Dynamic Kinetic Resolution: Synthesis of Optically Active α-Amino Acid Derivatives

Stuart Andrew Brown

The University of Edinburgh

A thesis submitted for the degree of Doctor of Philosophy **April 1999**

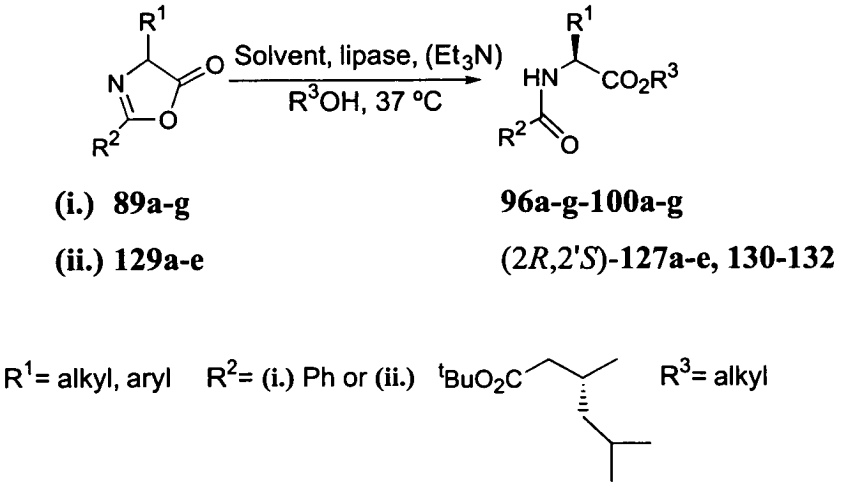
Acknowledgements

I would like to express my gratitude to Professor Nicholas J. Turner for his support and encouragement over the course my studies. I would like to thank Professor Herbert Waldmann of the Institut für Organische Chemie der Universität Karlsruhe for allowing me the opportunity to study in his research group for a period of three months, September-December 1997. I would also like to thank the following academic and technical staff; John Millar, Wesley Kerr, Dr Chandralal M. Hewage, Dr David Reed, Dr Ian Sadler, and Dr Emma Beattie for their running and help with nmr spectra; Alan Taylor and Harry McKenzie for the running of mass spectrometry samples; Stuart Franklin and Lorna Eades for CHN analysis; Dr Simon Parsons and Andrew Smith for X-ray crystallography; Donald Robertson and Patrick Hencher for use of their IR spectrometer; Derek Burgess, Raymond Borwick and Tim Calder for help and fun at chemical stores; and Alan Fisher for preliminary studies. I would like to thank Dr Gareth Jenkins for initial help, Dr James Dowden for proof reading this thesis, and all the members of the Turner-Flitsch group. Finally, I would like to thank Novo-Nordisk for the gift of Novozyme® and Lipozyme®, and the Biotechnology & Biological Sciences Research Council (BBSRC) for financial support.

Declaration

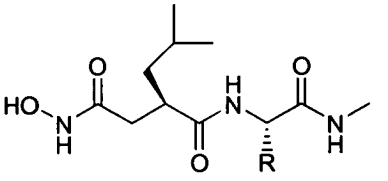
This thesis is submitted in part fulfilment of the requirement for the degree of Doctor of Philosophy at the University of Edinburgh. Unless otherwise stated, the work is original, and has not been previously submitted, in whole or in part, for any degree at this, or any other university.

Abstract



Scheme 1 *Dynamic kinetic resolution of 5(4*H*)-oxazolones*

The dynamic kinetic resolution of 2-phenyl-4-substituted-5(4*H*)-oxazolones **89a-g** has been investigated as a method for the synthesis of optically active α-amino acid derivatives. The effects of lipase, [either Novozyme® (*Candida antarctica* lipase B), or Lipozyme® (*Rhizomucor miehei* lipase)], solvent, nucleophile, and the addition of external triethylamine to the reaction is described. When R^1= Ph, an 88% yield and 98% enantiomeric excess (e.e.) of α-amino acid ester **96a** was obtained with Novozyme® in acetonitrile as solvent. The synthesis of novel 5(4*H*)-oxazolones **129a-e**, which are identified as key intermediates in the synthesis of a series of matrix metalloproteinase inhibitors **94**, is described. Application of the lipase catalysed dynamic kinetic resolution conditions to **129a-e**, afforded high yields (96%) and diastereomeric excesses (d.e.'s), (86%) of the resulting pseudodipeptides (2*R*,2'*S*)-**127a-e** and **130-132**, by careful selection of the reaction conditions.



94

Abbreviations

Ac	acetyl
a_w	water activity
Bn	benzyl
BnBr	benzyl bromide
BnOH	benzyl alcohol
Boc	*tert*-butoxycarbonyl
BOP	benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate
br	broad
Bu	butyl
^{t}BuOMe	*tert*-butyl methyl ether
Cbz	carboxybenzyl
$CDCl_3$	deuterochloroform
$CHCl_3$	chloroform
CI	chemical ionisation
CLEC	cross linked enzyme crystals
d	doublet
DCC	1,3-dicyclohexylcarbodiimide
DCM	dichloromethane
d.e.	diastereomeric excess
DIPE	diisopropylether
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DIPEA	diisopropylethylamine
EDCI	1(3-dimethylaminopropyl)-ethylcarbodiimide hydrochloride
e.e.	enantiomeric excess
ESMS	electrospray mass spectrometry
Et	ethyl
Et_3N	triethylamine
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
FAB	fast atom bombardment
HOBt	1-hydroxybenzotriazole hydrate
HPLC	high performance liquid chromatography
h	hour(s)
IPA	*iso*-propyl alcohol
Lipozyme®	*Rhizomucor miehei* lipase
m	multiplet
Me	methyl
MMPI	matrix metalloproteinase inhibitor
Mp	melting point
MS	mass spectrometry
NMM	*N*-methyl morpholine

nmr	nuclear magnetic resonance
Novozyme®	*Candida antarctica* lipase B
Ph	phenyl
Pn	pentyl
Pr	propyl
s	singlet
SAR	structure activity relationship
t	triplet
Tf	triflate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Ts	tosylate
TBTU	2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
ZBG	zinc binding group

Contents

1.0.0. Introduction

1.1.0. Lipases and their role in nature

1.1.1. Role of lipases in nature

In nature, lipases, or triacylglycerol hydrolases (E.C. 3.1.1.3) as they are systematically known, are classified as hydrolases that catalyse the hydrolysis of fatty acids and glycerol at the lipid/water interface. In contrast to proteases and esterases, lipases generally exhibit little or no catalytic activity at low substrate concentrations, and therefore do not follow normal Michaelis-Menten kinetics. Hydrolysis is only realised once the substrate concentration has reached its saturation limit, or critical micellar concentration (CMC), (Figure 1).[1]

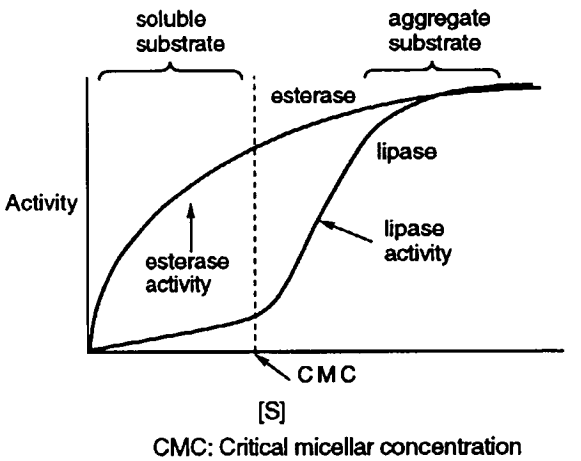
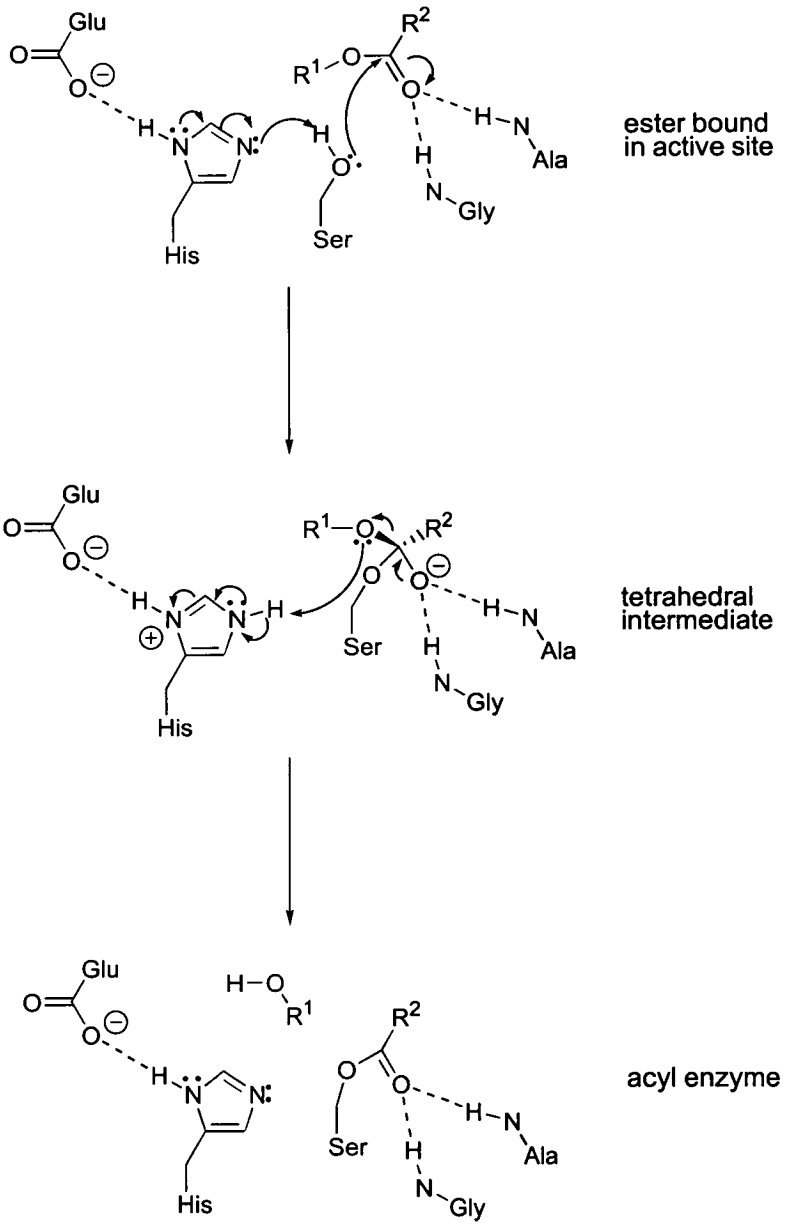


Figure 1 *Activity of lipases and esterases*[1]

In the early 1990's when the first X-ray crystal structures of lipases began to emerge, it became evident that the mechanism of interfacial activation was due, in part, to the presence of an amphiphilic peptidic loop, or lid, covering the active site of the enzyme.[2,3] The analysis of X-ray co-crystals between lipase and substrate analogues suggested that once CMC is reached, a conformational change occurs at the lipid/water interface, and the lid 'flips', thus allowing access to the previously buried

active site.[4] It should be noted however that not all lipases have a lid present in their tertiary structure, or show interfacial activation.[5]



Scheme 2 *Catalytic mechanism of lipases*

All of the lipases whose structures have been elucidated, (12 based on data up to 1998) contain a common architecture. Catalytic activity is realised by a triad consisting of a nucleophilic serine residue, a histidine residue, and an aspartate or glutamate residue (Scheme 2) similar to that found in the serine proteases. Also, in

over thirty cases of cloned lipases, a consensus sequence of -Gly/Ala-X-*Ser*-X-Glu- has been identified at the active site serine.[5]

Recently there has been some degree of confusion in the literature with regards to the naming of some lipases. For example, *Candida rugosa* was previously classified as *Candida cylindracea*, and *Pseudomonas cepacia* was previously classified as *Pseudomonas fluorescens*. To avoid any confusion, the name given in the cited article is used here.

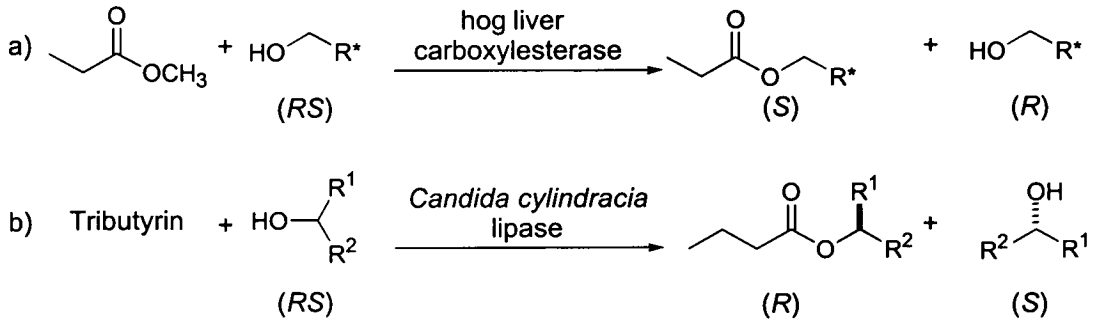
1.1.2. The introduction of organic solvent

There are several advantages to using an organic solvent as opposed to an aqueous system for enzymatic transformations, these include, (a) water insoluble substrates, and substrates and products that may be unstable in aqueous media can be investigated, (b) a broader range of nucleophiles can be used including *e.g.* alcohols, amines, ammonia, oximes, and hydrazines, (c) separation of reagents from the enzymes is greatly simplified as the enzymes are insoluble in organic solvents therefore only require filtration, and (d) the enzyme can be recovered and reused.

The use of organic media in enzymatic catalysis was first documented by Pottevin[6] in 1906 when he used a pancreatic extract in methanol to catalyse the formation of methyl oleate. It was not until the pioneering work of Klibanov and co-workers almost eighty years later in the mid 1980's, that biocatalysis in organic solvent was implemented in the synthetic chemistry laboratory. Initial perception was that enzymes would exhibit only low catalytic activity as the concentration of organic solvent was increased, due to denaturation of the enzyme.

To circumvent this problem, Klibanov and co-workers[7] encapsulated aqueous solutions of hog liver carboxylesterase and lipase from *Candida cylindracea* inside the porous supports Sepharose or Chromosorb. The resulting beads were suspended in a water-immiscible organic solvent, methyl propionate in the case of hog liver carboxylesterase, and tributyrin for lipase from *Candida cylindracea*. In both cases the solvent also served as substrate (Scheme 3a and b respectively). A number of

primary and secondary alcohols were tested as nucleophiles and both enzymes exhibited high transesterification activity and stereoselectivity.



Scheme 3

Expanding on these promising initial results, Klibanov and co-workers[8] utilised lyophilised *Candida cylindracea* and porcine pancreatic lipases in hexane in the esterification and transesterification of racemic carboxylic acids and esters respectively. Klibanov *et al.*,[9] were also the first to implement lipases in organic solvent for amide bond formation and peptide synthesis (see Section **(1.2.2.)** below).

A major problem with using lyophilised enzyme preparations in organic solvents is that the observed activity is often 10,000 fold less than that observed in aqueous systems due to denaturation.[10] Immobilisation of enzymes onto solid supports, *via* covalent attachment, or electrostatic interactions, and more recently the introduction of cross-linked enzyme crystals,[11,12] (CLECs, prepared by cross-linking the enzyme with glyceraldehyde), heralded a new approach to enzyme preparation and provided enzymes with increased stability and activity in organic solvents. The introduction of surfactants,[13] hydrophobic sol-gel materials,[14] solid state acid-base buffers[15] and control of the hydration level of enzymes[16] have all added to a greater understanding of the processes of enzyme activation in organic solvents. The advances in techniques for using biocatalysts in organic media have been reviewed in two informative articles.[17,18]

1.2.0. Resolution processes

1.2.1. Kinetic resolution

The general reaction is outlined in Figure 2. The success of a kinetic resolution relies on the fact that in the presence of a chiral environment, *e.g.* an enzyme, the rate of reaction, ($k_{(R)}$ and $k_{(S)}$) for each enantiomer, ((*R*) and (*S*)) of a racemic mixture differ significantly. In the ideal case, the rate of reaction of the slower reacting enantiomer would be 0, resulting in the reaction of a single enantiomer. As a result, a maximum inherent yield of 50% is obtainable in kinetic resolution processes.

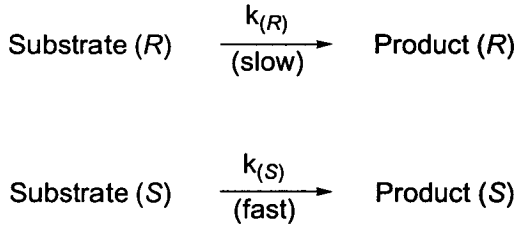


Figure 2

Kinetic resolution of alcohols and carboxylic acids are carried out routinely in the laboratory and are far too wide ranging to be covered in the required detail in this thesis. Kazlauskas and co-workers[19-21] have published an empirical rule predicting the chiral preference of lipases for the resolution of secondary alcohols. In all cases, the preferred enantiomer, drawn with the hydroxyl group pointing forward out of the plane of the paper, was as depicted in Figure 3a. Kazlauskas[22] also published a similar rule for the resolution of primary alcohols (with no oxygen substituent at the adjacent stereocentre) using lipase from *Pseudomonas cepacia*. Although the opposite enantiopreference is predicted, it is hypothesised that the substrate interacted with the lipase in a similar manner to secondary alcohols, with the substituents on the adjacent chiral carbon functioning as the large, L, and medium, M, sized groups. The addition of the methylene group allows the primary alcohol moiety the flexibility to adopt a similar conformation to that of the secondary alcohol (Figure 3b).

Figure 3 *a) Enantiopreference of a) lipases towards secondary alcohols, and b)* Pseudomonas cepacia *towards primary alcohols*

Similar rules have been predicted for the enantioselectivity of *Candida rugosa* lipase[23] towards carboxylic acids (Figure 4a), and *Aspergillus niger* lipase[20] for L-α-amino acids (Figure 4b). The predicative rules for carboxylic acids, however, are less reliable than those for secondary alcohols, and are best applied to reactions carried out with purified lipase instead of the crude commercial preparation which often contain contaminating enzymes.[24]

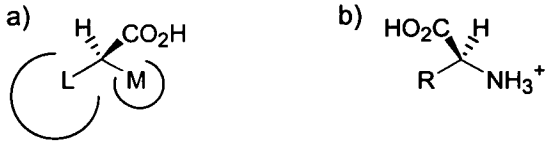
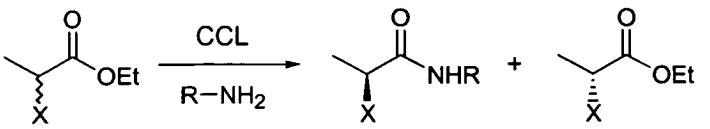


Figure 4 *a) Proposed enantiomer preference of a)* Candida rugosa *lipase towards carboxylic acids, and b) charged L-α-amino acids preferred by* Aspergillus niger *lipase*

A large number of excellent articles already exist in the literature documenting kinetic resolutions in both non-aqueous and aqueous media.[25-27] A number of unusual cases merit closer inspection.

1.2.2. Kinetic resolution with nitrogen nucleophiles

Although there are far fewer examples documenting the use of nitrogen nucleophiles in enzymatic processes in the literature, the area is growing rapidly. As indicated above, Klibanov was the first to document the use of amines of amino acid esters, as nucleophiles for lipase mediated biotransformations in organic solvent. However, Gotor and co-workers[28] were the first to implement the use of primary amines as nucleophiles in the aminolysis of ethyl (±)-2-halopropionate, **1** (X= Cl[28] and Br[29]) catalysed by *Candida cylindracea* lipase (Scheme 4).



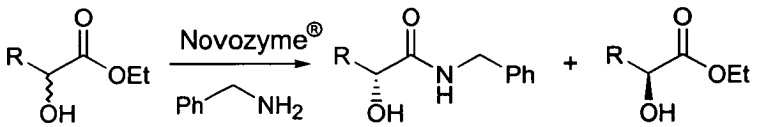
***rac*-1** **(*S*)-2**

X= Cl 40-90% e.e.

X= Br 5-90 % e.e.

Scheme 4

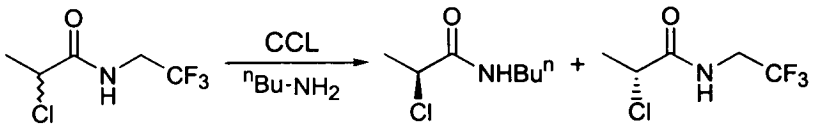
Greater enantioselectivity was obtained for the Novozyme®, (*Candida antarctica* lipase B), mediated resolution of ethyl 3-substituted-3-hydroxypropionates **3** in dioxan with benzylamine as the nucleophile (Scheme 5).[30,31] The resulting (*R*)-benzylamides **4** were obtained in >99% and 98% e.e. at 45% and 50% conversion when R= methyl and chloromethyl respectively. Also, in the case where R= chloromethyl the corresponding (*S*)-ethyl ester **3** was recovered in optically pure form. Both chiral β-hydroxy amides and esters are of interest as they serve as intermediates in the synthesis of β-aminoalcohols, which are themselves intermediates in the synthesis of a number of antibiotics and antidepressants.



rac-**3** (*R*)-**4** (*S*)-**3**

Scheme 5

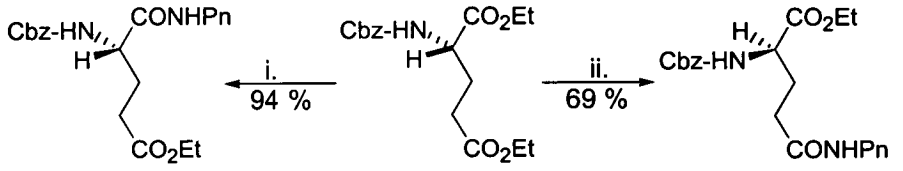
Gotor *et al.*[32] also introduced the transamidation reaction illustrated in Scheme 6. The activated racemic trifluoroethylamide **5** was reacted with *n*-butylamine in the presence of *Candida cylindracea* lipase (CCL) to give the (*S*)-*n*-butylamide **6** in 49% yield and 78% e.e.



rac-**5** (*S*)-**6** 78% e.e.

Scheme 6

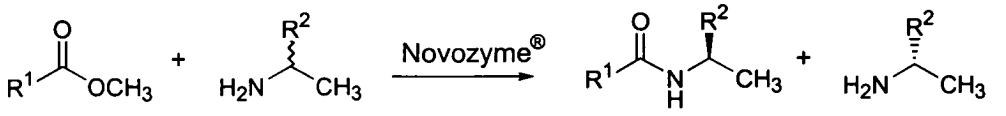
An excellent example of amide bond formation was achieved by Conde *et al.*[33] Separate exposure of each enantiomer of diethyl Cbz-glutamate **7** to Novozyme® in the presence of a variety of amines, afforded aminolysis predominantly at the α-position for the L-enantiomer, and predominantly at the γ-position for the D-enantiomer (Scheme 7). Best results were obtained at an elevated temperature of 45-60 °C.



L-**8** **7** D-**9**

Scheme 7 *reagents and conditions:* i. L-**7**, Novozyme®, DIPE, pentylamine, 1h, 60 °C, ii. D-**7**, Novozyme®, DIPE, pentylamine, 4 days, 45 °C.

Greater success has been achieved in the resolution of amines, although a number of issues must be addressed before carrying out such reactions. Amines, being more nucleophilic than alcohols, are prone to react non-enzymatically with activated esters resulting in low e.e.'s. The use of enol esters must also be avoided as the aldehydes or ketones liberated in these processes undergo Schiff-base reactions with the amine substrates to form imines. The synthesis of chiral (*R*)-propiolamides **12,** using 3-substituted-propylate methyl esters **10** as acyl donors, proceeded in good yield with e.e.'s ranging from 63-97% (Scheme 8).[34,35] The best results, collected in Table 1, were achieved with 1-phenylethylamine in diisopropylether. Lower e.e.'s were obtained (63-84%) when 2-propyl and 2-hexylamine were used.



10 *rac*-**11** (*R*)-**12**

Scheme 8

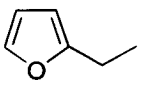
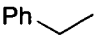
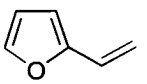
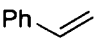
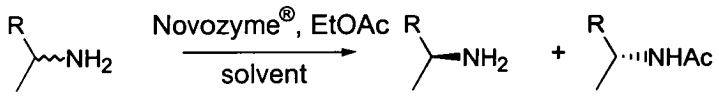
Entry	**10** R^1	**11** R^2	**12** Yield/ %	e.e./ %
1	CH_2=CH	C_5H_{11}	20	95
2	CH_2=C(CH_3)	C_2H_5	27	95
3		Ph	83	>95
4		Ph	84	>95
5		Ph	79	>95
6		Ph	78	>95

Table 1

Gotor *et al.*[36] expanded on these results publishing the resolution of 1-(heteroaryl)ethylamines **13** with Novozyme®, to furnish the desired (*R*)-amides **14** with excellent e.e.'s (Scheme 9) Hedenström[37] also used Novozyme® for the resolution of amines. Using a variety of organic solvents and temperatures, diastereomeric 2-methyloctanoic phenylethylamide was prepared from racemic phenylethylamine and racemic ethyl 2-methyloctanoate with moderate selectivity.



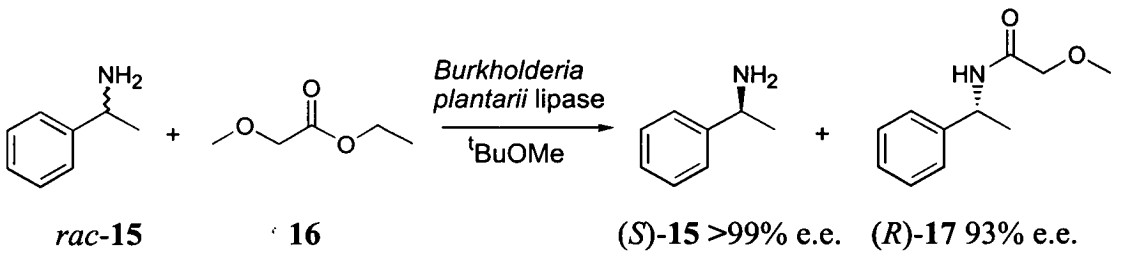
rac-**13** (*R*)-**14** 89-99% e.e.

R= a) furyl, b) thienyl, c) pyridyl,

Scheme 9

BASF AG (Germany) introduced the resolution of 1-phenylethylamine **15** as a commercial process utilising lipase from *Burkholderia plantarii* with ethyl

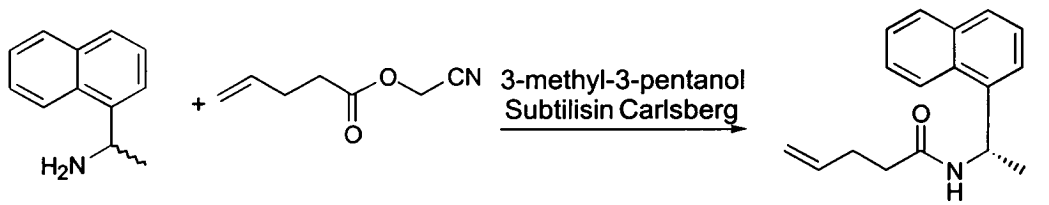
methoxyacetate **16** as acyl donor (Scheme 10).[38] Yields in excess of 45% of pure (*S*)-1-phenylethylamine **15** are reported.



rac-**15** **16** (*S*)-**15** >99% e.e. (*R*)-**17** 93% e.e.

Scheme 10

The use 3-methyl-3-pentanol as solvent has proved successful in suppressing non-enzymatic amidation. In the resolution of 1-(1-naphthyl)ethylamine **18** with cyanomethyl pent-4-enoate **19** as acyl donor, the use of THF, CH_2Cl_2, DMF and *tert*-butyl alcohol as solvent resulted in a degree of non-enzymatic amidation. On switching to 3-methyl-3-pentanol, (*S*)-amide **20** was isolated in 43% yield and 97% e.e. as depicted in Scheme 11.[39]



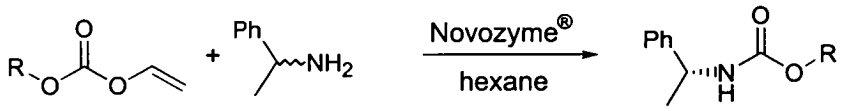
rac-**18** **19** (*S*)-**20**

43% yield, 97% e.e.

Scheme 11 *Enantioselective acylation of 1-(1-naphthyl)ethylamine*

A recent advance in enzymatic amidation reactions has been the introduction of symmetrical[40,41] and unsymmetrical[39,42-44] carbonates as acyl donors. The reactions are irreversible as the carbamates formed are not substrates for lipases or proteases. Carbamates also have the advantage of simple removal under mild conditions to furnish the valuable chiral amine.

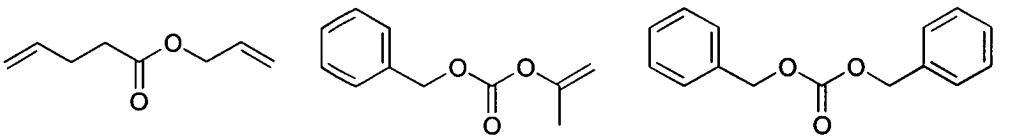
Gotor and co-workers[44] were the first to implement unsymmetrical vinyl carbonates in the resolution of amines (Scheme 12). The best results obtained were for the resolution of 1-phenylethylamine **22** with *n*-octyl vinyl carbonate in the presence of Novozyme®. The resulting (*R*)-carbamate **23** was obtained in 98% e.e. at 39% conversion. Lower e.e.'s were observed when *n*-butyl vinyl carbonate was used as acyl donor. Lower e.e.'s were also obtained in the resolution of 2-heptyl and 2-butyl amine.



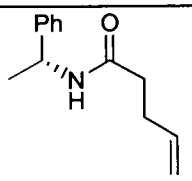
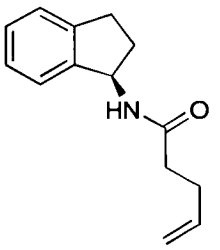
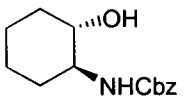
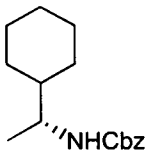
R= a) *n*-octyl, b) *n*-butyl **21** *rac*-**22** (*R*)-**23**

Scheme 12

Wong and co-workers[45] carried out a systematic study of amine protecting groups and categorised their utility. By investigating the amount of non-enzymatic background reaction in non-reaction suppressing solvents, such as toluene, or reaction suppressing solvent (3-methyl-3-pentanol) against the IR absorption of the carbonyl groups, it is possible to obtain an accurate estimation of reactivity of similar and dissimilar esters and carbonates. This is possible because the frequency of the carbonyl stretch reflects the bond length, and therefore the reactivity of the carbonyl group. A larger wavenumber indicates a shorter C=O bond, thus a more reactive carbonyl group. The study identified novel enzymatic protecting groups, namely **24**, and carbonate **25**, and the known dibenzyl carbonate **26**.[41] which were utilised in amine resolutions with yields of 33-46% and e.e. of 81-99% (Table 2).



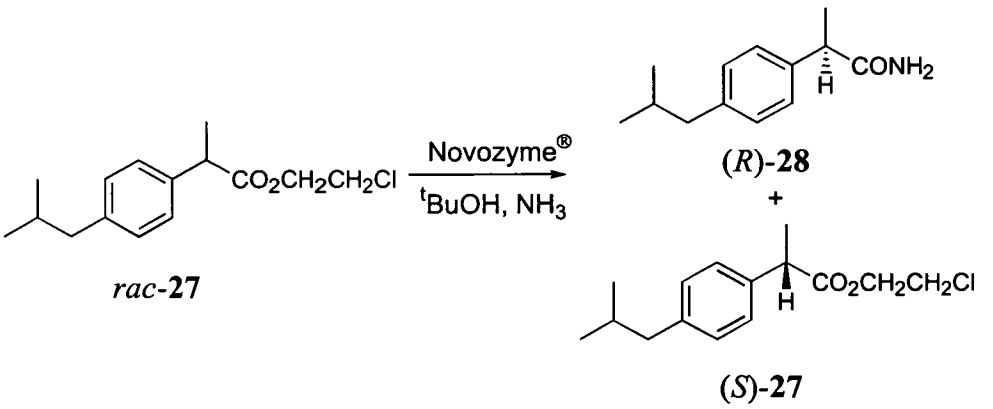
24 **25** **26**

Entry	Product[a]	Acyl Donor	Yield	e.e. %
1		**24**	43	(*R*) 99
2		**24**	46	(*R*) 99
3		**26**	36	(*S,S*) 82
4		**26**	41	(*R*) 81

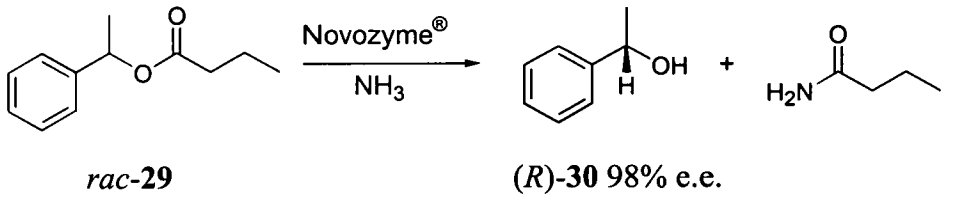
a) Reaction conditions: toluene (high concentration), Novozyme®

Table 2

The use of ammonia as the nucleophile was simultaneously reported by the groups of Sheldon[46] and Gotor.[47] Sheldon reported that by bubbling ammonia gas through a solution of the 2-chloroethyl ester of ibuprofen **27**, and adding Novozyme®, the resulting (*R*)-amide **28** was formed (Scheme 13).[48] At 56% conversion, the (*S*)-ester **27** was recovered in 93% e.e. The corresponding hydrolysis reaction in water furnished ester (*S*)-**27** in only 58% e.e. at 63% conversion. Similarly, in the ammoniolysis of α-methylbenzyl *n*-butyrate **29**, α-methylbenzyl alcohol **30** was recovered at 45% conversion in 98% e.e (Scheme 14). Enzymatic ammoniolysis has also been employed in a one pot, double resolution process for the synthesis of fatty acid amides and carboxylic amides.[49]



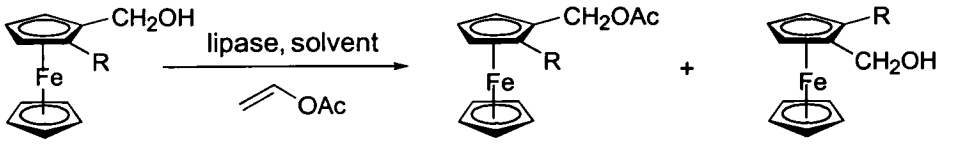
Scheme 13 *Ammoniolysis of Ibuprofen 2-chloroethyl ester*



Scheme 14 *Ammoniolysis of α-methylbenzy* n-*butyrate*

1.2.3. Kinetic resolution of organometallic substrates

Lipases have proved to be robust enzymes and their use in the kinetic resolution of organometallic compounds such as ferrocenes, (η^6-arene)-chromium and (η^4-diene)-iron tricarbonyl complexes is known. Chiral 1-hydroxymethyl-2-substituted ferrocenes, possessing planar chirality, are useful in asymmetric catalysis and therefore routes to optically pure ferrocenes are of great interest. 1-Hydroxymethyl-2-substituted ferrocenes have been resolved using a number of lipases with excellent selectivity, as depicted in Scheme 15, with a summary of the results presented in Table 3.[50-54] The results lead to a preferred enantiomer model (with regard to the size of the *ortho* substituent to the hydroxymethyl group) similar to that proposed by Kazlauskas[19] for the enantiopreference of lipases in the recognition of secondary alcohols.



rac-**31** **32** **33**

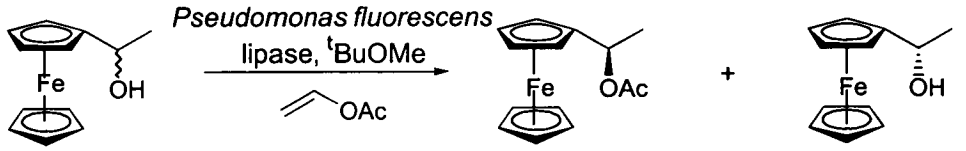
Scheme 15

Entry	Lipase	Solvent	**31** R	conv. / %	**32** e.e. / %	**33** e.e. / %
1	*Pseudomonas cepacia*[51]	benzene	CH_2OH	80	100(*1S*)	20
2	CCL[52]	^{t}BuOMe	$CH_2N(CH_3)_2$	65	-	>95 (32[a], 1*R*))
3	CCL[52]	^{t}BuOMe	$CH_2N(CH_3)$	42	92(*1S*)	-
4	Novozyme®[53]	DIPE	SCH_3	32	90(*1R*)	48
5	Lipozyme®[b][54]	DIPE	S^{t}Bu	55	-	95(43[a])
6	Novozyme®[c][54]	DCM	SPh	40	90(*1R*)	60
7	Novozyme®[50]	DCM	I	52	89 (*1S,2S*)	96

a) Isolated yield, b) *Rhizomucor miehei* lipase, c) Vinyl propionate used as acyl donor, product was corresponding propionate ester.

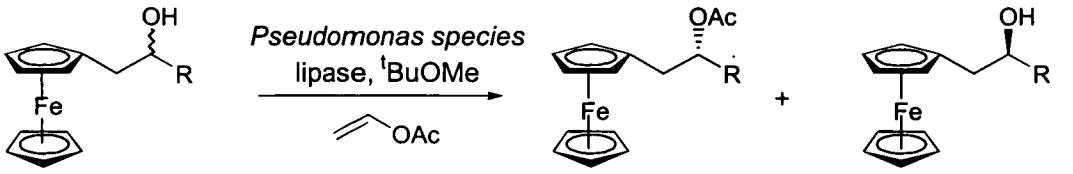
Table 3

Ferrocenes containing central chirality have also been resolved enzymatically. Boaz[55] resolved 1-ferrocenylethanol **34** into the (*R*)-acetate **35** and recovered (*S*)-alcohol **34** with a high degree of selectivity (Scheme 16). Similarly, Kim *et al.*[56] prepared the (*R*)-acetates **37** and recovered (*S*)-alcohols **36** of ferrocenylpropanol and ferrocenylbutanol (Scheme 17).



34 (*R*)-**35** 96%e.e. (*S*)-**34** 92% e.e.

Scheme 16

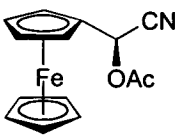


36 (*R*)-**37** >98% e.e. (*S*)-**36** 98% e.e.

R= a) Me, b) Et

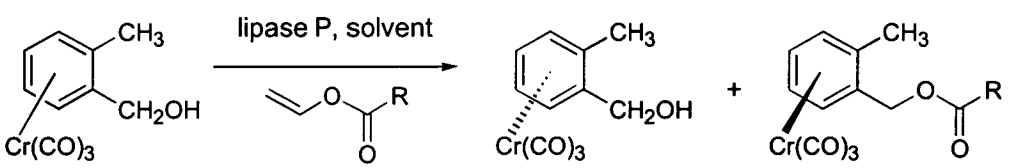
Scheme 17

The (*R*)-(+)-cyanohydrin acetate **38**, was prepared in 84% e.e. from the corresponding racemic cyanohydrin.[57]



(*R*)-**38**

Nakamura *et al.*[58] and Yamazaki[59] independently reported the resolution of (±)-tricarbonyl (η^6-2-methylbenzyl alcohol)-chromium **39** in isoproprenyl acetate and vinyl palmitate in toluene respectively. Excellent selectivity was observed, furnishing both the unreacted (*R*)-**39** and acetylated (*S*)-**40** products in optically pure form (Scheme 18). The 2-methoxy, 3-methyl[59] and 2-trimethylsily[58] derivatives were also prepared with equally impressive results.

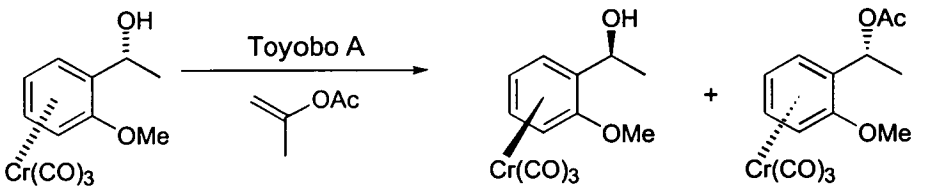


(*RS*)-**39** (*R*)-**39** (*S*)-**40**

R= CH_3	47% yield, 100% e.e.	48% yield, 98% e.e.[58]
R= $C_{15}H_{31}$	43% yield, 98% e.e.	49% yield, > 98% e.e.[59]

Scheme 18 *Resolution of (±)-tricarbonyl (η^6-2-methylbenzyl alcohol)-chromium* **39**

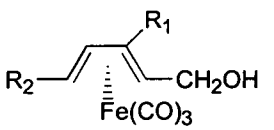
The resolution of (arene)$Cr(CO)_3$ complexes with central chirality has also been achieved. When **41** was exposed to lipase Toyobo A from *Pseudomonas aeruginosa*, acetate **43** and unreacted alcohol **42** were obtained in optically pure form in >40% yield (Scheme 19).[60]



41 (Ar1*R*2*S*, α*S*)-**42** (Ar1*S*2*R*, α*R*)-**43**

Scheme 19

The resolution of hydroxymethyl substituted (diene)$Fe(CO)_3$ complexes **44**, has also been realised (Table 4).[60] By careful choice of the lipase, it was also possible to obtain either enantiomer of the alcohol **44** (entries 2 and 3).



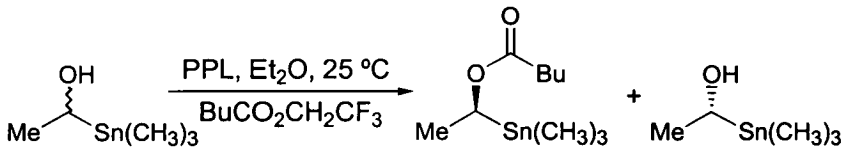
44

Entry	Lipase	R_1	R_2	yield/ %	e.e./ % (config.)
1	Amano PS	H	CH_3	22	97 (*S*)
2	Amano PS	H	Ph	36	96 (*S*)
3	Amano AY	H	Ph	18	99 (*R*)
4	Amano PS	CH_3	Ph	48	99 (*S*)
5	Amano PS	CH_3	^{n}Bu	47	92 (*S*)

Table 4

The resolution of a number of α-hydroxystannanes was achieved *via* formation of the corresponding butyl esters (Scheme 20). At 50% conversion, the butyl acetate

(*S*)-**46** was obtained in 38% yield, 98% e.e., and the unreacted alcohol (*R*)-**45** in 41% yield, 97% e.e.[61]

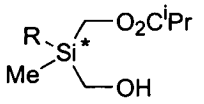


45 (*S*)-**46** (*R*)-**45**

98% e.e. 97% e.e.

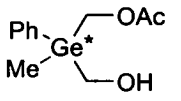
Scheme 20

Optically active silane[62] **47** and germane[63] **48** compounds have also been prepared. Both enantiomers of silane **47** were prepared by correct choice of lipase, CCL producing (+)-**47**, and lipase from *Chromobacterium viscosum* (-)-**47**.



R= Ph, *n*-octyl **47**

50-80% yield, 70-76% e.e.

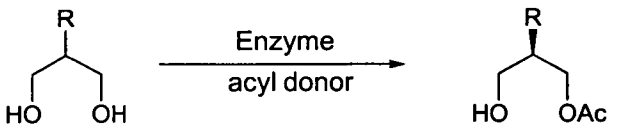


(-)-**48**

57% yield, 50% e.e.

1.2.4. The desymmetrisation of *meso* or prochiral substrates

Although kinetic resolution processes have proved very successful, they suffer from the limitation of a maximum yield of 50%. Close monitoring of the progress of the reaction is often required so as to maximise the e.e. of the desired compound. In general, if the product is required in optically pure form, the reaction is stopped at low conversion, 20-30%, whereas if the substrate is required, the reaction is stopped at high conversion, 70-80%. The end result in both instances is a reduction in yield to significantly less than the theoretical 50%. The use of *meso* or prochiral substrates is one method of overcoming this limitation (Scheme 21). As in a normal kinetic resolution, the enzyme will react with one alcohol group faster than the other, thereby creating a chiral product, in this case (*R*)-**50** with a theoretical yield and e.e. of 100%.



49 **(*R*)-50**

Scheme 21 *Desymmetrisation of prochiral 2-substituted-1,3-diols*

Through the desymmetrisation of *meso* or prochiral compounds a large number of acyclic and cyclic, mono and unsymmetrical diacetates and diacids have been prepared. A number of excellent reviews summarising the compounds prepared utilising *meso* or prochiral substrates exist in the literature.[25,64-66]

Johnson has carried out an extensive programme in the enzymatic preparation of 5, 6, 7, and 8 membered ring unsaturated diols, represented in Figure 5. These chiral cyclic intermediates have been used in the synthesis of a number of biologically important compounds, such as C-glycosides, several (deoxy)norjirimycins, conduritol derivatives, 3-deoxy-D-*arabino*-heptulosonic acid derivatives, and the tropane alkaloid calystegine A_3.[67]

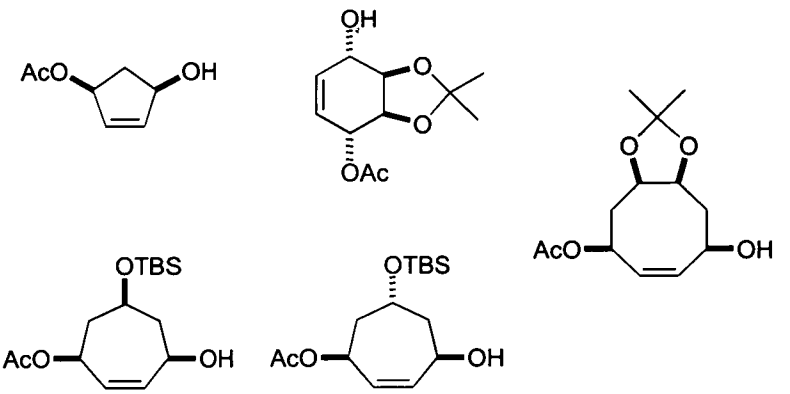
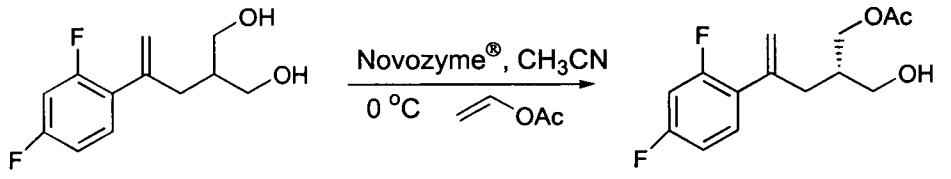


Figure 5

A comprehensive study on the effect of solvent, acyl donor, enzyme, temperature and concentration was carried out in the desymmetrisation of the 2-substituted-1,3-propanediol **51**, an intermediate in the synthesis of a potential antifungal agent SCH51408 developed by Schering-Plough.[68] Of the 205 commercial enzyme preparations tested, four furnished the desired (*S*)-acetate **52** with an acceptable e.e.

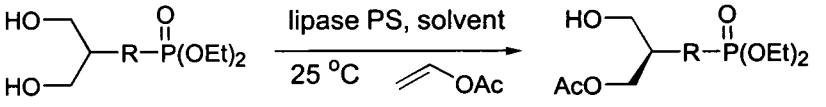
of >97%. Novozyme® was chosen for further examination and the optimised conditions (Scheme 22) were operated on pilot plant scale to furnish the product in 81% yield and 97% e.e.



51 (*S*)-**52** 97% e.e.

Scheme 22 *Resolution of 2-substituted-1,3-propanediol*

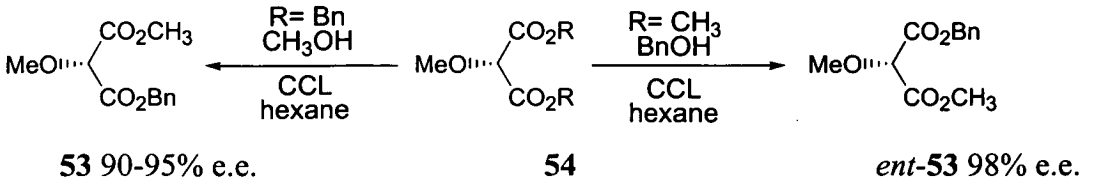
Chiral phosphonate derivatives have recently been prepared from prochiral phosphonates (Scheme 23).[69] Best results were obtained with Lipase PS in a range of ether solvents at 25 °C. The results were found to be in agreement with those predicted for the enantiopreference of chiral primary alcohols with lipases from *Pseudomonas cepacia* reported by Kazlauskas.[22]



R=a) CH_2, b) CH_2CH_2, c) CH_2CF_2 83-99% yield, 91-98% e.e.

Scheme 23

The desymmetrisation of mono-substituted malonates **54** was achieved under transesterification conditions by Gutman *et al.*[70,71] By the correct choice of nucleophile and substrate ester, both enantiomers of the unsymmetrical diester **53** were obtained with the same lipase. Although excellent e.e.'s were obtained, the reactions were stopped at moderate conversions (48-53%) to minimise the formation of diester by-product.



53 90-95% e.e. **54** *ent*-**53** 98% e.e.

Scheme 24

1.2.5. Dynamic kinetic resolution

Another method of maximising the obtainable yield is through a dynamic kinetic resolution process (Figure 6) which involves a classical kinetic resolution coupled with *in situ* substrate racemisation.[72-76] Substrate racemisation enables complete conversion to the desired product, resulting in a theoretical yield and e.e. of 100%. For a dynamic kinetic resolution to be effective, the rate of racemisation, $k_{(rac)}$ must be greater than (or at least equal to) the rate of enzymatic reaction $k_{(S)}$, for the faster reacting enantiomer (*S*). Also, the initial kinetic resolution must be selective in that $k_{(S)}$ is greater than $k_{(R)}$.

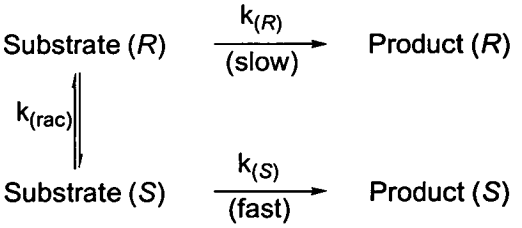
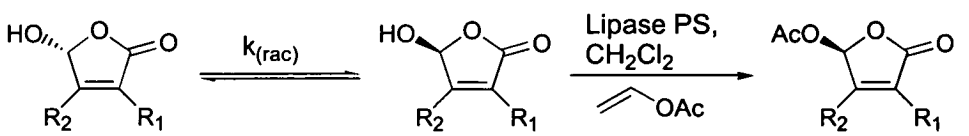


Figure 6 *Dynamic kinetic resolution*

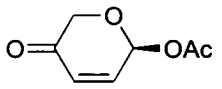
Zwanenburg and co-workers[77] utilised a dynamic kinetic resolution in the synthesis of the 5-acetyloxy-2(5*H*)-furanones **56** (Scheme 25). The stereogenic centre at C-5 of the substrate hydroxy-2(5*H*)-furanones **55** is labile due to mutarotation. Acylation of the faster reacting (*S*) enantiomer with lipase PS-30 furnished the stable 5-acetyl-2(5*H*)-furanones **56** in 78-86% e.e. at 100% conversion.



55 *ent*-**55** (*S*)-**56** 78-86% e.e.

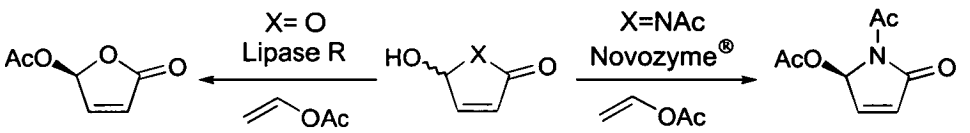
Scheme 25 *Dynamic resolution of 5-hydroxy-2(5H)-furanones*

In a subsequent report, the synthesis of 6-acetyloxy-2*H*-pyran-3(6*H*)-one **57** under identical conditions was achieved.[78]



(*S*)-**57** 76% e.e.

Kellog and Feringa[79] reported the synthesis of 5-acetyloxy-2(5*H*)-furanone **58,** and the corresponding pyrrolinone **60**, in optically pure form using Lipase R or Novozyme® respectively (Scheme 26).



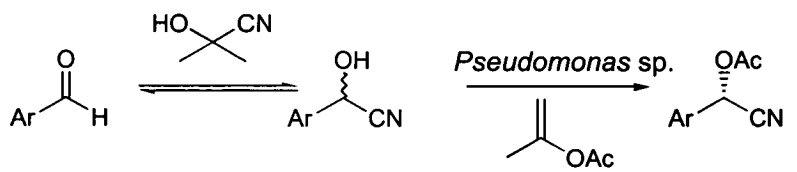
58 90% yield
>99% e.e.

59

60 100% conversion
>99% e.e.

Scheme 26

Oda and co-workers[8] employed cyanohydrins in the enzymatic synthesis of optically active aryl cyanohydrin acetates **62** (Scheme 27). Cyanohydrins are ideal substrates for dynamic kinetic resolution as they readily equilibrate under basic conditions to the corresponding aldehyde and cyanide. The racemic cyanohydrins **61** were prepared *in situ* through reversible transhydrocyanation with aryl aldehydes and acetone cyanohydrin as a mild source of HCN, catalysed by basic ion exchange resin. The subsequent transesterification catalysed by immobilised *Pseudomonas* species lipase furnished the stable aryl cyanohydrin acetates **62** in high yields and e.e. of 80-90%

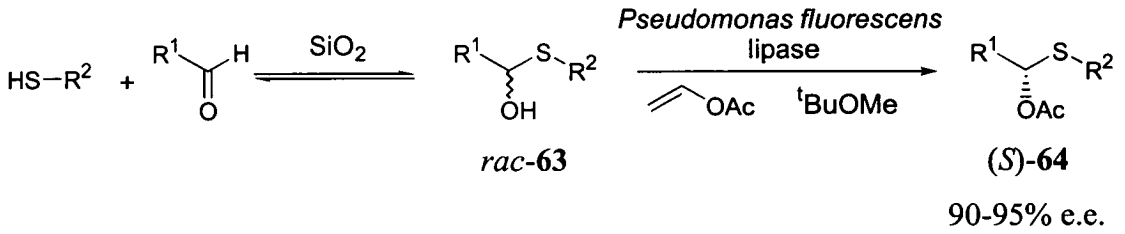


rac-**61**

(*S*)-**62**

Scheme 27 *Synthesis of optically active cyanohydrin acetates*

Rayner and co-workers[80] exploited reversible hemithioacetal formation and their conversion to stable thioacetates catalysed by *Pseudomonas fluorescens* lipase was achieved (Scheme 28). The racemic hemithioacetals **63** were prepared *in situ* by mixing an aldehyde and thiol, with dissociation of the hemithioacetals promoted by SiO_2. The one pot process gave yields of ~80% and e.e.'s of 90-95% for the acylated products **64**.

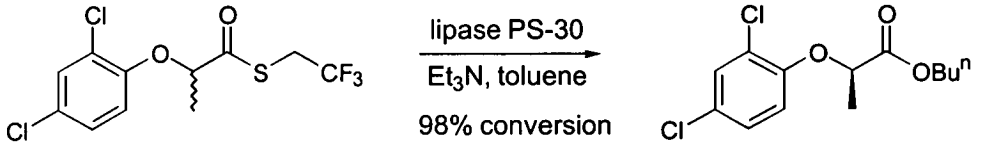


rac-**63** (*S*)-**64**

90-95% e.e.

Scheme 28

Thioesters have also proved fruitful substrates in dynamic resolution processes.[81] It has been shown that the α-protons of thioesters can be removed under basic conditions that do not deprotonate the corresponding oxoesters. Most of the resolutions were carried out in aqueous media, however, in the case of the thioesters of 2,4-dichlorophenoxypropionate **65**, it was discovered that non-enzymatic hydrolysis occurred, resulting in low e.e.'s.[82] To overcome unwanted hydrolysis the transesterification was performed in toluene with lipase PS-30. The *n*-butyl ester **66** was obtained in 75% e.e. at 98% conversion.

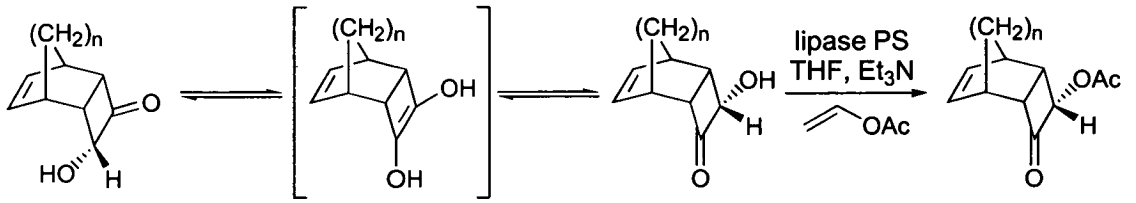


rac-**65** (*R*)-**66** (75% e.e.)

Scheme 29 *Dynamic resolution of 2,4-dichlorophenoxypropionate trifluoroethyl thioester*

Another example of base catalysed substrate racemisation was demonstrated by Ogasawara and co-workers[83,84] in the dynamic kinetic resolution of tricyclic acyloins **67**. In the presence of triethylamine, the racemic acyloins **67** formed the *meso*-1,2-

enediol intermediate **68**. On exposure to Lipase PS, the (-)-*endo*-acyloin acetates **70** were obtained in yields of 75 and 67% and e.e.'s of 97 and 99% when n= 1 and 2 respectively.

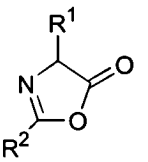


n= 1, 2

(+)-**67** *meso*-**68** (-)-**69** (-)-**70**

Scheme 30

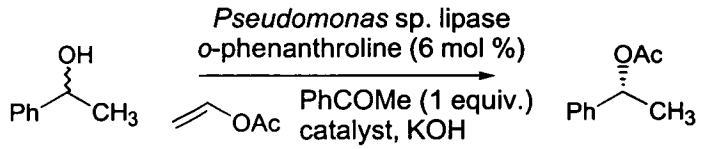
5(4*H*)-Oxazolones **71** have also been used as substrates for dynamic kinetic resolution and will be discussed in detail in Chapter 2.



71

1.2.6. Dynamic kinetic resolution with transition metals

An interesting area that has emerged over the last few years is the use of transition metals as racemisation agents, coupled with an enzymatic resolution. For this approach to be effective, the enzyme and the product must be unreactive to the transition metal used. Williams *et al.*[85] used a ruthenium catalyst and enzyme combination in the resolution of phenyl ethyl alcohol **72** as illustrated in Scheme 31. The role of the transition metal is to facilitate a temporary oxidation of the alcohol to the corresponding ketone, which in turn is reduced by hydrogen transfer catalysis. Coupling the racemisation reaction with an enzymatic acylation using *Pseudomonas fluorescens* lipase resulted in good to excellent e.e.'s for the product acetate **73** as shown in Table 5.



rac-**72** (*S*)-**73**

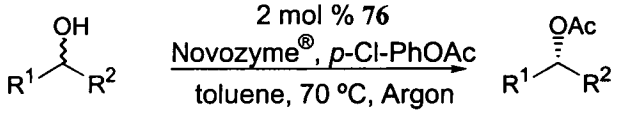
Scheme 31

Entry	Catalyst	Solvent	Temp./ C	Conv./ %	e.e./ %
1	3 mol %, $[Rh_2(cod)Cl]_2$	cyclohexane	50	76	80
2[a]	2 mol % $Rh_2(OAc)_4$	CH_2Cl_2	20	60	98

a) no KOH

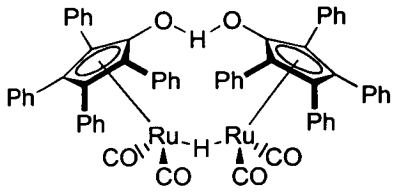
Table 5

Bäckvall[86,87] used ruthenium catalyst **76** in the synthesis of a number of optically pure acetates of secondary alcohols in the presence of the Novozyme® as illustrated in Scheme 32 and Table 6. The choice of acyl donor proved crucial for reaction success. When normal enol esters were employed, the resulting aldehydes or ketones were reduced to alcohols under the reaction conditions. These alcohols then participated in the acylation process reducing the yield of the desired product. The use of *p*-chlorophenyl acetate prevented this from occurring as the *p*-chlorophenol released does not contain an α-proton; therefore cannot interfere with the transition metal catalyst.

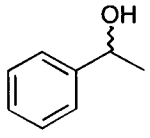
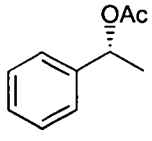
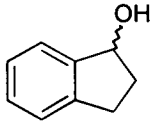
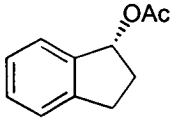
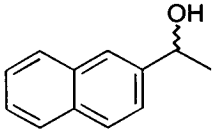
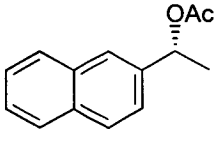
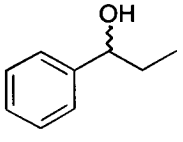
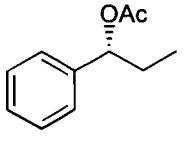
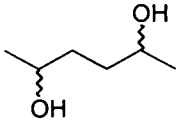
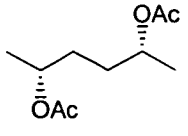


74 **75**

Scheme 32



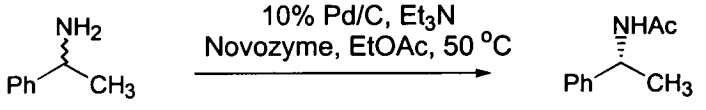
76

Substrate **74**	Product **75**	Yield/ %	e.e./ %
		80	>99
		77	>99
		79	>99
		80	98
		63	>99[a]

a) *R,R:meso* 86:14

Table 6

The use of enzymatic, transition metal assisted, dynamic kinetic resolution has also been extended to the production of chiral amides by Reetz *et al.*[88] Palladium on carbon racemised phenyl ethylamine **77** *via* transient oxidation to the corresponding imine. Using Novozyme® in triethylamine, amide **78** was isolated in 64% yield and 99% e.e. (Scheme 33).

