THE UNIVERSITY *of* LIVERPOOL

LIPASE B FROM *CANDIDA ANTARCTICA* - APPLICATIONS TO INDUSTRIAL POLYESTER SYNTHESIS

A thesis submitted to
The University of Liverpool for the degree of
Doctor of Philosophy in Chemistry
in the Faculty of Science

by

Paul Harffey

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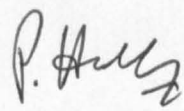
I certify that all material included in this thesis which is not my own work has been identified and that no material is included for which a degree has previously been conferred on me



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Paul Harffey
Robert Robinson Laboratories, December 1998

ABBREVIATIONS AND CONVENTIONS

Ac	acetyl
AIBN	2,2'-azobisisobutyronitrile
Ar	aryl
ax	axial
br.	broad
Bn	benzyl
b.pt.	boiling point
c.	concentration
CALB	lipase B from *Candida antarctica*
cat.	catalytic
CI	chemical ionisation
COSY	correlated spectroscopy
d	doublet
Da.	Dalton
DCC	1,3-dicyclohexylcarbodiimide
def.	deformation
DEPT	distortionless enhancement through polarisation transfer
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone
DMSO	dimethyl sulphoxide
ee	enantiomeric excess
EI	electron impact
eq	equatorial
FAB	fast atom bombardment
FTIR	Fourier transform infra-red spectroscopy
GC	gas chromatography
GPC	gel permeation chromatography
h	hour
HPLC	high performance liquid chromatography
lit.	literature
m	multiplet
MALDI-TOF	matrix-assisted laser desorption ionisation - time of flight
m-CPBA	*meta*-chloroperoxybenzoic acid
Me	methyl
min	minute
m.pt.	melting point
NMR	nuclear magnetic resonance
p-	*para*-
PDC	pyridinium dichromate
Ph	phenyl
PLE	pig liver esterase
PPL	porcine pancreas lipase

PTMEG	poly(tetramethylene ether) glycol
q	quartet
R_f	retention factor
s	singlet
str.	stretch
t	triplet
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDMSCl	*tert*-butyldimethylsilyl chloride
TBDPS	*tert*-butyldiphenylsilyl
TBDPSCl	*tert*-butyldiphenylsilyl chloride
tert-	*tertiary*-
THF	tetrahydrofuran
TLC	thin layer chromatography
UV	ultraviolet
vol.	volume
wrt	with respect to
wt.%	percent by weight

ABSTRACT

In the early 1990s, enzymatic polymerization studies on an unactivated adipic acid (A) / butane-1,4-diol (B) system in anhydrous organic media suggested that in solvent-free conditions a step-growth mechanism operates involving the effective sequential addition of an 'AB' unit. Subsequent optimisation work led to development of an efficient solvent-free process using lipase B from *Candida antarctica* as catalyst at temperatures of around 60 °C, producing polyesters with unique properties compared to their counterparts synthesised using conventional high temperature processes.

The present work describes the synthesis and unambiguous characterisation by GPC of a series of key oligomers formed during the enzymatic process, enabling a clearer understanding of the polymer assembly route. Use of semi-quantitative methods to compare the reactivity of these oligomeric species along the proposed enzymatic reaction pathway further clarifies the polymerisation mechanism and explains the differences between the final products of conventional and enzymatic polymerisations. Comparison of polyesterifications performed in solvent-free and toluene-based media reveals a change in lipase specificity from a simple esterification to an esterification/ transesterification mode.

Addition of a variety of compounds to the enzymatic polyesterification process led to an enhancement of lipase activity and sometimes enabled recovery of lipase from the system with no loss in activity. Explanations for these effects are proffered with supporting experimental evidence. A variety of studies on the effect of changing the polarity of reaction medium have been performed, an apparent trend of decreasing lipase activity with increasing solvent polarity having been noted.

The low temperatures employed in the enzymatic polymerisations enable access to a range of polyesters precluded by harsh conventional conditions. Studies on the polymerisation of novel polymers possessing unsaturation, labile groups such as epoxides and chirality are detailed, giving an indication of one of the potential advantages of an enzymatic system.

In addition, studies on the more conventional uses of enzymes in organic synthesis are included, where an apparent switch in lipase stereoselectivity occurs, in line with the findings of other workers in the field.

In completing one discovery we never fail to get an imperfect knowledge of others of which we could have no idea before, so that we cannot solve one doubt without creating several new ones.

Experiments and Observations on Different Kinds of Air [1775-1786]

Joseph Priestley (1733-1804)

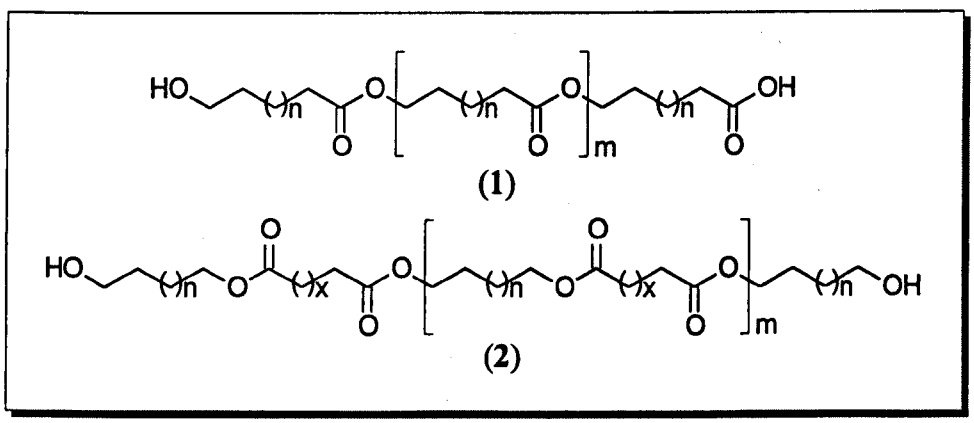
CHAPTER 1

INTRODUCTION

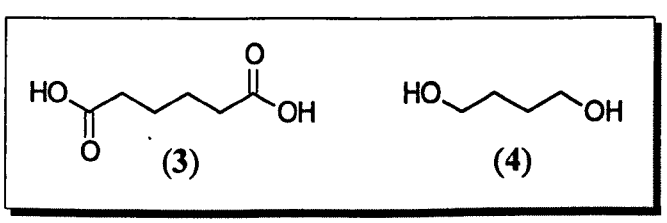
1.1 POLYESTERS

1.1.1 General background

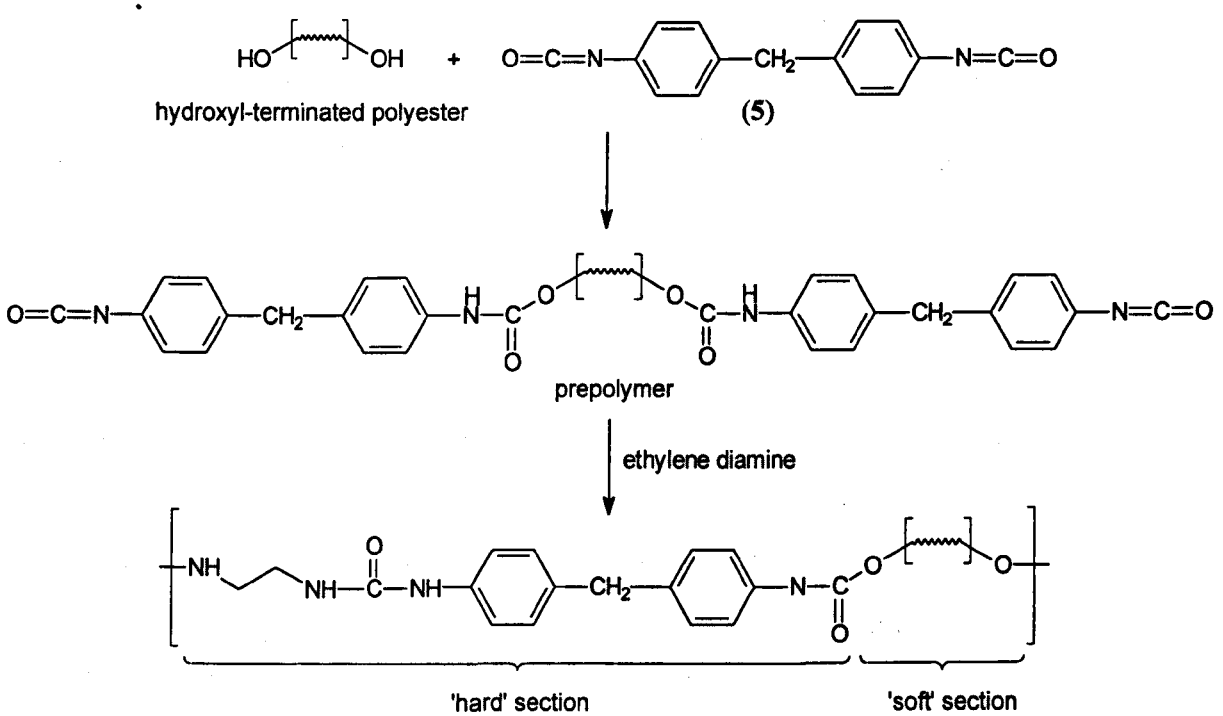
Polyesters are polymeric structures containing regularly repeating ester functions along the polymer backbone. Whilst aromatic (*e.g.* terephthalate esters) and branched polyesters are produced commercially and have uses in areas such as textile films and blow-moulding applications such as plastic bottle manufacture, the most important polyesters are based on linear aliphatic starting materials. The straight chain structures may be divided into two generic types, one where the carbonyl groups 'face' the same way along the length of the chain (**1**), the other where alternate carbonyls face one another (**2**). The former may be regarded as being composed of polycondensed hydroxyacids, the latter of dicarboxylic acids esterified at each end with diols.



Carothers was the first to investigate the formation and properties of linear aliphatic polyesters based on polymerisation of polyols with succinic, malonic or oxalic acids in the 1930s.[1] The materials formed were not exploited widely as their molecular weights and melting points were too low for practical applications. Subsequently, the use of adipic acid (**3**) as a substrate led to a wider acceptance of these linear aliphatic polyesters and applications such as hot-melt adhesives,[2] fibres and coatings.[3] The primary use for such polyadipates is in the manufacture of polyurethanes.



Polyurethanes were discovered in 1937 by Bayer and are formed from the addition of hydroxyl groups to isocyanate groups. Bayer used butane-1,4-diol (**4**) as the source of hydroxyl groups, but nowadays this has been superseded principally by polyester polyols (**2**) and polyethers. Typically, a diisocyanate species such as methylene-4,4'-diphenyl isocyanate (MDI) (**5**) is used to generate a prepolymer, a polyurethane species with isocyanate terminal groups. Subsequent reaction with a diamine leads to cross-linking of the polyurethane linear chains giving the finished polymer (Scheme 1).



Scheme 1

The section derived from the aliphatic polyester is flexible and gives the product elastic character; that derived from the MDI (**5**) is more rigid and possesses groups which may hydrogen bond to groups on other chains, preventing the chains from slipping over one another. Overall, the product is said to be a thermoplastic elastomer (synthetic rubber) by virtue of the action of these two opposing characteristics. The elastomeric properties of polyurethanes mean they will stretch on applying a stress, but will snap back into shape on releasing the force, giving them a myriad of applications, for instance in foams, fibres, adhesives and coatings.[4]

1.1.2 Conventional polyester production

Currently, polyesters are synthesised using long-standing high temperature processes. Typically, a diacid and diol (less expensive and more readily available than a hydroxyacid) are heated in the absence of an external solvent to temperatures in excess of 180 °C. Dry nitrogen is blown through the mixture (a technique known as sparging) in order to assist the removal of water formed during the esterification process. This technique is crucial; esterifications are equilibrium reactions, and water must be removed from the system in order to drive the equilibrium over to ester formation. The process is acid-catalysed, and the acid substrate itself usually acts as catalyst in the early part of the process; in the later stages of the polymerisation, an inorganic Lewis acid catalyst (usually tin- or titanium-based) is added to the mixture. Further aliquots of polyol are usually dosed throughout the reaction to compensate for lost volatilised material; also, these regular additions ensure that dihydroxyl-terminated polymer (*e.g.* (**2**)) of the desired molecular weight results. The presence of dihydroxyl functionality is critical to ensure effective cross-linking during polyurethane synthesis.

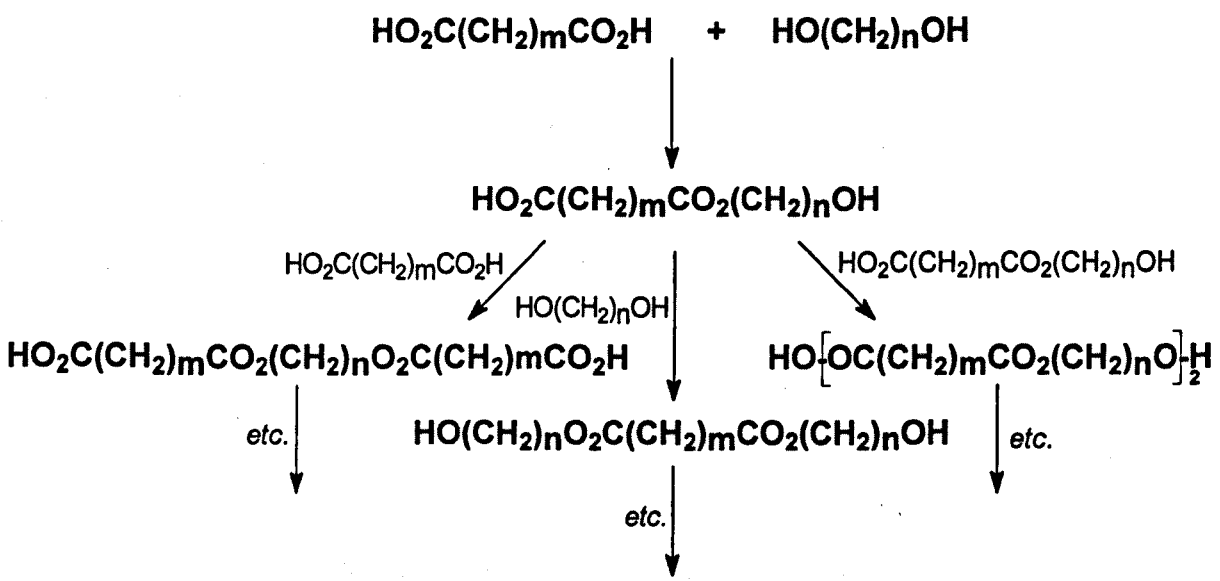
The kinetics of such conventional polyesterifications was intensively studied by Carothers[1] and subsequently by Flory.[5] Flory proposed that in the absence of an external acid catalyst an acid monomer would act as its own catalyst for esterification, giving a third order rate expression, the reaction being second order with respect to the acid. In the presence of an added acid catalyst, he proposed that the reaction was overall second order, first order with respect to both acid and polyol. Flory's work used equimolar amounts of the acid and diol, but the conventional solvent-free process consists of a heterogeneous mixture with much undissolved acid present in the early stages of the reaction. Further studies, in particular work by Lin and Hsieh,[6] using systems which mimic the process conditions have given results conflicting with those of Flory. The validity of the Flory kinetics for the uncatalysed system was supported by Moustafa *et al.*,[7] but in the presence of an added acid catalyst the kinetics of Lin and Hsieh were obeyed.

More recently, Chang and Karalis[8] performed further comprehensive studies into catalysed and uncatalysed adipic acid-based polyesterifications and concluded, in contrast to Flory and Lin, that the order of the catalysed reaction lay somewhere between second and

third order, due to a combination of catalytic activity of both the acid catalyst and the adipic acid itself.

Certainly the kinetics of the early stages of heterogeneous polyesterifications are still open to much debate and research, an area covered in an interesting review by Solomon and Jones,[9] who discuss the hurdles which must be overcome in order to topple long-standing theories such as that of Flory.

In contrast, the actual mechanism of polyesterification is well understood in such systems,[3] having been the subject of much study. High temperature polycondensation is an established example of a step-growth polymerisation, a reaction that proceeds with a slow increase in molecular weight of polymer. In this growth, the two monomers (diacid and polyol) react to form a monocondensation species. This may then react with either a polyol or diacid monomer, or with another monocondensation species. The products of these reactions may then react with any of the other oligomers present and so on giving a diverging series of possible reaction combinations which eventually form polymer (Scheme 2). These reactions characteristically show consumption of monomeric materials long before the formation of any truly polymeric material.



Scheme 2

Kinetic analysis of this system was much simplified by Flory[5] using assumptions referred to as the 'principle of equal functional group reactivity.' This states that the reactivity of a functional group of a bifunctional monomer is constant, regardless of whether the other

functional group has reacted or not, and that functional group reactivity is independent of the chain length of the oligomer to which it is attached. Hence, in a high temperature polyesterification, reactions between all oligomers / monomers are considered to be of equal energy, giving a highly random polymer assembly mechanism with a huge number of possible reactions occurring. In addition to these esterification reactions, chain transfer reactions (transesterifications) also occur *via* attack of alcohol groups on ester linkages in oligomers / polymer. These reactions lead to broadening of the range of molecules in the polymer product (referred to as broader dispersity), giving poorer crystallinity; this is a distinct disadvantage in applications such as hot melt adhesives.

The limitations of high temperature polyesterification processes are manifold. The high temperatures involved (often >200 °C), which often must be maintained for up to 2 days represent a high energy demand for the process which is obviously expensive for industry. Also, the range of substrates which may be polymerised under these conditions is limited - for example, oxalic and malonic acids undergo decarboxylation readily at 170 °C and alkene substrates may undergo *cis-trans* isomerism and participate in side reactions such as Diels-Alder cycloadditions.[10] Other documented side reactions are ether formation and chain cleavage *via* ester pyrolysis.[11,12] Such undesirable side reactions lead to a broadening of the polymer dispersity.

Other problems with high temperature polyesterification include the requirement of an inorganic catalyst in the final stages, which remains in the polymer subsequently, eventually being discarded into the environment. The harsh conditions also preclude the possibility of synthesising speciality polymers such as those incorporating acid-labile functional groups or even chiral centres, which are likely to be scrambled.

The many and varied problems associated with high temperature polycondensations have prompted research into lower temperature polycondensation systems. Some alternative chemically-catalysed systems have shown promise, such as the biphasic low temperature process developed by Saam[13] consisting of an acidic hydrophilic phase and an organic reactants phase. Contemporaneously, a huge amount of research has been undertaken into enzymatic polyesterification, often leading to highly promising alternative processes.

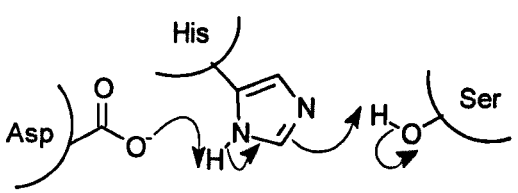
1.2 ENZYMATIC POLYESTERIFICATION

1.2.1 Early work

The pioneering work of Klibanov in the late 1970s led to the crucial discovery that enzymes, previously only used in aqueous media at highly specific temperatures at pH, could catalyse reactions in anhydrous organic media.[14] As many synthetic organic reactions require anhydrous conditions due to stability of reactants / products, the discovery led to an explosion in the amount of research on the use of biocatalysts to catalyse synthetic organic reactions. This growth in popularity has been thoroughly documented elsewhere[15] and will not be discussed further.

Probably the most widely researched enzyme-type during the 1980s was that of the lipases. Lipases are hydrolase enzymes which in nature catalyse the hydrolysis of triacylglycerols; in nonaqueous systems they can catalyse the reverse reaction, namely ester formation.

Lipases are believed to operate through a charge relay system, using a catalytic triad of amino acid residues in the active site to activate the enzyme for reaction. Deprotonation of a histidine residue by an aspartate ion leads to deprotonation of a serine hydroxyl residue (Scheme 3); it is this activated serine residue that performs nucleophilic attack on an acid / ester, forming a covalent acyl-enzyme complex. Nucleophilic attack of an alcohol / water breaks the lipase-substrate bond and releases the product from the active site.



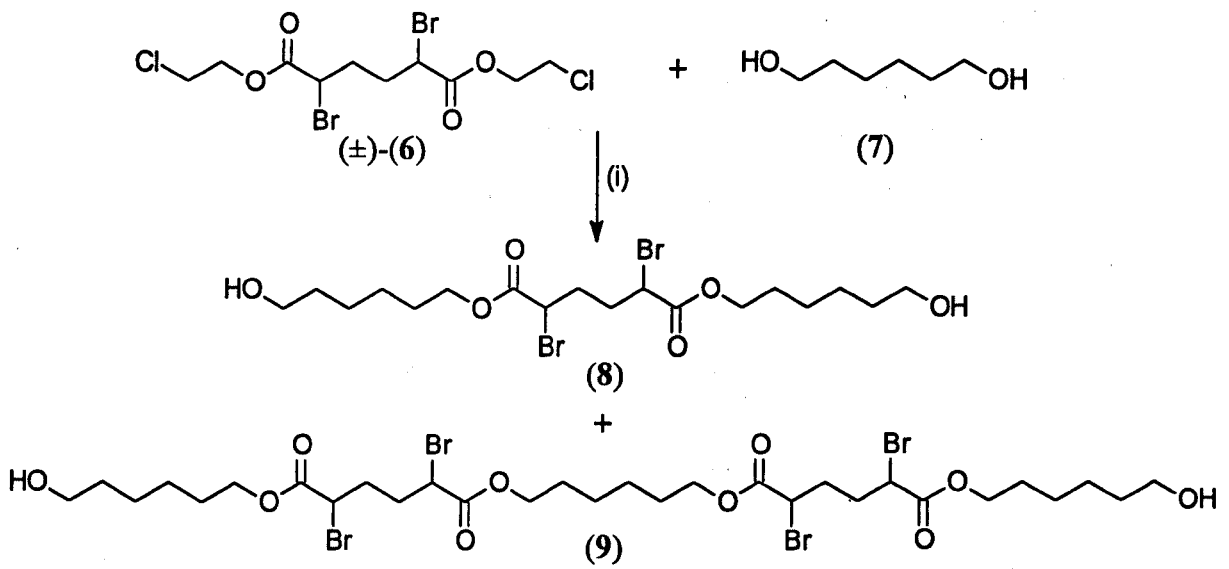
Scheme 3

Again, Klibanov and co-workers were pioneers of using lipases in organic synthesis[14(c),16] and extended their use, along with other groups, into polyester synthesis.

Okumura *et al.*[17] used the commercially available dicarboxylic acids and diols as starting materials, in line with the substrates of commerce. By employing an excess of the diol

as solvent and lipase from *Aspergillus niger*, they were able to produce low weight oligomeric species at 30 °C. The presence of large excesses of diol lead to rapid end-capping of the free acid ends of the oligomers and apparently stopped the reaction. A further limitation was that the higher weight oligomers precipitated out of solution, thus rendering them unable to further oligomerise.

More commonly, researchers used activated dicarboxylic acid substrates, usually esters. Because the carbonyl groups of such derivatives possess a good leaving group, attack of the serine residue on the carbonyl is facilitated, assuming steric effects of the leaving group do not outweigh this advantage. The overall process occurring is therefore transesterification, as the original ester group is thus replaced by the desired product ester.



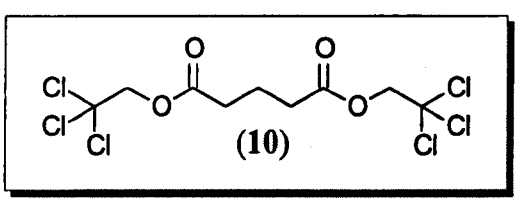
Reagents and conditions:[18] (i) toluene, *Chromobacterium* sp. or *Aspergillus niger*, 45 °C, 7 days.

Scheme 4

Klibanov *et al.*[18] made progress towards the synthesis of optically active polyesters in 1987, using lipases from *Chromobacterium* sp. and *Aspergillus niger* to catalyse the transesterification reaction of bis(2-chloroethyl) (±)-2,5-dibromoadipate (±)-(**6**) with hexane-1,6-diol (**7**); principal products were the trimer BAB (**8**) (where B = diol moiety, A = diacid moiety) and pentamer BABAB (**9**) (Scheme 4). In each case, the oligomers isolated showed optical activity. Repeating the work with bis(2,2,2-trichloroethyl) (±)-3-methyladipate, catalysed by PPL again gave selective incorporation of one enantiomer, confirming the ability of lipases stereoselectively to form oligomers. In this case, the diester is highly activated, as the electron-withdrawing chlorine groups remove further electron density from the carbonyl

carbons *via* the inductive effect; the trichloroethanol formed as a by-product is also poorly nucleophilic for the same reason, and would be unlikely to attack the products and promote reverse reaction. Nonetheless, only BAB- and BABAB-type species were formed once again.

Similar work was performed by Wallace and Morrow, who produced an optically active epoxyadipate polyester using PPL, a lipase found to be highly selective towards the substrate used.[19(a)] Further discussion on this work can be found in Section 2.3. In addition, other achiral substrates were polymerised, with substantially higher molecular weights being achieved than those of Klibanov or Okumura. Polymerisation of bis(2,2,2-trichloroethyl) glutarate (**10**) with butane-1,4-diol (**4**) in diethyl ether at 45 °C, catalysed by PPL gave polyester of molecular weight average (measured by GPC) M_w 11800.[19(b)] Such a high molecular weight was obtained by minimising the lipase-catalysed side reactions of lactone formation (by use of concentrated solutions) and reverse reaction / hydrolysis (using anhydrous solvents and forming polyhalogenated alcohols from transesterification). For a detailed explanation of the use of GPC in polymer molecular weight determination, see general experimental methods.

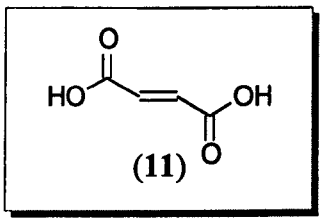


(10)

The high cost of halogenated starting esters such as (**10**) and the toxicity of the by-produced haloalcohols makes processes involving these species unlikely to achieve commercial recognition. However, such seminal work showed the potential applications of lipases in polyester synthesis; in particular, the lower temperatures compared to conventional processes was an attractive feature. The latter advantage spurs continued research to this day.

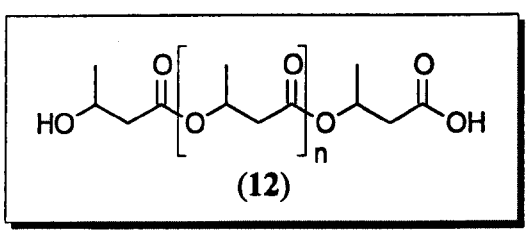
In the early 1990s, Geresh and Gilboa used biocatalytic methods to polymerise unsaturated substrates, taking advantage of the by then accepted advantages of low temperatures and mild conditions that lipase-catalysed polyesterification could offer. By using alkyl and activated haloalkyl esters of fumaric acid (**11**) with butane-1,4-diol (**4**) in the presence of a range of commercially available lipases in various solvents, all *trans* geometry polyester was produced, with molecular weight averages of up to M_w 1250.[20] Subsequently, the same research group successfully polymerised aromatic diacids and diols using various lipases, although again the products were predominantly oligomeric, M_w values typically only

reaching 1000 Da.[21] Whilst the weights achieved in fumarate polyester synthesis were low, the polymers were highly crystalline compared to similar commercial samples due to the presence of only *trans* geometry double bonds in the former case. This difference derives from the lower temperatures used which precluded *cis-trans* isomerisation.



(11)

In 1959 Doudoroff and Stanier conducted experiments which confirmed that the bacteria *Pseudomonas saccharophila* and *Rhodospirillum rubum* stored much of their assimilated carbon as D-poly(β-hydroxybutyrate) (PHB) (**12**).[22] This chiral polymer was then used as a source of energy and carbon by the bacteria. After this discovery, a wealth of study, pioneered by Agostini in the early 1970s, began on the characterisation and mechanism of bacterial synthesis of (**12**), often using achiral PHB mimic systems.[23,24] Differences in *in vivo* and *in vitro* synthesis were found to be a crucial issue.[24(b)]



(12)

Much interest was subsequently shown in using lipases to catalyse the ring-opening of substituted lactones to stereoselectively obtain substituted polyesters. The fact that many bacteria are able to synthesise (**12**) means that similar structures might be biodegraded by bacteria, a property which has become increasingly important over the last two decades, with the heightened public interest in environmental issues. Also, if the bacteria could only degrade polymer containing one enantiomer of the monomer, the other enantiomer could be polymerised separately in order to achieve similar physical properties but avoiding the possibility of biodegradation.

In the late 1980s, Marchessault studied chemical methods of synthesising β-hydroxybutyrate and β-hydroxyvalerate polyesters, comparing polymer containing both

enantiomers of the starting lactones and that containing predominantly a single enantiomer.[25] Significant differences in crystallinity of the samples was observed.

A decline in the amount of research into poly(hydroxybutyrates) occurred in the late 1980s. Probably, this was due to the commercial production of the optically active materials by ICI using a fermentation process, and the subsequent successful marketing of one of the products by the same company as Biopol®.[26]

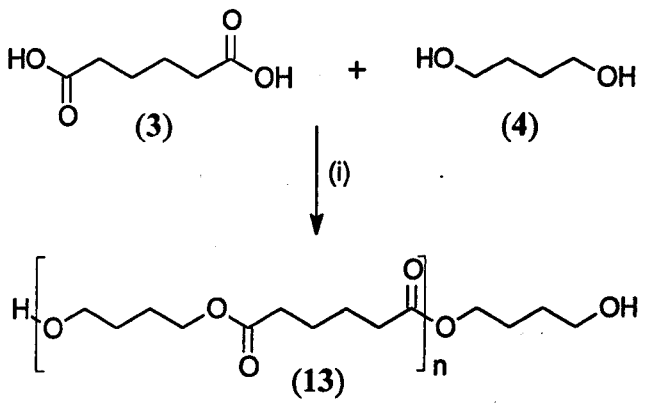
Gutman successfully generated chiral polyesters both *via* asymmetrisation of prochiral substrates during polymerisation (see Section 2.3.6.2)[27] and using racemic ω-hydroxyesters (effectively lactones ring-opened by water).[28]

1.2.2 Previous work on Baxenden enzymatic polyesterification process

In 1991, Baxenden Chemicals Ltd., in collaboration with Williams and Roberts at the University of Exeter, became interested in enzymatic polyesterification. Appreciation that activated esters were unlikely to be commercially viable substrates led to the use of unactivated diacids and diols. Also, low molecular weight materials were targeted, as these are suitable for further reaction to form polyurethanes, the most lucrative use for polyesters. The lack of side reactions and concomitant higher crystallinity observed by many authors suggested that such materials might impart new properties to the subsequently-formed polyurethane derivatives.

Previous testing of unactivated diol / diacid systems had been confined to that of Okumura *et al.*[17] Low weight oligomers were observed, since these authors failed to appreciate the need for close stoichiometry of substrates to achieve polymer formation. Application of this crucial feature, with optimisation of solvent, temperature and dehydration conditions by Williams *et al.* led to a process for the polymerisation of butane-1,4-diol (**4**) and adipic acid (**3**), using lipase from *Mucor miehei* (*Lipozyme® IM-20*) as catalyst.[29] *Lipozyme® IM-20* is an immobilised lipase, the free lipase being adsorbed onto a resin support and supplied in the form of small beads. This formulation allows easy handling of the material, and facilitates recovery of the enzyme at completion of the reaction. To drive the esterification equilibrium towards polyester formation, activated 4 Å molecular sieves were employed to remove the by-produced water; to avoid abrasion of the enzyme, a two chamber reaction vessel was used which physically separated the immobilised enzyme and sieves whilst

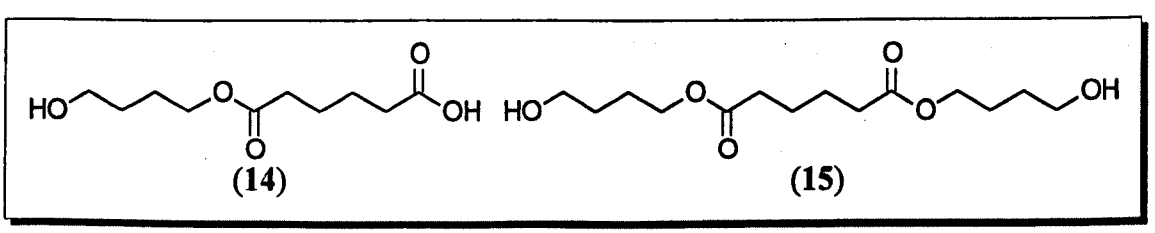
allowing free continuous circulation of solvent around both solid phases. A two stage process was used, where acid-ended species were removed at the mid-point of the reaction, allowing the dihydroxyl-terminated species remaining to transesterify and produce higher weight polymer. Typically, hydroxyl-terminated polymer (**13**) with molecular weight averages M_n 4200, M_w 4700 was achieved (Scheme 5).



Reagents and conditions:[29] (i) diisopropyl ether, *Lipozyme® IM-20*, remote 4 Å molecular sieves, 40-45 °C, 2 x 70 h.

Scheme 5

Curiously, few acid-terminated oligomeric species were observed, the only characterised examples being adipic acid (**3**) and the monocondensation product, referred to as AB (A = diacid species, B = diol species) (**14**), even though equimolar amounts of diol (**4**) and acid (**3**) were employed. The poor solubility of adipic acid in the comparatively non-polar solvent meant that an effective excess of diol was present in solution during the early stages of the reaction; the most likely fate of AB (**14**) therefore was further reaction with another diol moiety to produce the species referred to as BAB (**15**). As hydroxyl-terminated polyesters are required for polyurethane formation, the poor solubility of adipic acid (**3**) was actually beneficial to the process.



The process did not show the characteristic step-growth property of rapid consumption of the monomers prior to formation of polymeric material. An alternative polymer growth

mechanism was proposed, where the BAB (**15**) formed was the principal active species. An ester linkage in BAB (**15**) would acylate the lipase serine residue, in so doing releasing a butane-1,4-diol (**4**) molecule as a result of transesterification. A second BAB (**15**) species could then attack the acyl-enzyme complex releasing an oligomer effectively lengthened by an AB (**14**) unit, termed $B(AB)_2$. This process could be continued using BAB (**15**) to acylate the enzyme and higher weight oligomers to break the acyl-enzyme complex. The mechanism would then give elongation of the polymer chain in each instance by an AB (**14**) unit.

Much in-house work was performed by Binns at Baxenden Chemicals Ltd. during the three-four years following the completion of the work of Roberts *et al.*[29] and prior to the commencement of the present work. The two chamber solvent-based polymerisation system described above was the subject of a UK patent applcation in 1993.[30] Subsequently, a series of alterations to the process were made, all of which added further potential for the use of lipases in the industrial manufacture of polyesters:

- Difficulties with batches of lipase from *Mucor miehei* of inconsistent activity led to the use of a newly available immobilised lipase from *Candida antarctica* (CALB) (*Novozyme 435®*) which was found to be more active and the polymerisations more reproducible. Detailed information on *Novozyme 435®* is given in the general experimental methods section.
- Molecular sieves used to drive the reaction were omitted from the process. The enhanced activity of CALB plus natural water evaporation promoted polymerisation.
- Solvent minimisation experiments were performed until eventually the reaction proceeded in the absence of solvent. Hence, in the same fashion as high temperature polycondensations, the polyol can act as the solvent and substrate for the reaction. Similar solvent minimisation trials had also been performed by Kuhl *et al.*[31] in 1990 during enzymatic peptide synthesis studies.
- Application of partial vacuum at the final finishing stage promoted a greater degree of polymerisation.
- Recycling of the immobilised enzyme was a crucial issue determining economic viability, yet filtration from the final viscous polymer was foreseen as problematic. Therefore, filtration of the catalyst at the midpoint

was tested; surprisingly, the polymerisation continued. Subsequently, this apparently catalyst-free process was discovered to owe its reactivity to the presence of free enzyme leached off the immobilised support.

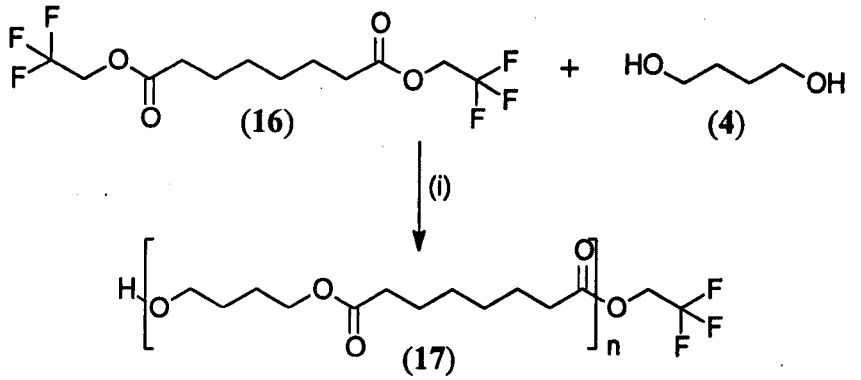
The discovery of the solvent-free conditions led to a second UK patent application on the process, no other lipase-catalysed systems having formed truly polymeric materials under solvent-free conditions.[32] The highly polar nature of the diol-based medium was considered inappropriate for retaining high lipase activity, less polar solvents such as DMSO having been shown to deactivate enzymes.[33] Hence, the system was considered exceptional, both from the aspect of enzyme stability and the commercial promise a system requiring neither activated starting materials nor solvents might show. Recent work by the group of Matsumoto[34] has shown lipases to be stabilised by the addition of polyols. A possible explanation is that the preferential exclusion of polyol from the surface of the protein preserves the hydration shell around the lipase giving enhanced conformational rigidity and therefore resilience.

Study of the solvent-free process forms the main body of this work and is detailed in Discussion Sections 2.1 and 2.2.

1.2.3 Recent developments in the field of enzymatic polyesterification

Much progress has been made in the enzymatic polymerisation field since the inception of the studies at Baxenden Chemicals Ltd. The growth in the amount of literature in this area during the course of the present work has been particularly strong.

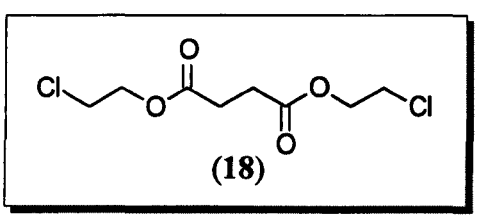
Work performed subsequently to 1990 often perpetuated the trend for using activated starting diols and diacids. Linko *et al.* focused on the polymerisation of substrates such as bis(2,2,2-trifluoroethyl) sebacate (**16**) and aliphatic diols such as butane-1,4-diol (**4**).[35] A high molecular weight polyester (**17**), with degree of polymerisation of 184 repeat units (M_w 46400 Da) was obtained when lipase from *Mucor miehei* was used in diphenyl ether (Scheme 6).



Reagents and conditions:[35] (i) *Mucor miehei* lipase, diphenyl ether, 37 °C, 7 days, periodic vacuum applications.

Scheme 6

When sebacic acid itself was employed in place of the activated diester (**16**) and a vacuum was used to remove water, a polymer with M_w 42000 was obtained, further demonstrating the promise that unactivated diacid / diol polymerisations might have.[36] High accuracy of weighing was used to ensure 1:1 stoichiometry of diester:diol and consequently allow high molecular weight polyester to form. Much lower molecular weights had been achieved by Linko a year earlier using bis(2-chloroethyl) succinate (**18**) and butane-1,4-diol (**4**) with a range of lipases in various solvents in the absence of vacuum. The low weights may be explained by the precipitation of the polymer at molecular weight average M_w~1500 Da.[37]



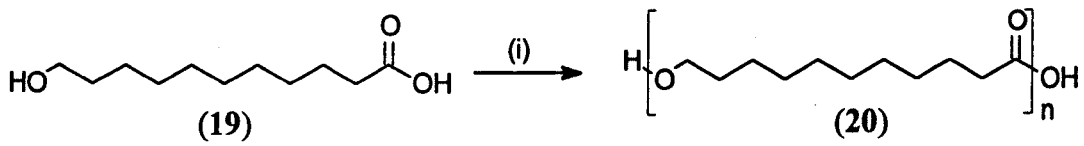
Morrow *et al.*[38] employed similar techniques to Linko (such as accurate weighing of reagents, use of trifluoroethyl esters to inhibit reverse reaction and application of high vacuum to remove by-produced trifluoroethanol) and also generated high molecular weight (M_w~4000 Da) polyesters, in this case polyglutarates. Rigorous drying of the PPL catalyst was also considered important to prevent hydrolysis caused by enzyme-associated water.

The group of Jarvie[39] has more recently continued to use polyhalogenated substrates. Interestingly, comparisons of the reaction of glutarate and adipate esters with butane-1,4-diol (**4**) catalysed by PPL showed that the lipase may become coated with polymer from the glutarate but not the adipate system.[39] The effect of molecular sieve on the early and later

stages of the reactions was studied; unfortunately, by this time, molecular sieves had all but been superseded by vacuum technology.

Enzymatic polyesterification of hydroxyacids / hydroxyesters in the early 1990s was a method which was not readily employed. A major advantage of such substrates is that they have a built-in perfect stoichiometry, meaning that high molecular weight polymers ought to be easily created. The disadvantage of free acid end groups in the final polymer could be rectified by addition of an aliquot of a polyol to end-cap the chains. However, the prohibitive expense of synthesising the starting materials probably led to little enthusiasm for this type of polymerisation.

O'Hagan and Zaidi[40] used achiral hydroxyacids such as 11-hydroxyundecanoic acid (**19**) and 10-hydroxydecanoic acid as substrates in solvent-based polymerisations, using the two chamber reaction system of Roberts *et al.*[29] Low dispersity, highly crystalline polymers were obtained, typically M_w 22400, M_n 20000.



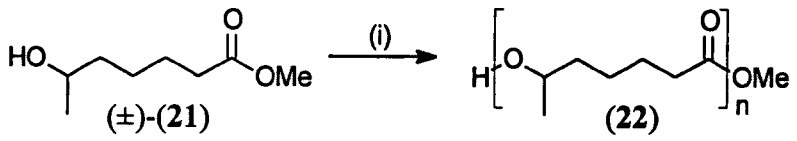
(**19**) (**20**)

Reagents and conditions:[40] (i) *Candida cylindracea* lipase, hexane, remote 4 Å molecular sieves, 55 °C, 103 h.

Scheme 7

Probing the mechanism of the polymer assembly led the research group to conclude that the early stages of the polymerisation consist of sequential addition of monomer onto the increasing length polymer chains followed subsequently (when all the monomer has been consumed) by a significantly slower oligomer polymerisation stage. The marked substrate specificity of lipases was observed by O'Hagan, C_8 and C_{12} equivalent hydroxyacids being very poor substrates for the lipase used.

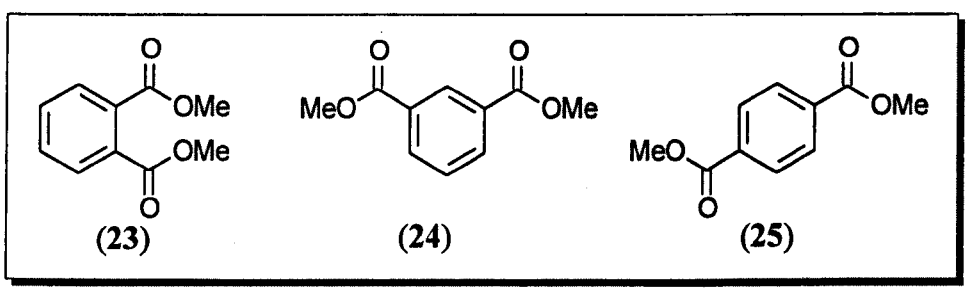
Knani and Kohn[41] showed continued interest in chiral polymers, starting from ε-hydroxyesters such as (±)-(**21**). The latter were prepared using a two-step synthesis, and were polymerised in the presence of PPL at 70 °C (Scheme 8). The individual oligomers formed (**22**) (up to hexamer) were isolated by chromatography and optical rotations measured. All showed optical activity.



Reagents and conditions:[41] (i) PPL, hexane, 69 °C, 400 h.

Scheme 8

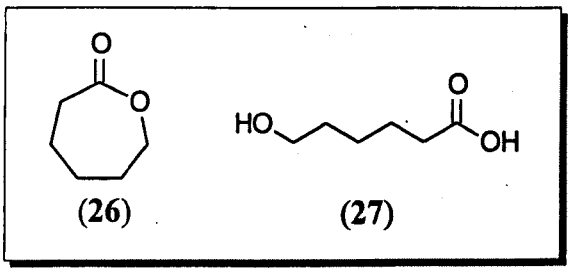
Brigodiot and Lalot *et al.* have entered the enzymatic polyesterification arena over recent years. Again, activated substrates are employed, although only alkyl esters rather than the non-commercial halogenated esters were used. Their work on unsaturated substrates[42] is scrutinised in Section 2.3.2.1. This group uses *Novozyme 435®* as catalyst for their polymerisations and has demonstrated recently the broad substrate specificity of CALB by polymerising the three isomers of dimethyl phthalate (**23**), (**24**) and (**25**) with hexane-1,6-diol (**7**); only the isophthalate (**23**) and terephthalate (**25**) polymerised, the *o*-phthalate (**24**) remaining unchanged.[43]



Since 1993, a large number of publications have been released using lactones as enzymatic polymerisation substrates. Like the hydroxyacids, these structures feature a built-in perfect 1:1 alcohol:acid group stoichiometry.

The most commonly used and one of the cheapest achiral lactones is ε-caprolactone (**26**). Enzymatic polymerisation of this substrate could provide important polymers, due to its ability to form useful materials on copolymerisation with a large variety of other substrates. Gutman *et al.*[44] used PPL to catalyse the ring-opening of (**26**) in hexane; a trace of nucleophile is required to start the ring-opening sequence, in this case methanol was used. As no water is formed in these reactions, the complication of water removal is precluded, a major advantage over diacid / diol polymerisations. In addition to polyester, some macrolactone formation was observed (<5mol%), although this is in line with the amounts seen in conventional polymerisations.

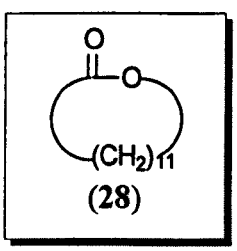
Two main groups began studies on enzymatic ring-opening polymerisations in the early-mid 1990s, both of which remain strong forces in this field - those of Kobayashi and Gross.



(26) **(27)**

Kobayashi began work on enzymatic ring-opening polymerisation of ε-caprolactone (**26**) in 1993. Using the substrate in the bulk phase (solvent-free), ring-opening polymerisation was catalysed by lipase from *Pseudomonas fluorescens* at 60 °C to give a 7000 Da M_n average molecular weight polymer, with the expected acid and alcohol group terminations.[45] Subsequently, the effects of temperature and reaction time were studied. The proposed mechanism for the polymerisation consisted of ring-opening initiation by water to give the ω-hydroxyacid (**27**), followed by either esterification of two molecules of ω-hydroxyacid (**27**) or a molecule of lactone with a molecule of (**27**); both reactions were considered to be likely to occur.[46]

Concurrently, Kobayashi successfully polymerised commercially available cyclic acid anhydrides with polyols, again using lipase from *Pseudomonas fluorescens* in a range of solvents.[47] This work was less successful, mostly giving rise to low weight materials in reactions which were highly sensitive to solvent.



(28)

In 1995, Kobayashi returned to lactone polymerisations.[48] Comparison of the ring-opening polymerisation of 12-dodecanolide (**28**) with that of ε-caprolactone (**26**) showed interesting differences. Alkaline hydrolysis of (**28**) proceeds much more slowly than (**26**) because of the higher ring strain in the latter molecule; hence the polymerisability of (**28**) would be expected to be lower, as the driving force for ring-opening is much lower. With both

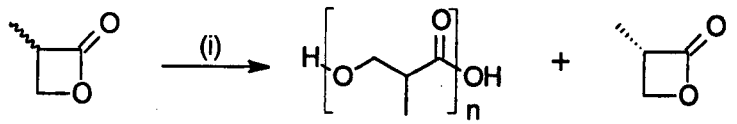
Pseudomonas fluorescens and *Candida cylindracea* lipases at a range of temperatures, (**28**) was more rapidly converted to polymer than (**26**). The contravention of the expected order of reactivity was explained by the selectivity of the lipases for the formation of acyl-enzyme complexes, the larger ring molecule being more readily recognised by the enzymes.

During the course of the present study, Kobayashi has produced further publications where macrolactones other than (**28**) have been polymerised.[49,50,51] Recently, the mechanism of lactone polymerisation has been further studied. The rate determining step is believed to be formation of the acyl-enzyme intermediate, verified by performing the polymerisation of (**28**) in the presence and absence of an initiator (1-octanol). In the absence of initiator, traces of water act in its place, but the rates of coversion in the two systems was approximately the same, implying that the formation of the complex rather than initiation is the rate determining step.[52,53]

Most recently, Kobayashi's group have explored aqueous polymerisations of lactones[54] and, more surprisingly, diacids and diols.[55] In the former case, lipases from *Pseudomonas cepacia* and *Candida cylindracea* catalysed the formation of polyesters, with M_n values between 400 and 2100 Da. No explanation has been found for the apparent opposing of equilibrium considerations occurring in the aqueous diacid / diol polymerisation.

Gross has produced a similar volume of publications to Kobayashi over the past 4 years. Initial work in 1996 was again based on ring-opening polymerisations of ε-caprolactone (**26**), stressing the potential commercial importance of enzymatically-produced polycaprolactone. Study of the propagation kinetics showed that despite rapid initiation by the added nucleophile butylamine, chain propagation was still negligible after 2 hours, corroborating the work of Kobayashi. If the polymerisation is living (*i.e.* no chain transfer or termination of reaction occurs), the propagation should follow a first order rate law. This was seen to be the case.[56,57]

Studies on stereoselective ring-opening reactions were subsequently performed by Gross *et al.*, notably on α-methyl-β-propiolactone (±)-(**29**).[58] Incorporation of predominantly the (*S*)-enantiomer of (**29**) occurred in the polymerisation to give (**30**), catalysed by lipase from *Pseudomonas fluorescens*, the highest selectivity being shown in toluene (Scheme 9). A sample of the polymer containing ~75% (*S*)-(**29**) showed a surprisingly broad melting range.

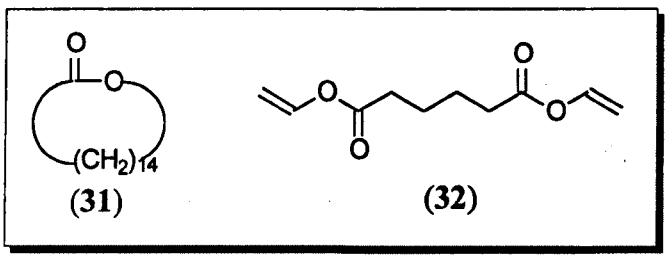


(±)-(29) (30) (*R*)-(29)

Reagents and conditions:[58] (i) *Pseudomonas fluorescens* lipase, toluene, 35 °C, 48-744 h.

Scheme 9

Recently, Gross has used bulk polymerisation conditions in place of the solvent-based systems to perform enzyme-catalysed ring-opening polymerisations on ω-pentadecalactone (**31**).[59] CALB in the form of *Novozyme 435®* and *Lipozyme® IM-20* were used as catalysts which tolerated the bulk conditions. Temperature effects were found to be important, the reaction rate increasing with heating between 60-70 °C, but remaining constant between 70-90 °C. If the temperature was taken over 90 °C, a lower average molecular weight was achieved, attributable to increasing importance of chain degradation reactions such as hydrolysis and increase in initiation reactions which would lead to more, shorter chains. Careful control of water is necessary, as rate of monomer consumption increases with an increase in water content, but this could lead to shorter chains due to formation of more propagation centres. The water activity would also be expected to affect the lipase activities.



(**31**) (**32**)

The majority of work on lipase-catalysed diol / diester polyesterifications performed during the course of the present research has been from the group of Russell. Substrates resembling those used in the Baxenden process have been used (divinyl adipate (**32**) and butane-1,4-diol (**4**)) in a transesterification process catalysed by *Novozyme 435®* in various solvents.[60] Little termination of the polymers formed with vinyl ester groups was seen, and the group deduced that in the presence of equimolar quantities of diol and diester the lipase would preferentially catalyse the reaction of diesters with oligomers. Problems with hydrolysis of the vinyl ester end groups by water formed during the process was found, adipic acid formation being observed. Divinyl adipate (**32**) was also hydrolysed in the absence of

diol, implying that the traces of water bound in the lipase could attack the ester. Russell appreciated that the hydrolysis was of only slight consequence early in the reaction, when the diester reacted rapidly with butane-1,4-diol (**4**). However, later in the reaction, the slower reacting oligomers allow the hydrolysis reaction to become more significant. MALDI-TOF mass spectrometry and titrimetric methods were used to study the amount and distribution of acid-ended polymer present. Again, the good fortune in the Baxenden solvent-free process of heterogeneity of the reaction mixture in the early stages of the reaction is apparent, only hydroxyl-terminated species being evolved above the low weight oligomers.

In 1997, Russell investigated a solvent-free polymerisation of the same substrates,[61] in a process similar to that which is the subject of one of the Baxenden patents.[32] Using divinyl adipate (**32**) in place of adipic acid (**3**), the problem of water removal was precluded, as the vinyl alcohol formed during transesterification tautomerises to acetaldehyde; thus, the reaction should not reach equilibrium, as the reverse reaction cannot occur. However, the hydrolysis seen in the previous publication means that the equilibrium position will be controlled by the competition between transesterification and hydrolysis near the end of the reaction. Clearly this method precludes the requirement of vacuum technology, although the starting material (**32**) would be prohibitively expensive for use on a commercial scale.

Minimising the effect of water from the lipase was again considered critical to the success of the process, and this was achieved by substantially lowering the enzyme dose. Russell also noted a drop in lipase activity when attempting to recycle lipase which had previously been used for the polymerisation. This is in accord with the findings of Binns,[62] the details of which are discussed in Section 2.2.

Earlier this year, Russell *et al.* developed a mathematical model to predict the change in reactivity of oligomers with molecular weight.[63] As mentioned previously, the Flory principle of equal functional group reactivity cannot apply in enzyme-catalysed processes, as the substrate specificity of the enzyme means the latter will interact with different oligomers at different rates. The model takes into account side reactions such as hydrolysis of divinyl adipate (**32**) and esterification of the acid groups formed during the hydrolysis. Such a model is unlikely to have great relevance to the Baxenden solvent-free system, due to the very different conditions used.

1.3 PROJECT OBJECTIVES

Clearly, the concept of lipase-catalysed polyesterification has been validated over the past decades, and a variety of different systems have shown promise. Nonetheless, the solvent-free process patented by Baxenden Chemicals Ltd.[32] still appears to be the favourite insofar as commercialisation is concerned. Since the completion of the present work, continued interest in nearly solvent-free processes has been shown, notably by Halling *et al.*[64] The latter group has also wrestled with the difficulties of understanding the controlling influences in reactions where much of the substrate remains undissolved in the early stages.

Whilst the work of Roberts *et al.* on the solvent-based procedure[29] gave useful information and led to theories about how the polymer chains are assembled, little mechanistic work on the solvent-free system had been performed up to 1995. Consequently, the principal purpose of the present work was to determine the mechanism of polymer growth for the enzymatic process and to judge whether or not this was the same as for the conventional high temperature processes. Examination of some difficulties regarding the use of certain polyols in the enzymatic process seen at Baxenden was also considered a priority. Having made progress in these areas, other methodology of interest was the synthesis of novel polyesters, including those containing unsaturation or acid-sensitive functional groups or even chiral centres.

The project was believed to hold much potential because of the unique position it held within the scope of chemistry. Disciplines to be studied and mastered included synthetic organic and polymer chemistry, as well as aspects of enzyme behaviour, still a comparatively poorly-understood area. The potential commercial exploitation was an added incentive to drive the research forward - an understanding of the way the lipase operates was considered critical to the success in industry of the entire project, enabling optimisation of conditions and possibly leading to eventual commercial viability.

CHAPTER 2

RESULTS AND DISCUSSION

2.1 MECHANISTIC WORK ON ENZYMATIC POLYESTERIFICATION PROCESS

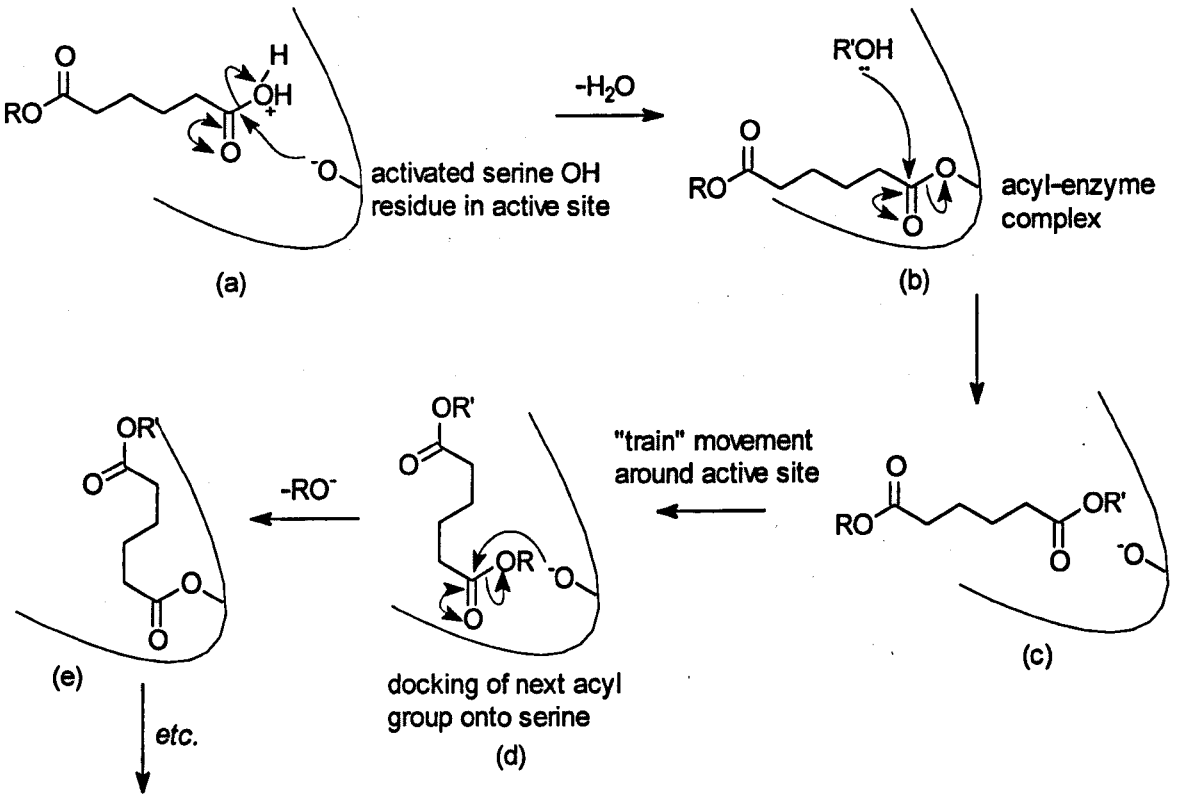
2.1.1 Introduction

Work performed previously on a solvent-based enzymatic polymerisation of butane-1,4-diol (**4**) and adipic acid (**3**) by Roberts *et al.*[29] indicated that the predominant chain assembly mechanism occurring involved the reaction of certain oligomeric species with one another, rather than reaction of oligomeric species with the monomers (**3**) and (**4**). In the conventional high temperature solvent-free process this is not the case, because of the application of the Flory principle of equal reactivity of functional groups.[5] Hence, the substrate specificity of lipases may overrule the Flory principle leading to a different, more controlled polymer chain assembly. The behaviour of different oligomers in the presence of lipase was therefore considered to be critical to understanding the lipase mode of action.

Two plausible chain assembly mechanisms were postulated for the Baxenden enzymatic solvent-free polyesterification process during the present work. Both rely on the acceptability of oligomeric species into the lipase active site. The first is the conventional "bi-bi ping-pong" mechanism,[65] where attack of the activated serine hydroxyl residue on the acid species gives the acyl-enzyme intermediate with the loss of water. Attack of the alcohol species then gives ester formation and reforms the activated enzyme (transformations (a) to (c) in Scheme 10). The ester formed will then leave the active site, allowing another acylating agent to enter.

An alternative mechanism postulated was a "train"-type assembly, where the ester formed after breaking of the acyl-enzyme intermediate moved around in the active site, and the next acyl group along the chain then acylated the enzyme and so on (transformations (c) to (e) in Scheme 10). If long chain oligomeric species were to be able to participate in the latter mechanism, transesterification of the molecules would occur along the length of the chain. Sterically, this may not be favoured. The active site of the lipase must also be large enough to accommodate the folded oligomers as they progress around the active site. Work by Jones *et al.*[66] suggests that the active site of CALB is not large enough for this to be the case. The classical bi-bi ping-pong mechanism, therefore, seems the most likely to be operational in the system studied here.

Investigation of the mechanism of chain assembly of the solvent-free polyesterification process developed at Baxenden Chemicals Ltd. was not easily amenable to conventional initial rate kinetic measurements, as the reaction mixture is heterogeneous at the start of the process: the vast majority of adipic acid (**3**) present is suspended in the diol, and whilst the overall stoichiometry of acid and diol is near to 1:1, the effective solution stoichiometry is much lower in acid.



Scheme 10

An alternative approach, and the method used in this work, was a semi-quantitative strategy which entailed studying the ease of polymerisation (under CALB catalysis) of a number of oligomers formed early on in the process. Of particular interest were the oligomer formed from one molecule of butane-1,4-diol (**4**) and adipic acid (**3**), termed AB (**14**) and that formed from one molecule of adipic acid (**3**) and two molecules of butane-1,4-diol (**4**), termed BAB (**15**): the former because earlier studies had shown that incremental growth with an AB (**14**) unit separating each predominant oligomer, and the latter because of its apparently high concentration in the early stages of the process. For consistency, the same polyester system was studied throughout the experiments, namely poly(butanediyl adipate) formation.

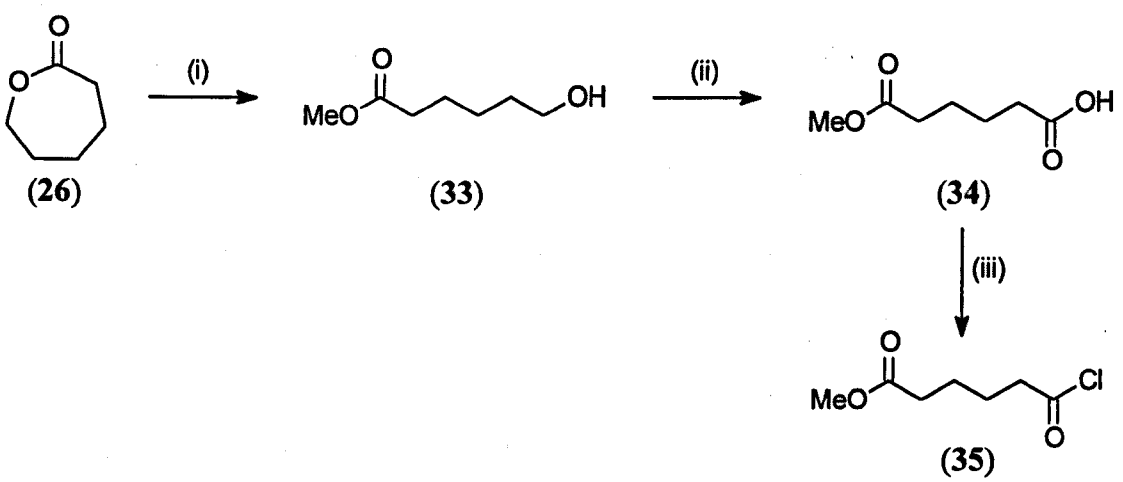
The investigation therefore necessitated the synthesis of these and other plausible oligomeric intermediates, both to unambiguously identify the individual oligomers and to shed light on their relative reactivity when polymerised in the presence of *Novozyme 435®* in a semi-quantitative fashion.

2.1.2 Synthesis of substrates for polymerisation

2.1.2.1 Synthesis of AB (14)

Simple coupling of adipic acid (**3**) and butane-1,4-diol (**4**) is not a suitable method for the synthesis of the monoester AB (**14**), as the difunctional nature of the diol and diacid means that polymeric species would necessarily form, leading to low yields and hampering purification. Roberts *et al.*[29] had previously synthesised (**14**) *via* synthesis of a monoprotected diol and monoprotected diacid species. Ester formation followed by deprotections gave AB (**14**) in milligram quantities (Schemes 11, 12 and 13).

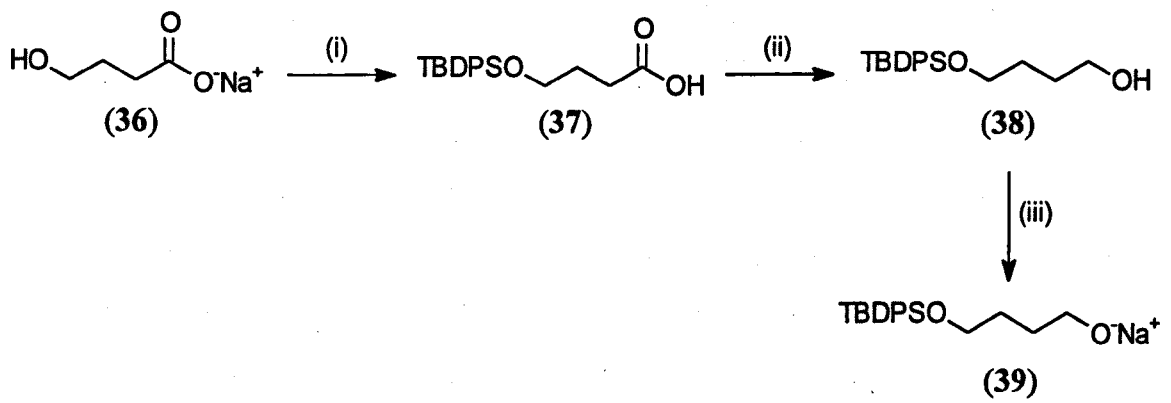
A monoprotected diacid species (**34**) was synthesised using sodium methoxide to ring-open ε-caprolactone (**26**) followed by Jones oxidation of the alcohol (**33**). Activation of (**34**) for reaction with activated alcohol (**39**) was achieved by formation of the acyl chloride (**35**) (Scheme 11).



Reagents and conditions:[29] (i) sodium methoxide, methanol, ambient temperature, 2 h, 75%; (ii) Jones reagent, acetone, 0 °C to ambient temperature, 90 min, 46%; (iii) oxalyl chloride, dichloromethane, DMF, 0 °C to ambient temperature, 17 h, (product not isolated).

Scheme 11

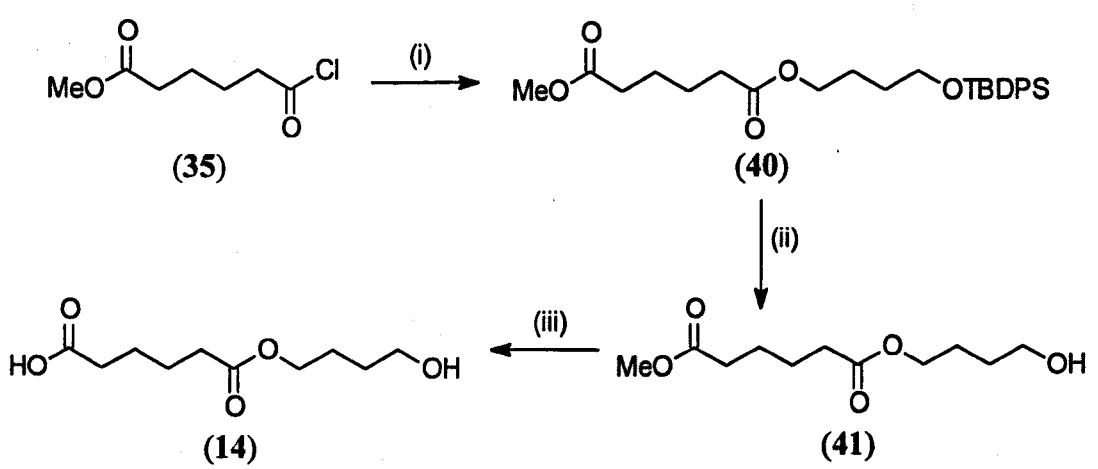
Protection of the alcohol function of the sodium salt of 4-hydroxybutanoic acid (**36**) using the TBDPS group gave acid (**37**), borane reduction of which gave monoprotected diol (**38**). Activation of (**38**) for coupling with (**35**) required formation of the alkoxide ion (**39**) (Scheme 12).



Reagents and conditions:[29] (i) TBDPSCl, DMF, imidazole, ambient temperature, 18 h, 55%; (ii) BH_3.THF, THF, ambient temperature, 19 h, 53%; (iii) sodium hydride, THF, imidazole, ambient temperature, 19 h, (product not isolated).

Scheme 12

Coupling of (**35**) and (**39**) proceeded in excellent yield to give ester (**40**). Unfortunately, deprotection of the alcohol to form (**41**) and subsequent cleavage of the methyl ester to give the free acid AB (**14**) were low yielding steps and led to isolation of much lower amounts of material than expected. In the latter case, much material was probably lost through cleavage of the central ester of (**41**) by lithium iodide.

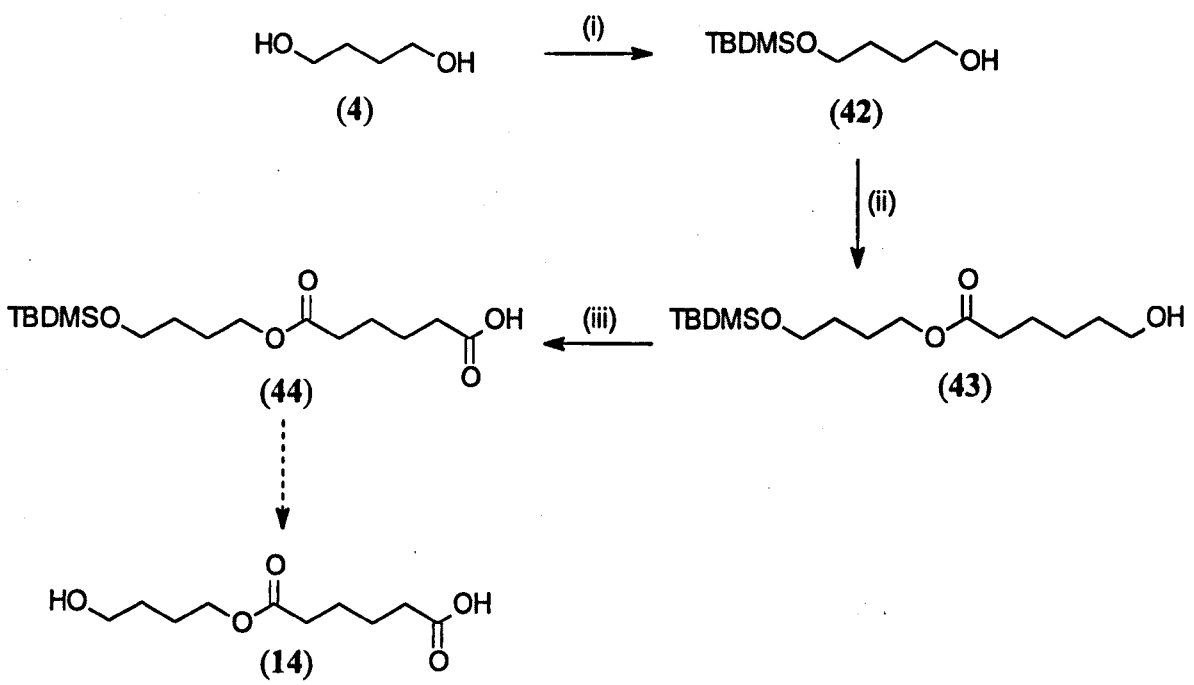


Reagents and conditions:[29] (i) (**39**), THF, reflux, 4 h, 98%; (ii) TBAF, THF, ambient temperature, 70 h, 40%; (iii) lithium iodide, pyridine, reflux, 20 h, 25%.

Scheme 13

In order to perform a series of polymerisation trials on AB (**14**), gram quantities were required. Whilst many steps of the aforementioned synthesis gave good product yields, the final two steps made the multistep synthesis inefficient and impractical for scale-up.

Efforts to lower the number of steps first of all led to a modified monoprotected diol preparation. Finding methods of synthesis from the free diol with a minimum formation of diprotected by-product has been the subject of much research. Statistically, a 1:2:1 distribution of recovered diol:monoprotected derivative:bisprotected diol is to be expected in the product mixture. Strategies employed to increase the yield of monoprotected species have included use of polymer-bound intermediates,[67] monosodium salts,[68] and excess diol[69] with varying degrees of success. Recently, Mash *et al.*[70] used dibutylstannylene intermediates in the synthesis of primary aliphatic monobenzylated diols, typically achieving yields of >50%. Use of excess diol is most appropriate where the starting diol is commercially available and inexpensive, and was the method of choice in this work. The TBDMS group was used in place of TBDPS, as cleavage of the former using TBAF is more facile; the hope was that this would raise the yield of the deprotection step at the end of the synthesis.



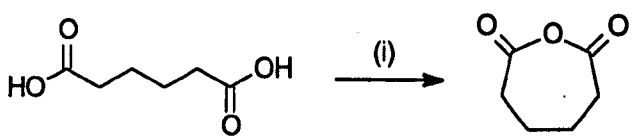
Reagents and conditions: (i) TBDMSCl, triethylamine, DMAP, dichloromethane, ambient temperature, 19 h, 80% based on TBDMSCl; (ii) a. sodium hydride, THF, 0 °C, 5 min; b. ε-caprolactone (**26**), THF, 0 °C to ambient temperature, 46%; (iii) PDC, DMF, ambient temperature, 18 h, 30%.

