Microbial Generation of (2*R*,3*S*)- and (2*S*,3*S*)-Ethyl 2-Benzamidomethyl-3-hydroxybutyrate, a Key Intermediate in the Synthesis of (3*S*,1′*R*)-3-(1′-Hydroxyethyl)azetidin-2-one

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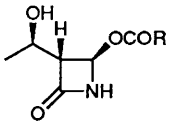
Microbial reduction of the carbonyl group of the substituted acetoacetate esters **3**–**6** affords directly, or after Ni-Raney desulfurization, the corresponding enantiomerically pure 3*S* carbinols of variable diastereoisomeric composition. These compounds are transformed into (3*S*,1′*R*)-3-(1′-hydroxyethyl)-azetidin-2-one, a useful intermediate in the synthesis of β-lactam antibiotics.

Since the importance of β-lactam antibiotics is well recognized,[1] a chiral intermediate such as (3*R*,1′*R*)-4-acyloxy-3-(1′-hydroxyethyl)azetidin-2-one **1** possessing the stereochemical features required for their preparation is of considerable interest. Such key intermediates are currently accessible from various chiral synthons such as L-aspartic acid,[2a] 2-benzamidomethyl-3-hydroxybutyrate[2b] and (3*R*)-hydroxybutrate[2c] or through synthetic sequences followed by classical resolution of the racemic intermediate.[2d] Since functionalization of the β-lactam nucleus at position 4 in compound **2** can be effected by radical oxidation,[3] compound **2** itself is, in fact, also to be considered as a key intermediate in the above mentioned synthetic path.

Compound **2** can be considered as derived from a suitable open-chain α-substituted β-hydroxy ester of type **14**, itself available from the keto ester of the corresponding structure. A recent patent report[4] refers to the utility of ethyl (2*RS*,2*S*)-2-benzamidomethyl-3-hydroxybutyrate **11**, obtained by baker's yeast (BY) treatment of **3**, in the synthesis of (3*S*,1′*R*)-3-(1′-hydroxyethyl)azetidin-2-one **2**. Product **2** is actually obtained from **14**, prepared from **11**, the actual product of the microbial reduction, by inversion of configuration at C-3 through conversion into ethyl *trans*-(5*S*,6*R*)-6-methyl-2-phenyl-5,6-dihydro-1,3-oxazine-5-carboxyate, followed by acid hydrolysis. The microbial reduction affords the required compound with *R* absolute configuration at C-3, the reverse of that in **2**.



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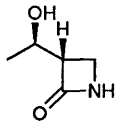
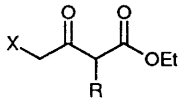


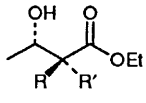
2

Microbial reduction of β-keto esters has been extensively studied, the 3-hydroxy esters of both enantiomeric forms being obtained by appropriate substrate modification at both ends of the molecule, or making use of additives in order to direct competitive enzymatic reactions.[5] In the case of racemic α-substituted β-keto esters of various origin, the prediction of the course of the reaction is less obvious, incomplete enantio- and/or diastereo-selection usually being achieved. In this particular field, however, excellent results have been obtained by catalytic asymmetric reduction.[6] With the aim of improving efficiency in obtaining **2**, we have investigated the mode of microbial reduction of a series of potential precursors, and devised an approach alternative to the one reported for inversion of configuration, if required, of the hydroxy ester obtained.

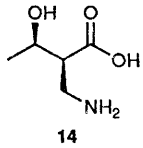
In the first instance we submitted **3** to the action of a set of growing microorganisms, including the two strains of *Geotrichum candidum* which reduce ethyl 3-oxobutyrate to (*S*)- and (*R*)-ethyl 3-hydroxybutyrate, respectively.[8] Identification of the transformation products was achieved by HPLC on a chiral column† and direct comparison with authentic (2*R*,3*S*)-**12** and (2*S*,3*S*)-**13**, obtained from **3** by BY treatment,[4] and *rac*-**11**, prepared from **3** by $NaBH_4$ reduction. The steric outcome of the microbial reduction of **3** is reported in Table 1. Inspection of the results indicates that two enantiomerically pure diastereoisomers are obtained, all possessing the 3*S* configuration. In only a few instances enhanced *syn* and *anti* diastereoselection is observed, whereas in most cases the two carbinols are obtained in ratios of nearly 1 : 1. In our hands, BY reduction of **3** gave **12** and **13** in a *ca.* 1 : 2 ratio.