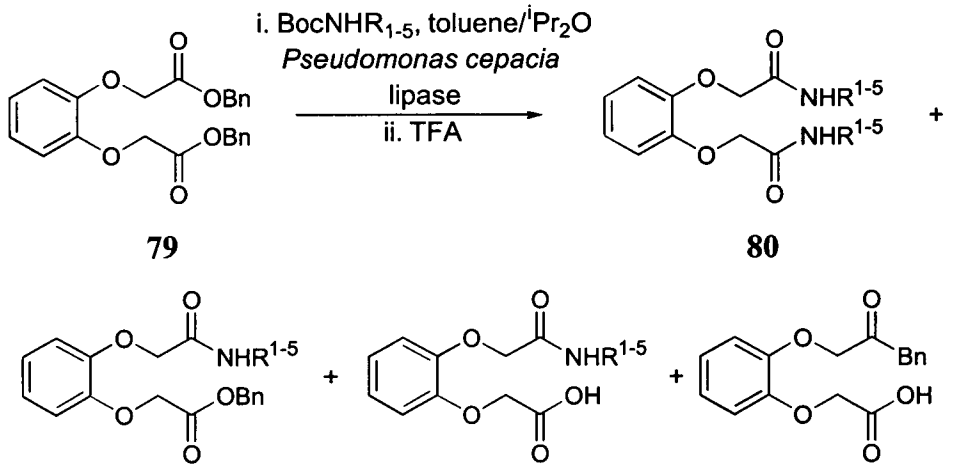


rac-**77** (*R*)-**78**

Scheme 33

1.3.0. The use of lipases in combinatorial chemistry

Over the past decade combinatorial chemistry has become extremely popular for generating molecular diversity to aid drug discovery. One method of generating diversity relies on elaborating a core substrate unit containing a number of functional groups with a selection of partners. However, until very recently, the derivatisation of polyfunctionalised core units without protection/deprotection protocols was problematic due to a lack of sufficiently selective chemical procedures. The inherent regioselectivity offered by enzymes proved attractive and was employed by Adamczyk and co-workers[89] in the lipase catalysed solution phase synthesis of a library of 26 compounds as shown in Scheme 34. When 1,2-phenylenedioxydiacetate **79** was simultaneously exposed to five mono Boc protected amines in the presence of lipase from *Pseudomonas cepacia* species, followed by deprotection of the resulting Boc-amides, a yield of 93% based on the average weight of the bis-amide TFA salt product was obtained. ESMS analysis identified twenty-six different products including all fifteen desired bis-amides **80**, five mono-amide mono-esters **81**, and five mono-amide mono-acid **82** products from their corresponding MH^+ peak (the hydrolysis products attributed to the presence of water in the hygroscopic monoprotected amine substrates).



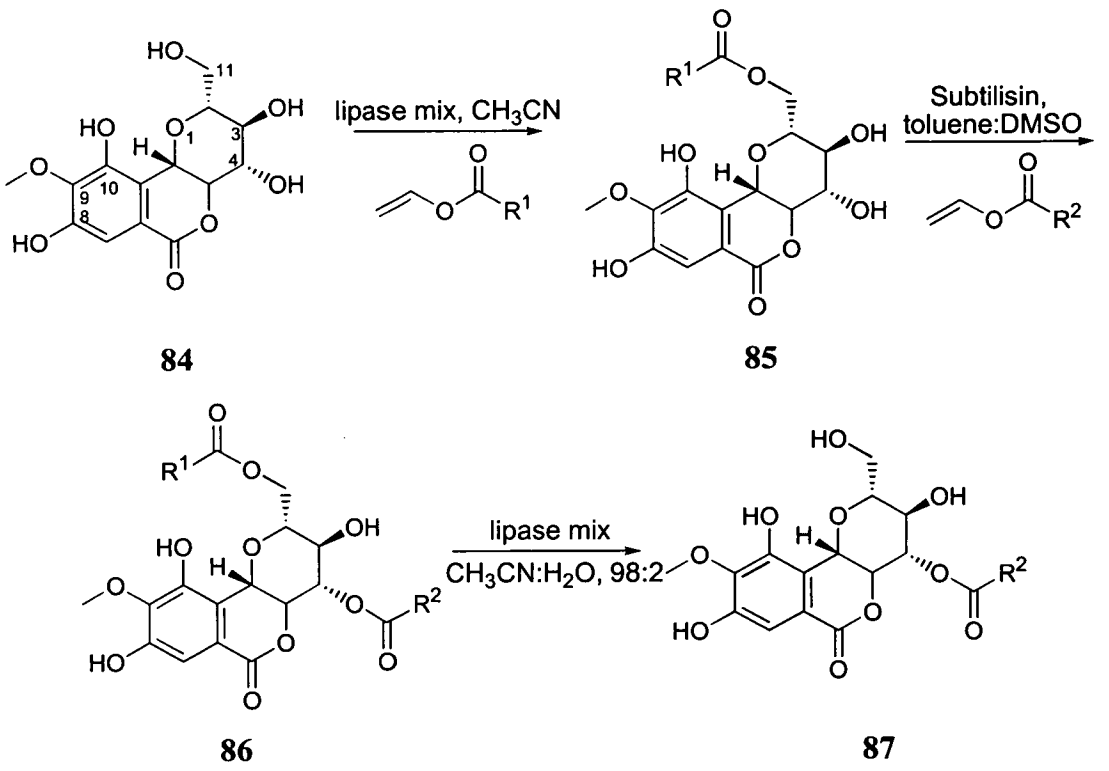
79 **80**

81 **82** **83**

R^1= $(CH_2)_2NH_2$; R^2= $(CH_2)_3NH_2$; R^3= $(CH_2)_5NH_2$; R^4= $(CH_2)_6NH_2$; R^5= p-$CH_2C_6H_4CH_2NH_2$

Scheme 34

Khmelnitsky *et al.*[90] synthesised a 167 member library of regioselectively acylated derivatives of the polyhydroxylated flavanoid, bergenin **84** (Scheme 35).



84 **85** **86** **87**

Scheme 35

A mixture of four lipases (Chirazymes L-2 and L-9, and lipases PS30 and FAP-15) was identified as the best catalyst for the initial regioselective acylation at position-11. Subtilisin was utilised in the second regioselective acylation step at position-4, followed by selective hydrolysis at position-11, again mediated by the lipase mixture. In all, twelve acyl donors were employed, resulting in a library of N^2+2N compound. All but one of the expected products was identified by HPLC/MS. The unaccounted product, a diacylation product from two bulky aromatic acyl donors, was discounted due to unfavourable steric interactions in the second acylation step.

1.4.0. Closing remarks

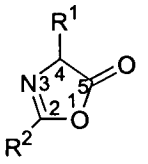
The utility of enzymes, and their diversity of applications is increasing in the field of chemistry. Aswell as the areas covered in this article, the rapidly growing area of directed evolution has the potential to provide enzymes designed for specific

substrates or reactions.[91] Industrially enzymes offer a clean, reusable, and with the advances in cloning and purification technologies, a cheap alternative to conventional approaches. Biocatalysis in non-aqueous media has grown into an independent discipline and due to the increased understanding of enzyme properties provides an invaluable tool to the synthetic chemist.

2.0.0. Results and Discussion I

2.1.0. Development of 5(4*H*)-oxazolone methodology

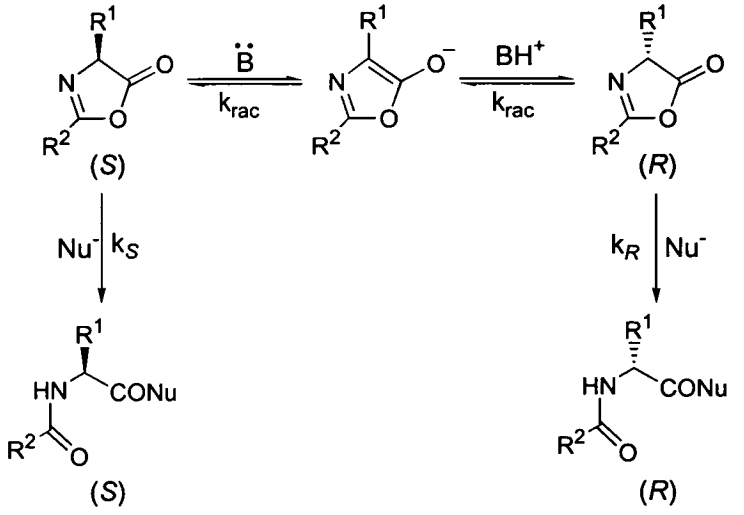
2.1.1. 5(4*H*)-Oxazolones



71

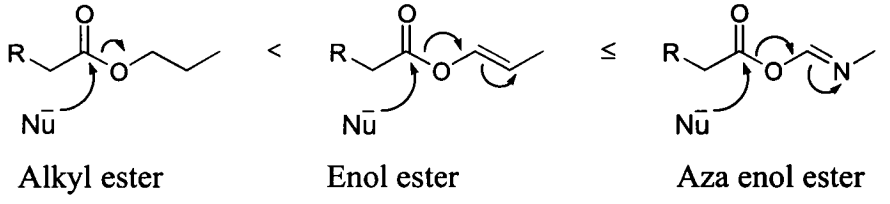
The formation of 5(4*H*)-oxazolones **71**, also known as oxazolin-5(4*H*)-ones or azlactones, in peptide synthesis has been documented as the main cause of racemisation.[92,93] Racemisation occurs as a result of the decrease in the pKa of the C-4 proton upon cyclisation from the activated α-amino acid residue. de Jersey *et al.*[94] showed that the pKa of the C-4 proton was 8.9-9.5 depending on the R^1 and R^2 substituents. Deprotonation at C-4 results in the formation of the stable pseudoaromatic anion intermediate as depicted in Scheme 36. Reprotonation of the pseudoaromatic intermediate can occur from either face to produce either oxazolone enantiomer.

In enzyme catalysed reactions involving serine proteases, the oxazolone can act as an acyl donor. One enantiomer, *e.g.* (*S*) preferentially acylates the enzyme to give an acyl-enzyme intermediate which is further attacked by a nucleophile (Nu^-), *i.e.* alcohol, amine, water *etc.* to produce the chiral α-amino acid derivative. The remaining enantiomer (*R*) is racemised (due to the pK_a of the C-4 proton) and the enzyme again preferentially reacts with the (*S*) enantiomer, thus dynamic resolution occurs. For the dynamic resolution process to be effective however, the rate of racemisation (k_{rac}), must be greater than the rate of enzymatic catalysis (k_{enz}), which in turn must be much greater than the rate of chemical reaction (k_{chem}). Furthermore, for the enzymatic catalysis to be enantioselective, the rates of enzymatic catalysis for each enantiomer must differ significantly, i.e. $k_{rac} > (k_{enz(S)} > k_{env(R)}) >> k_{chem}$.[95]



Scheme 36 *Racemisation of 5(4*H*)-oxazolones*

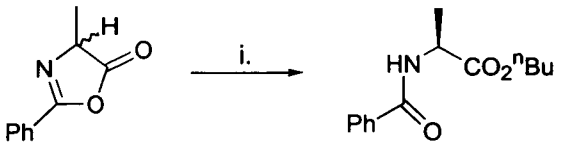
Bevinakatti *et al.*[96] described 5(4*H*)-oxazolones as cyclic aza enol esters (Scheme 37) and hypothesised that in organic solvents they would react in a similar manner to the enol esters in lipase catalysed transesterification studies. When a nucleophile attacks the activated carbonyl of an enol ester, the enol released tautomerises to the corresponding aldehyde or ketone, thus preventing reversible transesterification which can cause problems when normal alkyl esters are used as acyl donors.



Scheme 37 *Comparison of ester reactivity*

2.1.2. Biocatalytic ring opening of 5(4*H*)-oxazolones

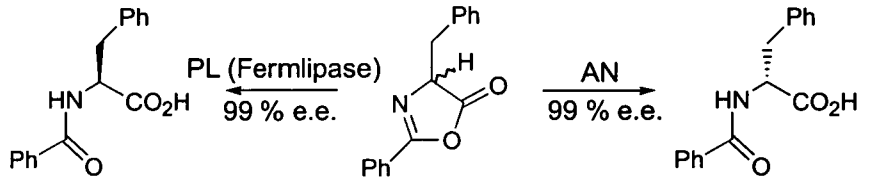
Bevinakatti[96] was the first to demonstrate the lipase catalysed ring opening of oxazolones in organic solvent to yield optically active α-amino acid derivatives, albeit with moderate enantioselectivity as illustrated in Scheme 38.



(*S*)-**88** 34% e.e.

Scheme 38 *Reagents and conditions*: i. DIPE, ^{n}BuOH, Lipozyme®[96]

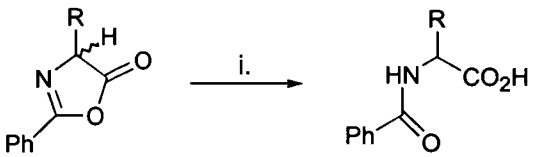
Sih *et al.*[95] expanded on the work of Bevinakatti and screened a number of lipases for the corresponding hydrolysis of phenylalanine derived oxazolone **89a** using phosphate buffer at pH 7.6 as the solvent and the source of the nucleophile. Of the ten lipases tested, two were found to give opposite enantiomers in optically pure form. Porcine pancreatic lipase (PL Fermlipase) catalysed the formation of **(*S*)-90a** while *Aspergillus niger* lipase (AN) yielded (*R*)-**90a** as shown in Scheme 39.



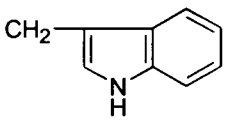
(*S*)-**90a** *rac*-**89a** (*R*)-**90a**

Scheme 39 *Enantioselective hydrolysis of 2-phenyl-4-benzyl-5(4*H*)-oxazolone **89a***[95]

Sih *et al.* also tested a selection of 2-phenyl-4-substituted-5(4*H*)-oxazolones using these two lipases (Scheme 40).[95] Moderate to good enantioselectivities, (Table 7) were observed but as with the work of Bevinakatti no yields were reported.



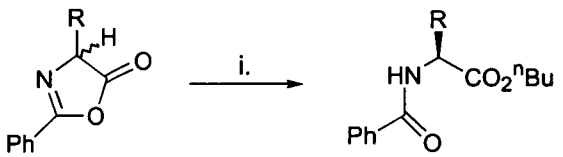
Scheme 40 *Reagents and conditions*: i. Lipase, phosphate buffer, pH 7.6

Entry	R	Lipase	time/ h	e.e./ %	Conf.
1	$CH_2CH_2SCH_3$	AP	5	83	R
		PL	16	80	S
2	p-$HOC_6H_4CH_2$	AP	20	37	R
		PL	20	19	S
3		AP	100	77 (34)[a]	R
		PL	100	99 (55)[a]	S
4	$CH_2CH(CH_3)_2$	AP	18	6	R
		PL	18	87	S
5	Ph	AP	20	80	R
		PL	20	76	S

a) results in parenthesis were obtained with new enzyme

Table 7 *Results obtained for hydrolysis of 5(4H)-oxazolones*[95]

Bevinakatti[97] also studied the effect of solvent in the reaction with Lipozyme® and *n*-butanol as the nucleophile (Table 8). It was shown that by careful screening of solvents the e.e. of the product could be dramatically increased. For example, when the reaction was carried out in DIPE an e.e. of 33% was obtained, whereas the same reaction in dichloromethane produced an e.e. of 69%. As a footnote a purified general yield of >90% was quoted.



rac-**a-d** (*S*)-**a-d**

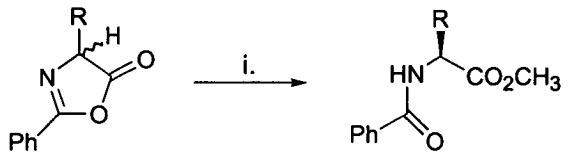
Reagents and conditions: i. Solvent Lipozyme®, ^{n}BuOH

Solvent	e.e./ %			
	a	**b**	**c**	**d**
DIPE	33	34	39	26
n-BuOH	59	42	66	39
DCM	69	47	61	43

R= (a) CH_2Ph, (b) CH_3, (c) $CH_2CH(CH_3)_2$, (d) $CH_2CH_2CH_3$

Table 8 *Results obtained for alcoholyses of 5(4H)-oxazolones in various solvents*[97]

A far more comprehensive study documenting the methanolysis of a number of 2-phenyl-5(4*H*)-oxazolone derivatives with *Pseudomonas cepacia* lipase (P-30) in *tert*-butyl methyl ether was conducted by Sih *et al.*[98] (Table 9). The lipase catalysed alcoholyses were carried out in the presence and absence of five equivalents of water. In both cases the formation of the preferred (*S*) enantiomer was observed. The rate of the reaction in the presence of water did increase but the yields of the products were generally lower due to the competing hydrolysis reaction. The e.e. of the product appeared to increase as the C-4 substituent increased in size, but also slightly decreased when the reactions were carried out in the presence of water.



Reagents and conditions: i. Lipase P-30, ^{t}BuOMe, CH_3OH (5 equiv.), (H_2O (5 equiv.)), 50 °C

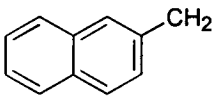
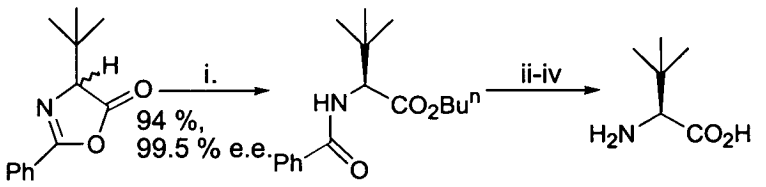
Entry	R	H_2O	Time/ h	Yield/ %	e.e./ %
1	$CH(CH_3)_2$	yes	130	47	77
2	$CH_2CH(CH_3)_2$	yes	72	85	90
		no	72	82	78
3	$CH_2CH_2SCH_3$	yes	48	31	82
		no	114	56	71
4		yes	91	83	49
		no	86	90	75
5	$CH_2C_6H_4(pCH_3)$	yes	84	78	63
		no	156	8	66
6	Ph	yes	84	46	75
7	CH_2Ph	yes	35	80	78
		no	22	93	69
8	CH_2CH_2Ph	yes	42	61	93
9	$CH_2CH_2CH_2Ph$	yes	72	76	95
		no	72	91	84

Table 9 *Results obtained for the methanolysis of 5(4*H*)-oxazolones*[98]

Turner *et al.*[99] applied the oxazolone methodology in the lipase catalysed synthesis of the non-proteinogenic α-amino acid L-(*S*)-*tert*-leucine **92g** as shown in Scheme 41.

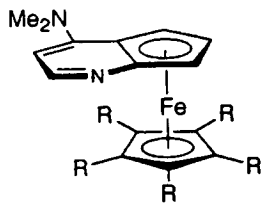


rac-**89g** (*S*)-**91** (*S*)-**92g**

Scheme 41 *Reagents and conditions*: i. Toluene, Lipozyme®, ^{n}BuOH, Et_3N (0.25 equiv.), 37 °C, ii. Alcalase®, iii. 6 N HCl, iv. Amberlite IRA-67.

2-Phenyl-4-*tert*-butyl-5(4*H*)-oxazolone **89g** was dissolved in toluene and Lipozyme®, *n*-butanol and a catalytic amount of triethylamine were added. The optically pure product was isolated by chromatography in 94% yield. Alcalase® mediated deprotection of the ester followed by acid hydrolysis to remove the *N*-benzoyl protecting group and finally ion exchange chromatography furnished the optically pure L-(*S*)-*tert*-leucine **92g**.

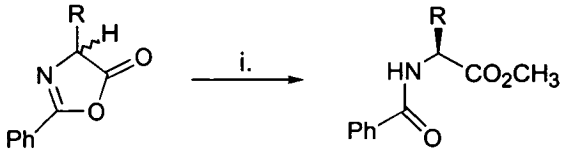
In 1998, Fu *et al.*[100] published a chemical dynamic kinetic resolution of 2-phenyl-4-substituted-5(4*H*)-oxazolone derivatives utilising a planar chiral DMAP based catalyst **93**.



(-)-**93** R= CH_3

Toluene was identified as the best solvent and the reaction proceeded with excellent yields and moderate e.e.'s, as illustrated in Table 10. It was also discovered that the reaction rate and e.e. of the product were both increased if 10% benzoic acid was

added to the reaction mixture. The e.e. of the product in entry 1 dropped to <2% in the absence of benzoic acid. No explanation for this effect was given.



Reagents and conditions: i. Toluene, 5% (-)-**93**, 10% $PhCO_2H$,, MeOH, room temp.

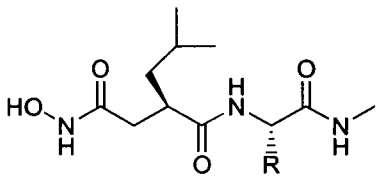
Entry	R	Yield/ %	e.e./ %
1	CH_3	98	54
2	CH_2=$CHCH_2$	94	61
3	$CH_2CH(CH_3)_2$	95	55
4	$CH_2CH_2SCH_3$	94	50
5	$CH(C_6C_{11})$	93	54

Table 10 *Results obtained for the chemical dynamic kinetic resolution of 5(4H)-oxazolones*[100]

2.2.0. Aim of project

As discussed above, Turner *et al.*[99] published a stereospecific high yielding route to L-(*S*)-*tert*-leucine **92g**. However, when the optimised conditions were applied to less sterically demanding substrates such as 2-phenyl-4-isopropyl-5(4*H*)-oxazolone, there was a dramatic drop in the observed e.e. of the product from 99% for the *tert*-leucine derivative to 0% for the valine derivative. Substituting a methyl group for a proton resulted in a complete loss of selectivity.[101]

The aim of the project was to expand this 5(4*H*)-oxazolone methodology to gain a more general route to optically active α-amino acid derivatives and later apply this to the synthesis of pertinent biologically active compounds. The target chosen was the matrix metalloproteinase inhibitors (MMPI's) of structure **94**. These will be discussed in detail in Chapter 3.

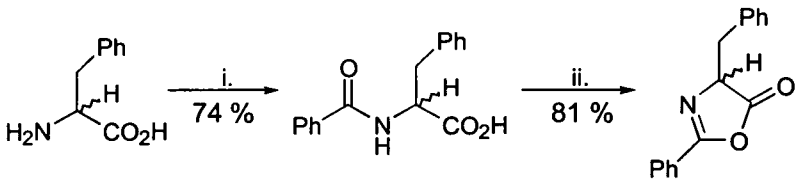


94 R= alkyl, aryl

2.3.0. Preliminary studies

2.3.1. Synthesis of substrates

2-Phenyl-4-benzyl-5(4*H*)-oxazolone **89a** was chosen as the substrate for the initial studies into the lipase catalyse dynamic kinetic resolution process. Oxazolone **89a** was synthesised in two steps from the DL-phenylalanine **92a**. *N*-Benzoylation under Schotten-Baumann conditions, followed by acetic anhydride mediated cyclodehydration proceeded in high yield to furnish the desired oxazolone as a colourless solid. Oxazolone **89a** decomposed readily at room temperature over a period of a few days. The oxazolone, and all other oxazolones subsequently prepared in this study were stored in the fridge or freezer until required.

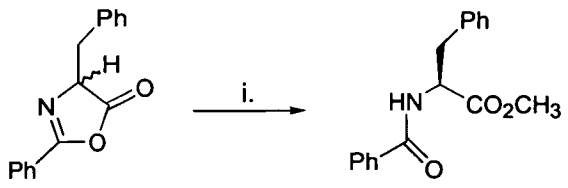


92a **95a** **89a**

Scheme 42 *Reagents and conditions*: i. 2M NaOH, PhCOCl, 0 °C, ii. 1,4-dioxane:acetic anhydride (1:1), 40 °C

2.3.2. Screening of lipases

As a starting point the Fluka Lipase Basic Kit[102] was tested, as illustrated in Scheme 43, with the results shown in Table 11. Small scale reactions (0.04 mmol substrate and 1 mL solvent) were incubated at 37 °C with methanol as the nucleophile, and the progress of the reactions monitored by chiral HPLC. A sample of the racemic methyl ester **96a,** (and all subsequent racemic derivatives), was prepared by acid catalysed alcoholysis of the corresponding oxazolone. The experimental details are given in Chapter 4.



89a **96a**

Scheme 43 *Reagents and conditions*: i. Toluene, lipase (see Table 11), Et_3N(0.25 mmol), CH_3OH (2 equiv.), 37°C

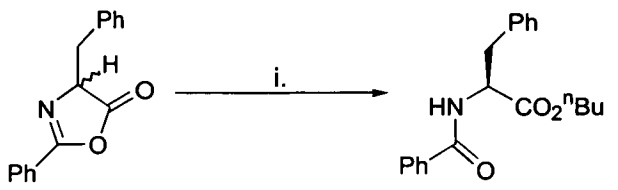
Entry	Lipase	Conversion	e.e./ %
1	*Aspergillius niger*	+	25
2	*Candida antarctica*	+	3
3	*Candida cylindracea*	++	3
4	*Rhizomucor miehei*	+	28
5	*Pseudomonas cepacia*	+	43
6	*Pseudomonas fluorescens*	+++	64
7	*Rhizopus arrhizus*	+	5[a]
8	*Rhizopus niveus*	++	3[a]
9	*Hog pancreas*	++	42

a) Opposite enantiomer

Table 11 *Results obtained for screening of Fluka Lipase Basic Kit*

Employing the optimised conditions found for the Lipozyme®/*tert*-leucine system, the immobilised lipases Lipozyme® (*Rhizomucor miehei* lipase immobilised on an anionic exchange resin) and Novozyme® (*Candida antarctica* lipase B immobilised on acrylic resin) were tested, and the results are shown in Table 12. The natural (*S*) stereochemistry of the product, and subsequent biotransformation products, was assigned by comparison with previous results obtained using Lipozyme® and optical rotation values in the literature.[101]

The results obtained with the Fluka Lipase Basic Kit (Table 11) were disappointing with only poor to moderate e.e. and conversions obtained. In contrast, the immobilised lipases produced interesting results. The Lipozyme® experiments, (entries 1 and 2, Table 12) were a repeat of previous experiments[101] for reference and showed again the enhancement of e.e. with the addition of external triethylamine.



89a **97a**

Reagents and conditions: i. Toluene, (Et_3N (0.25 equiv.)), lipase, CH_3OH (2.0 equiv.), 37 °C

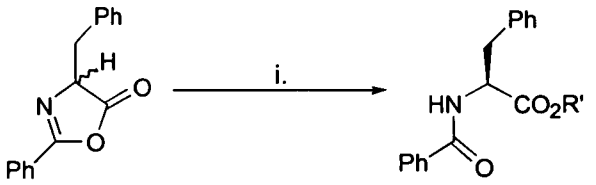
Entry	Lipase	Et_3N	Yield/ %	e.e./ %
1	Lipozyme®	no	59	55
2	Lipozyme®	yes	74	69
3	Novozyme®	no	40	64
4	Novozyme®	yes	53	95
5	Novozyme®	yes	81	95

Table 12 *Results obtained for screening Lipozyme® and Novozyme®*

The Novozyme® results also exhibit a dependence on triethylamine addition to increase the e.e. of the product (entries 3 and 4). Although the Novozyme® mediated biotransformation resulted in an excellent e.e. of 95%, (entry 4), only a moderate yield of 51% pure product was obtained. On closer examination of the ^1H nmr (200 MHz, $CDCl_3$) spectrum of the crude biotransformation product it could be seen that a 1:1 ratio of desired product to hydrolysis product, i.e. *N*-benzoyl phenylalanine was obtained. The ratio was calculated from the integrals corresponding to the α-C<u>H</u> of each amino acid derivative, δ 5.06 and 4.94 for the ester and acid respectively. To minimise the formation of the undesired hydrolysis product Novozyme® was crushed in a mortar and pestle and dried to constant weight. It was hoped that the use of phosphorus pentoxide as desiccant would remove any excess water trapped in the solid support, or associated with the surface of the lipase, but would not be powerful enough to remove the water held at the catalytic triad of the lipase that is essential for catalytic activity. Indeed the resulting powder, when subjected to the biotransformation, produced an identical e.e. of 95% while the yield increased to 81% (entry 5).

2.3.3. Effect of alkyl chain length of nucleophile

As an effective, and reproducible set of biotransformation conditions had been established, it seemed logical to probe the parameters of the reaction to achieve a greater understanding of the role of each component. The first variable to be investigated was the effect of the alcohol chain length and the results are shown in Table 13, with the previous results obtained for Lipozyme® also shown.[99,101]



89a **96a-100a**

Reagents and conditions: i. Toluene, (Et_3N (0.25 equiv.)), lipase, R'OH (2.0 equiv.), 37 °C

Compound	R'	Novozyme®		Lipozyme®	
		Yield/ %	e.e./ %	Yield/ %	e.e./ %
96a	CH_3	79	94	55	40
98a	CH_3CH_2	82	97	53	83
99a	$CH_3CH_2CH_2$	83	97	-	-
97a	$CH_3CH_2CH_2CH_2$	81	95	69	73
100a	$CH_3CH_2CH_2CH_2CH_2$	32	88	-	-

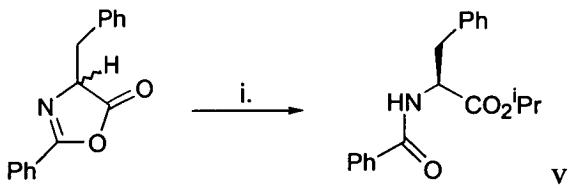
Table 13 *Effect of alkyl chain length of alcohol nucleophile for the alcoholysis of 5(4H)-oxazolone 89a*

The results show that there is very little effect on the resulting e.e. of the Novozyme® mediated biotransformation product when the length of the alkyl chain of the primary alcohol nucleophile is varied. This is in direct contrast to the erratic results obtained in the Lipozyme® mediated alcoholysis.

2.3.4. Secondary alcohols

The use of secondary alcohols as nucleophiles was also investigated. When *iso*-propanol was used as the nucleophile, (Scheme 44), the biotransformation proceeded at a very slow rate with a reaction time of 26 days required for 100% conversion. The isolated product yield of only 18% with an e.e. of 29% indicates that secondary alcohols are poor nucleophiles under the conditions studied. An explanation for this

result could be that in processes where Novozyme® has been used successfully with secondary alcohol nucleophiles, it has been to resolve the alcohol using achiral acyl donors. In the above reaction, the oxazolone is a relatively bulky chiral acyl donor. The resulting acyl enzyme intermediate formed will have considerable steric constraints in comparison to simple acyl enzyme intermediates, thus the secondary alcohol nucleophile has difficulty accessing the crowded active site of the enzyme.



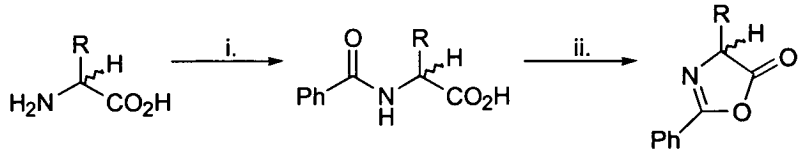
89a **101a**

Scheme 44 *Reagents and conditions*: i. Toluene, Novozyme®, Et_3N, ^iPrOH, 37°C

2.4.0. Expansion of substrate range

2.4.1. Synthesis of substrates

The next and most obvious variable to probe was the C-4 substituent (R) to examine the scope of the substrate range tolerated by Novozyme®. The substrates were all prepared in two steps as described in Section **(2.3.1.)** and illustrated in Scheme 45. They all proceeded smoothly in good to excellent yield with the results shown in Table 14. It was decided to carry out the biotransformations with methanol and *n*-propanol as the nucleophiles so that the two sets of results could be compared (see Section **(2.4.2.)**).



92b-g **95b-g** **89a-g**

Scheme 45 *Reagents and conditions*: i. 2M NaOH, PhCOCl, 0 °C, ii. 1,4-dioxane:acetic anhydride (1:1), 40 °C

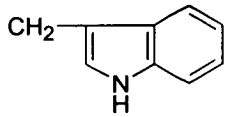
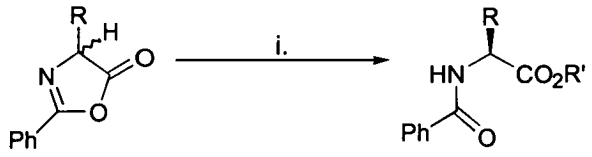
Compound	R	**95** Yield/ %	**89** Yield/ %
b	$CH_2CH(CH_3)_2$	78	81
c	$CH(CH_3)_2$	83	95
d	$CH_2CH_2SCH_3$	75	56
e		76	36
f	CH_3	70	99
g	$C(CH_3)_3$	81	82

Table 14 *Synthesis of 2-phenyl-4-substituted-5(4*H*)-oxazolones* ***89b-g***

2.4.2. Testing of substrates

Using the optimised reaction conditions each substrate was tested, Scheme 46, and the results are shown in Table 15.



89b-g

R'= Me **96b-g**

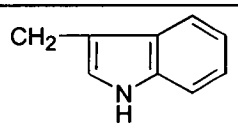
R'= Et **98f**

R'= ^{n}Pr **99b-g**

Scheme 46 *Reagents and conditions*: i. Toluene, Et_3N (0.25 equiv.), lipase, R'OH (2.0 equiv.), 37 °C

The results in Table 15 again indicate that there is only a small change in the e.e. of the product when the nucleophile is changed from methanol to *n*-propanol. They also indicate that Novozyme® prefers flexible alkyl side chains with the *iso*-propyl and *iso*-butyl side chains giving the highest e.e.'s. The small methyl side chain gave poor chiral induction, presumably due to lack of steric interactions with the lipase The bulky *tert*-butyl group gave only poor to moderate yields (after the recovery of starting oxazolone) and poor e.e.'s, perhaps due to excessive steric interactions with the lipase. As the flexibility of the C-4 side chain was increased, as in the case of the methyl thioethyl group, the e.e. began to fall to ~80%. The aromatic indolemethylene side chain also gave good e.e.'s of 80 and 90%. Although the indolemethylene group

is relatively bulky, the methylene spacer (as in the case of the benzyl side chain) allowed the substrate the flexibility to fit into the active site of the lipase.

Compound	R	R'	Yield/ %	e.e./ %
96b	$CH_2CH(CH_3)_2$	CH_3	96	97
99b		$CH_2CH_2CH_3$	89	98
96c	$CH(CH_3)_2$	CH_3	82	95
99c		$CH_2CH_2CH_3$	70	93
96d	$CH_2CH_2SCH_3$	CH_3	69	80
99d		$CH_2CH_2CH_3$	64	83
96e		CH_3	90	90
99e		$CH_2CH_2CH_3$	48	80
96f	CH_3	CH_3	-	-
98f		CH_2CH_3	60	14
99f		$CH_2CH_2CH_3$	72	14
96g	$C(CH_3)_3$	CH_3	40[a]	35
99g		$CH_2CH_2CH_3$	15[a]	19[b]

a) Yield based on recovered starting material, b) not baseline separation

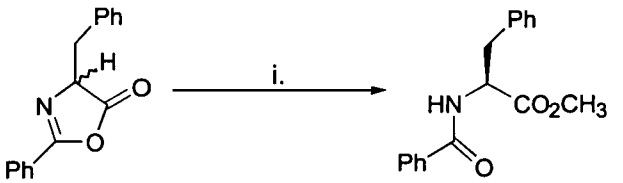
Table 15 *Results obtained for Novozyme® mediated alcoholysis of 2-phenyl-4-substituted 5(4H)-oxazolones* ***89b-g***

2.4.3. Studies on the effect of solvent

To gain a further understanding of the reaction parameters the solvent was varied. The methanolysis of **89a** was performed in the presence and absence of a catalytic amount of triethylamine to also examine the role of the base in the reaction. A number of conclusions can be drawn from the results shown in Table 16. Firstly, if we examine the results obtained when triethylamine was present, we can see that chlorinated solvents, (entries 1 and 2) gave poorer yields and e.e.'s. while ethers, (entries 3-5) gave excellent yields and e.e.'s comparable to the results obtained in toluene. The polar solvents of tetrahydrofuran and acetonitrile both produced products with excellent e.e.'s but with only moderate yields. Lower e.e.'s were observed when the same series of reactions were carried out in the absence of triethylamine. The effect was most prominent in the ether series (entries 3-5), where

the e.e. dropped from 96% to 33% in the case of DIPE. Examining the relative rates of reaction, it is evident that in the presence of triethylamine the reactions are much faster. The ethers gave relatively fast reactions both in the presence and absence of triethylamine and in all cases were complete after being left overnight. Chlorinated solvents resulted in the largest decrease in rate.

The results obtained in tetrahydrofuran and acetonitrile in the absence of triethylamine are the most interesting. In the absence of triethylamine the e.e. remained very high and the yield increased, (doubled in the acetonitrile case).



89a **96a**

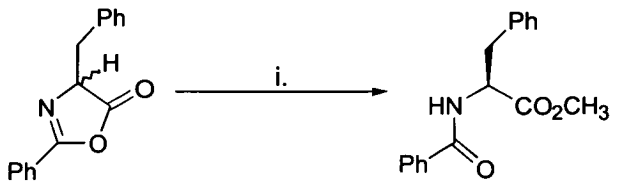
Reagents and conditions: i. Solvent, (Et_3N (0.25 equiv.)), Novozyme®, R'OH (2.0 equiv.), 37 °C

Entry	Solvent	Time/ h	Et_3N Present		Time/ h	Et_3N Absent	
			Yield/ %	e.e./ %		Yield/ %	e.e./ %
1	DCM	41	78	89	124	65	75
2	$CHCl_3$	39	66	75	124	63	83
3	THF	48	64	95	123	71	97
4	Et_2O	17	87	97	18	90	58
5	^{t}BuOMe	15	90	96	18	91	34
6	DIPE	15	86	96	18	90	33
7	toluene	26	82	94	58	82	71
8	acetonitrile	22	44	97	51	88	98

Table 16 *Solvents studies for the methanolysis of 2-phenyl-4-benzyl-5(4H)-oxazolone **89a** with Novozyme®*

2.4.4. Acetonitrile as solvent

To explore if the acetonitrile solvent effect discovered above was specific to Novozyme®, or was a more general phenomenon, the reaction was repeated with Lipozyme® as illustrated in Scheme 47.



89a **96a**

Scheme 47 *Reagents and conditions*: i. Solvent, (Et_3N, 0.25 equiv.)), Lipozyme®, CH_3OH, 37 °C

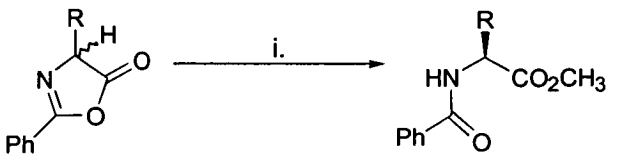
Entry	Solvent	Et_3N	Time/ h	Yield/ %	e.e./ %
1	toluene	No	-	48	19[101]
2	toluene	Yes	-	55	40[101]
3	acetonitrile	No	23	94	73
4	acetonitrile	Yes	23	61	73

Table 17 *Acetonitrile studies for the methanolysis of 2-phenyl-4-benzyl-5(4*H*)-oxazolone **89a** with Lipozyme®*

The results in Table 17 show that using acetonitrile as the solvent with Lipozyme® produced the same effect as observed with Novozyme®. Not only did the yield increase from 55% to 94% but the e.e. almost doubled from 40% to 73%.

2.4.5. Testing of substrates with acetonitrile as solvent

To expand on the results found with acetonitrile as solvent, the 2-phenyl-5(4*H*)-oxazolones tested in Section **(2.4.2.)** were also subjected to the acetonitrile conditions with both lipases, (Table 18).



89b-g **96b-g**

Reagents and conditions: i. Acetonitrile, lipase, CH_3OH, 37 °C

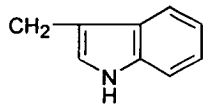
Compound **96**	R	Novozyme®		Lipozyme®	
		Yield/ %	e.e./ %	Yield/ %	e.e./ %
a	CH_2Ph	88	98	94	73
b	$CH_2CH(CH_3)_2$	96	97	89	62
c	$CH(CH_3)_2$	83	97	68	19
d		0	-	98	46
e	$CH_2CH_2SCH_3$	79	73	83	59
f	CH_3	94	10	79	39
g	$C(CH_3)_3$	0	0	0	0

Table 18 *Acetonitrile studies for the methanolysis of 2-phenyl-4-substituted-5(4H)-oxazolones* ***89a-g*** *with Novozyme® and Lipozyme®*

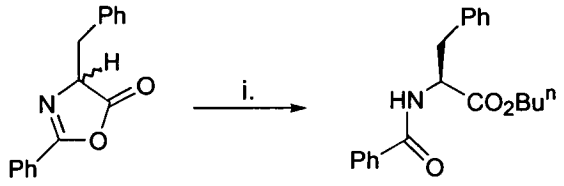
Examining the Novozyme® data first, and comparing it with the results in Section **(2.4.2.)**, Table 15, it can be clearly seen that for the C-4 alkyl side chains the results are almost identical in yield and e.e. In the case of the *tert*-butyl side chain the enzyme activity was entirely depressed and no desired product was isolated after several weeks. An identical result was obtained with the indolemethylene side chain (compared to a yield and e.e. of 90% when the reaction was carried out in toluene with triethylamine). An explanation for the above results could be that the use of acetonitrile as the solvent causes a conformational change in the lipase, resulting in a decrease in the accessibility of the active site of the lipase. The corresponding Lipozyme® reactions were generally high yielding, with only modest enhancement of the e.e. of the product when compared with the results obtained in toluene/triethylamine. The e.e. for the *iso*-propyl side chain increased from 0 to 19%. The *tert*-butyl side chain also gave a dramatic change in reactivity. Under the previous conditions a 47% yield and 80% e.e. was obtained. With acetonitrile as the solvent the activity was again entirely depressed. It appears that the best substrates for Novozyme® are flexible side chains *i.e. iso*-propyl or *iso*-butyl. It is also implied that the presence of a methylene spacer, as in the benzyl and methylsulfanyl-ethyl side chains can be advantageous as higher e.e. were observed with these substrates. Lipozyme® on the other hand appears to have a wider cavity at the active site and therefore is most stereoselective for sterically demanding side chains such as *tert*-butyl. In the absence of any steric bulk, only poor enantioselectivity was achieved. In

addition, once the lipase has been selected for the biotransformation the choice of solvent between toluene and acetonitrile can dramatically effect the enantioselectivity.

2.5.0. The role of the base

The intended role of the addition of triethylamine to the reaction system was to increase the rate of racemisation of the oxazolone substrates. However, during the course of our studies it was realised that the actual role of the triethylamine was more complex. To gain a greater understanding of the role of the base an in depth kinetic study was undertaken in our laboratory by Dr. M.-C. Parker.[103]

The most reliable predictor of enzyme catalytic activity in low water organic media is thermodynamic water activity (a_w). The hydration level was controlled by equilibrating the enzyme and solvent (over a period of 48-72 h) with the appropriate saturated salt solution of known a_w. For example, a low a_w system was one where the solvent was poorly hydrated, therefore the enzyme was similarly poorly hydrated. At high a_w the solvent was near water saturation and the enzyme was fully hydrated as in an aqueous system. The reaction depicted in Scheme 48 was performed in solvents with a range of known a_w.



89a **97a**

Scheme 48 *Reagents and conditions:* i. Solvent, (Et_3N (0.25 equiv.)), Novozyme®, ^{n}BuOH (2.0 equiv.), 37 °C

The effect of the level of hydration on the initial catalytic rate and enantioselectivity found in three different solvents in the presence and absence of triethylamine is shown in Table 19. In the absence of triethylamine, even low levels of hydration present in non-polar solvents such as hexane and toluene were detrimental to the rate and e.e. When the reactions were carried out in the presence of triethylamine, the rate

did drop, but to a lesser degree, while the e.e. remained high. The conditions which resulted in the optimum rate and e.e. were when the lipase, solvent and triethylamine were rigorously dried.

		No Et_3N		Et_3N	
Solvent	Water activity a_w	initial rate	e.e./ %	initial rate	e.e./ %
n-hexane	~0 (anhydrous)	26 (±1.5)	85 (± 3)	30 (± 1.5)	90 (± 3)
n-hexane	0.69	4 (±0.5)	55 (±2)	20 (±1)	87 (±3)
n-hexane	0.97	1.5 (±0.15)	30 (± 5)	18 (± 0.9)	80 (± 5)
toluene	~0	15 ((± 0.8)	85 ((± 4)	27 (±1.5)	93 (± 3)
toluene	0.22	3	61 (± 6)	17 (±1)	95 ((± 2)
acetonitrile	~0	15	>99	10	97 ((± 2)
acetonitrile	0.1 (0.5% v/v H_2O)	no reaction	-	5 (± 0.3)	90 (±4)
acetonitrile	0.4 (2% v/v H_2O)	no reaction	-	no reaction	-

Table 19 *Effect of water activity on initial catalytic rate and enantioselectivity as a function of hydration, in the presence and absence of triethylamine*

The effect of triethylamine addition to a reaction already proceeding with poor enantioselectivity was also examined. Triethylamine was added to the reaction in hexane (a_w = 0.69) after 140 min, resulting in an instantaneous increase in rate and e.e. as illustrated in Figure 7

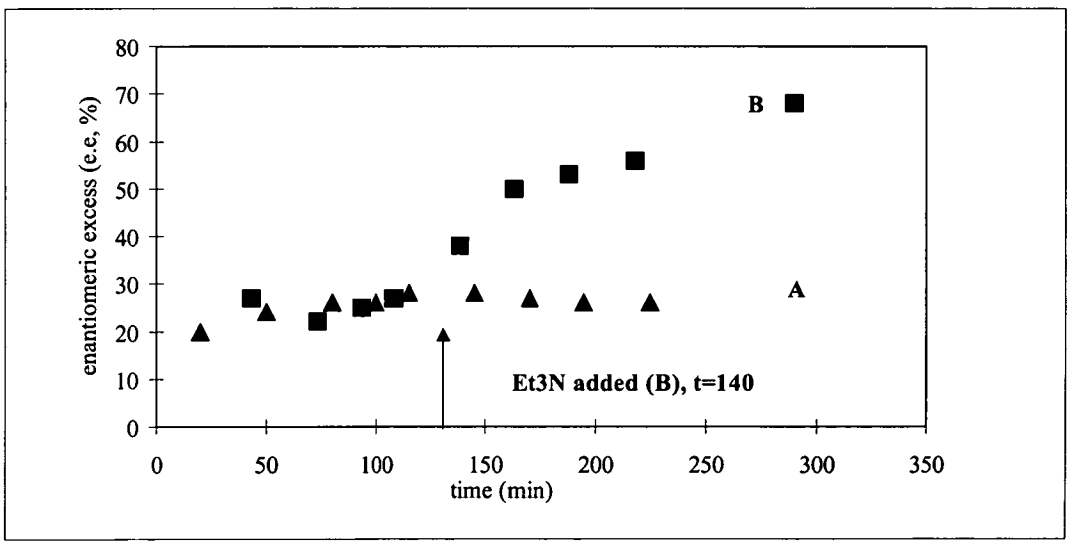


Figure 7 *Effect of triethylamine on e.e. Reactions A and B were carried out under identical conditions (a_w= 0.69). At t= 140 min, 14 mol % triethylamine was added to reaction B (arrow)*

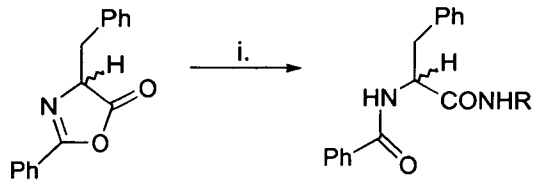
The addition of organic bases to increase the enantioselectivity of enzyme catalysed reactions carried out in organic solvents has been documented in the literature.[104-106] One hypothesis is that the addition of external base results in the formation of an ion-pair with any acid by-product formed during the reaction. As mentioned earlier in Section **(2.3.2.)**, the formation of *N*-benzoyl amino acids such as **95a** has been observed by ^{1}H nmr in the resolution of oxazolone **89a**. We have also found that addition of acid **95a** to an already hydrated system results in loss of activity. On subsequent addition of triethylamine the activity was regained, presumably *via* ion-pair formation. Ion-pair formation was observed in both low and high, non-hydrogen bonding dielectric solvents such as hexane and acetonitrile. The acid **95a** was found to be more soluble in acetonitrile than hexane. In the absence of triethylamine, the dissolved acid remains bound to the surface of the lipase through electrostatic interactions, thus altering the protonation state, and leading to protein deactivation. In polar solvents such as acetonitrile, the acid by-product is more soluble and therefore is not bound to the surface of the enzyme and no reduction in the e.e. is observed. However, for reactions carried out with rigorously dried reagents and low a_w (< 0.7) there was no evidence of hydrolysis over the initial rate measurements, yet the addition of triethylamine did enhance the enantioselectivity of the reaction. A role of the triethylamine in the reaction has been identified but does not give the complete answer. Investigations to further understand the activation process are currently under way.

2.6.0. Nitrogen nucleophiles

2.6.1. Amines

As well as testing alcohol nucleophiles, nitrogen based nucleophiles were investigated. As mentioned in Section **(2.2.0.)**, one aim of the project was to synthesise a number of MMPI derivatives **94**, all of which contain a methyl amide functionality in the position resulting from nucleophilic attack of the corresponding oxazolone. In control reactions 2-phenyl-4-benzyl-5(4*H*)-oxazolone **89a** was subjected to the amine nucleophiles benzylamine and allylamine in the absence of any lipase (Scheme 49). Unfortunately in both cases the amide product **102** was isolated in high yield indicating that amines are too nucleophilic and would by-pass

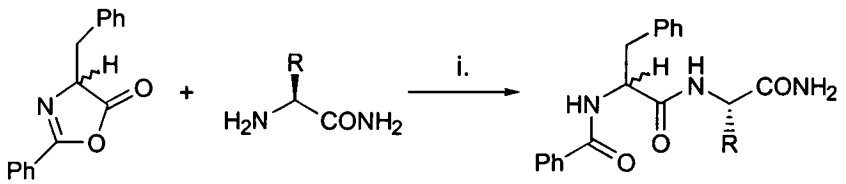
the lipase and produce only racemic amide products. Shriner *et al.*[107] observed amide formation in their studies involving the reactions of 2-phenyl-4-benzyl-5(4*H*)-oxazolone **89a** and secondary amines such as piperidine, morpholine, dimethylamine, diethylamine, methylaniline and ethylaniline.



89 **102a-b**

Scheme 49 *Reagents and conditions:* i. (a) Toluene, $PhCH_2NH_2$, 77%, (b) THF, $H_2NCH_2CH{=}CH_2$, 90%

The use of α-amino acid derivatives as nucleophiles was also investigated as an extension of the work carried out by Sih[108,109]. Sih subjected 2-phenyl-4-benzyl-5(4*H*)-oxazolone **89a** to nucleophilic attack by various α-amino acid derivatives in the presence of α-chymotrypsin or the cysteine protease papain. Either acetonitrile:phosphate buffer (1:1, pH 8) or DMF:phosphate buffer (1:1, pH 8.5) were used as solvent respectively, Scheme 50.



89a **103** **104**

Scheme 50 *Reagents and conditions:* i. (a) α-chymotrypsin, acetonitrile:phosphate buffer (1:1, pH 8), (b) papain, DMF:phosphate buffer (1:1, pH 8.5)

The experimental results obtained by Sih are shown in Table 20. They indicate that when optically pure oxazolone **89a** was used as the acyl donor only one diastereomeric dipeptide (L,L) was isolated, indicating that under the reaction conditions the L-oxazolone did not racemise. When racemic oxazolone was used, a mixture of (L,L) and (D,L) diastereomers of varying ratios were produced. These results suggest that the enzymes show a degree of enantioselectivity.

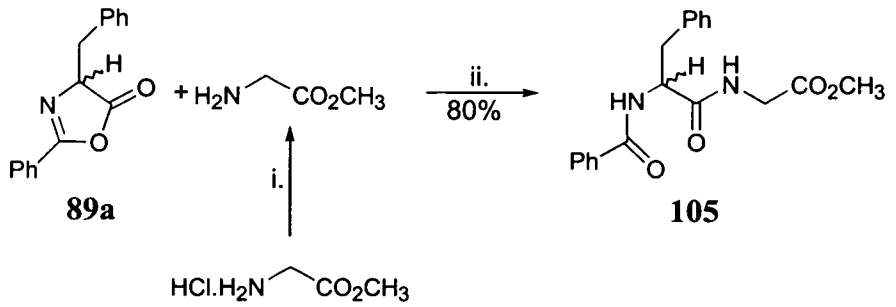
Entry	Oxazolone configuration	acyl acceptor **103**	Conditions	Time/ min	Yield/ % **104**
1	L	Phe-NH_2	a	30	64
2	L	Arg-NH_2	a	30	85
3	DL	Phe-NH_2	a	30	59 (L,L)
					13 (D,L)
4	DL	Arg-NH_2	a	5	70 (L,L)
					17 (D,L)
5	L	Ala-NH_2	b	30	62
6	L	Glu-NH_2	b	30	80
7	DL	Ala-NH_2	b	30	40(L,L)
					19 (D,L)
8	DL	Glu-NH_2	b	30	62 (L,L)
					18 (D,L)

Table 20 *Results obtained by Sih for the aminolysis of 2-phenyl-4-benzyl-5(4*H*)-oxazolone **89a** with α-chymotrypsin and papain*[108,109]

It is also clear that during the course of the reaction, the oxazolone partially re-racemised because the yield of the (L,L,) diastereomer is greater than 50%. However, no control experiment where the reagents were reacted in the absence of the enzyme is documented, therefore the optically pure diastereomers obtained in entries 1, 2, 5 and 6 could have been formed by direct reaction of the amino acid amide with the oxazolone. The diastereomeric ratios of the products in entries 3, 4, 7 and 8 could also have been a result of chemical diastereoselectivity because a chiral nucleophile was used. The yields in excess of 50% could result from racemisation of the relatively less reactive D-oxazolone (for steric reasons). A closer examination of the rate of reaction could shed light on this.

To provide more information on the mechanism of the above reaction we carried out the following control experiment. 2-Phenyl-4-benzyl-5(4*H*)-oxazolone **89a** was dissolved in toluene and freshly prepared glycine methyl ester added as shown in Scheme 51. The dipeptide product **105** precipitated almost instantly as a colourless solid, and was isolated in a yield of 80%. This is conclusive proof that these α-amino

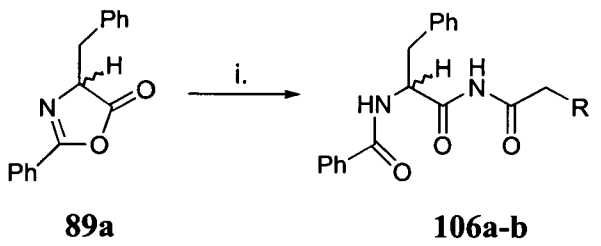
acid derivatives are too nucleophilic for use in enzymatic oxazolone aminolysis reactions.



Scheme 51 *Reagents and conditions:* i. Et_2O, $NH_{3(g)}$, 0°C, ii. Toluene, room temp.

2.6.2. Amides

In an attempt to lower the nucleophilicity of the nitrogen nucleophiles the use of amides was investigated. Unfortunately, the two attempts shown in Scheme 52 failed. It is hypothesised that in the case of phenylacetamide, (R= Ph), the amide was too weak a nucleophile due to the relatively poor electron withdrawing nature of the aromatic ring. Trifluoroacetamide, (R= CF_3) was tested in the hope that the greater electron withdrawing affect of the trifluoromethyl group would activate the amide sufficiently. Unfortunately no reaction was observed.

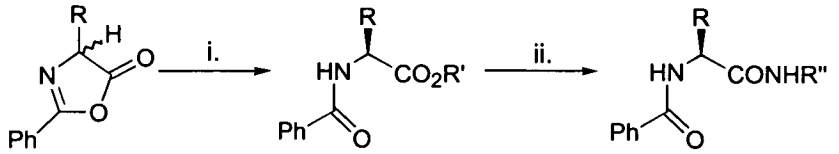


Scheme 52 *Reagents and conditions:* i. THF, Novozyme®, Et_3N, a) R= Ph, phenylacetamide, 0%, b) R= CF_3, trifluoroacetamide, 0%

2.6.3. Kinetic resolution with amines

Another route for preparing amides was tested. A double resolution process was envisaged as illustrated in Scheme 53. Ideally, both reactions would be carried out in the same reaction vessel. The initial dynamic kinetic resolution, upon completion, would be followed by amine addition to facilitate the enzymatic kinetic aminolysis utilising the first product as the new substrate. Not only would this provide the

desired functionality, but it could also potentially produce optically pure amide product.

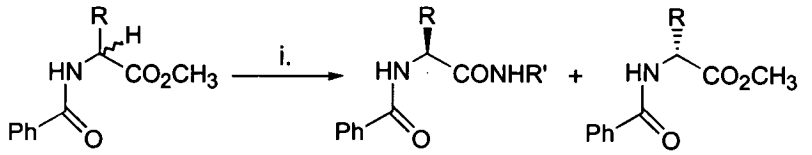


89 **107** **108**

Scheme 53 *Reagents and conditions*: i. Solvent, Novozyme®, (Et_3N), R'OH, 37 °C, ii. solvent, Novozyme®, H_2NR''

Conde *et al.*[33] (see Chapter 1, Section **(1.2.2.)**) have shown that when both enantiomers of diethyl Cbz-glutamate are separately exposed to Novozyme® in the presence of a variety of amines, aminolysis occurs predominantly at the α-position for the L-enantiomer, and predominantly at the γ-position for the D-enantiomer.

To test the utility of the aminolysis methodology, racemic *N*-benzoyl phenylalanine (R= benzyl) and *N*-benzoyl valine methyl esters (R= *iso*-propyl) were subjected to methyl and benzyl amine in various solvents as shown in Scheme 54 and Table 21. Unfortunately, no reaction was observed.



Scheme 54 *Reagents and conditions:* i. Solvent, Novozyme®, H_2NR'

Entry	Solvent	Temp/ °C	R	R'
1	toluene + Et_3N (0.25 equiv.)	37	CH_2Ph	CH_3
2	1,4-Dioxane	37	CH_2Ph	CH_2Ph
3	DIPE	37	CH_2Ph	CH_2Ph
4	DIPE	60	CH_2Ph	CH_2Ph
5	DIPE	37	$CH(CH_3)_2$	CH_2Ph
6	DIPE	60	$CH(CH_3)_2$	CH_2Ph

Table 21 *Attempted aminolysis of α-amino acid esters in the presence of Novozyme®*

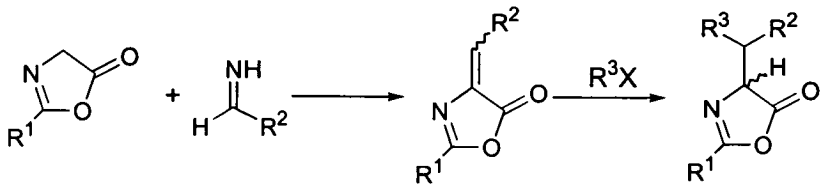
In a subsequent report, Conde[110] demonstrated that acyl protected amines react slower than their carbamate counterparts. It was hypothesised that the alkyl-oxygen atom of the carbamates interacted with the hydrogen bond network of the polar residues of the active site of the lipase. As a result, the energy required to bind the substrate to the active site will be significantly smaller than that for amines. Due to the unsuccessful results obtained in attempting to introduce nitrogen nucleophiles into the biotransformations, no further research on nitrogen nucleophiles was carried out.

2.7.0. Conclusions

To conclude, a reliable and reproducible assay has been developed utilising 2-phenyl-4-substituted-5(4*H*)-oxazolones **89a-g** as substrates in a lipase mediated dynamic kinetic resolution process in the synthesis of optically active L-α-amino acid derivatives. High enantioselectivities were achieved by the correct selection of lipase and solvent. It was found that Novozyme® preferred relatively flexible C-4 substituents, with the incorporation of a methylene spacer at C-4 proving advantageous for achieving a high degree of enantioselectivity. Lipozyme® on the other hand required sterically demanding C-4 substituents for high enentioselectivities to be achieved. The addition of triethylamine to the reaction proved crucial in obtaining high enantioselectivities, with the base acting as a counter ion to any hydrolysis product formed, thus removing any inhibitory effects. Acetonitrile was the solvent of choice for flexible C-4 side chains, whereas toluene and a catalytic volume of triethylamine were preferred for bulky C-4 side chains.

2.8.0. Future work

As there are already viable, economical routes to the proteinogenic α-amino acids, the utility of this methodology lies in the synthesis of unnatural α-amino acid derivatives. The oxazolone skeleton can provide a template for Knoevenagel condensation with various imines. Subsequent reduction or alkylation yields novel saturated 5(4*H*)-oxazolones **111** which can be utilised as the substrates in the biotransformation process as illustrated in Scheme 55.