Scheme 14

Methanolysis of ε-caprolactone (**26**) followed by oxidation was the method previously employed to prepare the monoprotected diacid (**34**). However, ring-opening of (**26**) directly by the monoprotected diol (**42**) followed by Jones oxidation and deprotection of the TBDMS group was expected to yield AB (**14**). The low-yielding methyl ester cleavage step from the previous synthesis[29] would thus be avoided (Scheme 14).

Thus, monosilylation of butane-1,4-diol (**4**) using excess diol using a modified procedure of Hermitage[71] gave alcohol (**42**) in excellent yield based on TBDMSCl. Activation of the alcohol by alkoxide formation was performed prior to ring-opening of ε-caprolactone (**26**). The alcohol (**43**) formed was then oxidised using PDC, a milder oxidising agent than Jones reagent, to form the acid (**44**).[72] Unfortunately, a yield of only 30% was achieved (Scheme 14). The low yield of (**44**) was attributed to the acid-lability of the TBDMS group; whilst certainly labile under the highly acidic conditions of Jones reagent, any traces of water present in the PDC oxidation might also render the mixture acidic enough for silyl ether cleavage. Use of the less acid-vulnerable TBDPS group was disfavoured due to the more forcing conditions required for its removal at the end of the synthesis.

The problematic oxidation step after ring-opening of ε-caprolactone (**26**) was precluded by ring-opening the corresponding acid anhydride (**45**). In this way, the acid functionality was already established prior to ring-opening. The corresponding anhydride, adipic anhydride (**45**), is not commercially available, but was prepared by reaction of adipic acid (**3**) and acetic anhydride, using the method of Hill[73] (Scheme 15).



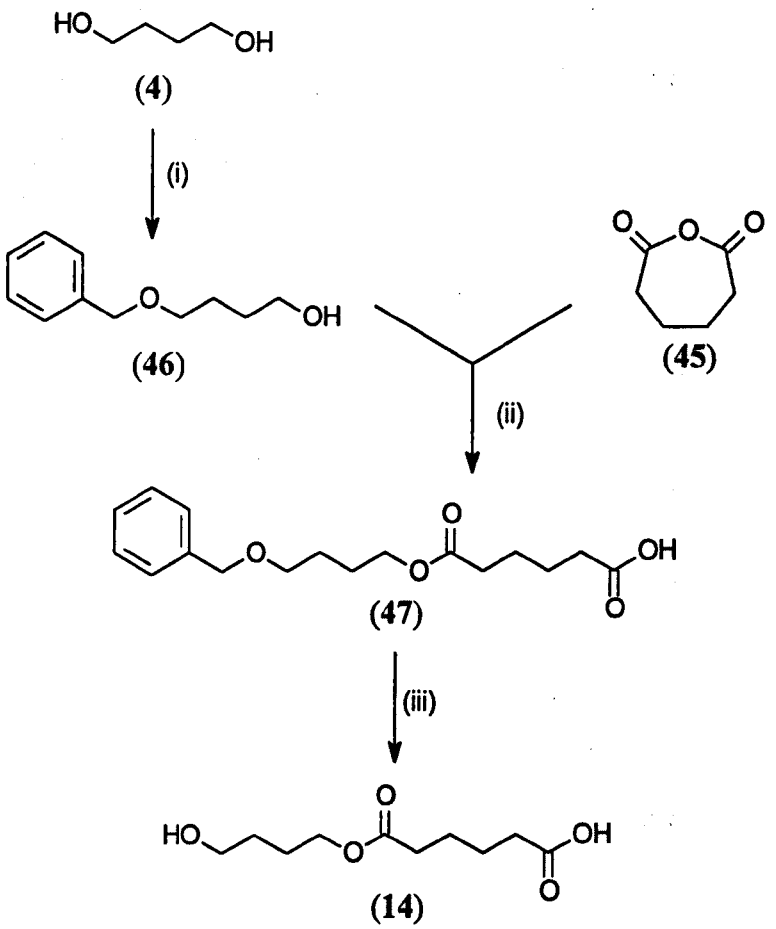
(**3**) (**45**)

Reagents and conditions: (i) acetic anhydride, reflux, 4 h; distillation under reduced pressure, 35%.

Scheme 15

As an acid-catalysed ring-opening of (**45**) was to be employed, monoprotection of butane-1,4-diol (**4**) with an acid-resistant protecting group was considered prudent. Benzylation using modified conditions of Saeki[74] gave alcohol (**46**) in very good yield based on benzyl chloride. Ring-opening of adipic anhydride (**45**) using alcohol (**46**) in the presence of *p*-toluenesulphonic acid under the conditions of Matsuyama *et al.*[75] gave an acceptable

yield of acid (**47**) after optimisation of work-up procedure. Catalytic hydrogenation of (**47**) furnished the hydroxyacid AB (**14**) in excellent yield (Scheme 16). It is worth noting at this point that purification of (**47**) and (**14**) using flash column chromatography gave very low yields of product, possibly because of their highly polar nature or, in the case of (**14**), through cyclic ester formation on the column. However, dry flash chromatography[76] on (**14**) gave a sufficiently pure product with minimal loss of material.



Reagents and conditions: (i) a. sodium hydroxide, DMSO; b. benzyl chloride, ambient temperature, 15 h, 81% based on benzyl chloride; (ii) *p*-toluenesulphonic acid, benzene, reflux, 18 h, 35%; (iii) H_2 / Pd on charcoal, ethyl acetate, ambient temperature, 24 h, 88%.

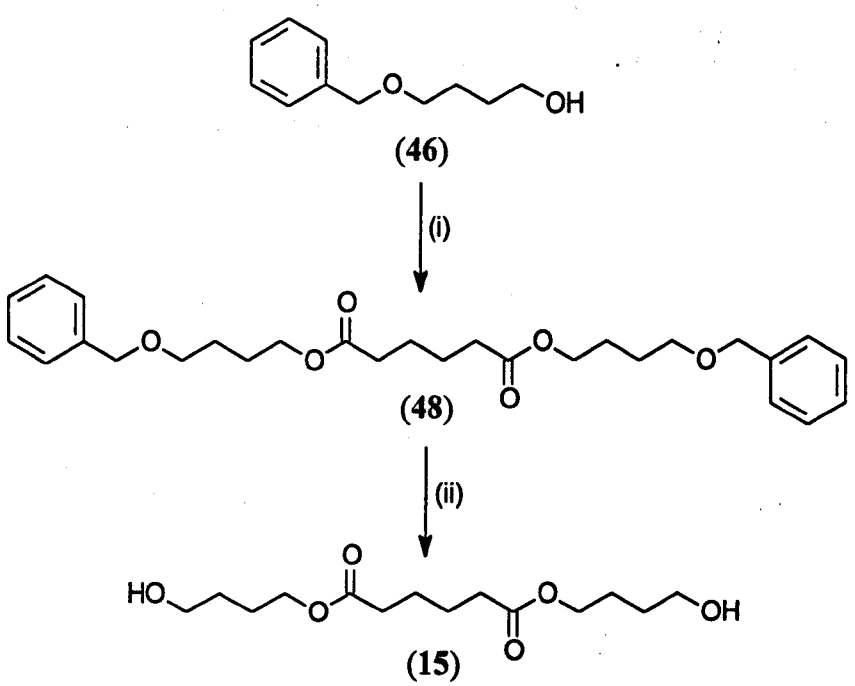
Scheme 16

Although *ca.* 200 mg AB (**14**) was initially synthesised, the synthesis in Scheme 16 was subsequently scaled-up to prepare the gram quantities needed.

2.1.2.2 Synthesis of BAB (15)

Synthesising BAB (**15**) provided less of a challenge than AB (**14**). The basis of our synthesis was to couple both acid groups of adipic acid (**3**) with a monoprotected diol, thus preventing further reaction of the diprotected species formed with the adipic acid present.

Coupling of (**46**) with adipic acid was accomplished in excellent yield using *Novozyme 435®* as catalyst and 4 Å molecular sieves to drive the esterification equilibrium over to diester (**48**) formation. Catalytic hydrogenation cleaved the benzyl ethers of (**48**) to give diol BAB (**15**) (Scheme 17). Again, dry flash chromatography[76] was required to purify (**15**) without major losses of material.



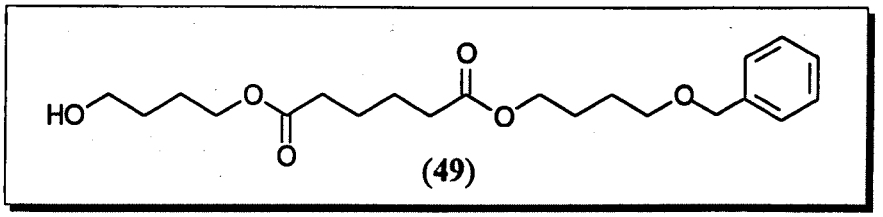
Reagents and conditions: (i) adipic acid (**3**), toluene, *Novozyme 435®*, 4 Å molecular sieves, 60 °C, 20 h, 81%; (ii) H_2 / Pd on charcoal, ethyl acetate, ambient temperature, 24 h, 63%.

Scheme 17

2.1.2.3 Synthesis of $(AB)_2$ (51)

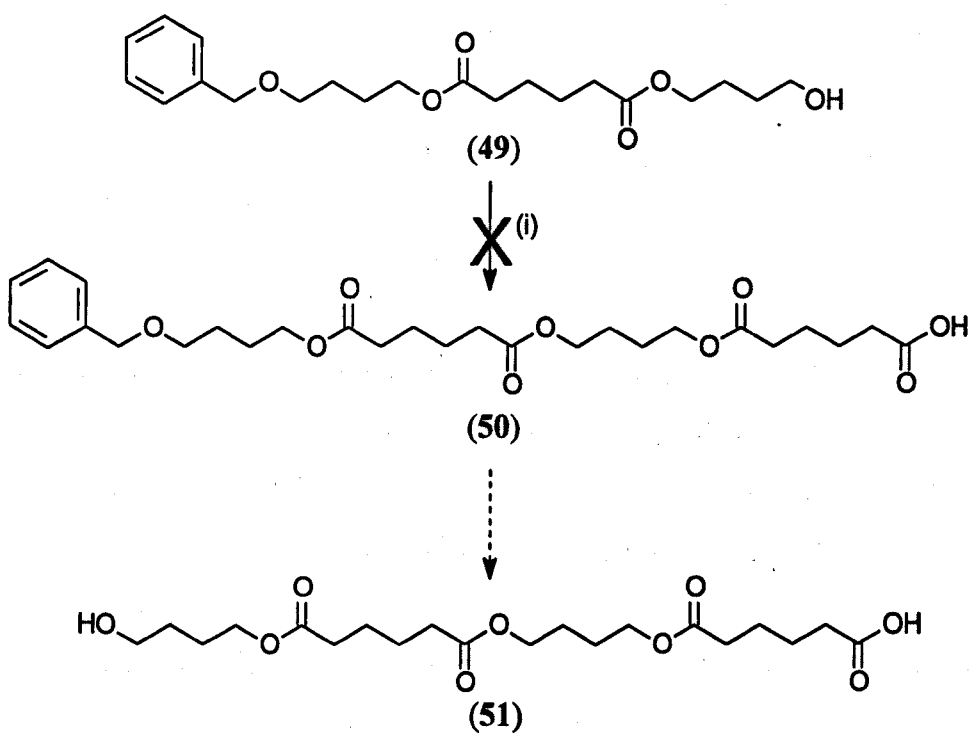
Deprotection of diester (**48**) proceeded through the monoprotected derivative (**49**), clearly seen by TLC with an R_f value intermediate between the diprotected species (**48**) and the diol BAB (**15**). Through monitoring by TLC, the reaction could be stopped when the

amount of (**49**) reached a maximum. In this way, isolation of (**49**) (amenable to flash column chromatography) in yields of up to 46% was possible with some recovery of starting material.



(**49**)

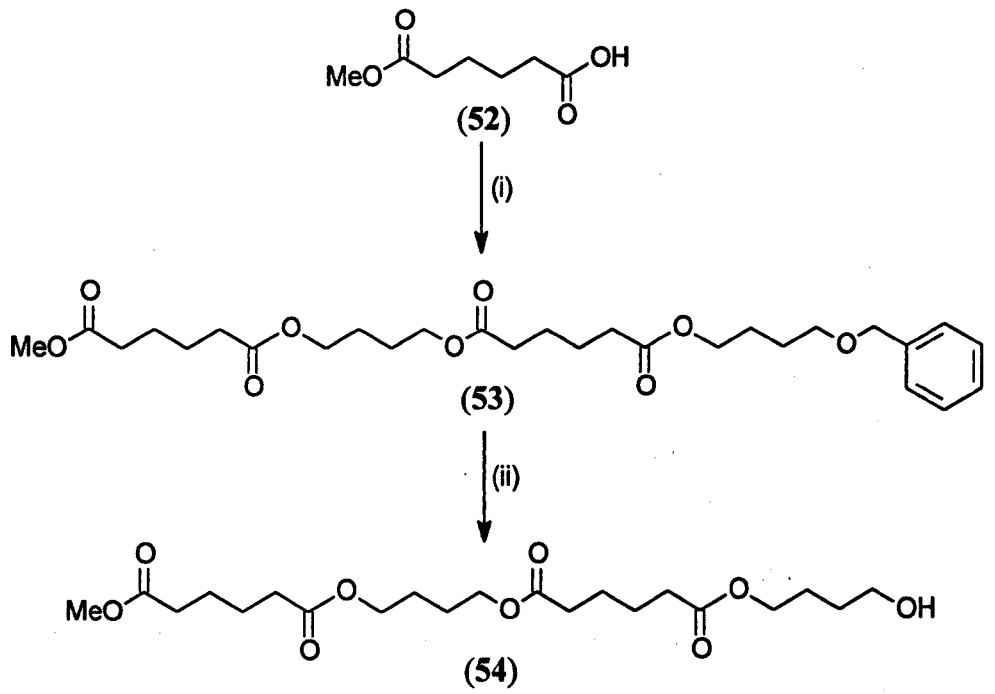
Alcohol (**49**) was viewed as a potential substrate for the production of higher weight oligomers in the butane-1,4-diol / adipic acid polymerisation process. Whilst the predominant oligomer formed of higher molecular weight than BAB was the hydroxyl terminated $B(AB)_2$ (**58**), $(AB)_2$ (**51**) was also of interest, not least because its ability to acylate the lipase was in question. Reaction of the monoprotected BAB species (**49**) with adipic anhydride was expected to produce a benzylated $(AB)_2$ (**50**) which could be deprotected using catalytic hydrogenation to yield $(AB)_2$ (**51**). Ring-opening of adipic anhydride (**45**) using alcohol (**49**) under Matsuyama conditions,[75] however, led to a mixture of several products by TLC. Attempts to extract the desired product (**50**) into aqueous base gave no significant amounts of material (Scheme 18).



Reagents and conditions: (i) adipic anhydride (**45**), *p*-toluenesulphonic acid, benzene, reflux, 18 h.

Scheme 18

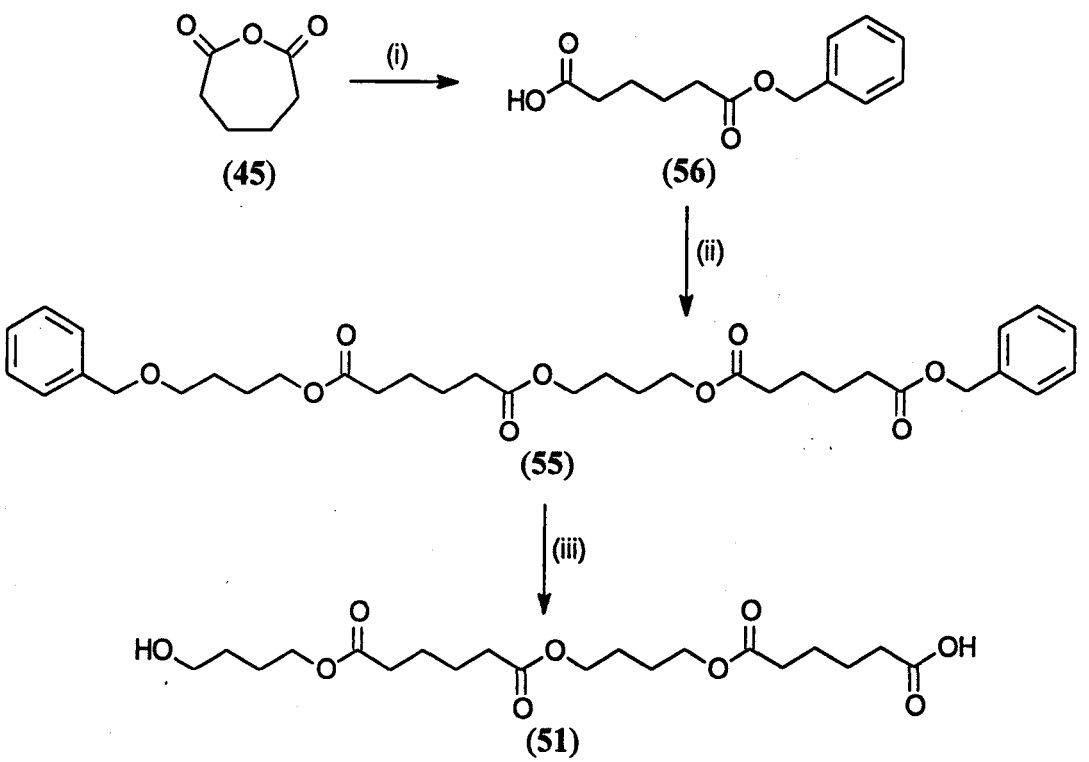
In order to authenticate the elution time of $(AB)_2$ (**51**), the methyl ester (**52**) was synthesised on a preparative scale. By effectively capping one of the acid functionalities of adipic acid (**3**) and reacting with alcohol (**49**), a clean reaction was expected with a single product. Hence, monomethyl adipate (**52**) was reacted with thionyl chloride to form the corresponding acyl halide. Reaction with monobenzylated BAB (**49**) gave protected methyl ester (**53**). Catalytic hydrogenation then proceeded quantitatively to give adequate amounts of $(AB)_2$ methyl ester (**54**) for characterisation and GPC authentication (Scheme 19).



Reagents and conditions: (i) a. thionyl chloride, 90 °C, 10 min; b. (**49**), dichloromethane, reflux, 15 h, 48%; (ii) H_2 / Pd on charcoal, ethyl acetate, ambient temperature, 20 h, 100%.

Scheme 19

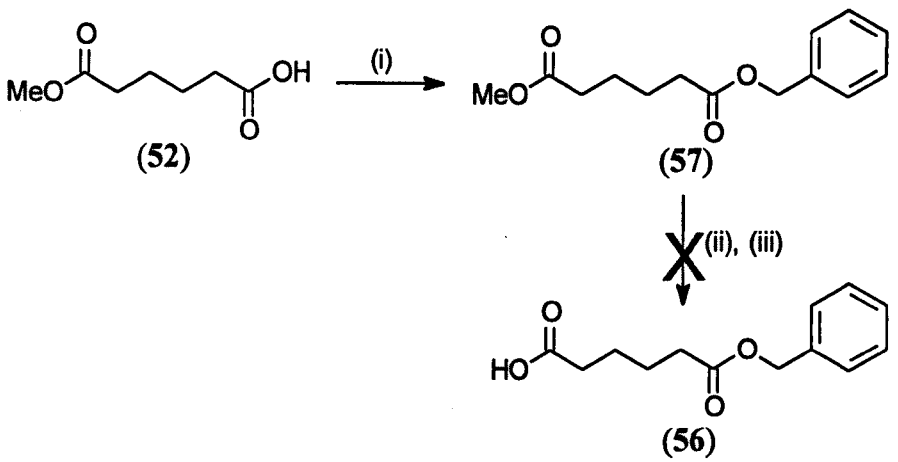
Subsequently, a diprotected $(AB)_2$ species (**55**) was synthesised by conventional DCC coupling of monoprotected diol (**49**) with monoprotected diacid (**56**), the latter being formed by ring-opening of adipic anhydride (**45**) using benzyl alcohol. Monoprotection of both the alcohol and acid led to an apparently clean, although low yielding, reaction. Catalytic hydrogenation rapidly cleaved both the benzyl ether and benzyl ester groups of (**55**) to give $(AB)_2$ (**51**) in high yield (Scheme 20). An adequate amount of material was prepared to allow a set of small scale polymerisation studies to be performed with *Novozyme 435®*.



Reagents and conditions: (i) *p*-toluenesulphonic acid, benzene, benzyl alcohol, reflux, 18 h, 20%; (ii) (**49**), DCC, DMAP, dichloromethane, ambient temperature, 20 h, 28%; (iii) H_2 / Pd on charcoal, THF, ambient temperature, 2 h, 86%.

Scheme 20

The low yield of monoprotected acid (**56**) achieved prompted efforts towards an alternative method of preparation. Benzylation of monomethyl adipate (**52**) was performed using benzyl alcohol and DCC in the presence of a catalytic amount of DMAP to furnish (**57**) in excellent yield. Haslam had shown that, in some instances, cleavage of methyl esters may proceed up to 70 times faster than the equivalent ethyl ester using lithium iodide in refluxing pyridine.[77] Similarly, preferential cleavage of the methyl ester over the benzyl ester of (**57**) to give acid (**56**) was attempted using lithium iodide, but only adipic acid (**3**) was isolated from the reaction mixture. Use of PLE to selectively hydrolyse the methyl ester of (**57**) in preference to the benzyl ester was also unsuccessful.[78] In this case, the diester (**57**) appeared not to be a substrate for the esterase (Scheme 21).

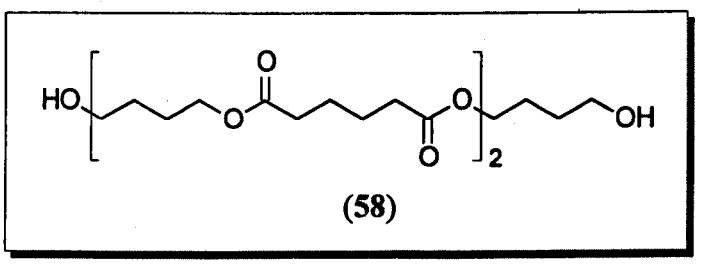


Reagents and conditions: (i) benzyl alcohol, DCC, DMAP, dichloromethane, ambient temperature, 18 h, 88%; (ii) lithium iodide, pyridine, reflux, 20 h; (iii) PLE, phosphate buffer, pH 7, DMSO, ambient temperature, 24 h.

Scheme 21

2.1.2.4 Synthesis of $B(AB)_2$ (58)

Rather than using synthetic chemistry for the synthesis of $B(AB)_2$ (**58**), a preparative GPC method was employed to provide enough material to perform a small-scale polymerisation trial.



A solvent-free polymerisation of adipic acid (**3**) and butane-1,4-diol (**4**) catalysed by *Novozyme 435*® was performed and halted after five hours; the GPC trace of the oligomeric mixture is shown in Figure 1(a). The mixture was dissolved in diethyl ether and extracted with potassium carbonate solution to remove all acid-ended species (product GPC trace shown in Figure 1(b)). Preparative GPC was used to elute BAB (**15**) from the mixture, leaving a mixture containing predominantly the next hydroxyl-terminated oligomer in the sequence, namely $B(AB)_2$ (**58**) (Figure 1(c)).

Having synthesised a range of oligomers from the butane-1,4-diol (**4**) / adipic acid (**3**) polymerisation process, their polymerisation in the presence of CALB was undertaken.

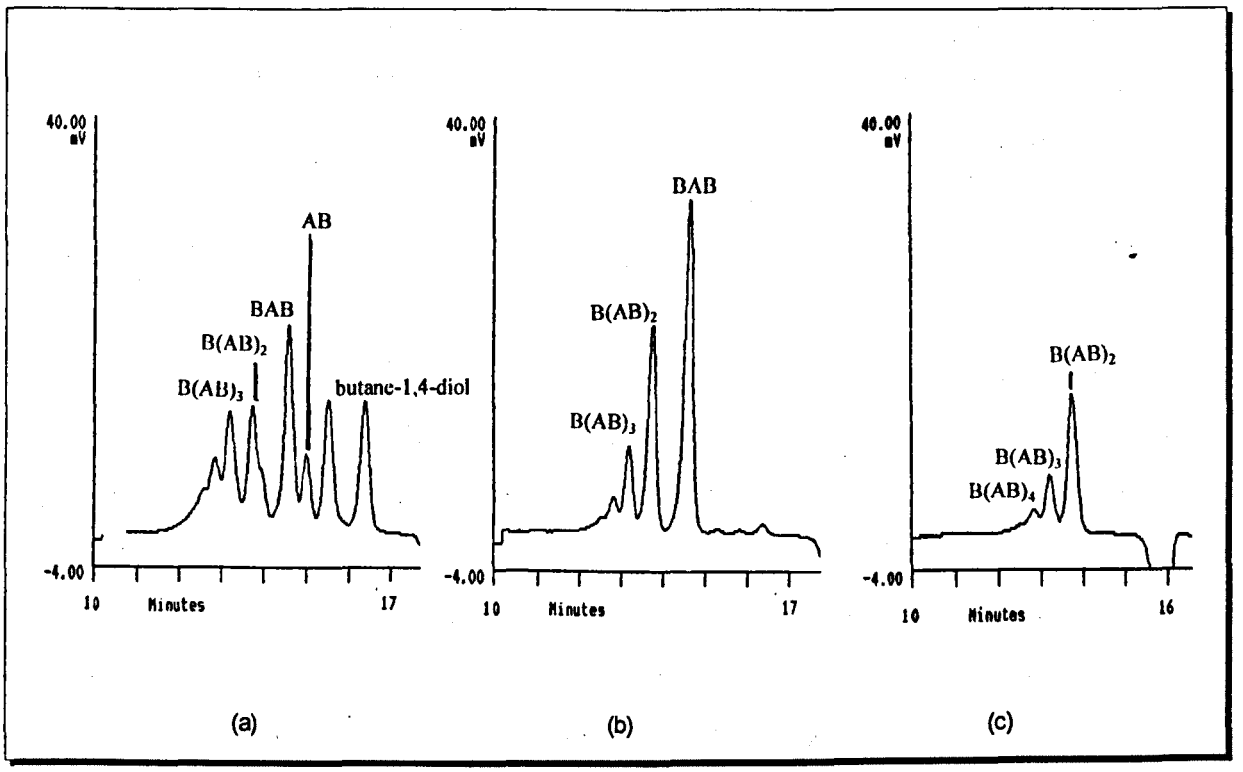


Figure 1

2.1.3 Polymerisation of key oligomers from the butane-1,4-diol / adipic acid system, catalysed by *Novozyme 435*®

2.1.3.1 Introduction

Oligomers AB (**14**) and BAB (**15**) were synthesised in gram quantities, enabling polymerisations on a similar scale. Oligomers $(AB)_2$ (**51**) and $B(AB)_2$ (**58**) were synthesised in only small amounts; in the latter case preparative GPC gave only *ca.* 6 mg. Because of the limited amount of material sometimes available, a standard solvent-free polymerisation was not always practicable. In these cases (and, for consistency, in cases where adequate material was also available for solvent-free polymerisation to be possible) a second system was devised, using anhydrous toluene as solvent in a modified Dean and Stark apparatus containing activated 4 Å molecular sieves. This solvent-based system comprised a pressure-equalising dropping funnel, the bottom of which was connected to the reaction vessel and the top to a Liebig condenser. Activated 4 Å molecular sieves were placed in the dropping funnel

with anhydrous toluene so as to fill the barrel to the top. In this way, water formed in the reaction would be removed as an azeotrope with the toluene in the reaction mixture and trapped by the molecular sieves. Any toluene lost through vaporisation would be returned by displacement of toluene from the reservoir in the dropping funnel. Removal of water in this way would drive the equilibrium of the esterification towards ester formation. As well as facilitating the study of substrate acceptability by *Novozyme 435®*, a comparison between anhydrous and water-containing systems could be made.

In order that meaningful semi-quantitative conclusions regarding extent of oligomer formation could be drawn by GPC, it was necessary to ensure that the starting materials and oligomers synthesised had the same response factor on GPC. The detector used depends on refractive index of the eluting molecules. By preparing samples of the individual oligomers of known concentration and injecting equivalent amounts of sample into the column, a quantification of each oligomer's response factor was determined. Adipic acid (**3**), butane-1,4-diol (**4**) and AB (**14**) were found to have nearly identical responses; BAB (**15**) gave a signal approximately 10% larger than that of AB (**14**), although this was considered insignificant and within experimental error. Thus, semi-quantitative treatment of the GPC traces of the polymerisations was validated.

The formation of large quantities of cyclic species within the system would lead to inaccuracies in average molecular weight determination as the correlation between molecular volume and molecular weight would be different to that of the corresponding linear polyesters. Binns has shown that, in the solvent-free system at least, the amount of cyclic esters formed is comparable to that formed in the conventional high temperature polymerisation, namely <0.5%.[79] GPC should therefore be a reliable guide to the molecular weight averages achieved.

2.1.3.2 Polymerisation of adipic acid (3) and butane-1,4-diol (4)

The standard solvent-free process used throughout this work consists of two stages. The first stage comprises:

- Initial dosing of acid, diol and aliquot of water, stirring at 40 °C for 1 hour to ensure saturation of the diol with acid, and equilibration of temperature throughout the mixture
- Dosing of enzyme and subsequent stirring for 4 hours at 40 °C
- Increase of temperature to 60 °C and application of a partial vacuum (100 ± 5 mbar) to remove water, stirring for 17 hours
- Filtering off immobilised lipase from molten oligomer / polymer.

The second stage comprises:

- Stirring at 60 °C at atmospheric pressure, typically for 7 hours
- Stirring at 60 °C under partial vacuum (10 ± 3 mbar) to remove by-produced water, stirring for *ca.* 17 hours.

Usually, only the first stage of the polymerisation was studied in comparisons of oligomer polymerisations with the acid / diol polymerisation, as GPC monitoring shows that the majority of oligomer and polymer formation occurs during stage 1.

A solvent-free polymerisation of adipic acid (**3**) and butane-1,4-diol (**4**) was performed, sampling the reaction for GPC analysis 2 and 4 hours after addition of enzyme. The partial vacuum (100 ± 5 mbar) was applied and the mixture sampled after another 4 and 17 hours.

Little difference in the extent of polymerisation exists between the samples taken at 2 and 4 hours. Subsequently, larger scale polymerisations have shown that the reaction effectively stops after 2 hours at atmospheric pressure, implying that equilibrium has rapidly been reached. The GPC trace of the sample taken 4 hours after application of partial vacuum (Figure 2) shows clearly the predominance of hydroxy-terminated oligomers, especially BAB (**15**) and $B(AB)_2$ (**58**) *etc.* in a discrete array; this indicates that incremental growth occurs, with an AB unit separating each oligomeric hydroxyl-terminated species. Adipic acid (**3**) appears to be present in large concentrations, but this is merely an artefact of sampling the heterogeneous mixture without filtering off the suspended acid prior to analysis. The important plausible "building block" AB (**14**) appears in comparatively low concentration; its dimer $(AB)_2$ (**51**) appears as a shoulder on the right hand side of the $B(AB)_2$ (**58**) peak. The low concentration of acid-ended species was believed to result from the excess of diol in the liquid phase in the early stages of the reaction;[29] the high concentration of butane-1,4-diol (**4**)

around the enzyme means that the most likely fate of the first formed oligomer AB (**14**) is a second esterification with butane-1,4-diol (**4**) to give BAB (**15**). As AB (**14**) never reaches a high concentration, it is fairly unlikely to be esterified with another AB (**14**) molecule to form $(AB)_2$ (**51**). The dominance of BAB (**15**) lends support to this theory.

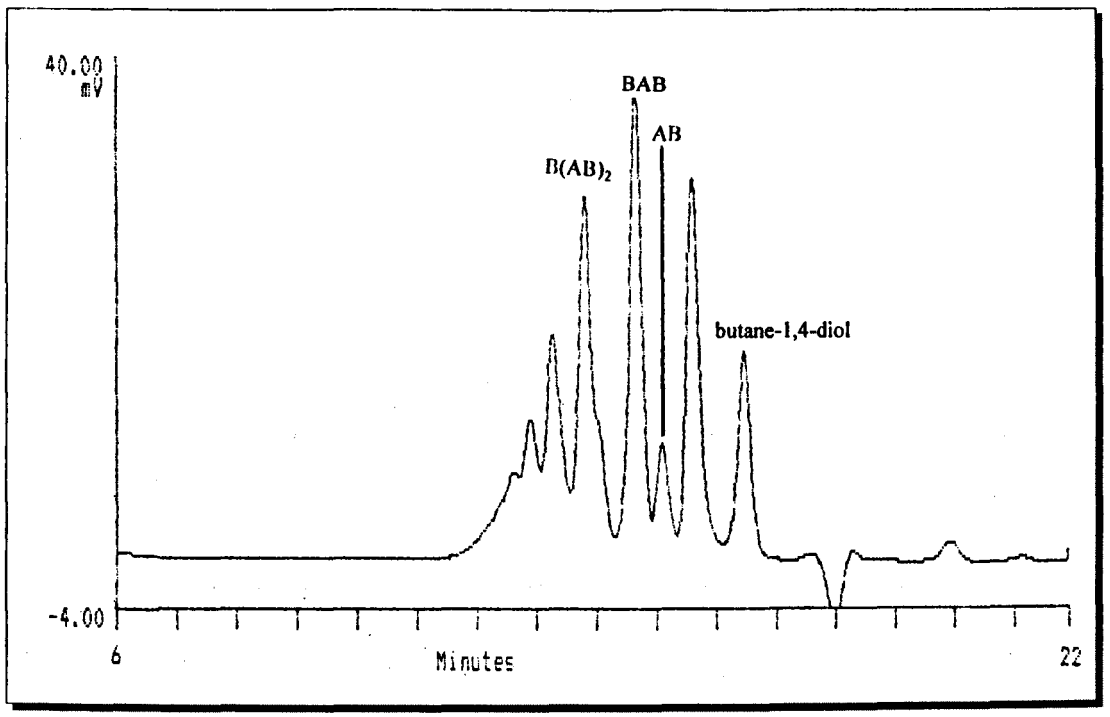


Figure 2

GPC on the reaction mixture after 17 hours at 100 mbar revealed that largely polymeric material was present and only small amounts of acid (**3**), diol (**4**) and AB (**14**) (approximately 0.3, 2.1 and 3.0% respectively) (Figure 3). The polyester evidently possesses a narrow dispersity (M_w / M_n 1.5). Interestingly, whilst the small amounts of residual low weight oligomers and monomers are consumed in the second stage of the process, the overall dispersity of the polymer tends to broaden, possibly indicating a change in assemby mechanism.

A control reaction run in the absence of lipase gave no reaction under identical conditions.

Toluene-based polymerisation of adipic acid (**3**) and butane-1,4-diol (**4**) was also performed, although the reaction was only sampled for GPC after 21 hours. Whilst a higher relative enzyme dose was used than for the solvent-free reaction, lower molecular weight material was produced and the mixture had a significantly larger dispersity (M_w/M_n 2.0). The low solubility of adipic acid (**3**) in toluene or saturation with water of the activated molecular

sieves used to drive the reaction might have caused the unexpectedly low weight. The reaction was not repeated so it was not possible to validate these suggestions.

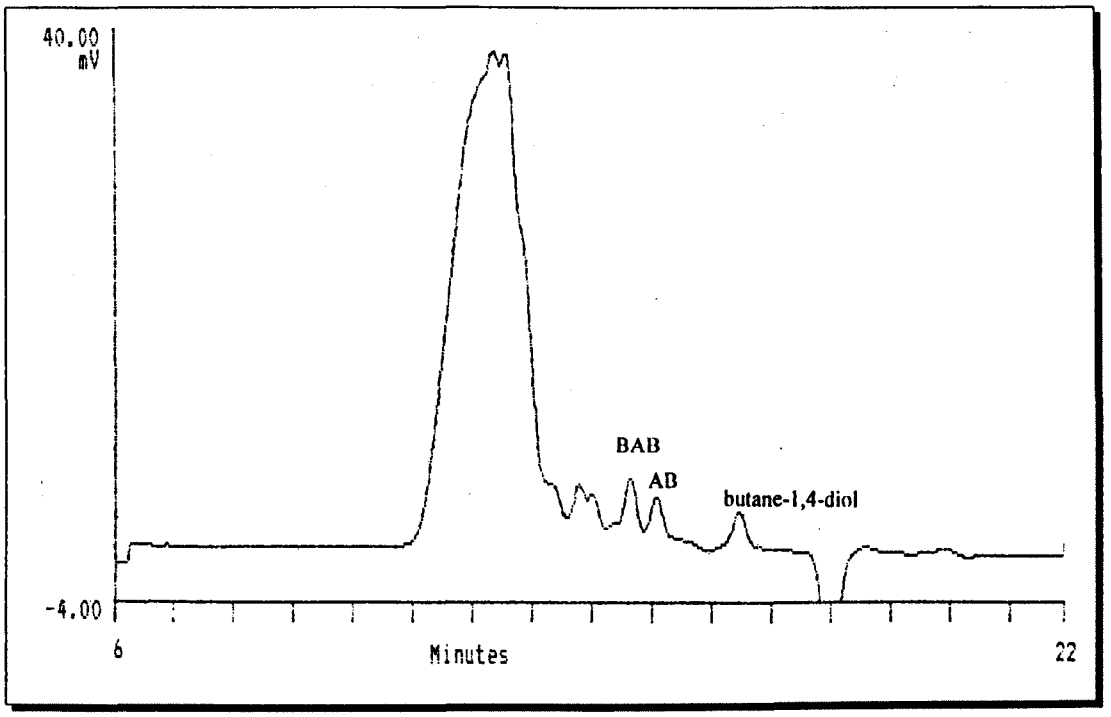


Figure 3

2.1.3.3 Polymerisation of AB (**14**)

AB (**14**) was polymerised in the presence of an equivalent amount of *Novozyme 435*® to that used for acid and diol in Section 2.1.3.2 in order that the rates of polymerisation of the two substrate types might be compared. AB (**14**) behaved similarly after 2 and 4 hours, with little increase in molecular weight average or change in oligomer distribution being observed. The absence of diol in the reaction led to formation of $(AB)_n$-type species, mainly $(AB)_2$ (**51**) at this early stage of the oligomerisation. As the reaction proceeded under partial vacuum conditions, a discreet array of higher weight $(AB)_n$ oligomers formed, although curiously, small amounts of intervening species presumed to be BAB (**15**) and ABA were observed. By the time the reaction was halted (17 hours after initial enzyme dose) substantial amounts of polymer were present (M_n 1008, M_w 1696, M_w / M_n 1.7), although the reaction had reached a significantly lower molecular weight than the diacid / diol system (Figure 4).

As the acid / diol system must form AB (**14**) as the initial step in the reaction, the AB polymerisation would be expected to reach a higher molecular weight, having effectively had a "head start". The paradox of the apparently conflicting reaction rates might be explained by

the difference between the systems: in the AB case AB (**14**) acts as solvent and monomer whereas the acid / diol system uses butane-1,4-diol (**4**) as solvent but both adipic acid (**3**) and butane-1,4-diol (**4**) as substrates. In the presence of a large excess of butane-1,4-diol (**4**), the process of ester formation may be heavily favoured, whereas in AB (**14**) alone the effect on position of equilibrium may not be so marked.

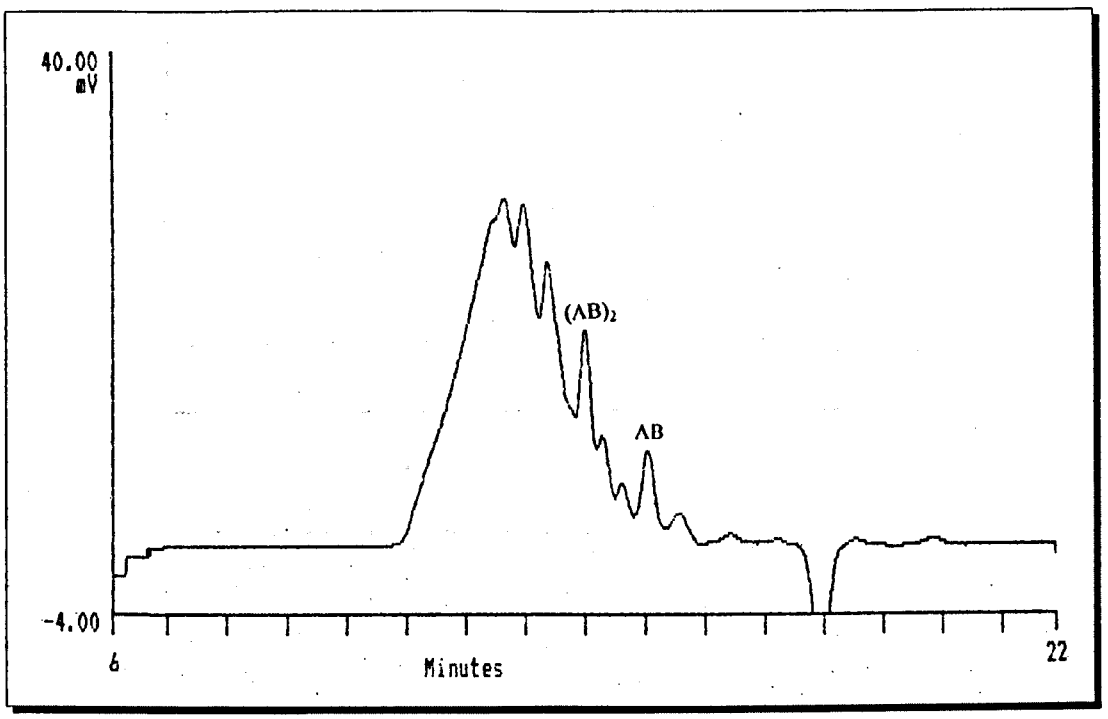


Figure 4

A study of the reaction of AB (**14**) with a 5-fold excess of butane-1,4-diol (**4**) was performed under standard first stage solvent-free conditions, to mimic the conditions in the standard acid / diol process. Although monitoring of the reaction progress was not performed, study of the GPC trace of the reaction after 6 hours at 60 °C / 100 mbar vacuum showed the expected predominance of BAB (**15**) and residual diol (**4**), only a tiny amount of AB (**14**) remaining. A significant amount of $B(AB)_2$ (**58**) and a trace of $B(AB)_3$ were also apparent (Figure 5). The statistical spread of these oligomers suggests, importantly, that chain transfer does not occur, that is transesterification *via* attack of an alcohol on an ester linkage. This may arise from the inability of CALB to catalyse the transesterification or from the fact that transesterification at an end ester linkage would release both a larger polymeric structure and butane-1,4-diol (**4**): the large excess of diol present would make the formation of more diol unfavourable. An example of the transesterification of BAB (**15**) with another BAB molecule

catalysed by *Novozyme 435*® is shown in Scheme 22; LIPASE-O$^-$ represents the serine hydroxyl residue.

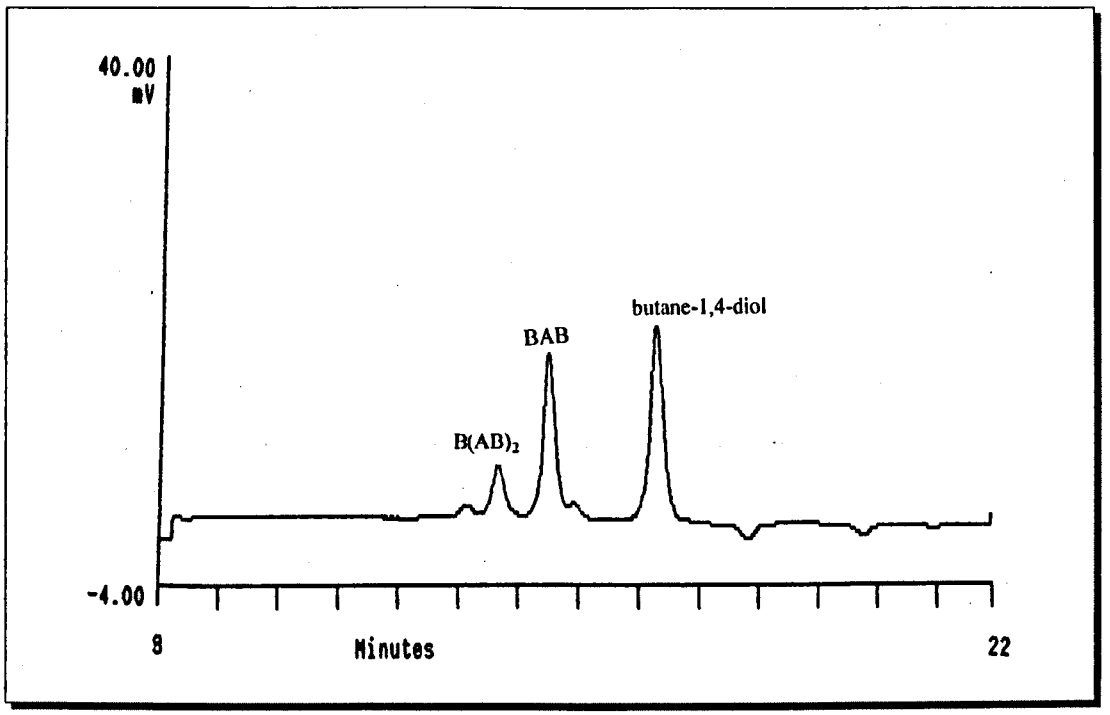
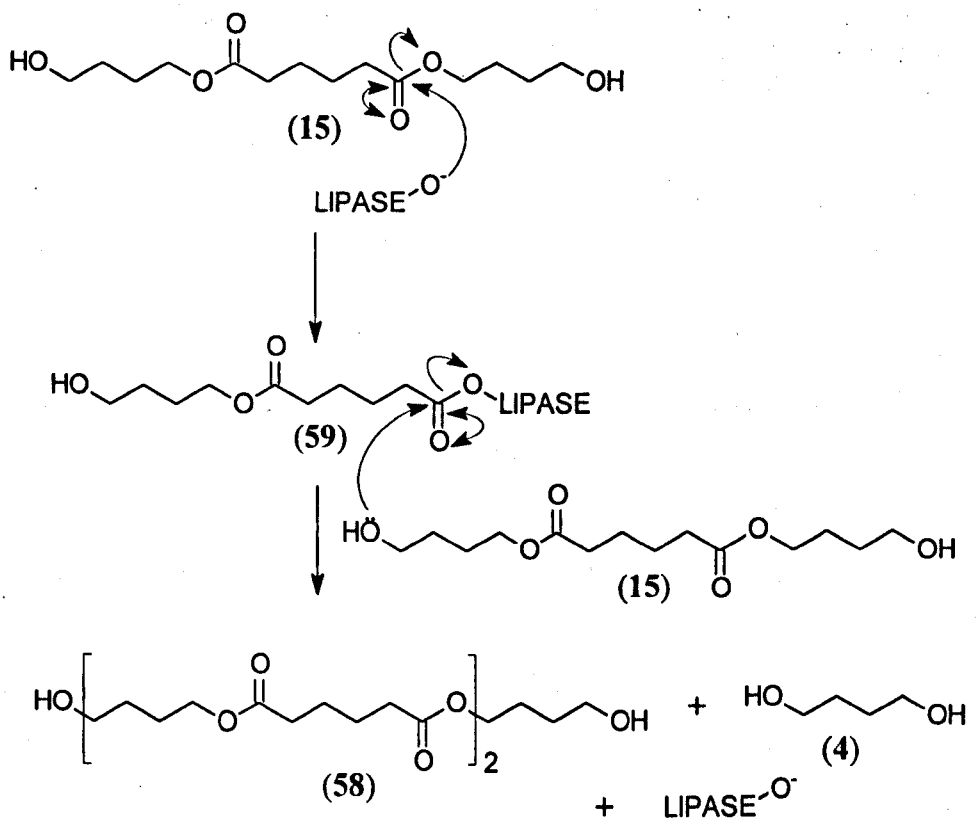


Figure 5



Scheme 22

Polymerisation of AB (**14**) in toluene under anhydrous conditions was also performed. As for the acid / diol system, a larger enzyme:substrate ratio was employed than for the solvent-free system, making direct comparison of extents of polymer formation invalid. However, differences in the polymer assembly mechanism would be significant.

The toluene-based AB (**14**) polymerisation was sampled after 1, 3, 7, 11 and 22 hours. The large quantities of lipase used led to formation of much polymeric material after just 1 hour (Figure 6(a)), although little change in the molecular weight occurred between 3 and 11 hours. At 22 hours, oligomers were still present in small amounts and the apparently small increase in molecular weight averages seen may be attributable to the GPC technique: a 50-100 Å column was employed, which is only suitable for lower weight polymers. At the upper limit, a near vertical cut-off of the polymer signal is seen (Figure 6(b)), leading to underestimation of the molecular weight averages. The latter are given in Table 1.

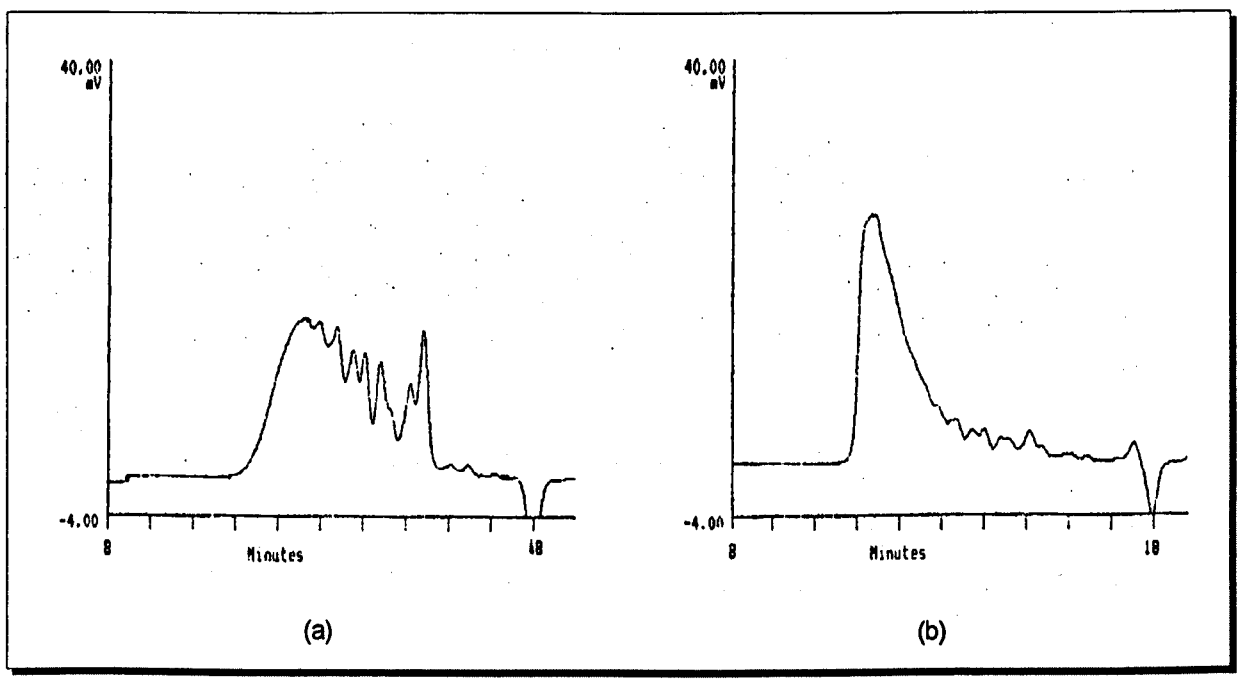


Figure 6

During the final stages of oligomer consumption the polydispersity of the polymer, having dropped during the middle stages, begins to climb again. This is in accord with the solvent-free polymerisation studies in Section 2.1.3.1.

Both solvent-free and toluene-based reactions using AB (**14**) were also run in the absence of lipase; neither showed any reaction, their GPCs remaining monodisperse. Again, this proves that AB (**14**) does not catalyse its own polymerisation to any extent.

sample time / hours	M_w	M_n	M_w / M_n
1	1304	644	2.0
3	1917	944	2.0
7	2189	1205	1.8
11	2270	1342	1.7
22	3088	1471	2.1

Table 1

2.1.3.4 Polymerisation of BAB (**15**)

Polymerisations of BAB (**15**) were performed in solvent and under solvent-free conditions. The solvent-free process used was identical to that used for AB (**14**) in order that meaningful comparisons could be drawn, but whilst anhydrous toluene was used in the solvent-based process, a modified Dean and Stark apparatus was not deemed necessary. As only transesterification can result when BAB (**15**) alone is present (the molecule having no acid ends), the presence of molecular sieves would be expected to have no effect on the position of equilibrium.

Under solvent-free conditions BAB (**15**) failed to react appreciably; sampling by GPC after 17 hours at 60 °C / 100 mbar showed a slight increase in the amount of material assumed by its elution time to be $B(AB)_2$ (**58**) (Figure 7(b)); a GPC trace of the starting material is shown in Figure 7(a).

Confirmation that the lipase was active at the end of the process was achieved by the addition of a small sample of AB (**14**) (~7 mol% wrt BAB (**15**)). Stirring for 4 hours at 60 °C led to formation of some higher weight oligomeric species.

Stirring BAB (**15**) in anhydrous toluene for 17 hours at 60 °C showed a very different picture. The same starting material as Figure 7(a) gave a large array of oligomeric species (Figure 8(a)). Transesterification was clearly occurring, confirmed by the presence of substantial quantities of butane-1,4-diol (**4**) in the GPC trace. Hence, the mechanism shown in Scheme 22 must indeed apply when anhydrous toluene is used as reaction medium. The GPC

trace from a control reaction in toluene in the absence of *Novozyme 435®* is given in Figure 8(b), and shows negligible reaction.

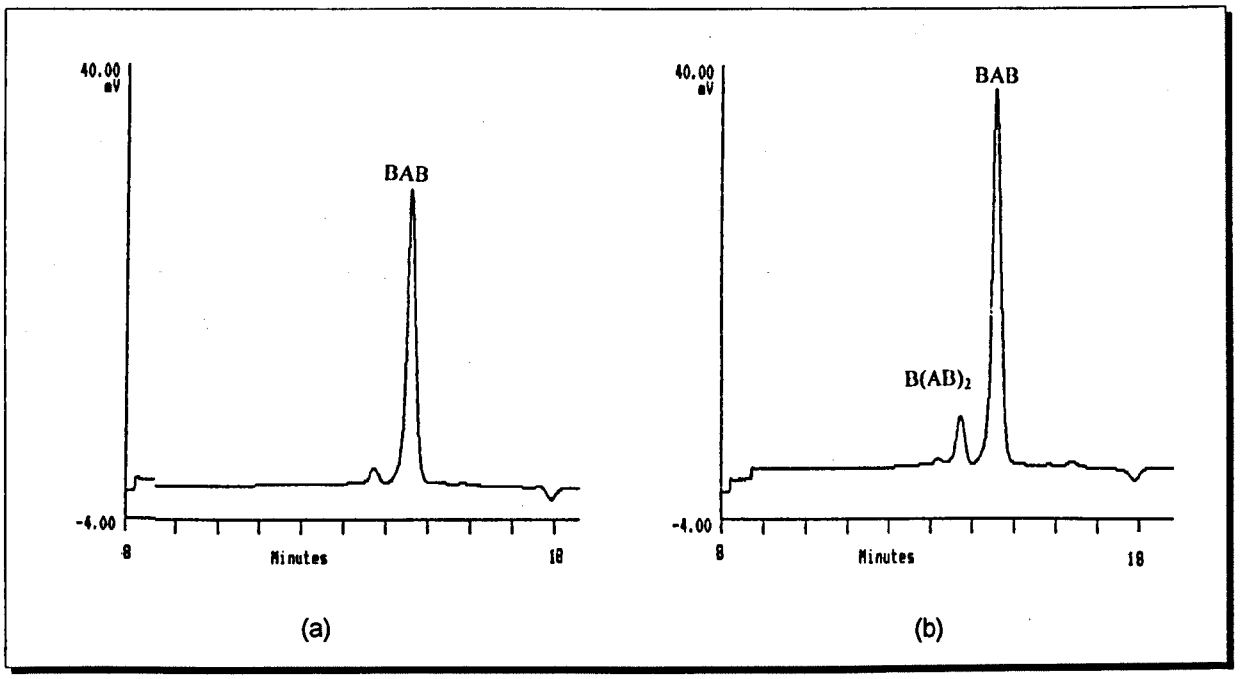


Figure 7

Direct comparison of the solvent-free and toluene-based reaction rates is not meaningful, as a much higher enzyme dose is used in the latter case; however, the change in lipase activity between the two systems is marked enough to be of significance. Transesterification may have taken place if larger quantities of lipase were added to the solvent-free system, but in the solvent-free diol / diacid process (which uses a low enzyme dose) the implication is that transesterification of BAB (**15**) is absent, or at least contributes very little to the polymer assembly mechanism. This is surprising, as esters are more activated for nucleophilic attack than the corresponding acids, the presence of a leaving group (in the case of BAB (**15**) this is the 4-hydroxybut-1-oxy group) making the δ^+ charge on the carbonyl carbon larger. The explanation may be based, therefore, on steric grounds, the lipase being less able to accommodate (and therefore be acylated by) the bulky ester groups than the relatively unhindered free acid. In toluene, there is the possibility that the lipase substrate specificity alters, possibly through a change in enzyme conformation. Such a rearrangement might not be obvious from polymerisation of AB (**14**), as the molecule is likely to be small enough to fit in the active site and acylate the serine residue using the acid function of AB

(14); BAB **(15)** offers no unhindered acylation site (acid end) and hence may require the expanded active site in toluene to be able to react.

The phenomenon of a change in the substrate specificity of a lipase in different solvents is not new, and has often been explained by the existence of two extreme forms of lipase, with closed and open conformations. The change of selectivity and reaction rate of a lipase with a change in polarity of solvent has also been documented.[80,81,82] Recent work has shown the desolvation energy of the substrates to be the key factor in determining their rates of reaction with the enzyme.[83,84]

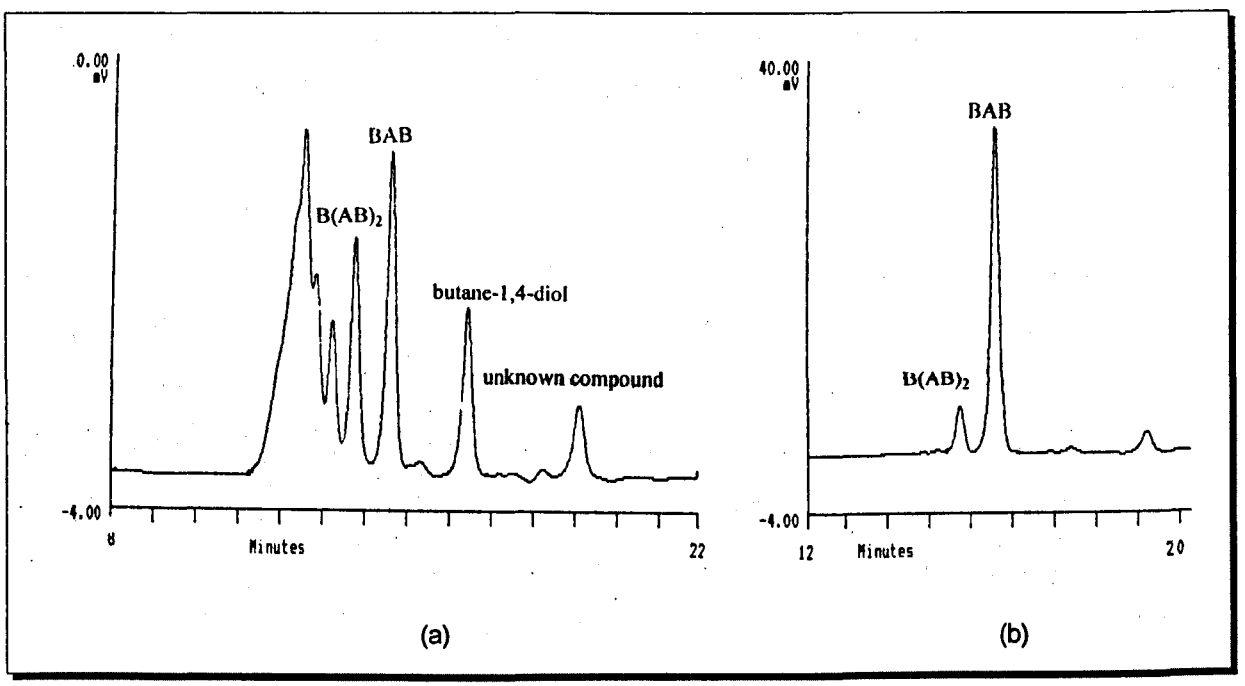


Figure 8

Comparison of the solvent-free and solvent-based systems used here reveals two possible important differences. The first is the solvent polarity, in the former case a highly polar diol, in the latter the comparatively non-polar toluene. The second difference, again in medium, is the water activity. In the solvent-free system, water is dosed at the start of the reaction (enzyme activity found to be significantly lower if not added) and builds up to *ca.* 8wt.% by the end of the first stage.[62] In the toluene-based system, the modified Dean and Stark apparatus used ought to ensure that the system remains anhydrous for at least the majority of the process time.

Changes in substrate specificity of α-chymotrypsin in aqueous *versus* anhydrous organic medium was recorded more than a decade ago by Klibanov.[85] More recently, the same

author found that Subtilisin Carlsberg also showed different substrate specificities in various solvents at constant water activity;[86] the deduction was that the log *P* value (partition coefficient) of the solvent was the key issue rather than water activity, and these conclusions led to the more recent examinations involving the ease of substrate desolvation.[83,84]

Lipases usually share the common structural feature of a lid over the active site, which opens when the enzyme undergoes interfacial activation at an oil-water interface,[87,88] often resulting from the presence of micelles. CALB, however, possesses only an incipient lid feature,[66] and has been shown not to participate in interfacial activation,[89] giving CALB properties more akin to those of an esterase.[90] For CALB to be virtually inactive towards BAB (**15**) in the neat conditions and then highly accessible in toluene, intermediate states of "openness" might need to operate, rather than just the accepted active and inactive extremes. The possibility of such transitional intermediates, especially in aqueous media, has been discussed by Schrag and Cygler.[91] Interestingly, they point out that lid-closed forms of classical lipases persist in aqueous media and in lid-open form concomitant exposure of a large hydrophobic surface occurs. The latter would be expected to be favoured by the non-polar anhydrous toluene medium.

An alternative explanation for the change in specificity of CALB may result from a difference in pH of the aqueous medium (solvent-free system) to that from which the enzyme was lyophilised. The solubilisation of adipic acid in the early stages of the process would give a solution phase of low pH. Russell and co-workers showed that the activity of Subtilisin Carlsberg suspended in organic solvents at varying water activities depended on pH, related to a change in polarity (ionisation) at the active site.[92] In addition, Jones *et al.* found that changing the pH of CALB crystals dislodged a detergent molecule at the entrance to the active site, giving rise to disorder in the lid helix.[66,93]

Otero[94,95] showed that lipases from *Candida rugosa* could exist in more conformations than just "closed" and "open" forms after treatment with reverse micellar, *n*-heptane and 2-propanol systems. Circular dichroism and fluorescence spectroscopy were employed to confirm that changes in conformation were occurring. A change in catalytic activity on treatment of *Candida rugosa* lipase with 2-propanol had been demonstrated by Kazlauskas *et al.*,[96] the enantioselectivity of the lipase towards chiral carboxylic esters being enhanced by up to 25-fold.

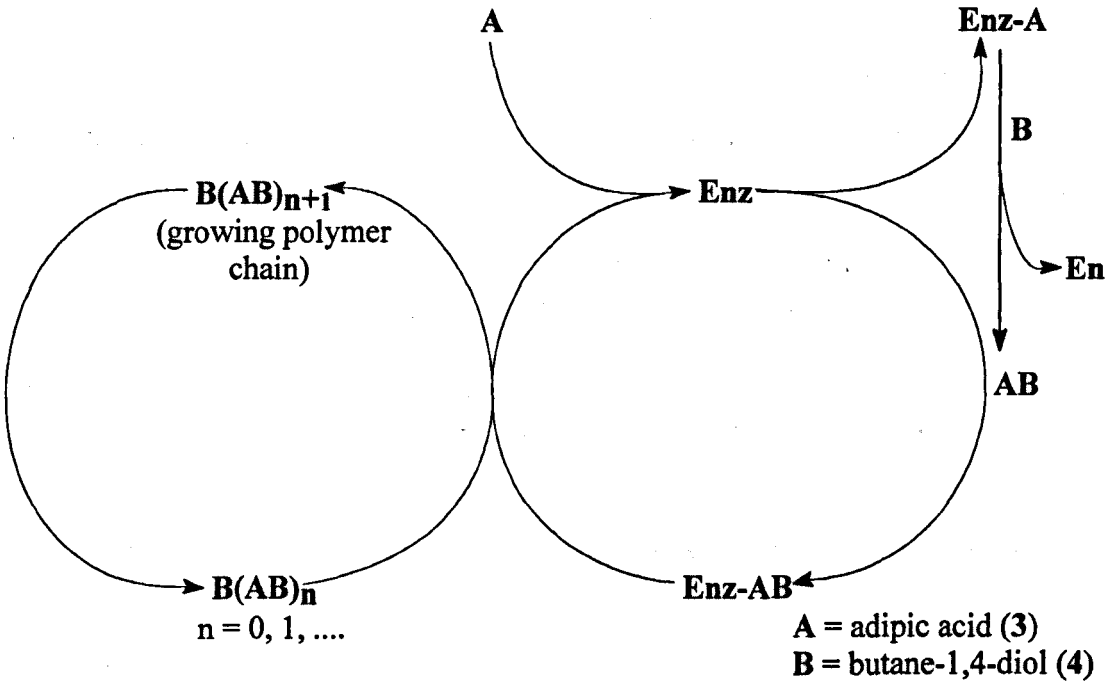
Evidence for possible conformation changes in thermolysin-catalysed peptide synthesis was given by Klibanov,[97,98] who showed that polyols such as glycerol may replace water in the enzyme structure to some extent; also, Farber showed that addition of 4% isopropanol to hexane surrounding crystals of γ-chymotrypsin gave rise to a doubling of the number of water molecules in the crystal structure.[99] Subtilisin Carlsberg had shown a large variation in activity on placing the enzyme in a variety of solvents. Klibanov recently showed that conformational changes in these solvents were not occurring, using an FTIR method to monitor secondary structure.[100] This result may conflict with the more established acceptance of changes in enzyme conformation being linked to enzyme activity and specificity.

The absence of transesterification of BAB (**15**) implies that AB (**14**), a structurally very similar molecule, would also not transesterify to an appreciable extent. The formation of butane-1,4-diol (**4**) would occur, and in the early stages of the solvent-free system, where butane-1,4-diol (**4**) is in excess in the reaction medium, transesterification would again be disfavoured by equilibrium considerations. A polymerisation of BAB (**15**) in a 5-fold excess of butane-1,4-diol (**4**), mimicking the conditions of the solvent-free acid / diol system, confirmed that only a trace of reaction occurred.

The lack of transesterification in the solvent-free polymerisation gives further confirmation that the postulated "train" mechanism idea cannot be operating, and that the classical bi-bi ping-pong assembly occurs (Section 2.1.1).

The notion that stepwise addition of an AB unit occurs as the main polymer chain construction route is not in doubt; however, the original thought that this occurs through transesterification of a BAB unit by another BAB (**15**) molecule[29] seems flawed. In fact, what must occur is simple esterification of AB with BAB, and thereafter of AB with $B(AB)_n$ as the polymer chains grow. The presence of acid and diol throughout the first stage of the process would serve to provide a constant supply of AB for the polymerisation to continue. A representation of this solvent-free process chain growth mechanism is shown in Scheme 23.

Esterification of AB (**14**) with another acid-ended species (*e.g.* another AB molecule) may also occur, although this is statistically less likely in the early stages of the process due to the predominance in solution of the diol.



Scheme 23

2.1.3.5 Polymerisation of $(AB)_2$ (**51**)

By synthesising (**51**) and its methyl ester (**54**), unambiguous confirmation of the elution time on GPC gave a fingerprint, which confirmed that $(AB)_2$ (**51**) is formed during the solvent-free enzymatic polymerisation process. The disappearance of (**51**) during the polymerisation confirms its ability to act as an alcohol, forming an ester with the enzyme acylated by AB (**14**). Whether or not $(AB)_2$ (**51**) can acylate the lipase itself is unclear. Certainly, if this were not possible, the finished polyester would contain substantial amounts of $(AB)_n$-type acid-ended species. Although MALDI-TOF mass spectrometry and acid number measurements had confirmed that the finished enzymatically-produced polyester contained only small quantities of acid-ended material, polymerisation of $(AB)_2$ (**51**) in the presence of CALB was considered an important experiment.

The small quantities of (**51**) prepared meant only a very small scale solvent-free polymerisation could be performed, making direct comparisons with the polymerisation of acid and diol and AB invalid. GPC showed the starting material to be ~85% pure with a single major impurity at an elution time (15.4 minutes) approximately corresponding to that of AB (**14**).

Polymerisation of $(AB)_2$ (**51**) under standard solvent-free process conditions produced a largely polymeric product after 17 hours at 60 °C / 100 mbar (M_w 2056, M_n 952, M_w / M_n 2.2) (Figure 9(b)). The signal at 15.4 minutes remained, and an estimation of the area of polymer *versus* area of signal at 15.4 minutes by GPC suggested that the latter constituted only 10-15% of the material, implying that no reaction of this component had occurred and that therefore it could not be AB (**14**). Probably it was a residue from the DCC coupling stage employed in the synthesis of (**51**).

Sampling of the reaction after 2 hours at 40 °C showed only accumulation of oligomeric species of higher weight than (**51**), although the "mystery" signal at 15.4 minutes would have obscured any AB (**14**) formed (Figure 9(a)). Certainly, no butane-1,4-diol (**4**) was formed at all, confirming the absence of transesterification at the terminal ester linkage. If esterification alone occurred, clearly the oligomeric species expected would be multiples of $(AB)_2$ (**51**), namely $(AB)_4$, $(AB)_6$ *etc.*, *i.e.* $(AB)_{2n}$ species. The presence of $(AB)_3$, $(AB)_5$ and so on would prove that transesterification occurred. The $(AB)_n$-type oligomers have not been perspicuously identified on GPC, meaning direct proof of absence of transesterification cannot be realised. However, the oligomers $B(AB)_2$, $B(AB)_3$ and $B(AB)_4$ have been identified by GPC (following preparative GPC on a purely hydroxy-terminated reaction mixture), having elution times of approximately 13.8, 13.2 and 12.8 minutes respectively (Figure 1(c)). Hence one minute separates $B(AB)_2$ and $B(AB)_4$. $(AB)_2$ (**51**) and the next oligomer formed in the solvent-free $(AB)_2$ polymerisation have elution times of 14.3 and 13.4 minutes respectively, a difference of 0.9 minutes. By analogy to the hydroxy-terminated oligomers case, the latter signal is therefore most likely to be $(AB)_4$ and not $(AB)_3$.

$(AB)_2$ (**51**) therefore will acylate CALB, and consequently in Scheme 23 AB might be replaced by $(AB)_n$, where n = 1,2. Transesterification has again been shown not operate in the solvent-free process and this is not because equilibrium disfavours the formation of butane-1,4-diol (**4**) as might be argued for the diol / diacid polymerisation.

