Biotransformations with Yeasts and their Applications in Organic Synthesis

By

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To Thomas.

"Time is a fake healer anyhow."

Malcolm Lowry 1947

Contents

Abbreviations

EPC	Enantiomerically pure compounds
NMR	Nuclear magnetic resonance
s	Singlet
d	Doublet
t	Triplet
q	Quartet
m	Multiplet
br	Broad
J	Coupling constant
ppm	Parts per million
TMS	Tetramethylsilane
IR	Infra red
s	Strong
m	Medium
w	Weak
sh	Shoulder
MS	Mass spectrum
CD	Circular dichroism
[α]	Specific rotation
c	Concentration (g/100ml)
ee	Enantiomeric excess
de	Diastereomeric excess
GLC	Gas-liquid chromatography
HPLC	High pressure liquid chromatography
TLC	Thin-layer chromatography
mp	Melting point
bp	Boiling point
PN	Productivity number
OD	Optical density
h	Hour
d	Days
DME	1,2-Dimethoxyethane
THF	Tetrahydrofuran
DMSO	Dimethylsulfoxide
DMF	Dimethylformamide
AN	Acetonitrile
DIBAL	Diisobutylaluminiumhydride
CDI	Carbonyldiimidazole
TMEDA	Tetramethylethylenediamine
DMAP	Dimethylaminopyridine

mCPBA	meta-Chloroperbenzoicacid
HMPA	Hexamethylphosphoramide
NBS	N-Bromosuccinimide
NCS	N-Chlorosuccinimide
AIBN	Azoisobutyronitrile
Me	Methyl
Et	Ethyl
Pr	Propyl
i-Pr	iso-Propyl
Ph	Phenyl
pClPh	para-Chlorophenyl
R	Alkyl or Aryl
Ac	Acetyl
Bu	Butyryl
DNB	3,5-Dinitrobenzoyl
MTPA	(-)-2-Methoxy-2-trifluromethylphenylacetyl
Bzl	Benzyl
Boc	N-tert-Butoxycarbonyl
MEM	Methoxyethoxymethoxy
THP	Tetrahydropyranyl
MTM	Methylthiomethyl
TBDMS	tert-Butyldimethylsilyl
NADH	Dihydronicotinamideadeninedinucleotide
NADPH	Dihydronicotinamideadeninedinucleotide phosphate
HLADH	Horseliver alcohol dehydrogenase
TBADH	*Thermoanaerobicum brockii* alcohol dehydrogenase
GGDH	*Geotrichum candidum* alcohol dehydrogenase
HSDH	3α,20β-Hydroxysteroid dehydrogenase
YADH	Yeast alcohol dehydrogenase
GDH	Glucose dehydrogenase
FDH	Formate dehydrogenase
PAN	Polyacrylamide gel
MEEC	Membrane enclosed enzymatic catalysis
LDH	L-Lactate dehydrogenase
PEG	Polyethyleneglycol
LeuDH	L-Leucine dehydrogenase
NCYC	National collection of yeast cultures
HMG-CoA	3-Hydroxy-3-methylglutaryl coenzyme A
PLE	Pigliver esterase

Acknowledgements

I would like to thank Professor D.H.G.Crout for his enthusiasm and advice which he has provided throughout the course of this work. Further I want to thank Professor S.M.Roberts and Dr. P.Myers for helpful suggestions and discussions.

The financial support of Glaxo plc is gratefully acknowledged.

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Declaration

The work described in this thesis is the original of the author, except where acknowledgement has been made to results and ideas previously published. It was carried out in the Department of Chemistry, University of Warwick between October 1985 and September 1988 and has not been submitted previously for a degree at any institution.

Publications

Parts of the research described in this thesis have appeared in the scientific literature as follows:

Biotransformation in Organic Synthesis: Applications of Yeast Reduction in the Synthesis of 3,5-Dihydroxy Esters of High Optical Purity.
Christen,M., Crout,D.H.G., *J.Chem.Soc.Chem.Commun.*, **1988**, 264-266.

Enzymatic Reduction of β-Ketoesters using immobilised yeasts.
Christen,M., Crout,D.H.G., Bioreactors and Biotransformations: Ed. Moody G.W., Baker P.B., Elsevier, **1987**, 213.

Summary

This thesis describes the synthesis of a new chiral synthon *via* biotransformation and the applications of this chiron in organic synthesis.

It was possible to reduce a variety of β-ketoesters with a range of yeast species to the corresponding β-hydroxyesters. We selected from these screening results methyl 4-(p-chlorophenylthio)-3-oxobutanoate as the substrate of choice and the two yeast strains *Saccharomyces cerevisiae* and *Candida guilliermondii* as the biocatalysts. Immobilisation of the yeasts proved to be a successful method for improving the yield and enantiomeric excess in the biotransformation, and we obtained the product methyl 4-(p-chlorophenylthio)-3-hydroxybutanoate in both enantiomeric forms, depending on the choice of the biocatalyst. *S.cerevisiae*, immobilised on alginate, gave the L-product in 60% yield and 80% ee, whilst *C.guilliermondii* afforded the D-product in 60% yield and 87% ee. Both enantiomers could be recrystallised to an optical purity of ee > 95%.

We then proceeded to explore the reactivity pattern of our new chiron. We found that it was possible to protect the hydroxyl group with a variety of protecting groups under acid or base catalysed reaction conditions. We chose the tert-butyldimethylsilyl group as the most suitable one for our purposes. Hydrolysis of the protected ester, followed by conversion into the imidazolide and treatment with methyl magnesium malonate resulted in the formation of a new β-ketoester, which in turn could be deprotected with a overall yield of 68% to methyl 4-(p-chorophenylthio)-5-hydroxy-3-oxohexanoate. This ester was subsequently reduced with tetramethylammonium triacetoxyborohydride to the *anti* diastereomer and with methoxydiethylborane / sodium borohydride to the *syn* diastereomer. We succeeded therefore in synthesizing all four stereoisomers of methyl 6-(p-chlorophenylthio)-3,5-dihydroxyhexanoate in enantiomerically pure form.

Additionally we found that it was possible to chlorinate the original β-hydroxyester at C-4 under radical reaction conditions. This allowed us to form a new carbon-carbon bond by treatment of the chlorinated product with a silylenolether under Lewis acid catalysis. Further manipulation of this addition product allowed the synthesis of 3-hydroxytetradecanoic acid with high enantiomeric excess. The alkylation at C-2 was easily achieved *via* the corresponding dioxanone by deprotonation and diastereoselective methylation.

Aim

This thesis is divided into three parts. The first one will introduce the reader to the field of biotransformations, in particular conversions with whole cells and applications with oxidoreductases. The second part describes the synthesis of enantiomerically pure compounds (EPC) starting from the biotransformation products. In the third part are listed all the experimental details and spectroscopic data of the new compounds mentioned in this thesis.

Leading references are given to relevant, but non-essential topics, and should enable the interested reader to follow up on these themes. Throughout the whole text the older D,L-nomenclature is used. This has the advantage that merely changing the substituent at the γ-position of the β-hydroxyester does not change the nomenclature, which is not necessarily the case with the R,S-nomenclature. The latter, however, is included with the systematic names in the experimental part.

Part 1: Biotransformations with Yeasts.

1.1. Introduction.

Traditional biochemistry, as the study of the molecular basis of life, long ago revealed the tremendous power of enzymes.[1] Many chemists and biochemists have devoted their energies to the elucidation of the structure and mechanism of nature's catalysts, and the work continues still.[2,3] It is only recently that enzymes have been rediscovered by the organic chemist and are now increasingly becoming a useful tool in synthesis. This is documented by the publication of many good reviews in the last few years.[4,5,6,7,8] The conversion of organic substrates by enzymes has been termed biotransformation. It has become so important, that the editors of "Biotechnology", a comprehensive treatise in eight volumes, have devoted to it a whole volume.[9]

We have defined biotransformations as:

> **Selective enzymatic conversions of natural or chemically synthesized substrates into defined products on a preparative scale with whole cells or isolated enzymes.**

It is important to notice that every stage in the purification process of an enzyme, whole cell, cell extract or pure enzyme, can be used in a biotransformation, and that the biocatalyst plays basically the same role as a chemical reagent. Biotransformations are capable of performing every reaction which lies in the scope of enzymatic transformations listed below, with the class number as

assigned by the Nomenclature committee of the International Union of Biochemistry:

-Oxidoreductases	Class 1
-Transferases	Class 2
-Hydrolases	Class 3
-Lyases	Class 4
-Isomerases	Class 5
-Ligases	Class 6

We will restrict ourselves in this introduction to dehydrogenases, a subclass of class 1, the oxidoreductases.

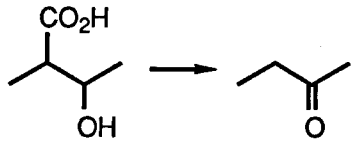
Dehydrogenases are the largest subclass of oxidoreductases and a lot of information is available on mechanistic aspects and substrate specificities. By contrast with other enzymes, such as for instance, the hydrolytic enzymes, dehydrogenases require a cofactor, which acts as acceptor or donor for the hydride transfer. The complex of enzyme and coenzyme is called the holoenzyme; the free enzyme, the apoenzyme. They can be categorised into two classes according to cofactor requirements: NAD(P)H-dependent or flavin coenzyme-dependent. The most important dehydrogenase catalysed reaction types are listed in Scheme 1, together with examples.

Scheme 1.

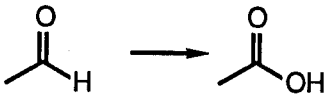
<u>NAD(P)H-dependent:</u>

alcohol dehydrogenase

glutamate dehydrogenase



isocitrate dehydrogenase



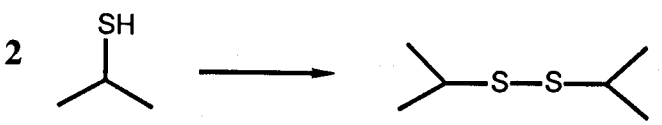
aldehyde dehydrogenase

dihydrosteroid dehydrogenase

dihydrofolate dehydrogenase

FAD-dependent:

D-lactate dehydrogenase



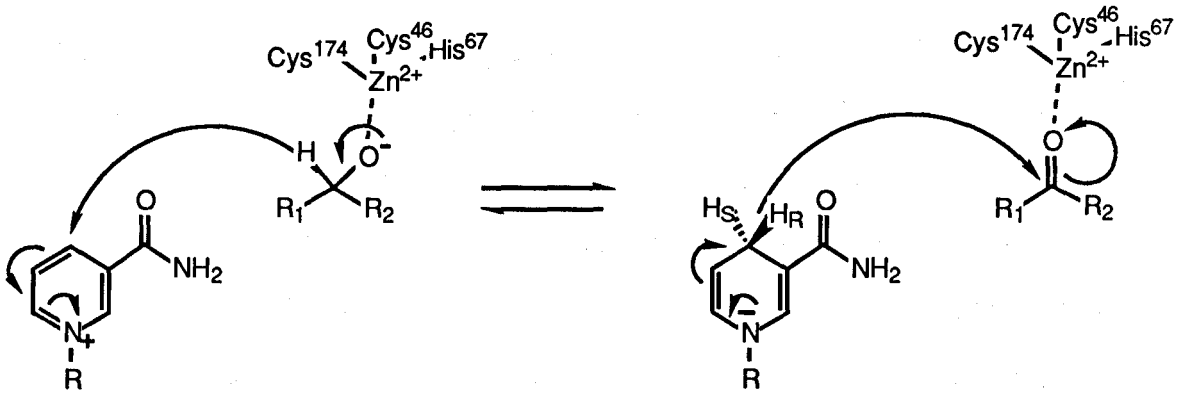
lipoamide dehydrogenase

For the synthetic organic chemist, alcohol dehydrogenases are the most interesting. They are well studied and the mechanisms of several individual enzymes are well understood.[10]

Though it is by no means clear and obvious what type of dehydrogenase is actually involved in the whole cell reduction described later in this thesis, we want to discuss briefly the reaction mechanism of a typical alcohol dehydrogenase from horse liver HLADH. It is a zinc-requiring enzyme and classical studies on the enzyme stereochemistry and the recognition of prochiral centres have been performed on it. The catalytic zinc which is bound to two cysteine and one histidine residues, is coordinated to

the oxygen atom of the substrate. This facilitates the direct hydride transfer. The nicotinamide ring of the NADH is bound close to the zinc ion at the hydrophobic active site pocket.

Scheme 2.



It was discovered that the hydride transfer between the substrate and NAD^+ is stereospecific, and that HLADH uses the pro-*R* hydrogen of NADH as indicated in scheme 2 and transfers the hydride ion to the *re*-face of NAD^+. It therefore belongs to the stereospecificity class A.

A special problem in the application of dehydrogenases in biotransformations is the recycling of cofactors as they are very expensive and can not be used in equimolar amounts. Basically there are five different possibilities open for cofactor regeneration:

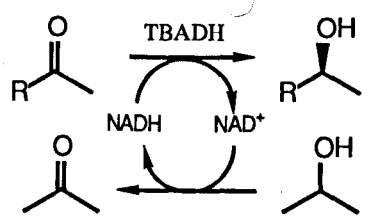
1. chemical
2. photochemical
3. electrochemical
4. enzymatic
5. whole cell system

None of the first three have so far been used with much success. However the enzymatic method has been used successfully, either

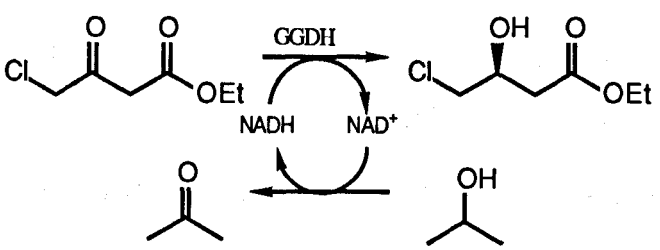
in the coupled substrate approach (the same enzyme reduces the substrate and recycles the cofactor at the expense of a cheap alcohol) or with two different enzymes for reduction and regeneration.

The first method has been applied in the reduction of acyclic ketones with the alcohol dehydrogenase from *Thermoanaerobicum brockii* (TBADH)[11,12] or glycerol dehydrogenase from *Geotrichum candidum* (GGDH)[13] and in many reductions of cyclic ketones with horse liver alcohol dehydrogenase (HLADH).[14,15] The alcohols most often used for delivering the hydride equivalents are isopropanol or ethanol, because the products, acetone or acetaldehyde, are easily removed.

Scheme 3.



[11,12]



[13]

More often, however, a second enzyme is employed for the regeneration of the cofactor. In the reduction of bicyclic ketones by 3α,20β-hydroxysteroid dehydrogenase (HSDH) the cofactor NADH has been recycled either by oxidation of ethanol by yeast alcoholdehydrogenase (YADH) or by HLADH. An alternative

approach is to use the oxidation of glucose with glucose dehydrogenase (GDH).[16] The best solution for cofactor recycling is probably the formate/formate dehydrogenase (FDH) system. It has the great advantage that the by-product CO_2 is easily removed, and does not interfere with the reaction itself. This system has been scaled up to multigram quantities of substrate by the following methods:

-coimmobilisation of reaction- and recycling-enzyme in polyacrylamide gel PAN.[17]

-membrane enclosed reactor system.[18]

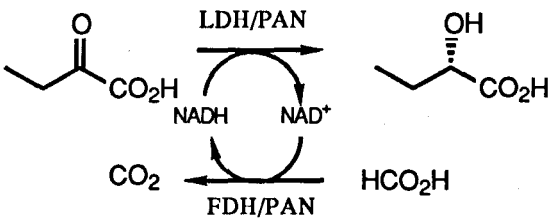
-membrane enclosed enzymatic catalysis MEEC.[19]

The PAN immobilised method has been used for the reduction of a large range of α-ketoesters with L-lactate dehydrogenase LDH to the corresponding (S)-α-hydroxyesters in considerable quantities (up to 100 mmol).

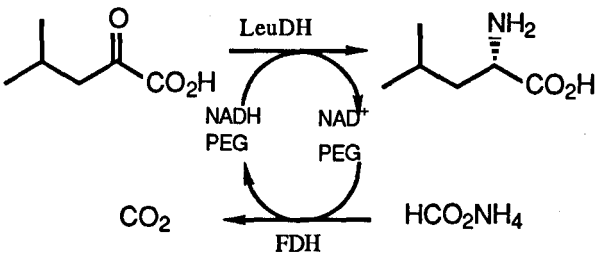
The membrane enclosed reactor system in turn has great potential for continuous processes, which produce large amounts of product automatically removed from the reactor system. To prevent NADH leaking it can be modified by covalent attachment to a water soluble polymer such as polyethyleneglycol PEG. In this way it was possible to synthesize L-leucine from α-ketocaproic acid with L-leucine dehydrogenase LeuDH over 48 days, in a conversion of 99.7% and a yield of $42.5 g/l_{x day}$.

The MEEC technique will be the method of choice for relatively unreactive substrates and for large amounts of enzymes. It is used in a two-phase system where the enzymes are kept in the aqueous phase in a dialysis membrane and the product is extracted into the organic phase.

Scheme 4.



[17]



[18]

In spite of the progress that has been made with cofactor recycling using isolated enzymes, this method still has some serious drawbacks, mostly related to the expense and instability of enzymes and cofactors.

Accordingly, the use of whole cells, incorporating a built-in cofactor recycling system, has been preferred by many workers. Its advantages and disadvantages are listed below:

Advantages:	Disadvantages:
-inherent recycling	-work up and scale up
-large substrate range	-side reactions
-cheap	-limited access to enzymes
-stable enzymes	
-large reaction range	

Biotransformations with whole cells have the advantage that they are cheap, the enzymes are stable in their natural environment, cofactor recycling is effected automatically and they have a broad substrate and large reaction range. Practical problems, especially in work up and scale up have been reduced recently by the introduction of immobilised cells in biotransformations. However there are some drawbacks.

Cells often exhibit low enantioselectivity, because of the presence of several enzymes which act on the same substrate with opposite stereospecificities. Additionally it is possible that further metabolism of the substrate greatly reduces the yield. But the great number of publications over the last eight years proves that the advantages of using whole cells in oxidoreductions outweigh the disadvantages.

Generally speaking, every microorganism displaying oxidoreductase activity can be used in a biotransformation. However, the literature indicates a strong preference for yeasts, particularly the species *Saccharomyces cerevisiae* referred to as "baker's yeast". We give, in the following pages, a short overview of the history and major developments in the use of this and other yeast strains.

As far back as 1874 Dumas[20] reported that a suspension of brewer's yeast liberated hydrogen sulfide on addition of powdered sulfur, and concluded that the yeast acted as in a hydrogenation. Neuberg and his coworkers[21] then set out at the beginning of this century and showed that fermenting yeast not only reduces acetaldehyde to ethanol, but also a whole range of other aldehydes and carbonyl-substrates. They termed this kind of conversion "phytochemical reduction" and later

"bioreduction".[22] They proved in 1914 that the reaction had to be enzymatic and they can therefore be credited with the first planned biotransformations with oxidoreductases.[23]

In the following years Neuberg and others reduced a large amount of aldehydes, hydroxyaldehydes, ketones, diketones, quinones, ketoacids, polycarbonyl compounds (e.g. triketopentane), conjugated alkenes and nitro compounds. In some cases, for instance in the reduction of trichloroacetaldehyde to trichloroethanol, the conversion proceeded so smoothly and with such high yields that the biotransformation was preferred to conventional chemical reduction methods.[24] By the 1930's the following facts were clearly established:

-fermenting yeasts show a great reductive potential, with broad substrate specificity, often superior to traditional chemical methods.

-the reduction of racemic material can lead to optically enriched products.

-the reduction of achiral ketones can lead to optically active alcohols, even though enantiomeric excess and absolute configurations could not be determined.

-the reduction is enzymatic and requires a coenzyme with a nicotinamide residue as the hydrogen acceptor of the sugar fermentation.

-it is possible to obtain the same product with opposite optical rotation by screening a range of microorganisms.

That means that by this time the basic principles of biotransformations with oxidoreductases were clearly established, with groups all over the world actively engaged in research. The

developments of this early period are reviewed by Neuberg in his article "Biochemical reduction at expense of sugars".[25]

Unfortunately, interest in yeast reductions declined, almost to vanishing point after the second world war. It was not until the end of the seventies that there was a true revival of yeast reductions, probably best documented by the acceptance of the first biotransformation by "Organic Synthesis", a baker's yeast reduction of ethyl acetoacetate in 1985.[26]

We will now highlight the major developments between the second world war and today. In 1951 it was Lemieux,[27] who determined for the first time the absolute configuration of β–hydroxybutyric acid, the reduction product of the baker's yeast reduction of β-ketobutyric acid and showed it to be L in the nomenclature then used or S when one applies Kahn-Ingold-Prelog rules. Later on in 1964, Mosher[28] concluded for the first time that the formation of only partially enantiomerically pure products could result from the presence of several reductases with different kinetic properties and stereospecificities in yeasts. In 1975 Fuganti[29] showed for the first time the stereochemical course of a double bond reduction by fermenting baker's yeast and one year later Ridley[30] established that β-ketoesters and β-ketoacids were particularly good substrates for reduction with *S.cerevisiae* and published a procedure, which was followed subsequently by many workers. The work up until 1980 has been reviewed by Fischli[31] and we wish to concentrate now on a summary of yeast reductions carried out during the last few years.

Since 1980 interest in biotransformations with baker's yeast and other yeasts has grown tremendously. The reason for this virtual

explosion in the number of publications is the urgent need for the production of optically active chiral synthons, so called chirons,[32] in the chemical industry. Yeast reduction can provide in one step, starting from cheap, achiral material, optically active products, without the cofactor regeneration problem.

Of all the yeasts, *S.cerevisiae* is the easiest to obtain and is therefore the ideal starting point for a chemist not so familiar with enzymology or microbiology. One of the best investigated biotransformations is the reduction of β-ketoesters to the corresponding β-hydroxyesters. This reaction found entry in the Organic Synthesis series, as mentioned above, and has been scaled up and optimised in industry.[33] Many other β-ketoesters with different substituents in the 4-position have been reduced with *S.cerevisiae*. The most important ones are summarised in table 1.

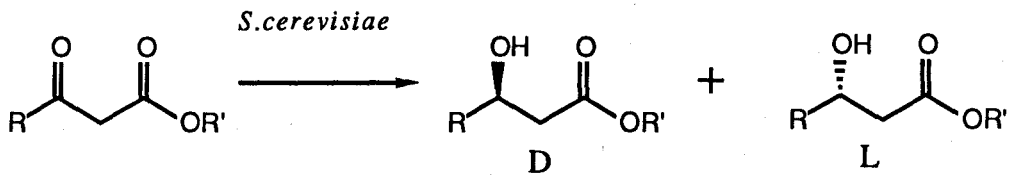
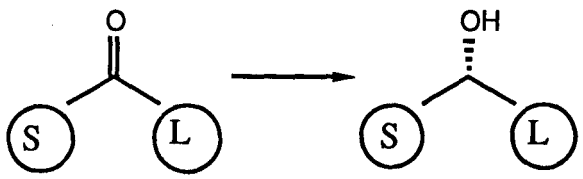


Table 1. Reduction of β-ketoesters with *S.cerevisiae*(Baker's yeast).

R	R'	Abs. config.	%ee	%yield/Lit.
CH_3	CH_2CH_3	L	>97	60 [34]
$PhCH_2CHNHBoc$	CH_3	D	92	95 [35]
n-$C_{15}H_{31}$	K	D	>98	40 [36]
n-C_5H_{11}	CH_3	D	>98	32 [37]
$PhCH_2OCH_2CH_2$	n-C_4H_9	L	70	55 [38]
"	$CH_2CH(CH_3)_2$	L	90	70 [38]
"	$CH_2C(CH_3)_3$	L	96	35 [38]
"	CH_3	D	60	58 [39]
$CH_3OCH_2CH_2$	n-C_4H_9	L	82	48 [39]
$HOCH_2CH_2$	n-C_5H_{11}	L	87	59 [39]
$CH_3CONHCH_2$	CH_2CH_3	D	80	60 [40]
$CF_3CONHCH_2$	CH_2CH_3	D	80	60 [40]
$(CH_3)_3COCH_2$	CH_2CH_3	L	97	72 [41]
$PhSCH_2CH_2$	K	L	42	88 [42]
N_3CH_2	CH_2CH_3	L	80	70 [43]
"	$(CH_2)_2Ph$	L	95	70 [43]
"	n-C_8H_{17}	L	>98	70 [43]
$PhSO_2CH_2$	CH_3	D	98	80 [44]
Cl_3C	CH_2CH_3	D	84	70 [45]
F_3C	CH_2CH_3	L	50	75 [45]
$CH_2=CHCH_2CH_2$	K	D	>98	38 [46]
$CH_2=CCH_3CH_2CH_2$	K	D	>98	55 [46]
$(CH_3)_2C=CHCH_2CH_2$	K	D	>98	59 [46]
n-C_3H_7	CH_2CH_3	D	90	50 [47]

For predicting the stereoselectivity of *S.cerevisiae* reductions an, old model developed for the reduction of decalones by *Curvularia lunata* proved to be quite useful, and is generally referred to as "Prelog's Rule".[48] This generalization states that the predominant oxidoreductase in *S.cerevisiae* delivers the hydride to the re-face of a prochiral ketone.

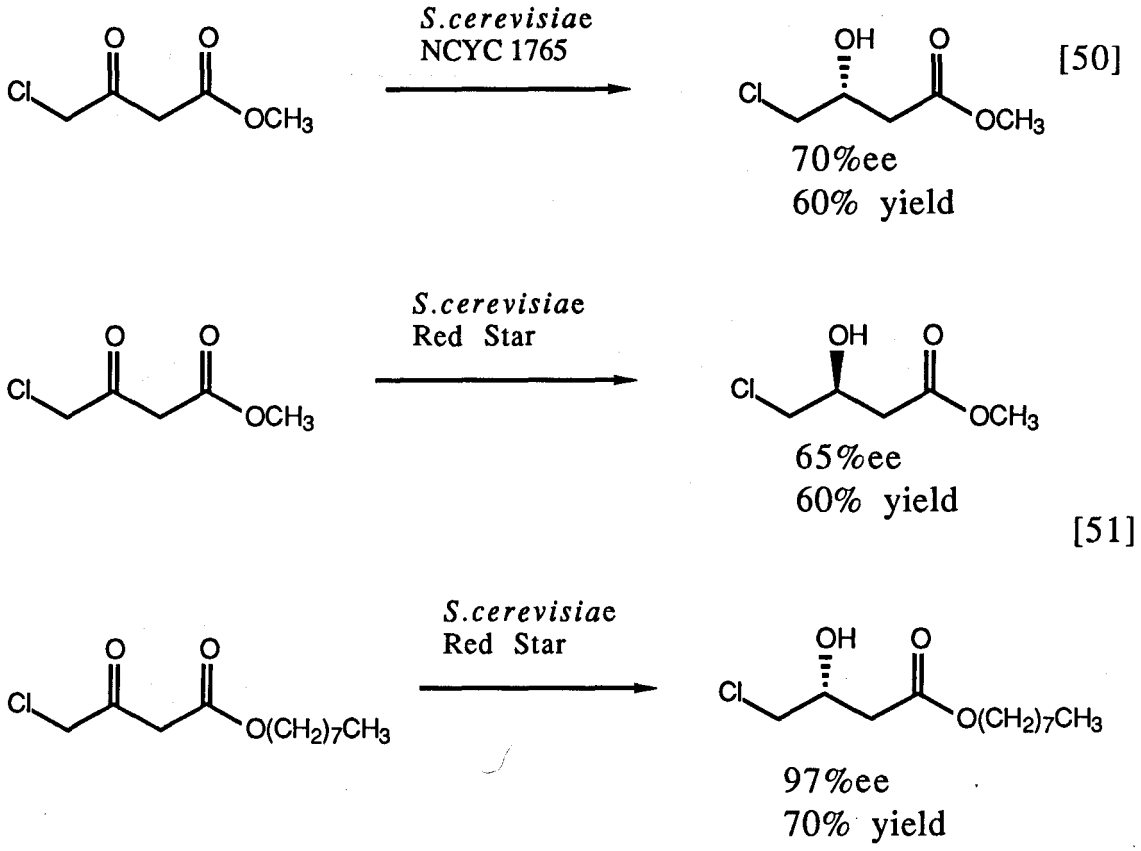
Scheme 5.



S: Small
L: Large

However the rule has to be applied very carefully. Partial stereoselectivity can have different origins. The substrate could be reduced by a single reductase with different transition states for the two competing faces. Or alternatively the yeast may contain different oxidoreductases producing products with opposite configurations. Furthermore, the results can be influenced by transport phenomena and side reactions, for example the selective hydrolysis of one product. Thus it can happen that different strains of the same species show opposite enantioselectivities.[49] For example, most strains of *S.cerevisiae* reduce methyl-4-chloroacetoacetate to the D-hydroxyester. However we found, that our locally obtained yeast reduces the same substrate to the L-hydroxyester.[50] Having decided on a given strain, it is possible to influence optical purity and absolute configuration by variation of the size of the substituents.[51]

Scheme 6.



Considering all the available data, we can summarise the possible methods of control for the stereochemical outcome of yeast reductions as:

-very slow addition of substrate, thereby taking maximum advantage of the different relative rates of catalysis by competing enzymes.[52]

-selective inhibition of one of the competing enzymes.[53]

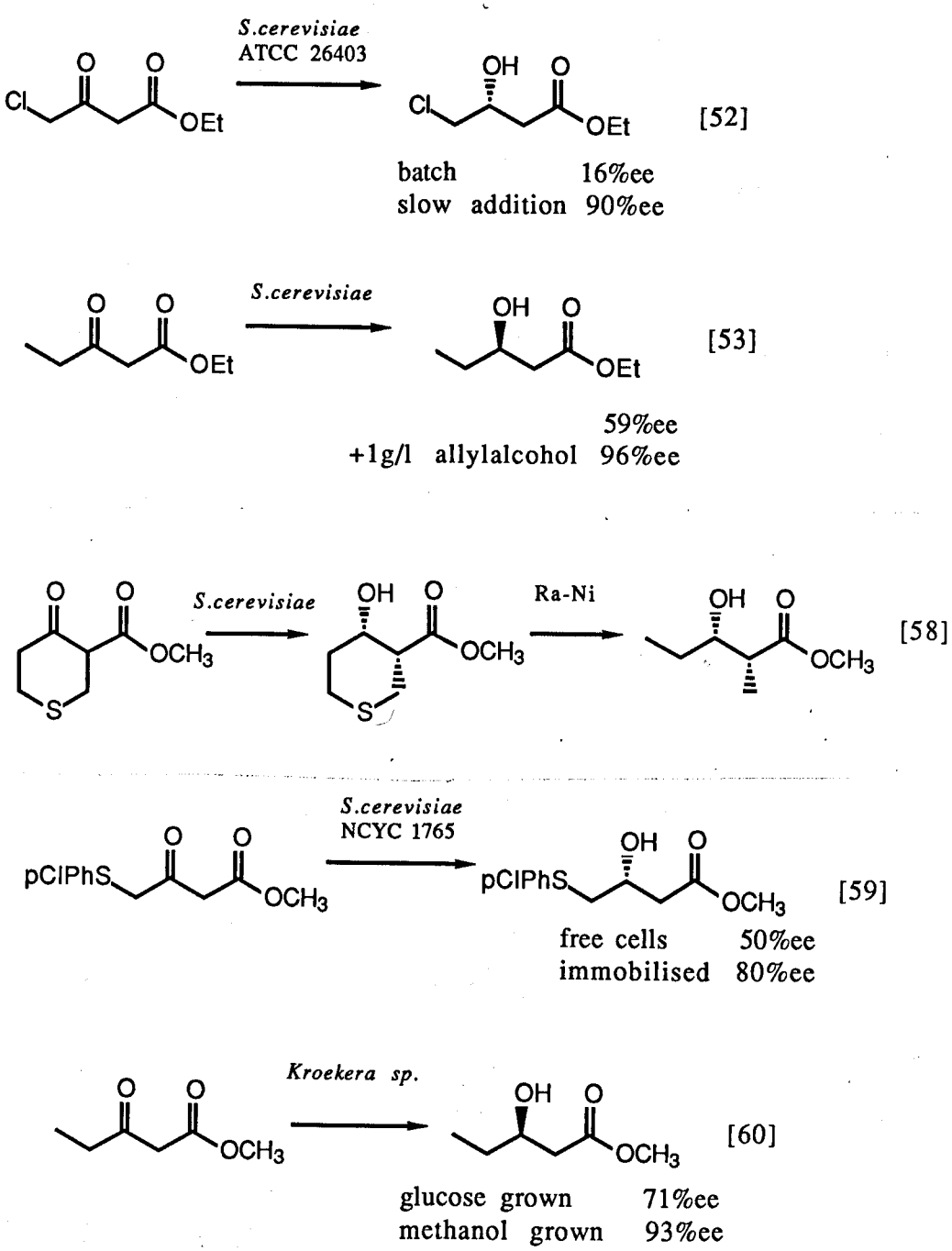
-screening of microorganisms.[54]

-redesign of substrate or addition of removable auxiliary group.[55,56,57,58]

-immobilisation of cells.[59]

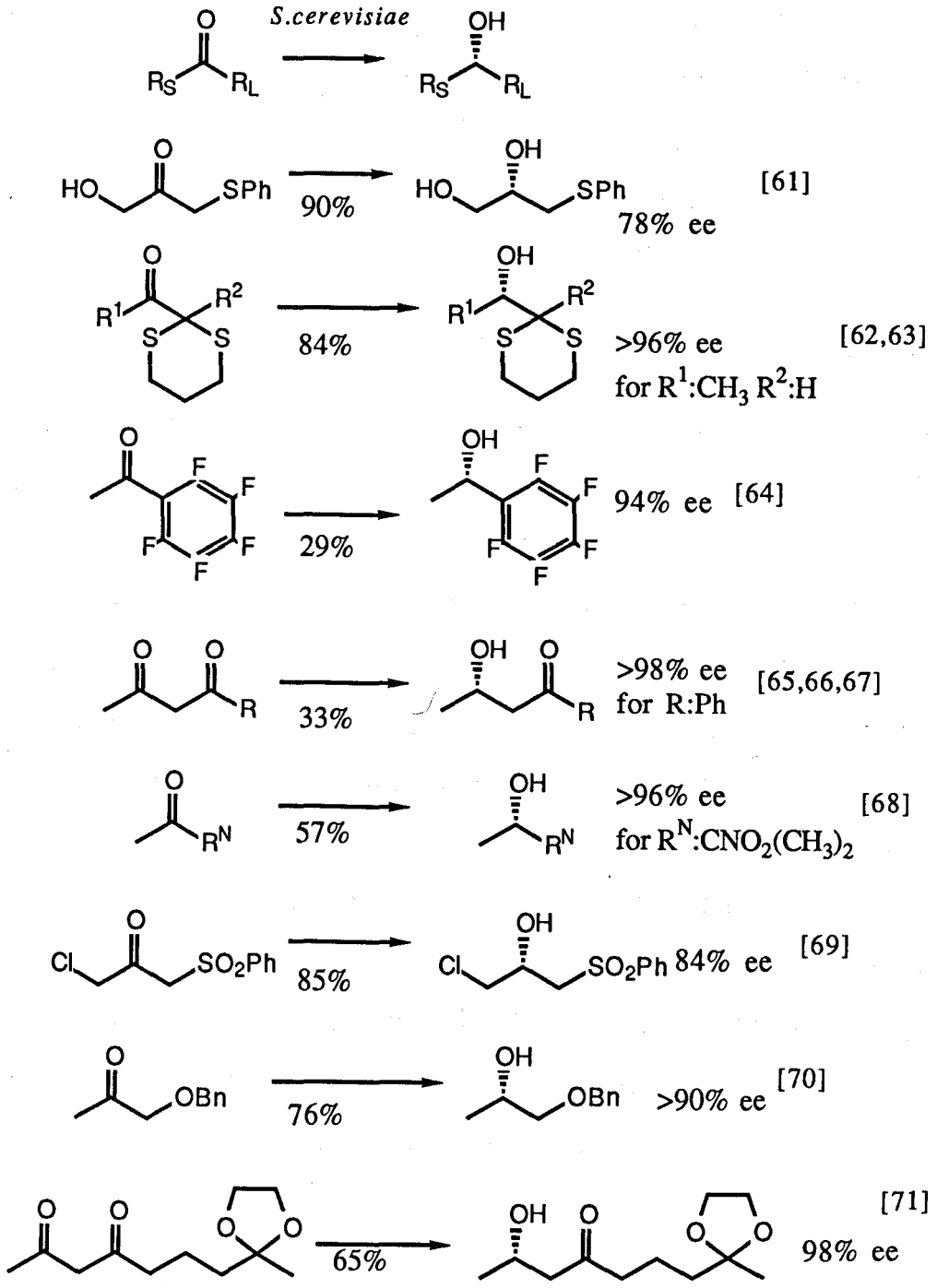
-variation of growing conditions.[60]

Scheme 7.



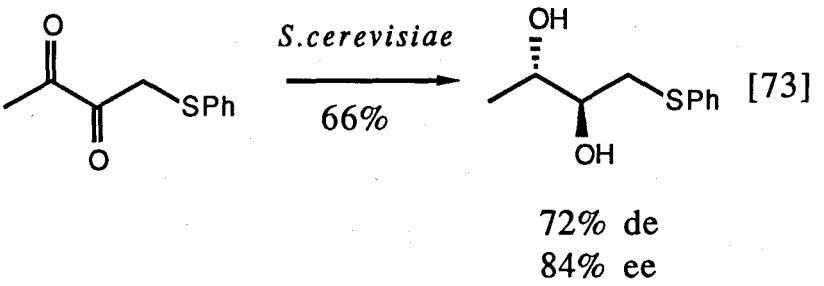
Employing these techniques, yeast reductions have not only been used with β-ketoesters as substrates, but with a large variety of other substrates as well. We have summarised some examples in scheme 8.[61,62,63,64,65,66,67,68,69,70,71]

Scheme 8.



It is remarkable that in the cited reduction of 1,3-diketones only one carbonyl function is reduced, whereas in the reduction of 1,2-diketones both carbonyl groups are reduced yielding predominantly one of the four possible diastereomers.[72,73]

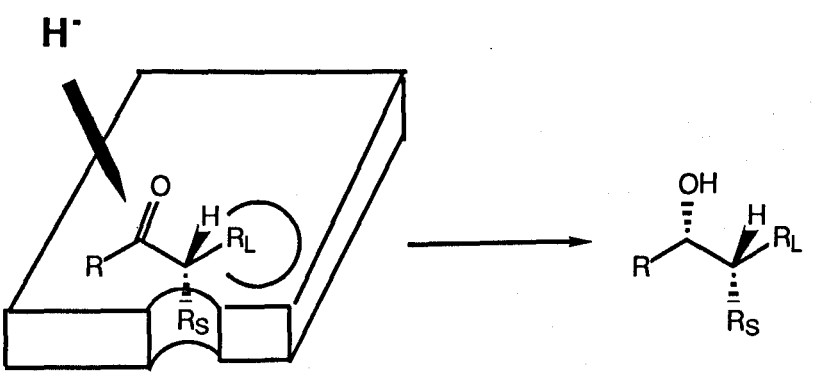
Scheme 9.



72% de
84% ee

This example also introduces a new and exciting application of yeast reductions. The enantioselective and diastereoselective reduction often makes it possible to obtain one of the stereoisomers in a single step. Basically it is a kinetic resolution by an enantioselective reduction and provides two stereogenic centres *via* one reaction. There are now many examples of this type of reaction and it is possible to draw up a model for the diastereoselective reduction with *S.cerevisiae*.[74] In this the size and hydrophobicity of the α-substituent is compared to that of the ester ligand. During the reduction the larger ligand would reside in the same plane as the carbonyl, whilst the smaller ligand would fit in a smaller site. The hydride is then delivered from the opposite direction to the re-face of the ketone, resulting in the formation of the L-alcohol and the *syn* diastereomer.

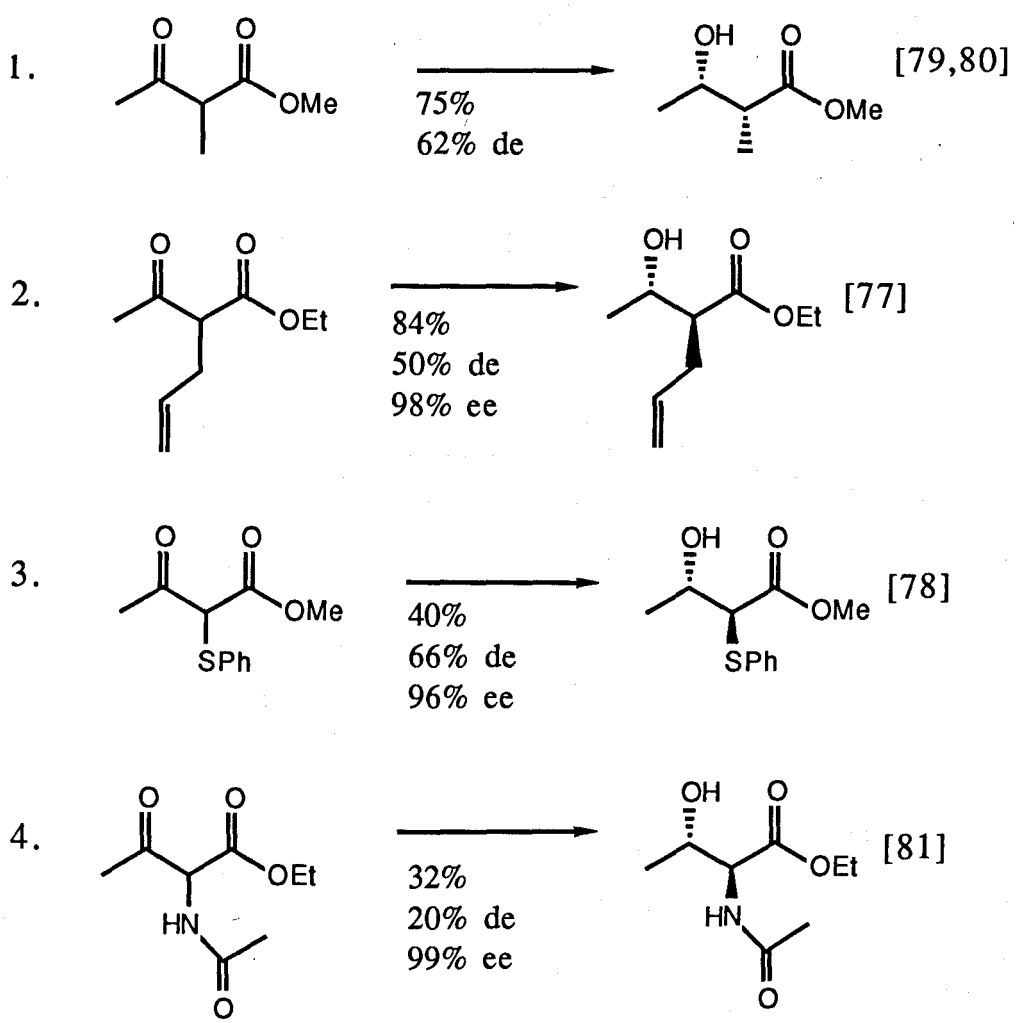
Scheme 10.