109 **110** **111**

Scheme 55 *Proposed synthesis of novel 4-substituted-5(4H)-oxazolones*

3.0.0. Results and Discussion II

3.1.0. Application of developed methodology: The synthesis of potential matrix metalloproteinase inhibitors

3.1.1. Matrix metalloproteinases and their inhibitors

Matrix metalloproteinases (MMPs) are a family of closely related zinc containing endopeptidases. Their role in the body is to cleave large biomolecules such as collagens, proteoglycans, and gelatins. They have also been implicated in the remodelling of the extracellular matrix.[111] To date there are 14 known MMPs, and a high degree of sequence homology has been identified. All the MMPs have been found to contain a zinc (II) metal-ion at the active site and are inhibited by metal ion chelating agents.[112]

The role of MMPs in nature is to cleave molecules such as collagens, (the most abundant protein in the body and the major structural component of many organs and tissues). Their activity in the body must be precisely regulated. Expression of MMPs is tightly controlled by pro- and anti-inflammatory cytokines and growth factors. On production, the MMPs are generally secreted as inactive zymogens, or pro-enzymes.[113] To activate these zymogens the *N*-terminal must be modified or cleaved, which results in the freeing of the zinc (II) containing active site. This is achieved by cleaving the bond between the Cys73 residue and the Zn (II) as illustrated in Figure 8. In the inactive form, the pro-domain is folded over, allowing the Cys73 residue to interact with the Zn (II), thereby preventing access to the active site by the water molecules that are required for the cleavage mechanism.[114] The active forms of the MMPs are also regulated by natural inhibitors in the body, the tissue inhibitors of metalloproteinases, or TIMPs, and general plasma proteinase inhibitors such as α_2-macroglobulin.[113]

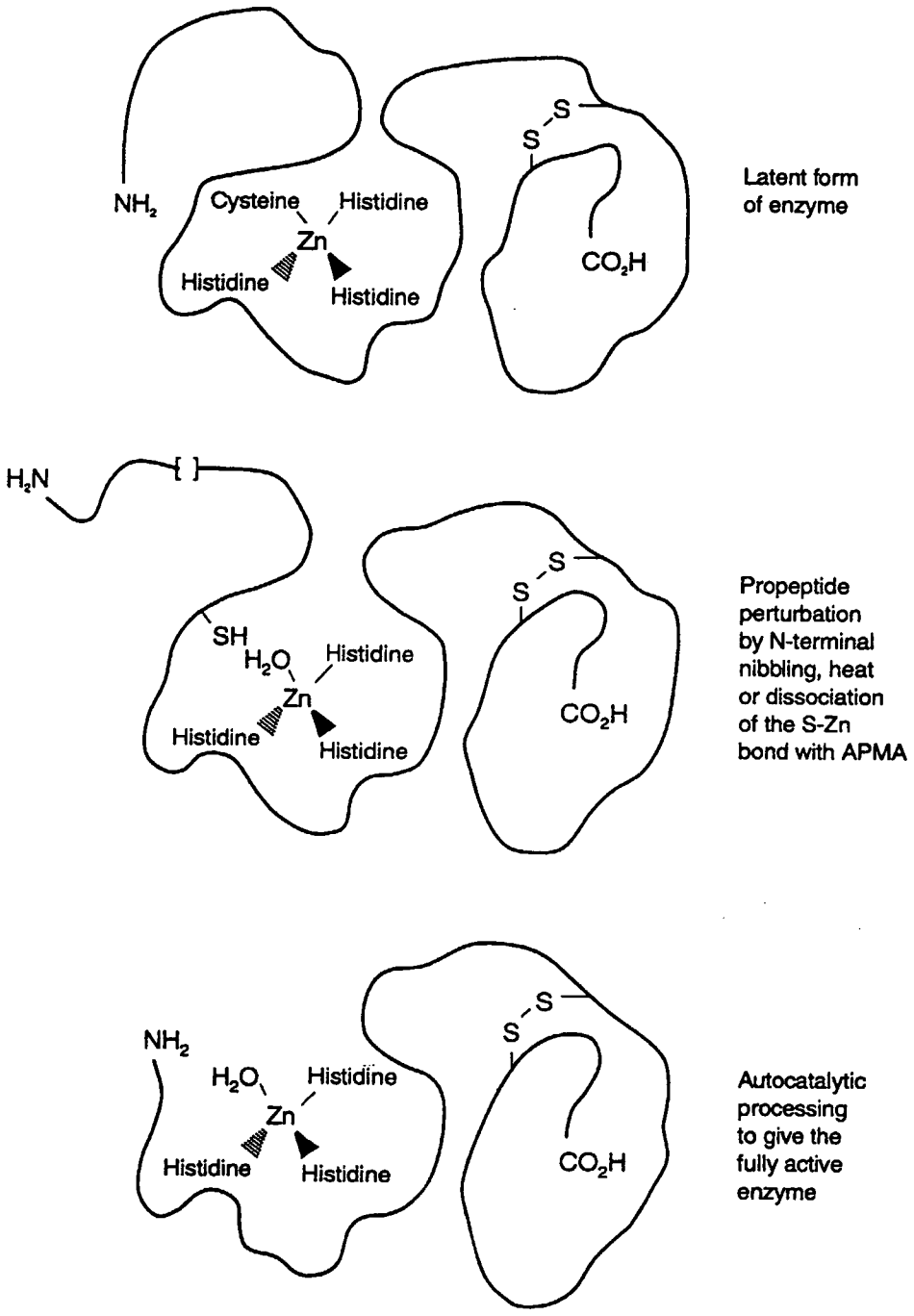


Figure 8 *Activation of matrix metalloproteinases*[111]

3.1.2. Matrix metalloproteinases and disease

In a number of pathological conditions such as cancers and arthritis, there is an imbalance between the levels of the active MMPs and the native inhibitors. The increased levels of MMPs result in increased degradation of the extracellular matrix,

which in turn, cause irreversible damage to the body. For example, in various (but not all) cancers, abnormally high levels of MMPs cause the breakdown of the extracellular matrix of the cancer tumour, allowing proliferation and local invasion of the primary tumour. As the tumour cells travel through the blood vessels, they adhere to the blood vessel wall. Again MMP activity causes a breakdown of the cell wall allowing tumour cells to cross the vascular basement membrane, culminating in secondary tumour growth. In addition, there is evidence that MMP activity contributes to the invasive growth of new blood cells, (angiogenesis) which allows the tumour to grow.[112,115] This process is illustrated in Figure 9.

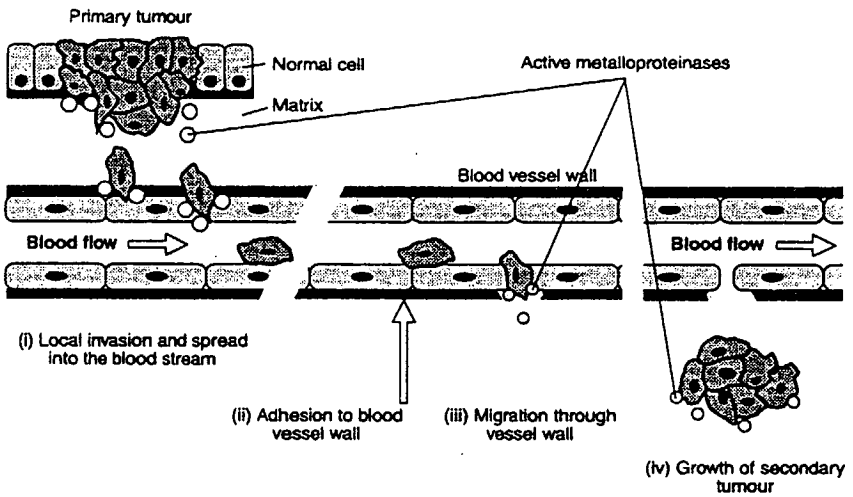


Figure 9 *Potential sites of action of matrix metalloproteinases in tumour growth*[115]

3.1.3. Synthetic matrix metalloproteinase inhibitors

A number of pharmaceutical companies currently have active research projects directed towards finding an orally active matrix metalloproteinase inhibitor (MMPI).[114] The strategy of treatment is the continuous administration of low toxicity therapeutics that will stabilise malignant disease and prevent further growth. To this end, the MMPI's do not constitute a magic bullet or a preventative treatment, but are better regarded as a quality of life enhancing tool for patients. Two approaches in MMPI development have been followed, namely the screening of natural compounds and the substrate-based design of pseudo-peptide derivatives. The second approach has been by far the most successful. The starting point for structure based design lay

with the sequence around the glycine-isoleucine and glycine-leucine cleavage sites of collagen, which frees the zinc (II) containing active site as indicated in Figure 10. Of the three resulting substructures, it has been MMPI's based on the right hand side (RHS) of the sequence that have proved the most potent.

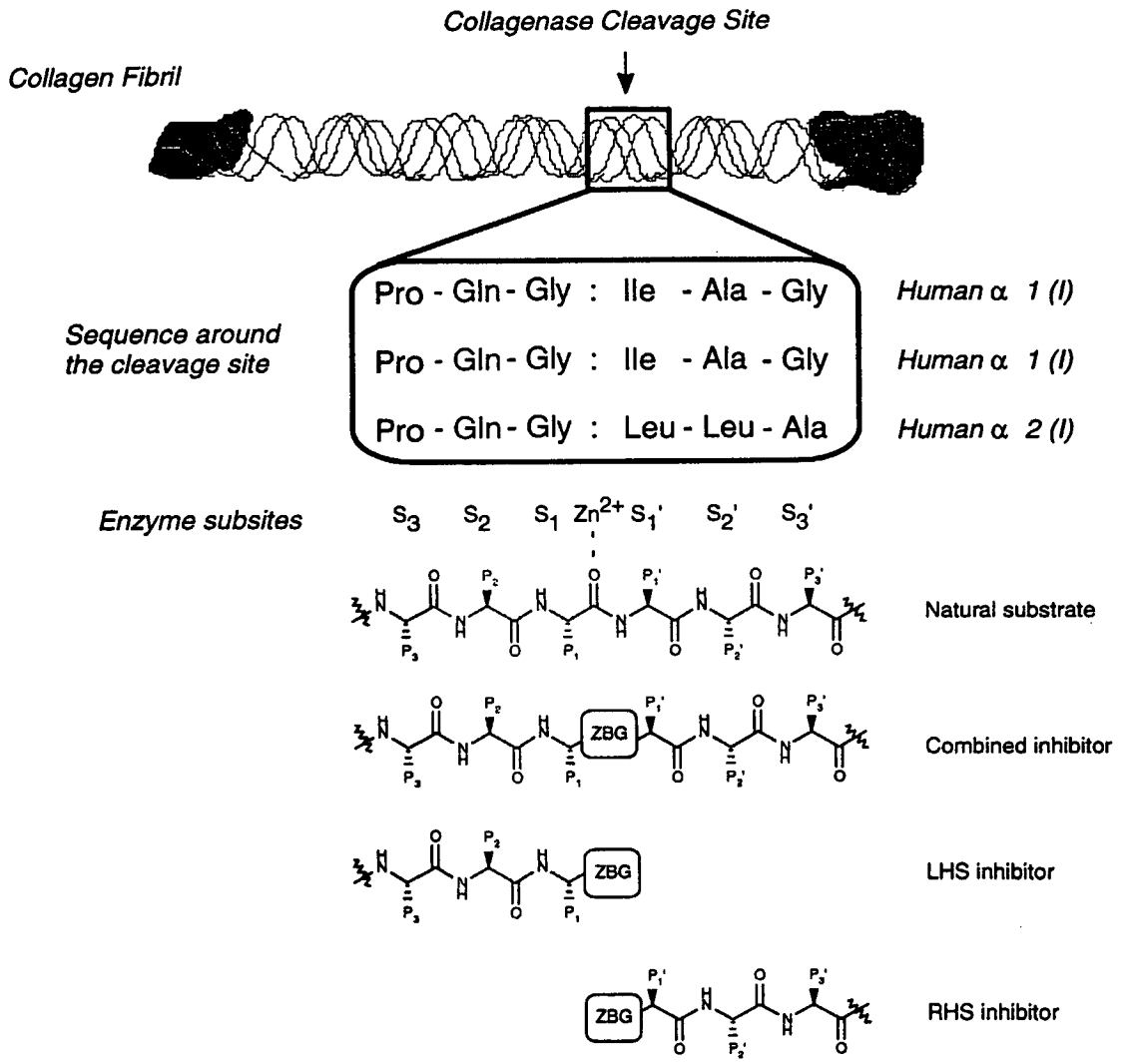


Figure 10 *Design of matrix metalloproteinase inhibitors on the basis of the cleavage site of collagen*[113]

A summary of the structure activity relationship (SAR) found for MMPI's is given in Figure 11. The incorporation of a zinc (II) binding group, (ZBG) to chelate to the active site of the MMPs proved essential for potent inhibitory levels. Of the zinc binding groups (ZBG's) tested, including carboxylate, aminocarboxylate, sulfhydryl,

derivatives of phosphorus acids and hydroxamic acids, it has been the hydroxamic acids that have been incorporated most frequently. From an X-ray crystal structure of a hydroxamate substrate binding to matrilysin (MMP7) it is evident that the hydroxamate acts as a bidentate ligand, with each oxygen at an optimum distance (1.9-2.3 Å) from the active Zn(II) ion. The P_1' group fits into the S_1' pocket of the enzyme and offers the greatest opportunity for selective inhibitor design. A large number of the developed inhibitors have incorporated an *iso*-butyl group which results in non-selective inhibition. The P_2' and P_3' groups can tolerate a variety of α-amino acid residues which shows that the P_2' side chain does not play a major role in enzyme binding. Bulky R^3 groups such as *tert*-butyl, as in the Roche compound Ro 31-9790, and an indolemethylene as in Glycomed's Galardin, have shown enhanced inhibition in comparison with other P_2' alkyl groups. The use of bulky R^3 groups also prevents amide hydrolysis *in vivo*. It has also been discovered that the P_1'-P_2' and the P_2'-P_3' C=O and N-H are all involved in hydrogen bonding interactions with the enzyme.[113]

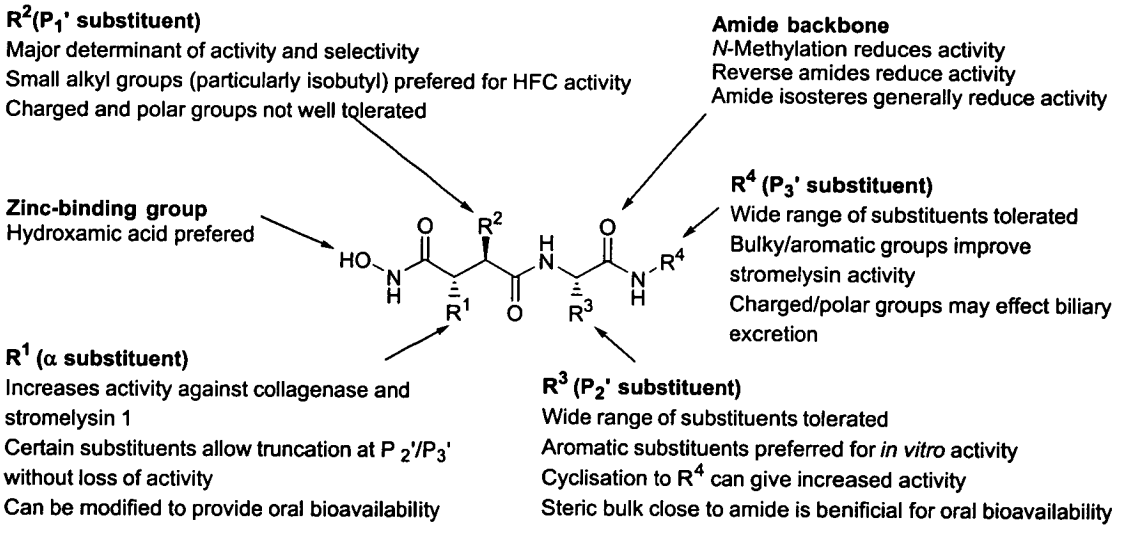
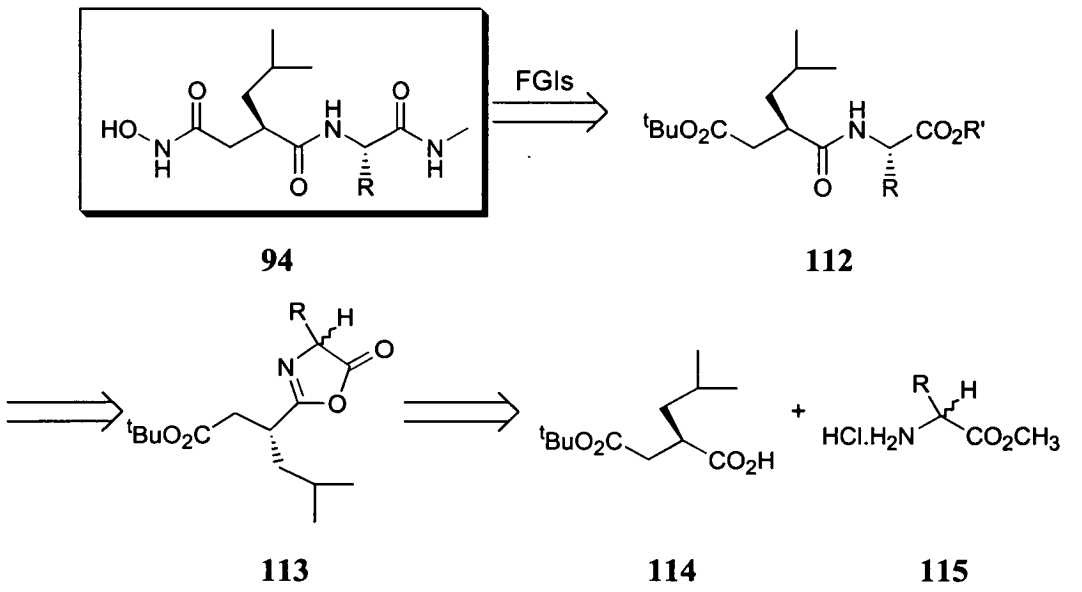


Figure 11 *Summary of the SAR for RHS matrix metalloproteinase inhibitors. HFC- human fibroplast collagenase*[113]

3.2.0. Retrosynthetic analysis

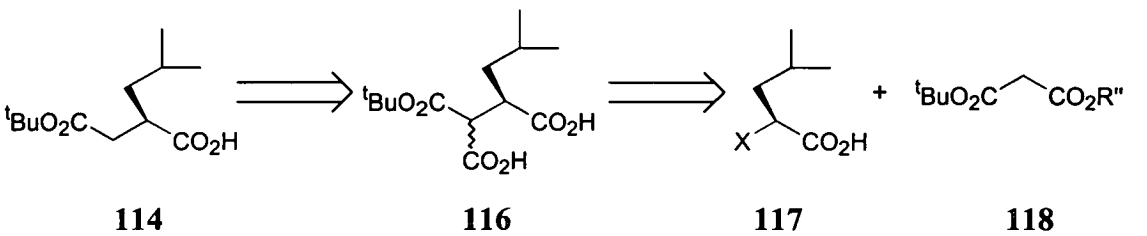
Through retrosynthetic analysis, (Scheme 56), it can clearly be illustrated how the oxazolone methodology developed in the previous chapter can be utilised in the

synthesis of the MMPI's such as Galardin (R= indolemethylene) and Ro 31 9790 (R= *tert*-butyl). Through a number of simple functional group interconversions, the diester **112** is obtained, which can be considered as the dynamic kinetic resolution product from the corresponding oxazolone **113**. Synthesis of oxazolone **113** from racemic α-amino acid methyl esters and the substituted succinate monoester **114** was envisaged. The first goal was therefore the synthesis of the substituted succinate monoester **114**.



Scheme 56 *Retrosynthetic analysis of matrix metalloproteinase inhibitors*

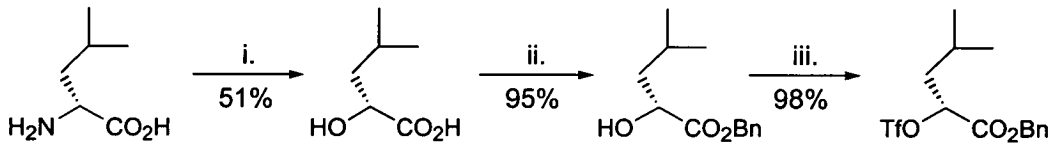
Succinate **114** can be considered as the downstream product from the alkylation of a unsymmetrical malonate **118** with the acceptor molecule **117** (Scheme 57). The acceptor molecule **117**, where X = I, Br or TfO, TsO, could be synthesised from either enantiomer of leucine.



Scheme 57 *Retrosynthetic analysis of succinate 114*

3.3.0. Synthesis of substituted succinate mono ester 114

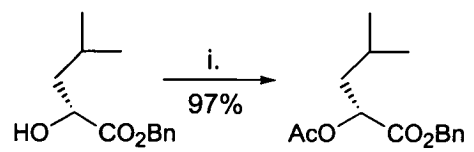
Starting from D-leucine, as illustrated in Scheme 58, the amine group was converted to a hydroxyl group *via* diazotisation to form (*R*)-leucic acid **119** according to the procedure detailed by Mori.[116] The literature yield was moderate for this reaction and could not be improved on. Acid catalysed benzylation furnished the α-hydroxy ester **120** in excellent yield after column chromatography; which was used to form the triflate **121** as described by Degerbeck.[117] Evidence for the formation of the triflate was obtained from the ^{1}H and ^{13}C nmr spectra. In the ^{1}H nmr the α-proton was shifted downfield from δ 4.24 for the hydroxy ester **120** to δ 5.19 due to the electron withdrawing effect of the trifluoromethyl group. In the ^{13}C nmr spectrum of **121,** a quartet at δ 118.29 with a coupling constant of 319 Hz was observed which is characteristic for the trifluoromethyl group of a triflate. The triflate was stable to chromatography but readily decomposed on standing and was therefore used immediately in the next stage of the synthesis. All the above reactions were carried out on at least a 70 mmol scale providing multigram quantities (~24 g) of pure triflate **121**.



D-Leucine **119** **120** **121**

Scheme 58 *Reagents and conditions*: i. 1M H_2SO_4, $NaNO_2$, 0 °C, ii. toluene, BnOH (2.0 equiv.), *p*-toluenesulfonic acid (5 mol %), iii. DCM, 2,6-lutidine, $(CF_3SO)_2O$, -78 °C

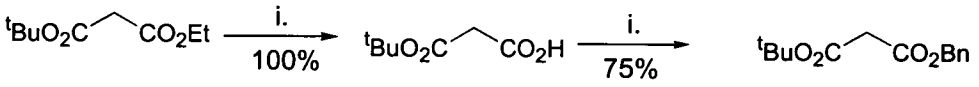
The optically purity of α-hydroxy ester **120** was also determined by HPLC analysis of the acetate **122** and found to be 97% e.e. (Scheme 59). This was in agreement with the optical purity of the D-leucine (97% e.e. commercially available) used at the beginning of the synthesis.



120 (*R*)-**122** 97% e.e.

Scheme 59 *Reagents and conditions*: i. Pyridine, acetic anhydride, room temp.

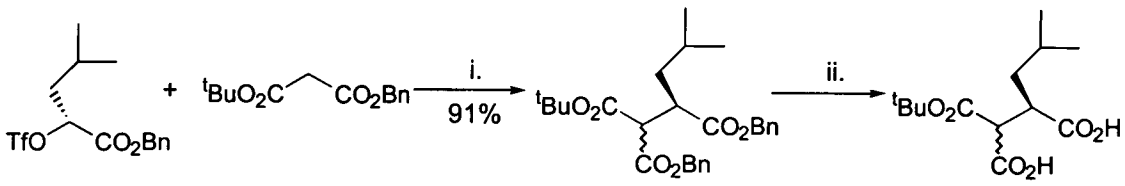
The unsymmetrical malonate **125** was synthesised in high yield from the commercially available *tert*-butyl ethyl malonate **123** as illustrated in Scheme 60. Base hydrolysis of **123** proceeded quantitatively yielding mono *tert*-butyl malonate **124**. Next, the potassium salt was formed and dried overnight under vacuum before being used in the alkylation reaction with benzyl bromide in DMF, to furnish *tert*-butyl benzyl malonate **125** in 75% yield on an 80 mmol scale.



123 **124** **125**

Scheme 60 *Reagents and conditions*: i. THF:H_2O (1:1), LiOH, room temp., ii. THF:H_2O (1:1), KOH, iii. DMF, BnBr

With the two fragments in hand, the carbon-carbon bond forming reaction was attempted. The S_N2 displacement of the triflate group with the enolate derived from malonate **125** proceeded in an excellent yield of 91% on a 60 mmol scale (Scheme 61). The triester product **126** was isolated as a 1:1 mixture of diastereomers, as calculated from the integrals from the ^1H nmr of the OCCHCH proton. Deprotection of the benzyl esters to furnish diacid **116** proceeded quantitatively using 10% Pd/C and hydrogen at atmospheric pressure. Diacid **116** was not purified but used directly in the subsequent decarboxylation step.

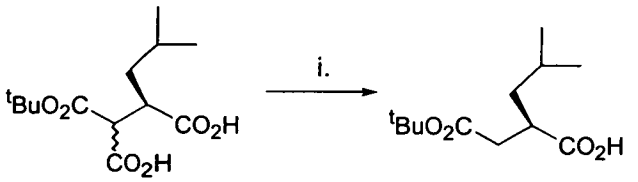


121 **125** **126** **116**

Scheme 61 *Reagents and conditions*: i. THF, NaH, -10 °C, ii. THF, 10% Pd/C, H_2, room temp.

A number of conditions were screened for the decarboxylation step outlined in Scheme 62 in order to obtain the optimum yield. Due to the *tert*-butyl ester

functionality, acid catalysed decarboxylation was avoided. The results are summarised in Table 22.



116 **114**

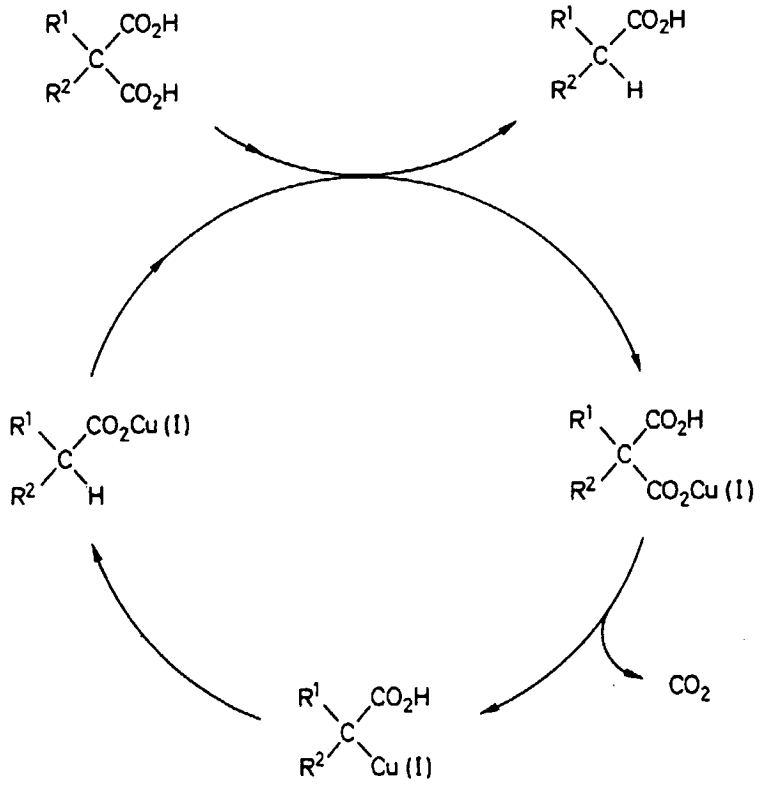
Scheme 62 *Reagents and conditions: i. See Table 22*

Entry	Conditions	Crude Yield/ %	Purified Yield/ %	Comment, ratio **116**:**114**
1	toluene reflux, 20 h		60	desired product
2	THF, reflux	-	-	recovered starting material
3	THF Cu_2O, 65 °C			
4	THF, Et_3N, room temp.	-	-	recovered starting material
5	THF, Et_3N, reflux 5 h	98	-	1:4
6	CH_3CN, Cu_2O, 70 °C	0	0	recovered starting material
7	CH_3CN, reflux 20 h	-	-	1:1
8	CH_3CN, Cu_2O, reflux, 1.5 h	78	-	2:7
9	CH_3CN Cu_2O, reflux, 5 h	92	67	desired product
10	CH_3CN, Cu_2O, reflux, 24 h	51	-	desired product
11	CH_3CN, Et_3N, reflux, 5 h	90	-	1:2
12	CH_3CN, Et_3N, reflux, 19 h	-	85	desired product

Table 22 *Decarboxylation studies for diacid **116***

Petit[118] utilised catalytic (0.1 equiv.) copper (I) oxide in the decarboxylation of chiral diacids similar to **116** in an extension of the procedure developed by Maumy.[119,120] Maumy proposed that the reaction proceeded by an ionic mechanism involving copper (I) carboxylates as shown in Scheme 63. Brunner *et al.*[121] carried out a number of experiments to disprove the participation of the proposed copper (I) carboxylates and discovered that the monoanionic malonate derivatives were the reactive species undergoing decarboxylation. The effect of copper (I) was attributed to basicity influence, therefore any compound that increases the concentration of the

monoanionic species will also increase the rate of decarboxylation. Brunner applied these experimental findings and used chiral amine alkaloids such as cinchonine, and cinchonidine in catalytic quantities as base to furnish chiral ethyl 2-phenyl propionate from mono ethyl methylphenylmalonate in excellent yields, albeit with only moderate enantioselectivity (11-34% e.e.).

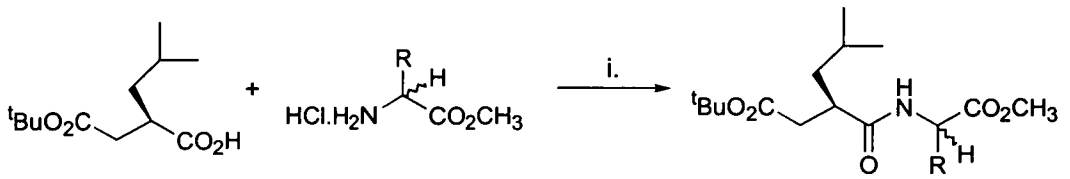


Scheme 63 *Proposed catalytic cycle for Cu(I) catalysed decarboxylation of malonic acids*[119]

The results in Table 22 for the decarboxylation of diacid **116** show that thermal decarboxylation required high temperatures and proceeded with moderate yields of 60% in toluene, entry 1. The conditions described by Petit and Maumy were also screened. When tetrahydrofuran was employed as the solvent, only starting material was recovered, entry 3. With acetonitrile and Cu (I), 5 h under reflux was required for complete decarboxylation, with the desired product isolated in a 67% yield, entry 9. The use of catalytic triethylamine as base resulted in complete decarboxylation after 19 h, and furnished the desired succinate **114** in 85% (~10 g) yield from triester **126**, entry 11.

3.4.0. Synthesis of novel 5(4*H*)-oxazolones for lipase catalysed dynamic kinetic resolution

To produce the required 4-substituted-5(4*H*)-oxazolones, the succinate **114** was coupled to racemic α-amino acids to produce a 1:1 mixture of diastereomeric amides **127** using either of two sets of peptide coupling conditions illustrated in Scheme 64. The second set of conditions, (ii.), were required because uneven ratios of the resulting diastereomers were obtained when the reaction conditions (i.) were employed with leucine, tryptophan and *tert*-leucine methyl esters. The results in Table 23 indicate the good to excellent yields of amides (2*R*,2'*RS*)-**127a-e** obtained.



114 *rac*-**115a-e** (2*R*,2'*RS*)-**127a-e**

Scheme 64 *Reagents and conditions*: i. (i.) DCM, HOBt, EDCI, Et_3N, room temp., or (ii.) DMF, HOBt, TBTU, DIPEA, room temp.

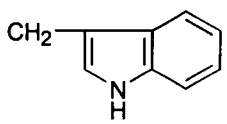
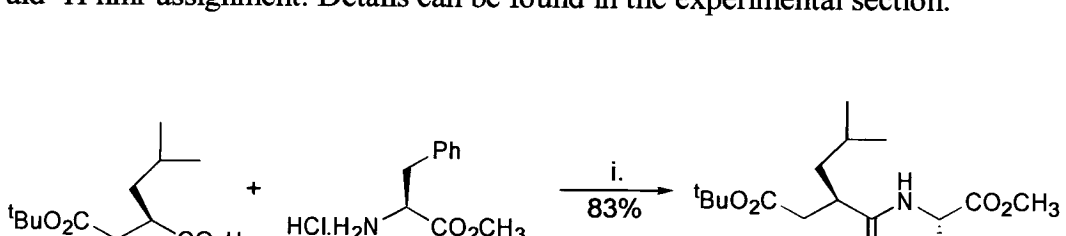
Compound (2*R*,2'*RS*)-**127**	R	Method	Yield/ %
a	CH_2Ph	i.	80
b	$CH(CH_3)_2$	i.	77
c	$CH_2CH(CH_3)_2$	ii.	77
d		ii.	78
e	$C(CH_3)_3$	ii.	62

Table 23 *Synthesis of 1:1 mixture of diastereomeric amides* (2*R*,2'*RS*)-**127a-e**

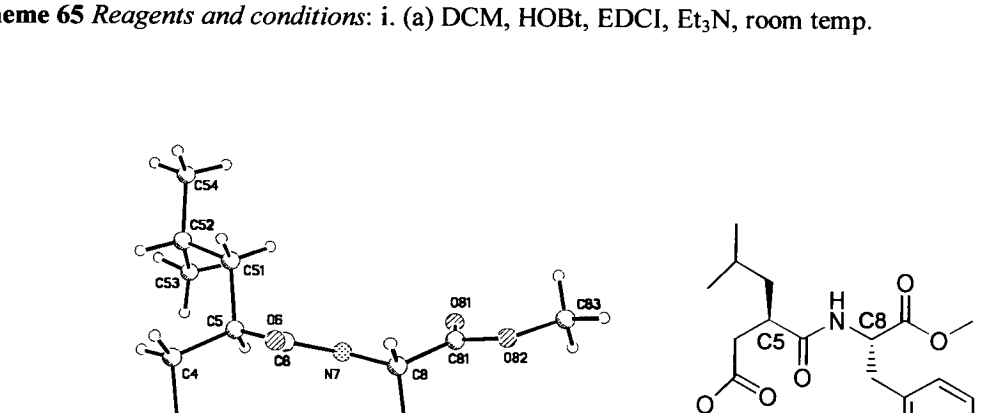
Succinate **114** was also coupled to L-phenylalanine methyl ester as shown in Scheme 65. The product was isolated in 95% d.e. as calculated from the 600 MHz ^1H nmr spectrum by comparison of the signals corresponding to the methyl ester protons, δ 3.69 and 3.67 for the minor and major diastereomers respectively. The crystal structure of the amide **127a** was also obtained, (see Appendix I for details), and is

represented in Figure 12. The structure clearly indicates that C-5 (as numbered in Figure 12) has an (*R*) configuration since the C-8 configuration was fixed as (*S*) due to the use of L-phenylalanine methyl ester. This result confirms that the synthesis of succinate **114** proceeded with the desired stereochemical integrity, and that the carbon-carbon bond forming reaction involving malonate **125** and triflate **121** did proceed *via* an S_N2 inversion mechanism. As the e.e. of α-hydroxy ester **120** was determined to be 97% (Scheme 59), and amide **127a** was obtained with 95% d.e. when reacted with optically pure L-phenylalanine, it can be concluded that (*R*)-succinate **114** was obtained in 97% e.e. It should be noted that samples of the C-5-(*R*)-C-8-(*S*)-pseudodipeptides for all five C-8 substituents were prepared in high yield and d.e. from coupling of succinate **114** and the corresponding L-α-amino acid ester to aid ^{1}H nmr assignment. Details can be found in the experimental section.



(*R*)-**114** (*S*)-**115a** (2*R*,2'*S*)-**127a**

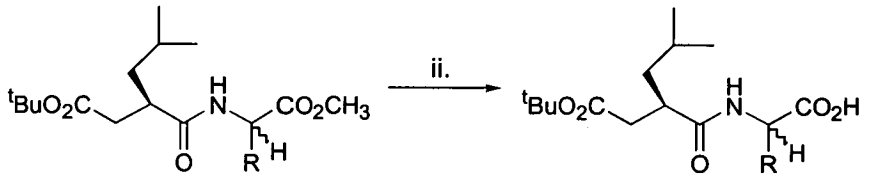
Scheme 65 *Reagents and conditions*: i. (a) DCM, HOBt, EDCI, Et_3N, room temp.



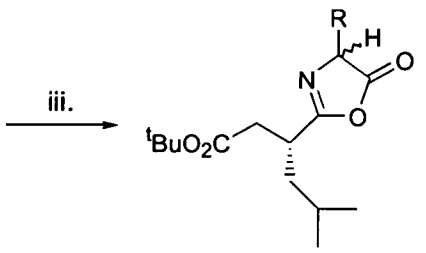
(2*R*,2'*S*)-**127a**

Figure 12 *X-ray crystal structure obtained for* (2*R*,2'*S*)-***127a*** *synthesised chemically*

As illustrated in Scheme 66, base catalysed hydrolysis of the diastereomeric amides (2*R*,2'*RS*)-**127a-e** proceeded in quantitative yield; subsequent cyclisation using EDCI in acetonitrile furnished the desired oxazolone substrates **129a-e** in good to excellent yield (Table 24). The characteristic carbonyl stretch at ~1818 cm^{-1} was observed in the infrared spectra of all oxazolone products. In the ^1H nmr spectra, oxazolone formation was accompanied by the loss of the N-H signal. These oxazolones were considered to be relatively unstable and were used immediately, or stored in the freezer at -26 °C. The low yield obtained for the cyclisation of the *tert*-leucine derived acid **128e** appeared to be due to cleavage of the *tert*-butyl ester as a results of prolonged reaction conditions (see experimental). From the ^1H and ^{13}C nmr of the crude hydrolysis product **128e,** it was evident that a mixture of four compounds (two sets of diastereomers) was obtained in an almost equal ratio. A pure sample of acid **128e** was obtained by repeated trituration of the undesired diacid. Cyclisation of crude hydrolysis product **128e**, followed by purification by column chromatography furnished pure oxazolone **129e**.

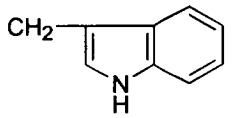


(2*R*,2'*RS*)-**127a-e** **128a-e**



129a-e

Scheme 66 *Reagents and conditions*: ii. THF:H_2O (1:1), LiOH (2.0 equiv.), room temp., iii. CH_3CN, EDCI, room temp.

Compound	R	**128** Yield/ %	**129** Yield/ %
a	CH_2Ph	100	86
b	$CH(CH_3)_2$	100	81
c	$CH_2CH(CH_3)_2$	100	75
d		100	91
e	$C(CH_3)_3$	100[a]	52

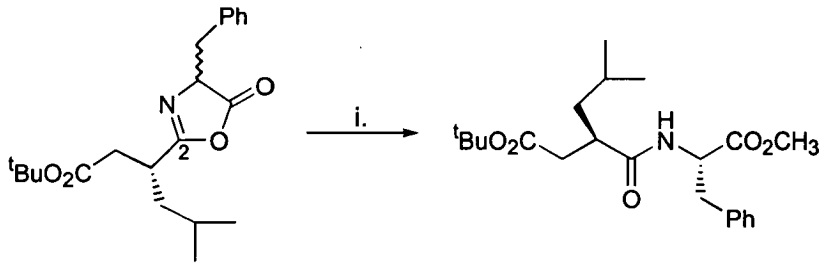
a) Product was a mixture of desired product and *tert*-butyl ester cleavage product (1:1)

Table 24 *Synthesis of 2-alkyl-5(4H)-oxazolone substrates* ***129a-e***

3.5.0. Dynamic kinetic resolution of novel 5(4*H*)-oxazolones

3.5.1. Initial studies

The first substrate tested was the phenylalanine derived oxazolone **129a** (Scheme 67). The compound was subjected to both sets of optimised conditions developed in Chapter 2, and to both the lipases Novozyme® and Lipozyme®. The results in Table 25. can be directly compared with those obtained in Chapter 2, where the C-2 substituent was a phenyl group.



129a (2*R*,2'*S*)-**127a**

Scheme 67 *Reagents and conditions*: i. Solvent, (Et_3N, (0.25 equiv.)), lipase, CH_3OH (2.0 equiv.), 37 °C

The use of acetonitrile as solvent was detrimental to the rate of reaction. In the case of Novozyme®, entry 2, a good yield of 88% was obtained but this figure is based on the recovery of 25% starting oxazolone **129a**. Under identical reaction conditions, but using Lipozyme®, entry 5, only a 47% yield was obtained with 24% recovery of starting material after a period of 48 days These results reinforce the hypothesis from Chapter 2 that the use of acetonitrile as solvent causes a conformational change in

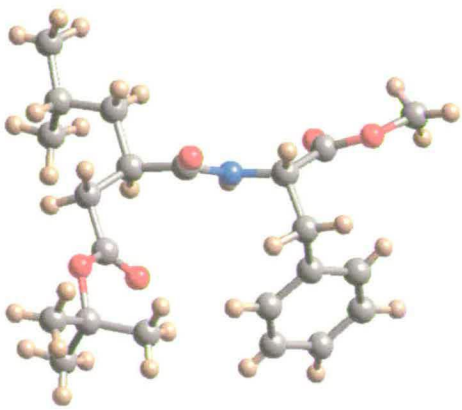
the lipase that results in a decrease in the size of the active site of the lipases. The introduction of a more complex C-2 substituent, which also contains an additional chiral centre, imposes greater restrictions on the fit of the substrate into the active site of the enzyme.

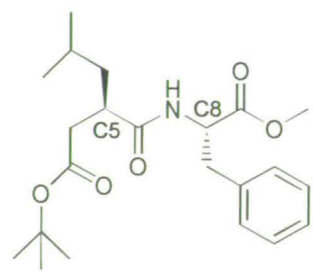
Entry	Solvent	Et_3N	Lipase	Time/ days	Yield/ %	d.e./ %
1	toluene	yes	Novozyme®	4	85	81
2	acetonitrile	no	Novozyme®	10	88[a]	78
3	^{t}BuOMe	yes	Novozyme®	2	90	79
4	toluene	yes	Lipozyme®	1	73	58
5	acetonitrile	no	Lipozyme®	48	47[a]	55

a) Yield based on recovered starting material

Table 25 *Results obtained for the methanolysis of 5(4H)-oxazolone* ***129a*** *with Novozyme® and Lipozyme®*

The d.e. for the Novozyme® and Lipozyme® reactions were consistent under the conditions studied, within the error of analysis (±5% by nmr); with Novozyme® producing the higher d.e. of 80% compared to 55% for Lipozyme®. The use of *tert*-butyl methyl ester as solvent with Novozyme® did enhance the rate, as predicted from the results in Chapter 2, but had no positive effect on the d.e. of the product. By comparison of the ^{1}H nmr of the methyl esters obtained in Table 25 with the ^{1}H nmr of (2*R*,2'*S*)-**127a** synthesised chemically, (Scheme 65), it was ascertained that the products had the (*S*) conformation at C-8. This was proved conclusively by obtaining a X-ray crystal structure (See Appendix II for data) of the Novozyme® mediated methanolysis product shown in Figure 13.



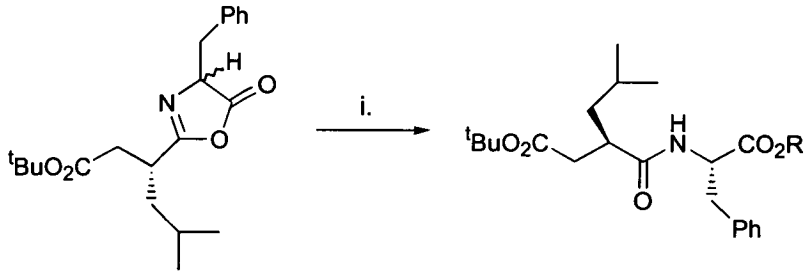


(2*R*,2'*S*)-**127a**

Figure 13 *X-ray crystal structure obtained for enzymatic methanolysis product* (2*R*,2'*S*)-**127a**

3.5.2. The effect of alkyl chain length of the nucleophile

In a similar process as described in Chapter 2, the alcohol nucleophile chain length was varied in the biotransformation as illustrated in Scheme 68. All the reactions were carried out with Novozyme® and the results reported in Table 26. Again, as discovered in Chapter 2, the d.e. of the product varied very little in going from a methyl to *n*-butyl alkyl chain. A range of ± 4% was observed which is within the accuracy of the ^{1}H nmr measurements (± 5%). The rate and high yield for each reaction also remained constant throughout the series.



129a (2*R*,2'*S*)-**127a, 130-132**

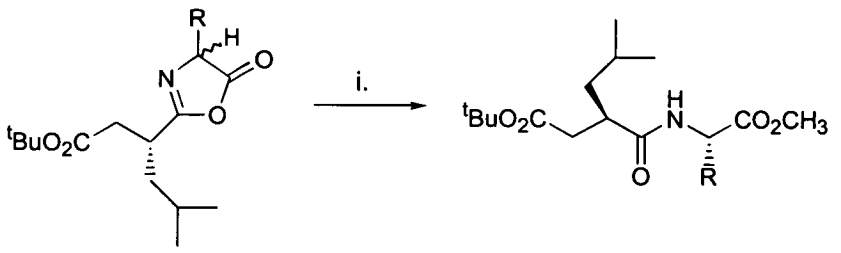
Scheme 68 *Reagents and conditions*: i. Toluene, Et_3N, (0.25 equiv.), Novozyme®, ROH (2.0 equiv.), 37 °C

Compound	R	Time/ days	Yield/ %	d.e./ %
(2*R*,2'*S*)-**127a**	CH_3	4	85	81
130	CH_3CH_2	3	76	84
131	$CH_3CH_2CH_2$	3.5	84	84
132c	$CH_3CH_2CH_2CH_2$	3.5	85	80

Table 26 *Effect of alkyl chain length of the nucleophile on d.e. of the product*

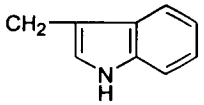
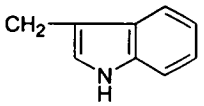
3.5.3. Testing of remaining substrates

The remaining 5(4*H*)-oxazolone substrates **129b-e** were tested with both Lipozyme® and Novozyme® (Scheme 69) and the results, collected in Table 27. The results indicate the following trends. From analysis of the results obtained for the simple C-4 alkyl substituents, where R= *iso*-propyl and *iso*-butyl, entries 1-4, it can be seen that the Novozyme® mediated reactions proceeded at a faster rate than the corresponding Lipozyme® reactions. The choice of enzyme had little effect on the yield. In all four reactions, excellent yields of 87-96% were obtained.



129b-e (2*R*,2'*S*)-**127b-e**

Scheme 69 *Reagents and conditions*: i. Toluene, Et_3N, (0.25 equiv.), lipase, CH_3OH (2.0 equiv.), 37 °C

Compound (2*R*,2'*S*)-127	R	Lipase	Time/ days	Yield/ %	d.e./ %
b	$CH_2CH(CH_3)_2$	Novozyme®	2	96	86
b	$CH_2CH(CH_3)_2$	Lipozyme®	12	96	54
c	$CH(CH_3)_2$	Novozyme®	11	93	75
c	$CH(CH_3)_2$	Lipozyme®	29	87	27
d		Novozyme®	13	84	74
d		Lipozyme®	13	89	7
e	$C(CH_3)_3$	Lipozyme®	28	12(44)[a]	72
e	$C(CH_3)_3$	Lipozyme®[b]	28	5[c]	-

a) Yields in parentheses are based on recovered 5(4*H*)-oxazolones, b) *n*-Butanol (2.0 equiv.) as nucleophile, c) yield based on conversion as calculated from ^1H nmr integrals.

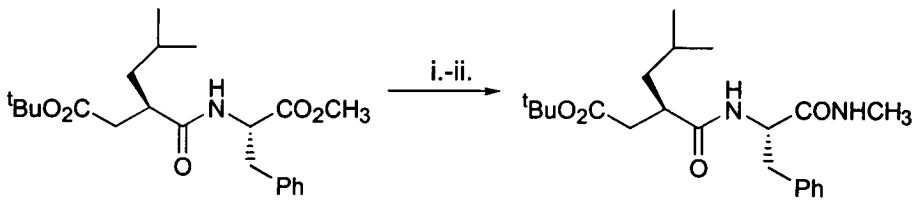
Table 27 *Results obtained for the methanolysis of 2-alkyl-4-substituted-5(4*H*)-oxazolones* ***129b-e***

On examination of the d.e. of each product, the Novozyme® mediated reactions again produced the best optical resolution product. In the case of the indolemethylene side chain, high yield and good d.e. was also obtained with Novozyme®, in contrast to Lipozyme® which produced almost racemic material (entries 5 and 6 respectively). In the case of *tert*-butyl side chain it was decided not to attempt the Novozyme® mediated reaction due to the extremely poor results obtained with the equivalent substrate where the C-2 substituent was a phenyl ring. Instead, the Lipozyme® reaction was investigated with methanol and *n*-butanol as the nucleophile. A d.e. of

72% was obtained for the reaction with methanol, but only a 12% yield (44% based on recovered starting oxazolone) was achieved after 28 days (entry 7). In the case of the *n*-butanol reaction, only 5% conversion was observed by examination of the crude ^{1}H nmr (200 MHz, $CDCl_3$) after 28 days, therefore the reaction was abandoned. If all the d.e. results obtained in Table 27 are compared with there counterparts in Chapter 2, it can be seen that the chiral induction obtained at C-4 in reduced by 10-20% with the introduction of the chiral C-2 substituent. It is speculated that this effect is due to an increase in the steric interactions of the C-2 substituent with the active site of the lipase.

3.6.0. Introduction of methyl amide functionality

The desired MMPI targets **94** all contain a methylamide functionality adjacent to the stereocentre at C-5 (numbered as in Figure 12). Using the biotransformation product (2*R*,2'*S*)-**127a** from Table 25, entry 1, and the previously employed hydrolysis and peptide coupling conditions, (method (ii.)) with methyl amine as the nucleophile, the methyl amide **134** was synthesised in 85% for the two steps. On examination of the resulting ^{1}H nmr (600 MHz) it was discovered that the d.e. had fallen from 81% to 42%. It is hypothesised that the cause of epimerisation was the reformation of the oxazolone **129a**.

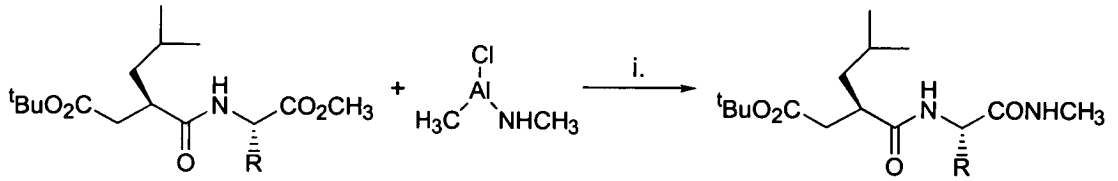


(2*R*,2'*S*)-**127a** **134a** 42% d.e.

Scheme 70 *Reagents and conditions*: i. THF:H_2O (1:1), LiOH, room temp., ii. DMF, HOBt, TBTU, DIPEA, CH_3NH_2.HCl, room temp.

In an attempt to prevent reformation of the oxazolone, the Weinreb[122,123] amide approach was considered. Under Weinreb conditions, the ester (2*R*,2'*S*)-**127a** is reacted with the desired amine in the presence of trimethylaluminium to furnish the diamide **134a** as illustrated in Scheme 71. It has recently been demonstrated that when such conditions were employed in the synthesis of peptides where the amine is

an unprotected α-amino acid, there is a 10-20% racemisation of the chiral centre alpha to the substrate ester. The cause of racemisation is attributed to the formation of the corresponding oxazolone.[124] Unfortunately, due to lack of time, no further attempts to synthesise the desired amide **134a** were possible.



(2*R*,2'*S*)-**127a-e** **134a-e**

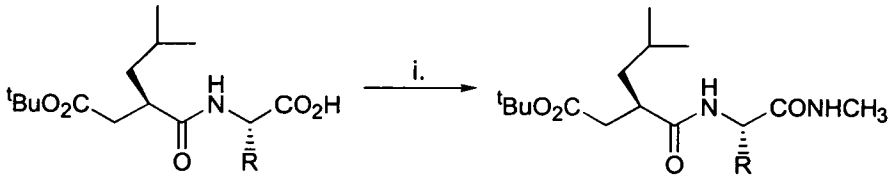
Scheme 71 *Reagents and conditions*: Benzene, reflux

3.7.0. Conclusions

A high yielding synthesis of novel, chiral C-2-substituted-4-substituted-5(4*H*)-oxazolones **129a-e** was achieved, and their application in a dynamic kinetic resolution process under lipase mediated conditions in organic solvent proved successful. Excellent yields as high as 96%, and high diastereomeric excesses up to 86% were achieved for a range of C-4 substituents with the use of Novozyme®. The best results were obtained when the C-2 substituent was either benzyl or *iso*-butyl, again demonstrating the apparent advantage of the incorporation of a methylene spacer into the C-4 side chain to be resolved by the lipase. The choice of solvent also proved important. The use of acetonitrile resulted in a large decrease in the rate of reaction compared to the corresponding reaction in toluene with triethylamine. It is speculated that the decreased reaction rate in acetonitrile is a result of a conformational change in the lipase, tightening the active site channel, thus limiting access to the substrate.

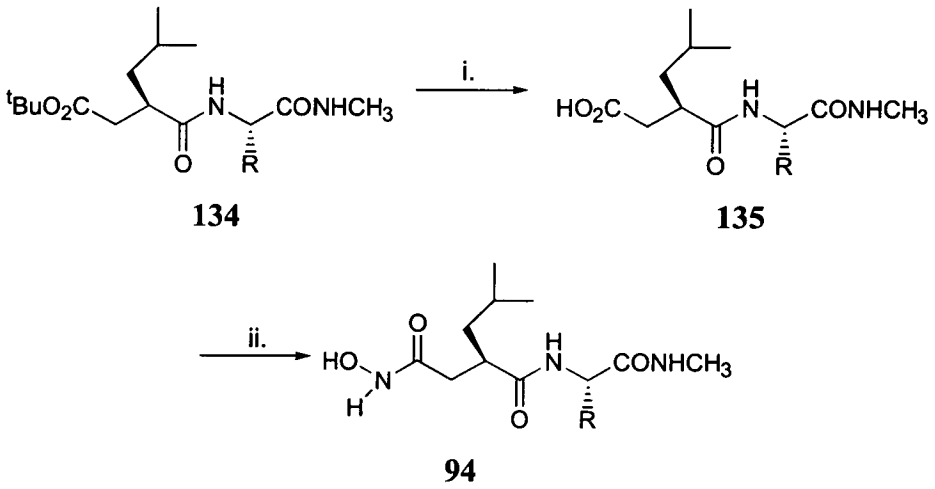
3.8.0. Future work

Obviously future work would involve the completion of the synthesis of the MMPI's. The introduction of the methyl amide bond could be realised utilising an enzyme such as papain or Subtilisin. Instead of using the methyl ester as the substrate more success may be obtained utilising the corresponding acid as depicted in Scheme 72.



Scheme 72 *Reagents and conditions*: i. Solvent, enzyme, CH_3NH_2

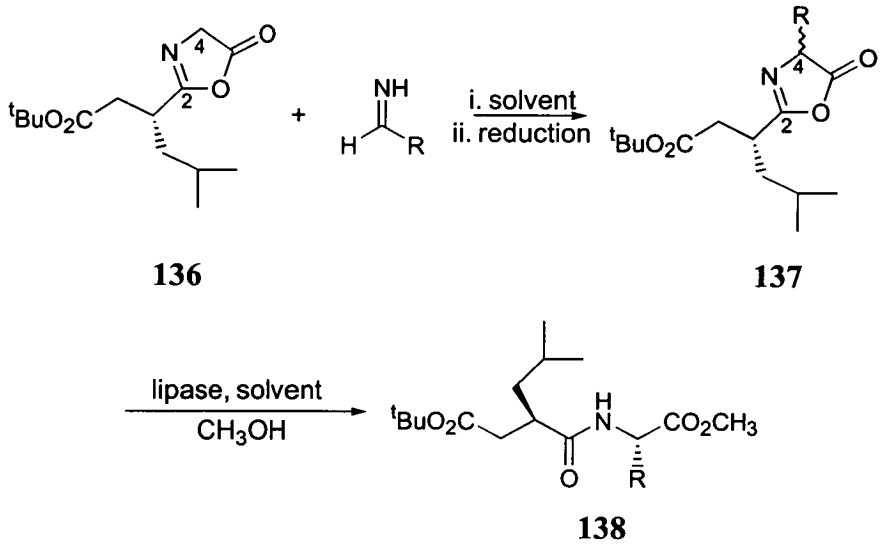
The synthesis of the MMPI's could then be completed using published procedures for the introduction of the hydroxamic acid moiety as illustrated in Scheme 73.[125]



134 **135** **94**

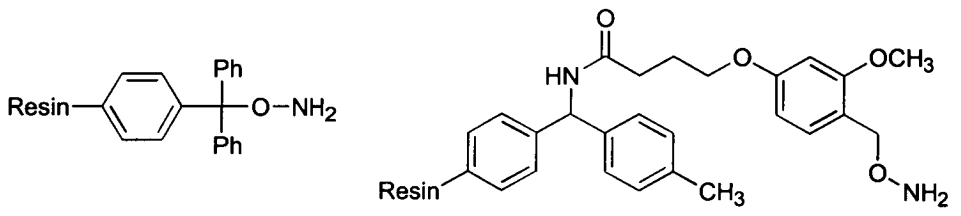
Scheme 73 *reagents and conditions*: i. TFA, room temp., ii. DMF, TBTU, HOBt, hydroxylamine, NMM or (a) BOP, Et_3N, benzyl hydroxylamine, CH_2Cl_2, 0-22 °C, (b) H_2, 5% Pd on $BaSO_4$, CH_3OH, room temp.

On a broader level, the novel 2-substituted-5(4*H*)-oxazolone **136** could be utilised in alkylation/reduction reactions to generate oxazolones **137** with increased diversity at the C-4 position as discussed in Chapter 2. Subsequent dynamic kinetic resolution would furnish potentially new MMPI's. The approach outlined in Scheme 74 could also be used to generate a combinatorial library of potential MMPI's on solid support. The *tert*-butyl ester functionality of oxazolone **136** could be used to tether a linker and resin. *O*-Hydroxyamines **139**[126] and **140**[127] have recently been used as linkers to solid supports; cleavage resulting in the formation of the desirable hydroxamic acids.



136 **137** **138**

Scheme 74 *Proposed synthesis of novel MMPI's*



139 **140**

4.0.0. Experimental

4.1.0. General experimental

^{1}H and ^{13}C nmr were recorded on Bruker AC 200, Varian Gemini 200, Bruker AC 250, Bruker WH 360 or Varian UNITY INOVA 600 instruments. Chemical shifts (δ_H, δ_C) are reported in ppm and coupling constants (J) are in Hertz (Hz). Chemical shifts were referenced to residual undeuterated solvent present in the deuterated sample, *i.e.* $CHCl_3$ in $CDCl_3$.

Electron Impact (EI) and Chemical Ionisation (CI) mass spectrometry were carried out on a Finnegan 4500 mass spectrometer. Fast atom bombardment (FAB) was performed on a Kratos MS50TC.

Infra-red spectra were recorded on a Biorad FTS-7 or a Perkin Elmer Paragon 1000 FT-IR spectrophotometer with the frequencies (ν) measured in wavenumbers (cm^{-1}). Samples were measured on disposable IR Cards (Type 61 3M, polyethylene, 19 mm aperture), in $CHCl_3$ or as nujol mulls.

Melting points were measured on a Gallenkamp melting point apparatus, and are quoted in °C and uncorrected.

Chiral HPLC analysis was carried out using a Waters 486 Tunable Absorbance Detector and a Waters 600E Pump and Controller. Waters Millennium Chromatography Manager software package was used to analyse the results. A Chiracel OD column was used as the stationary phase eluting with hexane:*iso*-propanol (9:1) with a flow rate of 0.5 mL min^{-1} unless otherwise stated. Retention times (R_t) are quoted in minutes.

Elemental analysis (CHN) was performed on a Perkin Elmer 2400 CHN Elemental Analyser.

Optical rotations were measured on an Optical Activity AA-1000 polarimeter (sodium 589 nm detection). Sample concentration was measured in g/100 mL and $[\alpha]_D^{20}$ are quoted in 10^{-1} deg cm^2 g^{-1}.

Thin layer chromatography (tlc) was carried out on 2.5 mm glass plates coated with silica gel 60 F254, with detection by UV (254 nm) fluorescence, ammonium molybdate, bromocresol, ninhydrin or potassium permanganate dips. Chromatography was carried out using silica gel 60 (Merck 9385).

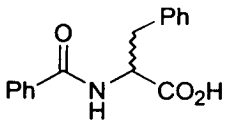
All reagents were used as supplied from commercial sources unless stated. Lipozyme® and Novozyme® were received as gifts from Novo-Nordisk and the Fluka Lipase Basic Kit was a gift from Fluka. Dichloromethane was distilled from calcium hydride while tetrahydrofuran was pre dried over sodium wire and distilled from sodium benzophenone ketal. Toluene was dried over sodium wire for 24 h before use. *n*-Butanol was dried, and distilled from sodium metal and stored over 4 Å molecular sieves. Novozyme® was crushed with a mortar and pestle and dried to constant weight over phosphorus pentoxide.

4.2.0. Development of 5(4*H*)-oxazolone methodology

4.2.1. General procedure for *N*-benzoyl DL-amino acids 95a-g

Benzoyl chloride (1.05 equiv.) and aqueous sodium hydroxide (2M, 100 mL) were simultaneously added to a stirring solution of DL-amino acid (1.00 equiv.) in aqueous sodium hydroxide (2M, 10 mL per 1.00 g of amino acid) at 0 °C over a period of 10 min. The resulting solution was stirred at room temperature for 30 min. The reaction mixture was cooled to -10 °C and conc. hydrochloric acid added until precipitation was complete. The mixture was stirred for a further 1 h, filtered, dried, and recrystallised from ethanol:water (2:1) to furnish the desired product as a colourless solid.