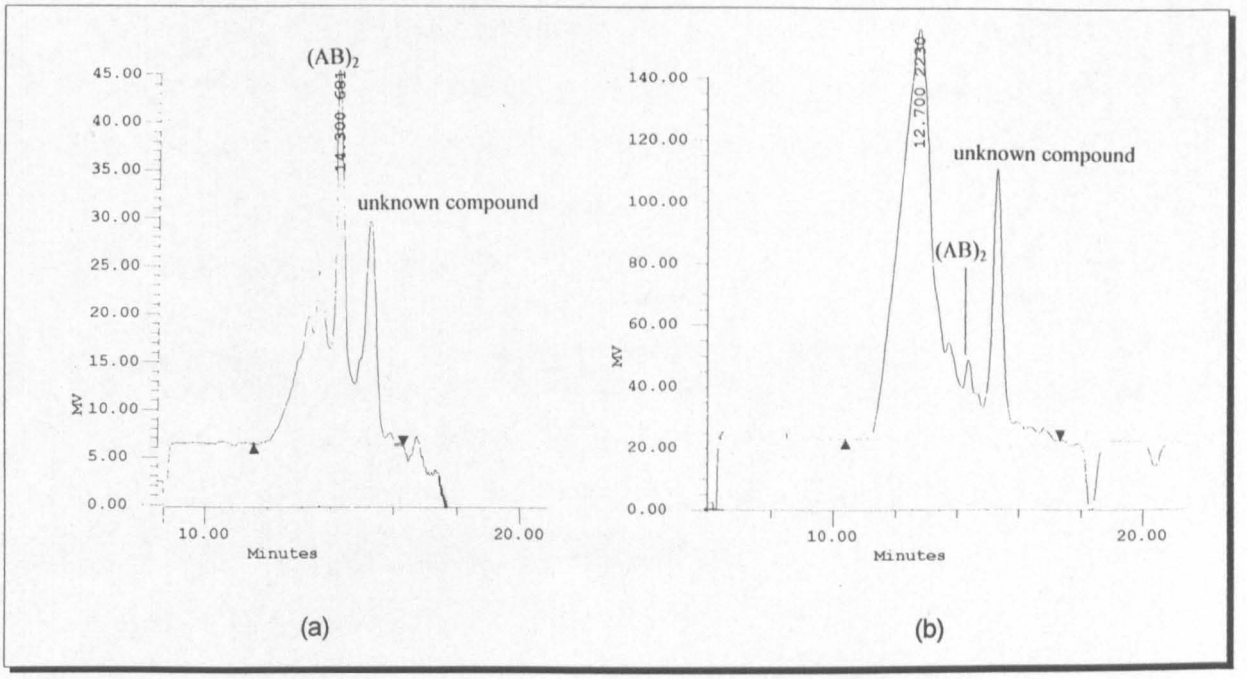


Figure 9

Polymerisation of $(AB)_2$ (**51**) in anhydrous toluene catalysed by *Novozyme 435*® also proceeded to give polymeric material (time course sampling results shown in Table 2). Again, the unknown contaminant appeared to have remained unused at the end of the polymerisation. In addition to formation of higher weight oligomers and polymer, a new product formed with elution time 14.7 minutes, supposed to be BAB (**15**). Transesterification of $(AB)_2$ (**51**) at the ester adjacent to the acid end of the molecule would be expected to yield BAB (**15**) and a new acid-ended species. Curiously, a much smaller signal was observed in the region of elution of butane-1,4-diol (**4**), the product of transesterification at the ester group adjacent to the alcohol end of $(AB)_2$ (**51**). Transesterification of BAB (**15**) in anhydrous toluene was found to occur readily to form significant amounts of diol; transesterification of $(AB)_2$ (**51**) appears to occur almost exclusively at the ester linkage adjacent to the acid end of the molecule, unless the diol is formed and consumed rapidly.

sample time / h	$\mathbf{M_w}$	$\mathbf{M_n}$	$\mathbf{M_w / M_n}$
1	1029	587	1.8
2	1471	675	2.2
17	2129	764	2.8

Table 2

The difference in substrate specificity of *Novozyme 435®* in aqueous diol medium and anhydrous toluene has again been corroborated by studies on $(AB)_2$ (**51**), although it is again not clear whether the effect is a result of solvent polarity change or water activity.

2.1.3.6 Polymerisation of $B(AB)_2$ (**58**)

The 6 mg of $B(AB)_2$ (**58**) from preparative GPC was polymerised in anhydrous toluene; as for the BAB (**15**) polymerisation, a modified Dean and Stark apparatus was deemed unnecessary. Stirring at 60 °C for 6 hours gave an oligomeric mix (M_n 774, M_w 1348, M_w / M_n 1.7) (Figure 10).

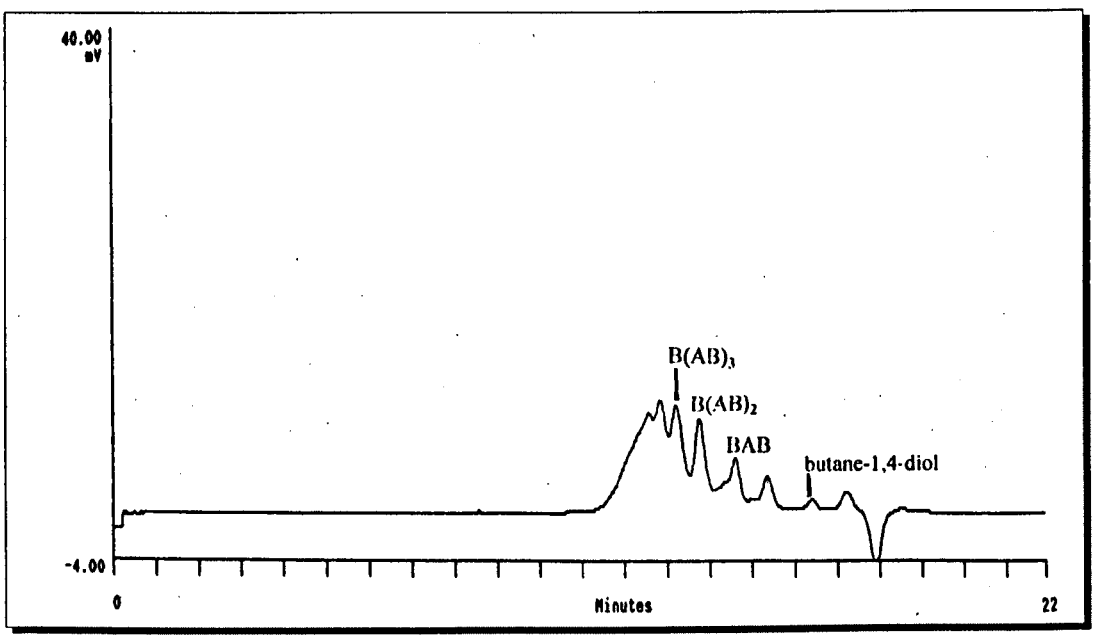


Figure 10

Discrete signals at elution times 12.8, 13.2 and 13.8 minutes corresponding to starting $B(AB)_2$ (**58**) and $B(AB)_3$ and $B(AB)_4$ can be discerned as well as higher weight material for which the individual signals coalesce. Material of lower weight was seen with elution times 14.7, 15.4 and 16.4. The first is due to BAB (**15**) and the latter to butane-1,4-diol (**4**), however the other does not correspond to a plausible linear product, and might be a cyclic ester formed from intramolecular transesterification of an intermediate.

$B(AB)_2$ (**58**) possesses two different ester environments, a "terminal" and "central" position, shown in Figure 11 by the symbols * and ** respectively.

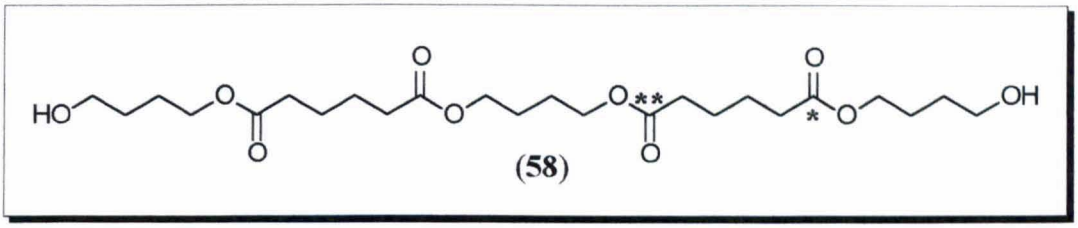
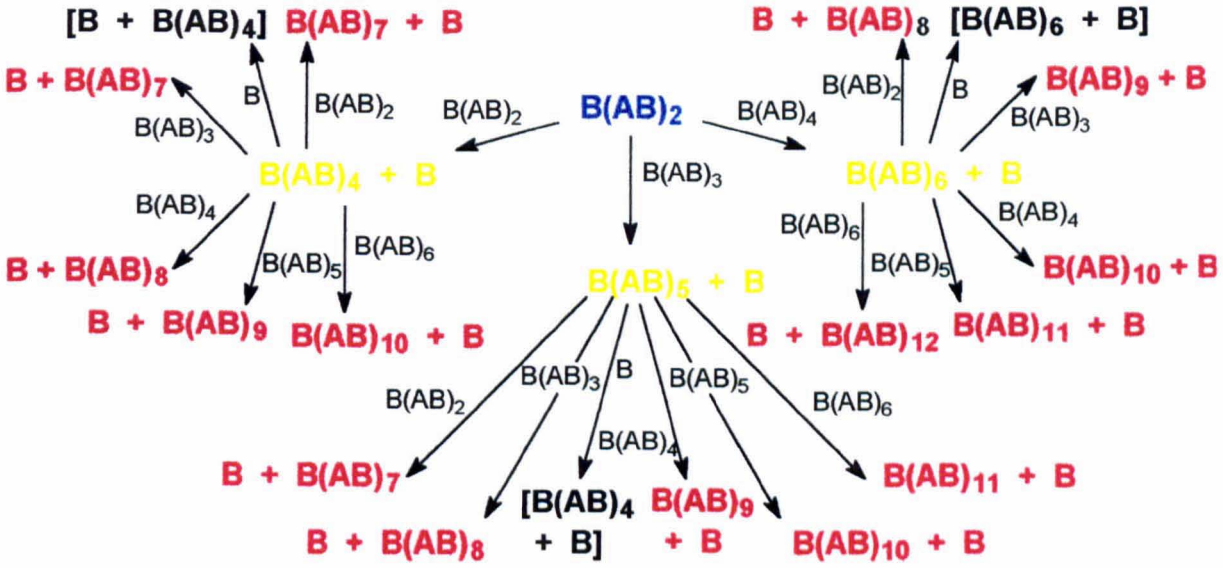


Figure 11

If transesterification occurs only at the terminal ester linkage of $B(AB)_2$ (**58**) and larger oligomers, a limited number of low weight oligomers can be generated. Scheme 24 shows the possible transformations which may occur in the early stages, starting with the oligomeric mix of mainly $B(AB)_2$ (**58**) with small amounts of $B(AB)_3$ and $B(AB)_4$. The first generation of products are shown in yellow, the second generation in red. Black products imply no net change occurs.



Scheme 24

The species formed from terminal transesterification are B (butane-1,4-diol (**4**)), $B(AB)_4$, $B(AB)_5$, $B(AB)_6$, $B(AB)_7$, $B(AB)_8$, $B(AB)_9$, $B(AB)_{10}$, $B(AB)_{11}$ and $B(AB)_{12}$ *etc.* $B(AB)_2$ (**58**) is never reformed by any steps and neither BAB (**15**) nor $B(AB)_3$ will be formed. In Figure 10, $B(AB)_3$ and BAB (**15**) have both clearly been formed, implying that central transesterification is also occurring. All steps in terminal transesterification involve the formation of butane-1,4-diol (**4**) which can then never be reconsumed, meaning a stockpiling of (**4**) should result; in fact, only traces of (**4**) are seen by GPC. Central transesterification of $B(AB)_2$ (**58**) and larger oligomers forms $B(AB)_n$ oligomers, where n = 1,2,3,4,*etc.* These

transformations may then incorporate butane-1,4-diol (**4**) forming other oligomers in the process, thus depleting (**4**). Butane-1,4-diol (**4**) is only seen in small quantities, but this may be due to its rapid consumption in central transesterification on formation or possibly because central transesterification dominates over that involving the terminal position. Unfortunately, the two issues cannot be separated.

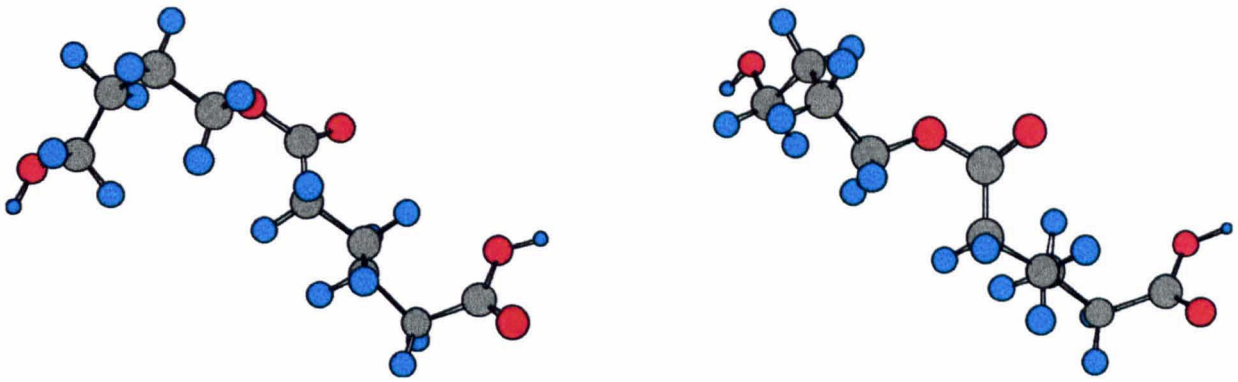
Many of the results shown in this section, along with explanations of relative reactivities towards CALB have recently been published by us.[101]

2.1.4 Studies on AB mimics

2.1.3.1 Introduction

Jones *et al.* have shown that a crystal structure of CALB possesses a narrow channel approximately 10 x 4 Å wide and 12 Å deep, which allows access of solvent to the active site.[66] The serine residue which is acylated during the lipase catalysis lies buried at the bottom of the tunnel. The solvent accessibility suggests that the lipase crystallised in its active "open" conformation. By use of the crude energy minimisation programs MM2 and CS MOPAC (molecular modelling programs in CS Chem3D Pro) the two conformations for AB (**14**) shown in Figure 12 were generated. MM2 gave Figure 12(a), CS MOPAC Figure 12(b). An approximate estimation of the dimensions of AB (**14**) from these structures suggests that (**14**) could be contained in a cylinder of length 11-12 Å and diameter 6 Å. AB (**14**) is therefore of similar dimensions to the lipase channel. The author appreciates the highly approximate nature of these structure conformation determinations.

As well as giving the dimensions of the active site tunnel, the crystal structure determination showed that the tunnel is lined with hydrophobic residues which are mainly aliphatic in nature,[66] and would give no potential hydrogen bonding sites for incoming substrates. The presence of the polar ester linkage and alcohol function in AB (**14**) might be responsible for the apparent lack of reactivity of this molecule towards *Novozyme 435®*. If, however, a hydrophilic residue were present at the outer region of the tunnel, the hydroxyl group of AB (**14**) might have a positive hydrogen bonding interaction *via* the free alcohol end which is likely to lie close to the outside of the tunnel.



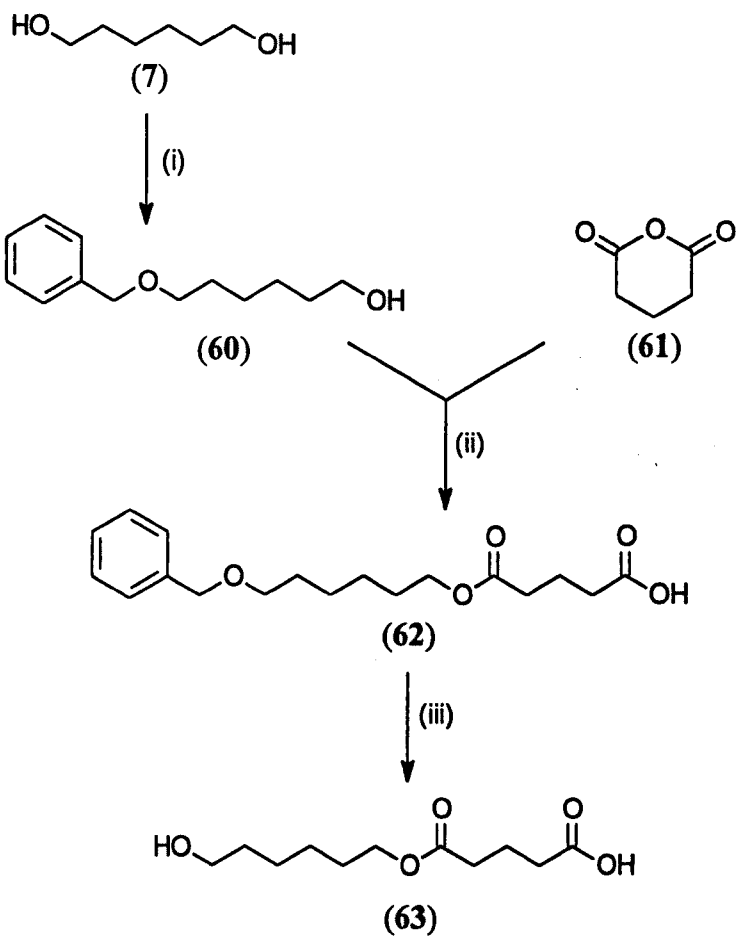
(a) (b)

Figure 12

By synthesising mimics of AB (**14**), with slight variations in chain length or position of the central ester linkage, a difference in the extent of polymerisation was possible. Whilst the AB species itself may lie completely in the active site tunnel on acylating CALB, $(AB)_2$ and higher oligomers will lie partially outside the active site entrance; ester groups along the chain length of these oligomers, or perhaps more likely, of the long chain hydroxyl terminated oligomers attacking the acyl-enzyme intermediate, may have hydrogen bonding interactions with polar residues adjacent to the active site entrance. Again, alteration of ester position might disrupt such interactions, leading to poorer recognition and slower polymerisation.

2.1.3.2 Synthesis of AB mimics

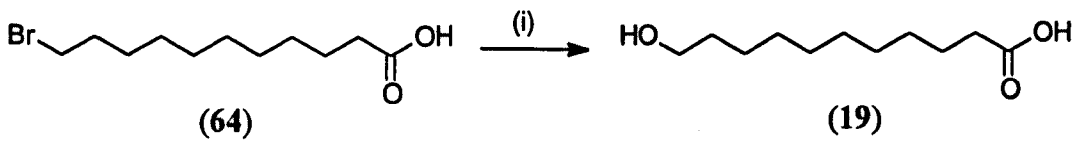
The hexane-1,6-diol / glutaric acid analogue of AB (**63**) was prepared using a modified version of the method used to form AB (**14**). Thus, hexane-1,6-diol (**7**) was monobenzylated, giving (**60**) in acceptable yield. Acid-catalysed ring-opening of glutaric anhydride (**61**) using (**60**) gave the protected AB mimic (**62**) in moderate yield, which, under catalytic hydrogenation conditions was cleanly deprotected in good yield to furnish AB mimic (**63**) (Scheme 25).



Reagents and conditions: (i) a. sodium hydroxide, DMSO, ambient temperature, 15 min; b. benzyl chloride, ambient temperature, 16 h, 42%; (ii) glutaric anhydride (**61**), *p*-toluenesulphonic acid, benzene, reflux, 15 h, 52%; (iii) H_2 / Pd on charcoal, ethyl acetate, ambient temperature, 24 h, 72%.

Scheme 25

The AB mimic 11-hydroxyundecanoic acid (**19**) was prepared using the literature procedure of Kinoshita *et al.*[102] The action of aqueous potassium hydroxide on 11-bromoundecanoic acid (**64**) gave the desired hydroxyacid (**19**) in excellent yield (Scheme 26).



(**64**) (**19**)

Reagents and conditions: (i) potassium hydroxide, water, reflux, 120 h, 95%.

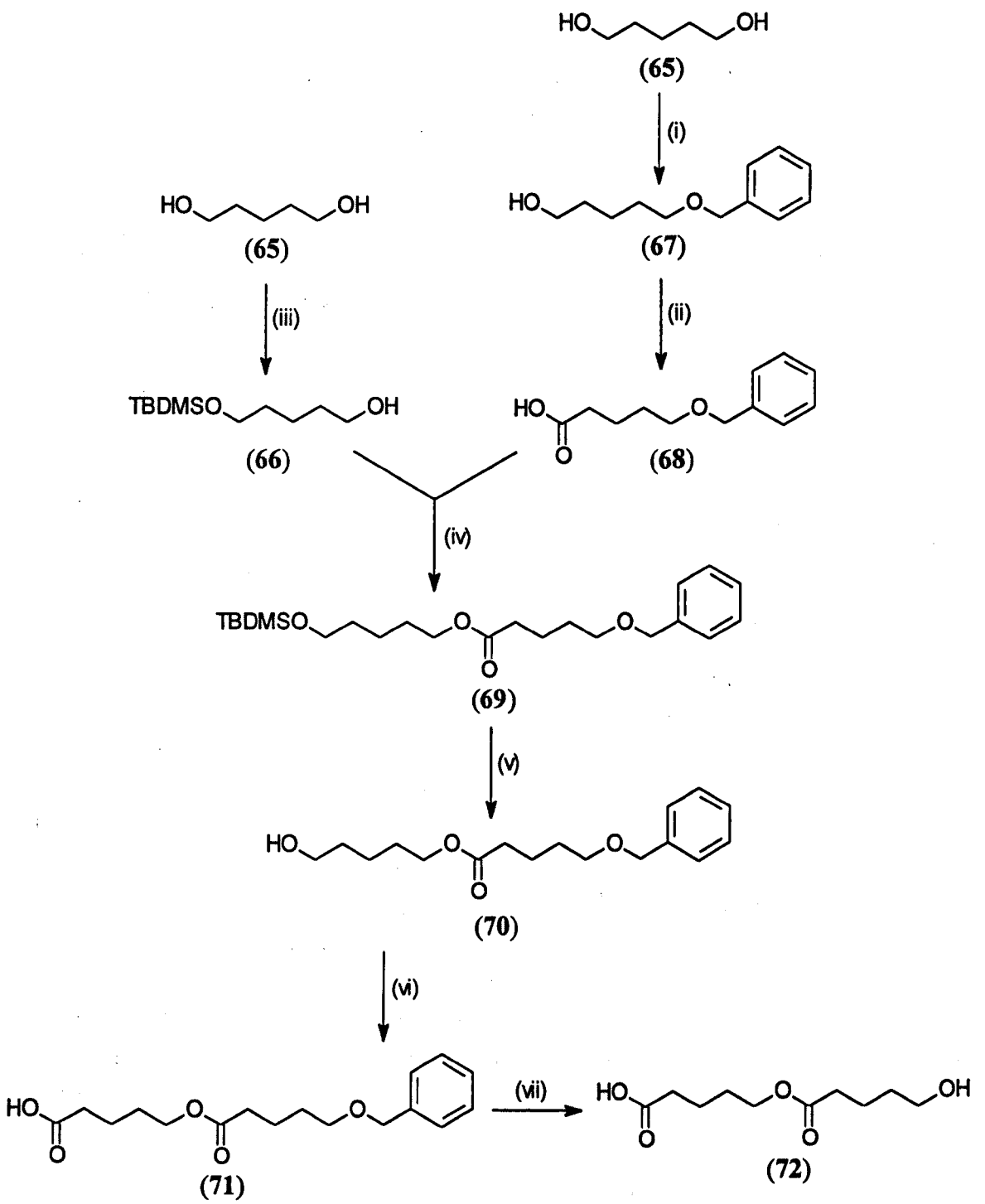
Scheme 26

A multistep synthesis was required to prepare the AB mimic 11-hydroxy-6-oxa-7-oxoundecanoic acid (**72**) which is identical to AB (**14**) but for the switching of the position of

the oxa and oxo groups in the ester linkage; compound (**72**) is thus the dimer of 5-hydroxypentanoic acid (**77**).

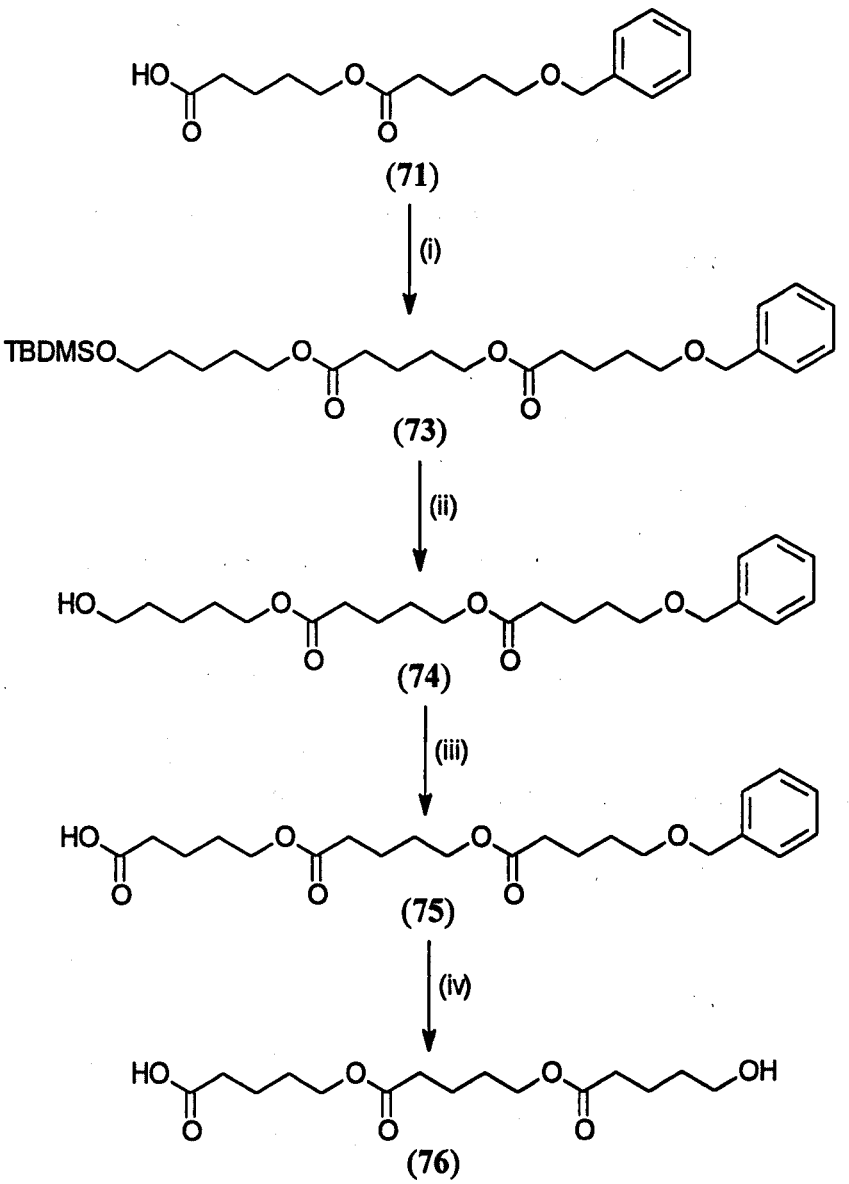
Protection of pentane-1,5-diol (**65**) as both the TBDMS and benzyl monoethers, (**66**) and (**67**) respectively, was accomplished using excess diol as in the synthesis of AB (**14**); good yields (based on the protecting agent) were achieved in each case. Oxidation of (**67**) using Jones reagent proceeded in good yield to furnish acid (**68**) which was successfully esterified with alcohol (**66**), giving (**69**) in excellent yield. Unmasking the alcohol group of (**69**) using TBAF was also high yielding to give the free alcohol (**70**). Oxidation of (**70**) to carboxylic acid (**71**), again with Jones reagent, was accomplished in acceptable yield. Finally, catalytic hydrogenation of (**71**) furnished the desired AB mimic 11-hydroxy-6-oxa-7-oxoundecanoic acid (**72**), albeit in low yield after dry flash chromatography (Scheme 27).

Synthesis of the trimer of 5-hydroxypentanoic acid, 17-hydroxy-6,12-dioxa-7,13-dioxoheptadecanoic acid (**76**) was achieved by chain extension of (**71**). A preparative scale synthesis was used, as the molecule was required for GPC authentication alone. Thus, DCC coupling of benzyl-protected AB mimic (**71**) with alcohol (**66**) gave diester (**73**) in good yield. Deprotection of the TBDMS ether gave alcohol (**74**) which was subsequently oxidised to acid (**75**) using Jones reagent and debenzylated using catalytic hydrogenation, furnishing trimer (**76**) in moderate yield (Scheme 28).



Reagents and conditions: (i) a. sodium hydroxide, DMSO, ambient temperature, 30 min; b. benzyl chloride, ambient temperature, 15 h, 73%; (ii) Jones reagent, acetone, 0 °C to ambient temperature, 45 min, 62%; (iii) TBDMSCl, triethylamine, DMAP, dichloromethane, 0 °C to ambient temperature, 16 h, 62%; (iv) DCC, DMAP, dichloromethane, ambient temperature, 20 h, 83%; (v) TBAF, THF, ambient temperature, 5 h, 80%; (vi) Jones reagent, acetone, 0 °C to ambient temperature, 1 h, 42%; (vii) H_2 / Pd on charcoal, ethyl acetate, ambient temperature, 60 h, 35%.

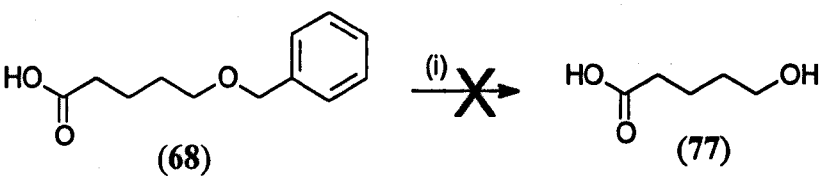
Scheme 27



Reagents and conditions: (i) (66), DCC, DMAP, dichloromethane, ambient temperature, 20 h, 71%; (ii) TBAF, THF, ambient temperature, 19 h, 77%; (iii) Jones reagent, acetone, 0 °C to ambient temperature, 30 min, 84%; (iv) H_2 / Pd on charcoal, ethyl acetate, ambient temperature, 25 h, 50%.

Scheme 28

Attempts to prepare the monomer of (76), 5-hydroxypentanoic acid (77), were unsuccessful. Catalytic hydrogenation of benzyl ether (68) gave a mixture of highly polar and non-polar components by TLC. Efforts to isolate and characterise these components, again using dry flash chromatography, gave only a non-polar compound, probably resulting from the lactonisation of (77) (Scheme 29).



Reagents and conditions: (i) H_2 / Pd on charcoal, ethyl acetate, ambient temperature, 40 h.

Scheme 29

2.1.3.3 Polymerisation of AB mimics

Polymerisation of the hexane-1,6-diol / glutaric acid AB mimic (**63**) was run in tandem with that of AB (**14**). The small amounts of (**63**) synthesised meant only a toluene-based polymerisation seemed viable. The anhydrous reaction (performed in a modified Dean and Stark apparatus) was sampled at regular intervals; the GPC results of this polymerisation compared to the tandem AB (**14**) polymerisation are shown in Table 3 (note AB (**14**) data is also seen in Table 1). The mimic (**63**) possesses an extra carbon atom in the chain compared to AB (**14**), which may have an impact on the position of the free hydroxyl end in relation to the hydrophobic active site entrance.

	AB mimic (63)			**AB (14)**		
sample time / h	**M_w**	**M_n**	**M_w / M_n**	**M_w**	**M_n**	**M_w / M_n**
1	1630	774	2.1	1304	644	2.0
3	2204	918	2.4	1917	944	2.0
7	2289	912	2.5	2189	1205	1.8
11	2319	963	2.4	2270	1342	1.7
22	3175	1658	1.9	3088	1471	2.1

Table 3

Polymer growth is rapid in the first hour for both substrates because of the large enzyme dose used. Both systems then show a slowing of the polymerisation, especially between 3 and 11 hours, where both M_w values increase by 100-200 Da. The mimic (**63**) slows more than AB itself (**14**), allowing the latter system to catch up in terms of its molecular

weight. However, the weights and polydispersities of the mimic system are significantly higher throughout, suggesting better enzyme recognition, possibly for the glutatic acid section in the active site or the hexane-1,6-diol section through interactions on the surface of the lipase. The reactions appear to slow over the 3-11 hour stages, suggesting on first inspection that equilibrium may have been reached, the molecular sieves having been saturated with water. However, a substantial amount of further polymerisation occurs in both systems over the second half of the reaction, up to 22 hours. At this point, the dispersities of the glutarate mimic system dropped to almost the same level as the AB system, the M_n value of the former being ~200 Da greater than the latter. Hence, some minor substrate recognition seems to have been operation. Work by Jarvie *et al.* using PPL as catalyst showed that this lipase also had a preference for polymerisation of glutarate esters over adipates.[39]

Compared to AB (**14**), 11-hydroxyundecanoic acid (**19**) has the same chain length, but lacks a central ester linkage, giving the latter a more hydrophobic character. Polymerisation of (**19**) was performed in toluene on the same scale as AB (**14**) and the AB mimic (**63**), but an oversight meant that no molecular sieves were used. The reaction proceeded to only M_n 533, M_w 1336, M_w / M_n 2.5; the likelihood is that the reaction came to equilibrium in the absence of sieves, explaining the comparatively low molecular weights obtained.

A solvent-free polymerisation of (**19**) was also performed on a similar scale to the neat AB (**14**) polymerisation; a variant was that the reaction had to be performed at 70 °C to ensure the molecule was in the molten phase. Novo Nordisk claim a 10% initial rate enhancement for CALB on raising the temperature from 60 to 70 °C,[103] which must be accounted for in the analysis of the results. After the same first stage, AB (**14**) had reached M_n 1008, M_w 1696, M_w / M_n 1.7, whilst the 11-hydroxyundecanoic acid (**19**) had formed polymer which on GPC analysis showed M_n 1437, M_w 2372, M_w / M_n 1.7 (Figure 13(a)). The substantial difference in molecular weight averages is unlikely to be explained by the modest temperature difference between the two systems, and is more likely to result from the presence of unfavourable interactions of the ester linkage of AB (**14**) with the hydrophobic active site tunnel in the (**14**) case. Interestingly, ^{1}H MNR spectroscopy on the (**19**) polymerisation product showed a CH_2OH:CH_2OCOR ratio of ~1:4, suggesting that the mean (M_n) molecular length corresponded to an $(AB)_5$-type oligomer, molecular weight 940 Da. Clearly, however,

comparison of the GPC data for AB (**14**) and (**19**) shows a significant rate enhancement for the latter system.

The lengthy synthesis required to prepare the AB mimic 11-hydroxy-6-oxa-7-oxoundecanoic acid (**72**) was deemed worthwhile, as further evidence for the absence of transesterification in highly polar media could be gained: as the molecule is a dimer of 5-hydroxypentanoic acid (77), transesterification at the ester linkage of (**72**) would lead to formation of (**77**). Unfortunately, preparation of the monomer (**77**) for GPC authentification was not possible, and the trimer (**76**) was used instead (Scheme 28), eluting at 14.8 minutes on GPC. Transesterification of the dimer (**72**) with another molecule of (**72**) would be expected to produce trimer (**76**) and monomer (**77**), which should be clearly visible on GPC. By analogy with AB (**14**), transesterification was expected to play only a minor rôle in polymer assembly.

In toluene, the AB mimic (**72**) proceeded to a predominantly polymeric mixture, with a high dispersity due to traces of oligomeric species remaining (M_w 2347, M_n 797, M_w / M_n 2.9). The elution time separation of the oligomers in the mixture is small enough to suggest that transesterification had occurred to a great extent.

The polyesterification reaction of 11-hydroxy-6-oxa-7-oxoundecanoic acid (**72**) in solvent-free conditions was sampled after 1, 2, 4, 8 and 22 hours, the standard temperature increase (40 to 60 °C) and partial vacuum (100 ± 5 mbar) being applied after 4 hours. The GPC sampling data are given in Table 4.

sample time / hours	M_w	M_n	M_w / M_n
1	387	307	1.3
2	503	335	1.5
4	608	374	1.6
8	1090	581	1.9
22	1565	887	1.8

Table 4

The GPC trace of the product of solvent-free polymerisation of 11-hydroxyundecanoic acid (**19**) is shown in Figure 13(a), displaying discrete low weight oligomers. As no central

ester linkage exists in (**19**), transesterification to form oligomers intermediate between the monomer, dimer, trimer *etc.* is not possible; hence, the oligomers eluting at 15.4, 14.3, 13.6 and 13.1 minutes correspond to monomer AB, dimer $(AB)_2$, trimer $(AB)_3$ and tetramer $(AB)_4$ respectively. If transesterification were not occurring in the solvent-free (**72**) polymerisation, signals at approximately the same elution times would be expected, corresponding to the starting material 11-hydroxy-6-oxa-7-oxoundecanoic acid (**72**) (dimer), tetramer, hexamer and octamer respectively, with no intervening signals corresponding to molecules with odd numbers of monomer (**77**) condensations. The trace for solvent-free (**72**) polymerisation after 22 hours is shown in Figure 13(b). A signal corresponding to that of the trimer (**76**) is seen, along with a signal located between the expected positions of the tetramer and hexamer. No trimer (**77**) or pentamer would be formed by simple esterification, implying that tranesterification must occur to a significant degree.

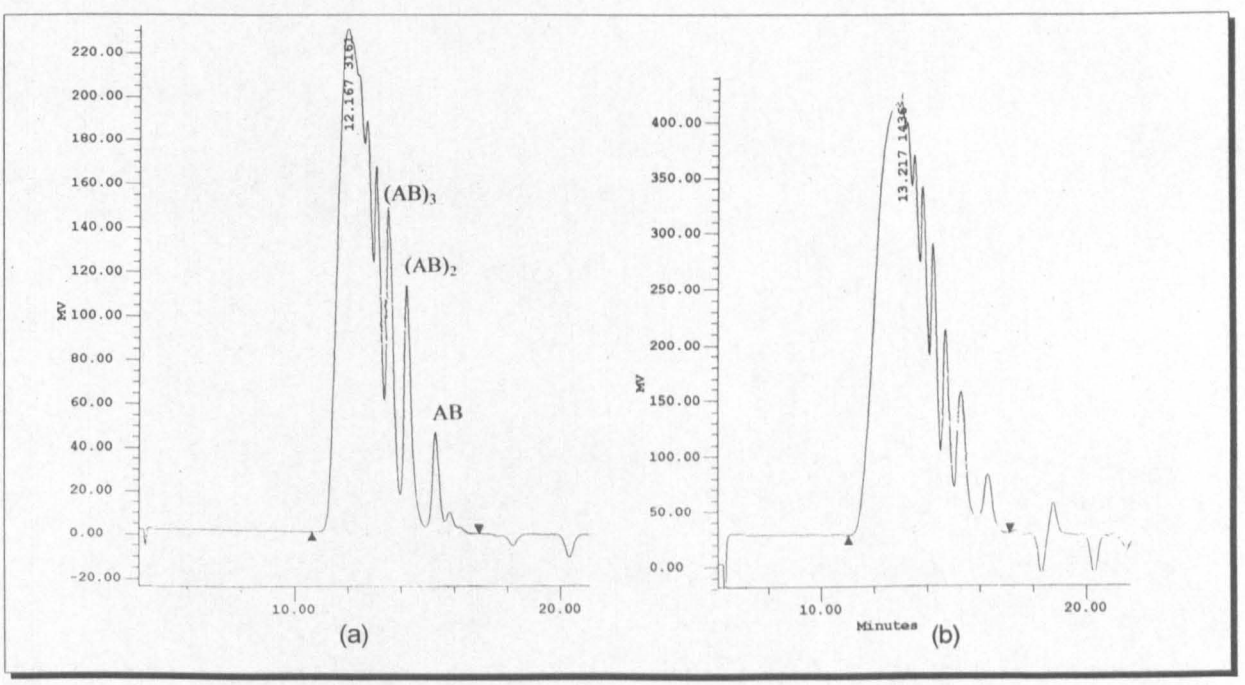
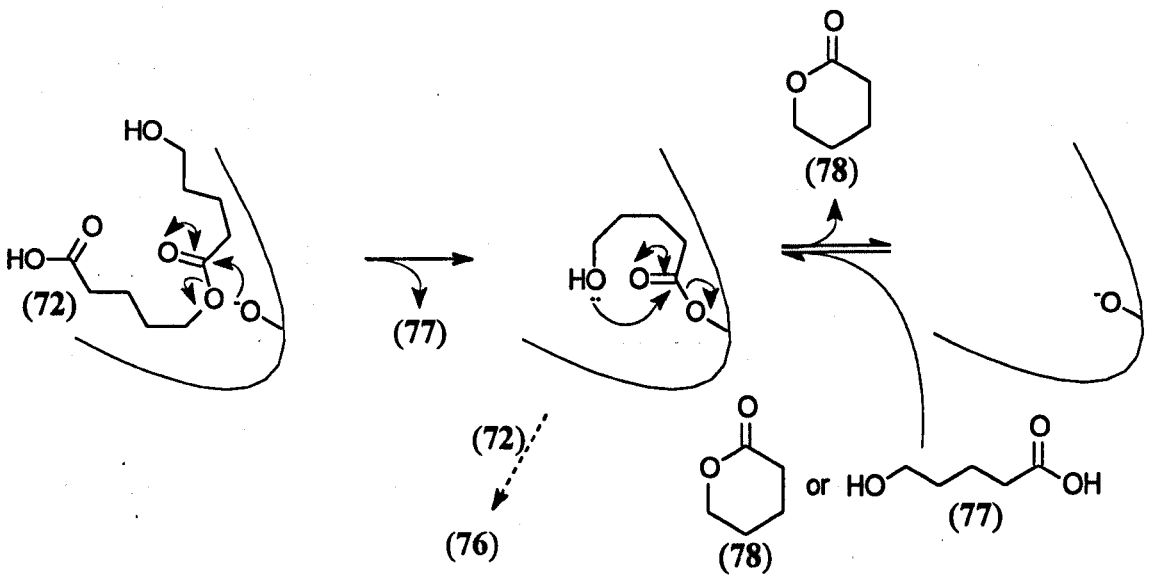


Figure 13

Inspection of the GPC trace after polymerisation of (**72**) over 1 hour shows a spread of oligomers with ~2 times more tetramer than trimer. If transesterification of the starting dimer (**72**) alone were possible, the products formed would be trimer (**76**) and monomer (**77**). Esterification of these species with (**72**) (the latter acting as the alcohol species) would give more trimer and pentamer, a similar situation to that shown by GPC. However, this would

mean that interaction of the ester linkage with the lipase was strongly favoured over acylation by the acid group. No evidence is available to confirm this postulate.

An alternative but related mechanism whereby trimer (**76**) and thence pentamer *etc.* could form involves δ-valerolactone (**78**) formation (Scheme 30). However, under the highly concentrated partially-aqueous conditions used, lactone formation would be expected to be minimal, intermolecular reactions being more likely than intramolecular. On the other hand, the unique environment of the lipase active site may hold the acyl-enzyme intermediate in a position where intramolecular attack may occur. There is no corroborating evidence for the mechanistic postulate detailed in Scheme 30.



Scheme 30

2.1.5 Other work exploring fundamentals of solvent-free polymerisation

2.1.4.1 MALDI-TOF mass spectrometry studies

MALDI-TOF mass spectrometry has become an important tool in determining the composition of polydisperse polymeric species in the past few years. Researchers have been quick, however, to use the technique in combination with the more usual NMR, GPC and titrimetric procedures. MALDI-TOF is a "soft" ionising technique, which uses laser light to bring about desorption-ionisation of the analyte molecules. In order to prevent fragmentation of the molecules, the analyte is diluted with a low weight solid or liquid matrix which absorbs much of the laser energy; the remaining energy is soft enough to ionise the molecules under

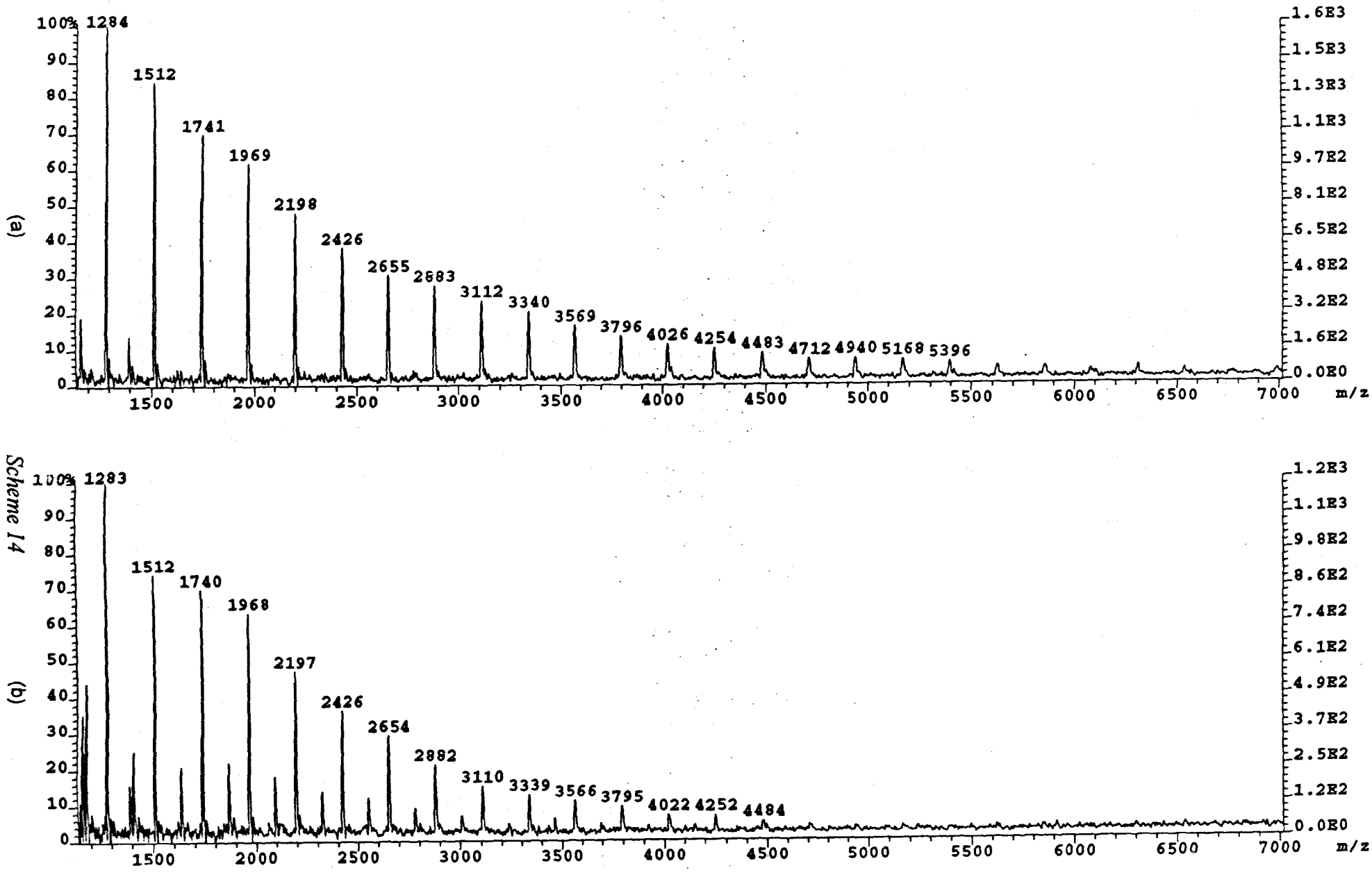
test with a minimum of fragmentation. Hence, polymeric and oligomeric species of weights >*ca.* 500 Da may be seen in the MALDI-TOF trace. Russell and co-workers used MALDI-TOF to analyse end-groups of enzymatically-produced polyesters, and demonstrated by correlation with titrimetric methods that no significant difference in desorption occurred between acid or hydroxyl-ended polyesters.[60,104] In this way, they verified that MALDI-TOF may be used quantitatively under such circumstances.

Comparison of the properties of polyadipates synthesised commercially by Baxenden Chemicals Ltd. with their enzymatically-produced counterparts was a key part of this study. MALDI-TOF was therefore used as a probe of the differences in polydispersity and end-groups in addition to the titrimetric and GPC methods used.

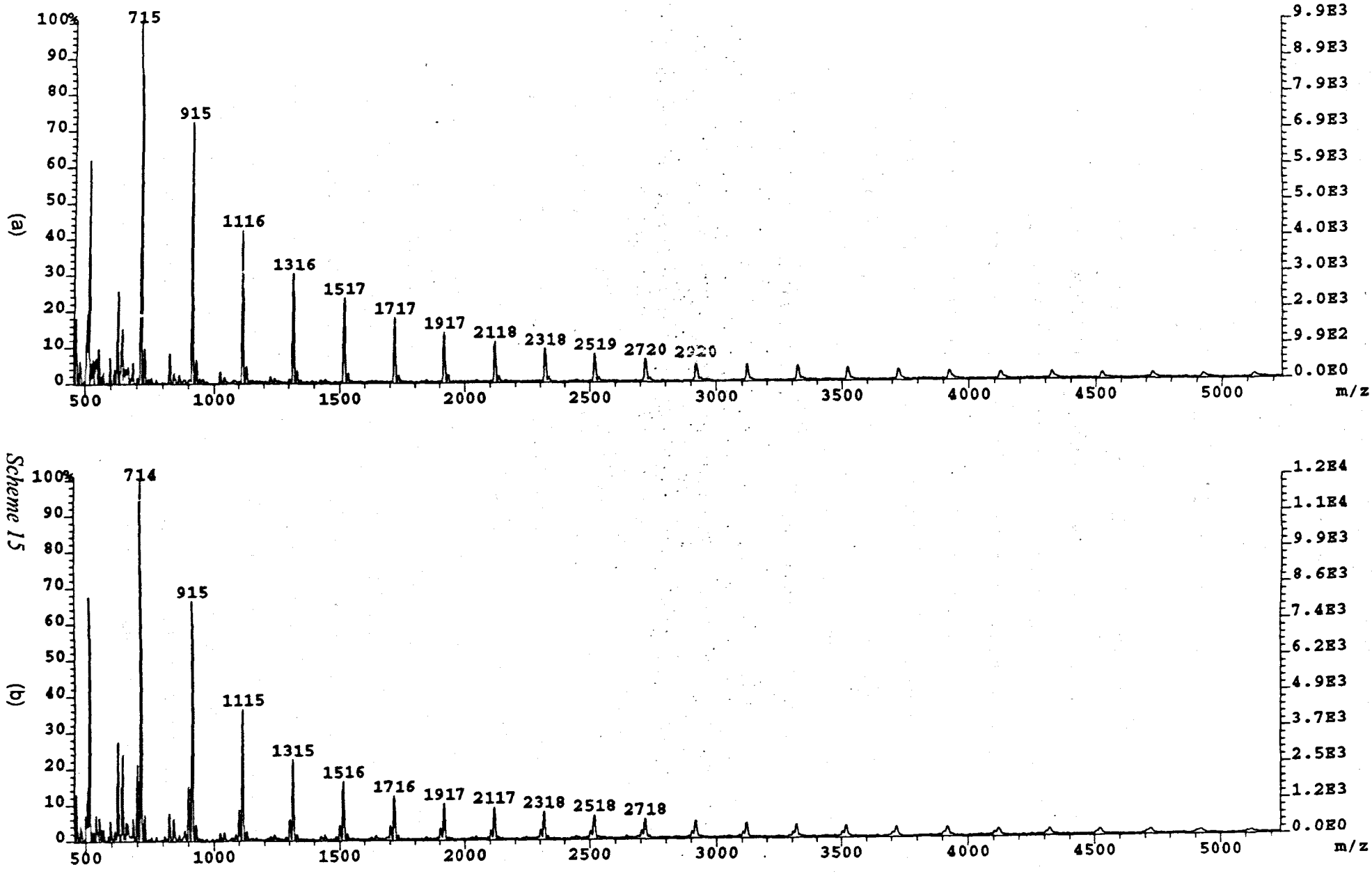
MALDI-TOF analysis on a sample of commercially-synthesised poly(hexane-1,6-diyl adipate) is shown in Figure 14(b); the enzymatically-produced counterpart (after both stages of solvent-free hexane-1,6-diol / adipic acid polymerisation) gives a spectrum as shown in Figure 14(a). Likewise, poly(butane-1,4-diyl adipate) samples produced by conventional Lewis acid-catalysed procedures (Figure 15(a)) and *via* enzymatic solvent-free polymerisation (Figure 15(b)) were also subjected to MALDI-TOF mass spectrometry.

The structures of the poly(butane-1,4-diyl adipates) appear to be very similar, both showing exclusively hydroxyl-terminated $[M + Na]^+$ ions; oligomers possessing an hydroxyl and an acid end would be seen between these *bis*-hydroxyl-ended species; they are conspicuously absent, certainly above 900 Da. No obvious difference in dispersity is seen either, both methods of synthesis showing only traces of polymeric species above 3000 Da, and showing maximum absorption for the oligomer at 715 Da ($B(AB)_3$).

Differences are seen with exquisite clarity on comparison of the two poly(hexane-1,6-diyl adipate) traces. Whilst the enzymatic product has a slightly broader spread of *bis*-hydroxyl-ended species than the conventional material, the latter contains a large number of acid-terminated species of molecular weight >1000 Da, seen as smaller intermediate $[M + Na]^+$ signals of lower intensity. By analogy to the work of Russell,[104] *ca.* one quarter of the middle weight oligomers are acid ended, a severe disadvantage if the polyester is to be reacted on to form polyurethanes. Titrimetric methods showed that the polyester from the enzymatic and conventional processes had similar numbers of acid ends, implying that the acid-ended species in the former type must be of low molecular weight only.


(a)
1284
1512
1741
1969
2198
2426
2655
2883
3112
3340
3569
3796
4026
4254
4483
4712
4940
5168
5396
m/z
(b)
1283
1512
1740
1968
2197
2426
2654
2882
3110
3339
3566
3795
4022
4252
4484
m/z

Scheme 14


(a)
715
915
1116
1316
1517
1717
1917
2118
2318
2519
2720
2920
m/z
(b)
714
915
1115
1315
1516
1716
1917
2117
2318
2518
2718
m/z

Scheme 15

The lack of acid-ended species in the enzymatic poly(hexane-1,6-diyl adipate) gives rise to unique properties, especially rapid crystallisation over a narrow temperature range,[105] an important property in hot-melt adhesive applications.

2.1.4.2 Studies on interchange between 1st and 2nd stages of solvent-free process

Experiments have shown that CALB is substantially deactivated by the end of the first stage of the butane-1,4-diol / adipic acid process (see Section 2.2 for detailed discussion). Addition of water at the start of the process to ensure high lipase activity, along with formation of water *via* esterification during the first four hours of the process leads to a cessation of the reaction after *ca.* two hours; our belief is that the reaction reaches equilibrium. A partial vacuum (100 ± 5 mbar) is then applied and the oil bath temperature increased from 40 to 60 °C. Use of a pressure-temperature nomograph gives a boiling point for water at this pressure of 50 °C. Hence, 100 mbar is adequate to remove much of the water present, and indeed much bubbling of the reaction mixture is seen on application of the vacuum. However, Binns[62] has shown that after 17 hours at this pressure, the reaction paradoxically contains approximately 8wt.% water. The inference from this result is that because of surface area effects, the water removal does not keep pace with the esterification rate. The majority of polymer assembly takes place over the 17 hours, and the reaction rate is likely to be rapid over this period. On filtering off the lipase and application of a higher vacuum, the polymer dispersity actually broadens, although final traces of low weight oligomers are used up in this 2nd stage. The reaction continues to be catalysed over the second stage by small amounts of free lipase which have leached from the immobilisation support (see Section 2.2 for validation). However, the reaction rate appears to reduce after the 1st stage, the recovered enzyme usually possessing about 35% activity compared to unused *Novozyme 435®*. As the lipase is still active to a degree, this reduction in rate is probably due to the reaction again reaching equilibrium. Alternatively, a possible activation of the lipase present in solution may occur when the higher vacuum is applied, then facilitating continuation of polymer assembly.

A standard solvent-free polymerisation of butane-1,4-diol (**4**) and adipic acid (**3**) was performed over the two stages, sampling at the end of the first (low-vacuum) stage, then after 8 hours at atmospheric pressure and then after the higher vacuum stage (Table 5, entries 1, 2 and 4 respectively). The covalently immobilised CALB *Chirazyme®* was employed, so as to

avoid the complication of leaching effects (see Section 2.2.2.3), but was left in the mixture during the second stage to act as catalyst. The expected GPC trace of oligomers was seen after the first stage, a slight increase in molecular weight average being observed on stirring at atmospheric pressure for 8 hours, implying that the enzyme was still active and possibly that the reaction was near equilibrium. Half an hour after application of the higher vacuum, a jump in M_w of ~100 Da is seen (entry 3). Sampling after 17 hours revealed a growth in the amount of polymeric material (entry 4), although the weight achieved was lower than normally expected. Half an hour after addition of 8wt.% water and stirring at atmospheric pressure, the molecular weight averages had dropped significantly as hydrolysis of the oligomers took place (entry 5). No further hydrolysis appeared to take place, the reaction being sampled several times over 24 hours, an actual increase in weight being seen after 24 hours (entry 6). Reapplication of the 2nd stage vacuum conditions gave a slow progressive increase in molecular weight after 0.5, 2, 4 and 24 hours (entries 7-10).

sample no.	process duration / h	step change	M_w	M_n	M_w / M_n
1	21	oligomerisation and 1st vacuum stage	601	311	1.9
2	29	60 °C atmospheric pressure stage	695	349	2.0
3	29.5	2nd vacuum stage (0.5 h after vacuum application)	780	406	1.9
4	46	2nd vacuum stage (at end of vacuum stage)	1041	523	2.0
5	46.5	0.5 h after water dose, at atmospheric pressure	885	377	2.3
6	70	24 h after water dose, at atmospheric pressure	990	456	2.2
7	70.5	0.5 h after reapplication of 2nd stage vacuum conditions	919	444	2.1
8	72	2 h after reapplication of 2nd stage vacuum conditions	950	484	2.0
9	74	4 h after reapplication of 2nd stage vacuum conditions	963	490	2.0
10	94	24 h after reapplication of 2nd stage vacuum conditions	1024	502	2.0

Table 5

A tandem reaction was conducted in which *Chirazyme®* was filtered off after the 2nd stage. With no leached enzyme no hydrolysis of the oligomers was expected on addition of

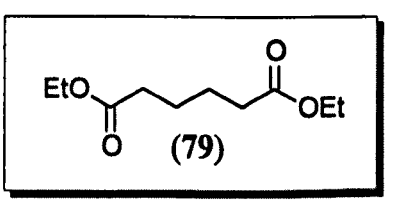
water; this was indeed the case, confirming the fully covalently-bound nature of CALB in *Chirazyme®*.

These experiments suggest that the reaction does indeed reach equilibrium after the first stage, although the fact that a substantial amount of polyester was not formed is a source of concern. The experiment is worthy of repeating on a much larger and more reproducible scale.

Another potential method of determining whether the enzyme specificity changes on entering the high vacuum 2nd stage was to add a low weight oligomer or monomer to the mixture after the 2nd stage. Hence, addition of adipic acid (**3**) to a mixture of poly(butane-1,4-diyl adipate) (M_w 856, M_n 449, M_w / M_n 1.9) containing immobilised *Novozyme 435®* which had been used throughout the process gave no significant increase in molecular weight (M_w 887, M_n 464, M_w / M_n 1.9) after stirring for 17 hours under 2nd stage conditions, suggesting that the lipase could not link chains together using adipic acid (**3**). Roberts *et al.*[29] had similar findings using *Lipozyme® IM-20* as catalyst, hydrolysis of the polyester giving low weight diol oligomers which then condensed to a small extent with adipic acid (**3**).

2.1.4.3 Polymerisation of diethyl adipate (79) with butane-1,4-diol (4)

Polymerisation of the diester (**79**) with butane-1,4-diol (**4**) under solvent-free conditions was performed using *Novozyme 435®* as the catalyst. Clearly, for the polymerisation to proceed, transesterification of the diester (**79**) would have to occur, although solvent-free conditions had precluded the transesterification of BAB (**15**); the latter is effectively diester (**79**) but with 4-hydroxybutyl groups in place of the ethyl unit. In the presence of butane-1,4-diol (**4**), the system was expected to mimic the standard adipic acid / butane-1,4-diol process in terms of the polarity of the medium.



(79)

In a standard run, after the 1st stage, GPC showed that polymerisation had occurred, against expectations (M_w 834, M_n 426, M_w / M_n 2.0). The result suggests that some key element of the diacid / diol system is absent, enabling the lipase to move into

transesterification mode. Possibly, the acidic aqueous low pH conditions of the diacid / diol system are vital to affect the conformation of the lipase and so prevent transesterification. Alternatively, the steric incumberance of the ethyl group may not be large enough to prevent transesterification occurring. Once the reaction starts, the by-produced ethanol would be removed much more rapidly than water from the acid / diol system due to its higher volatility. The equilibrium would thus be driven rapidily towards further transesterification.

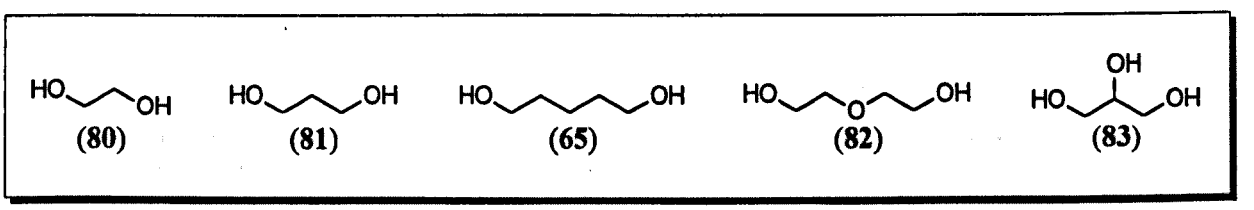
2.2 PROCESS STUDIES

2.2.1 Introduction

Several issues remained unresolved after the studies detailed in Section 2.1 on the Baxenden solvent-free polyesterification process, and these will be dealt with in Section 2.2. Of the phenomena found at Baxenden Chemicals, the key ones requiring theoretical resolution were as follows:

- Failure of *Novozyme 435®* to readily catalyse the formation of polyester from adipic acid with glycerol (**83**) or diethylene glycol (**82**)
- Different extents of deactivation of *Novozyme 435®* by butane-1,4-diol (**4**) and hexane-1,6-diol (**7**) / adipic acid (**3**) systems
- Prevention of deactivation of *Novozyme 435®* in adipic acid (**3**) / butane-1,4-diol (**4**) system by doping with triethylamine and other additives

These areas of research will be dealt with separately in this section.



2.2.2 Polyol variation experiments

2.2.2.1 Background

Work performed at Baxenden showed that *Novozyme 435®* could be filtered off after the first (low-vacuum) stage for both the hexane-1,6-diol (**7**) and butane-1,4-diol (**4**) / adipic acid (**3**) polyesterification processes. The second stage of the process may then be catalysed by small amounts of free leached lipase dissolved in the polymer. The viscous liquid polymer is filtered through a nylon mesh at 60 °C, thus collecting the immobilised lipase which is then washed with hot acetone until all traces of polyester have been removed. Anthonsen has shown that acetone does not alter the conformation of CALB, and hence the washing should have no deleterious effect on CALB activity,[81] a fact subsequently confirmed by Binns.[62]

Finally the lipase is dried under high vacuum at ambient temperature. Curiously, the lipase recovered from the butane-1,4-diol (**4**) system is generally found to be *ca.* 65% deactivated whereas that from the hexane-1,6-diol (**7**) system (which is run at higher temperatures due to the higher melting point of (**7**)) loses only 10% of its activity. Whilst both diols are highly polar molecules, butane-1,4-diol (**4**) would be expected to have the higher polarity because of the shorter, less lipophilic carbon chain. Hence, high substrate (solvent) polarity was deemed a possible cause of the deactivation; alternatively the deactivation may stem from the higher water contamination of diol (**4**) compared to (**7**) (again resulting from the more hydrophilic nature of (**4**)).

Efforts to polymerise glycerol (**83**) and diethylene glycol (**82**) with adipic acid (**3**) at Baxenden had led to moderate success. Glycerol gave only very low weight oligomeric species, whilst diethylene glycol, although forming some high molecular weight material, had been shown to deactivate the lipase substantially. Again, an effect due to polyol polarity / hydrophilicity seemed plausible and represented a phenomenon worthy of investigation.

2.2.2.2 Small scale polyol trials

polyol substrate	M_w	M_n	M_w / M_n	**comments**
ethylene glycol (**80**)	200	164	1.2	no reaction
propane-1,3-diol (**81**)	582	324	1.8	extensive oligomer formation
butane-1,4-diol (**4**)	627	338	1.9	extensive oligomer formation
pentane-1,5-diol (**65**)	929	543	1.7	extensive polymer formation
hexane-1,6-diol (**7**)	642	311	2.1	extensive oligomer formation†
diethylene glycol (**82**)	610	299	2.0	extensive oligomer formation
glycerol (**83**)	282	190	1.5	some oligomer formation

†Artificially low molecular weight averages, due to insufficient mixing of (**7**) and adipic acid (**3**) at 40 °C

Table 6

In an effort to probe the effect of varying polyol structure on enzyme activity, small scale solvent-free polymerisations of the homologous series of diols, from ethylene glycol (**82**) through to hexane-1,6-diol (**7**), with adipic acid (**3**) using *Novozyme 435®* as catalyst were performed. All reactions were run under the standard butane-1,4-diol / adipic acid system conditions, over the first low-vacuum stage only. In addition, trials of diethylene glycol (**82**) and glycerol (**83**) with adipic acid were performed (Table 6).

A trend reflecting an increasing extent of polymerisation with increase in diol hydrophobicity is evident (hexane-1,6-diol (**7**) shows a lower than expected molecular weight because of solidification of the reaction mixture in the early stages at 40 °C), suggesting a correlation of increased lipase deactivation with increasing diol polarity does exist. Diethylene glycol (**82**) has a polarity similar to butane-1,4-diol (**4**) and the two systems gave products with similar molecular weights. Ethylene glycol (**80**) showed no formation of oligomeric species at all, suggesting that either it is not a substrate for CALB or that its high polarity leads to almost instantaneous deactivation of the lipase. The results for the glycerol (**83**) polymerisation confirmed those found at Baxenden, little oligomer formation having been observed. Glycerol would be expected to be one of the most polar of all the diols, the apparent lack of reactivity suggesting prompt deactivation of the lipase by this polyol.

system from which lipase was recovered	M_w	M_n	M_w / M_n	**comments**
virgin enzyme (∴n/a)	1447	507	2.9	extensive polymer formation
ethylene glycol (**80**)	171	151	1.1	no reaction
propane-1,3-diol (**81**)	448	256	1.7	extensive oligomer formation
butane-1,4-diol (**4**)	950	443	2.1	extensive oligomer formation
pentane-1,5-diol (**65**)	964	551	1.7	extensive polymer formation
diethylene glycol (**82**)	186	160	1.2	slight oligomer formation

Table 7

To understand whether the differing extents of reaction seen in Table 6 truly resulted from variation of lipase activity in the different systems, a series of recycling trials was performed. Filtration and washing of the lipase from the polymerisation of the homologous

series of polyols (**80**) to (**65**) and also (**82**) was performed as described previously, to give the recovered immobilised lipase. By running these samples through a second set of trials (using the butane-1,4-diol (**4**) / adipic acid (**3**) system), a measure of the relative activity of the recovered lipases was possible. A control reaction was run in tandem on the same scale, using "virgin" *Novozyme 435®* to give an idea of how much deactivation the lipases from the various systems had suffered.

Table 7 shows that the lipase recovered from the ethylene glycol (**80**) and diethylene glycol (**82**) systems was almost totally inactive, only trace amounts of oligomers from butane-1,4-diol (**4**) and adipic acid (**3**) being observed; however, lipase recovered from the butane-1,4-diol (**3**) and pentane-1,5-diol (**65**) systems was still highly active, reaching M_w values in excess of 900 Da. Enzyme which had not been used previously (virgin enzyme) gave a largely polymeric mixture with M_w 1447.

These results confirm the apparent trend of falling lipase activity with increasing polarity of the medium. As well as solvating greater quantities of water, the more polar polyols would also be expected to dissolve more adipic acid (**3**). The increase in acid concentration in the semi-aqueous reaction medium could lead to lowering of the pH and consequently greater levels of deactivation. Taylor has recently discovered that variations in pH of the medium in the solvent-free system can have dramatic effects on the activity of CALB, adding credence to such a possibility.[106]

Polymerisation of ethylene glycol (**80**) with adipic acid (**3**) in anhydrous toluene was attempted in the presence of *Novozyme 435®*. In this case the medium would be substantially less polar than the solvent-free medium and hence, if polymerisation occurred to an appreciable extent, the nature of the medium rather than an intrinsic lack of compatability of (**80**) with CALB could be confirmed as culpable. After stirring at 60 °C for 22 hours, an extensively oligomerised material was isolated (M_w 855, M_n 458, M_w / M_n 1.9), confirming that diol (**80**) can act as a substrate for CALB and that rapid deactivation of the lipase was the reason for lack of oligomerisation in the solvent-free method. A difference in lipase conformation in the anhydrous toluene *versus* solvent-free conditions would not explain the lack of reactivity of ethylene glycol (**80**) in the latter system: clearly, the larger polyols are not precluded entry to the active site so the smaller ethylene glycol (**80**) moiety is unlikely to be barred by any conformational change.

A standard butane-1,4-diol (**4**) / adipic acid (**3**) polymerisation was performed in tandem with an equivalent reaction containing 20mol% ethylene glycol (**80**) and another containing an additional 50mol% of (**80**). GPC data for the reactions are shown in Table 8. Clearly, the presence of just 20mol% ethylene glycol (**80**) is enough to slow the reaction dramatically. On dosing 50mol% (**80**), reaction is negligible. No enzyme recycling studies were undertaken in these systems, but the assumption that deactivation by (**80**) was the root cause of the low molecular weights achieved seems valid. Again, these results point towards a polarity change being the key cause of deactivation.

system	M_w	M_n	M_w / M_n	comments
butane-1,4-diol (**4**) / adipic acid (**3**)	1587	653	2.4	extensive polymer formation
butane-1,4-diol (**4**) / adipic acid (**3**) / 20mol% ethylene glycol (**80**)	627	338	1.9	extensive oligomer formation
butane-1,4-diol (**4**) / adipic acid (**3**) / 50mol% ethylene glycol (**80**)	250	194	1.3	slight oligomer formation

Table 8

2.2.2.3 Large scale polyol trials / enzyme recyclability trials

The work performed in Section 2.2.2.2 used a method of lipase recycling where the recovered immobilised enzyme was added to a second polymerisation process ("process recycling method"). An alternative method of enzyme activity measurement involves a kinetic assay. This method uses an esterification reaction, that of oleic acid with hexan-1-ol. By measurement of the rate of loss of acid with time for virgin and recycled lipase (using a titrimetric method), linear plots may be obtained. The ratio of the gradient of the line for recycled enzyme to that for virgin enzyme gives the relative activity of the recovered enzyme.[107]

The two methods have advantages and disadvantages associated with them. The process recycling method uses a similar medium to that from which the recovered enzyme has been removed, whereas the kinetic assay method has a very different medium; hence the latter case may not give an activity exactly representative of the lipase behaviour in the polar polyol

media. Conversely, by removing *Novozyme 435®* from one polyol environment and adding it to a different polyol reaction mixture (as in process recycle), the medium is again changing. However, a combination of the two techniques should give reliable indications of the true enzyme activity.

polyol substrate	**sample time / h**	**M_w**	**M_n**	**M_w / M_n**	**relative activity of lipase at end of process / %**
ethylene glycol **(80)**	2	321	199	1.6	-
"	4	292	203	1.4	-
"	21	194	146	1.3	6
propane-1,3-diol **(81)**	2	389	205	1.9	-
"	4	319	172	1.9	-
"	21	536	300	1.8	8
butane-1,4-diol **(4)**	2	159	122	1.3	-
"	4	196	134	1.5	-
"	21	606	314	1.9	26
pentane-1,5-diol **(65)**	2	387	227	1.7	-
"	4	318	198	1.6	-
"	21	846	442	1.9	103†
hexane-1,6-diol **(7)**	2	206	172	1.2	-
"	4	436	229	1.9	-
"	21	784	326	2.4	88
diethylene glycol **(82)**	2	330	169	1.9	-
"	4	331	180	1.8	-
"	21	416	229	1.8	30

†within experimental error - assume recovered lipase fully active

Table 9

By repeating the first six reactions shown in Table 6 on a larger scale, adequate amounts of *Novozyme 435®* were recovered for kinetic assay activity measurements. Sampling of the reactions for GPC was also performed two and four hours after lipase dosing and after a further 17 hours under 1st stage vacuum conditions, in order to build up a picture of how rapidly polymer assembly occurs and possibly at what point deactivation of the enzyme begins. The GPC data from these reactions is given in Table 9. Also shown is the activity of

Novozyme 435® recovered at the end of the processes (after 21 hours), determined using the kinetic assay method. The hexane-1,6-diol (**7**) / adipic acid (**3**) reaction was performed at 60 °C throughout, to ensure that the mixture was molten at all times.

Comparison of the molecular weight averages for each polyol system after 21 hours shows the expected trend of increasing molecular weight average with increasing chain length, although a drop is noted on moving from pentane-1,5-diol (**65**) to hexane-1,6-diol (**7**). Diethylene glycol (**82**) shows quite low molecular weight averages in line with those of the propane-1,3-diol (**81**) system.

Correlation of lipase activity with diol polarity is seen by comparison of the trend with literature polarity measurements. Whilst log *P* values (representing the partition coefficient of the molecule between octan-1-ol and water) are often used, a useful set of empirical polarity parameters (E_T values) was published 20 years ago by Reichardt,[108] and may be a good guide in the present work. The data are based upon effects that various solvents have upon solvent-dependent equilibrium constants, rate constants and absorption maxima employing a range of spectroscopic techniques. All of the polyols tested within the present work have very high polarities, data tables placing them far above DMSO in polarity; the latter is known to deactivate enzymes by solubilisation of the protein.[33] The general trend glycerol (**83**) > ethylene glycol (**80**) > propane-1,3-diol (**81**) > diethylene glycol (**82**) ≈ ethanol:water (80:20 vol.%) is seen in Reichardt's work. None of the other polyols tested here are listed.

Ethylene glycol (**80**) and propane-1,3-diol (**81**) are seen to nearly deactivate *Novozyme 435*® entirely, whilst diethylene glycol (**82**) and butane-1,4-diol (**4**) leave the lipase with *ca.* 30% of its original activity. A maximum activity is reached (*ca.* 100%) for the pentane-1,5-diol system and drops again for hexane-1,6-diol (**7**) (correlating with the final molecular weight averages trend), although process temperature differences for the latter system may account for the slight difference. The assumption that longer chain aliphatic primary diols than (**7**) would leave the lipase 90-100% active appears reasonable.