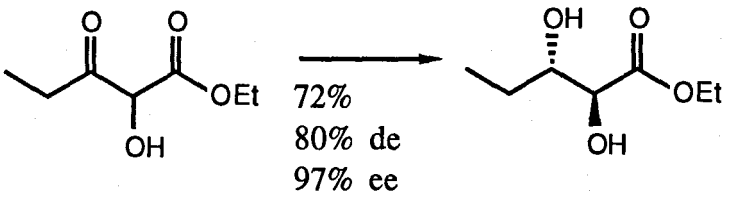


This is illustrated by the first example in scheme 11. Large α-substituents tend to occupy the ester binding pocket and the *anti* diastereomer is formed preferentially (examples 2 and 3). When the two substituents are comparable in size and hydrophobicity low diastereoselectivity is observed (example 4).

The substrates for diastereoselective yeast reductions are often α-substituted 1,3-dicarbonyl compounds and we have illustrated it for both acyclic (scheme 11)[75,76,77,78,79,80,81] and cyclic substrates (scheme 12)[82,83,84,85,86,87,88] with yield, diastereomeric and enantiomeric excess shown.

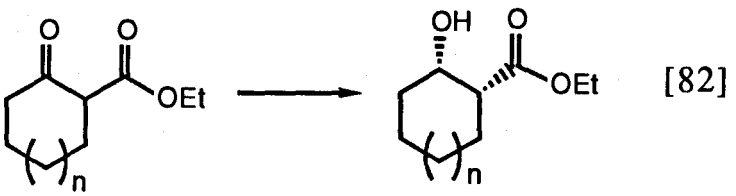
Scheme 11.



5. 

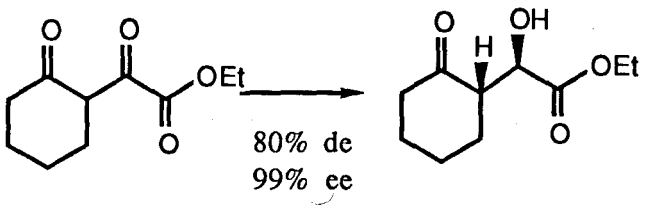
[75,76]

Scheme 12.

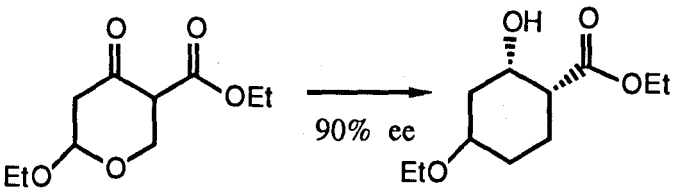


[82]

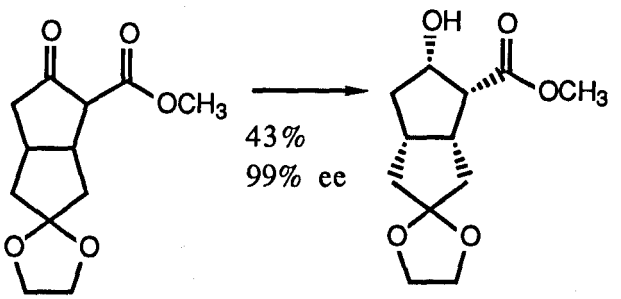
n=1 44%, 99% de, 99% ee
n=2 80%, 99% de, 99% ee



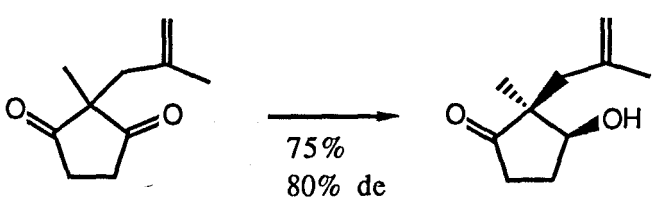
[83,84,85]



[86]



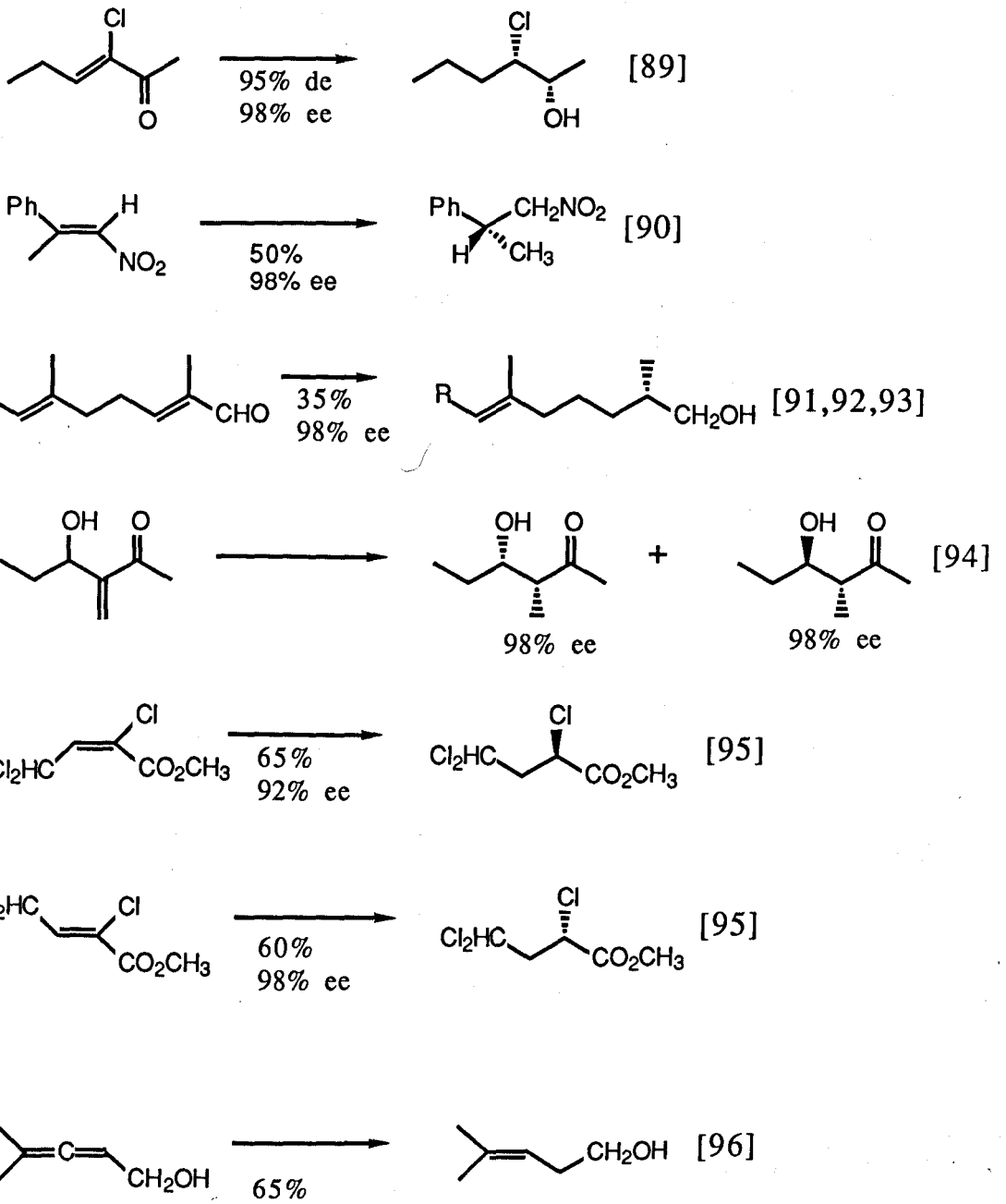
[87]



[88]

However, yeasts are also able to perform reactions other than the reduction of carbonyl groups. Thus is it possible to reduce activated double bonds with high diastereo- and enantioselectivity.

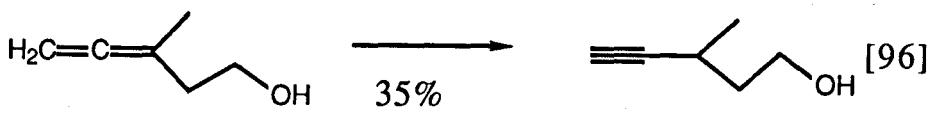
Scheme 13.[89,90,91,92,93,94,95,96]



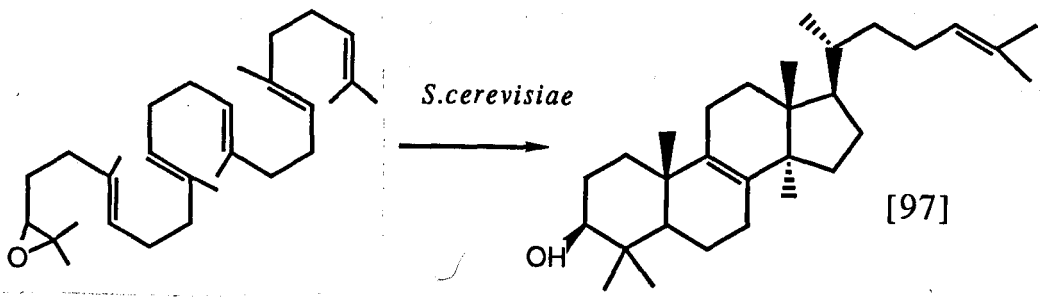
Other examples of yeast activity are the isomerization of allenic alcohols to optically active acetylenic alcohols[96] (scheme 14), the

cyclisation of racemic squalene oxide to enantiomerically pure lanosterol with ultrasonically treated yeast[97] (scheme 15) and the desaturation of dithiastearic acid[98] (scheme 16).

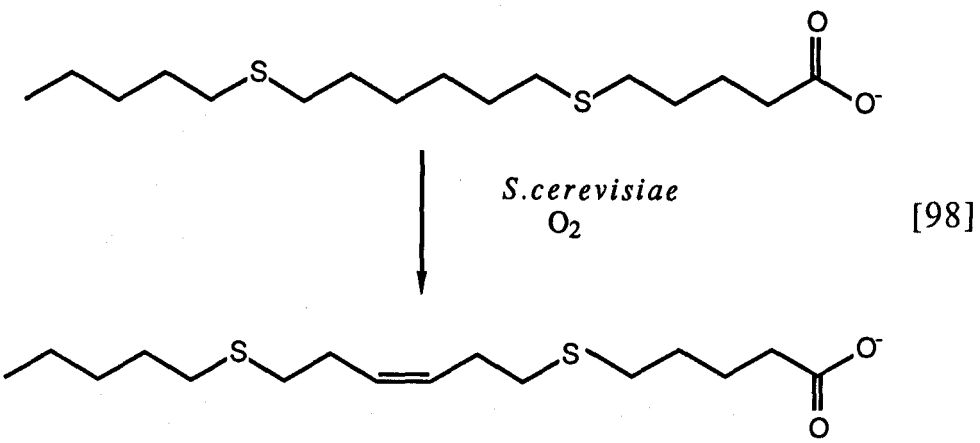
Scheme 14.



Scheme 15.



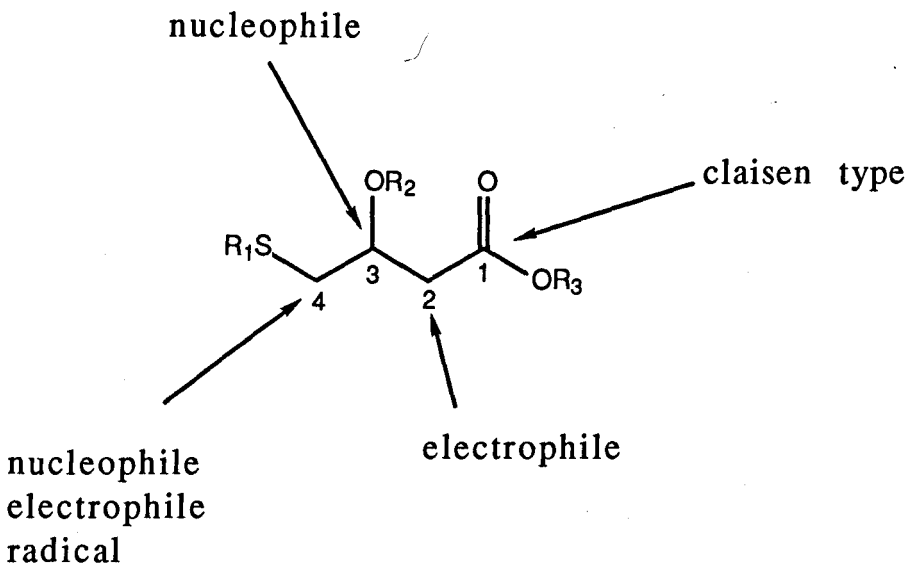
Scheme 16.



However yeasts are not the only microorganisms displaying oxidoreductase activity. Many other microorganisms can be used to solve a special reduction problem. In fact most of the reductions mentioned above have parallels in other microbial systems.

1.2. Reduction of 4-substituted β-ketoesters with yeasts.

In spite of all the work done on the reduction of β-ketoesters mentioned in the introduction, we were convinced that there was room for further improvement. In the past, it was normal to develop for every special target molecule the corresponding chiron. This approach we found unsatisfactory and therefore decided to investigate the possibility of producing a chiron which would embody selective reactivity on all carbon atoms and would be available by biotransformation using yeasts. For this purpose we chose to introduce a sulfur atom in 4-position of β-ketoesters. Scheme 17.



The resulting β-ketoester has many advantages:

Carbon 1: reduction, hydrolysis, Claisen type chain extension

Carbon 2: electrophilic addition

Carbon 3: nucleophilic addition after conversion into leaving group

Carbon 4: electrophilic addition after oxidation of sulfur;

nucleophilic addition after conversion into a sulfonium species;

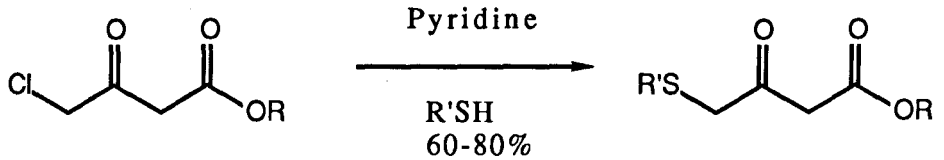
radical reactions;

desulfurisation.

In particular, desulfurisation proved to be important as it allowed us to determine easily the absolute configuration of our biotransformation products by reducing them to the known β-hydroxybutanoates.

It was decided to introduce substituents with four different chain lengths starting from two to five carbon atoms, and two aromatic substituents, one of which produced crystalline products. These substrates **3-9** could easily be synthesized in high yields by nucleophilic displacement of chlorine in 4-chloroacetoacetate **1** and **2**, by a sulfur nucleophile with pyridine as solvent.[99]

Scheme 18.

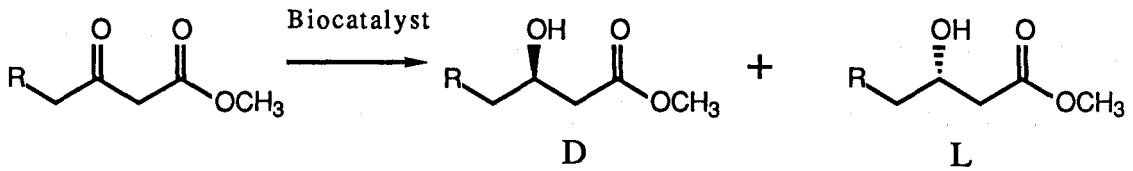


1 - 2 **3 -9**

R:		R':	R:	
Me	**1**	n-C_2H_5S	Me	**3**
Et	**2**	n-C_3H_7S	Me	**4**
		n-C_4H_9S	Me	**5**
		n-$C_5H_{11}S$	Me	**6**
		PhS	Me	**7**
		pClPhS	Me	**8**
		PhS	Et	**9**

It was proposed to study the influence of substitution on yield, enantiomeric excess and absolute configuration of the products and to compare the results with those obtained by biotransformation of methyl 4-chloroacetoacetate **1,** a well known substrate (see scheme 6).

Scheme 19.



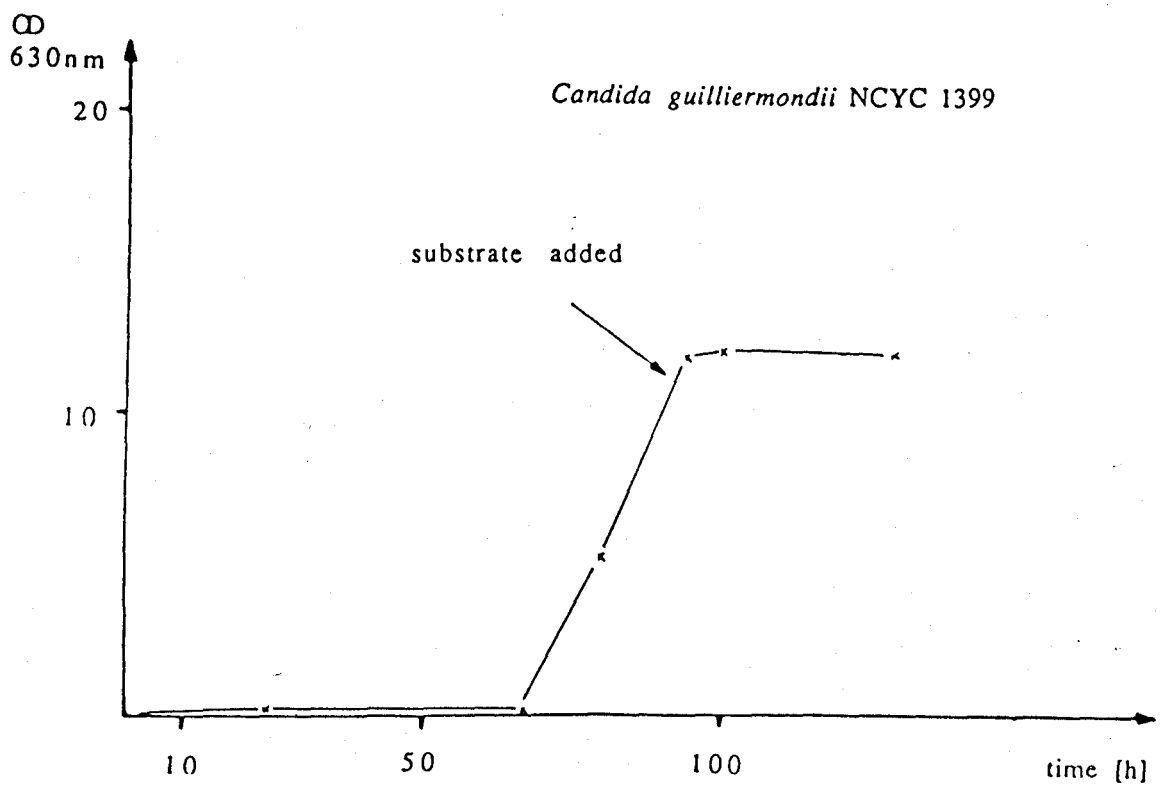
R=Cl	**1**	**10**
R=n-C_2H_5S	**3**	**11**
R=n-C_3H_7S	**4**	**12**
R=n-C_4H_9S	**5**	**13**
R=n-$C_5H_{11}S$	**6**	**14**
R=PhS	**7**	**15**
R=pClPhS	**8**	**16**

We resort here to the older L/D nomenclature for assigning absolute configuration, because it is more convenient to use with changing substituents at C-4. Following Kahn-Ingold-Prelog rules the same absolute configuration L in a β-hydroxyester would be assigned S with a proton as substituent, but R with a chlorine or sulfur as substituent. This leads often to confusion, when one applies the Prelog rule, which assigns substituents priority in terms of size, and assigns the configuration with the Kahn-Ingold-Prelog rule, which has the atomic number as priority.

As biocatalysts we chose different yeast strains which were easily obtainable from the National Collection of Yeast Cultures NCYC at the Food Research Institute Norwich, and a baker's yeast strain, which we bought from the local Sainsbury's superstore and deposited as NCYC strain 1765.

The yeasts were cultivated in conical flasks in a rotary shaker with Difco YM broth as medium, and the growth was followed by determination of the optical density at 630nm. Normally the substrate was added at the beginning of the resting phase. The conditions and growth pattern for a given set of experiments were very reproducible. A typical growth curve of the control flask is depicted in figure 1.

Figure 1. Growth curve of *Candida guilliermondii* NCYC 1399.

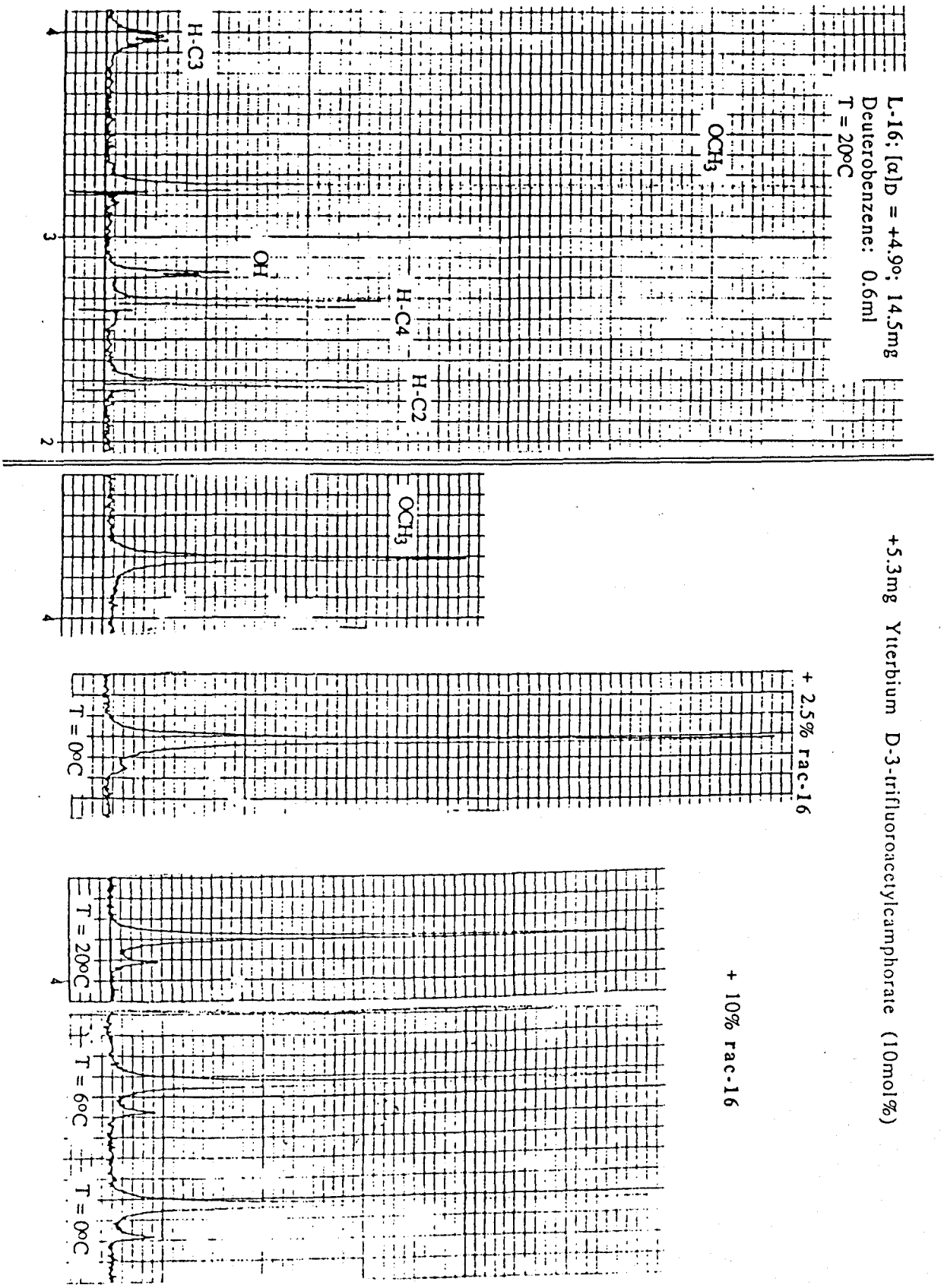


Because the lag phase of the same strain varied from experiment to experiment, it was necessary to follow the growth for each set to achieve maximum reproducibility of the results. Assuming that enzyme activity changes during the growing phase, it is advisable to add the substrate always at the same stage of the growing phase and not after a certain amount of time as often suggested in the literature.

In screening experiments the substrate was added as a solution in ethanol and the reaction was followed by thin layer chromatography. When the reaction was judged to be finished, the products were isolated by filtration, extraction and flash chromatography on silica gel.

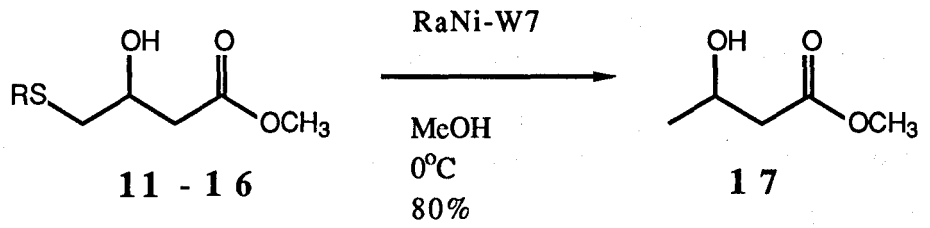
The enantiomeric excess was determined by 220MHz ^{1}H-NMR with chiral lanthanide shift reagents. All commercially available shift reagents were tested in deuterochloroform, carbon tetrachloride and deuterobenzene at various temperatures. The best combination was selected and a typical experiment for the determination of the detection limit is depicted in figure 2. It was found that the shift reagents Europium D-3-trifluoroacetylcamphorate and Ytterbium D-3-trifluoroacetylcamphorate in deuterobenzene gave the best results. The observed signal was the singlet of the methylester protons shifted about 0.9 ppm downfield. The detection limit was found to be about 2.5% under optimal conditions. An enantiomeric excess of 95% indicates therefore, that the other enantiomer could not be detected. It was important that the compound, shift reagent and solvent were absolutely anhydrous, otherwise immediate line broadening was observed.

Figure 2. Determination of detection limit with Ytterbium D-3-trifluorocamphorate on β-hydroxyester **16**.



The absolute configuration was determined by desulfurisation of the first series of products with Raney-Nickel W7 in methanol at 0°C to the known methyl 3-hydroxybutanoate **17**. For the rest of the screening experiments the peaks in the NMR spectra could be correlated.

Scheme 20.



11 - 16 **17**

Compound **17** is well known and its absolute configuration has been determined unambiguously. It was necessary to desulfurise at low temperature, otherwise a small amount of racemisation was observed (about 15%). The measurement of the optical rotations of the products was not found to be a suitable means for the determination of absolute configuration and enantiomeric excess. The rotation even of optically pure material is very low and small impurities from the yeast extraction could lead to false results.

All products were independently synthesized as racemic mixtures by sodium borohydride reduction at -20°C, and fully characterised. Generally, we assigned the protons at lower field to C-4, the ones at higher field to C-2. The rest of the spectral interpretation is trivial.

The results of the screening experiments are summarised in Table 2.

Table 2. Reduction of 4-substituted acetoacetates **1** and **3-8** by different yeast strains; % Yield (%ee, abs.conf.) of products **10-16**.

	10	**11**	**12**	**13**	**14**	**15**	**16**
S.cerevisiae NCYC 1769	60 (70,L)	55 (70,L)	49 (65,L)	67 (70,L)	30 (58,L)	42 (73,L)	40 (50,L)
Candida guilliermondii NCYC 1399		41 (85,D)	36 (85,D)	53 (80,D)	28 (85,D)	50 (30,D)	30 (80,D)
C.guilliermondii NCYC 973	50 (78D)	8 (90,D)	12 (90,D)	30 (90,D)	14 (80,D)	32 (88,D)	38 (80,D)
Hansenula polymorpha NCYC 1456		60 (60,D)	61 (50,D)	70 (60,D)	35 (66,D)	70 (18,D)	45 (25,D)
H.polymorpha NCYC 1459		70 (30,D)	45 (35,D)	60 (50,D)	42 (60,D)	56 (25,D)	50 (20,D)
Pichia membranaefaciens NCYC 333		80 (60,D)	76 (64,D)	80 (60,D)	42 (70,D)	86 (50,D)	70 (45,D)
P.membranaefaciens NCYC 765		75 (54,D)	70 (50,D)	73 (60,D)	55 (65,D)	60 (53,D)	65 (40,D)

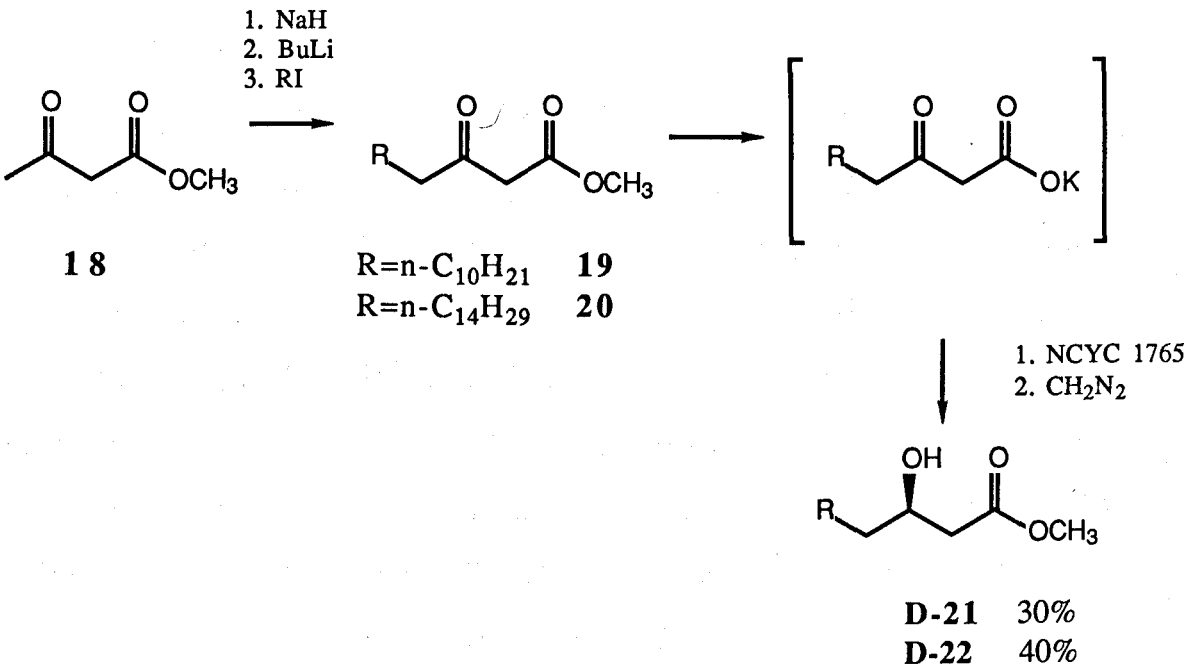
It was found that only *S.cerevisiae* afforded predominantly the L-enantiomer, whilst all the other species reduced the starting material to the corresponding D-enantiomer. There is no obvious relationship between chain length and enantiomeric excess or yield. However there is a slight trend for compounds with the aromatic substituents to be of lower enantiomeric purity.

The result obtained with methyl 4-chloroacetoacetate **1** is noteworthy. By contrast with the report of Sih and coworkers,[51] we obtained the L-enantiomer as the major product (see scheme 6). This underlines once more the common experience that the outcome of a microbial biotransformation may depend not only on the species, but also on the particular strain used. Our results

agree with those obtained by Sih, in so far as it seems difficult to find a yeast strain which reduces the β-ketoester **1** to the L-product.[55]

To prove that there is an influence of chainlength on absolute configuration, attention was directed to the biotransformation of fatty acids with *S.cerevisiae* NCYC 1765. Syntheses of the long chain β-ketoesters **19** and **20** were accomplished *via* double deprotonation of **18** and quenching with the corresponding alkyl halide. The ester was hydrolysed, and the resulting potassium salt was subjected without isolation to yeast reduction.

Scheme 21.



18

R=n-$C_{10}H_{21}$ **19**
R=n-$C_{14}H_{29}$ **20**

D-21 30%
D-22 40%

The products were directly treated with diazomethane and purified by flash chromatography. Both products **D-21** and **D-22** were optically pure and were shown to have the D absolute configuration. This was determined by chiral shift reagent experiments and comparison of the optical rotation with known

examples. Furthermore, **L-21** was synthesized independently and proved to be of opposite configuration (see part 2, schemes 75 and 76). It was necessary to use the potassium salt in the biotransformation because of the low solubility of the esters **19** and **20** in water. This however resulted in low yields, probably owing to decarboxylation.

Overall this survey provided a useful range of chiral synthons. The yeast *S.cerevisiae* NCYC 1765 seemed to have an exceptional tolerance towards the size of substituents in 4-position, and reduced even substrates with large substituents like chlorine from the re-face. Only with very long chains was the direction of reduction switched, with delivery of the hydride from the si-face of the trigonal system.

Studies by Sih and coworkers[49] indicate the action of two different enzymes, provisionally designated as the D-enzyme and L-enzyme, with two different rates of reaction. This would mean that in our case the L-enzyme shows quite large tolerance to substituents in 4-position and has its rate significantly reduced only by very long chains. However little is known about these enzymes. It has been shown that yeast alcohol dehydrogenase (EC 1.1.1.1.) does not reduce β-ketoesters, and Sih has reported in 1985 the isolation of 3 enzymes from *S.cerevisiae* (Red Star) with L- and D-specificity.[52] Two of them require NADPH as the cofactor, and the D-enzyme seemed to be associated with the fatty acid synthase complex. In 1988 Tressl and coworkers[100] reported the purification of two enzymes from *S.cerevisiae* with opposite enantioselectivity. Again both enzymes required NADPH as cofactor and again the D-enzyme was identical with a subunit of fatty acid synthase. However purified, intact fatty acid synthase

did not reduce β-ketoesters. Both enzymes were inactive against ketones, which are substrates of baker's yeast as shown in the introduction. These two publications are the only ones which have addressed this particular problem.

One can conclude that there are indeed at least two enzymes present with opposite enantioselectivities, which reduce β-ketoesters. The rate of reaction is determined by size and probably also by polarity of the substituent on the keto function. Ketones lacking the polar acid or ester function are not substrates. It would be of great interest to know more about these enzymes in *S.cerevisiae* and especially about the obviously abundant D-enzyme in other yeast strains such as *C.guilliermondii.*

We concluded this part of our investigations by selecting methyl 4-(p-chlorophenylthio)-3-hydroxybutanoate **16** as the product to be developed into a useful chiron. This choice was based on the fact that it provided the only crystalline products in the series, and that it was possible to accumulate the major enantiomer by recrystallisation. However the enantiomeric purity of the biotransformation product was unsatisfactory and we set out to improve the reduction of this substrate by immobilisation.

1.3. Effect of immobilisation on yeast biotransformations.

A publication by Ohno and coworkers[101] in 1985 suggested that immobilisation of yeasts could alter the stereospecificity of the biotransformation dramatically. Intrigued by this, we decided to investigate the influence of immobilisation on our biotransformation system. It should greatly facilitate the handling and workup of the sometimes rather messy reactions and provide the chemist with a storable and reusable biocatalyst. Since we started to work on this problem seven publications appeared using immobilised yeasts in biotransformations including our own one.[59,102,103,104,105] The popular immobilisation matrixes are alginate beads, polyurethane or carrageenan. Biocatalyst prepared in this manner can be stored for several months, reused up to eight times[106] and even show activity in organic solvents such as hexane.[107]

We chose our normal substrate range, and the two yeasts *S.cerevisiae* NCYC 1765 and *C.guilliermondii* NCYC 973 as the biotransformation systems. This was compared to two immobilisation techniques, active entrapment in alginate beads[108,109] and passive absorption on celite.[110] The results are summarised in table 3.

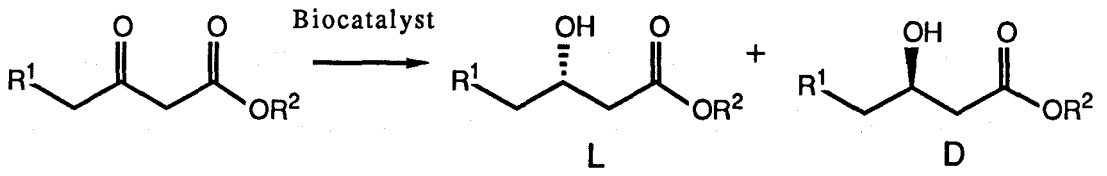


Table 3. Reduction of β-ketoesters with free and immobilised yeasts; yields/enantiomeric excess,absolute configuration.

R^1	R^2	Subs.	Prod.	*S.cerevisiae*			*C.guilliermondii*		
				free cells	alginate immob.	celite immob.	free cells	alginate immob.	celite immob.
Cl	CH_3	1	10	60/70L	70/75L	75/77L	50/78D	53/80D	55/83D
C_2H_5S	CH_3	3	11	55/70L	60/80L	65/80L	8/90D	30/90D	35/90D
C_3H_7S	CH_3	4	12	49/56L	60/70L	60/73L	12/90D	48/95D	50/94D
C_4H_9S	CH_3	5	13	67/70L	70/80L	60/75L	30/90D	52/93D	55/95D
$C_5H_{11}S$	CH_3	6	14	30/58L	45/80L	50/78L	14/80D	55/88D	53/93D
PhS	CH_3	7	15	42/73L	50/80L	50/75L	32/88D	58/90D	60/92D
ClPhS	CH_3	8	16	40/70L	60/80L	60/82L	38/80D	60/87D	65/85D
H	CH_3	18	17	75/70L	70/80L	70/78L	55/70D	60/75D	60/78D
H	C_2H_5	23	24	80/80L	75/90L	75/92L	40/35D	43/50D	50/50D

In all cases studied we obtained an increase in yield with immobilised cells compared to free cells. This is not surprising as the workup of the reactions is much facilitated by immobilisation. But much more important is the observed increase of the enantiomeric purity in the proportion of the major enantiomer compared to the free cell system. These results were at the time in strong contrast to those reported by Ohno,[101] but have in the meantime been underlined by similar observations in other groups, where a switch in the direction of reduction as reported by Ohno[101] was never observed.

Encouraged by these results, we set out to investigate the influence of cosolvents on the reduction by *S.cerevisiae* of substrate 8. As mentioned above, this substrate is highly crystalline and rather insoluble in water. We chose for study the solvents ethanol, dimethylsulfoxide, dimethylformamide, dimethoxyethanol and acetonitrile. The different systems were compared by their productivity numbers PN:

$$PN = \frac{\text{amount of product [mmol]}}{\text{dry weight of catalyst [kg] x time [h]}}$$

The reactions were followed by reverse phase HPLC and the concentrations were determined using a calibration curve. A typical reaction curve is depicted in figure 3. It shows the formation of the product for the cosolvent acetonitrile. Figure 4 then compares the different immobilisation techniques for the same system.

Figure 3. Reduction with alginate immobilised yeast.

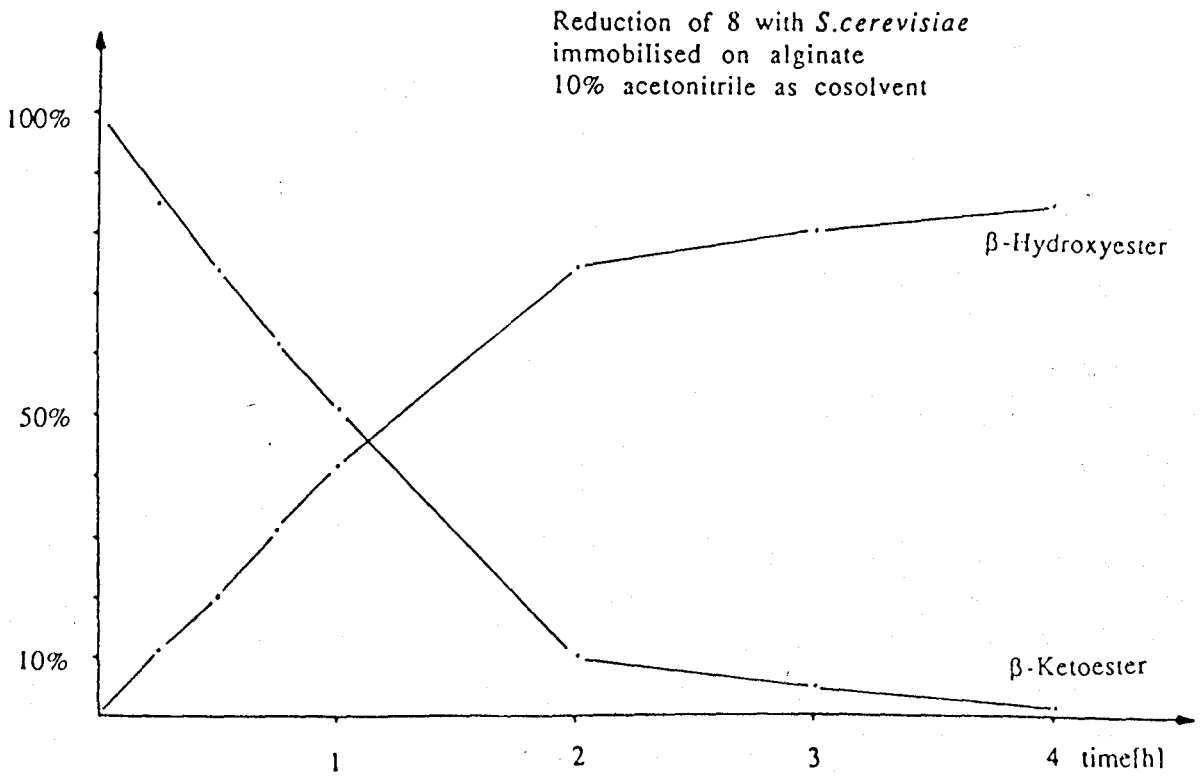
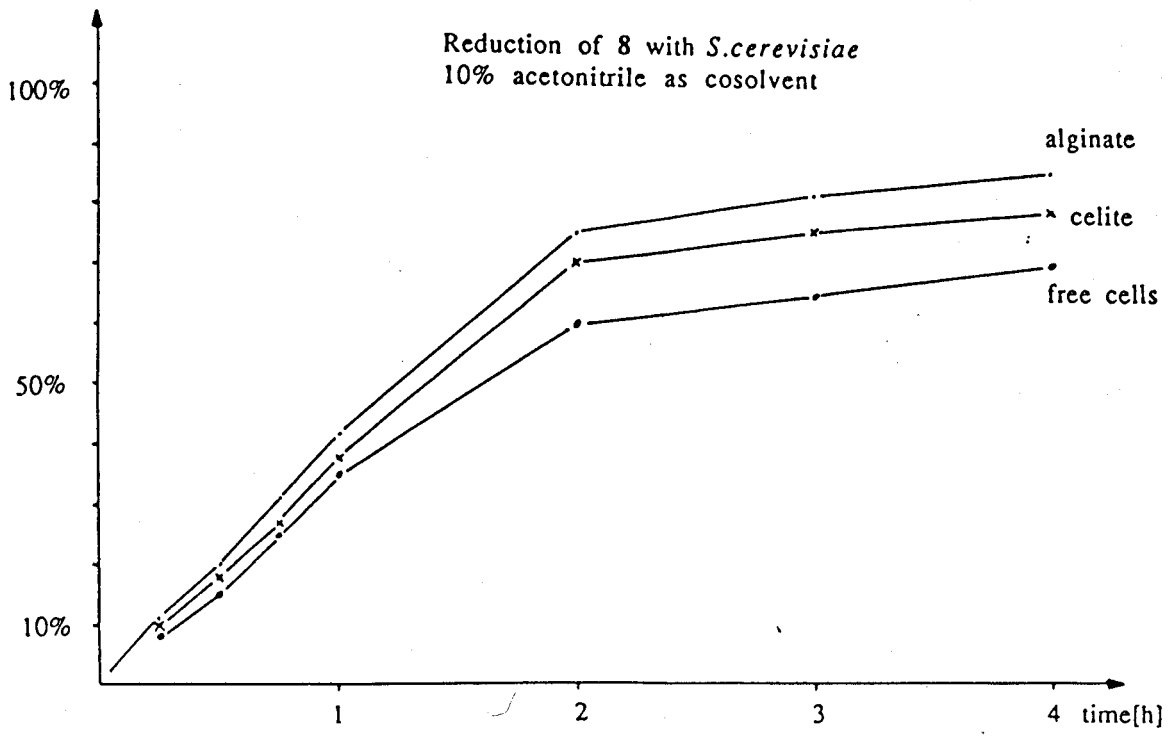


Figure 4. Comparison of reduction with different biocatalysts.