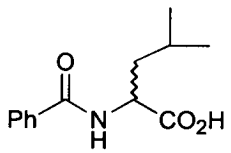
4.2.2. *N*-Benzoyl-DL-phenylalanine 95a



The general procedure outlined above **(4.2.1)** with DL-phenylalanine (10.00 g, 60.0 mmol) and benzoyl chloride (7.38 mL, 63.6 mmol, 1.05 equiv.) was followed and gave the title compound as a colourless solid (12.1 g, 74%).

Mp 186-188 °C (EtOH:water), Lit.[128] 187-189 °C; υ_{max}(nujol)/cm^{-1} 3322 (NH), 2800-2500 (acid OH), 1717 (acid C=O), 1612, 1536 (CONH); δ_H ($(CD_3)_2SO$; 200 MHz), 12.76 (1 H, br s, O<u>H</u>), 8.73 (1 H, d, *J* 8.0, N<u>H</u>), 7.83-7.77 (2 H, m, C<u>H</u>$_{ar}$), 7.71-7.41 (3 H, m, C<u>H</u>$_{ar}$), 7.31-7.12 (5 H, m, C<u>H</u>$_{ar}$), 4.60 (1 H, ddd, *J* 11.0, 8.0 and 5.0, NHC<u>H</u>), 3.18 (1 H, dd, *J* 16.5, 11.0, C<u>H</u>$_A$H$_B$Ph), 3.08 (1 H, dd, *J* 16.5, 5.0, CH$_A$<u>H</u>$_B$Ph); δ_C ($(CD_3)_2SO$; 63 MHz) 173.3, 166.6 (<u>C</u>O$_2$H, <u>C</u>ONH), 138.9, 134.1 (*ipso*-Ar), 131.5, 129.2, 128.4, 128.3, 127.5, 126.5 (<u>C</u>H$_{ar}$), 54.4 (HN<u>C</u>H), 36.4 (<u>C</u>H$_2$Ph); *m/z* (FAB) 270 (90%, MH$^+$), 224 (82, M-CO$_2$H), 105 (85, 224-NHCHCH$_2$Ph), 77 (72, 105-CO).

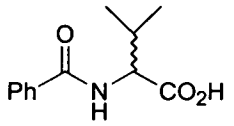
4.2.3. *N*-Benzoyl-DL-leucine 95b



The general procedure outlined above **(4.2.1)** with DL-leucine (10.00 g, 76 mmol) and benzoyl chloride (9.29 mL, 80 mmol, 1.05 equiv.) was followed and gave the title compound as a colourless solid (14.07 g, 78%).

Mp 140-142 °C, (EtOH:water), Lit.[129] 141-143°C; υ_{max}(nujol)/cm^{-1}, 3271 (NH), 2800-2500 (acid OH), 1720 (acid C=O), 1634, 1536 (CONH); δ_H ($(CD_3)_2SO$; 200 MHz) 8.59 (1 H, d, *J* 8.0, <u>N</u>H), 7.92-7.86 (2 H, m, C<u>H</u>$_{ar}$), 7.58-7.42 (3 H, m C<u>H</u>$_{ar}$), 4.44 (1 H, m, NHC<u>H</u>), 1.85-1.55 (3 H, m, C<u>H</u>$_2$CH(CH$_3$)$_2$ and CH$_2$C<u>H</u>(CH$_3$)$_2$), 0.92 (3 H, d, *J* 6.0, C<u>H</u>$_{3A}$CHCH$_{3B}$), 0.88 (3 H, d, *J* 6.0, CH$_{3A}$CHC<u>H</u>$_{3B}$); δ_C ($(CD_3)_2SO$; 63 MHz) 174.4, 166.6 (<u>C</u>O$_2$H, <u>C</u>ONH), 134.1 (*ipso*-Ar), 131.5, 128.3, 127.6 (<u>C</u>H$_{ar}$), 51.0 (HN<u>C</u>H), 24.7 (<u>C</u>H(CH$_3$)$_2$) 23.1, 21.3 (CH(<u>C</u>H$_3$)$_2$); *m/z* (FAB), 236 (94%, MH$^+$), 190 (82, M-CO$_2$H,), 105 (94, 190-NHCHCH$_2$CH(CH$_3$)$_2$,), 77 (76, 105-CO,).

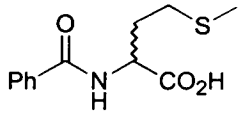
4.2.4. *N*-Benzoyl-DL-valine 95c



The general procedure outlined above **(4.2.1)** with DL-valine (10.00 g, 85 mmol) and benzoyl chloride (10.4 mL, 87 mmol, 1.05 equiv.) was followed and gave the title compound as a colourless solid (15.75 g, 83%).

Mp 127-129 °C, (EtOH:water), Lit.[130] 129.5-130.5 °C; υ_{max}(nujol)/cm^{-1} 3330 (OH), 2800-2475 (acid OH), 1727 (acid C=O), 1626, 1537 (CONH); δ_H, ($(CD_3)_2SO$; 250 MHz) 12.51 (1 H, br s, O<u>H</u>), 8.44 (1 H, d, *J* 8.0, N<u>H</u>), 7.89 (2 H, d, *J* 8.0, C<u>H</u>$_{ar}$), 7.57-7.43 (3 H, m, C<u>H</u>$_{ar}$), 4.28 (1 H, dd, *J* 8.0, 7.0, NHC<u>H</u>), 2.19 (1 H, dq, *J* 7.0, C<u>H</u>$(CH_3)_2$), 0.97 (3 H, d, *J* 7.0, C<u>H</u>$_{3A}$CHC<u>H</u>$_{3B}$) 0.95 (3 H, d, *J* 7.0, CH_{3A}CHC<u>H</u>$_{3B}$); δ_C ($(CD_3)_2SO$; 63 MHz) 173.3, 167.1 (<u>C</u>O_2H, <u>C</u>ONH), 134.3 (*ipso*-Ar), 131.4 128.3, 127.8 (<u>C</u>H$_{ar}$), 58.5 (HN<u>C</u>H), 29.6 (<u>C</u>H$(CH_3)_2$, 19.5 18.9 (2 x <u>C</u>H_3); m/z (FAB) 222 (80%, MH^+), 176 (97, M-CO_2H, 97), 105 (100, 176-NHCHCHCH$_3$), 77 (88, 105-CO).

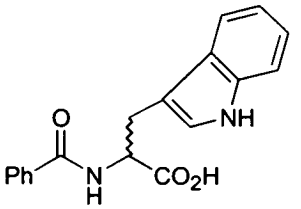
4.2.5. *N*-Benzoyl-DL-methionine 95d



The general procedure outlined above **(4.2.1)** with DL-methionine (10.00 g, 67 mmol) and benzoyl chloride (8.17 mL, 70 mmol, 1.05 equiv.) was followed and gave the title compound as a colourless solid (12.78 g, 75%).

Mp 146-148 °C, (EtOH:water), Lit.[131] 146-149 °C; υ_{max}(nujol)/cm^{-1} 3320 (NH), 2800-2500 (acid OH), 1717 (acid C=O), 1626, 1537 (CONH); δ_H ($(CD_3)_2SO$; 200 MHz) 8.63 (1 H, d, *J* 8.0, NH), 7.90-7.84 (2 H, m, C<u>H</u>$_{ar}$), 7.53-7.41 (3 H, m, C<u>H</u>$_{ar}$), 4.51 (1 H, ddd, *J* 8.0, 7.5, 7.0, NHC<u>H</u>), 2.59-2.47 (2 H, m partially obscured by DMSO peak, C<u>H</u>$_2$C<u>H</u>$_2$S), 2.05 (2 H, dd, *J* 7.5, C<u>H</u>$_2$S), 2.03 (3H, s, SC<u>H</u>$_3$); δ_C ($(CD_3)_2SO$; 63 MHz) 173.6, 166.9 (<u>C</u>O_2H, <u>C</u>ONH), 134.1 (*ipso*-Ar), 131.6, 128.4, 127.6 (<u>C</u>H$_{ar}$), 51.8 (HN<u>C</u>H), 30. 4, 30.3 (<u>C</u>H$_2$<u>C</u>H$_2$S), 14.7 (<u>C</u>H_3); *m/z* (FAB) 254 (100%, MH^+), 206 (26, M-SCH_3), 105 (85, 206-NHCH$(CH_2CH_2SCH_3)CO_2$H), 77 (50, 105-CO).

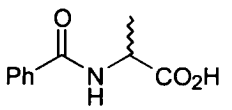
4.2.6. N^{α}-Benzoyl-DL-tryptophan 95e



The general procedure outlined above **(4.2.1)** with DL-tryptophan (7.70 g, 37.7 mmol) and benzoyl chloride (4.60 mL, 40 mmol, 1.05 equiv.) was followed and gave the title compound as a colourless solid (8.85 g, 76%).

Mp 192-194 °C, (EtOH:water), Lit.[132] 192-193 °C; υ_{max}(nujol)/cm^{-1} 3392 (indole NH), 3359 (amide NH), 2800-2500 (acid OH), 1729 (acid C=O), 1628, 1547 (CONH); δ_H ($(CD_3)_2SO$; 250 MHz); 10.85 (1 H, d, *J* 2.0, N$\underline{H}_{indole}$), 8.67(1 H, d, *J* 8.0, N$\underline{H}_{amide}$), 7.86-7.80 (2 H, m, C$\underline{H}_{ar}$), 7.62-7.59 (1 H, m, C$\underline{H}_{ar}$), 7.56-7.31 (4 H, m, C$\underline{H}_{ar}$), 7.22 (1 H, d, *J* 2.5, C$\underline{H}_{ar}$), 7.10-6.96 (2 H, m, C$\underline{H}_{ar}$), 4.68 (1H, ddd, *J* 10.0, 8.0, 5.0, NHC$\underline{H}$), 3.31 (1 H, dd, *J* 14.5, 5.0, C$\underline{H}_A$H$_B$Indole), 3.23 (1 H, dd, *J* 14.5, 10.0, CH$_A\underline{H}_B$Indole); δ_C ($(CD_3)_2SO$; 63 MHz) 173.8, 166.6 ($\underline{C}O_2H$, $\underline{C}$ONH), 136.3, 134.1 (*ipso*-Ar), 131.4, 128.4, 127.5 ($\underline{C}H_{ar}$), 127.3 (*ipso*-Ar), 123.7, 121.1, 118.5, 118.3, 111.6 ($\underline{C}H_{ar}$), 110.6 (*ipso*-Ar), 53.9 (HN$\underline{C}$H), 26.8 ($\underline{C}H_2$); m/z (FAB) 331 (100%,[M+Na]$^+$), 309 (57, MH$^+$), 263 (51, M-CO$_2$H), 105 (62, 263-NHCHCH$_2$Indole), 77 (54, 105-CO).

4.2.7. *N*-Benzoyl-DL-alanine 95f

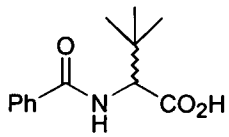


The general procedure outlined above **(4.2.1)** with DL-alanine (10.00 g, 112 mmol) and benzoyl chloride (13.7 mL, 118 mmol, 1.05 equiv.) was followed and gave the title compound as a colourless solid (15.28 g, 70%).

Mp 164-165 °C, (EtOH:water), 165-166 °C[133]; υ_{max}(nujol)/cm^{-1} 3367 (NH), 2800-2500 (acid OH), 1723 (acid C=O), 1662, 1546 (CONH); δ_H ($(CD_3)_2SO$; 200 MHz) 8.65 (1 H, d, *J* 7.5, N$\underline{H}$), 7.89-7.84 (2 H, m, C$\underline{H}_{ar}$), 7.53-7.40 (3H, m, C$\underline{H}_{ar}$), 4.43 (1 H, dq, *J* 7.5, NHC$\underline{H}$), 1.37 (3 H, d, *J* 7.5 C$\underline{H}_3$); δ_C ($(CD_3)_2SO$; 63 MHz) 174.4, 166.4 ($\underline{C}O_2H$, $\underline{C}$ONH), 134.1 (*ipso*-Ar), 131.6, 128.4, 127.6 ($\underline{C}H_{ar}$), 48.3 (HN$\underline{C}$H), 14.7 (

$\underline{C}H_3$); m/z (FAB) 194 (100%, MH^+), 148 (35, M-CO_2H), 105 (97, 148-$NHCHCH_3$), 77 (97, 105-CO).

4.2.8. *N*-Benzoyl-DL-*tert*-leucine 95g



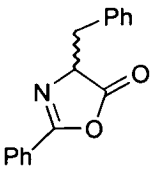
The general procedure outlined above **(4.2.1)** with DL-*tert*-leucine (1.00 g, 7.6 mmol) and benzoyl chloride (1.43 mL, 8.4 mmol, 1.10 equiv.) was followed with recrystallisation from ethanol:water (1:1) and furnished the title compound as a colourless solid (1.46 g, 81%).

Mp 165-166 °C, (EtOH:water), Lit.[134] 165-166 °C; υ_{max}(nujol)/cm^{-1} 3362(NH), 1724 (acid C=O), 1625, 1540 (CONH); δ_H ($(CD_3)_2SO$; 200 MHz) 8.13 (1 H, d, *J* 8.0, N$\underline{H}$), 7.86-7.81 (2 H, m, C$\underline{H}_{ar}$), 7.56-7.39 (3 H, m, C$\underline{H}_{ar}$), 4.34 (1 H, d, *J* 8.0, NHC$\underline{H}$), 1.02 (9 H, s, C$\underline{H}_3$); δ_C ($(CD_3)_2SO$; 63 MHz) 172.6, 167.2 ($\underline{C}O_2H$, $\underline{C}ONH$), 134.4 (*ipso*-Ar), 131.4, 128.3, 127.8 ($\underline{C}H_{ar}$), 61.1 (HN$\underline{C}$H), 33.7 ($\underline{C}(CH_3)_3$), 27.0 ($C(\underline{C}H_3)_3$); m/z (FAB) 236 (100%, MH^+), 190 (64, M-CO_2H), 105 (34, 190-$NHCHC(CH_3)_3$), 77 (11, 105-CO).

4.2.9. General procedure for (*SR*)-2-phenyl-4-substituted-5(4*H*)-oxazolones 89a-g

A solution of *N*-benzoyl DL-amino acid in 1,4-dioxane:acetic anhydride (1:1, 10 mL per 1.00 g of *N*-benzoyl amino acid) was stirred at 22-60 °C until a clear solution was obtained. On cooling, the solution was filtered and concentrated under reduced pressure. The excess acetic anhydride was co-evaporated with toluene to give the crude product which was purified by recrystallisation or column chromatography as described.

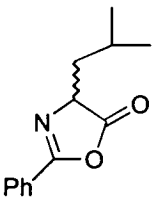
4.2.10. (*SR*)-2-Phenyl-4-benzyl-5(4*H*)-oxazolone 89a



The general procedure outlined above **(4.2.9.)** with *N*-benzoyl-DL-phenylalanine (5.17 g, 19.2 mmol) was followed with recrystallisation from hexane:diethyl ether (1:1) to give the title compound as a colourless solid (4.30 g, 89%).

R_f (Hexane:EtOAc, 4:1) 0.62; Mp 67-69 °C, Lit.[135] 68-70 °C; $\upsilon_{max}(CHCl_3)/cm^{-1}$ 1819 (C=0), 1652 (C=N); δ_H ($CDCl_3$; 250 MHz) 7.94 (2 H, m, C$\underline{H}_{ar}$), 7.58-7.40 (3 H, m, C$\underline{H}_{ar}$), 7.29-7.20 (5 H, m, C$\underline{H}_{ar}$), 4.68 (1 H, dd, *J* 6.5, 5.0, C$\underline{H}CH_2$), 3.32 (1 H, dd, *J* 14.0, 5.0, C$\underline{H}_A H_B$Ph), 3.25 (1 H, dd, *J* 14.0, 6.5, $CH_A\underline{H}_B$Ph); δ_C ($CDCl_3$; 63 MHz) 177.3 (C=O), 161.7 (C=N), 135.0 (*ipso*-Ar), 132.7, 129.4, 128.6, 128.3, 127.8, 127.1 (CH_{ar}), 125.5 (*ipso*-Ar), 66.3 (CH), 37.1 (CH_2Ph); *m/z* (FAB) 252 (100%, MH^+), 224 (49, MH^+-CO).

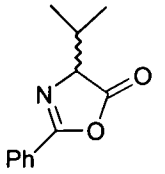
4.2.11. (*SR*)-2-Phenyl-4-*iso*-butyl-5(4*H*)-oxazolone 89b



The general procedure outlined above **(4.2.9.)** with *N*-benzoyl-DL-leucine (2.10 g, 8.9 mmol) was followed with recrystallisation from hexane:diethyl ether (1:1) to give the title compound as a colourless solid (1.58 g, 81%).

R_f (Hexane:EtOAc, 8:1) 0.66; Mp 54-55 °C, Lit.[135] 54-56 °C; υ_{max}(nujol)/cm^{-1} 1812 (C=O), 1653 (C=N); δ_H ($CDCl_3$; 200 MHz) 8.02-7.97 (2 H, m, C$\underline{H}_{ar}$), 7.61-7.43 (3 H, m, C$\underline{H}_{ar}$), 4.41 (1 H, dd, *J* 9.0, 5.5, NC$\underline{H}$), 2.06 (1 H, ddq, *J* 7.0, 6.5, 6.0, C$\underline{H}(CH_3)_2$), 1.84 (1 H, ddd, *J* 13.5, 7.0, 5.5, CHC$\underline{H}_A H_B$), 1.69 (1 H, ddd, *J* 13.5, 9.0, 6.5, $CHCH_A\underline{H}_B$), 1.03 (3 H, d, *J* 6.5, C$\underline{H}_{3A}$CHCH_{3B}), 1.00 (3 H, d, *J* 6.5, $CH_3CH\underline{CH}_{3B}$); δ_C ($CDCl_3$; 63 MHz) 178.9 (C=O), 161.2 (C=N), 132.5, 128.6, 127.7, (CH_{ar}), 125.9 (*ipso*-Ar) 63.8 (NCH), 40.7 ($CH\underline{C}H_2$), 25.1 ($C\underline{H}(CH_3)_2$), 22.6, 21.9 (2x $\underline{C}H_3$); *m/z* (FAB) 218 (12%, MH^+).

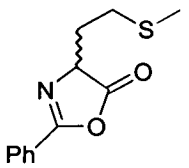
4.2.12. (*SR*)-2-Phenyl-4-*iso*-propyl-5(4*H*)-oxazolone 89c



The general procedure outlined above **(4.2.9.)** with *N*-benzoyl-DL-valine (2.00 g, 9.0 mmol) was followed with recrystallisation from hexane:diethyl ether (1:1) to give the title compound as a colourless solid (1.74 g, 95%).

R_f (Hexane:EtOAc, 4:1) 0.61; Mp 48-51 °C, Lit.[135] 48-51 °C; $\upsilon_{max}(CHCl_3)/cm^{-1}$ 1821 (C=O), 1652 (C=N); δ_H ($CDCl_3$; 200 MHz) 8.05-7.99 (2 H, m, C<u>H</u>$_{ar}$), 7.61-7.44 (3 H, m, C<u>H</u>$_{ar}$), 4.29 (1 H, d, *J* 4.5, NC<u>H</u>), 2.39 (1 H, dqq, *J* 7.0, 4.5, C<u>H</u>$(CH_3)_2$), 1.15 (3 H, d, *J* 7.0 C<u>H</u>$_{3A}$CHCH_{3B}), 1.12 (3 H, d, *J* 7.0 CH_{3A}CHC<u>H</u>$_{3B}$); δ_C ($CDCl_3$; 63 MHz) 177.7 (<u>C</u>=O), 161.5 (<u>C</u>=N), 132.5, 128.6, 127.7, (<u>C</u>H$_{ar}$), 125.8 (*ipso*-Ar) 70.6 (N<u>C</u>H), 31.1 (NCH<u>C</u>H), 18.6, 17.4 (2 x <u>C</u>H_3); *m/z* (FAB) 204 (74%, MH^+), 176 (47, MH^+-CO).

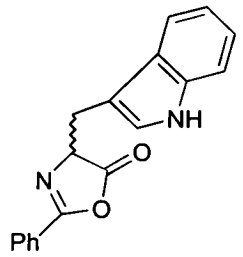
4.2.13. (*SR*)-2-Phenyl-4-(2-methylsulfanyl-ethyl)-5(4*H*)-oxazolone 89d



The general procedure outlined above **(4.2.9.)** with *N*-benzoyl-DL-methionine (2.50 g, 9.9 mmol) was followed and the crude product purified by column chromatography, eluting with hexane:diethyl ether (7:1) to give the title compound as a colourless oil (1.31 g, 56%). Spectroscopic data was in agreement with previously reported results.[136]

R_f (Hexane:EtOAc, 1:1) 0.40; $\upsilon_{max}(CHCl_3)/cm^{-1}$ 1825 (C=O), 1653 (C=N); δ_H ($CDCl_3$; 200 MHz) 8.02-7.99 (2 H, m, C<u>H</u>$_{ar}$), 7.98-7.43 (3H, m, C<u>H</u>$_{ar}$), 4.69 (1 H, dd, *J* 7.5, 6.0, NC<u>H</u>), 2.74 (2 H, t, *J* 7.0, C<u>H</u>$_2$S), 2.29 (1 H, ddd, *J* 14.5, 7.0, 6.0, CHC<u>H</u>$_A$H$_B$CH$_2$), 2.22 (1H, ddd, *J* 14.5, 7.5, 7.0, CHCH$_A$<u>H</u>$_B$CH$_2$), 2.11 (3 H, s, SC<u>H</u>$_3$); δ_C ($CDCl_3$; 63 MHz) 178.26 (<u>C</u>=O), 161.8 (<u>C</u>=N), 132.6, 128.6, 127.7, (<u>C</u>H$_{ar}$), 125.6 (*ipso*-Ar) 63.5 (N<u>C</u>H), 30.2, 29.9 (<u>C</u>H$_2$<u>C</u>H$_2$S), 14.9 (<u>C</u>H$_3$); *m/z* (FAB) 236 (42%, MH^+), 208 (32, MH^+-CO).

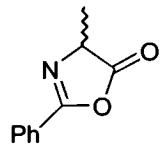
4.2.14. (*SR*)-2-Phenyl-4-(1*H*-indol-3-ylmethyl)-5(4*H*)-oxazolone 89e



The general procedure outlined above **(4.2.9.)** with *N*-benzoyl-DL-tryptophan (2.00 g, 6.5 mmol) was followed with recrystallisation from ethyl acetate to give the title compound as a colourless solid (669 mg, 36%).

R_f (Hexane:EtOAc, 1:1) 0.75; Mp139-142 °C, Lit.[137] 142 °C; $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3477 (NH), 3018 (CH_{ar}), 1817 (C=O), 1651 (C=N); δ_H ($CDCl_3$; 200 MHz) 8.05 (1 H, br s, $N\underline{H}_{indole}$) 7.89 (2 H, d, *J* 7.0, $C\underline{H}_{Ph}$), 7.74-7.70 (1 H, m, $C\underline{H}_{indole}$), 7.56-7.37 (3 H, m, $C\underline{H}_{Ph}$), 7.3-7.25 (2 H, m, $C\underline{H}_{indole}$), 7.18-7.08 (2 H, m, $C\underline{H}_{indole}$), 4.76 (1 H, dd, *J* 6.0, 5.5, NC$\underline{H}$), 3.51 (1 H, dd, *J* 15.0, 5.5, CHC$\underline{H}_A$H$_B$), 3.42 (1H, dd, *J* 15.0, 6.0, CHCH$_A\underline{H}_B$); δ_C ($CDCl_3$; 63 MHz) 177.9 ($\underline{C}$=O), 161.6 ($\underline{C}$=N), 135.7 (*ipso*-Ar), 132.4, 128.5, 127.65 ($\underline{C}H_{ar}$), 127.2, 125.6 (*ipso*-Ar), 123.3, 121.8, 119.3, 118.9, 110.9 ($\underline{C}H_{ar}$), 66.4 (N$\underline{C}$H), 27.1 ($\underline{C}H_2$); *m/z* (FAB) 291 (67%, MH^+), 263 (33, MH-CO), 130 (100, CH_2Indole), 105 (51 (PhCO).

4.2.15. (*SR*)-2-Phenyl-4-methyl-5(4*H*)-oxazolone 89f

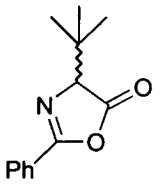


The general procedure outlined above **(4.2.9.)** with *N*-benzoyl-DL-alanine (2.00 g, 10.3 mmol) was followed and the crude product purified by column chromatography, eluting with hexane:ethyl acetate (4:1) to give the title compound as a colourless solid (1.79 g, 99%).

R_f (Hexane:EtOAc, 4:1) 0.52; Mp 36 °C, Lit.[135] 37-38 °C; $\upsilon_{max}(CHCl_3)/cm^{-1}$ 1825 (C=O), 1653 (C=N);δ_H ($CDCl_3$; 200 MHz) 8.02-7.96 (2 H, m $C\underline{H}_{ar}$), 7.61-7.43 (3 H, m, $C\underline{H}_{ar}$), 4.44 (1 H, q, *J* 7.5, NC$\underline{H}$), 1.58 (3 H, d, *J* 7.5, $C\underline{H}_3$); δ_C ($CDCl_3$; 63 MHz) 178.6 ($\underline{C}$=O), 161.3 ($\underline{C}$=N), 132.5, 128.5, 127.6 ($\underline{C}H_{ar}$), 125.4 (*ipso*-Ar), 60.7 ($\underline{C}$H),

16.6 (CH_3); *m/z* (FAB) 176 (31%, MH^+), 148 (11, MH^+-CO), 105 (100, 148-NHCHCH_3).

4.2.16. (*SR*)-2-Phenyl-4-*tert*-butyl-5(4*H*)-oxazolone 89g



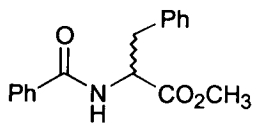
The general procedure outlined above **(4.2.9.)** with *N*-benzoyl-DL-*tert*-leucine (1.25 g, 5.3 mmol) was followed and the crude product purified by column chromatography, eluting with hexane:diethyl ether (14:1) to give the title compound as a colourless solid (0.94 g, 82%).

R_f (Hexane:EtOAc, 6:1) 0.70; Mp 72-73 °C, Lit.[134] 73-74 °C; $\upsilon_{max}(CHCl_3)/cm^{-1}$; 1820 (C=O), 1656 (C=N)$\delta_H$ ($CDCl_3$; 200 MHz) 8.01 (2 H, d, *J* 7.0, CH_{ar}), 7.60-7.43 (3 H, m, CH_{ar}), 4.07 (1 H, s, NCH), 1.13 (9 H, s, CH_3); δ_C ($CDCl_3$; 63 MHz) 176.8 (C=O), 161.1 (C=N), 132.5, 128.6, 127.8 (CH_{ar}), 125.9 (*ipso*-Ar), 73.9 (NCH), 35.8 (C$(CH_3)_3$), 26.1 (C$(CH_3)_3$); *m/z* (FAB) 218 (56%, MH^+), 190 (24, MH^+-CO), 105 (100, 190-NHCHC$(CH_3)_3$).

4.2.17. General procedure for *N*-benzoyl-DL-amino acid esters

To a solution of (*RS*)-2-phenyl-4-substituted-5(4*H*)-oxazolones **89a-g** (100 mg) in alcohol (5 mL) was added a catalytic volume of conc. hydrochloric acid (30 μL). The solution was heated under reflux overnight, cooled and the solvent removed under reduced pressure. The crude product was purified by column chromatography as described, furnishing the desired ester as a colourless solid unless stated.

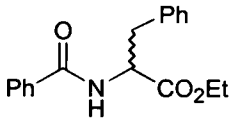
4.2.18. *N*-Benzoyl-DL-phenylalanine methyl ester 96a



The general procedure outlined above **(4.2.17.)** with oxazolone **89a** in methanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (4:1), (96 mg, 85%).

R_f (Hexane:EtOAc, 6:1) 0.12; R_t 19.57 and 25.72; Mp 85-87 °C, Lit.[138] 86.5-87.5 °C; $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3410 (NH), 3029 (CH_{ar}) 1740 ester C=O), 1644, 1526 (CONH); δ_H ($CDCl_3$; 250 MHz) 7.74-7.70 (2 H, m, C<u>H</u>$_{ar}$), 7.53-7.28 (3 H, m, C<u>H</u>$_{ar}$), 7.27-7.21 (3 H, m, C<u>H</u>$_{ar}$), 7.15-7.11 (2 H, m, C<u>H</u>$_{ar}$), 6.63 (1 H, d, *J* 7.0, N<u>H</u>), 5.09 (1 H, ddd, *J* 7.0, 6.0, 5.5, NHC<u>H</u>), 3.75 (3 H, s, OC<u>H</u>$_3$), 3.29 (1 H, dd, *J* 14.0, 6.0, CHC<u>H</u>$_A$CH$_B$), 3.23 (1 H, dd, *J* 14.0, 5.5, CHCH$_A$C<u>H</u>$_B$); δ_C ($CDCl_3$; 63 MHz) 171.9, 166.7 (<u>C</u>O$_2$CH$_3$, <u>C</u>ONH), 135.7 133.7 (*ipso*-Ar), 131.6, 129.1, 128.4, 127.0, 126.8 (<u>C</u>H$_{ar}$), 53.4 (HN<u>C</u>H), 52.3 (O<u>C</u>H$_3$), 37.7 (<u>C</u>H$_2$Ph); *m/z* (FAB) 284 (68%, MH^+), 268 (13, M-Me), 224 (73, 268-CO_2), 105 (100, 224-NHCHCH$_2$Ph), 77 (37, 105-CO).

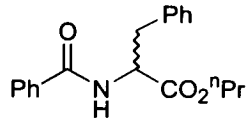
4.2.19. *N*-Benzoyl-DL-phenylalanine ethyl ester 98a



The general procedure outlined above **(4.2.17.)** with oxazolone **89a** in ethanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (4:1), (96 mg, 85%).

R_f (Hexane:EtOAc, 6:1) 0.18; R_t 12.13 and 16.78; Mp 90-92 °C, Lit.[139] 94-95 °C; $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3321 (NH), 3029 (CH_{ar}), 1740 (ester C=O), 1641, 1534 (CONH); δ_H ($CDCl_3$; 250 MHz) 7.75-7.70 (2 H, m, C<u>H</u>$_{ar}$), 7.53-7.37 (3 H, m, C<u>H</u>$_{ar}$) 7.32-7.20 (3 H, m, C<u>H</u>$_{ar}$), 7.16-7.12 (2 H, m, C<u>H</u>$_{ar}$), 6.65 (1 H, d, *J* 7.0, N<u>H</u>), 5.06 (1 H, ddd, *J*, 6.0, 5.5, NHC<u>H</u>), 4.22 (2 H, q, *J* 7.5, OC<u>H</u>$_2$), 3.28 (1 H, dd, *J* 14.0, 6.0, C<u>H</u>$_A$CH$_B$Ph), 3.24 (1 H, dd, *J* 14.0, 5.5, CH$_A$C<u>H</u>$_B$Ph); 1.28 (3 H, t, *J* 7.0, CH$_2$C<u>H</u>$_3$); δ_C ($CDCl_3$; 63 MHz) 171.4, 166.6 (<u>C</u>O$_2$CH$_2$, <u>C</u>ONH), 135.7 133.8 (*ipso*-Ar), 131.6, 129.2, 128.4, 128.4, 127.0, 126.8 (<u>C</u>H$_{ar}$), 61.5 (O<u>C</u>H$_2$), 53.4 (HN<u>C</u>H), 37.7 (<u>C</u>H$_2$Ph), 14.0 (CH$_2$<u>C</u>H$_3$); *m/z* (FAB) 298 (49%, MH^+), 252 (30, M-OEt), 224 (73, 252-CO), 105 (100, 224-NHCHCH$_2$Ph), 77 (45, 105-CO).

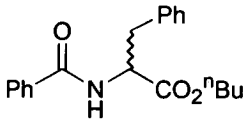
4.2.20. *N*-Benzoyl-DL-phenylalanine propyl ester 99a



The general procedure outlined above **(4.2.17.)** with oxazolone **89a** in *n*-propanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (8:1), (109 mg, 88%).

R_f (Hexane:EtOAc, 8:1) 0.18; R_t 11.67 and 17.47; Mp 69-69 °C; $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3332 (NH), 3030 (CH_{ar}), 1736 (ester C=O), 1647, 1522 (CONH); δ_H ($CDCl_3$; 200 MHz) 7.75 (2 H, m, C<u>H</u>$_{ar}$), 7.55-7.40 (3 H, m, C<u>H</u>$_{ar}$), 7.38-7.23 (3 H, m, C<u>H</u>$_{ar}$), 7.21-7.12 (2 H, m, C<u>H</u>$_{ar}$), 6.6 (1 H, d, *J* 7.0, N<u>H</u>), 5.08 (1 H, ddd, *J* 7.0, 6.0, 5.5, NHC<u>H</u>), 4.11 (1 H, dt, *J* 10.5, 6.5, OC<u>H</u>$_A$H$_B$CH$_2$), 4.10 (1 H, dt, *J* 10.5, 6.5, OCH$_A$<u>H</u>$_B$CH$_2$). 3.28 (1 H, dd, *J* 13.5, 6.0, C<u>H</u>$_A$H$_B$Ph), 3.24 (1 H, dd, *J* 13.5, 5.5, CH$_A$<u>H</u>$_B$Ph), 1.66 (2 H, tq, *J* 7.5, OCH$_2$C<u>H</u>$_2$), 0.93 (3 H, t, *J* 7.5, C<u>H</u>$_3$); δ_C ($CDCl_3$; 63 MHz) 171.5, 166.7 (<u>C</u>O$_2$CH$_2$, <u>C</u>ONH), 135.7 133.8 (*ipso*-Ar), 131.5, 129.2, 128.4, 126.9, 126.8 (<u>C</u>H$_{ar}$), 67.0 (O<u>C</u>H$_2$), 53.4 (HN<u>C</u>H), 37.8 (<u>C</u>H$_2$Ph), 21.7 (0CH$_2$<u>C</u>H$_2$), 10.2 (CH$_2$C<u>H</u>$_3$); *m/z* (FAB) 312 (81%, MH^+), 252 (30, M-OPr), 224 (76, 252-CO), 105 (100, 224-NHCHCH$_2$Ph), 77 (46, 105-CO), Found (FAB) 312.1592, $C_{19}H_{22}NO_3$ requires 312.1600.

4.2.21. *N*-Benzoyl-DL-phenylalanine butyl ester 97a

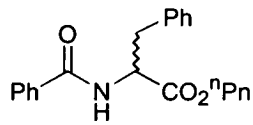


The general procedure outlined above **(4.2.17.)** with oxazolone **89a** in *n*-butanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (9:1), (121 mg, 93%).

R_f (Hexane:EtOAc, 9:1) 0.29; R_t 13.70 and 17.12; $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3325 (NH), 3030 (CH_{ar}), 1736 ester C=O), 1654, 1517 (CONH); δ_H ($CDCl_3$; 200 MHz) 7.74-7.70 (2 H, m, C<u>H</u>$_{ar}$), 7.51-7.40 (3 H, m, C<u>H</u>$_{ar}$), 7.38-7.20 (3 H, m, C<u>H</u>$_{ar}$), 7.19- 7.11 (2 H, m, C<u>H</u>$_{ar}$), 6.64 (1 H, d, *J* 7.5, N<u>H</u>), 5.07 (1 H, ddd, *J* 7.5, 6.0, 5.5, NHC<u>H</u>), 4.14 (1 H, dt, *J* 11.0, 6.5, OC<u>H</u>$_A$H$_B$CH$_2$), 4.11 (1 H, dt, *J* 11.0, 6.5, OCH$_A$<u>H</u>$_B$CH$_2$), 3.26 (1 H, dd, *J* 14.0, 6.0, C<u>H</u>$_A$H$_B$Ph), 3.24 (1 H, dd, *J* 14.0, 5.5, CH$_A$<u>H</u>$_B$Ph), 1.68-1.54 (2 H, m,

$OCH_2C\underline{H}_2$), 1.34 (2 H, septet, *J* 7.5, $C\underline{H}_2CH_3$), 0.92 (3 H, t, *J* 7.5, $C\underline{H}_3$); δ_C ($CDCl_3$; 63 MHz) 171.5, 166.6 ($\underline{C}O_2CH_2$, $\underline{C}ONH$), 135.8, 133.8 (*ipso*-Ar), 131.5, 129.2, 128.4, 128.4, 126.9, 126.8 ($\underline{C}H_{ar}$), 65.3 ($O\underline{C}H_2$), 53.4 ($HN\underline{C}H$), 37.8 ($\underline{C}H_2Ph$), 30.3, 18.9 ($0CH_2\underline{C}H_2\underline{C}H_2$), 10.2 ($CH_2C\underline{H}_3$); m/z (FAB) 326 (94%, MH^+), 252 (25, M-OBu), 224 (53, 252-CO), 105 (100, 224-$NHCHCH_2Ph$), 77 (35, 105-CO), Found (FAB) 326.1579, $C_{20}H_{24}NO_3$ requires 326.1756.

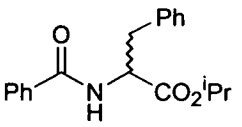
4.2.22. *N*-Benzoyl-DL-phenylalanine pentyl ester 100a



n-Pentanol (87 μl, 0.80 mmol, 2.0 equiv.) was added to a solution of oxazolone **89a** (100 mg, 0.40 mmol) in toluene (8 mL). Conc. hydrochloric acid (30 μL) was added, and the solution heated under reflux for 6 h. On cooling, the solvent was removed under reduced pressure and the crude product purified by column chromatography, eluting with hexane:ethyl acetate (10:1), to furnish the desired product as a colourless wax (129 mg, 95%).

R_f (Hexane:EtOAc, 8:1) 0.48; R_t 11.20 and 13.08; $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3432 (NH), 3030 (CH_{ar}), 1735 (ester C=O), 1658, 1515 (CONH); δ_H ($CDCl_3$; 200 MHz) 7.77-7.70 (2 H, m $C\underline{H}_{ar}$), 7.62-7.37 (3 H, m, $C\underline{H}_{ar}$), 7.34-7.21 (3 H, m, $C\underline{H}_{ar}$), 7.19-7.11 (2 H, m $C\underline{H}_{ar}$), 6.62 (1 H, d, *J* 7.5, $N\underline{H}$), 5.08 (1 H, ddd, *J* 7.5, 6.0, 5.5, $NHC\underline{H}$), 4.14 (1 H, dt, *J*, 10.5, 6.5, $OC\underline{H}_ACH_BCH_2$), 4.12 (1 H, dt, *J*, 10.5, 6.5, $OCH_AC\underline{H}_BCH_2$), 3.26 (1 H, dd, *J* 14.0, 6.0, $C\underline{H}_AH_BPh$), 3.24 (1 H, dd, *J*, 14.0, 5.5, $CH_A\underline{H}_BPh$), 1.63 (2 H, m, $OCH_2C\underline{H}_2$), 1.32 (4 H, m, $CH_2(C\underline{H}_2)_2CH_3$), 0.90 (3 H, t, *J* 6.5, $C\underline{H}_3$); δ_C ($CDCl_3$; 63 MHz) 171.5, 166.6 ($\underline{C}O_2CH_2$, $\underline{C}ONH$), 135.8, 133.8 (*ipso*-Ar), 131.6, 129.2, 128.4, 128.4, 126.9, 126.8 ($\underline{C}H_{ar}$), 65.6 ($O\underline{C}H_2$), 53.4 ($HN\underline{C}H$), 37.8 ($\underline{C}H_2Ph$), 28.0, 27.8, 22.1 ($OCH_2\underline{C}H_2\underline{C}H_2\underline{C}H_2$), 13.8 ($CH_2C\underline{H}_3$); *m/z* (FAB) 340 (93%, MH^+), 252 (53, M-OPen), 224 (77, 252-CO), 120 (100, 224-$CHCH_2Ph$), 105 (68, 120-NH), 77 (21, 105-CO), Found (FAB) 340.1903, $C_{21}H_{26}NO_3$ requires 340.1913.

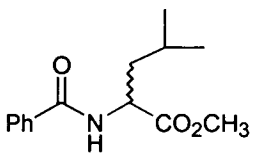
4.2.23. *N*-Benzoyl-DL-phenylalanine *iso*-propyl ester 101a



The general procedure outlined above **(4.2.17.)** withoxazolone **89a** in *iso*-propanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (8:1), (114 mg, 92%).

R_f (Hexane:EtOAc, 4:1) 0.50; R_t 13.00 and 17.00; Mp 97-99 °C; $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3332 (NH), 3030 (CH_{ar}), 1737 (ester C=O), 1644, 1533 (CONH); δ_H ($CDCl_3$; 200 MHz) 7.75-7.70 (2 H, m, C<u>H</u>$_{ar}$), 7.61-7.37 (3 H, m, C<u>H</u>$_{ar}$), 7.33-7.20 (3 H, m, C<u>H</u>$_{ar}$), 7.18-7.13 (2 H, m, C<u>H</u>$_{ar}$), 6.62 (1 H, d, *J* 7.5, N<u>H</u>), 5.11-4.95 (2 H, m, NHC<u>H</u> and C<u>H</u>$(CH_3)_2$), 3.26 (1 H, dd, *J* 6.0, 13.5, C<u>H</u>$_A$H$_B$Ph), 3.23 (1 H, dd, *J* 13.5, 6.5, CH$_A$<u>H</u>$_B$Ph), 1.25 (3 H, d, *J* 6.5, CHC<u>H</u>$_3$), 1.24 (3 H, d, *J* 6.5, CHC<u>H</u>$_3$); δ_C ($CDCl_3$; 63 MHz) 171.0, 166.6 (<u>C</u>O_2CH, <u>C</u>ONH), 135.8, 133.9 (*ipso*-Ar), 131.5, 129.3, 128.4, 128.4, 126.9, 126.8 (<u>C</u>H$_{ar}$), 69.4 (O<u>C</u>H), 53.5 (HN<u>C</u>H), 37.8 (<u>C</u>H_2Ph), 21.6, 21.5 (CH(<u>C</u>$H_3)_2$); *m/z* (FAB) 312 (63%, MH^+), 252 (25, M-OPr^i), 224 (80, 252-CO), 120 (88, 224-CHCH_2Ph), 105 (100, 120-NH), 77 (69, 105-CO), Found (FAB) 312.1594, $C_{19}H_{22}NO_3$ requires 312.1600.

4.2.24. *N*-Benzoyl-DL-leucine methyl ester 96b

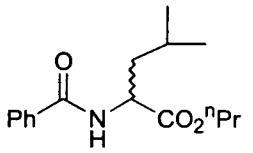


The general procedure outlined above **(4.2.17.)** with oxazolone **89b** in methanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (6:1), (92 mg, 80%).

R_f (Hexane:EtOAc, 8:1) 0.14; R_t 9.63 and 13.28; Mp 94-96 °C, Lit.[138] 95-96 °C; υ_{max}(Card)/cm^{-1} 3317 (NH), 3030 (CH_{ar}), 1747 (ester C=O), 1641, 1534 (CONH); δ_H ($CDCl_3$; 200 MHz) 7.82-7.76 (2 H, m, C<u>H</u>$_{ar}$), 7.54-7.37 (3 H, m, C<u>H</u>$_{ar}$), 6.59 (1 H, d, *J* 8.5, N<u>H</u>), 4.55 (1H, ddd, 8.5, 5.5, NHC<u>H</u>), 3.74 (3 H, s, OC<u>H</u>$_3$), 1.69 (3 H, m, C<u>H</u>$_2$CH and CH$_2$C<u>H</u>), 0.97 (3 H, d, *J* 6.5, C<u>H</u>$_{3A}$CHCH$_{3B}$), 0.96 (3 H, d, *J* 6.5, CH$_{3A}$CHC<u>H</u>$_{3B}$); δ_C ($CDCl_3$; 63 MHz) 173.5, 167.0 (<u>C</u>O_2CH$_3$, <u>C</u>ONH), 133.8 (*ipso*-

Ar), 131.5, 128.4 126.90 ($\underline{C}H_{ar}$), 52.1 ($O\underline{C}H_3$), 51.0 ($HN\underline{C}H$), 41.6 ($\underline{C}H_2CH$), 24.8 ($\underline{C}H(CH_3)_2$), 22.6, 21.8 ($CH(\underline{C}H_3)_2$), *m/z* (FAB) 250 (100%, MH^+), 234 (11, M-CH_3), 190 (67, 234-CO_2), 105 (53, 190-$NHCHCH_2CH(CH_3)_2$), 77 (37, 105-CO).

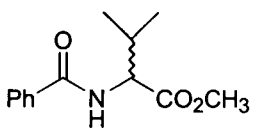
4.2.25. *N*-Benzoyl-DL-leucine propyl ester 99b



The general procedure outlined above **(4.2.17.)** with oxazolone **89b** in *n*-propanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (8:1), (114 mg, 93%).

R_f (Hexane:EtOAc, 8:1) 0.32; R_t 8.22 and 16.02; Mp 62-63 °C; υ_{max}(Card)/cm^{-1}; 3329 (NH), 3029 (CH_{ar}), 1743 ester (CO), 1642, 1535, (CONH); δ_H ($CDCl_3$; 200 MHz) 7.82-7.76 (2 H, m, $C\underline{H}_{ar}$), 7.54-7.37 (3 H, m, $C\underline{H}_{ar}$), 6.58, (1 H, d, *J* 7.5, N$\underline{H}$), 4.84 (1H, ddd, 8.5, 7.5, 5.5, NHC$\underline{H}$), 4.11 (2 H, t, *J* 6.5 $OC\underline{H}_2$), 1.79-1.59 (5 H, m, $CHC\underline{H}_2CH$, $CHCH_2C\underline{H}$, and $C\underline{H}_2CH_3$), 0.99 (3 H, d, *J* 6.0, $C\underline{H}_{3A}CHCH_{3B}$), 0.97 (3 H, d, *J* 6.0, $CH_{3A}CHC\underline{H}_{3B}$), 0.95 (3 H, t, *J* 7.5, $CH_2C\underline{H}_3$); δ_C ($CDCl_3$; 63 MHz) 173.2, 166.9 ($\underline{C}O_2CH_2$, $\underline{C}ONH$), 133.9 (*ipso*-Ar), 131.5, 128.4, 126.9 ($\underline{C}H_{ar}$), 66.8 ($O\underline{C}H_2$), 51.1 ($HN\underline{C}H$), 41.8 ($CH\underline{C}H_2$), 24.9 ($\underline{C}H(CH_3)_2$), 22.6, 22.0 ($CH(\underline{C}H_3)_2$), 21.8 ($\underline{C}H_2CH_3$), 10.2 ($CH_2\underline{C}H_3$); *m/z* (FAB) 278 (81%, MH^+), 218 (16, M-OPr), 190 (66, 218-CO), 105 (100, 190-$NHCHCH_2CH(CH_3)_2$), 77 (12, 105-CO), Found (FAB) 278.1744, $C_{16}H_{23}NO_3$ requires 278.1756.

4.2.26.*N*-Benzoyl-DL-valine methyl ester 96c

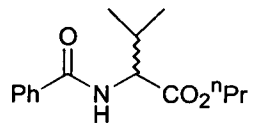


The general procedure outlined above **(4.2.17.)** with oxazolone **89c** in methanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (6:1), (87 mg, 75%).

R_f (Hexane:EtOAc, 4:1) 0.23; R_t 9.55 and 11.38; Mp 84-86 °C, Lit.[140] 86 °C; υ_{max}(nujol)/cm^{-1} 3436 9NH), 3018 (CH), 1734 (ester C=O), 1663, 1517 (CONH); δ_H

($CDCl_3$; 200 MHz) 7.84 (2 H, m, C<u>H</u>$_{ar}$), 7.55-7.39 (3 H, m, C<u>H</u>$_{ar}$), 6.63 (1 H, d, *J* 7.0, N<u>H</u>), 4.76 (1 H, dd, *J* 7.0, 8.5, NHC<u>H</u>), 3.76 (3 H, s, OC<u>H</u>$_3$), 2.26 (1 H, dqq, *J* 7.0, 8.5 C<u>H</u>CH$_3$), 1.00 (3 H, d, *J* 7.0 CHC<u>H</u>$_3$), 0.98 (3 H, d, *J* 7.0 CHC<u>H</u>$_3$); δ_C ($CDCl_3$; 63 MHz) 172.5, 167.1 (<u>C</u>O$_2$CH$_3$, <u>C</u>ONH), 134.0 (*ipso*-Ar), 131.6, 128.5, 126.9 (<u>C</u>H$_{ar}$), 57.3, (NH<u>C</u>H), 52.1 (O<u>C</u>H$_3$), 31.5 (<u>C</u>H(CH$_3$)$_2$), 18.9, 17.8 (CH(<u>C</u>H$_3$)$_2$); *m/z* (FAB) 236 (81%, MH$^+$), 204 (3, M-OMe), 176 (30, 204-CO), 105 (100, 176-NHCHCH(CH$_3$)$_2$), 77 (25, 105-CO).

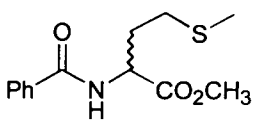
4.2.27. *N*-Benzoyl-DL-valine propyl ester 99c



The general procedure outlined above **(4.2.17.)** with oxazolone **89c** in *n*-propanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (10:1), (105 mg, 81%).

R_f (Hexane:EtOAc, 10:1) 0.18; R_t 6.43 and 8.35; Mp 53-54 °C; υ_{max}(nujol)/cm^{-1} 3432 (NH), 3017 (CH), 1730 (ester C=O), 1663, 1517 (CONH); δ_H ($CDCl_3$; 200 MHz) 7.78 (2 H, M, C<u>H</u>$_{ar}$), 7.50-7.33 (3 H, m, C<u>H</u>$_{ar}$), 6.59 (1 H, d, *J* 9.0, N<u>H</u>), 4.72 (1 H, dd, *J* 9.0, 7.0, NHC<u>H</u>), 4.10 (1 H, dt, *J* 12.1, 6.5, OC<u>H</u>$_A$H$_B$), 4.04 (1 H, dt, *J* 12.1, 6.5, OCH$_A$<u>H</u>$_B$)2.21 (1 H, ddq, *J* 7.0, 6.5, C<u>H</u>(CH$_3$)$_2$) 1.63 (2 H, tq, *J* 7.0, OCH$_2$C<u>H</u>$_2$), 0.96 (3 H, d, *J* 7.0, C<u>H</u>$_{3A}$CHCH$_{3B}$), 0.92 (3 H, d, *J* 7.0, CH$_{3A}$CHC<u>H</u>$_{3B}$), 0.90 (3 H, t, *J* 7.5, CH$_2$C<u>H</u>$_3$); δ_C ($CDCl_3$; 63 MHz) 172.1, 167.1 (<u>C</u>O$_2$CH$_2$, <u>C</u>ONH), 134.1 (*ipso*-Ar), 131.6, 128.5, 126.9, (<u>C</u>H$_{ar}$), 66.9 ((O<u>C</u>H$_2$), 57.2 (NH<u>C</u>H), 31.6 (<u>C</u>H(CH$_3$)$_2$), 21.8 (OCH$_2$C<u>H</u>$_2$), 18.9, 17.8 (CH(C<u>H</u>$_3$)$_2$), 10.3 (CH$_2$C<u>H</u>$_3$); *m/z* (FAB) 264 (71%, MH+), 204 (39, M-O^{n}Pr) 176 (78, 204-CO), 105 (100, 176-NHCHCH(CH$_3$)$_2$), 77 (46, 105-CO), Found (FAB) 264.1599, $C_{15}H_{22}NO_3$ requires 264.1600.

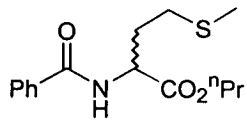
4.2.28. *N*-Benzoyl-DL-methionine methyl ester 96d



The general procedure outlined above **(4.2.17.)** with oxazolone **89d** in methanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (6:1), (98 mg, 83%).

R_f (Hexane:EtOAc, 8:1) 0.10; R_t 15.08 and 20.70; Mp 85-87 °C, Lit.[131] 87.5-88 °C; υ_{max}(Card)/cm^{-1} 3322 (NH), 3030 (CH_{ar}), 1734 (ester C=O), 1642, 1534 (CONH); δ_H ($CDCl_3$; 200 MHz) 7.84-7.77 (2 H, m, C<u>H</u>$_{ar}$), 7.55-7.38 (3 H, m, C<u>H</u>$_{ar}$), 6.99 (1 H, d, *J* 6.5, N<u>H</u>), 4.92 (1 H, ddd, *J* 7.5, 7.0, 6.5, NHC<u>H</u>), 3.78 (3 H, s, OC<u>H</u>$_3$), 2.57 (2 H, dd, *J* 8.0, 7.5, C<u>H</u>$_2$S), 2.36-2.21 (2 H, m, C<u>H</u>$_A$H$_B$CH$_2$S and CH$_A$<u>H</u>$_B$CH$_2$S), 2.09 (3 H, s, SC<u>H</u>$_3$); δ_C ($CDCl_3$; 63 MHz) 172.4, 166.9 (<u>C</u>O$_2$CH$_3$, <u>C</u>ONH), 133.5 (*ipso*-Ar), 131.6, 128.4, 126.9 (<u>C</u>H$_{ar}$), 52.4 (O<u>C</u>H$_3$), 51.9 (HN<u>C</u>H), 31.4, 29.9 (<u>C</u>H$_2$<u>C</u>H$_2$S), 15.3 (S<u>C</u>H$_3$); *m/z* (FAB) 268 (96%, MH$^+$), 236 (19, M-OMe), 208 (51, 236-CO), 105 (83, 208-NHCHCH$_2$CH$_2$SCH$_3$), 77 (50, 105-CO).

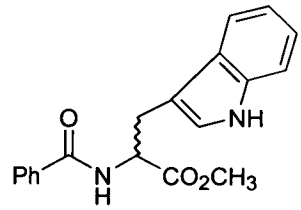
4.2.29. *N*-Benzoyl-DL-methionine propyl ester 99d



The general procedure outlined above **(4.2.17.)** with oxazolone **89d** in *n*-propanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (8:1), (107 mg, 86%).

R_f (Hexane:EtOAc, 8:1) 0.29; R_t 11.57 and 20.03; Mp 76-78 °C; υ_{max}(Card)/cm^{-1} 3326 (NH), 3029 (CH_{ar}), 1740 (ester C=O), 1643, 1534 (CONH); δ_H ($CDCl_3$; 200 MHz) 7.82-7.77 (2 H, m, C<u>H</u>$_{ar}$), 7.54-7.38 (3 H, m, C<u>H</u>$_{ar}$), 6.98 (1 H, d, *J* 7.5, N<u>H</u>), 4.91 (1 H, ddd, *J* 7.5, 7.0, 5.0, NHC<u>H</u>), 4.13 (2 H, t, *J* 7.0, OC<u>H</u>$_2$), 2.58 (2 H, m, C<u>H</u>$_2$S), 2.34-2.04 (2 H, m, CHC<u>H</u>$_A$H$_B$CH$_2$ and CHCH$_A$<u>H</u>$_B$CH$_2$), 2.09 (3 H, s, SC<u>H</u>$_3$), 1.69 (2 H, tq, *J* 7.0, 7.5, OCH$_2$C<u>H</u>$_2$), 0.95 (3 H, t, *J* 7.5, CH$_2$C<u>H</u>$_3$); δ_C ($CDCl_3$; 63 MHz) 172.0, 166.9 (<u>C</u>O$_2$CH$_2$, <u>C</u>ONH), 133.6 (*ipso*-Ar), 131.6, 128.4, 126.9 (<u>C</u>H$_{ar}$), 67.1 (O<u>C</u>H$_2$), 52.0 (HN<u>C</u>H), 31.6, 29.9 (<u>C</u>H$_2$CH$_2$S and CH$_2$<u>C</u>H$_2$S), 21.7 (OCH$_2$<u>C</u>H$_2$), 15.3 (S<u>C</u>H$_3$), 10.2 (CH$_2$<u>C</u>H$_3$); *m/z* (FAB) 296 (100%, MH$^+$), 280 (7, M-CH$_3$), 248 (63, 280-S), 236 (17, M-OPr), 208 (33, 236-CO), 105 (31, 208-NHCHCH$_2$CH$_2$SCH$_3$), 77 (30, 105-CO), Found (FAB) 296.1328, $C_{15}H_{22}NO_3S$ requires 296.1320.

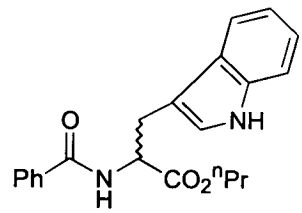
4.2.30. N^{α}-Benzoyl-DL-tryptophan methyl ester 96e



The general procedure outlined above **(4.2.17.)** with oxazolone **89e** (60 mg, 0.21 mmol) in methanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (2:1), (65 mg, 98%).

R_f (Hexane:EtOAc, 1:1) 0.46; R_t (Hexane:EtOH, 9:1) 50.10 and 53.92 (not baseline separation); Mp 105-107 °C, Lit.[141] 109-110 °C; $\upsilon_{max}(CHCl_3)/cm^{-1}$; 3475 (indole NH), 3424 (amide NH), 3017 (CH_{ar}), 1738 (ester C=O), 1656, 1519 (CONH); δ_H ($CDCl_3$; 200 MHz) 8.27 (1 H, br s, $N\underline{H}_{(Indole)}$), 7.62-7.57 (2 H, m, $C\underline{H}_{ar}$), 7.49-7.24 (5 H, m, $C\underline{H}_{ar}$), 7.17-6.95 (2 H, m, $C\underline{H}_{ar}$), 6.90 (1 H, d, *J* 2.0, $C\underline{H}_{ar}$), 6.64 (1 H, d, *J* 7.5, N$\underline{H}$), 5.07 (1 H, ddd, *J* 7.5, 5.5, 5.0, NHC$\underline{H}$), 3.63 (3 H, S, $OC\underline{H}_3$), 3.45 (1 H, dd, *J* 15.0 5.5, $C\underline{H}_ACH_B$Indole) 3.43 (1 H, dd, *J* 15.0, 5.0 $CH_AC\underline{H}_B$Indole); δ_C ($CDCl_3$; 63 MHz) 172.3, 167.0 ($\underline{C}O_2CH_3$, $\underline{C}$ONH), 136.0, 133.5 (*ipso*-Ar), 131.6, 128.4 ($\underline{C}H_{ar}$), 127.4 (*ipso*-Ar), 126.9, 122.8, 122.0, 119.4, 118.3, 111.3, ($\underline{C}H_{ar}$) 109.5 (*ipso*-Ar), 53.4 (HN$\underline{C}$H), 52.3 ($O\underline{C}H_3$), 27.4 ($CH\underline{C}H_2$); *m/z* (FAB) 323 (28%, MH^+), 291 (2, M-OMe), 263 (13, 291-CO), 130 (100, CH_2Indole), 105 (69, 263-NHCHCH_2Indole), 77 (15, 105-CO).

4.2.31. N^{α}-Benzoyl-DL-tryptophan propyl ester 99e

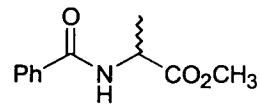


The general procedure outlined above **(4.2.17.)** with oxazolone **89e** (60 mg, 0.21 mmol) in *n*-propanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (1:1), (65 mg, 89%).

R_f (Hexane:Et_2O, 1:1) 0.16; R_t (Hexane:EtOH, 9:1) 38.75 and 41.73 (not baseline separation); Mp 104-106 °C; $\upsilon_{max}(CHCl_3)/cm^{-1}$; 3476 (indole NH), 3421 (amine NH),

3017 (CH_{ar}), 1736 (ester C=O), 1656, 1519; δ_H ($CDCl_3$; 200 MHz) 8.46 (1 H, br s, $N\underline{H}_{(Indole)}$), 7.70-7.66 (2 H, m, $C\underline{H}_{ar}$), 7.59-7.21 (5 H, m, $C\underline{H}_{ar}$), 7.17-7.03 (2 H, m, $C\underline{H}_{ar}$), 6.98 (1 H, d, *J* 2.0, $NHC\underline{H}_{ar}$), 6.74 (1 H, d, *J* 7.5 N$\underline{H}$), 5.15 (1 H, ddd, *J* 7.5, 5.5, NHC$\underline{H}$), 4.07 (1 H, dt, *J* 10.5, 6.5, $OC\underline{H}_ACH_BCH_2$), 4.04 (1 H, dt, *J* 10.5, 6.5, $OCH_AC\underline{H}_BCH_2$), 3.45 (1 H, dd, *J* 15.5, 5.5, $C\underline{H}_ACH_B$Indole) 3.43 (1 H, dd, *J* 15.5, 5.5, $CH_AC\underline{H}_B$Indole); 1.63 (2 H, tq, *J* 7.5, 7.0, $OCH_2C\underline{H}_2$), 0.89 (3 H, t, *J* 7.5, $CH_2C\underline{H}_3$); δ_C ($CDCl_3$; 63 MHz) 171.9, 166.9 ($\underline{C}O_2CH_3$, $\underline{C}$ONH), 136.0, 133.7 (*ipso*-Ar), 131.5, 128.4 ($\underline{C}H_{ar}$), 127.5 (*ipso*-Ar), 126.9, 122.7, 122.0, 119.4, 118.4, 111.2, ($\underline{C}H_{ar}$), 109.7 (*ipso*-Ar), 53.4 (HN$\underline{C}$H), 27.6, 21.7 ($OCH_2\underline{C}H_2$ and $CH\underline{C}H_2$), 10.1 ($CH_2\underline{C}H_3$); m/z (FAB) 351 (15%, MH^+), 291 (3, M-OPr), 263 (15, 291-CO), 130 (100, CH_2Indole), 105 (62, 263-NHCHCH$_2$Indole), 77 (13, 105-CO), Found (FAB) 351.1705, $C_{21}H_{23}N_2O_3$ requires 351.1709.