3 X = H, R = CH_2NHBz
4 X = SCH_2Ph, R = CH_2NHBz
5 X = H, R = >CHNHBz
6 X = SCH_2Ph, R = >CHNHBz
7 X = Cl, R = 2H
8 X = SCH_2Ph, R = 2H
9 X = SCH_2Ph, R = >CHOEt
10 X = SCH_2Ph, R = $>CH_2NH_2$



11 R, R′ = H, CH_2NHBz
12 R = H, R′ = CH_2NHBz
13 R = CH_2NHBz, R′ = H



14

The unsaturated keto ester **5** was then selected as an alternative substrate with the aim of increasing the diastereoselection and possibly inverting the absolute configuration at C-3. The mode of microbial reduction of **5**, however, was identical with that of **3**. It is likely that the reduction of **5** involves initially saturation of the double bond, as suggested by the presence of **3** in the reaction mixture. Moreover, we were unable to detect at any point in the reduction process the

† Compounds **11**, **12**, **13**: CHIRALCEL OD (Daicel), hexane/EtOH 97/3, 0.6 cm^3 min^{-1}, UV 254 mm.

Table 1 Product distribution in the microbial reduction of **3**

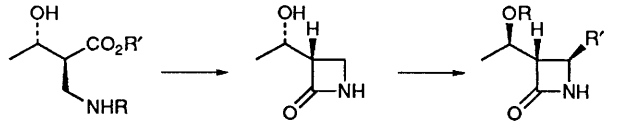
Entry	Microorganism[a]	Ratio **12** (2*R*,3*S*) : **13** (2*S*,3*S*)	GC¶ yields (%)
1	*Candida guilliermondii* (NCYC 1399) A	4.4:1	68*
2	*Candida guilliermondii* (NCYC 973) A	1:1	70*
3	*Candida lipolytica* (CBS 2074) C	6.5:1	52*
4	*Geotrichum candidum* (CBS 109.12) A	1.5:1	65†
5	*Geotrichum candidum* (CBS 233.76) B	2:1	60†
6	*Kloeckera saturnus* (CBS 5761) A	1:6.3	67*
7	*Pichia ohmeri* (CBS 5367) A	7.7:1	80*
8	*Rhodotorula glutinis* (CBS 20) C	11.3:1	90*
9	*Saccharomyces cerevisiae* (NCYC 739) A	1:2	60*
10	*Sporobolomyces sp.* (CBS 6322) C	8.9:1	21‡

[a] *Culture medium:* A = GYP: glucose (30 g dm^{-3}), yeast extract (10 g dm^{-3}), peptone (10 g dm^{-3}); B = modified GYP: glucose (50 g dm^{-3}), yeast extract (10 g dm^{-3}), peptone (10 g dm^{-3}); C = MPGB: glucose (20 g dm^{-3}), malt extract (20 g dm^{-3}), peptone (5 g dm^{-3}). * = Substrate completely converted. † = 96 h, substrate not completely converted. ‡ = 244 h, substrate not completely converted. ¶ GC conditions: Dani 8610 apparatus. Fused silica capillary column (MEGA) 30 m × 0.25 i.d. coated with 0.25 μm of OV-1. He, 0.8 bar: initially at 40 °C (1 min); raised at 20 °C min^{-1} to 190 °C (1 min); raised at 3 °C min^{-1} to 220 °C (2 min); raised at 15 °C min^{-1} to 260 °C: R_t **12** = 14.64 min and **13** = 14.92 min.