The complete results are summarised in table 4:

Table 4. Effect of cosolvent on the productivity number PN in the reduction of β-ketoester 8 with *S.cerevisiae*.

	PN		
Cosolvent	Free cells	Alginate immob. cells	Celite immob. cells
10% EtOH	190	180	170
10% DMSO	170	120	140
10% DMF	550	500	420
10% DME	320	340	340
10% AN	570	600	590

Surprisingly, there were hardly any differences in the reaction rates between the free cells and the immmobilised systems. Unfortunately it was not possible to determine a productivity

number in the absence of cosolvent, because the ester **8** was not soluble enough in water. However the reduction of ethyl-acetoacetate **23**, amply described in literature, proceeded with a productivity number of about 850, depending on the amount of sucrose used and using *S.cerevisiae* immobilised on alginate. Based on these results, acetonitrile was selected as the most suitable cosolvent for use with our biocatalyst. It afforded perfectly clear solutions and permitted for the first time, scale-up of the biotransformation of substrate **8** in good yield and enantiomeric excess.

However we know nothing about the state of the cells. There are strong suggestions in the literature that immobilised cells treated with such a high percentage of water miscible organic solvent are permeabilized.[111] Cells can be permeabilized without lysis of cells or destruction of enzyme systems. The morphology of the cells remains intact, but low molecular weight molecules can freely enter and leave the cell. After permeabilization with organic solvents the cells most often are no longer viable, which can be useful as energy is no longer wasted for the synthesis of cell mass. The viability depends largely on the concentration of the agent used and the time of its application.

We have not gone further into this question, but we did investigate reuse of our biocatalyst. Working with *S.cerevisiae* and *C.guilliermondii* immobilised on celite, the reaction mixture was filtered and the catalyst was reused directly, without regeneration, for the next batch of substrate. The results are summarised in table 5.

Table 5. Productivity number in three consecutive experiments with celite immobilised biocatalysts.

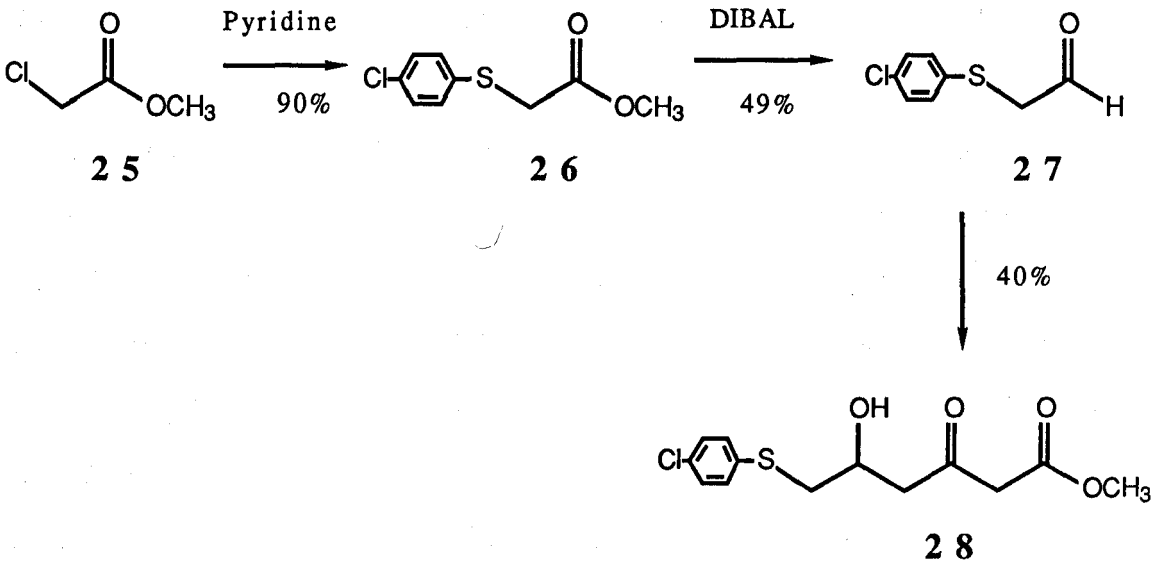
Number of consecutive reductions	PN *S.cerevisiae* on celite	PN *C.guilliermondii* on celite
1	590	620
2	320	410
3	90	80

It is clear that both catalysts lost most of their activity after their second use. This is probably attributable to permeabilization, but there is no definite proof of this. Overall we were satisfied that the use of immobilised cells allowed us to overcome most of the disadvantages associated with the use of free cell reductions. It provides for easy work up, controlled reaction conditions, acceptable productivity numbers and good reproducibility. Given that yeasts generally are easy to grow, we have in immobilised yeasts a very useful biocatalyst which should permit ready scale up - one of the traditional drawbacks of yeast reductions.

1.4. Diastereoselective reduction of a β-ketoester with yeasts.

Because of our great interest in the synthesis of 3,5-dihydroxyesters, we turned our attention to the diastereoselective yeast reduction of 5-hydroxy-3-ketoesters. The starting material was synthesized by nucleophilic displacement of chlorine in chloroacetate **25** by p-chlorothiophenol and subsequent reduction of product **26** with DIBAL to aldehyde **27**.

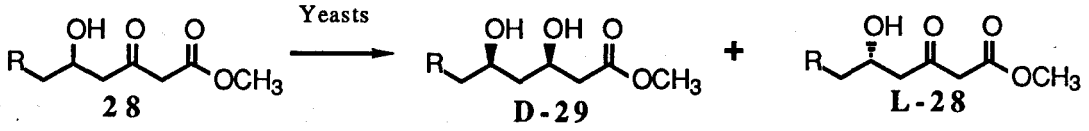
Scheme 22.



25 **26** **27** **28**

Aldehyde **27** was then reacted with the dianion of methyl-acetoacetate **18** to give, albeit in poor yield, substrate **28**. This route was chosen to synthesize large amounts of racemic 5-hydroxy-3-ketoester and cannot compare with the route developed for the synthesis of optically pure material described in part 2 (see scheme 54).

Ester **28** was then subjected to the reduction with eleven yeast species. The results are summarised in table 6:



R=pClPhS

Table 6. Diastereoselective reduction of 5-hydroxy-3-ketoester **28** by different yeast strains.

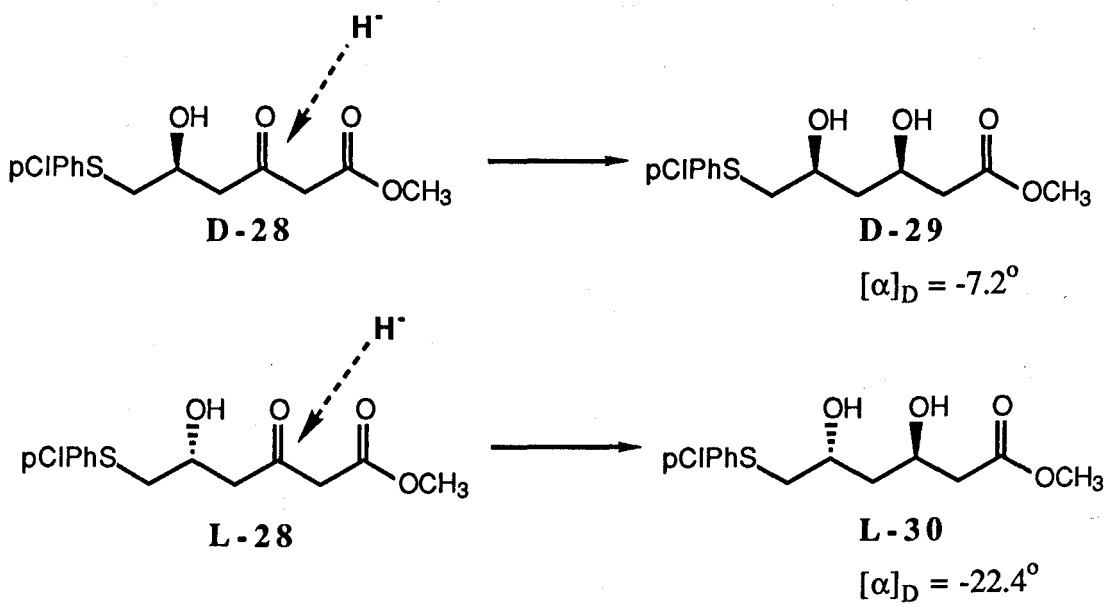
				D-29			L-28	
Name	NCYC	time	yield	de%	ee%	$[\alpha]_D \pm 1.0^o$	yield	ee%
S.cerevisiae	667	6h	42%	78	-	-4.6°	46%	36
S.cerevisiae (Wine)	463	4d	-	-	-	-	31%	9
S.cerevisiae	1765	6h	14%	>95	>95	-7.2°	49%	19
Rhodotorula glutinis	974	5h	25%	80	-	-8.9°	32%	17
R.rubra	796	27h	10%	56	-	-7.2°	30%	7
C.guilliermondii	973	5h	41%	>95	>95	-7.6°	32%	59
C.guilliermondii	1399	4.5h	37%	42	-	-9.4°	-	-
H.polymorpha	1456	5.5h	37%	92	-	-6.8°	36%	59
H.polymorpha	1459	5h	20%	58	-	-7.9°	20%	51
P.membranaefaciens	333	9h	30%	88	-	-4.3°	20%	15
P.membranaefaciens	795	6h	32%	>95	>95	-7.1°	39%	41

The reactions could easily be followed by reverse phase HPLC and were stopped after approximately 50% conversion. Filtration, extraction and separation by flash chromatography afforded the products. They were then analysed for diastereomeric excess by HPLC and for enantiomeric excess by comparison of the optical rotations with independently synthesized compounds (see part 2, scheme 54). Unfortunately it was not possible to separate the mixtures of diastereomers into their two antipodes by preparative

HPLC and thereby determine the enantiomeric excess of products not diastereomerically pure.

It was astonishing to find that all yeasts preferred the D-enantiomer of the starting material and reduced it to the *syn* diastereomer, leaving the L-enantiomer untouched. As all previous work on diastereoselective yeast reductions has been done on α-substituted β-ketoesters, it is very difficult to find literature precedent for our results. However, it can be suggested by analogy with Van Middlesworth,[74] that there is one enzyme responsible for the reduction, with different affinities for the two enantiomers. This is supported by the observation that low enantiomeric excess of the residual starting material parallels low diastereomeric excess of the product.

Scheme 23.



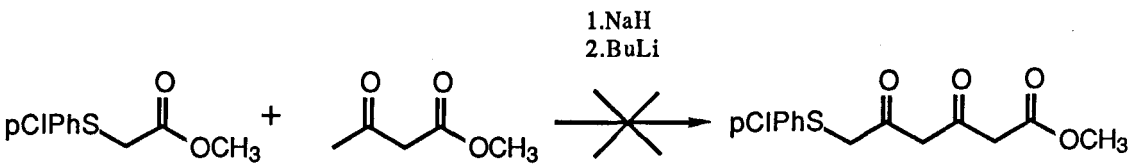
Because we were never able to isolate the *anti* component, we could not determine its absolute configuration. The observed increase of the negative rotation in cases with low diastereomeric excess, however, indicates strongly that the *anti* diastereomer **30**

formed in the biotransformation, is of **L** absolute configuration, because independently synthesized **L-30** has a rotation of $[\alpha]_D = -22.4^o \pm 1.5^o$.

It was pleasing to observe in three cases the formation of optically pure **D-29** in reasonable yield (see table 6), remembering that the maximum yield possible is 50%.

The work on the diastereomeric yeast reduction was just in its starting stages, when the practical work on this thesis was concluded. There are still a lot of unanswered questions left. It is, for instance, quite surprising that our two preferred yeasts *S.cerevisiae* NCYC 1765 and *C.guillliermondii* NCYC 973 give products of the same diastereomeric and enantiomeric excess, whilst in the reduction of β-ketoester **8**, they show opposite enantioselectivity. Furthermore, it would be of great interest to investigate the reduction of a 3,5-diketoester. Unfortunately, experiments to synthesize the required substrate were not successful.

Scheme 24.



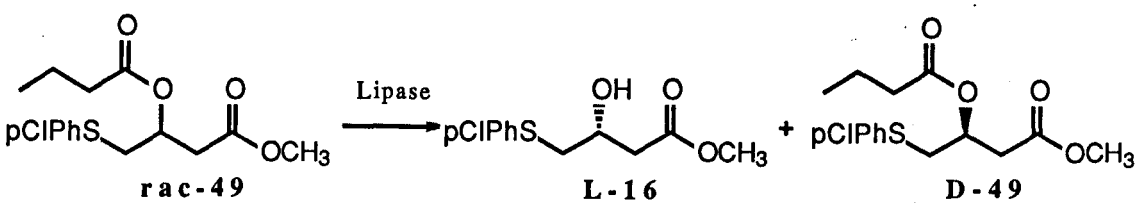
26 **18**

1.5. Resolution with Lipase.

Lipases are hydrolytic enzymes which have, as their natural substrates, glycerides of long-chain fatty acids. They find a wide range of applications in biotransformations and are readily available. Stimulated by the fact that the natural reaction of lipases is the hydrolysis of esters of chiral alcohols, rather than of esters of chiral acids, we attempted the resolution of butyrate **49** to find a complementary synthesis of chiron **16**.

A screening experiment quickly established the Amano *Pseudomonas* lipase as the most suitable one. It afforded the L-enantiomer in about 80% enantiomeric excess and 20% yield.

Scheme 25.



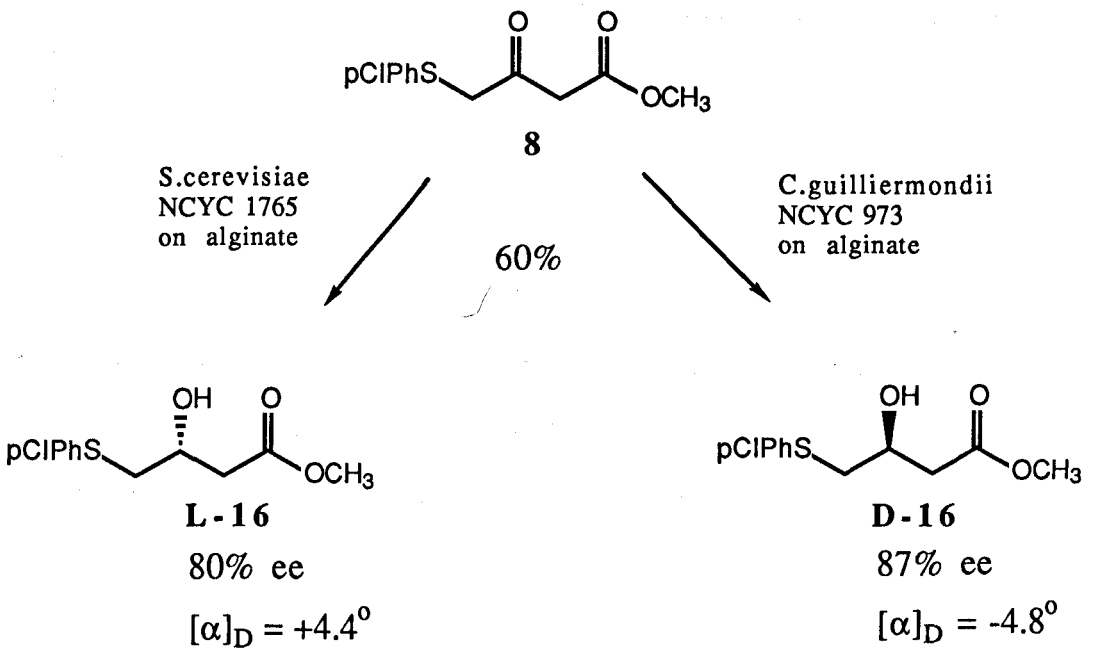
Yield:	20%	30%
$[a]_D \pm 1.0^o$:	$+4.5^o$	$+8.5^o$
ee:	80%	54%

This experiment remains unoptimised and will not be further discussed, as it is not in the scope of this thesis. We were satisfied, however, to have found an additional route to chiron **16**.

1.6. Conclusions.

It was possible with a simple screening of substrates and yeast strains to produce a new optically active starting material for organic synthesis. Employing the basic rules for conducting yeast reductions, summarised on page 25, it was possible to optimise the biotransformation via immobilisation.

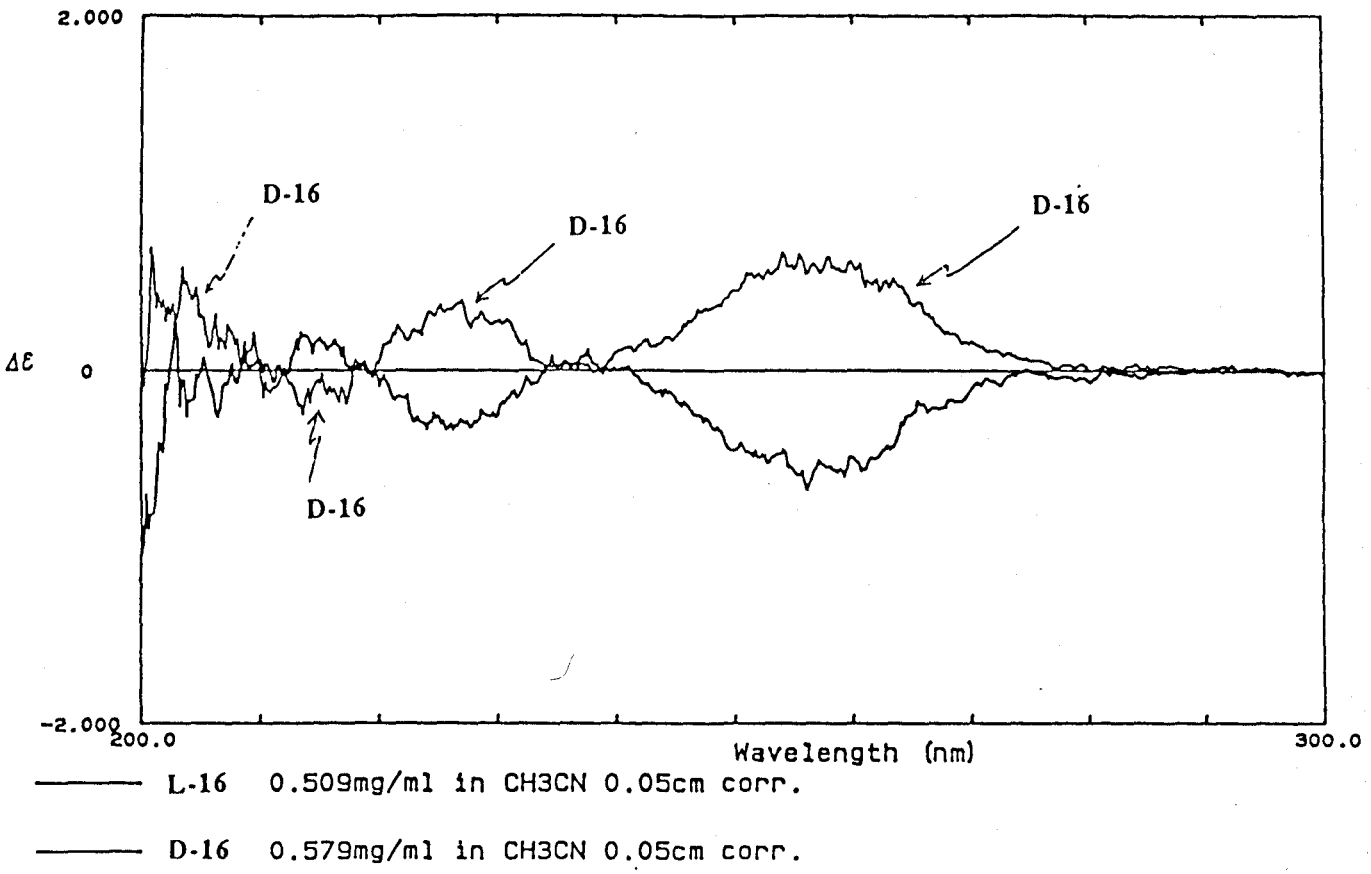
Scheme 26.



Both products could be recrystallised to optical purity (>95%ee) and have been fully characterised. The CD-spectra of both enantiomers have been measured and are depicted on the next page (figure 5).

The reduction of the racemic 5-hydroxy-3-ketoester **28** opened up new possibilities for the synthesis of optically pure polyhydroxyesters *via* biotransformation.

Figure 5. CD-spectra of **D-16** and **L-16**.



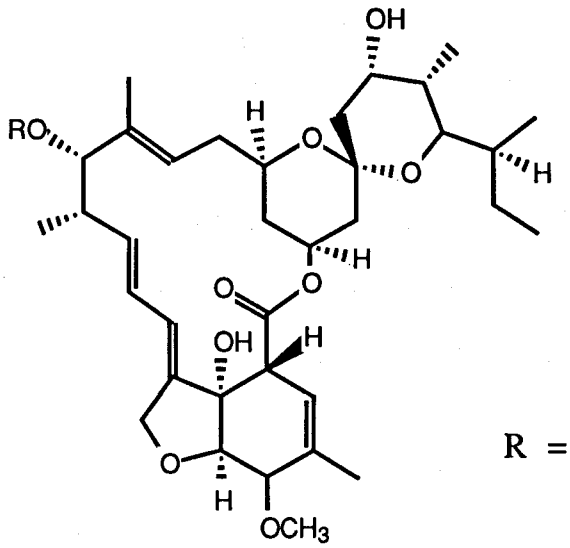
Part 2: Synthesis of EPC from biotransformation products.

2.1. Introduction.

Our interest in polyhydroxyesters was very much stimulated from beginning by the occurrence of polyoxygenated units in many polyether and macrolide antibiotics. So far, more than 150 macrolide lactones, such as erythromycin, and more than 70 polyethers, such as monensin, have been described. Both classes are important antibiotics produced by actinomycetes, and can be classified as polyoxygenated carboxylic lactones, and polyoxygenated carboxylic acids respectively. In spite of the diversity of structures it was possible to construct for both classes a general stereochemical model, for macrolides by Celmer[112] and for polyethers by Cane, Celmer and Westley.[113]

Special attention in recent years has been paid to the avermectin family of macrolide-like antibiotics. Numerous attempts to synthesize compounds like the milbemycins and avermectins have been published.[114] One member of this group is shown in scheme 27.

Scheme 27.

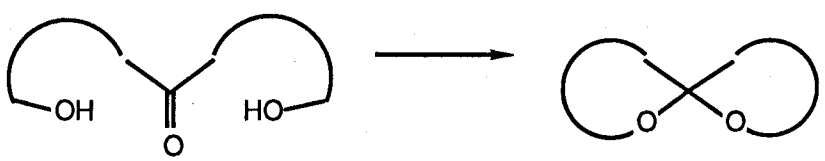


R = disaccharide

Avermectin A_{2a}

One particular problem in the synthesis of these compounds is the construction of the spiroketal moiety. It has been the subject of much recent work,[115,116,117,118,119,120,121,122,123,124,125,126,127,128] and many approaches have been used to tackle it. We were most interested in the ketalisation of the corresponding keto diol **A** to **B** under thermodynamically controlled conditions.[129]

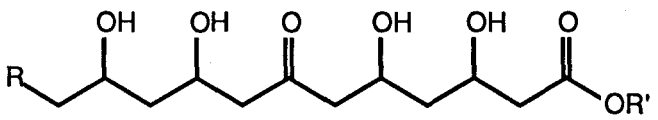
Scheme 28.



A **B**

This reduces the synthetic problem to a general approach to enantiomerically pure, polyoxygenated carboxylic esters of the structure depicted in scheme 29.

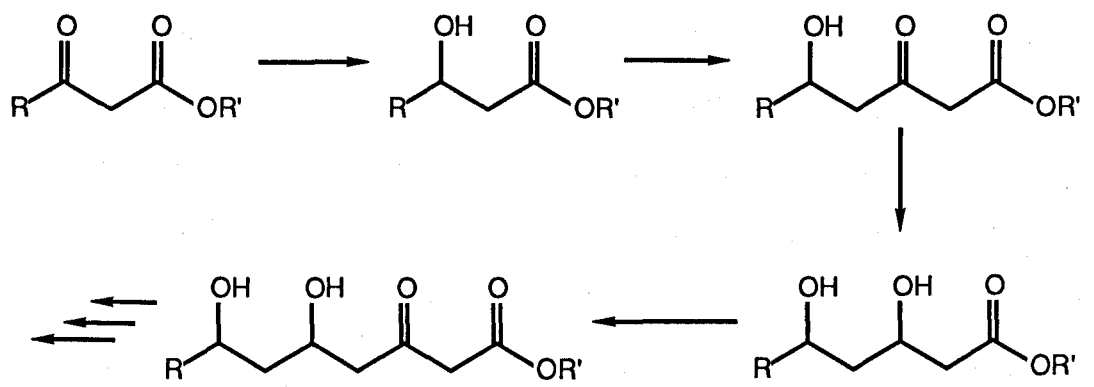
Scheme 29.



Of course the different functionalities require selective protection. In particular, the use of the dithioacetal allows, on deprotection with mercuric chloride, spontaneous cyclisation.[130]

The construction of the 1,3-dihydroxy relationship has often been solved via a stereo controlled aldol reaction.[131] However, we wanted to solve the problem in a completely different way, allowing us to synthesize, by the same basic procedure, any stereoisomer. This approach would include as major components a chain extension and a stereoselective reduction, both of which could be repeatedly used. It is outlined in scheme 30:

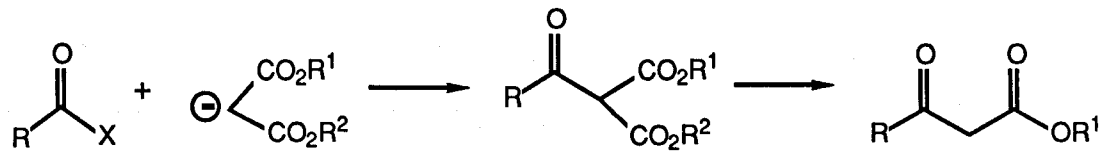
Scheme 30.



Since the first publication on ester condensations by Claisen[132] in 1887, 3-ketoesters have had a prominent place in organic synthesis. With the alternating reactivity pattern of their carbon chains, they enjoy great popularity as starting materials. Although there are a great number of preparations known,[133] they are often

afflicted with the problem of product mixtures, as usually obtained in cross-Claisen condensations. One possibility for overcoming this drawback is the acylation version of the malonic acid synthesis (scheme 31).

Scheme 31.



X: Cl, $O(CO)OC_2H_5$, $PO(C_6H_5)_2$, imidazole

R^1: alkyl

R^2: alkyl, benzyl, silyl, H

This version of 3-keto ester synthesis requires special conditions to avoid side reactions such as, for instance, diacylation. Magnesium, as introduced by Lundt, is usually the most suitable counterion.[134] As the acylating agent any form of activated carboxylic acid derivative can be used. Examples are acid chlorides, mixed anhydrides, phosphine oxides, imidazolides etc. Fair to good yields have been reported for this first step (R^2 = R^1).[135] However, this route suffers from the requirement for selective ester hydrolysis of one of the two malonic ester groups. If both of them are hydrolysed, double decarboxylation leads to the formation of a methyl ketone as a side product.[136] The use of mixed malonates (R^2 = alkyl or benzyl) can partially overcome this problem, especially when the two ester groups react to selective cleavage conditions, such as hydrogenolysis. The use of ethyl

trimethylsilylmalonates offers an attractive modern alternative and gives 3-ketoesters in good yields.[137]

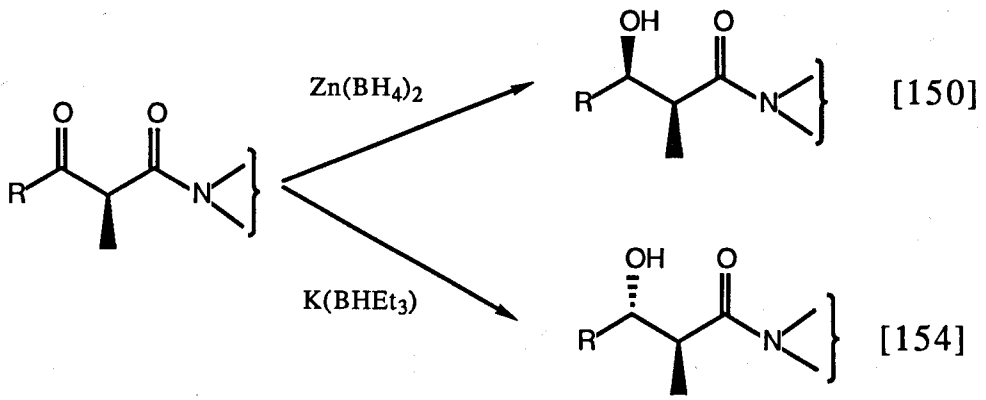
Since all mixed malonates are synthesized from the corresponding ethyl hydrogenmalonate[138] (R^1 = Et, R^2 = H), this compound itself has been extensively investigated. It has been shown that it can be used indeed as its magnesium chelate in tetrahydrofuran, together with mild acylation species such as imidazolides.[139] With more reactive acyl donors, deacylation was observed.[140] This method has recently received a lot of attention and has been used with success in a range of organic syntheses.[141,142,143,144,145] The great advantage of this procedure is the instant decarboxylation upon acidic workup, which permits a one-pot synthesis of 3-ketoesters.

Further development has been achieved by the use of Meldrum's acid (2,2-dimethyl-1,3-dioxan-4,6-dione) together with pyridine as a base.[146] The resulting product undergoes facile alcoholysis to 3-ketoesters under fairly mild conditions, and a range of products has been prepared by this route.[147,148]

The second problem which needs to be solved is the stereoselective reduction of β-hydroxyketones. A literature search astonishingly yielded poor results. This may be due to the assumption that high 1,3-asymmetric induction can hardly be expected. It is only in the last two years that major progress has been reported. We subsequently applied this to our own work with success. It is therefore not surprising that a lot of effort has gone into the 1,2-asymmetric induction.[149,150,151,152,153,154,155] Thus it was possible selectively to reduce β-keto-α-methylcarboxylic acid derivatives with $Zn(BH_4)_2$ to the *syn*, and

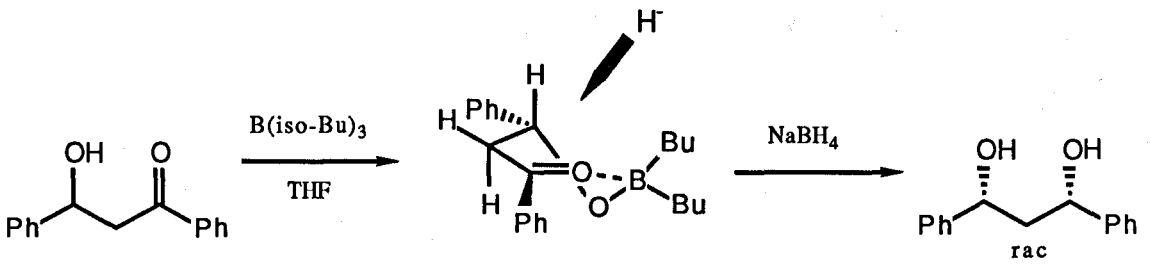
with $K(BHEt_3)$ to the *anti* β-hydroxy-α-methylcarboxylic acid derivatives.

Scheme 32.



The few early reports on 1,3-asymmetric induction are summarised in the following references.[156,157,158,159,160,161] However they are not highly stereoselective and have limited utility. Much more promising was the method published by Narasaka in 1984. There the 3-hydroxyketones were first chelated by tri-isobutylborane, before being reduced with high stereoselectivity with sodium borohydride. The *syn* diastereomer was the major one obtained and the stereoselectivity is explained by the formation of a cyclic, chair-like boron chelate in which the axial proton prevents the approach of the reducing agent. Hence the preferred diastereomer formed is *syn*.[162]

Scheme 33.



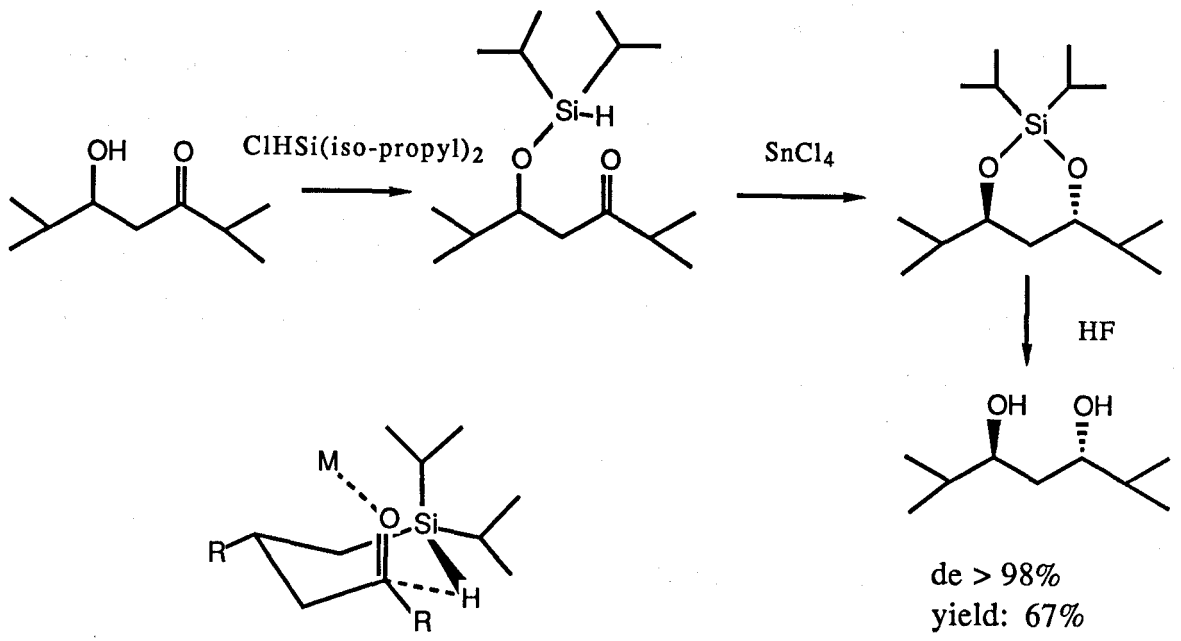
de: 96%
yield: 94%

In the alternative $Zn(BH_4)_2$ method, which also affords the *syn* diastereomer as the major product the equivalent zinc chelate is proposed.[163] However the reagent is not readily available and has a very low solubility in ether. It was indeed found by Prasad and coworkers that a simple hydrogen bond could be sufficient to direct hydrogenation of β-hydroxyketones, and they obtained the *syn* diastereomer in good diastereomeric excess.[164] The same group later published an improved boron chelate method, in which alkoxydialkylboronates form the intermediate without any activation. The subsequent reduction gave selectively the desired *syn* diol.[165]

The alternative *anti* diol can be obtained by two methods. In both a covalent bond is formed between the reducing agent itself and the β-hydroxyketone. The hydride ion is delivered in a chairlike transition state.

Davis and Anwar form first a silyloxy ketone, which upon treatment with Lewis acid forms preferentially the *anti* siladioxanes.[166]

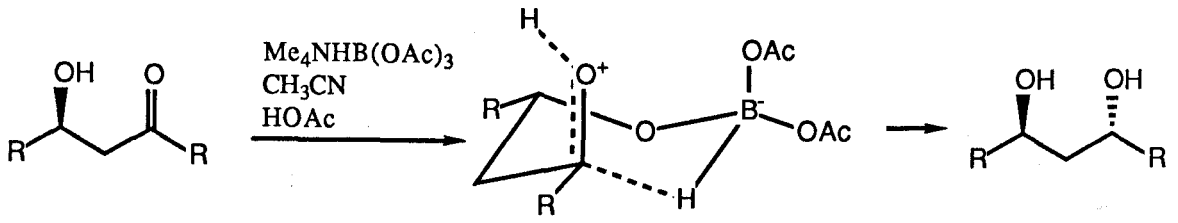
Scheme 34.



However this method involves three separate chemical steps and the overall yield is therefore reduced.

More promising is the use of tetramethylammonium triacetoxyborohydride, which reduces β-hydroxyketones in one step to the corresponding *anti* diol.[167,168]

Scheme 35.



Again, a chairlike transition state is postulated. This mechanism is supported by the finding that the reaction requires the presence of either acids or metal ions (such as Na^{+}) if β-hydroxyketones are to be reduced. Additionally, it is possible to reduce the starting

material in the presence of a simple ketones such as acetone, without reducing the ketone.

Considering these results, we can summarise the factors directing diastereoselective reductions as follows:

For 1,2-diastereoselective reduction:

-nucleophilic, non-chelating reagent - Cram selectivity - **syn**

-electrophilic, chelating reagent - chelation control - **anti**

For 1,3-diastereoselective reduction:

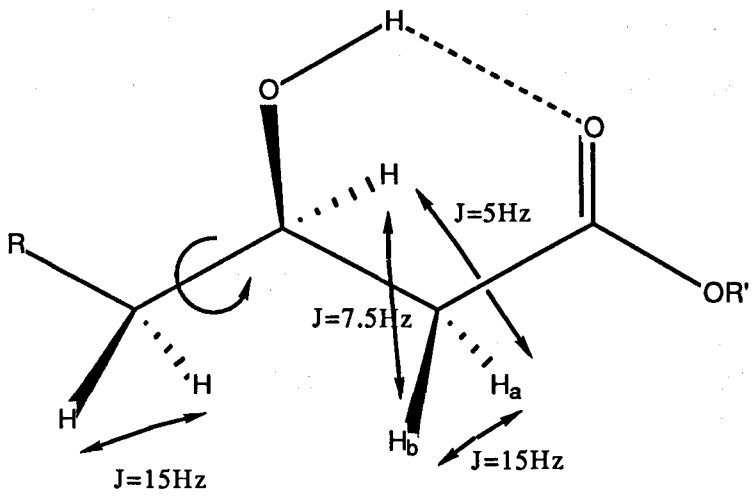
-chelating reagent, external hydride delivery - **syn**

-chelating reagent(covalent bond), internal hydride delivery- **anti**

2.2. Synthesis of the 3,5-dihydroxyester.

We decided to concentrate our efforts on the synthesis of all four stereoisomers of 3,5-dihydroxyhexanoate substituted at carbon 6. All synthetic work was carried out on racemic material and only in the cases of the p-chlorophenylthio substituent repeated with optically active starting material. The starting β-ketoesters were synthesized as mentioned on page 34 and subsequently reduced with sodium borohydride. It was necessary to work at low temperature, because even at 0°C partial reduction of the ester group was observed. The resulting β-hydroxyesters were easily characterised and showed, if substituted in the 4-position with sulfur or chloride, a shift to lower field of the protons at carbon 4 in the ^{1}H-NMR spectrum. The two protons H_a and H_b on C-2 and C-4 are separated in most products. They show a geminal coupling constant of J_{ab}=15 Hz and a coupling constant between the protons on C-2 and C-3 of J_{32a}=5 Hz and J_{32b}=7.5 Hz. These coupling constants vary little from compound to compound and support the postulated existence of strong intramolecular hydrogen bonds in β-hydroxyesters. In contrast, the protons on C-4 show distinct variations in their coupling pattern between different compounds. The interpretation of the spectra is illustrated in figure 6.

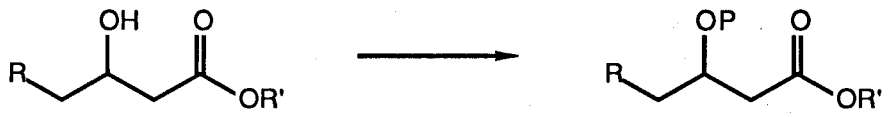
Figure 6. Coupling constants of β-hydroxyesters.



All IR-spectra show a strong band at 1735 cm^{-1} for the carbonyl absorption. The ^{13}C-NMR spectra, proton decoupled, agree completely with the proposed structures. The carbonyl carbon appears at 172 ppm, C-3 at 66.9 ppm, C-4 at about 40 ppm and C-2 at slightly higher field at about 38 ppm. The assignment of C-4 and C-2 has been made tentatively from increment calculations. All spectra agree with literature data of similar compounds.

In the course of our synthetic work with β-hydroxyesters we soon realised the need for protection of the hydroxy group and therefore synthesized a whole range of protected esters. The products are summarised in table 7.

Table 7. Protection of β-hydroxyesters.