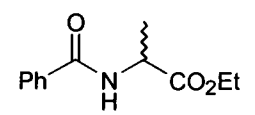
4.2.32. *N*-Benzoyl-DL-alanine methyl ester 96f



The general procedure outlined above **(4.2.17.)** with oxazolone **89f** in methanol was followed Product purified by column chromatography, eluting with hexane:ethyl acetate (6:1), (97 mg, 82%).

R_f (Hexane:EtOAc, 4:1) 0.13; R_t 10.61 and 14.21; Mp 80-82 °C, Lit.[142] 80-82 °C; υ_{max}(nujol)/cm^{-1} 3431 (NH), 3017 (CH), 1740 (ester C=O), 1653, 1517 (CONH); δ_H ($CDCl_3$; 200 MHz) 7.78 (2 H, m, $C\underline{H}_{ar}$), 7.52-7.37 (3 H, m, $C\underline{H}_{ar}$), 6.81 (1 H, b d, *J* 5.5, N$\underline{H}$), 4.63 (1 H, dq, *J* 7.0, NHC$\underline{H}$), 3.76 (3 H, s, $OC\underline{H}_3$), 1.50 (3 H, d, *J* 7.0, $CHC\underline{H}_3$); δ_C ($CDCl_3$; 63 MHz) 173.5, 160.7 ($\underline{C}O_2C$, $\underline{C}$ONH), 133.7 (*ipso*-Ar), 131.6, 128.4, 126.9 ($\underline{C}H_{ar}$), 52.4 ($O\underline{C}H_3$), 48.3 ($\underline{C}$H), 18.4 ($CH\underline{C}H_3$); *m/z* (FAB) 208 (100%, MH^+), 176 (6, M-OMe), 148 (13, 176-CO), 105 (55, 148-NHCHCH$_3$).

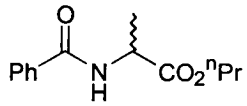
4.2.33. *N*-Benzoyl-DL-alanine ethyl ester 98f



The general procedure outlined above **(4.2.17.)** with oxazolone **89f** in methanol was followed Product purified by column chromatography, eluting with hexane:ethyl acetate (6:1), (107 mg, 85%).

R_f (Hexane:EtOAc, 6:1) 0.15; R_t 10.97 and 14.07; Mp 75 °C; υ_{max}(nujol)/cm^{-1} 3431 (NH), 3018 (CH), 1734 (ester C=O), 1653, 1517 (CONH); δ_H ($CDCl_3$; 200 MHz) 7.83 (2 H, m, C$\underline{H}_{ar}$), 7.54-7.38 (3 H, m, C$\underline{H}_{ar}$), 6.77 (1 H, d, *J* 6.0, N$\underline{H}$),4.75 (1 H, dq, *J* 7.0, NHC$\underline{H}$), 4.23 (2 H, q, *J* 7.0, OC$\underline{H}_2$), 1.51, (3 H, d, *J* 7.0, C$\underline{H}$C$\underline{H}_3$), 1.30, (3 H, t, *J* 7.0, $CH_2C\underline{H}_3$); δ_C ($CDCl_3$; 63 MHz) 173.1, 166.6 ($\underline{C}O_2CH_2$, $\underline{C}$ONH), 133.8 (*ipso*-Ar), 131.5, 128.4, 126.9 ($\underline{C}H_{ar}$), 61.5 (O$\underline{C}H_2$), 48.4 (CH), 18.6 (CH$\underline{C}H_3$), 14.01 ($CH_2\underline{C}H_3$); *m/z* (FAB) 222 (100%, MH$^+$), 176 (92, M-OEt), 148 (99, 176-CO), 105 (48, 148-NHCHCH$_3$), 77 (37, 105-CO), Found 222.1136, $C_{12}H_{16}NO_3$ requires 222.1130.

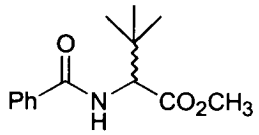
4.2.34. *N*-Benzoyl-DL-alanine propyl ester 99f



The general procedure outlined above **(4.2.17.)** with **89f** in *n*-propanol was followed Product purified by column chromatography, eluting with hexane:ethyl acetate (6:1), furnished the desired product (95 mg, 71%).

R_f (Hexane:EtOAc, 6:1) 0.18; R_t 10.43 and 13.90; Mp 60-61 °C; υ_{max}(nujol)/cm^{-1} 3429 (NH), 3016 (CH), 1731 (ester C=O), 1655, 1518 (CONH); δ_H ($CDCl_3$; 200 MHz) 7.83-7.77 (2 H, m, C$\underline{H}_{ar}$), 7.54-7.38 (3 H, m, C$\underline{H}_{ar}$), 6.78 (1 H, d, *J* 6.0, N$\underline{H}$), 4.79 (1 H, dq, *J* 7.0, NHC$\underline{H}$), 4.17 (1 H, dt, *J* 11.5, 6.5, OC$\underline{H}_A$H$_B$CH$_2$), 4.10 (1 H, dt, *J* 11.5, 6.5, OCH$_A\underline{H}_B$CH$_2$), 1.78-1.60 (2 H, m, OC$\underline{H}_2$CH$_2$), 1.52 (3 H, d, *J* 7.0, CHC$\underline{H}_3$), 0.95 (3 H, t, *J* 7.5, $CH_2C\underline{H}_3$); δ_C ($CDCl_3$; 63 MHz) 173.2, 166.7 ($\underline{C}O_2CH_2$, $\underline{C}$ONH), 133.8 (*ipso*-Ar), 131.5, 128.4, 126.9 ($\underline{C}H_{ar}$), 67.0 (O$\underline{C}H_2$), 48.4 (NH$\underline{C}$H), 21.8 (OCH$_2\underline{C}$H$_2$), 18.6 (CH$\underline{C}$H$_3$), 10.2 ($CH_2C\underline{H}_3$); *m/z* (FAB) 236 (100%, MH$^+$) 176 (78, M-OEt), 148 (81, 176-CO), 105 (39, 148-NHCHCH$_3$), 77 (29, 105-CO), Found 236.1290, $C_{12}H_{18}NO_3$ requires 236.1286.

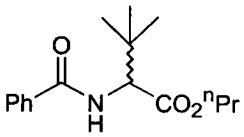
4.2.35. *N*-Benzoyl-DL-*tert*-leucine methyl ester 96g



The general procedure outlined above **(4.2.17.)** with oxazolone **89g** in methanol was followed Product purified by column chromatography, eluting with hexane:ethyl acetate (6:1), (96 mg, 84%).

R_f (Hexane:EtOAc, 4:1) 0.28; R_t (Heptane:IPA, 98:2, not baseline separation) 48.13 and 51.50; Mp 65-66 °C, Lit.[143] 66 °C; $\upsilon_{max}(CHCl_3)/cm^{-1}$; 3437 (NH), 3018 ($CH_{ar}$), 1736 (ester C=O),1664, 1518 (CONH); δ_H ($CDCl_3$; 200 MHz) 7.80-7.76 (2 H, m, C<u>H</u>$_{ar}$), 7.50-7.37 (3 H, m, C<u>H</u>$_{ar}$), 6.67 (1 H, d, *J* 9.0 N<u>H</u>), 4.76 (1 H, d, *J* 9.0, NHC<u>H</u>), 3.73 (3 H, s, OC<u>H</u>$_3$), 1.04 (9 H, s, C(C<u>H</u>$_3$)$_3$); δ_C ($CDCl_3$; 63 MHz) 172.0, 166.9 (<u>C</u>O$_2$CH$_3$, <u>C</u>ONH), 134.0 (*ipso*-Ar), 131.5, 128.4, 126.8 (<u>C</u>H$_{ar}$), 60.0 (NH<u>C</u>H), 51.7 (O<u>C</u>H$_3$), 34.9 (<u>C</u>(CH$_3$)$_3$), 26.4 (C(<u>C</u>H$_3$)$_3$); *m/z* (FAB) 250 (100%, MH^+), 105 (36, M-NHCH(C(CH$_3$)$_3$)CO$_2$CH$_3$), 77 (5, 105-CO).

4.2.36. *N*-Benzoyl-DL-*tert*-leucine propyl ester 99g



The general procedure outlined above **(4.2.17.)** with **89g** in *n*-propanol was followed Product purified by column chromatography, eluting with hexane:ethyl acetate (8:1), (95 mg, 74%).

R_f (Hexane:EtOAc, 6:1) 0.41; R_t (Heptane:IPA, 98:2) 40.35 and 43.52; Mp 60-62 °C; $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3435 (NH), 3018 (CH_{ar}), 1725 (ester C=O), 1663, 1517 (CONH); δ_H ($CDCl_3$; 200 MHz) 7.81-7.77 (2 H, m, <u>C</u>H$_{ar}$), 7.54-7.39 (3 H, m, <u>C</u>H$_{ar}$), 6.69 (1 H, d, *J* 9.0, N<u>H</u>), 4.69 (1 H, d, *J* 9.0, NHC<u>H</u>), 4.10 (2 H, t, *J* 7.0, OC<u>H</u>$_2$), 1.69 (2 H, tq, *J* 7.5, 7.0, OCH$_2$C<u>H</u>$_2$), 1.04 (9 H, s, C(C<u>H</u>$_3$)$_3$), 0.95 (3 H, t, *J* 7.5, CH$_2$C<u>H</u>$_3$); δ_C ($CDCl_3$; 63 MHz) 171.7, 166.9 (<u>C</u>O$_2$CH$_2$, <u>C</u>ONH), 134.1 (*ipso*-Ar), 131.5, 128.4, 126.8 (<u>C</u>H$_{ar}$), 66.6 (O<u>C</u>H$_2$), 60.1 (NH<u>C</u>H), 35.1 (<u>C</u>(CH$_3$)$_3$), 26.5 (C(<u>C</u>H$_3$)$_3$), 21.7 (<u>C</u>H$_2$CH$_3$), 10.3 (CH$_2$<u>C</u>H$_3$); *m/z* (FAB) 278 (100%, MH^+), 105 (67, M-NHCH(C(CH$_3$)$_3$)CO$_2$Pr), Found (FAB) 278.1764, $C_{16}H_{24}NO_3$ requires 278.1756.

4.2.37. General procedure for the lipase catalysed ring opening of (*RS*)-2-phenyl-4-substituted-5(4*H*)-oxazolones 89a-g

i. In the presence of triethylamine

Catalytic triethylamine (0.25 equiv.), lipase (crushed and dried Novozyme®, 100 mg pre-dried weight, or Lipozyme®, 100 mg) and alcohol (2.0 equiv.) were added to a solution of (*RS*)-2-phenyl-DL-4-substituted-5(4*H*)-oxazolone **89a-g** (100 mg) in solvent (8 mL). The flask was stoppered and placed in an orbital incubator at 37 °C and 200 rpm. The reactions were monitored by tlc and on complete consumption of the starting 5(4*H*)-oxazolone the lipase was filtered, washed with solvent (2x 10 mL) and the combined organic fractions concentrated under reduced pressure. Purification by column chromatography, as described for the corresponding racemate, furnished the desired product as a colourless solid unless otherwise stated. Spectroscopic data for all purified products were in agreement with the corresponding racemic sample.

ii. In the absence of triethylamine

As above with the elimination of triethylamine.

4.2.38. *N*-Benzoyl-L-phenylalanine methyl ester 96a

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89a**, toluene, methanol, triethylamine, and Novozyme®. Reaction time 26 h, (93 mg, 82%).

R_t 25.72, e.e. 94%; $[\alpha]_D^{20}$ +96.4 (c 1.00, $CHCl_3$); Mp 86-88 °C.

4.2.39. *N*-Benzoyl-L-phenylalanine ethyl ester 98a

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89a**, toluene, ethanol, triethylamine and Novozyme®. Reaction time 26 h, (94 mg, 79%).

R_t 16.78, e.e. 97%; $[\alpha]_D^{20}$ +92.0 (c 1.00, $CHCl_3$); Mp 95-97 °C.

4.2.40. *N*-Benzoyl-L-phenylalanine propyl ester 99a

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89a**, toluene, with *n*-propanol, triethylamine and Novozyme®. Reaction time 26 h, (103 mg, 83%).

R_t 17.47, e.e. 97%; $[\alpha]_D^{20}$ +85.8 (c 1.00, $CHCl_3$); Mp 78-79 °C.

4.2.41. *N*-Benzoyl-L-phenylalanine butyl ester 97a

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89a**, toluene, *n*-butanol, triethylamine and Novozyme® (100 mg as supplied). Reaction time 2 days, (69 mg, 53%).

R_t 17.12, e.e. 95%; $[\alpha]_D^{20}$ +78.6 (c 1.00, $CHCl_3$).

4.2.42. *N*-Benzoyl-L-phenylalanine butyl ester 97a

The general procedure outlined above **(4.2.37.ii.)** was followed using oxazolone **89a**, toluene, *n*-butanol, and Novozyme® (100 mg as supplied). Reaction time 6 days, (51 mg, 40%).

R_t 17.12, e.e. 64%; $[\alpha]_D^{20}$ +54.0 (c 1.00, $CHCl_3$).

4.2.43. *N*-Benzoyl-L-phenylalanine butyl ester 97a

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89a**, toluene, with *n*-butanol, triethylamine, and Novozyme®. Reaction time 2 days, (104 mg, 81%).

R_t 17.12, e.e. 95%; $[\alpha]_D^{20}$ +78.6 (c 1.00, $CHCl_3$); Mp 68-70 °C.

4.2.44. *N*-Benzoyl-L-phenylalanine butyl ester 97a

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89a**, toluene, *n*-butanol, triethylamine, and Lipozyme®. Reaction time 2 days, (96 mg, 74%).

R_t 17.12, e.e. 69%; $[\alpha]_D^{20}$ +52.7 (c 1.00, $CHCl_3$).

4.2.45. *N*-Benzoyl-L-phenylalanine butyl ester 97a

The general procedure outlined above **(4.2.37.ii.)** was followed using oxazolone **89a**, toluene, *n*-butanol, and Lipozyme®. Reaction time 3 days, (76 mg, 59%).

R_t 17.12, e.e. 55%.

4.2.46. *N*-Benzoyl-L-phenylalanine pentyl ester 100a

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89a**, toluene, *n*-pentanol, triethylamine, and Novozyme®. Reaction time 26 h, (43 mg, 32%).

R_t 13.08, e.e. 88%; $[\alpha]_D^{20}$ +75.4 (c 1.00, $CHCl_3$); Mp 55-58 °C.

4.2.47. *N*-Benzoyl-L-phenylalanine *iso*-propyl ester 101a

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89a**, toluene, *iso*-propanol, triethylamine, and Novozyme®. Reaction time 26 days, (22 mg, 18%).

R_t 17.00, e.e. 29%; Mp 95-97 °C.

4.2.48. *N*-Benzoyl-L-leucine methyl ester 96b

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89b**, toluene, methanol, triethylamine, and Novozyme®. Reaction time 2 days, (110 mg, 96%).

R_t 13.28, e.e. 97%; $[\alpha]_D^{20}$ -22.1 (c 0.98, CH_3OH), Lit.[144] $[\alpha]_D^{25}$ -22.8 (c 0.80, CH_3OH); Mp 101-103 °C.

4.2.49. *N*-Benzoyl-L-leucine propyl ester 99b

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89b**, toluene, methanol, triethylamine, and Novozyme®. Reaction time 2 days. Product obtained as a colourless oil (114 mg, 89%).

R_t 16.02, e.e. 98%; $[\alpha]_D^{20}$ -21.4 (c 1.20, CH_3OH).

4.2.50. *N*-Benzoyl-L-valine methyl ester 96c

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89c**, toluene, methanol, triethylamine, and Novozyme®. Reaction time 6 days, (95 mg, 82%).

R_t 11.38, e.e. 95%; $[\alpha]_D^{20}$ +40.2 (c 1.02, $CHCl_3$), Lit.[145] $[\alpha]_D^{20}$ +35 (c 0.4, $CHCl_3$); Mp 109-110 °C.

4.2.51. *N*-Benzoyl-L-valine propyl ester 99c

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89c**, toluene, *n*-propanol, triethylamine, and Novozyme®. Reaction time 6 days, (91 mg, 70%).

R_t 8.35, e.e. 93%; $[\alpha]_D^{20}$ +36.4 (c 0.52, $CHCl_3$); Mp 59-60 °C.

4.2.52. *N*-Benzoyl-L-methionine methyl ester 96d

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89d**, toluene, methanol, triethylamine, Novozyme®. Reaction time 26 h, (79 mg, 69%).

R_t 20.70, e.e. 80%; $[\alpha]_D^{20}$ +27.9 (c 1.12, $CHCl_3$); Mp 79-81 °C.

4.2.53. *N*-Benzoyl-L-methionine propyl ester 99d

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89d**, toluene, *n*-propanol, triethylamine, and Novozyme®. Reaction time 26 h, (80 mg, 64%).

R_t 20.03, e.e. 83%; $[\alpha]_D^{20}$ +27.0 (c 1.18, $CHCl_3$); Mp 51-53 °C.

4.2.54. N^{α}-Benzoyl-L-tryptophan methyl ester 96e

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89e**, toluene, methanol, triethylamine, and Novozyme®. Reaction time 2 days, (100 mg, 90%).

R_t 50.10, 90%; $[\alpha]_D^{20}$ -39.7 (c 0.70, CH_3OH), Lit.[146] $[\alpha]_D^{20}$ - 45 (c 0.175, CH_3OH); Mp 102-104 °C.

4.2.55. N^{α}-Benzoyl-L-tryptophan propyl ester 99e

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89e**, toluene, *n*-propanol, triethylamine, and Novozyme®. Reaction time 7 days, (58 mg, 48%).

R_t 38.73, e.e. 80%; $[\alpha]_D^{20}$ -30.9 (c 0.80, CH_3OH); Mp 107-109 °C.

4.2.56. *N*-Benzoyl-L-alanine ethyl ester 98f

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89f**, toluene, ethanol, triethylamine, and Novozyme®. Reaction time 6 days, (76 mg, 60%).

R_t 14.07, e.e. 14%; $[\alpha]_D^{20}$ -1.7 (c 0.24, $CHCl_3$); Mp 74-76 °C.

4.2.57. *N*-Benzoyl-L-alanine propyl ester 99f

The general procedure outlined above **(4.2.38.i)** was followed using oxazolone **89f**, toluene, *n*-propanol, triethylamine, and Novozyme®. Reaction time 6 days, (97 mg, 72%).

R_t 13.90, e.e. 14%; $[\alpha]_D^{20}$ +0.4 (c 0.54, $CHCl_3$); Mp 45-46 °C.

4.2.58. *N*-Benzoyl-L-*tert*-leucine methyl ester 96g

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89g**, toluene, methanol, triethylamine, and Novozyme®. After 21 days, the incomplete reaction was worked up. Purification by column chromatography, eluting with hexane:ethyl acetate (6:1) furnished recovered starting oxazolone, (22 mg, 22%) and desired product, (36 mg, 31%, or 40% based on recovered oxazolone.

R_t (Heptane:IPA 98:2, not baseline separation) 51.50, e.e. 35%.

4.2.59. *N*-Benzoyl-L-*tert*-leucine propyl ester 99g

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89g**, toluene, *n*-propanol, triethylamine, and Novozyme®. After 21 days, the incomplete reaction was worked up. Purification by column chromatography, eluting with hexane:ethyl acetate (8:1) furnished recovered starting oxazolone (42 mg, 42%, and desired product, (11 mg, 11%, or 15% based on recovered oxazolone).
R_t (Heptane:IPA, 98:2), 43.52, e.e. 19%; Mp 57-58 °C.

4.2.60. Solvent studies: *N*-benzoyl-L-phenylalanine methyl ester 96a in the presence of triethylamine

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89a** in (a) dichloromethane, (b) chloroform, (c) tetrahydrofuran, (d) diethyl ether, (e) *tert*-butyl methyl ether (f), diisopropylether, and (g) acetonitrile, with methanol, triethylamine, and Novozyme®. Reaction times are quoted after the yield. Products were purified by column chromatography, eluting with hexane:ethyl acetate (8:1). R_t 25.72; (a) (88 mg, 78%, e.e. 89%), 41 h, (b) (74 mg, 66%, e.e. 75%), 39 h, (c) (72 mg, 64%, e.e. 95%), 48 h, (d) (98 mg, 87%, e.e. 97%), 17 h (e) (102 mg, 90%, e.e. 96%) 15 h, (f) (97 mg, 86%, e.e. 96%), 15 h, (g) (49 mg, 44%, e.e. 97%), 22 h.

4.2.61. Solvent studies: *N*-benzoyl-L-phenylalanine methyl ester 96a in the absence of triethylamine

The general procedure outlined above **(4.2.37.ii.)** was followed using oxazolone **89a** in (a) dichloromethane, (b) chloroform, (c) tetrahydrofuran, (d) diethyl ether, (e) *tert*-butyl methyl ether (f), diisopropylether, and (g) acetonitrile, with methanol and Novozyme®. Reaction times are quoted after the yield. Products were purified by column chromatography, eluting with hexane:ethyl acetate (8:1). R_t 25.72; (a) (73 mg, 65%, e.e. 75%), 124 h, (b) (71 mg, 63%, e.e. 83%), 124 h, (c) (80 mg, 71%, e.e. 97%) 123 h, (d) (102 mg, 90%, e.e. 58%), 18 h, (e) (103 mg 91%, e.e. 34%), 18 h, (f) (102 mg, 90%, e.e. 33%), 18 h, (g) (99 mg, 88%, e.e. 98%), 51 h.

4.2.62. *N*-Benzoyl-L-phenylalanine methyl ester 96a

The general procedure outlined above **(4.2.37.i.)** was followed using oxazolone **89a**, acetonitrile, triethylamine, methanol, and Lipozyme®. Reaction time 23 h, (69 mg, 61%).

R_t 25.72, e.e. 73%

4.2.63. *N*-Benzoyl-L-phenylalanine methyl ester 96a

The general procedure outlined above **(4.2.37.ii.)** was followed using oxazolone **89a**, acetonitrile, methanol, and Lipozyme®. Reaction time 23 h, (106 mg, 94%).

R_t 25.72, e.e. 73%

4.2.64. *N*-Benzoyl-L-leucine methyl ester 96b

The general procedure outlined above **(4.2.37.ii.)** was followed using oxazolone **89b**, acetonitrile, methanol, and Novozyme®. Reaction time 19 h, (110 mg, 96%).

R_t 13.28, e.e. 97%

4.2.65. *N*-Benzoyl-L-leucine methyl ester 96b

The general procedure outlined above **(4.2.37.ii.)** was followed using oxazolone **89b**, acetonitrile, methanol, and Lipozyme®. Reaction time 4 days, (102 mg, 89%).

R_t 13.28, e.e. 62%

4.2.66. *N*-Benzoyl-L-valine methyl ester 96c

The general procedure outlined above **(4.2.37.ii.)** was followed using oxazolone **89c**, acetonitrile, methanol, Novozyme®. Reaction time 4 days, (96 mg, 83%).

R_t 11.38, e.e. 97%; $[\alpha]_D^{20}$ + 1.63 (c 1.66, $CHCl_3$).

4.2.67. *N*-Benzoyl-L-valine methyl ester 96c

The general procedure outlined above **(4.2.37.ii.)** was followed using oxazolone **89c**, acetonitrile, methanol, and Lipozyme®. After 21 days, the incomplete reaction was worked up. Purification by column chromatography, eluting with hexane:ethyl

acetate (4:1), afforded starting oxazolone, (34 mg, 34%) and desired product (52 mg, 45%, or 68%, yield based on recovered starting oxazolone).
R_t 11.38, e.e. 19%

4.2.68. *N*-Benzoyl-L-methionine methyl ester 96d

The general procedure outlined above **(4.2.37.ii.)** was followed using oxazolone **89d**, acetonitrile, with methanol, and Novozyme®. Reaction time 16 h, (90 mg, 79%).
R_t 20.70, e.e. 73%

4.2.69. *N*-Benzoyl-L-methionine methyl ester 96d

The general procedure outlined above **(4.2.37.ii.)** was followed using oxazolone **89d**, acetonitrile, methanol, and Lipozyme®. Reaction time 2 days, (94 mg, 83%).
R_t 20.70, e.e. 59%

4.2.70. *N*-Benzoyl-L-tryptophan methyl ester 96e

The general procedure outlined above **(4.2.37.ii.)** was followed using oxazolone **89e**, acetonitrile, methanol, and Novozyme®. Reaction time 16 days. Column chromatography, eluting with hexane:ethyl acetate (2:1), (0 mg, 0%).

4.2.71. *N*-Benzoyl-L-tryptophan methyl ester 96e

The general procedure outlined above **(4.2.37.ii.)** was followed using oxazolone **89e**, acetonitrile, methanol, and Lipozyme®. Reaction time 4 days, (109 mg, 98%).
R_t 50.10, e.e. 46%

4.2.72. *N*-Benzoyl-L-alanine methyl ester 96f

The general procedure outlined above **(4.2.37.ii.)** was followed using oxazolone **89f**, acetonitrile, methanol, and Novozyme®. Reaction time 19 h, (111 mg, 94%).
R_t 14.21, e.e. 10%

4.2.73. *N*-Benzoyl-L-alanine methyl ester 96f

The general procedure outlined above **(4.2.37.ii.)** was followed using oxazolone **89f**, acetonitrile, methanol, and Lipozyme®. Reaction time 4 days, (94 mg, 79%).
R_t 14.21, e.e. 39%; Mp 79-81 °C.

4.2.74. *N*-Benzoyl-L-*tert*-leucine methyl ester 96g

The general procedure outlined above **(4.2.37.ii.)** was followed using oxazolone **89g**, acetonitrile, methanol, and Novozyme®. Reaction time 22 days. No product formation was observed by tlc, hexane:ethyl acetate (6:1), or ^{1}H nmr (200 MHz, $CDCl_3$). (0 mg, 0%).

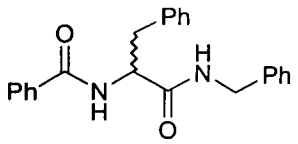
4.2.75. *N*-Benzoyl-L-*tert*-leucine methyl ester 96g

The general procedure outlined above **(4.2.37.ii.)** was followed using oxazolone **89g**, acetonitrile, methanol, and Lipozyme®. Reaction time 22 days. No product formation was observed by tlc, hexane:ethyl acetate (6:1), or ^{1}H nmr (200 MHz, $CDCl_3$). (0 mg, 0%).

4.2.76. Nitrogen based nucleophiles

4.2.77. Amine nucleophiles

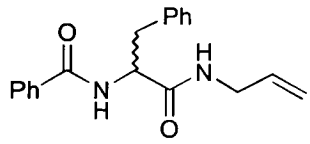
4.2.78. *N*-Benzyl-DL-phenylalanine benzylamide 102a



To a solution of oxazolone **89a** (50 mg, 0.20 mmol) in toluene (5 mL) was added triethylamine (8 μL, 0.25 equiv.) and benzylamine (44 μL, 0.4 mmol, 2.0 equiv.). The solution was stirred at room temperature and almost instantly a white precipitate formed. The solid was filtered, washed with toluene (2 x 10 mL) and dried under vacuum overnight to give the product (55 mg, 77%).
R_f (Hexane:EtOAc, 8:1) 0.31; Mp 210-211 °C Lit.[144] 211-212 °C; υ_{max}(IR Card)/cm^{-1} 3239 (NH), 1664, 1633, 1570, 1537 (CONH); δ_H ($(CD_3)_2SO$; 200 MHz) 8.64 (1 H, d, *J* 8.0, NHCH), 8.62 (1H, d, 5.5, $NHCH_2$), 7.81 (2 H, m, CH_{ar}), 7.72-7.16 (13 H,

C$\underline{H}_{ar}$), 4.75 (1 H, ddd, 10.0, 8.0, 5.0, NHC$\underline{H}$), 4.32 (2 H, d, *J* 5.5, NHC$\underline{H}_2$), 3.13 (1 H, dd, *J* 13.5, 5.0, C$\underline{H}_A$H$_B$Ph), 3.03 (1 H, dd, *J* 13.5, 10.0, CH$_A\underline{H}_B$Ph); δ_C ((CD$_3$)$_2$SO; 63 MHz) 171.51, 166.47 (C$\underline{O}$NH), 139.41, 138.51, 134.17 (*ipso*-Ar), 131.42, 129.31, 128.37, 128.29, 128.23, 127.58, 127.20, 126.85, 126.39 (C$\underline{H}_{ar}$), 55.25 (NH$\underline{C}$H), 42.25 (NH$\underline{C}$H$_2$), 37.41 ($\underline{C}$H$_2$Ph); *m/z* (FAB) 359 (75%, MH$^+$), 252 (45, M-NHBn), 224 (100, 252-CO), 105 (224-NHCHCH$_2$Ph).

4.2.79. *N*-Benzoyl-DL-phenylalanine allylamide 102b

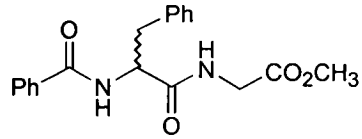


To a solution of oxazolone **89a** (100 mg, 0.40 mmol) in tetrahydrofuran (3 mL) was added allylamine (30 μL, 0.4 mmol, 1.0 equiv.) and the solution stirred at room temperature. The solvent was evaporated under reduced pressure and the resulting colourless solid purified by column chromatography, eluting with hexane:ethyl acetate (1:1) to furnish the product (110 mg, 90%).

R_f (Hexane:EtOAc, 1:1) 0.50; Mp 160-161°C, Lit.[129] 164-166 °C; υ_{max}(CHCl$_3$)/cm^{-1} 3435 (NH), 3018 (CH), 1653, 1646, 1517 (CONH); δ_H (CDCl$_3$; 200 MHz); 7.83 (2 H, m, C$\underline{H}_{ar}$), 7.65-7.40 (3 H, m, C$\underline{H}_{ar}$), 7.39-7.25 (5 H, m, C$\underline{H}_{ar}$) 5.85 (1 H, ddt *J* 17.0, 10.0, 5,5, C$\underline{H}$=CH$_2$), 5.19 (1 H, ddt, *J* 17.0, 2.0, CH=CH$\underline{H}_{(trans)}$), 5.14 (1 H, ddt, *J* 10.0, 1.5, C=CH$\underline{H}_{(cis)}$), 4.91 (1 H, dd, *J*, 8.5, 6.5, NHC$\underline{H}$), 3.87 (2 H, ddd, *J* 5.5, 2.0, 1.5, OC$\underline{H}_2$CH), 3.30 (1 H, dd, *J* 13.5, 6.5, C$\underline{H}_A$H$_B$Ph), 3.16 (1 H, dd, *J* 13.5, 8.5, CH$_A\underline{H}_B$Ph); δ_C (CDCl$_3$; 63 MHz) 171.58, 168.17 (C$\underline{O}$NH), 136.64, 133.36 (*ipso*-Ar), 133.20, 120.90, 128.46, 127.57, 126.53 (C$\underline{H}_{ar}$), 125.89 (C$\underline{H}$=CH$_2$), 114.37 (CH=C$\underline{H}_2$), 54.94 (NH$\underline{C}$H), 40.80 and 37.16 ($\underline{C}$H$_2$Ph and $\underline{C}$H$_2$CH=CH$_2$); *m/z* (FAB) 309 (55%, MH$^+$), 252 (100, M-CH$_2$CH=CH$_2$), 224 (48, 252-CO), 105 (87, 224-NHCHCH$_2$Ph), 77 (15, 105-CO).

4.2.80. Amino acid ester nucleophiles

4.2.81. *N*-benzoyl-DL-phenylalanine glycine methyl ester 104



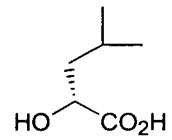
Glycine methyl ester was prepared following the procedure outlined by Frankel.[147] Glycine methyl ester hydrochloride (2.70 g, 21.5 mmol) was suspended in ether (25 ml) and ammonia gas bubbled through the vigorously stirred mixture for a period of 1 h. Nitrogen gas was bubbled through the resulting suspension to remove any excess ammonia and the precipitated ammonium chloride was filtered. The solvent was removed by distillation at 40 °C and the crude product was distilled, collecting the fraction boiling at 62 °C, 15 mm Hg to give the desired product as a colourless oil (0.99 g, 51%). ^{1}H nmr (200 MHz, $CDCl_3$) indicated the desired product. The product was not characterised further but used directly in the next step of the synthesis.

To a solution of oxazolone **89a** (50 mg, 0.20 mmol) in toluene (3 mL) was added freshly prepared glycine methyl ester (20 mg, 0.22 mmol, 1.1 equiv.) and the solution stirred at room temperature. A colourless precipitate formed almost immediately which was filtered, washed with ether (2 x 5 mL) and dried under vacuum to give the product (54 mg, 80%).

R_f (EtOAc:Hexane, 2:1) 0.37; Mp 155-157 °C; $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3317 (NH), 3018 (CH), 1750 (ester C=O), 1651, 1627, 1577, 1551 (CONH); δ_H ($CDCl_3$; 200 MHz) 7.69 (2 H, m C<u>H</u>$_{ar}$), 7.52-7.31 (3 H, m, C<u>H</u>$_{ar}$), 7.25 (5 H, s, C<u>H</u>$_{ar}$), 7.06 (1 H, d, 7.5 N<u>H</u>CH), 6.88 (1 H, brt, N<u>H</u>CH_2), 4.98 (1 H, ddd, *J* 7.5, NHC<u>H</u>), 3.99 (1 H, dd, *J* 18.5, 6.0, C<u>H</u>$_A$H$_B$Ph), 3.95 (1 H, dd, *J* 18.5, 6.0, CH$_A$<u>H</u>$_B$Ph), 3.68 (3 H, s, OC<u>H</u>$_3$), 3.19 (2 H, d, *J* 7.0, NHC<u>H</u>$_2$); δ_C ($CDCl_3$; 63 MHz) 171.26, 169.65 (<u>C</u>ONH), 167.27 (<u>C</u>O$_2$C), 136.40, 133.53 (*ipso*-Ar), 131.70, 129.20, 128.51, 128.43, 126.98, 126.88 (<u>C</u>H$_{ar}$), 54.49 (<u>C</u>HCONH), 52.19 (O<u>C</u>H$_3$), 41.09 and 38.07 (<u>C</u>H$_2$CO$_2$ and <u>C</u>H$_2$Ph); *m/z* (FAB) 341 (100%, MH$^+$), 252 (30, M-NHCH$_2$CO$_2$CH$_3$), 224 (32, 252-CO), 105 (44, 252-NHCHCH$_2$Ph).

4.3.0. Application of 5(4*H*)-oxazolone methodology: Synthesis of matrix metalloproteinase inhibitors

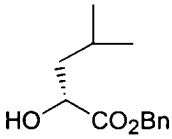
4.3.1. (*R*)-2-Hydroxy-4-methyl-hexanoic acid 119



The title compound was prepared by the method outlined by Mori.[116] To a solution of D-leucine (50.0 g, 0.38 mol) in aqueous sulfuric acid (0.5M, 250 mL) at 0 °C was added a solution of aqueous sodium nitrite (41.98 g, 0.61 mol, 1.6 equiv.) in water (150 mL) over a period of 3 h. The resulting suspension was stirred at 0 °C for a further 2 h, and overnight at room temperature. The resulting clear aqueous solution was extracted with ether (3 x 200 mL), and the combined organic phase dried (Na_2SO_4), filtered and evaporated under reduced pressure to give a pale yellow solid. Recrystallisation three times from petroleum ether (40:60):diethyl ether (1:1) furnished the desired product as a colourless solid (25.70 g, 51%).

Mp 78-79 °C, Lit.[116] 80-81 °C; $[\alpha]_D^{20}$ +26.2 (c 1.0 in 1 N NaOH), Lit.[116] $[\alpha]_D^{20}$ +26.5 (c 1.52, 1 N NaOH); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3420 (OH), 3200-2400 (acid OH), 1718 (acid C=O); δ_H ($CDCl_3$; 250 MHz) 6.99 (1 H, br s, O<u>H</u>), 4.28 (1 H, dd, *J* 7.5 7.0, HOC<u>H</u>), 1.89 (1 H, ddq, *J* 6.5, C<u>H</u>(CH_3)$_2$), 1.63, (1 H, ddd, *J* 14.0, 7.0, 6.5, CHC<u>H</u>$_A$H$_B$CH), 1.61 (1 H, ddd, *J* 14.0, 7.5, 6.5, CHCH$_A$<u>H</u>$_B$CH) 0.95 (6 H, d, *J* 6.514.0, 7.5, C<u>H</u>$_{3A}$CHCH$_{3B}$ and CH$_{3A}$CHC<u>H</u>$_{3B}$); δ_C ($CDCl_3$; 63 MHz) 188.2 (<u>C</u>O$_2$H), 68.8 (HO<u>C</u>H), 43.0 (<u>C</u>H$_2$CH), 24.3 (<u>C</u>H(CH_3)$_2$), 23.1, 21.3 (CH(<u>C</u>H$_3$)$_2$; *m/z* (FAB) 113 (91%, MH^+), 115 (42, M-OH), 87 (69, 115-CO).

4.3.2. (*R*)-Benzyl 2-hydroxy-4-methyl pentanoate 120

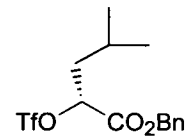


D-Leucic acid (10.00 g, 75.67 mmol) was suspended in toluene (100 mL). Benzyl alcohol (11.75 mL, 113.50 mmol) and *p*-toluenesulfonic acid (720 mg, 3.78 mmol, 0.05 equiv.) were added and the mixture heated under reflux using Dean-Stark conditions for 2.5 h. The reaction was quenched with saturated aqueous sodium

hydrogen carbonate (100 mL) and the aqueous layer extracted with ethyl acetate (2 x 70 mL). The combined organic extracts were washed with brine (2 x 50 mL), dried (Na_2SO_4), filtered, and evaporation under reduced pressure produced a yellow oil. Purification by column chromatography, eluting with hexane:ethyl acetate (10:1) furnished the title compound as a colourless oil (16.02 g, 95%).

R_f (Hexane:EtOAc, 4:1) 0.50; $[\alpha]_D^{20}$ +16.1 (c 1.0, $CHCl_3$), Lit.[117] $[\alpha]_D^{20}$ +14.4 (c 2.98, $CHCl_3$); υ_{max}(neat)/cm^{-1} 3470 (OH), 2955 (saturated CH), 1738 (ester C=O); δ_H ($CDCl_3$; 250 MHz) 7.36 (5 H, s, C<u>H</u>$_{ar}$), 5.20 (2 H, s, C<u>H</u>$_2$Ph), 4.24 (1 H, ddd, *J* 7.5, 7.0, 6.0, HOC<u>H</u>), 2.79 (1 H, d, *J* 6.0 O<u>H</u>), 1.87 (1H, ddq, *J* 6.5, C<u>H</u>$(CH_3)_2$), 1.60 (1 H, ddd, *J* 14.0, 7.0, 6.5, CHC<u>H</u>$_A$H$_B$CH), 1.51 (1 H, ddd, *J* 14.0, 7.5, 6.5, CHCH$_A$<u>H</u>$_B$CH), 0.93 (3 H, d, *J* 6.6 C<u>H</u>$_{3A}$CHCH$_{3B}$) 0.92 (3 H, d, *J* 6.6 CH$_{3A}$CHC<u>H</u>$_{3B}$); δ_C ($CDCl_3$; 63 MHz) 175.6 (<u>C</u>O$_2$CH$_2$), 135.1 (*ipso*-Ar), 128.5, 128.4, 128.2 (<u>C</u>H$_{ar}$), 69.0 (HO<u>C</u>H), 67.1 (<u>C</u>H$_2$Ph), 43.2 (<u>C</u>H$_2$CH), 24.2 (<u>C</u>H$(CH_3)_2$), 23.1, 21.4 (CH(<u>C</u>H$_3$)$_2$); *m/z* (FAB) 223 (42%, MH^+), 91 (100, CH_2Ph), Found (FAB) MH^+ 233.1342, $C_{13}H_{19}O_3$ requires 233.1334.

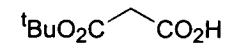
4.3.3. (*R*)-Benzyl 2-trifluoromethanesulfonyl-4-methyl pentanoate 121



The title compound was prepared according to the procedure outlined by Degerbeck.[117] To a solution of α-hydroxy ester **120** (15.75 g, 70.86 mmol) in DCM (160 mL) and 2,6-lutidine (10.73 mL, 92.11 mmol, 1.3 equiv.) cooled to -78 °C was added trifluoromethanesulfonic anhydride (13.71 mL, 81.48 mmol, 1.1 equiv.) over a period of 30 minutes. The solution was stirred at -78 °C for a further 1 h, followed by warming to -10 °C over 40 minutes, and 40 minutes stirring at room temperature. The reaction was quenched with water (100 mL) and the aqueous layer extracted with DCM (2 x 100 mL). The combined organic fractions were dried (Na_2SO_4), filtered and evaporated under reduced pressure. The crude brown product was filtered through a plug of silica, eluting with hexane:DCM (3:1) to furnish the title compound as a colourless oil (24.62 g, 98%).

R_f (Hexane:DCM, 1:1) 0.76; $[\alpha]_D^{20}$ +42.8 (c 1.8, CH_2Cl_2), Lit.[117] $[\alpha]_D^{20}$ +43.8 (c 1.0, CH_2Cl_2); υ_{max}(neat)/cm^{-1} 2963 (CH), 1763 (ester C=O), 1419 (SO_2-O), 1208 (C-F), 1043 (S=O); δ_H ($CDCl_3$; 250 MHz) 7.37 (5 H, s, C$\underline{H}_{ar}$), 5.25 (2 H, s, C$\underline{H}_2$Ph), 5.19 (1 H, dd, *J* 9.0, 3.5, OC$\underline{H}$), 1.94 (1 H, obscured ddq, C$\underline{H}$(CH_3)$_2$), 1.85-1.69 (2 H, m, CHC$\underline{H}_A\underline{H}_B$CH, and CHC$H_A\underline{H}_B$CH), 0.96 (3 H, d, *J* 6.0, C$\underline{H}_{3A}$CHCH_{3B}), 0.95 (3 H, d, *J* 6.0, CH_{3A}CHC$\underline{H}_{3B}$); δ_C ($CDCl_3$; 63 MHz) 167.4 ($\underline{C}O_2CH_2$); 134.2 (*ipso*-Ar), 128.7, 128.6, 128.4 (CH_{ar}); 118.3 (1 C, q, *J* 319, $F_3\underline{C}$), 82.2 (HO$\underline{C}$H), 68.1 ($\underline{C}H_2$Ph), 40.5 ($\underline{C}H_2$CH), 23.9 ($\underline{C}$H(CH_3)$_2$), 22.7, 20.9 (CH($\underline{C}H_3$)$_2$).

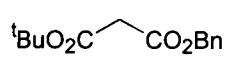
4.3.4. Mono *tert*-butyl malonate 124



tert-Butyl ethyl malonate (15.10 g, 80.23 mmol) was suspended in a solution of THF:water (1:1, 350 mL) and lithium hydroxide monohydrate (3.70 g, 88.25 mmol, 1.1 equiv.) added. The suspension was stirred at room temperature for 20 minutes until a clear solution was obtained. The solution was acidified to pH= 3 with aqueous citric acid (1M, 260 mL) and extracted with ethyl acetate (3 x 250 mL). The combined organic extracts were washed with brine (2 x 150 mL), dried (Na_2SO_4), filtered, and evaporation under reduced pressure gave the desired product as a colourless oil (12.85 g, 100%).

υ_{max}(neat)/cm^{-1} 3204-2400 (acid OH), 1734 (ester C=O and acid C=O); δ_H ($CDCl_3$; 250 MHz) 10.42 (1 H, br s, O$\underline{H}$), 3.34 (2 H, s, C$\underline{H}_2$), 1.48 (9 H, s, C(C$\underline{H}_3$)$_3$); δ_C ($CDCl_3$; 63 MHz) 171.3, 166.3 ($\underline{C}O_2H$, $\underline{C}O_2C$), 82.9 ($\underline{C}$(CH_3)$_3$), 41.7 ($\underline{C}H_2$), 27.7 (C($\underline{C}H_3$)$_3$); m/z (FAB) 161 (52%, MH^+), 57 (100, ^{t}Bu), Found (FAB) 161.0815, $C_7H_{13}O_4$ requires 161.0813.

4.3.5. *tert*-Butyl benzyl malonate 125

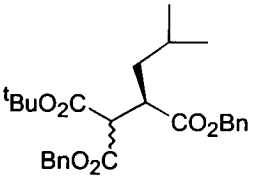


Mono acid **124** (12.85 g, 80.23 mmol) was suspended in THF:water (1:1, 200 mL) and potassium hydroxide (4.95 g, 88.24 mmol, 1.1 equiv.) added. Once complete dissolution had occurred the solvent was removed under reduced pressure to give a colourless solid which was dried under vacuum overnight. The solid was suspended

in DMF (300 mL); benzyl bromide (10.50 mL, 88.25 mmol, 1.1 equiv.) was added and the suspension stirred at room temperature for 7 h. The resulting mixture was quenched with saturated aqueous ammonium chloride (200 mL) and the aqueous solution extracted with ethyl acetate (4 x 200 mL). The combined organic extracts were washed with saturated aqueous ammonium chloride (2 x 200 mL), brine (2 x 150 mL), dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give a yellow oil. Purification by column chromatography, eluting with hexane:diethyl ether (20:1) furnished the title compound as a colourless oil (15.06 g, 75%).

R_f (Hexane:EtOAc, 4:1) 0.65; υ_{max}(neat)/cm^{-1} 2978 (saturated CH), 1729 (ester C=O); δ_H ($CDCl_3$; 250 MHz) 7.34 (5 H, s, C$\underline{H}_{ar}$), 5.16 (2 H, s, C$\underline{H}_2$Ph), 3.32 (2 H, s, C$\underline{H}_2CO_2$), 1.42 (9 H, s, C (C$\underline{H}_3)_3$); δ_C ($CDCl_3$; 63 MHz) 166.6, 165.3 (2 x $\underline{C}O_2C$), 135.2 (*ipso*-Ar), 128.3, 128.2, 128.1, (CH_{ar}), 81.8 ($\underline{C}(CH_3)_3$), 66.8 ($\underline{C}H_2Ph$), 42.7 (C$\underline{C}H_2C$), 27.6 (C($\underline{C}H_3)_3$); *m/z* (CI) 251 (56%, MH^+), Found (EI) 250.1202, $C_{14}H_{18}O_4$ requires 250.1205.

4.3.6. (2*RS*,3*R*)-4-Benzyl 1-*tert*-butyl 2-benzyloxycarbonyl-3-*iso*-butyl-succinate ester 126

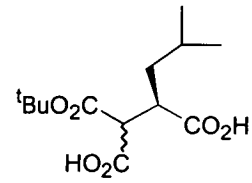


Malonate **125** was dissolved in THF (150 mL), cooled to -78 °C and added to a suspension of sodium hydride (1.45 g, 60.17 mmol, 1.0 equiv.) in THF (150 mL) at -78 °C *via* cannulation over 15 minutes. The suspension was stirred for 40 minutes and a solution of triflate **121** (22.39 g, 63.18 mmol, 1.05 equiv.) was added over a period of 15 minutes. The reaction mixture was stirred at -78 °C for 1 h followed by warming to -10 °C and stirring for a further 1 h until a clear solution was obtained. The solution was allowed to warm to room temperature for a further 1 h and quenched with saturated aqueous ammonium chloride (100 mL). The aqueous layer was extracted with ethyl acetate (3 x 200 mL), washed with brine (2 x 100 mL), water (2 x 100 mL), dried (Na_2SO_4), filtered, and evaporated to give a colourless oil. Purification by column chromatography, eluting with hexane:DCM (1:3) produced

the title product as a colourless oil.^{1}H nmr indicated a 1:1 mix of diastereomers (24.99 g, 91%).

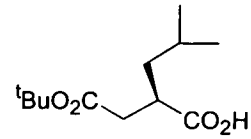
R_f (Hexane:EtOAc, 4:1) 0.68; $[\alpha]_D^{20}$ +22.8 (c 1.0, $CHCl_3$); υ_{max}(neat)/cm^{-1} 2959 (saturated CH), 1733 (ester C=O); δ_H ($CDCl_3$; 360 MHz) 7.34 (2.5 H, s, C$\underline{H}_{ar}$), 7.33 (5 H, s, C$\underline{H}_{ar}$), 7.32 (2.5 H, s, C$\underline{H}_{ar}$), 5.20, 5.17, 5.15, 5.13, 5.11, 5.09, 5.05 and 4.99 (total of 2 H, 8 x d, *J* 12.4, 12.2 and 12.0, 2 x C$\underline{H}_A$H$_B$Ph and CH$_A\underline{H}_B$Ph of each diastereomer), 3.69 (0.5 H, d, *J* 10.2, C$\underline{H}^A$CHCO$_2$), 3.66 (0.5 H, d, *J* 10.4, C$\underline{H}^B$CHCO$_2$), 3.18 and 3.17 (total of 1 H, 2 x m, CHC$\underline{H}$CO$_2$, of each diastereomer), 1.7201.52 (total of 2 H, 2 x m, CHC$\underline{H}_A$H$_B$CH and CHCH$_A\underline{H}_B$CH of each diastereomer), 1.34, 1.33 (total of 9 H, 2 x s, C(CH$_3$)$_3$ of each diastereomer), 1.26, 1.16 (total of 1 H, 2 x m, CH(CH$_3$)$_2$ of each diastereomer), 0.90, 0.85, 0.83, 0.78 (total of 6 H, 4 x d, J 6.4, C$\underline{H}_{3A}$CHCH$_{3B}$ and CH$_{3A}$CHC$\underline{H}_{3B}$ of each diastereomer); δ_C ($CDCl_3$; 90 MHz) 173.6, 173,4, 167.9, 167.8, 167.5, 167.3, (C$\underline{O}_2$C), 135.5, 135.1 (*ipso*-Ar), 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0 (C$\underline{H}_{ar}$), 82.3, 82.2, (C$\underline{C}$(CH$_3$)$_3$), 66.9, 66.4, 66.3 (C$\underline{H}_2$Ph), 55.6, 55.4 (O$_2$C$\underline{C}$H$_2$CO$_2$), 42.7, (CH$\underline{C}$HCH$_2$), 39.3, 39.2 (C$\underline{H}_2$CH(CH$_3$)$_2$), 27.5 (C$\underline{C}$(CH$_3$)$_3$), 25.5 (C$\underline{H}$(CH$_3$)$_2$), 23.4, 23.2, 21.1, 21.0 (CH(C$\underline{H}$$_3$)$_2$); m/z (FAB) 455 (9%, MH$^+$), 399 (78, MH$^+$-^{t}Bu), 91 (100, CH$_2$Ph), 57 (23, ^{t}Bu), Found (FAB) 455.2424, $C_{27}H_{35}O_6$ requires 455.2433.

4.3.7. (2*RS*,3*R*)-4-*tert*-Butyl 2-carboxy-3-*iso*-butyl-succinic acid 116



10% Palladium on carbon (2.50 g, 10% w) was added to a solution of triester **126** (24.99 g, 54.98 mmol) in THF (400 mL) and the reaction vessel placed under a atmosphere of hydrogen. The mixture was stirred at room temperature for 2 h until the required volume of hydrogen had adsorbed (~2.5 L). The catalyst was filtered through celite and washed with THF (2 x 100 mL). The combined organic fractions were evaporated under reduced pressure to give a colourless solid which was not further purified but carried directly to the next step.

4.3.8. (*R*)-2-*iso*-butyl-succinic acid 4-*tert*-butyl ester 114



Triethylamine (756 L, 5.43 mmol, 0.1 equiv.) was added to a suspension of crude diacid **116** (14.88 g, 54.25 mmol expected yield) in acetonitrile (250 mL) and the mixture heated under reflux for 14 h under and atmosphere of nitrogen. On cooling, the clear solution was filtered through a plug of silica, eluting with acetonitrile. The solvent was removed under reduced pressure to give the title compound as a pale yellow oil (10.76 g, 85% from triester **126**).

$[\alpha]_D^{20}$ +14.0 (c 2.20, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3400-2500 (acid OH), 1733 (ester C=O), 1710 (acid C=O); δ_H ($CDCl_3$; 360 MHz) 1.16 (1 H, br s, O$\underline{H}$), 2.83 (1 H, m, C$\underline{H}CO_2H$), 2.56 (1 H, dd, *J* 16.4, 9.2, $O_2CC\underline{H}_AH_BCH$) 2.36 (1 H, dd, *J* 16.4, 6.4, $O_2CCH_A\underline{H}_BCH$) 1.65-1.54 (2 H, 2 x m, $CHC\underline{H}_AH_BCH$ and $CHCH_A\underline{H}_BCH$), 1.41 (9 H, s, $C(C\underline{H}_3)_3$), 1.26 (1 H, m, $C\underline{H}(CH_3)_2$), 0.92 (3 H, d, *J* 6.4, $C\underline{H}_{3A}CHCH_{3B}$), 0.88 (3 H, d, *J* 6.4, $CH_{3A}CHC\underline{H}_{3B}$); δ_C ($CDCl_3$; 63 MHz) 181.7, 170.9 ($\underline{C}O_2H$, $\underline{C}O_2C$), 80.8 ($\underline{C}(CH_3)_3$), 40.7 ($O_2C\underline{C}H_2$), 39.5 ($\underline{C}HCO_2H$), 37.5 ($CH\underline{C}H_2CH$), 27.8 ($\underline{C}(CH_3)_3$), 25.6 ($\underline{C}H(CH_3)_2$), 22.4, 22.1 ($CH(\underline{C}H_3)_2$); *m/z* (FAB) 231 (82%, MH^+), 175 (100, MH^+-^{t}Bu), 57 (71, ^{t}Bu), Found (FAB) 231.1592, $C_{12}H_{23}O_4$ requires 231.1596.

4.3.9. General procedure for the coupling of α-amino acid esters to (*R*)-2-*iso*-butyl-succinic acid 4-*tert*-butyl ester 114

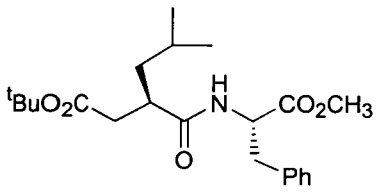
Method (i)

EDCI (1.2 equiv.), HOBt (1.2 equiv.) and triethylamine (2.4 equiv.) were added to a solution of succinate **114** (1.0 equiv.) in DCM (40 mL). Once complete dissolution occurred α-amino acid ester hydrochloride (1.2 equiv.) was added and the solution stirred at room temperature for 6 h. The reaction mixture was diluted with DCM (50 mL), washed with saturated aqueous ammonium chloride (2 x 50 mL), water (50 mL), saturated aqueous sodium hydrogen carbonate (2 x 50 mL), water (50 mL), dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give the crude product. Purification by column chromatography furnished the desired product.

Method (ii)

TBTU (1.2 equiv.), HOBt (1.2 equiv.) and DIPEA (3.0 equiv.) were added to a solution of succinate **114** (1.0 equiv.) in DMF (40 mL). α-Amino acid ester hydrochloride (1.2 equiv.) was added and the resulting solution stirred at room temperature under an atmosphere of nitrogen for 1.5 h. After quenching with saturated aqueous ammonium chloride (50 mL), the aqueous layer was extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were washed with saturated aqueous ammonium chloride (50 mL), saturated aqueous sodium hydrogen carbonate (2 x 50 mL), brine (50 mL), dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give the crude product. Purification by column chromatography furnished the desired product.

4.3.10. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a

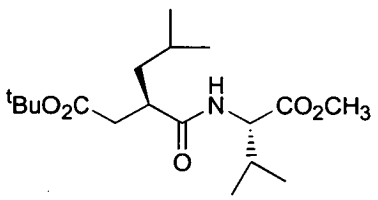


The procedure outlined in Section **(4.3.9.i.)** was followed using EDCI (68 mg, 0.36 mmol, 1.2 equiv.), HOBt (48 mg, 0.36 mmol, 1.2 equiv.), triethylamine (99 μL, 0.72 mmol, 2.4 equiv.), succinate **114** (70 mg, 0.30 mmol, 1.0 equiv.) and L-phenylalanine methyl ester hydrochloride (78 mg, 0.36 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title compound as a colourless solid (98 mg, 83%). ^{1}H nmr indicated a d.e. of 95%. The spectroscopic data given is for the diastereomer whose absolute stereochemistry was determined from the crystal structure (see appendix 1).

R_f (Hexane:EtOAc, 6:1) 0.27; d.e. 95%; Mp 97-99 °C; (Found: C, 67.58; H, 8.50; N, 3.75; $C_{22}H_{33}NO_5$ requires C, 67.49; H, 8.49; N 3.58); $[\alpha]_D^{20}$ +62.3 (c 0.82, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3430 (amide NH), 3018 (saturated CH), 1726 (ester C=O), 1673, 1510 (CONH); δ_H ($CDCl_3$; 600 MHz) 7.27 (2 H, tt, *J* 7.3, C$\underline{H}_{ar(meta)}$), 7.22 (1 H, tt, *J* 7.3, C$\underline{H}_{ar(para)}$),7.16 (2 H, dt, *J* 7.3, C$\underline{H}_{ar(ortho)}$), 6.81 (1 H, d, *J* 7.9 N$\underline{H}$), 4.85 (1 H, ddd, *J* 7.9, 6.0, 5.9, NHC$\underline{H}$), 3.67 (3 H, s, OC$\underline{H}_3$), 3.09 (1 H, dd, *J* 14.3, 5.9, C$\underline{H}_A$H$_B$Ph)

3.08 (1 H, dd, *J* 14.3, 6.0, $CH_A\underline{H}_BPh$), 2.61 (1 H, m, C<u>H</u>CONH), 2.52 (1 H, dd, *J* 16.6, 8.9, $O_2CC\underline{H}_AH_BCHCO$), 2.27 (1 H, dd, 16.6, 4.9, $O_2CCH_A\underline{H}_BCHCO$), 1.55 (1 H, ddd, *J* 13.6, 8.9, 5.9, $CHC\underline{H}_AH_BCH$), 1.53 (1 H, m, C<u>H</u>$(CH_3)_2$), 1.42 (9 H, s, C(C<u>H</u>$_3)_3$), 1.15 (1 H, ddd, *J* 13.6, 7.6, 5.6, $CH_A\underline{H}_BCH(CH_3)_2$), 0.85 (3 H, d, *J* 6.4, $C\underline{H}_{3A}CHCH_{3B}$), 0.84 (3 H, d, *J* 6.4, $CH_{3A}CHC\underline{H}_{3B}$); δ_C ($CDCl_3$; 63 MHz) 174.3, 171.8, 171.5 (<u>C</u>ONH, <u>C</u>O_2R); 135.8, (*ipso*-Ar); 129.2 128.4, 126.9 (<u>C</u>H_{ar}); 80 6 (<u>C</u>$(CH_3)_3$); 53.0 (NH<u>C</u>H); 52.0 ($CO_2\underline{C}H_3$); 41.2 ($O_2C\underline{C}H_2$); 40.7 (<u>C</u>HCONH); 38.0, 37.9 (<u>C</u>H_2Ph and <u>C</u>H_2CH); 27.9 (C(<u>C</u>$H_3)_3$), 25.4 (<u>C</u>H$(CH_3)_2$); 22.8, 22.0 (CH(<u>C</u>$H_3)_2$); *m/z* (FAB) 392 (34%, MH^+), 91 (100, CH_2Ph, 57 (35, ^{t}Bu), Found (FAB) 392.2419, $C_{22}H_{34}NO_5$ requires 392.2437.

4.3.11. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-valine methyl ester 127b

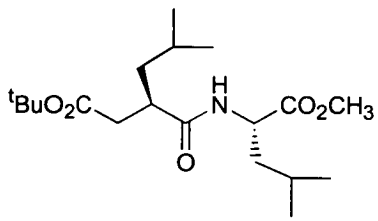


The procedure outlined in Section **(4.3.9.i.)** was followed using EDCI (70 mg, 0.36 mmol, 1.2 equiv.), HOBt (49 mg, 0.36 mmol, 1.2 equiv.), triethylamine (102 μL, 0.73 mmol, 2.4 equiv.), succinate **114** (70 mg, 0.30 mmol, 1.0 equiv.) and L-valine methyl ester hydrochloride (61 mg, 0.36 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title compound as a colourless solid (74 mg, 71%).

R_f (Hexane:EtOAc, 6:1) 0.20; d.e. 86%; Mp 75-76 °C; (Found: C, 63.05; H, 9.60; N, 3.98; $C_{18}H_{34}NO_5$ requires C, 62.95; H, 9.68; N 4.08); $[\alpha]_D^{20}$ +12.7 (c 1.26, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3428 (amide NH), 3018 (saturated CH), 1734 (ester C=O), 1675, 1512 (CONH); δ_H ($CDCl_3$; 600 MHz) 6.21 (1 H, d, *J* 8.9, N<u>H</u>), 4.53 (1 H, dd, *J* 8.9, 4.9, NHC<u>H</u>), 2.68 (1 H, m, C<u>H</u>CONH), 2.58 (1 H, dd, *J* 17.0, 9.6, $OCC\underline{H}_AH_BCH$), 2.29 (1 H, dd, *J* 17.0 4.0, $OCCH_A\underline{H}_BCH$), 2.14 (1 H, dqq, *J* 6.9, 4.9, CHC<u>H</u>$(CH_3)_2$), 1.60 (1 H, ddd, *J* 14.6, 9.0, 5.6, $CHC\underline{H}_AH_BCH$), 1.55 (1 H, m, $CH_2C\underline{H}(CH_3)_3$), 1.40 (9 H, s, C(C<u>H</u>$_3)_3$), 1.15 (1 H, ddd, *J* 14.6, 8.0, 5.6, $CHCH_A\underline{H}_BCH$), 0.90 (6 H, d, *J* 6.8, CH(C<u>H</u>$_{3A}$)CHCH$_{3B}$ and CH(CH$_{3A}$)CHC<u>H</u>$_{3B}$), 0.89, (3 H, d, *J* 6.8, $CH_2(C\underline{H}_{3A})CHCH_{3B}$), 0.86 (3 H, d, *J* 6.8, $CH_2(CH_{3A})CHC\underline{H}_{3B}$); δ_C ($CDCl_3$; 63 MHz)

174.56, 172.3, 171.7 (CONH, CO_2C), 80.6 ($C(CH_3)_3$), 56.7 (NHCH), 51.9 (OCH_3), 41.2 ($OCCH_2CH$), 40.7 (CHCONH), 38.3 ($CH_2CH(CH_3)_2$), 31.2 ($CHCH(CH_3)_2$), 27.9 ($C(CH_3)_3$), 25.5 ($CH_2CH(CH_3)_2$), 22.8, 22.0 ($CH_2CH(CH_3)_2$), 18.8, 17.5 ($CHCH(CH_3)_2$); *m/z* (FAB), 344 (70%, MH^+), 288 (100, MH_2-^{t}Bu), 270 (11, 288-H_2O), 132 (43), 72 (57) Found (FAB), 358.2595 $C_{19}H_{36}NO_5$ requires 3518.2594.