Another point of great interest in Table 9 is that the reactions appear by GPC to stick after 2 hours, indeed the molecular weight averages often drop again between 2 and 4 hour sampling times. This phenomenon suggests that the reactions rapidly reach equilibrium, as in the first 4 hours of the process water removal is not carried out. On application of the 1st stage vacuum (100 ± 5 mbar), water is removed and the process of ester formation is again driven through displacement of the equilibrium position. Some studies on this area have already been

described in Section 2.1.4.2. An experiment was performed to determine the lipase activity in the early stages of the polymerisation. Butane-1,4-diol (**4**) / adipic acid (**3**) polymerisations catalysed by *Novozyme 435*® were performed, and the immobilised lipase filtered off after 1 and 2 hours and washed and dried in the usual way. The extent of polymerisation at these times was monitored (entries 1 and 2, Table 10). Process recycles in the butane-1,4-diol (**4**) / adipic acid (**3**) system were then performed using the two batches of recovered lipase, taking the reactions over the first stage of the process (entries 3 and 4, Table 10). The extents of polymerisation for the initial runs and for the recyle runs are shown in Table 10.

entry no.	process run number	recycled enzyme used	sample time / h	M_w	M_n	M_w / M_n
1	1	n/a	1	606	322	1.9
2	1	n/a	2	685	370	1.9
3	2	from entry 1	21	288	204	1.4
4	2	from entry 2	21	400	233	1.7

Table 10

Comparison of entries 1 and 2 shows again the characteristic slowing of the reaction in the early stages, little molecular weight gain being observed in the 1 to 2 hour interval. Use of the lipase removed at these times over the first stage of the second process led to only oligomer formation, with molecular weights much lower than normally expected, suggesting that the lipase was highly deactivated during the first 2 hours of the process. Application of the vacuum usually reactivates the lipase, but if removed from the deactivating environment and replaced in a "reactivating" process, the deactivation appears irreversible. Halling has also observed a sharp drop in lipase activity with increasing water content in polar solvents,[109] which is suggested to be related to a closing of the lid structure; although the lid in CALB is largely undeveloped, this same phenomenon may be the cause of the reversible deactivation seen here.

2.2.2.4 Separation of enzyme leaching *versus* deactivation issues

The strongly deactivated nature of the immobilised lipase in the early stages of the butane-1,4-diol (**4**) / adipic acid (**3**) system led to a questioning of the nature of the active catalytic species in the early stages of the reaction. In both the butane-1,4-diol (**4**) and hexane-1,6-diol (**7**) processes, the immobilised lipase is filtered off prior to the 2nd stage. Fine particles of immobilised lipase formed by abrasion of the enzyme beads during agitation would be unlikely to pass through the fine mesh filter used to remove the beads, implying that free lipase leached from the support must catalyse the 2nd stage of the polymerisations. Other losses of activity may arise from handling losses between runs (not recovering all the enzyme from the reaction vessel, spillages *etc.*) or, more importantly, may be due to deactivation of the lipase which is still bound to the support. In the hexane-1,6-diol (**7**) / adipic acid (**3**) case, overall deactivation of the recovered immobilised lipase is ~10%, hence only a small quantity of free lipase is present in the final stage, although it is clearly adequate to catalyse final polymer assembly steps. In the butane-1,4-diol (**4**) / adipic acid (**3**) system, however, deactivation of the lipase after the 1st stage is ~65%; clarification of whether all this deactivation arises from leaching or principally from "on-support" deactivation was necessary in order to decide whether free lipase was the dominant catalytic species in the process.

Scale-up studies at Baxenden have focused on the hexane-1,6-diol (**7**) / adipic acid (**3**) system, principally because recyclability of *Novozyme 435®* in this system is unproblematic compared to the butane-1,4-diol (**4**) / adipic acid (**3**) system; the former also has greater commercial interest. The loss of activity of *ca.* 10% seen during a normal run can be compensated for during recycles by addition of 10wt.% of virgin enzyme to that which is recovered. Loss of 65% activity in the butane-1,4-diol (**4**) / adipic acid (**3**) process cannot economically be compensated for in the same way, making the latter system economically unworkable. The financial incentive led to two key discoveries by Binns at Baxenden:[62] firstly, that a preparation of free CALB (*Novozyme 525®*) could be employed throughout the butane-1,4-diol (**4**) / adipic acid (**3**) process in place of *Novozyme 435®* and in much reduced amounts in spite of the lower "weight-for-weight" activity of the former preparation; secondly, that by running 65% deactivated *Novozyme 435®* through a butane-1,4-diol (**4**) / adipic acid (**3**) process doped with triethylamine, the lipase could be recovered with an

activity of *ca.* 90% compared to virgin *Novozyme 435®*. The effect of triethylamine will be discussed fully in Section 2.2.3.

Thus Binns had confirmed that free lipase was indeed able to efficiently catalyse the first stage of oligomer assembly as well as the final poymerisation stages. Also, the 65% deactivation of *Novozyme 435®* was mostly reversible to give a 90% active material, implying that the leaching in the butane-1,4-diol (**4**) / adipic acid (**3**) system was of a similar order of magnitude to that seen in the hexane-1,6-diol (**7**) / adipic acid (**3**) process, suggesting that the amount of lipase available for leaching was *ca.* 10%, regardless of the polarity of diol used.

polyol substrate	**sample time / h**	**M_w**	**M_n**	**M_w / M_n**	**relative activity of lipase at end of process / %**
ethylene glycol (**80**)	2	219	163	1.3	-
"	4	207	145	1.4	-
"	21	255	179	1.4	20
propane-1,3-diol (**81**)	2	501	281	1.8	-
"	4	594	318	1.9	-
"	21	523	292	1.8	25
butane-1,4-diol (**4**)	2	423	227	1.9	-
"	4	282	168	1.7	-
"	21	1292	610	2.1	45
pentane-1,5-diol (**65**)	2	193	155	1.2	-
"	4	146	136	1.1	-
"	21	869	461	1.9	91
hexane-1,6-diol (**7**)	2	170	155	1.1	-
"	4	161	153	1.0	-
"	21	1356	591	2.3	89
diethylene glycol (**82**)	2	381	206	1.9	-
"	4	388	209	1.9	-
"	21	294	187	1.6	41

Table 11

Confirmation of the latter theory was sought by repeating the series of trials detailed in Table 9, but using *Chirazyme®* in place of *Novozyme 435®*. *Chirazyme®* is also an immobilised form of CALB, but the immobilisation technique forms covalent bonds between

the lipase and the support; consequently, leaching should not be possible when this preparation is employed. As the specific activity of the *Chirazyme®* preparation used is 1.5x greater than that of the *Novozyme 435®* used, the amount of lipase used was scaled down accordingly. GPC data from the polymerisations (again sampling after 2, 4 and 21 hours) and final activity measurements (kinetic assay method) are shown in Table 11. If leaching is the main cause of deactivation in the case of the short chain, highly deactivating diols (*e.g.* **(80)**, **(81)** and **(4)**), then lipase recovered from these systems should have a much higher activity than *Novozyme 435®* recovered from the same systems.

Again, little change is seen in molecular weight averages over the period 2-4 hours, weights actually dropping in some cases; after 17 hours under partial vacuum, all but the ethylene glycol **(80)**, propane-1,3-diol **(81)** and diethylene glycol **(82)** cases proceeded to higher molecular weights, although curiously in this set of results, the butane-1,4-diol **(4)** system gave higher weight material than the pentane-1,5-diol **(65)**, even though the lipase from the latter system was found to be twice as active at the end of the process. This result suggests that in the pentane-1,5-diol / *Novozyme 435®* system, the high molecular weight average achieved may be due to activation of the free leached lipase which is then the dominant catalytic species; when *Chirazyme®* is employed, no free lipase is available for activation and the reaction proceeds more slowly and consequently reaches a lower molecular weight.

Examination of lipase activities reveals little difference from the *Novozyme 435®* trials (Table 9). For the most polar diols **(80)**, **(81)** and **(4)**, the activity is ~10% higher than for the corresponding experiment using *Novozyme 435®*, which might account for the leach observed implying that a constant 10% loss in activity arises from leaching throughout the series. However, this is contradicted by the **(65)** and **(7)** systems, where activites are still ~90%, no increase to 100% being observed due to lack of enzyme leach. Overall, however, these results suggest that leaching plays only a small part in the deactivation phenomenon, and that "on-support" deactivation is the larger contributor.

Proof that *Chirazyme®* does not leach out lipase was given in Section 2.1.4.2, where poly(butane-1,4-diyl adipate) was synthesised using *Chirazyme®*. After the first stage of the reaction *Chirazyme®* was removed from the mixture; no change in oligomer spread or molecular weight average was observed when the mixture was taken over the 2nd stage of the process confirming the absence of free lipase.

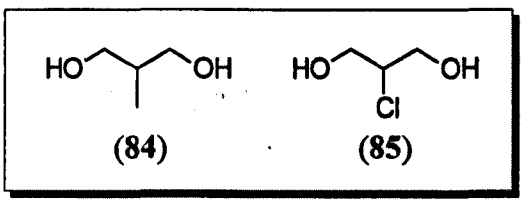
Although it is now possible to say that there are likely to be only small amounts of free lipase in the oligomeric mixture, quantification of the activity of this lipase relative to the immobilised lipase also present in the first stage is impossible. Solubilisation and activation of enzymes in polymers has been documented in the literature in recent years, but normally in relation to solubilising the enzyme in an apolar medium.[110,111] In these instances, non-covalent complex formation allows polymer aggregation around the enzyme, thus shielding the latter from the apolar solvent. In the solvent-free system described herein, an opposing effect might occur. Once the polyester chains begin to form, they could hydrogen bond with the polar lipase enabling solubilisation. Alternatively, the polar diols present at the start may have a similar effect. An idea of when leaching is occurring would be of interest in understanding more fully how such solubilisation occurs.

2.2.2.5 Glycerol and glycerol mimics polymerisation studies

Glycerol (**83**) oligomerised with adipic acid (**3**) in the presence of *Novozyme 435®* (Table 6). However, uncertainty existed over whether the oligomers formed early in the reaction, were themselves substrates for CALB. Confirmation was sought by running a glycerol (**83**) / adipic acid (**3**) reaction in tandem with a butane-1,4-diol (**4**) / adipic acid (**3**) reaction and sampling at intervals during the early stages of the reactions. The former system showed some low weight oligomers after two hours, but showed no further oligomerisation five, eight and twenty-two hours into the reaction suggesting that the lipase had been deactivated under the system conditions; the latter system continued to increase in molecular weight until the end of the reaction. Addition of 0.5 mole equivalents of butane-1,4-diol (**4**) to the glycerol / adipic acid system after twenty-two hours and stirring for five hours gave no increase in molecular weight of the mixture, confirming that the enzyme had indeed been completely deactivated by the glycerol system.

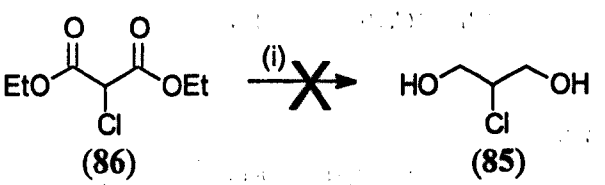
The results in Table 6 suggest that the polarity of glycerol (**83**), diethylene glycol (**82**) and ethylene glycol (**80**) was the root cause of deactivation, although the secondary alcohol functionality of glycerol (**83**) makes it structurally distinct from all the other polyols used in Table 6. A strong, perhaps irreversible interaction of the secondary alcohol unit of glycerol with a functional group in the lipase active site was considered a plausible alternative mechanism of deactivation. To verify whether polarity was the cause of deactivation,

polymerisation of the commercially available 2-methylpropane-1,3-diol (**84**) with adipic acid (**3**) was considered worthwhile, the diol having a similar steric bulk at the 2-position to glycerol,[112] but a significantly lower polarity. Also, the synthesis of 2-chloropropane-1,3-diol (**85**) was undertaken, as this was deemed a good glycerol mimic in terms of polarity and steric bulk at the 2-position[112], however, the compound lacks a secondary alcohol group.



(**84**) (**85**)

Synthesis of the apparently simple molecule (**85**) proved non-trivial. The first method employed had been used by Eliel[113] and involved the reduction of diethyl chloromalonate (**86**) using aluminium hydride very cautiously generated *in situ* from lithium aluminium hydride and 100% sulphuric acid. However, ^{1}H NMR spectroscopy suggested that in our hands a mixture of products had formed and that loss of the chlorine atom had occurred, possibly giving mainly propane-1,3-diol (**81**) (Scheme 31).

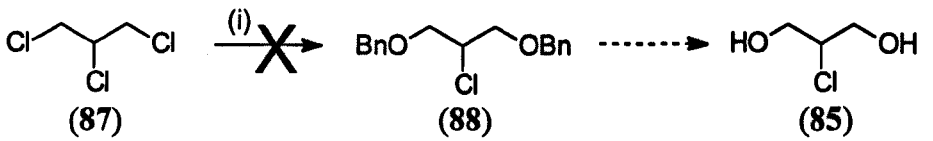


(**86**) (**85**)

Reagents and conditions: (i) lithium aluminium hydride, 100% sulphuric acid, THF, 0 °C to ambient temperature, 14 h.

Scheme 31

The failure of this reaction was surprising, as examples exist of the use of aluminium hydride to reduce esters in the presence of halogens.[114] However, an alternative synthesis was attempted; substitution of the two primary chloro groups of 1,2,3-trichloropropane (**87**) by benzyl ethers was supposed to give (**88**); catalytic hydrogenation would then yield the desired diol (**85**). However, attempted displacement of chloride using the benzyloxy anion in DMF[115] was unsuccessful even after stirring under reflux at 150 °C for 14 hours, TLC indicating the presence of starting material alone (Scheme 32).

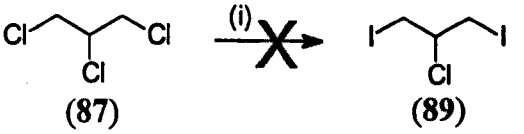


(87) (88) (85)

Reagents and conditions: (i) sodium hydride, benzyl alcohol, DMF, 150 °C, 14 h.

Scheme 32

To make the displacement of halide more favourable, a Finkelstein reaction was attempted, with the aim of displacing the primary chlorine groups with iodide to give diiodide (**89**). Reaction of (**89**) with the benzyloxy anion would then yield the desired ether (**88**). However, no product was observed by TLC after refluxing sodium iodide with 1,2,3-trichloropropane (**87**) in acetone for 16 hours;[116] a portion of the crude material was worked-up and subjected to ^{1}H NMR spectroscopy. No downfield shift of the proton at the 1-position was seen, implying that no incorporation of iodine had occurred (Scheme 33).

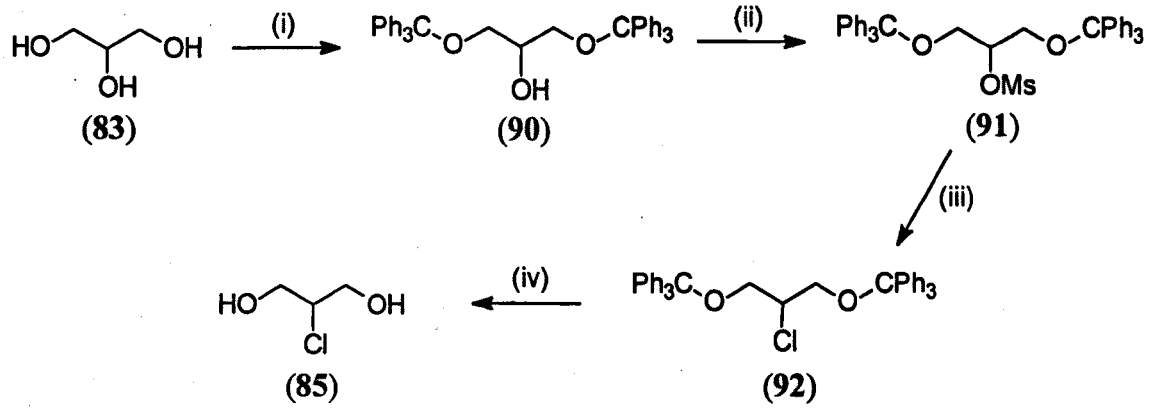


(87) (89)

Reagents and conditions: (i) sodium iodide, acetone, 60 °C, 16 h.

Scheme 33

A new strategy was adopted for the synthesis of (**85**) due to the failure of the above attempts. Glycerol (**83**) was treated with triethylamine, two mole equivalents of trityl chloride and a catalytic amount of DMAP in DMF according to the method of Hernandez[117] to provide the ditritylated alcohol (**90**) in good yield. Mesylation[118] of the secondary alcohol group of (**90**) proceeded quantitatively at ambient temperature to give mesylate (**91**). Displacement of the mesylate anion using anhydrous lithium chloride and 12-crown-4 in DMF gave the chlorinated derivative (**92**) in very good yield after recrystallisation. Following the protocol of Kraft *et al.*[119] deprotection of the tritylated alcohol (**92**) furnished the desired diol (**85**) in good yield (Scheme 34). ^{1}H NMR spectroscopy showed that no further purification of (**85**) was required.



Reagents and conditions: (i) trityl chloride, triethylamine, DMAP (cat.), DMF, ambient temperature, 12h, 40 °C, 1.5 h, 78%; (ii) mesyl chloride, pyridine, 0 °C to ambient temperature, 15 h, 100%; (iii) anhydrous lithium chloride, 12-crown-4, DMF, 100 °C, 36 h, 85%; (iv) 2 M hydrochloric acid (aq.), ethanol, 90 °C, 22 h, 83%.

Scheme 34

Glycerol (**83**) and the two glycerol mimics (**84**) and (**85**) were reacted in tandem with adipic acid (**3**). The GPC analysis results of the polycondensations after a first stage run are shown in Table 12. These data follow the trend that highly polar polyol substrates cause deactivation of *Novozyme 435®* under solvent-free reaction conditions, the highly polar 2-chloropropane-1,3-diol (**85**) apparently deactivating the CALB before any reaction with adipic acid (**3**) could occur. The possibility that (**85**) might not be a substrate for *Novozyme 435®* was considered, in which case the enzyme may not have been deactivated by (**85**); this was not verified, as the scale of the reaction was too small to enable recycling of the immobilised enzyme (Table 12).

system	M_w	M_n	M_w / M_n	comments
glycerol (**83**) / adipic acid (**3**)	254	189	1.35	little oligomer formation
2-methylpropane-1,3-diol (**84**) / adipic acid (**3**)	420	251	1.67	some oligomer formation
2-chloropropane-1,3-diol (**85**) / adipic acid (**3**)	183	172	1.07	no reaction

Table 12

2.2.3 Studies directed towards preventing lipase deactivation

2.2.3.1 Effect of triethylamine

Binns has shown[62] that addition of 2mol% triethylamine wrt adipic acid (**3**) to the butane-1,4-diol (**4**) / adipic acid (**3**) polymerisation system catalysed by *Novozyme 435®* has a profound effect on the activity of the recovered lipase. In the absence of amine, deactivation is usually ~65%; with amine addition the lipase is ~90% active on recovery after the first stage, in line with that from the hexane-1,6-diol (**7**) / adipic acid (**3**) process. By supplementing each recovery of lipase with a 10wt.% addition of virgin lipase and adding amine each time, Binns was able to recycle the same batch of lipase 13 times through the butane-1,4-diol (**4**) / adipic acid (**3**) system, making the process much more attractive commercially. The higher activity of the lipase led to higher molecular weight polymer formation after the first stage of the process.

Addition of amines to enzymatic reactions is not novel, although it has not been performed before in a solvent-free system. Numerous examples of triethylamine addition to lipase-catalysed resolutions in organic solvents exist,[120] although reasons for the addition are somewhat harder to come by. Often in resolutions employing vinyl acetate, the explanation given is that triethylamine acts as a sponge to soak up any free acetic acid liberated during the process, thus preventing deactivation. However, the selectivity of some resolutions in the presence of triethylamine was found to change suggesting either a direct interaction between the amine and enzyme or an indirect effect of the change in the medium. Schick[120(a)] found that additions of up to 0.5mol% triethylamine to asymmetrisations of *meso* diols was necessary to substantially increase the rate of reaction.

Repeating the triethylamine addition work confirmed the results of Binns. Comparative polymerisation of butane-1,4-diol (**4**) and adipic acid (**3**) in the presence of *Novozyme 435®* with and without a 2% addition of triethylamine gave greatly different extents of reaction after the 1st stage (21 hours) (Table 13). The extent of reaction achieved in the amine-free system is lower than usually expected, but the two systems were run in tandem on the same vacuum line, so any change in vacuum / temperature during the overnight process ought to affect both systems equally.

entry no.	system	M_w	M_n	M_w / M_n	comments
1	butane-1,4-diol (4) / adipic acid (3)	536	283	1.9	heterogeneous mixture
2	butane-1,4-diol (4) / adipic acid (3) / triethylamine	1177	508	2.3	homogeneous clear solution

Table 13

The lipase from each system was removed by filtration and that from entry 1 (amine-free) split into two equal portions. One portion was used in a scaled down reaction identical to entry 1 (entry 1, Table 14); the other was used in a scaled down reaction identical to entry 2 (entry 2, Table 14). In so doing, it was hoped that confirmation that triethylamine could reactivate deactivated *Novozyme 435®* would be found, in accord with the findings of Binns.[62] The lipase recovered from entry 2 was recycled through a repeat of entry 1 (entry 3, Table 14); there was no need to split the entry 2 recovered lipase and process it through a repeat of entry 2, as the amine had clearly had an activating effect, and the work of Binns had demonstrated conclusively that the activation could be maintained by triethylamine addition to each successive polymerisation.

Entry 1 (Table 14) shows that lipase which has been used twice in the butane-1,4-diol (4) / adipic acid (3) process is largely deactivated, a lower weight average being attained than that found in the first run. Performing the polymerisation in the presence of triethylamine (entry 2) shows a modest increase in molecular weight average, although it is not clear whether the increase is significant; from the work of Binns, a more dramatic increase in reaction rate is to be expected if reactivation of the lipase is occurring. Entry 3 shows that the effect of the amine on the lipase is not permanent, and hence deactivation will occur if the lipase is put in a non-activating (amine-free) medium. The extent of polymerisation is approximately the same as in entry 2, Table 13 which is to be expected. Hence, the validity of these experiments is debatable, as a rather higher molecular weight average would be expected in entry 2, Table 13 if reactivation of the lipase were occurring. Repetition on a larger, more reproducible scale is necessary.

entry no.	system	recycled enzyme "history"	M_w	M_n	M_w / M_n
1	butane-1,4-diol (**4**) / adipic acid (**3**)	from entry 1, Table 13	426	236	1.8
2	butane-1,4-diol (**4**) / adipic acid (**3**) / triethylamine	from entry 1, Table 13	479	254	1.9
3	butane-1,4-diol (**4**) / adipic acid (**3**)	from entry 2, Table 13	504	261	1.9

Table 14

Addition of triethylamine to a reaction mixture containing a carboxylic acid might give rise to an amine salt with the acid which would have some solubility in the semi-aqueous medium used in the solvent-free polymerisation process. The salt would be in equilibrium with the free acid and base. Whilst the salt would remain in the mixture during the atmospheric pressure stage at 40 °C, application of the partial vacuum and increasing the temperature to 60 °C would be expected to remove triethylamine quite rapidly (b.pt. 89 °C at atmospheric pressure). The equilibrium would adjust to compensate for the loss of free amine, thus depleting the organic salt concentration. Therefore, by the end of the first stage, no amine would be expected to remain in the mixture.

To study whether the amine effect could be perpetuated or even enhanced, a much less volatile amine than triethylamine was added. In this way, the molecule would remain in the reaction mixture throughout the two stages of the reaction. The amine of choice was trioctylamine, as the latter would be expected to possess a similar basicity to triethylamine (both possess three aliphatic unbranched alkyl substituents) and has a boiling point of 365 °C at atmospheric pressure. Polymerisations were performed in the presence of *Novozyme 435®* in the butane-1,4-diol (**4**) / adipic acid (**3**) system over the first stage. One reaction was doped with triethylamine, the other with an equal number of moles of trioctylamine. The immobilised lipase was filtered off from each reaction and each sample was used to catalyse an amine-free butane-1,4-diol (**4**) / adipic acid (**3**) polymerisation (process recycle); in this way, a measure of the enhancement of activity over the initial reaction and in a recycle could be ascertained (Table 15).

entry no.	system	recycled enzyme "history"	M_w	M_n	M_w / M_n
1	butane-1,4-diol (**4**) / adipic acid (**3**) / triethylamine	virgin enzyme	1327	731	1.8
2	butane-1,4-diol (**4**) / adipic acid (**3**) / trioctylamine	virgin enzyme	1101	624	1.8
3	butane-1,4-diol (**4**) / adipic acid (**3**)	from entry 1	387	229	1.7
4	butane-1,4-diol (**4**) / adipic acid (**3**)	from entry 2	314	208	1.5

Table 15

Addition of trioctylamine gives a slightly lower activation than triethylamine; whether this is significant is uncertain, but clearly there is no advantage to retaining amine throughout the process. Elemental analysis was performed on samples of poly(butane-1,4-diyl adipate) prepared under amine-free and triethylamine- and trioctylamine-doped conditions. The threshold minimum detectable amount of nitrogen for this technique is 0.5%; all samples contained undetectable amounts of nitrogen, making this method of detection invalid. However, the fact that the triethylamine-doped product has no odour of triethylamine suggests that it has indeed evaporated during the course of the process; the involatility of trioctylamine means its presence in the final polymer is implicit. Recycling supports the GPC results, the lipase from the triethylamine-doped system reaching a marginally greater molecular weight average than that from the trioctylamine-doped system.

The upshot of these trials is that the amine effect is only crucial in the early stages of the process; after the first few hours, much of the adipic acid (**3**) and AB (**14**) are consumed to form hydroxyl-terminated oligomers, so the effect may involve interaction with the acid in solution to form the amine salt. However, as only 2mol% amine wrt adipic acid (**3**) is present, it seems unlikely that a macro effect is operating. Further work by Binns[62] indicated that no further activation is achieved above this dosage, but dropping below *ca.* 1mol% wrt acid leads to a drop in the activation caused.

The most likely effect operating is a control of pH in the semi-aqueous medium. Recent work by Taylor has shown CALB to be sensitive to adipic acid concentrations and hence pH.[106] Change in pH can dramatically affect enzyme activity by changing the ionisation state of key polarisable groups within the protein, *e.g.* the catalytic triad in the case of lipases.

Controlling ionisation state has been studied intensively by various groups,[121,122,123] but most notably by that of Halling.

Halling *et al.* have employed hydrophobic acids and their sodium salts[124] and acids and bases and their salts[125,126] as "organic phase buffers" in anhydrous organic media. The systems rely on both the neutral and ion-pair forms of the buffer being soluble in the organic phase rather than partitioning into an aqueous phase. By altering the ratio of free acid and base to salt, the ionisation state of the enzyme can be controlled, thus regulating / maintaining the activity of the catalyst. Crucially, the pH "memory" of an enzyme for the conditions from which it was lyophilised can be overridden in this way. Interestingly, triisooctylamine and its hydrochloride salt have also been used as a buffering system by Halling.[127]

The systems described above operate most effectively in low water media, where the counter ions of enzyme ionisation are associated strongly with the protein; where water is present, the effects are influenced less by the counter ions, which are more weakly associated with the protein, and more by the pH of the medium. In the Baxenden solvent-free processes, a significant amount of water is present throughout, perhaps making the system vulnerable to pH control. If so, trapping of some of the adipic acid as the amine salt would lead to a raising of pH, perhaps to a near optimal level for enzyme activity; addition of large amounts of amine could take the pH too high, over the other side of the bell-shaped pH / activity curve.

Very recently, Maugard *et al.*[128] have found interesting chemoselectivity changes on adding a base to the reaction of oleic acid with *N*-methylglucamine, catalysed by immobilised CALB. Where acid is in excess, the initial rate of ester synthesis increases and that of amide synthesis decreases; where base is in excess, the rates for the two reactions switch over.

2.2.3.2 Effect of toluene

Anhydrous toluene was employed as solvent where only small quantities of substrate were available for testing (Section 2.1). The phenomenon of enzyme leaching in the solvent-free system led to inspection of the toluene-based system for a similar effect. A toluene-based polymerisation of butane-1,4-diol (**4**) with adipic acid (**3**) was performed at 60 °C, catalysed by *Novozyme 435*®, filtering off the lipase after 2 hours. The solution was stirred for a further 22 hours at 60 °C, however GPC indicated that very little reaction had occurred (M_w 157, M_n 125, M_w / M_n 1.3), implying that negligible amounts of free lipase were present in the

solution. The apolar nature of toluene would mean that solubilisation of the polar lipase is unfavourable.

As well as apparently preventing leaching of the free lipase, it was considered that the apolarity of toluene might prevent deactivation of the immobilised lipase if used as an additive to the butane-1,4-diol (**4**) / adipic acid (**3**) system. A series of trials were performed, therefore, adding 0, 1, 5 and 20 mol% toluene wrt adipic acid (**3**) to the process. Difficulties with the volatility of toluene meant that the reactions could only feasibly be performed on a lab scale over the first 4 hours of the process. Recovery of the lipase from each system and kinetic assay of the lipase activity gave the trend shown in Table 16.

mol% toluene added (wrt adipic acid (3))	**recovered enzyme activity (% wrt virgin *Novozyme 435*®)**
0	66
1	74
5	100
20	100

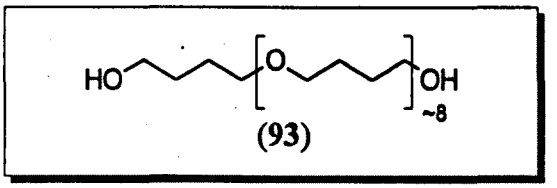
Table 16

No GPC data were obtained for these trials, to verify whether polymerisation did occur in the toluene-doped reactions. However, on the assumption that polymerisation occurs at a comparable rate in the four systems, the optimal amount of toluene additive lies between 1 and 5 mol% wrt adipic acid (**3**). Toluene has the advantage of easier commercial / large scale application compared to triethylamine; hence, further work on this system may have implications on the future process. The assumption remains that the apolar character of toluene is the virtue which prevents deactivation.

2.2.3.3 Effect of PTMEG (**93**)

PTMEG (**93**) is a common polyether monomer of *ca.* 650 Da molecular weight used extensively at Baxenden Chemicals. Attempts to polymerise (**93**) with adipic acid (**3**) using *Novozyme 435*® were successful, indicating the acceptability of such a polyether substrate for

CALB. More interesting was the fact that the lipase recovered from this reaction was fully active, suggesting the absence of any leaching as well as on-support deactivation.



(93)

Again, the result prompted trials into the possible use of PTMEG (**93**) as an additive in the butane-1,4-diol (**4**) / adipic acid (**3**) process. Clearly, in this instance the additive itself will compete for the adipic acid (**3**) and become incorporated in the polyester product. Low concentrations would not be expected to alter the product properties greatly.

Interest had been shown in the literature in adding polyethers and crown and thiacrown ethers to lipase-catalysed reactions, seminal work having been performed a decade ago by Reinhoudt.[129] He showed that addition of macrocyclic polyethers to α-chymotrypsin led to an acceleration of the transesterification of *N*-acetyl-L-phenylalanine, which, it was postulated, results from the ability of the polyether to solubilise salts in the protein by complexation of the cation. More recent work has used crown ethers to dry enzymes prior to use, using their ability to complex water in organic solvents.[130] Takagi *et al.* have recently used thiacrown ethers in lipase-catalysed resolutions of secondary alcohols,[131] however in this case uncertainty exists as to whether the additive interacts with the acylating agent, the lipase or both. Here too, considerable enhancements of lipase activity (up to 4-fold) were observed.

A series of butane-1,4-diol (**4**) / adipic acid (**3**) polymerisations were performed with addition of supplements of PTMEG (**93**) in various doses. The reactions were run over the first stage of the process (21 hours) and the lipase from each trial was removed by filtration, washed and dried in the usual way. Kinetic assays on the lipase samples showed the activities to be those shown in Table 17; the GPC data for samples of the mixture taken after 21 hours are also given.

If the PTMEG (**93**) affects the lipase directly, *via* a protective chaperoning interaction, thus protecting the latter from the effects of the polar medium, the addition of 1-2mol% of (**93**) wrt adipic acid (**3**) would be expected to give a dramatic enhancement of activity at the end of the process, as the effect would operate on a "micro" level. If, however, large doses were required for enhancement to be observed, the conclusion that the effect is change in medium property or "macro" effect could be drawn.

mol% PTMEG (93) added (wrt adipic acid)	M_w	M_n	M_w / M_n	recovered enzyme activity (% wrt virgin *Novozyme 435®*)
0	716	353	2.0	24
1	816	392	2.1	43
2	780	361	2.2	51
5	1335	522	2.6	61
10	935	359	2.6	83

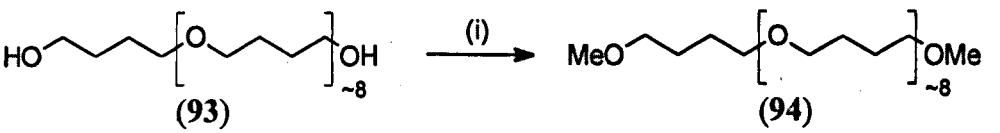
Table 17

Table 17 shows a progressive increase in recovered lipase activity through the various additions. Oddly, the molecular weight averages are highest for the 5% not 10% PTMEG (**93**) additions. However, as PTMEG (**93**) will be consumed in the reactions, the activity enhancement in the 1 and 2% additions may be lost rapidly as PTMEG (**93**) is used up and the protectant effect also is lost. Initial inspection of the results suggests that the predominant effect is probably the lowering of the polarity of the medium, possibly leading to lower solubility of adipic acid and hence higher pH. In this way, deactivation effects would be lessened. Clearly, the effect is not as dramatic as that observed following the addition of toluene.

A further study was undertaken in which a related polyether was used that was prevented from reacting with the adipic acid. This was made possible by "end-capping" the polyether *via* methylation of the free hydroxyl groups of PTMEG (**93**). Addition of such a molecule would enable the polyether to protect the lipase, but would prevent consumption of the polyether. Again addition of 1 and 10 mol% wrt adipic acid was expected to demonstrate whether the effect was on a molecular scale or was an effect of solvent engineering.

Synthesis of the dimethyl ether of PTMEG (**94**) was achieved using the method of Finch *et al.*[132] Treatment of PTMEG (**93**) of ~650 molecular weight with silver (I) oxide and methyl iodide in acetonitrile gave the dimethyl ether (**94**), confirmed by the loss of the CH_2OH signal at 3.6 ppm in the ^1H NMR spectrum (Scheme 34). Unfortunately, purification of the crude product was troublesome, highly coloured impurities being present which could not be removed using sodium thiosulphate washes and Norit® activated charcoal. Use of such

material in an enzymatic reaction was considered imprudent. Passing the material through neutral alumina gave colourless material, pure by ^{1}H NMR spectroscopy; unfortunately, much material was lost during this process enabling only a scaled-down series of enzyme trials.



(93) (94)

Reagents and conditions: (i) methyl iodide, silver (I) oxide, acetonitrile, reflux, 20 h, 13%.

Scheme 35

Butane-1,4-diol (**4**) / adipic acid (**3**) trials with addition of 1 and 10mol% (**94**) wrt adipic acid (**3**) were performed in tandem with a standard additive-free process. GPC was used to monitor the extent of polymerisation after 21 hours. Unfortunately, the small scale used meant that meaningful kinetic assays on the recovered lipase samples could not be obtained (Table 18).

mol% (94) added (wrt adipic acid)	M_w	M_n	M_w / M_n
0	1942	926	2.1
1	1914	913	2.1
10	578	302	1.9

Table 18

The GPC data alone suggest that no apparent enhancement of rate occurs on addition of 1mol% (**94**), although without enzyme activity measurements there is uncertainty as to the relative activities of the recovered lipases. However, a peculiar effect appears to operate on adding larger quantities of (**94**) - a much lower extent of polymerisation is seen, in spite of the fact that all reactions were run in tandem (same temperature and pressure). Repeat of these reactions on a larger, more reproducible scale is necessary to determine whether the apparent detrimental effect of adding large quantities of (**94**) is real.

2.3 ENZYMATIC SYNTHESIS OF NOVEL POLYESTERS

2.3.1 Introduction

Sections 2.1 and 2.2 show *Novozyme 435®* capable of the production of polyadipates which are conventionally made under high temperature conditions. The quality of the finished polyester is better in the enzymatic case, since high weight acid ended moieties are conspicuously absent; in the conventional polymerisation, chain transfer reactions lead to the presence of acid-ended species. In the case of polyadipates, side reactions at 220 °C such as acid decarboxylation[12] and ether formation do not occur to any great extent. Unfortunately, the presence of acid or temperature sensitive functional groups in the substrate(s) is precluded as a result of the many side reactions which may occur. Use of a lipase in a lower temperature polymerisation in these instances would avoid the competing reactions and enable a low dispersity polyester to be produced which may possess different properties to conventional polyesters.

Two possible functional groups that would be interesting to include in an enzymatic polyesterification substrate are epoxide and alkene, neither of which would be expected to be stable under conventional conditions. Epoxides would be expected to ring open under the strongly acidic conditions and high temperatures of conventional polyesterification methods. Alkenes may undergo *cis-trans* isomerism as well as participating in side reactions such as Diels-Alder cycloadditions[10] under conventional high temperature conditions. Losses of *ca.* 10% of double bonds during a conventional polyesterification are not uncommon due to attack of free hydroxyl groups on the double bonds to give branched structures.[10]

Both alkenes and epoxides would be expected to be stable under the conditions used in the enzymatic polymerisation, although employing free diacids would be imprudent in the presence of epoxide groups due to the possibility of acid-catalysed ring opening. Conservation of the two functionalities would leave them free to participate in a second reaction subsequent to polymerisation, *e.g.* crosslinking of the polymer chains.

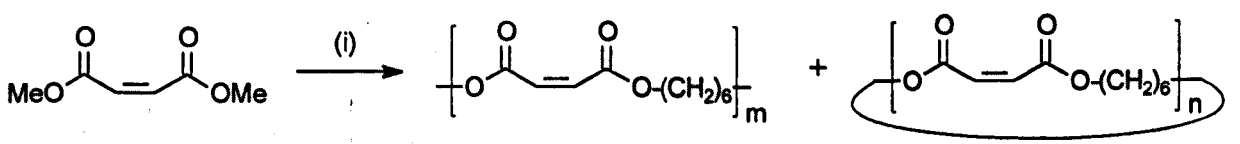
The functionality may be present in the backbone of the linear polymer chain or may be on a branched chain off the backbone of the polymer. Studies on both these substrate types will be discussed separately.

The intention of Section 2.3 is to provide an insight into the substrate range accepted by CALB, by examining a small number of substrates with a variety of different structure types, to give an indication of which may be of industrial use. These may then be more closely examined and the conditions optimised accordingly.

2.3.2 Use of substrates with unsaturation in the backbone

2.3.2.1 Unsaturated acid trials

Brigodiot and co-workers studied lipase-catalysed polytransesterification of the diesters dimethyl maleate (**95**) and dimethyl fumarate with hexane-1,6-diol (**7**),[42] and found that at 60 °C both poly(hexane-1,6-diyl maleate) (**96**) and poly(hexane-1,6-diyl fumarate) could be prepared using *Novozyme 435®* in toluene (Scheme 36); ^{1}H NMR spectroscopy showed in the former case that no isomerisation of the *cis* double bonds to the *trans* geometry had occurred, evidenced by the absence of a signal at 6.8 ppm, characteristic of *trans* alkene protons. Presumably due to the low concentration of substrates in solution, up to 24wt.% of the product was composed of lactones (**97**) in the maleate case. In the fumarate case, only 1wt.% of macrocycles were formed, suggesting that cyclisation is unsurprisingly favoured by the *cis* double bond geometry.



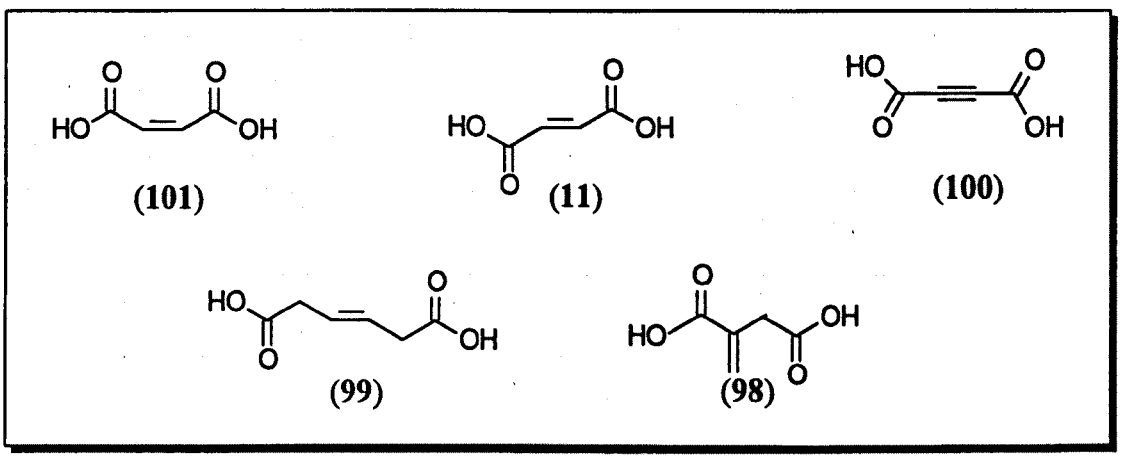
(95) **(96)** **(97)**

Reagents and conditions:[42] (i) hexane-1,6-diol (**7**), toluene, *Novozyme 435®*, 60 °C, 15 days.

Scheme 36

In spite of the success of these reactions, an unactivated diol / diacid system would be commercially more viable, due to the ready availability and cheapness of the starting materials. As Brigodiot had shown the potential of *Novozyme 435®* to catalyse the formation of poly(hexane-1,6-diyl maleate) (**96**) and poly(hexane-1,6-diyl fumarate), verification as to whether the reaction could proceed under the solvent-free conditions was considered to be important. Again, a solvent-free process would be far more likely to realise commercial use than an equivalent solvent-based process. The reactions of itaconic acid (**98**) and *trans*-3-

hexenedioic acid (**99**) with butane-1,4-diol (**4**) were also studied. The latter may be considered as an unsaturated adipic acid mimic, having a six-carbon chain. As a further test of the ability of *Novozyme 435*® to tolerate unsaturation and concomitant rigidity in acid substrates, the commercially available acetylene dicarboxylic acid (**100**) was also tested. Acid (**100**) may be regarded as a conformationally restrained analogue of compounds (**101**) or (**11**).



The GPC results of the polyesterifications with butane-1,4-diol under standard solvent-free conditions over 21 hours are shown in Table 19.

substrate	M_w	M_n	M_w / M_n	comments
maleic acid (**101**)	367	268	1.4	some oligomer formation
fumaric acid (**11**)	150	140	1.1	no reaction
acetylene dicarboxylic acid (**100**)	574	117	4.9	much oligomer formation
trans-3-hexenedioic acid (**99**)	251	182	1.4	some oligomer formation
itaconic acid (**98**)	138	128	1.1	slight oligomer formation

Table 19

The reactions were also performed in the presence of dry toluene in a modified Dean and Stark apparatus at 60 °C as discussed previously. The latter system was expected to give results analogous to those of Brigodiot.[42] These GPC results are shown in Table 20.

The GPC data in Table 20 suggest that, in contrast to the work of Brigodiot, fumaric acid (**11**) is not a substrate for the lipase under the conditions used, whereas the other four acids are accepted as substrates for CALB. Comparison of the reaction of maleic acid (**101**)

and fumaric acid (**11**) with butane-1,4-diol (**4**) in toluene reveals the same story, the *trans* diacid again not reacting; itaconic acid (**98**), *trans*-3-hexenedioic acid (**99**) and acetylene dicarboxylic acid (**100**) showed some oligomer formation in the toluene system. Fumaric acid (**11**) may have a much lower solubility in both the diol and toluene than maleic acid (**101**), and probably, as a consequence, the lipase is not acylated by the acid. The low dispersities seen in almost every case attest to the slowness of the polymerisations. In the hands of Brigodiot a period of 15 days was required to produce poly(hexane-1,6-diyl maleate) (**96**) with M_n ~5810, again indicating the reaction is slow.

substrate	M_w	M_n	M_w / M_n	comments
maleic acid (**101**)	374	281	1.3	some oligomer formation
fumaric acid (**11**)	132	122	1.1	no reaction
acetylene dicarboxylic acid (**100**)	1166	496	2.3	much oligomer formation
trans-3-hexenedioic acid (**99**)	603	229	2.7	some oligomer formation
itaconic acid (**98**)	259	152	1.7	some oligomer formation

Table 20

A source of concern was that the acids themselves may have catalysed the polymerisation; whilst control reactions using adipic acid (**3**) and butane-1,4-diol (**4**) under standard solvent-free polymerisation conditions in the absence of CALB had given no reaction, Binns[62] had shown that oxalic acid and butane-1,4-diol (**4**) could form poly(butane-1,4-diyl oxalate) in the absence of an added catalyst. A repeat of the reactions of acids (**98**), (**99**), (**100**) and (**101**) with butane-1,4-diol (**4**) under solvent free conditions, but in the absence of enzyme gave the GPC results shown in Table 21.

The average molecular weights shown in Table 21 for enzyme-free polymerisation of maleic acid (**101**), acetylene dicarboxylic acid (**100**) and itaconic acid (**98**) with butane-1,4-diol (**4**) are almost identical to those of the corresponding acids in Table 19. This implies that the acids are not acceptable substrates for *Novozyme 435®*, and that by virtue of their strengths as acids they catalyse their own condensation with the polyol. Geresh and Gilboa[20] had also found that maleate esters are not substrates for lipases, although CALB was not among the lipases screened.

substrate	M_w	M_n	M_w / M_n	comments
maleic acid (**101**)	388	279	1.4	some oligomer formation
acetylene dicarboxylic acid (**100**)	582	285	2.1	much oligomer formation
trans-3-hexenedioic acid (**99**)	177	158	1.1	no reaction
itaconic acid (**98**)	139	129	1.1	slight oligomer formation

Table 21

^{1}H NMR spectra of the oligomers formed from butane-1,4-diol (**4**) and maleic acid (**101**) indicated the presence of only *cis* geometry double bonds, the signal at 6.8 ppm characteristic of *trans* protons being absent. On heating this polyester for two hours at 130 °C, (conditions adequate to isomerise molten maleic acid (**101**) to fumaric acid (**11**)[133]), still no isomerisation was seen by ^{1}H NMR spectroscopy. Hence, polymerisation of butane-1,4-diol (**4**) and maleic acid (**101**) at 130 °C using a conventional nitrogen sparge to remove by-produced water could provide a method of unsaturated polyester production without the need for a lipase or an added acid catalyst. The polymer would presumably contain only *cis* geometry double bonds.

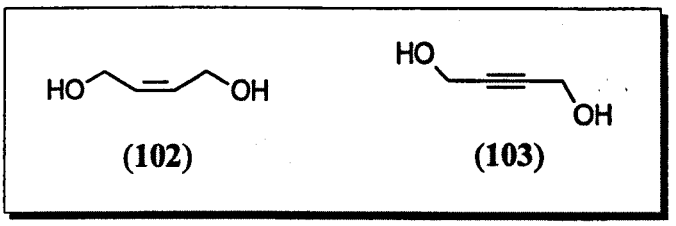
Although *Novozyme 435®* appears to play no part in catalysing the reaction of the unsaturated diacids (**98**), (**100**) and (**101**) with butane-1,4-diol (**4**), the methods of Geresh and Gilboa[20] and Brigodiot[42] can provide access to both poly(diyl fumarates) and poly(diyl maleates).

The unsaturated acid substrate of choice for process development using *Novozyme 435®* appears to be *trans*-3-hexenedioic acid (**99**), which showed significant oligomer formation in both solvent-based and solvent-free conditions but no reaction with butane-1,4-diol (**4**) in the absence of lipase. However, NMR spectroscopic studies are required to ascertain that no loss of functional group integrity occurs during the polymerisation.

2.3.2.2 Unsaturated alcohol trials

In order to test the alcohol substrate range of CALB, in particular the tolerance of rigidity in the backbone of the diol, two unsaturated diols were polymerised with adipic acid (**3**) in the

presence of *Novozyme 435*®, *cis*-but-2-ene-1,4-diol (**102**) and but-2-yne-1,4-diol (**103**). Both represent unsaturated butane-1,4-diol (**4**) mimics. As the latter polymerises well under solvent-free conditions, the unsaturated mimics were expected to be acceptable substrates for CALB under these conditions. The GPC results are shown in Table 22. Trials based in toluene were also performed - GPC results of these trials are detailed in Table 23.



(102) **(103)**

substrate	M_w	M_n	M_w / M_n	comments
cis-but-2-ene-1,4-diol (**102**)	358	207	1.7	some oligomer formation
but-2-yne-1,4-diol (**103**)	175	158	1.1	no reaction

Table 22

substrate	M_w	M_n	M_w / M_n	comments
cis-but-2-ene-1,4-diol (**102**)	841	315	2.7	extensive oligomer formation
but-2-yne-1,4-diol (**103**)	326	145	2.3	little reaction

Table 23

A trend of extent of reaction with degree of diol unsaturation is evident, *cis*-but-2-ene-1,4-diol (**102**) producing a much lower molecular weight polyadipate than would the fully saturated butane-1,4-diol. The former, however, showed a wide spread of oligomeric material in the GPC trace in the toluene-based system and may have potential for commercial development. Tables 22 and 23 suggest that but-2-yne-1,4-diol (**103**) is a very poor substrate for CALB.

Both (**102**) and (**103**) are held conformationally rigid at the C2-C3 bond, but may rotate along the C1-C2 (C3-C4) and the C1-O (C4-O) bond; alkyne (**103**), however, has a linear carbon chain, so rotation along the C1-C2 bond will give a more limited change in molecular shape / volume compared to alkene (**102**). This decreasing flexibility (butane-1,4-

diol (**4**) > *cis*-but-2-ene-1,4-diol (**102**) > but-2-yne-1,4-diol (**103**)) may lead to steric interactions between the diol and the lipase active site, and hence a lowered reaction rate with increasing unsaturation. Electronic effects of the double or triple C-C bonds on the nucleophilicity of the alcohol functionality are not likely to be influential, as such inductive effects decrease rapidly with distance along the molecule. Confirmation that steric effects are participating could be sought by polymerising *trans*-but-2-ene-1,4-diol (not commercially available), which would impose similar electronic effects to *cis*-but-2-ene-1,4-diol (**102**) on the hydroxyl group, but would possess a different geometry at the double bond.

2.3.3 Use of substrates with unsaturation pendant from the backbone

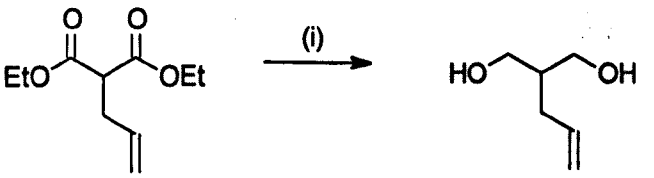
2.3.3.1 Choice and synthesis of substrates

Incorporating unsaturation into a side chain which is, itself, branched off the polyester backbone appeared a logical alternative to the use of substrates with unsaturation in the polymer chain itself. The presence of a double bond some distance from the backbone could potentially be used for cross-linking with other polyester chains following enzymatic polymerisation. Such cross-linked structures may be well-defined as a result of the retention of all the substrate double bonds during polymerisation. Two criteria were key to the selection of suitable substrates:

- The substrate should mimic the substrates known to be well accepted by CALB, *e.g.* adipic acid (**3**), butane-1,4-diol (**4**)
- The substrate should be symmetrical, that is with branching at a central carbon atom. Although the substrate will be prochiral, a chiral centre will not be present prior to reaction.

The molecule chosen for study was diethyl allylmalonate (**104**), which fits the above criteria well. Moreover, reduction of the diester (**104**) using a literature procedure[134] could provide the diol 2-allylpropane-1,3-diol (**105**) which should mimic butane-1,4-diol (**4**) well in terms of polarity.

Treatment of diester (**104**) with lithium aluminium hydride gave the desired diol (**105**) in excellent yield after purification (Scheme 37).



(104) **(105)**

Reagents and conditions: lithium aluminium hydride, diethyl ether, 0 °C to ambient temperature, 18 h, 80%.

Scheme 37

The two CH_2OH groups in (**105**) are enantiotopic, that is, alteration of one group (*e.g.* by acetylation) would give one enantiomer of a chiral compound, whilst alteration of the other group would give the opposite enantiomer. Enzymes may preferentially select between two enantiotopic groups to give an enantiomerically enriched product, and studies on acetylations of (**105**) using *Novozyme 435*® are dealt with in Section 2.4 of this chapter.

2.3.3.2 Polymerisations of substrates

Polymerisation of diester (**104**) with butane-1,4-diol (**4**) catalysed by *Novozyme 435*® was performed in standard solvent-free and anhydrous tolune-based conditions. The same conditions were employed to polymerise diol (**105**) with adipic acid (**3**). Details of the reactions and GPC data found are shown in Table 24.

The results in Table 24 show that 2-allylpropane-1,3-diol (**105**) is an excellent substrate for CALB. The reactions were all run over a single night (~20 h), and in the case of the solvent-free reactions no second (10 mbar) polymerisation stage was performed, which would normally lead to a higher molecular weight polymer. Although malonate (**104**) was a substrate for CALB, the GPC data indicate that only low weight oligomers formed in both the toluene-based and solvent-free systems.

^{1}H NMR spectroscopy confirmed that the allyl double bond had remained intact in both 2-allylpropane-1,3-diol (**105**) / adipic acid (**3**) reaction systems, as expected, due to the mildness of the reaction conditions. End-group analysis was used in addition to GPC to estimate the degree of polymerisation for the solvent-free and toluene-based 2-allyl-propane-1,3-diol (**105**) / adipic acid (**3**) reaction systems. Integration of the CH_2OH and CH_2OCOR signals for the solvent-free system gave a CH_2OH:CH_2OCOR ratio of ~1:4, suggesting that the mean (M_n) molecular length corresponded to a $B(AB)_4$-type oligomer. The ^{13}C NMR

spectrum showed no discernable signal in the region around 177 ppm, the area in which COOH resonances would appear, suggesting that the hydroxyl end-capped species $B(AB)_4$ was indeed a good approximation for the mean oligomeric species (molecular weight 1020 Da). For the toluene-based system the CH_2OH:CH_2OCOR ratio was ~1:8, implying that the mean oligomeric species was $B(AB)_8$, molecular weight ~1700. However, end group analysis normally only gives accurate estimations of mean molecular weight for low weight oligomers, but beyond ~4 repeat units inaccuracy in integration leads to errors of >1 repeat unit.

substrates	conditions	M_w	M_n	M_w / M_n	comments
diethyl allylmalonate (**104**) / butane-1,4-diol (**4**)	solvent-free	276	141	2.0	some oligomer formation
diethyl allylmalonate (**104**) / butane-1,4-diol (**4**)	toluene-based	447	131	3.4	some oligomer formation
2-allyl-propane-1,3-diol (**105**) / adipic acid (**3**)	solvent-free	1810	863	2.1	much polymer formation
2-allyl-propane-1,3-diol (**105**) / adipic acid (**3**)	toluene-based	1375	878	1.6	much polymer formation

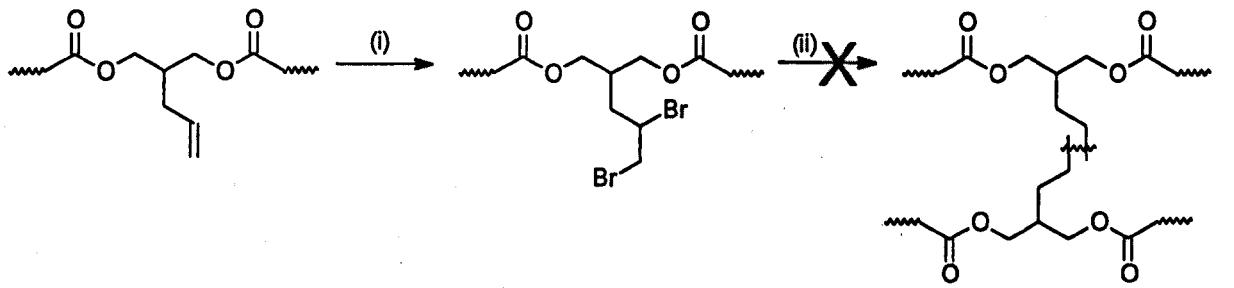
Table 24

The products of the enzymatic bulk (solvent-free) polymerisation of 2-allyl-propane-1,3-diol (**105**) and adipic acid (**3**) were compared with those of a conventional high temperature polyesterification. The latter was performed in a 1 mL reactor using a catalytic quantity of titanium tetraisopropoxide, heating to 220 °C under a flow of nitrogen; subsequently, a partial vacuum was applied whilst maintaining the temperature. Analysis of the product by GPC revealed that a high molecular weight polymer had formed (M_n 2091, M_w 5204, M_w / M_n 2.5). The polymeric material was isolated as a yellow gel, suggesting that side reactions or some decomposition had occurred. However, ^{1}H NMR spectroscopy showed only major signals in the expected regions and that the allyl group had been left intact during the polymerisation. In this instance, enzymatic polymerisation was only advantageous by virtue of the lack of discolouration and absence of residual inorganic catalyst in the final polymer.

2.3.3.3 Attempt to cross-link poly(2-allyl-1,3-diyl adipate)

Enzymatic synthesis of poly(2-allyl-1,3-diyl adipate) catalysed by *Novozyme 435*® has been shown to be an efficient method. The next objective was to attempt to cross-link the

polyester chains using the built-in unsaturation of the pendant allyl group. Formation of the free radicals required for cross-linking could not be achieved from the allyl group itself; however, addition of bromine across the double bond followed by radical initiation was considered a possible route to cross-linked material. The method used is shown in Scheme 38.



Reagents and conditions: (i) Br_2, chloroform, ambient temperature, 30 min; (ii) AIBN, tributyltin hydride, toluene, reflux, 17 h

Scheme 38

^{1}H NMR spectroscopy on the bromination product showed the loss of the allylic proton resonances and appearance of resonances at 3.6 and 3.9 ppm of equal intensity (CH_2Br), implying that bromination had occurred. The $CHBr$ signal would be masked under those of CH_2OCOR at 4.3 ppm.

Treatment of the crude dibromo polymer with the radical initiator AIBN and tributyltin hydride in refluxing toluene was expected to give a mixture of debrominated material and some cross-linked polymer. Inspection of the GPC trace of the crude product showed a slight decrease in molecular weight averages, suggesting that the loss of the bulky bromine atoms and no cross-linking had occurred. A new predominant component appeared in the GPC trace (elution time 15.90 min), which may be residual tributyltin hydride. This gave the material an artificially high dispersity by lowering the M_n value.

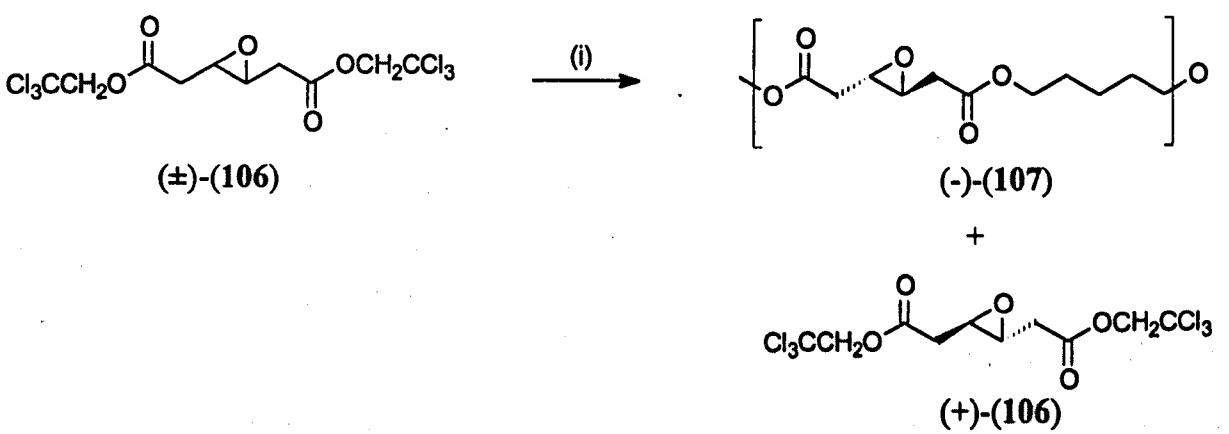
No further efforts were directed towards cross-linking the dibromo polymer, although the observation that bromination of poly(2-allyl-propane-1,3-diyl adipate) proceeds cleanly was a useful pointer. Use of a molecule with two nucleophilic groups (*e.g.* a diamine) which could displace bromide as a leaving group on two polymer chains could be an alternative route to cross-linked polyesters.

2.3.4 Trials of substrates containing an epoxide group

2.3.4.1 Choice and synthesis of substrates

Again, the choice of substrates was selected to be closely analogous in structure to other molecules known to be good substrates for CALB. That incorporation of epoxides into polyester chains could be important was demonstrated very recently by Lenz *et al.* It was shown that epoxide-containing polyesters have considerably different properties to their unsaturated counterparts[136] and that crosslinking of the polymer chains may be achieved using succinic anhydride as linking agent.[137]

Morrow had polymerised the racemic diester (±)-bis(2,2,2-trichloroethyl) *trans*-3,4-epoxyadipate (±)-(**106**) with butane-1,4-diol (**4**) using PPL as catalyst.[19] The lipase proved to be highly enantioselective, incorporating almost exclusively one enantiomer of the diester (**106**) to give (-)-(**107**). The unused enantiomer (+)-(**106**) was recovered in >90% yield and high (95%) optical purity (Scheme 39).



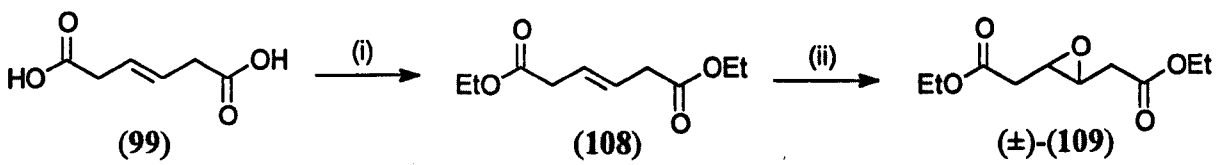
Reagents and conditions:[19] butane-1,4-diol (**4**), diethyl ether, PPL, ambient temperature, 3.5 days.

Scheme 39

The reaction in Scheme 39 is of great academic interest and represents one of the few syntheses of optically active polyesters[18,19,41] where the product is not based on the naturally occurring polyhydroxybutyrates (PHBs). Unfortunately, the use of a highly flammable and volatile solvent such as diethyl ether and the formation of the harmful by-product 2,2,2-trichloroethanol means the system does not lend itself to commercial exploitation. Once again, the solvent-free system had potential to provide a plausible alternative procedure. The

formation of halogenated by-products was precluded by use of (±)-diethyl 3,4-epoxyadipate (±)-(**109**) (a close mimic of adipic acid (**3**)), and CALB was used in place of PPL. CALB was expected to show a preference for one enantiomer of (±)-(**109**), similarly to PPL, which would be of general interest in probing the substrate range of the lipase.

The synthesis of (±)-diethyl 3,4-epoxyadipate (±)-(**109**) was analogous to Morrow's preparation of (2,2,2-trichloroethyl) *trans*-3,4-epoxyadipate (**106**). *trans*-3-Hexenedioic acid (**99**) was treated with ethanol and DCC in the presence of DMAP in dichloromethane to give diethyl *trans*-3-hexenedioate (**108**) which was epoxidised using *m*-CPBA to yield the desired (±)-diethyl 3,4-epoxyadipate (±)-(**109**) (Scheme 40).

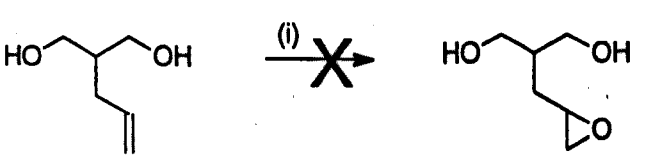


(**99**) (**108**) (±)-(**109**)

Reagents and conditions: (i) ethanol, DCC, DMAP, dichloromethane, ambient temperature, 18 h, 29%; (ii) *m*-CPBA, dichloromethane, 0°C to ambient temperature, 91 h, 81%.

Scheme 40

Use of a polyol containing an epoxide group was also desirable in order that the tolerance of CALB to epoxide groups in both esters and alcohols could be judged. As 2-allylpropane-1,3-diol (**105**) had been shown to be an excellent substrate for CALB, the logical diol to use was (±)-2-(2',3'-epoxypropyl)propane-1,3-diol (±)-(**110**). Preparation was attempted by treating 2-allylpropane-1,3-diol (**105**) with *m*-CPBA (Scheme 41). Unfortunately, in the acidic conditions of the reaction, ring opening of the epoxide must have occurred *via* attack by the nucleophilic alcohol groups. A mass spectrum (CI^+) confirmed the absence of a molecular ion for the desired epoxide (**110**) and the presence of many higher mass impurity peaks.

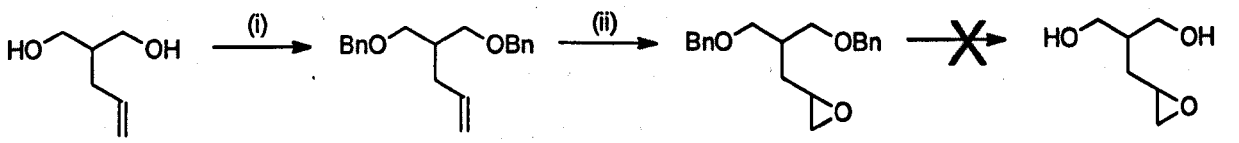


(**105**) (±)-(**110**)

Reagents and conditions: (i) *m*-CPBA, dichloromethane, 0°C to ambient temperature, 48 h.

Scheme 41

An alternative route was devised, whereby the alcohol groups of (**105**) were protected as benzyl ethers (**111**). During subsequent *m*-CPBA epoxidation of the allyl double bond, no nucleophilic attack on the epoxide formed could occur and (±)-(**112**) was isolated in moderate yield. Catalytic hydrogenation of (±)-(**112**) was expected to yield the epoxydiol (±)-(**110**), the benzyl groups being more susceptible than the epoxide (Scheme 42). However, after stirring the reaction overnight, the epoxide appeared to have been hydrogenated also. However, the result of this reaction should be to form an alcohol group, hence the molecule formed would be a triol. A ^{1}H COSY NMR experiment and mass spectrometry suggested that the molecule formed was 2-propylpropane-1,3-diol. A ^{13}C DEPT NMR experiment confirmed the presence of $\underline{C}H_2\underline{Me}$ signals expected from complete saturation of a terminal epoxide.



(**105**) (**111**) (±)-(**112**) (±)-(**110**)

Reagents and conditions: (i) sodium hydride, THF, DMF, benzyl chloride, 0 °C to ambient temperature, 15 h, 76%; (ii) *m*-CPBA, dichloromethane, 0 °C to ambient temperature, 43 h, 52%; (iii) H_2, palladium on charcoal, ethyl acetate, ambient temperature, 21 h.

Scheme 42

2.3.4.2 Polymerisation studies

Enzymatic polymerisation of (±)-diethyl 3,4-epoxyadipate (±)-(**109**) with butane-1,4-diol (**4**) using *Novozyme 435*® as catalyst was performed at 60 °C as a test of the compatability of the substrate. Solvent-free conditions were used, with butane-1,4-diol (**4**) in a slight excess.

^{1}H NMR spectroscopy was performed on the reaction mixture in deuteriochloroform. A signal at ~4.2 ppm consisted of resonances from ester linkages formed during polymerisation and the residual $COOC\underline{H}_2Me$ signal in the starting diester (**109**). By assuming that the polymeric material is only hydroxyl terminated and that all butane-1,4-diol (**4**) has been consumed, an estimation of the degree of polymerisation may be obtained using the Me signal of the ethyl ester as a marker. The ratio of $C\underline{H}_2OCOR$:$Me\underline{C}H_2OCOR$ was estimated to be 1.00:0.17; therefore, of the protons resonating at ~4.2 ppm, 0.12 is due to $MeC\underline{H}_2OCOR$, leaving the remaining 0.88 as ester signals in the polymer. End group analysis then gives the

ratio of CH_2OCOR:CH_2OH as 0.88:0.19 or ~5:1, implying that the mean structure of the polymer is a $B(AB)_5$-type oligomer with molecular weight of 1160 Da.

A second solvent-free polymerisation of (±)-diethyl 3,4-epoxyadipate (±)-(**109**) with butane-1,4-diol (**4**) was performed at 30 °C, with a view to studying the enantioselectivity of CALB towards diethyl 3,4-epoxyadipate (±)-(**109**) at lower temperature. The diester (±)-(**109**) was present in two-fold excess in order that polymeric material could form, even if the lipase catalysed the reaction of one enantiomer only.

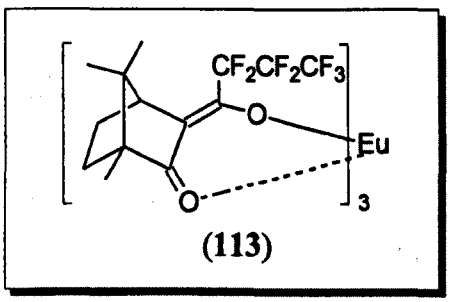
After polymerisation for 19 hours at a pressure of 10 ± 3 mbar, the reaction mixture was fractionated by washing the viscous pale yellow oil with hexane. ^{1}H NMR spectroscopy on the colourless oil recovered from the hexane extract indicated that it consisted entirely of diester (**109**). Morrow[19] had developed an NMR chiral shift method for determining the ee of recovered bis(2,2,2-trichloroethyl) *trans*-3,4-epoxyadipate (**106**) which was envisaged also to be applicable to diethyl 3,4-epoxyadipate (**109**). Chiral shift experiments were therefore performed on the latter material and on racemic diester (±)-(**109**) to confirm whether the polymerisation was enantioselective.

Chiral shift reagents are usually complexes of rare earth metals and a chiral enantiomerically-pure beta diketone. A chemical bond exists between the metal and the beta diketone through an oxygen atom *via* enolisation of one of the ketone functionalities. The most commonly employed shift reagent is europium tris(3-(heptafluoropropylhydroxy-methylene)-(+)-camphorate) ($Eu(hfc)_3$) (**113**). Complexation of this enantiomerically pure complex with two enantiomeric molecules will lead to two diastereomeric complexes, which may then be resolved by ^{1}H NMR spectroscopy. Addition of the shift reagent is performed in aliquots until adequate separation of signals is observed. By comparison of a mixture of unknown optical purity with the racemate, signal integration gives the relative abundance of the two enantiomers.[137]

Addition of 10 mol% ($Eu(hfc)_3$) (**113**) to racemic diethyl 3,4-epoxyadipate (±)-(**109**) gave a splitting in two of the $CH(O)CH_2COOCH_2Me$ signal and a shift from ~3 to ~4 ppm. Adding the same quantity of shift reagent to the hexane extract of diethyl 3,4-epoxyadipate (**109**) from the polymerisation of diethyl 3,4-epoxyadipate (±)-(**109**) with butane-1,4-diol (**4**) gave the same extent of separation and shift of signals; the material was still racemic. A measurement of the optical rotation of another sample of the hexane extract of diethyl 3,4-epoxyadipate (**109**) showed no rotation, confirming that the material was racemic.

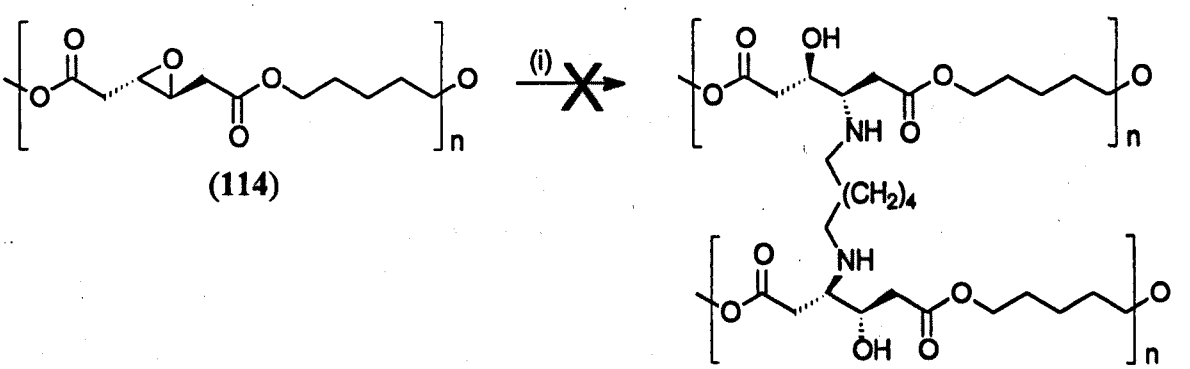
GPC confirmed that polymerisation had occurred, the material recovered after washing with hexane showing a range of oligomeric species (M_w 629, M_n 316, M_w / M_n 2.0).

Clearly, the selectivity of CALB towards the diester (**109**) was negligible and both enantiomers would be incorporated into the polyester. The method of Morrow[19] is therefore the choice for producing optically active polyester (-)-(**107**). The polymer synthesised using CALB could nonetheless be used in a cross-linking experiment.



(113)

Opening of the epoxide groups in poly(butane-1,4-diyl 3,4-epoxyadipate) (**114**) using a diamine could potentially lead to the linking of polyester chains at points along the backbone. Using a modified method of Hudlicky,[138] hexane-washed poly(butane-1,4-diyl 3,4-epoxyadipate) (**114**) was heated with hexamethylenediamine under mildly acidic conditions. A representation of the reaction is shown in Scheme 43. GPC data on the isolated product indicated that reaction had not taken place. More forcing conditions might give the desired cross-linking reaction.