Starting material:

R	C_2H_5S	C_3H_7S	C_4H_9S	$C_5H_{11}S$	PhS	pClPhS	H	H	PhS
R'	Me	Me	Me	Me	Me	Me	Me	Et	Et
Nr.	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**24**	**31**

Mosher esters:

R	H	C_2H_5S	C_3H_7S	C_4H_9S	$C_5H_{11}S$	PhS	PhS
R'	Et	Me	Me	Me	Me	Me	Et
P	MTPA	MTPA	MTPA	MTPA	MTPA	MTPA	MTPA
Nr.	**32**	**33**	**34**	**35**	**36**	**37**	**38**

Products:

R	H	H	H	H	PhS	PhS	PhS	PhS
R'	Et	Et	Et	Et	Et	Et	Et	Et
P	DNB	Bzl	MEM	MTM	MEM	TMS	THP	MTM
Nr.	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**

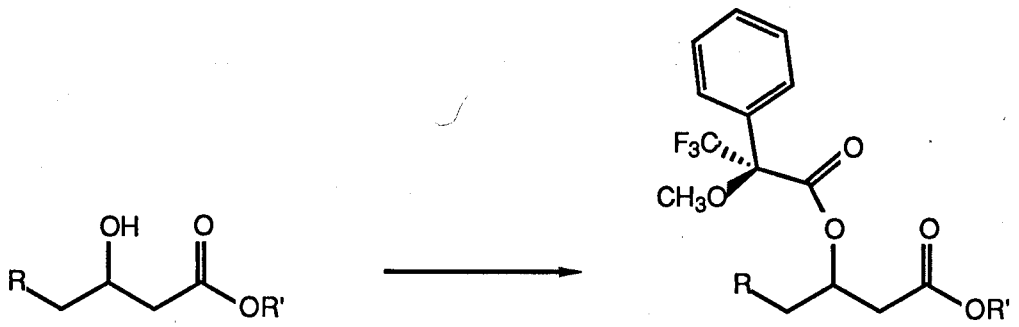
R	pClPhS	pClPhS	pClPhS	pClPhS
R'	Me	Me	Me	Me
P	THP	Ac	Bu	TBDMS
Nr.	**47**	**48**	**49**	**50**

The number of compounds may look slightly confusing, but it is quite easy to explain. Generally we set out on all our investigations with the ethyl esters. It was only after some time that we realised how convenient the determination of the

enantiomeric excess was when working with the methyl ester signal in the ^{1}H-NMR spectrum.

We therefore started out to determine all enantiomeric purities from the ^{19}F-NMR spectra of the Mosher-ester derivatives **32 - 38**. They were prepared from the corresponding S-(-)-2-methoxy-2-trifluoromethyl-2-phenylaceticacid chloride, which in turn was synthesized from the acid and thionylchloride as described by Mosher.[169] Treatment of the β-hydroxyesters with the acid chloride in carbon tetrachloride and pyridine gave the Mosher-esters **32 - 38** in excellent yield.

Scheme 36.

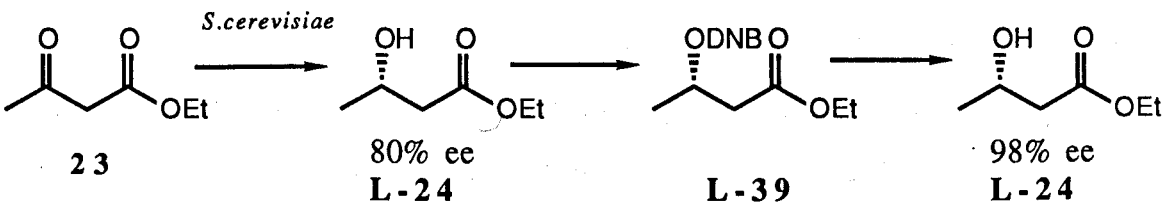


11-16,24,31 **32-38**

They were purified by flash chromatography (no separation of the diastereomers) and analysed by ^{19}F-NMR. With $CFCl_3$ as reference the signal due to the D-enantiomer appeared at 71.34 ppm and that due to the L-enantiomer at 71.16 ppm, with CF_3COOH as reference the signal due to the D-enantiomer appeared at 7.09 ppm and that due to the L-enantiomer at 7.21 ppm. However this method proved to be very tedious, as for every single compound a derivatisation was needed and the corresponding racemate had to be synthesized. In a large screening programme this would have consumed too much time and we therefore decided to look for an

alternative method. A solution was found by using the methyl ester signal and lanthanide shift reagents as illustrated in figure 2. All our preliminary studies were concerned with ethyl 3-hydroxybutanoate **24**. It was obtained by biotransformation with *S.cerevisiae*, derivatised with dinitrobenzoicacid to the 3,5-dinitrobenzoate **39**, which in turn could be recrystallised to an enantiomeric excess of >95%.[170] This recrystallisation in large amounts was extremely tedious and required as a first stage the separation of racemic crystals from enantiomerically pure crystals by hand. Hydrolysis then afforded the optically pure **L-24**.

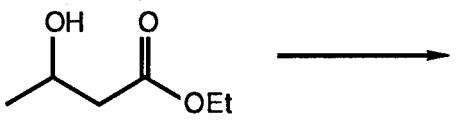
Scheme 37.



β-Hydroxyester **24** could be converted into a variety of protected derivatives. We considered the benzyl group to be the most suitable one for our purposes, because it could be removed under mild conditions. However, we encountered, in the synthesis of benzylether **40** with benzyl bromide and sodium hydride, unexpected difficulties. After refluxing the reaction mixture for three days we obtained the product in only 45% yield.

The corresponding methoxyethoxymethylether **41** could be obtained in 85% yield by treatment with MEM-chloride and triethylamine in dichloromethane. Formation of methylthiomethylether **42** with dimethylsulfoxide and acetic anhydride proceeded in only about 60% yield.[171]

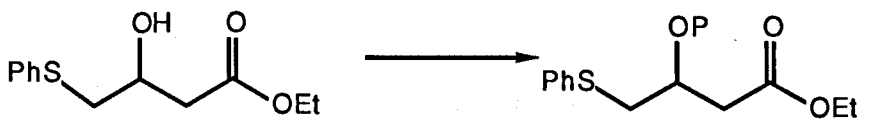
Scheme 38.



24

P:	Nr:	Yield:
Bzl	**40**	45%
MEM	**41**	85%
MTM	**42**	60%

In a similar fashion we protected ethyl 3-hydroxy-4-phenylthiobutanoate **31**, as its methoxyethoxymethylether **43** and as its trimethylsilylether **44**. The latter slowly decomposed at room temperature. Under astonishingly mild reaction conditions (room temperature, 10min) the tetrahydropyranyl derivative **45** could be formed. However the resulting two diastereomers were a complication we wanted to avoid at the beginning of the synthesis. Scheme 39.



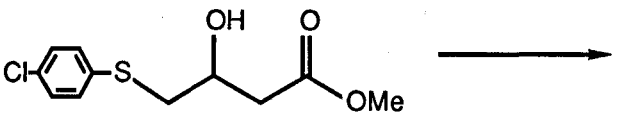
31

P:	Nr:	Yield:
MEM	**43**	70%
TMS	**44**	85%
THP	**45**	90%
MTM	**46**	45%

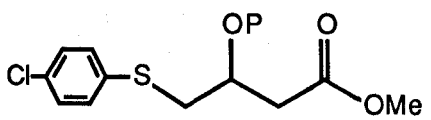
The compound with which we later carried out most of our synthetic work was methyl (p-chlorophenylthio)-3-hydroxybutanoate **16**. It could be protected as its THP-derivative **47**, but also as the acetate or butyrate **48** and **49**. The protecting

group which was most successful, however, was the tert-butyldimethylsilylgroup (cf. **50**).

Scheme 40.



16



P:	Nr:	Yield:
THP	**47**	89%
Ac	**48**	75%
Bu	**49**	80%
TBDMS	**50**	93%

All compounds gave satisfactory spectral data and agreed with similar compounds in literature. The only remarkable feature was the shift of the proton on C-3 to lower field, when the acetate is introduced (see table 8).

Table 8. Comparison of ^{1}H-NMR chemical shift of H-C3 in ppm

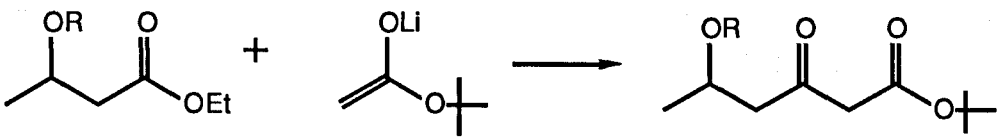
Nr:	**16**	**47**	**48**	**49**	**50**
H-C3(ppm)	4.20	4.20+4.15	5.35	5.20	4.25

The introduction of an ester group at C-3 seems to deshield the proton H-C3 sufficiently to cause a shift of about 1ppm to lower field. This makes the identification of the acetate and butyrate quite easy and allows for a facile detection of impurities.

We first started our synthesis of β-ketoesters with a method published by Rathke.[172,173,174] Direct treatment of ethyl esters **24**, **40** and **41** with the stable lithium enolate of tert-butylacetate

afforded the 3-ketoesters **51, 52** and **53** in poor to moderate yields.

Scheme 41.

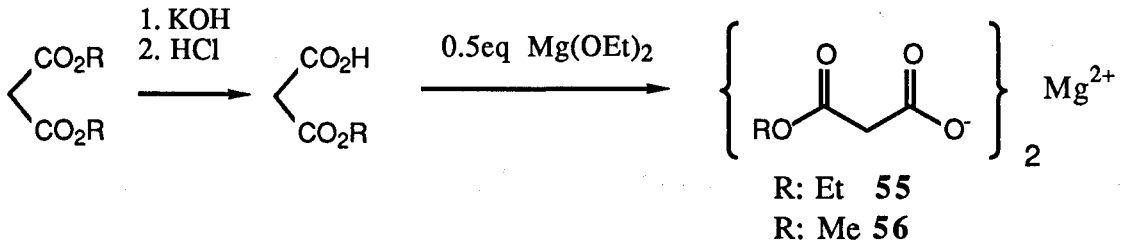


R:	
H	**24**
Bzl	**40**
MEM	**41**

Yield:	
51	8%
52	68%
53	35%

The new 3-ketoesters show two strong absorptions in their IR-spectra at 1745 cm^{-1} and 1720 cm^{-1}. The ^1H-NMR-spectrum shows a distinctive singlet at 3.40 ppm for the protons at C-2. The protons at C-4 appear as a doublet of doublets between 2.5 ppm and 2.9 ppm and show the same coupling patterns as in the starting material, a geminal coupling constant J_{ab}=15 Hz and two coupling constants of 5 Hz and 7.5 Hz to the proton at C-5. The three protons at C-6 appear as a doublet at 1.25 ppm. This method, however, did not satisfy our requirements for mild, high yielding reaction conditions, and we therefore started to work on the acylation version of the malonic synthesis mentioned in the introduction. We synthesized the two reagents **55** and **56** from the corresponding malonic esters by partial hydrolysis, and treated the half malonic esters with magnesium ethoxide. Evaporation and drying under reduced pressure overnight afforded the two magnesium salts, which could be stored under nitrogen in the dark at 0°C.

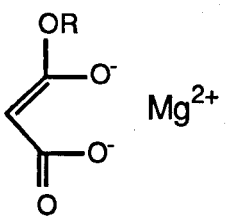
Scheme 42.



R: Et **55**
R: Me **56**

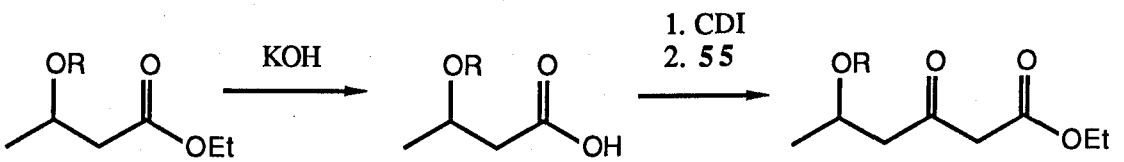
These reagents have a distinct advantage over previously reported magnesium salts, where one equivalent of magnesium leads to the formation of a basic compound (see figure 7), and should allow virtually neutral reaction conditions.

Figure 7. Basic magnesium malonate.



We prepared the acylation agent by hydrolysis of the 3-oxyesters to their acids and activation of the acids with carbonyldiimidazole CDI.

Scheme 43.



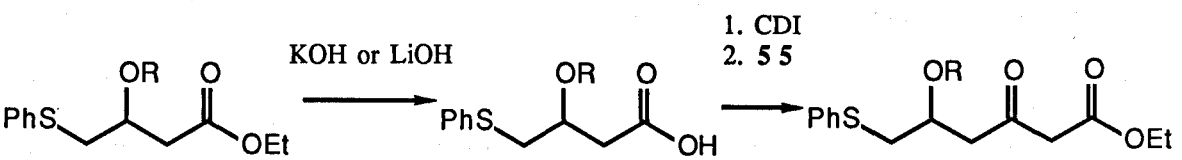
R: H **24** **57** 90% **59** 40%
R: MEM **41** **58** 95% **60** 90%

The use of potassium hydroxide in THF and water was quite successful in this case, and yielded the acids in nearly quantitative yield and excellent purity. We observed, however, that when

working with the THP protecting group some elimination occurred. This could be prevented by switching to lithium hydroxide in methanol and water. Hence we used these hydrolysis conditions for all further reactions.

The chain extension proceeded smoothly in anhydrous THF at room temperature. The acid was first treated with one equivalent of CDI and the resulting imidazolide reacted *in situ* with the magnesium salt. Acidic workup resulted in decarboxylation and the product could be purified by flash chromatography. We observed in all cases with free hydroxyl groups a lower yield compared to the protected case even when an excess of reagents was used. This is surprising because the secondary hydroxyl group exhibits quite low reactivity towards basic conditions, as was discovered when we tried to protect it. However it seemed to influence the reaction sufficiently to reduce the yield significantly. Similar results were obtained when applying these reaction conditions to the substrates **31, 43** and **45**.

Scheme 44.



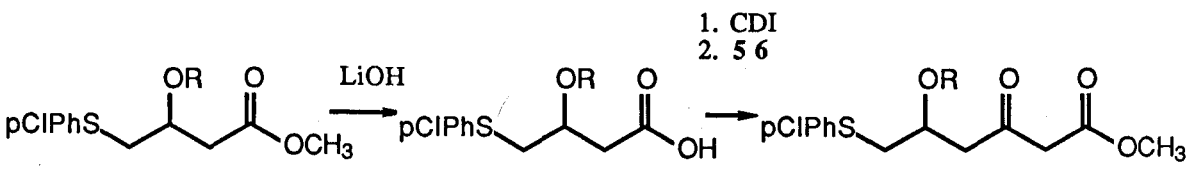
R: H	**31**	**61**	91%	**64**	60%
R: MEM	**43**	**62**	87%	**65**	80%
R: THP	**45**	**63**	90%	**66**	90%

It was no problem to hydrolyse the 4-substituted esters to the corresponding acids **61, 62** and **63**. But on attempting to recrystallise acid **63** we could only recover the deprotected acid **61**. It seems that **63** is acidic enough to catalyse its own

deprotection with traces of water present. As recrystallisation was not necessary this did not influence significantly the reaction sequence. Again, in the unprotected case a lower yield was obtained, which dropped even further when an attempt was made to scale the reaction up.

We then applied this reaction sequence to our target starting material, methyl 4-(p-chlorophenylthio)-3-hydroxybutanoate **16** and its protected derivatives **47** and **50**. As expected, the reaction proceeded smoothly with a low yield in the case of the unprotected acid **67**.

Scheme 45.



R: H	**16**	**67**	95%	**28**	40%
R: THP	**47**	**68**	95%	**70**	70%
R: TBDMS	**50**	**69**	90%	**71**	96%

All the products were characterised and showed the expected spectral data. As in the tert-butyl ester case, two typical carbonyl absorptions at 1745 cm^{-1} and 1715 cm^{-1} were observed in the IR spectrum. The ^{1}H-NMR spectra showed a singlet at 3.50 ppm for the protons at C-2. Again the protons at C-4 and C-6 showed the typical doublet of doublets coupling pattern with a strong Dach-effect. The geminal coupling is about 15 Hz, the coupling to H-C5 between 5 Hz and 8 Hz. In case of THP derivatives the formation of two diastereomers in a nearly one to one ratio was observed. They were never separated physically and are only distinguishable in the 400MHz ^{1}NMR spectrum.

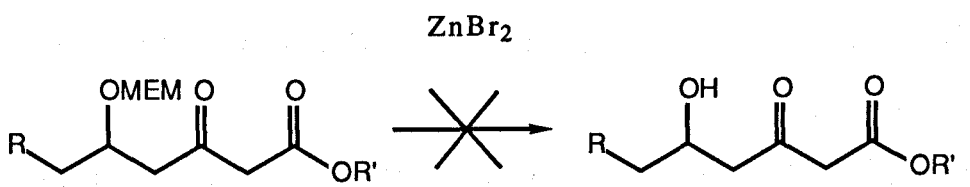
This series of experiments demonstrated that it is possible to synthesize β-ketoesters *via* acylation of malonic half esters. From the experimental point of view the reactions are quite demanding and have to be conducted under absolutely anhydrous conditions and under nitrogen. The following points have to be observed:

- the starting acid was dried overnight under high vacuum.
- THF was freshly distilled from K/Na/benzophenone.
- CDI was treated with activated charcoal and recrystallised from anhydrous THF. It was stored at -10°C under nitrogen.
- Hydrogen methylmalonate was freshly distilled and the magnesium reagent was best prepared a day in advance and dried overnight under high vacuum.

Only by observing these rules was it possible to scale up the reaction to multigram quantities without a dramatic loss in yield and with high reproducibility.

We then turned our attention to the deprotection of the different 3-keto-5-oxyesters and encountered unexpected difficulties. The deprotection of the MEM derivatives proved to be absolutely impossible.

Scheme 46.

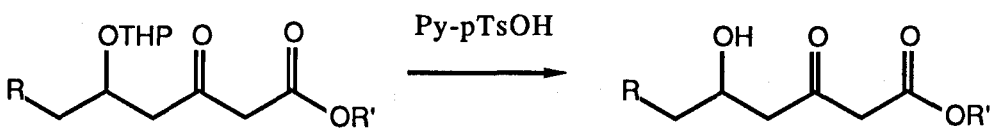


R:	R':		
H	tert-butyl	**53**	**51**
H	Et	**60**	**59**
PhS	Et	**65**	**64**

Treatment of the MEM-ether with powdered, dried zinc bromide in anhydrous dichloromethane led to a mixture of products from which the desired 5-hydroxyester could only be isolated in low yield (about 20%). It is possible that the bidentate coordination of the MEM-group to the Lewis acid is severely disturbed by the presence of the β-ketoester moiety, and that this prevents a ready cleavage. The only byproducts isolated were recovered starting material and a small amount of the lactone. Other lewis acids ($TiCl_4$, $ZnCl_2$, $SnCl_4$) did not improve the results. As, in the mean time, the progress with other protective groups looked promising, work on the MEM group was stopped.

The deprotection of the THP-derivatives proceeded in reasonable yield.

Scheme 47.



R:	R':			
PhS	Et	**66**	**64**	71%
pClPhS	Me	**70**	**28**	75%

Treatment of the THP ether with pyridinium p-toluenesulfonate in methanol afforded the 5-hydroxyesters along with a small amount of the δ-lactone. Interestingly, during the deprotection of **70** formation of a byproduct was observed, which upon treatment with aqueous acid equilibrated to the desired 5-hydroxy-3-ketoester **28**. We propose the formation of a hemiacetal dimer, but cannot offer definite spectroscopic proof. The ^{1}H-NMR spectrum indicates the presence of the methyl ester and the other

signals are slightly shifted. In the MS analysis, however, it was not possible to find a molecular ion with twice the mass of **28**. The yield on deprotection was not high enough to be a useful alternative to the chain extension without protection.

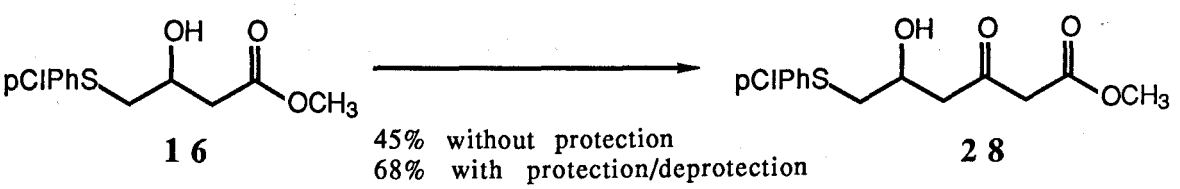
Finally, the best solution was found in the deprotection of the TBDMS ether **71**.

Scheme 48.

7 1 **28** 80-85%

There are a variety of conditions under which silylethers can be cleaved. Treatment of **71** with either aqueous hydrofluoric acid in acetonitrile or tetrabutylammonium fluoride easily afforded the product **28** in good yield, depending on the reagent used and the scale of the reaction. We were satisfied that the use of the TBDMS protecting group was a better alternative to the chain extension reaction without protection, providing us with the desired product **28** in 68% overall yield compared to 40-45% in the unprotected case.

Scheme 49.

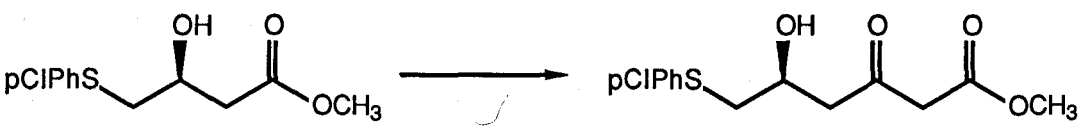


1 6 **2 8**

The reaction with optically active material was shown to proceed without any racemisation. The enantiomeric purity of **L-28** or **D-**

28 was determined by 400MHz ^{1}H-NMR with the chiral solvating agent (R)-(-)-2,2,2-trifluoro-1-(9-anthryl)ethanol in carbon tetrachloride and deuterobenzene. Again we found the methylester singlet to be the most suitable one for the determination of the enantiomeric excess. We could not detect a change in optical purity of **28** compared with the starting material **16**. The absolute configuration of **28** is assumed to be the same as in **16**. We have no independent proof for this, but it is difficult to conceive of a mechanism for the complete inversion of the stereocenter at C-5 under the prevailing reaction conditions.

Scheme 50.



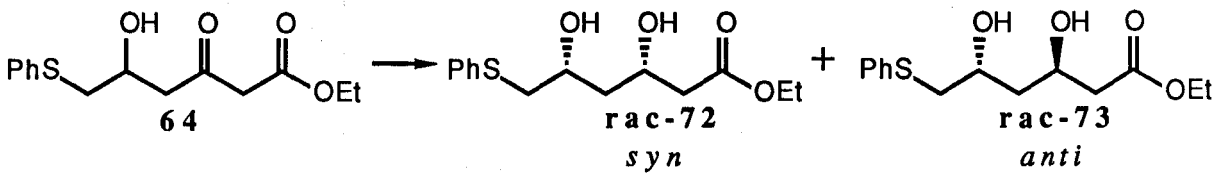
D-16
ee>95%

60%

D-28
ee>95%

The remaining problem was now the reduction of the 5-hydroxy-3-ketoesters to the corresponding 3,5-dihydroxyesters. A variety of conditions were first tried for the reduction with sodium borohydride. However, changes in temperature and cosolvent did not have an effect on the diastereomeric excess of the product. We then tried other reaction conditions until we found the solution in two recently published procedures. The results are summarised in table 9.

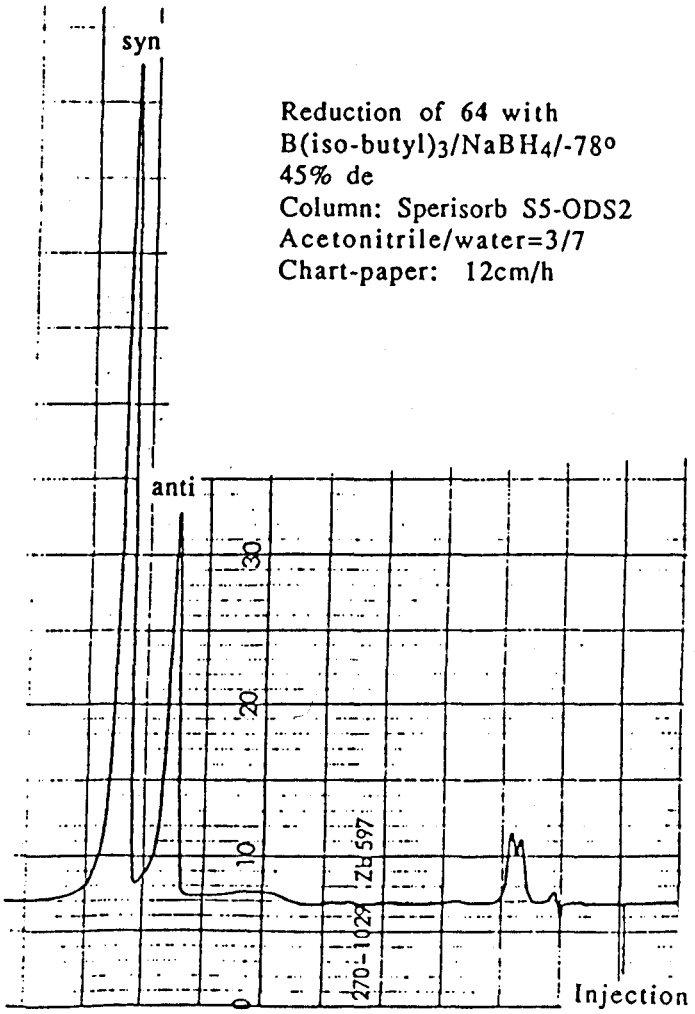
Table 9. Diastereoselective reduction of 5-hydroxy-3-ketoester **64** by various chemical methods.



Method	%Yield	%de
$NaBH_4$, THF, 0°C	70	0
$NaBH_4$, THF/MeOH = 4/1, 0°C	85	0
$NaBH_4$, THF/MeOH = 4/1, -20°C	90	10 *syn*
$NaBH_4$, THF/i-PrOH = 4/1, -20°C	90	20 *syn*
$Zn(BH_4)_2$, Ether, 0°C	76	22 *anti*
1. Chlorodiisopropylsilane, 2. $SnCl_4$, -78°C	20	70 *anti*
$Al(i\text{-}PrO)_3$, toluene	30	24 *anti*
1. B(iso-butyl)$_3$, 2. $NaBH_4$, -78°C	82	45 *syn*
1. Et_2BOMe 2. $NaBH_4$, -78°C	95	95 *syn*
$Me_4NBH(OAc)_3$, AcOH, Acetonitrile, -40°C	95	95 *anti*

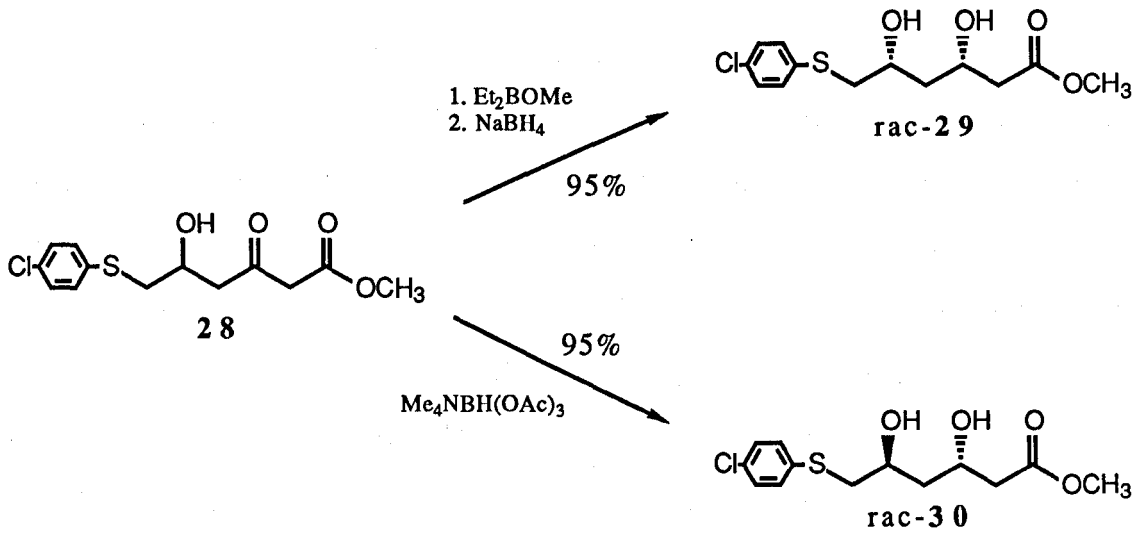
The product mixtures were analysed by reverse phase HPLC. The two diastereomers could easily be separated into two peaks and the diastereomeric excess calculated by gravimetric integration. An alternative method is the analysis of the ^{13}C-NMR spectrum. The two diastereomers give rise to two different sets of signals for C-3 and C-5. Comparison of the chemical shifts of our compounds with literature data confirmed our assignment of the relative configuration. A typical determination of diastereomeric excess by HPLC is shown in figure 8.

Figure 8. Determination of diastereomeric excess by HPLC.



We were therefore able to synthesize both diastereomers of our target molecule in pure form and in nearly quantitative yield.

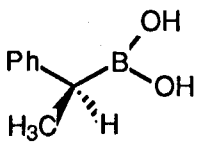
Scheme 51.



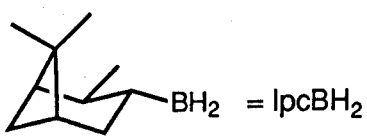
Both diastereomers have been fully characterised. They show a strong carbonyl absorption at 1730 cm^{-1} in the IR spectrum. In the ^{1}H-NMR spectrum the protons of the methylester give rise to a singlet at 3.85 ppm, whilst the protons at C-3 and C-5 appear as multiplets at 4.05 ppm and 4.37 ppm respectively. The two protons at C-6 give a multiplet at 3.12 ppm and the two protons at C-2 a multiplet at 2.69 ppm. Finally, the protons at C-4 show a multiplet at 2.0-1.6 ppm. The ^{13}C-NMR spectrum shows for both diastereomers a peak at 173 ppm for the carbonyl carbon atom and a peak at 52.2 ppm for the methylester carbon atom. The two stereogenic carbons at C-3 and C-5 show quite a distinct difference . In the *syn*-diastereomer **29** they appear at 70.3 ppm and at 68.6 ppm, whilst in the *anti*-diastereomer **30** they appear at 67.1 ppm and at 65.5 ppm. Carbon atoms C-2, C-4 and C-6 show peaks between 41.1 ppm to 42.0 ppm and are not easily distinguishable in the spectrum of the diastereomers.

The chiral analysis of the four optically active products **L-29**, **D-29**, **L-30** and **D-30** proved to be a problem. All attempts to analyse the racemates with chiral lanthanide shift reagents or chiral solvating reagents did not succeed or showed an insufficient splitting of the signals. The derivatisation of both hydroxyl groups with an optically pure compound (i.e. di-Mosher esters) seemed to us not to be a feasible method for the determination of the enantiomeric excess. One would expect at least five separate peaks for the methylester in the ^{1}H-NMR spectrum and four peaks in the ^{19}F-NMR spectrum for the trifluoromethyl group. This is not an advantageous situation for an accurate analysis, and we therefore tried to find a derivatisation which introduced only one new stereogenic center and at the same time would combine the two hydroxyl groups in a more rigid ring structure. A solution was proposed in the formation of a boronic ester with an chiral substituent and we set out to synthesize the optically active (S)-1-phenylethaneboronicacid **75**[175] and the mono-isopinocampheylborane **76**.[176]

Scheme 52.



75



= $IpcBH_2$

76

$IpcBH_2 \cdot TMEDA \cdot H_2BIpc$

77

Unfortunately the synthesis of **75** on a large scale (10g) resulted in an explosion, and we therefore stopped work on this synthesis. There were no problems in synthesizing **76** from the corresponding (1R)-(+)-α-pinene, and we isolated it as its

crystalline TMEDA dimer **77**. Treatment of **77** with two equivalents of 3,5-dihydroxyester led to the formation of the desired boronic ester **78**. But neither of the two diastereomers *syn* or *anti* **78** showed any splitting of signals in the ^{1}H-NMR or ^{13}C-NMR spectra.

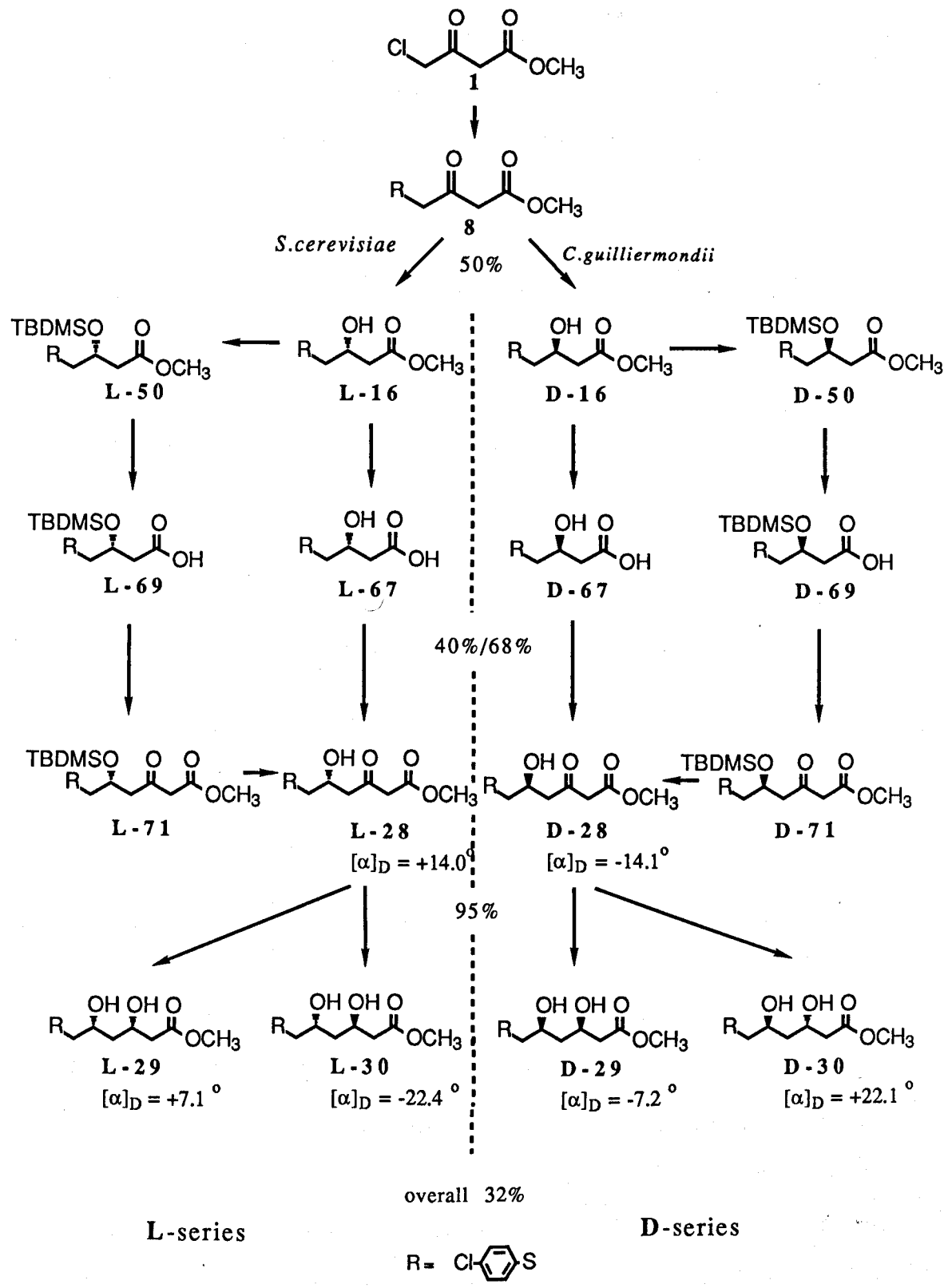
Scheme 53.

29 and **30** **78**

We have therefore no definite proof for the enantiomeric purity of the four dihydroxyesters. However starting from enantiomerically pure 5-hydroxy-3-ketoester **28**, it seems likely that the reduction proceeds without racemisation and we can argue a strong case for the products being enantiomerically pure.

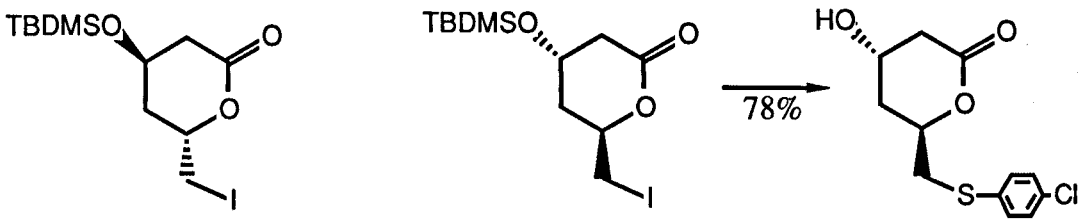
We conclude that wehave succeeded in finding a new and short route to all four stereoisomers of 6-substituted 3,5-dihydroxyhexanoates in an overall yield of 32% starting from **8**, and have summarised this in scheme 54.

Scheme 54.

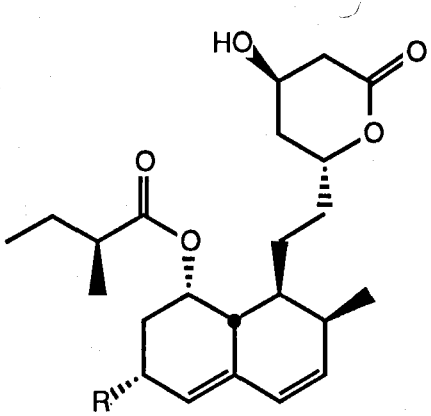

1
8
S.cerevisiae
50%
C.guilliermondii
L-50
L-16
D-16
D-50
L-69
L-67
D-67
D-69
40%/68%
L-71
L-28
[α]D = +14.0°
D-28
[α]D = -14.1°
D-71
95%
L-29
[α]D = +7.1 °
L-30
[α]D = -22.4 °
D-29
[α]D = -7.2 °
D-30
[α]D = +22.1 °
overall 32%
L-series
D-series
R=

This compares favourably with two recently published syntheses of the iodolactones **D-79** and **L-79**.[177,178] They are of great interest as building blocks for structurally simplified analogues of compactin and mevinolin, two potent inhibitors of HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis.

Scheme 55.



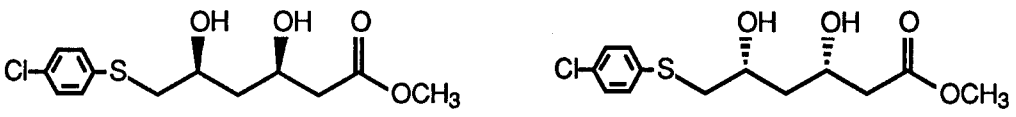
D-79 **L-79** **L-80**



R = H Compactin
R = CH_3 Mevinolin

It was shown that the biological activity is largely retained when the hexahydronaphtalene moiety is replaced by suitably substituted achiral aromatic rings and the connection with the lactone is chemically modified by the presence of an oxygen or sulfur atom. Thus the synthesis of derivative **L-80** was described. This is the δ-lactone of our **L-29.** We have compared the syntheses in scheme 56.

Scheme 56.



D-29 32% overall **L-29**
6 steps from **8**
>95% de

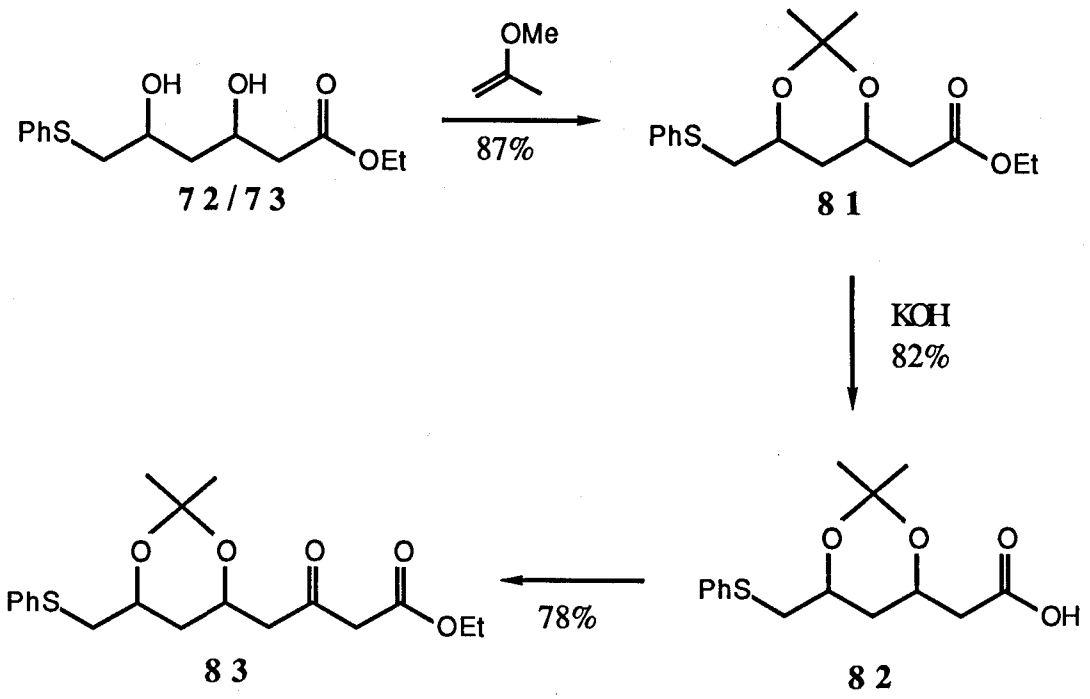
D-79
17% from glucose
17 steps
99% ee

L-80
37% with PLE hydrolysis
9 steps
70% ee

L-80 has been synthesized in 28% overall yield and 70% enantiomeric excess. This compares with 32% for its direct precursor **L-29** in >95% enantiomeric excess. Furthermore it is possible to obtain **D-29**, the direct precursor of the naturally occurring lactone, in one step, optically pure, from the racemic 5-hydroxy-3-ketoester **28** by yeast biotransformation as outlined in part 1, table 6. Both antipodal lactones had to be prepared by completely different routes, as compared to our synthesis, which uses the basic three reactions; biotransformation, chain extension and reduction. We are convinced that this comparison demonstrates the potential of our approach.

To underline the remarks made above the following compounds were synthesized, indicating a possible route to the spiroketal precursor shown in scheme 29.

Scheme 57.

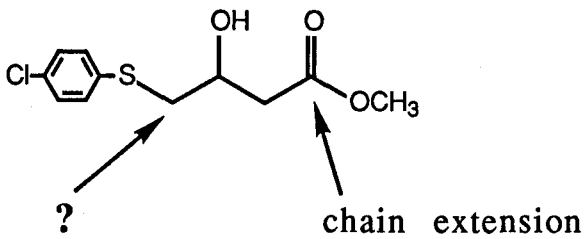


Simple protection with methoxypropene of the 3,5-dihydroxyester **72/73** afforded the isopropylidene derivative **81**, which in turn was hydrolysed to the acid **82** with potassium hydroxide in THF and converted in the usual manner to the new β-ketoester **83**. All compounds have been characterised by ^{1}H-NMR and IR and show the expected properties.

2.3. Synthesis of long chain acids.

Having already demonstrated the synthetic potential of utilising the reactivity at C-4 of our new chiral synthon **16**, we now wished to do the same for the C-4 centre.

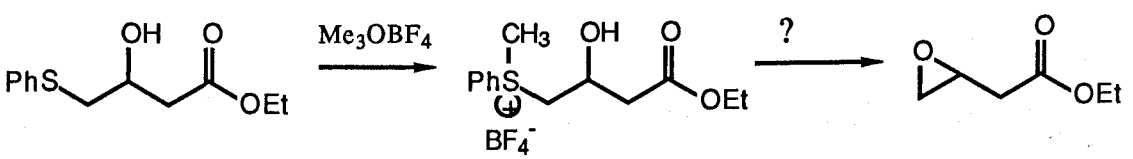
Scheme 58.



