Biotransformation in Organic Synthesis: Applications of Yeast Reduction in the Synthesis of 3,5-Dihydroxy Esters of High Optical Purity

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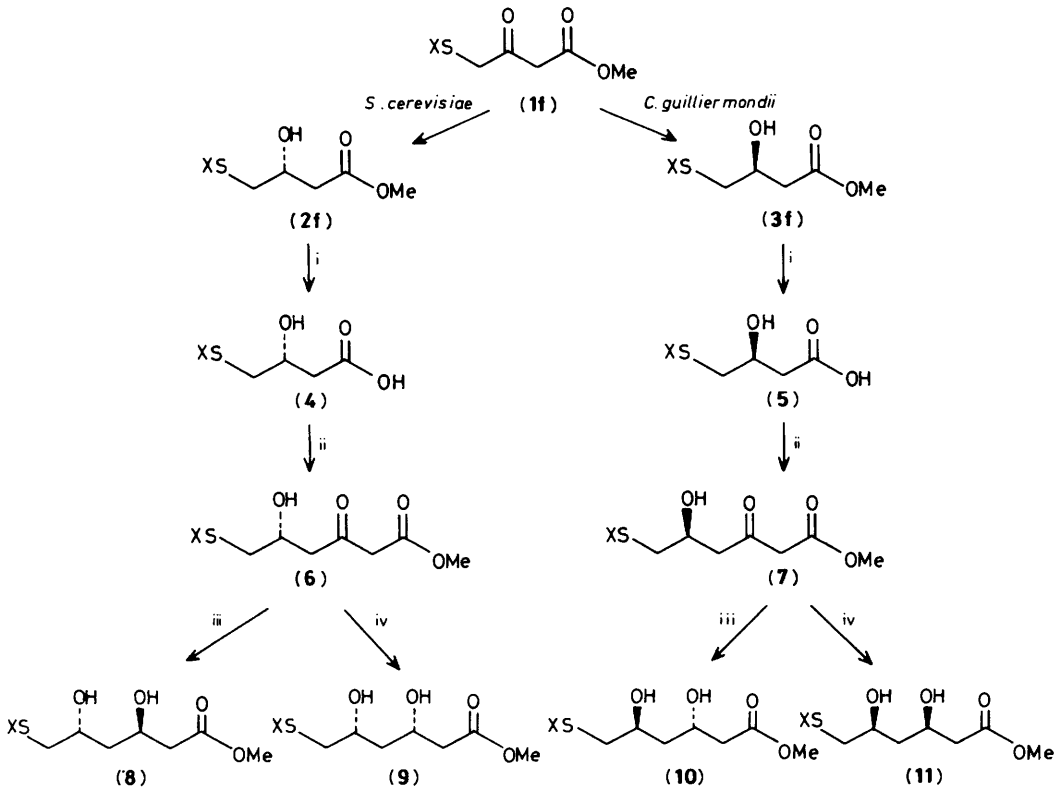
All four stereoisomers of methyl 6-(*p*-chlorophenylthio)-3,5-dihydroxyhexanoate have been synthesised by a route in which the key introduction of chirality was effected by an appropriate yeast reduction.

Because polyoxygenated units occur in many polyether and macrolide antibiotics[1] their synthesis has attracted much attention. We have investigated an approach which would provide an iterative route to such systems. As outlined in Scheme 1, this was envisaged as beginning with stereospecific biological conversion of a β-keto ester into a β-hydroxy ester followed by chain extension to generate a 3-oxo-5-hydroxy ester. Reduction of the chain-extended system could then be effected either by chemical procedures or by a further biotransformation. We have found that such a sequence,

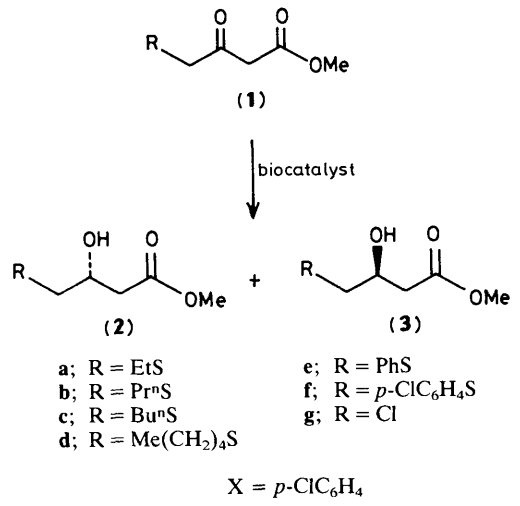
Table 1. Reduction of 4-substituted methyl acetoacetates (**1a**—**g**) by different yeast strains.[a]

	% Yield and stereochemistry of product (**2**) or (**3**)						
	a	**b**	**c**	**d**	**e**	**f**	**g**
Saccharomyces cerevisiae NCYC[b] 1765	55[c] (70, *R*)[d]	49 (65, *R*)	67 (70, *R*)	30 (58, *R*)	42 (73, *R*)	40 (50, *R*)	60 (70, *R*)
Candida guilliermondii NCYC 1399	41 (85, *S*)	36 (85, *S*)	53 (80, *S*)	28 (85, *S*)	50 (30, *S*)	30 (80, *S*)	
C. guilliermondii NCYC 973	8 (90, *S*)	12 (90, *S*)	30 (90, *S*)	14 (80, *S*)	32 (88, *S*)	38 (80, *S*)	
Hansenula polymorpha NCYC 1456	60 (60, *S*)	61 (50, *S*)	70 (60, *S*)	35 (66, *S*)	70 (18, *S*)	45 (25, *S*)	
H. polymorpha NCYC 1459	70 (30, *S*)	45 (35, *S*)	60 (50, *S*)	42 (60, *S*)	56 (25, *S*)	50 (20, *S*)	
Pichia membranaefaciens NCYC 333	80 (60, *S*)	76 (64, *S*)	80 (60, *S*)	42 (70, *S*)	86 (50, *S*)	70 (45, *S*)	
P. membranaefaciens NCYC 795	75 (54, *S*)	70 (50, *S*)	73 (60, *S*)	55 (65, *S*)	60 (53, *S*)	65 (40, *S*)	