4.3.12. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-leucine methyl ester 127c

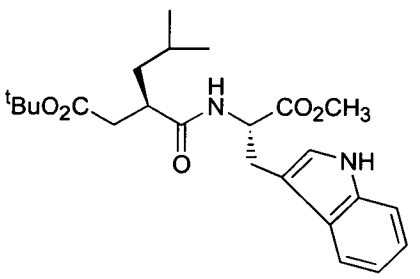


The procedure outlined in Section **(4.3.9.ii.)** was followed using TBTU (117 mg, 0.36 mmol, 1.2 equiv.), HOBt (49 mg, 0.36 mmol, 1.2 equiv.), diisopropylethylamine (159 μL, 0.91 mmol, 3.0 equiv.), succinate **114** (70 mg, 0.30 mmol, 1.0 equiv.) and L-leucine methyl ester hydrochloride (66 mg, 0.36 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title compound as a colourless solid (80 mg, 74%).

R_f (Hexane:EtOAc, 6:1) 0.55; d.e. 90%; Mp 106-108 °C; (Found: C, 63.25; H, 9.74; N, 3.79; $C_{19}H_{34}NO_5$ requires C, 63.48; H, 9.87; N 3.94); $[\alpha]_D^{20}$ +3.2 (c 1.44, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3431 (amide NH), 3018 (saturated CH), 1736 (ester C=O), 1673, 1513 (CONH); δ_H ($CDCl_3$; 600 MHz) 6.08 (1 H, d, *J* 8.3 NH), 4.9 (1 H, ddd, *J* 13.6, 8.3, 5.0, NHCH), 3.69 (3 H, s, OCH_3), 2.64 (1 H, m, $OCCH_2CH$), 2.57 (1 H, dd, *J* 16.9, 9.6, $OCCH_AH_BCH$), 2.28 (1 H, dd, *J* 16.9, 4.0, $OCCH_AH_BCH$), 1.68-1.49 (total of 5 H, m, $CH_2CHCH_AH_BCH$, CH_2CHCH_2CH, $NHCHCH_AH_BCH$, $NHCHCH_AH_BCH$ and $NHCHCH_2CH$), 1.40 (9 H, s, $C(CH_3)_3$), 1.13 (1 H, ddd, *J* 13.3, 7.9, 5.6 $OCCH_2CHCH_AH_B$), 0.90 (3 H, 2 d, *J* 6.3, $CH_{3A}CHCH_{3B(leucine)}$), 0.89 (3 H, 2 d, *J* 6.6, $CH_{3A}CHCH_{3B(succinate)}$), 0.88 (3 H, d, *J* 6.3, $CH_{3A}CHCH_{3B(leucine)}$), 0.86 (3 H, d, *J* 6.6, $CH_{3A}CHCH_{3B(succinate)}$); δ_C ($CDCl_3$; 63 MHz) 174.6, 173.4, 171.7 (CONH, CO_2C), 80.6 ($C(CH_3)_3$), 52.1 (OCH_3), 50.4 (NHCH), 41.6, 41.3 ($OCCH_2CH$ and $NHCHCH_2$), 40.6 (CHCONH), 38.3 ($OCCH_2CHCH_2$), 28.0 ($C(CH_3)_3$), 25.5, 24.5 ($CH(CH_3)_2$), 23.0, 22.8 ($CH(CH_3)_{2(succinate)}$), 21.8, 21.7 ($CH(CH_3)_{2(leucine)}$); *m/z* (FAB) 357 (24%, MH^+),

302 (100, MH_2-^{t}Bu), 284 (7, 302-H_2O), 146 (20), 86 (38), 57 (54, ^{t}Bu), Found (FAB) 344.2439, $C_{18}H_{36}NO_5$ requires 344.2437.

4.3.13. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-tryptophan methyl ester 127d

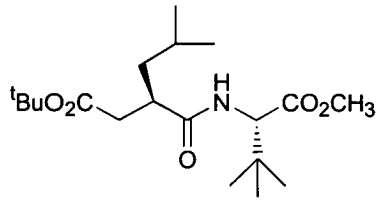


The procedure outlined in Section **(4.3.9.ii.)** was followed using TBTU (119 mg, 0.37 mmol, 1.2 equiv.), HOBt (50 mg, 0.37 mmol, 1.2 equiv.), diisopropylethylamine (161uL, 0.92 mmol, 3.0 equiv.), succinate **114** (71 mg, 0.31 mmol, 1.0 equiv.) and L-tryptophan methyl ester hydrochloride (94 mg, 0.37 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:diethyl ether (1:1) furnished the title compound as a colourless glass (109 mg, 82%).

R_f (Hexane:Et_2O, 1:1) 0.20; d.e. 90%; Mp 96-97 °C; (Found: C, 66.45; H, 7.93; N, 6.33; $C_{24}H_{34}N_2O_5$ requires C, 66.95; H, 7.96; N 6.51); $[\alpha]_D^{20}$ +64.9 (c 1.96, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3480 (indole NH), 3428 (amide NH), 3020 (saturated CH), 1726 (ester C=O), 1668, 1513 (CONH); δ_H ($CDCl_3$; 600 MHz) 8.29 (1 H, brs, $N\underline{H}_{(indole)}$)7.56 (1 H, d, *J* 7.6,$C\underline{H}_{ar}$), 7.32 (1 H, d, *J* 8.0, $C\underline{H}_{ar}$), 7.15 (1 H, m, $C\underline{H}_{ar}$), 7.13 (1 H, d, *J*, 2.4, $C\underline{H}NH_{(indole)}$), 7.09 (total of 1 H, m, $C\underline{H}_{ar}$), 6.27 (1 H, d, *J* 7.8, $N\underline{H}$), 4.92 (1 H, ddd, *J* 7.8, 5.6, 5.4, NHC$\underline{H}$), 3.64 (3 H, s, $OC\underline{H}_3$), 3.34 (1 H, dd, *J* 14.8, 5.4, $CHC\underline{H}_AH_B$Indole), 3.24 (1 H, dd, *J* 14.8, 5.6, $CHCH_A\underline{H}_B$Indole), 2.61 (1 H, m, C$\underline{H}$CONH), 2.58 (1 H, dd, *J* 15.8, 9.0, $OCC\underline{H}_AH_BCH$), 2.29 (1 H, dd, *J* 15.8, 4.0, $OCCH_A\underline{H}_BCH$), 1.58-1.50 (2 H, m, $C\underline{H}(CH_3)_2$ and $CH_2CHC\underline{H}_AH_BCH$), 1.40 (9 H, s, $C(C\underline{H}_3)_3$), 1.17 (1 H, ddd, *J* 15.4, 9.8, 5.4 $CH_2CHCH_A\underline{H}_BCH$), 0.84 (3 H, d, *J* 6.4, $C\underline{H}_{3A}CHCH_{3B}$), 0.82 (3 H, d, *J* 6.4, $CH_{3A}CHC\underline{H}_{3B}$); δ_C ($CDCl_3$; 63 MHz) 174.4, 172.2, 171.6 ($\underline{C}$ONH, $\underline{C}O_2C$), 136.0, 127.4 (*ipso*-Ar), 123.1, 121.9, 119.4, 118.4, 111.1 ($\underline{C}H_{ar}$), 109.6 (*ipso*-Ar), 80.5 ($\underline{C}(CH_3)_3$), 52.5 (NH$\underline{C}$H), 52.1 ($OC\underline{H}_3$), 41.3 ($OC\underline{C}H_2CH$), 40.7 ($\underline{C}$HCONH), 37.9 ($CH\underline{C}H_2CH$), 27.9 ($C(\underline{C}H_3)_3$), 27.6

($\underline{C}H_2$Indole), 25.4 ($\underline{C}H(CH_3)_2$), 22.7, 22.0 ($CH(\underline{C}H_3)_2$); *m/z* (FAB) 431 (1%, MH^+), 375 (12, MH_2^+-^{t}Bu), 202 (24), 130 (100, CH_2Indole), 57 (41, ^{t}Bu), Found (FAB) 431.2550, $C_{24}H_{35}N_2O_5$ requires 431.2546.

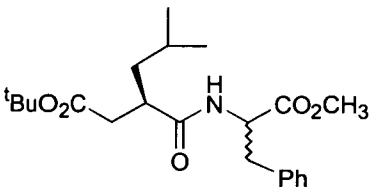
4.3.14. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-*tert*-leucine methyl ester 127e



The procedure outlined in Section **(4.3.9.ii.)** was followed using TBTU (119 mg, 0.37 mmol, 1.2 equiv.), HOBt (50 mg, 0.37 mmol, 1.2 equiv.), diisopropylethylamine (161 μL, 0.92 mmol, 3.0 equiv.), succinate **114** (71 mg, 0.32 mmol, 1.0 equiv.) and L-*tert*-leucine methyl ester hydrochloride (67 mg, 0.37 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title compound as a colourless solid (84 mg, 77%).

R_f (Hexane:EtOAc, 6:1) 0.38; d.e. 86%; Mp 104-106 °C; (Found: C, 63.82; H, 9.67; N, 3.91; $C_{19}H_{35}NO_5$ requires C, 63.84; H, 9.87; N 3.92); $[\alpha]_D^{20}$ +5.3 (c 1.48, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3433 (amide NH), 3018 (saturated CH), 1729 (ester C=O), 1675, 1512 (CONH); δ_H ($CDCl_3$; 600 MHz) 6.29 (1 H, d, *J* 9.3, N$\underline{H}$), 4.42 (1 H, d, *J* 9.3, NHC$\underline{H}$), 3.69 (3 H, s, OC$\underline{H}_3$), 2.67 (1 H, m, C$\underline{H}$CONH), 2.58 (1 H, dd, *J* 17.0, 9.6, OCC$\underline{H}_A$H$_B$CH), 2.29 (1 H, dd, *J* 17.0, 4.0, OCCH$_A\underline{H}_B$CH), 1.59 (1 H, ddd, *J* 13.6, 8.9, 5.9, CHC$\underline{H}_A$H$_B$CH), 1.51 (1 H, m, C$\underline{H}(CH_3)_2$), 1.14 (1 H, ddd, *J* 13.6, 7.9, 5.9, CHCH$_A\underline{H}_B$CH), 0.88 (3 H, d, *J* 6.6, C$\underline{H}_{3A}$CHCH$_{3B}$), 0.84 (3 H, d, *J* 6.6, CH$_{3A}$CHC$\underline{H}_{3B}$); δ_C ($CDCl_3$; 63 MHz) 174.3, 171.9, 171.7 ($\underline{C}$ONH, $\underline{C}O_2C$), 80.6 (O$\underline{C}(CH_3)_3$), 59.7 (NH$\underline{C}$H), 51.6 (O$\underline{C}H_3$), 41.1 (OC$\underline{C}H_2$CH) 40.7 ($\underline{C}$HCONH), 38.1 (CH$\underline{C}H_2$CH), 34.7 (CH$\underline{C}(CH_3)_3$), 27.9 (OC($\underline{C}H_3)_3$), 26.4 (CHC($\underline{C}H_3)_3$), 25.5 ($\underline{C}H(CH_3)_2$),. 22.8, 22.1 ($CH(\underline{C}H_3)_2$); *m/z* (FAB) 358 (24%, MH^+), 302 (100, MH_2^+-^{t}Bu), 242 (5), 146 (12), 86 (44), 57 (^{t}Bu), Found (FAB) 358.2609, $CHNO_5$ requires 358.2593.

4.3.15. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester and (2*R*,2'*R*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a

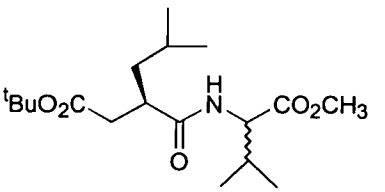


The procedure outlined in Section **(4.3.9.i.)** was followed using EDCI (1.05 g, 5.47 mmol, 1.2 equiv.), HOBt (739 mg, 5.47 mmol, 1.2 equiv.), triethylamine (1.53 mL, 10.94 mmol, 2.4 equiv.), succinate **114** (1.05 g, 4.56 mmol, 1.0 equiv.) and DL-phenylalanine methyl ester hydrochloride (1.18 g, 5.47 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title compound as a colourless solid (1.42 g, 80%). ^{1}H nmr indicated a (1:1) ratio of diastereomers.

R_f (Hexane:EtOAc, 6:1) 0.27, 0.23; Mp 69-72 °C (Found: C, 67.52; H, 8.49; N, 3.49; $C_{22}H_{33}NO_5$ requires C, 67.49; H, 8.49; N 3.58); $[\alpha]_D^{20}$ +7.9 (c 1.34, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3429 (amide NH), 3019 (saturated CH), 1737, 1732 (ester C=O), 1668, 1510 (CONH); δ_H ($CDCl_3$; 600 MHz) 7.25 (1 H, tt, *J* 7.3, C$\underline{H}^{A}_{ar\ (meta)}$), 7.23 (1 H, tt, *J* 7.3, C$\underline{H}^{B}_{ar\ (meta)}$), 7.21-7.17 (total of 1 H, m, C$\underline{H}_{ar(para)}$ of each diastereomer), 7.13 (1 H, brt, *J* 7.3, C$\underline{H}^{A}_{ar\ (ortho)}$), 7.08 (1 H, brt, *J* 7.3, C$\underline{H}^{B}_{ar\ (ortho)}$), 6.18 (total of 1 H, br d, N$\underline{H}$ of each diastereomer), 4.84 (total of 1 H, 2 x m, NHC$\underline{H}$, of each diastereomer), 3.66 (1.5 H, s, OC$\underline{H}^{A}_{3}$), 3.64 (1.5 H, s, OCH$^{B}_{3}$), 3.11 (0.5 H, dd, *J* 14.0, 5.9, C$\underline{H}^{B}_{A}H_B$Ph) 3.06 (0.5 H, dd, *J* 14.3, 6.0, C$\underline{H}^{A}_{A}H_B$Ph) 3.05 (0.5 H, dd, *J* 14.3, 5.9 $CH_A\underline{H}^{A}_{B}$Ph), 3.00 (0.5 H, dd, *J* 14.0, 6.9, $CH_A\underline{H}^{B}_{B}$Ph), 2.57 (total of 2 H, m, C$\underline{H}$CONH of each diastereomer), 2.50 (0.5 H, dd, *J* 16.3, 9.0, OCC$\underline{H}^{B}_{A}H_B$CH), 2.49 (0.5 H, dd, *J* 16.6, 8.9, OCC$\underline{H}^{A}_{A}H_B$CH), 2.23 (0.5 H, dd, *J* 16.6, 4.9, OCC$H_A\underline{H}^{A}_{B}$CH) 2.22 (0.5 H, dd, *J* 16.3, 5.0, OCC$H_A\underline{H}^{B}_{B}$CH), 1.55-1.47 (total of 1.5 H, m, CHC$\underline{H}^{A}_{A}H_B$CH, C$\underline{H}^{A}$(CH$_3$)$_2$ and CHC$\underline{H}^{B}_{A}H_B$CH) 1.39 (4.5 H, s, C(C$\underline{H}^{A}_{3}$)$_3$), 1.36 (4.5 H, s, C(C$\underline{H}^{B}_{3}$)$_3$), 1.32 (0.5 H, m, C$\underline{H}^{B}$(CH$_3$)$_2$), 1.13 (0.5 H, ddd, *J* 13.3, 7.6, 5.6,

$CHCH_A\underline{H}^A_BCH$), 1.06 (0.5 H, ddd, *J* 13.6, 8.6, 5.3, $CHCH_A\underline{H}^B_BCH$), 0.83 (1.5 H, d, *J* 6.6, $C\underline{H}^A_{3A}CHCH_3$), 0.81 (1.5 H, d, *J* 6.6, $CH_3CHC\underline{H}^A_{3B}$), 0.79 (1.5 H, d *J* 6.6 $CH_3CHC\underline{H}^B_{3A}$) 0.79 (1.5 H, d *J* 6.6 $CH_3CHC\underline{H}^B_{3B}$); δ_C ($CDCl_3$; 63 MHz) 174.3, 174.2, 171.8, 171.5, 171.3 (<u>C</u>ONH, <u>C</u>O_2R), 135.8 (*ipso*-Ar), 129.1, 129.0, 128.3, 126.9, (<u>C</u>H_{ar}), 80.6, (<u>C</u>$(CH_3)_3$), 52.9, 52.8 (NH<u>C</u>H), 52.0 ($CO_2\underline{C}H_3$), 41.2, 41.0 ($O_2C\underline{C}H_2$), 40.6, 40.5 (<u>C</u>HCONH), 38.2, 37.9, 37.8 ($\underline{C}H_2CH(CH_3)_2$ and $\underline{C}H_2Ph$), 27.9, 27.8 ($C(\underline{C}H_3)_3$), 25.4 ($\underline{C}H(CH_3)_2$), 22.9, 22.7, 22.0, 21.9 ($CH(\underline{C}H_3)_2$); *m/z* (FAB) 392 (62%, MH^+), 336 (100, MH_2^+-^{t}Bu), 258 (13), 180 (52), 120 (30), 91 (18, CH_2Ph), 57 (25, ^{t}Bu), Found (FAB), 392.24124, $C_{22}H_{34}NO_5$ requires 392.2437.

4.3.16. (2*R*,2'*R*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-valine methyl ester and (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-valine methyl ester 127b

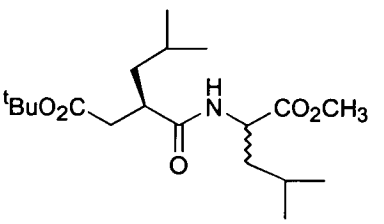


The procedure outlined in Section **(4.3.9.i.)** was followed using EDCI (1.04 g, 5.42 mmol, 1.2 equiv.), HOBt (732 mg, 5.42 mmol, 1.2 equiv.), triethylamine (1.51 mL, 10.84 mmol, 2.4 equiv.), succinate **114** (1.04 g, 4.52 mmol, 1.0 equiv.) and DL-valine methyl ester hydrochloride (1.18 g, 5.47 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title compound as a colourless wax (1.20 g, 77%). ^{1}H nmr indicated a (1:1.5) ratio of diastereomers.

R_f (Hexane:EtOAc, 6:1) 0.20, 0.18;(Found: C, 62.97; H, 9.98; N, 4.01; $C_{18}H_{33}NO_5$ requires C, 62.95; H, 9.68; N 4.08); $[\alpha]_D^{20}$ + 4.74 (c 1.16, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3428 (amide NH), 1734, 1730 (ester C=O), 1671, 1507 (CONH); δ_H ($CDCl_3$; 600 MHz) 6.30 (0.6 H, d, *J* 8.3, N<u>H</u>^{A}), 6.22 (0.4 H, d, *J* 8.9, N<u>H</u>^{B}), 4.53 (0.4 H, dd, *J* 8.9, 4.9, NHC<u>H</u>^{B}), 4.48 (0.6 H, dd, *J* 8.3, 4.9 NHC<u>H</u>^{A}), 3.70 (1.8 H, s, $OC\underline{H}^B_3$), 3.68 (1.2 H, s, $OC\underline{H}^A_3$), 2.86 (total of 1 H, m, C<u>H</u>CONH of each diastereomer), 2.57 (0.4 H, dd, *J* 17.0, 9.6, $OCC\underline{H}^B_AH_BCH$), 2.54 (0.6 H, dd, *J* 16.9, 8.6, $OCC\underline{H}^A_AH_BCH$), 2.29

(0.4 H, dd, *J* 17.0, 4.0, $OCCH_A\underline{H}_B^BCH$), 2.2.8 (0.6 H, dd, *J* 16.9, 5.0, $OCCH_A\underline{H}_B^ACH$), 2.12 (total of 1 H, m, $CHC\underline{H}(CH_3)_2$ of each diastereomer), 1.64 (0.6 H, ddd, *J* 15.0, 9.6, 5.6, $CHC\underline{H}_A^AH_BCH$), 1.60 (0.4 H, ddd, *J* 14.6, 9.0, 5.6, $CHCH_A\underline{H}_B^BCH$), 1.54 (total of 1 H, m, $CHCH_A\underline{H}_BCH$ of each diastereomer), 1.40 (3.6 H, s, $C(C\underline{H}_3^A)_3$), 1.39 (2.4 H, $C(C\underline{H}_3^B)_3$),1.14 (0.6 H, ddd, J 15.0, 8.6, 5.6 $CHCH_A\underline{H}_B^ACH$), 1.13 (0.4 H, ddd, J 14.6, 8.0, 5.6 $CHCH_A\underline{H}_B^BCH$), 0.90 (1.8 H, d, *J* 6.6, $CH_2(C\underline{H}_{3A}^A)CHCH_{3B}$), 0.89 (3 H, d, *J* 6.9, $CH(C\underline{H}_{3A})CHCH_{3B}$ of each diastereomer), 0.88 (3 H, d, *J* 6.9, $CH(C\underline{H}_{3A})CHCH_{3B}$ of each diastereomer), 0.88 (1.2 H, d, *J* 6.6, $CH_2(CH_{3A})CHC\underline{H}_{3B}^A$), 0.87 (1.8 H, d, *J* 6.6 $CH_2(C\underline{H}_{3A}^B)CHCH_{3B}$), 0.85 (1.2 H, d, *J* 6.6, $CH_2(CH_{3A})CHCH_{3B}^B$); δ_C ($CDCl_3$; 63 MHz) 174.7, 174.6, 172.3, 172.1, 171.7, 171.6 (<u>C</u>ONH, <u>C</u>O_2R), 80.7, 80.6 ($\underline{C}(CH_3)_3$), 56.9, 56.7 (NH<u>C</u>H), 51.9 ($CO_2\underline{C}H_3$), 41.2, 41.0 ($O_2C\underline{C}H_2$), 40.7, 40.6 (<u>C</u>HCONH), 38.4, 38.2 ($CH\underline{C}H_2CH$), 31.1, 30.9 ($CH\underline{C}H(CH_3)_2$), 27.9 ($C(\underline{C}H_3)_3$), 25.6, 25.5 ($CH_2\underline{C}H(CH_3)_2$), 23.0, 22.8, 22.0, 21.8 ($CH_2CH(\underline{C}H_3)_2$), 18.8, 18.8, 17.7, 17.5 ($CHCH(\underline{C}H_3)_2$); *m/z* (FAB) 344 (49%, MH^+), 288 (100, MH_2^+-^{t}Bu), 210 (17), 157 (12), 132 (59), 72 (100), 57 (55, ^{t}Bu), Found (FAB), 344.2432, $C_{18}H_{34}NO_5$ requires 344.2437.

4.3.17. (2*R*,2'*R*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-leucine methyl ester and (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-leucine methyl ester 127c

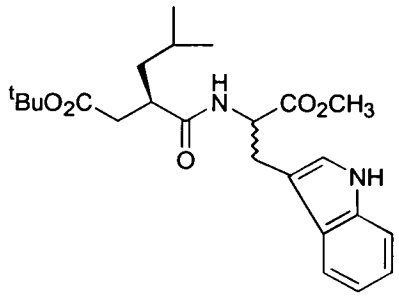


The procedure outlined in Section **(4.3.9.ii.)** was followed using TBTU (1.74 g, 5.42 mmol, 1.2 equiv.), HOBt (732 mg, 5.42 mmol, 1.2 equiv.), diisopropylethylamine (2.36 mL, 13.55 mmol, 3.0 equiv.), succinate **114** (1.04 g, 4.52 mmol, 1.0 equiv.) and DL-leucine methyl ester hydrochloride (984 mg, 5.42 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1)

furnished the title compound as a colourless solid (1.25 g, 77%). ^{1}H nmr indicated a (1:1) mixture of diastereomers.

R_f (Hexane:EtOAc, 6:1) 0.55, 0.46; Mp 63-66 °C, (Found: C, 64.15; H, 10.02; N, 3.68; $C_{19}H_{35}NO_5$ requires C, 63.84; H, 9.87; N 3.94); $[\alpha]_D^{20}$ + 6.08 (c 1.20, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$; 3434 (amide NH), 1740, 1734 (ester C=O), 1675, 1672, 1517, 1512 (CONH); δ_H ($CDCl_3$; 600 MHz) 6.21 (0.5 H, d, *J* 8.0, N$\underline{H}^A$), 6.10 (0.5 H, d, *J* 8.3 N$\underline{H}^B$),4.60 (0.5 H, ddd, *J* 13.6, 8.3, 5.0, NHC$\underline{H}^B$), 4.54 (0.5 H, ddd, *J* 13.6, 8.0, 5.0, NHC$\underline{H}^A$), 3.69 (1.5 H, s, OC$\underline{H}_3^B$), 3.67 (1.5 H, s, OC$\underline{H}_3^A$), 2.65 (total of 1 H, m, C$\underline{H}$CONH of each diastereomer), 2.56 (0.5 H, dd, *J* 16.9, 9.6, OCC$\underline{H}_A^B H_B$CH), 2.53 (0.5 H, dd, *J* 16.9, 8.6, OCC$\underline{H}_A^A H_B$CH) 2.28 (0.5 H, dd, *J* 16.9, 5.3, OCCH$_A\underline{H}_B^A$CH), 2.27 (0.5 H, dd, *J* 16.9, 4.0, OCCH$_A\underline{H}_B^B$CH), 1.66-1.48 (total of 5 H, m, $CH_2CHC\underline{H}_AH_BCH$, $CH_2CHCH_2C\underline{H}$, $NHCHC\underline{H}_AH_BCH$, $NHCHCH_A\underline{H}_BCH$, and $NHCHCH_2C\underline{H}$, of each diastereomer), 1.40 (4.5 H, s, C(C$\underline{H}_3^A$)$_3$), 1.39 (4.5 H, s, C(C$\underline{H}_3^B$)$_3$), 1.14 (0.5 H, ddd, *J* 13.6, 8.3, 5.6, $CH_2CHCH_A\underline{H}_B^A$CH), 1.13 (0.5 H, ddd, *J* 13.6, 8.3, 5.6, $CH_2CHCH_A\underline{H}_B^B$CH), 0.91 (1.5 H, d, *J* 6.3, C$\underline{H}_{3A}^A$ $CHCH_{3B(leucine)}$), 0.90 (1.5 H, d, *J* 6.3, $CH_{3A}CHC\underline{H}_{3B\,(leucine)}^A$) 0.89 (1.5 H, d, *J* 6.3, C$\underline{H}_{3A}^B$ $CHCH_{3B(leucine)}$), 0.88 (1.5 H, d, *J* 6.6, C$\underline{H}_{3A}^B$ $CHCH_{3B(succinate)}$), 0.87 (1.5 H, d, *J* 6.3, $CH_{3A}CHC\underline{H}_{3B\,(leucine)}^B$), 0.87 (1.5 H, d, *J* 6.6, C$\underline{H}_{3A}^A$ $CHCH_{3A(succinate)}$), 0.86 (1.5 H, d, *J* 6.6, $CH_{3A}CHC\underline{H}_{3B\,(succinate)}^A$) 0.85 (1.5 H, d, *J* 6.6, $CH_{3A}CHC\underline{H}_{3B\,(succinate)}^B$); δ_C ($CDCl_3$; 63 MHz) 174.6, 174.5 (<u>C</u>ONH), 173.3, 173.2, 171.7, 171.6 (<u>C</u>O$_2$R), 80.7, 80.6 (<u>C</u>(CH$_3$)$_3$, 52.0 (CO$_2$<u>C</u>H$_3$), 50.6, 50.3 (NH<u>C</u>H), 41.5, 41.3, 41.2, 40.9, (O$_2$C<u>C</u>H$_2$ and <u>C</u>H$_2$CH(CH$_3$)$_{2(leucine)}$), 40.5, 40.4 (<u>C</u>HCONH), 38.3, 38.2 (<u>C</u>H$_2$CH(CH$_3$)$_{2(succinate)}$), 27.9 (C(<u>C</u>H$_3$)$_3$), 25.6, 25.4, 24.7, 24.4 (2 x <u>C</u>H(CH$_3$)$_2$), 22.9, 22.8, 22.7, 22.6, 22.0, 21.9, 21.7, 21.6 (2 x CH(<u>C</u>H$_3$)$_2$); *m/z* (FAB) 358 (55%, MH$^+$), 302 (100, MH$_2^+$-^{t}Bu), 284 (26, 284-H$_2$O), 154 (26), 146 (44), 86 (52), 57 (38, ^{t}Bu), Found (FAB), 358.2588, $C_{19}H_{36}NO_5$ requires 358.2593.

4.3.18. (2*R*,2'*R*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-tryptophan methyl ester and (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-tryptophan methyl ester 127d

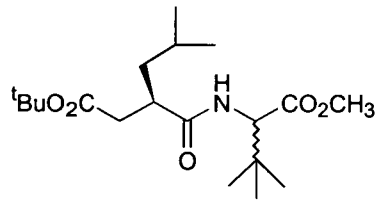


The procedure outlined in Section **(4.3.9.ii.)** was followed using TBTU (1.82 g, 5.68 mmol, 1.2 equiv.), HOBt (767 mg, 5.68 mmol, 1.2 equiv.), diisopropylethylamine (2.47 mL, 14.20 mmol, 3.0 equiv.), succinate **114** (1.09 g, 4.73 mmol, 1.0 equiv.) and DL-tryptophan methyl ester hydrochloride (1.45 g, 5.68 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (2:1) furnished the title compound as a colourless solid (1.55 g, 78%). ^{1}H nmr indicated a (1:1) ratio of diastereomers.

R_f (Hexane:EtOAc, 1:1) 0.76, 0.76; Mp 51-53 °C, (Found: C, 66.93; H, 8.09; N, 6.48; $C_{24}H_{34}N_2O_5$ requires C, 66.95; H, 7.95; N 6.51); $[\alpha]_D^{20}$ + 21.47 (c 1.36, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$; 3476 (indole NH), 3433 (amide NH), 3017 (saturated CH), 1734, 1725 (ester C=O), 1675, 1669, 1512, 1507 (CONH); δ_H ($CDCl_3$; 600 MHz) 8.29 (total of 1 H, brs, $N\underline{H}_{(indole)}$ of each diastereomer)7.56 (total of 1 H, m, $C\underline{H}_{ar}$ of each diastereomer), 7.32 (total of 1 H, m, $C\underline{H}_{ar}$ of each diastereomer), 7.15 (total of 1 H, m, $C\underline{H}_{ar}$ of each diastereomer), 7.13 (total of 1 H, m, $C\underline{H}NH_{(indole)}$ of each diastereomer), 7.09 (total of 1 H, m, $C\underline{H}_{ar}$ of each diastereomer), 6.27 (0.5 H, d, *J* 7.8, $N\underline{H}^B$), 6.25 (0.5 H, d, *J* 7.8, $N\underline{H}^A$), 4.92 (0.5 H, ddd, *J* 7.8, 5.6, 5.4, $NHC\underline{H}^B$), 4.91 (0.5 H, ddd, *J* 7.8, 6.0, 5.8, $NHC\underline{H}^A$), 3.64 (1.5 H, s, $OC\underline{H}_3^A$), 3.61 (.5 H, s, $OC\underline{H}_3^B$), 3.34 (0.5 H, dd, *J* 14.8, 5.4, $CHC\underline{H}_A^B H_B$Indole), 3.28 (0.5 H, dd, *J* 5.8, $CHC\underline{H}_A^A H_B$Indole), 3.27 (0.5 H, dd, *J* 5.8, $CHCH_A\underline{H}_B^A$Indole), 3.24 (0.5 H, dd, *J* 14.8, 5.6, $CHCH_A\underline{H}_B^B$Indole), 2.63-2.56 (1 H, m, C<u>H</u>CONH of each diastereomer), 2.58 (0.5 H, dd, *J* 15.8, 9.0, $OCC\underline{H}_A^B H_B CH$), 2.53 (0.5 H, dd, *J* 16.2,5.6, $OCC\underline{H}_A^A H_B CH$), 2.29 (0.5 H, dd, *J* 15.8, 4.0, $OCCH_A\underline{H}_B^B CH$), 2.26 (0.5 H, dd, J 16.2, 5.2, $OCCH_A\underline{H}_B^A CH$), 1.58-1.48 (total of 1.5 H, m, $C\underline{H}^A(CH_3)_2$, $C\underline{H}^B(CH_3)_2$,

$CH_2CHC\underline{H}^{A}_{A}H_BCH$ and $CH_2CHC\underline{H}^{B}_{A}H_BCH$), 1.40 (4.5 H, s, $C(C\underline{H}^{A}_{B})_3$), 1.38 (4.5 H, s, $C(C\underline{H}^{A}_{3})_3$), 1.37 (0.5 H, m, obscured by ^{t}Bu peaks, $C\underline{H}^{B}(CH_3)_2$), 1.17 (0.5 H, ddd, *J* 15.4, 9.8, 5.4 $CH_2CHCH_A\underline{H}^{B}_{B}CH$), 1.11 (0.5 H, ddd, *J* 13.8, 8.4, 5.8 $CH_2CHCH_A\underline{H}^{A}_{B}CH$), 0.84 (1.5 H, d, *J* 6.4, $C\underline{H}^{B}_{3A}CHCH_{3B}$), 0.82 (1.5 H, d, *J* 6.4, $CH_{3A}CHC\underline{H}^{B}_{3B}$), 0.76 (1.5 H, d, *J* 6.4, $C\underline{H}^{A}_{3A}CHCH_{3B}$), 0.75 (1.5 H, d, *J* 6.4, $CH_{3A}CHC\underline{H}^{A}_{3B}$); δ_C ($CDCl_3$; 63 MHz) 174.5, 174.4, 172.2, 171.6, 171.4 (<u>C</u>ONH, <u>C</u>O_2R), 136.0, 136.0, 127.4 (*ipso*-Ar), 123.1, 122.7, 122.0, 121.9, 119.4, 119.3, 118.4, 118.3, 111.1, 111.0 (<u>C</u>H_{ar}), 109.8, 109.6 (*ipso*-Ar), 80.6, 80.6 (<u>C</u>$(CH_3)_3$), 52.6, 52.5 (NH<u>C</u>H), 52.0 ($O_2C\underline{C}H_3$), 41.3, 40.9 ($O_2C\underline{C}H_2$), 40.8, 40.5 (<u>C</u>HCONH), 38.2, 37.9, ($\underline{C}H_2CH(CH_3)_2$), 27.9, 27.8 ($C(\underline{C}H_3)_3$), 27.6 (<u>C</u>$H_2$Indole), 25.4, 25.3 ($\underline{C}H(CH_3)_2$), 22.7, 22.6, 22.0, 21.9 ($CH(\underline{C}H_3)_2$); *m/z* (FAB) 431 (50, MH^+), 375 (88, MH_2^+-^{t}Bu), 201(83), 159 (42), 130 (100, CH_2Indole), 57 (58, ^{t}Bu), Found (FAB), 431.2533, $C_{24}H_{35}N_2O_5$ requires 431.2546.

4.3.19. (2*R*,2'*R*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-*tert*-leucine methyl ester and (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-*tert*-leucine methyl ester 127e



The procedure outlined in Section **(4.3.9.ii.)** was followed using TBTU (1.52 g, 4.73 mmol, 1.2 equiv.), HOBt (639 mg, 4.73 mmol, 1.2 equiv.), diisopropylethylamine (2.06 mL, 11.83 mmol, 3.0 equiv.), succinate **114** (908 mg, 3.94 mmol, 1.0 equiv.) and DL-*tert*-leucine methyl ester hydrochloride (788 g, 4.34 mmol, 1.1 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title compound as a colourless wax (873 g, 62%). ^{1}H nmr indicated a (1:1). ratio of diastereomers.

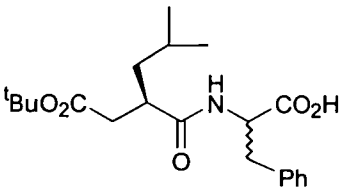
R_f (Hexane:EtOAc, 6:1) 0.38, 0.32; $[\alpha]_D^{20}$ + 5.78 (c 1.16, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3432 (amide NH), 3010 (saturated CH), 1731 (ester C=O), 1673, 1513 (CONH); δ_H ($CDCl_3$; 600 MHz) 6.35 (0.5 H, d, *J* 9.3, N<u>H</u>^{A}), 6.29 (0.5 H, d, *J* 9.3, N<u>H</u>^{B}), 4.42 (0.5

H, d, *J* 9.3, NHC$\underline{H}^{B}$), 4.38 (0.5 H, d, *J* 9.3, NHC$\underline{H}^{A}$), 3.69 (1.5 H, s, OC$\underline{H}_3^{B}$), 3.67 (1.5 H, s, OC$\underline{H}_3^{A}$), 2.68 (total of 1 H, m, C$\underline{H}$CONH of each diastereomer), 2.58 (0.5 H, dd, *J* 17.0, 9.6, OCC$\underline{H}_A^{B}$ H_BCH), 2.51 (0.5 H, dd, *J* 16.9, 9.0, OCC$\underline{H}_A^{A}$ H_BCH), 2.29 (0.5 H, dd, *J* 17.0, 4.0, OCC$H_A\underline{H}_B^{B}$CH), 2.28 (0.5 H, dd, *J* 16.9, 5.0, OCC$H_A\underline{H}_B^{A}$CH), 1.65 (0.5 H, ddd, *J* 13.6, 9.3, 5.6, CHC$\underline{H}_A^{A}$ H_BCH), 1.59 (0.5 H, ddd, *J* 13.6, 8.9, 5.9, CHC$\underline{H}_A^{B}$ H_BCH), 1.58-142 (total of 1 H, m, C$\underline{H}(CH_3)_2$ of each diastereomer), 1.14 (0.5 H, ddd, *J* 13.6, 7.9, 5.9, CHC$H_A\underline{H}_B^{B}$CH), 1.13 (0.5 H, ddd, *J* 13.6, 8.3, 5.6, CHC$\underline{H}_A^{A}$ H_BCH), 0.90 (1.5 H, d, *J* 6.6, C$\underline{H}_{3A}^{A}$ $CHCH_{3B}$), 0.88 (1.5 H, d, *J* 6.6, C$\underline{H}_{3A}^{B}$ $CHCH_{3B}$), 0.87 (1.5 H, d, *J* 6.6, CH_{3A}CHC$\underline{H}_{3B}^{A}$), 0.84 (1.5 H, d, *J* 6.6, CH_{3A}CHC$\underline{H}_{3B}^{B}$); δ_C ($CDCl_3$; 63 MHz) 174.6, 174.3, 171.9, 171.7, 171.6 (C$\underline{O}$NH, $\underline{C}O_2$C), 80.7, 80.6 (O$\underline{C}(CH_3)_3$), 60.0, 59.7 (NH$\underline{C}$H), 51.5, 51.5 (O$\underline{C}H_3$), 41.1, 41.0 ($O_2C\underline{C}H_2$), 40.7 ($\underline{C}$HCONH), 38.4, 38.2 (CH$\underline{C}H_2$CH), 34.7, 34.3 (CH$\underline{C}(CH_3)_3$), 27.9, 27.9 (OCC($\underline{C}H_3)_3$), 26.5, 26.4 (CHC($\underline{C}H_3)_3$), 25.7, 25.5 ($\underline{C}H(CH_3)_2$), 23.0, 22.8, 22.1, 21.8 (CH($\underline{C}H_3)_2$); m/z (FAB), 358 (24%, MH^+), 302 (100, MH_2^+-^{t}Bu), 284 (16, 302-H_2O), 242 (12), 86 (30), 57 (30, ^{t}Bu), Found (FAB) 358.2593, $C_{19}H_{36}NO_5$ requires 358.2594.

4.3.20. General procedure for the hydrolysis of (2*R*,2'*RS*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-α-amino acid methyl esters 127a-e

To amide **127a-e** suspended in THF:water (1:1, 20 mL), was added lithium hydroxide monohydrate (2.0 equiv.) and the resulting suspension stirred for 30 minutes at room temperature until a clear solution was obtained. The reaction mixture was acidified to pH 3 with aqueous citric acid (1M), and the aqueous layer extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were washed with brine (30 mL), dried (Na_2SO4), filtered, and evaporated under reduced pressure to give the desired product.

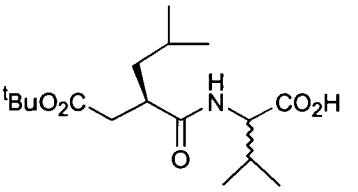
4.3.21. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine and (2*R*,2'*R*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine 128a



The general procedure outlined in Section **(4.3.20.)** was followed with DL-phenylalanine derived amide **127a** (1.03 g, 2.63 mmol) and lithium hydroxide monohydrate (221 mg, 5.26 mmol, 2.0 equiv.) to furnish the title compound as a colourless gum (0.99 g, quantitative). ^{1}H nmr indicated a (1:1) ratio of diastereomers. $[\alpha]_D^{20}$ + 11.02 (c 1.18, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$; 3425 (amide NH), 3200-2400 (acid OH), 3019 (saturated CH), 1723 (ester C=O, and acid C=O), 1671, 1516 (CONH); δ_H ($CDCl_3$; 600 MHz) 7.29-7.25 (total of 2 H, m, C$\underline{H}_{ar}$ of each diastereomer), 7.24-7.21 (total of 2 H, m, C$\underline{H}_{ar}$ of each diastereomer), 7.19-7.17 (total of 1 H, m, C$\underline{H}_{ar}$ of each diastereomer), 6.46 (0.5 H, d, *J* 7.8., N$\underline{H}^A$), 6.41 (0.5 H, d, *J* 7.6, N$\underline{H}^B$), 4.87 (0.5 H, ddd, *J* 7.8, 5.4, NHC$\underline{H}^A$), 4.84 (0.5 H, ddd, *J* 7.6, 6.4, NHC$\underline{H}^B$), 3.24 (0.5 H, d, *J* 14.2, 5.4, C$\underline{H}^A_A$H$_B$Ph), 3.17 (0.5 H, d, *J* 14.0, 5.6, C$\underline{H}^B_A$H$_B$Ph), 3.12 (0.5 H, d, *J* 14.0, 6.4, CH$_A\underline{H}^B_B$Ph), 3.03 (0.5 H, d, *J* 14.2, 8.0, CH$_A\underline{H}^A_B$Ph), 2.67-2.57 (total of 1 H, m, C$\underline{H}$CONH of each diastereomer), 2.54 and 2.50 (total of 1 H, 2 x dd, *J* 16.0, 9.0, and 16.6, 8.8, OCC$\underline{H}_A$H$_B$CH of each diastereomer), 2.29 and 2.25 (total of 1 H, 2 x dd, *J* 16.6, 5.0, and 16.0, 4.2, OCCH$_A\underline{H}_B$CH of each diastereomer), 1.58-1.41 (total of 1.5 H, C$\underline{H}_A$H$_B$CH(CH$_3$)$_2$ of each diastereomer, and C$\underline{H}^A$(CH$_3$)$_2$), 1.41 (4.5 H, s, C(C$\underline{H}^B_3$)$_3$), 1.38 (4.5 H, s, C(C$\underline{H}^A_3$)$_3$), 1.23 (0.5 H, m, C$\underline{H}^B$(CH$_3$)$_2$), 1.15 (0.5 H, ddd, *J* 13.6, 8.0, 6.2, CHCH$_A$C$\underline{H}^A_B$CH), 1.04 (0.5 H, ddd, *J* 13.6, 8.8, 5.4, CHCH$_A$C$\underline{H}^B_B$CH), 0.83, (1.5 H, d, *J* 6.4 C$\underline{H}^B_{3A}$ CHCH$_{3B}$), 0.82 (1.5 H, d, *J* 6.4 CH$_{3A}$CHC$\underline{H}^B_{3B}$), 0.76 (1.5 H, d, *J* 6.4 C$\underline{H}^A_{3A}$ CHCH$_{3B}$), 0.75 (1.5 H, d, *J* 6.4 CH$_{3A}$CHC$\underline{H}^A_{3B}$); δ_C ($CDCl_3$; 63 MHz) 175.6, 175.2, 174.5, 174.3, 172.2, 171.8 (C$\underline{O}$NH, C$\underline{O}_2$H, C$\underline{O}_2$C), 135.9, 135.8, (*ipso*-Ar), 129.3, 129.1, 128.5, 126.97 (C$\underline{H}_{ar}$), 81.8, 81.0 (C$\underline{(CH_3)_3}$), 53.2, 53.01 (NHC$\underline{H}$), 41.0 (O$_2$C$\underline{C}$H$_2$), 40.7, 40.5 (C$\underline{H}$CONH), 38.1, 38.0 (C$\underline{H}_2$CH(CH$_3$)$_2$), 37.3, 37.2 (C$\underline{H}_2$Ph), 27.9, 27.9 (C(C$\underline{H}_3$)$_3$), 25.5, 25.4 (C$\underline{H}$(CH$_3$)$_2$), 22.9, 22.7, 22.0, 21.9 (CH(C$\underline{H}_3$)$_2$); *m/z* (FAB) 378 (41%, MH$^+$), 322 (100, MH$_2^+$-^{t}Bu), 258 (8), 166 (38),

120 (67), 91 (34, CH_2Ph), 57 (62, ^tBu), Found (FAB), 378.2263, $C_{21}H_{32}NO_5$ requires 378.2280.

4.3.22. (2*R*,2'*R*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-valine and (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-valine 128b

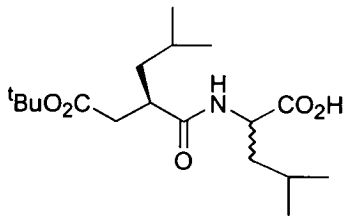


The general procedure outlined in Section **(4.3.20.)** was followed with DL-valine derived amide **127b** (1.02 g, 2.97 mmol) and lithium hydroxide monohydrate (249 mg, 5.94 mmol, 2.0 equiv.) to furnish the title compound as a colourless gum (0.98 g, quantitative). ^1H nmr indicated a (1.5:1) ratio of diastereomers.

$[\alpha]_D^{20}$ + 6.67 (c 1.08, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$; 3496 (amide NH), 3400-2400 (acid OH), 3020 (saturated CH), 1752 (ester C=O) 1718 (acid C=O), 1654, 1519 (CONH); δ_H ($CDCl_3$; 600 MHz) 9.88 (total of 1 H, br s, O$\underline{H}$ of each diastereomer), 6.62 (0.6 H, d, *J* 8.6, N$\underline{H}^A$), 6.50 (0.4 H, d, *J* 8.9, N$\underline{H}^B$), 4.56 (0.4 H, dd, *J* 8.9, 4.6, NHC$\underline{H}^A$), 4.51 (0.6 H, dd, *J* 8.6, 4.6, NHC$\underline{H}^B$), 2.73 (total of 1 H, m, C$\underline{H}$CONH of each diastereomer), 2.58 (0.4 H, dd, *J* 17.3, 9.6, OCC$\underline{H}^B_A H_B$CH), 2.55(0.6 H, dd, 16.6, 8.6, OCC$\underline{H}^A_A H_B$CH), 2.31 (0.4 H, dd, *J* 17.3, 4.3, OCCH$_A\underline{H}^B_B$CH), 2.30 (0.6 H, dd, *J* 16.6, 5.3, OCCH$_A\underline{H}^A_B$CH), 2.21 (total of 1 H, m, CHC$\underline{H}$(CH$_3$)$_2$ of each diastereomer), 1.64 (0.6 H, ddd, *J* 13.3, 9.6, 5.3, CHC$\underline{H}^A_A H_B$CH), 1.60 (0.4 H, ddd, *J* 13.3, 9.0, 5.9, CHC$\underline{H}^B_A H_B$CH), 1.56-1.49 (total of 1 H, 2 x m, CH$_2$C$\underline{H}$(CH$_3$)$_2$ of each diastereomer), 1.39 (3.6 H, s, C(C$\underline{H}_3^B$)$_3$), 1.38 (5.4 H, s, C(C$\underline{H}_3^A$)$_3$), 1.1 (0.6 H, ddd, *J* 13.3, 8.6, 5.3, CHCH$_A\underline{H}^A_B$CH), 1.13 (0.4 H, ddd, *J* 13.3, 8.0, 5.9, CHCH$_A\underline{H}^B_B$CH), 0.95 and 0.92 (total of 6 H, 2 x d, *J* 6.6, CH(C$\underline{H}_{3A}$)CHCH$_{3B}$ and CH(CH$_{3A}$)CHC$\underline{H}_{3B}$ of each diastereomer), 0.89, 0.87, 0.86, 0.83 (total of 6 H, 4 x d, *J* 6.6, CH$_2$(C$\underline{H}_{3A}$)CHCH$_{3B}$ and CH$_2$(CH$_{3A}$)CHC$\underline{H}_{3B}$ of each diastereomer); δ_C ($CDCl_3$; 63 MHz) 175.5, 175.3, 175.2, 172.1, 171.9 (C$\underline{O}$NH, C$\underline{O}_2$H, C$\underline{O}_2$C), 81.1, 80.9 (C$\underline{(CH_3)_3}$), 57.0, 56.8 (NHC$\underline{H}$), 41.0, 40.9 (O$_2$C$\underline{C}$H$_2$), 40.7, 40.6 (C$\underline{H}$CONH), 38.3, 38.2 (C$\underline{H}_2$CH(CH$_3$)$_2$), 30.9, 30.6 (C$\underline{H}$(CH$_3$)$_{2(valine)}$), 27.9, 27.8 (C(C$\underline{H}_3$)$_3$), 25.6, 25.5 (C$\underline{H}$(CH$_3$)$_{2(succinate)}$), 22.9,

22.8, 22.1, 22.0, 21.8, ($CH(\underline{C}H_3)_{2(succinate)}$), 18.9, 18.8, 17.5, 17.3 ($CH(\underline{C}H_3)_{2(valine)}$); *m/z* (FAB), 330 (25%, MH^+), 274 (100, MH_2^+-^{t}Bu), 256 (16, 274-H_2O), 210 (7), 157 (9), 118 (37), 72 (46), 57 (30, ^{t}Bu), Found (FAB), 330.2283, $C_{17}H_{32}NO_5$ requires 330.2280.

4.3.23. (2*R*,2'*R*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-leucine and (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-leucine 128c

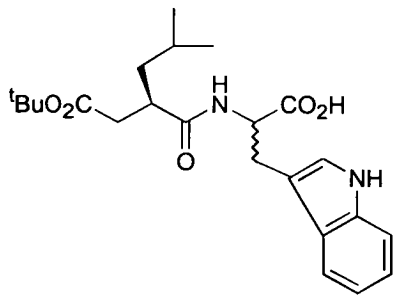


The general procedure outlined in Section **(4.3.20.)** was followed with DL-leucine derived amide **127c** (1.12 g, 3.13 mmol) and lithium hydroxide monohydrate (263 mg, 6.26 mmol, 2.0 equiv.) to furnish the title compound as a colourless gum (1.08 g, quantitative). ^{1}H nmr indicated a (1:1) ratio of diastereomers.

$[\alpha]_D^{20}$ + 8.56 (c 1.32, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$; 3442 (amide NH), 3200-2450 (acid OH), 3019 (saturated CH), 1720 (ester C=O and acid C=O), 1655, 1522 (CONH); δ_H ($CDCl_3$; 600 MHz) 8.16 (total of 1 H, br s, O$\underline{H}$ of each diastereomer), 6.49 (0.5 H, d, *J* 8.0, N$\underline{H}^A$), 6.35 (0.5 H, d, *J* 8.0, N$\underline{H}^B$), 4.58 (0.5 H, ddd, *J* 9.6, 8.0, 4.9, NHC$\underline{H}^B$), 4.53 (0.5 H, ddd, *J* 9.3, 8.0, 4.6, NHC$\underline{H}^A$), 2.62 (total of 1 H, m, C$\underline{H}$CONH), 2.58 (0.5 H, dd, *J* 16.9 10.0, OCC$\underline{H}_A^B H_B$CH), 2.56 (0.5 H, dd, *J* 16.9, 9.0, OCC$\underline{H}_A^A H_B$CH) 2.31 (0.5 H, dd, *J* 16.9 5.1, OCCH$_A\underline{H}_B^A$CH), 2.30 (16.9, 4.3, OCCH$_A\underline{H}_B^B$CH), 1.71-1.51 (total of 5 H, m, $CH_2CHC\underline{H}_2CH$, $CH_2CHCH_2C\underline{H}$, $NHCHCH_2C\underline{H}$, and $NHCHC\underline{H}_AH_BCH$, of each diastereomer), 1.40 (total of 9 H, s, $C(C\underline{H}_3)_3$ of each diastereomer), 1.15 (0.5 H, ddd, *J* 13.6, 8.3, 5.6, $CH_2CHCH_ACH_B^A C\underline{H}(CH_3)_2$), 1.14 (0.5 H, ddd, *J* 13.6, 7.9, 6.0, $CH_2CHCH_ACH_B^B C\underline{H}(CH_3)_2$), 0.94, 0.92, 0.91, 0.90, 0.89(2), 0.87, 0.85 (total of 12 H, 8 x d, *J* 6.6, $C\underline{H}_{3A}CHCH_{3B}$ and $CH_{3A}CHC\underline{H}_{3B}$ of each *iso*-butyl group of each diastereomer); δ_C ($CDCl_3$; 63 MHz) 176.5, 176.2, 175.8, 175.3, 172.1, 171.9 ($\underline{C}$ONH, $\underline{C}O_2$H, $\underline{C}O_2$C), 81.2, 80.9 ($\underline{C}(CH_3)_3$), 50.9, 50.8 (NH$\underline{C}$H), 41.1 ($O_2C\underline{C}H_2$), 40.9, 40.7 ($\underline{C}H_2CH(CH_3)_{2(leucine)}$), 40.6, 40.5 ($\underline{C}$HCONH), 38.3, 38.1 ($\underline{C}H_2CH(CH_3)_{2(succinate)}$), 27.9 ($C(\underline{C}H_3)_3$), 25.7, 25.4 ($\underline{C}H(CH_3)_{2(succinate)}$), 24.8,

24.5 ($\underline{C}H(CH_3)_{2(leucine)}$), 22.9, 22.8, 22.7, 22.6, 22.0, 21.9, 21.6 (2 x $CH(\underline{C}H_3)_2$); *m/z* (FAB), 344 (23%, MH^+). 288 (100, MH_2^+-^{t}Bu), 270 (18, 288-H_2O), 229 (30), 157 (17), 132 (53), 86 (81), 57 (72, ^{t}Bu), Found (FAB), 344.2445, $C_{18}H_{34}NO_5$ requires 344.2437.

4.3.24. (2*R*,2'*R*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-tryptophan and (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-tryptophan 128d

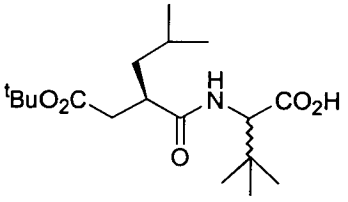


The general procedure outlined in Section **(4.3.20.)** was followed with DL-tryptophan derived amide **127d** (1.35 g, 3.14 mmol) and lithium hydroxide monohydrate (263 mg, 6.27 mmol, 2.0 equiv.) to furnish the title compound as a colourless foam (1.31 g, quantitative). ^{1}H nmr indicated a (1:1) ratio of diastereomers.

Mp 77-79°C; $[\alpha]_D^{20}$ + 22.82 (c 1.10, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$; 3476 (amide NH and indole NH), 3400-2400 (acid OH), 3019 (saturated CH), 1720 (ester C=O and acid C=O), 1669, 1519 (CONH); δ_H ($CDCl_3$; 600 MHz) 8.46 (total of 1 H, ,br s, $N\underline{H}_{(indole)}$ of each diastereomer),7.63 and 7.55 (total of 1 H, 2 x d, *J* 7.9, $C\underline{H}_{ar}$ of each diastereomer), 7.32 and 7.31 (total of 1 H, 2 x d, *J* 7.3, $C\underline{H}_{ar}$ of each diastereomer), 7.16 (total of 1 H, m, $C\underline{H}_{ar}$ of each diastereomer), 7.08 (total of 1.0 H, m, $C\underline{H}_{ar}$ of each diastereomer) 7.06 (0.5 H, d, *J* 2.3, $C\underline{H}_{ar}$), 6.97 (0.5 H, d, *J* 2.3, $C\underline{H}_{ar}$), 6.45 and 6.42 (total of 1 H, 2 x d, *J* 7.6, $NH_{(amide)}$ of each diastereomer), 4.92 and 4.90 (total of 1 H, 2 ddd, *J* 7.6, 5.6, and 7.6, 5.8, NHC$\underline{H}$ of each diastereomer), 3.33 (0.5 H, dd, *J*, 15.0, 6.6, $CHC\underline{H}_A^B H_B$Indole), 3.30 (total of 1.0 H, m, $CHC\underline{H}_A^A H_B$Indole and $CHC\underline{H}_A H_B^A$Indole), 3.26 (0.5 dd, *J* 15.0, 6.6, $NHCHCH_A\underline{H}_B^B$), 2.61 (0.5 H, m, $C\underline{H}^B$CONH), 2.52 (total of 1.5 H, m, CHCONH, $OCC\underline{H}_A^B H_B$CH and $OCCH_A^A H_B$CH), 2.28 (0.5 H, dd, *J* 16.6, 5.3, $OCCH_A\underline{H}_B^B$CH) 2.24 (0.5 H, dd, *J* 20.2, 8.6,

$OCCH_A\underline{H}^A_B$), 1.49 (total of 1 H, m, $C\underline{H}(CH_3)_2$ of each diastereomer), 1.43 (0.5 H, m, $CHC\underline{H}_AH_BCH$), 1.39 (4.5 H, s, $C(C\underline{H}^B_3)_3$), 1.35 (4.5 H, s, $C(C\underline{H}^A_3)_3$), 1.25 (0.5 H, m, $CHC\underline{H}_AH_BCH$), 1.14 (0.5 H, ddd, *J* 13.0, 9.6, 5.6, $CHCH_A\underline{H}^B_B CH$), 1.07 (0.5 H, ddd, J 13.6, 8.3, 5.3, $CHCH_A\underline{H}^A_B CH$), 0.77, 0.76 and 0.70, 0.68 (total of 6 H, 4 x d, *J* 6.3, $C\underline{H}_{3A}CHCH_{3B}$ and $CH_{3A}CHC\underline{H}_{3B}$ of each diastereomer); δ_C ($CDCl_3$; 63 MHz) 175.7, 175.4, 174.9, 174.7, 172.1, 171.9 (CONH, $\underline{C}O_2H$, $\underline{C}O_2C$), 136.1, 136.0, 127.5, 127.5 (*ipso*-Ar), 123.4, 123.2, 122.0, 121.9, 119.5, 119.4, 118.4, 118.3, 111.2, ($\underline{C}H_{ar}$), 109.4 (*ipso*-Ar), 81.3, 80.9 ($\underline{C}(CH_3)_3$), 53.0 ($NH\underline{C}H$), 41.1, 40.9 ($O_2C\underline{C}H_2$), 40.7, 40.5 ($\underline{C}HCONH$), 38.1, 37.9 ($\underline{C}H_2CH(CH_3)_2$), 27.9, 27.8 ($C(\underline{C}H_3)_3$), 27.2, 26.9 ($\underline{C}H_2$Indole), 25.4 ($\underline{C}H(CH_3)_2$), 22.6, 22.0, 21.9 ($CH(\underline{C}H_3)_2$); *m/z* (FAB), 417 (3%, MH^+), 361 (21, MH_2^+-^{t}Bu), 288 (10), 229 (23), 188 (19), 130 (30 , CH_2Indole), 88 (19), 57 (100, ^{t}Bu), Found (FAB), 417.2372, $C_{23}H_{33}N_2O_5$ requires 417.2389.

4.3.25. (2*R*,2'*R*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-*tert*-leucine and (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-*tert*-leucine 128e



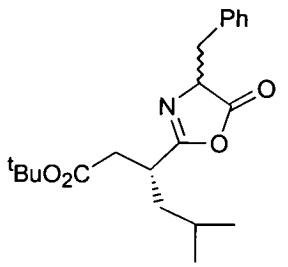
The general procedure outlined in Section **(4.3.20.)** was followed with DL-*tert*-leucine derived amide **127e** (843 mg, 3.36 mmol) and lithium hydroxide monohydrate (396 mg, 9.43 mmol, 4.0 equiv.). The reaction mixture was stirred for 2 days. The product was obtained as a colourless gum (810 mg, 100%). ^{1}H nmr (200 MHz) indicated a mixture of two products, the desired acid and the corresponding diacid amide resulting from concomitant cleavage of the *tert*-butyl ester in an almost 1:1 ratio. The bulk crude product was not further purified but was used immediately in the next step (see Section **(4.3.28.)**). A portion of the crude product was purified by repeated trituration, (Et_2O:hexane) to remove the diacid amide. The mother liquor was evaporated under reduced pressure to furnish a pure sample of the desired product. ^{1}H nmr indicated a (1:1) ratio of diastereomers.