One obvious method to achieve this would be the formation of an epoxide, which then in turn could be opened by a range of nucleophiles. It is a well known fact that 2-hydroxysulfides can be converted into epoxides via sulfonium ion formation and treatment with base.[61,179]

We found that we could easily convert the starting β-hydroxyester **31** into the corresponding tetrafluoroborate sulfonium salt **84** by treatment with the Meerwein reagent trimethyloxonium tetrafluoroborate in dichloromethane.

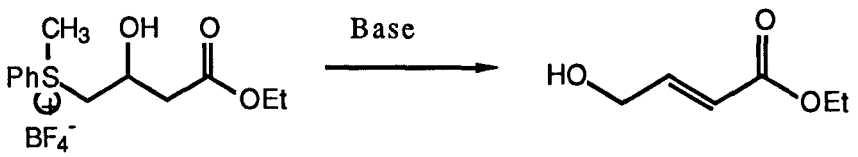
Scheme 59.



31 **84**

Isolation of **84** was possible by simply removing the solvent. However it was never possible to get the compound completely free of solvent and there were considerable difficulties in determining its exact structure. However hydrolysis of **84** yielded the starting material **31**. This excluded one of the possible side products, the formation of the methylether. We then proceeded to treat the sulfonium salt **84** *in situ* with a variety of bases summarised in table 12.

Table 12. Attempted epoxide formation *via* base treatment of the sulfoniumsalt **84**.



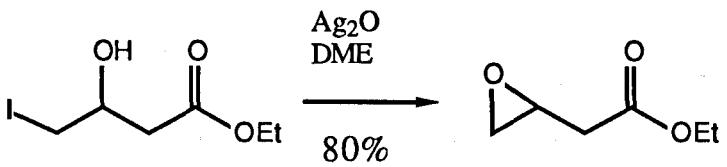
84 **85**

Base:	Product:
1M NaOH	decomposition
Et_3N	**31** (previous page)
$(i\text{-}Pr)_2NEt$	**31**
Pyridine	**31**
DMAP	**31**
1M EtONa/EtOH	**85** (10%)
Ag_2O/DME	decomposition

Unfortunately it was impossible to isolate the desired epoxide or to detect it by glc or tlc by comparison with an independently synthesized sample (from vinylaceticacid, via esterification and epoxidation with mCPBA). We either observed decomposition,

meaning that we were unable to isolate, purify and characterise a product from the reaction mixture, or reisolated the starting material **31** or ethyl 4-hydroxy-2-butenoate **85** in low yield. The formation of the α,β-unsaturated ester probably involves the epoxide as an intermediate. The extreme sensitivity of the epoxide towards base was also noted by Henrot and Larcheveque in the attempted cyclisation of the corresponding iodohydrin.[180]

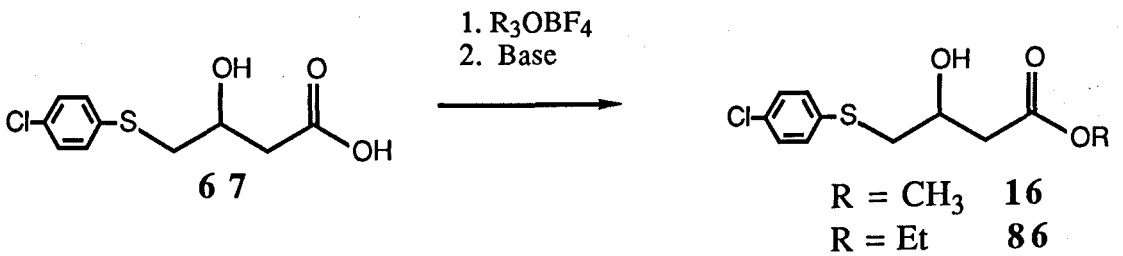
Scheme 60.



The authors only succeeded by using silver oxide in dimethoxyethanol. However we were not able to repeat this with our compound.

When we tried to render the protons on C-2 less acidic by cleaving the ester and then applying the same reaction conditions, we only observed the formation of esters **16** or **86**, or reisolated the acid **67** depending on the strength of the base used.

Scheme 61.

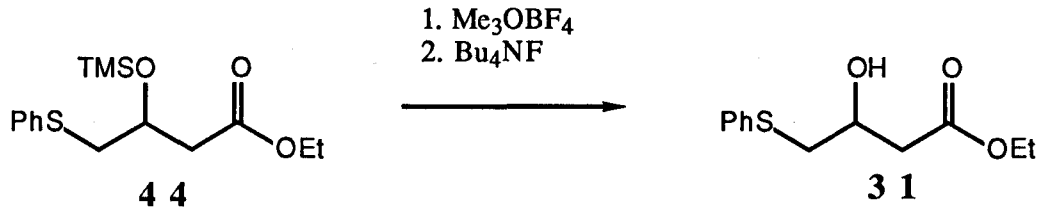


6 7

R = CH_3 **16**
R = Et **86**

In a last attempt we took the silylether **44**, converted it into the sulfonium salt and treated it with tetrabutylammonium fluoride. However we could only isolate **31**.

Scheme 62.



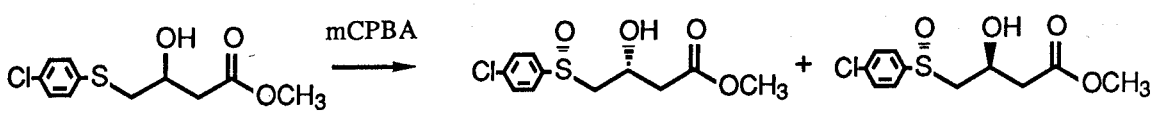
44 **31**

At the same time Tamm and coworkers[181] published the synthesis of the enantiomerically pure epoxide *via* esterase catalysed hydrolysis of the racemic ester. It was at this time that we decided to abandon this approach and to tackle the problem from a different angle.

It is well known that the dianions of β-hydroxysulfones can easily be alkylated by a variety of electrophiles.[182,183,184,185] Similar results have been obtained for β-hydroxysulfoxides,[186,187] and the stereochemical consequences of this reaction have been carefully investigated.

We hoped to create a stable carbanion on C-4 of the sulfoxide or sulfone derivative of our starting material **16**. Therefore we decided to prepare a range of derivatives of **16** with different oxidation states, protected and unprotected, as esters and acids.

Scheme 63.



16 rac-**87** rac-**88**

It was possible to oxidise the sulfide **16** with one equivalent of mCPBA at low temperature to the two diastereomeric sulfoxides **87** and **88**. One single recrystallisation afforded a separation of the product mixture and the two diastereomers virtually pure. The relative configurations of the obtained products has not yet been assigned, and they have been temporarily named A and B. Table 13 summarises the available data on the two diastereomers.

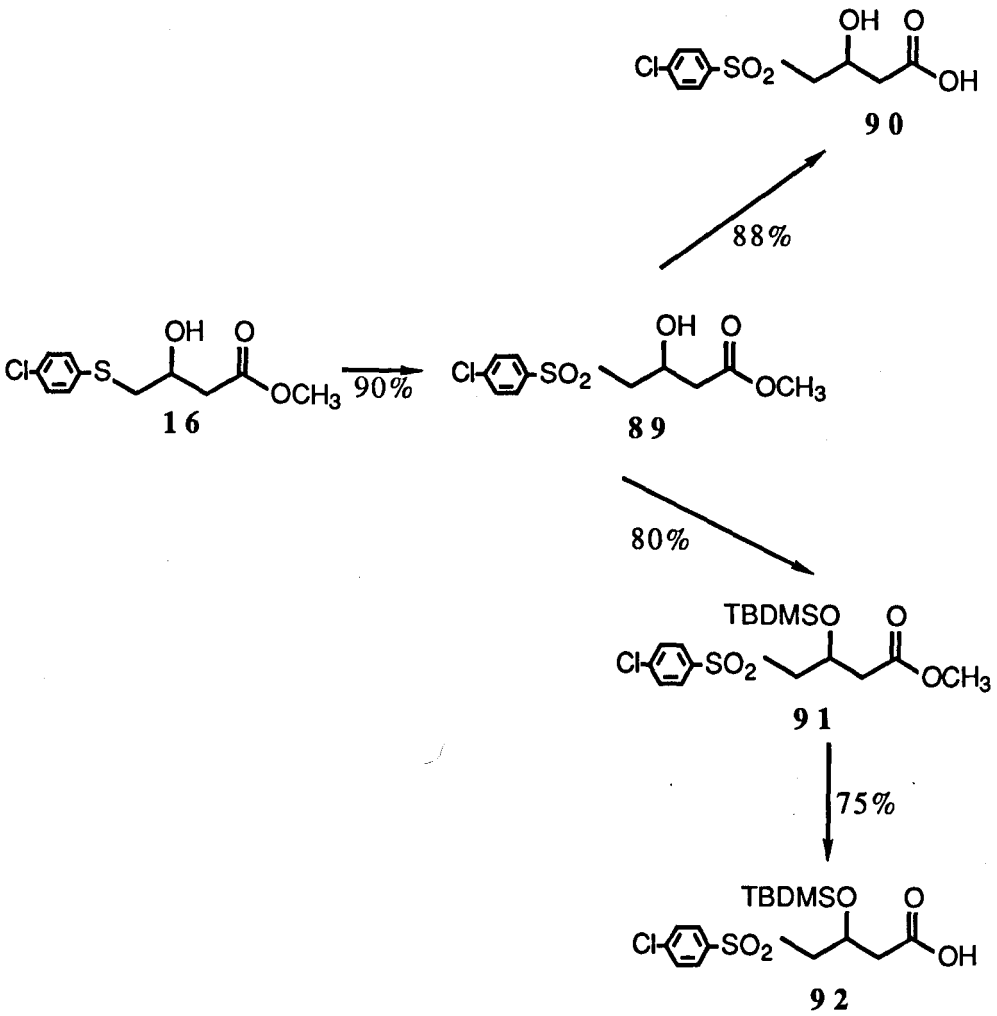
Table 13. Physical data of the two diastereomeric sulfoxide **87** and **88**.

	Solubility in ether	IR carbonyl	^{13}C-NMR C-3	^{1}H-NMR OH	^{1}H-NMR H-C3
A	high	1735cm^{-1}	64.5	4.02	4.58
B	low	1735cm^{-1}	63.0	4.29	4.68

Unfortunately it is not possible to make a definite assignment of the relative configuration from this data, but we still hope to answer this question with an X-ray crystal structure of one of the two diastereomers.

The oxidation to the sulfone proceeded smoothly and afforded a highly crystalline product, **89**. It was converted into the acid **90** or protected as the tert-butyldimethylsilylether **91**, which in turn was hydrolysed to the acid **92**.

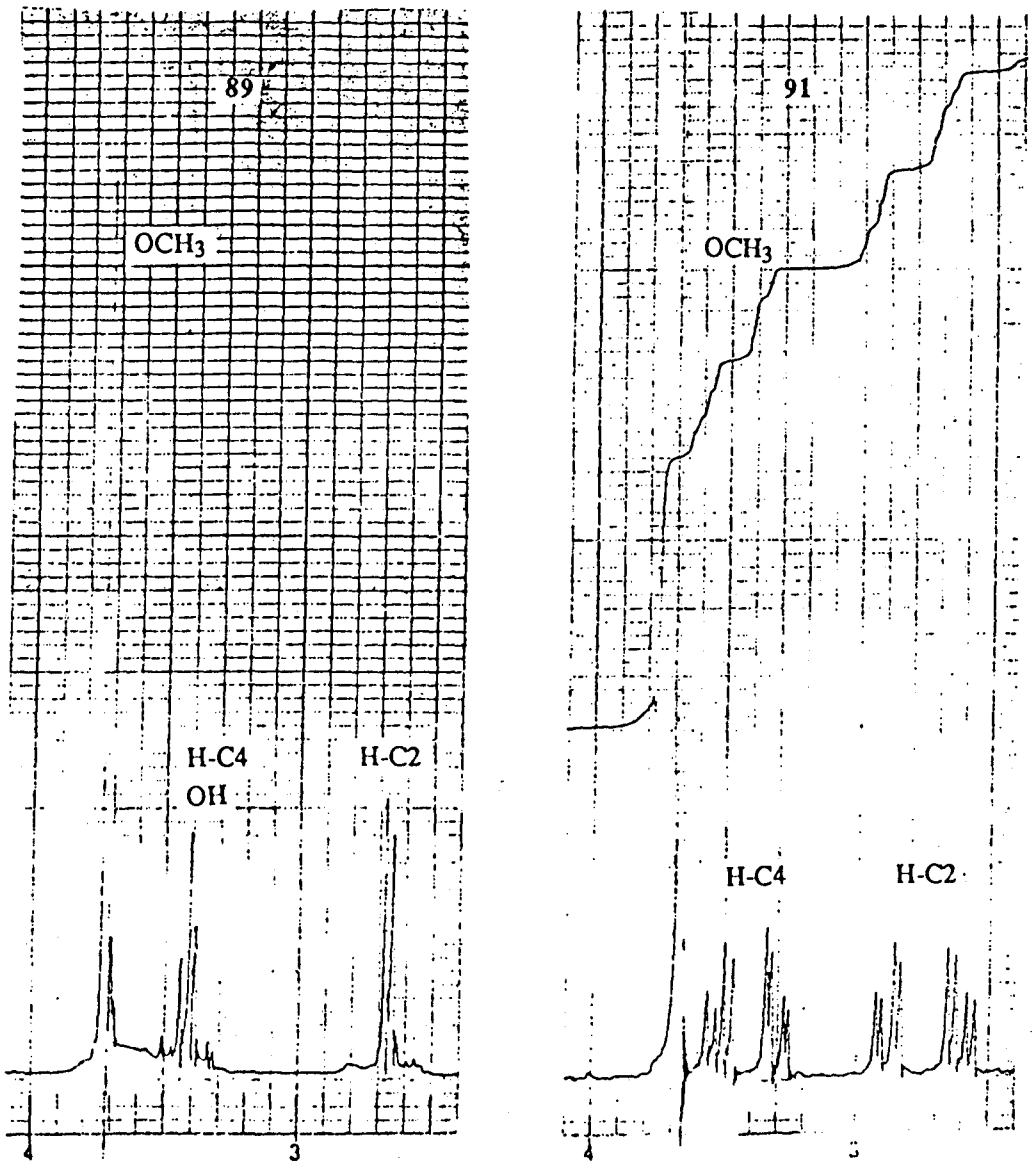
Scheme 64.



All reactions proceeded without problems in good to very good yields under standard conditions. The resulting sulfones all showed in their IR spectra a strong absorption at 1735 cm^{-1} for the carbonyl bond and strong absorptions at 1320 cm^{-1} and 1155 cm^{-1} for the asymmetric and symmetric S-O vibrations. The proton at C-3 is deshielded, and moves about 0.5 ppm downfield to 4.60 ppm in the ^{1}H-NMR. The two protons at C-2 appear at 2.65 ppm, whilst the two protons at C-4 appear at 3.40 ppm. In the unprotected derivatives **89** and **90** the coupling pattern cannot be determined, because the peaks overlap, but in the protected

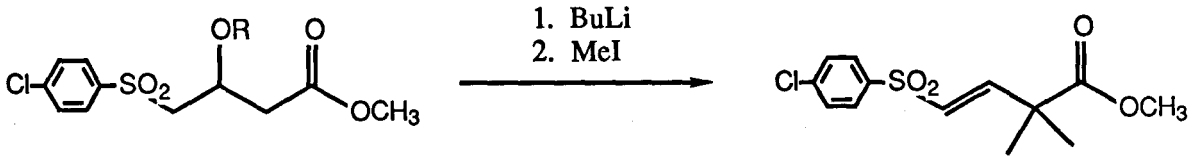
derivatives **91** and **92** the doublet of doublets is clearly visible. Because this change of the magnetic environment with protection for the two protons at the same carbon is general for **16** and its derivatives, we have illustrated it in figure 9.

Figure 9. ^{1}H-NMR spectra of **89** and **91**.



We then treated the two esters **89** and **91** with two and one equivalents respectively of butyllithium at -78°C and quenched the reaction with methyl iodide.

Scheme 65.



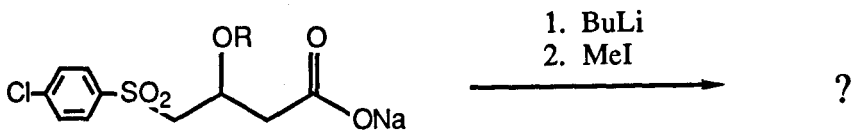
R = H **89**
R = TBDMS **91**

93

In all cases the starting material was reisolated in considerable amounts and formation of the α-adduct **93** was observed, accompanied by elimination. This indicated that the desired anion had not been formed. But when, as suggested in literature, the solution was warmed up or left longer at -78°C, the starting material rapidly decomposed and no product could then be isolated. The addition of HMPA or TMEDA did not change this behaviour.

The same experiments were performed with the sodium salts of the acids **90** and **92**. Again we either reisolated the starting material or observed a gradual decomposition when warming up the solutions. In case of the acid **90** the insolubility of the trianion in all the solvents used contributed to its unreactivity.

Scheme 66.



R = H **90**
R = TBDMS **92**

It seemed at this point that an electrophile attack on this type of molecule was definitely not the right approach and consequently all experiments on sulfones were abandoned.

The next attempt to produce useful reactivity at C-4 was the conversion of the sulfide into the iodide. This normally routine displacement of sulfur with halogen is well described in the literature.[188]

Scheme 67.

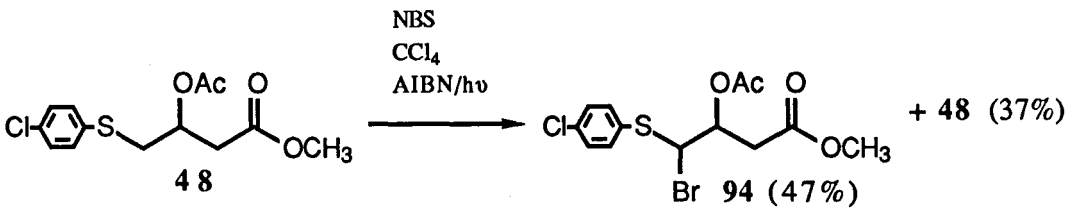
47 **16**

However, upon treatment of **47** with an excess of methyl iodide and sodium iodide in DMF, only the formation of the deprotected ester **16** in 55% yield was observed. A change in the protecting group did not result in an improvement. In the case of the acetate **48** only the starting material could be reisolated from the reaction mixture.

Here it was decided to abandon the electrophilic and nucleophilic modes of attack and to change to a radical-oriented approach. With a radical reaction on substrate **16**, or one of its derivatives, it should be possible to avoid all the problems associated with the previously mentioned reactions.

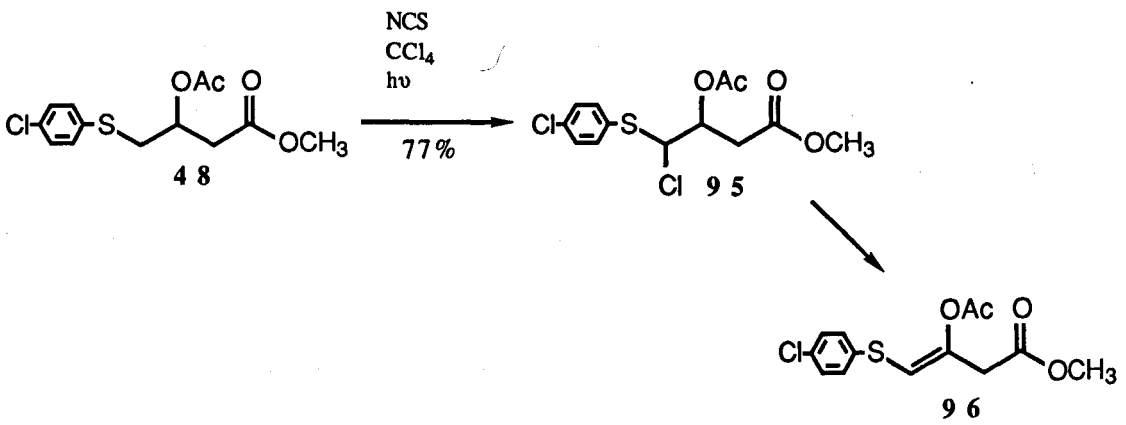
We therefore submitted the unprotected hydroxyester **16** and its acetate **48** to common radical reaction conditions.[189,190]

Scheme 68.



The acetate **48** could be brominated with N-bromosuccinimide in 47% yield. It was not possible to increase the yield by variation of the reaction conditions and starting material was always reisolated. However the corresponding chlorination gave a yield of 77% of isolated product.

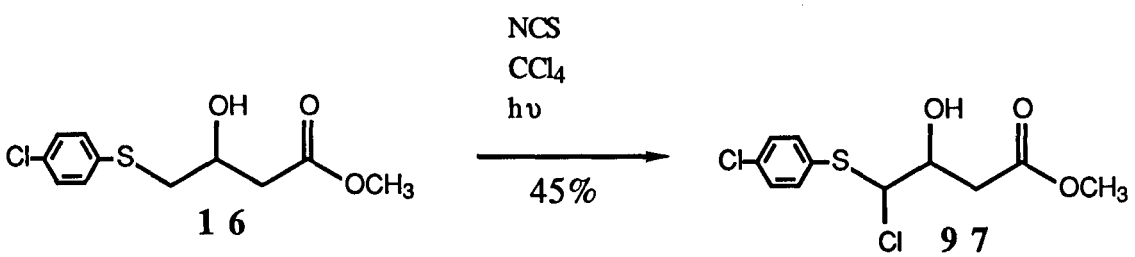
Scheme 69.



It was found that the halogenated esters were extremely difficult to isolate, decomposing on silica gel columns to **96**. Only when kept in the dark under nitrogen at low temperature was it possible to store **95** for several days without decomposition. We were not able to obtain satisfactory microanalytical and mass spectrometric data, but were satisfied with the spectral data described later.

The reaction with unprotected ester **16** proceeded much faster then with the protected one.

Scheme 70.



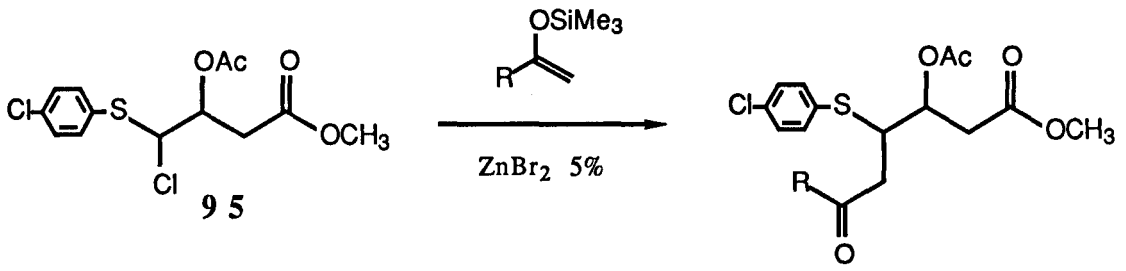
The product **97** was even more unstable and it was difficult to reproduce the reaction with constant yields. It was decided therefore to optimise the chlorination of acetate **48**. The optimal reaction conditions were achieved in anhydrous carbon tetrachloride, 1.3 equivalents of N-chlorosuccinimide, a 100W bulb as light source and a temperature of 55°C. The reaction mixture was cooled overnight to -10°C, filtered and then worked up. If carefully controlled, yields up to 90% could be achieved.

The chlorinated products show a strong absorption at 1745 cm^{-1} in their IR spectrum, and two doublets at 5.03 ppm and at 5.08 ppm in the ^{1}H-NMR spectrum for H-C4 in a ratio of about 1:2 and with a coupling constant of J_{34}=4 Hz. The structure of the byproduct **96** can only be assigned tentatively. The IR spectrum shows the typical 1745 cm^{-1} carbonyl absorption, whilst the ^{1}H-NMR spectrum clearly has peaks for the aromatic protons, the methylester and the acetate protons. The H-C4 olefinic proton appears at 5.95 ppm as expected as a doublet of doublets with one coupling constant of J_{2a4}=2 Hz, and the other one of J_{2b4}=7 Hz. This is very large for an allylic coupling constant. H_a-C2 appears at 3.35

ppm and H_b-C2 at 2.98 ppm with a geminal coupling constant of J_{ab}=14 Hz.

The chlorinated acetate **95** was then coupled with 2-silylenolethers under zinc bromide catalysis to yield the 6-keto esters **98** and **99**.

Scheme 71.

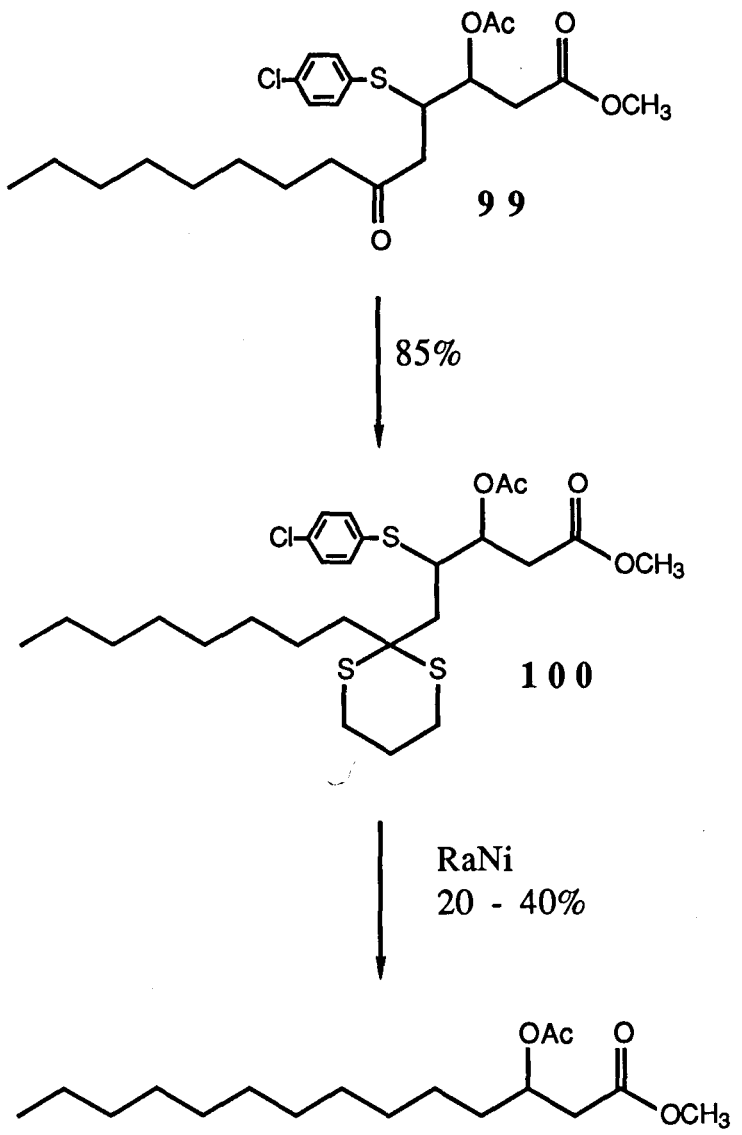


R = Ph **98** 45%
R = n-C_8H_{17} **99** 80%

With this reaction we succeeded at last in forming a new carbon-carbon bond at C-4. The products **98** and **99** showed a 1745 cm^{-1} and 1720 cm^{-1} carbonyl absorption in the IR spectrum, and the ^{1}H-NMR spectrum showed a chemical shift of 5.5 ppm for H-C3 and 4.0 ppm for H-C4. The four protons at C-2 and C-5 appear between 2.7 ppm and 2.9 ppm.

The adduct **99** was then converted into its cyclic thioacetal **100** with propane dithiol in 85% yield and desulfurised with RaNi-W7 to the β-acetoxyester **101**.

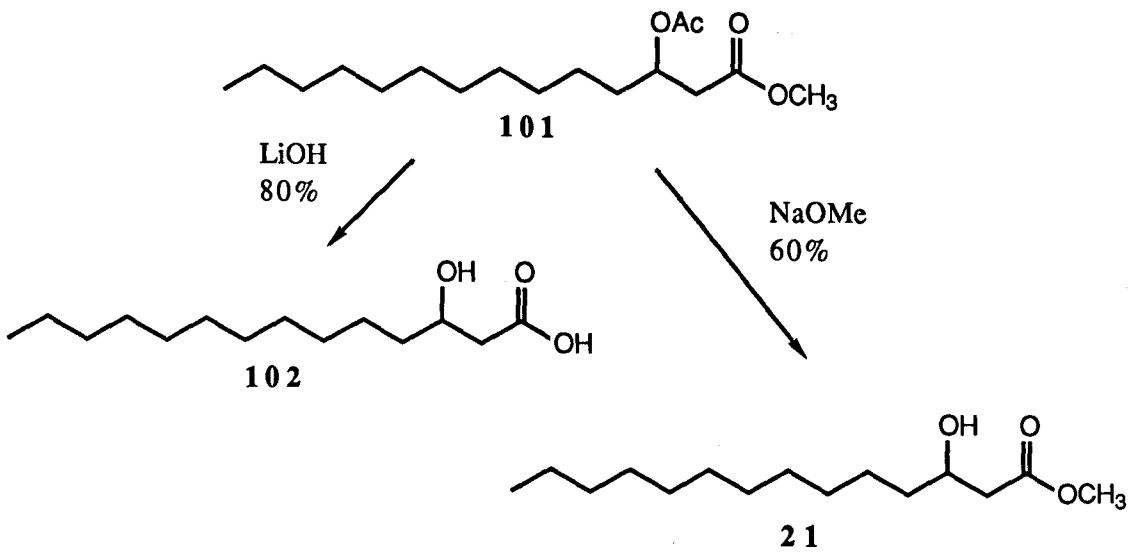
Scheme 72.



99 **100** **101**

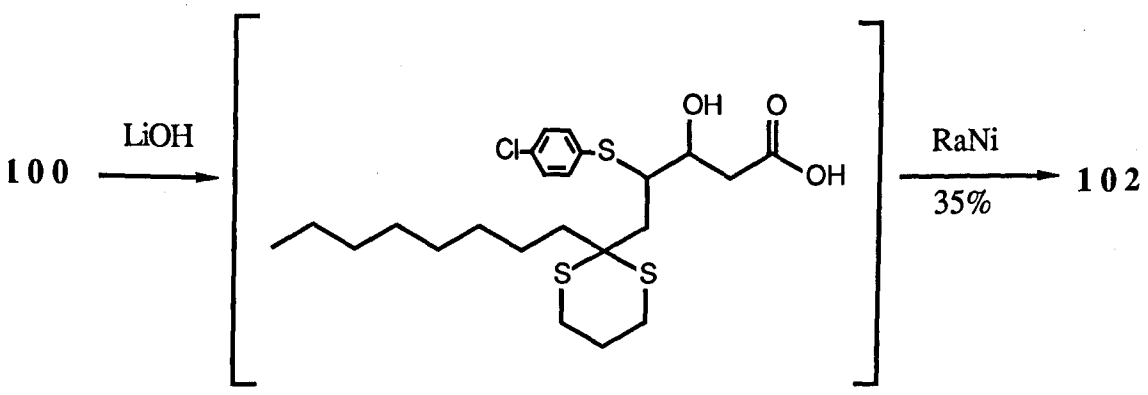
101 could then be hydrolysed to the β-hydroxyacid **102** or selectively deprotected to **21**.

Scheme 73.



The problematic step in this synthesis is the desulfurisation of **100**, which proceeds only in very low yield probably owing its low solubility in methanol. We therefore decided to deprotect **100** first and then desulfurise it in aqueous sodium bicarbonate. This reaction sequence proceeded in slightly higher yield, but was still not entirely satisfactory.

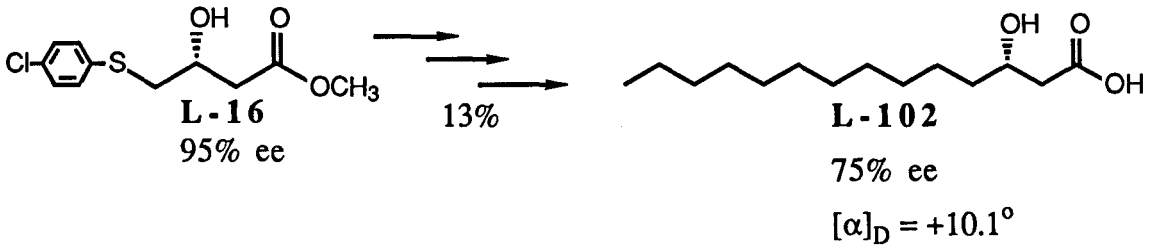
Scheme 74.



This reaction sequence, especially the desulfurisation, remains unoptimised, as we were mainly interested in demonstrating the formation of a new carbon-carbon bond and in obtaining a small

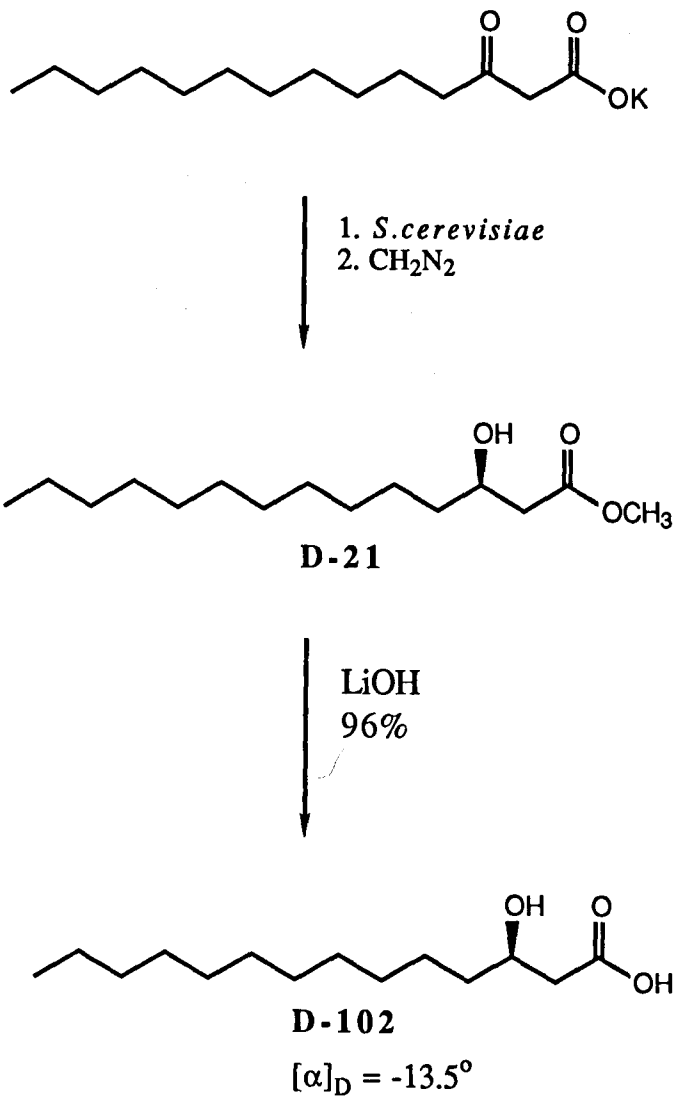
sample of the optically active acid **102**. This was done starting from **L-16** in the manner described and gave a 13% overall yield of the acid **L-102**.

Scheme 75.



The enantiomeric excess was determined with europium D-3-trifluoroacetylcamphorate in deuterobenzene on the β-hydroxyester **L-21**. The observed partial racemisation could have occurred either in the chlorination or in the desulfurisation steps. As we are not able to determine the enantiomeric purity of the intermediates, we cannot give a definite answer to this question. The sign of the optical rotation is opposite to that of the compound obtained by yeast reduction and hydrolysis (see schemes 21 and 76), and corresponds to examples of similar compounds in literature.[53]

Scheme 76.

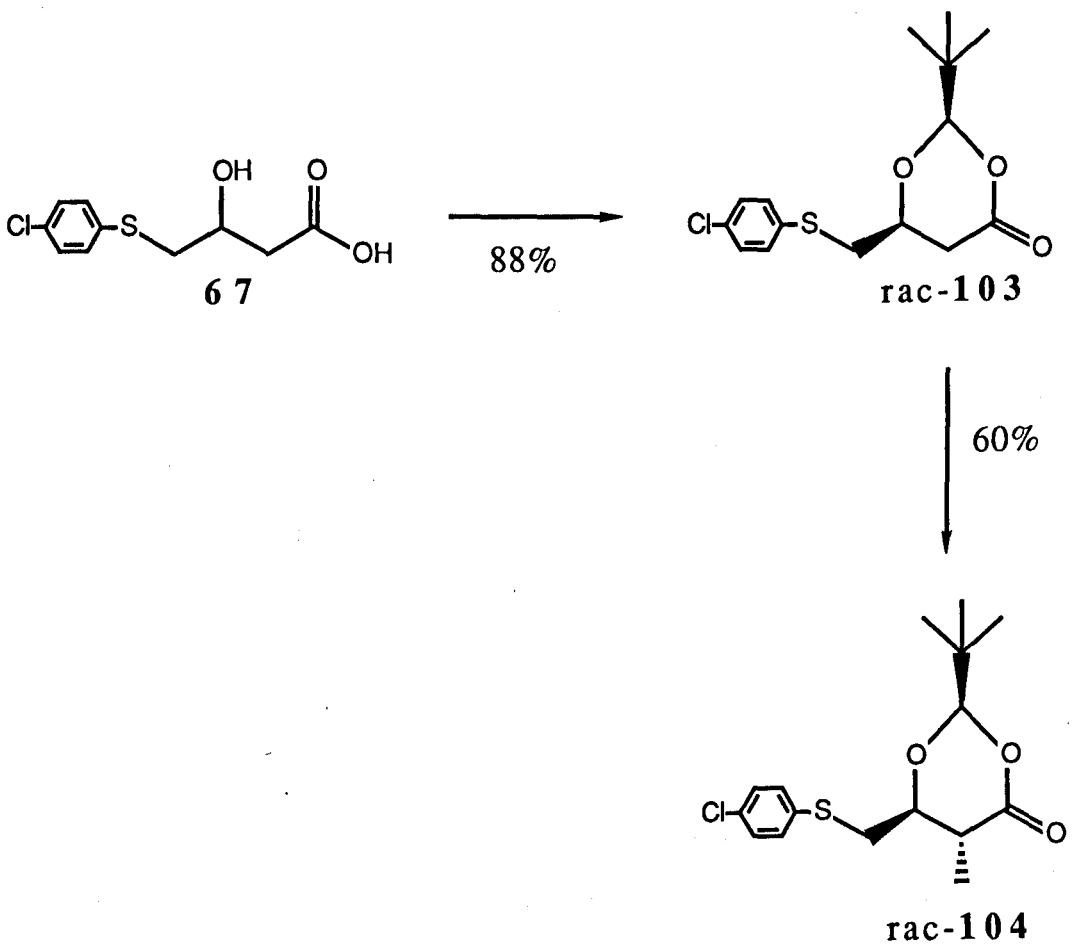


This type of fatty acid is of considerable interest, and appears as a substructure in a range of natural products. The D-enantiomer, for instance, forms the sole fatty acid substituent in Lipid X, a monosaccharide with antibacterial activity.[191] **102** has also been found to be the main fatty acid constituent of surfactin, the most efficient biosurfactant sofar known.[192] This is a cyclic peptide, which consists out of 7 aminoacids and one β-hydroxyacid of unknown absolute configuration.

We are convinced that the intermediate α-chlorosulfide, an aldehyde equivalent, possesses great potential in organic synthesis and were satisfied to have found a solution to the problem of the reactivity at C-4.

The only remaining problem was now a controlled addition on C-2. As mentioned before, we had already observed electrophilic addition at C-2 leading to byproducts, and we therefore anticipated no problems in the deprotonation at C-2 of a suitable intermediate. This reaction is known for ethyl β-hydroxybutanoate, and we adapted an improved procedure by Seebach and coworkers.[193,194,195] The acid **67** was converted with pivaldehyde into the corresponding 1,3-dioxanone **103**.

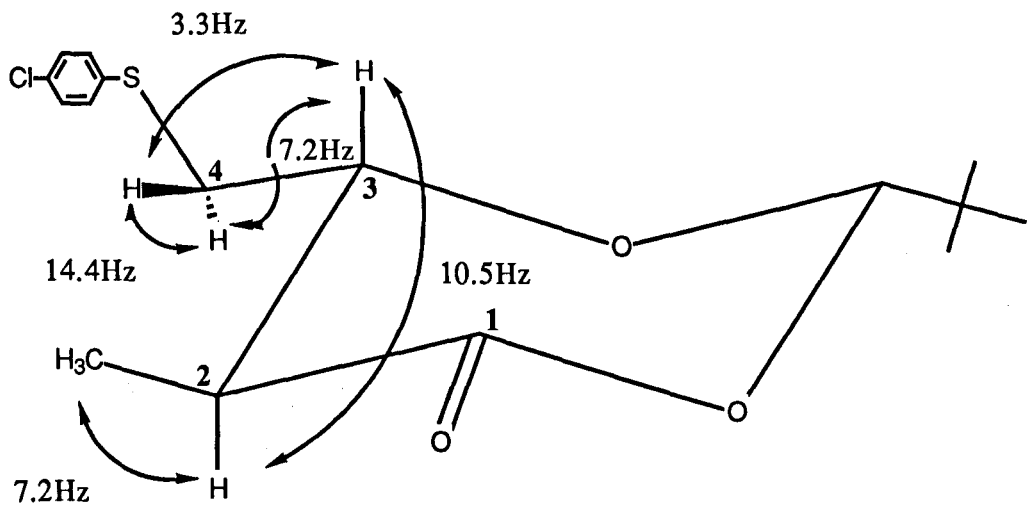
Scheme 77.



67 rac-**103** rac-**104**

The 2-(tert-butyl)-1,3-dioxanone **103** is a stable, highly crystalline compound, which was found to be diastereomerically pure after one recrystallisation. It could easily be deprotonated at the α-position and alkylated with methyl iodide. All products were completely characterised. They showed a strong carbonyl absorption at 1745 cm^{-1} in their IR Spectra. Their ^{1}H-NMR spectra have been analysed in the following way. H-C3 appears at 4.01 ppm for **103** and is shifted in **104** slightly upfield to 3.70 ppm as a doublet of doublets of doublets with J=3.3, 7.2 and 10.5 Hz. The two protons at C-4 appear at 3.09 ppm in **103** and at 3.14 ppm for **104**. The two protons at C-2 in **103** have a shift of 2.56 ppm and are reduced to one at 2.59 ppm in **104**. Additionally there is a methyl group in **104** at 1.23 ppm as a doublet with J=7.2 Hz. The coupling constants for **104** are illustrated in figure 10.

Figure 10. Coupling constants of dioxanone **104**.