(114)

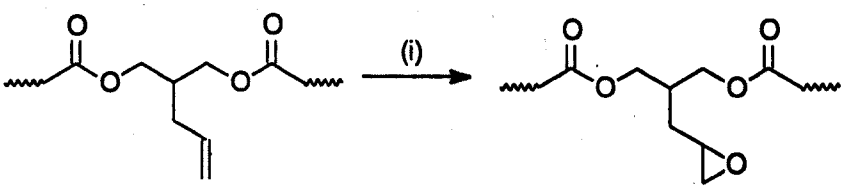
Reagents and conditions: (i) hexamethylenediamine, ammonium chloride, 1,2-dimethoxyethane:ethanol:water 3:3:2, reflux, 3 h.

Scheme 43

A conventional polymerisation of diethyl 3,4-epoxyadipate (±)-(**109**) with butane-1,4-diol (**4**) was performed, to confirm the instability of epoxide groups under the high

temperatures involved. Stirring the substrates with a catalytic amount of titanium tetraisopropoxide at 220 °C for 30 minutes was adequate to cause dark red discolouration of the mixture. ^{1}H NMR spectroscopy on this material showed the presence of several impurity signals between 4.5 and 7 ppm and a very complex set of signals between 2 and 3 ppm, indicating some loss of epoxide functionalities. The utility of enzymatic synthesis has therefore been demonstrated in this instance.

Whilst synthesis of epoxydiol (**110**) had proven difficult, the preparation of poly(2-(2',3'-epoxypropyl)propane-1,3-diyl adipate) was achieved *via* epoxidation of poly(2-allylpropane-1,3-diyl adipate) (Scheme 44). Hydroxyl terminated poly(2-allylpropane-1,3-diyl adipate) was used as the work-up requires a base wash which would remove any acid-ended polymer in addition to the *m*-chlorobenzoic acid formed in the reaction.



Reagents and conditions: *m*-CPBA, dichloromethane, ambient temperature, 48 h.

Scheme 44

^{1}H NMR spectroscopy verified that the allyl double bond had reacted. Appearance of one proton signals at 2.4, 2.7 and 2.9 ppm indicated that a terminal epoxide group had indeed been introduced. End group analysis gave a CH_2OH:CH_2OCOR ratio of 1:9, corresponding to a molecular weight of >2000 Da. GPC analysis gave weight averages M_n 943, M_w 1757, M_w / M_n 1.9, showing the divergence of end group and GPC analyses at higher molecular weights.

The epoxypolymer may potentially be crosslinked using a diamine, and the pendant nature of the epoxide groups means they should not be sterically encumbered by the polymer chain. The cross-linking would therefore be expected to proceed more easily than the cross-linking of poly(butane-1,4-diyl 3,4-epoxyadipate) (**114**).

2.3.5 Cyclohexane-based diols and diacids - attempts to form chiral polyesters

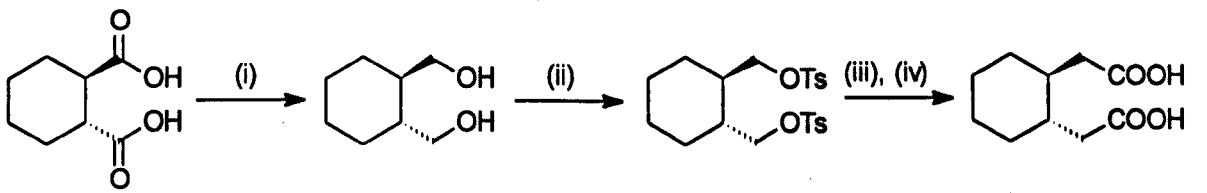
2.3.5.1 Introduction

The work in this section describes the synthesis and polymerisation of a series of related 1,2-disubstituted cyclohexane diacids and diols. Where the substituents are *trans* to one another, the molecules are chiral, and by use of the innate selectivity of CALB, the hope was that incorporation of predominantly one enantiomer of the molecules would occur. With *cis* geometry, the achiral molecules might have very different acceptability into the lipase active site, possibly leading to useful polymerisations which would separate a mixture of *cis* and *trans* isomers.

The molecules used are related to either succinic or adipic acid (**3**) or butane-1,4-diol (**4**) and hexane-1,6-diol (**7**), by virtue of the carbon chain length between the two acid or two alcohol groups. This set of chiral substrates analogous to known substrates for CALB, but with greater backbone rigidity would be likely also to be good substrates for CALB-catalysed polyesterification.

2.3.5.2 Synthesis of starting materials

The commercially available racemic *trans*-1,2-cyclohexanedicarboxylic acid (±)-(**115**) was taken as the starting point for the preparation of all the chiral adipic acid / diol mimics. The literature procedure of Roberts *et al.*[139] was used to reduce (**115**) to (±)-*trans*-1,2-cyclohexanedimethanol (±)-(**116**) using lithium aluminium hydride. Homologation of the two chains was achieved *via* modification of the literature procedures of Ali and Owen[140] and Fujita *et al.*[141] Thus, tosylation of (±)-(**116**) gave (±)-(**117**) in very good yield; displacement of the tosyl groups of (±)-(**117**) using aqueous potassium cyanide gave the dinitrile which was not characterised. Hydrolysis then gave the free diacid (±)-(**118**), the homologue of (±)-(**115**) (Scheme 45).

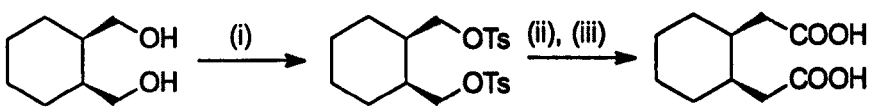


(±)-(**115**) (±)-(**116**) (±)-(**117**) (±)-(**118**)

Reagents and conditions: (i) lithium aluminium hydride, THF, reflux, 18 h, 83%; (ii) *p*-toluenesulphonyl chloride, pyridine, 0 °C to ambient temperature, 5 h, 84%; (iii) potassium cyanide, DMSO, 90 °C, 4 h; (iv) 9 M potassium hydroxide (aq.), ethanol, 90 °C (reflux), 100 h, 88% over 2 steps.

Scheme 45

The *cis* diacid (**121**) was likewise prepared, although in this case the commercially available *cis*-1,2-cyclohexanedimethanol (**119**) was used as starting material, as well as being a suitable candidate for polymerisation studies itself (Scheme 46).

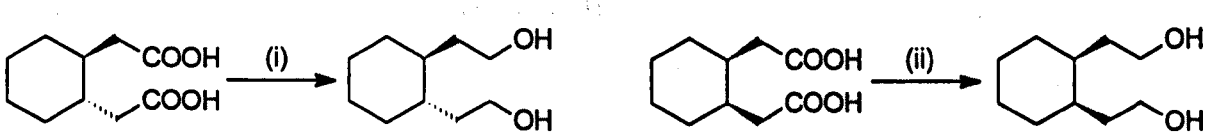


(**119**) (**120**) (**121**)

Reagents and conditions: (i) *p*-toluenesulphonyl chloride, pyridine, 0 °C to ambient temperature, 3 h, 56%; (ii) potassium cyanide, DMSO, 90 °C, 4 h; (iii) 9 M potassium hydroxide (aq.), ethanol, 90 °C (reflux), 110 h, 82% over 2 steps.

Scheme 46

Reduction of both diacids (±)-(**118**) and (**121**), again using lithium aluminium hydride, gave the homologated diols (±)-(**122**) and (**123**), which may be viewed as hexane-1,6-diol (**7**) mimics (Scheme 47).



(±)-(**118**) (±)-(**122**) (**121**) (**123**)

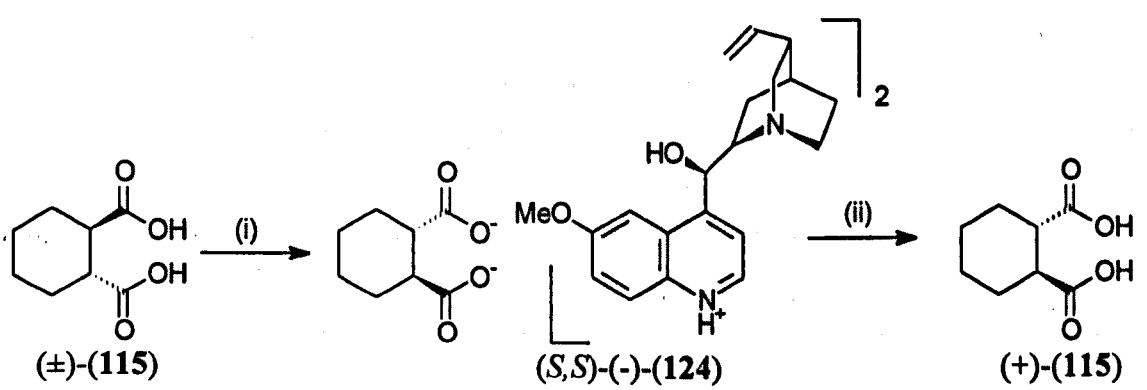
Reagents and conditions: (i) lithium aluminium hydride, THF, reflux, 18 h, 72%; (ii) lithium aluminium hydride, THF, reflux, 18 h, 52%.

Scheme 47

2.3.5.3 Polymerisation studies on 1,2-cyclohexane diols and diacids

A method for chiral GC analysis of the recovered starting material from the polymerisation of (±)-*trans*-1,2-cyclohexane dicarboxylic acid (±)-(**115**) with butane-1,4-diol (**4**) was developed. Derivatisation of the racemic diacid (±)-(**115**) as the dimethyl ester was achieved conveniently, cleanly and rapidly using the derivatising agent trimethylsilyl diazomethane.[142] The latter is a safer and much more convenient alternative to diazomethane, although it has generally only found favour in analytical uses, possibly because of its comparatively high cost. By performing a classical resolution using the procedure of Applequist and Werner,[143] the (*S,S*) enantiomer of (±)-(**115**) was isolated in good optical purity. Thus, recrystallisation of (±)-(**115**) with (-)-quinine from ethanol, followed by several further recrystallisations gave quinine salt (*S,S*)-(-)-(**124**). Hydrolysis using refluxing aqueous sulphuric acid in diethyl ether gave predominantly the (*S,S*) diacid (+)-(**115**) (Scheme 48).

The dimethyl ester of the racemic diacid (±)-(**115**) was separated by chiral GC (Lipodex E column, oven temperature 100 °C, detector temperature 250 °C, injector temperature 250 °C, elution times 31.9 and 33.2 minutes). By likewise derivatising the resolved diacid (+)-(**115**) and separating under the same GC conditions, the component eluting at 31.9 minutes was shown to be the (+)-(*S,S*)-(**115**) enantiomer.



(±)-(**115**) (*S,S*)-(-)-(**124**) (+)-(**115**)

Reagents and conditions: (i) (-)-quinine, ethanol, reflux, 10 min; recrystallise; (ii) 1:4 sulphuric acid:water, diethyl ether, reflux, 50 °C, 17 h, 17% over 2 steps.

Scheme 48

Polymerisation of diacid (±)-(**115**) with butane-1,4-diol (**4**) was performed under solvent-based conditions at 60 °C in toluene using the modified Dean and Stark system, in the presence of *Novozyme 435*®. Sampling at intervals up to 22 hours and monitoring by TLC

revealed that the reaction was very slow, which was subsequently confirmed by GPC analysis of the samples; a final confirmation by derivatisation as the methyl ester as before showed the recovered starting material (**115**) to be racemic.

Small-scale solvent-based polymerisations of diols (±)-(**116**) and (**119**) with adipic acid and acids (±)-(**118**) and (**121**) with butane-1,4-diol (**4**) were performed in the presence of *Novozyme 435*® to verify which were substrates for polymerisation by CALB. All were found to be substrates for the enzyme, both the diols (±)-(**116**) and (**119**) proceeding to high molecular weights. Interestingly, the latter two polymerisations had almost identical molecular weight averages, suggesting that the two diols are polymerised at similar rates to one another by CALB (Table 25). This could be confirmed by time-course sampling of the reactions, to check that the reactions are not at equilibrium at the point of sampling, the apparent similarity of rates then not being valid. The *cis* diacid (**121**) polymerised much more rapidly than the *trans* diacid (±)-(**118**), meaning a potential method of separation of the two may have been found.

Small-scale toluene-based polymerisation of the hexane-1,6-diol mimics (±)-(**122**) and (**123**) with adipic acid led to the formation of tiny amounts of oligomeric material (M_w 167, M_n 123, M_w / M_n 1.4 and M_w 188, M_n 170, M_w / M_n 1.1 respectively), suggesting that the size or shape of these diols was incompatible with the active site of CALB. No further polymerisation studies were therefore performed on these two substrates.

substrate 1	substrate 2	M_w	M_n	M_w / M_n
(±)-*trans*-1,2-cyclohexane diacetic acid (±)-(**118**)	butane-1,4-diol (**4**)	612	224	2.7
cis-1,2-cyclohexane diacetic acid(**121**)	butane-1,4-diol (**4**)	1690	830	2.0
(±)-*trans*-1,2-cyclohexane dimethanol (±)-(**116**)	adipic acid (**3**)	1697	815	2.1
cis-1,2-cyclohexane dimethanol (**119**)	adipic acid (**3**)	1537	463	3.3

Table 25

As all the substrates tested in Table 25 were substrates for CALB, the two chiral molecules (±)-(**118**) and (±)-(**116**) were polymerised again, this time sampling for chiral GC

analysis, to verify whether *Novozyme 435®* was enantioselective towards either of the molecules. Chiral GC separation systems were developed. (±)-*trans*-1,2-Cyclohexane dimethanol (±)-(**116**) was derivatised as the diacetate using acetic anhydride and pyridine. After purification by flash column chromatography, the two enantiomers were successfully separated (CP-Chirasil-Dex-CB column, oven temperature 150 °C, detector temperature 250 °C, injector temperature 250 °C, elution times 30.0 and 31.2 minutes). Similarly, by derivatisating the diacid (±)-(**118**) as its methyl ester using trimethylsilyl diazomethane, separation by chiral GC was also achieved (CP-Chirasil-Dex-CB column, oven temperature 130 °C, detector temperature 250 °C, injector temperature 250 °C, elution times 52.4 and 53.2 minutes).

system	sample time / h	M_w	M_n	M_w / M_n†	ratio of peak areas - 31.2:30.0 min
solvent-free	0.5	184	138	1.3	1:1.01
solvent-free	1	179	137	1.3	1:1.02
solvent-free	2.25	217	156	1.4	1.01:1
solvent-free	4	227	152	1.5	1:1.02
solvent-free	22	568	310	1.8	not determined
solvent-free	48	1173	544	2.2	1.01:1
toluene-based	0.5	385	313	1.2	not determined
toluene-based	1	482	338	1.4	1.03:1
toluene-based	2.25	467	388	1.2	1.02:1
toluene-based	4	509	424	1.2	1.19:1
toluene-based	9	613	495	1.2	1.22:1
toluene-based	20	958	760	1.3	not determined

†low dispersities apparent are due to narrower-than-usual measurement of molecular weight averages, avoiding influence of starting materials.

Table 26

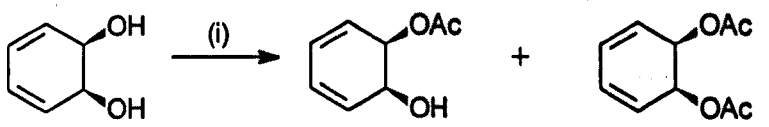
Polymerisation of (±)-*trans*-1,2-cyclohexane dimethanol (±)-(**116**) with adipic acid (**3**) was undertaken in solvent-free and solvent-based conditions. In the former case, the reaction was performed at 70 °C throughout, as the melting point of the diol (**116**) is approximately 60 °C. Both systems used larger doses of *Novozyme 435®* than for the standard processes, to allow rapid reaction and sampling to be required over a single day. For the toluene system, a set of six identical reactions at 60 °C were performed in sealed vials, in order that each could be halted after the desired interval, the unreacted starting material recovered and isolated prior to derivatisation. For the neat reaction, aliquots were sampled at the appropriate intervals and the starting diol isolated and purified prior to derivatisation. The results are shown in Table 26.

Clearly, the selectivity of *Novozyme 435®* towards diol (±)-(**116**) is very low at the elevated temperatures used in both systems, although the extent to which polymerisation occurs in both is high. In an effort to raise the enzyme selectivity, the toluene-based reactions were re-run at ambient temperature. The results are shown in Table 27, demonstrating that the reaction slows to an almost negligible rate, despite the presence of the same amount of catalyst as for the elevated temperature solvent-based runs (10wt.%). In view of the very low conversion achieved, no determination of the ee of recovered starting material was made.

sample time / h	M_w	M_n	M_w / M_n	**ratio of peak areas - 31.2:30.0 min**
16.5	114	107	1.1	not determined
23	128	110	1.2	not determined
47	119	109	1.1	not determined
90	128	112	1.1	not determined

Table 27

CALB has, however, been shown to asymmetrise the *meso* diol *cis*-1,2-dihydroxycyclohexa-3,5-diene (**125**) with moderate discrimination, producing monoacetate (*R*)-(**126**) in 89% yield and 70% ee along with small amounts of diacetate (Scheme 49).[144]



(125) **(-)-(126)** **(127)**

Reagents and conditions:[144] (i) *Novozyme 435®*, *tert*-butyl methyl ether, vinyl acetate, 40 °C.

Scheme 49

Clearly, (±)-*trans*-1,2-Cyclohexane dimethanol (±)-(**116**) is not resolved to any significant degree by CALB when adipic acid is used as acylating agent. Although (**125**) may have greater rigidity in the ring, the key factor may be the homologated chains of diol (±)-(**116**) which impart greater flexibility to the molecule and perhaps enables a good fit into the active site for both enantiomers. Whilst the alcohol-accepting part of the lipase active site may not be selective towards the diol (±)-(**116**), the acid section was believed to be more discriminating. Hence, the polymerisation of the diacid (±)-(**118**) with butane-1,4-diol (**4**) was performed despite the results obtained from the diol (±)-(**116**) polymerisation trials.

The polymerisation of diacid (±)-(**118**) with butane-1,4-diol (**4**) was performed in toluene in sealed vials at 60 °C, an inadequate amount of material being available for solvent-free studies. Sampling of the reaction after 0.5, 1, 2 and 4 hours for GPC and chiral GC analysis indicated that the reaction was proceeding very slowly, the samples remaining monodisperse. Whilst a high enzyme dose was used, it appears that a much higher level was needed in order to sample the reaction early on in the process. Time precluded further polymerisation studies on diacid (±)-(**118**).

Very recently, Kobayashi used CALB to perform bulk phase ring-opening polymerisation reactions on racemic lactones.[53] Whilst the lipase was an efficient catalyst for the transformations, no resolution of the lactones was observed during the course of the reaction.

2.3.6 Multibranched polymers

2.3.6.1 Introduction

Multifunctional polycondensations are reactions in which at least one of the substrates contains at least three reactive functionalities. The simplest example involves self-

polycondensation of a single substrate of AB_2 structure, where A = acid function, B = alcohol function, for example. Other examples are polycondensation of A_2 and B_3, A_3 and B_3, *etc.*

Hyperbranched and dendritic polymers are both types of multibranched polymers. The former are prepared from AB_2 substrates, either alone or in combination with AB substrates and are branched irregularly. An hypothetical example is shown in Figure 16.[145]

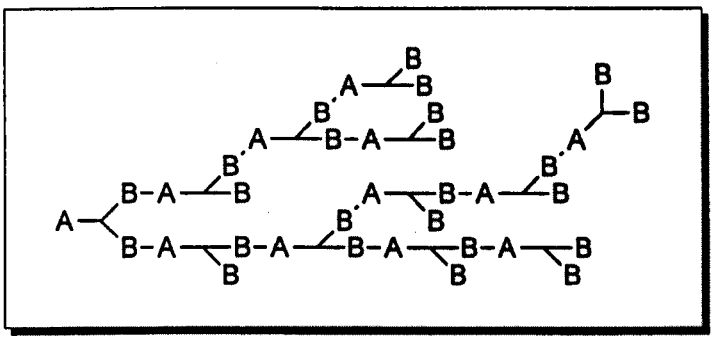


Figure 16

On every addition of a monomer unit further branching occurs, that is the polymer never becomes cross-linked. Such hyperbranched polymers may be synthesised in one-pot reactions.

Dendritic polymers are synthesised *via* stepwise condensations, often involving protection / deprotection methodology to give a very uniform structure. Consequently, they are not formed in multifunctional polymerisations, but by a series of bifunctional reactions using multifunctional monomers. Protection strategies can be avoided if the monomer is employed in large excess in each sequential synthetic step, the unused material being recovered; this leads to equal branch lengths and hence uniformity of constitution. A beautiful example of a dendrimer formed from three successive etherification steps ("third generation dendrimer") of 3,5-dihydroxybenzyl alcohol is shown in Figure 17,[146] the hydroxyl end groups capped as benzyl ethers.

Multibranched polymers have aroused considerable interest as a result of their unusual physical[147] and, more recently, biological[148] properties. Also, the ability of acid functionalised polybenzylethers to act as organic buffers has been demonstrated.[124] Importantly, hyperbranched polymers have been shown to possess many of the physical characteristics of dendrimers, but may be prepared at a fraction of the cost of multi-stage dendrimer synthesis.

Lipases, with their powerful substrate specificities might lend themselves to the creation of dendritic polyesters: the restricted size of the active site of the enzyme could lead

to an upper limit in molecular size / shape of acceptable substrate. In this way, a uniform polymer with equal branch lengths might be obtained, rendering protection / deprotection methodologies unnecessary.

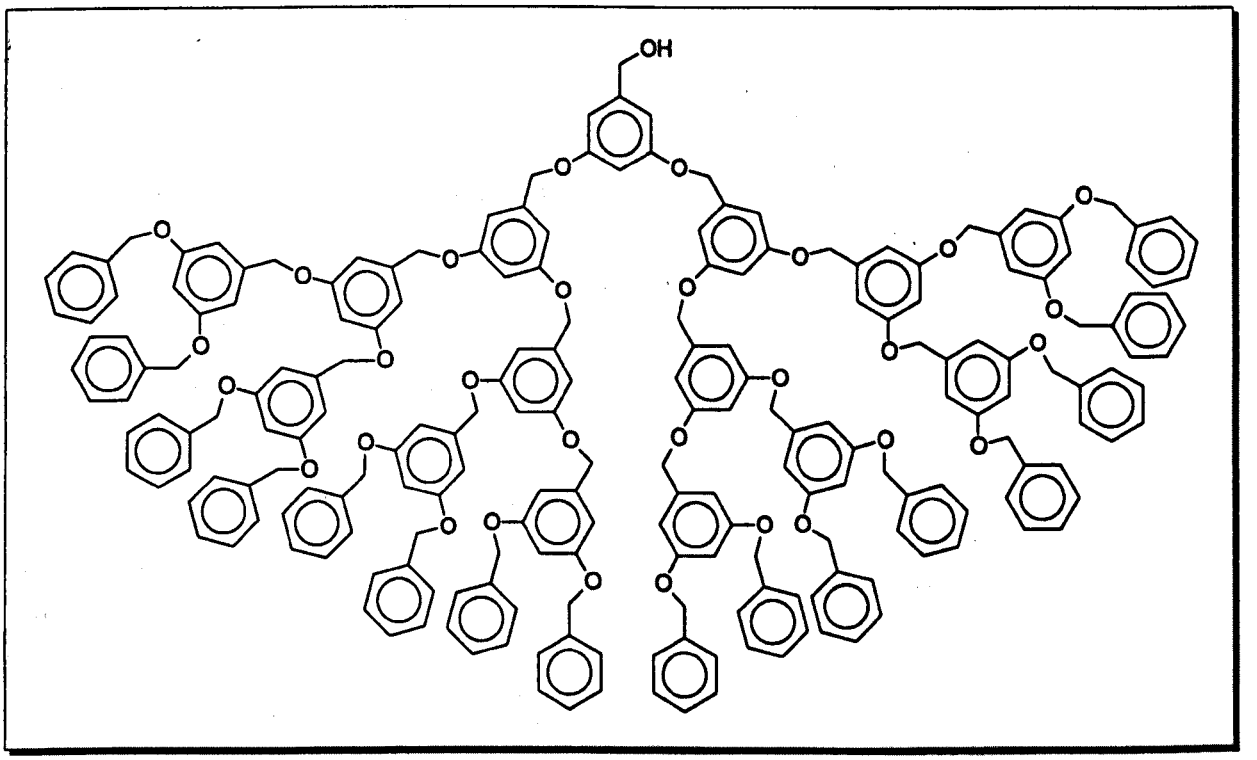
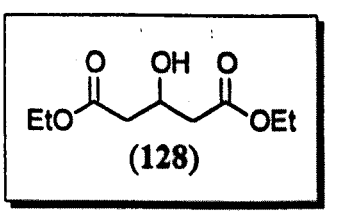


Figure 17

2.3.6.2 Choice and synthesis of substrates for trials

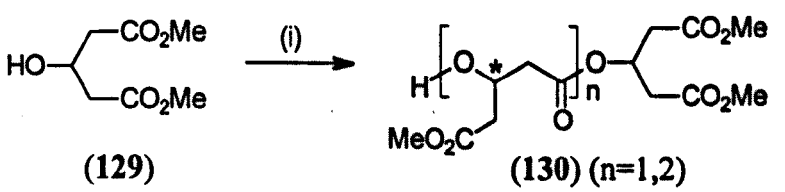
A simple and commonly used substrate for hyperbranched polyester synthesis is the commercially available diethyl 3-hydroxyglutarate (**128**). This is an AB_2-type substrate, containing a secondary hydroxyl group. Conventional methods have been employed to self-polymerise (**128**), whilst an enzymatic method has been employed to polymerise the dimethyl ester (**129**).



(128)

Feast[149] recently used a conventional titanium catalyst in high temperature processes and characterised the products formed from **(128)** using MALDI-TOF spectrometry as well as the more usual NMR spectroscopy and GPC analysis. Even at high temperatures and in the presence of 5wt.% titanium tetrabutoxide, the monomer was converted into low weight oligomers only. Also, considerable amounts of exchange of alkoxide ligands in the catalyst with those of the terminal ester groups competed with the polymerisation.

Gutman had studied enzymatic oligomerisation of **(129)** as early as 1989,[27] screening a variety of lipases and proteases for activity. Horse and pig liver acetone powders and the protease from *Streptomyces griceus* were found to be most active. The work was directed towards asymmetrisation of the prochiral diester **(129)**, hoping to achieve the theoretical 100% incorporation of a single enantiomer attainable from prochiral substrates. The polymerisations were found to be exceedingly slow, 10 days being required to form predominantly a trimer of **(129)**. The dimers and trimers **(130)** (n=1 and 2) prepared showed optical activity resulting from the formation of the new chiral centre(s) (asterisked) (Scheme 50).



(129) **(130)** (n=1,2)

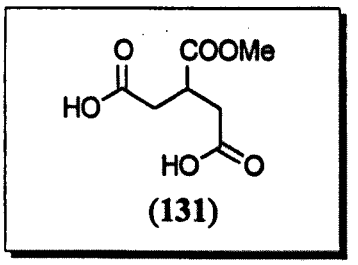
Reagents and conditions:[27] (i) enzyme, 40-69 °C, 10 days.

Scheme 50

However, in a recent review by Gutman,[150] no further advances or exploitation of this methodology were mentioned.

The apparent lack of powerful prochiral selectivity (see Section 2.4) and enantioselectivity shown by CALB towards many substrates suggested that such a lipase might catalyse the formation of higher molecular weight oligomers than those seen by Feast or Gutman and more rapidly than in the latter case. The polymerisation work performed is reviewed in Section 2.3.6.3.

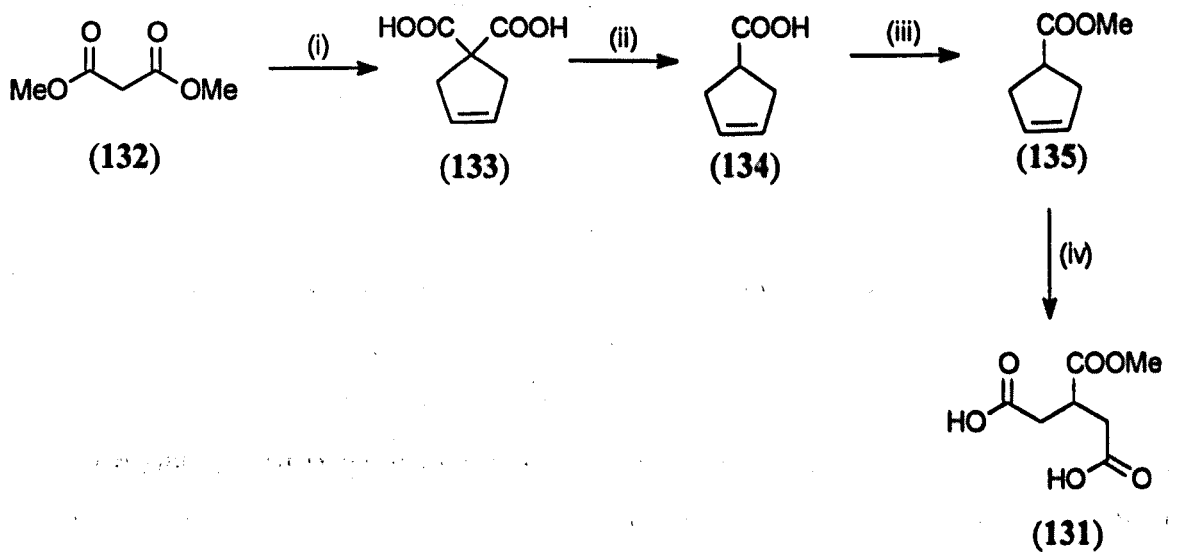
Another molecule chosen for hyperbranching study is the ester diacid **(131)** which has a glutaric acid backbone with an ester group branch at the 3-position.



(131)

Although the free triacid of **(131)**, tricarballylic acid, is commercially available, an indication of whether esterification on the branch acyl group was occurring in the presence of CALB was desired. Clearly, this acid group is not equivalent to the other two which are a methylene unit further from the central CH unit. By polymerising the methyl ester **(131)**, loss of the methyl singlet in the ^{1}H NMR spectrum would indicate that the branch group was capable of acylating CALB and therefore reacting with a polyol.

The synthesis of **(131)** cannot be performed by starting with the corresponding free triacid, as all three acid groups could potentially be esterified. An alternative synthesis was therefore chosen, the pivotal step of which was oxidative cleavage of methyl 3-cyclopentene-1-carboxylate **(135)**.



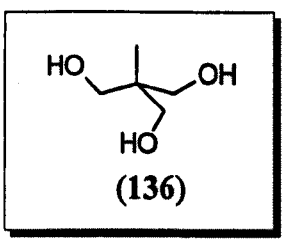
(132) **(133)** **(134)** **(135)** **(131)**

Reagents and conditions: (i) a. lithium hydride, DMPU, THF, 0 °C to ambient temperature, 4 h; b. *cis*-1,4-dichloro-2-butene, 40 °C, 22h; c. water, lithium hydroxide monohydrate, ambient temperature, 20 h, 91% overall; (ii) 170 °C, 1.5 h; distil, 75%; (iii) DCC, methanol, DMAP, ambient temperature, 21 h, 57%; (iv) potassium permanganate, Aliquat® 336, toluene, water, 0 °C to ambient temperature, 1.5 h, 61%.

Scheme 51

3-Cyclopentene-1,1-dicarboxylic acid (**133**) was prepared in excellent yield according to the method of Greene,[151] by treating dimethyl malonate (**132**) with lithium hydride and DMPU followed by *cis*-1,4-dichloro-2-butene. Heating (**133**) to melting brought about decarboxylation to give 3-cyclopentene-1-carboxylic acid (**134**) in good yield after distillation;[151] the methyl ester (**135**) was then formed using standard DCC coupling methodology. Oxidative cleavage of the C-C double bond of (**135**) was accomplished using a modified method of Starks;[152] treatment of ester (**135**) with a fourfold excess of potassium permanganate in toluene / water in the presence of a catalytic amount of Aliquat® 336 gave the diacid (**131**) in acceptable yield. No further purification of (**131**) was required (Scheme 51).

The final choice of substrate, in this case for dendrimer formation, was another commercially available starting material, the triol 1,1,1-tris(hydroxymethyl)ethane (**136**).



(136)

Polymerisation of (**136**) with adipic acid (**3**) would be expected to proceed fairly rapidly, as (**136**) contains only primary hydroxyl groups. The fact that the alcohol functionalities are close to a quaternary carbon centre, however, might sterically encumber the molecule enough to prevent its entry to the lipase active site. If several esterifications are possible before the molecule becomes too large to fit into the active site, a regular 'star' polymer might result. Clearly, though, there is the possibility that the molecule may be too large at a point where the three branches are not all of the same length; in this case, the resulting oligomer would not be dendritic.

2.3.6.3 Enzymatic polymerisations of substrates

Self-polymerisation of diethyl 3-hydroxyglutarate (**128**) was performed under solvent-free and toluene-based conditions, catalysed by *Novozyme 435*®. Inspection of ^{1}H NMR spectra from the solvent-free system showed a CHOH:CH_2OCOR ratio of precisely 1:4, indicating that no reaction had occurred. If only a dimer were formed, this ratio would change

to 1:6 (excluding the CHOCOR signal). GPC confirmed that only a minimal formation of an oligomeric species occurred in the toluene case (M_w 199, M_n 155, M_w / M_n 1.3)

The absence of self-polymerisation suggested that either the ester groups of (**128**) could not acylate CALB or that the secondary alcohol in (**128**) was incapable of attacking the acyl-enzyme complex formed. However, Gotor and co-workers had shown that in solvent-based conditions monoaminolysis of (**128**) would take place in the presence of CALB.[153] The options were distinguished though, by performing a solvent-free polymerisation of (**128**) with butane-1,4-diol (**4**). Any reaction shown in this instance would confirm that (**128**) could acylate CALB and that the sterically-hindered secondary alcohol of (**128**) was the factor preventing self-polymerisation.

GPC analysis on the reaction mixture (M_w 199, M_n 155, M_w/M_n 1.3) after a 17 hour standard polymerisation showed the formation of a new component presumed to be the monoester of (**128**) and (**4**) (AB). A small amount of another higher mass component was also seen, indicating that acylation of the enzyme by (**128**) was indeed occurring, although the reaction was very slow, even in the presence of a primary alcohol. ^{1}H NMR spectroscopy indicated that only a small amount of oligomeric material had formed.

Polymerisation of diacid (**131**) with butane-1,4-diol (**4**) was undertaken in solvent-free conditions using *Novozyme 435*® as catalyst. After 17 hours, ^{1}H NMR spectroscopy gave a resonance at 4.15 ppm, indicative of ester formation. The ratio of signals CH_2OCOR:$CHCH_2COOH$ was ~1:1 implying that approximately half the starting diacid had reacted. That the methyl ester had not reacted at all could not be determined, due to the coincidence of the MeOCOR signal with the CH_2OH signal of butane-1,4-diol (**4**). Repeating this work with larger quantities of lipase and for extended periods could presumably give higher oligomeric species.

Polymerisation of triol (**136**) with adipic acid (**3**) was attempted under toluene-based conditions only, in a modified Dean and Stark apparatus over a 22 hour period; solvent-free methods could not be employed due to the high melting point of the triol. Unfortunately, GPC analysis of the reaction mixture indicated that no reaction had occurred (M_w 200, M_n 195, M_w/M_n 1.0). The proximity of the hydroxyl groups to the quaternary centre of (**136**) may have prevented entry of the material into the active site of CALB. Use of a triol with only a tertiary

carbon centre or where the alcohol groups are displaced further away from the quaternary centre may allow entry to the active site.

2.3.7 Concluding remarks

Efforts towards the synthesis of new polyesters containing sensitive functional groups has led to some success, although clearly *Novozyme 435®* shows little enantioselectivity towards the substrates studied in this section. Whilst only modest interest appears to have been shown in the literature for incorporating unsaturation and pendant functional groups into polyesterification substrates, Russell and co-workers[154] have very recently further demonstrated the potential that biocatalysis may have for polymerisation of such substrates. Polyesterification of various triols with divinyl adipate led to polyesters with pendant hydroxyl groups along the polymer chain of molecular weight averages (M_w) up to 13000 Da.

2.4 ASYMMETRISATIONS AND RESOLUTIONS OF 2-ALLYLPROPANE-1,3-DIOL-BASED SUBSTRATES CATALYSED BY CALB

2.4.1 Introduction

Asymmetric synthesis is the key area in which lipases have found applications over the past twenty years. The use of lipases in synthetic organic chemistry has been perpetuated and extended principally because of the high selectivity lipases show towards enantiomeric and prochiral molecules. In the former instance, (kinetic resolution) a lipase will catalyse the transformation of one enantiomer of a racemic mixture more rapidly than the other, in the ideal case giving complete conversion of one enantiomer and leaving the other untouched. Even in such a case, a maximum yield of 50% of the desired enantiomer can be obtained. Prochiral molecules may either have a mirror plane and contain an even number of chiral centres of opposite sense, in which case the molecule is said to be *meso*. Otherwise, it may possess no stereogenic centre, but have two identical groups, the transformation of one or the other of which leads to two enantiomeric molecules. Desymmetrisation of prochiral molecules is the more attractive technique in organic synthesis as a theoretical maximum 100% yield, 100% ee may be attained.

So much work has been performed using lipases in organic synthesis that it is impossible to provide an exhaustive review within the scope of this work. However, Xie[155] and more recently Theil,[156] Johnson,[157] Schmid[158] and Boland[159] have written useful reviews detailing many applications of lipases in asymmetric transformations.

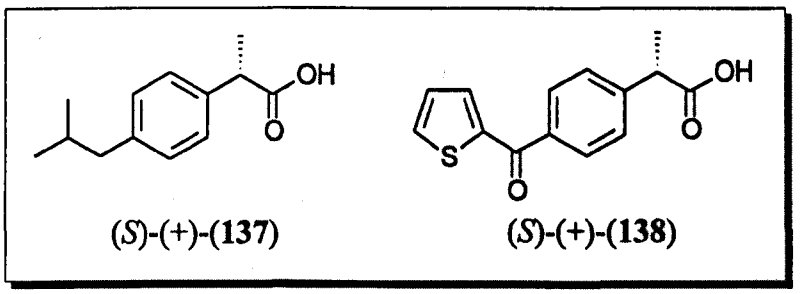
Because commercial preparations of CALB have only been available comparatively recently, exploitation of this enzyme in organic synthesis is not particularly well documented in the literature.

2.4.2 Applications of CALB in asymmetric synthesis

2.4.2.1 Kinetic resolutions

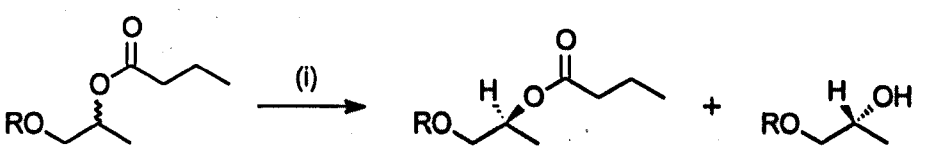
A major area of work using CALB has been to prepare the non-steroidal anti-inflammatory drugs (*S*)-ibuprofen (*S*)-(+)-(**137**)[160,161] and (*S*)-suprofen (*S*)-(+)-(**138**)[162] in

enantiomerically pure form *via* selective esterification of the (*R*)-isomer of the racemic mixture with aliphatic primary alcohols. In addition to performing the resolutions in anhydrous organic solvents, Lortie *et al.*[163] used a solvent-free system with molten substrates at 55 °C and salt solutions to control the water activity of the mixture, once again attesting to the resilience of CALB under seemingly harsh conditions.



(*S*)-(+)-(**137**) (*S*)-(+)-(**138**)

An attractive and often more convenient approach is use of a lipase to catalyse the resolution of the racemic ester *via* hydrolysis. Hydrolytic methods are commonly used as they often lead to higher enantioselectivities than esterifications; the aqueous medium drives the equilibrium towards acid formation; in esterification procedures, the water formed must usually be removed in order to prevent the reverse reaction occurring. Anthonsen and co-workers used *Novozyme 435*® to catalyse the hydrolysis of the butanoates of 1-phenoxy, 1-phenylmethoxy- and 1-(2-phenylethoxy)-2-propanol[164] (**139a-c**) (Scheme 52), giving resolved (*S*)-(**139a-c**) and free alcohol (**140a-c**).



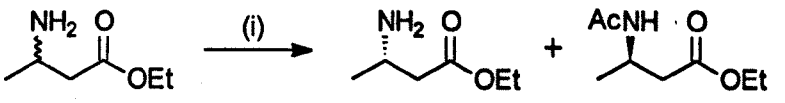
(±)-(**139a-c**) (*S*)-(**139a-c**) (*R*)-(**140a-c**)

Reagents and conditions:[164] (i) CALB, 0.05 M phosphate buffer (pH 7), ambient temperature, overnight. [(**a**) R = Ph, (**b**) R = CH_2Ph, (**c**) R = CH_2CH_2Ph].

Scheme 52

In all cases the reactions stopped at 50% conversion, none of the 'wrong' enantiomer being hydrolysed, indicative of the high selectivity CALB shows towards esters of secondary alcohols.

Gotor *et al.*[165] resolved (±)-ethyl 3-aminobutyrate (±)-(**141**) using CALB by selective acetylation of the amino group rather than hydrolysis of the ester group (Scheme 53).



(±)-(**141**) (*S*)-(**141**) (*R*)-(**142**)

Reagents and conditions:[165] (i) CALB, ethyl acetate.

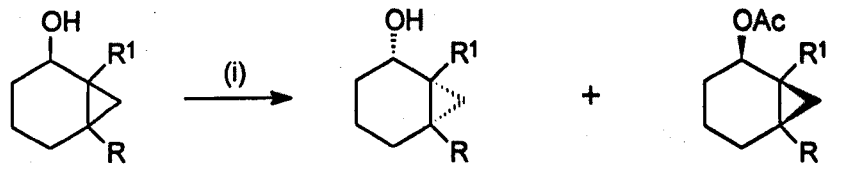
Scheme 53

With ethyl acetate used as acetylating agent and solvent alike and stopping the reaction at 53% conversion, the amidoester (*R*)-(**142**) was found to have 95% ee. Derivatisation of the remaining (*S*)-(**141**) aminoester using benzoxycarbonyl chloride was required to determine its ee, which was found to be 99%.

Resolution of secondary alcohols using *Novozyme 435*® has recently been thoroughly studied by Naoshima and co-workers, using both hydrolysis and esterification methods.[166] In the hydrolysis of the acetates of twelve different secondary alcohols, containing a variety of saturated, unsaturated and aromatic functionalities, CALB was seen to be highly enantioselective: indeed, an isolated yield of alcohol product of 30-40% and ee of >98% may be taken as representative of their findings. Comparison of CALB with other lipases of *Candida* sp. and *Pseudomonas* sp. showed CALB to be more highly enantioselective in almost every instance.

Esterification of some of the corresponding free alcohols with vinyl acetate in the presence of *Novozyme 435*® again verified the high enantioselectivity of CALB towards secondary alcohols, although the selectivity of hydrolysis was higher than esterification for corresponding substrates. Naoshima suggested that this may be attributed to a difference in enzyme conformation in the two different solvent systems used.

Blanco *et al.*[167] used *Novozyme 435*® to resolve a series of racemic endo-bicyclo[4.1.0]heptan-2-ols (±)-(**143a-c**) *via* esterification of the free alcohols. Lipases from *Pseudomonas cepacia* and *Mucor miehei* were found to be less efficient and selective than CALB for the transformations, the latter giving ees of recovered alcohol (*1R, 2S, 6S*)-(**143a-c**) of >97% at just over 50% conversion (Scheme 54).



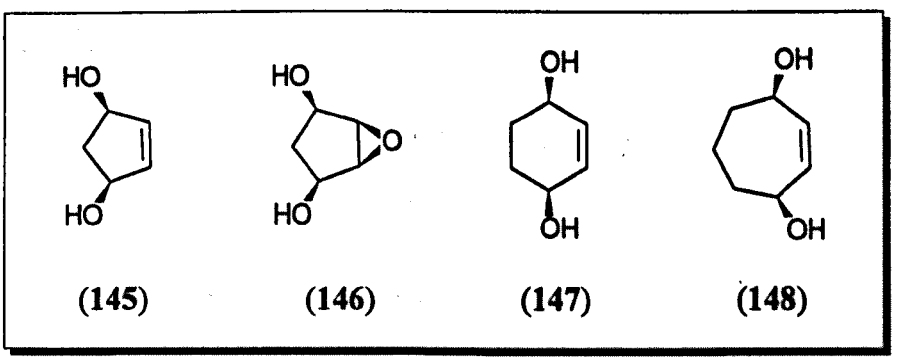
(±)-(**143a-c**) (*1R, 2S, 6S*)-(**143a-c**) (*1S, 2R, 6R*)-(**144a-c**)

Reagents and conditions:[167] (i) isopropenyl acetate, *tert*-butylmethyl ether, CALB, 37 °C, 0.75-1.25 h. [(**a**) R = H, R^1 = H, (**b**) R = H, R^1 = Me, (**c**) R = Me, R^1 = H].

Scheme 54

2.4.2.2 Asymmetrisations

Theil[168] has reviewed enantioselective acylation of diols catalysed by lipases, including asymmetrisations of prochiral diols. Of the many transformations detailed, CALB had only been shown to be efficaceous in the asymmetrisation of the *meso* diols (**145**), (**146**), (**147**) and (**148**).



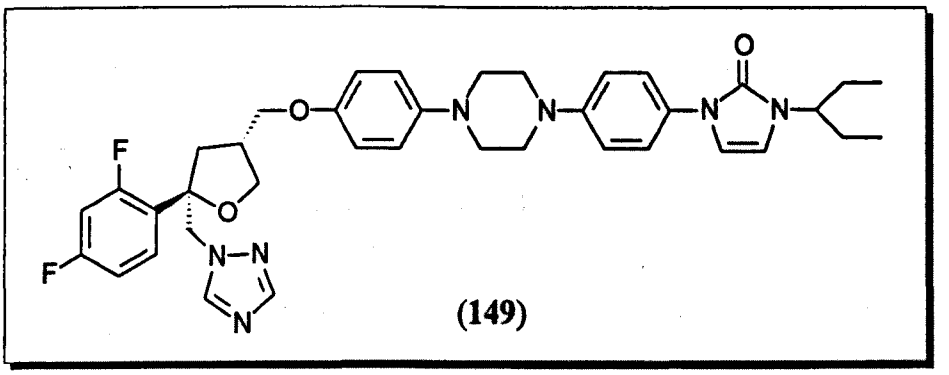
(**145**) (**146**) (**147**) (**148**)

The scarceness of asymmetrisations catalysed by CALB is further supported by Drauz and Waldmann[169] whose review of hydrolysis- and esterification-based asymmetrisations demonstrates the dominant position of PLE and PPL in the two reaction types.

The other predominant class of asymmetrisations studied in the literature are the 2-(mono- or di-)substituted propane-1,3-diols, which on asymmetrisation become important chiral building blocks for asymmetric synthesis. In the last decade, chemoenzymatic syntheses of the analgesics (-)-aphanorphine and (+)-eptazocine[170] and the aryloxypropylamine-type beta-blocker (*S*)-propranolol[171] from 2-substituted propane-1,3-diols have been developed. CALB has been used to asymmetrise pyridyl-substituted propane-1,3-diols by acetylation, the lipase showing moderate selectivity. However, PPL-catalysed acetylation of the diol and

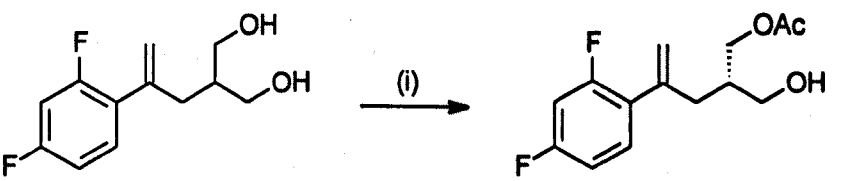
hydrolysis of the corresponding diacetate proceeded in a more selctive fashion, giving ees of up to 98% with 81% yield of monoacetate.[172]

Recently, Morgan *et al.*[173] used CALB in the chemoenzymatic synthesis of the broad-spectrum antifungal agent SCH51048 (**149**).



(149)

Novozyme 435® was found to desymmetrise the prochiral diol (**150**), a precursor to (**149**). Optimised scaled-up conditions used vinyl acetate as acetylating agent and acetonitrile as solvent at 0 °C to give monoacetate (*S*)-(**151**) (81%) and diacetate (17%); the monacetate (*S*)-(**151**) had an ee of 97% (Scheme 55).



(150) **(*S*)-(151)**

Reagents and conditions:[173] (i) vinyl acetate, acetonitrile, *Novozyme 435®*, 0 °C.

Scheme 55

Comparison of the work of Riva[172] and Morgan[173] indicates the variation of selectivity of CALB towards 2-substituted propane-1,3-diols. As work performed in Section 2.3.3.2 showed 2-allylpropane-1,3-diol (**105**) to be an excellent substrate for CALB, it was decided to perform studies on some asymmetrisations and resolutions of substrates based on (**105**).

2.4.3 Choice of reaction conditions

2.4.3.1 Effect of solvent

The addition of different co-solvents to the aqueous medium is now recognised to profoundly affect the enantioselectivity of enzyme-catalysed hydrolyses. Klibanov and co-workers studied the variation of enantioselctivity with added co-solvent of some proteolytic enzymes in the hydrolysis of esters of *N*-acetyl-L- and D-amino acids a decade ago.[174] Subsequently, the same group have made progress in rationalising the dependence of prochiral selectivities in enzyme-catalysed esterification and hydrolysis reactions; both experimental[83] and molecular modelling work[84] was used. The authors conclude that prochiral selectivity (and indeed any other type of selectivity) is a function of the ease of desolvation of the substrate, in this instance the desolvation of the transition states of *pro-S* and *pro-R* orientations of the prochiral substrate. In forming the acyl-enzyme complex, the two orientations would displace different amounts of bound water from the enzyme into the surrounding medium. An apolar medium would favour repulsion of the water molecules more than a polar medium. Hence, the medium would favour the desolvation of the faster reacting orientation to different degrees, depending on its ability to dissolve the water released.

Anthonsen studied the effect of adding various co-solvents in the hydrolysis of racemic secondary butanoate esters catalysed by CALB.[81] Addition of many co-solvents of varying polarity led mostly to a decrease in the enantioselectivity of the reaction. However, certain solvents, in particular acetone and *tert*-butanol gave great enhancement of enantioselectivity. For both the latter solvents, addition of 25-30% solvent to the buffered reaction mixture gave optimal selectivity. Addition of 30% acetone to the esterification of the corresponding free alcohol with vinylbutyrate and CALB led to a significant increase in enantioselectivity. This work has subsequently been reviewed[175] by Anthonsen and Jongejan, although no further explanations for the observed changes in selectivity was offered.

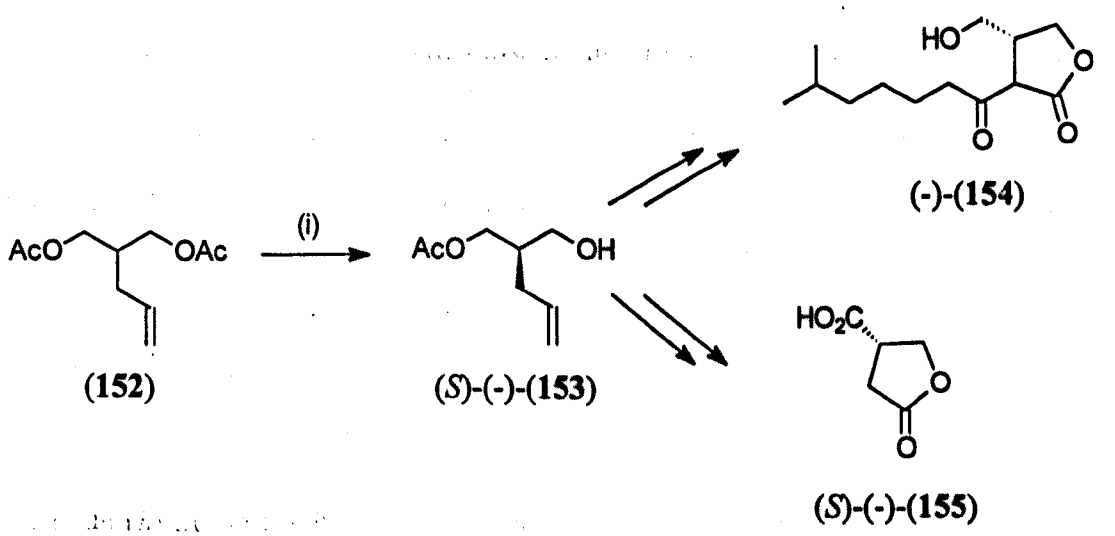
2.4.3.2 Choice of acylating agent

The process of esterification is reversible. In a resolution, the reaction of the faster reacting enantiomer approaches equilibrium more rapidly than the slower reacting enantiomer.

The net rate of reaction therefore slows, the concentration of the slower reacting enantiomer continuing to slowly decrease. When the faster reaction reaches equilibrium, the enantiomeric excess of the ester product will begin to drop as incorporation of the undesired ester enantiomer continues. Prolonged reaction will see hydrolysis of the ester and incorporation of the undesired enantiomer of the alcohol. Avoiding this reversal of reaction may be achieved either by using a large excess of acyl donor or, more commonly, an acyl donor which circumvents reversal of reaction.[176] The most commonly used irreversable acyl donor is vinyl acetate. On acetylation of an alcohol, the by-product formed is vinyl alcohol, which immediately tautomerises to acetaldehyde, so preventing attack of the alcohol formed on the product ester. Consequently, vinyl acetate was used in all cases in this work as acyl donor.

2.4.4 Asymmetrisations of 2-allyl-propane-1,3-diol (105)

Monoacetylation of 2-allyl-propane-1,3-diol has been accomplished in the literature; both methods *via* lipase-catalysed hydrolysis of the diacetate (**152**). Sih *et al.*[177] used (*S*)-(-)-(**153**) which formed to prepare microbial growth factor (-)-A-factor (-)-(**154**) whilst Mori and Chiba[178] subsequently employed the asymmetrisation as the key step in their synthesis of (*S*)-(-)-paraconic acid (*S*)-(-)-(**155**), a related compound to (-)-(**154**) (Scheme 56).



(**152**) (*S*)-(-)-(**153**) (-)-(**154**) (*S*)-(-)-(**155**)

Reagents and conditions: (i) PPL, 0.1 M phosphate buffer (pH 7);[177] lipase MY, acetone, Triton X-100, water, sodium hydroxide aliquots (pH 7).[178]

Scheme 56

Studies on the asymmetrisation of 2-allylpropane-1,3-diol (**105**) used a much higher wt.% of *Novozyme 435*® than the polymerisation reactions to limit reaction times to within a few hours. As Anthonsen[81] had shown that addition of *tert*-butanol as co-solvent to a *Novozyme 435*®-catalysed hydrolysis improved enantioselectivity, it was decided that *tert*-butanol would be used as a co-solvent. Also, as much of our polymerisation work has compared substrate-only (solvent-free) and toluene-based systems, a system using toluene as solvent and another where vinyl acetate acted as solvent and acylating agent were used. Other solvents tested were THF and, in view of the effects seen in polymerisations on addition of triethylamine, the latter was used both as solvent and as an additive in catalytic amounts. Indeed, Schick *et al.* had shown that without addition of triethylamine to the asymmetrisation of *cis*-2-cyclopentene-1,4-diol, the reaction proceeded very slowly.[120(a)] Also, Turner and co-workers had found that, as well as a drop in yield, *Lipozyme*® showed a lower enantioselectivity for the ring-opening of 2-phenyl-4-benzyl oxazolin-5(*4H*)-one in the absence of a catalytic amount of triethylamine.[80]

The reactions detailed in Table 28 were stirred until no further 2-allylpropane-1,3-diol (**105**) remained, whereupon the reactions were stopped and the monoacetates (**153**) and diacetate (**152**) were isolated using flash column chromatography. All reactions performed used the same amounts of diol (**105**), *Novozyme 435*® and vinyl acetate as acylating agent.

Inspection of the first three entries in Table 28 indicates that the progressive addition of triethylamine to the reaction in THF has an effect. Whilst all required approximately one hour for complete consumption of the diol (**105**), the presence of triethylamine clearly allows more rapid progression to the diacetate (**152**), even though the rate of formation of the monoacetate (**153**) appears largely unchanged. Triethylamine may act either by activating the lipase or by preventing deactivation of the lipase, the two cases being subtly different. Acetaldehyde, the tautomerised by-product of acetylation using vinyl acetate may act as an alkylating agent by forming Schiff's bases on ε-amino groups of lysine residues.[179] In so doing, a positive charge is removed from the enzyme, leading to possible deactivation. The triethylamine might prevent this deactivation, an effect manifested as a faster reaction rate.

Addition of toluene, *tert*-butyl methyl ether or no solvent (entries 4-6) lead to a similar situation to the use of THF alone (entry 1), where there is an approximately 2:1 excess of monoacetate (**153**) over diacetate (**152**) when diol (**105**) has been completely consumed,

indicating that the rates of formation of monoacetate in each case and diacetate in each case are very similar.

At 0 °C the rate of reaction slows dramatically, as expected, but the formation of much more diacetate (**152**) than monoacetate (**153**) suggests that the second acetylation is more rapid than the asymmetrisation step (entries 7-9).

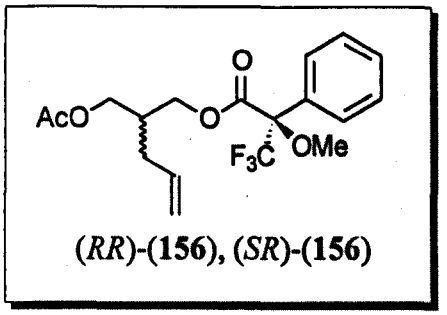
solvent system	**reaction time / h**	**temp. / °C**	**% yield of monoacetate (153)**	**% yield of diacetate (152)**	**overall % yield**
THF	1.0	25	40	24	64
THF / cat. triethylamine	1.0	25	20	42	62
THF / triethylamine	1.0	25	11	56	67
THF / *tert*-butyl methyl ether	1.2	25	33	18	51†
toluene	0.75	20	42	19	61
no added solvent	1.0	20	39	22	61
THF	6.0	0	30	40	70
THF / *tert*-butyl methyl ether	6.0	0	26	41	67
THF / *tert*-butanol	7.0‡	0	11	20	31

†artificially low yield, due to some spillage of reaction mixture;
‡starting material still present.

Table 28

The recovered monoacetates (**153**) were derivatised using enantiomerically pure (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride in the presence of DMAP, forming the two diastereomeric esters (*RR*)- and (*SR*)-(**156**).

Mosher[180] first suggested using (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride as a derivatising agent for chiral alcohols; the (*RR*) and (*SR*) diastereomers formed usually have non-equivalent NMR spectra, and use of ^{1}H or, more usually and simply, ^{19}F NMR spectroscopy may show the ratio of the two diastereomers present and hence the ratio of enantiomers present prior to derivatisation.



(*RR*)-(**156**), (*SR*)-(**156**)

Synthesis of the racemic alcohol (±)-(**153**) using acetic anhydride in pyridine followed by derivatisation with (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride gave a standard for NMR studies. ^{19}F NMR spectroscopy showed resonances for the CF_3 nuclei of (*RR*)-(**156**) and (*SR*)-(**156**) in the ^{19}F NMR spectrum at -70.8 and -71.9 ppm. A third smaller signal at -71.4 ppm was attributed to the slight excess of the derivatising agent used. Ratios of the two ester signals for the various reactions are given in Table 29.

solvent system	**temperature / °C**	**area of signal at -70.8 ppm**	**area of signal at -71.9 ppm**	**% ee of (153)**
THF	25	1.00	2.33	40
THF / cat. triethylamine	25	1.00	2.53	43
THF / triethylamine	25	1.00	1.73	27
THF / *tert*-butyl methyl ether	25	1.00	2.79	47
toluene	20	2.86	1.00	48
no added solvent	20	1.00	1.78	28
THF	0	1.11	1.00	5
THF / *tert*-butyl methyl ether	0	1.32	1.00	14
THF / *tert*-butanol	0	5.00	1.00	67

Table 29

Interestingly, despite the formation of increasing amounts of diacetate (**152**) with increase in triethylamine dose, the optical purity of the monoacetate (**153**) decreases, implying that the more rapidly formed enantiomer of (**153**) is also the more rapidly consumed for

diacetate (**152**) formation. Discrete study of the selectivity of the second acetylation step could be performed by resolving the racemic monoacetate (±)-(**153**) under identical conditions to those used in entries 1-3, halting each reaction at the same conversion. Derivatisation and analysis of the remaining starting material could then be accomplished as above.

Addition of *tert*-butyl methyl ether and the absence of solvent (Figure 16(a)) at ambient temperature appear to give similar selectivity for CALB as when THF alone is used.

Most interesting, however, is the apparent reversal of prochiral selectivity on use of toluene alone as the reaction medium. The result could be viewed as an artefact if inadequate amounts of derivatising agent were present; as the chiral monoacetate (**153**) reacts with a chiral agent, the rates of reaction of each enantiomer of (**153**) need not be identical. Therefore, faster reaction of the less abundant enantiomer could lead to the artificial appearance of reverse selectivity. This seems not to be the case, as the signal at -71.4 ppm can clearly be seen (Figure 16(b)). The apparent reversal of selectivity could otherwise be explained by different extents of conversion of the monoacetate (**153**) into diactetate (**152**) between systems: if the less abundant enantiomer of (**153**) formed reacts more slowly in the second acetylation step (as appears to be the case from entries 1-3) and much more diacetate is present in one system than another, the apparent selectivity towards that enantiomer is higher. However, when entries 5 and 6 are compared, it is seen that the extent of reaction in both cases is almost identical, precluding this possibility.

Reverse selectivity appears to have occurred again in entries 7-9, when the temperature was lowered to 0 °C, possibly indicating that the effect of toluene and lowering of temperature on the enzyme are similar. The two THF systems (entries 1 and 7) and *tert*-butyl methyl ether systems (entries 4 and 8) show a virtual swapping over of the ratio of monoacetate:diacetate making comparison of selectivity impossible. That is, the apparent reversal of selectivity with temperature may be due to:

- Change in prochiral selectivity, enantioselectivity remaining the same
- Same prochirality, the enantiopreference swapping
- Complimentary change in both prochiral selectivity and enantiopreference

Again, separation of these issues could be achieved by studying the resolution of racemic monoacetate (±)-(**153**) at different temperatures, and halting the reaction at the same conversion. Unfortunately, time did not allow such a set of trials to be carried out.

The concept of a switching of enantiopreference is not novel, the effect having been documented by Klibanov *et al.* recently.[82] γ-Chymotrypsin was used to catalyse the transesterification of methyl 3-hydroxy-2-phenylpropionate with propanol. In comparatively non-polar solvents such as toluene, hexane and octane γ-chymotrypsin showed a strong preference for the (*S*) enantiomer of methyl 3-hydroxy-2-phenylpropionate whilst in polar solvents such as acetone and propanol, the same enzyme showed a moderate preference for the (*R*) enantiomer. The result is linked again by the authors to the desolvation energies of the transition states of the reactions.

The acetylation performed at 0 °C in the presence of *tert*-butanol was incomplete after 7 hours. Isolation of the monoacetate (**153**) and diacetate (**152**) confirmed this, an overall yield of 31% being achieved. No further reaction appeared to occur within the last 2 hours of the reaction, suggesting that the lipase had been slowly deactivated over the course of the reaction, presumably by the *tert*-butanol. However, again the diacetate was present in about a two-fold excess, making the system unviable in spite of the comparatively high enantiomeric excess of (**153**) (Figure 16(c)).

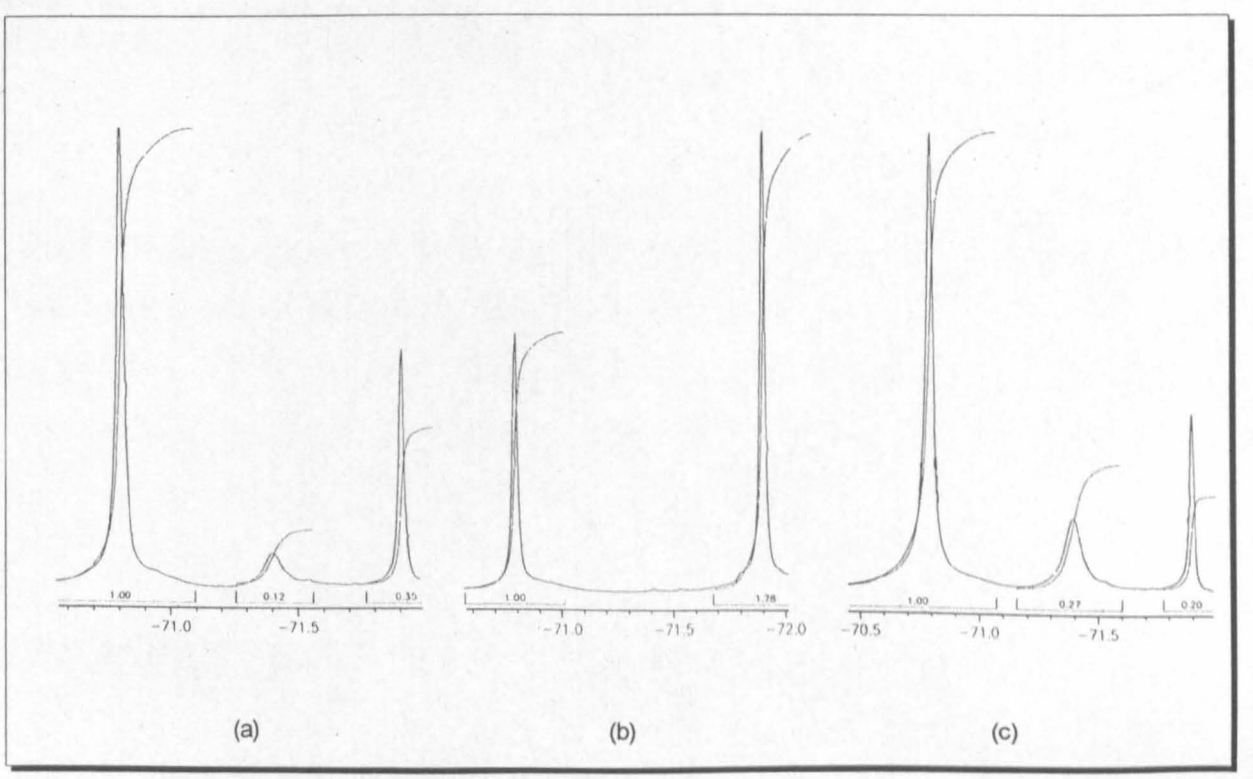
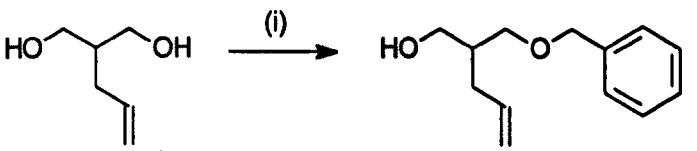


Figure 16

2.4.5 Resolution of (±)-2-Allyl-3-benzyloxypropan-1-ol (157)

2.4.5.1 Synthesis of substrate (157)

During investigations into the synthesis of (±)-2-(2',3'-epoxypropyl)propane-1,3-diol (**110**), 2-allyl-1,3-dibenzyloxypropane (**111**) was synthesised in order to prevent ring opening of the epoxide in (**110**) by attack of the nucleophilic alcohol groups present. By using less sodium hydride in the procedure, deprotonation of predominantly one alcohol group of (**105**) prior to benzylation with benzyl chloride was targeted, giving the monoprotected derivative (±)-(**157**) as the main product (Scheme 57).

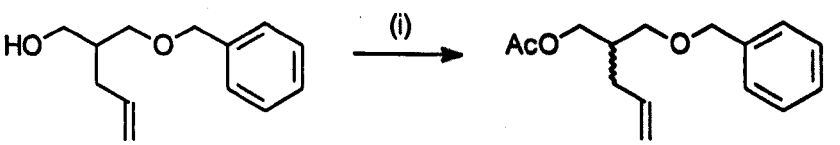


(**105**) (±)-(**157**)

Reagents and conditions: (i) a. sodium hydride, THF, 0 °C to ambient temperature, 30 mins; b. DMF, benzyl chloride, 0 °C to ambient temperature, 15 h, 71%.

Scheme 57

The racemic alcohol (±)-(**157**) was envisioned to be a good substrate for CALB, and suitable for attempting kinetic reolution by acetylation. Time permitted a single foray into this field, *i.e.* a resolution of (±)-(**157**) catalysed by *Novozyme 435®* using vinyl acetate in THF (Scheme 58).



(±)-(**157**) (*R*)-(**158**), (*S*)-(**158**)

Reagents and conditions: (i) vinyl acetate, THF, *Novozyme 435®*, ambient temperature, 1 h.

Scheme 58

The reaction was halted at ~50% conversion (estimated by TLC). Isolation and characterisation gave 50 μmol of acetate (**158**) and 53 μmol of recovered alcohol. Determination of ee was in this instance performed by chiral shift NMR using [Eu(hfc)$_3$] (**113**). Addition of 10 mol% [Eu(hfc)$_3$] (**113**) to the recovered alcohol (**157**) gave a separation

in the ^{1}H NMR spectrum of the signal at ~5.8 ppm (Figure 17(b)) corresponding to the $RCHCH_2C\underline{H}CH_2$ proton. Integration of the two signals showed a ratio of ~3.5:1 (Figure 17(a)), implying that the resolved alcohol had an ee of 56%. As the conversion was ~50%, no attempt was made to determine the ee of the acetate (**158**).

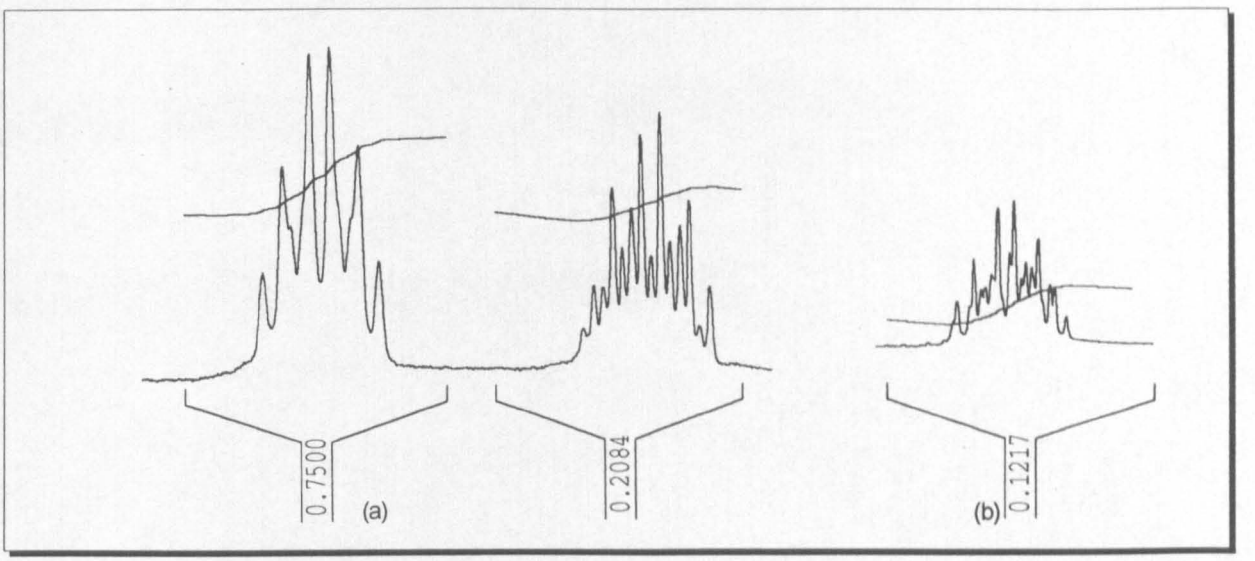


Figure 17

2.4.6 Concluding remarks

The work performed here has thrown light on some potentially interesting properties of CALB under different temperature and solvent conditions, although much remains unanswered. To fully understand the apparent changes in selectivity, further work mentioned earlier in this chapter needs to be performed. Trials in a wider range of solvents are also required in order to see trends in selectivity.

Unfortunately, whilst the variation in selectivity of CALB seems great, no asymmetrisations or resolutions attempted were selective enough to be synthetically useful.

CHAPTER 3

CONCLUSIONS AND FUTURE WORK

3.1 CONCLUSIONS

The prime objective of the present work was the elucidation of the mechanism of polymer assembly operating during the solvent-free enzymatic polyesterification of adipic acid (**3**) with butane-1,4-diol (**4**), catalysed by lipase B from *Candida antarctica*. The synthesis and polymerisation of key oligomers formed during the process in the presence of the lipase led to the conclusion that transesterification is disfavoured under the solvent-free conditions used; comparison with a solvent-based procedure showed that transesterification could occur. In this way, a suggested predominant pathway for polymer formation was proposed (Scheme 23), with a more limited and regulated assembly than the accepted "step" polymerisation route of conventional high temperature systems (Scheme 2).

Differences in the physical characteristics of polymer synthesised using the enzymatic method compared to the conventional high temperature processes have also been explained by examination of the polymer using techniques such as MALDI-TOF mass spectrometry.