Table 2 Product distribution in the microbial reduction of **4** and **6**, after Ni-Raney treatment of the crude mixture

Microorganism[a]	Substrate	**12** (2*R*,3*S*)	**13** (2*S*,3*S*)	(2*R*,3*R*)-stereoisomer	GC yields (%)
Candida guilliermondii (CBS 973)	**4**	100	0	0	35*
Saccharomyces cerevisae (NCYC 739)	**6**	69	17	14	66*

[a] *Culture medium:* GYP: glucose (30 g), yeast extract (10 g dm^{-3}), peptone (10 g dm^{-3}). * = 96 h, substrate not completely converted.



15 R = Bz, R' = Et
16 R = H, R' = H

17

2 R, R' = H
18 R = Bz, R' = H
19 R = Bz, R' = OAc
20 R = TBDMS, R' = OAc

Scheme 1

unsaturated carbinol. A reference sample of the latter was obtained by reducing **5** $NaBH_4$ [(1 mol equiv.) in Pr^iOH at −15 °C]. The use of higher temperatures and/or of an excess of hydride leads to *rac*-**11**.

Since the above approaches failed to give 3*R* reduction products, we decided to explore the possibility of inverting the steric course of the enzymic reduction process by introducing a bulky substituent at C-4 of the acetoacetate framework, in order to change, temporarily, the relative dimensions of the carbonyl group substituents; $PhCH_2S$ was chosen as the group on the basis of literatures precedent.[9] Accordingly, compound **6** was prepared from ethyl 4-chloroacetoacetate **7** *via* intermediates **8**–**10**.[10] Catalytic reduction (10% Pd/C, 4 atm H_2, EtOH) of highly purified **6** afforded **4**. Products **6** and **4**, poorly soluble in most organic solvents, were added to the growing cultures of several microorganisms as DMSO or DMF solutions. In most instances, however, no transformation was observed. The results relative to *Candida guilliermondii* (NCYC 973) (the strain NCYC 1399[9] did not transform the substrates) and BY are reported in Table 2. In each case, the crude transformation mixture was submitted to HPLC on a chiral column before and after treatment in refluxing ethanol with Raney Ni.‡ This treatment caused no loss of optical purity, since the enantiomeric composition of the resulting ethyl 2-benzamidomethyl-3-hydroxybutyrates, determined through HPLC analysis (Table 2), was identical with that of the sulfur-containing precursors. In the case of BY, **6** afforded enantiomerically pure *syn* **12**, **13** and the 2*R*,3*R* diastereoisomer in a 5:1:1 ratio.

The above results, seen together, indicate the limits of the experimental techniques (use of different microorganisms and/or substrate structural modifications) for control of the mode of enzymic carbonyl reduction; in this respect, in only one instance was a minor quantity of 3*R* material identified. However, it is noteworthy that the results of entries 3, 6, 7, 8 and 10 of Table 1 in which transformation products definitely enriched in the *syn* and *anti* diastereoisomers were obtained.§ Moreover, the microbial reduction of **4** gave **12** as one single enantiomer, no trace of the diastereoisomer being detected. Our results compare favourably with that recently reported[4] in that they allow the preparation of a precursor to **2** requiring the inversion of only one chiral centre. Its transformation to the targer azetidinone is described in Scheme 1. Compound (2*S*,3*S*)-**15** was hydrolysed to **16** ($[\alpha]_D^{20}$ −29) and on treatment with $Ph_3P/(pyS_2)$[11] this gave **17** of the same absolute configuration. Inversion at C-1′ was effected with Ph_3P/DEAD to afford **18** from which the target compound **2** was accessible from **15** (total yield 43%). The absolute configuration and the enantiomeric excess of compounds **2** was established by correlation through **20** with **21** of commercial origin. In conclusion, the microbial reduction of α-substituted β-keto esters coupled with an efficient procedure for the inversion of configuration at the carbon bearing the newly formed hydroxy group, proves useful in the synthesis of the enantiomerically pure azetidinone **2**.