Of course, the assignment of the coupling constants between H-C4 and H-C3 is purely arbitrary. It is not possible, from the available data, to decide whether the pro-*S* or the pro-*R* proton has the large or small coupling. In none of the ^{1}H-NMR or ^{13}C-NMR spectra

is there any indication of the other diastereomer, which has presumably distinctly different properties. Although we did not attempt to hydrolyse our dioxanone **104**, the hydrolysis of the dioxanone to the acid is well described in the literature[193,194,195] and proceeds under mild acid catalysis. Furthermore it is possible, according to the same reference, to deprotonate the alkylated product, quench the anion with trimethylsilylchloride and hydrolyse the resulting silylenolether selectively to yield the *syn* diastereomer in about 85% de. We did not, however, attempt this with our dioxanone **104**.

2.4. Conclusions.

We have demonstrated that it is possible to determine rationally the outcome of a biotransformation with yeasts. By observing the basic rules and considering the criteria listed below, which influence the stereochemical course, one can easily optimise a biotransformation with oxidoreductases in whole cells:

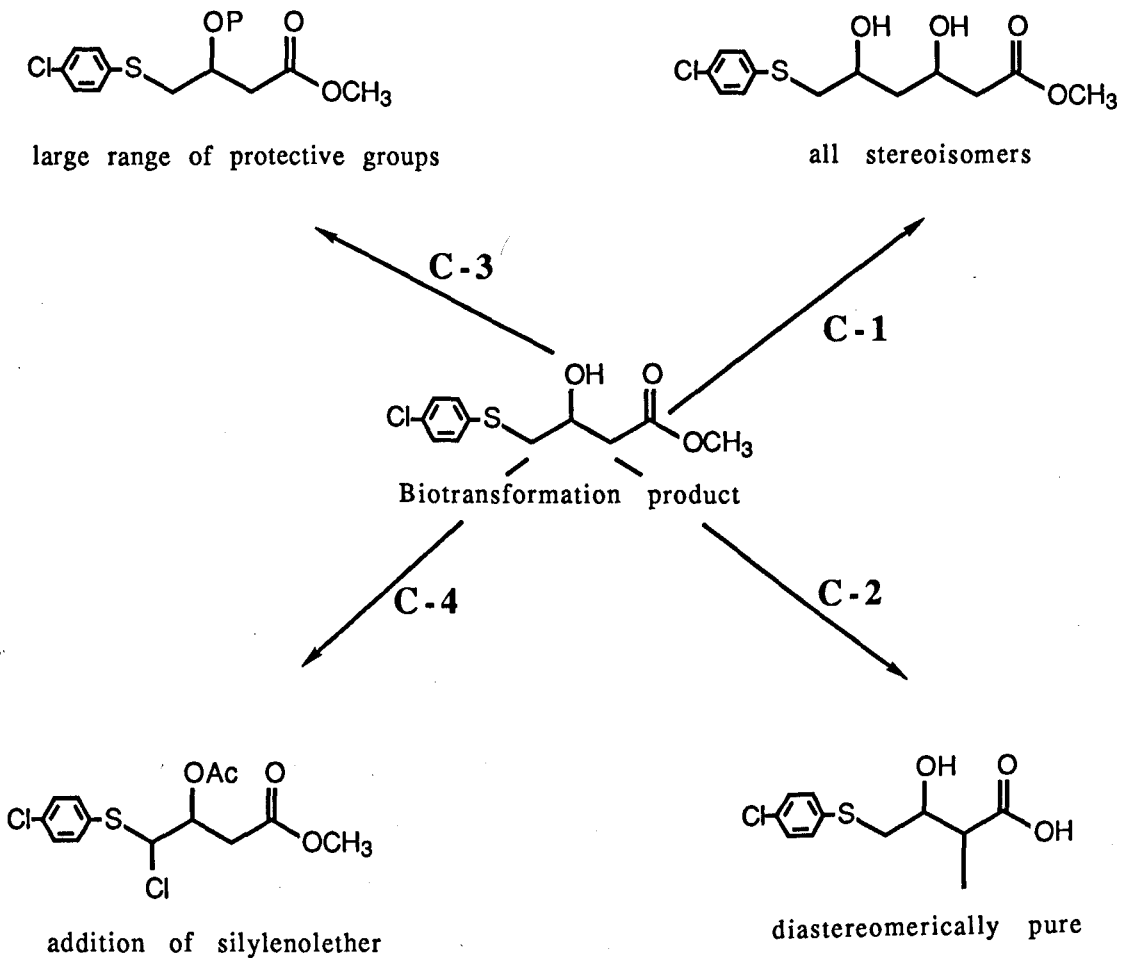
- -screening of microorganism
- -redesign of substrate
- -introduction of auxiliary groups
- -increasing the difference in rates of reaction of competing enzymes by:
  - -slow addition of substrate
  - -immobilisation of cells
  - -selective inhibition of one enzyme
- -variation of growing condition

However yeast reductions are still very much treated as a black box chemistry, a situation hopefully changing rapidly since the isolation of enzymes from *S.cerevisiae* with the desired activity. We were able to produce a new chiral synthon in both enantiomeric forms and in good enantiomeric purity by using two different yeast strains. This chiron was then converted into a range of products displaying reactivity on all carbon atoms.

We synthesized all four stereoisomers of methyl 3,5-dihydroxyhexanoate, a useful intermediate in the synthesis of spiroacetals. This approach is general, and can be extended to the synthesis of long-chain polyoxygenated esters. We have further shown that chlorination at the 4-position introduces a new

dimension to the reactivity pattern of the starting material. The full potential of α-chlorosulfides, an aldehyde equivalent, has not yet been completely explored, but the formation of a new carbon-carbon bond has been accomplished. The resultant product was further converted into a useful natural product. Alkylation at C-2 was easily achieved by the formation of a dioxanone as described in literature. We have summarised the different routes in scheme 78.

Scheme 78.



Part 3: Experimental details.

3.1. Introduction.

All chemicals, including solvents were purified according to literature methods.[196] Nuclear magnetic resonance spectra were recorded using the instruments listed below operating at the frequencies given in the table 14.

Table 14. Operating frequencies of spectrometer.

Spectrometer	Frequency/MHz	
	^{1}H	^{13}C
Bruker WH 400	400.13	100.62
Bruker WH 180		45.28
Bruker WH 90		22.63
Perkin-Elmer R34	220	

Chemical shifts, unless otherwise stated, are quoted in ppm downfield from a tetramethylsilane internal reference. Mass spectra were recorded using a Kratos MS 80 spectrometer. Infra red spectra were recorded using a Perkin-Elmer 580-B spectrophotometer and a sample concentration of 5%. Ultra violet spectra were recorded using a Shimadzu UV-365 spectrophotometer. Optical rotations were measured on an "Optical Activity Ltd." AA-1000 polarimeter at 589nm in a 2dm path length cell. Circular dichroism spectra were recorded by Dr A.F.Drake, Birkbeck College, London, using a Jasco J600 spectropolarimeter. Melting points were determined using a Gallenkamp apparatus and are quoted uncorrected. Gas-liquid chromatographic analysis was performed using a Pye 204 gas chromatograph. The columns used were 1.8 meters in length.

Nitrogen was used as the carrier gas at a flow rate of 30ml/min. High pressure liquid chromatography was performed using a Gilson system comprising the following components; a model 302 piston pump, a model 802C manometric module and an HM holochrome UV/VIS detector. Thin layer chromatography was performed on Merck Kieselgel F_{254} 0.2mm pre-coated glass plates. Spot detection was by one or more of the following methods:[197]

- UV fluorescence quenching
- exposure to iodine vapour
- 5% potassium permanganate in water followed by gentle heating
- 10% phosphomolybdic acid in ethanol followed by heating to 150°C
- 10% vanillin in a solution of 5% sulfuric acid in ethanol (95%) followed by gentle heating (β-ketoesters)
- saturated solution of o-dianisidine in acetic acid (aldehydes)
- methyl red (0.02g) in ethanol (60ml) and water (40ml) (acids)

Flash chromatography was performed on Merck Kieselgel 60 silica gel (230-400mesh).[198] Ether refers to diethyl ether and petrol to the petroleum fraction boiling in the range 40-60°C. The concentration of butyllithium in hexane solutions was determined according to Juaristi.[199,200] Solvents were removed in a Buchi rotary evaporator at a maximum temperature of 45°C.

3.2. Experiments of part 1.

General procedure for the synthesis of 4-sulfur substituted β-ketoesters:

Freshly distilled 4-chloroacetoacetate **1** (27.5g, 0.25mol) was added dropwise to a stirred solution of the thiol (0.25mol) in dry pyridine (30ml, 0.37mol). The mixture was stirred for two hours followed by addition of ether (100ml) and water (75ml). The organic layer was washed with 1M aqueous hydrochloric acid (4x50ml) and dried ($MgSO_4$). Evaporation of the solvent afforded the product as a light yellow oil. Purification was achieved by flash chromatography (ethyl acetate:petrol=1:9) or distillation.

Methyl 4-(ethylthio)-3-oxobutanoate **3**:

Yield: 70%

bp: 105°C (0.1mmHg)

IR($CHCl_3$): 2980w, 2960w, 2940w, 1750s, 1715s, 1660w, 1630w, 1455w, 1440m, 1410w, 1326m, 1150m, 1085w, 1015w

^{1}H-NMR(220MHz,$CDCl_3$): 3.79(s,3H,OCH_3), 3.74(s,2H,H-C4), 3.39(s,2H,H-C2), 2.53(q,J=7.3,2H,H-C1'), 1.26(t,J=7.3,3H,H-C2')

^{13}C-NMR(45.28MHz,$CDCl_3$): 197.8(C3), 167.5(C1), 52.2(OCH_3), 45.8(C2), 40.7(C4), 25.9(C1'), 14.0(C2')

MS(EI): m/z = 176$(M)^{+\cdot}$, 145$(M\text{-}OCH_3)^+$, 116, 102, 75

Elemental analysis: Found: C 47.95 H 7.14 S 18.14

$C_7H_{12}O_3S$ Calc: C 47.71 H 6.86 S 18.19

Methyl 4-(propylthio)-3-oxobutanoate **4**:

Yield: 46%

bp: 115°C (0.1mmHg)

IR($CHCl_3$): 2970m, 2940w, 2880w, 1750s, 1715s, 1660w, 1630w, 1450w, 1440m, 1410w, 1325m, 1150m, 1090w, 1015w

^{1}H-NMR(220MHz,$CDCl_3$): 3.78(s,3H,OCH_3), 3.71(s,2H,H-C4), 3.35(s,2H,H-C2), 2.47(t,J=7.3,2H,H-C1'), 1.61(qt,J=7.3,2H,H-C2'), 0.98(t,J=7.3,3H,H-C3')

^{13}C-NMR(45.28MHz,$CDCl_3$): 197.8(C3), 167.6(C1), 52.3(OCH_3), 45.9(C2), 41.1(C4), 34.0(C1'), 22.2(C2'), 13.2(C3')

MS(EI): m/z = 190$(M)^{+\cdot}$, 159$(M\text{-}OCH_3)^+$, 116, 101, 89

Elemental analysis: Found: C 50.62 H 7.62 S 17.09

$C_8H_{14}O_3S$ Calc: C 50.50 H 7.47 S 16.85

Methyl 4-(butylthio)-3-oxobutanoate **5**:

Yield: 66%

IR($CHCl_3$): 2965m, 2940m, 2880w, 1750s, 1715s, 1660w, 1630w, 1440m, 1410w, 1330m, 1020w

^{1}H-NMR(220MHz,$CDCl_3$): 3.78(s,3H,OCH_3), 3.72(s,2H,H-C4), 3.36(s,2H,H-C2), 2.49(t,J=7.4,2H,H-C1'), 1.56(m,2H,H-C2'), 1.41(m,2H,H-C3') 0.91(t,J=7.2,3H,H-C4')

^{13}C-NMR(45.28MHz,$CDCl_3$): 197.8(C3), 167.6(C1), 52.3(OCH_3), 45.9(C2), 41.2(C4), 31.8(C1'), 31.0(C2'), 21.9(C3'), 13.6(C4')

MS(EI): m/z = 204$(M)^{+\cdot}$, 173$(M\text{-}OCH_3)^+$, 130, 116, 101

Elemental analysis: Found: C 53.06 H 7.80 S 15.70

$C_9H_{16}O_3S$ Calc: C 52.92 H 7.89 S 15.65

Methyl 4-(pentylthio)-3-oxobutanoate **6**:

Yield: 42%

IR($CHCl_3$): 2980m, 2940m, 2865w, 1750s, 1715s, 1660w, 1630w, 1440m, 1410w, 1330m, 1015w

^{1}H-NMR(220MHz,$CDCl_3$): 3.79(s,3H,OCH_3), 3.73(s,2H,H-C4), 3.38(s,2H,H-C2), 2.50(t,J=7.2,2H,H-C1'), 1.60(m,2H,H-C2'), 1.36(m,4H,H-C3',C4'), 0.91(t,3H,H-C5')

^{13}C-NMR(45.28MHz,$CDCl_3$): 197.8(C3), 167.6(C1), 52.3(OCH_3), 45.9(C2), 41.2(C4), 32.0, 31.9, 28.6, 22.2, 13.9

MS(EI): m/z = 218$(M)^{+\cdot}$, 187$(M-OCH_3)^+$, 144, 116, 101

Methyl 4-(phenylthio)-3-oxobutanoate **7**:

Yield: 70%

IR($CHCl_3$): 2960w, 1750s, 1720s, 1660w, 1630w, 1485w, 1445m, 1405w, 1330m, 1030w

^{1}H-NMR(220MHz,$CDCl_3$): 7.43-7.28(m,5H,H-aromat), 3.83(s,2H,H-C4), 3.73(s,3H,OCH_3), 3.68(s,2H,H-C2)

^{13}C-NMR(45.28MHz,$CDCl_3$): 197.8(C3), 167.4(C1), 133.8, 129.8, 129.2, 127.2(C-aromat), 52.3(OCH_3), 46.3(C2), 43.9(C4)

MS(EI): m/z = 224$(M)^{+\cdot}$, 193$(M-OCH_3)^+$, 150, 123, 109

Methyl 4-(p-chlorophenylthio)-3-oxobutanoate **8**:

Yield: 85%

mp: 49-50°C

IR($CHCl_3$): 2940w, 1745s, 1715s, 1650w, 1620w, 1480m, 1325m, 1095s, 1015s

^{1}H-NMR(220MHz,$CDCl_3$): 7.32(s,4H,H-aromat), 3.83(s,2H,H-C4), 3.78(s,3H,OCH_3), 2.69(s,2H,H-C2)

^{13}C-NMR(45.28MHz,$CDCl_3$): 197.0(C3), 167.0(C1), 133.1, 132.4, 131.1, 129.1(C-aromat), 52.2(OCH_3), 46.1(C2), 43.8(C4)

MS(EI): m/z= 258$(M)^{+\cdot}$, 226$(M-OCH_3)^+$, 200, 184, 157

Elemental analysis: Found: C 51.31 H 4.33 Cl 14.58 S 12.29

$C_{11}H_{11}O_3ClS$ Calc: C 51.07 H 4.29 Cl 13.70 S 12.39

UV(Acetonitrile): 220(7200), 257(10500)

Ethyl 4-(phenylthio)-3-oxobutanoate **9**:

Yield: 73%

IR($CHCl_3$): 2990w, 1735s, 1720s, 1660w, 1630w, 1590w, 1485w, 1445w, 1375w, 1325m, 1030m

^{1}H-NMR(220MHz,$CDCl_3$): 7.45-7.20(m,5H,H-aromat), 4.19(q,J=7.6,2H,OCH_2CH_3), 3.82(s,2H,H-C4), 3.66(s,2H,H-C2), 1.28(t,J=7.6,3H,OCH_2CH_3)

^{13}C-NMR(45.28MHz,$CDCl_3$): 197.8(C3), 167.0(C1), 134.1, 129.8, 129.2, 127.1(C-aromat), 61.4(OCH_2CH_3), 46.5(C2), 44.0(C4), 14.0(OCH_2CH_3)

General Procedure for the reduction of β-ketoester with sodium borohydride:

To a solution of the β-ketoester (10mmol) in THF (100ml) and methanol (25ml) at -20°C (CCl_4/CO_2) was added sodium borohydride (5mmol). After 2h the reaction mixture was quenched with 50ml 1M aqueous hydrochloric acid and extracted with ether. The organic phase was dried ($MgSO_4$) and the solvent removed in vacuo. Purification was achieved by recrystallisation or flash chromatography.

General procedure for the reduction of β-ketoesters with free or immobilised cells:

Microorganisms and cultivation conditions:

A pure strain of *S.cerevisiae* was obtained locally and deposited in the national collection of yeast cultures as NCYC 1765. All other

strains were obtained from the NCYC. Typically cultivation was carried out at 30°C in conical flasks in a rotary shaker at 100 r.p.m. in Difco YM broth. Growth was followed by determination of the optical density at 630 nm. The cells were immobilised in the last stage of the growth phase.

Immobilisation on alginate:

To 100ml of culture broth containing about 0.5g of wet cells, was added sodium alginate (0.5g). The suspension was dropped into calcium chloride solution (100ml, 10% w/v). The gel beads (20g, 4-5mm in diameter) were collected and washed with water. The beads could stored at 4°C in 5mM calcium chloride for several months, if the calcium chloride solution was regulary replaced (every two weeks) with freshly sterilised solutions.

Immobilisation on celite:

To 100ml of culture broth containing about 0.5g of wet cells, was added Celite 630 (2.5g) and the suspension was stirred for 3h. The celite was collected by filtration, washed with water and stored in phophate buffer (0.1M, pH 6.0) at 4°C. The buffer was replaced with freshly sterilised solution every two weeks.

Reduction with free cells:

To 100ml of the culture broth (0.5g of wet cells) was added sucrose (100mg, 0.3mmol), Triton X-100 (50mg, 0.08mmol) and substrate (0.1mmol) in 5ml of cosolvent. When the reaction was judged to be complete (by tlc), celite 535 (2g) was added. The resulting suspension was filtered and the filtrate was extracted three times with ether. The combined organic extracts were dried

($MgSO_4$), filtered and the solvent removed *in vacuo*. The residue was dried under high vacuum. If necessary, products were separated and purified by flash chromatography.

Reductions with immobilised cells:

To alginate beads (20g) or celite 630 (2.5g) in 45ml of 5mM calcium chloride solution or 0.1M phosphate buffer (pH6) respectively, was added sucrose (100mg, 0.3mmol), Triton X-100 (50mg, 0.08mmol) and substrate (0.1mmol) in cosolvent (5ml). On completion of the reaction, the mixture was filtered, evaporated and dried as above.

Further experimental details:

Reactions were monitored by thin layer chromatography on silica gel using the solvent system ethyl acetate:petrol=1:1. HPLC analysis was carried out on a C-18 reversed-phase column (Spherisorb S5 ODS2) in acetonitrile water (7:3). Concentrations were determined using a calibration curve constructed at 254nm. Enantiomeric excesses were determined by ^{1}H-NMR in deuterobenzene using the chiral shift reagents ytterbium or europium (D-3-trifluoroacetyl camphorate)$_3$. Additionally, optical rotations were determined in those cases in which the rotations of the pure enantiomers were known.

Results: see table 2 (page 40) and table 3 (page 45).

Methyl 4-(ethylthio)-3-hydroxybutanoate **11**:

IR($CHCl_3$): 3530br, 2980w, 2960w, 2940w, 1735s, 1440s, 1335m, 1050m

^{1}H-NMR(220MHz,$CDCl_3$): 4.16(m,1H,H-C3), 3.75(s,3H,OCH_3), 3.20(d,1H,OH), 2.8-2.5(m,6H), 1.29(t,J=7.8,3H,H-C2')

^{13}C-NMR(45.28MHz,$CDCl_3$): 172.5(C1), 66.9(C3), 51.8(OCH_3), 40.2(C2), 38.3(C4), 26.4(C1'), 14.8(C2')

Methyl 4-(propylthio)-3-hydroxybutanoate **12**:

IR($CHCl_3$): 3530br, 2980w, 2960w, 2940w, 1730s, 1440s, 1330m, 1050m

^{1}H-NMR(220MHz,$CDCl_3$): 4.16(m,1H,H-C3), 3.75(s,3H,OCH_3), 3.20(d,J=4.8,1H,OH), 2.8-2.5(m,6H), 1.63(qt,J=7.4,2H,H-C2'), 1.01(t,J=7.4,3H,H-C3')

^{13}C-NMR(45.28MHz,$CDCl_3$): 172.7(C1), 67.1(C3), 52.1(OCH_3), 40.4(C2), 38.9(C4), 34.8(C1'), 23.3(C2'), 13.6(C3')

Methyl 4-(butylthio)-3-hydroxybutanoate **13**:

IR($CHCl_3$): 3530br, 2980m, 2960w, 2930w, 1735s, 1440s, 1340m, 1050m

^{1}H-NMR(220MHz,$CDCl_3$): 4.17(m,1H,H-C3), 3.76(s,3H,OCH_3), 3.20(s,1H,OH), 2.8-2.5(m,6H), 1.7-1.3(m,4H), 0.98(t,3H,H-C4')

^{13}C-NMR(45.28MHz,$CDCl_3$): 172.5(C1), 66.9(C3), 51.8(OCH_3), 40.2(C2), 38.9(C4), 32.2(C1'), 31.8(C2'), 21.9(C3'), 13.6(C4')

Methyl 4-(pentylthio)-3-hydroxybutanoate **14**:

IR($CHCl_3$): 3510br, 2980m, 2960w, 2930w, 1730s, 1440s, 1340m, 1050m

^{1}H-NMR(220MHz,$CDCl_3$): 4.13(m,1H,H-C3), 3.75(s,3H,OCH_3), 3.20(d,J=4.5,1H,OH), 2.78-2.5(m,6H), 1.60(m,2H,H-C2'), 1.40-1.20(m,4H), 0.90(t,J=7.4,3H,H-C5')

^{13}C-NMR(45.28MHz,$CDCl_3$): 172.5(C1), 66.8(C3), 51.8(OCH_3), 40.1(C2), 38.7(C4), 32.5(C1'), 31.0(C2'), 29.4(C3'), 22.3(C4'), 14.0(C5')

Methyl 4-(phenylthio)-3-hydroxybutanoate **15**:

IR($CHCl_3$): 3550br, 2970w, , 2930w, 1730s, 1475s, 1440s, 1390w, 1100s, 1050m, 1010m

^{1}H-NMR(220MHz,$CDCl_3$): 7.7-7.5(m,5H,H-aromat), 4.67(m,1H,H-C3), 3.71(s,3H,OCH_3), 3.20(br,1H,OH), 3.1(m,2H,H-C4), 2.64(m,2H,H-C2)

^{13}C-NMR(45.28MHz,$CDCl_3$): 172.3(C1), 135.3, 129.5, 129.0, 126.4(C-aromat), 66.6(C3), 51.8(OCH_3), 40.1(C4), 39.9(C2)

MS(EI): m/z = 226$(M)^{+\cdot}$, 208$(M-H_2O)^{+\cdot}$, 149$(M-Ph)^+$, 135, 124, 103

Elemental analysis: Found: C 58.56 H 6.45 S 13.89

$C_{11}H_{14}O_3S$ Calc: C 58.38 H 6.24 S 14.17

Reduction with resting cells:

A suspension of fresh baker's yeast (10g) and sucrose (10g) in water (200ml) was stirred at 30°C for 30min. Then the substrate **8** (200mg) in ethanol (5ml) was added and the reaction followed by t.l.c. (ethyl acetate:petrol=1:1). When the reaction was complete, celite (20g) was added and the mixture was stirred for a further 30 min. The suspension was then filtered and the filtrate extracted with ether (4x50ml). The celite was also washed with ether (50ml), the combined organic extracts were dried ($MgSO_4$) and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (ethyl acetate:petrol=4:6) and the product was recrystallised from dichloromethane/pentane to obtain pure methyl (R)-4-(p-chlorophenylthio)-3-hydroxybutanoate.

Methyl 4-(p-chlorophenylthio)-3-hydroxybutanoate **16:**

Yield: 50%

mp: 50.0-53.0°C

IR($CHCl_3$): 3550br, 2960w, 2930w, 1732s, 1480s, 1440s, 1390w, 1100s, 1050m, 1010m

^{1}H-NMR(220MHz,$CDCl_3$): 7.45-7.35(m,4H,H-aromat), 4.20(m,1H,H-C3), 3.78(s,3H,OCH_3), 3.21(d,J=4.8,1H,OH), 3.10(m,2H,H-C4), 2.18(m,2H,H-C2)

^{13}C-NMR(45.28MHz,$CDCl_3$): 172.7(C1), 134.3, 133.0, 131.4, 129.5(C-aromat), 66.9(C3), 52.2(OCH_3), 40.8(C4), 40.0(C2)

MS(EI): m/z=260$(M)^{+\cdot}$, 242$(M-H_2O)^{+\cdot}$, 183, 158, 143

UV(Acetonitrile): 218($7000M^{-1}cm^{-1}$), 260($12000M^{-1}cm^{-1}$)

Elemental analysis: Found: C 50.82 H 4.78 Cl 13.30 S 12.20

$C_{11}H_{13}O_3ClS$ Calc: C 50.67 H 5.03 Cl 13.60 S 12.30

Methyl (R)-4-(p-chlorophenylthio)-3-hydroxybutanoate **L-16:**

The spectral data were identical to those reported for **rac-16.**

Recrystallised from dichloromethane/pentane.

mp: 58.5-59.0°C

$[\alpha]_D$ = +5.5° ±1.0° (c=1.0,$CHCl_3$)

Methyl (S)-4-(p-chlorophenylthio)-3-hydroxybutanoate **D-16:**

The spectral data were identical to those reported for **rac-16.**

Recrystallised from ether/pentane.

mp: 56.0-57.0°C

$[\alpha]_D$ = -5.9° ±1.0° (c=1.0,$CHCl_3$)

Ethyl 4-(phenylthio)-3-hydroxybutanoate **31**:

IR($CHCl_3$): 3500br, 3000w, 1730s, 1450m, 1335m, 1030m

^{1}H-NMR(220MHz,$CDCl_3$): 7.45-7.20(m,5H,H-aromat), 4.18(qm,J=7.3,3H,H-C3,H-OCH_2CH_3), 3.25(d,J=4.3,1H,OH), 3.09(m,2H,H-C4), 2.62(m,2H,H-C2), 1.26(t,J=7.3,3H,OCH_2CH_3)

^{13}C-NMR(45.28MHz,$CDCl_3$): 172.0(C1), 135.4, 129.6, 129.0, 126.4(C-aromat), 66.7(C3), 60.8(OCH_2CH_3), 40.2(C4), 40.1(C2), 14.1(OCH_2CH_3)

MS(EI): m/z = 240$(M)^{+\cdot}$, 222$(M-H_2O)^{+\cdot}$, 195$(M-OEt)^+$, 177, 149, 124

General procedure for the reductive desulfurisation with RaNi-W7:

To a solution of sodium hydroxide (1.28g,32mmol) in water (5ml) was carefully added nickel-aluminium alloy (1g) and the suspension stirred for 30 min. The mixture was decanted and the RaNi was washed with water (5ml portions) until the washings were neutral, followed by ethanol (3x10ml) and anhydrous methanol (1x10ml). To the thus prepared RaNi was added a solution of the sulfide (0.1g) in methanol (10ml) at 0°C and the reaction mixture was stirred for 4h. The RaNi was filtered off and the filtrate was concentrated *in vacuo*. Flash chromatography (ethyl acetate:petrol=3:7) of the residue afforded the product **17** in approximately 80% yield.

General procedure for the alkylation of methyl 3-oxobutanoate at the 4-position:

To a suspension of sodium hydride (1.29g,43mmol) in dry THF (100ml) at 0°C was slowly added methyl acetoacetate (5g,43mmol). After 10min butyllithium in hexane (43mmol) was slowly added and after a further 15min the freshly distilled alkyl

bromide (43mmol) was added. The reaction mixture was stirred for 30min, quenched with water, washed with 1M aqueous hydrochloric acid (2x50ml) and then with 1M aqueous sodium hydrogen carbonate (1x50ml). The organic phase was dried (Na_2SO_4), the solvent was removed *in vacuo* and the product was purified by flash chromatography (ethyl acetate:petrol=1:19).

Methyl 3-oxotetradecanoate **19**:

Yield: 40%

mp: 30.5-31.5°C

IR($CHCl_3$): 2960m, 2930s, 2860s, 1745s, 1715s, 1440m, 1410w, 1320m

^{1}H-NMR(220MHz,$CDCl_3$): 3.76(s,3H,OCH_3), 3.48(s,2H,H-C2), 2.54(m,2H,H-C4), 1.60(m,2H,H-C5), 1.29(m,16H,H-C6-C13), 0.89(m,3H,H-C14)

^{13}C-NMR(45.28MHz,$CDCl_3$): 202.7(C3), 167.7(C1), 52.2(OCH_3), 49.0(C2), 43.1(C4), 32.0(C5), 29.7, 29.5, 29.4, 29.1(C6-C11), 23.5(C12), 22.8(C13), 14.1(C14)

MS(EI): m/z = 256$(M)^{+\cdot}$, 225$(M\text{-}OCH_3)^+$, 183, 129, 116

Elemental analysis: Found: C 70.01 H 10.96

$C_{15}H_{28}O_3$ Calc: C 70.27 H 11.01

Methyl 3-oxooctadecanoate **20**:

Yield: 45%

mp: 50.5-52.0°C

IR($CHCl_3$): 2960m, 2930s, 2860s, 1745s, 1715s, 1440m, 1410w, 1320m

^{1}H-NMR(220MHz,$CDCl_3$): 3.86(s,3H,OCH_3), 3.67(s,2H,H-C2), 2.65(m,2H,H-C4), 1.70(m,2H,H-C5), 1.40(m,24H,H-C6-C17), 1.00(m,3H,H-C18)

^{13}C-NMR(45.28MHz,$CDCl_3$): 202.8(C3), 167.7(C1), 52.3(OCH_3), 49.0(C2), 43.1(C4), 32.0(C5), 29.7, 29.5, 29.4, 29.1(C6-C15), 23.5(C16), 22.7(C17), 14.1(C18)

MS(EI): m/z = 312$(M)^{+\cdot}$, 239, 129, 116

Elemental analysis: Found: C 73.29 H 11.53

$C_{19}H_{28}O_3$ Calc: C 73.03 H 11.61

Reduction of longchain carboxylic acids with *S.cerevisiae* NCYC 1765:

A solution of β-ketoester **19** or **20** (2.7mmol), lithium hydroxide (3.6mmol,150mg) in methanol (25ml) and water (15ml) was stirred at RT for 18h. Then the methanol was removed *in vacuo* and the residue added to a suspension of fermenting yeast (6g,wet weight), sucrose (3g) in water (40ml) at 30°C. The pH was adjusted to 5.8 and the mixture was stirred for 2 days. Celite (12g) was added and the reaction mixture was filtered after a further 2h of stirring. The filtrate was acidified to pH 2 and extracted with ethyl acetate. The celite was washed with ethyl acetate and the combined organic fractions were dried ($MgSO_4$) and the solvent was removed *in vacuo*. The residue was redissolved in ether and treated with diazomethane to give the crude ester. Purification was achieved by flash chromatography (ethyl acetate:petrol=1:9).

(R)-Methyl 3-hydroxytetradecanoate **D-21**:

Yield: 30%

mp: 34.0-36.0°C

$[\alpha]_D$= -13.9° ±1.0° (c=0.9,$CHCl_3$)

IR($CHCl_3$): 2930s, 2860s, 1715m, 1470w, 1045w

^{1}H-NMR(220MHz,$CDCl_3$): 4.03(m,1H,H-C3), 3.74(s,3H,OCH_3), 3.04(br,1H,OH), 2.55(dd,J=14.3,3.0,1H,H-C2), 2.43(dd,J=14.3,6.8,1H,H-C2), 1.7-1.1(br,m,20H,H-C4-C13), 0.90(m,3H,H-C14)

^{13}C-NMR(45.28MHz,$CDCl_3$): 173.5(C1), 68.1(C3), 51.7(OCH_3), 41.1,36.6(C2,C4), 31.9(C5), 29.6, 25.5, 22.7(C6-C13), 14.1(C14)

MS(CI): m/z = 276$(M+NH_4)^+$, 259$(M+H)^+$, 241$(259-H_2O)^+$, 208, 166, 110, 103

Elemental analysis: Found: C 70.01 H 11.83

$C_{15}H_{30}O_3$ Calc: C 69.72 H 11.70

(R)-Methyl 3-hydroxyoctadecanoate **D-22**:

Yield: 40%

mp: 55-56°C

$[\alpha]_D$= -13.3° ±1.0° (c=1.1,$CHCl_3$)

Literature[35]: $[\alpha]_D$= -13.2° (c=1.9,$CHCl_3$)

IR($CHCl_3$): 2930s, 2860s, 1715m, 1470w, 1045w

^{1}H-NMR(220MHz,$CDCl_3$): 4.05(m,1H,H-C3), 3.75(s,3H,OCH_3), 2.94(br,1H,OH), 2.55(dd,J=14.6,3.1,1H,H-C2), 2.42(dd,J=14.6,7.1,1H,H-C2), 1.6-1.1(br,m,28H,H-C4-C17), 0.89(m,3H,H-C18)

^{13}C-NMR(45.28MHz,$CDCl_3$): 173.5(C1), 68.0(C3), 51.7(OCH_3), 41.2(C2), 36.6(C4), 32.0(C5), 29.7, 29.4, 25.5, 22.8(C17), 14.1(C18)

MS(EI): m/z = 296$(M-H_2O)^{+\cdot}$, 264, 222, 180, 103

Elemental analysis: Found: C 72.68 H 12.05

$C_{19}H_{38}O_3$ Calc: C 72.56 H 12.18

Methyl 2-(p-chlorophenylthio)-acetate **26**:

Following the general procedure for the synthesis of 4-sulfur substituted β-ketoesters, **26** was prepared from methyl 2-chloroacetate **25**.

Yield: 90%

bp: 120°C (0.06mmHg)

IR($CHCl_3$): 2960w, 1735s, 1480s, 1440m, 1390w, 1285m, 1095s, 1015m

^{1}H-NMR(220MHz,$CDCl_3$): 7.45-7.25(m,4H,H-aromat), 3.74(s,3H,OCH_3), 3.65(s,2H,H-C2)

^{13}C-NMR(45.28MHz,$CDCl_3$): 169.8(C1), 133.5, 133.0, 131.2, 129.1(C-aromat), 52.5(OCH_3), 36.4(C2)

MS(EI): m/z = 216$(M)^{+\cdot}$, 157, 103, 75

Elemental analysis: Found: C 49.96 H 4.28 Cl 16.01 S 14.36

$C_9H_9O_2ClS$ Calc: C 49.89 H 4.19 Cl 16.36 S 14.80

2-(p-Chlorophenylthio)-acetaldehyde **27**:

To a solution of DIBAL (658mg,0.82ml,4.63mmol) in dry pentane (10ml) at -100°C (ethanol/liquid nitrogen) was slowly added a solution of **26** (1g,4.62mmol) in dry ether (10ml). After the reaction mixture was stirred at -100°C for 2h, it was poured into 1M aqueous hydrochloric acid (30ml) and extracted with ether (3x20ml). The organic phase was washed with 1M aqueous sodium hydrogen carbonate, dried ($MgSO_4$) and the solvent removed *in vacuo*. Flash chromatography (ethyl acetate:petrol=2:8) of the residue afforded **27**.

Yield: 0.42g (49%)

^{1}H-NMR(220MHz,$CDCl_3$): 9.56(t,J=2.5,1H,CHO), 7.40-7.20(m,4H,H-aromat), 3.59(d,J=2.5,2H,H-C2)

Methyl 6-(p-chlorophenylthio)-5-hydroxy-3-oxohexanoate **28**:

To a stirred suspension of sodium hydride (74mg,2.6mmol) in THF (2ml) at 0°C under argon, was added methyl acetoacetate (287mg,2.5mmol). The solution was stirred at 0°C for 10min, cooled to -20°C and butyllithium in hexane (2.6mmol) added dropwise. The resulting deep yellow solution of the dianion was stirred at -20°C for 10min, cooled to -78°C and aldehyde **27** (420mg,2.3mmol) in THF (1ml) added dropwise. The mixture was allowed to warm to 0°C over 30min, poured into saturated aqueous ammonium chloride (6ml) and extracted with ether (3x10ml). The combined organic extracts were dried (Na_2SO_4), the solvent was removed *in vacuo* and the residue was purified by flash chromatography (ethyl acetate:petrol=4:6).

Yield: 278mg(40%)

mp: 48-49°C

IR($CHCl_3$): 5380br, 2960w, 1750s, 1720s, 1480s, 1440m, 1330m, 1100s, 1015m

^{1}H-NMR(220MHz,$CDCl_3$): 7.40-7.25(m,4H,H-aromat), 4.20(m,1H,H-C5), 3.76(s,3H,OCH_3), 3.50(s,2H,H-C2), 3.04(m,3H,H-C6,OH), 2.88(m,2H,H-C4)

^{13}C-NMR(45.28MHz,$CDCl_3$): 202.3(C3), 167.2(C1), 133.7, 132.6, 130.9, 129.2(C-aromat), 66.0(C5), 52.5(OCH_3), 49.6(C4), 48.1(C2), 40.4(C6)

MS(EI): m/z = 302$(M)^{+\cdot}$, 284$(M-H_2O)^{+\cdot}$, 242, 210, 186, 157

Elemental analysis: Found: C 51.83 H 4.78 Cl 11.20 S 10.31

$C_{13}H_{15}O_4ClS$ Calc: C 51.57 H 4.99 Cl 11.71 S 10.59

Reduction of **28** to **D-29** by yeasts:

To 100ml of the culture broth was added **28** (100mg,0.33mmol) in ethanol (2ml). When the reaction was judged to be 50% complete (tlc or HPLC), celite (2g) was added. The resulting suspension was filtered and the filtrate was extracted with ether (3x50ml). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed *in vacuo*. Flash chromatography (ethyl acetate:petrol=4:6) of the residue gave the products.

Results:

			D-29				**L-28**	
Name	NCYC	time	yield	de%	ee%	$[\alpha]_D \pm 1.0^o$	yield	ee%
S.cerevisiae	667	6h	42%	78	-	-4.6°	46%	36
S.cerevisiae (W)	463	4d	-	-	-	-	31%	9
S.cerevisiae	1765	6h	14%	>95	>95	-7.2°	49%	19
Rhodotorula glutinis	974	5h	25%	80	-	-8.9°	32%	17
R.rubra	796	27h	10%	56	-	-7.2°	30%	7
C.guilliermondii	973	5h	41%	>95	>95	-7.6°	32%	59
C.guilliermondii	1399	4.5h	37%	42	-	-9.4°	-	-
H.polymorpha	1456	5.5h	37%	92	-	-6.8°	36%	59
H.polymorpha	1459	5h	20%	58	-	-7.9°	20%	51
P.membranaefaciens	333	9h	30%	88	-	-4.3°	20%	15
P.membranaefaciens	795	6h	32%	>95	>95	-7.1°	39%	41

Methyl (3R,5S)-6-(p-chlorophenylthio)-3,5-dihydroxyhexanoate **D-29**:

IR($CHCl_3$): 3500br, 2970w, 2930w, 1730s, 1480s, 1445m, 1280m, 1100s, 1020m

^{1}H-NMR(220MHz,$CDCl_3$): 7.20(m,4H,H-aromat), 4.19(m,1H,H-C5), 3.87(m,1H,H-C3), 3.62(s,3H,OCH_3), 2.91(m,2H,H-C6), 2.48(m,2H,H-C2), 1.80-1.50(m,2H,H-C4)

^{13}C-NMR(45.28MHz,$CDCl_3$): 173.0(C1), 134.4, 132.7, 129.5(C-aromat), 70.3(C5), 68.6(C3), 52.2(OCH_3), 41.8, 41.7, 41.4(C2,C4,C6)

Resolution of Methyl 4-(p-chlorophenylthio)-3-O-butanoylbutanoate **49**:

To a solution of methyl 4-(p-chlorophenylthio)-3-O-butyratebutanoate **49** (1g,3mmol) in 0.2M pH 7 phophate buffer, methanol (15ml) and Triton X-100 (50mg) was added Amano *Pseudomonas* Lipase (200mg). The reaction was maintained at pH 7 by titrating with 0.5M sodium hydroxide. After 8h at RT the pH was raised to 10 and the reaction was extracted with ether (4x300ml). The combined organic fractions were dried ($MgSO_4$), the solvent removed *in vacuo* and the products isolated by flash chromatography (ethyl acetate:petrol=2:8)

Methyl (R)-4-(p-chlorophenylthio)-3-hydroxybutanoate **L-16**:

The spectral data were identical to those reported for **rac-16**.

Yield: 160mg(20%)

$[\alpha]_D = +4.5^o \pm 1.0^o$ (c=3.0,$CHCl_3$)

Methyl (S)-4-(p-chlorophenylthio)-3-O-butanoylbutanoate **D-49**:

The spectral data were identical to those reported for **rac-49**.

Yield:297mg(30%)

$[\alpha]_D = +8.5^o \pm 1.0^o$ (c=1.0,$CHCl_3$)

3.3. Experiments of part 2.

(-)-2-Methoxy-2-trifluoromethylphenylacetic acid chloride:

(-)-2-Methoxy-2-trifluoromethylphenylacetic acid (5g,21.4mmol), freshly distilled thionyl chloride (14.7g,9ml,123mmol) and sodium chloride (80mg,1.4mmol) were refluxed for 3 days. The excess of thionyl chloride was removed *in vacuo* and the residue was distilled under high vacuum.

Yield: 4.4g (81%)

bp: 160°C (1.5mmHg), 70°C (0.05mmHg)

IR($CHCl_3$): 2960w, 2860w, 1790s, 1500w, 1460m, 1270s, 1200s, 1180s, 1140m, 1005m

^{1}H-NMR(220MHz,$CDCl_3$): 7.80-7.40(m,5H,H-aromat), 3.78(s,3H,OCH_3)

$[\alpha]_D$= -110.1° (c=2.6,$CHCl_3$)

General procedure for the formation of 2-Methoxy-2-trifluoromethylphenylacetates:

To dry pyridine (3ml,37mmol) in a dry flask was added under nitrogen 2-methoxy-2-trifluoromethylphenylacetic acid chloride (378mg,1.5mmol), dry carbon tetrachloride (3ml) and the β-hydroxyester (1mmol). The reaction mixture was stirred at RT and the formation of the product monitored by tlc (ethyl acetate:petrol=3:7). When the reaction was complete, water was added and it was extracted with chloroform. The combined organic extracts were washed with 0.5M aqueous hydrochloric acid, 1M aqueous sodium carbonate and dried ($MgSO_4$). Evaporation of the solvent *in vacuo*, and purification by flash chromatography yielded the product.