$[\alpha]_D^{20}$ +6.04 (c 1.44, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$; 3428 (amide NH), 3200-2400 (acid OH), 3020 (saturated CH), 1719 (ester C=O and acid C=O), 1676, 1515 (CONH); δ_H ($CDCl_3$; 600 MHz) 10.19 (total of 1 H, br s, OH), 6.69 (0.5 H, d, *J* 9.0, $N\underline{H}^A$), 6.57 (0.5 H, d, *J* 9.3, $N\underline{H}^B$), 4.44 (0.5 H, d, *J* 9.3, $NHC\underline{H}^B$), 4.39 (0.5 H, d, *J* 9.0, $NHC\underline{H}^A$), 2.75 (total of 1 H, m, C<u>H</u>CONH of each diastereomer), 2.58 (0.5 H, dd, *J* 17.0, 9.6, $CHC\underline{H}^B_A H_BCH$), 2.53 (0.5 H, dd, *J* 16.9, 8.6, $CHC\underline{H}^A_A H_BCH$), 2.31 (0.5 H, dd, *J* 17.1 4.2, $CHCH_A\underline{H}^B_B CH$), 2.30 (0.5 H, dd, *J* 16.9, 5.0, $CHCH_A\underline{H}^A_B CH$), 1.64 (0.5 H, m, $C\underline{H}(CH_3)_2$ of one diastereomer), 1.61-1.49 (total of 1.5 H, m, $CHC\underline{H}^B_A H_BCH$, $C\underline{H}(CH_3)_2$ of each diastereomer), 1.39 ($OC(C\underline{H}^A_3)_3$), 1.38 (4.5 H, s, $OC(C\underline{H}^B_3)_3$), 1.15 (total of 1 H, m, $CHCH_A\underline{H}_BCH$ of each diastereomer), 0.99 (4.5 H, s, $CHC(C\underline{H}^A_3)_3$), 0.92 (4.5 H, s, $CHC(C\underline{H}^B_3)_3$), 0.89 (1.5 H, d, *J* 6.6, $C\underline{H}^A_{3A}CHCH_{3B}$), 0.87 (1.5 H, d, *J* 6.6, $C\underline{H}^B_{3A}CHCH_{3B}$), 0.86 (1.5 H, d, *J* 6.6, $C\underline{H}_{3B}CHCH^A_{3B}$), 0.83 (1.5 H, d, *J* 6.6, $C\underline{H}_{3B}CHCH^B_{3B}$); δ_C ($CDCl_3$; 63 MHz) 175.3, 175.2, 175.1, 175.0, 172.1, 172.0 (<u>C</u>ONH, <u>C</u>O_2H, <u>C</u>O_2C), 81.2, 80.9 ($O\underline{C}(CH_3)_3$), 60.2, 59.9 (NH<u>C</u>H), 41.0, 40.8 ($O_2C\underline{C}H_2$), 40.6, 40.5 (<u>C</u>HCONH), 38.2, 38.0 ($CH\underline{C}H_2CH$), 34.6, 34.1 ($CH\underline{C}(CH_3)_3$), 27.9, 27.8 ($OCC(\underline{C}H_3)_3$), 26.5, 26.4 ($CHC(\underline{C}H_3)_3$), 25.6, 25.4 ($\underline{C}H(CH_3)_2$), 22.9, 22.8, 22.0, 21.8 ($CH(\underline{C}H_3)_2$); *m/z* (FAB), 344 (26%, MH^+), 288 (100, MH_2-^{t}Bu), 270 (39, 288-$H_2$0), 242 (9, 270-CO), 132 (20), 86 (32), 57 (40, ^{t}Bu), (FAB) 344.2436, $C_{18}H_{34}NO_5$ requires 344.2437.

4.3.26. General procedure for (3*R*,4'*RS*)-2'-substituted-4'-substituted-5'(4'*H*)-oxazolones 129a-e

EDCI (1.1 equiv.) was added to a solution of acid (1.0 equiv.) in acetonitrile (30 mL) and the solution stirred at room temperature under an atmosphere of nitrogen for 90 minutes. The reaction was quenched with saturated aqueous ammonium chloride (20 mL) and the aqueous layer extracted with diethyl ether (3 x 30 mL). The combined organic extracts were washed with brine (2 x 20 mL), dried (Na_2SO_4), filtered, and evaporated under reduced pressure with a water bath temperature of no greater than 30 °C. Purification by column chromatography yielded the desired product as a colourless oil.

4.3.27. (3*R*,4'*RS*)-5-Methyl-3-(4'-benzyl-5'-oxo-4',5'-dihydro-oxazol-2'-yl)-hexanoic acid *tert*-butyl ester 129a

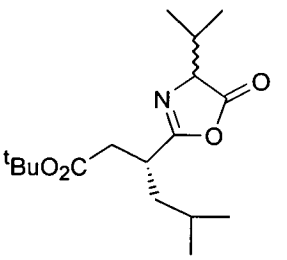


The procedure outlined in Section **(4.3.26.)** was followed using DL-phenylalanine derived acid **128a** (1.09 g, 2.89 mmol) and EDCI (1.09 g, 3.18 mmol, 1.1 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title product (896 mg, 86%). ^{1}H nmr indicated a (1:1) ratio of diastereomers.

R_f (Hexane:EtOAc, 6:1) 0.27; $[\alpha]_D^{20}$ -1.03 (c 2.34, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3019 (saturated CH), 1817 (oxazolone C=O), 1724 (ester C=O), 1671 (C=N); δ_H ($CDCl_3$; 600 MHz) 7.26-7.18 (total of 3 H, m, C$\underline{H}_{ar}$ of each diastereomer), 7.17-7.15 (total of 2 H, m, C$\underline{H}_{ar}$ of each diastereomer), 4.42 (total of 1 H, m, NC$\underline{H}$ of each diastereomer), 3.22 (0.5 H, dd, *J* 13.6, 5.2, C$\underline{H}_A$H$_B$Ph of one diastereomer), 3.21 (0.5 H, dd, *J* 13.6, 5.2, C$\underline{H}_A$H$_B$Ph of one diastereomer), 3.13 (0.5 H, dd, *J* 13.6, 5.4, CH$_A\underline{H}_B$Ph of one diastereomer), 3.11 (0.5 H, dd, *J* 13.6, 5.8, CH$_A\underline{H}_B$Ph of one diastereomer), 2.93 (0.5 H, m, C$\underline{H}$C=N of one diastereomer), 2.86 (0.5 H, m, C$\underline{H}$C=N of one diastereomer), 2.45 (0.5 H, dd, *J* 16.0, 7.8, OCC$\underline{H}_A$H$_B$CH of one diastereomer), 2.42 (0.5 H, dd, *J* 16.6, 8.0, OCC$\underline{H}_A$H$_B$CH of one diastereomer), 2.29 (0.5 H, dd, *J* 16.6, 6.4, OCCH$_A\underline{H}_B$CH of one diastereomer), 2.26 (0.5 H, dd, *J* 16.6, 6.4, OCCH$_A\underline{H}_B$CH of one diastereomer), 1.40 (total of 9 H, s, C(C$\underline{H}_3$)$_3$ of each diastereomer), 1.37 (0.5 H, ddd, obscured by ^{t}Butyl peak, CHC$\underline{H}_A$HBCH of one diastereomer), 1.30 (0.5 H, ddd, *J* 13.2, 8.6, 6.0, CHC$\underline{H}_A$HBCH of one diastereomer), 1.25 (0.5 H, m, C$\underline{H}$(CH$_3$)$_2$ of one diastereomer), 1.12 (0.5 H, ddd, *J* 12.8, 8.8, 5.8, CHCH$_A\underline{H}_B$CH of one diastereomer), 1.10 (0.5 H, ddd, *J* 13.2, 8.0, 6.0, CHCH$_A\underline{H}_B$CH of one diastereomer), 0.93 (0.5 H, m, C$\underline{H}$(CH$_3$)$_2$ of one diastereomer), 0.80, 0.77, 0.77, 0.74 (total of 6 H, 4 x d, *J* 6.6, C$\underline{H}_{3A}$CHCH$_{3B}$ and CH$_{3A}$CHC$\underline{H}_{3B}$ of each diastereomer); δ_C ($CDCl_3$; 63 MHz) 177.8, 177.7 (NCH$\underline{C}$O$_2$C), 170.2, 170.1 ($\underline{C}$O$_2$C), 167.8, 167.1 ($\underline{C}$=N), 135.0, 134.7 (*ipso*-Ar), 129.6, 129.5, 128.3, 128.1, 127.0 ($\underline{C}$H$_{ar}$), 80.8, 80.7 ($\underline{C}$(CH$_3$)$_3$), 65.5, 65.4 (N$\underline{C}$HCO), 40.5, 40.1 (O$_2$C$\underline{C}$H$_2$), 37.6,

37.4 ($\underline{C}H_2CH(CH_3)_2$), 36.6, ($\underline{C}H_2Ph$), 34.8, 34.1 ($CH_2\underline{C}HCN$), 27.9 ($C(\underline{C}H_3)_3$), 25.2, 25.0 ($\underline{C}H(CH_3)_2$), 22.7, 22.6, 21.8, 21,7 ($CH(\underline{C}H_3)_2$); *m/z* (FAB) 360 (69%, MH^+), 304 (100, MH_2^+-^{t}Bu), 286 (29, 304-H_2O), 258 (96, 286-CO), 212 (10), 154 (33), 120 (77), 91 (51, CH_2Ph), 57 (74, ^{t}Bu), Found (FAB) 360. 2174, $C_{21}H_{30}NO_4$ requires 360.2174.

4.3.28. (3*R*,4'*RS*)-4-Methyl-2-(4'-*iso*-propyl-5'-oxo-4',5'-dihydro-oxazol-2'-yl)-hexanoic acid *tert*-butyl ester 129b

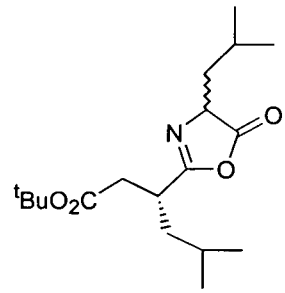


The procedure outlined in Section **(4.3.26.)** was followed using DL-valine derived acid **128b** (587 mg, 1.81 mmol) and EDCI (382 mg, 1.99 mmol, 1.1 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title product (486 mg, 81%). ^{1}H nmr indicated a (1:1) ratio of diastereomers.

R_f (Hexane:EtOAc, 6:1) 0.54; $[\alpha]_D^{20}$-7.5 (c 1.16, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 1819 (oxazolone C=O), 1725 (ester C=O), 1673 (C=N); δ_H ($CDCl_3$; 600 MHz) 4.01 (0.5 H, d, J 5.4, NC$\underline{H}$ of one diastereomer), 4.00 (0.5 H, d, J 5.0, NC$\underline{H}$ of one diastereomer), 3.06 (total of 1 H, m, C$\underline{H}$C=N of each diastereomer), 2.64 (0.5 H, dd, *J* 16.2, 9.2, OCC$\underline{H}_A$CH_BCH of one diastereomer), 2.61 (0.5 H, dd, *J* 16.0, 8.2, OCC$\underline{H}_A$CH_BCH of one diastereomer), 2.44 (0.5 H, dd, *J* 16.0, 6.0, OCCH_AC$\underline{H}_B$CH of one diastereomer), 2.42 ((0.5 H, dd, *J* 16.2, 5.6, OCCH_AC$\underline{H}_B$CH of one diastereomer), 2.25 (0.5 H, m, CHC$\underline{H}(CH_2)_3$ of one diastereomer), 2.24 (0.5 H, m, CHC$\underline{H}(CH_2)_3$ of one diastereomer), 1.66- 1.58, (total of 2 H, m, $CH_2C\underline{H}(CH_3)_2$ and CHC$\underline{H}_A$H$_B$CH of each diastereomer), 1.42 (4.5 H, s, C($C\underline{H}_3$)$_3$ of one diastereomer), 1.41 (4.5 H, s, C($C\underline{H}_3$)$_3$ of one diastereomer), 1.34 and 1.33 (total of 1 H, m, CHC$H_A\underline{H}_B$CH of each diastereomer), 1.05, 1.04, 0.93(2), 0.92(2), 0.90, 0.89 (total of 12 H, 8 x d, *J* 6.6, C$\underline{H}_{3A}$CHCH_{3B} and CH_{3A}CHC$\underline{H}_{3B}$ of the *iso*-butyl and *iso*-propyl groups of each diastereomer); δ_C ($CDCl_3$; 63 MHz) 177.9 (NCH$\underline{C}O_2C$), 170.4, 170.2 ($\underline{C}O_2C$), 167.7, 167,5 ($\underline{C}$=N), 80.9, 80.8 (C(CH_3)$_3$), 69.8, 69.7 (H$\underline{C}$HCO), 40.6 ($O_2C\underline{C}H_2$), 37.5, 37.3

(CH$\underline{C}$H$_2$CH), 34.6, 34.5 (CH$_2\underline{C}$HN), 30.6, 30.5 (CH$\underline{C}$H(CH$_3$)$_2$), 27.9, 27.8 (C(CH$_3$)$_3$), 25.6, 25.5 (CH$_2\underline{C}$H(CH$_3$)$_2$), 22.6, 22.50, 22.1, 22.0 (CH$_2$CH($\underline{C}$H$_3$)$_2$), 18.6, 18.5, 17.3, 17.2 (CHCH($\underline{C}$H$_3$)$_2$); *m/z* (FAB) 312 (49%, MH$^+$), 256 (100, MH$_2^+$-^{t}Bu), 210 (74, 256-H$_2$O-CO), 157 (9), 111 (7), 83 (9), 72 (39), 57 (33, ^{t}Bu), Found (FAB) 312.2171, $C_{17}H_{22}NO_4$ requires 312.2174.

4.3.29. (3*R*,4'*RS*)-5-Methyl-3-(4'-*iso*-butyl-5'-oxo-4',5'-dihydro-oxazol-2'-yl)-hexanoic acid *tert*-butyl ester 129c

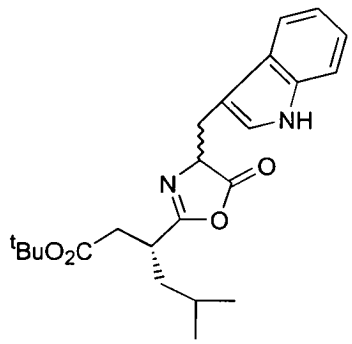


The procedure outlined in Section **(4.3.26.)** was followed using DL-leucine derived acid **128c** (753 mg, 2.19 mmol) and EDCI (462 mg, 2.41 mmol, 1.1 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title product (533 mg, 75%). ^{1}H nmr indicated a (1:1) ratio of diastereomers.

R_f (Hexane:EtOAc, 6:1) 0.45; $[\alpha]_D^{20}$ -4.1 (c 1.22, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 1822 (oxazolone C=O), 1723 (ester C=O), 1670 (C=N); δ_H ($CDCl_3$; 600 MHz) 4.13 (0.5 H, dd, *J* 8.6, 5.8 HC$\underline{H}$ of one diastereomer), 4.12 (0.5 H, dd, *J* 9.0, 5.8 HC$\underline{H}$ of one diastereomer), 3.03 (total of 1 H, m, C$\underline{H}$C=N of each diastereomer), 2.64 (0.5 H, dd, *J* 16.0, 9.0, CH$_2$CHC$\underline{H}_A$H$_B$CH of one diastereomer), 2.60 (0.5 H, dd, *J* 15.8, 8.4, CH$_2$CHC$\underline{H}_A$H$_B$CH of one diastereomer), 2.44 (0.5 H, dd, *J* 15.8, 6.2, CH$_2$CHCH$_A\underline{H}_B$CH of one diastereomer), 2.42 (0.5 H, dd, *J* 16.0, 5.6, CH$_2$CHCH$_A\underline{H}_B$CH of one diastereomer), 1.96-1.90 (total of 1 H, m, CH$_2$CHCH$_2$C$\underline{H}$(CH$_3$)$_2$ of each diastereomer), 1.69 (0.5 H, ddd, *J* 13.4, 7.4, 5.6, CHC$\underline{H}$HCH of one diastereomer), 1.68 (0.5 H, ddd, *J* 13.4, 7.6, 5.8, CHC$\underline{H}$HCH of one diastereomer), 1.65-1.58 (total of 2 H, NCHCH$_2$CH(CH$_3$)$_2$ of one diastereomer, and 3 x CHC$\underline{H}$HCH of either diastereomer), 1.52 (0.5 H, ddd, *J* 13.6, 8.8, 6.4, CHC$\underline{H}$HCH of one diastereomer), 1.49 (0.5 H, ddd, *J* 13.6, 9.0, 6.4, CHC$\underline{H}$HCH of one diastereomer), 1.41 (4.5 H, s, C(CH$_3$)$_3$ of one diastereomer), 1.40 (4.5 H, s,

$C(CH_3)_3$ of one diastereomer), 1.37-1.32 (total of 1 H, m, $NCHCH_2C\underline{H}(CH_3)_2$ of one diastereomer and $CHC\underline{H}HCH$ of either diastereomer), 0.96, 0.95, 0.93, 0.92, 0.90 0.89 (total of 12 H, 8 x d, *J* 6.6, $C\underline{H}_{3A}CHCH_{3B}$ and $CH_{3A}CHC\underline{H}_{3B}$ of each *iso*-butyl group of each diastereomer); δ_C ($CDCl_3$; 63 MHz) 179.2 ($NCH\underline{C}O_2C$), 170.2, 170.0 ($\underline{C}O_2C$), 167.3, 167,0 ($\underline{C}$=N), 80.8 ($\underline{C}(CH_3)_3$), 63.0, 62.9 ($H\underline{C}HCO$), 40.6, 40.4 40.3, 40.2 ($O_2C\underline{C}H_2$ and $NCH\underline{C}H_2$), 37.5, 37.3 ($O_2CCH_2CH\underline{C}H_2$), 36.6, 34.4 ($CH_2\underline{C}HN$), 27.9 ($C(\underline{C}H_3)_3$), 25.6, 25.5, 24.9 (2 x $\underline{C}H(CH_3)_2$), 22.5, 22.1, 22.0, 21.9, 21.9 (2 x $CH(\underline{C}H_3)_2$); *m/z* (FAB) 326 (80%, MH^+), 270 (86, MH_2^+-^{t}Bu), 252 (27, 270-H_2O), 224 (68, 252-CO), 182 (29), 154 (96), 136 (59), 91 (23, CH_2Ph), 57 (43, ^{t}Bu), Found (FAB) 326.2345, $C_{18}H_{32}NO_4$ requires 326.2331.

4.3.30. (3*R*,4'*RS*)-4-Methyl-2-[4'-(1*H*-indol-3-ylmethyl)-5'-oxo-4',5'-dihydro-oxazol-2'-yl]-hexanoic acid *tert*-butyl ester 129d

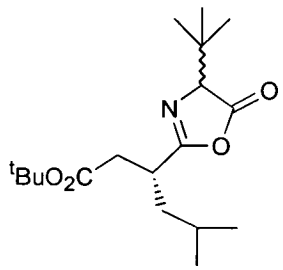


The procedure outlined in Section **(4.3.26.)** was followed using DL-tryptophan derived acid **128d** (914 mg, 2.19 mmol) and EDCI (463 mg, 2.41 mmol, 1.1 equiv.). Column chromatography, eluting with hexane:ethyl acetate (3:1) furnished the title product (900 mg, 91%). ^{1}H nmr indicated a (1:1) ratio of diastereomers.

R_f (Hexane:EtOAc, 2:1) 0.60, 0.53; $[\alpha]_D^{20}$ +14.9 (c 1.20, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3479 (indole NH), 1815 (oxazolone C=O), 1724 (ester C=O), 1672 (C=N); δ_H ($CDCl_3$; 600 MHz)8.15 (total of 1 H, s, $N\underline{H}_{(indole)}$ of each diastereomer), 7.64 (0.5 H, d, *J* 7.2, $C\underline{H}_{ar}$ of one diastereomer), 7.63 (0.5 H, d, *J* 7.0, $C\underline{H}_{ar}$ of one diastereomer), 7.29 (total of 1 H, m, $C\underline{H}_{ar}$ of each diastereomer), 7.15 (total of 1 H, m, $C\underline{H}_{ar}$ of each diastereomer), 7.10 (total of 1 H, m, $C\underline{H}_{ar}$ of each diastereomer), 7.03 (total of 1 H, m, $C\underline{H}_{ar}$ of each diastereomer), 4.51 (0.5 H, dd, *J* 5.0 $NC\underline{H}$ on one diastereomer), 4.49 (0.5 H, dd, *J* 5.0 $NC\underline{H}$ on one diastereomer), 3.45 (0.5 H dd, *J* 15.0, 5.0,

C$\underline{H}_A$H$_B$Indole of one diastereomer), 3.44 (45 (0.5 H dd, *J* 15.0, 5.0, C$\underline{H}_A$H$_B$Indole of one diastereomer), 3.30 (45 (0.5 H dd, *J* 15.0, 5.0, CH$_A\underline{H}_B$Indole of one diastereomer), 3.29 (45 (0.5 H dd, *J* 15.0, 5.0, CH$_A\underline{H}_B$Indole of one diastereomer), 2.87 (0.5 H, m, C$\underline{H}$C=N of one diastereomer), 2.79 (0.5 H, m, C$\underline{H}$C=N of one diastereomer), 2.32 (0.5 H, dd *J* 16.0, 8.0, OCC$\underline{H}_A$H$_B$CH of one diastereomer), 2.26 (0.5 H, dd *J* 16.2, 7.8, OCC$\underline{H}_A$H$_B$CH of one diastereomer), 2.16 (0.5 H, dd *J* 16.2, 6.4, OCCH$_A\underline{H}_B$CH of one diastereomer), 2.14 (0.5 H, dd *J* 16.0, 6.6, OCCH$_A\underline{H}_B$CH of one diastereomer), 1.40 (4.5 H, s, C(C$\underline{H}_3$)$_3$ of one diastereomer), 1.39 (4.5 H, s, C(C$\underline{H}_3$)$_3$ of one diastereomer), 1.25 (0.5 H, ddd, *J* 13.6, 9.0, 5.8, CHC$\underline{H}_A$H$_B$CH of one diastereomer), 1.14 (0.5 H, m, C$\underline{H}$(CH$_3$)$_2$ of one diastereomer), 1.03 (0.5 H, ddd, *J* 14.2, 8.4, 6.0, CHC$\underline{H}_A$H$_B$CH of one diastereomer), 1.01 (0.5 H, ddd, 13.6, *J* 8.4, 6.4, CHCH$_A\underline{H}_B$CH of one diastereomer), 0.95 (0.5 H, m, C$\underline{H}$(CH$_3$)$_2$ of one diastereomer), 0.82 (0.5 H, ddd, *J* 14.2, 8.0, 6.4, OCCH$_A\underline{H}_B$CH of one diastereomer), 0.70, 0.66, 0.64, 0.63 (total of 6 H, 4 x d, J 6.6, C$\underline{H}_{3A}$CHCH$_{3B}$ and CH$_{3A}$CHC$\underline{H}_{3B}$ of each diastereomer); δ_C (CDCl$_3$; 63 MHz) 178.6, 178.5 (NCH$\underline{C}$O$_2$C), 170.4, 170.2 ($\underline{C}$O$_2$C), 167.7, 167,1 ($\underline{C}$=N), 137.7, 127.5, 127.4 (*ipso*-Ar), 123.2, 123.1, 122.0, 121.9, 119.5, 119.3, 119.2, 110.9 (C$\underline{H}_{ar}$), 109.3, 109.1 (*ipso*-Ar), 80.8, 80.7 ($\underline{C}$(CH$_3$)$_3$), 66.1, 66.0 (H$\underline{C}$HCO), 40.0, 39.9 (O$_2$C$\underline{C}$H$_2$), 37.4, 37.0 ($\underline{C}$H$_2$CH(CH$_3$)$_2$), 34.6, 33.9 (CH$_2\underline{C}$HN), 27.9, 27.8 ($\underline{C}$(CH$_3$)$_3$), 26.6, 26.5 ($\underline{C}$H$_2$Indole), 25.1, 24.9 (CH$_2\underline{C}$H(CH$_3$)$_2$), 22.5, 22.3, 21.9, 21.5 (CH$_2$CH($\underline{C}$H$_3$)$_2$); *m/z* (FAB) 399 (36%, MH$^+$), 361 (71), 343 (17, (MH$_2^+$-^{t}Bu), 297 (6, 343-H$_2$O-CO), 277 (16), 205 (12), 188 (48), 159 (82), 130 (100, CH$_2$Indole), 57 (36, ^{t}Bu), Found (FAB) 399.2266, C$_{23}$H$_{31}$N$_2$O$_4$ requires 399.2283.

4.3.31. (3*R*,4'*RS*)-4-Methyl-2-(4'-*tert*-butyl-5'-oxo-4',5'-dihydro-oxazol-2-yl)-hexanoic acid *tert*-butyl ester 129e



The procedure outlined in Section **(4.3.26.)** was followed using crude DL-*tert*-leucine derived acid **128e** (500 mg, 1.60 mmol) and EDCI (367 mg, 1.91 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title compound as a colourless oil in a diastereomeric ratio of 2:1 as calculated from the integrals of the ^{1}H nmr (244 mg, 52%). ^{1}H nmr indicated a (1:1) ratio of diastereomers.

R_f (Hexane:EtOAc, 6:1) 0.60; $[\alpha]_D^{20}$ -17.3 (c 0.74, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$; 3018 (saturated CH), 1821 (oxazolone C=O), 1729 (ester C=O), 1675 (C=N); δ_H ($CDCl_3$; 600 MHz) 3.80 (0.5 H, s, NC<u>H</u> of one diastereomer), 3.79 (0.5 H, s, NC<u>H</u> of one diastereomer), 3.06 (total of 1 H, m, C<u>H</u>C=N of each diastereomer), 2.65 (0.5 H, dd, *J* 16.3 9.0, OCC<u>H</u>$_A$H$_B$CH of one diastereomer), 2.60 (0.5 H, dd, *J* 16.3 8.0, OCC<u>H</u>$_A$H$_B$CH of one diastereomer) 2.44 (0.5 H, dd, *J* 16.3 6.0, OCCH$_A$<u>H</u>$_B$CH of one diastereomer) 2.42 (0.5 H, dd, *J* 16.3 5.6, OCCH$_A$<u>H</u>$_B$CH of one diastereomer), 1.66-1.52 (total of 2 H, m, CHC<u>H</u>$_A$CH$_B$CH of each diastereomer, CHCH$_A$C<u>H</u>$_B$CH of one diastereomer, and C<u>H</u>(CH$_3$)$_2$ of one diastereomer), 1.42 (4.5 H, s, OC(C<u>H</u>$_3$)$_3$ of one diastereomer), 1.41 (4.5 H, s, OC(C<u>H</u>$_3$)$_3$ of one diastereomer), 1.33 (total of 1 H, m, CHCH$_A$<u>H</u>$_B$CH of one diastereomer, and C<u>H</u>(CH$_3$)$_2$ of one diastereomer), 1.04 (4.5 H, s, CHC(C<u>H</u>$_3$)$_3$ of one diastereomer), 1.03 (4.5 H, s, CHC(C<u>H</u>$_3$)$_3$ of one diastereomer), 0.93, 0.92, 0.90, 0.89 (total of 6 H, 4 x d, *J* 6.4, C<u>H</u>$_{3A}$CHCH$_{3B}$ and CH$_{3A}$CHC<u>H</u>$_{3B}$ of each diastereomer); δ_C ($CDCl_3$; 63 MHz) 177.1 (NCH<u>C</u>O$_2$C), 170.4, 170.2 (<u>C</u>O$_2$C), 167.3, 167.0 (<u>C</u>=N), 80.8 (O<u>C</u>(CH$_3$)$_3$), 73.1 (N<u>C</u>HCO), 40.6, 40.5 (OC<u>C</u>H$_2$), 37.5, 37.1 (CH<u>C</u>H$_2$CH), 35.2 (<u>C</u>HC(CH$_3$)$_3$), 34.5, 34.3 (CH$_2$<u>C</u>HCN), 28.0 and 27.9 (OC(<u>C</u>H$_3$)$_3$ and CHC(<u>C</u>H$_3$)$_3$), 26.0 (CHC(<u>C</u>H$_3$)$_2$), 25.6, 25.5 (<u>C</u>H(CH$_3$)$_2$) 22.5, 22.4, 22.1, 22.0 (CH(<u>C</u>H$_3$)$_2$); *m/z* (FAB) 326 (100%, MH$^+$), 270 (81, MH$_2^+$-^{t}Bu) 252 (11, 270-H$_2$O), 224 (30, 252-CO), 137 (10), 86 (26), 57 (50, ^{t}Bu), Found (FAB) 326.2328, $C_{18}H_{32}NO_4$ requires 326.2331.

4.3.32. General procedure for the lipase catalysed ring opening of (4*RS*)-2-substituted-4-substituted-5(4*H*)-oxazolones 129a-e

i. In the presence of triethylamine

Triethylamine (0.25 equiv.), lipase (crushed dried Novozyme®,100 mg pre-dried weight, or dried Lipozyme®, 100 mg pre-dried weight) and alcohol (2.0 equiv.) were

added to a solution of oxazolone **129a-e** (100 mg) dissolved in solvent (8 mL). The flask was stoppered and placed in an orbital incubator at 37 °C at 200 rpm. The reactions were monitored by tlc and on complete consumption of the starting 5(4*H*)-oxazolone the lipase was filtered, washed with solvent (2x 10 mL), and the combined organic fractions concentrated under reduced pressure. Purification by column chromatography as described for the corresponding 1:1 mix of diastereomers afforded the desired product as a colourless solid.

ii. In the absence of triethylamine

As above with the elimination of triethylamine.

4.3.33. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a

The procedure outlined in Section **(4.3.32.i.)** was followed using oxazolone **129a**, triethylamine, Novozyme®, methanol, and toluene. Reaction time 4 days, (93 mg, 85%).

R_f (Hexane:EtOAc, 6:1) 0.27; d.e. 81%; Mp 94-96 °C; $[\alpha]_D^{20}$ +54.7 (c 1.90, $CHCl_3$).

4.3.34. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a

The procedure outlined in Section **(4.3.32.ii.)** was followed using oxazolone **129a**, Novozyme®, methanol, and acetonitrile. Reaction stopped after 10 days. Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished starting oxazolone (25 mg, 25%), and the desired product as a colourless solid (72 mg, 66%, 88% based on recovered starting material).

R_f (Hexane:EtOAc, 6:1) 0.27; d.e. 78%; Mp 92-95 °C; $[\alpha]_D^{20}$ +60.2 (c 1.38, $CHCl_3$).

4.3.35. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a

The procedure outlined in Section **(4.3.32.i.)** was followed using oxazolone **129a**, triethylamine, Novozyme®, methanol, and *tert*-butyl methyl ether. Reaction time 2 days, (98 mg, 90%).

R_f (Hexane:EtOAc, 6:1) 0.27; d.e. 79%; Mp 94-96 °C; $[\alpha]_D^{20}$ + 52.6 (c 1.80, $CHCl_3$).

4.3.36. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a

The procedure outlined in Section **(4.3.32.i.)** was followed using oxazolone **129a**, triethylamine, Lipozyme®, methanol, and toluene. Reaction time 1 day, (80 mg, 73%).

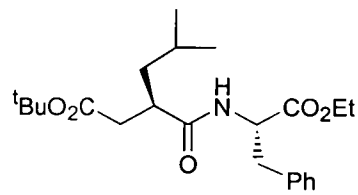
R_f (Hexane:EtOAc, 6:1) 0.27; d.e. 58%; Mp 89-91 °C; $[\alpha]_D^{20}$ +42.6 (c 1.52, $CHCl_3$).

4.3.37. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a

The procedure outlined in Section **(4.3.32.ii.)** was followed using oxazolone **129a**, Lipozyme®, methanol, and acetonitrile. Reaction stopped after 48 days. Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished starting oxazolone (24 mg, 24%) and desired product, (39 mg, 36%, 47% based on recovered oxazolone).

R_f (Hexane:EtOAc, 6:1) 0.27; d.e. 55%; Mp 88-90 °C; $[\alpha]_D^{20}$ +37.76 (c 0.76, $CHCl_3$).

4.3.38. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine ethyl ester 130

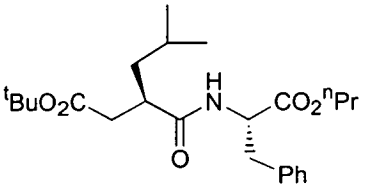


The procedure outlined in Section **(4.3.32.i.)** was followed using oxazolone **129a**, triethylamine, Novozyme®, ethanol, and toluene. Reaction time 3 days. Column chromatography eluting, with hexane:ethyl acetate (6:1) furnished the desired product as a colourless solid (85 mg, 76%). Spectroscopic data given is for the major product diastereomer.

R_f (Hexane:Et_2O, 2:1) 0.38 (major), 0.29; d.e. 84%; Mp 65-67 °C; (Found: C, 68.09; H, 9.00; N, 3.30; $C_{23}H_{35}NO_5$ requires C, 68.12; H, 8.70; N, 3.45); $[\alpha]_D^{20}$ +49.6 (c

1.00, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3435 (amide NH), 3018 (saturated CH), 1732 (ester C=O), 1682, 1511 (CONH); δ_H ($CDCl_3$; 600 MHz) 7.20 (2 H, d, *J* 7.0, C$\underline{H}_{ar}$), 7.15 (1 H, d, *J* 7.0, C$\underline{H}_{ar}$), 7.11 (2 H, d, *J* 7.0 C$\underline{H}_{ar}$), 6.14 (1 H, d, *J* 7.9, N$\underline{H}$), 4.78 (1 H, ddd, *J* 7.9, 6.0, NHC$\underline{H}$), 4.03 (2 H, q, *J* 7.0, OC$\underline{H}_2$), 3.04 (1 H, dd, *J* 13.6, 5.9, C$\underline{H}_A$H$_B$Ph), 3.01 (1 H, dd, 13.6, 6.0, CH$_A\underline{H}_B$Ph), 2.55 (1 H, m, C$\underline{H}$CONH), 2.47 (1 H, dd, *J* 16.6, 8.9, OCC$\underline{H}_A$H$_B$CH), 2.21 (1 H, dd, *J* 16.6, 4.9, OCCH$_A\underline{H}_B$CH), 1.50 (1 H, ddd, *J* 13.6, 8.9, 5.9, CHC$\underline{H}_A$H$_B$CH), 1.40 (1 H, m, C$\underline{H}$(CH$_3$)$_2$), 1.36 (9 H, s, C(C$\underline{H}_3$)$_3$), 1.13 (3 H, t, *J* 7.3, CH$_2$C$\underline{H}_3$), 1.09 (1 H, ddd, obscured by CH_3 ethyl ester peak, C$\underline{H}$(CH$_3$)$_2$), 0.80 (3 H, d, *J* 6.6, C$\underline{H}_{3A}$CHCH$_{3B}$), 0.78 (3 H, d, *J* 6.5, CH$_{3A}$CHC$\underline{H}_{3B}$); δ_C ($CDCl_3$; 63 MHz) 174.2, 171.5, 171.3 (C$\underline{O}$NH, C$\underline{O}_2$C), 135.9 (*ipso*-Ar), 129.3, 128.3, 126.8 (C$\underline{H}_{ar}$), 80.5 (C$\underline{(CH_3)_3}$), 60.2 (OC$\underline{H}_2$CH$_3$), 53.0 (NHC$\underline{H}$), 41.2 (O$_2$CC$\underline{H}_2$), 40.7 (C$\underline{H}$CONH), 38.0 (C$\underline{H}_2$CH(CH$_3$)$_2$ and C$\underline{H}_2$Ph), 27.9 (C(C$\underline{H}_3$)$_3$), 25.4 (C$\underline{H}$(CH$_3$)$_2$), 22.8, 22.0 (CH(C$\underline{H}_3$)$_2$), 13.9 (CH$_2$C$\underline{H}_3$); *m/z* (FAB) 406 (27%, MH$^+$), 378 (38, MH$^+$-Et) 350 (74, MH$_2^+$-^{t}Bu), 332 (15, 350-H$_2$O), 304 (12, 332-CO), 258 (25), 222 (14), 194 (35), 157 (12), 120 (100), 91 (14, CH$_2$Ph), 57 (30, ^{t}Bu), Found (FAB), 406.2577, $C_{23}H_{36}NO_5$ requires 406.2593.

4.3.39. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine *n*-propyl ester 131

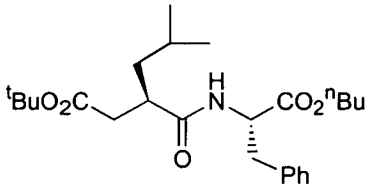


The procedure outlined in Section **(4.3.32.i.)** was followed using **129a**, triethylamine, Novozyme®, *n*-propanol, and toluene. Reaction time 3.5 days. Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the desired product as a colourless solid (98 mg, 84%). Spectroscopic data given is for the major product diastereomer.

R_f (Hexane:EtOAc, 2:1) 0.52; d.e. 84%; Mp 50-53 °C; (Found: C, 68.46; H, 9.11; N, 3.21; $C_{24}H_{37}NO_5$ requires C, 68.68; H, 8.89; N, 3.38); $[\alpha]_D^{20}$ +47.3 (c 1.76, $CHCl_3$). $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3429 (amide NH), 3017 (saturated CH), 1728 (ester C=O), 1669, 1513 (CONH); δ_H ($CDCl_3$; 600 MHz) 7.21 (2 H, d, 7.0, C$\underline{H}_{ar}$), 7.16 (1 H, d, 7.0,

C$\underline{H}_{ar}$), 7.06 (2 H, d, 7.0, C$\underline{H}_{ar}$), 6.13 (1 H, d, *J* 7.9, N$\underline{H}$), 4.80 (1 H, ddd, *J* 7.9, 6.3, NHC$\underline{H}$), 3.96 (1 H, dd, *J* 10.6, 6.9, OC$\underline{H}_A$H$_B$CH$_2$), 3.95 (1 H, dd, *J* 10.6, 6.6 OCH$_A\underline{H}_B$CH$_2$), 3.04 (1 H, dd, *J* 13.9, 5.9, C$\underline{H}_A$H$_B$Ph), 3.01 (1 H, dd, *J* 13.9, 6.3, CH$_A\underline{H}_B$Ph), 2.55 (1 H, m, C$\underline{H}$CONH), 2.47 (1 H, dd, *J* 16.6, 9.0, OCC$\underline{H}_A$H$_B$CH) 2.22 (1 H, dd, *J* 16.6, 4.9, OCCH$_A\underline{H}_B$CH), 1.57-1.42 (total of 4 H, m, OCH$_2$C$\underline{H}_2$, CHC$\underline{H}_A$H$_B$CH, and C$\underline{H}$(CH$_3$)$_2$), 1.36 (9 H, s, C(C$\underline{H}_3$)$_3$), 1.10 (1 H, ddd, J 13.6, 7.6, 5.9, CHCH$_A\underline{H}_B$CH), 0.81, (3 H, d, *J* 6.4, C$\underline{H}_{3A}$CHCH$_{3B}$), 0.79 (3 H, d, *J* 6.4, CH$_{3A}$CHC$\underline{H}_{3B}$), 0.80 (3 H, t, *J* 7.3, CH$_2$C$\underline{H}_3$); δ_C ($CDCl_3$; 63 MHz) 174.4, 171.7, 171.6 (C$\underline{O}$NH, C$\underline{O}_2$C), 136.0 (*ipso*-Ar), 129.4, 128.5 127.0 (C$\underline{H}_{ar}$), 80.7 (C$\underline{(CH_3)_3}$), 66.9 (OC$\underline{H}_2$CH$_2$), 53.1 (NHC$\underline{H}$), 41.3 (O$_2$CC$\underline{H}_2$), 40.8 (C$\underline{H}$CONH), 38.2, 38.1 (C$\underline{H}_2$CH(CH$_3$)$_2$ and C$\underline{H}_2$Ph), 28.5, (C(C$\underline{H}_3$)$_3$), 25.5 (C$\underline{H}$(CH$_3$)$_2$), 22.9 (OC$\underline{H}_2$CH$_2$), 22.2, 21.8 (CH(C$\underline{H}_3$)$_2$), 10.3 (CH$_2$C$\underline{H}_3$); *m/z* (FAB) 420 (23%, MH$^+$), 365 (100, MH$_2^+$-^{t}Bu), 346 (6, 365-H$_2$O), 258 (7), 208 (20), 120 (26), 91 (5, CH$_2$Ph), 57 (9, ^{t}Bu), Found (FAB), 420.2736, $C_{24}H_{38}NO_5$ requires 420.2750.

4.3.40. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine *n*-butyl ester 132



The procedure outlined in Section **(4.3.32.i.)** was followed using **129a**, triethylamine, Novozyme®, *n*-butanol, and toluene. Reaction time 3.5 days. Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the desired product as a colourless solid (103 mg, 85%). Spectroscopic data given is for the major product diastereomer.

R_f (Hexane:EtOAc, 2:1) 0.55; d.e. 80%,; Mp 53-56 °C; (Found: C, 68.84; H, 9.14; N, 3.14; $C_{26}H_{37}NO_5$ requires C, 69.21; H, 8.99; N 3.23); $[\alpha]_D^{20}$ +45.2 (c 1.76, $CHCl_3$). $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3427 (amide NH), 3018 (saturated CH), 1726 (ester C=O), 1675, 1507 (CONH); δ_H ($CDCl_3$; 600 MHz) 7.20 (2 H, d, *J* 7.0, C$\underline{H}_{ar}$), 7.16 (1 H, d, *J* 7.0, C$\underline{H}_{ar}$), 7.11 (2 H, d, *J* 7.0, C$\underline{H}_{ar}$), 6.14 (1 H, d, *J* 7.9, N$\underline{H}$), 4.79 (1 H, ddd, *J* 7.9, 6.0, NHC$\underline{H}$), 4.00 (1 H, dt, *J* 10.6, 6.6, OC$\underline{H}_A$H$_B$CH$_2$), 3.99 (1 H, dt, *J* 10.6, 6.6,

$OCH_A\underline{H}_BCH_2$), 3.04 (1 H, dd, *J* 13.9, 5.6, $C\underline{H}_AH_BPh$), 3.01 (1 H, dd, *J* 13.9, 6.3, $CH_A\underline{H}_BPh$), 2.55 (1 H, m, C<u>H</u>CONH), 2.48 (1 H, dd, *J* 16.6, 9.0, $OCC\underline{H}_AH_BCH$), 2.22 (1 H, dd, *J* 16.6, 4.9, $OCCH_A\underline{H}_BCH$), 1.53-1.44 (total of 4 H, m, $CHC\underline{H}_AH_BCH$, $C\underline{H}(CH_3)_2$, and $C\underline{H}_2CH_2CH_3$), 1.36 (9 H, s, $C(C\underline{H}_3)_3$), 1.23 (2 H, q, *J* 7.3, $C\underline{H}_2CH_3$), 1.10 (1 H, ddd, *J* 13.6, 7.6, 5.9, $CHCHA\underline{H}_BH$), 0.83 (3 H, t, *J* 7.3, $CH_2C\underline{H}_3$), 0.80 (3 H, d, *J* 6.4, $C\underline{H}_{3A}CHCH_{3B}$), 0.78 (3 H, d, *J* 6.4, $CH_{3A}CHC\underline{H}_{3B}$); δ_C ($CDCl_3$; 63 MHz) 174.3, 171.7, 171.6 (<u>C</u>ONH, <u>C</u>O_2C), 136.0 (*ipso*-Ar), 129.4, 128.4 127.0 ($\underline{C}H_{ar}$), 80.7 ($\underline{C}(CH_3)_3$), 65.2 ($O\underline{C}H_2CH_2$), 53.1 (NH<u>C</u>H), 41.3 ($O_2C\underline{C}H_2$), 40.8 (<u>C</u>HCONH), 38.2, 38.1 ($\underline{C}H_2CH(CH_3)_2$ and $\underline{C}H_2Ph$), 30.4 ($OCH_2\underline{C}H_2$), 28.1, ($C(\underline{C}H_3)_3$), 25.6 ($\underline{C}H(CH_3)_2$), 22.9, 22.2 ($CH(\underline{C}H_3)_2$), 19.0 ($\underline{C}H_2CH_3$), 10.3 ($CH_2\underline{C}H_3$); *m/z* (FAB) 434 (32%, MH^+), 378 (100, MH_2^+-^{t}Bu), 360 (20, 378-H_2O), 304 (9), 258 (17), 222 (24), 157 (6), 120 (48), 91 (5, CH_2Ph), 57 (1, ^{t}Bu), Found (FAB), 434.2896, $C_{25}H_{40}NO_5$ requires 434.2906.

4.3.41. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-valine methyl ester 127b

The procedure outlined in Section **(4.3.32.i.)** was followed using oxazolone **129b**, triethylamine, Novozyme®, methanol, and toluene. Reaction time 11 days, (103 mg, 93%).

R_f (Hexane:EtOAc, 6:1) 0.20; d.e. 75%; Mp 70-73 °C; $[\alpha]_D^{20}$ +11.36 (c 1.98, $CHCl_3$)

4.3.42. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-valine methyl ester 127b

The procedure outlined in Section **(4.3.32.i.)** was followed using oxazolone **129b**, triethylamine, Lipozyme®, methanol, and toluene. Reaction time 29 days, (96 mg, 87%).

R_f (Hexane:EtOAc, 6:1) 0.20; d.e. 27%; Mp 58-59 °C; $[\alpha]_D^{20}$ +7.14 (c 1.68, $CHCl_3$)

4.3.43. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-leucine methyl ester 127c

The procedure outlined in Section **(4.3.32.i.)** was followed using oxazolone **129c**, triethylamine, Novozyme, methanol, and toluene. Reaction time 2 days, (105 mg, 96%).

R_f (Hexane:EtOAc, 6:1) 0.55; d.e. 86%; Mp 102-103 °C; $[\alpha]_D^{20}$ +2.48 (c 2.06, $CHCl_3$)

4.3.44. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-leucine methyl ester 127c

The procedure outlined in Section **(4.3.32.i.)** was followed using oxazolone **129c**, triethylamine, Lipozyme®, methanol, and toluene. Reaction time 12 days, (105 mg, 96%).

R_f (Hexane:EtOAc, 6:1) 0.55; d.e. 54%; Mp 94-96 °C; $[\alpha]_D^{20}$ +1.44(c 1.88, $CHCl_3$)

4.3.45. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-tryptophan methyl ester 127d

The procedure outlined in Section **(4.3.32.i.)** was followed using oxazolone **129d**, triethylamine, Novozyme®, methanol, and toluene. Reaction stopped after 13 days, (91 mg, 84%).

R_f (Hexane:Et_2O, 1:1) 0.20; d.e. 74%; Mp 71-73 °C; $[\alpha]_D^{20}$ +60.1 (c 1.76, $CHCl_3$)

4.3.46. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-tryptophan methyl ester 127d

The procedure outlined in Section **(4.3.32.i.)** was followed using oxazolone **129d**, triethylamine, Lipozyme®, methanol, and toluene. Reaction stopped after 13 days, (96 mg, 89%).

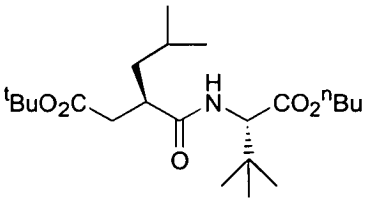
R_f (Hexane:Et_2O, 1:1) 0.20; d.e. 7%; Mp 54-56 °C; $[\alpha]_D^{20}$ +15.72 (c 1.80, $CHCl_3$)

4.3.47. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-*tert*-leucine methyl ester 127e

The procedure outlined in Section **(4.3.32.i.)** was followed using oxazolone **129e**, triethylamine, Lipozyme®, methanol, and toluene. Reaction time 28 days. Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished starting oxazolone (73 mg, 73%) and the desired product as a colourless solid (13 mg, 12%, 44% based on recovered starting material).

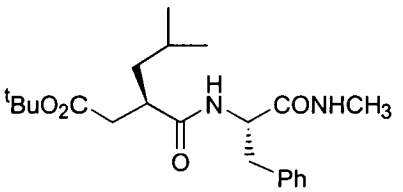
R_f (Hexane:EtOAc, 6:1) 0.38; d.e. 72%; Mp 100-102 °C; $[\alpha]_D^{20}$ +3.75 (c 0.24, $CHCl_3$)

4.3.48. (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-*tert*-leucine *n*-butyl ester 132e



The procedure outlined in Section **(4.3.32.i.)** was followed using oxazolone **129e**, triethylamine, Lipozyme®, *n*-butanol, and toluene. Reaction time 28 days. ^1H nmr (200 MHz, $CDCl_3$) of the crude product indicated mainly starting oxazolone. The title compound appeared to be present in only 5% calculated by comparison of the integrals at δ_H 4.45 (NHCH) of product and δ_H 3.81 (NCH) of each diastereomer of starting oxazolone. Due to the low conversion no further purification was attempted. R_f (Hexane:EtOAc, 6:1) 0.48.

4.3.49. (2*R*,2'*R*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine-*N*-methylamide and (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine-*N*-methylamide 134a



The general procedure outlined in Section **(4.3.20.)** was followed with the biotransformation product obtained in Section **(4.3.33.)** (88 mg, 0.22 mmol) and lithium hydroxide monohydrate (19 mg, 0.45 mmol, 2.0 equiv.) to furnish the hydrolysis product as a colourless gum (85 mg, quantitative). The product was not characterised but used directly in the next step of the synthesis.

The procedure outlined in Section **(4.3.9.ii.)** was followed using TBTU (87 mg, 0.27 mmol, 1.2 equiv.), HOBt (37 mg, 0.27 mmol, 1.2 equiv.), diisopropylethylamine (118 μL, 0.67 mmol, 3.0 equiv.), the acid obtained above (85 mg, 0.22 mmol, 1.0 equiv.) and methylamine hydrochloride (18 mg, 0.26 mmol, 1.1 equiv.). Column chromatography, eluting with ethyl acetate:hexane (2:1) furnished the title compound as a colourless solid (78 mg, 88% from starting methyl ester). ^{1}H nmr indicated a (1:2.75). ratio of diastereomers.

R_f (EtOAc, 100%) 0.50; d.e. 42%; Mp 168-171 °C; (Found: C, 67.35; H, 9.05; N, 7.00; $C_{22}H_{34}N_2O_4$ requires C, 67.66; H, 8.78; N, 7.17); $[\alpha]_D^{20}$ +46.1 (c 1.56, $CHCl_3$); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3384 (amide NH), 3019 (saturated CH), 1712 (ester C=O), 1664, 1542 (CONH); δ_H ($CDCl_3$; 600 MHz)7.27-7.25 (total of 2 H, m, C$\underline{H}_{ar}$ of each diastereomer), 7.22-7.18 (total of 3 H, m, C$\underline{H}_{ar}$ of each diastereomer), 6.37 (0.71 H, br q N$\underline{H}^A$CH$_3$), 6.13 (0.29 H, br q, N$\underline{H}^B$CH), 5.83 (total of 1 H, d, *J* 8.9, N$\underline{H}$CH of each diastereomer), 4.84 (0.71 H, ddd, *J* 8.9, 4.9, NHC$\underline{H}^A$), 4.54 (0.29 H, ddd, NHC$\underline{H}^B$), 3.30 (0. 71 H, dd, *J* 14.3, 5.0, C$\underline{H}^A_A$H$_B$Ph), 3.10 (0. 29 H, dd, *J* 13.6, 6.6, C$\underline{H}^B_A$H$_B$Ph), 3.06 (0. 29 H, dd, *J* 13.6, 7.6, CH$_B\underline{H}^B_B$Ph), 3.05 (0. 29 H, dd, *J* 14.3, 8.6, CH$_B\underline{H}^A_B$Ph), 2.74 (2.13 H, d, *J* 4.6, NHC$\underline{H}^A_3$), 2.72 (0.71 H, dd, *J* 17.7, 11.3, OCH^A_AH$_B$CH), 2.68 (0.87 H, d, *J* 4.9, NHC$\underline{H}^B_3$), 2.57 (0.29 H, m, CH$_2$C$\underline{H}^B$CONH), 2.45 (0.29 H, dd, *J* 16.3, 8.9, OC$\underline{H}^B_A$H$_B$CH), 2.34 (0.29 H, dd, *J* 16.3, 4.9, OCH$_B\underline{H}^B_B$CH), 2.32 (0.71 H, m, CH$_2$C$\underline{H}^A$CONH), 2.18 (0.71 H, dd, *J* 17.6, 3.3, OCCH$_A\underline{H}^A_B$CH), 1.45 (0.29 H, ddd, *J* 14.6, 8.6, 6.0, CHC$\underline{H}^B_A$H$_B$CH), 1.40 (2.61 H, s, C(CH$^A_3$)$_3$), 1.39 (0.71 H, ddd, obscured by ^{t}Bu ester peaks, CHC$\underline{H}^A_A$H$_B$CH), 1.38 (6.39 H, s, C(CH$^B_3$)$_3$), 1.15 (0.29 H, m, C$\underline{H}^B$(CH$_3$)$_2$), 0.98 (0.71 H, m, C$\underline{H}^A$(CH$_3$)$_2$), 0.93 (0.71 H, ddd, 13.6, 9.6, 4.6, CHCH$_A\underline{H}^A_B$CH), 0.92 (0.29 H, ddd, *J* 14.6, 9.6, 4.9, CHCH$_A\underline{H}^A_B$CH), 0.84 (0.87, d, *J* 6.3, C$\underline{H}^B_{3A}$CHCH$_{3B}$), 0.80 (0.87, d, *J* 6.3, CH$_{3A}$CHC$\underline{H}^B_{3B}$), 0.70 (2.13, d, *J* 6.3, C$\underline{H}^A_{3A}$CHCH$_{3B}$), 0.68 (2.13, d, *J* 6.3, CH$_{3A}$CHC$\underline{H}^A_{3B}$); δ_C ($CDCl_3$; 63 MHz) 174.8, 174.6, 173.1, 171.6, 171.3, 171.1 (C$\underline{O}_2$C and $\underline{C}$ONH), 136.9 (*Ipso*-Ar), 129.1, 129.0, 128.5, 128.4, 126.7 (C$\underline{H}_{ar}$), 81.3, 80.9 ($\underline{C}$(CH$_3$)$_3$), 54.7, 53.6 (NH$\underline{C}$H), 41.5, (O$_2$C$\underline{C}$H$_2$), 41.0, 40.9 ($\underline{C}$HCONH), 38.3, 38.0,

37.8, 37.1 ($\underline{C}H_2Ph$ and $CH\underline{C}H_2CH$), 27.9 ($C(\underline{C}H_3)_3$), 26.1, 26.0 ($NH\underline{C}H_3$), 25,5, 24.9 ($\underline{C}H(CH_3)_2$), 23.1, 22.6, 22.2, 21.7 ($CH(\underline{C}H_3)_2$); *m/z* (FAB) 391 (52%, MH^+), 335 (69, MH_2^+-^{t}Bu), 304 (335-NH_2CH_3), 279 (33), 179 (27), 91 (40, CH_2Ph), 57 (54, ^{t}Bu), Found (FAB) 391.2595, $C_{22}H_{35}N_2O_4$ requires 391.2597.

5.0.0. Bibliography

1 C.-H. Wong and G. M. Whitesides, *Enzymes in Synthetic Organic Chemistry*, Elsevier Science Ltd, 1994.

2 L. Brady, A. M. Brozozowski, Z. S. Derewenda, E. Dodson, G. Dodson, S. Tolley, J. P. Turkenburg, L. Christiansen, B. Huge-Jensen, L. Norscov, L. Thim, and U. Menge, *Nature*, 1990, **343**, 676.

3 F. K. Winkler, A. D'Arcy, and W. Hunziker, *Nature*, 1990, **343**.

4 A. M. Brozowski, U. Derewenda, Z. S. Derewenda, G. G. Dodson, D. M. Lawson, J. P. Turkenburg, F. Bjorkling, B. Huge-Jensen, S. A. Patkar, and L. Thim, *Nature*, 1991, **351**, 491.

5 R. D. Schmid and R. Verger, *Angew. Chem. Int. Ed. Engl.*, 1998, **37**, 1608.

6 H. Pottevin, *Annales de l' Institute Pasteur*, 1906, **20**, 901.

7 B. Cambou and A. M. Klibanov, *J. Am. Chem. Soc.*, 1984, **106**, 2687.

8 M. Inagaki, J. Hiratake, T. Nishioka, and J. Oda, *J. Am. Chem. Soc.*, 1991, **113**, 9369.

9 A. L. Margolin and A. M. Klibanov, *J. Am. Chem. Soc.*, 1987, **109**, 3802.

10 K. Ryu and J. S. Dordick, *Biochemistry*, 1992, **31**, 2588.

11 N. L. S. Clair and M. A. Navia, *J. Am. Chem. Soc.*, 1992, **114**, 7314.

12 T. Zelinski and H. Waldmann, *Angew. Chem. Int. Ed. Engl.*, 1997, **36**, 722.

13 P. P. Wangikar, P. C. Michels, D. S. Clark, and J. S. Dordick, *J. Am. Chem. Soc.*, 1997, **119**, 70.

14 M. T. Reetz, A. Zonta, J. Simpelkamp, and W. Könen, *J. Chem. Soc., Chem Commun.*, 1996, 1397.

15 E. Zacharis, B. D. Moore, and P. J. Halling, *J. Am. Chem. Soc.*, 1997, **119**, 12396.

16 J. Partridge, P. J. Halling, and B. D. Moore, *J. Chem. Soc., Chem. Commun.*, 1998, 841.

17 R. J. Kazlauskas and H. K. Weber, *Curr. Opin. Chem. Biol.*, 1998, **2**, 121.

18 Y. L. Khmelnitsky and J. O. Rich, *Curr. Opin. Chem. Biol.*, 1999, **3**, 47.

19 R. J. Kazlauskas, A. N. E. Weissfloch, A. T. Rappaport, and L. A. Luccia, *J. Org. Chem.*, 1991, **56**, 2656.

20 L. E. Janes and R. J. Kazlauskas, *Tetrahedron: Asymmetry*, 1997, **8**, 3719.

21 M. Cygler, P. Grochulski, R. J. Kazlauskas, J. D. Schrag, F. Bouthillier, B. Rubin, A. N. Serreqi, and A. K. Gupta, *J. Am. Chem. Soc.*, 1994, **116**, 3180.

22 A. N. E. Weissfloch and R. J. Kazlauskas, *J. Org. Chem.*, 1995, **60**, 6959.

23 S. N. Ahmed, R. J. Kazlauskas, A. H. Morinville, P. Grochulski, J. D. Schrag, and M. Cygler, *Biocatalysis*, 1994, **9**, 209.

24 H. K. Weber, H. Stecher, and K. Faber, *Preparative Biotransformations: Some Properties of Commertially Available Crude Lipase Preparations*, Wiley, 1995.

25 W. Boland, C. Frößl, and M. Lorenz, *Synthesis*, 1991, 1049.

26 C.-S. Chen and C. J. Sih, *Angew. Chem., Int. Ed. Engl.*, 1989, **28**, 695.

27 Z.-F. Xie, *Tetrahedron: Asymmetry*, 1991, **2**, 733.

28 V. Gotor, R. Brieva, and F. Rebolledo, *Tetrahedron Lett.*, 1988, **29**, 6973.

29 M. Quiros, V. M. Sanchez, R. Brieva, F. Rebolledo, and V. Gotor, *Tetrahedron: Asymmetry*, 1993, **4**, 1105.

30 M. J. Garcia, F. Rebolledo, and V. Gotor, *Tetrahedron: Asymmetry*, 1992, **3**, 1519.

31 M. J. Garcia, F. Rebolledo, and V. Gotor, *Tetrahedron: Asymmetry*, 1993, **4**, 2199.

32 V. Gotor, R. Brieva, C. Gonzalez, and F. Rebolledo, *Tetrahedron*, 1991, **47**, 9207.

33 C. Chamorro, R. Gonzalez-Muniz, and S. Conde, *Tetrahedron: Asymmetry*, 1995, **6**, 2343.

34 V. Gotor, E. Menendez, Z. Mouloungui, and A. Gaset, *J. Chem. Soc., Perkin Trans. 1*, 1993, 2453.

35 S. Puertas, R. Brieva, F. Rebolledo, and V. Gotor, *Tetrahedron*, 1993, **49**, 4007.

36 L. E. Iglesias, V. M. Sanchez, F. Rebolledo, and V. Gotor, *Tetrahedron: Asymmetry*, 1997, **8**, 2675.

37 C. Vörde, H.-E. Högberg, and E. Hendenström, *Tetrahedron: Asymmetry*, 1996, **7**, 1507.

38 F. Balkenhohl, K. Ditrich, B. Hauer, and W. Lander, *J. Prakt. Chem.*, 1997, **339**, 381.

39 S. Takayama, W. J. Moree, and C.-H. Wong, *Tetrahedron Lett.*, 1996, **37**, 6287.

40 B. Orsat, P. B. Alper, W. Moree, C.-P. Mac, and C.-H. Wong, *J. Am. Chem. Soc.*, 1996, **118**, 712.

41 A. Maestro, C. Astorga, and V. Gotor, *Tetrahedron: Asymmetry*, 1997, **8**, 3153.

42 M. Pozo, R. Pulido, and V. Gotor, *Tetrahedron*, 1992, **48**, 6477.

43 M. Pozo and V. Gotor, *Tetrahedron*, 1993, **49**, 10725.

44 M. Pozo and V. Gotor, *Tetrahedron*, 1993, **49**, 4321.

45 S. Takayama, S. T. Lee, S.-C. Hung, and C.-H. Wong, *J. Chem. Soc., Chem Commun.*, 1999, 127.

46 M. C. de Zoete, A. C. Kock-van Dalen, F. van Rantwijk, and R. A. Sheldon, *J. Chem. Soc., Chem. Commun.*, 1993, 1831.

47 M. J. Garcia, F. Rebolledo, and V. Gotor, *Tetrahedron Lett.*, 1993, **34**, 6141.

48 M. C. de Zoete, A. C. Kock-van Dalen, F. van Rantwijk, and R. A. Sheldon, *Biocatalysis*, 1994, **10**, 307.

49 M. C. de Zoete, A. C. Kock-van Dalen, F. van Rantwisk, and R. A. Sheldon, *J. Mol. Cat. B: Enzymatic*, 1996, **2**, 19.

