H), 2.64 (bs, D_2O exchangeable, 1H), 5.06 (q, *J*=6 Hz, 1H), 7.4–7.55 (3H), 7.7–7.83 (m, 4H); MS *m/z* (rel. int. %): 172 (M^+, 16.2), 157 (15.9), 154 (100), 139 (10.7), 128 (64.8), 115 (16.2), 101 (11.4), 87 (8.6), 77 (32), 76 (57.5), 63 (26.1).

3.2.27. 6b.[13c,f,g] White solid; $[\alpha]_D^{23}=-47.4$ (*c* 1.64, $CHCl_3$); IR: 3365, 1060, 879, 809 cm^{-1}; ^1H NMR: δ 1.46 (d, *J*=6 Hz, 3H), 2.16 (bs, D_2O exchangeable, 1H), 5.03 (q, *J*=6 Hz, 1H), 7.0–7.3 (5H), 7.7–7.83 (m, 2H); MS *m/z* (rel. int. %): 172 (M^+, 50.6), 155 (83), 154 (57), 127 (100), 115 (16.2), 101 (11.4), 87 (7.2), 77 (32), 76 (47.3), 63 (34.2).

3.2.28. 6c. Light yellow solid; $[\alpha]_D^{27}=-43.2$ (*c* 0.24, $CHCl_3$); IR: 3320, 1060, 836, 740 cm^{-1}; ^1H NMR: δ 1.66 (d, *J*=6 Hz, 3H), 2.03 (bs, D_2O exchangeable, 1H), 4.03 (s, 2H), 5.14 (q, *J*=6 Hz, 1H), 7.3–8.0 (m, 7H); MS *m/z* (rel. int. %): 210 (M^+, 9.3), 192 (100), 165 (81.8), 152 (6.4), 139 (8.1), 115 (7.2), 96 (13.4), 94 (25.3), 82 (17.1), 63 (9.6). Anal. Calcd. for $C_{15}H_{14}O$: C 85.68, H 6.71; Found: C 85.50, H 6.95.

References

1. Singh, V. K. *Synthesis* **1992**, 605–617.
2. (a) Jones, J. B.; Beck, J. F. In *Application of Biochemical Systems in Organic Chemistry. Part 1*; Jones, J. B., Sih, C. J., Perlman, D., Eds.; Wiley: New York, 1976; Vol. X, pp 236–401. (b) Faber, K. *Biotransformations in Organic Chemistry*; 2nd ed.; Springer: Berlin, 1995. (c) Roberts, S. M.; Turner, A. J.; Willetts, A. J.; Turner, M. K. *Introduction to Biocatalysis Using Enzymes and Microorganisms*; Cambridge University Press: New York, 1995.
3. (a) Servi, S. *Synthesis* **1990**, 1–25. (b) Csuk, R.; Glanzer, B. I. *Chem. Rev.* **1991**, *91*, 49–97.
4. (a) Nakamura, K.; Matsuda, T. *J. Org. Chem.* **1998**, *63*, 8957–8964. (b) Ema, T.; Sugiyama, Y.; Fukumoto, M.; Moriya, H.;

Cui, J.-N.; Sakai, T.; Utaka, M. *J. Org. Chem.* **1998**, *63*, 4996–5000. (c). Kayser, M. M.; Mihovilovic, M. D.; Kearns, J.; Feicht, A.; Stewart, J. D. *J. Org. Chem.* **1999**, *64*, 6603–6608.
5. Salvi, N. A.; Patil, P. N.; Udupa, S. R.; Banerji, A. *Tetrahedron: Asymmetry* **1995**, *6*, 2287–2290.
6. Salvi, N. A.; Udupa, S. R.; Banerji, A. *Biotechnol. Lett.* **1998**, *20*, 201–203.
7. Dhami, P. S.; Gopalakrishnan, V.; Kannan, R.; Ramanujam, A.; Salvi, N. A.; Udupa, S. R. *Biotechnol. Lett.* **1998**, *20*, 225–228.
8. Dhami, P. S.; Kannan, R.; Gopalakrishnan, V.; Ramanujam, A.; Salvi, N. A.; Udupa, S. R. *Biotechnol. Lett.* **1998**, *20*, 869–872.
9. Roshsen, T.; Heathcock, C. H. *J. Am. Chem. Soc.* **1985**, *107*, 3731–3733.
10. Ito, Y.; Hayashi, T. *Japan, Kokai Tokyo, Koho JP* 02, 264 736 (*Chem. Abstr.* **1991**, *114*, 142856e).
11. Prema, B. R.; Bhattacharya, P. K. *Appl. Microb.* **1962**, *10*, 524–528.
12. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519.
13. (a) Nakamura, K.; Kawasaki, M.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 1079–1085. (b) Ziffer, H.; Kawai, K.; Kasai, M.; Imuta, M.; Froussios, C. *J. Org. Chem.* **1983**, *48*, 3017–3021. (c) Naemura, K.; Fukuda, R.; Murata, M.; Konishi, M.; Hirose, K.; Tobe, Y. *Tetrahedron: Asymmetry* **1995**, *6*, 2385–2394. (d) Brown, S. M.; Davies, S. G.; de Sousa, J. A. A. *Tetrahedron: Asymmetry* **1993**, *4*, 813–822. (e) Basavaiah, D.; Raju, S. B. *Synth. Commun.* **1991**, *21*, 1859–1863. (f) Itsuno, S.; Hirao, A.; Nakahama, S.; Yamazaki, N. *J. Chem. Soc. Perkin Trans. 1* **1983**, 1673–1676. (g) Fujii, A.; Hashiguchi, S.; Uematsu, N.; Ikariya, T.; Noyori, R. *J. Am. Chem. Soc.* **1996**, *118*, 2521–2522. (h) Takemoto, M.; Moriyasu, Y.; Achiwa, K. *Chem. Pharm. Bull.* **1995**, *43*, 1458–1461.
14. (a) Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119–130. (b) Jones, J. B.; Sih, C. J.; Perlman, D. *Technol. Chem. (NY) Part 1* **1976**, *10*, 295–310.