Experimental

General Procedure for the Bioconversion of Substrates **3**, **4** *and* **6**.—Each microorganism, maintained on an agar slant, was transferred to 300 cm^3 Erlenmeyer flask containing a liquid medium (50 cm^3) (see Tables). The flask was stirred during 24 h on an orbital shaker at 200 rpm at 30 °C. 10% Of the culture was further transferred to a 300 cm^3 Erlenmeyer flask containing the same liquid medium (50 cm^3) and stirred during 48 h on an orbital shaker at 200 rpm at 30 °C. The substrate (50 mg) dissolved in EtOH or DMSO (0.5 cm^3) was then added to the flask which was further stirred at 30 °C during 72–244 h. The

‡ Product recovered from **4** and **6** after biotransformation: CHIRALCEL OD, hexane/EtOH 90/10, 0.6 cm^3 min^{-1}, UV 254 mm.
§ (2*R*,3*S*)-**1b**, oil, $[\alpha]_D^{20}$ −386 (*c* 1, $CHCl_3$); (2*S*,3*S*)-**1c**, m.p. 72–73 °C, $[\alpha]_D^{20}$ −68 (*c* 1, $CHCl_3$).

broth was then extracted with ethyl acetate and analysed by GC and HPLC. Diastereoisomer separation was effected by flash chromatography (hexane–ethyl acetate, 1:2).

Ethyl 2-benzamidomethylene-3-oxobutyrate **5**. This compound was prepared as described in the literature.[10]

Ethyl 2-benzamidomethyl-3-oxobutyrate **3**. This compound was obtained by catalytic hydrogenation of **5** (EtOH, 10% Pd/C) in quantitative yield as white crystals, m.p. 62–63 °C (EtOAc–hexane); $\delta_H(CDCl_3)$ 1.25 (3 H, CH_3, t), 2.35 (3 H, CH_3, s), 3.9 (3 H, CH + CH_2, m), 4.2 (2 H, CH_2, q), 6.85 (1 H, NH, br m) and 7–8 (5 H, ArH, m).

Ethyl 4-benzylthio-3-oxobutyrate **8**. To a stirred solution of toluene-α-thiol (7.2 cm^3, 60 mmol) and triethylamine (8.5 cm^3, 60 mmol) in methylene dichloride (50 cm^3), ethyl 4-chloro-3-oxobutyrate (8.3 cm^3, 60 mmol) was added; the reaction mixture was maintained at 25 °C with an ice-bath. After 4 h, the mixture was poured in water (120 cm^3) and the organic phase was separated, washed with 5% HCl and saturated aqueous $NaHCO_3$, dried (Na_2SO_4) and evaporated to dryness. The residue was purified by column chromatography to give **8** as a colourless oil (11.8 g, 77%); $\delta_H(CDCl_3)$ 1.25 (3 H, CH_3, t), 3.2 (2 H, CH_2, s), 3.6 (2 H, CH_2, s), 3.7 (2 H, CH_2, s), 4.2 (2 H, CH_2, q) and 7.3 (5 H, ArH, s).

Ethyl 2-benzamidomethylene-4-benzylthio-3-oxobutyrate **6**. Compound **8** (10 g, 40 mmol) was heated at reflux with ethyl orthoformate (6.6 cm^3, 40 mmol) and acetic anhydride (7.5 cm^3, 80 mmol) after which the latter was distilled off *in vacuo* to give a dark oil. This was dissolved in EtOH (10 cm^3) and the solution cooled to 2 °C (ice-bath) when an equal volume of EtOH saturated with dry ammonia was added to it. The mixture was stirred at room temperature until the reaction was complete (TLC) after which it was evaporated under reduced pressure to give an oil. This was treated with an equimolar proportion of benzoyl chloride and triethylamine in methylene dichloride, the temperature being maintained at 5 °C; disappearance of the starting material was followed by TLC. At the end of the reaction the mixture was washed with water, 5% HCl and saturated aqueous $NaHCO_3$. The solution was then dried (Na_2SO_4) and evaporated to dryness. The residue was purified by column chromatography to give **6** as a white crystalline material (m.p. 115–117 °C, EtOH); $\delta_H(CDCl_3)$ 1.35 (3 H, CH_3, t), 3.7 (2 H, CH_2, s), 3.8 (2 H, CH_2, s), 4.25 (2 H, CH_2, q), 7–8 (10 H, ArH, m), 8.8 (1 H, CH, d) and 12.9 (1 H, NH, d).