50 A. Patti, D. Lambusta, M. Piattelli, and G. Nicolosi, *Tetrahedron: Asymmetry*, 1998, **9**, 3073.

51 G. Nicolosi, R. Morrone, A. Patti, and M. Piattelli, *Tetrahedron: Asymmetry*, 1992, **3**, 753.

52 G. Nicolosi, A. Patti, R. Morrone, and M. Piattelli, *Tetrahedron: Asymmetry*, 1994, **5**, 1275.

53 D. Lambusta, G. Nicolosi, A. Patti, and M. Piattelli, *Tetrahedron Lett.*, 1996, **37**, 127.

54 A. Patti, D. Lambusta, M. Piattelli, G. Nicolosi, P. McArdle, D. Cunningham, and M. Walsh, *Tetrahedron*, 1997, **53**, 1361.

55 N. W. Boaz, *Tetrahedron Lett.*, 1989, **30**, 2061.

56 M.-J. Kim, H. Cho, and Y. K. Choi, *J. Chem. Soc., Perkin Trans. 1.*, 1991, 2270.

57 J. A. S. Howell, K. Humphries, P. McArdle, D. Cunningham, G. Nicolosi, A. Patti, and M. A. Walsh, *Tetrahedron: Asymmetry*, 1997, **8**, 1027.

58 K. Nakamura, K. Ishihara, A. Ohno, M. Uemura, H. Nishimura, and Y. Hayashi, *Tetrahedron Lett.*, 1990, **31**, 3603.

59 Y. Yamazaki and K. Hosono, *Tetrahedron Lett.*, 1990, **31**, 3895.

60 M. Uemura, H. Nishimura, S. Yamada, Y. Hayashi, K. Nakamura, K. Ishihara, and A. Ohno, *Tetrahedron: Asymmetry*, 1994, **5**, 1673.

61 J. M. Chong and E. K. Mar, *Tetrahedron Lett.*, 1991, **32**, 5683.

62 A. H. Djerourou and L. Blanco, *Tetrahedron Lett.*, 1991, **32**, 6325.

63 R. Tacke, S. A. Wagner, and J. Sperlich, *Chem. Ber.*, 1994, **127**, 639.

64 F. Theil, *Chem. Rev.*, 1995, **95**, 2203.

65 E. Schoffers, A. Golebiowski, and C. R. Johnson, *Tetrahedron*, 1996, **52**, 3769.

66 E. Santaniello, P. Ferraboschi, P. Grisenti, and A. Manzocchi, *Chem. Rev.*, 1992, **92**, 1017.

67 C. R. Johnson, *Acc. Chem. Res.*, 1998, **31**, 333.

68 B. Morgan, D. R. Dodds, A. Zaks, D. R. Andrews, and R. Klesse, *J. Org. Chem.*, 1997, **62**, 7736.

69 T. Yokomatsu, M. Sato, and S. Shibuya, *Tetrahedron: Asymmetry*, 1996, **7**, 2743.

70 A. L. Gutman, M. Shapira, and A. Boltanski, *J. Org. Chem.*, 1992, **57**, 1063.

71 M. Shapira and A. L. Gutman, *Tetrahedron: Asymmetry*, 1994, **5**, 1689.

72 H. Stecher and K. Faber, *Synthesis*, 1997, 1.

73 S. Caddick and K. Jenkins, *Chem. Soc. Rev.*, 1996, 447.

74 R. Noyori, M. Tokungaga, and M. Kitamura, *Bull. Chem. Soc. Jpn.*, 1995, **68**, 36.

75 R. S. Ward, *Tetrahedron: Asymmetry*, 1995, **6**, 1475.

76 M. L. E. Gihani and J. M. J. Williams, *Curr. Opin. Chem. Biol.*, 1999, **3**, 11.

77 J. W. J. F. Thuring, A. J. H. Klunder, G. H. L. Nefkens, M. A. Wegman, and B. Zwanenburg, *Tetrahedron Tett.*, 1996, **37**, 4759.

78 M. van den Heuvel, A. D. Cuiper, H. van der Deen, R. M. Kellog, and B. L. Feringa, *Tetrahedron Lett.*, 1997, **38**, 1655.

79 H. van der Deen, A. D. Cuiper, R. P. Hof, A. van Oeveren, B. L. Feringa, and R. M. Kellog, *J. Am. Chem. Soc.*, 1996, **118**, 3801.

80 S. Brand, M. F. Jones, and C. M. Rayner, *Tetrahedron Lett.*, 1995, **36**, 8493.

81 D. S. Tan, M. M. Gunter, and D. G. Drueckhammer, *J. Am. Chem. Soc.*, 1995, **117**, 9093.

82 P.-J. Um and D. G. Drueckhammer, *J. Am. Chem. Soc.*, 1998, **120**, 6505.

83 T. Taniguchi, R. M. Kanada, and K. Ogasawara, *Tetrahedron: Asymmetry*, 1997, **8**, 2773.

84 T. Taniguchi and K. Ogasawara, *J. Chem. Soc., Chem. Commun.*, 1997, 1399.

85 P. M. Dinh, J. A. Howarth, A. R. Hudnott, and J. M. J. Williams, *Tetrahedron Lett.*, 1996, **37**, 7623.

86 A. L. E. Larsson, B. A. Persson, and J.-E. Bäckvall, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 1211.

87 B. A. Persson, A. L. E. Larsson, M. L. Ray, and J.-E. Bäckvall, *J. Am. Chem. Soc.*, 1999, **121**, 1645.

88 M. T. Reetz and C. Schimossek, *Chimia*, 1996, 668.

89 M. Adamczyk, J. C. Gebler, and J. Grote, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1027.

90 V. V. Mozhaev, C. L. Budde, J. O. Rich, A. Y. Usyatinsky, P. C. Michels, Y. L. Khmelnitsky, D. S. Clark, and J. S. Dordick, *Tetrahedron*, 1998, **54**, 3971.

91 F. H. Arnold, *Chem. Eng. Sci.*, 1996, **51**, 5091.

92 M. Goodman and L. Levine, *J. Am. Chem. Soc.*, 1964, **86**, 2918.

93 W. J. McGahren and M. Goodman, *Tetrahedron*, 1967, **23**, 2017.

94 J. de Jersey and B. Zerner, *Biochemistry*, 1969, **8**, 1967.

95 R.-L. Gu, I. Lee, and C. J. Sih, *Tetrahedron Lett.*, 1992, **33**, 1953.

96 H. S. Bevinakatti, R. V. Newadkar, and A. A. Banerji, *J. Chem. Soc., Chem. Commun.*, 1990, 1091.

97 H. S. Bevinakatti, A. A. Banerji, R. V. Newadkar, and A. A. Mokashi, *Tetrahedron: Asymmetry*, 1992, **3**, 1505.

98 J. Z. Crich, R. Brieva, P. Marquart, R.-L. Gu, S. Flemming, and C. J. Sih, *J. Org. Chem.*, 1993, **58**, 3252.

99 N. J. Turner, J. R. Winterman, R. McCague, J. S. Parratt, and S. J. C. Taylor, *Tetrahedron Lett.*, 1995, **36**, 1113.

100 J. Liang, J. C. Ruble, and G. C. Fu, *J. Org. Chem.*, 1998, **63**, 3154.

101 J. R. Winterman, *Thesis: Chemo-Enzymic Methods for The Synthesis of Optically Active α-Amino Acids*, Ph. D, University of Exeter, 1996.

102 The Fluka Lipase Basic Kit was a gift from Fluka.

103 M.-C. Parker, S. A. Brown, L. Robertson, and N. J. Turner, *J. Chem. Soc., Chem. Commun.*, 1998, 2247.

104 B. Berger, C. G. Rabiller, K. Königsberger, K. Faber, and H. Griengel, *Tetrahedron: Asymmetry*, 1990, **1**, 541.

105 T. Maugard, M. Remaud-Simeon, D. Petre, and P.Monsan, *Tetrahedron*, 1997, **53**, 7629.

106 N. W. Boaz and R. L. Zimmerman, *Tetrahedron: Asymmetry*, 1994, **5**, 153.

107 D. K. Barnes, E. Campaigne, and R. L. Shriner, *J. Am. Chem. Soc.*, 1948, **70**, 1769.

108 B. K. Hwang, Q.-M. Gu, and C. J. Sih, *J. Am. Chem. Soc.*, 1993, **115**, 7912.

109 B. K. Hwang, Q.-M. Gu, and C. J. Sih, *Tetrahedron Lett.*, 1994, **35**, 2317.

110 S. Conde, P. Lopez-Serrano, M. Fierros, M. I. Biezama, A. Martinez, and M. I. Rodriguez-Franco, *Tetrahedron*, 1997, **53**, 11745.

111 N. R. A. Beeley, P. R. J. Ansell, and A. J. P. Docherty, *Curr. Opin. Ther. Patents*, 1994, 7.

112 A. H. Davidson, A. H. Drummond, W. A. Galloway, and M. Whittaker, *Chemistry & Industry*, 1997, 258.

113 R. P. Beckett, A. H. Davidson, A. H. Drummond, P. Huxley, and M. Whittaker, *Drug Discovery Today*, 1996, **1**, 16.

114 A. Zask, J. I. Levin, L. M. Killar, and J. S. Skotnicki, *Curr. Pharm. Design*, 1996, **2**, 624.

115 P. D. Brown, *Curr. Opin. Invest. Drugs*, 1993, 617.

116 K. Mori, *Tetrahedron*, 1976, **32**, 1101.

117 F. Degerbeck, B. Fransson, L. Grehn, and U. Ragnarsson, *J. Chem. Soc., Perkin Trans. 1*, 1993, 11.

118 M. Larcheveque and Y. Petit, *Synthesis*, 1991, 162.

119 O. Toussaint, P. Capdevielle, and M. Maumy, *Synthesis*, 1986, 1029.

120 O. Toussaint, P. Capdevielle, and M. Maumy, *Tetrahedron Lett.*, 1987, **28**, 539.

121 H. Brunner, J. Mueller, and J. Spitzer, *Monatshefte für Chemie*, 1996, **127**, 845.

122 A. Basha, M. Lipton, and S. M. Weinreb, *Tetrahedron Lett.*, 1977, **38**, 4171.

123 J. I. Levin, E. Turos, and S. M. Weinreb, *Synth. Commun.*, 1982, **13**, 989.

124 S. F. Martin, M. P. Dwyer, and C. L. Lynch, *Tetrahedron Lett.*, 1998, **39**, 1517.

125 K. F. McClure and M. Z. Axt, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 143.

126 U. Bauer, W. B. Ho, and A. M. P. Koskinen, *Tetrahedron Lett.*, 1997, **38**, 7233.

127 C. D. Floyd, C. N. Lewis, S. R. Patel, and M. Whittaker, *Tetrahedron Lett.*, 1996, **37**, 8045.

128 G. M. Devasia and C. J. Rajasekharan, *Tetrahedron Lett.*, 1975, **16**, 4051.

129 W. O. Foye and R. A. Mode, *J. Am. Pharm. Assoc*, 1955, **76**, 441.

130 G. Stork, A. Y. W. Leong, and A. M. Touzin, *J. Org. Chem.*, 1976, **21**, 3491.

131 M. Nakagawa, T. Suzuki, T. Kawashima, and T. Hino, *Chem. Pharm. Bull.*, 1972, **20**, 2413.

132 N. F. Albertson, S. Archer, and C. M. Sutter, *J. Am. Chem. Soc.*, 1945, **67**, 36.

133 E. Fischer, *Chem. Ber.*, 1899, **32**, 2454.

134 A. Kjaer, *Acta Chem. Scand*, 1953, 900.

135 F. M. F. Chen, K. Kurodo, and N. L. Benoiton, *Synthesis*, 1979, 230.

136 G. Schulz, P. Gruber, and W. Steglich, *Chem. Ber.*, 1979, **112**, 3221.

137 K. Suda, F. Hino, and C. Yijima, *Chem. Pharm. Bull*, 1985, **33**, 882.

138 J. Max, *Liebigs Ann. Chem.*, 1909, 276.

139 A. P. Krapcho and E. A. Dundulis, *Tetrahedron Lett.*, 1976, **16**, 2205.

140 J.-I. Oh-Hashi and K. Harada, *Bull. Chem. Soc. Jpn.*, 1966, **39**, 2287.

141 T. Lakhilifi, N. D. An, G. Schmitt, B. Lande, and M.-F. Mercier, *Bull. Soc. Chim. Belg.*, 1984, **103**, 169.

142 D. B. Berkowitz, *Synth. Commun*, 1990, **20**, 1819.

143 T. Bretschneider, W. Miltz, P. Muenster, and W. Steglich, *Tetrahedron*, 1988, **17**, 5403.

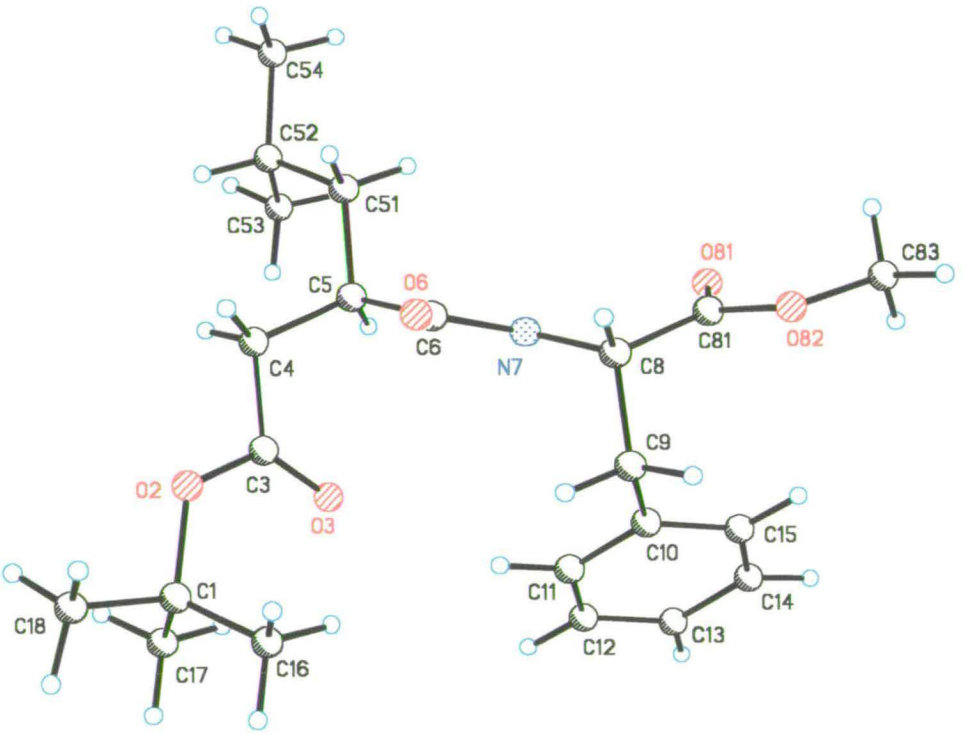
144 J. W. Cornforth, *Chemistry of Penicillin*, Princeton Univ. Press, 1949.

145 T. H. Applewhite, H. Waite, and C. Neimann, *J. Am. Chem. Soc.*, 1958, **80**, 1465.

146 K. Sato and A. P. Kozikowski, *Tetrahedron Lett.*, 1989, **30**, 4073.

147 M. Frankel and E. Katchalski, *J. Am. Chem. Soc.*, 1942, **65**, 2264.

6.0.0. Appendix I



X-ray crystal structure for (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a synthesised chemically

Crystal data and structure refinement for (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a synthesised chemically

A. Crystal data

Empirical formula	$C_{22}H_{33}NO_5$	
Formula weight	391.51	
Wavelength	1.54180 Å	
Temperature	150 K	
Crystal system	Monoclinic	
Space group	P21	
Unit cell dimensions	a = 12.401(5) Å	α = 90°
	b = 6.238(3) Å	β = 104.43(3)°
	c = 14.793(5) Å	γ = 90°
Volume	1108.3 $Å^3$	
Number of reflections for cell	32 ($20 < \theta < 22°$)	
Z	2	
Density (calculated)	1.17 Mg/m^3	
Absorption coefficient	0.63 mm^{-1}	
F(000)	425.22	

B. Data collection

Crystal description	colourless needle
Crystal size	0.44 x 0.06 x 0.08 mm
θ range for data collection	0.00 to 0.00°
Index ranges	$-15 \leq h \leq 14, -7 \leq k \leq 7, \leq l \leq 18$
Reflections collected	3011
Independent reflections	3011 [R(int) = 0.04]
Scan type	Omega-theta

C. Solution and refinement

Solution	Direct Methods (SIR92)
Refinement type	Full-matrix least-squares on F
Program used for refinement	CRYSTALS version 10
Hydrogen atom placement	Geometric
Hydrogen atom treatment	Placed geometrically after each cycle
Data / parameters	1463/259
Goodness-of-fit on F^2	1.1623
R	0.0511
R_w	0.0360
Observed criterion	>2.00\s(I)
Absolute structure parameter	0.0(1)
Extinction coefficient	31.4(43)
Final maximum δ/σ	0.112713
Weighting scheme	calc
Largest diff. peak and hole	0.31 and -0.33 e. $Å^{-3}$

Table1 Atomic co-ordinates (x 10^4), equivalent isotropic displacement parameters ($Å^2$ x 10^3) and site occupancies for **(2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a** synthesised chemically. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

Atom	x	y	z	U(eq)	Occ
C1	6286(4)	7985(12)	8729(4)	31	1.00
O2	5862(2)	7576(7)	7718(2)	28	1.00
O3	4059(3)	7503(8)	7758(2)	36	1.00
C3	4768(4)	7492(11)	7332(4)	28	1.00
C4	4534(4)	7300(10)	6283(3)	20	1.00
C5	3407(4)	6275(9)	5873(4)	21	1.00
C51	3185(4)	5940(10)	4807(4)	25	1.00
C52	3280(4)	7915(11)	4233(3)	21	1.00
C53	2540(5)	9747(12)	4409(4)	41	1.00
C54	2990(5)	7339(12)	3194(4)	40	1.00
O6	4143(3)	2874(7)	6518(3)	34	1.00
C6	3350(5)	4092(9)	6314(4)	22	1.00
N7	2337(4)	2337(4)	6453(3)	25	1.00
H7	1720(60)	4790(140)	6320(50)	110(30)	1.00
C8	2176(4)	1548(11)	6868(4)	26	1.00
O81	254(3)	1737(7)	6008(3)	38	1.00
C81	1010(5)	703(12)	6470(4)	26	1.00
O82	933(3)	-1385(7)	6681(3)	34	1.00
C83	-160(4)	-2290(13)	6390(4)	39	1.00
C9	2442(4)	1630(10)	7946(4)	26	1.00
C10	1684(4)	3100(10)	8319(3)	20	1.00
C11	2006(5)	5140(10)	8605(4)	28	1.00
C12	1344(5)	6496(12)	8969(4)	39	1.00

Table1 cont.

Atom	x	y	z	U(eq)	Occ
C13	306(5)	5786(13)	9058(4)	43	1.00
C14	-31(5)	3729(12)	8762(4)	38	1.00
C15	643(4)	2376(11)	8409(3)	29	1.00
C16	5991(7)	6161(13)	9273(5)	65	1.00
C17	5862(6)	10077(12)	8985(5)	53	1.00
C18	7528(5)	8065(16)	8831(4)	62	1.00
H41	4544	8764	6009	19	1.00
H42	5126	6398	6122	19	1.00
H51	2833	7277	6002	21	1.00
H511	3733	4856	4695	25	1.00
H512	2413	5358	4578	25	1.00
H521	4068	8427	4430	21	1.00
H531	2629	11009	4018	39	1.00
H532	2763	10154	5083	39	1.00
H533	1745	9270	4241	39	1.00
H541	3054	8649	2820	39	1.00
H542	3516	6218	3080	39	1.00
H543	2211	6781	3004	39	1.00
H81	2723	538	6705	25	1.00
H831	-134	-3833	6576	36	1.00
H832	-675	-1496	6695	36	1.00
H833	-434	-2169	5697	36	1.00
H91	3225	2146	8186	25	1.00
H92	2370	154	8183	25	1.00
H111	2747	5659	8547	28	1.00
H121	1603	7981	9169	36	1.00

Table1 cont.

Atom	x	y	z	U(eq)	Occ
H131	-181	6737	9329	39	1.00
H141	-781	3215	8804	35	1.00
H151	393	882	8219	29	1.00
H161	6281	6452	9956	57	1.00
H162	6334	4814	9107	57	1.00
H163	5164	5997	9123	57	1.00
H171	6154	10332	9670	49	1.00
H172	6115	11257	8629	49	1.00
H173	5029	10047	8827	49	1.00
H181	7918	8337	9499	49	1.00
H182	7707	9240	8433	49	1.00
H183	7785	6661	8630	49	1.00

Table 2 Bond lengths [Å] for **(2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a** synthesised chemically.

Bond	Bond Lengths [Å]	Bond	Bond Lengths [Å]
C1-O2	1.478(6)	C8-H81	0.999(6)
C1-C16	1.491(9)	O81-C81	1.202(7)
C1-C17	1.490(9)	C81-O82	1.348(8)
C1-C18	1.511(7)	O82-C83	1.432(6)
O2-C3	1.336(5)	C83-H831	1.000(8)
O3-C3	1.202(6)	C83-H832	1.000(7)
C3-C4	1.510(7)	C83-H833	1.000(6)
C4-C5	1.519(7)	C9-C10	1.512(7)
C4-H41	1.001(6)	C9-H91	0.999(5)
C4-H42	1.000(5)	C9-H92	1.000(6)
C5-C51	1.546(7)	C10-C11	1.364(8)
C5-C6	1.520(7)	C10-C15	1.406(7)
C5-H51	1.000(5)	C11-C12	1.381(8)
C51-C52	1.519(8)	C11-H111	0.999(6)
C51-H511	1.000(5)	C12-C13	1.397(9)
C51-H512	1.000(5)	C12-H121	1.001(7)
C52-C53	1.528(8)	C13-C14	1.386(9)
C52-C54	1.531(7)	C13-H131	0.999(6)
C52-H521	1.000(5)	C14-C15	1.379(8)
C53-H531	0.999(7)	C14-H141	1.000(6)
C53-H532	1.000(6)	C15-H151	1.000(6)
C53-H533	1.000(6)	C16-H161	1.001(7)
C54-H541	1.001(7)	C16-H162	1.000(8)
C54-H542	0.999(7)	C16-H163	0.998(8)
C54-H543	1.000(6)	C17-H171	1.000(6)
O6-C6	1.219(6)	C17-H172	1.000(8)

Table 2 cont.

Bond	Bond Lengths [Å]	Bond	Bond Lengths [Å]
C6-N7	1.365(6)	C17-H173	1.001(7)
N7-H7	1.07(8)	C18-H181	1.001(6)
N7-C8	1.430(7)	C18-H182	0.999(8)
C8-C81	1.513(7)	C18-H183	1.001(9)
C8-C9	1.547(7)		

Table 3 Bond Angles (°) for **(2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a** synthesised chemically.

Angle	Angle Size (°)	Angle	Angle Size (°)
O2-C1-C16	109.9(5)	C81-C8-H81	109.1(5)
O2-C1-C17	110.4(5)	C9-C8-H81	106.8(5)
C16-C1-C17	112.4(6)	C8-C81-O81	124.7(7)
O2-C1-C18	101.7(4)	C8-C81-O82	111.3(5)
C16-C1-C18	110.6(6)	O81-C81-O82	124.1(6)
C17-C1-C18	111.2(6)	C81-O82-C83	115.3(5)
C1-O2-C3	120.4(4)	O82-C83-H831	109.4(5)
O2-C3-O3	125.0(5)	O82-C83-H832	109.4(6)
O2-C3-C4	111.0(4)	H831-C83-H832	109.5(6)
O3-C3-C4	124.0(4)	O82-C83-H833	109.4(5)
C3-C4-C5	111.5(4)	H831-C83-H833	109.5(7)
C3-C4-H41	108.9(5)	H832-C83-H833	109.5(6)
C5-C4-H41	109.0(4)	C8-C9-C10	113.9(5)
C3-C4-H42	109.0(4)	C8-C9-H91	108.4(5)
C5-C4-H42	109.0(5)	C10-C9-H91	108.3(5)
H41-C4-H42	109.4(5)	C8-C9-H92	108.3(5)
C4-C5-C51	112.1(4)	C10-C9-H92	108.3(5)
C4-C5-C6	110.0(4)	H91-C9-H92	109.5(5)
C51-C5-C6	107.5(5)	C9-C10-C11	121.0(5)
C4-C5-H51	106.7(5)	C9-C10-C15	120.5(5)
C51-C5-H51	109.1(4)	C11-C10-C15	118.4(5)
C6-C5-H51	111.5(5)	C10-C11-C12	122.4(6)
C5-C51-C52	116.1(5)	C10-C11-H111	118.8(6)
C5-C51-H511	107.8(5)	C12-C11-H111	118.8(6)
C52-C51-H511	107.8(5)	C11-C12-C13	119.5(7)
C5-C51-H512	107.8(5)	C11-C12-H121	120.3(6)

Table 3 cont.

Angle	Angle Size (°)	Angle	Angle Size (°)
C52-C51-H512	107.8(5)	C13-C12-H121	120.1(7)
H511-C51-H512	109.5(5)	C12-C13-C14	118.4(6)
C51-C52-C53	112.3(4)	C12-C13-H131	120.8(8)
C51-C52-C54	109.8(5)	C14-C13-H131	120.8(7)
C53-C52-C54	110.2(4)	C13-C14-C15	121.5(6)
C51-C52-H521	107.6(5)	C13-C14-H141	119.3(7)
C53-C52-H521	107.2(6)	C15-C14-H141	119.2(7)
C54-C52-H521	109.6(4)	C10-C15-C14	119.7(6)
C52-C53-H531	109.5(6)	C10-C15-H151	120.2(5)
C52-C53-H532	109.4(5)	C14-C15-H151	120.1(6)
H531-C53-H532	109.6(7)	C1-C16-H161	109.4(7)
C52-C53-H533	109.4(6)	C1-C16-H162	109.4(7)
H531-C53-H533	109.5(6)	H161-C16-H162	109.4(7)
H532-C53-H533	109.5(6)	C1-C16-H163	109.5(6)
C52-C54-H541	109.4(6)	H161-C16-H163	109.6(8)
C52-C54-H542	109.5(5)	H162-C16-H163	109.6(7)
H541-C54-H542	109.5(5)	C1-C17-H171	109.5(6)
C52-C54-H543	109.5(5)	C1-C17-H172	109.5(6)
H541-C54-H543	109.4(5)	H171-C17-H172	109.5(7)
H542-C54-H543	109.6(7)	C1-C17-H173	109.5(6)
C5-C6-O6	123.0(5)	H171-C17-H173	109.4(7)
C5-C6-N7	115.3(5)	H172-C17-H173	109.4(7)
O6-C6-N7	121.7(5)	C1-C18-H181	109.5(5)
C6-N7-H7	116.1(43)	C1-C18-H182	109.6(6)
C6-N7-C8	120.8(5)	H181-C18-H182	109.5(8)
H7-N7-C8	122.6(42)	C1-C18-H183	109.4(7)

Table 3 cont.

Angle	Angle Size (°)	Angle	Angle Size (°)
N7-C8-C81	111.1(5)	H181-C18-H183	109.3(7)
N7-C8-C9	113.3(5)	H182-C18-H183	109.5(6)
C81-C8-C9	110.5(4)		
N7-C8-H81	105.8(5)		

Symmetry transformations used to generate equivalent atoms.

Table 4 Anisotropic displacement parameters (A^2 x 10^3) for **(2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a** synthesised chemically.

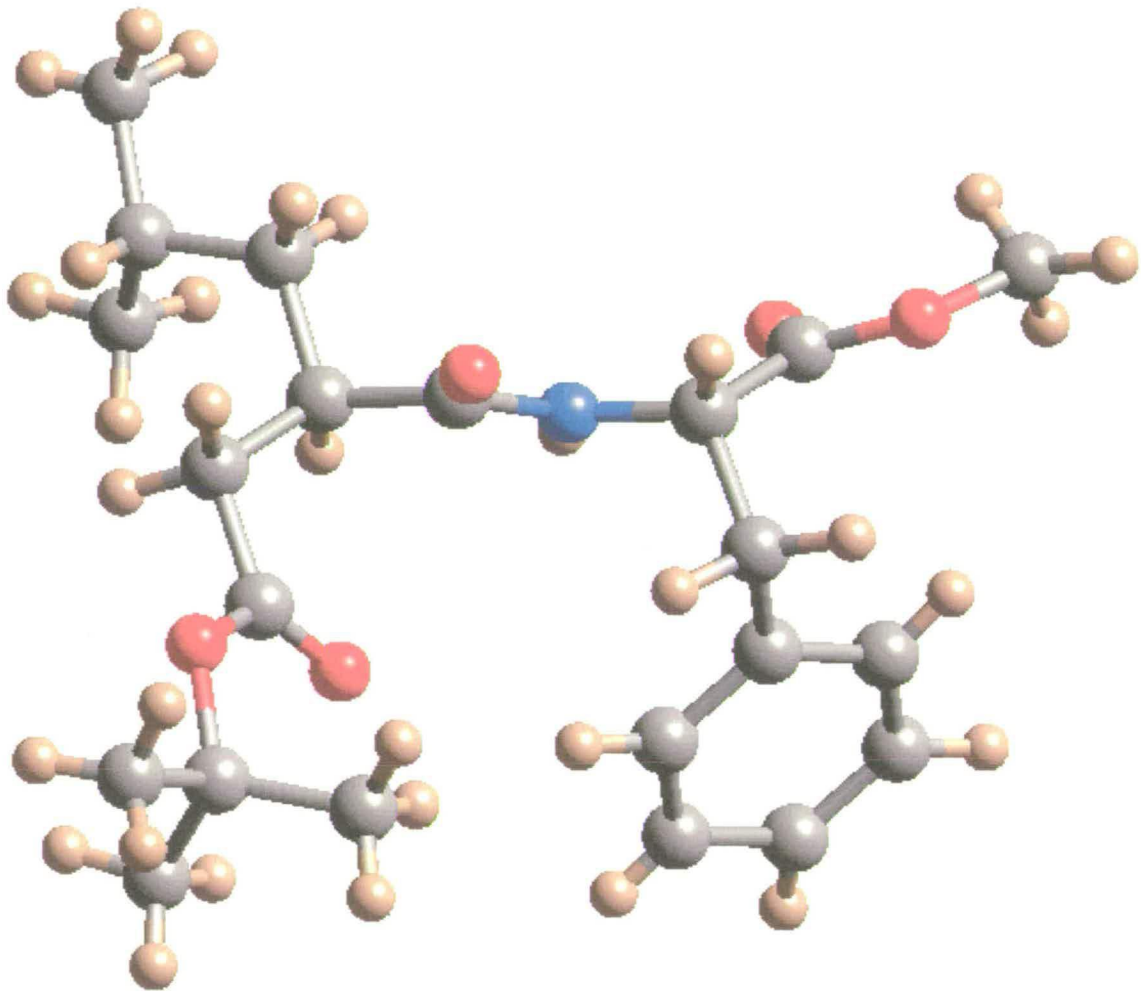
The anisotropic displacement factor exponent takes the form: $-2\pi^2$ [h^2 a^{*2} U11 + ... + 2 h k a* b* U12]

Atom	U11	U22	U33	U23	U13	U12
C1	24(3)	39(5)	26(3)	-1(4)	0(2)	-4(4)
O2	22(2)	39(3)	22(2)	-8(2)	3(2)	4(2)
O3	23(2)	55(4)	31(2)	-4(2)	10(2)	-6(2)
C3	20(3)	26(4)	36(4)	8(4)	4(3)	-6(3)
C4	24(3)	13(4)	22(3)	-7(3)	3(2)	-1(3)
C5	21(3)	17(4)	26(3)	0(3)	7(2)	4(3)
C51	20(3)	26(5)	30(3)	-15(3)	7(3)	-4(3)
C52	20(3)	22(4)	22(3)	7(3)	7(2)	5(3)
C53	41(4)	39(5)	39(4)	3(4)	0(3)	-8(4)
C54	44(4)	43(5)	31(4)	7(4)	7(3)	3(4)
O6	21(2)	28(3)	53(3)	15(3)	11(2)	5(2)
C6	26(3)	15(4)	25(4)	-1(3)	8(3)	-1(3)
N7	23(3)	29(4)	27(3)	12(3)	13(2)	6(3)
C8	18(3)	34(5)	27(3)	-5(3)	11(2)	1(3)
O81	27(2)	44(3)	39(3)	4(2)	2(2)	-2(2)
C81	25(3)	32(5)	20(3)	-3(3)	3(3)	-7(3)
O82	29(2)	18(3)	52(3)	12(2)	3(2)	-8(2)
C83	27(3)	36(5)	48(4)	8(4)	0(3)	-15(4)
C9	25(3)	21(4)	31(4)	-1(3)	4(3)	0(3)
C10	28(3)	24(5)	11(3)	-4(3)	7(2)	6(3)
C11	31(3)	27(5)	26(4)	-6(3)	7(3)	5(3)

Table 4 cont.

Atom	U11	U22	U33	U23	U13	U12
C12	46(4)	51(6)	20(4)	-1(4)	8(3)	0(4)
C13	39(4)	63(6)	24(4)	-5(4)	6(3)	14(4)
C14	21(3)	61(6)	33(4)	0(4)	9(3)	6(4)
C15	33(3)	27(4)	27(3)	-13(3)	6(2)	-4(3)
C16	89(6)	52(7)	41(5)	15(5)	-8(4)	-8(5)
C17	65(5)	41(6)	43(5)	-19(4)	-8(4)	13(4)
C18	32(3)	115(8)	32(4)	-12(5)	-6(3)	1(5)

6.1.0. Appendix II



X-ray crystal structure for (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a synthesised enzymatically

Crystal data and structure refinement for (2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a synthesised enzymatically

A. Crystal data

Empirical formula	$C_{22}H_{33}NO_5$	
Formula weight	391.49	
Wavelength	1.54178 Å	
Temperature	220(2) K	
Crystal system	Monoclinic	
Space group	P 2(1)	
Unit cell dimensions	a = 12.453(3) Å	α = 90°
	b = 6.2652(13) Å	β = 104.06(2)°
	c = 14.866(3) Å	γ = 90°
Volume	1125.1(4) $Å^3$	
Number of reflections for cell	43 ($20 < \theta < 22°$)	
Z	2	
Density (calculated)	1.156 Mg/m^3	
Absorption coefficient	0.656 mm^{-1}	
F(000)	424	

B. Data collection

Crystal description	Colourless rod
Crystal size	0.86 x 0.08 x 0.08 mm
θ range for data collection	3.06 to 60.03°
Index ranges	$-13 \leq h \leq 13, 0 \leq k \leq 6, 0 \leq l \leq 16$
Reflections collected	2216
Independent reflections	1813 [R(int) = 0.0521]
Scan type	Omega-theta
Absorption correction	Psi-scans (T_{min}= 0.555, T_{max}=0.782)

C. Solution and refinement

Solution	Direct (SHELXS-97 (Sheldrick, 1990))
Refinement type	Full-matrix least-squares on F^2
Program used for refinement	SHELXL-97
Hydrogen atom placement	difmap and geometric
Hydrogen atom treatment	mixed
Data / restraints / parameters	1813/1/368
Goodness-of-fit on F^2	1.014
Conventional R [F>4σ(F)]	R1 = 0.0433 [1393 data]
Weighted R (F^2 and all data)	wR2 = 0.1144
Absolute structure parameter	0.2(5)
Extinction coefficient	0.0048(9)
Final maximum δ/σ	0.187
Weighting scheme	calc w=1/[\s^2^(Fo^2^)+(0.0672P)^2^+0.0250P] where P=(Fo^2^+2Fc^2^)/3
Largest diff. peak and hole	0.155 and -0.157 e. $Å^{-3}$

Table 1 Atomic co-ordinates (x 10^4), equivalent isotropic displacement parameters (Å^2 x 10^3) and site occupancies for **(2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a** synthesised enzymatically. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

Atom	x	y	z	U(eq)	Occ
C(1)	8708(4)	3940(10)	1281(3)	55(1)	1
C(16)	9059(12)	2230(20)	717(8)	99(4)	0.80
C(17)	9090(9)	6117(18)	1056(7)	71(3)	0.80
C(18)	7470(20)	3940(40)	1183(19)	111(9)	0.80
C(16')	8710(50)	1760(90)	840(40)	77(17)	0.20
C(17')	9310(40)	5590(60)	830(30)	50(10)	0.20
C(18')	7530(70)	4260(100)	1220(60)	49(13)	0.20
O(2)	9146(2)	3593(6)	2286(2)	51(1)	1
C(3)	10227(3)	3447(9)	2656(3)	46(1)	1
O(3)	10937(2)	3486(8)	2234(2)	68(1)	1
C(4)	10464(4)	3258(9)	3703(3)	44(1)	1
C(5)	11587(3)	2221(8)	4112(3)	41(1)	1
C(51)	11815(4)	1926(9)	5171(3)	44(1)	1
C(52)	11739(4)	3891(9)	5745(3)	46(1)	1
C(53)	12487(6)	5683(12)	5572(5)	68(2)	1
C(54)	11989(6)	3330(14)	6761(4)	69(2)	1
C(6)	11648(3)	79(8)	3676(3)	43(1)	1
O(6)	10862(3)	-1148(6)	3488(3)	63(1)	1
N(7)	12649(4)	-466(7)	3522(3)	45(1)	1
C(8)	12808(4)	-2470(9)	3114(3)	44(1)	1
C(81)	13969(4)	-3277(9)	47(1)	3532(3)	1
O(81)	14714(3)	-2226(6)	3979(2)	62(1)	1
O(82)	14049(3)	-5329(6)	3322(2)	58(1)	1
C(83)	15138(5)	-6289(14)	3619(5)	70(2)	1

Table 1 cont.

Atom	x	y	z	U(eq)	Occ
C(9)	12565(4)	-2392(10)	2040(3)	48(1)	1
C(10)	13318(4)	-926(9)	1668(3)	47(1)	1
C(11)	13012(4)	1127(10)	1398(4)	53(1)	1
C(12)	13670(5)	2448(11)	1025(4)	61(2)	1
C(13)	14705(4)	1710(12)	953(4)	63(2)	1
C(14)	15035(4)	-303(12)	1230(4)	66(2)	1
C(15)	14350(4)	-1646(10)	1581(4)	54(1)	1

Table 2 Bond lengths [Å] for **(2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a** synthesised enzymatically.

Bond	Bond Length [Å]	Bond	Bond Length [Å]
C(1)-O(2)	1.477(5)	C(6)-O(6)	1.223(6)
C(1)-C(16)	1.492(14)	C(6)-N(7)	1.364(6)
C(1)-C(18')	1.47(8)	N(7)-C(8)	1.429(7)
C(1)-C(18)	1.51(2)	C(8)-C(81)	1.516(7)
C(1)-C(17')	1.52(4)	C(8)-C(9)	1.552(6)
C(1)-C(17)	1.509(11)	1.552(6)	1.198(6)
C(1)-C(16')	1.52(6)	C(81)-O(82)	1.332(6)
O(2)-C(3)	1.328(5)	O(82)-C(83)	1.451(6)
C(3)-O(3)	1.202(5)	C(9)-C(10)	1.510(7)
C(3)-C(4)	1.517(6)	C(10)-C(11)	1.373(8)
C(4)-C(5)	1.528(6)	C(10)-C(15)	1.396(7)
C(5)-C(6)	1.501(7)	C(11)-C(12)	1.373(8)
C(5)-C(51)	1.541(7)	C(12)-C(13)	1.399(8)
C(51)-C(52)	1.514(7)	C(13)-C(14)	1.359(9)
C(52)-C(54)	1.508(7)	C(14)-C(15)	1.388(8)
C(52)-C(53)	1.520(8)		

Table 3 Bond Angles[Å] for **(2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a** synthesised enzymatically.

Angle	Angle Size (°)	Angle	Angle Size (°)
O(2)-C(1)-C(16)	112.1(6)	C(6)-C(5)-C(51)	108.5(4)
O(2)-C(1)-C(18')	101(4)	C(4)-C(5)-C(51)	112.4(4)
C(16)-C(1)-C(18')	120(3)	C(52)-C(51)-C(5)	117.2(4)
O(2)-C(1)-C(18)	102.5(11)	C(54)-C(52)-C(51)	110.4(5)
C(16)-C(1)-C(18)	112.2(12)	C(54)-C(52)-C(53)	110.8(5)
C(18')-C(1)-C(18)	8(3)	C(51)-C(52)-C(53)	112.4(4)
O(2)-C(1)-C(17')	116.3(17)	O(6)-C(6)-N(7)	121.2(5)
C(16)-C(1)-C(17')	90.0(13)	O(6)-C(6)-C(5)	122.6(4)
C(18')-C(1)-C(17')	118(3)	N(7)-C(6)-C(5)	116.2(4)
C(18)-C(1)-C(17')	124(2)	C(6)-N(7)-C(8)	121.2(4)
O(2)-C(1)-C(17)	107.4(5)	N(7)-C(8)-C(81)	109.6(4)
C(16)-C(1)-C(17)	112.0(7)	N(7)-C(8)-C(9)	113.4(4)
C(18')-C(1)-C(17))	103(3	C(81)-C(8)-C(9)	111.1(4)
C(18)-C(1)-C(17)	110.3(11)	O(81)-C(81)-O(82)	124.7(5)
C(17')-C(1)-C(17)	22.1(13)	O(81)-C(81)-C(8)	125.2(5)
O(2)-C(1)-C(16')	105(2)	O(82)-C(81)-C(8)	110.1(4)
C(16)-C(1)-C(16')	23(2)	C(81)-O(82)-C(83)	116.5(5)
C(18')-C(1)-C(16')	102(3)	C(10)-C(9)-C(8)	114.4(4)
C(18)-C(1)-C(16')	94(2)	C(11)-C(10)-C(15)	118.2(5)
C(17')-C(1)-C(16')	112(2)	C(11)-C(10)-C(9)	121.6(4)
C(17)-C(1)-C(16')	133(2)	C(15)-C(10)-C(9)	120.3(5)
C(3)-O(2)-C(1)	121.2(3)	C(12)-C(11)-C(10)	122.1(5)
O(3)-C(3)-O(2)	125.6(4)	C(11)-C(12)-C(13)	118.9(7)
O(3)-C(3)-C(4)	123.6(4)	C(14)-C(13)-C(12)	120.0(6)
O(2)-C(3)-C(4)	110.8(4)	C(13)-C(14)-C(15)	120.6(5)
C(3)-C(4)-C(5)	111.9(4)	C(14)-C(15)-C(10)	120.2(6)
C(6)-C(5)-C(4)	110.2(4)		

Symmetry transformations used to generate equivalent atoms

Table 4 Anisotropic displacement parameters (A^2 x 10^3) for **(2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a** synthesised enzymatically.

The anisotropic displacement factor exponent takes the form: $-2\pi^2$ [h^2 a^{*2} U11 + ... + 2 h k a* b* U12]

Atom	U11	U22	U33	U23	U13	U12
C(1)	47(3)	77(4)	36(3)	0(3)	2(2)	-8(3)
C(16)	122(11)	108(10)	55(7)	-22(7)	-1(7)	-2(9)
C(17)	78(7)	85(7)	45(6)	15(5)	10(5)	-2(6)
C(18)	47(5)	210(20)	65(7)	33(12)	-11(4)	-7(10)
O(2)	35(2)	76(2)	39(2)	9(2)	4(1)	-2(2)
C(3)	34(2)	54(3)	49(3)	5(3)	7(2)	5(2)
O(3)	43(2)	120(4)	46(2)	13(2)	19(1)	9(2)
C(4)	41(2)	56(3)	35(2)	6(2)	11(2)	2(2)
C(54)	80(4)	92(5)	35(3)	-13(4)	12(3)	-4(4)
C(6)	36(2)	51(3)	44(3)	4(2)	14(2)	1(2)
O(6)	41(2)	66(2)	88(3)	-16(2)	26(2)	-9(2)
N(7)	38(2)	51(3)	48(3)	-6(2)	14(2)	1(2)
C(8)	41(3)	52(3)	41(3)	1(2)	11(2)	-1(2)
C(81)	42(3)	57(3)	41(3)	-2(3)	11(2)	-2(2)
O(81)	44(2)	76(3)	62(2)	-13(2)	7(2)	-2(2)
O(82)	44(2)	65(3)	61(2)	-8(2)	8(2)	6(2)
C(83)	49(3)	80(5)	77(5)	1(4)	9(3)	24(3)
C(9)	39(3)	67(4)	39(3)	-1(3)	11(2)	-3(2)
C(10)	41(2)	63(4)	37(3)	-5(2)	11(2)	-8(2)
C(11)	48(3)	64(4)	48(3)	-6(3)	18(2)	-1(3)
C(12)	70(4)	65(4)	47(3)	0(3)	15(3)	-5(3)
C(13)	57(3)	87(5)	49(3)	3(3)	21(3)	-20(3)
C(14)	48(3)	93(5)	60(3)	2(4)	21(3)	-7(3)
C(15)	45(3)	64(4)	55(3)	5(3)	16(2)	0(3)

Table 5 Hydrogen co-ordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for **(2*R*,2'*S*)-[4-(*tert*-butyl)-2-*iso*-butyl-succinyl]-phenylalanine methyl ester 127a** synthesised enzymatically.

Atom	x	y	z	U(eq)
H(161)	8813	852	892	148
H(162)	9859	2227	829	148
H(163)	8733	2484	65	148
H(171)	9884	6092	1120	106
H(172)	8914	7163	1479	106
H(173)	8719	6495	424	106
H(181)	7277	5107	1543	167
H(182)	7251	2598	1406	167
H(183)	7094	4126	535	167
H(164)	8430	1878	173	116
H(165)	8239	794	1084	116
H(166)	9458	1200	978	116
H(174)	8871	5909	212	74
H(175)	10023	5031	796	74
H(176)	9414	6886	1201	74
H(184)	7400	5737	1369	73
H(185)	7295	3329	1660	73
H(186)	7102	3940	599	73
H(42)	9800(40)	2140(80)	3850(30)	49(13)
H(41)	10420(30)	4900(80)	3870(30)	30(11)
H(5)	12150(30)	3280(70)	4000(30)	35(11)
H(511)	11360(40)	790(80)	5260(30)	42(13)
H(512)	12600(50)	1430(90)	5430(40)	63(16)
H(521)	11000(30)	4230(70)	5530(30)	39(12)
H(531)	12260(60)	6340(140)	4920(50)	110(30)
H(532)	12500(50)	6950(110)	6040(40)	80(18)
H(533)	13250(50)	5160(100)	5690(40)	75(18)

Table 5 cont.

Atom	x	y	z	U(eq)
H(541)	11550(50)	1980(120)	6880(40)	100(20)
H(542)	12820(50)	2670(90)	6990(40)	72(17)
H(543)	11950(40)	4370(100)	7120(40)	62(18)
H(71)	13080(60)	260(120)	3580(50)	110(30)
H(81)	12320(40)	-3520(80)	3240(30)	48(14)
H(831)	7840(140)	-7840(140)	3320(50)	110(30)
H(832)	15380(50)	-5840(120)	4270(50)	90(20)
H(833)	15660(50)	-5550(120)	3400(50)	100(20)
H(91)	11770(30)	-1630(70)	1810(30)	30(10)
H(92)	12580(40)	-4010(90)	1780(30)	45(13)
H(111)	12290(40)	1770(80)	1420(30)	45(13)
H(121)	13470(40)	3720(110)	840(40)	60(18)
H(131)	15100(40)	2880(80)	720(30)	52(14)
H(141)	15750(40)	-960(90)	1170(30)	64(15)
H(151)	14530(30)	-3010(80)	1770(30)	29(11)

6.2.0. Appendix III

Publications

Enhancement of *Candida antarctica* lipase B enantioselectivity and activity in organic solvents

Marie-Claire Parker,*[a]† Stuart A. Brown,[b] Lindsey Robertson[b] and Nicholas J. Turner[b]

[a] *Department of Chemistry, Joseph Black Building, University of Glasgow, Glasgow, UK G12 8QQ*
[b] *Edinburgh Centre for Protein Technology, Department of Chemistry, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh, UK EH9 3JJ*

The enantioselectivity and catalytic activity of Novozym 435® [*Candida antarctica* lipase B (CALB)] in organic solvents was found to dramatically increase upon the addition of a non-reactive organic base, such as Et_3N, to the reaction system.

It has been shown that the unusual microenvironment of enzymes in organic solvents can affect a number of parameters, including the degree of protein hydration,[1,2] secondary structure,[3] the susceptibility of the protein to inactivation and variations in the ionisation state[4] of side-chain residues. Frequently, these differences have been shown to result in interesting changes in the enzymes, including reversal of substrate specificity and changes in stereoselectivity, although the underlying reasons remain poorly understood.

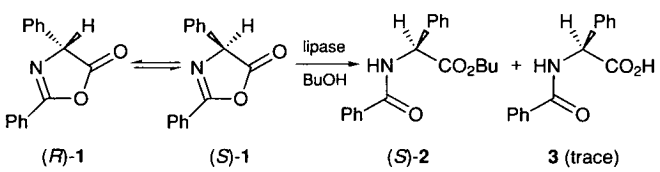
It is commonly accepted that the best predictor of enzyme catalytic activity in low water organic media is thermodynamic water activity (a_w).[1]‡ Over the past few years although much has been reported on enzyme enantioselectivity in organic media there are as yet no predictive rules available. Crude lipase preparations have proved to be simple and effective biocatalysts for kinetic resolutions, *e.g.* chiral carboxylic acids and alcohols. However, the low purity of these preparations (presence of other lipases and competing hydrolases) can, in specific reactions, lead to low and unpredictable enantioselective behaviour. This effect can be compounded when using organic solvents, due to the effect of different solvent properties on catalytic activity.

The starting point for the work described herein was the lipase (Lipozyme® *Mucor miehei*) catalysed dynamic resolution of 4-substituted oxazol-5(4*H*)-ones, a reaction we have previously employed for the synthesis of enantiomerically pure (*S*)-L-*tert*-leucine.[5] It was previously found that the modest enantioselectivity in toluene (*ca.* 68% ee) could be enhanced (*ca.* 97% ee) by the addition of a catalytic amount of Et_3N to the reaction; the role of Et_3N is not to facilitate racemisation of the substrate.

We decided to investigate this effect in more detail by using a commercially available immobilised lipase,§ Novozym 435 (*Candida antarctica* lipase B[6] (CALB), since a larger substrate range could be tested with this enzyme. The catalytic activity and enantioselectivity of the alcoholysis of (±)-2-phenyl-4-benzyloxazol-5(4*H*)-one **1** using butan-1-ol as the nucleophile (Scheme 1) was monitored¶ under a range of reaction conditions, including controlled water activity. Hydration was controlled by equilibrating‖ enzyme and solvent with the appropriate saturated salt solution[7] of known thermodynamic water activity a_w. Therefore a low a_w system will be one in which the solvent is poorly hydrated and the enzyme, similarly, has a low level of hydration, and at high a_w (*e.g.* 0.97) the solvent is near water saturation and the enzyme is fully hydrated (as would be found in an aqueous system). Table 1 shows the effect of hydration on the initial catalytic rate and enantioselectivity, in three different solvents, n-hexane, toluene and MeCN, either with or without Et_3N.**

It can immediately be seen that the lipase-catalysed reaction is very sensitive to water activity. The addition of a non-reactive organic base,†† Et_3N, to the reaction enhances significantly both the enantioselectivity and catalytic activity of the enzyme. Even low levels of hydration, present in the more nonpolar solvents such as n-hexane and toluene, are detrimental to the overall catalytic performance of CALB. We find that generally for optimum yield and enantioselectivity, both the enzyme and solvent should be rigorously dried prior to addition of Et_3N. We were interested to see if addition of Et_3N to a reaction already in progress and of poor enantioselectivity, could reverse this effect. As can be seen from Fig. 1, the addition of Et_3N after 140 min immediately results in enhanced catalytic rate and enantioselectivity.

In order to examine the generality of the effect of Et_3N we investigated a second reaction, namely the CALB-catalysed



Scheme 1

Table1 Effect of water activity on initial catalytic rate[a,b] and enantiospecificity as a function of hydration, with and without Et_3N

		No Et_3N		Et_3N	
Solvent[c]	a_w	Initial rate/nmol min^{-1} mg^{-1}	Ee (%)	Initial rate/nmol min^{-1} mg^{-1}	Ee (%)
n-hexane	~0 (anhydrous)	26 (± 1.5)	85 (± 3)	30 (± 1.5)	90 (± 3)
n-hexane	0.69	4 (± 0.5)	55 (± 2)	20 (± 1)	87 (± 3)
n-hexane	0.97	1.5 (± 0.15)	30 (± 5)	18 (± 0.9)	80 (± 5)
toluene	~0	15 (± 0.8)	85 (± 4)	27 (± 1.5)	93 (± 3)
toluene	0.22	3	61 (± 6)	17 (± 1)	95 (± 2)
MeCN[d]	~0	15	>99	10	97 (± 2)
MeCN[d]	0.1 (0.5% v/v H_2O)	NR[e]	—	5 (± 0.3)	90 (± 4)
MeCN[d]	0.4 (2% v/v H_2O)	NR[e]	—	NR[e]	—

[a] Initial rate for (*S*)-butyl ester enantiomer **2**. [b] Results reported are the average of three separate measurements. [c] Note ‖. [d] Ref. 8. [e] No reaction.

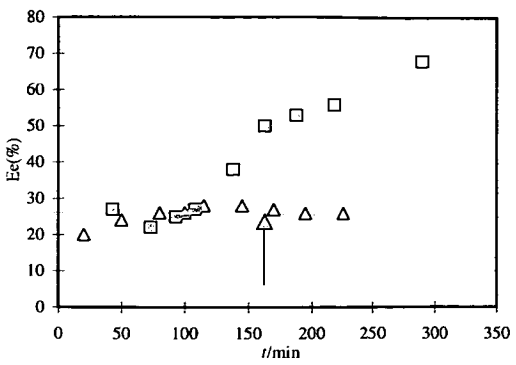


Fig. 1 Effect of Et_3N on ee. Reactions A (△) and B (□) were carried out under identical conditions (a_w = 0.69). At t = 140 min, 14 mol% Et_3N was added to reaction B (arrow).

reaction between 1-phenylacetoxy-2-methylcyclohexene and butanol yielding 2-methylcyclohexanone and butyl phenylacetate.[9,15] Using n-hexane (a_w = 0) and MeCN (0.5% H_2O, a_w = 0.1) as the solvents, we observed that the addition of Et_3N to the solvent resulted in a dramatic increase in the catalytic activity. An approximate 200-fold increase in activity was observed in MeCN (a_w = 0.1) and a 700-fold one for that in n-hexane (a_w = 0.97). The higher activity found in n-hexane is presumably due to a more intimate contact between the enzyme and Et_3N in a more nonpolar environment. Similarly, the activation effect for (±)-2-phenyl-4-benzyloxazol-5(4*H*)-one ring-opening in MeCN is similar to that described above and is expected to be a result of less Et_3N adsorption to the enzyme in MeCN.

The ability of organic bases to increase the enantioselectivity of lipase-catalysed reactions in water-saturated organic solvents has previously been reported.[10–13] In some cases[11,12] this effect has been attributed to the formation of an ion-pair between the base and any by-product acid. Using electrospray ionisation mass spectrometry (ESI-MS)‡‡ we have detected the formation of carboxylic acid **3** during the course of the oxazolone reaction at intermediate to high water activities (*e.g.* a_w = 0.69–0.97). We have also found that addition of acid **3** to an already hydrated system results in loss of activity, which can be fully recovered upon addition of an organic base, presumably *via* formation of an ion pair. Ion pair formation is observed in both low and high dielectric non-hydrogen bonding solvents such as n-hexane and MeCN. In a high dielectric, non-hydrogen bonding solvent such as MeCN, where the acid was found to be more soluble, we find experimentally that dissolution of acid **3** in n-hexane and MeCN occurs upon addition of Et_3N, thus removing acid from the immediate microenvironment of the enzyme. However, the enhancement of catalytic performance and enantioselectivity for rigorously dried samples, and those of low water activity (a_w < 0.7) where we find no evidence for hydrolysis over the course of the initial rate measurement, cannot be explained in terms of hydrolysis products affecting enantioselectivity, since for an unrelated substrate, an activating effect on the catalytic activity has been demonstrated.

The addition of co-solvents, such as DMF and DMSO, was found to solubilise the acid and thus it was anticipated that they would perform a similar role to Et_3N in removing any acid from the immediate vicinity of the enzyme. Both DMF and DMSO were chosen as additives to the bulk organic solvent (toluene at a_w = 0.22). Although both DMF and DMSO increased the enantioselectivity of the reaction to 85% ee, there was no significant effect on the catalytic rate as found with Et_3N. Since the solvation of the carboxylic acid by these co-solvents occurs by a different mechanism to that of Et_3N, *i.e.* the additives are unable to form ion-pairs, they have limited use in reducing the overall effect.

The role of Et_3N therefore appears to be dual in nature, *i.e.* increasing both the enantioselectivity and catalytic activity of lipase-catalysed reactions. The addition of Et_3N therefore provides an additional strategy for improving the enantioselectivity of lipase-catalysed reactions. We are currently investigating this effect with other lipolytic enzymes.

We are grateful to Boehringer Mannheim, Germany, for the generous gift of lipase samples. The BBSRC is acknowledged for a David Phillips Fellowship (M. C. P.) and a studentship (S. A. B.).

Notes and References

† E-mail: m.parker@chem.gla.ac.uk

‡ The thermodynamic water activity (a_w) describes the mass action effect of water on hydrolytic equilibria and also describes the partitioning of various water phases that can compete for water binding (ref. 1).

§ Polyacrylamide gel electrophoresis of CALB desorbed from the solid support exhibited a single band corresponding to the reported molecular weight of CALB (33 KDa) (ref. 6).

¶ (±)-2-Phenyl-4-benzyloxazol-5(4*H*)-one **1** (0.16 mmol) was placed in a 4 ml screw top vial together with the solvent, (either anhydrous or hydrated), butan-1-ol (0.24 mmol, 1.5 equiv.) CALB (40 mg) and Et_3N (14 mol%). The reaction vial was shaken at 250 rpm on a rotary shaker at 37 °C and the progress and ee (%) of the reaction were monitored by chiral HPLC (Chiralcel-OD, 250 × 4.6 mm, Mallinckrodt Baker, n-hexane–Pr^iOH (90:10 v/v), UV detection λ = 254 nm).

‖ *Candida antarctica* lipase B (CALB) was received as an immobilised preparation (Novozym 435, Boehringer Mannheim, Germany) and was dehydrated over P_2O_5 (at room temp.) for 2–3 days. Rehydration of dried lipase to the desired water activity (a_w) was carried out using saturated salt solutions (equilibration period 48–72 h). (±)-2-Phenyl-4-benzyloxazol-5(4*H*)-one **1** was stored over P_2O_5 at 0 °C; anhydrous solvents were stored over freshly reactivated 3 Å or 4 Å molecular sieves. The water content of dried solvents was measured using Karl Fischer water titration (ref. 15) and found to be <0.001 wt%. Solvents were hydrated separately from the enzyme using the same water equilibration procedure as described above, approximately 24 h before use.

** Control reactions showed that no detectable ester (as judged by HPLC) was formed in the absence of enzyme, either with or without Et_3N, over a 48 h analysis period.

†† Other organic bases give very similar results to Et_3N, *e.g.* DABCO and lutidine. Insoluble inorganic bases, *e.g.* $KHCO_3$ and K_2CO_3, had no effect and did not result in the high catalytic rate and enantioselectivity observed with the soluble organic bases.

‡‡ Electrospray ionisation mass spectrometry (ESI-MS) and atmospheric chemical ionisation (APCI) were performed on a Micromass Platform II spectrometer (cone voltage 20 V).

1 P. J. Halling, *Enzyme Microb. Technol.*, 1994, **16**, 178.
2 M. C. Parker, A. J. Blacker and B. D. Moore, *Biotechnol. Bioeng.*, 1995, **46**, 452.
3 D. S. Hartsough and K. M. Merz, *J. Am. Chem. Soc.*, 1993, **115**, 6529.
4 E. Zacharis, B. D. Moore and P. J. Halling, *J. Am. Chem. Soc.*, 1997, **119** 12 396.
5 N. J. Turner, J. R. Winterman, R. McCague, J. S. Parratt and S. J. C. Taylor, *Tetrahedron. Lett.*, 1995, **36**, 1113.
6 J. Uppenberg, N. Öhrner, M. Norin, K. Hult, G. J. Kleywegt, S. Patkar, V. Waagen, T. Anthonsen and T. Jones, *Biochemistry*, 1995, **34**, 16 838.
7 G. A. Hutcheon, P. J. Halling and B. D. Moore, in *Methods in Enzymology*: *Lipases Part B*, ed. B. Rubins and E.A. Dennis, Academic Press, NY, 1997, vol. 286, p. 465.
8 J. Gmehling and V. Onken, *Vapour-Liquid Equilibrium Data Collection*, Dechema, Frankfurt, 1977, vol. 1.
9 K. Matsumoto, T. Oishi, T. Nakata, T. Shibata and K. Ohta, *Biocatalysis*, 1994, **9**, 97.
10 B. Berger, C. G. Rabiller, K. Konigsberger, K. Faber and H. Griengl, *Tetrahedron*: *Asymmetry*, 1990, **1**, 541.
11 J. L. L. Rakels, A. J. J. Straathof and J. J. Heijnen, *Tetrahedron*: *Asymmetry*, 1994, **5**, 93.
12 T. Maugard, M. Remaud-Simeon, D. Petre and P. Monsan, *Tetrahedron*, 1997, **53**, 7629.
13 P. Stead, H. Marley, M. Mahmoudian, G. Webb, D. Noble, Y. T. Ip, E. Piga, T. Rossi, S. M. Roberts and M. J. Dawson, *Tetrahedron*: *Asymmetry*, 1996, **7**, 2247.
14 H. A. Laitennen and W. E. Harris, *Chemical Analysis*, 2nd edn, McGraw-Hill, 1975, p. 361.
15 A. J. Carnell, J. Barkley and A. Singh, *Tetrahedron Lett.*, 1997, **38**, 7781.

Received in Liverpool, UK, 10th August 1998; 8/06332J