Further studies on the solvent-free enzymatic process led to employment of new additives which appear to protect the lipase from the harmful deactivating effects of the highly polar reaction medium, enabling recovery and subsequent recycling of the immobilised lipase. The phenomenon of leaching of free enzyme off its support has been shown to occur and it is highly probable that a small amount (*ca.* 10%) only is available for leaching, regardless of the polarity of the surrounding medium. A trend of heightened deactivation of the lipase with increasing medium polarity has been shown to exist and possible explanations for this trend have been provided.

Exploiting the mild temperature conditions of the solvent-free enzymatic polyesterification procedure, attempts were made to synthesise polyesters containing functionality which would be lost in the conventional high temperature process. Attempts to polymerise unsaturated acids revealed that they could act as their own acid catalyst at 60 °C, giving polyesters. Unfortunately, the lipase did not further catalyse the polymerisation, possibly because of the high acidity of the substrates.

Diols and diacids / diesters containing epoxide groups and pendant unsaturation were shown to be substrates for CALB, indicating the potential use of lipases in speciality polymer synthesis. Attempts to form regulated dendritic and chiral polyesters were less successful, in the former case because of the slowness of the polymerisations due to poor substrate acceptability and in the latter case because of the poor enantioselectivity of CALB.

Further studies on the selectivity of CALB revealed only a modest selectivity in the asymmetrisation of a 2-substituted propane-1,3-diol; interestingly, this selectivity appears also to switch from *pro-R* to *pro-S* in the presence of different solvents. Explanations for this behaviour from various authors has been cited.

In summary, the faith shown by Baxenden Chemicals Ltd. in their enzymatic process appears to have been well-founded. Certainly, the substrate range for CALB has been shown to be wide, and the additive experiments performed suggest that substrates which polymerise but deactivate the lipase (*e.g.* diethylene glycol (**82**)) may still have potential applicability to the enzymatic system.

The much lower temperatures used are still the predominant advantage of the enzymatic system, and along with the lack of inorganic catalyst provides a process which is much more environmentally sound than the conventional systems. The presence of free leached lipase in the final product may be a disadvantage, as this may catalyse the hydrolysis of the polyester, leading to poor product stability; overcoming this obstacle is facile, however: flash heating of the product at 200 °C denatures the lipase completely, leading to good hydrolytic stability for the materials tested so far. The presence of lipase in the product might have a distinct advantage in applications where biodegradability was deemed an advantage: why not exploit the lipase in the preparation and disposal of the polymer?

3.2 Future Work

The semi-quantitative oligomer polymerisation studies have provided an insight into the mechanism of polymerisation catalysed by CALB. The poor initial solubility of adipic

acid (3) in the diol at the start of the process make conventional kinetic analysis of the rates of polymerisation of the various oligomers impractical. However, by employing a large excess of diol in the reaction (which is effectively the situation at the start of the process) a mimicking of the conditions in the solution phase of the reaction may be obtained. As the mixture is homogeneous, classical Michaelis-Menten kinetics[181] may be used to determine the initial rate of reaction. This work is currently being studied by A. Taylor, and may give further useful information on the key early steps of the enzymatic polyesterification process.

Whilst *Novozyme 435®* has proven useful in the synthesis of unusual polymers possessing additional functionality both on and pendant from the backbone, the low enantioselectivity of CALB for chiral primary alcohols has also been demonstrated. Clearly, other lipases could be employed to attempt combined polymerisation-resolution reactions.

Scale-up of the successful preparations of epoxy-functionalised and unsaturated polyesters could give enough material for applications testing and a more thorough range of cross-linking experiments.

Whilst the use of CALB in the synthesis of dendrimers was unsuccessful in the preliminary trials attempted in the present work, use of starting monomers where the functional groups for polyester formation lay further from the sterically-hindered branching centre might prove better substrates for the lipase. Such materials could be generated simply *via* homologation of shorter chained substrates.

CHAPTER 4

EXPERIMENTAL

General Experimental Methods

All starting materials were purchased directly from commercial suppliers and used without further purification unless otherwise stated. Butane-1,4-diol and hexane-1,6-diol were gifts from Baxenden Chemicals Ltd.

Novozyme 435® contains Lipase B from the fungus *Candida antarctica*, an organism which lives near hot springs in Antarctica, hence the tolerance of the lipase for high temperatures. *Novozyme 435®* is prepared using recombinant DNA technology; the gene coding for the lipase is transferred from a strain of *Candida antarctica* into the host organism *Aspergillus oryzae*. The enzyme produced is immobilised by adsorption onto a macroporous acrylic resin.[103] *Novozyme 435®* was purchased from Novo Nordisk Ltd., Denmark.

Chirazyme® was purchased from Boehringer Mannheim GmbH.

All aqueous inorganic reagents were previously prepared to the stated concentrations or as saturated aqueous (sat. aq.) solutions. Brine refers to a saturated aqueous solution of sodium chloride.

Petrol refers to the light petroleum fraction of b.pt. 40-60 °C, and was distilled prior to use; diethyl ether and THF were distilled from sodium benzophenone ketyl; dichloromethane was distilled from calcium hydride. Acetonitrile, DMF, DMSO, methanol, pyridine and toluene were purchased from Aldrich Chemical Co. as anhydrous solvents and stored under nitrogen. Triethylamine was distilled from potassium hydroxide and stored over potassium hydroxide pellets under nitrogen. Benzene was dried over sodium wire.

TLC was performed on Merck 60F - 254 (0.25 mm thickness) glass-backed silica plates; visualisation was achieved using either ethanolic *p*-anisaldehyde, ceric ammonium molybdate or potassium permanganate followed by heating or by UV light. Flash column chromatography was performed using Merck 60 - silica gel (0.040-0.063 mm particle size).

^{1}H and ^{13}C NMR spectra were recorded on Bruker AC200, Varian 300 Gemini 2000, Bruker AMX400 or Varian Inova 600 spectrometers. Chemical shifts are referenced to

tetramethylsilane (TMS) or the residual protonated solvent peak. Coupling constants are in Hz and quoted to the nearest 0.5 Hz. Where necessary, spectra were assigned on the basis of DEPT and COSY 2-D NMR experiments. ^{19}F NMR spectra were recorded on a Bruker WM250 spectrometer.

Low resolution mass spectra were obtained from double-focussing magnetic sector VG7070E (CI, FAB) or Trio 1000 (EI) mass spectrometers. For CI spectra, the carrier gas is specified. High resolution mass spectra were obtained on a VG7070E mass spectrometer. MALDI-TOF spectra were recorded on a micromass TofSpec machine, using an alpha cyano layered matrix, polyester concentration in solution added ~1 $mgmL^{-1}$ in THF.

Optical rotations were obtained using an AA-1000 'optical activity' polarimeter.

Infra-red spectra were recorded on a Perkin Elmer 881 spectrophotometer by the method stated.

Elemental analysis was performed on a Carlo Erba Model 1106 CHN elemental analyser.

Melting points were obtained on a Gallenkamp apparatus and are uncorrected.

m-CPBA purification: disodium hydrogen phosphate (3.58 g, 25.2 mmol) and potassium dihydrogen phosphate (0.39 g, 2.9 mmol) were dissolved in water (330 mL). Half of the solution was added to 57-86% pure *m*-CPBA (8.30 g, < 41 mmol) and the mixture filtered. The residue was dissolved in dichloromethane (100 mL) and the organic solution washed with the remainder of the phosphate buffer solution, dried (magnesium sulphate), filtered and concentrated *in vacuo* to give pure *m*-CPBA as a white fluffy solid (5.13 g, 29.7 mmol).

Methyl iodide purification: methyl iodide was passed through a plug of activated alumina and distilled at atmospheric pressure (b.pt. 42 °C) then stored at -40 °C under argon, protected from light.

Jones reagent preparation: chromium (VI) oxide (20.0 g, 0.20 mol) was added slowly to 8 M sulphuric acid (100 mL, 0.80 mol) with stirring until it had fully dissolved.

GPC was performed at Baxenden Chemicals Ltd. using a Waters 510 series HPLC pump with THF as the mobile phase. The columns (Polymer Laboratories Ltd.) were of dimensions 600 x 7.5 mm and packed with PL gel (polystyrene / divinylbenzene material, particle size 10 μm and pore size 50 / 100 Å (unless 500 Å specified)). The detector was a Waters model 401 differential refractometer, and estimates of molecular weight data were made using Waters Millennium GPC software from calibration against commercial polystyrene samples.

A solution of the polymer in THF is passed down the GPC column, which is packed with a polymer gel. Smaller molecules tend to spend more time in pores in the gel than the larger components, giving the former a longer elution time; hence, the technique relies on separation of species by virtue of their molecular volume. A differential refractometer monitors the elution of the components, giving a plot of intensity *versus* retention time. Because the relationship of molecular volume with retention time is logarithmic, the smaller components are much better resolved than the larger molecules. Discrete small oligomers can therefore be individually identified. As molecular weight is approximately proportional to molecular volume for structurally similar oligomers / polymer the correlation may be made in the systems studied in the present work.

Estimates of the number average (M_n) and weight average (M_w) molecular weights are obtained by calibration against commercial polystyrene samples. As the correlation is approximate, an experimental error of ± 10% is usually to be expected. M_n is effectively the mean molecular weight, whereas M_w is the modal average. A further value obtained from GPC is the M_w / M_n value, referred to as dispersity. This gives a measure of the spread of different weight components; hence, if $M_w = M_n$, the dispersity is 1, and the material is said to be monodisperse.

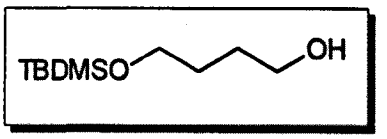
M_w is defined as $\Sigma M_i n_i / \Sigma n_i$

M_n is defined as $\Sigma M_i^2 n_i / \Sigma M_i n_i$

where i molecular species of mass M are each present in abundance n.

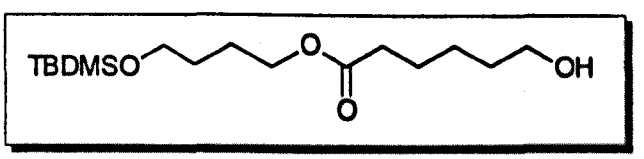
Synthesis of key oligomers (Section 2.1.2).

4-(*tert*-Butyldimethylsilyloxy)butan-1-ol (42).



Triethylamine (10.5 mL) and DMAP (820 mg, 6.71 mmol) were added to a stirred solution of butane-1,4-diol **4** (12.1 g, 134 mmol) in dichloromethane (100 mL) under an atmosphere of nitrogen at 0 °C. After 5 minutes, a solution of TBDMSCl (11.14 g, 73.9 mmol) in dichloromethane (60 mL) was added dropwise at 0 °C and the mixture stirred at ambient temperature for 16 hours. Volatile components were removed *in vacuo* and the resulting colourless oil was purified by flash column chromatography using gradient elution (1:9 - 1:3 ethyl acetate:hexane) to give the monoprotected derivative **42** as a colourless oil (12.1 g, 59.3 mmol, 80% based on TBDMSCl); R_f (1:1 ethyl acetate:hexane) 0.45; (Found: MH^+, 205.1625. $C_{10}H_{24}O_2Si$ requires *MH*, 205.1624); ν_{max} (thin film)/cm^{-1} 3354 (br., O-H str.), 2934, 2860 (C-H str.), 1471, 1255, 1101 (C-O str.), 837 ($SiMe_2$ Si-C def.); δ_H (200 MHz; $CDCl_3$) 0.03 (6 H, s, $SiMe_2$), 0.85 (9 H, s, $SiCMe_3$), 1.57-1.60 (4 H, m, $SiOCH_2CH_2CH_2CH_2$-OH), 2.85 (1 H, br. s, OH), 3.58-3.61 (4 H, m, CH_2-OH, CH_2-OSi); δ_C (75.5 MHz; $CDCl_3$) -5.58 ($SiMe_2$), 18.15 ($SiCMe_3$), 25.78 ($SiCMe_3$), 29.66, 29.93 (CH_2CH_2-OH, CH_2CH_2-OSi), 62.57, 63.26 (CH_2-OH, CH_2-OSi); *m/z* (CI^+, NH_3) 205 (MH^+, 100%), 91 (12).

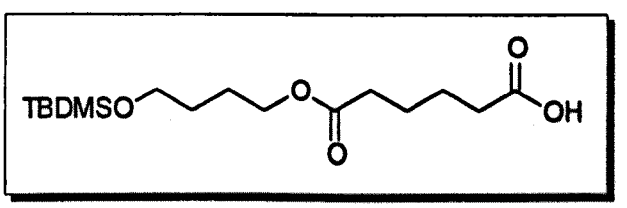
4'-(*tert*-Butyldimethylsilyloxy)butyl 6-hydroxyhexanoate (43).



THF (3 x 8 mL) was added with stirring to sodium hydride (60% dispersion in mineral oil, 0.43 g, 10.8 mmol) under an atmosphere of nitrogen and the supernatant liquid withdrawn after each addition. THF (2 mL) was then added and a solution of **42** (2.00 g, 9.8 mmol) in THF (8 mL) was added dropwise at 0 °C. After 5 minutes, a solution of ε-caprolactone **26**

(750 mg, 6.57 mmol) in THF (3 mL) was added at 0 °C and the solution stirred for 3 hours at ambient temperature. Water (20 mL) was added and the solution extracted with diethyl ether (3 x 50 mL). The combined ethereal layers were dried (magnesium sulphate) and filtered through Celite®. Evaporation of the solvent *in vacuo* gave the crude material as a colourless oil. Purification by flash column chromatography using gradient elution (1:4 - 1:2 ethyl acetate:petrol) gave the alcohol **43** as a colourless oil (953 mg, 3.00 mmol, 46%); R_f (1:1 ethyl acetate:petrol) 0.35; (Found: MH^+, 319.2303. $C_{16}H_{34}O_4Si$ requires *MH*, 319.2304); ν_{max} (thin film)/cm^{-1} 3449 (br., O-H str.), 2935, 2861 (C-H str.), 1732 (C=O str.), 1471, 1255, 1097 (C-O str.), 837 ($SiMe_2$ Si-C def.); δ_H (200 MHz; $CDCl_3$) 0.03 (6 H, s, $SiMe_2$), 0.83 (9 H, s, $SiCMe_3$), 1.49-1.64 (10 H, m, $HOCH_2(CH_2)_3CH_2COOR$, $SiOCH_2(CH_2)_2CH_2OCOR$), 2.24 (2 H, t, *J* 7.5, CH_2COOR), 3.54 -3.61 (4 H, m, CH_2OH, CH_2OSi), 4.00 (2 H, t, *J* 6.5, CH_2OCOR); δ_C (75.5 MHz; $CDCl_3$) -5.51 ($SiMe_2$) 18.17 ($SiCMe_3$), 25.81 ($SiCMe_3$), 24.47, 24.57, 25.20, 29.12, 32.20 ($HOCH_2(CH_2)_3CH_2COOR$, $SiOCH_2(CH_2)_2CH_2OCOR$), 34.15 (CH_2COOR), 62.43, 62.56 (CH_2OH, CH_2OSi), 64.26 (CH_2OCOR), 173.94 (COOR); *m/z* (CI^+, NH_3) 319 (MH^+, 10%), 205 (MH^+-$SiCH_2(Me)(CMe_3)$, 100), 187 (MH^+- $SiCH_2(Me)(CMe_3)$-H_2O, 100), 115 ($SiMe_2(CMe_3)^+$, 69).

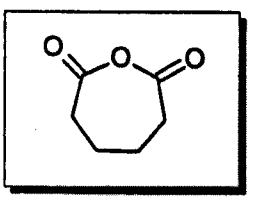
11-(*tert*-Butyldimethylsilyloxy)-7-oxa-6-oxo undecanoic acid (44).



A solution of PDC (418 mg, 1.11 mmol) in DMF (1.5 mL) was added to a solution of **43** (100 mg, 0.32 mmol) in DMF (0.5 mL) under an atmosphere of nitrogen and the mixture stirred for 18 hours at ambient temperature. Water (20 mL) was added to the reaction mixture and the solution extracted with diethyl ether (3 x 50 mL). The ethereal extracts were combined, dried (magnesium sulphate), filtered through Celite® and volatile components were removed *in vacuo*. Dissolution into diethyl ether (100 mL) and further washing with ammonium chloride solution (sat. aq.) (10 mL) was required to remove DMF. The ethereal solution was dried (magnesium sulphate), filtered through Celite® and the solvent removed *in vacuo* to give the

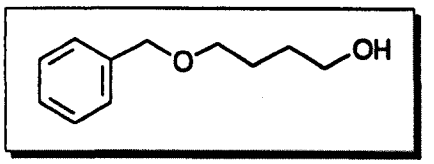
acid **44** as a colourless oil (30.2 mg, 95 μmol, 30%); R_f (4:1 ethyl acetate:petrol) 0.7; (Found: MH^+, 333.2098. $C_{16}H_{32}O_4Si$ requires *MH*, 333.2097); ν_{max} (thin film)/cm^{-1} 3600-2400 (br., O-H str.), 2954, 2860 (C-H str.), 1732, (C=O str.), 1462, 1255, 1097 (C-O str.), 837 ($SiMe_2$ Si-C def.); δ_H (200 MHz; $CDCl_3$) 0.03 (6 H, s, $SiMe_2$) 0.84 (9 H, s, $SiCMe_3$), 1.36-1.61 (8 H, m, $HOOCCH_2(CH_2)_2CH_2COOR$, $SiOCH_2(CH_2)_2CH_2OCOR$), 2.21-2.38 (4 H, m, $HOOCCH_2CH_2CH_2CH_2COOR$), 3.58 (2 H, t, *J* 6.5, CH_2OSi), 4.04 (2 H, t, *J* 6.5, CH_2OCOR); δ_C (75.5 MHz; $CDCl_3$) -5.46 ($SiMe_2$) 18.18 ($SiCMe_3$), 25.81 ($SiCMe_3$), 24.21, 25.15, 28.23, 29.10 ($HOOCCH_2(CH_2)_2CH_2COOR$, $SiOCH_2(CH_2)_2CH_2OCOR$), 33.39, 33.81 ($HOOCCH_2CH_2CH_2CH_2COOR$) 62.57 (CH_2OSi), 64.33 (CH_2OCOR), 173.51 (COOR), 178.36 (COOH); *m/z* (CI^+, NH_3) 333 (MH^+, 22%), 187 ($SiCH_2(Me)(CMe_3)OC_4H_9^+$, 100).

Adipic anhydride (45).[73]



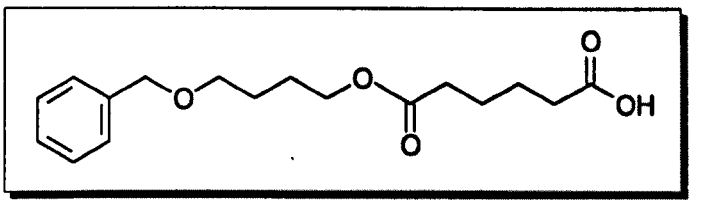
Acetic anhydride (150 mL, 1.59 mol) was added to adipic acid **3** (50.0 g, 342 mmol) under an atmosphere of argon, and the mixture heated under reflux for 4 hours. Vacuum distillation at 150 mbar was then used to remove *ca.* 100 mL of distillate, b.pt. 78-90 °C. Short-path distillation at 5 mbar using a Bunsen flame was then employed to remove *ca.* 30 mL of further excess starting material and by-products, b.pt. 50-60 °C. Heating was maintained until the stillhead temperature reached 80 °C. The crude product was then distilled at 5 mbar, using a Bunsen flame, collecting the fraction b.pt. <160 °C as a pale yellow-brown viscous liquid (*ca.* 30 mL). Purification by vacuum distillation (b.pt. 110 °C / 5 mbar [lit.[73] 98-100 °C / 0.1 mmHg]) gave adipic anhydride **45** as a colourless viscous liquid (11 mL, 15 g, 120 mmol, 35%); δ_H (200 MHz; $CDCl_3$) 1.88-2.09 (4 H, m, 2 x CH_2CH_2COOR), 2.65-2.86 (4 H, m, 2 x CH_2CH_2COOR). The anhydride **45** was stored under argon at -40 °C and used within 2 days.

4-Benzyloxybutan-1-ol (46).



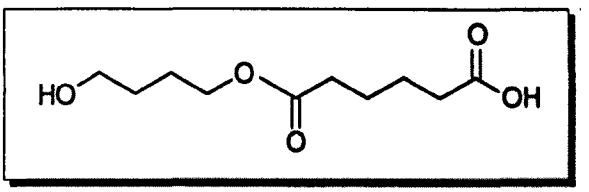
DMSO (200 mL) was added to sodium hydroxide pellets (6.38 g, 160 mmol) and stirred under an atmosphere of nitrogen for 30 minutes, giving a faintly yellow solution with a little suspended solid. A solution of butane-1,4-diol **4** (13.1 g, 145 mmol) in DMSO (50 mL) was added dropwise over 10 minutes at ambient temperature and stirring maintained for 1 hour. A solution of benzyl chloride (9.0 mL, 80 mmol) in DMSO (30 mL) was added dropwise and the mixture stirred for 15 hours at ambient temperature. The mixture was poured into ice-water (400 mL) and extracted with diethyl ether (3 x 200 mL). The combined ethereal layers were dried (magnesium sulphate), filtered through Celite® and the solvent was removed *in vacuo* to give a colourless oil. Purification by flash column chromatography (3:2 ethyl acetate:hexane) gave the monoprotected derivative **46** as a colourless oil (11.8 g, 65.2 mmol, 81% based on benzyl chloride); R_f (3:2 ethyl acetate:hexane) 0.35; (Found: MH^+, 181.1231. $C_{11}H_{16}O_2$ requires *MH*, 181.1229); ν_{max} (thin film)/cm^{-1} 3355 (br., O-H str.), 2932, 2885, 2859 (C-H str.), 1256, 1102 (C-O str.), 776, 712 (aromatic C-H def.); δ_H (200 MHz; $CDCl_3$) 1.53-1.63 (4 H, m, $BnOCH_2(CH_2)_2CH_2OH$) 2.90 (1 H, s, OH), 3.37-3.49 (4 H, m, $BnOCH_2CH_2CH_2CH_2OH$), 4.41 (2 H, s, $ArCH_2OR$), 7.14-7.25 (5 H, m, ArH); δ_C (75.5 MHz; $CDCl_3$) 26.54, 29.87 ($BnOCH_2(CH_2)_2CH_2OH$), 62.54 (CH_2OH), 70.36 (CH_2OBn), 73.06 ($ArCH_2OR$), 127.74, 128.48, 129.62, 138.24 (aromatic C); *m/z* (CI^+, NH_3) 181 (MH^+, 30%), 107 (MNH_4^+-C_7H_7, 61), 91 ($C_7H_7^+$, 100).

11-Benzyloxy-7-oxa-6-oxoundecanoic acid (47).



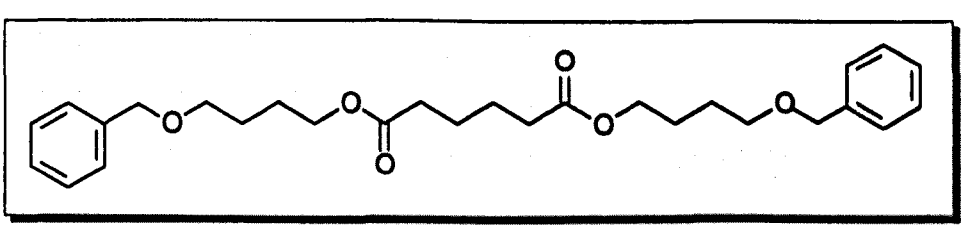
Adipic anhydride **45** (12.4 g, 97 mmol) and *p*-toluenesulphonic acid (5.0 mg, 30 mmol) were added to a solution of **46** (17.5 g, 97.1 mmol) in benzene (15 mL) under an atmosphere of argon, and the mixture heated under reflux for 18 hours. One half of the mixture was separated and concentrated *in vacuo*. The crude material was purified by flash column chromatography (2:1 ethyl acetate:petrol) to give the acid **47** as a colourless oil (1.97 g, 6.39 mmol, 7%). Volatile components were removed *in vacuo* from the second half of the mixture, and the crude material dissolved in diethyl ether (50 mL) and extracted with potassium carbonate solution (sat. aq.) (150 mL). The aqueous layer was acidified to ~ pH 5 using 2M hydrochloric acid and back-extracted into diethyl ether (200 mL). The organic layer was dried (magnesium sulphate), filtered through Celite® and concentrated *in vacuo* to give the acid **47** as a colourless oil (10.4 g, 33.7 mmol, 35%); R_f (4:1 ethyl acetate:petrol) 0.6; (Found: MH^+, 309.1702. $C_{17}H_{24}O_5$ requires *MH*, 309.1702); ν_{max} (thin film)/cm^{-1} 3600-2400 (br., O-H str.), 2950, 2867 (C-H str.), 1740-1710 (br., C=O str.), 740, 700 (aromatic C-H def.); δ_H (200 MHz; $CDCl_3$) 1.63-1.74 (8 H, m, $BnOCH_2(CH_2)_2CH_2OCOR$, $HOOCCH_2(CH_2)_2CH_2COOR$) 2.26-2.40 (4 H, m, $HOOCCH_2CH_2CH_2CH_2COOR$), 3.47 (2 H, t, *J* 6, CH_2OBn), 4.06 (2 H, t, *J* 6, CH_2OCOR), 4.48 (2 H, s, $ArCH_2OR$), 7.26-7.32 (5 H, m, ArH), 9.14 (1 H, br. s, OH); δ_C (75.5 MHz; $CDCl_3$) 23.93, 24.13, 25.34, 26.05 ($BnOCH_2(CH_2)_2CH_2OCOR$, $HOOCCH_2(CH_2)_2CH_2COOR$), 33.44, 33.67 $HOOCCH_2CH_2CH_2CH_2COOR$), 64.18 (CH_2OBn), 69.61 (CH_2OCOR), 72.80 ($ArCH_2OR$), 127.58, 127.64, 128.37 (aromatic C), 173.52, 178.92 (COOR, COOH); *m/z* (CI^+, NH_3) 326 (MNH_4^+, 8%), 309 (MH^+, 2%), 203 (39), 163 (MH^+-$C_6H_{10}O_4$, 80), 128 ($C_6H_8O_3^+$, 43), 108 ($C_7H_7OH^+$, 94), 91 ($C_7H_7^+$, 27).

11-Hydroxy-7-oxa-6-oxoundecanoic acid (AB) (14).



10% Palladium on charcoal (0.56 g) was added to a solution of **47** (3.73 g, 12.1 mmol) in ethyl acetate (100 mL) and the suspension stirred at ambient temperature under an atmosphere of hydrogen at atmospheric pressure for 48 hours. The mixture was filtered through Celite® and the solvent removed *in vacuo*. Purification by dry flash chromatography[76] (ethyl acetate) gave hydroxyacid **14** as a colourless oil (2.32 g, 10.6 mmol, 88%); R_f (4:1 ethyl acetate:petrol) 0.15 (Found: MNH_4^+, 236.1500. $C_{10}H_{18}O_5$ requires *MNH*$_4$, 236.1498); (Found: C, 54.85; H, 8.35. Calculated for $C_{10}H_{18}O_5$: C, 55.0; H, 8.3%); ν_{max} (thin film)/cm^{-1} 3600-2400 (br., O-H str.), 2954, 2880 (C-H str.), 1728 (br., C=O str.); δ_H (200 MHz; $CDCl_3$) 1.57-1.73 (8 H, m, $HOCH_2(CH_2)_2CH_2OCOR$, $HOOCCH_2(CH_2)_2CH_2COOR$), 2.29-2.40 (4 H, m, $HOOCCH_2CH_2CH_2CH_2COOR$), 3.66 (2 H, t, *J* 6, CH_2OH), 4.09 (2 H, t, *J* 6, CH_2OCOR), 6.28 (2 H, br. s, OH); δ_C (75.5 MHz; $CDCl_3$) 23.91, 24.10, 24.83, 28.63 ($HOCH_2(\underline{C}H_2)_2CH_2OCOR$, $HOOCCH_2(\underline{C}H_2)_2CH_2COOR$), 33.71, 33.36 ($HOOC\underline{C}H_2CH_2CH_2\underline{C}H_2COOR$), 61.87 ($\underline{C}H_2OH$), 64.12 ($\underline{C}H_2OCOR$), 173.67 177.93, ($\underline{C}OOR$, $\underline{C}OOH$); *m/z* (CI^+, NH_3) 236 (MNH_4^+, 100%), 218 (MNH_4^+-H_2O, 53), 175 (MH^+-CO_2, 12), 145 ($C_6H_9O_4^+$, 52), 128 ($C_6H_8O_3^+$, 43); GPC analysis: elution time 15.2 minutes.

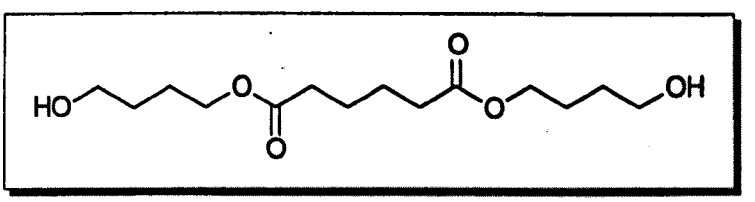
1,6-Hexanedioic acid di(4'-benzyloxybutyl) ester (48).



A solution of alcohol **46** (1.69 g, 9.38 mmol) in toluene (5 mL) and *Novozyme 435*® (35 mg) were added to adipic acid **3** (0.70 g, 4.79 mmol) in a modified Dean and Stark apparatus and the mixture stirred at 60 °C for 20 hours. Volatile components were removed *in vacuo* and the

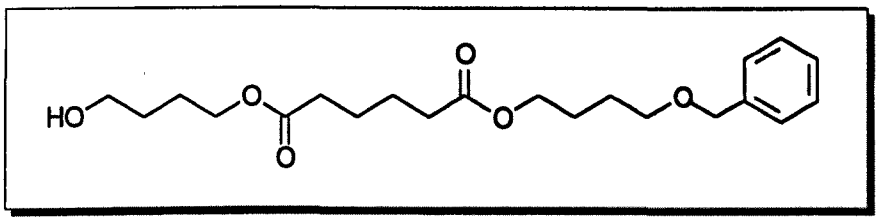
crude material purified by flash column chromatography (2:3 ethyl acetate:petrol) to give the diester **48** as a colourless oil (1.82 g, 3.87 mmol, 81%); R_f (3:2 ethyl acetate:petrol) 0.8; (Found: MNH_4^+ 488.3012. $C_{28}H_{38}O_6$ requires *MNH*$_4$ 488.3012); ν_{max} (thin film)/cm^{-1} 2950, 2860 (C-H str.), 1736 (br., C=O str.), 1099 (C-O str.), 737, 698 (aromatic C-H def.); δ_H (200 MHz; $CDCl_3$) 1.59-1.88 (12 H, m, 2 x $BnOCH_2(CH_2)_2CH_2OCOR$, 2 x CH_2CH_2COOR), 2.30 (4 H, t, *J* 6, 2 x CH_2COOR), 3.48 (4 H, t, *J* 6, 2 x CH_2OBn), 4.08 (4 H, t, *J* 6.5, 2 x CH_2OCOR), 4.48 (4 H, s, 2 x $ArCH_2OR$), 7.25-7.47 (10 H, m, ArH); δ_C (75.5 MHz; $CDCl_3$) 24.26, 25.40, 26.13 ($BnOCH_2(CH_2)_2CH_2OCOR$, CH_2CH_2COOR), 33.75 (CH_2COOR), 64.09 (CH_2OBn), 69.65 (CH_2OCOR), 72.84 ($ArCH_2OR$), 127.59, 128.37, 129.59 (aromatic C), 173.39 (COOR); *m/z* (CI^+, NH_3) 488 (MNH_4^+, 17%), 181 ($C_7H_7OC_4H_8OH_2^+$, 73), 163 ($C_7H_7OC_4H_8^+$, 38), 108 ($C_7H_7OH^+$, 64), 91 ($C_7H_7^+$, 23).

1,6-Hexanedioic acid di(4'-hydroxybutyl) ester (15).



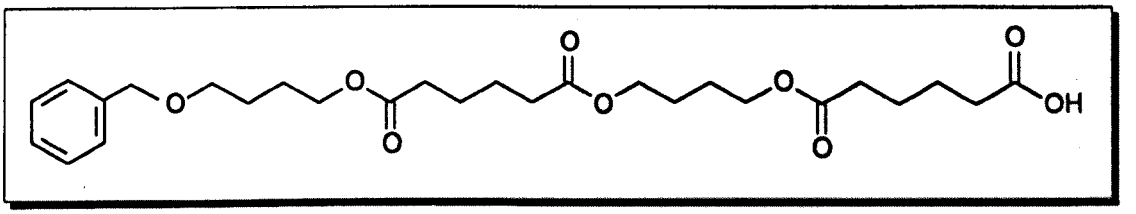
10% Palladium on charcoal (3.0 mg) was added to a solution of **48** (24.0 mg, 51 μmol) in THF (0.5 mL) and stirred at ambient temperature under an atmosphere of hydrogen at atmospheric pressure for 19 hours. The solution was filtered through Celite® and the solvent removed *in vacuo*. Purification by dry flash chromatography[76] (ethyl acetate) gave the diol **15** as a colourless oil (9.4 mg, 32 μmol, 63%); R_f (ethyl acetate) 0.35; (Found: MH^+, 291.1807. $C_{14}H_{26}O_6$ requires *MH*, 291.1808); ν_{max} (thin film)/cm^{-1} 3427 (O-H str.), 2945, 2873 (C-H str.), 1735 (C=O str.), 1060 (C-O str.); δ_H (200 MHz; $CDCl_3$) 1.60-1.89 (12 H, m, 2 x $HOCH_2(CH_2)_2CH_2OCOR$, 2 x CH_2CH_2COOR) 2.28-2.41 (4 H, m, 2 x CH_2CH_2COOR), 3.68 (4 H, t, *J* 6, 2 x CH_2OH), 4.13 (4 H, t, *J* 6.5, CH_2OCOR); δ_C (75.5 MHz; $CDCl_3$) 24.25, 24.97, 29.01, ($HOCH_2(CH_2)_2CH_2OCOR$, CH_2CH_2COOR), 33.78 (CH_2COOR), 62.24 (CH_2OH), 64.07 (CH_2OCOR), 173.44 (COOR); *m/z* (CI^+, NH_3) 308 (MNH_4^+, 9%), 291 (MH^+, 16), 218 (MH^+-C_4H_8OH, 45), 145 ($HO_2CC_4H_8CO_2^+$, 23), 91 ($HOC_4H_8OH_2^+$, 78); GPC analysis: elution time 14.7 minutes.

16-Benzyloxy-5,12-dioxa-6,11-dioxohexadecan-1-ol (49).



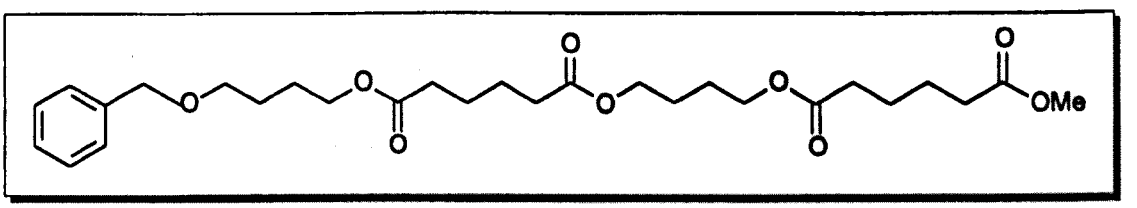
10% Palladium on charcoal (640 mg) was added to a solution of **48** (4.25 g, 9.03 mmol) in ethyl acetate (100 mL) and the suspension stirred at ambient temperature under an atmosphere of hydrogen at atmospheric pressure for 9 hours. The solution was filtered through Celite® and the solvent removed *in vacuo*. Purification by flash column chromatography (1:1 ethyl acetate:petrol) gave recovered starting material **48** (562 mg, 1.19 mmol, 13%) and the alcohol **49** as a colourless oil (1.59 g, 4.18 mmol, 46%); R_f (3:2 ethyl acetate:petrol) 0.45; (Found: MNH_4^+, 398.2543. $C_{21}H_{32}O_6$ requires *MNH*$_4$, 398.2542); ν_{max} (thin film)/cm^{-1} 3452 (O-H str.), 2947, 2869 (C-H str.), 1731 (C=O str.), 1076 (C-O str.), 738, 699 (aromatic C-H def.); δ_H (200 MHz; $CDCl_3$) 1.63-1.72 (12 H, m, $BnOCH_2(C\underline{H}_2)_2CH_2OCOR$, $HOCH_2(C\underline{H}_2)_2CH_2OCOR$, $ROOCCH_2(C\underline{H}_2)_2CH_2COOR$), 2.25-2.45 (4 H, m, $ROOCC\underline{H}_2(CH_2)_2C\underline{H}_2COOR$), 3.47 (2 H, t, *J* 6, $C\underline{H}_2OBn$), 3.64 (2 H, t, *J* 6, $C\underline{H}_2OH$), 4.05-4.26 (4 H, m, 2 x $C\underline{H}_2OCOR$), 4.50 (2 H, s, $ArC\underline{H}_2OR$), 7.23-7.46 (5 H, m, Ar$\underline{H}$); δ_C (75.5 MHz; $CDCl_3$) 24.31, 24.34, 25.05, 25.46, 26.19, 29.07 ($BnOCH_2(\underline{C}H_2)_2CH_2OCOR$, $HOCH_2(\underline{C}H_2)_2CH_2OCOR$, $ROOCCH_2(\underline{C}H_2)_2CH_2COOR$), 33.83, 33.86 ($ROOC\underline{C}H_2(CH_2)_2\underline{C}H_2COOR$), 62.26 ($\underline{C}H_2OH$), 64.28 ($\underline{C}H_2OBn$), 69.74 (2 x $\underline{C}H_2OCOR$), 72.94 ($Ar\underline{C}H_2OR$), 127.70, 128.46, 129.64, 138.59 (aromatic $\underline{C}$), 173.58 (2 x $\underline{C}OOR$); *m/z* (CI^+, NH_3) 398 (MNH_4^+, 8%), 381 (MH^+, 1), 292 (MH^+-OC_4H_8OH, 11), 181 ($C_7H_7OC_4H_8OH_2^+$, 18), 163 ($C_7H_7OC_4H_8^+$, 47), 108 ($C_7H_6O^+$, 64).

23-Benzyloxy-7,12,19-trioxa-6,13,18-trioxotricosanoic acid (50).



Alcohol **49** (560 mg, 1.47 mmol) and *p*-toluenesulphonic acid (5.3 mg, 28 μmol) were added to sodium-dried toluene (1 mL). Adipic anhydride **45** (135 μL, 1.48 mmol) was added and the stirred mixture heated under reflux for 18 hours. TLC indicated a mixture of at least two products. The mixture was dissolved in diethyl ether (10 mL) and extracted with potassium carbonate solution (sat. aq.) (15 mL) but TLC showed no products in the aqueous layer. The solvent was removed *in vacuo* and attempted recrystallisation from several solvents gave no identifiable products.

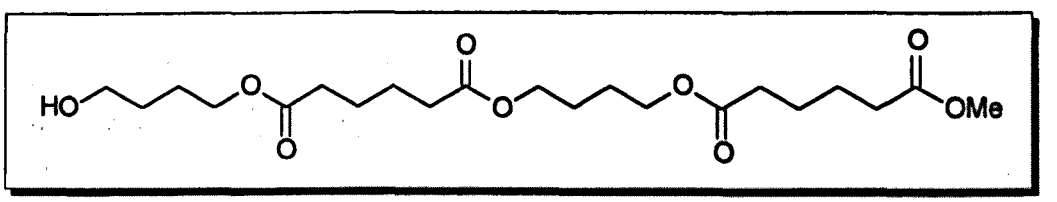
23-Benzyloxy-6,13,18-trioxo-7,12,19-trioxatricosanoic acid methyl ester (53).



Freshly distilled thionyl chloride (10 μL, 140 μmol) was added to adipic acid monomethyl ester **52** (8.0 mg, 50 μmol) under an atmosphere of nitrogen, and the mixture heated under reflux for 10 minutes. Excess thionyl chloride was removed *in vacuo*, and a solution of alcohol **49** (15.6 mg, 0.04 mmol) in dichloromethane (0.75 mL) was added. The mixture was heated under reflux for 15 hours. Further dichloromethane (5 mL) was added and the mixture washed with sodium bicarbonate solution (sat. aq.) (10 mL), brine (10 mL) and water (10 mL). The organic phase was dried (magnesium sulphate) and filtered through Celite®. Volatile components were removed *in vacuo* and the crude material purified by elution through a plug of silica (1:4 ethyl acetate:petrol) to give ester **53** as a colourless oil (10.3 mg, 20 μmol, 48%); R_f (1:1 ethyl acetate:petrol) 0.45; (Found: MH^+, 523.2885. $C_{28}H_{42}O_9$ requires *MH*, 523.2907); ν_{max} ($CHCl_3$)/cm^{-1} 2956, 2869 (C-H str.), 1730 (C=O str.), 1080 (C-O str.), 753, 699 (aromatic

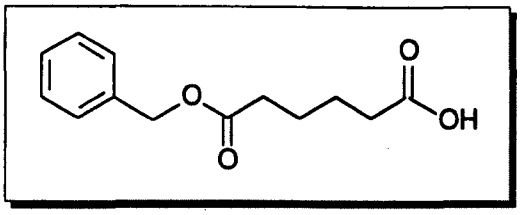
C-H def.); δ_H (200 MHz; $CDCl_3$) 1.57-1.83 (16 H, m, 3 x $\underline{C}H_2CH_2OCOR$, 4 x CH_2CH_2COOR, $BnOCH_2CH_2$), 2.24-2.41 (8 H, m, 4 x $\underline{C}H_2COOR$), 3.49 (2 H, t, *J* 6, $\underline{C}H_2OBn$), 3.66 (3H, s, $\underline{Me}OCOR$), 4.05-4.17 (6 H, m, 3 x $\underline{C}H_2OCOR$), 4.53 (2 H, s, $ArCH_2OR$), 7.25-7.48 (5 H, m, Ar$\underline{H}$); δ_C (75.5 MHz; $CDCl_3$) 24.21, 25.14, 26.11 (3 x $\underline{C}H_2CH_2OCOR$, 4 x $\underline{C}H_2CH_2COOR$, $BnOCH_2\underline{C}H_2$), 33.49, 33.70 (4 x $\underline{C}H_2COOR$), 51.34 ($\underline{Me}O$), 63.71, 64.10, 69.64 (3 x $\underline{C}H_2OCOR$), 72.84 ($Ar\underline{C}H_2OR$), 127.60, 128.38, 128.80 (aromatic $\underline{C}$), 173.44, 173.50 (4 x $\underline{C}OOR$); *m/z* (FAB^+) 523 (MH^+, 3%), 215 ($MeO_2CC_4H_8CO_2C_4H_8^+$, 53). 201 ($HO_2CC_4H_8CO_2C_4H_8^+$, 28), 55 ($C_4H_7^+$, 24).

23-Hydroxy-7,12,19-trioxa-6,13,18-trioxotricosanoic acid methyl ester (54).



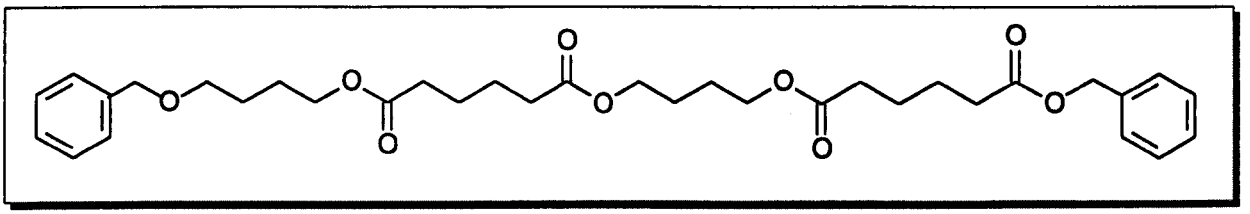
10% Palladium on charcoal (3.0 mg) was added to a solution of **53** (3.4 mg, 6.5 μmol) in EtOAc (1 mL) and stirred at ambient temperature under an atmosphere of hydrogen at atmospheric pressure for 18 hours. The solution was then filtered through Celite® and volatile components removed *in vacuo* to give the alcohol **54** as a colourless oil (2.8 mg, 6.5 μmol, 100%); R_f (1:1 ethyl acetate:petrol) 0.25; (Found: MH^+, 433.2423. $C_{21}H_{36}O_9$ requires *MH*, 433.2438); ν_{max} (thin film)/cm^{-1} 3529 (br., O-H str.), 2932, 2864 (C-H str.), 1731 (br., C=O str.), 1076 (C-O str.); δ_H (200 MHz; $CDCl_3$) 1.62-1.75 (16 H, m, 3 x $\underline{C}H_2CH_2OCOR$, 4 x CH_2CH_2COOR, $HOCH_2CH_2$), 2.28-2.48 (8 H, m, 4 x $\underline{C}H_2COOR$), 3.67 (2 H, t, *J* 6, $\underline{C}H_2OH$ and 3 H, s, $\underline{Me}O$), 4.08-4.14 (6 H, m, 3 x $\underline{C}H_2OCOR$); δ_C (100.6 MHz; $CDCl_3$) 24.38, 24.43, 25.14, 25.31, 25.45, 29.12, 29.69 (3 x $\underline{C}H_2CH_2OCOR$, 4 x $\underline{C}H_2CH_2COOR$, $HOCH_2\underline{C}H_2$), 33.67, 33.88, 33.94 (4 x $\underline{C}H_2COOR$), 51.56 ($\underline{Me}O$), 62.22, 63.87, 64.19 (3 x $\underline{C}H_2OCOR$, $HO\underline{C}H_2$), 173.31, 173.35, 173.38, 173.80 (4 x $\underline{C}OOR$); *m/z* (FAB^+) 433 (MH^+, 12%), 343 (MH^+-HOC_4H_8OH, 10), 215 ($MeO_2CC_4H_8CO_2C_4H_8^+$, 29); GPC analysis: elution time 13.9 minutes.

Monobenzyl adipate (56).



Adipic anhydride **45** (1.65 mL, 17.5 mmol) and *p*-toluenesulphonic acid (5.0 mg, 32 μmol) were added to a solution of benzyl alcohol (2.00 g, 18.5 mmol) in benzene (5 mL) under an atmosphere of nitrogen, and the mixture heated under reflux for 18 hours. Volatile components were removed *in vacuo*, and the crude material dissolved in diethyl ether (20 mL). The product was extracted with potassium carbonate solution (sat. aq.) (30 mL) and the aqueous phase acidified to ~ pH 5 using 5M hydrochloric acid then back-extracted with diethyl ether (100 mL). The combined organic phases were dried (magnesium sulphate), filtered through Celite® and concentrated *in vacuo* to give the acid **56** as a colourless oil (835 mg, 3.53 mmol, 20%); R_f (1:1 ethyl acetate:petrol) 0.35; (Found: MH^+, 237.1128. $C_{13}H_{16}O_4$ requires *MH*, 237.1127); ν_{max} (thin film)/cm^{-1} 3500-2400 (br., O-H str.), 2930, 2853 (C-H str.), 1738 (C=O str., ester), 1710 (C=O str., acid), 1085 (C-O str.), 749, 697 (aromatic C-H def.); δ_H (200 MHz; $CDCl_3$) 1.62-1.79 (4 H, m, $HOOCCH_2(CH_2)_2CH_2COOBn$) 2.29-2.50 (4 H, m, $HOOCCH_2CH_2CH_2CH_2COOBn$), 5.11 (2 H, s, $ArCH_2OCOR$), 7.28-7.45 (5 H, m, ArH), 9.78 (1 H, br. s, OH); δ_C (75.5 MHz; $CDCl_3$) 23.90, 24.11 ($HOOCCH_2(CH_2)_2CH_2COOBn$), 33.47, 33.72 ($HOOCCH_2CH_2CH_2CH_2COOBn$), 66.18 ($ArCH_2OCOR$) 128.24, 128.58, 129.06, 136.05 (aromatic C), 173.29, 179.58 (COOBn, COOH); *m/z* (CI^+, NH_3) 254 (MNH_4^+, 100%), 237 (MH^+, 33), 146 (MH^+-C_7H_7, 9), 108 ($C_7H_7OH^+$, 34), 91 ($C_7H_7^+$, 9).

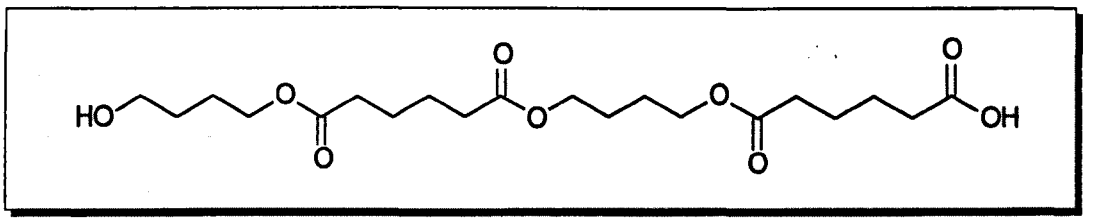
23-Benzyloxy-7,12,19-trioxa-6,13,18-trioxotricosanoic acid benzyl ester (55).



Method 1. 4 Å molecular sieves, *Novozyme 435®* (0.020 g) and a solution of acid **56** (310 mg, 1.31 mmol) in benzene (1.5 mL) were added to a solution of alcohol **49** (476 mg, 1.25 mmol) in benzene (1.5 mL) under an atmosphere of nitrogen, and the mixture stirred for 14 hours at 60 °C. TLC indicated many components and attempted isolation by flash column chromatography proved fruitless.

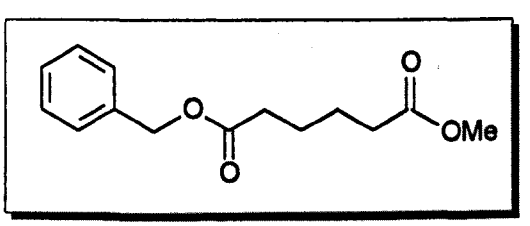
Method 2. A solution of alcohol **49** (323 mg, 0.85 mmol) in dichloromethane (5 mL) was added to a solution of DCC (186 mg, 0.90 mmol), DMAP (12 mg, 0.10 mmol) and acid **56** (200 mg, 0.85 mmol) in dichloromethane (5 mL) and the mixture stirred at ambient temperature for 20 hours. Volatile components were removed *in vacuo* and purification by flash column chromatography using gradient elution (1:10 - 1:2 ethyl acetate:petrol) gave diprotected derivative **55** as a colourless oil (146 mg, 0.24 mmol, 28%); R_f (1:1 ethyl acetate:petrol) 0.9; (Found: MH^+, 599.3230. $C_{34}H_{46}O_9$ requires *MH*, 599.3220); ν_{max} (thin film)/cm^{-1} 2951, 2860 (C-H str.), 1734 (br., C=O str.), 738, 699 (aromatic C-H def.); δ_H (200 MHz; $CDCl_3$) 1.62-1.75 (16 H, m, 3 x $C\underline{H}_2CH_2OCOR$, 4 x $C\underline{H}_2CH_2COOR$, $BnOCH_2C\underline{H}_2$), 2.25-2.44 (8 H, m, 4 x $C\underline{H}_2COOR$), 3.49 (2 H, t, *J* 6, $C\underline{H}_2OBn$), 4.06-4.14 (6 H, m, 3 x $C\underline{H}_2OCOR$), 4.50 (2 H, s, $ArC\underline{H}_2OR$), 5.11 (2 H, s, $ArC\underline{H}_2OCOR$), 7.20-7.38 (10 H, m, Ar<u>H</u>); δ_C (75.5 MHz; $CDCl_3$) 24.36, 25.29, 25.53, 26.26 (3 x $\underline{C}H_2CH_2OCOR$, 4 x $\underline{C}H_2CH_2COOR$, $BnOCH_2\underline{C}H_2$), 33.86, ($\underline{C}H_2COOBn$, 3 x $\underline{C}H_2COOR$), 63.81, 64.21, 66.19, 69.78 ($BnO\underline{C}H_2$, 3 x $\underline{C}H_2OCOR$), 72.98 ($ArC\underline{H}_2OR$), 77.20 ($ArC\underline{H}_2OCOR$), 127.62, 128.23, 128.40, 128.59, 136.15, 138.58 (aromatic <u>C</u>), 173.32, (4 x <u>C</u>OOR); *m/z* (FAB^+) 599 (MH^+, 1%), 201 ($HO_2CC_4H_8CO_2C_4H_8^+$, 3), 91 ($C_7H_7^+$, 100).

23-Hydroxy-7,12,19-trioxa-6,13,18-trioxotricosanoic acid $(AB)_2$ (51).



10% Palladium on charcoal (20 mg) was added to a solution of **55** (120 mg, 0.20 mmol) in dry THF (3 mL) and the suspension stirred at ambient temperature under an atmosphere of hydrogen at atmospheric pressure for 2 hours. The mixture was filtered through Celite® and the solvent removed *in vacuo* to give hydroxyacid **51** as a colourless oil (72.4 mg, 173 μmol, 86%) which required no further purification; R_f (4:1 ethyl acetate:petrol) 0.2; (Found: MH^+, 419.2291. $C_{20}H_{34}O_9$ requires *MH*, 419.2281); ν_{max} (thin film)/cm^{-1} 3600-2400 (br., O-H str.), 2959, 2922, 2854 (C-H str.), 1732 (C=O str., ester), 1713 (C=O str., acid), 1077 (C-O str.); δ_H (400 MHz; $CDCl_3$) 1.61-1.72 (16 H, m, $C\underline{H}_2CH_2COOH$, 3 x $C\underline{H}_2CH_2OCOR$, 3 x $C\underline{H}_2CH_2COOR$, $HOCH_2C\underline{H}_2$), 2.24-2.40 (8 H, m, $C\underline{H}_2COOH$, 3 x $C\underline{H}_2COOR$), 3.68 (2 H, t, *J* 6, $C\underline{H}_2OH$), 4.06-4.13 (6 H, m, 3 x $C\underline{H}_2OCOR$); δ_C (100.6 MHz; $CDCl_3$) 24.12, 24.30, 24.35, 24.39, 25.09, 25.31, 29.06, 29.68 ($\underline{C}H_2CH_2COOH$, 3 x $\underline{C}H_2CH_2OCOR$, 3 x $\underline{C}H_2CH_2COOR$, $HOCH_2\underline{C}H_2$), 33.41, ($\underline{C}H_2COOH$), 33.87, 33.92 (3 x $\underline{C}H_2COOR$), 62.31 ($HO\underline{C}H_2$), 63.89, 64.21 (3 x $\underline{C}H_2OCOR$), 173.32, 173.45, 173.49 (3 x $\underline{C}OOR$); *m/z* (FAB^+) 419 (MH^+, 22%), 401 (MH^+-H_2O, 7), 353 (15), 201 ($HO_2CC_4H_8CO_2C_4H_8^+$, 23), 147 ($HO_2CC_4H_8CO_2H_2^+$, 7), 55 ($C_4H_7^+$, 100); GPC analysis: elution time 14.3 minutes.

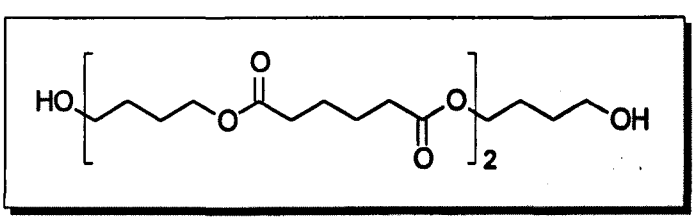
Benzyl methyl adipate (57).



Benzyl alcohol (1.35 mL, 13.0 mmol) was added to a solution of adipic acid monomethyl ester **52** (2.00 g, 12.5 mmol), DCC (3.35 g, 16.3 mmol) and DMAP (170 mg, 1.39 mmol) in

dichloromethane (100 mL) under an atmosphere of argon at ambient temperature and the mixture stirred for 18 hours. The mixture was filtered and concentrated *in vacuo*. Purification by flash column chromatography using gradient elution (1:10 - 1:4 ethyl acetate:petrol) gave the diester **57** as a colourless oil (2.74 g, 10.9 mmol, 88%); R_f (1:4 ethyl acetate:petrol) 0.75; (Found: MH^+, 251.1286. $C_{14}H_{18}O_4$ requires *MH*, 251.1283); ν_{max} (thin film)/cm^{-1} 2953 (C-H str.), 1737 (C=O str.), 1080 (C-O str.), 737, 697 (aromatic C-H def.); δ_H (200 MHz; $CDCl_3$) 1.62-1.73 (4 H, m, $BnOOCCH_2(CH_2)_2CH_2COOMe$), 2.32-2.46 (4 H, m, $BnOOCCH_2CH_2CH_2CH_2COOMe$), 3.66 (3 H, s, COOMe), 5.11 (2 H, s, $ArCH_2OCOR$), 7.28-7.42 (5 H, m, ArH); δ_C (75.5 MHz; $CDCl_3$) 24.33 ($BnOOCCH_2(CH_2)_2CH_2COOMe$), 33.63, 33.87 ($BnOOCCH_2CH_2CH_2CH_2COOMe$), 51.47 (COOMe), 66.19 ($ArCH_2OCOR$) 128.23, 128.59, 136.09 (aromatic C), 173.16, 173.76 (COOMe, COOBn); *m/z* (CI^+, NH_3) 268 (MNH_4^+, 42%), 251 (MH^+, 100), 160 (MH^+-C_7H_7, 5), 145 (MH^+-C_7H_7-Me, 7), 128 (MH^+-C_7H_7OH-Me, 8), 108 ($C_7H_7OH^+$, 34), 91 ($C_7H_7^+$, 28).

7,12-Dioxa-6,13-dioxooctadecanedioic acid di(4-hydroxybutyl) ester (B(AB)$_2$) (58).



Butane-1,4-diol **4** (1.04 g, 11.5 mmol) and water (48 μL, 2.7 mmol ≡ 4.68 g/mol adipic acid) were added to adipic acid **3** (1.50 g, 10.3 mmol) and the mixture stirred at 40 °C for 1 hour. *Novozyme 435*® (8.6 mg, ≡ 0.835 g/mol adipic acid) was added and the mixture stirred for a further 2 hours at 40 °C. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 5.5 hours, whereupon a clear solution had formed; GPC analysis: M_n 307, M_w 678, M_w / M_n 2.2. The mixture was extracted into diethyl ether (50 mL) and the solution decanted from the insoluble adipic acid. The ethereal solution was washed with potassium carbonate solution (sat. aq.) (2 x 10 mL) and water (5 mL), dried (magnesium sulphate), filtered and volatile components removed *in vacuo* to give a colourless oil; GPC analysis: M_n 526, M_w 714, M_w / M_n 1.4. Preparative GPC was employed to remove BAB **15**, giving a colourless oil, which GPC showed consisted of B(AB)$_2$ **58** plus small

amounts of higher weight $B(AB)_n$-type oligomers; GPC analysis: elution time 13.7 minutes; M_n 880, M_w 969, M_w / M_n 1.1.

Comparative polycondensation reactions (Section 2.1.3).

Method 1: solvent-free systems

Section 2.1.3.2

Butane-1,4-diol **4** (1.04 g, 11.5 mmol) and water (48 μL, 2.7 mmol, 1.9% w/w butane-1,4-diol /adipic acid, ≡ 4.68 g/mol adipic acid) were added to adipic acid **3** (1.50 g, 10.3 mmol) and the mixture stirred at 40 °C for 1 hour. *Novozyme 435®* (8.6 mg, 0.34% w/w butane-1,4-diol/adipic acid, ≡ 0.835 g/mol adipic acid) was added and the mixture stirred for a further 4 hours at 40 °C. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 17 hours, whereupon a clear, homogeneous material was formed; GPC analysis: M_n 1490, M_w 2227, M_w / M_n 1.5; constitution (by GPC) of AB **14**: 3.0%, adipic acid **3**: 0.3, butane-1,4-diol **4**: 2.1.

Section 2.1.3.3

AB **14** (1.70 g, 7.8 mmol) and water (37 μL, 2.1 mmol, 4.68 g/mol AB) were stirred for 1 hour at 40 °C. *Novozyme 435®* (6.5 mg, ≡ 0.835 g/mol AB) was added and the mixture stirred for a further 4 hours at 40 °C. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 17 hours, whereupon a viscous, clear, homogeneous mixture had formed; GPC analysis: M_n 1008, M_w 1696, M_w / M_n 1.7; constitution of AB **14**: 6.0%.

AB **14** (25 mg, 0.12 mmol), water (~1 μL, ~55 μmol, ≅ 4.68 g/mol AB) and butane-1,4-diol **4** (52 mg, 0.58 mmol) were stirred for 1 hour at 40 °C. *Novozyme 435®* (1.0 mg, ≡ 0.835 g/mol AB) was added and the mixture stirred for a further 4 hours at 40 °C. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 6 hours, whereupon a viscous, clear, homogeneous mixture had formed; GPC analysis: M_n 180, M_w 336, M_w / M_n 1.9.

Section 2.1.3.4

BAB **15** (1.19 g, 4.10 mmol) and water (19 μL, 1.05 mmol, ≡ 4.68 g/mol BAB) were stirred for 1 hour at 40 °C. *Novozyme 435®* (3.4 mg, ≡ 0.835 g/mol BAB) was added and the mixture stirred for a further 4 hours at 40 °C. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 10 hours, giving a clear, homogeneous material; GPC analysis: starting material M_n 421, M_w 432, M_w / M_n 1.0; product M_n 432, M_w 459, M_w / M_n 1.1. AB **14** (60 mg, 0.28 mmol) was added and the mixture stirred at 60 °C for 4 hours to give a clear colourless oil; GPC analysis: M_n 484, M_w 566, M_w / M_n 1.2.

BAB **15** (33.4 mg, 0.012 mmol) and water (~0.5 mg, ~0.028 mmol, ≅ 4.68 g/mol BAB) were stirred in butane-1,4-diol **4** (51.8 mg, 0.058 mmol) for 1 hour at 40 °C. *Novozyme 435®* (~0.1 mg, ≅ 0.835 g/mol BAB) was added and the mixture stirred for a further 4 hours at 40 °C. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 6 hours, whereupon a clear reaction mixture had formed. GPC analysis M_n 165.92, M_w 295.63, M_w / M_n 1.78.

Section 2.1.3.5

$(AB)_2$ **51** (50 mg, 0.119 mmol) and water (*ca.* 1 μL, 56 μmol) were stirred for 1 hour at 40 °C. *Novozyme 435®* (0.1 mg, ≅ 0.835 g/mol $(AB)_2$) was added and the mixture stirred for a further 4 hours at 40 °C. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 17 hours, giving a clear, homogeneous material; δ_H (300 MHz; $CDCl_3$) 1.50-1.75 (~11 H, m, $HOCH_2\underline{CH_2}$, $\underline{CH_2}CH_2COOR$, $\underline{CH_2}CH_2OCOR$), 2.18-2.42 (~6 H, m, $\underline{CH_2}COOR$), 3.68 (1 H, t, *J* 6, $\underline{CH_2}OH$), 3.96-4.18 (~6 H, m, $\underline{CH_2}OCOR$); δ_C (75.5 MHz; $CDCl_3$) 24.11, 24.33, 25.10, 25.28 ($HOCH_2\underline{CH_2}$, $\underline{CH_2}CH_2COOR$, $\underline{CH_2}CH_2OCOR$), 33.45, 33.81, ($\underline{CH_2}COOR$), 62.34 ($\underline{CH_2}OH$), 63.86, 64.04 ($\underline{CH_2}OCOR$), 173.39 ($\underline{C}OOR$); GPC analysis: M_n 952, M_w 2056, M_w / M_n 2.2.

Method 2: solvent-based systems

Section 2.1.3.2:

Adipic acid **3** (80.4 mg, 0.55 mmol) and *Novozyme 435®* (3.0 mg) were added to a solution of butane-1,4-diol **4** (49.6 mg, 0.55 mmol) in toluene (5 mL) and stirred at 60 °C in a modified

Dean and Stark apparatus with activated 4 Å molecular sieves for 21 hours. Removal of the volatile components *in vacuo* gave a semi-crystalline white solid; GPC analysis: M_n 905, M_w 1763, M_w / M_n 2.0.

Section 2.1.3.3:

AB **14** (120 mg, 0.55 mmol) and *Novozyme 435®* (3.0 mg) were added to sodium-dried toluene (5 mL) and stirred at 60 °C for 22 hours in a modified Dean and Stark apparatus with activated 4 Å molecular sieves. Volatile components were removed *in vacuo* to give a semi-crystalline white solid; GPC analysis: M_n 1471, M_w 3088, M_w / M_n 2.1.

Section 2.1.3.4:

BAB **15** (50 mg, 0.17 mmol) and *Novozyme 435®* (1.1 mg) were added to toluene (1 mL) and stirred at 60 °C for 17 hours. Volatile components were removed *in vacuo* to give a semi-crystalline colourless oil; GPC analysis: starting material M_n 421, M_w 432, M_w / M_n 1.0; product M_n 477, M_w 1358, M_w / M_n 2.9.

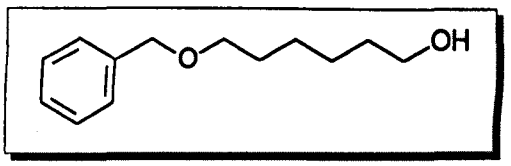
Section 2.1.3.5:

$(AB)_2$ **51** (12.1 mg, 29 μmol) and *Novozyme 435®* (0.1 mg) were added to toluene (0.3 mL) and stirred at 60 °C for 17 hours in a modified Dean and Stark apparatus. Volatile components were removed *in vacuo* to give a semi-crystalline colourless oil; GPC analysis: starting material M_n 629, M_w 507, M_w / M_n 1.2; product M_n 764, M_w 2129, M_w / M_n 2.8.

Section 2.1.3.6:

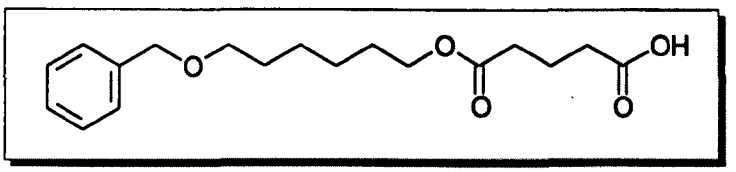
Novozyme 435® (0.1 mg) was added to a solution of $B(AB)_2$ **58** (6 mg) in toluene (0.5 mL) and the mixture stirred at 60 °C for 6 hours. Removal of volatile components *in vacuo* gave a semi-crystalline solid; GPC analysis: M_n 774, M_w 1348, M_w / M_n 1.7.

6-Benzyloxyhexan-1-ol (60).



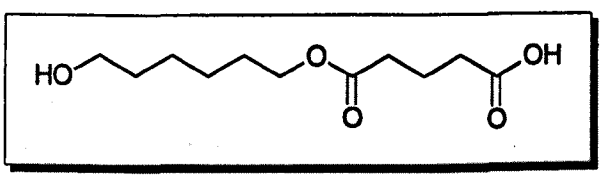
DMSO (60 mL) was added to sodium hydroxide pellets (6.96 g, 174 mmol) and stirred under an atmosphere of nitrogen for 15 minutes, giving a faintly yellow solution with some suspended solid. A solution of hexane-1,6-diol **7** (17.1 g, 145 mmol) in DMSO (120 mL) was added over 10 minutes at ambient temperature and stirring maintained for a further 45 minutes. A solution of benzyl chloride (9.2 mL, 80 mmol) in DMSO (30 mL) was added dropwise and the mixture stirred for 15 hours at ambient temperature. The mixture was poured into ice-water (400 mL) and extracted with diethyl ether (5 x 100 mL). The combined ethereal extracts were dried (magnesium sulphate), filtered through Celite® and the solvent was removed *in vacuo* giving the crude material as a colourless oil. Purification by flash column chromatography (3:2 ethyl acetate:hexane) gave the monoprotected derivative **60** as a colourless oil (6.95 g, 33.4 mmol, 42% based on benzyl chloride); R_f (3:2 ethyl acetate:hexane) 0.35; (Found: MH^+, 209.1545. $C_{13}H_{20}O_2$ requires *MH*, 209.1541); ν_{max} (thin film)/cm^{-1} 3378 (br., O-H str.), 2935, 2860 (C-H str.), 736, 698 (aromatic C-H def.); δ_H (200 MHz, $CDCl_3$) 1.14-1.46 (8 H, m, $BnOCH_2(CH_2)_4CH_2OH$), 2.18 (1 H, s, OH), 3.29 (2 H, t, *J* 7, CH_2OBn), 3.40 (2 H, t, *J* 7.5, CH_2OH), 4.32 (2 H, s, $ArCH_2OR$), 7.08-7.17 (5 H, m, ArH); δ_C (75.5 MHz, $CDCl_3$) 22.85, 23.43, 26.44, 29.78 ($BnOCH_2(CH_2)_4CH_2OH$), 63.02 (CH_2OBn), 70.56 (CH_2OH), 72.98 ($ArCH_2OR$), 127.54, 128.27, 129.51, 138.43 (aromatic C); *m/z* (CI^+, NH_3) 226 (MNH_4^+, 55%), 209 (MH^+, 100), 108 (C_7H_7OH, 33), 91 ($C_7H_7^+$, 15).

12-Benzyloxy-6-oxa-5-oxododecanoic acid (62).



Glutaric anhydride **61** (1.01 g, 8.85 mmol) and *p*-toluenesulphonic acid (5 mg, 30 μmol) were added to a stirred solution of **60** (2.02 g, 9.70 mmol) in benzene (30 mL) under an atmosphere of nitrogen, and the mixture heated under reflux for 18 hours. Volatile components were removed *in vacuo* and the crude product purified by flash column chromatography (3:7 ethyl acetate:petrol) to give the acid **62** as a colourless oil (1.46 g, 4.53 mmol, 52%); R_f (1:1 ethyl acetate:petrol) 0.4; (Found: MH^+, 323.1863. $C_{18}H_{26}O_5$ requires *MH*, 323.1858); ν_{max} (thin film)/cm^{-1} 3600-3100 (br., O-H str.), 2938, 2860 (C-H str.), 1734 (C=O str.), 737, 714 (aromatic C-H def.); δ_H (200 MHz; $CDCl_3$) 1.27-1.37 (4 H, m, $BnOCH_2CH_2(C\underline{H}_2)_2CH_2CH_2OCOR$), 1.52-1.63 (4 H, m, $BnOCH_2C\underline{H}_2(CH_2)_2C\underline{H}_2CH_2OCOR$), 1.88-1.95 (2 H, m, $ROOCCH_2C\underline{H}_2CH_2COOH$), 2.32-2.43 (4 H, m, $ROOCC\underline{H}_2CH_2C\underline{H}_2COOH$), 3.45 (2 H, t, *J* 6.5, $C\underline{H}_2OBn$), 4.04 (2 H, t, *J* 6.5, $C\underline{H}_2OCOR$), 4.49 (2 H, s, $ArC\underline{H}_2OR$), 7.21-7.39 (5 H, m, $Ar\underline{H}$); δ_C (75.5 MHz; $CDCl_3$) 20.07, 20.26 ($BnOCH_2CH_2\underline{C}H_2\underline{C}H_2CH_2CH_2OCOR$), 25.50, 25.59 ($BnOCH_2\underline{C}H_2(CH_2)_2\underline{C}H_2CH_2OCOR$), 28.45 ($ROOCCH_2\underline{C}H_2CH_2COOH$), 33.51, 33.06 ($ROOC\underline{C}H_2CH_2\underline{C}H_2COOH$), 64.31 ($\underline{C}H_2OBn$), 68.94 ($\underline{C}H_2OCOR$), 72.53 ($Ar\underline{C}H_2OR$), 127.34, 128.50, 129.67 (aromatic $\underline{C}$), 177.53, 173.82 ($\underline{C}OOH$, $\underline{C}OOR$); *m/z* (CI^+, NH_3) 340 (MNH_4^+, 28%), 323 (MH^+, 17), 232 (19), 114 ($C_5H_6O_3^+$, 16), 108 ($C_7H_7OH^+$, 100), 91 ($C_7H_7^+$, 28).

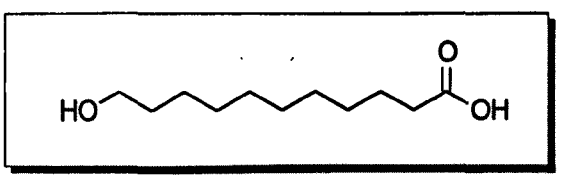
12-Hydroxy-6-oxa-5-oxododecanoic acid (63).



10% Palladium on charcoal (15 mg) was added to a solution of **62** (329 mg, 1.02 mmol) in ethyl acetate (10 mL) and stirred at ambient temperature under an atmosphere of hydrogen at

atmospheric pressure for 19 hours. The solution was filtered through Celite® and volatile components removed *in vacuo*. Purification by flash column chromatography (4:1 ethyl acetate:petrol) gave acid **63** as a viscous colourless oil (169 mg, 0.73 mmol, 72%); R_f (4:1 ethyl acetate:petrol) 0.45; (Found MNH_4^+, 250.1653. $C_{11}H_{20}O_5$ requires *MNH*$_4$, 250.1655); ν_{max} (thin film)/cm^{-1} 3600-2400 (br., O-H str.), 2940, 2863 (C-H str.), 1731 (br., C=O str.); δ_H (200 MHz; $CDCl_3$) 1.31-1.68 (8 H, m, $HOCH_2(CH_2)_4CH_2OCOR$), 1.86-1.95 (2 H, m, $ROOCCH_2CH_2CH_2COOH$), 2.33-2.40 (4 H, m, $ROOCCH_2CH_2CH_2COOH$), 3.63 (2 H, t, *J* 6.5, CH_2OH), 4.04 (2 H, t, *J* 6.5, CH_2OCOR), 8.07 (2 H, br. s, OH); δ_C (75.5 MHz; $CDCl_3$) 19.93, 20.16 ($HOCH_2CH_2(CH_2)_2CH_2CH_2OCOR$), 25.52, 25.65 $HOCH_2CH_2CH_2CH_2CH_2CH_2OCOR$), 28.42 ($ROOCCH_2CH_2CH_2COOH$), 32.95, 33.24 ($ROOCCH_2CH_2CH_2COOH$), 62.55, 64.45 ($HOCH_2CH_2CH_2CH_2CH_2CH_2OCOR$) 173.26, 177.39 (COOH, COOR); *m/z* (CI^+, NH_3) 250 (MNH_4^+, 100%), 232 (MNH_4^+-H_2O, 48), 116 ($C_5H_8O_3^+$, 18); GPC analysis: elution time 15.3 minutes.