Ethyl 2-benzamidomethyl-4-benzylthio-3-oxobutyrate **4**. Compound **6** was catalytically reduced (EtOH, 10% Pd/c, 4 atm H_2) to give **4** as white crystalline material (m.p. 75–76 °C, EtOH); $\delta_H(CDCl_3)$ 1.25 (3 H, CH_3, t), 3.3 (2 H, CH_2, s), 3.6 (2 H, CH_2, s), 3.9 (2 H, CH_2, m), 4.2 (2 H, CH_2, q), 4.35 (1 H, CH, t), 6.9 (1 H, NH, br t) and 7–8 (10 H, ArH).

(2S,3S)-2-*Aminomethyl-3-hydroxybutyric acid* **16**. (2*S*,3*S*)-Ethyl 2-benzamidomethyl-3-hydroxybutyrate **15** (1.64 g, 6.2 mmol) in 10% HCl (12 cm^3) was refluxed for 4 h after which the solution was cooled to room temperature and washed twice with ethyl acetate. The organic layer was discarded and the aqueous solution was evaporated under reduced pressure to afford an oily residue. This was taken up in acetonitrile (10 cm^3) and triethylamine (1 cm^3) was added to it. The mixture was then stirred overnight to give a white solid which was filtered off and washed with acetonitrile and acetone to afford a white amorphous solid (0.8 g, 97%); $\delta_H(D_2O$, ref. $H_2O)$ 1.01 (3 H, CH_3, d, *J* 6), 2.56 (1 H, $CHCO_2H$, m), 3.03 (CH_2NH_2, m) and 4.07 (1 H, CHOH, *J* 4.8, 6.6, dq); *m/z* (FD): 133 (M^+); $[\alpha]_D^{20}$ −42 (*c* 0.12, H_2O).

(3S)-3-[(1R)-1-*Benzoyloxyethyl*]*azetidin-2-one* **18**. Benzoic acid (0.75 g, 6 mmol), triphenylphosphine (2.34 g, 9 mmol) and diethyl azodicarboxylate (1.44 cm^3, 9 mmol) were sequentially added at 0–5 °C to a THF solution (90 cm^3) of (3*S*)-3-[(1*S*)-1-hydroxyethyl]azetidin-2-one **17** (0.53 g, 4.5 mmol). The solution was then warmed to room temperature and stirred for 1 h. After this, the solvent was removed under reduced pressure and the residue was purified by column chromatography to afford an amorphous solid (0.75 g, 76%); δ_H(DMSO) 1.35 (3 H, CH_3, d, *J* 6.3), 3.2, 3.3 (CH_2NH, 2 H, 2 m), 3.51 (1 H, 3-H, m), 5.30 (1 H, 1-H, *J* 6.3, dq), 7.4–8.0 (5 H, ArH, m) and 7.82 (1 H, NH, br); *m/z* (FD) 219 (M^+); $\nu(CHCl_3)/cm^{-1}$ 1760 and 1735; $[\alpha]_D^{20}$ −45 (*c* 0.1, dioxane).

(3S)-3-[(1R)-1-*Hydroxyethyl*]*azetidin-2-one* **2**. A solution of the azetidinone **18** (0.58 g, 5 mmol) in methanol (100 cm^3) containing 0.5 mol dm^{-3} NaOH (10.5 cm^3) was stirred at room temperature overnight and then quenched with an excess of acetic acid. The solvent was removed under reduced pressure to give compound **2** which was purified by column chromatography; this afforded an amorphous solid (0.21 g, 69%; 43% overall from **15**); $\delta_H(CDCl_3)$ 1.28 (3 H, CH_3, d, *J* 6.4), 3.32 (1 H, 3-H, m), 3.35 (CH_2NH_2, 2 H, m), 4.23 (1 H, 5-H, *J* 4.7, 6.4, dq) and 6.12 (1 H, NH, br); *m/z* (FD) 115 (M^+); $\nu(CHCl_3)/cm^{-1}$ 3420, 1760; $[\alpha]_D^{20}$ 62 (*c* 0.1, dioxane).

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