Ethyl 3-(2-methoxy-2-trifluoromethylphenylacetoxy)butanoate **32:**

^{19}F-NMR(84.67MHz,$CDCl_3$/CF_3COOH): 7.092, 7.208

Methyl 4-ethylthio-3-(2-methoxy-2-trifluoromethylphenylacetoxy)butanoate **33:**

^{19}F-NMR(84.67MHz,$CDCl_3$/$CFCl_3$): 71.36

Methyl 4-propylthio-3-(2-methoxy-2-trifluoromethylphenylacetoxy)butanoate **34:**

^{19}F-NMR(84.67MHz,$CDCl_3$/$CFCl_3$): 71.34

Methyl 4-butylthio-3-(2-methoxy-2-trifluoromethylphenylacetoxy)butanoate **35:**

^{19}F-NMR(84.67MHz,$CDCl_3$/$CFCl_3$): 71.34, 71.16

Methyl 4-pentylthio-3-(2-methoxy-2-trifluoromethylphenylacetoxy)butanoate **36:**

^{19}F-NMR(84.67MHz,$CDCl_3$/$CFCl_3$): 71.34

Methyl 4-phenylthio-3-(2-methoxy-2-trifluoromethylphenylacetoxy)butanoate **37:**

^{19}F-NMR(84.67MHz,$CDCl_3$/$CFCl_3$): 71.25, 71.04

Ethyl 4-phenylthio-3-(2-methoxy-2-trifluoromethylphenylacetoxy)butanoate **38:**

^{19}F-NMR(84.67MHz,$CDCl_3$/$CFCl_3$): 71.25

(S)-Ethyl 3-(3',5'-dinitrobenzoyloxy)butanoate **L-39:**

To a solution of **L-24** (85%ee,3g,23mmol), dicyclohexylcarbodiimide (5.7g,27mmol) and N,N-dimethylaminopyridine (227mg) in dichloromethane (45ml) was added 3,5-dinitrobenzoic acid (7.2g,34mmol). The mixture was stirred for 16h at RT, diluted with n-pentane (15ml) and filtered. The filtrate was concentrated *in vacuo*. The residue was allowed to stand in dichloromethane:pentane=1:1 (45ml) at 0°C for 3h. The precipitate was filtered off and the filtrate was concentrated *in vacuo*. This process was repeated three times to afford a yellow oil which was purified by flash chromatography (ether:petrol=3:7) to give crude **L-39** (2.7g,90%). This was recrystallised from pentane:ether=4:1 to give pure **L-39**.

Yield: 1.7g(55%)

mp: 40-41°C

$[\alpha]_D$ = +26.0° (c=1.5,$CHCl_3$)

Literature[171]: $[\alpha]_D$ = +26° (c=1.37,$CHCl_3$)

The spectral data were identical to those reported in literature.[171]

(S)-Ethyl 3-hydroxybutanoate **L-24:**

1M Aqueous potassium hydroxide (1.1ml) was added dropwise over a period of 30min to a stirred solution of **L-39** (326mg,1mmol) in THF:ethanol=1:1 at 0°C. The mixture was then stirred for 30min at 0°C and diluted with ether (20ml) and saturated aqueous potassium hydrogen carbonate (3ml). The ether layer was separated and the aqueous layer extracted with dichloromethane. The combined organic fractions were washed with brine, dried (Na_2SO_4) and the solvent removed *in vacuo*. The residue was distilled to give pure **L-24**.

Yield: 100mg (80%)

bp: 71°C (10mmHg)

$[\alpha]_D$ = +43° (c=1.5,$CHCl_3$) Literature:[201] $[\alpha]_D$ = +43.9° (c=1.4,$CHCl_3$)

IR($CHCl_3$): 3400m, 2960m, 2920m, 1735s, 1380m, 1300m, 1180m, 1090m, 1070m, 1025m

^{1}H-NMR(400MHz,$CDCl_3$): 4.16(m,1H,H-C3), 4.14(q,J=7.1,2H,H-OCH_2CH_3), 3.1(s,1H,OH), 2.46(dd,J=16.4,3.7,1H,H-C2), 2.38(dd,J=16.4,8.6,1H,H-C2), 1.24(t,J=7.2,3H,H-OCH_2CH_3), 1.20(d,J=6.3,3H,H-C4)

^{13}C-NMR(22.63MHz,$CDCl_3$): 172.6(C1), 64.1(C3), 60.4($O\underline{C}H_2CH_3$), 42.8(C2), 22.3(C4), 14.0($OCH_2\underline{C}H_3$)

General procedure for the formation of methoxyethoxymethylether:

A solution of the β-hydroxyester (30mmol), MEM-chloride (7.56g,60mmol) and triethylamine (12g,120mmol) in dry THF (100ml) was heated under reflux for 14h. The reaction mixture was poured into 100ml of water and extracted with ethyl acetate. Drying of the organic phase ($MgSO_4$), evaporation of the solvent *in vacuo* and flash chromatography afforded the MEM-ether.

Ethyl 3-methoxyethoxymethoxybutanoate **41**:

Yield: 85%

bp: 100-110°C

IR($CHCl_3$): 2990m, 2950m, 2900m, 1735s, 1460w, 1390m, 1310m, 1205m, 1170m, 1140m, 1105m, 1050s

^{1}H-NMR(220MHz,$CDCl_3$): 4.80(s,2H,H-C1'), 4.20(m,1H,H-C3), 4.18(q,J=7.2,2H,H-OCH_2CH_3), 3.72(m,2H,H-C2'), 3.60(m,2H,H-C3'), 3.43(s,3H,H-C4'), 2.61(dd,J=14.7,7.3,1H,H-C2),

2.42(dd,J=14.7,5.4,1H,H-C2), 1.29(t,J=6.8,3H,H-OCH_2CH_3), 1.24(d,J=5.0,3H,H-C4)

^{13}C-NMR(45.28MHz,$CDCl_3$): 171.1(C1), 94.2(C1'), 71.8(C2'), 70.3(C3'), 66.9(OCH_3), 60.3(C4'), 58.9(OCH_2CH_3), 42.3(C2), 20.4(OCH_2CH_3, 14.2(C4)

MS(CI): m/z = 238$(M+NH_4)^+$, 221$(M+H)^+$, 175$(M-OEt)^+$, 145, 115, 89

Elemental analysis: Found: C 55.70 H 9.54

$C_{10}H_{20}O_5$ Calc: C 54.53 H 9.15

Ethyl 3-methoxyethoxymethoxy-4-(phenylthio)butanoate **43**:

Yield: 70%

^{1}H-NMR(220MHz,$CDCl_3$): 7.45-7.18(m,5H,H-aromat), 4.80(m,2H,H-C1'), 4.20(m,1H,H-C3), 4.18(q,J=7.5,2H,H-OCH_2CH_3), 3.74(m,2H,H-C2'), 3.55(m,2H,H-C3'), 3.38(s,3H,H-C4'), 3.32(dd,J=14.0,4.2,1H,H-C4), 3.15(dd,J=14.0,6.1,1H,H-C4), 2.80(dd,J=14.7,3.9,1H,H-C2), 2.63(dd,J=14.7,7.9,1H,H-C2), 1.25(t,J=7.5,3H,H-OCH_2CH_3)

Ethyl 3-benzyloxybutanoate **40**:

A solution of **24** (7.0g,53mmol), sodium hydride (2g,64mmol) and benzyl chloride (40g,316mmol) in dry DMF (120ml) was refluxed for three days. The reaction mixture was poured into 100ml of water and extracted with ether. The combined organic fractions were dried ($MgSO_4$), the solvent was removed *in vacuo* and most of the remaining benzyl chloride removed by distillation at 100°C (0.05mmHg). Flash chromatography (ether:petrol =1:9) of the residue afforded **40**.

Yield: 5g (45%)

bp: 150°C (0.05mmHg)

^{1}H-NMR(220MHz,$CDCl_3$): 7.36(m,5H,H-aromat), 4.58(m,2H,H-benzyl), 4.17(q,J=6.8,2H,H-OCH_2CH_3), 4.04(q,J=6.4,H-C3), 2.68(dd,J=14.7,6.3,1H,H-C2), 2.43(dd,J=14.7,5.4,1H,H-C2), 1.29(d,J=6.4,3H,H-C4), 1.28(t,J=6.8,3H,H-OCH_2CH_3)

General procedure for the formation of methylthiomethylethers:

To a solution of the β-hydroxyester (38mmol) in dimethylsulfoxide (150ml) was added a mixture of acetic anhydride (100ml) and acetic acid (20ml). The mixture was stirred for 2 days and then poured carefully into cold 1M aqueous sodium hydrogen carbonate (1500ml) and stirred for 1h. The pH at this stage was approximately 7 to 8. The mixture was extracted with chloroform (3x500ml) and the combined organic fractions were washed with 1M aqueous sodium hydrogen carbonate (500ml) and water (5x100ml). The organic fraction was dried ($MgSO_4$) and the solvent was evaporated *in vacuo*. The residue was then purified by distillation or flash chromatography (ethyl acetate: petrol=2:8).

Ethyl 3-methylthiomethoxybutanoate **42:**

Yield: 60%

bp: 110°C (1mmHg)

^{1}H-NMR(220MHz,$CDCl_3$): 4.68(m,2H,H-C1'), 4.20(m,1H,H-C3), 4.15(q,J=7.0,2H,OCH_2CH_3), 2.57(dd,J=14.5,7.0,1H,H-C2), 2.41(dd,J=14.5,3.8,1H,H-C2), 2.15(s,3H,H-C2'), 1.28(t,J=7.0,3H,H-OCH_2CH_3), 1.23(d,J=6.0,3H,H-C4)

Ethyl 3-methylthiomethoxy-4-phenylthiobutanoate **46:**

Yield: 45%

^{1}H-NMR(220MHz,$CDCl_3$): 7.48-7.20(m,5H,H-aromat), 4.69(m,2H,H-C1'), 4.26(m,1H,H-C3), 4.15(q,J=7.0,2H,OCH_2CH_3), 3.28(dd,J=14.0,4.0,1H,H-C4), 3.11(dd,J=14.0,6.1,1H,H-C4), 2.79(dd,J=14.7,3.5,1H,H-C2), 2.65(dd,J=14.7,7.5,1H,H-C2), 2.15(s,3H,H-C2'), 1.26(t,J=7.0,3H,H-OCH_2CH_3)

Ethyl 4-phenylthio-3-(trimethylsilyloxy)butanoate **44:**

A solution of **31** (1.03g,4.3mmol), triethylamine (3.0g,4.2ml,30mmol) and trimethylsilyl chloride (2.3g,2.7ml,21mmol) in dry dichloromethane (20ml) was stirred for 30min and the water (20ml) was added and the two phases were separated. The aqueous phase was extracted with ether (3x10ml), the combined organic fractions were dried ($MgSO_4$) and the solvent was removed *in vacuo*. The product was obtained by distillation.

Yield: 1.1g (85%)

bp: 100°C (0.1mmHg)

^{1}H-NMR(220MHz,$CDCl_3$): 7.40-7.10(m,5H,H-aromat), 4.20(m,1H,H-C3), 4.08(q,J=6.9,2H,H-OCH_2CH_3), 2.98(m,2H,H-C4), 2.67(dd,J=13.7,3.8,1H,H-C2), 2.43(dd,J=13.7,7.3,1H,H-C2), 1.19(t,J=6.9,3H,H-OCH_2CH_3)

General procedure for the formation of tetrahydropyranylethers:

To a stirred solution of the β-hydroxyester (5mmol) and 3,4-dihydro-2H-pyran (0.46g,0.5ml,5.5mmol) in dry dichloromethane (15ml) was added p-toluenesulfonic acid (10mg). After 30min, 1M aqueous sodium hydrogen carbonate (10ml) was added and the

mixture was extracted with dichloromethane (3x10ml). The combined organic fractions were dried ($MgSO_4$) and the solvent removed *in vacuo*. The product was purified by flash chromatography.

Ethyl 4-phenylthio-3-tetrahydropyranyloxybutanoate **45:**

Yield: 90%

^{1}H-NMR(220MHz,$CDCl_3$): 7.43-7.10(m,5H,H-aromat), 4.73(br,1H,H-C1'), 4.30-4.10(m,1H,H-C3), 4.13(q,J=8.0,2H,H-OCH_2CH_3), 3.90-3.30(br,3H,2H-C5',H-C4), 3.13(m,1H,H-C4), 2.80-2.50(m,2H,H-C2), 1.80-1.30(br,6H,2H-C2',2H-C3',2H-C4'), 1.23(t,3H,OCH_2CH_3)

Methyl 4-(p-chlorophenylthio)-3-tetrahydropyranyloxybutanoate **47:**

Yield: 89%

IR($CHCl_3$):2920m, 2870w, 1735s, 1480s, 1440m, 1310w, 1100m, 1070m

^{1}H-NMR(400MHz,$CDCl_3$): 7.29-7.17(m,4H,H-aromat), 4.66(m,1H,H-C1'), 4.19,4.13(m,1H,H-C3), 3.95-3.40(br,m,2H,H-C5'), 3.63,3.62(s,3H,OCH_3), 3.40(dd,J=14.0,4.2,1H,H-C4), 3.10(dd,J=14.0,5.0,1H,H-C4), 3.06(dd,J=14.0,6.0,1H,H-C4), 2.95(dd,J=13.8,8.0,1H,H-C4), 2.80-2.65(m,1H,H-C2), 2.52(dd,J=16.0,8.0,1H,H-C2), 1.80-1.40(m,6H,2H-C2',C3',C4')

^{13}C-NMR(100.62MHz,$CDCl_3$): 171.4,171.2(C1), 134.8,134.7(C-Cl), 131.9,131.6(C-aromat), 130.4,129.8,128.8(4C-aromat), 99.9,98.5(C1'), 73.7,72.7(C3), 62.9,62.4(C5'), 51.4(OCH_3), 39.6, 38.4, 38.04, 38.0(C2,C4), 30.7, 30.4(C2'), 25.1(C4'), 19.6, 19.2(C3')

MS(EI): m/z = 344, 346$(M)^{+\cdot}$, 260$(M\text{-}DHP)^+$, 242

Elemental analysis: Found: C 55.92 H 6.18 S 9.51

$C_{16}H_{21}O_4ClS$ Calc: C 55.73 H 6.14 S 9.30

General procedure for the formation of acetates and butyrates:

A solution of **16** (5g,19.2mmol), the anhydride (57.5mmol), pyridine (7.6g,7.8ml,96.0mmol) and DMAP (2.34g,19.2mmol) in dry dichloromethane was refluxed for 2h. The reaction mixture was washed with 1M aqueous hydrochloric acid (3x50ml) and 1M aqueous sodium carbonate (2x50ml). The organic phase was dried ($MgSO_4$) and the solvent was removed *in vacuo*. Flash chromatography of the residue afforded the product.

Methyl 3-acetoxy-4-(p-chlorophenylthio)-butanoate **48**:

Flash chromatography: ethyl acetate:petrol=2:8

Yield: 75%

IR($CHCl_3$): 2970w, 1745s, 1480m, 1445m, 1380m, 1100m, 1035m, 1015m

^{1}H-NMR(220MHz,$CDCl_3$): 7.50-7.30(m,4H,H-aromat), 5.35(m,1H,H-C3), 3.71(s,3H,OCH_3), 3.21(m,2H,H-C4), 2.85(dd,J=13.5,4.7,1H,H-C2), 2.72(dd,J=13.5,7.2,1H,H-C2), 1.99(s,3H,H-$COCH_3$)

^{13}C-NMR(45.28MHz,$CDCl_3$): 170.4, 170.2(C1,$COCH_3$), 134.1, 132.7, 131.2, 129.3(C-aromat), 69.4(C3), 52.0(OCH_3), 37.6, 37.2(C2,C4), 21.0($COCH_3$)

Elemental analysis: Found: C 52.63 H 5.26 S 11.33

$C_{13}H_{15}O_4ClS$ Calc: C 51.57 H 4.99 S 10.59

Methyl 3-butyroxy-4-(p-chlorophenylthio)-butanoate **49**:

Flash chromatography: ethyl acetate:petrol=2:8

Yield: 82%

IR($CHCl_3$): 2960w, 2930, 2880w, 1740s, 1735s, 1480m, 1440m, 1390w, 1310w, 1100m, 1015m

^{1}H-NMR(220MHz,$CDCl_3$): 7.45-7.30(m,4H,H-aromat), 5.35(m,1H,H-C3), 3.70(s,3H,OCH_3), 3.25(dd,J=14.3,6.1,1H,H-C4), 3.15(dd,J=14.3,6.1,1H,H-C4), 2.85(dd,J=14.7,5.8,1H,H-C2), 2.70(dd,J=14.7,6.8,1H,H-C2), 2.20(m,2H,H-C2'), 1.60(m,2H,H-C3'), 0.92(t,J=6.0,3H,H-C4')

^{13}C-NMR(45.28MHz,$CDCl_3$): 172.6, 170.3(C1,C1'), 133.9, 132.5, 130.9, 129.1(C-aromat), 68.9(C3), 51.8(OCH_3), 37.5, 37.0, 36.0(C2, C4,C2'), 18.3(C3'), 13.5(C4')

MS(EI): m/z = 330$(M)^{+\cdot}$, 299$(M\text{-}OCH_3)^+$, 242(M-butyricacid)$^{+\cdot}$, 211, 183

Elemental analysis: Found: C 54.55 H 5.94 Cl 10.98 S 9.57

$C_{15}H_{19}O_4ClS$ Calc: C 54.46 H 5.79 Cl 10.72 S 9.69

Methyl 4-(p-chlorophenylthio)-3-tert-butyldimethylsilyloxy-butanoate **50**:

A solution of **16** (2.78g,10.7mmol), DBU (2.45g,16.1mmol) and tert-butyldimethylsilyl chloride (1.9g,12.84mmol) in dry dichloromethane (100ml) was stirred at RT for 23h. The reaction mixture was washed with icecold 0.5M aqueous hydrochloric acid (2x70ml) and 1M aqueous sodium hydrogen carbonate. The organic phase was dried ($MgSO_4$) and the solvent was removed *in vacuo*. Distillation of the residue afforded the product.

Yield: 3.8g(93%)

bp:190°C (0.1mmHg)

IR($CHCl_3$): 2980m, 2970m, 2880m, 1735s, 1480s, 1445m, 1260m, 1015m

^{1}H-NMR(220MHz,$CHCl_3$): 7.30(m,4H,H-aromat), 4.25(m,1H,H-C3), 3.69(s,3H,OCH_3), 3.05(m,2H,H-C4), 2.77(dd,J=14.2,3.9,1H,H-C2), 2.53(dd,J=14.2,6.5,1H,H-C2), 0.94(s,9H,H-tert-butyl), 0.02(s,3H,H-$SiCH_3$), -0.01(s,3H,H-$SiCH_3$)

^{13}C-NMR(45.28MHz,$CDCl_3$): 171.9(C1), 135.2, 132.5, 131.2, 129.4(C-aromat), 68.9(C3), 51.9(OCH_3), 41.8, 41.4(C2,C4), 26.0($SiC(CH_3)_3$), 18.3($SiC(CH_3)_3$), -4.2($SiCH_3$)

MS(CI): m/z = 394$(M+NH_4)^+$, 375$(M)^+$, 317, 243

Elemental analysis: Found: C 54.64 H 7.23 S 8.57

$C_{17}H_{27}O_3ClSSi$ Calc: C 54.45 H 7.26 S 8.55

General procedure for the synthesis of 3-ketoesters *via* their lithium salts:

To a solution of the ethylester (1mmol) in dry toluene (10ml) at 0°C was added lithio-tert-butylacetate[173] (2mmol) as a solid. After stirring at 0°C for 12h, the reaction mixture was washed with 0.5M aqueous hydrochloric acid (3x10ml), the organic phase was dried (Na_2SO_4) and the solvent was removed *in vacuo*. Flash chromatography (ethyl acetate:petrol=4:6) afforded the pure products.

tert-Butyl 5-hydroxy-3-oxohexanoate **51**:

Yield: 17mg(8%)

^{1}H-NMR(220MHz,$CDCl_3$): 4.18(m,1H,H-C5), 3.41(s,2H,H-C2), 2.70(m,2H,H-C4), 1.50(s,9H,H-tert-butyl), 1.23(d,J=5.4,3H,H-C6)

tert-Butyl 5-benzyloxy-3-oxohexanoate **52**:

Yield: 250mg (68%)

^{1}H-NMR(220MHz,$CDCl_3$): 7.35(m,5H,H-aromat), 4.53(m,2H,H-benzyl), 4.08(m,1H,H-C5), 3.40(s,2H,H-C2), 2.89(dd,J=15.1,7.3,1H,H-C4), 2.62(dd,J=15.1,4.9,1H,H-C4), 1.49(s,9H,H-tert-butyl), 1.27(d,J=5.4,3H,H-C6)

tert-Butyl 5-methoxyethoxymethoxy-3-oxobutanoate **53**:

Yield: 100mg (35%)

IR($CHCl_3$): 2990m, 2945m, 2900w, 1740s, 1720, 1660w, 1465w, 1400w, 1375m, 1330w, 1155s, 1110m, 1050s

^{1}H-NMR(220MHz,$CDCl_3$): 4.75(m,2H,H-C1'), 4.18(m,1H,H-C5), 3.68(m,2H,H-C2'), 3.58(m,2H,H-C3'), 3.40(s,2H,H-C2), 3.39(s,3H,H-C4'), 2.86(dd,J=15.2,7.3,1H,H-C4), 2.58(dd,J=15.2,4.9,1H,H-C4), 1.48(s,9H,H-tert-butyl), 1.22(d,J=5.9,3H,H-C6)

Synthesis of the Magnesium-salts **55** and **56**:

Magnesium ethoxide (0.49g,5mmol) was added to a solution of freshly distilled hydrogen ethyl (methyl)malonate (10mmol) in dry THF (25ml, and the mixture was stirred for 2h. The solvent was removed *in vacuo* to give a hygroscopic white solid which was dried overnight under reduced pressure.

Elemental analysis: Found: C 41.85 H 4.94 Mg 7.55

for **55** $C_8H_{10}O_8Mg$ Calc: C 41.92 H 4.92 Mg 8.48

General procedure for the hydrolysis of β-hydroxyesters:

Procedure A: To a solution of the ester (1mmol) in THF (3ml) was added 1M aqueous potassium hydroxide (1.1ml), and the resulting suspension was stirred at RT until all the starting material was consumed. Water (2ml) was then added and the mixture was

washed with ethyl acetate (1x2ml). The aqueous phase was acidified to pH 2 and extracted with ethyl acetate (3x3ml). The combined organic extracts were dried ($MgSO_4$), the solvent was removed *in vacuo* and the residue was dried under reduced pressure.

Procedure B: To a solution of the ester (1mmol) in methanol (3ml) and water (3ml) was added lithium hydroxide (1.5mmol) and the resulting suspension was stirred at RT until all the starting material was consumed. Water (3ml) was then added and the mixture was washed with ether (1x3ml). The aqueous phase was acidified to pH 2 and extracted with ether (3x4ml). The combined organic extracts were dried ($MgSO_4$), the solvent was removed *in vacuo* and the residue was dried under reduced pressure.

3-Hydroxybutyric acid **57**:

Procedure A

Yield : 90%

^{1}H-NMR(220MHz,$CDCl_3$): 6.0(br,2H,OH), 4.26(m,1H,H-C3), 2.55(m,2H,H-C2), 1.29(d,J=7.7,3H,H-C4)

3-Methoxyethoxymethoxybutyric acid **58**:

Procedure A

Yield: 95%

^{1}H-NMR(220MHz,$CDCl_3$): 10.0-9.0(br,1H,OH), 4.80(m,2H,H-C1'), 4.23(m,1H,H-C3), 3.72(m,2H,H-C2'), 3.59(m,2H,H-C3'), 3.42(s,3H,H-C4'), 2.65(dd,J=14.7,6.2,1H,H-C2), 2.50(dd,J=14.7,4.7,1H,H-C2), 1.29(d,J=7.3,3H,H-C4)

^{13}C-NMR(22.63MHz,$CDCl_3$): 176.4(C1), 94.1(C1'), 71.7(C2'), 70.0(C3), 66.9(C3'), 58.8(C4'), 41.9(C2), 20.3(C4)

MS(CI): m/z = 210$(M+NH_4)^+$, 193$(M+H)^+$, 134, 117, 89

3-Hydroxy-4-(phenylthio)-butyric acid **61**:

Procedure B

Yield: 91%

^{1}H-NMR(220MHz,$CDCl_3$): 7.50-7.25(m,5H,H-aromat), 4.15(m,1H,H-C3), 3.10(m,2H,H-C4), 2.75(dd,J=13.9,4.1,1H,H-C2), 2.13(dd,J=13.9,6.5,1H,H-C2)

3-Methoxyethoxymethoxy-4-(phenylthio)-butyric acid **62**:

Procedure A

Yield: 87%

4-Phenylthio-3-tetrahydropyranyloxybutyric acid **63**:

Procedure B

Yield: 90%

^{1}H-NMR(220MHz,$CDCl_3$): 7.50-7.25(m,5H,H-aromat), 4.75(br,1H,H-C1'), 4.22(m,1H,H-C3), 4.05-2.25(6H,H-C2,H-C4,H-C5'), 1.90-1.40(br,6H,H-C2',C3',C4')

4-p-Chlorophenylthio-3-hydroxybutyric acid **67**:

Procedure B

Yield: 95%

IR($CHCl_3$): 2920w, 2850w, 1705s, 1475s, 1095m

^{1}H-NMR(220MHz,$CDCl_3$): 7.41-7.28(m,4H,H-aromat), 4.14(m,1H,H-C3), 3.09(m,2H,H-C4), 2.75(dd,J=14.2,4.4,1H,H-C2), 2.62(dd,J=14.2,6.1,1H,H-C2)

^{13}C-NMR(22.63MHz,$CDCl_3$): 176.7(C1), 131.4, 129.3(C-aromat), 66.5(C3), 40.7, 39.8(C2,C4)

MS(EI): m/z = 246$(M)^{+\cdot}$, 228$(M-H_2O)^{+\cdot}$, 186, 158

UV(Acetonitrile): 225(6800), 260(12500)

4-p-Chlorophenylthio-3-tetrahydropyranyloxybutyric acid **68:**

Procedure B

Yield: 95%

IR($CHCl_3$): 3100br, 2980m, 2890w, 1710s, 1480s, 1100s, 1080m, 1030m

^{1}H-NMR(220MHz,$CDCl_3$): 7.50-7.20(m,4H,H-aromat), 4.75(m,1H,H-C1'), 4.3-2.5(7H, H-C3,C4,C2,C5'), 1.8-1.4(6H,H-C2',C3',C4')

MS(EI): m/z = 330$(M)^{+\cdot}$, 246(M-pyranyl)$^{+\cdot}$, 228, 183

4-p-Chlorophenylthio-3-tert-butyldimethylsilyloxybutyric acid **69:**

Procedure B

Yield: 90%

mp: 56-57°C

IR($CHCl_3$): 2970m, 2940m, 2870w, 1715s, 1480m, 1260m, 1100s, 1020w

^{1}H-NMR(220MHz,$CDCl_3$): 7.45-7.28(m,4H,H-aromat), 4.25(m,1H,H-C3), 3.07(m,2H,H-C4), 2.85(dd,J=14.8,4.0,1H,H-C2), 2.57(dd,J=14.8,8.1,1H,H-C2), 0.87(s,9H,H-tert-butyl), 0.03(s,3H,H-$SiCH_3$), 0.00(s,3H,H-$SiCH_3$)

^{13}C-NMR(45.28MHz,$CDCl_3$): 178.0(C1), 134.9, 132.7, 131.3, 129.4(C-aromat), 68.8(C3), 41.7, 41.3(C2,C4), 26.0($SiC(CH_3)_3$), 18.3($SiC(CH_3)_3$), -4.3, -4.6($SiCH_3$)

Elemental analysis: Found: C 53.21 H 6.81 S 9.88

$C_{16}H_{25}O_3ClSSi$ Calc: C 53.24 H 6.98 S 8.88

General procedure for the synthesis of β-ketoesters *via* acylation:

Carbonyldiimidazole (180mg,1.1mmol) was added to a solution of the acid (1.0mmol) in dry THF (5ml). After stirring at room temperature for 5h, the magnesium salt was added. The mixture was stirred for 18h at RT and then ethyl acetate (5ml) was added. The reaction mixture was washed with 0.5M aqueous hydrochloric acid (1x10ml) and 1M aqueous sodium hydrogen carbonate (1x10ml). The organic phase was then dried (Na_2SO_4) and the solvent was removed *in vacuo*. When necessary the residue was purified by flash chromatography.

Ethyl 5-hydroxy-3-oxohexanoate **59**:

Yield: 40%

bp: 110°C (0.05mmHg,decomp)

IR($CHCl_3$): 2990m, 2940w, 2910w, 1745s, 1715s, 1660w, 1420m, 1390m, 1380m, 1150m, 1055w, 1030m

^{1}H-NMR(220MHz,$CDCl_3$): 4.23(m,1H,H-C5), 4.22(q,J=7.3,2H,OCH_2CH_3), 3.49(s,2H,H-C2), 2.90(s,1H,OH), 2.70(m,2H,H-C4), 1.30(t,J=7.3,3H,OCH_2CH_3), 1.23(d,J=6.4,3H,H-C6)

Ethyl 5-methoxyethoxymethoxy-3-oxohexanoate **60**:

Yield: 90%

IR($CHCl_3$): 2990m, 2910w, 2890w, 1750s, 1725s, 1650w, 1450w, 1420w, 1380m, 1320m, 1100br, 1030m

^{1}H-NMR(220MHz,$CDCl_3$): 4.75(m,2H,H-C1'), 4.21(m,1H,H-C5), 4.20(q,J=7.1,2H,OCH_2CH_3), 3.69(m,2H,H-C2'), 3.58(m,2H,H-C3'),

3.50(s,2H,H-C2), 3.41(s,3H,H-C4'), 2.86(dd,J=14.9,6.8,1H,H-C4), 2.60(dd,J=14.9,4.1,1H,H-C4), 1.28(t,J=7.1,3H,OCH_2CH_3), 1.23(d,J=6.2,3H,H-C6)

Ethyl 5-hydroxy-3-oxo-6-(phenylthio)hexanoate **64**:

Yield: 60%

^{1}H-NMR(220MHz,$CDCl_3$): 7.48-7.20(m,5H,H-aromat), 4.21(m,q,J=7.3,3H,H-C5,OCH_2CH_3), 3.49(s,2H,H-C2), 3.09(m,2H,H-C6), 2.93(dd,J=13.2,3.8,1H,H-C4), 2.80(dd,J=13.2,7.9,1H,H-C4), 1.29(t,J=7.3,3H,OCH_2CH_3)

Methyl 5-methoxyethoxymethoxy-3-oxo-6-(phenylthio)-hexanoate **65**:

Yield: 80%

^{1}H-NMR(220MHz,$CDCl_3$): 7.43-7.15(m,5H,H-aromat), 4.76(m,2H,H-C1'), 4.26(m,1H,H-C5), 3.72(s,3H,OCH_3), 3.70(m,2H,H-C2'), 3.50(m,2H,H-C3'), 3.46(s,2H,H-C2), 3.38(s,3H,H-C4'), 3.27(dd,J=14.4,4.8,1H,H-C6), 3.08(dd,J=14.4,8.1,1H,H-C6), 2.93(m,2H,H-C4)

Ethyl 3-oxo-6-(phenylthio)-5-tetrahydropyranyloxyhexanoate **66**:

Yield: 90%

^{1}H-NMR(220MHz,$CDCl_3$): 7.45-7.10(m,5H,H-aromat), 4.65,4.55(m,1H,H-C1'), 4.15(m,3H,H-C5,OCH_2CH_3), 3.55-2.70(6H,H-C4,H-C6,H-C5'), 3.35,3.30(s,2H,H-C2), 1.90-1.50(br,6H,H-C2',C3',C4'), 1.30(t,J=7.5,3H,OCH_2CH_3)

Methyl 6-(p-chlorophenylthio)-5-hydroxy-3-oxohexanoate **28**:

The spectral data were identical to those reported for **rac-28**.

Yield: 40%

Methyl (R)-6-(p-chlorophenylthio)-5-hydroxy-3-oxohexanoate **L-28:**

The spectral data were identical to those reported for **rac-28**.

Recrystallised from dichloromethane/pentane.

Yield: 42%

mp: 50.0-51.0°C

$[\alpha]_D$ = +14.0° ±1.0° (c=1.2,$CHCl_3$)

Methyl (S)-6-(p-chlorophenylthio)-5-hydroxy-3-oxohexanoate **D-28:**

The spectral data were identical to those reported for **rac-28**.

Recrystallised from ether/pentane.

Yield: 35%

mp: 51-52°C

$[\alpha]_D$ = -14.1° ±1.0° (c=1.0,$CHCl_3$)

Methyl 6-(p-chlorophenylthio)-3-oxo-5-tetrahydropyranyloxyhexanoate **70:**

Yield: 70%

^{1}H-NMR(220MHz,$CDCl_3$): 7.50-7.25(m,4H,H-aromat), 4.70, 4.15(m,1H,H-C1'), 4.30(br,1H,H-C5), 3.80, 3.78(s,5H,H-C2,OCH_3), 3.50(br,4H,H-C6,H-C5'), 3.0(br,2H,H-C4), 1.80-1.40(m,6H,H-C2',C3',C4')

Methyl 6-(p-chlorophenylthio)-3-oxo-5-tert-butyldimethylsilyloxyhexanoate **71:**

Yield: 96%

IR($CHCl_3$): 2970m, 2940m, 2870m, 1750s, 1720s, 1660w, 1635w, 1480s, 1100s, 1020m

^{1}H-NMR(220MHz,$CDCl_3$): 7.53(4H,H-aromat), 4.31(m,1H,H-C5), 3.78(s,3H,OCH_3), 3.39(s,2H,H-C2), 2.95(dd,J=13.9,6.1,1H,H-C6), 2.77(dd,J=13.9,6.0,1H,H-C6), 2.65(dd,J=14.3,6.2,1H,H-C4), 2.36(dd,J=14.3,6.2,1H,H-C4), 0.85(s,9H,H-tert-butyl), 0.02(s,6H,H-$SiCH_3$)

General procedure for the cleavage of MEM-ethers:

To a solution of the MEM-ether (1mmol) in dry dichloromethane (5ml) was added the Lewis acid (2.5mmol) and the mixture was stirred under nitrogen at RT. The reaction mixture was washed with 1M aqueous sodium carbonate, the organic phase was dried (Na_2SO_4) and the solvent removed *in vacuo*. The product was obtained by flash chromatography.

Yields in %:	$ZnBr_2$	$TiCl_4$	$ZnCl_2$	$SnCl_4$
51	20	18	15	10
59	15	19	12	--
64	5	--	10	--

General procedure for the cleavage of THP-ethers:

Procedure A:

A solution of THP-ether (1mmol) in acetic acid:THF:water=4:2:1 (20ml) was stirred at 50°C until the reaction was complete. The solution was adjusted to pH 8 and then extracted with ethyl acetate. The combined organic extracts were dried (Na_2SO_4) and the solvent was removed *in vacuo*.

Procedure B:

To a solution of the THP-ether (1mmol) in methanol (8ml) was added pyridinium p-toluenesulfonate (0.1mmol) and the reaction mixture was stirred at 60°C for 1h. The solvent was removed *in vacuo* and the product purified by flash chromatography.

Product:	Method:	Yield:
64	A	71%
28	B	75%

Procedure for the cleavage of silyl-ethers:

To a solution of silyl-ether **71** (1mmol) in THF was added tetrabutylammonium fluoride (1.3mmol), and the reaction was stirred at RT. When the reaction was complete, 0.5M aqueous hydrochloric acid was added and the mixture was extracted with ethyl acetate. The combined organic extracts were dried (Na_2SO_4) and the solvent removed *in vacuo*.

Yield: 82%

Diastereoselective reduction of 5-hydroxy-3-oxoesters:

With sodium borohydride:

To a solution of ester **64** (1mmol) in the solvent (5ml) at the specified temperature was added solid sodium borohydride (0.5mmol). When the reaction was complete, 1M aqueous hydrochloric acid (2ml) was added and the reaction mixture was extracted with ethyl acetate (3x3ml). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed *in vacuo*.

Solvent	Temp.	%yield	%de
THF	0°C	70	0
THF/MeOH=4/1	0°C	85	0
THF/MeOH=4/1	-20°C	90	10 *syn*
THF/iPrOH=4/1	-20°C	90	20 *syn*

With zinc borohydride:

To a solution of ester **64** (1mmol) in ether (5ml) at 0°C was added a solution of zinc borohydride (approx. 1.5mmol) in ether[202] and the mixture was stirred under nitrogen. When the reaction was complete 1M aqueous hydrochloric acid (2ml) was added and the reaction mixture was extracted with ether (3x3ml). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed *in vacuo*.

Yield: 76% 22%de *anti*

With chlorodiisopropylsilane:

Ethyl 5-diisopropylsilyloxy-3-oxo-6-(phenylthio)hexanoate:

Chlorodiisopropylsilane[203] (593mg,3.3mmol), pyridine (261mg,3.3mmol) and petrol (10ml) were placed in a 50ml two necked flask equiped with a reflux condenser and a magnetic stirrer. A solution of hydroxyester **64** (550mg,1.95mmol) in THF (5ml) was added *via* a syringe. The reaction mixture was heated under reflux for 6h and was stirred at RT overnight. After filtration, the solvent was removed *in vacuo* and the product was obtained by flash chromatography (ethyl acetate:petrol=1:19).

Yield: 405mg (52%)

IR($CHCl_3$): 2960s, 2870s, 2100m(Si-H), 1750s, 1720s, 1460m, 1370m, 1080m, 1050s

^{1}H-NMR(220MHz,$CDCl_3$): 7.40-7.10(m,5H,H-aromat), 4.36(m,1H,H-C3), 4.20(m,3H,Si-H,OCH_2CH_3), 3.42(s,2H,H-C2), 3.20-2.73(m,4H,H-C4,H-C6), 1.26(t,J=7.8,3H,OCH_2CH_3), 0.99-0.95(m,14H,H-isopropyl)

Ethyl 3,5-diisopropylsiladioxane-6-(phenylthio)hexanoate:

The silylated hydroxyketone (380mg,0.96mmol) in dichloromethane (5ml) was treated with tin tetrachloride (11µl) at -70°C under nitrogen over a period of 2h. The reaction mixture was then quenched with 1M aqueous sodium hydrogen carbonate (0.5ml), allowed to warm gradually to RT and extracted with ether. The organic extract was dried (Na_2SO_4) and the solvent was removed *in vacuo*.

Yield: 228mg (60%)

^{1}H-NMR(220MHz,$CDCl_3$): 7.5-7.2(m,5H,H-aromat), 4.6-4.2(m,2H,H-C3,H-C5), 4.18(q,2H,OCH_2CH_3), 3.3-2.3(m,4H,H-C2,H-C6), 2.05(m,2H,H-C4), 1.31(t,3H,OCH_2CH_3), 1.00(m,14H,H-isopropyl)

Cleavage of the siladioxane:

To a solution of siladioxane (200mg,0.5mmol) in acetonitrile (4ml) was added aqueous hydrofluoric acid (48%,5drops) and the mixture was stirred at RT for 30 min. The reaction mixture was partitioned between chloroform and water, the organic phase was dried ($MgSO_4$) and the solvent removed *in vacuo*.

Yield: 70% 70%de *anti*

Complexation with triisobutylborane:

Through a solution of hydroxyester **64** (1mmol) and triisobutylborane (1.1mmol) in THF (5ml) was bubbled a catalytic quantity of air and the reaction stirred at RT. The mixture was

cooled to -78°C and sodium borohydride (0.5mmol) was added. After quenching with a mixture of 30% hydrogen peroxide, pH 7 buffer and methanol the diol was obtained by extraction with dichloromethane, drying ($MgSO_4$) and removal of the solvent *in vacuo*.

Yield: 82% 45%de *syn*

Complexation with methoxydiethylborane:

To a solution of the hydroxyester (1mmol) in THF (8ml) and anhydrous methanol (2ml) at -70°C under argon was added dropwise methoxydiethylborane[204] (1.1mmol) and the resulting mixture was stirred for 15min. Then sodium borohydride (1mmol) was added and the mixture stirred for 3-5h, followed by the addition of acetic acid (1ml). The quenched reaction mixture was diluted with ethyl acetate, washed with 1M aqueous sodium carbonate, dried ($MgSO_4$) and the solvent removed *in vacuo*. The residue thus obtained was azeotroped with methanol until the hydrolysis of the boronate was complete.

Yield: 95% 95%de *syn*

With tetramethylammonium triacetoxyborohydride:

To a solution of tetramethylammonium triacetoxyborohydride (5mmol) in dry acetonitrile (4ml) and dry acetic acid (4ml) at -40°C was added a solution of the hydroxyester (1mmol) in dry acetonitrile (1ml). After the reaction had stirred for 5h it was quenched by the addition of 0.5M aqueous sodium potassium tartrate (15ml) and stirred vigorously for 30min. The mixture was then diluted with dichloromethane and washed with 1M aqueous sodium carbonate. The aqueous layer was back-extracted with

dichloromethane, the combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo*.

Yield: 95% 95%de *anti*

Analysis of de's for dihydroxyhexanoates by HPLC:

Column: S5 ODS2 C-18 reverse phase

Detection: 254nm

Flow rate: 0.9ml/min

Solvent system: acetonitrile:water = 32:68

Concentration sample: 1mg/ml 5µl injected

Retention times: *anti* 27.6min

syn 30.7min

keto 49.9min

Ethyl 3,5-dihydroxy-6-(phenylthio)hexanoate **72** and **73**:

^{1}H-NMR(400MHz,$CDCl_3$): 7.40-7.15(m,5H,H-aromat), 4.32(m,1H,H-C5*syn*), 4.22(m,1H,H-C5*anti*), 4.15(q,J=6.2,2H,OCH_2CH_3), 3.97(m,1H,H-C3), 3.81, 3.68, 3.47, 3.15(2H,OH), 3.00(m,2H,H-C6), 2.46(m,2H,H-C2), 1.87-1.59(m,2H,H-C4), 1.25(t,J=6.2,3H,OCH_2CH_3)

^{13}C-NMR(100.62MHz,$CDCl_3$): 172.1(C1), 143.5(C-aromat,*anti*), 135.3(C-aromat,*syn*), 129.4, 128.8(C-aromat), 69.7, 68.04(C5,C3,*syn*), 66.8, 65,2(C5,C3,*anti*), 60.5(OCH_2CH_3), 41.5, 41.1, 41.0(C2,C4,C6), 13.9(OCH_2CH_3)

Methyl 6-(p-chlorophenylthio)-3,5-dihydroxyhexanoate **29**:

Recrystallised from dichloromethane/pentane at -10°C.

mp: 45.0-46.0°C

IR($CHCl_3$): 3500br, 2970w, 2930w, 1730s, 1480s, 1445m, 1280m, 1100s, 1020m

^{1}H-NMR(220MHz,$CDCl_3$): 7.20(m,4H,H-aromat), 4.19(m,1H,H-C5), 3.87(m,1H,H-C3), 3.62(s,3H,OCH_3), 2.91(m,2H,H-C6), 2.48(m,2H,H-C2), 1.80-1.50(m,2H,H-C4)

^{13}C-NMR(45.28MHz,$CDCl_3$): 173.0(C1), 134.4, 132.7, 129.5(C-aromat), 70.3(C5), 68.6(C3), 52.2(OCH_3), 41.8, 41.7, 41.4(C2,C4,C6)

Elemental analysis: Found: C 51.48 H 5.31 Cl 11.35 S 10.82

$C_{13}H_{17}O_4ClS$ Calc: C 51.23 H 5.62 Cl 11.63 S 10.52

Methyl 6-(p-chlorophenylthio)-3(S),5(R)-dihydroxyhexanoate **L-29:**

The spectral data were identical to those reported for **rac-29**.