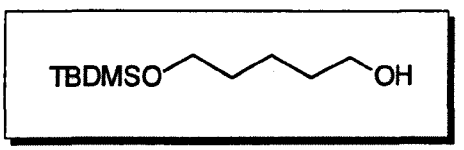
11-Hydroxyundecanoic acid (19).[102]

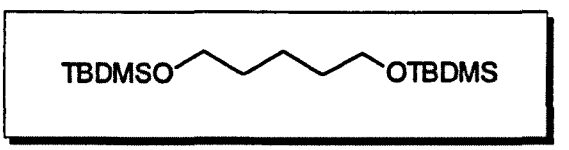


11-Bromoundecanoic acid **64** (6.00 g, 22.6 mmol) and potassium hydroxide pellets (3.82 g, 68.1 mmol) were dissolved in water (60 mL) and heated under reflux for 120 hours. The cooled solution was acidified to ~ pH 1 using 4 M hydrochloric acid and the resultant white precipitate extracted into diethyl ether (2 x 100 mL). The combined ethereal layers were dried (magnesium sulphate), filtered and the solvent removed *in vacuo* to afford the hydroxyacid **19** as a white amorphous solid which required no further purification (4.35 g, 21.5 mmol, 95%), m.pt. 66.5-68.5 °C (lit.[102] 65-67 °C); R_f (ethyl acetate) 0.25; (Found: MNH_4^+, 220.1916. $C_{11}H_{22}O_3$ requires *MNH*$_4$, 220.1913); (Found: C, 65.45; H, 11.05. Calculated for $C_{11}H_{22}O_3$: C, 65.3; H, 10.95%); ν_{max} (Nujol)/cm^{-1} 3300-2400 (br., O-H str.), 1713 (C=O str.), 1109 (C-O str.), 1069 (C-H str.); δ_H (200 MHz; $CDCl_3$) 1.26-1.48 (12 H, m, $HOCH_2CH_2(CH_2)_6CH_2CH_2COOH$), 1.48-1.75 (4 H, m, $HOCH_2CH_2$, CH_2CH_2COOH), 2.34 (2 H, t, *J* 7.5, CH_2COOH), 3.65 (2 H, t, *J* 6.5, CH_2OH); δ_C (75.5 MHz; $CDCl_3$) 24.62, 25.63,

28.95, 29.09, 29.26, 29.38 ($HOCH_2CH_2(\underline{C}H_2)_7CH_2COOH$), 32.67, 33.87 ($HOCH_2\underline{C}H_2$, $\underline{C}H_2COOH$), 63.03 ($\underline{C}H_2OH$), 179.04 ($\underline{C}OOH$); *m/z* (CI^+, NH_3) 220 (MNH_4^+, 100%), 202 (MNH_4^+-H_2O, 18); GPC analysis: elution time 15.3 minutes.

5-(*tert*-Butyldimethylsilyloxy)pentan-1-ol (66) and 1,5-di(*tert*-Butyldimethylsilyloxy) pentane.

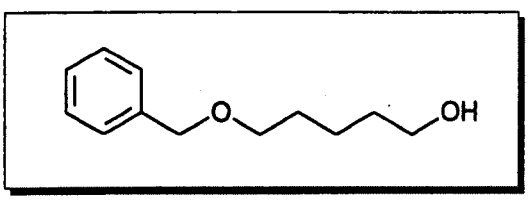


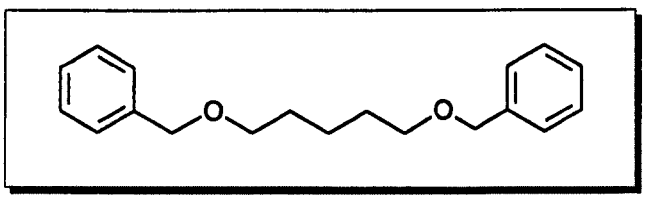


Triethylamine (10.3 mL, 74 mmol) and DMAP (820 mg, 6.71 mmol) were added to a stirred solution of pentane-1,5-diol **65** (14.0 g, 134 mmol) in dichloromethane (100 mL) under an atmosphere of nitrogen at 0 °C. After 5 minutes a solution of TBDMSCl (11.1 g, 73.9 mmol) in dichloromethane (60 mL) was added dropwise at 0 °C and the mixture stirred at ambient temperature for 16 hours. Volatile components were removed *in vacuo* and the resulting mixture purified by flash column chromatography using gradient elution (1:3 - 1:1 ethyl acetate:petrol) to give the monoprotected derivative **66** as a colourless oil (10.1 g, 46.2 mmol, 62% based on TBDMSCl) and diprotected derivative as a colourless oil (2.83 g, 8.51 mmol, 23% based on TBDMSCl); Monoprotected derivative **66**: R_f (1:1 ethyl acetate:petrol) 0.5; (Found: MH^+, 219.1781. $C_{11}H_{26}O_2Si$ requires *MH*, 219.1780); ν_{max} (thin film)/cm^{-1} 3347 (br., O-H str.), 2933, 2870 (C-H str.), 1471, 1255, 1100 (C-O str.), 837 ($SiMe_2$ Si-C def.); δ_H (200 MHz; $CDCl_3$) 0.02 (6 H, s, $Si\underline{Me}_2$), 0.87 (9 H, s, $SiC\underline{Me}_3$), 1.35-1.64 (6 H, m, $SiOCH_2(C\underline{H}_2)_3CH_2OH$), 3.55-3.65 (4 H, m, $C\underline{H}_2OH$, $C\underline{H}_2OSi$); δ_C (75.5 MHz; $CDCl_3$) -5.38 ($Si\underline{Me}_2$), 18.29 ($Si\underline{C}Me_3$), 21.99 ($\underline{C}H_2CH_2CH_2OSi$), 25.91 ($SiC\underline{Me}_3$), 32.47 ($\underline{C}H_2CH_2OH$, $\underline{C}H_2CH_2OSi$), 62.91, 63.08 ($\underline{C}H_2OH$, $\underline{C}H_2OSi$); *m/z* (CI^+, NH_3) 219 (MH^+, 100%), 92 (12). Diprotected derivative: R_f (1:1 ethyl acetate:petrol) 0.9; (Found: MH^+, 333.2643. $C_{17}H_{40}O_2Si_2$ requires *MH*, 333.2645); ν_{max} (thin film)/cm^{-1} 2932 (C-H str.), 1471, 1255, 1100 (C-O str.), 835 ($SiMe_2$ Si-C def.); δ_H (200 MHz; $CDCl_3$) 0.02 (12 H, s, 2 x $Si\underline{Me}_2$), 0.87 (18 H, s, 2 x $SiC\underline{Me}_3$), 1.32-1.60 (6 H, m, $SiOCH_2(C\underline{H}_2)_3CH_2OSi$), 3.58 (4 H, t, *J* 6.5, 2 x $C\underline{H}_2OSi$); δ_C (75.5 MHz; $CDCl_3$) -5.33 ($Si\underline{Me}_2$), 18.32 ($Si\underline{C}Me_3$), 22.13 ($\underline{C}H_2CH_2CH_2OSi$), 25.94

(SiC<u>Me</u>$_3$), 32.66 (<u>C</u>H$_2$CH$_2$OSi), 63.21 (<u>C</u>H$_2$OSi); *m/z* (CI$^+$, NH$_3$) 333 (MH$^+$, 100%), 201 (MH$^+$-HOSiMe$_2$(CMe$_3$), 18).

5-Benzyloxypentan-1-ol (67) and 1,5-di(benzyloxy)pentane.

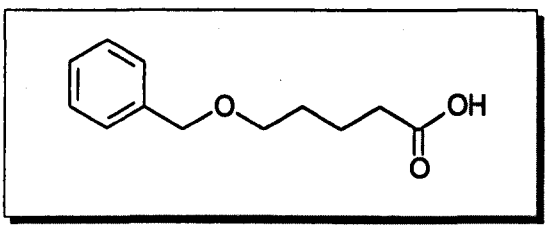




DMSO (200 mL) was added to sodium hydroxide pellets (6.38 g, 160 mmol) and stirred under an atmosphere of nitrogen for 30 minutes, giving a faintly yellow solution. A solution of pentane-1,5-diol **65** (15.1 g, 145 mmol) in DMSO (50 mL) was added dropwise over 10 minutes at ambient temperature and stirring maintained for 1 hour. A solution of benzyl chloride (10.2 mL, 89 mmol) in DMSO (30 mL) was added dropwise and the mixture stirred for 15 hours at ambient temperature. The mixture was poured into ice-water (400 mL) and extracted with diethyl ether (3 x 200 mL). The combined ethereal layers were dried (anhydrous magnesium sulphate), filtered through Celite® and the solvent was removed *in vacuo* to give a colourless oil. Purification by flash column chromatography (3:2 ethyl acetate:hexane) gave monoprotected derivative **67** (12.6 g, 64.6 mmol, 73% based on benzyl chloride) and diprotected derivative (3.03 g, 10.6 mmol, 24% based on benzyl chloride) as colourless oils; Monoprotected derivative **67**: R_f (1:1 ethyl acetate:petrol) 0.4; (Found: MH$^+$, 195 1386. $C_{12}H_{18}O_2$ requires *MH*, 195.1385); ν_{max} (thin film)/cm^{-1} 3398 (br., O-H str.), 2937, 2862, (C-H str.), 1243, 1098 (C-O str.), 735, 698 (aromatic C-H def.); δ_H (300 MHz; $CDCl_3$) 1.40-1.50 (2 H, m, C<u>H</u>$_2$CH$_2$CH$_2$OH), 1.54-1.70 (4 H, m, BnOCH$_2$C<u>H</u>$_2$CH$_2$C<u>H</u>$_2$CH$_2$OH), 2.16 (1 H, s, O<u>H</u>), 3.49 (2 H, t, *J* 6.5, BnOC<u>H</u>$_2$), 3.63 (2 H, t, *J* 6.5, C<u>H</u>$_2$OH), 4.50 (2 H, s, ArC<u>H</u>$_2$OR), 7.26-7.35 (5 H, m, Ar<u>H</u>); δ_C (75.5 MHz; $CDCl_3$) 22.39 (<u>C</u>H$_2$CH$_2$CH$_2$OH), 29.41, 32.47 (BnOCH$_2$<u>C</u>H$_2$CH$_2$<u>C</u>H$_2$CH$_2$OH), 62.78 (<u>C</u>H$_2$OBn), 70.28 (<u>C</u>H$_2$OH), 72.91 (Ar<u>C</u>H$_2$OR), 127.51, 127.64, 128.36, 138.65 (aromatic <u>C</u>); *m/z* (CI$^+$, NH$_3$) 195 (MH$^+$, 100%), 108 ($C_7H_7OH^+$, 23), 91 ($C_7H_7^+$, 18). Diprotected derivative: R_f (1:1 ethyl acetate:petrol) 0.95; (Found: MH$^+$, 285.1864. $C_{19}H_{24}O_2$ requires *MH*, 285.1855); ν_{max} (thin film)/cm^{-1} 3031, 2937, 2858, (C-H str.), 1240, 1099 (C-O str.), 735, 697 (aromatic C-H def.); δ_H (300 MHz; $CDCl_3$)

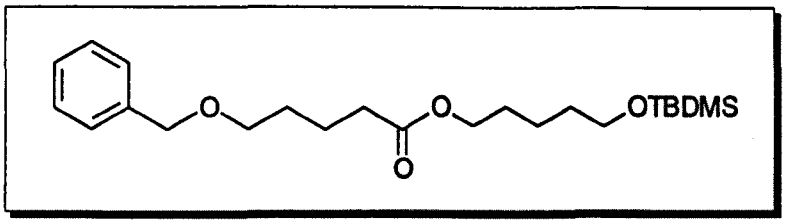
1.41-1.50 (2 H, m, $BnOCH_2CH_2C\underline{H}_2$), 1.59-1.68 (4 H, m, 2 x $BnOCH_2C\underline{H}_2$), 3.46 (4 H, t, *J* 6.5, 2 x $BnOC\underline{H}_2$), 4.48 (4 H, s, 2 x $ArC\underline{H}_2OR$), 7.25-7.36 (10 H, m, Ar<u>H</u>); δ_C (75.5 MHz; $CDCl_3$) 22.79 ($BnOCH_2CH_2\underline{C}H_2$), 29.59 ($BnOCH_2\underline{C}H_2$), 70.28 ($BnOC\underline{H}_2$), 72.81 ($Ar\underline{C}H_2OR$), 127.43, 127.73, 128.31, 138.72 (aromatic <u>C</u>); *m/z* (CI^+, NH_3) 285 (MH^+, 100%), 108 ($C_7H_7OH^+$, 28), 91 ($C_7H_7^+$, 42).

5-Benzyloxypentanoic acid (68).



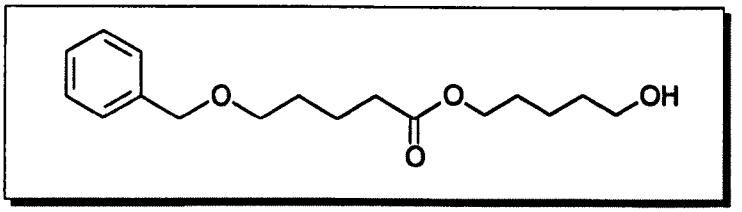
Jones reagent was added dropwise over 15 minutes to a solution of alcohol **67** (8.00 g, 41.2 mmol) in acetone (150 mL) at 0 °C until an orange colour just persisted. The mixture was stirred for 30 minutes at ambient temperature and the excess reagent destroyed by dropwise addition of methanol until a turquoise colour persisted. Volatile components were removed *in vacuo*, water (40 mL) was added to the residue and the mixture was extracted with ethyl acetate (2 x 200 mL). The combined organic layers were extracted with sodium bicarbonate solution (sat. aq.) (3 x 100 mL), and the combined aqueous layers acidified to ~ pH 4 using 2 M hydrochloric acid, saturated with sodium chloride and extracted with ethyl acetate (3 x 150 mL). The combined organic layers were dried (magnesium sulphate), filtered and concentrated *in vacuo* to give the acid **68** as a colourless oil (5.29 g, 25.4 mmol, 62%); R_f (1:1 ethyl acetate:petrol) 0.45; (Found: MH^+, 209.1181. $C_{12}H_{16}O_3$ requires *MH*, 209.1178); ν_{max} (thin film)/cm^{-1} 3700-2400 (br., O-H str.), 2940, 2870 (C-H str.), 1702 (br., C=O str.), 1102 (C-O str.), 736, 698 (aromatic C-H def.); δ_H (300 MHz; $CDCl_3$) 1.65-1.78 (4 H, m, $BnOCH_2(C\underline{H}_2)_2$), 2.36-2.41 (2 H, t, *J* 7, $C\underline{H}_2COOH$), 3.48-3.52 (2 H, t, *J* 6, $BnOC\underline{H}_2$), 4.50 (2 H, s, $ArC\underline{H}_2OR$), 7.27-7.34 (5 H, m, Ar<u>H</u>); δ_C (75.5 MHz; $CDCl_3$) 21.49 ($BnOCH_2CH_2\underline{C}H_2$), 28.98 ($BnOCH_2\underline{C}H_2$), 33.65 ($\underline{C}H_2COOH$), 69.73 ($\underline{C}H_2OBn$), 72.91 ($Ar\underline{C}H_2OR$), 127.57, 127.65, 128.39, 138.50 (aromatic <u>C</u>), 179.47 (<u>C</u>OOH); *m/z* (CI^+, NH_3) 209 (MH^+, 81%), 191 (MH^+-H_2O, 12), 118 (MH^+-C_7H_7, 100), 108 ($C_7H_7OH^+$, 23), 91 ($C_7H_7^+$, 17).

5-Benzyloxypentanoic acid, 5'-(*tert*-butyldimethylsilyloxy)pentyl ester (69).



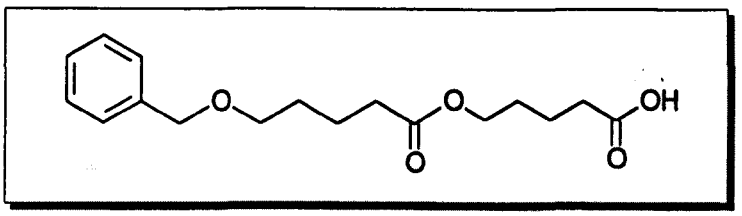
A solution of alcohol **66** (4.76 g, 21.8 mmol) in dichloromethane (40 mL) was added to a suspension of acid **68** (4.54 g, 21.8 mmol), DCC (4.95 g, 24.0 mmol) and DMAP (27 mg, 2.2 mmol) in dichloromethane (40 mL) at ambient temperature under an atmosphere of nitrogen, and the mixture stirred for 20 hours at ambient temperature. The mixture was filtered and volatile components removed *in vacuo*. Purification by flash column chromatography (1:8 ethyl acetate:petrol) gave the ester **69** as a colourless oil (7.39 g, 18.1 mmol, 83%); R_f (1:4 ethyl acetate:petrol) 0.65; (Found: MH^+, 409.2774. $C_{23}H_{40}O_4Si$ requires *MH*, 409.2774); ν_{max} (thin film)/cm^{-1} 2933, 2860 (C-H str.), 1738 (C=O str.), 1255, 1097 (C-O str.), 835 ($SiMe_2$ Si-C def.), 735, 697 (aromatic C-H def.); δ_H (300 MHz; $CDCl_3$) 0.04 (6 H, s, $SiMe_2$), 0.89 (9 H, s, $SiCMe_3$), 1.36-1.41 (2 H, m, $SiOCH_2CH_2CH_2$), 1.54-1.57 (2 H, m, $SiOCH_2CH_2$), 1.61-1.73 (6 H, m, CH_2CH_2OCOR, $BnOCH_2(CH_2)_2$), 2.32 (2 H, t, *J* 7, CH_2COOR), 3.48 (2 H, t, *J* 6, CH_2OBn), 3.61 (2 H, t, *J* 6.5, CH_2OSi), 4.06 (2 H, t, *J* 6.5, CH_2OCOR), 4.50 (2 H, s, $ArCH_2OR$), 7.27-7.35 (5 H, m, ArH); δ_C (75.5 MHz; $CDCl_3$) -5.36 ($SiMe_2$), 18.29 ($SiCMe_3$), 21.77, 22.27 ($SiOCH_2(CH_2)_2$), 25.91 ($SiCMe_3$), 28.43, 29.16, 32.37 (CH_2CH_2OCOR, $BnOCH_2(CH_2)_2$), 34.04 (CH_2COOR), 62.90, 64.34 (CH_2OBn, CH_2OSi), 69.85 (CH_2OCOR), 72.91 ($ArCH_2OR$), 127.53, 127.62, 128.38, 138.65 (aromatic C), 173.67 (COOR); *m/z* (CI^+, NH_3) 409 (MH^+, 100%), 201 ($C_5H_{10}OSiMe_2(CMe_3)^+$, 40), 191 ($MH^+$-$HOC_5H_{10}OSiMe_2(CMe_3)$, 24), 118 (22), 108 ($C_7H_7OH^+$, 49), 91 ($C_7H_7^+$, 59).

5-Benzyloxypentanoic acid, 5'-hydroxypentyl ester (70).



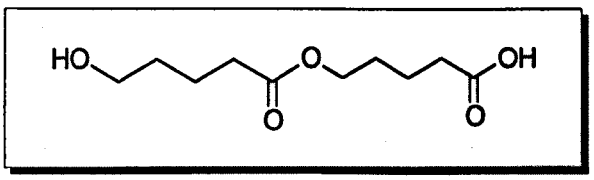
TBAF (1.0 M solution in THF, 23.5 mL, 23.5 mmol) was added to a solution of **69** (7.39 g, 18.1 mmol) in THF (120 mL) at ambient temperature, and the solution stirred for 5 hours at ambient temperature. Diethyl ether (200 mL) was added and the solution washed with brine (40 mL). The organic phase was dried (magnesium sulphate), filtered and concentrated *in vacuo* to give a pale yellow oil. Purification by flash column chromatography using gradient elution (1:3 - 1:1 ethyl acetate:petrol) gave alcohol **70** as a colourless oil (4.26 g, 14.5 mmol, 80%); R_f (1:4 ethyl acetate:petrol) 0.1; (Found: MH^+, 295.1912. $C_{17}H_{26}O_4$ requires *MH*, 295.1909); (Found: C, 69.25; H, 9.05. Calculated for $C_{17}H_{26}O_4$: C, 69.35; H, 8.9%); ν_{max} (thin film)/cm^{-1} 3431 (br., O-H str.), 2940, 2863 (C-H str.), 1732 (C=O str.), 1240, 1098 (C-O str.), 736, 698 (aromatic C-H def.); δ_H (300 MHz; $CDCl_3$) 1.40-1.48 (2 H, m, $HOCH_2CH_2\underline{CH_2}$), 1.55-1.78 (8 H, m, $HOCH_2\underline{CH_2}CH_2\underline{CH_2}CH_2OCOR$, $BnOCH_2(\underline{CH_2})_2$), 2.33 (2 H, t, *J* 7.5, $\underline{CH_2}COOR$), 3.49 (2 H, t, *J* 6, $\underline{CH_2}OBn$), 3.63 (2 H, t, *J* 6, $\underline{CH_2}OH$), 4.08 (2 H, t, *J* 6.5, $\underline{CH_2}OCOR$), 4.50 (2 H, s, $ArCH_2OR$), 7.27-7.35 (5 H, m, Ar<u>H</u>); δ_C (75.5 MHz; $CDCl_3$) 21.75, 22.18, 28.40, 29.12, 32.22 ($HOCH_2(\underline{CH_2})_3CH_2OCOR$, $BnOCH_2(\underline{CH_2})_2$), 34.01 ($\underline{CH_2}COOR$), 62.59 ($\underline{CH_2}OH$), 64.17 ($\underline{CH_2}OBn$,), 69.82 ($\underline{CH_2}OCOR$), 72.88 ($Ar\underline{CH_2}OR$), 127.53, 127.61, 128.36, 138.56 (aromatic <u>C</u>), 173.68 (<u>C</u>OOR); *m/z* (CI^+, NH_3) 295 (MH^+, 100%), 187 (MH^+-C_7H_7OH, 23), 118 (MH^+-C_7H_7-$C_5H_{10}O$, 92), 108 ($C_7H_7OH^+$, 17).

11-Benzyloxy-6-oxa-7-oxoundecanoic acid (71).



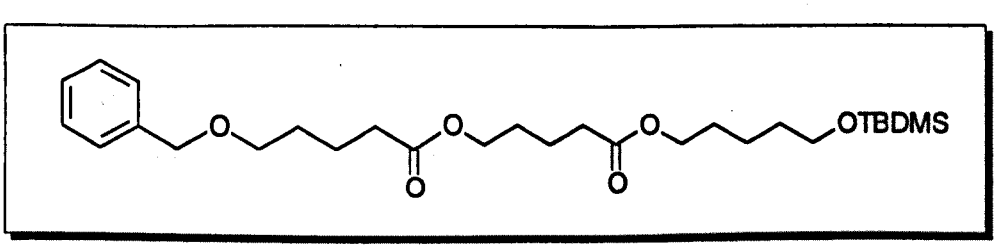
Jones reagent was added dropwise over 30 minutes to a solution of alcohol **70** (4.09 g, 13.9 mmol) in acetone (100 mL) at 0 °C until an orange colour just persisted. The mixture was stirred for 30 minutes at ambient temperature and the excess reagent destroyed by dropwise addition of methanol until a turquoise colour persisted. Volatile components were removed *in vacuo*, water (20 mL) was added to the residue and the mixture was extracted with ethyl acetate (2 x 200 mL). The combined organic layers were extracted with sodium bicarbonate solution (sat. aq.) (3 x 100 mL), and the combined aqueous layers acidified to ~ pH 4 using 2 M sulphuric acid, saturated with sodium chloride and extracted with ethyl acetate (3 x 200 mL). The combined organic layers were dried (magnesium sulphate), filtered and concentrated *in vacuo* to give the acid **71** as a colourless oil (1.79 g, 5.86 mmol, 42%); R_f (1:1 ethyl acetate:petrol) 0.3; (Found: MH^+, 309.1706. $C_{17}H_{24}O_5$ requires *MH*, 309.1702); ν_{max} (thin film)/cm^{-1} 3500-2400 (br., O-H str.), 2954, 2870 (C-H str.), 1733 (C=O str., ester), 1708 (C=O str., acid), 1098 (C-O str.), 737, 698 (aromatic C-H def.); δ_H (300 MHz; $CDCl_3$) 1.66-1.78 (8 H, m, $BnOCH_2(CH_2)_2$, $RCOOCH_2(CH_2)_2$), 2.31-2.45 (4 H, m, CH_2COOH, CH_2COOR), 3.49 (2 H, t, *J* 6, $BnOCH_2$), 4.08 (2 H, t, *J* 6, CH_2OCOR), 4.50 (2 H, s, $ArCH_2OR$), 7.26-7.34 (5 H, m, ArH); δ_C (75.5 MHz; $CDCl_3$) 21.16, 21.68 ($BnOCH_2CH_2CH_2$, CH_2CH_2COOH), 27.94, 29.05 ($BnOCH_2CH_2$, $RCOOCH_2CH_2$), 33.38, 33.93 (CH_2COOR, CH_2COOH), 63.71 (CH_2OBn), 69.75 (CH_2OCOR), 72.83 ($ArCH_2OR$), 127.52, 127.61, 128.34, 138.49 (aromatic C), 173.67 (COOR), 179.03 (COOH); *m/z* (CI^+, NH_3) 309 (MH^+, 4%), 201 (MH^+-C_7H_7OH, 3), 191 (MH^+-$HOOCC_4H_8OH$, 6), 91 ($C_7H_7^+$, 100).

11-Hydroxy-6-oxa-7-oxoundecanoic acid (72).



10% Palladium on charcoal (0.30g) was added to a solution of **71** (1.33 g, 4.31 mmol) in ethyl acetate (30 mL) and the suspension stirred at ambient temperature under an atmosphere of hydrogen at atmospheric pressure for 60 hours. The mixture was filtered through Celite® and the solvent removed *in vacuo*. Purification by dry flash chromatography[76] using gradient elution (1:1 ethyl acetate:petrol - ethyl acetate) gave hydroxyacid **72** as a colourless oil (328 mg, 1.50 mmol, 35%); R_f (1:10 glacial acetic acid:ethyl acetate) 0.25; (Found: MH^+, 219.1233. $C_{10}H_{18}O_5$ requires *MH*, 219.1233); ν_{max} (thin film)/cm^{-1} 3500-2400 (br., O-H str.), 2951 (C-H str.), 1729 (C=O str., ester), 1713 (C=O str., acid); δ_H (400 MHz; $CDCl_3$) 1.57-1.75 (8 H, m, $HOCH_2(CH_2)_2$, $(CH_2)_2CH_2OCOR$), 2.29-2.40 (4 H, m, CH_2COOH, CH_2COOR), 3.66 (2 H, t, *J* 6.5, CH_2OH), 4.09 (2 H, t, *J* 6.5, CH_2OCOR), 6.28 (2 H, br. s, OH); δ_C (100.6 MHz; $CDCl_3$) 21.13, 21.28, 28.00, 31.84 ($HOCH_2(CH_2)_2$, $(CH_2)_2CH_2OCOR$), 33.26, 33.91 (CH_2COOH, CH_2COOR), 62.28 (CH_2OH), 63.83 (CH_2OCOR), 173.73, 177.83, (COOR, COOH); *m/z* (CI^+, NH_3) 236 (MNH_4^+, 24%), 219 (MH^+, 10), 175 (MH^+-CO_2, 8), 118 ($HOC_4H_8CO_2H^+$, 100), 101 ($HOC_4H_8CO^+$, 24); GPC analysis: elution time 15.4 minutes.

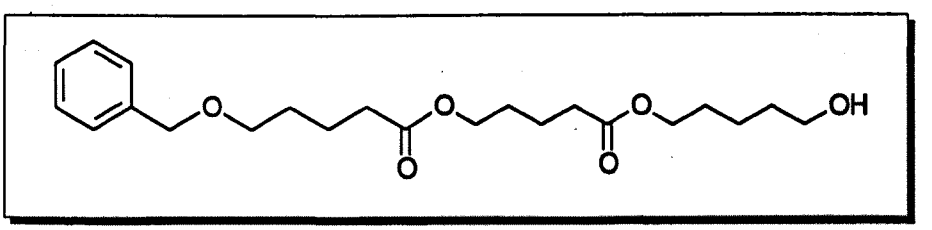
11-Benzyloxy-6-oxa-7-oxoundecanoic acid, 5'-(*tert*-butyldimethylsilyloxy)pentyl ester (73).



A solution of alcohol **66** (29.8 mg, 136 μmol) in dichloromethane (2 mL) was added to a solution of acid **71** (50.0 mg, 162 μmol), DCC (37.1 mg, 180 μmol) and DMAP (2.5 mg, 20 μmol) in dichloromethane (2 mL) at ambient temperature under an atmosphere of nitrogen,

and the mixture stirred for 20 hours at this temperature. The mixture was filtered and volatile components removed *in vacuo*. Purification by flash column chromatography using gradient elution (petrol - 1:6 ethyl acetate:petrol) gave the diester **73** as a colourless oil (49.3 mg, 97 μmol, 71%); R_f (1:4 ethyl acetate:petrol) 0.45; (Found: MH^+, 509.3303. $C_{28}H_{48}O_6Si$ requires *MH*, 509.3298); ν_{max} (thin film)/cm^{-1} 2956, 2860 (C-H str.), 1738 (C=O str.), 1249, 1097 (C-O str.), 835 ($SiMe_2$ Si-C def.), 734, 697 (aromatic C-H def.); δ_H (300 MHz; $CDCl_3$) 0.05 (6 H, s, $SiMe_2$), 0.91 (9 H, s, $SiCMe_3$), 1.37-1.45 (2 H, m), 1.50-1.57 (2 H, m) and 1.63-1.78 (10 H, m, $CH_2CH_2CH_2OCOR$, 2 x CH_2CH_2OCOR, 2 x CH_2CH_2COOR, $BnOCH_2CH_2$, $SiOCH_2CH_2$), 2.31-2.36 (4 H, m, 2 x CH_2COOR), 3.49 (2 H, t, *J* 6, CH_2OBn), 3.62 (2 H, t, *J* 6.5, CH_2OSi), 4.06-4.10 (4 H, m, 2 x CH_2OCOR), 4.50 (2 H, s, $ArCH_2OR$), 7.28-7.35 (5 H, m, ArH); δ_C (75.5 MHz; $CDCl_3$) -5.39 ($SiMe_2$), 18.25 ($SiCMe_3$), 21.43, 21.71, 22.23, 28.06, 28.39, 29.12, 32.32 ($SiOCH_2(CH_2)_2$, 2 x CH_2CH_2OCOR, 2 x CH_2CH_2COOR, $BnOCH_2CH_2$), 25.88 ($SiCMe_3$), 33.71, 33.94 (2 x CH_2COOR), 62.85 (CH_2OBn), 63.73 (CH_2OSi), 64.41, 69.79 (2 x CH_2OCOR), 72.86 ($ArCH_2OR$), 127.50, 127.58, 128.34, 138.60 (aromatic C), 173.33, 173.57 (2 x COOR); *m/z* (FAB^+) 509 (MH^+, 2%), 215 (4), 115 ($SiMe_2(CMe_3)^+$, 14), 91 ($C_7H_7^+$, 98).

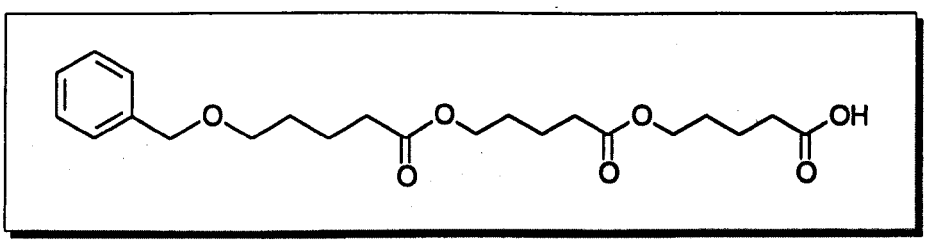
11-Benzyloxy-6-oxa-7-oxoundecanoic acid, 5'-hydroxypentyl ester (74).



TBAF (1.0 M solution in THF, 80 μL, 80 μmol) was added to a solution of **73** (31.2 mg, 61 μmol) in THF (1.5 mL) at ambient temperature, and the solution stirred for 19 hours at ambient temperature. Diethyl ether (20 mL) was added and the solution washed with water (3 mL). The organic phase was dried (magnesium sulphate), filtered and concentrated *in vacuo* to give a colourless oil. Purification by flash column chromatography (1:1 ethyl acetate:petrol) gave alcohol **74** as a colourless oil (18.6 mg, 47 μmol, 77%); R_f (1:1 ethyl acetate:petrol) 0.55; (Found: MNH_4^+, 412.2715. $C_{22}H_{34}O_6$ requires MNH_4, 412.2699); ν_{max} (thin film)/cm^{-1} 3446 (br., O-H str.), 2939, 2865 (C-H str.), 1733 (C=O str.), 1244, 1098 (C-O

str.), 736, 698 (aromatic C-H def.); δ_H (300 MHz; $CDCl_3$) 1.40-1.76 (14 H, m, 2 x $C\underline{H}_2CH_2COOR$, 2 x $C\underline{H}_2CH_2OCOR$, $HOCH_2(C\underline{H}_2)_2$, $BnOCH_2C\underline{H}_2$), 2.31-2.35 (4 H, m, 2 x $C\underline{H}_2COOR$), 3.49 (2 H, t, *J* 6, $BnOC\underline{H}_2$), 3.65 (2 H, t, *J* 6.5, $C\underline{H}_2OH$), 4.06-4.11 (4 H, m, 2 x $C\underline{H}_2OCOR$), 4.50 (2 H, s, $ArC\underline{H}_2OR$), 7.28-7.35 (5 H, m, Ar<u>H</u>); δ_C (75.5 MHz; $CDCl_3$) 21.44, 21.74, 22.22, 28.08, 28.41, 29.13, 32.25, 33.75, 33.98 (2 x $\underline{C}H_2CH_2OCOR$, $HOCH_2(\underline{C}H_2)_2$, 2 x $(\underline{C}H_2)_2COOR$, $BnOCH_2\underline{C}H_2$), 62.64 ($\underline{C}H_2OH$), 63.77 ($\underline{C}H_2OBn$,), 64.29, 69.82 (2 x $\underline{C}H_2OCOR$), 72.90 ($Ar\underline{C}H_2OR$), 127.54, 127.62, 128.37, 138.59 (aromatic <u>C</u>), 173.37, 173.65 (2 x $\underline{C}OOR$); *m/z* (CI^+, NH_3) 412 (MNH_4^+, 3%), 395 (MH^+, 1), 265 (1), 187 (MH^+-$C_7H_7OC_4H_8COOH$, 3), 118 ($HOC_4H_8COOH^+$, 100%).

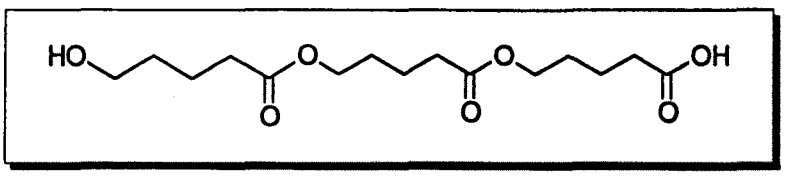
17-Benzyloxy-6,12-dioxa-7,13-dioxoheptadecanoic acid (75).



Jones reagent (2 drops) was added to a solution of alcohol **74** (15.2 mg, 39 μmol) in acetone (1 mL) at 0 °C whereupon an orange colour just persisted. The mixture was stirred for 15 minutes at 0 °C and 15 minutes at ambient temperature and the excess reagent destroyed by dropwise addition of methanol (2 drops) whereupon a turquoise colour persisted. Volatile components were removed *in vacuo*, water (2 mL) was added and the mixture extracted with ethyl acetate (15 mL). The organic layer was dried (magnesium sulphate), filtered and concentrated *in vacuo* to give acid **75** as a colourless oil (13.2 mg, 32 μmol, 84%); R_f (ethyl acetate) 0.45; (Found: MH^+, 409.2229. $C_{22}H_{32}O_7$ requires *MH*, 409.2226); ν_{max} (thin film)/cm^{-1} 3600-2400 (br., O-H str.), 2958, 2874 (C-H str.), 1735 (C=O str., ester), 1708 (C=O str., acid), 1098 (C-O str.), 739, 698 (aromatic C-H def.); δ_H (300 MHz; $CDCl_3$) 1.64-1.73 (12 H, m, $BnOCH_2C\underline{H}_2$, 2 x $C\underline{H}_2CH_2COOR$, $C\underline{H}_2CH_2COOH$, 2 x $C\underline{H}_2CH_2OCOR$), 2.30-2.41 (6 H, m, 2 x $C\underline{H}_2COOR$, $C\underline{H}_2COOH$), 3.49 (2 H, t, *J* 6, $BnOC\underline{H}_2$), 4.05-4.12 (4 H, m, 2 x $C\underline{H}_2OCOR$), 4.50 (2 H, s, $ArC\underline{H}_2OR$), 7.26-7.35 (5 H, m, Ar<u>H</u>); δ_C (75.5 MHz; $CDCl_3$) 21.17, 21.38, 21.66, 27.91, 28.01, 29.04 ($BnOCH_2\underline{C}H_2$, 2 x $\underline{C}H_2CH_2COOR$, $\underline{C}H_2CH_2COOH$, 2 x $\underline{C}H_2CH_2OCOR$), 33.17, 33.68, 33.93 (2 x $\underline{C}H_2COOR$, $\underline{C}H_2COOH$), 63.76, 63.80, 69.79

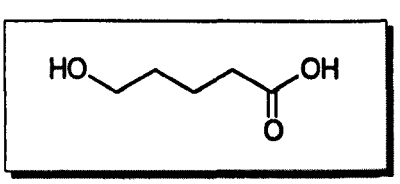
($\underline{C}H_2OBn$, 2 x $\underline{C}H_2OCOR$), 72.87 ($Ar\underline{C}H_2OR$), 127.63, 127.72, 128.44, 138.56 (aromatic $\underline{C}$), 173.43, 173.78 (2 x $\underline{C}OOR$), 177.73 ($\underline{C}OOH$); *m/z* (CI^+, NH_3) 409 (MH^+, 0.25%), 265 (0.5), 218 (MH^+-$C_7H_7OC_4H_8CO$, 0.8), 191 ($C_7H_7OC_4H_8CO^+$, 1.2), 118 ($HOC_4H_8COOH^+$, 100).

17-Hydroxy-6,12-dioxa-7,13-dioxoheptadecanoic acid (76).



10% Palladium on charcoal (10 mg) was added to a solution of **75** (12.1 mg, 30 μmol) in ethyl acetate (0.5 mL) and the suspension stirred at ambient temperature under an atmosphere of hydrogen at atmospheric pressure for 25 hours. The mixture was filtered through Celite® and the solvent removed *in vacuo* to give hydroxyacid **76** as a colourless oil (5.5 mg, 17 μmol, 57%); R_f (1:10 glacial acetic acid:ethyl acetate) 0.3; δ_H (200 MHz; $CDCl_3$) 1.64-1.75 (12 H, m, $HOCH_2\underline{C}H_2$, 2 x $\underline{C}H_2CH_2COOR$, $\underline{C}H_2CH_2COOH$, 2 x $\underline{C}H_2CH_2OCOR$), 2.30-2.48 (6 H, m, 2 x $\underline{C}H_2COOR$, $\underline{C}H_2COOH$), 3.69 (2 H, t, *J* 6.5, $\underline{C}H_2OH$), 4.06-4.16 (4 H, m, 2 x $\underline{C}H_2OCOR$); δ_C (100.6 MHz; $CDCl_3$) 20.94, 21.27, 21.45, 28.05, 29.69, 31.87 ($HOCH_2\underline{C}H_2$, 2 x $\underline{C}H_2CH_2COOR$, $\underline{C}H_2CH_2COOH$, 2 x $\underline{C}H_2CH_2OCOR$), 33.00, 33.15, 33.74 (2 x $\underline{C}H_2COOR$, $\underline{C}H_2COOH$), 62.30 ($\underline{C}H_2OH$), 63.85, 63.98 (2 x $\underline{C}H_2OCOR$); *m/z* (CI^+, NH_3) 118 ($HOC_4H_8COOH^+$, 100%). GPC analysis: elution time 14.8 minutes.

5-Hydroxypentanoic acid (77).



10% Palladium on charcoal (40.0 mg) was added to a solution of **68** (300 mg, 1.44 mmol) in ethyl acetate (10 mL) which had been passed through a plug of neutral alumina. The suspension was stirred at ambient temperature under an atmosphere of hydrogen at atmospheric pressure for 40 hours. TLC indicated the formation of a large amount of two non-

polar components as well as some highly polar material. The mixture was filtered through Celite® and the solvent removed *in vacuo*. Purification by dry flash chromatography[76] using gradient elution (1:1 ethyl acetate:petrol - ethyl acetate) gave only mixed fractions of non-polar materials which were not characterised.

Poly(12-hydroxy-6-oxa-5-oxododecanoic acid).

AB mimic **63** (128 mg, 0.55 mmol) and *Novozyme 435®* (3.0 mg) were added to sodium-dried toluene (5 mL) and stirred at 60 °C for 22 hours in a modified Dean and Stark apparatus with activated 4 Å molecular sieves. Volatile components were removed *in vacuo* to give a semi-crystalline white solid; GPC analysis: M_n 1657, M_w 3174, M_w / M_n 1.9.

Poly(11-hydroxyundecanoic acid).

Method 1: 11-Hydroxyundecanoic acid **19** (2.00 g, 9.89 mmol) and water (40 μL) were stirred at 70 °C for 30 minutes. *Novozyme 435®* (7.4 mg) was added and the mixture stirred at 70 °C for 4 hours. Stirring was then maintained under reduced pressure (100 ± 5 mbar) at 70 °C for a further 17 hours to give a colourless liquid which solidified to a hard white waxy solid; δ_H (200 MHz; $CDCl_3$) 1.20-1.45 (~9 H, m, $(CH_2)_6CH_2CH_2COOH$, $(CH_2)_6CH_2CH_2COOR$), 1.50-1.75 (~3 H, m, $HOCH_2CH_2$, CH_2CH_2COOH, CH_2CH_2COOR), 2.25-2.38 (1.4 H, m, CH_2COOH, CH_2COOR), 3.64 (0.27 H, t, *J* 6.5, CH_2OH), 4.05 (1.00 H, t, *J* 6.5, CH_2OCOR); δ_C (75.5 MHz; $CDCl_3$) 24.67, 24.97, 25.88, 28.63, 29.19, 29.33 ($(CH_2)_7CH_2COOH$, $(CH_2)_7CH_2COOR$), 33.64, 34.37 ($HOCH_2CH_2$, CH_2COOH, CH_2COOR), 63.05 (CH_2OH), 64.38 (CH_2OCOR), 174.04 (COOR); GPC analysis: M_n 1437, M_w 2372, M_w / M_n 1.7.

Method 2: *Novozyme 435®* (3.0 mg) was added to 11-hydroxyundecanoic acid **19** (111 mg, 0.55 mmol) and toluene (5 mL) and the mixture stirred at 60 °C for 22 hours. Volatile components were removed *in vacuo* to give a white waxy solid; GPC analysis: M_n 533, M_w 1336, M_w / M_n 2.5.

Poly(11-hydroxy-6-oxa-7-oxoundecanoic acid).

Method 1: 11-hydroxy-6-oxa-7-oxoundecanoic acid **72** (52.8 mg, 0.24 mmol) was dissolved in toluene (2.2 mL) and stirred at 60 °C for 30 minutes. *Novozyme 435®* (1.3 mg) was added and the mixture stirred at 60 °C for 22 hours. Volatile components were removed *in vacuo* to give a colourless semi-crystalline material; GPC analysis: M_n 797, M_w 2347, M_w / M_n 2.9.
Method 2: 11-hydroxy-6-oxa-7-oxoundecanoic acid **72** (95 mg, 0.44 mmol) and water (3 μL) were stirred at 40 °C for 1 hour. *Novozyme 435®* (0.4 mg) was added and the mixture stirred at 40 °C for 4 hours. Stirring was then maintained under reduced pressure (100 ± 5 mbar) at 60 °C for a further 17 hours to give a colourless liquid. Samples were taken 1, 2, 4, 8 and 22 hours after enzyme dosing; GPC analysis (22 hours): M_n 887, M_w 1565, M_w / M_n 1.8.

Equilibrium test experiment (Section 2.1.4.2).

Butane-1,4-diol **4** (31.26 g, 0.346 mol) and water (1.46 mL, 0.08 mol) were added to adipic acid **3** (45.19 g, 0.309 mol) and the mixture stirred at 40 °C for 1 hour. *Chirazyme®* (157 mg, 0.21% w/w butane-1,4-diol/adipic acid, ≡ 0.508 g/mol adipic acid) was added and the mixture stirred for a further 4 hours at 40 °C. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 17 hours. The mixture was stirred at 60 °C for a further 8 hours at atmospheric pressure, then a partial vacuum (10 ± 3 mbar) was applied for 17 hours. Water (6.0 mL, 0.33 mol, 8% w/w butane-1,4-diol/adipic acid) was added and the mixture stirred at 60 °C at atmospheric pressure for a further 24 hours. A partial vacuum (10 ± 3 mbar) was again applied for 24 hours. For GPC analyses, see Section 2.1.4.2.

Polymerisation of diethyl adipate (79) with butane-1,4-diol (4) (Section 2.1.4.3).

Butane-1,4-diol **4** (2.12 g, 23.5 mmol) and water (100 μL) were added to diethyl adipate **79** (4.25 g, 21.0 mmol) and the mixture stirred for 30 minutes at 40 °C. *Novozyme 435®* (17.5 mg) was added and the mixture stirred at 40 °C for 4 hours. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 17 hours; GPC analysis: M_w 834, M_n 426, M_w / M_n 2.0.

Comparative polyol trials (Section 2.2.2.2).

Adipic acid **3** (3.00 g, 21 mmol) and water (100 μL) were added to the polyol under test (23.5 mmol) and the mixture stirred at 40 °C for 1 hour. *Novozyme 435®* (17.5 mg) was added and the mixture stirred at 40 °C for 4 hours. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 17 hours.

Lipase recycling trials (Section 2.2.2.2).

Adipic acid **3** (0.94 g, 6.6 mmol) and water (100 μL) were added to butane-1,4-diol **4** (0.67 g, 7.4 mmol) and the mixture stirred at 40 °C for 1 hour. A sample of *Novozyme 435®* recovered from polyol trials (5.5 mg) was added and the mixture stirred at 40 °C for 4 hours. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 17 hours. A control reaction using virgin *Novozyme 435®* was run in tandem with the recycled lipases under test.

Large scale polyol trials (Section 2.2.2.3).

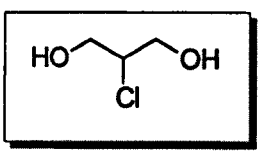
The polyol under test (0.346 mol) and water (1.46 mL, 0.08 mol) were added to adipic acid **3** (45.19 g, 0.309 mol) and the mixture stirred at 40 °C for 1 hour. *Chirazyme®* (157 mg) or *Novozyme 435®* (230 mg) was added and the mixture stirred at 40 °C for 4 hours. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 17 hours. The lipase used was removed by filtration and washed with hot acetone and dried under high vacuum for 2 hours at ambient temperature. The recovered lipase samples were subjected to kinetic assay activity measurements.

Enzyme kinetic assay method (Section 2.2.2.3).[107]

Two vials, each containing oleic acid (4.000 g, 14.2 mmol) and hexan-1-ol (20 mL, 160 mmol) were stirred at 40 °C for 1 hour. To one was added a control dose of lipase and to the other the lipase sample under test (200 mg of each unless otherwise stated). The mixtures were stirred at 40 °C for 1 hour. A 500 μL sample of each mixture was taken at this point and

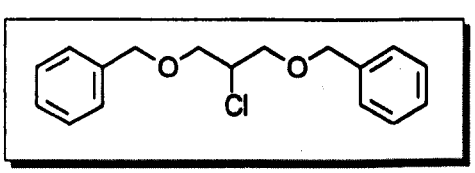
then 15, 30, 45 and 60 minutes afterwards. Each sample was added to a solution of phenolphthalein (1 mL of a solution of 1 wt.% phenolphthalein in isopropanol) in isopropanol (20 mL) and titrated against 0.02 M sodium hydroxide solution. A plot of titre against time gives a straight line for each sample; the ratio of the two gradients gives the activity of the test sample relative to the control.

2-Chloro-1,3-propanediol (85).[113]



Lithium aluminium hydride (2.74 g, 72.2 mmol) was added to THF (100 mL) at 0 °C with stirring under an atmosphere of nitrogen and stirring maintained for 30 minutes at 0 °C. 100% sulphuric acid (1.9 mL, 35.5 mmol) was added with extreme caution at 0 °C over 15 minutes. A solution of diethyl chloromalonate **86** (5.00 g, 26 mmol) in THF (30 mL) was then added dropwise at 0 °C. Stirring was maintained for 30 minutes at 0 °C and 14 hours at ambient temperature, whereupon TLC indicated the absence of starting material. Sodium sulphate solution (sat. aq.) (6 mL) was added and the inorganic salts were removed by filtration and washed with boiling THF (100 mL). The combined organic layers were dried (magnesium sulphate), filtered and concentrated *in vacuo* to give a colourless oil. ^{1}H NMR spectroscopy indicated that the diol **85** had not been formed.

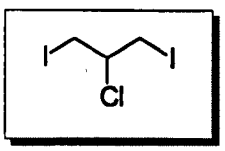
2-Chloro-1,3-dibenzyloxypropane (88).



Benzyl alcohol (7.0 mL, 68 mmol) was added to a solution of sodium hydride (60% dispersion in mineral oil, 2.85 g, 71.2 mmol) in DMF (100 mL) at 0 °C with stirring under an atmosphere of nitrogen, and stirring maintained for 30 minutes at ambient temperature. 1,2,3-

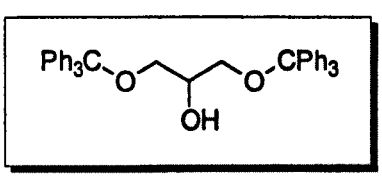
trichloropropane **87** (3.6 mL, 34 mmol) was added and the mixture heated under reflux for 11 hours, whereupon TLC indicated only the presence of starting material.

2-Chloro-1,3-diiodopropane (89).



Sodium iodide (50.5 g, 34 mmol) was added to a solution of 1,2,3-trichloropropane **87** (5.00 g, 34 mmol) in acetone (250 mL) at ambient temperature under an atmosphere of nitrogen. The mixture was heated under reflux for 16 hours, whereupon the mixture had become orange-red. ^{1}H NMR spectroscopy on the crude reaction mixture indicated that no iodine had been incorporated into the product.

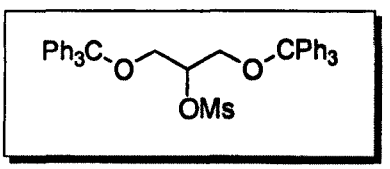
1,3-Di(trityloxy)-2-propanol (90).



A solution of glycerol **83** (3.87g, 42.0 mmol) in DMF (20 mL) and triethylamine (12.5 mL, 90 mmol) were added to a stirred solution of trityl chloride (24.0 g, 86.1 mmol) and DMAP (240 mg, 1.96 mmol) in DMF (150 mL) under an atmosphere of nitrogen at 0 °C. The solution was stirred at 0 °C for 30 minutes then at ambient temperature for 12 hours; completion of reaction was achieved by warming the mixture to 40 °C for 90 minutes. The mixture was poured onto ice-water (200 mL) and extracted into dichloromethane (2 x 250 mL). The combined organic layers were washed with ammonium chloride solution (sat. aq.) (200 mL), water (600 mL) and dried (magnesium sulphate), filtered and concentrated *in vacuo* to give a pale yellow solid. Recrystallisation (ethyl acetate) gave the diprotected derivative **90** as white plates (18.9 g, 32.7 mmol, 78%), m.pt. 171 °C; R_f (1:1 ethyl acetate:hexane) 0.8; (Found: C, 85.25; H, 6.3. Calculated for $C_{41}H_{36}O_3$: C, 85.4; H, 6.3%); ν_{max} (Nujol)/cm^{-1} 3486 (O-H str.), 3055, (C-H

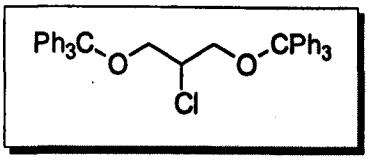
str.), 1458, 1286, 1079 (C-O str.), 710, 628 (C-H def., aromatic); δ_H (400 MHz; $CDCl_3$) 2.28 (1 H, d, *J* 5.5, OH), 3.24-3.26, 3.29-3.33, (2 x 2 H, m, 2 x CH_2OCPh_3), 3.93-3.97 (1 H, m, CHOH), 7.21-7.26 (18 H, m, ArH), 7.37-7.39 (12 H, m, ArH); δ_C (100.6 MHz; $CDCl_3$) 64.53 (CH_2OCPh_3), 70.24 (CHOH), 86.65 (Ph_3COR), 127.02, 127.83, 128.70, 143.86 (aromatic C); *m/z* (FAB$^-$) 577 (MH^+, 0.09%), 243 (Ph_3C^+, 100), 165 ($C_{13}H_9^+$, 32), 105 ($C_6H_5CO^+$, 13).

2-Methanesulphonyloxy-1,3-di(trityloxy)propane (91).



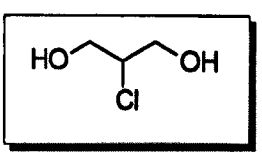
Methanesulphonyl chloride (2.60 g, 22.7 mmol) was added to a stirred solution of **90** (10.0 g, 17.2 mmol) in pyridine (80 mL) under an atmosphere of nitrogen at 0 °C and the solution stirred at 0 °C for 30 minutes then at ambient temperature for 15 hours. The mixture was poured into ice-water (10 mL) stirred for 30 minutes at 0 °C, concentrated *in vacuo* and the residue dissolved in diethyl ether (2 x 150 mL). The combined organic layers were washed with copper sulphate solution (sat. aq.) (3 x 30 mL) and water (20 mL) and the aqueous layers were back-extracted with diethyl ether (50 mL). The combined organic layers were dried (magnesium sulphate), filtered and concentrated *in vacuo* to give mesylate **91** as a white foam (11.3 g, 17.2 mmol, 100%) which required no further purification; R_f (3:7 ethyl acetate:hexane) 0.45; (Found: C, 76.9; H, 5.95. Calculated for $C_{42}H_{38}O_5S$: C, 77.0; H, 5.85%); ν_{max} (Nujol)/cm^{-1} 3058 (C-H str.), 1448, 1353, (S=O str.), 1092 (C-O str.), 706, 632 (C-H def., aromatic); δ_H (400 MHz; $CDCl_3$) 2.95 (3 H, s, $MeSO_2OR$), 3.34-3.42, (4 H, m, CH_2OCPh_3), 4.86 (1 H, quintet, *J* 5, $CHOSO_2Me$), 7.24-7.37 (30 H, m, ArH); δ_C (100.6 MHz; $CDCl_3$) 38.68 ($MeSO_2OR$), 63.01 (CH_2OCPh_3), 80.77 ($CHOSO_2Me$), 87.20 (Ph_3C), 127.24, 127.97, 128.65, 143.39 (aromatic C); *m/z* (FAB$^+$) 654 (M^+, 1.32%), 577 (MH_2^+-SO_2Me, 8), 411 (M^+-Ph_3C, 8), 243 (Ph_3C^+, 100), 165 ($C_{13}H_9^+$, 100), 105 ($C_6H_5CO^+$, 99).

2-Chloro-1,3-di(trityloxy)propane (92).



Anhydrous lithium chloride (1.95 g, 45.9 mmol) and 12-crown-4 (2.45 mL, 15.3 mmol) were added to a stirred solution of **91** (10.0 g, 15.3 mmol) in DMF (75 mL) under an atmosphere of nitrogen and the solution stirred at 100 °C for 36 hours. The mixture was poured into ice-water (250 mL) and extracted into dichloromethane (2 x 200 mL). The combined organic layers were washed with brine (100 mL), water (150 mL) and dried (magnesium sulphate), filtered and concentrated *in vacuo* to give a pale yellow solid. Recrystallisation (dichloromethane) gave the chlorinated derivative **92** as white needles (7.76 g, 13.0 mmol, 85%), m.pt. 181 °C; R_f (1:4 ethyl acetate:hexane) 0.8; (Found: C, 82.6; H, 6.15. Calculated for $C_{41}H_{35}ClO_2$: C, 82.8; H, 5.95%); ν_{max} ($CHCl_3$)/cm^{-1} 3063 (C-H str.), 1448, 1072 (C-O str.), 704, 632 (C-H def., aromatic); δ_H (300 MHz; $CDCl_3$) 3.34-3.42 (4 H, m, CH_2OCPh_3), 3.98 (1 H, quintet, *J* 5.5, CHCl), 7.24-7.37 (30 H, m, ArH); δ_C (75.5 MHz; $CDCl_3$) 58.65 (CHCl), 64.30 (CH_2OCPh_3), 86.82 (Ph_3C), 127.12 127.91, 128.78, 143.82 (aromatic C); *m/z* (FAB^+) 594/596 (M^+, 0.3/0.1%), 517/519 (M^+-C_6H_5, 1.5/0.5), 243 (Ph_3C^+, 100), 165 ($C_{13}H_9^+$, 40), 105 ($C_6H_5CO^+$, 21).

2-Chloro-1,3-propanediol (85).[119]



2 M Hydrochloric acid (21 mL, 42 mmol) was added to a solution of **92** (2.70 g, 4.54 mmol) in ethanol (100 mL) and the solution stirred vigorously under reflux for 22 hours. Ethanol was removed *in vacuo* then the aqueous residue was saturated with sodium chloride and extracted with ethyl acetate (3 x 100 mL). The combined organic layers were dried (magnesium sulphate), filtered and concentrated *in vacuo* to give diol **85** as a very pale yellow oil (412 mg,

3.73 mmol, 83%); R_f (1:1 ethyl acetate:hexane) 0.2; ν_{max} (thin film)/cm^{-1} 3353 (br., O-H str.), 2917, 2873 (C-H str.), 1097 (C-O str.), 695 (C-Cl str.); δ_H (300 MHz, DMSO-d_6) 3.49-3.67 (4 H, m, 2 x C$\underline{H}_2$OH), 3.90 (1 H, quintet, *J* 5.5, C$\underline{H}$Cl), 4.98, (2 H, t, *J* 6, O$\underline{H}$); δ_C (75.5 MHz, DMSO-d_6) 62.59 (C$\underline{H}_2$OH), 64.71 (C$\underline{H}$Cl).

Polymerisation of diols (83), (84) and (85) (Section 2.2.2.5).

Adipic acid **3** (0.43 g, 2.9 mmol) and water (20 μL) were added to the diol under test (3.3 mmol) and the mixture stirred for 30 minutes at 40 °C. *Novozyme 435®* (2.5 mg) was added and the mixtures stirred for 4 hours at 40 °C. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 7 hours.

Triethylamine dosing experiments (Section 2.2.3.1)

Adipic acid **3** (10.00 g, 69 mmol), triethylamine (0.19 mL, 1.4 mmol) and water (0.32 mL) were added to butane-1,4-diol **4** (6.92 g, 77 mmol) and the mixture stirred for 1 hour at 40 °C. *Novozyme 435®* (51 mg) was added and the mixture stirred for 4 hours at 40 °C. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 17 hours. An identical reaction was performed in tandem in the absence of triethylamine. The lipase from each reaction was removed by filtration, and that from the amine-free reaction split into two equal portions, and used in repeat half-scale reactions of the above; the lipase recovered from the amine-doped reaction was used in the same scale amine-doped process.

Comparative triethylamine / trioctylamine doped polymerisations.

Adipic acid **3** (3.00 g, 21 mmol), triethylamine (0.06 mL, 0.4 mmol) and water (0.1 mL) were added to butane-1,4-diol **4** (2.09 g, 23.5 mmol) and the mixture stirred for 1 hour at 40 °C. *Novozyme 435®* (17.5 mg) was added and the mixture stirred for 4 hours at 40 °C. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 17 hours. An identical reaction was performed in tandem in the presence of trioctylamine (0.19 mL, 0.4 mmol) instead of triethylamine. The lipase from each reaction

was removed by filtration, washed and dried and used in repeat reactions of the above in the absence of amine.

Test for free lipase leach in toluene (Section 2.2.3.2).

Novozyme 435® (3.0 mg) was stirred in anhydrous toluene (15 mL) at 60 °C for 2 hours. The mixture was filtered and added to adipic acid **3** (500 mg, 3.4 mmol) and butane-1,4-diol **4** (345 mg, 3.8 mmol) and the mixture stirred for 22 hours at 60 °C.

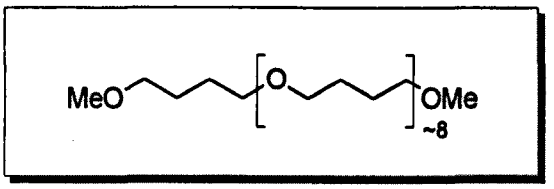
Toluene doping experiments.

Adipic acid **3** (45.19 g, 309 mmol) and water (1.46 mL) were added to butane-1,4-diol **4** (31.26 g, 347 mmol) and the mixture stirred for 1 hour at 40 °C. *Novozyme 435*® (230 mg) was added and the mixture stirred for 4 hours at 40 °C. Identical reactions were performed in tandem in the presence of toluene (0.32 mL, 1mol% wrt adipic acid; 1.65 mL, 5mol%; 6.60 mL, 20mol%). The lipase samples were recovered and subjected to kinetic assay activity measurement.

PTMEG (93) doping experiments (Section 2.2.3.3).

Adipic acid **3** (45.19 g, 309 mmol) and water (1.46 mL) were added to butane-1,4-diol **4** (31.26 g, 347 mmol) and the mixture stirred for 1 hour at 40 °C. *Novozyme 435*® (230 mg) was added and the mixture stirred for 4 hours at 40 °C. Identical reactions were performed in tandem in the presence of PTMEG **93** (2.00 g, ~1mol% wrt adipic acid; 4.00 g, ~2mol%; 10.00 g, ~5mol%; 20.00 g, ~10mol%). The lipase samples were recovered and subjected to kinetic assay activity measurement.

Polytetramethylene ether dimethyl ether (94).



Methyl iodide (25 mL, 0.40 mol) and silver (I) oxide (40.5 g, 0.18 mol) were added to a solution of ~650 molecular weight PTMEG **93** (30.0 g, ~50 mmol) in acetonitrile (170 mL) under an atmosphere of nitrogen. The mixture was heated under reflux for 20 hours, cooled and filtered through Celite®, washing with ethyl acetate (2 x 20 mL). The solution was washed with sodium thiosulphate solution (sat. aq.), (20 mL) and dried (magnesium sulphate). Attempted decolourisation using Norit® followed by filtration was unsuccessful. Concentration of the solution *in vacuo* gave an orange-brown oil (26 g). Hexane (100 mL) was added and the solution decanted from the brown oily residue. The solution was filtered twice through Celite® and twice through a pad of activated alumina to give a colourless solution which on concentrating *in vacuo* gave polyether **94** as a colourless oil (4.48 g, ~6.6 mmol, ~13%); ν_{max} (thin film)/cm^{-1} 2944, 2857, 2738 (C-H str.), 1117 (C-O str.); δ_H (300 MHz; $CDCl_3$) 1.58-1.64 (36 H, m, 2 x $MeOCH_2(\underline{CH_2})_2CH_2OR$, 7 x $ROCH_2(\underline{CH_2})_2CH_2OR$), 3.33 (6 H, s, 2 x $\underline{Me}O$), 3.34-3.44 (36 H, m, 18 x $\underline{CH_2}OR$); δ_C (75.5 MHz; $CDCl_3$) 26.42 ($MeOCH_2\underline{C}H_2$, $\underline{C}H_2CH_2OR$), 58.38 ($\underline{Me}O$), 70.53 ($\underline{C}H_2OR$), 72.61 ($MeO\underline{C}H_2$).

Polytetramethylene ether dimethyl ether (94) doping experiments.

Adipic acid **3** (8.66 g, 59.2 mmol) and water (0.28 mL) were added to butane-1,4-diol **4** (5.99 g, 66.3 mmol) and the mixture stirred for 1 hour at 40 °C. *Novozyme 435®* (44.1 mg) was added and the mixture stirred for 4 hours at 40 °C. Identical reactions were performed in tandem in the presence of polytetramethylene ether dimethyl ether **94** (0.39 g, ~1mol% wrt adipic acid; 3.85 g, ~10mol%). The lipase samples were recovered and subjected to kinetic assay activity measurement, although meaningful results could not be obtained.

Unsaturated acid polymerisation trials (Section 2.3.2.1).

Method 1: Butane-1,4-diol **4** (2.12 g, 23.5 mmol) and water (0.10 cm^3, 2.7 mmol) were added to the acid substrate (21.0 mmol) and the mixture stirred at 40 °C for 1 hour. *Novozyme 435®* (17.5 mg) was added and the mixture stirred for a further 4 hours at 40 °C. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 17 hours (Table 30).

substrate	weight used / g	M_w	M_n	M_w / M_n
maleic acid (**101**)	2.44	367	268	1.4
fumaric acid (**11**)	2.44	150	140	1.1
acetylene dicarboxylic acid (**100**)	2.40	574	117	4.9
trans-3-hexenedioic acid (**99**)	3.03	251	182	1.4
itaconic acid (**98**)	2.73	138	128	1.1

Table 30

substrate	weight used / mg	M_w	M_n	M_w / M_n
maleic acid (**101**)	79	374	281	1.3
fumaric acid (**11**)	79	132	122	1.1
acetylene dicarboxylic acid (**100**)	76	1166	496	2.3
trans-3-hexendioic acid (**99**)	98	603	229	2.7
itaconic acid (**98**)	88	259	152	1.7

Table 31

Method 2: The acid substrate (0.68 mmol) was added to a solution of butane-1,4-diol **4** (69 mg, 0.76 mmol) in toluene (5 mL) and stirred at 60 °C for 1 hour in a modified Dean and Stark apparatus with 4 Å molecular sieves. *Novozyme 435®* (0.6 mg) was added and the mixture stirred for 22 hours. The mixture was concentrated *in vacuo* (Table 31).

Unsaturated diol polymerisation trials (Section 2.3.2.2).

Method 1: The diol (23.5 mmol) and water (100 μL, 2.7 mmol) were added to adipic acid **3** (3.00 g, 21.0 mmol) and the mixture stirred at 40 °C for 1 hour. *Novozyme 435®* (17.5 mg) was added and the mixture stirred for a further 4 hours at 40 °C. The temperature was then increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 17 hours (Table 32).

substrate	weight used / g	M_w	M_n	M_w / M_n
cis-but-2-ene-1,4-diol (**102**)	2.07	358	207	1.7
but-2-yne-1,4-diol (**103**)	2.02	175	158	1.1

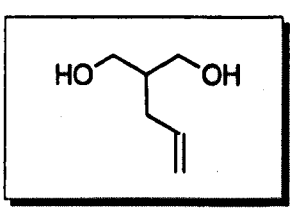
Table 32

Method 2: Adipic acid **3** (100 mg, 0.68 mmol) was added to a solution of the polyol (0.76 mmol) in toluene (5 mL) and stirred at 60 °C for 1 hour in a modified Dean and Stark apparatus with 4 Å molecular sieves. *Novozyme 435®* (0.6 mg) was added and the mixture stirred for 22 hours. The mixture was concentrated *in vacuo* (Table 33).

substrate	weight used / mg	M_w	M_n	M_w / M_n
cis-but-2-ene-1,4-diol (**102**)	67	841	315	2.7
but-2-yne-1,4-diol (**103**)	65	326	145	2.3

Table 33

2-Allylpropane-1,3-diol (105).[134]



Lithium aluminium hydride (1.90 g, 50.1 mmol) was added to diethyl ether (50 mL) under an atmosphere of nitrogen at 0 °C and stirred for 30 minutes. A solution of diethyl allylmalonate

104 (8.00 g, 40.0 mmol) in diethyl ether (30 mL) was added dropwise to the suspension at 0 °C and the mixture stirred at ambient temperature for 18 hours. Sodium sulphate solution (sat. aq.) (4 mL) was added dropwise to the vigorously stirred mixture at 0 °C and the inorganic salts were removed by filtration and dissolved in water (40 mL). The aqueous mixture was saturated with sodium chloride and extracted with diethyl ether (2 x 250 mL). The combined organic layers were dried (magnesium sulphate), filtered and concentrated *in vacuo* to give a colourless oil. Purification by flash column chromatography using gradient elution (1:1 ethyl acetate:hexane - ethyl acetate) gave the diol **105** as a colourless oil (3.52 g, 30.3 mmol, 76%); R_f (1:1 ethyl acetate:hexane) 0.25; (Found: MH^+, 117.0918. $C_6H_{12}O_2$ requires *MH*, 117.0916); ν_{max} (thin film)/cm^{-1} 3600-3100 (br., O-H str.), 3078, 2925 (C-H str.), 1640 (C=C str.), 1090 (C-O str.); δ_H (200 MHz; $CDCl_3$) 1.81-1.95 (1 H, m, C<u>H</u>CH_2OH), 2.03-2.10 (2 H, m, $C\underline{H}_2CH=CH_2$), 2.77 (2 H, s, 2 x O<u>H</u>), 3.60-3.86 (4 H, m, 2 x $C\underline{H}_2OH$), 5.01-5.04 (1 H, m) and 5.06-5.13 (1 H, m, $CH_2CH=C\underline{H}_2$), 5.80 (1 H, ddt, *J* 17, 10, 7, $CH_2C\underline{H}=CH_2$); δ_C (75.5 MHz; $CDCl_3$) 32.52 ($\underline{C}H_2CH=CH_2$), 41.83 ($\underline{C}HCH_2OH$), 65.71 ($\underline{C}H_2OH$), 116.62 ($CH_2CH=\underline{C}H_2$), 136.29 ($CH_2\underline{C}H=CH_2$); *m/z* (CI^+, NH_3) 134 (MNH_4^+, 100%), 117 (MH^+, 57), 99 (MH^+-H_2O, 42), 81 (MH^+-$2H_2O$, 28).

Polyesterifications using diethyl allylmalonate (104) (Section 2.3.3.2).

<u>Method 1</u>: Diethyl allylmalonate **104** (502 mg, 25.1 mmol), butane-1,4-diol **4** (250 mg, 27.7 mmol) and water (100 μL, 2.7 mmol) were stirred at 40 °C for 1 hour. *Novozyme 435®* (2.1 mg) was added and the mixture stirred for a further 4 hours at 40 °C. The temperature was then increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 17 hours (Table 34).

substrates	system	M_w	M_n	M_w / M_n
diethyl allylmalonate (**104**) / butane-1,4-diol (**4**)	Method 1 (solvent-free)	276	141	2.0
diethyl allylmalonate (**104**) / butane-1,4-diol (**4**)	Method 2 (toluene-based)	447	131	3.4

Table 34

<u>Method 2</u>: Diethyl allylmalonate **104** (110 mg, 0.55 mmol) and butane-1,4-diol **4** (49.6 mg, 0.55 mmol) were dissolved in toluene (5 mL) and stirred at 60 °C for 1 hour in a modified

Dean and Stark apparatus with 4 Å molecular sieves. *Novozyme 435®* (3.0 mg) was added and the mixture stirred for 22 hours. The mixture was concentrated *in vacuo* (Table 34).

Poly(2-allylpropanediyl adipate) (Section 2.3.3.2).

Method 1 (enzymatic): Adipic acid **3** (570 mg, 3.90 mmol), 2-allypropane-1,3-diol **105** (500 mg, 4.30 mmol) and water (~20 μL) were stirred at 40 °C for 30 minutes. *Novozyme 435®* (6.5 mg) was added and the mixture stirred for a further 4 hours at 40 °C. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 17 hours to give a viscous clear colourless mixture; δ_H (200 MHz; $CDCl_3$) 1.62-2.38 (~12 H, m, $(CH_2)_2COOR$, $CHCH_2OCOR$, $CH_2CH=CH_2$), 3.48-3.72 (~1 H, m, CH_2OH), 3.96-4.24 (~4 H, m, CH_2OCOR), 5.05-5.11 (2 H, m, $CH_2CH=CH_2$), 5.72-5.85 (1 H, m, $CH_2CH=CH_2$); δ_C (100.6 MHz; $CDCl_3$) 24.39, 32.58, 32.72, 33.75, 33.85, 37.06, 37.12, 40.26 ($(CH_2)_2COOR$, $CHCH_2OCOR$, $CH_2CH=CH_2$), 60.30, 62.38, 63.75, 64.09 (CH_2OH, CH_2OCOR), 117.00, 117.48 ($CH_2CH=CH_2$), 134.86, 135.70 ($CH=CH_2$), 173.10 (COOR); GPC analysis: M_n 863, M_w 1810, M_w / M_n 2.1.