[a] Reduction with resting cells and a substrate concentration of 1 g l^{-1}. [b] NCYC: National Collection of Yeast Cultures. [c] % Isolated yield of (**2**) or (**3**) after purification by flash chromatography. [d] % E.e. and configuration of major product as determined by 220 MHz n.m.r. studies with tris-[(+)-3-trifluoroacetylcamphorato]europium (III).



Scheme 1. *Reagents and conditions*: i, KOH (1.1 equiv.), tetrahydrofuran (THF), 90%; ii, *N*,*N*′-carbonyldi-imidazole (1.1 equiv.), then methyl magnesium malonate, THF, 40%; iii, $Me_4NBH(OAc)_3$, −40 °C, 95%; iv, Et_2BOMe–$NaBH_4$, −78 °C, 95%.



a; R = EtS
b; R = Pr^{n}S
c; R = Bu^{n}S
d; R = Me(CH$_2$)$_4$S
e; R = PhS
f; R = *p*-ClC$_6$H$_4$S
g; R = Cl

X = *p*-ClC$_6$H$_4$

embracing both final variants, provides an effective route to 3,5-dihydroxy esters of high optical purity. We here report a synthesis, using the first variant of this approach, of all four stereoisomers (**8**)—(**11**) of methyl 6-(*p*-chlorophenylthio)-3,5-dihydroxyhexanoate in optically pure form, starting from methyl 4-(*p*-chlorophenylthio)-3-oxobutanoate (Scheme 1).

Although biotransformations,[2] and yeast reductions in particular,[3] have been widely used to produce chiral building blocks for organic synthesis, their applications have been hampered by lack of reproducibility and by the frequent formation of products of inadequate optical purity. Accordingly, for the present work we have systematically studied the effect of varying the nature of the C-4 substituent in the β-keto ester system, using a selection of yeast strains. Sulphur-containing substituents were mainly studied as these allow for the introduction of functionality in the products that would permit chain extension from this terminus. The results are summarised in Table 1.

Although reductions of β-keto esters with yeasts have been extensively studied, it remains difficult to predict either the absolute configurations of product or, in particular, the enantiomeric excess (e.e.) likely to be achieved.[4] Thus we find that only *Saccharomyces cerevisiae* affords predominantly the (*R*)-enantiomer of the products (**2**), whilst the other species examined reduced keto esters (**1**) predominantly to the corresponding (*S*)-enantiomers (**3**). The absolute configurations of the products were determined by Raney nickel desulphurisation to the corresponding methyl 3-hydroxybutanoates, which could be effected typically in 80% yield.

Our results contrast with those of Sih on the reduction of 4-chloroacetoacetate (**1g**) using *S. cerevisiae*.[5] In our experiments, the (*R*)-enantiomer (**2g**) was the major product (70% e.e.). This underlines the common experience that the outcome of a microbial biotransformation may depend not only on the species, but also on the particular strain used.[6] Characterisations such as 'baker's yeast' are not sufficient to guarantee reproducibility. The *S. cerevisiae* used in our experiments was a pure strain obtained from a local supermarket. It has been deposited with the National Collection of Yeast Cultures as NCYC 1765.

From the result of the survey (Table 1), the 4-*p*-chlorophenylthio substituent was selected as the most suitable for our purposes. It affords a crystalline substrate (**1f**)[7] which is readily reduced by the organisms chosen for further study, *S. cerevisiae* and *C. guilliermondii*, to optically active products (**2f**) and (**3f**) respectively. The products are crystalline and readily brought to >95% e.e. (as determined by n.m.r.) by recrystallisation. Hydrolysis of the β-hydroxyesters (**2f**) and (**3f**) gave in 90% yield the corresponding acids (**4**) and (**5**) (Scheme 1). These were converted into the corresponding crystalline 5-hydroxy-3-oxo esters (**6**) and (**7**) which were obtained in a state of high optical purity (>95% e.e.) as determined by ^{1}H n.m.r. (400 MHz) in the presence of the chiral solvating agent (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)-ethanol (1.8 mol per mol substrate in CCl_4).[8]

Diastereoselective reduction of the β-ketoesters (**6**) and (**7**) to the corresponding 1,3-*anti*-diols (**8**) and (**10**), respectively, was accomplished using tetramethylammonium triacetoxyborohydride[9] with a diastereoisomeric excess (d.e.) of >95% as shown by h.p.l.c. and by ^{13}C n.m.r. Alternatively, the esters (**6**) and (**7**) were treated with methoxydiethylborane to form boron chelates which were reduced ($NaBH_4$) to give the corresponding 1,3-*syn*-diols (**9**) and (**11**) in >95% d.e.[10]

The route described above provides for ready access to all stereoisomers of the chosen 3,5-dihydroxy ester system. It illustrates the flexibility of an appropriate combination of biological and chemical transformations in the synthesis of stereoisomeric target molecules.

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