Recrystallised from dichloromethane/pentane at -10°C.

mp: 42.0-45.0°C

$[\alpha]_D$ = +7.1° ±1.0° (c=1.0,$CHCl_3$)

Methyl 6-(p-chlorophenylthio)-3(R),5(S)-dihydroxyhexanoate **D-29:**

The spectral data were identical to those reported for **rac-29**.

Recrystallised from dichloromethane/pentane at -10°C.

mp: 43.0-47.0°C

$[\alpha]_D$ = -7.2° ±1.0° (c=1.3,$CHCl_3$)

Methyl 6-(p-chlorophenylthio)-3,5-dihydroxyhexanoate **30:**

Recrystallised from ether/pentane.

mp: 86.8-87.2°C

IR($CHCl_3$): 3500br, 2970w, 2930w, 1730s, 1480s, 1445m, 1270m, 1100s, 1020m

^{1}H-NMR(220MHz,$CDCl_3$): 7.20(m,4H,H-aromat), 4.19(m,1H,H-C5), 3.87(m,1H,H-C3), 3.62(s,3H,OCH_3), 2.91(m,2H,H-C6), 2.48(m,2H,H-

C2), 1.80-1.50(m,2H,H-C4)

^{13}C-NMR(45.28MHz,$CDCl_3$): 173.3(C1), 134.4, 132.7, 131.3, 129.4(C-aromat), 67.1(C5), 65.5(C3), 52.1(OCH_3), 42.0, 41.5(C2,C4,C6)

Elemental analysis: Found: C 51.55 H 5.94 Cl 11.19 S 10.24

$C_{13}H_{17}O_4ClS$ Calc: C 51.23 H 5.62 Cl 11.63 S 10.52

Methyl 6-(p-chlorophenylthio)-3(R),5(R)-dihydroxyhexanoate **L-30:**

The spectral data were identical to those reported for **rac-30**.

Recrystallised from ether/pentane.

mp: 84.0-86.0°C

$[\alpha]_D$ = -22.4° ±1.5° (c=1.0,$CHCl_3$)

Methyl 6-(p-chlorophenylthio)-3(S),5(S)-dihydroxyhexanoate **D-30:**

The spectral data were identical to those reported for **rac-30**.

Recrystallised from ether/pentane.

mp: 88.0-89.0°C

$[\alpha]_D$ = +22.1° ±1.5° (c=1.1,$CHCl_3$)

Synthesis of boronic ester **78:**

To a solution of isopinocampheylboronate-TMEDA complex **77** (181mg,0.43mmol) in dry THF (10ml) was added the 3,5-dihydroxyester (265mg,0.87mmol) in THF (5ml), and the reaction was stirred overnight under argon. The solvent was then removed *in vacuo* and the product dried under reduced pressure for 24h.

Yield: 390mg (95%)

^{1}H-NMR(220MHz,$CDCl_3$): 7.40-7.25(m,4H,H-aromat), 4.37(m,1H,H-C5), 4.15(m,1H,H-C5), 3.69(s,3H,OCH_3), 3.28(dd,J=14.8,4.2,1H,H-C6),

2.91(dd,J=14.8,6.9,1H,H-C6), 2.60(dd,J=14.5,6.2,1H,H-C2), 2.39(dd,J=14.5,6.3,1H,H-C2), 2.1-1.0(18H,H-camphoyl,H-C4)

^{13}C-NMR(45.28MHz,$CDCl_3$): 171.4(C1), 135.1, 132.6, 131.2, 129.4(C-aromat), 70.6(C5), 68.3(C3), 52.1(OCH_3), 48.4, 42.3, 41.0, (C2,C4,C6), 38.9, 38.5, 37.5, 34.1, 28.8, 23.6, 23.1

Ethyl 3,5-isopropylidene-6-(phenylthio)-hexanoate **81:**

To a solution of 3,5-dihydroxyester **72/73** (180mg,0.63mmol) in dry dichloromethane at -10°C was added 2-methoxypropene (54.5mg,72.4µl,0.76mmol) and p-toluenesulfonic acid (5mg). The reaction mixture was stirred for 30min. and then quenched with 1M aqueous sodium hydrogen carbonate (2ml). The organic phase was dried ($MgSO_4$) and the solvent removed *in vacuo*. Flash chromatography (ethyl acetate:petrol=1:9) of the residue afforded the product.

Yield: 174mg (85%)

^{1}H-NMR(220MHz,$CDCl_3$): 7.45-7.20(m,5H,H-aromat), 4.30(m,1H,H-C5), 4.17(q,J=8.2,2H,OCH_2CH_3), 4.02(m,1H,H-C3), 3.00(m,2H,H-C6), 2.48(m,2H,H-C2), 1.80(m,2H,H-C4), 1.43, 1.40, 1.39, 1.32(s,6H,H-$C(CH_3)_2$), 1.27(t,J=8.2,3H,OCH_2CH_3)

^{13}C-NMR(100.62MHz,$CDCl_3$), *syn*-diastereomer: 170.5(C1), 136.2, 129.2, 128.7, 125.9(C-aromat), 99.0($C(CH_3)_2$), 68.1(C5), 65.7(C3), 60.2(OCH_2CH_3), 46.2, 39.3, 35.5(C2,C4,C6), 29.7, 19.5($C(CH_3)_2$), 14.0(OCH_2CH_3)

^{13}C-NMR(100.62MHz,$CDCl_3$), *anti*-diastereomer: 170.5(C1), 136.2, 129.4, 128.7, 126.0(C-aromat), 100.7($C(CH_3)_2$), 65.6(C5), 63.3(C3), 60.2(OCH_2CH_3), 40.6, 39.1, 36.9(C2,C4,C6), 24.5, 24.4($C(CH_3)_2$), 14.0(OCH_2CH_3)

3,5-isopropylidene-6-(phenylthio)hexanoic acid **82**:

Hydrolysis of **81** by method A.

Yield: 261mg (82%)

^{1}H-NMR(220MHz,$CDCl_3$): 7.45-7.20(m,5H,H-aromat), 4.30(m,1H,H-C5), 4.01(m,1H,H-C3), 3.20-2.85(m,2H,H-C6), 2.65(m,2H,H-C2), 1.90-1.70(m,2H,H-C4), 1.45, 1.41, 1.39, 1.33(s,6H,H-C(CH_3)$_2$)

Ethyl 5,7-isopropylidene-3-oxo-8(phenylthio)octanoate **83**:

83 was synthesised following the general procedure for acylation.

Yield: 251mg (78%)

IR($CHCl_3$): 3000m, 2940w, 1740s, 1715s, 1650w, 1585w, 1480w, 1440w, 1380m, 1370m, 1160m, 1030m

^{1}H-NMR(220MHz,$CDCl_3$): 7.35-7.05(m,5H,H-aromat), 4.25(m,1H,H-C7), 4.10(q,J=7.8,2H,O**CH$_2$**CH_3), 3.95(m,1H,H-C5), 3.40(s,2H,H-C2), 2.90(m,2H,H-C8), 2.60(m,2H,H-C4), 1.70(m,2H,H-C6), 1.32, 1.29(s,6H,H-C(CH_3)$_2$), 1.25(t,J=7.8,3H,OCH_2**CH_3**)

^{13}C-NMR(45.28MHz,$CDCl_3$): 200.9(C3), 167.0(C1), 136.2, 129.5, 129.3, 128.9, 126.1(C-aromat), 101.0, 99.2(**C**(CH_3)$_2$), 68.1(C2), 65.7, 65.5, 63.0, 61.3(C5,C7), 50.2(O$CH_2$$CH_3$), 50.0, 49.2, 48.6, 39.4, 35.8, 29.9, 19.6, 14.1(O$CH_2$$CH_3$)

Attempted cyclisation of **31** to the epoxide:

To a solution of **31** (700mg,3.2mmol) in dry dichloromethane (25ml) was added trimethyloxonium tetrafluoroborate (8.50mg,5.7mmol), and the mixture was stirred under nitrogen at RT for 3h. Then 1 to 3 equivalents of the base were added and the reaction was monitored by tlc (ethyl acetate:petrol=2:8). Workup included quenching with 0.5M aqueous hydrochloric acid, drying ($MgSO_4$) and evaporation of the solvent *in vacuo*. Purification by flash chromatography afforded the products. The reaction could easily be monitored by the appearence of methylphenylsulfide.

Methylphenylsulfide:

^{1}H-NMR(220MHz,$CDCl_3$): 7.30(m,5H,H-aromat), 2.50(s,3H,H-CH_3)

Ethyl 4-hydroxy-2-butenoate **85**:

^{1}H-NMR(220MHz,$CDCl_3$): 7.06(td,J=15.2,3.9,1H.H-C3), 6.11(td,J=15.2,2.1,1H,H-C2), 4.36(m,2H,H-C4), 4.22(q,J=6.4,2H,OCH_2CH_3), 2.6(br,1H,OH), 1.30(t,J=6.4,3H,OCH_2CH_3)

Base:	eq.	Product:	Yield:	Reaction time:
1M NaOH	3	-	-	3h
Et_3N	3	**31**	69%	24h
$(i\text{-}Pr)_2NEt$	3	**31**	71%	24h
pyridine	3	**31**	80%	24h
DMAP	3	**31**	65%	24h
1M EtONa/EtOH	1	**85**	10%	2h
Ag_2O/DME	2	-	-	12h

Attempted cyclisation of **67** to the epoxide:

To a solution of **67** (500mg,2mmol) in dry dichloromethane (30ml) was added the trialkyloxonium tetrafluoroborate (2.4mmol), and the reaction mixture was stirred at RT under nitrogen for 3h. Then 2 equivalent of the base were added and the reaction was monitored by tlc (ethyl acetate:petrol=2:8) by appearence of the alkylphenylsulfide byproduct. Workup included quenching with 0.5M aqueous hydrochloric acid, drying ($MgSO_4$) and evaporation of the solvent *in vacuo*.

Ethyl 4-(p-chlorophenylthio)-3-hydroxybutanoate **86**:

^{1}H-NMR(220MHz,$CDCl_3$): 7.45-7.25(m,4H,H-aromat), 4.19(m,q,J=7.5,3H,H-C3,OCH_2CH_3), 3.24(d,J=3.7,1H,OH), 3.08(m,2H,H-C4), 2.61(m,2H,H-C2), 1.28(t,J=7.5,3H,OCH2CH_3)

Meerwein reagent	Base	Product	Yield
$(CH_3)_3OBF_4$	ET_3N	**67**	60%
$(CH_3)_3OBF_4$	0.1M KOH	**16**	50%
$(CH_3CH_2)_3OBF_4$	ET_3N	**67**	80%
$(CH_3CH_2)_3OBF_4$	0.1M KOH	**86**	70%

Attempted cyclisation *via* the silylether:

To a solution of **44** (50mg,0.16mmol) in dry dichloromethane (5ml) was added trimethyloxonium tetrafluoroborate (28mg,0.19mmol) and the reaction mixture was stirred at RT under nitrogen for 3h. Then tetrabutylammonium fluoride (0.19mmol) was added and the reaction was stirred over night. Workup included quenching with 0.5M aqueous hydrochloric acid, drying ($MgSO_4$) and evaporation of the solvent *in vacuo*. The

products were purified by flash chromatography (ethyl acetate:petrol=3:7).

Ethyl 3-hydroxy-4-(phenylthio)butanoate **31**: Yield: 40%

Ethyl 3-trimethylsilyloxy-4-(phenylthio)butanoate **44**: Yield: 30%

Methyl 4-(p-chlorophenylsulfinyl)-3-hydroxybutanoate **87** and **88**:

To a solution of **16** (5g,14mmol) in dry dichloromethane (20ml) at 0°C was slowly added a solution of mCPBA (15mmol) in dichloromethane (25ml) and the reaction was stirred for 7h. Then 1M aqueous sodium carbonate was added (40ml), the mixture extracted with dichloromethane (3x20ml), the combined organic extracts dried ($MgSO_4$) and the solvent removed *in vacuo*. Recrystallisation from ether/pentane afforded the two diastereomers, which in turn were purified by recrystallisation.

Diastereomer A:

Solubility in ether: high

Yield: 1.2g (30%)

IR($CHCl_3$): 3450br, 2960w, 1735s, 1580w, 1480s, 1445s, 1395m, 1100s, 1050br, 1015s

^{1}H-NMR(220MHz,$CDCl_3$): 7.80-7.50(m,4H,H-aromat), 4.58(m,1H,H-C3), 4.01(d,J=2.1,1H,OH), 3.75(s,3H,OCH_3), 3.11(dd,J=12.9,5.2,1H,H-C4), 2.99(dd,J=12.9,3.3,1H,H-C4), 2.78(dd,J=13.1,5.2,1H,H-C2), 2.65(dd,J=13.1,4.1,1H,H-C2)

^{13}C-NMR(45.28MHz,$CDCl_3$): 171.6(C1), 141.8, 137.5, 129.7, 125.6(C-aromat), 64.5(C3), 62.5(C4), 51.9(OCH_3), 40.7(C2)

Elemental analysis: Found: C 47.69 H 4.92 Cl 12.86 S 11.92

$C_{11}H_{13}O_4ClS$ Calc: C 47.74 H 4.74 Cl 12.81 S 11.59

Diastereomer B:

Solubility in ether: low

Yield: 1.1g (28%)

IR($CHCl_3$): 3450br, 2960w, 1735s, 1580w, 1480s, 1445s, 1395m, 1100s, 1050br, 1015s

^{1}H-NMR(220MHz,$CDCl_3$): 7.73-7.55(m,4H,H-aromat), 4.67(m,1H,H-C3), 4.29(d,J=2.1,1H,OH), 3.72(s,3H,OCH_3), 3.10(dd,J=14.8,6.5,1H,H-C4), 2.85(dd,J=14.8,3.1,1H,H-C4), 2.60(m,2H,H-C2)

^{13}C-NMR(45.28MHz,$CDCl_3$): 171.4(C1), 141.7, 137.4, 129.7, 125.4(C-aromat), 63.0(C3), 62.7(C4), 51.9(OCH_3), 40.9(C2)

Methyl 4-(p-chlorophenylsulfonyl)-3-hydroxybutanoate **89**:

To a solution of **16** (5g,14mmol) in dry dichloromethane (20ml) at 0°C was slowly added a solution of mCPBA (30mmol) in dichloromethane (50ml) (exothermic reaction) and the reaction was stirred overnight at 30°C. Then 1M aqueous sodium carbonate was added (80ml), the mixture extracted with dichloromethane (3x40ml), the combined organic extracts dried ($MgSO_4$) and the solvent removed *in vacuo*. The product was purified by recrystallisation from ether/pentane.

Yield: 3.7g (90%)

mp: 69-70°C

IR($CHCl_3$): 3550br, 2960w, 1735s, 1585m, 1480m, 1440s, 1320s, 1155s, 1095s, 1015m

^{1}H-NMR(220MHz,$CDCl_3$): 7.93(m,2H,H-aromat), 7.69(m,2H,H-aromat), 4.55(m,1H,H-C3), 3.71(s,3H,OCH_3), 3.50(m,2H,H-C4), 2.64(m,2H,H-C2)

^{13}C-NMR(45.28MHz,$CDCl_3$): 171.4(C1), 140.5, 138.1, 129.7, 129.6(C-aromat), 63.2(C3), 61.3(C4), 51.9(OCH_3), 40.5(C2)

MS(CI): m/z = 310$(M+NH_4)^+$, 293$(M+H)^+$, 277, 243

Elemental analysis: Found: C 45.01 H 4.42 Cl 12.34 S 11.08

$C_{11}H_{13}O_5ClS$ Calc: C 45.13 H 4.48 Cl 12.11 S 10.95

4-(p-Chlorophenylsulfonyl)-3-hydroxybutyric acid **90**:

89 was hydrolysed by procedure B to **90**.

Yield: 88%

mp: 105-108°C

^{1}H-NMR(220MHz,$CDCl_3$): 7.92(m,2H,H-aromat), 7.62(m,2H,H-aromat), 4.60(m,1H,H-C3), 3.40(m,2H,H-C4), 2.70(m,2H,H-C2)

MS(CI): m/z = 296$(M+NH_4)^+$, 279$(M+H)^+$, 261$(279-H_2O)^+$, 243, 175, 128

Methyl 3-tert-butyldimethylsilyloxy-4-(p-chlorophenylsulfonyl)-butanoate **91**:

Following the general procedure, **91** was synthesized from **89**.

Yield: 80%

IR($CHCl_3$): 2970s, 2940s, 2870m, 1740s, 1590w, 1480m, 1445m, 1325s, 1160s, 1095s, 1020m

^{1}H-NMR(220MHz, $CDCl_3$): 7.92(m,2H,H-aromat), 7.60(m,2H,H-aromat), 4.58(m,1H,H-C3), 3.68(s,3H,OCH_3), 3.51(dd,J=14.3,5.8,1H,H-C4), 3.30(dd,J=14.3,3.9,1H,H-C4), 2.89(dd,J=13.9,4.0,1H,H-C2), 2.61(dd,J=13.9,6.1,1H,H-C2), 0.79(s,9H,H-tert-butyl), -0.02(s,3H,H-$SiCH_3$), -0.05(s,3H,H-$SiCH_3$)

^{13}C-NMR(45.28MHz,$CDCl_3$): 171.0(C1), 140.8, 138.8, 129.9, 129.7(C-aromat), 64.4(C3), 62.2(C4), 51.9(OCH_3), 42.2(C2), 25.8($SiC(CH_3)_3$), 18.0($SiC(CH_3)_3$), -4.7, -4.9($SiCH_3$)

MS(CI): m/z = 424$(M+NH_4)^+$, 407$(M)^+$, 349$(M\text{-tert-butyl})^+$, 159

3-tert-Butyldimethylsilyloxy-4-(p-chlorophenylsulfonyl)butyric acid **92:**

91 was hydrolysed by procedure B to **92.**

Yield: 75%

IR($CHCl_3$): 3500br, 2960s, 2940s, 2870m, 1720s, 1590w, 1480w, 1400w, 1320s, 1155s, 1095vs, 1020m

^{1}H-NMR(220MHz,$CDCl_3$): 7.91(m,2H,H-aromat), 7.62(m,2H,H-aromat), 4.58(m,1H,H-C3), 3.50(dd,J=14.8,6.2,1H,H-C4), 3.31(dd,J=14.8,3.8,1H,H-C4), 2.97(dd,J=14.2,4.1,1H,H-C2), 2.65(dd,J=14.2,5.5,1H,H-C2), 0.79(s,9H,H-tert-butyl), -0.01(s,3H,H-$SiCH_3$), -0.05(s,3H,H-$SiCH_3$)

General procedure for the attempted deprotonation of sulfones **89-92:**

To a solution of the sulfone (1mmol) in dry THF (10ml) and HMPA (360mg,348µl,2mmol) or TMEDA (232mg,300µl,2mmol) at -78°C was slowly added butyllithium (2.2mmol) in hexane, and the reaction stirred for 10 min. Methyl iodide (199mg,87µl,1.4mmol) was then added and the reaction gradually warmed to RT. After 12h 0.5M aqueous hydrochloric acid (5ml) was added and the mixture extracted with ethyl acetate. The combined organic extracts were dried ($MgSO_4$), the solvent removed *in vacuo* and the residue purified by flash chromatography.

Yields:

starting material:	reisolated starting material:	**93:**
89	50%	30%
90	95%	-
91	45%	28%
92	80%	-

Methyl 4-(p-chlorophenylsulfonyl)-2,2-dimethyl-3-butenoate **93:**
^{1}H-NMR(220MHz,$CDCl_3$): 7.79(m,2H,H-aromat), 7.56(m,2H,H-aromat), 7.06(d,J=15.7,1H,H-C4), 5.84(d,J=15.7,1H,H-C3), 3.80(s,3H,OCH_3), 1.53(s,6H,H-CH_3)

Attempted nucleophilic displacement of sulfide by iodide:
A solution of **47** (690mg,2mmol), sodium iodide (360mg,2.4mmol) and methyl iodide 92.84g,1.25ml,20mmol) in dry DMF (3ml) was stirred at 75°C for 4h. Water (3ml) was then added and the mixture was extracted with dichloromethane (3x3ml). The organic extracts were washed with saturated aqueous sodium chloride (1x5ml), dried ($MgSO_4$) and the solvent removed *in vacuo*. Flash chromatography (ethyl acetate:petrol=2:8) afforded 7 products of which only one could be characterised.

Methyl 4-(p-chlorophenylthio)-3-hydroxybutanoate **16:**
The spectral data were identical to those reported for **rac-16.**
Yield: 55%

Methyl 3-acetoxy-4-bromo-4-(p-chlorophenylthio)butanoate **94**:

A solution of acetate **48** (302mg,1mmol), N-bromosuccinimide (196mg,1.1mmol) and AIBN (8mg,0.05mmol) in carbon tetrachloride (10ml) was refluxed for 12h. The reaction mixture was then allowed to cool to RT, filtered and the solvent removed *in vacuo*. The residue was purified by flash chromatography (ethyl acetate:petrol=1:9).

Yield: 179mg (47%)

^{1}H-NMR(220MHz,$CDCl_3$): 7.60-7.40(m,4H,H-aromat), 5.61(m,2H,H-C3,H-C4), 3.75(s,3H,H-OCH_3), 2.98(m,2H,H-C2), 2.12(s,3H,H-$COCH_3$)

Methyl 3-acetoxy-4-chloro-4-(p-chlorophenylthio)butanoate **95**:

A solution of acetate **48** (302mg,1mmol) and N-chlorosuccinimide (187mg,1.4mmol) in dry carbon tetrachloride (40ml) was stirred under nitrogen and irradiated with a 100W bulb for 23h, which increased the reaction temperature to 55°C. The mixture was cooled to -10°C for a further 12h and then filtered. The filtrate was washed with 0.5M aqueous hydrochloric acid (1x5ml), dried (Na_2SO_4) and the solvent removed *in vacuo*. For analytical purpose a sample was purified by flash chromatography.

Yield: 77-90%

IR($CHCl_3$): 2960w, 2940w, 2860w, 1750s, 1480m, 1440m, 1380m, 1100m, 1020m

^{1}H-NMR(220MHz,$CDCl_3$): 7.62-7.40(m,4H,H-aromat), 5.70-5.50(m,2H,H-C3,H-C4), 3.76(s,3H,OCH_3), 2.95(m,2H,H-C2), 2.13(s,3H,H-$COCH_3$)

Acc.Mass: $C_{13}H_{14}O_4SCl_2$ 335.99798 -0.7%

Methyl 3-acetoxy-4-(p-chlorophenylthio)-3-butenoate **96**:

IR($CHCl_3$): 2970w, 1760s, 1580w, 1480m, 1445m, 1380m, 1100m, 1020m

^{1}H-NMR(220MHz,$CDCl_3$): 7.80-7.45(m,4H,H-aromat), 5.96(dd,J=8.8,2.3,1H,H-C4), 3.76(s,3H.OCH_3), 3.35(dd,J=13.4,2.3),1H,H-C2), 2.98(dd,J=13.4,8.8,1H,H-C2), 2.18(s,3H,H-$COCH_3$)

Methyl 4-chloro-4-(p-chlorophenylthio)-3-hydroxybutanoate **97**:

A solution of hydroxyester **16** (260mg,1mmol) and N-chlorosuccinimide (160mg,1.2mmol) in dry carbon tetrachloride (40ml) was stirred under nitrogen and irradiated with a 100W bulb for 1h, which increased the reaction temperature to 30°C. The mixture was cooled to -10°C for a further 6h and then filtered. The filtrate was washed with 0.5M aqueous hydrochloric acid (1x5ml), dried (Na_2SO_4) and the solvent removed *in vacuo*.

Yield: 132mg (45%)

^{1}H-NMR(220MHz,$CDCl_3$): 7.60-7.30(m,4H,H-aromat), 5.31(m,1H,H-C4), 4.35(m,1H,H-C3), 3.55(s,3H,OCH_3), 2.90-2.65(m,3H,H-C2,OH)

General procedure for the addition of silylenolether:

To a solution of the chlorosulfide **95** (50mg,0.44mmol) and the silylenolether (0.5mmol) in dry dichloromethane (5ml) was added dry zinc bromide (5mg,0.02mmol) and the reaction was stirred at RT for 2h. The solvent was then removed *in vacuo* and the residue purified by flash chromatography.

Methyl 3-acetoxy-4-(p-chlorophenylthio)-6-oxo-6-phenylhexanoate **98**:

Yield: 84mg (45%)

^{1}H-NMR(220MHz,$CDCl_3$): 7.98(d,J=8.3,2H,H-aromat), 7.70-7.30(m,7H,H-aromat), 5.58(m,1H,H-C3), 4.22(m,1H,H-C4), 3.69(s,3H,OCH_3), 3.31(m,2H,H-C5), 3.01(dd,J=14.4,3.8,1H,H-C2), 2.82(dd,J=14.4,6.9,1H,H-C2), 2.03(s,3H,H-$COCH_3$)

Methyl 3-acetoxy-4-(p-chlorophenylthio)-6-oxotetradecanoate **99**:

Yield: 80%

$[\alpha]_D$ = +5.2° ±1.0° (c=1.0,$CHCl_3$) for **L-99**

IR($CHCl_3$): 2980m, 2940m, 2860w, 1745s, 1715m, 1480m, 1440w, 1100m, 1020w

^{1}H-NMR(220MHz,$CDCl_3$): 7.60-7.45(m,4H,H-aromat), 5.51(m,1H,H-C3), 4.02(m,1H,H-C4), 3.70(s,3H,OCH_3), 3.00-2.69(m,4H,H-C2,H-C5), 2.45(m,2H,H-C7), 2.04(s,3H,H-$COCH_3$), 1.60(m,2H,H-C8), 1.29(m,10H,H-C9,C10,C11,C12,C13), 0.89(m,3H,H-C14)

MS(EI): m/z = 456$(M)^{+\cdot}$, 396, 364, 253

Methyl 3-acetoxy-4-(p-chlorophenylthio)-6-dithianetetradecanoate **100**:

To a solution of **99** (246mg,0.54mmol) and propanedithiol (87mg,81ml,0.81mmol) in dry dichloromethane (5ml) was added freshly distilled borontrifluoride etherate (153mg,133ml,0.81mmol) and the reaction was stirred at RT for 5h. It was then quenched with 5% aqueous sodium hydroxide (2ml), the organic phase was separated, dried ($MgSO_4$) and the

solvent removed *in vacuo*. The product was obtained by flash chromatography (ethyl acetate:petrol=1:9).

Yield: 244mg (85%)

$[\alpha]_D$ = -17.9° ±1.0° (c=3.0,$CHCl_3$) for **L-100**

IR($CHCl_3$): 2970s, 2940s, 2860m, 1735s, 1480s, 1440m, 1100s, 1020m

^{1}H-NMR(220MHz,$CDCl_3$): 7.60-7.38(m,4H,H-aromat), 3.90(m,1H,H-C3), 3.85(m,1H,H-C4), 3.80(s,3H,OCH_3), 3.3-2.3(10H), 2.10-1.75(6H), 1.50(m,2H,H-C8), 1.30(m,10H,H-C9,C10,C11,C12,C13), 0.90(m,3H,H-C14)

^{13}C-NMR(45.26MHz,$CDCl_3$): 171.6(C1), 134.1, 129.6(C-aromat), 53.7, 52.8, 52.3, 46.0, 40.0, 39.2, 36.0, 33.4, 32.2, 31.1, 30.0, 29.7, 29.6, 26.7, 25.4, 24.3, 23.7, 23.0, 14.6

Methyl 3-acetoxybutanoate **101**:

To a solution of sodium hydroxide (1.28g,32mg) in water (5ml) was carefully added nickel-aluminium alloy (1g) and the suspension was stirred for 30 min at 50°C. The liquid was then decanted and the RaNi washed with water (5ml portions until neutral) and ethanol (3x5ml). To the so-prepared RaNi was added a solution of **100** in methanol (5ml) or ethyl acetate (5ml) and the mixture refluxed over night. The suspension was filtered and the solvent removed *in vacuo*. Flash chromatography of the residue afforded the product.

Yield 25%

IR($CHCl_3$): 2930s, 2860s, 1735m, 1470w, 1045w

^{1}H-NMR(220MHz,$CDCl_3$): 5.15(m,1H,H-C3), 3.74(s,3H,OCH_3), 2.55(dd,J=14.3,3.0,1H,H-C2), 2.43(dd,J=14.3,6.8,1H,H-C2), 1.99(s,3H,H-$COCH_3$), 1.7-1.1(br,m,20H,H-C4-C13), 0.90(m,3H,H-C14)

^{13}C-NMR(45.26MHz,$CDCl_3$): 172.3($COCH_3$), 170.4(C1), 69.3(C3), 52.0(OCH_3), 41.0, 36.6(C2,C4), 31.9(C5), 29.6, 29.4, 25.4, 22.7(C6-C13), 21.0($COCH_3$), 14.1(C14)

3-Hydroxytetradecanoic acid **102:**

101 was hydrolysed by procedure B to **102.**

Yield: 80%

$[\alpha]_D$ = -13.5° ±1.0° (c=0.7,$CHCl_3$) for **D-102**

$[\alpha]_D$ = +10.1° ±1.0°(c=1.0,$CHCl_3$) for **L-102**

Literature:[205] $[\alpha]_D$ = -13.8° (c=1.0,$CHCl_3$) for (R)-D-3-hydroxyhexadecanoic acid

IR($CHCl_3$): 3500br, 2940s, 2860s, 1710s, 1420w, 1050w

^{1}H-NMR(220MHz,$CDCl_3$): 6.8(br,1H,OH), 4.09(m,1H,H-C3), 2.63(dd,J=13.8,3.2,1H,H-C2), 2.49(dd,J=13.8,6.4,1H,H-C2), 1.7-1.2(m,20H,H-C4-C13), 0.90(m,3H,H-C4)

^{13}C-NMR(45,26MHz,$CDCl_3$): 178.0(C1), 68.5(C3), 41.5(C2), 36.8(C4), 32.3(C5), 30.0(C6-C11), 25.8(C12), 23.1(C13), 14.5(C14)

2-tert-Butyl-1,3-dioxanone **103:**

3-Hydroxyacid **67** (8g,32.4mmol), pivaldehyde (4.8g,56.2mmol) and pyridinium p-toluenesulfonate (804mg,3.2mmol) were refluxed in benzene (200ml) in a Dean-Stark apparatus for 12h. Then dichloromethane (200ml) was added and the mixture was washed with 1M aqueous sodium carbonate, which in turn was extracted with dichloromethane. The combined organic extracts were dried ($MgSO_4$), the solvent removed *in vacuo* and the product purified by flash chromatography (ethyl acetate:petrol=2:8). The product was recrystallised from ether/pentane.

Yield: 88%

mp: 55°C

IR($CHCl_3$): 2970w, 1745s, 1440m, 1380m, 1035s

^{1}H-NMR(220MHz,$CDCl_3$): 7.40-7.25(m,4H,H-aromat), 4.83(s,1H,H-C**H**C(CH_3)$_3$), 4.02(m,1H,H-C3), 3.15(dd,J=13.8,7.1,1H,H-C4), 2.99(dd,J=13.8,7.1,1H,H-C4), 2.66(dd,J=14.6,4.9,1H,H-C2), 2.46(dd,J=14.6,7.5,1H,H-C2), 0.95(s,9H,H-CHC(C**H$_3$**)$_3$)

^{13}C-NMR(45.26MHz,$CDCl_3$): 167.4(C1), 133.9, 131.2, 129.1(C-aromat), 108.1(**C**HC(CH_3)$_3$), 73.1(C3), 38.3(C4), 35.1(C2), 23.8(CHC(**C**H$_3$)$_3$)

Elemental analysis: Found: C 57.01 H 6.24 Cl 11.41 S 9.96

$C_{15}H_{19}O_3ClS$ Calc: C 57.23 H 6.08 Cl 11.26 S 10.18

2-tert-Butyl-5-methyl-1,3-dioxanone **104**:

To a solution of diisopropylamine (169mg,1.67mmol) in dry THF (5ml) at -10°C was slowly added butyllithium (1.67mmol) in hexane and the mixture stirred for 15min. The reaction was then cooled to -78°C and a solution of **103** (500mg,1.59mmol) in THF (5ml) was slowly added. Again the mixture was stirred for 15min, and then freshly distilled methyl iodide (1.13g,7.95mmol) was added. The reaction was stirred for 12h and then quenched with 3M aqueous ammonium chloride. The mixture was extracted with ethyl acetate, the combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo*. The product was purified by flash chromatography (ethyl acetate:petrol=1:9).

Yield: 303mg (58%)

mp: 54-55°C

IR($CHCl_3$): 2990m, 2880w, 1740s, 1480s, 1350m, 1100s, 1060m, 1040m, 1015s

^{1}H-NMR(220MHz,$CDCl_3$): 7.40-7.30(m,4H,H-aromat), 4.84(s,1H,H-CHC(CH_3)$_3$), 3.71(ddd,J=10.5,7.2,3.3,1H,H-C3), 3.25(dd,J=14.4,3.3,1H,H-C4), 3.05(dd,J=14.4,7.2,1H,H-C4), 2.59(qd,J=10.5,7.2,1H,H-C2), 1.23(d,J=7.2,3H,H-CH_3), 0.93(s,9H,H-C(CH_3)$_3$)

Elemental analysis: Found: C 59.90 H 6.58 Cl 11.24 S 9.05

$C_{16}H_{21}O_3ClS$ Calc: C 58.44 H 6.44 Cl 10.78 S 9.75

4. Appendix.

Molecular structure of sulfoxide A, **rac-87**:

The X-ray crystal structure of diastereomer A shows a ***syn*** relationship between the hydroxyl group on C-3 and the oxygen on the sulfur.

Crystal data: orthorhombic, spacegroup $Pbca$, a = 8.146(2), b = 11.036(4), c = 28.781(7) Å, U = 2587(1) Å, Z = 8, R = 0.052 for 1792 unique observed [$I/\sigma(I)\geq 2.0$] reflections.

Atomic coordinates, bond lenghts and bond angles:

Table 1. Atomic coordinates ($x10^4$) and equivalent isotropic displacement parameters ($Å^2x10^3$)

	x	y	z	U(eq)
S	6670.6(12)	696.7(9)	4510.0(3)	37(1)
Cl	8553(2)	2550(2)	2527(1)	84(1)
O(1)	5577(4)	647(3)	6314(1)	54(1)
O(2)	8179(4)	1201(4)	6385(1)	76(1)
O(3)	8063(4)	-306(3)	5398(1)	45(1)
O(4)	5054(3)	1282(3)	4624(1)	43(1)
C(1)	6946(5)	1134(4)	6160(1)	44(1)
C(2)	6794(5)	1561(4)	5666(1)	43(1)
C(3)	8140(5)	976(3)	5374(1)	38(1)
C(4)	8194(5)	1407(3)	4873(1)	39(1)
C(5)	7339(5)	1324(4)	3966(1)	37(1)
C(6)	6414(5)	2212(4)	3762(1)	44(1)
C(7)	6818(6)	2620(4)	3317(1)	52(1)
C(8)	8129(6)	2102(5)	3094(1)	53(2)
C(9)	9082(6)	1227(5)	3296(2)	61(2)
C(10)	8676(6)	830(5)	3744(2)	55(2)
C(11)	5657(8)	153(6)	6782(2)	74(2)

* Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor

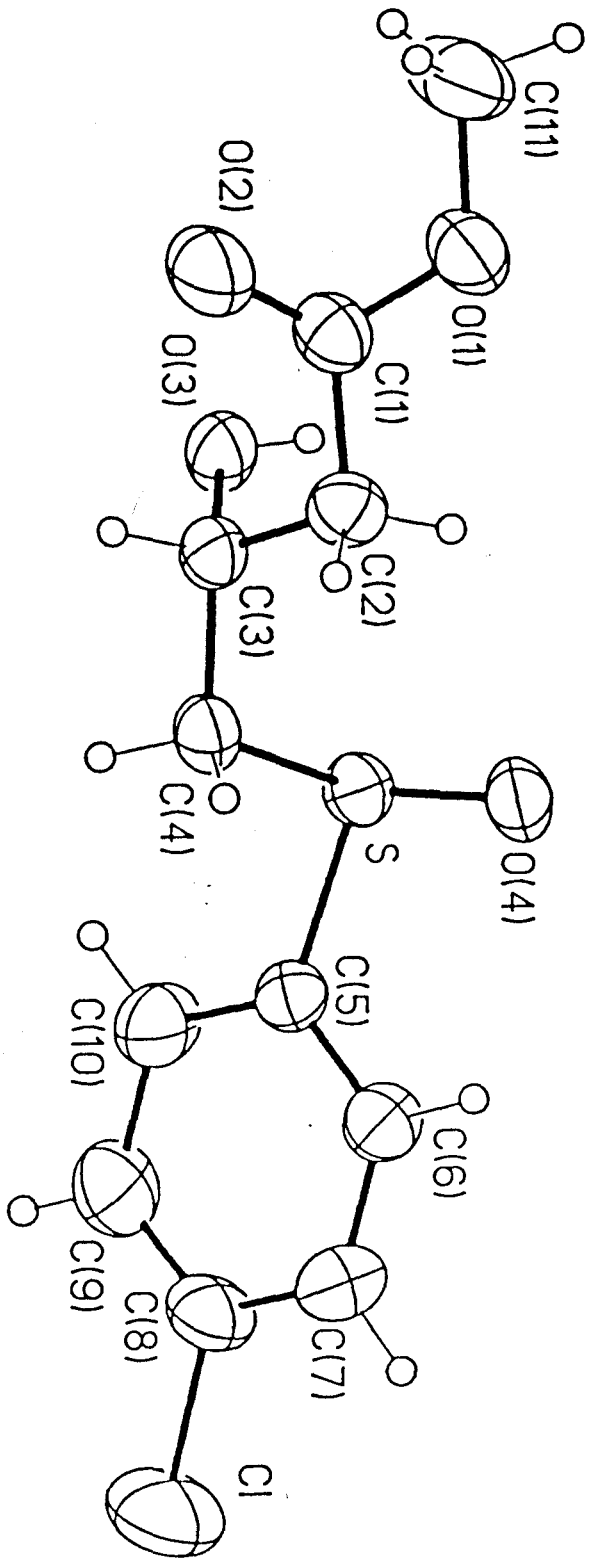
Table 2. Bond lengths (Å)

S-O(4)	1.503 (3)	S-C(4)	1.802 (4)
S-C(5)	1.796 (4)	Cl-C(8)	1.741 (4)
O(1)-C(1)	1.315 (5)	O(1)-C(11)	1.454 (6)
O(2)-C(1)	1.196 (6)	O(3)-C(3)	1.417 (5)
C(1)-C(2)	1.502 (6)	C(2)-C(3)	1.525 (6)
C(3)-C(4)	1.520 (5)	C(5)-C(6)	1.368 (6)
C(5)-C(10)	1.376 (6)	C(6)-C(7)	1.397 (6)
C(7)-C(8)	1.371 (7)	C(8)-C(9)	1.369 (7)
C(9)-C(10)	1.401 (6)		

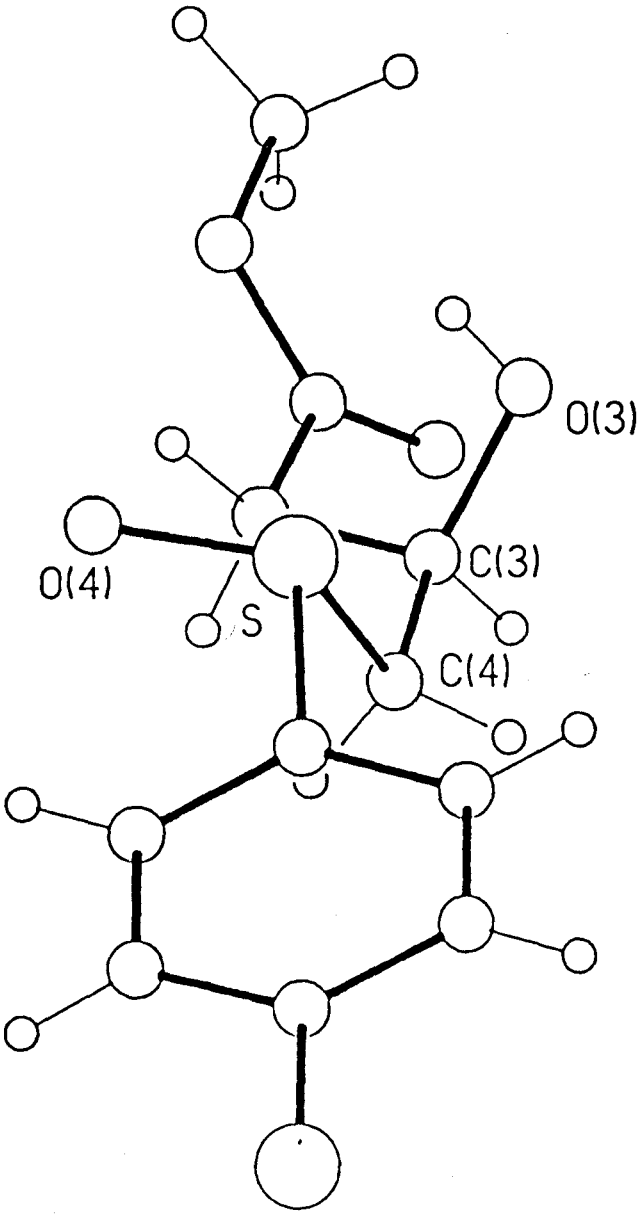
Table 3. Bond angles (°)

O(4)-S-C(4)	106.8(2)	O(4)-S-C(5)	106.9(2)
C(4)-S-C(5)	97.4(2)	C(1)-O(1)-C(11)	115.3(4)
O(1)-C(1)-O(2)	123.7(4)	O(1)-C(1)-C(2)	112.1(4)
O(2)-C(1)-C(2)	124.2(4)	C(1)-C(2)-C(3)	109.2(3)
O(3)-C(3)-C(2)	111.4(3)	O(3)-C(3)-C(4)	111.1(3)
C(2)-C(3)-C(4)	114.3(3)	S-C(4)-C(3)	113.2(3)
S-C(5)-C(6)	118.9(3)	S-C(5)-C(10)	119.5(3)
C(6)-C(5)-C(10)	121.4(4)	C(5)-C(6)-C(7)	119.6(4)
C(6)-C(7)-C(8)	118.6(4)	Cl-C(8)-C(7)	118.4(4)
Cl-C(8)-C(9)	119.1(4)	C(7)-C(8)-C(9)	122.4(4)
C(8)-C(9)-C(10)	118.6(4)	C(5)-C(10)-C(9)	119.3(4)

General view of the molecule:



View along the S-C4-C3 axis:



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