Method 2 (enzymatic): Adipic acid **3** (100 mg, 0.68 mmol) was added to a solution of 2-allylpropane-1,3-diol **105** (79.0 mg, 0.68 mmol) in toluene (5 mL) and stirred at 60 °C under nitrogen in a modified Dean and Stark apparatus with 4 Å molecular sieves for 30 minutes. *Novozyme 435®* (3.7 mg) was added and the mixture stirred for a further 17 hours at 60 °C. Removal of the volatile components *in vacuo* gave a semi-crystalline white solid; δ_H (200 MHz; $CDCl_3$) 1.572.52 (~15 H, m, CH_2CH_2COOR, $CHCH_2OCOR$, $CH_2CH=CH_2$), 3.45-3.67 (~0.5 H, m, CH_2OH), 3.94-4.26 (~4 H, m, CH_2OCOR), 5.02-5.16 (2 H, m, $CH_2CH=CH_2$), 5.64-5.91 (1 H, m, $CH=CH_2$); GPC analysis: M_n 878, M_w 1375, M_w / M_n 1.6.

Method 3 (conventional): Adipic acid (126 mg, 0.86 mmol) **3** and 2-allylpropane-1,3-diol (150 mg, 1.29 mmol) **105** were charged to a 1 mL reactor. Titanium tetraisopropoxide (~12 mg, ~40 μmol, cat.) was added under an atmosphere of nitrogen, and the mixture stirred at 200 °C for 2 hours under a stream of nitrogen. The mixture was then stirred at 100 mbar at 200 °C for a further 4 hours, to give a yellow gel; δ_H (300 MHz; $CDCl_3$) 1.63-2.36 (~11 H, m, $(CH_2)_2COOR$, $CHCH_2OCOR$, $CH_2CH=CH_2$), 3.98-4.12 (~3.5 H, m, CH_2OCOR), 5.04-5.10 (2 H, m, $CH_2CH=CH_2$), 5.69-5.83 (1 H, m, $CH=CH_2$); δ_C (75.5 MHz; $CDCl_3$) 24.31

($ROOCCH_2(\underline{C}H_2)_2CH_2COOR$), 32.72, 33.74, 37.16 ($\underline{C}H_2COOR$, $\underline{C}HCH_2OCOR$, $\underline{C}H_2CH{=}CH_2$), 63.87 ($\underline{C}H_2OCOR$), 117.51 ($CH_2CH{=}\underline{C}H_2$), 134.93 ($\underline{C}H{=}CH_2$), 173.14 ($\underline{C}OOR$); GPC analysis: M_n 2091, M_w 5204, M_w / M_n 2.5.

Poly(2-(1',2'-dibromopropyl)propanediyl adipate) (Section 2.3.3.3).

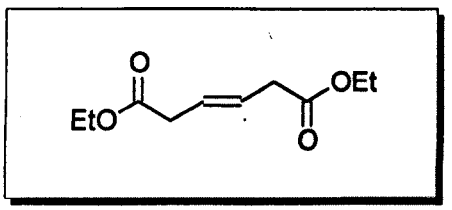
Poly(2-allylpropanediyl adipate) (189 mg) was dissolved in ethyl acetate (20 mL) and the solution washed with sodium bicarbonate solution (sat. aq.) (2 x 4 mL), dried (magnesium sulphate), filtered and the solvent removed *in vacuo* to give hydroxyl-capped poly(2-allylpropanediyl adipate) as a colourless oil (158 mg); GPC analysis: M_n 741, M_w 1702, M_w / M_n 2.3. This hydroxyl terminated material (49 mg) was dissolved in chloroform (2 mL) and a solution of bromine in chloroform (0.6 mL, 12 mmol in 10 mL) was added dropwise at ambient temperature until an orange colour persisted. The mixture was stirred for a further 15 minutes. Chloroform (3 mL) was added, the solution washed with sodium thiosulphate solution (sat. aq.) (2 mL), dried (magnesium sulphate), filtered and the solvent removed *in vacuo* to give a pale orange oil (115 mg); δ_H (300 MHz; $CDCl_3$) 1.66-2.36 (~11 H, m, $(\underline{CH}_2)_2COOR$, $C\underline{H}CH_2OCOR$, $C\underline{H}_2CH(Br)CH_2Br$), 3.59-3.66 (1 H, m) and 3.89-3.93 (1 H, m, $CH_2CH(Br)C\underline{H}_2Br$), 4.00-4.27 (~4.5 H, m, $C\underline{H}(Br)CH_2Br$, $C\underline{H}_2OCOR$); δ_C (75.5 MHz; $CDCl_3$) 24.31 ($\underline{C}H_2CH_2COOR$), 33.75, 35.57, 36.02, 36.50 ($\underline{C}H_2COOR$, $\underline{C}HCH_2OCOR$, $\underline{C}H_2CH(Br)CH_2Br$), 49.83 ($CH_2CH(Br)\underline{C}H_2Br$), 62.59, 64.49 ($\underline{C}H(Br)CH_2Br$, $\underline{C}H_2OCOR$), 173.09 ($\underline{C}OOR$); GPC analysis: M_n 692, M_w 1656, M_w / M_n 2.4.

Attempted cross-linking of Poly(2-(1',2'-dibromopropyl)propane-1,3-diyl adipate) (Section 2.3.3.3).

Tributyltin hydride (0.18 mL, 0.65 mmol) was added to a stirred solution of poly(2-(1'2'-dibromopropyl)propane-1,3-diyl adipate) (114 mg) and AIBN (15.0 mg, 0.13 mmol) in toluene (5 mL) under an atmosphere of nitrogen at ambient temperature. The mixture was heated under reflux for 17 hours, whereupon a small amount of a black suspension was observed. The mixture was filtered and volatiles removed *in vacuo*. The residue was partitioned between acetonitrile (8 mL) and hexane (5 mL) and the acetonitrile layer washed with hexane (2 x 10 mL). Removal of acetonitrile *in vacuo* gave a colourless oil (64 mg); δ_H

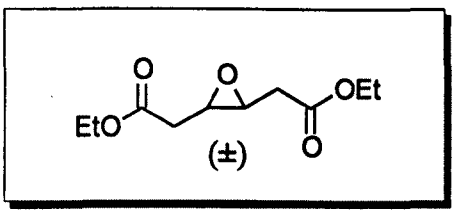
(300 MHz; $CDCl_3$) 1.10-2.45, (~19 H, m), 3.46-4.20 (~6 H, m, $C\underline{H}_2OCOR$), 5.04-5.10 (2 H, m, $CH_2CH{=}C\underline{H}_2$), 5.64-5.91 (1 H, m, $C\underline{H}{=}CH_2$); GPC analysis: M_n 358, M_w 1152, M_w / M_n 3.2.

Diethyl *trans*-3-hexenedioate (108).



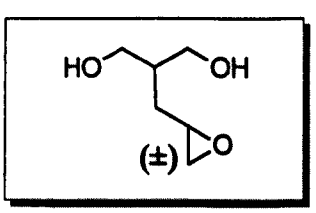
DCC (7.22g, 35.0 mmol) and DMAP (100 mg, 0.82 mmol) were added to a solution of *trans*-3-hexenedioic acid **99** (5.00 g, 34.7 mmol) and anhydrous ethanol (4.7 mL, 80 mmol) in dry dichloromethane (50 mL) under nitrogen at ambient temperature and the mixture stirred for 18 hours. The mixture was filtered, washing with dichloromethane (20 mL). The organic solution was washed with 2 M hydrochloric acid (50 mL), sodium bicarbonate solution (sat. aq.) (50 mL) and brine (50 mL), dried (magnesium sulphate), filtered and the solvent removed *in vacuo* to give the crude material as a pale yellow oil. Purification by flash column chromatography using gradient elution (hexane - 1:3 ethyl acetate:hexane) gave the diester **108** as a colourless oil (2.04 g, 10.2 mmol, 29%); R_f (1:4 ethyl acetate:hexane) 0.65; (Found: M^+, 200.1051. $C_{10}H_{18}O_4$ requires *M*, 200.1049); ν_{max} (thin film)/cm^{-1} 2986, 2942, 2909 (C-H str.), 1738 (br., C=O str.), 1097 (C-O str.), 1025 (C-H str.); δ_H (200 MHz; $CDCl_3$) 1.26 (6 H, t, *J* 7, 2 x $COOCH_2\underline{Me}$), 3.09 (4 H, dd, *J* 4, 1.5, 2 x $C\underline{H}_2COOEt$), 4.14 (4 H, q, *J* 7, 2 x $COOC\underline{H}_2Me$), 5.70 (2 H, tt, *J* 4, 1.5, 2 x $C\underline{H}CH_2COOEt$); δ_C (75.5 MHz; $CDCl_3$) 14.04 ($COOCH_2\underline{Me}$), 37.83 ($\underline{C}H_2COOEt$), 60.61 ($COO\underline{C}H_2Me$), 126.03 ($\underline{C}HCH_2COOEt$), 171.68 ($\underline{C}OOEt$); *m/z* (EI^+) 200 (M^+, 6%), 154 (M^+-C_2H_5OH, 94), 127 (M^+-CO_2Et, 94), 108 (M^+-$2C_2H_5OH$, 58).

(±)-Diethyl *trans*-3,4-epoxyhexanedioate (109).



m-CPBA (2.07 g, 12.0 mmol) was added to a solution of alkene **108** (1.59 g, 7.94 mmol) in dry dichloromethane (20 mL) at 0 °C under an atmosphere of argon, and the mixture stirred at ambient temperature for 91 hours. Dichloromethane (15 mL) was added and the mixture filtered. The solution was washed with sodium bisulphite solution (sat. aq.) (10 mL), sodium bicarbonate solution (sat. aq.) (40 mL) and brine (40 mL). The organic phase was dried (magnesium sulphate), filtered and the solvent removed *in vacuo* to give **109** as a pale yellow oil which gave yellow plates on standing (1.39 g, 6.43 mmol, 81%); R_f (1:4 ethyl acetate:petrol) 0.4; (Found: MH^+, 217.1077. $C_{10}H_{16}O_5$ requires *MH*, 217.1076); (Found: C, 55.55; H, 7.5. Calculated for $C_{10}H_{16}O_5$: C, 55.55; H, 7.5%); ν_{max} (thin film)/cm^{-1} 2987 (C-H str.), 1728 (C=O str.), 1250 (C-O str., epoxide), 1097 (C-O str.); δ_H (300 MHz; $CDCl_3$) 1.28 (6 H, t, *J* 7, 2 x $COOCH_2\underline{Me}$), 2.57-2.61 (4 H, m, 2 x $C\underline{H}_2COOEt$), 3.14 (2 H, ddd, *J* 7, 2, 2, 2 x $C\underline{H}(O)CH_2COOEt$), 4.19 (4 H, q, *J* 7, 2 x $COOC\underline{H}_2Me$); δ_C (75.5 MHz; $CDCl_3$) 14.03 ($COOCH_2\underline{Me}$), 37.36 ($\underline{C}H_2COOEt$), 53.71 ($\underline{C}H(O)CH_2COOEt$), 60.85 ($COO\underline{C}H_2Me$), 170.23 ($\underline{C}OOEt$); *m/z* ($CI^+$, NH_3) 234 (MNH_4^+, 22%), 217 (MH^+, 79), 199 (MH^+-H_2O, 100), 188 (MH^+-C_2H_5, 37).

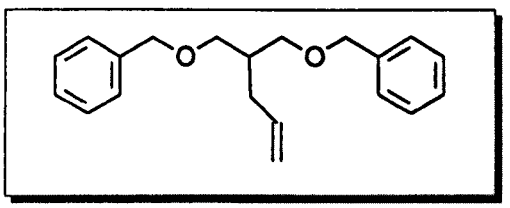
(±)-2-(2',3'-Epoxypropyl)propane-1,3-diol (110).



Method 1: *m*-CPBA (430 mg, 2.50 mmol) was added to a solution of 2-allylpropane-1,3-diol **105** (230.8 mg, 1.99 mmol) in dichloromethane (10 mL) at 0 °C and stirred at ambient temperature for 48 hours. Dichloromethane (40 mL) was added and the mixture washed with

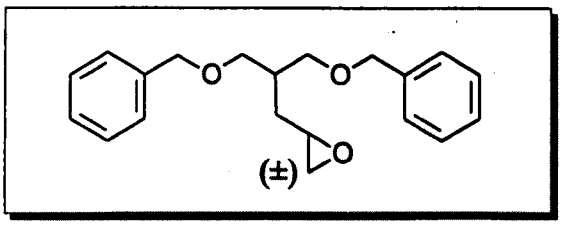
sodium bisulphite solution (sat. aq.) (5 mL), sodium bicarbonate solution (sat. aq.) (2 x 10 mL) and brine (5 mL), dried (magnesium sulphate), filtered and the solvent removed *in vacuo* to give a yellow oil. Mass spectrometry (CI^+) indicated a large number of components of higher weight than the desired epoxide **110**.

2-Allyl-1,3-dibenzyloxypropane (111).



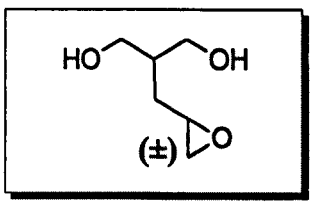
THF (4 mL) was added to sodium hydride (60% dispersion in mineral oil, 207 mg, 5.12 mmol) under an atmosphere of argon at 0 °C. A solution of diol **105** (150 mg, 1.29 mmol) in THF (2 mL) was added at 0 °C and the mixture stirred for 5 minutes at 0 °C and 30 minutes at ambient temperature. The mixture was cooled to 0 °C, then dry DMF (2 mL) was added followed by benzyl chloride (370 μL, 3.2 mmol) dropwise. The mixture was then stirred at ambient temperature for 15 hours. Water (1.5 mL) was added at 0 °C and the mixture extracted with diethyl ether (2 x 20 mL). The combined organic layers were dried (magnesium sulphate), filtered and concentrated *in vacuo* to give a yellow oil. Purification by flash column chromatography using gradient elution (petrol - 1:4 ethyl acetate:petrol) gave the dibenzylated derivative **111** as a colourless oil (293 mg, 0.99 mmol, 77%); R_f (1:3 ethyl acetate:petrol) 0.9; (Found: MH^+, 297.1854. $C_{20}H_{24}O_2$ requires *MH*, 297.1855); ν_{max} (thin film)/cm^{-1} 3067, 3031, 2857 (C-H str.), 1640 (C=C str.), 1097 (C-O str.), 734, 695 (aromatic C-H def.); δ_H (200 MHz; $CDCl_3$) 1.96-2.12 (1 H, m, C<u>H</u>CH_2OBn), 2.20 (2 H, m, $C\underline{H}_2CH=CH_2$), 3.47 (2 H, d, *J* 1.5) and 3.50 (2 H, d, *J* 1.5, $C\underline{H}_2OBn$), 4.49 (4 H, s, $ArC\underline{H}_2O$), 4.93-5.09 (2 H, m, $CH_2CH=C\underline{H}_2$), 5.76 (1 H, ddt, *J* 17, 11.5, 7, $CH_2C\underline{H}=CH_2$), 7.24-7.35 (10 H, m, Ar<u>H</u>); δ_C (75.5 MHz; $CDCl_3$) 33.18 ($\underline{C}H_2CH=CH_2$), 39.38 ($\underline{C}HCH_2OBn$), 70.59 ($\underline{C}H_2OBn$), 73.16, ($Ar\underline{C}H_2O$), 116.25 ($CH_2CH=\underline{C}H_2$), 127.49, 127.59, 128.36 (aromatic <u>C</u>), 136.74 ($CH_2\underline{C}H=CH_2$), 138.83 (aromatic <u>C</u>); *m/z* (CI^+, NH_3) 314 (MNH_4^+, 22%), 297 (MH^+, 100), 205 (M^+-C_7H_7, 13), 108 ($C_7H_8O^+$, 15), 91 ($C_7H_7^+$, 4).

(±)-1,3-Dibenzyloxy-2-(2',3'-epoxypropyl) propane (112).



m-CPBA (88.0 mg, 0.51 mmol) was added to a solution of **111** (100 mg, 0.34 mmol) in dry dichloromethane (5 mL) at 0 °C under an atmosphere of nitrogen and the mixture stirred at 0 °C for 1 hour then at ambient temperature for a further 42 hours. Dichloromethane (30 mL) was added and the solution washed with sodium bisulphite solution (sat. aq.) (5 mL), sodium hydrogen carbonate solution (sat. aq.) (10 mL), and brine (5 mL). The organic solution was dried (magnesium sulphate), filtered and concentrated *in vacuo* to yield crude epoxide **112** as a pale yellow oil. Purification by flash column chromatography (1:3 ethyl acetate:petrol) gave **112** as a colourless oil (56.1 mg, 0.18 mmol, 53%); R_f (1:3 ethyl acetate:petrol) 0.65; (Found: MH^+, 313.1808. $C_{20}H_{24}O_3$ requires *MH*, 313.1804); ν_{max} (thin film)/cm^{-1} 3087, 3064, 3032, 2856 (C-H str.), 1258, 914, 820 (C-O str., epoxide), 1093 (C-O str.), 736, 697 (aromatic C-H def.); δ_H (300 MHz; $CDCl_3$) 1.54-1.81 (2 H, m, $C\underline{H}_2CH(O)CH_2$), 2.20 (1 H, quintet, *J* 6, $C\underline{H}CH_2OBn$), 2.41-2.44 (1 H, m) and 2.72 (1 H, dd, *J* 5, 4, $CH_2CH(O)C\underline{H}_2$), 2.94-3.02 (1 H, m, $CH_2C\underline{H}(O)CH_2$), 3.46-3.61 (4 H, m, 2 x $C\underline{H}_2OBn$), 4.50 (4 H, s, $ArC\underline{H}_2O$), 7.24-7.34 (10 H, m, Ar$\underline{H}$); δ_C (75.5 MHz; $CDCl_3$) 32.24 ($\underline{C}H_2CH(O)CH_2$), 37.78 ($\underline{C}HCH_2OBn$), 47.19 ($CH_2CH(O)\underline{C}H_2$), 50.93 ($CH_2\underline{C}H(O)CH_2$), 70.66 ($\underline{C}H_2OBn$), 73.16, ($Ar\underline{C}H_2O$), 127.57, 128.36, 138.86 (aromatic $\underline{C}$); *m/z* (CI^+, NH_3) 330 (MNH_4^+, 78%), 313 (MH^+, 58), 205 (MH^+-$C_7H_7OH_2$, 100), 108 ($C_7H_8O^+$, 47), 91 ($C_7H_7^+$, 52).

(±)-2-(2',3'-Epoxypropyl)propane-1,3-diol (110).



Method 2: 10% Palladium on charcoal (10 mg) was added to a solution of (±)-1,3-dibenzyloxy-2-(2',3'-epoxypropyl)propane **112** (53.9 mg, 173 μmol) in ethyl acetate (3 mL) and the suspension stirred under an atmosphere of hydrogen at atmospheric pressure at ambient temperature for 21 hours. The mixture was filtered through Celite®, eluting with ethyl acetate (5 mL) and the volatile components removed *in vacuo* to give pale yellow oil (21.6 mg); R_f (1:1 petrol:ethyl acetate 0.2). Spectroscopic data indicated epoxide had been fully hydrogenated to give 2-propylpropane-1,3-diol; δ_H (300 MHz; $CDCl_3$) 0.92 (3 H, t, *J* 7, $CHCH_2CH_2\underline{Me}$), 1.18-1.27 (2 H, m, $CHC\underline{H}_2CH_2Me$), 1.29-1.42 (2 H, m, $CHCH_2C\underline{H}_2Me$), 1.73-1.83 (1 H, m, $C\underline{H}CH_2CH_2Me$), 2.74 (2 H, br. s, 2 x $CHCH_2O\underline{H}$), 3.61-3.67 (2 H, m) and 3.78-3.83 (2 H, m, 2 x $CHC\underline{H}_2OH$); δ_C (75.5 MHz; $CDCl_3$) 14.19 ($CHCH_2CH_2\underline{Me}$), 20.26 ($CH\underline{C}H_2CH_2Me$), 29.92 ($CHCH_2\underline{C}H_2Me$), 41.73 ($\underline{C}HCH_2CH_2Me$), 66.47 (2 x $CH\underline{C}H_2OH$); *m/z* (CI^+, NH_3) 136 (100%), 119 (19).

Poly(butane-1,4-diyl 3,4-epoxyadipate) (114) (Section 2.3.4.2).

Method 1 (enzymatic): Butane-1,4-diol **4** (138 mg, 1.53 mmol) and water (~20 μL) were added to diethyl 3,4-epoxyadipate **109** (300 mg, 1.39 mmol), and the mixture stirred at 40 °C for 1 hour. *Novozyme 435*® (5.0 mg) was added and the mixture stirred for a further 4 hours at 40 °C. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for 17 hours, whereupon a pale yellow clear, homogeneous material was formed; δ_H (200 MHz; $CDCl_3$) 1.28 (0.17 H, t, *J* 7, $\underline{Me}CH_2OCOR$), 1.53-1.96 (1.30 H, m, $RCOOCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OH$), 2.52-2.72 (1.00 H, m, $C\underline{H}_2COOR$), 3.14 (0.45 H, t, *J* 5, $C\underline{H}(O)CH_2COOCH_2Me$), 3.68 (0.19 H, t, *J* 6, $C\underline{H}_2OH$), 4.08-4.24 (1.00 H, m, $COOC\underline{H}_2Me$, $RCOOC\underline{H}_2$); δ_C (75.5 MHz; $CDCl_3$) 14.12 ($\underline{Me}CH_2OCOR$), 25.15 ($RCOOCH_2\underline{C}H_2$,

$\underline{C}H_2CH_2OH$), 37.27 ($\underline{C}H_2COOR$), 53.65 ($\underline{C}H(O)CH_2COOCH_2Me$), 64.34 ($RCOO\underline{C}H_2$), 170.16 ($\underline{C}OOR$).

Method 2 (enzymatic): Butane-1,4-diol **4** (31.3 mg, 0.35 mmol) was added to diethyl 3,4-epoxyadipate **109** (150 mg, 0.69 mmol), and the mixture stirred at 30 °C for 0.5 hour. *Novozyme 435*® (0.6 mg) was added and the mixture stirred for a further 19 hours at 30 °C under reduced pressure (10 ± 3 mbar), whereupon a pale yellow clear, homogeneous material was formed; GPC analysis: M_n 316, M_w 629, M_w / M_n 2.0.

Method 3 (conventional): Butane-1,4-diol **4** (62.5 mg, 0.69 mmol), diethyl 3,4-epoxyadipate **109** (100 mg, 0.46 mmol) and titanium tetraisopropoxide (~7 mg, ~20 μmol) were charged to a 1 mL reactor and the mixture stirred at 220 °C for 0.5 hours under a stream of nitrogen, whereupon a dark red colouration was observed. ^{1}H NMR spectroscopy showed impurity signals at ~4.7, 6.1 and 6.9 ppm in addition to signals observed in Method 1.

Cross-linking of poly(butane-1,4-diyl 3,4-epoxyadipate) (Section 2.3.4.2).

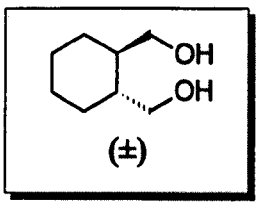
Hexamethylenediamine (14.0 mg, 0.12 mmol) and ammonium chloride (53.0 mg, 0.99 mmol) were added to a solution of hexane washed poly(butane-1,4-diyl 3,4-epoxyadipate) **114** (117 mg ~0.12 mmol) in 1,2-dimethoxyethane:ethanol:water 3:3:2 (2 mL) and the stirred mixture heated under reflux for 3 hours. ^{1}H NMR spectroscopy showed the presence of the epoxide signal at 3.15 ppm, indicating that little or no cross-linking had taken place; GPC analysis: M_n 890, M_w 1843, M_w / M_n 2.1.

Poly(2-(1',2'-epoxypropyl)propane-1,3-diyl adipate) (Section 2.3.4.2).

Hydroxyl terminated poly(2-allylpropane-1,3-diyl adipate) (150 mg) (GPC analysis: M_n 741, M_w 1702, M_w / M_n 2.3) was dissolved in dry dichloromethane (8 mL) under an atmosphere of nitrogen, *m*-CPBA (200 mg, 1.16 mmol) was added and the mixture stirred at ambient temperature for 48 hours. Dichloromethane (40 mL) was added and the mixture washed with sodium bisulphite solution (sat. aq.) (2 mL), sodium bicarbonate solution (sat. aq.) (15 mL) and brine (3 mL). The organic phase was dried (magnesium sulphate), filtered and the solvent

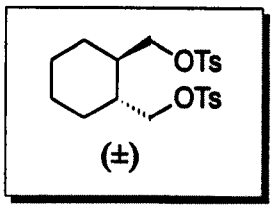
removed *in vacuo* to give a colourless oil (125 mg); δ_H (200 MHz; $CDCl_3$) 1.10-1.85 (~10 H, m, CH_2CH_2COOR, $CHCH_2OCOR$, $CH_2CH(O)CH_2$), 2.14-2.45 (8 H, m, CH_2COOR, $CH_2CH(O)CH(H)$), 2.70-2.76 (1 H, m, $CH_2CH(O)CH(H)$), 2.86-2.98 (1 H, m, $CH_2CH(O)CH_2$), 3.36-3.70 (1 H, m, CH_2OH), 3.85-4.45 (~8 H, m, CH_2OCOR); GPC analysis: M_n 943, M_w 1757, M_w / M_n 1.9.

(±)-*trans*-Cyclohexane-1,2-dimethanol (116).[139]



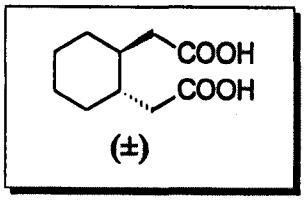
Lithium aluminium hydride (4.40 g, 116 mmol) was added to THF (150 mL) under an atmosphere of argon at 0 °C and stirred for 30 minutes. A solution of **115** (5.00 g, 29.0 mmol) in THF (120 mL) was added to the suspension over 10 minutes and the mixture stirred under reflux for 18 hours. Sodium sulphate solution (sat. aq.) (2.5 mL) was added dropwise to the vigorously stirred mixture at 0 °C and the inorganic salts removed by filtration then washed with boiling THF (2 x 100 mL). The combined organic layers were dried (magnesium sulphate), filtered and concentrated *in vacuo* to give a white amorphous solid. Flash column chromatography (ethyl acetate) gave the diol **116** as white plates (3.49 g, 24.2 mmol, 83%), m.pt. 60 °C (lit.[182] 61-62 °C); R_f (1:1 ethyl acetate:hexane) 0.2; (Found: M^+, 144.1149. $C_8H_{16}O_2$ requires *M*, 144.1150); (Found: C, 66.75; H, 11.25. Calculated for $C_8H_{16}O_2$: C, 66.6; H, 11.2%); ν_{max} (Nujol)/cm^{-1} 3293 (O-H str.), 1052 (C-O str.), 1020, 960 (C-H def.); δ_H (300 MHz; $CDCl_3$) 1.02-1.09 (2 H, m, 2 x $CH_{ax}HCH_2CHCH_2OH$), 1.17-1.36 (4 H, m, 2 x $CH_{eq}HCH_2CHCH_2OH$, 2 x $CH_{ax}HCHCH_2OH$), 1.61-1.76 (4 H, m, 2 x $CHCH_2OH$, $CH_{eq}HCHCH_2OH$), 3.06 (2 H, br. s, OH), 3.50-3.65 (4 H, m, 2 x CH_2OH); δ_C (75.5 MHz; $CDCl_3$) 26.08 ($CH_2CH_2CHCH_2OH$), 29.87 (CH_2CHCH_2OH), 44.65 ($CHCH_2OH$), 67.81 (CH_2OH); *m/z* (EI^+) 144 (M^+, 0.77%), 126 (M^+-H_2O, 23), 108 (M^+-$2H_2O$, 10), 81 (72).

(±)-*trans*-Cyclohexane-1,2-dimethanol ditosylate (117).[140]



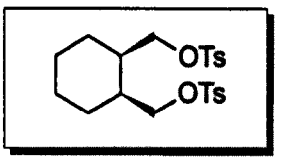
A solution of **116** (800 mg, 5.55 mmol) in pyridine (7 mL) was added dropwise over 20 minutes at 0 °C to a stirred solution of freshly recrystallised *p*-toluenesulphonyl chloride[183] (3.23 g, 16.9 mmol) in pyridine (5 mL) under an atmosphere of nitrogen and the mixture stirred at 0 °C for 3 hours then at ambient temperature for 2 hours. The mixture was poured onto ice-water (50 mL) and extracted with ethyl acetate (2 x 50 mL). The combined organic layers were washed with 1 M hydrochloric acid (150 mL), copper sulphate solution (sat. aq.) (15 mL) and brine (30 mL) and were dried (magnesium sulphate), filtered and concentrated *in vacuo* to give a colourless oil which yielded ditosylate **117** as white plates on standing (2.10 g, 4.64 mmol, 84%), m.pt. 108 °C (lit.[182] 108 °C); R_f (1:1 ethyl acetate:petrol) 0.75; (Found: MNH_4^+, 470.1682. $C_{22}H_{28}O_6S_2$ requires *MNH*$_4$, 470.1671); (Found: C, 58.4; H, 6.25. Calculated for $C_{22}H_{28}O_6S_2$: C, 58.4; H, 6.25%); ν_{max} (Nujol)/cm^{-1} 1596, 1492 (C=C str., aromatic), 1352, 1177 (S=O str.), 1096 (C-O str.), 814 (C-H def., aromatic); δ_H (300 MHz; $CDCl_3$) 1.14-1.16 (4 H, m, 2 x C$\underline{H}_{ax}$HCH$_2$CHCH$_2$OSO$_2$Ar, 2 x C$\underline{H}_{ax}$HCHCH$_2$OSO$_2$Ar), 1.54 (2 H, m, 2 x C$\underline{H}_{eq}$HCH$_2$CHCH$_2$OSO$_2$Ar), 1.61-1.65, (4 H, m, 2 x C$\underline{H}$CH$_2$OSO$_2$Ar, 2 x C$\underline{H}_{eq}$HCHCH$_2$OSO$_2$Ar), 2.46 (6 H, s, 2 x Ar$\underline{Me}$), 3.83-3.94 (4 H, m, 2 x C$\underline{H}_2$OSO$_2$Ar), 7.36 (4 H, d, *J* 8.5) and 7.75 (4 H, d, *J* 8.5, Ar$\underline{H}$); δ_C (75.5 MHz; $CDCl_3$) 21.61 (Ar$\underline{Me}$), 25.19, 28.94 (C$\underline{H}_2$C$\underline{H}_2$CHCH$_2$OSO$_2$Ar), 37.89 (C$\underline{H}$CH$_2$OSO$_2$Ar), 72.15 (C$\underline{H}_2$OSO$_2$Ar), 127.93, 129.98, 132.91, 144.96 (aromatic C); *m/z* (CI$^+$, NH_3) 470 (MNH_4^+, 6%), 298 (MNH_4^+-$C_7H_8SO_3$, 48), 126 (MNH_4^+-2$C_7H_8SO_3$, 23), 109 (MH^+-2$C_7H_8SO_3$, 100).

(±)-*trans*-Cyclohexane-1,2-diacetic acid (118).[141]



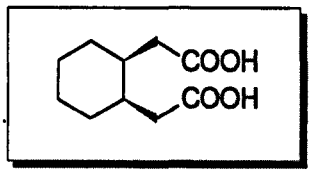
Potassium cyanide (640 mg, 9.83 mmol) was added to a solution of **117** (1.50 g, 3.31 mmol) in DMSO (20 mL) under an atmosphere of nitrogen at ambient temperature and the mixture stirred at 90 °C for 4 hours. The mixture was poured into ammonium chloride solution (sat. aq.) (20 mL) and extracted with dichloromethane (3 x 50 mL). The combined organic layers were washed with water (3 x 80 mL) and dried (magnesium sulphate), filtered and concentrated *in vacuo* to give the crude dinitrile as a pale yellow oil (0.54 g); R_f (1:3 ethyl acetate:petrol) 0.3. The crude material was dissolved in ethanol (20 mL), 9 M potassium hydroxide (aq.) (4 mL) was added and the solution stirred vigorously at 90 °C under reflux for 100 hours. Ethanol was removed *in vacuo* and the aqueous solution acidified to pH ~ 5 using 2 M hydrochloric acid then extracted with ethyl acetate (2 x 40 mL). The combined organic layers were dried (magnesium sulphate), filtered and concentrated *in vacuo* to give diacid **118** as a white amorphous solid (585 mg, 2.92 mmol, 88%), m.pt. 163-164 °C (lit.[184] 167 °C); R_f (1:3 ethyl acetate:hexane) 0.15; (Found: MNH_4^+, 218.1394. $C_{10}H_{16}O_4$ requires *MNH*$_4$, 218.1392); (Found: C, 59.9; H, 8.0. Calculated for $C_{10}H_{16}O_4$: C, 60.0; H, 8.05%); ν_{max} (Nujol)/cm^{-1} 3300-2400 (br., O-H str.), 1697 (C=O str.), 1078 (C-O str.); δ_H (300 MHz; DMSO-d_6) 0.97-1.09 (2 H, m, 2 x $CH_{ax}HCH_2CHCH_2COOH$), 1.10-1.24 (2 H, m, 2 x $CH_{ax}HCHCH_2COOH$), 1.44-1.53 (2 H, m, 2 x $CHCH_2COOH$), 1.59-1.75 (4 H, m, 2 x $CH_{eq}HCH_2CHCH_2COOH$, 2 x $CH_{eq}HCHCH_2COOH$), 1.87-1.96 (2 H, m) and 2.38-2.47 (2 H, m, CH_2COOH), 11.95 (2 H, br. s, OH); δ_C (75.5 MHz; DMSO-d_6) 25.56 ($CH_2CH_2CHCH_2COOH$), 31.96 (CH_2CHCH_2COOH), 38.46 ($CHCH_2COOH$), 38.90 (CH_2COOH), 174.26 (COOH); *m/z* (CI$^+$, NH_3) 218 (MNH_4^+, 100%), 200 (MNH_4^+-H_2O, 59), 182 (MNH_4^+-$2H_2O$, 20).

cis-Cyclohexane-1,2-dimethanol ditosylate (120).[140]



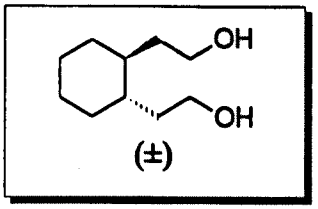
A solution of **119** (795 mg, 5.51 mmol) in pyridine (10 mL) was added dropwise over 10 minutes to a vigorously stirred solution of freshly recrystallised *p*-toluenesulphonyl chloride[183] (4.00 g, 21.0 mmol) in pyridine (10 mL) at 0 °C under an atmosphere of nitrogen. Stirring was maintained at 0 °C for 2 hours then at ambient temperature for 1 hour. The mixture was poured into ice-water (25 mL) and extracted with ethyl acetate (2 x 25 mL). The combined organic layers were washed with 1 M hydrochloric acid (2 x 60 mL), copper sulphate solution (sat. aq.) (2 x 3 mL) and brine (10 mL) then dried (magnesium sulphate), filtered and concentrated *in vacuo* to give a very pale yellow oil which yielded ditosylate **120** as colourless plates on cooling (1.39 g, 3.07 mmol, 56%), m.pt. 86-87 °C (lit.[182] 84-85 °C); R_f (1:3 ethyl acetate:petrol) 0.4; (Found: MH^+, 453.1405. $C_{22}H_{28}O_6S_2$ requires *MH*, 453.1406); (Found: C, 58.65; H, 6.3. Calculated for $C_{22}H_{28}O_6S_2$: C, 58.4; H, 6.25%); ν_{max} (Nujol)/cm^{-1} 1595, 1492 (C=C str., aromatic), 1348, 1171 (S=O str.), 1096 (C-O str.), 817 (C-H def., aromatic); δ_H (300 MHz; $CDCl_3$) 1.32-1.51 (8 H, m, 2 x $CH_2CH_2CHCH_2OSO_2Ar$), 1.98-2.12 (2 H, m, 2 x $CHCH_2OSO_2Ar$), 2.46 (6 H, s, 2 x ArMe), 3.90 (4 H, d, *J* 7.0, 2 x CH_2OSO_2Ar), 7.77 (4 H, d, *J* 8.5) and 7.35 (4 H, d, *J* 8.5, ArH); δ_C (75.5 MHz; $CDCl_3$) 21.63 (ArMe), 22.87, 25.79 ($CH_2CH_2CHCH_2OSO_2Ar$), 36.59 ($CHCH_2OSO_2Ar$), 70.35 (CH_2OSO_2Ar), 127.93, 130.00, 133.02 144.96 (aromatic C); *m/z* (CI^+, NH_3) 453 (MH^+, 4%), 281 (MH^+-$C_7H_8SO_3$, 18), 155 ($C_7H_7SO_2^+$, 23), 109 (MH^+- $2C_7H_8SO_3$, 100), 91 ($C_7H_7^+$, 41).

cis-Cyclohexane-1,2-diacetic acid (121).[141]



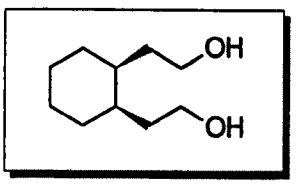
Potassium cyanide (530 mg, 8.11 mmol) was added to a solution of **120** (1.25 g, 2.70 mmol) in DMSO (10 mL) under an atmosphere of nitrogen at ambient temperature and the mixture stirred at 90 °C for 4 hours. The mixture was poured into ammonium chloride solution (sat. aq.) (20 mL) and extracted with dichloromethane (2 x 40 mL). The combined organic layers were washed with water (2 x 50 mL) then dried (magnesium sulphate), filtered and concentrated *in vacuo* to give the crude dinitrile as a pale yellow oil (450 mg); R_f (1:3 ethyl acetate:petrol) 0.3. The crude material was dissolved in ethanol (10 mL), 9 M potassium hydroxide (aq.) (2 mL) was added and the solution stirred vigorously under reflux for 110 hours. Ethanol was removed *in vacuo* and the aqueous solution acidified to pH ~ 5 using 2 M hydrochloric acid and extracted with ethyl acetate (2 x 30 mL). The combined organic layers were dried (magnesium sulphate), filtered and concentrated *in vacuo* to give diacid **121** as a white amorphous solid (443 mg, 2.21 mmol, 82%), m.pt. 161-162 °C (lit.[185] 160-161 °C); R_f (1:3 ethyl acetate:hexane) 0.15; (Found: MNH_4^+, 218.1396. $C_{10}H_{16}O_4$ requires *MNH_4*, 218.1392); ν_{max} (Nujol)/cm^{-1} 3400-2600 (br., O-H str.), 1691 (C=O str.), 1084 (C-O str.); δ_H (300 MHz; DMSO-d_6) 1.24-1.52 (8 H, m, 2 x $C\underline{H}_2C\underline{H}_2CHCH_2COOH$), 1.96-2.09 (2 H, m, $C\underline{H}CH_2COOH$), 2.12 (2 H, d, *J* 1.5) and 2.14 (2 H, d, *J* 1.5, 2 x $C\underline{H}_2COOH$), 11.99 (2 H, br. s, O$\underline{H}$); δ_C (75.5 MHz; DMSO-d_6) 22.93 ($\underline{C}H_2CH_2CHCH_2COOH$), 28.48 ($\underline{C}H_2CHCH_2COOH$), 35.04 ($\underline{C}H_2COOH$), 35.22 ($\underline{C}HCH_2COOH$), 174.23 ($\underline{C}OOH$); *m/z* ($CI^+$, NH_3) 218 (MNH_4^+, 100%), 200 (MNH_4^+-H_2O, 77), 182 (MNH_4^+-$2H_2O$, 29).

(±)-*trans*-Cyclohexane-1,2-diethanol (122).



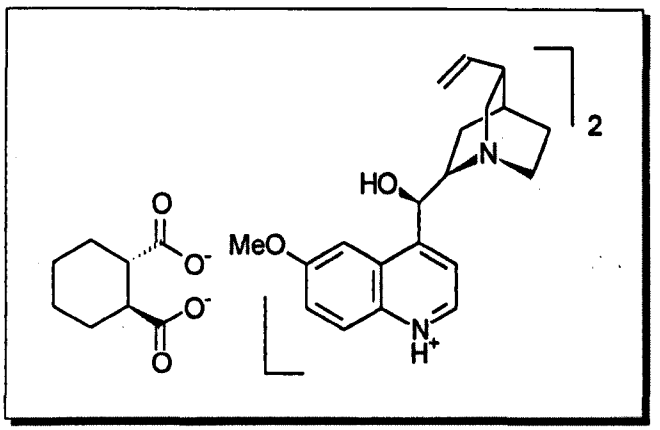
Lithium aluminium hydride (119 mg, 3.14 mmol) was added to THF (4 mL) under an atmosphere of nitrogen at ambient temperature and stirred for 20 minutes. A solution of **118** (150 mg, 0.75 mmol) in THF (4 mL) was added to the suspension over 10 minutes and the mixture stirred under reflux for 18 hours. Sodium sulphate solution (sat.aq.) (0.12 mL) was added dropwise to the vigorously stirred mixture at 0 °C. The inorganic salts were removed by filtration and washed with hot THF (10 mL). The combined organic layers were dried (magnesium sulphate), filtered and concentrated *in vacuo* to give a colourless oil. Flash column chromatography (2:1 ethyl acetate:petrol) gave the diol **122** as a colourless oil (93 mg, 0.54 mmol, 72%); R_f (4:1 ethyl acetate:petrol) 0.15; (Found: MH^+, 173.1543. $C_{10}H_{20}O_2$ requires *MH*, 173.1542); ν_{max} (thin film)/cm^{-1} 3328 (br., O-H str.), 2920, 2853 (C-H str.), 1445, 1051 (C-O str.); δ_H (600 MHz; $CDCl_3$) 0.95-1.02 (2 H, m, 2 x $C\underline{H}_{ax}HCHCH_2CH_2OH$), 1.16-1.24 (4 H, m, 2 x $CH_{ax}HCH_2C\underline{H}CH_2CH_2OH$), 1.34-1.41 (2 H, m, 2 x $C\underline{H}HCH_2OH$), 1.62-1.68 (2 H, m, 2 x $C\underline{H}_{eq}HCHCH_2CH_2OH$), 1.76 (2 H, dt, *J* 13.5, 2.5, 2 x $C\underline{H}_{eq}HCH_2CHCH_2CH_2OH$), 1.84 (2 H, ddt, *J* 14.5, 7.5, 2.5, 2 x $C\underline{H}HCH_2OH$), 2.46 (2 H, s, O<u>H</u>), 3.60-3.65 (2 H, m) and 3.68-3.73 (2 H, m, 2 x $C\underline{H}_2OH$); δ_C (100.6 MHz; $CDCl_3$) 25.95 ($\underline{C}H_2CH_2CHCH_2CH_2OH$), 31.97 ($\underline{C}H_2CHCH_2CH_2OH$), 36.24 ($\underline{C}H_2CH_2OH$), 38.34 ($\underline{C}HCH_2CH_2OH$), 60.60 ($\underline{C}H_2OH$); *m/z* ($CI^+$, NH_3) 173 (MH^+, 100%), 155 (MH^+-H_2O, 37), 137 (MH^+-$2H_2O$, 24).

***cis*-Cyclohexane-1,2-diethanol (123).**



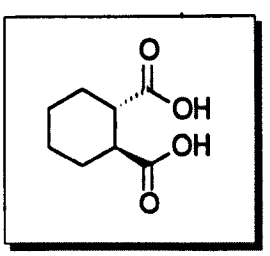
Lithium aluminium hydride (77.0 mg, 2.03 mmol) was added to THF (4 mL) under an atmosphere of nitrogen at ambient temperature and stirred for 20 minutes. A solution of **121** (97.2 mg, 0.49 mmol) in THF (4 mL) was added to the suspension over 10 minutes and the mixture stirred under reflux for 18 hours. Sodium sulphate solution (sat. aq.) (0.1 mL) was added dropwise to the vigorously stirred mixture at 0 °C and the inorganic salts were removed by filtration and washed with hot THF (10 mL). The combined organic layers were dried (magnesium sulphate), filtered and concentrated *in vacuo* to give a colourless oil. Flash column chromatography (ethyl acetate) gave the diol **123** as a colourless oil (45.3 mg, 0.27 mmol, 55%); R_f (4:1 ethyl acetate:petrol) 0.2; (Found: MNH_4^+, 190.1807. $C_{10}H_{20}O_2$ requires *MNH_4*, 190.1807); ν_{max} (thin film)/cm^{-1} 3321 (br., O-H str.), 2926, 2856 (C-H str.), 1054 (C-O str.); δ_H (400 MHz; $CDCl_3$) 1.37-1.42 (6 H, m, 2 x $C\underline{H}_2C\underline{H}_{ax}HCHCH_2CH_2OH$), 1.50-1.58 (6 H, m, 2 x $C\underline{H}_{eq}HCHC\underline{H}_2CH_2OH$), 1.68-1.72 (2 H, m, 2 x $C\underline{H}CH_2CH_2OH$), 2.01 (2 H, br. s, O$\underline{H}$), 3.59-3.74 (4 H, m, 2 x $C\underline{H}_2OH$); δ_C (100.6 MHz; $CDCl_3$) 24.19 ($\underline{C}H_2CH_2CHCH_2CH_2OH$), 29.36 ($\underline{C}H_2CHCH_2CH_2OH$), 33.17 ($\underline{C}H_2CH_2OH$), 36.26 ($\underline{C}HCH_2CH_2OH$), 62.21 ($\underline{C}H_2OH$); *m/z* ($CI^+$, NH_3) 190 (MNH_4^+, 100%), 173 (MH^+, 19).

(+)-(*S,S*)-*trans*-Cyclohexane-1,2-dicarboxylic acid di-(-)-quinine salt (124).[143]



A boiling solution of (±)-*trans*-cyclohexane-1,2-dicarboxylic acid **115** (2.00 g, 11.6 mmol) in ethanol (20 mL) was slowly added to a hot solution of (-)-quinine (7.53 g, 23.2 mmol) in ethanol (45 mL). The solution was boiled and reduced to 40 mL, filtered through a fluted filter paper and allowed to cool to ambient temperature for 2 hours then cooled to 4 °C for 15 hours. The white precipitate formed was separated by filtration and dried to give the crude salt (4.40 g). A second recrystallisation gave the salt **124** as a white solid (1.91 g, 2.33 mmol, 20%).

(+)-(*S,S*)-*trans*-Cyclohexane-1,2-dicarboxylic acid (115).[143]



Quinine salt **124** (1.91 g, 2.33 mmol) was slowly added to an ice-cooled solution of sulphuric acid in water (1:4 v/v) (20 mL). Diethyl ether (30 mL) was added and the biphasic mixture stirred vigorously under reflux at 50 °C for 17 hours. The ethereal extract was dried (magnesium sulphate), filtered and concentrated *in vacuo* to give resolved diacid **115** as a white amorphous solid (340 mg, 1.97 mmol, 85%, 17% from racemic diacid); $[\alpha]_D^{25}$ +16.3 (acetone, c. 4.7) (73 % ee); lit.[143] $[\alpha]_D^{30}$ +22.3 (acetone, c. 5.3).

Polymerisation of 1,2-cyclohexane diacids / diols.

Method 1: The diacid (116 μmol) was added to a solution of diol (116 μmol) in toluene (1 mL) in a modified Dean and Stark apparatus. *Novozyme 435®* (3.0 mg) was added and the mixtures stirred at 60 °C for 17 hours. Volatile components were removed *in vacuo* and the residue analysed by GPC.

diacid	diol	M_w	M_n	M_w / M_n
(±)-*trans*-1,2-cyclohexane diacetic acid (±)-(**118**)	butane-1,4-diol (**4**)	612	224	2.7
cis-1,2-cyclohexane diacetic acid(**121**)	butane-1,4-diol (**4**)	1690	830	2.0
adipic acid (**3**)	(±)-*trans*-1,2-cyclohexane dimethanol (±)-(**116**)	1697	815	2.1
adipic acid (**3**)	*cis*-1,2-cyclohexane dimethanol (±)-(**119**)	1537	463	3.3

Table 35

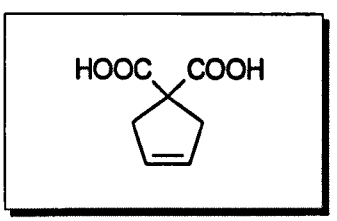
Method 2: Adipic acid **3** (30.4 mg, 208 μmol) was added to a solution of the diol (±)-**116** (30.0 mg, 208 μmol) in toluene (2 mL); 5 further identical reaction mixtures were made up. *Novozyme 435®* (3.0 mg) was added to each mixture, and the mixtures stirred at 60 °C. Reactions were halted after 0.5, 1, 2.25, 4, 9 and 20 hours and sampled for GPC analysis (50 μL). The remaining mixtures were filtered and the residual starting diol **116** isolated by flash column chromatography using gradient elution (1:3 - 3:1 ethyl acetate:hexane). Acetic anhydride (2 drops) was added to solutions of the recovered diol (~5 mg, ~35 μmol) in pyridine (0.5 mL) and the mixtures stirred for 15 hours at ambient temperature. Volatile components were removed *in vacuo* and the diacetates analysed by chiral GC.

Method 3: Adipic acid **3** (1.52 g, 10.4 mmol) and water (50 μL) were added to the diol (±)-**116** (1.50 g, 10.4 mmol). The mixture was stirred for 30 minutes at 70 °C and then *Novozyme 435®* (9.0 mg) was added and the mixture stirred at 70 °C for 48 hours at 100 ± 5 mbar, sampling for GPC and GC analysis after 0.5, 1, 2.25, 4, 22 and 48 hours. Isolation and derivatisation as for method 2.

Method 4: As method 2, but reactions stirred at ambient temperature.

Method 5: Diacid (±)-**118** (52.0 mg, 260 μmol) was added to a solution of butane-1,4-diol **4** (23.4 mg, 260 μmol) in toluene (2 mL) and stirred for 30 minutes at 60 °C. *Novozyme 435*® (3.0 mg) was added and the mixture stirred for 4 hours at 60 °C, sampling after 0.5, 1,2 and 4 hours for GPC and chiral GC analysis. Trimethylsilyl diazomethane (2 drops of a 2.0 M solution in hexane) was added to the GC samples (~5 mg) in toluene (0.5 mL) and left to stand for 30 minutes. Volatiles were removed *in vacuo* and the residues analysed by chiral GC.

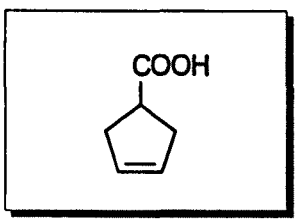
3-Cyclopentene-1,1-dicarboxylic acid (133).[151]



Lithium hydride (1.34 g, 168 mmol) was added in one portion to a stirred solution of dimethyl malonate **132** (8.80 g, 67 mmol) and DMPU (15 mL) in dry THF (100 mL) at 0 °C under an atmosphere of nitrogen. The nitrogen supply was disconnected and a gas bubbler attached. The mixture was stirred at 0 °C for 15 minutes then at ambient temperature for 3.5 hours when evolution of hydrogen had ceased. *cis*-1,4-Dichloro-2-butene (7.6 mL, 72 mmol) was added under an atmosphere of nitrogen and the mixture stirred at 40 °C for 22 hours. Water (20 mL) was added dropwise at 0 °C followed by lithium hydroxide monohydrate (8.39 g, 200 mmol) and the mixture stirred at ambient temperature for 20 hours. Water (150 mL) was added and the mixture stirred for 10 minutes then washed with ethyl acetate (4 x 200 mL). The organic phases were each back-extracted with brine (15 mL). The combined aqueous layers were acidified to ~ pH 1 using 6 M hydrochloric acid (40 mL) and extracted with ethyl acetate (2 x 150 mL). The combined organic phases were washed with 2 M hydrochloric acid (2 x 100 mL) and brine (2 x 100 mL), dried (magnesium sulphate), filtered and concentrated *in vacuo* to give diacid **133** as a pale yellow amorphous solid (9.53 g, 61.0 mmol, 92%), m.pt. 160-164 °C (lit. 160-163 °C,[151] 164-169 °C,[186] 162-165 °C[187]); R_f (1:9

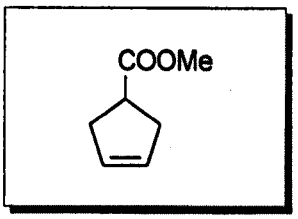
methanol:dichloromethane) 0.1; (Found: MNH_4^+, 174.0765. $C_7H_8O_4$ requires *MNH*$_4$, 174.0766); (Found: C, 53.8; H, 5.15. Calculated for $C_7H_8O_4$: C, 53.85; H, 5.15%); ν_{max} (Nujol)/cm^{-1} 2926, 2856 (C-H str.), 1710 (br., C=O str.), 1463, 1062 (C-O str.); δ_H (200 MHz; $CDCl_3$) 3.09 (4 H, s, 2 x $CH_2C(COOH)_2$), 5.64 (2 H, s, 2 x $CHCH_2C(COOH)_2$), 7.39 (2 H, br. s, 2 x COOH); δ_C (75.5 MHz; $CDCl_3$) 41.26 ($CH_2C(COOH)_2$), 127.79 ($CHCH_2C(COOH)_2$), 171.68 (COOH); *m/z* (CI^+, NH_3) 174 (MNH_4^+, 100%), 129 (MNH_4^+-CO_2H, 65), 95 ($C_5H_6COH^+$, 78).

3-Cyclopentenecarboxylic acid (134).[151]



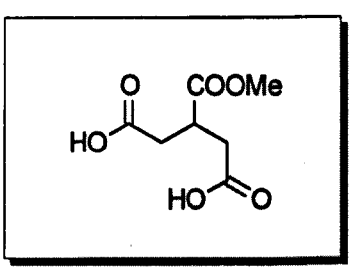
Diacid **133** (15.6 g, 99.9 mmol) was heated under reflux using a condenser fitted with a bubbler at 170 °C for 1.5 hours, whereupon a brown liquid had formed and evolution of gas had ceased. The brown oil was then distilled under reduced pressure (b.pt. 82-84 °C / 3 mmHg) (lit. 81-83 °C / 1.7 mmHg[151], 83-84 °C / 2 mmHg[186], 70 °C / 1 mmHg[187]) using a short-path apparatus to give acid **134** as a clear, colourless oil (8.43 g, 75.2 mmol, 75%); R_f (ethyl acetate) 0.85; (Found: M^+, 112.0526. $C_6H_8O_2$ requires *M*, 112.0524); ν_{max} (thin film)/cm^{-1} 3600-2300 (br., O-H str.), 2922 (C-H str.), 1705 (br., C=O str.), 1620 (C=C str.), 1070 (C-O str.); δ_H (300 MHz; $CDCl_3$) 2.68-2.71 (4 H, m, 2 x $CH_2CHCOOH$), 3.17 (1 H, quintet, *J* 7.5, CHCOOH), 5.68 (2 H, s, 2 x $CHCH_2CHCOOH$); δ_C (75.5 MHz; $CDCl_3$) 36.21 ($CH_2CHCOOH$), 41.35 (CHCOOH), 128.99 ($CHCH_2CHCOOH$), 182.31 (COOH); *m/z* (EI^+) 112 (M^+, 21%), 97 ($C_5H_6C(H)OH_2^+$, 23), 67 (M^+-CO_2H, 100).

Methyl 3-cyclopentenecarboxylate (135).



DCC (4.78 g, 23.2 mmol) and DMAP (220 mg, 1.80 mmol) were added to a solution of acid **134** (2.00 g, 17.8 mmol) in methanol (15 mL) under an atmosphere of argon at ambient temperature and the mixture stirred for 21 hours. The mixture was filtered, washing with methanol (20 mL) and volatile components removed *in vacuo*. Purification by flash column chromatography (1:10 ethyl acetate:hexane) gave the ester **135** as a colourless oil (1.27 g, 10.1 mmol, 56%); R_f (1:10 ethyl acetate:hexane) 0.6; (Found: M^+, 126.0680. $C_7H_{10}O_2$ requires *M*, 126.0681); ν_{max} (thin film)/cm^{-1} 3063 (C-H str., C=C), 2998, 2937, 2859 (C-H str.), 1742 (C=O str.), 1619 (C=C str.), 1107 (C-O str.); δ_H (200 MHz; $CDCl_3$) 2.63-2.68 (4 H, m, 2 x CH_2CHCOOMe), 3.13 (1 H, quintet, *J* 7.5, CHCOOMe), 3.70 (3 H, s, COOMe), 5.66 (2 H, s, 2 x CHCH$_2$CHCOOMe); δ_C (75.5 MHz; $CDCl_3$) 36.27 (CH_2CHCOOMe), 41.43 (CHCOOMe), 51.72 (COOMe), 128.99 (CHCH$_2$CHCOOMe), 176.83 (COOMe); *m/z* (EI^+) 126 (M^+, 24%), 111 (M^+-Me, 16), 95 (M^+-MeO, 20), 67 (M^+-CO_2Me, 100).

3-(Carbomethoxy)pentanedioic acid (131).



A solution of ester **135** (400 mg, 3.17 mmol) in toluene (3 mL) was added dropwise to a solution of potassium permanganate (2.00 g, 12.7 mmol) and Aliquat® 336 (60 mg, 0.15 mmol) in water (4 mL) at 0 °C. The mixture was stirred for 30 minutes at 0 °C then warmed to ambient temperature and stirred for a further 1 hour. Sodium sulphite (sat. aq.) (20 mL) was added and the brown precipitate formed filtered off and washed with hot ethyl acetate (50

mL). The aqueous phase was acidified with 2 M hydrochloric acid, saturated with sodium chloride and extracted using the same ethyl acetate used to wash the precipitate. The organic phase was dried (magnesium sulphate), filtered and concentrated *in vacuo* to give the diacid **131** as a colourless oil which formed white plates on standing (366 mg, 1.92 mmol, 61%); R_f (1:9 methanol:dichloromethane) 0.15; (Found: MNH_4^+, 208.0823. $C_7H_{10}O_6$ requires *MNH*$_4$, 208.0821); ν_{max} (Nujol)/cm^{-1} 3400-2200 (br., O-H str.), 2677 (C-H str.), 1706 (br., C=O str.), 1057 (C-O str.); δ_H (300 MHz; $CDCl_3$) 2.68 (2 H, dd, *J* 17, 6.5) and 2.88 (2 H, dd, *J* 17, 7, 2 x CH_2COOH), 3.28 (1 H, quintet, *J* 6.5, CHCOOMe), 3.74 (3 H, s, COOMe); δ_C (75.5 MHz; $CDCl_3$) 34.87 (CH_2COOH), 36.96 (CHCOOMe), 52.46 (COOMe), 173.19 (COOMe), 176.73 (COOH); *m/z* (CI^+, NH_3) 208 (MNH_4^+, 100%), 190 (MNH_4^+-H_2O, 44).

Multibranched polymerisation experiments (Section 2.3.6.3).

substrate1	substrate2	wt. substrate 1 / mg (no. mmol)	wt. substrate 2 / mg (no. moles)	method (vol. of solvent)	wt. *Novozyme 435*® / mg
diethyl 3-hydroxyglutarate (**128**)	-	1000	-	Method 1 (-)	20
diethyl 3-hydroxyglutarate (**128**)	-	150 (0.73)	-	Method 2 (5 mL)	3.0
diethyl 3-hydroxyglutarate (**128**)	butane-1,4-diol (**4**)	906 (4.44)	440 (4.88)	Method 1 (-)	3.7
3-(carbomethoxy) pentanedioic acid (**131**)	butane-1,4-diol (**4**)	150 (0.79)	107 (1.19)	Method 1 (-)	5.0
1,1,1-tris (hydroxymethyl) ethane (**136**)	adipic acid (**3**)	54.0 (0.76)	100.0 (0.68)	Method 2 (2 mL)	0.6

Table 36

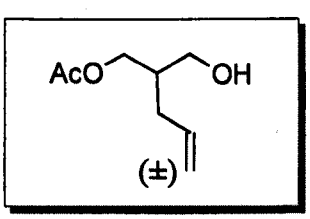
Method 1: The substrates were stirred at 40 °C for 30 minutes. *Novozyme 435*® was added and the mixture stirred for a further 4 hours at 40 °C. The temperature was increased to 60 °C and stirring maintained under reduced pressure (100 ± 5 mbar) for17 hours (Tables 36, 37).

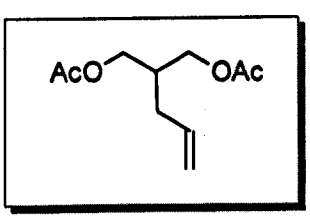
Method 2: The substrates were mixed with toluene and stirred at 60 °C for 30 minutes. *Novozyme 435®* was added and the mixture stirred for a further 22 hours at 60 °C (Tables 36, 37).

substrate1	substrate2	method	M_w	M_n	M_w / M_n
diethyl 3-hydroxyglutarate (**128**)	-	Method 1 (solvent-free)	-	-	-
diethyl 3-hydroxyglutarate (**128**)	-	Method 2 (toluene-based)	199	155	1.3
diethyl 3-hydroxyglutarate (**128**)	butane-1,4-diol (**4**)	Method 1 (solvent-free)	493	289	1.7
3-(carbomethoxy) pentanedioic acid (**131**)	butane-1,4-diol (**4**)	Method 1 (solvent-free)			
1,1,1-tris (hydroxymethyl) ethane (**136**)	adipic acid (**3**)	Method 2 (toluene-based)	200	195	1.0

Table 37

3-Acetoxy-2-allylpropan-1-ol (153) and 1,3-diacetoxy-2-allylpropane (152).





Vinyl acetate (0.22 mL, 2.4 mmol) and *Novozyme 435®* (5.0 mg) were added to a solution of 2-allylpropane-1,3-diol **105** (40.0 mg, 344 μmol) in the chosen solvent system and stirred until TLC indicated that all the diol had been consumed; monoacetate **153**: R_f (1:1 ethyl acetate:petrol) 0.4; (Found: MH^+, 159.1021. $C_8H_{14}O_3$ requires *MH*, 159.1021); ν_{max} (thin film)/cm^{-1} 3423 (br., O-H str.), 3082, 2934 (C-H str.), 1642 (C=C str.), 1095 (C-O str.); δ_H (400 MHz; $CDCl_3$) 1.84-1.96 (1 H, m, C<u>H</u>CH_2OAc), 2.04-2.16 (3 H, m, $CHCH_2O$<u>H</u>, <u>CH_2</u>$CH=CH_2$), 2.07 (3 H, s, <u>Me</u>COOR), 3.52 (1 H, dd, *J* 11, 5) and 3.60 (1 H, dd, *J* 11, 6, $CHCH_2O$H), 4.08 (1 H, dd, *J* 11, 6.5) and 4.18 (1 H, dd, *J* 11, 5, $CHCH_2O$Ac), 5.03-5.10 (2 H, m, $CH_2CH=CH_2$), 5.78 (1 H, ddt, *J* 17, 10, 7, $CH_2CH=CH_2$); δ_C (100.6 MHz; $CDCl_3$) 20.90 (<u>Me</u>COOR), 32.59 (<u>C</u>$H_2CH=CH_2$), 40.23 (<u>C</u>HCH_2OH), 62.42 (CH<u>C</u>H_2OH), 64.24

($\underline{C}H_2OAc$), 117.03 ($CH_2CH=\underline{C}H_2$), 135.70 ($CH_2\underline{C}H=CH_2$), 171.63 ($Me\underline{C}OOR$); *m/z* (CI^+, NH_3) 176 (MNH_4^+, 100%), 159 (MH^+, 85), 141 (MH^+-H_2O, 17), 99 (MH^+-CH_2CO-H_2O, 5), 81 (MH^+-AcOH-H_2O, 5); diacetate **152**: R_f (1:1 ethyl acetate:petrol) 0.85; (Found: MNH_4^+, 218.1395. $C_{10}H_{16}O_4$ requires *MNH_4*, 218.1392); ν_{max} (thin film)/cm^{-1} 3032, 2865 (C-H str.), 1639 (C=C str.), 1095 (C-O str.); δ_H (200 MHz; $CDCl_3$) 2.06 (6 H, s, 2 x $\underline{Me}COOR$), 2.07-2.22 (3 H, m, $C\underline{H}CH_2OAc$, $C\underline{H}_2CH=CH_2$), 3.96-4.14 (4 H, m 2 x $C\underline{H}_2OAc$), 5.02-5.07 (1 H, m) and 5.08-5.15 (1 H, m, $CH_2CH=C\underline{H}_2$), 5.76 (1 H, m, $CH_2C\underline{H}=CH_2$); δ_C (75.5 MHz; $CDCl_3$) 20.70 ($\underline{Me}COOR$), 32.65 ($\underline{C}H_2CH=CH_2$), 37.01 ($\underline{C}HCH_2OH$), 63.85 (2 x $\underline{C}H_2OAc$), 117.39 ($CH_2CH=\underline{C}H_2$), 134.90 ($CH_2\underline{C}H=CH_2$), 170.89 ($Me\underline{C}OOR$); *m/z* (CI^+, NH_3) 218 (MNH_4^+, 100%), 141 (MH^+-AcOH, 45), 81 (MH^+-2AcOH, 5).

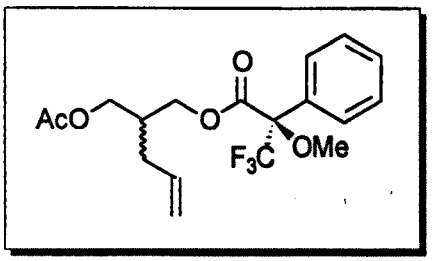
Asymmetrisation of 2-allylpropane-1,3-diol (105).

solvent (volume / mL)	reaction time / h	temp. / °C	% yield of (153)	% yield of (152)	overall % yield	% ee of (153)
THF (0.50)	1.0	25	40	24	64	40
THF (0.50) / triethylamine (0.03)	1.0	25	20	42	62	43
THF (0.50) / triethylamine (0.17)	1.0	25	11	56	67	27
THF (0.50) / *tert*-butyl methyl ether (0.14)	1.2	25	33	18	51†	47
toluene (0.50)	0.75	20	42	19	61	48
no added solvent	1.0	20	39	22	61	28
THF (0.50)	6.0	0	30	40	70	5
THF (0.50) / *tert*-butyl methyl ether (0.14)	6.0	0	26	41	67	14
THF (0.50) / *tert*-butanol (0.11)	7.0‡	0	11	20	31	67

†artificially low yield, due to some spillage of reaction mixture;
‡starting material still present.

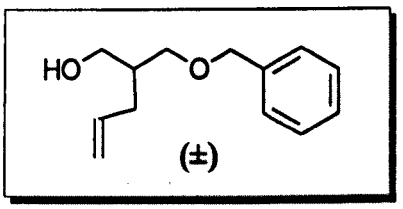
Table 38

3-Acetoxy-2-allylpropan-1-ol, (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid ester (*RR*)-(156) and (*SR*)-(156).



(*R*)-(+)-α-Methoxy-α-trifluoromethylphenylacetyl chloride (18.0 mg, 71 μmol) and DMAP (20.0 mg, 164 μmol) were added to a solution of alcohol **153** (8.0 mg, 51 μmol) in deuteriated chloroform (0.50 mL) in an NMR tube under an atmosphere of argon. The mixture was allowed to stand for 24 hours at ambient temperature. ^{19}F NMR spectroscopy showed resonances from the CF_3 nuclei at -70.8 and -71.9 ppm; a further signal at -71.4 ppm was presumed to be from the excess of (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride.

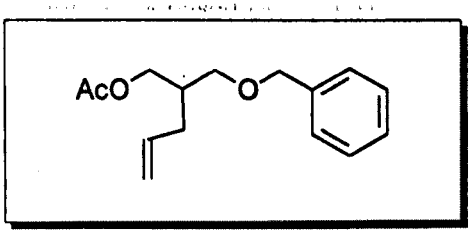
(±)-2-Allyl-3-benzyloxypropan-1-ol (157).



THF (4 mL) was added to sodium hydride (60% dispersion in mineral oil 114 mg, 2.84 mmol) under an atmosphere of argon at 0 °C. A solution of diol **105** (150 mg, 1.29 mmol) in THF (2 mL) was added at 0 °C and the mixture stirred for 5 minutes at 0 °C then for 30 minutes at ambient temperature. The mixture was cooled to 0 °C, and DMF (2 mL) was added followed by benzyl chloride (370 μL, 3.24 mmol) dropwise. The mixture was then stirred at ambient temperature for 15 hours. Water (1 mL) was added at 0 °C and the mixture extracted with diethyl ether (2 x 15 mL). The combined organic layers were dried (magnesium sulphate), filtered and concentrated *in vacuo* to give a yellow oil. Purification by flash column chromatography (1:2 ethyl acetate:petrol) gave the monobenzylated derivative **157** as a

colourless oil (190 mg, 0.92 mmol, 71% based on diol **105**); R_f (1:1 ethyl acetate:petrol) 0.6; (Found: MH^+, 207.1386. $C_{13}H_{18}O_2$ requires *MH*, 207.1385); ν_{max} (thin film)/cm^{-1} 3428 (br., O-H str.), 3066, 3032, 2863 (C-H str.), 1639 (C=C str.), 1096 (C-O str.), 736, 697 (aromatic C-H def.); δ_H (300 MHz; $CDCl_3$) 1.93-2.03 (1 H, m, C<u>H</u>CH_2OH), 2.09 (2 H, t, *J* 7, C<u>H</u>$_2CH{=}CH_2$), 2.37-2.46 (1 H, s, O<u>H</u>), 3.46-3.77 (4 H, m, C<u>H</u>$_2OH$, C<u>H</u>$_2OBn$), 4.48-4.53 (2 H, m, Ar<u>CH</u>$_2O$), 5.00-5.08 (2 H, m, $CH_2CH{=}C\underline{H}_2$), 5.78 (1 H, ddt, *J* 17, 10, 7, $CH_2C\underline{H}{=}CH_2$), 7.26-7.38 (5 H, m, Ar<u>H</u>); δ_C (75.5 MHz; $CDCl_3$) 32.79 (<u>C</u>$H_2CH{=}CH_2$), 40.47 (<u>C</u>HCH_2OH), 65.64 (<u>C</u>H_2OH), 73.24, 73.50 (<u>C</u>H_2OBn, Ar<u>C</u>H_2O), 116.52 ($CH_2CH{=}\underline{C}H_2$), 127.65, 127.77, 128.51 (aromatic <u>C</u>), 136.32 ($CH_2\underline{C}H{=}CH_2$), 138.13 (aromatic <u>C</u>); *m/z* (CI^+, NH_3) 224 (MNH_4^+, 48%), 207 (MH^+, 100), 115 (M^+-C_7H_7, 12), 108 ($C_7H_8O^+$, 27), 91 ($C_7H_7^+$, 23).

1-Acetoxy-2-allyl-3-benzyloxypropane (158).



Vinyl acetate (108 mg, 1.25 mmol) and *Novozyme 435*® (2.0 mg) were added to a solution of (±)-2-allyl-3-benzyloxypropan-1-ol **157** (40.0 mg, 194 μmol) in THF (0.80 mL) and stirred at ambient temperature for 1 hour, whereupon TLC indicated that approximately half the diol had been consumed; R_f (1:1 ethyl acetate:petrol) 0.85; (Found: MH^+, 249.1494. $C_{15}H_{20}O_3$ requires *MH*, 249.1491); ν_{max} (thin film)/cm^{-1} 3032, 2865 (C-H str.), 1639 (C=C str.), 1097 (C-O str.), 735, 695 (aromatic C-H def.); δ_H (200 MHz; $CDCl_3$) 2.02 (3 H, s, <u>Me</u>COOR), 2.06-2.17 (3 H, m, C<u>H</u>$_2CH{=}CH_2$, <u>C</u>HCH_2OBn), 3.43 (2 H, d, *J* 5.5, C<u>H</u>$_2OBn$), 4.10 (2 H, d, *J* 5.5, C<u>H</u>$_2OAc$), 4.49 (2 H, m, Ar<u>CH</u>$_2O$), 5.00-5.08 (2 H, m, $CH_2CH{=}C\underline{H}_2$), 5.78 (1 H, m, $CH_2C\underline{H}{=}CH_2$), 7.25-7.38 (5 H, m, Ar<u>H</u>); δ_C (75.5 MHz; $CDCl_3$) 20.81 (<u>Me</u>COOR), 32.89 (<u>C</u>$H_2CH{=}CH_2$), 38.17 (<u>C</u>HCH_2OH), 64.58 (<u>C</u>H_2OAc), 69.87 (<u>C</u>H_2OBn), 73.14 (Ar<u>C</u>H_2O), 116.82 ($CH_2CH{=}\underline{C}H_2$), 126.94, 127.58, 128.36 (aromatic <u>C</u>), 135.81 ($CH_2\underline{C}H{=}CH_2$), 138.48 (aromatic <u>C</u>), 171.11 (Me<u>C</u>OOR); *m/z* (CI^+, NH_3) 266 (MNH_4^+, 34%), 249 (MH^+, 81), 207 (MH^+-CH_2CO, 45), 108 ($C_7H_8O^+$, 100), 98 (MH^+-MeCO-C_7H_7OH, 19), 91 ($C_7H_7^+$, 43).

CHAPTER 5

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