SYNTHESIS OF FOUR STEREOISOMERS OF A NOVEL ANTIBACTERIAL AGENT

by

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Synthesis of four stereoisomers of a novel antibacterial agent

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Abstract

The novel compound 2,2-dimethyl-3-(2'-hydroxypropyl)-5-carboxy-1,4-thiazine (**1**) has been designed as a potential inhibitor of the penicillin receptor, and found to possess antibacterial activity.

All four stereoisomers of (**1**) were synthesized by a multistep route. Commercially available (S)-ethyl lactate was processed in two different ways to yield either (R)- or (S)-propylene oxide (**18a** and **18b**). The (R)- and (S)-5-methyl-4-hexen-2-ols (**4a** and **4b**) were then obtained by reaction of (R)- and (S)-propylene oxide with an organometallic reagent prepared from 1-bromo-2-methylpropene. Alternatively, kinetic resolution of racemic **4** with Porcine Pancreatic Lipase allowed the (R)-alcohol (**4a**) to be obtained with high enantiomeric excess (ee). The optically active alcohols **4a** and **4b** were protected with the t-butyldimethylsilyl group, oxidized with potassium permanganate under special conditions to the 5-t-butyldimethylsiloxy-2-hydroxy-2-methyl-3-hexanones **6a** and **6b**, and converted, via their mesylates, to the mercaptans **8a** and **8b**.

Condensation of the (R)- and (S)-mercaptans **8a** and **8b** with the (R)- and (S)-β-lactones **24a** and **24b**, prepared from (D)- and (L)-serine by the method of Vederas, produced the four stereoisomers **10a**, **10b**, **10c** and **10d**. Deprotection and cyclization yielded the four products **1a**, **1b**, **1c** and **1d**.

Bioassay of **1a**, **1b**, **1c** and **1d** revealed that the (5**S**,8**S**)- and (5**S**,8**R**)-2,2-dimethyl-3-(2'-hydroxypropyl)-5-carboxy-1,4-thiazines (**1a** and **1c**) inhibit the growth of S. *aureus*. The (5**R**,8**S**)- and (5**R**,8**R**)-2,2-dimethyl-3-(2'-hydroxypropyl)-5-carboxy-1,4-thiazines (**1b** and **1d**) are much less active.

Dedication

To my wife and my daughter

Acknowledgments

I wish to express my sincere gratitude to my supervisor Professor Saul Wolfe for his continual encouragement, financial support and subtle stimulation during the course of this study, especially his patience in guiding a foreign student with a big language problem.

The following people also deserve my gratitude: Dr. B. D. Johnston, who showed me how to undertake a multi-step organic synthesis properly; Dr. C. K. Kim and Dr. N. Weinberg, who helped me to understand the theoretical section; M. Tracey and Dr. A. Tracey, who provided excellent NMR spectra and assignments; M. K. Yang, who carried out the microanalyses and helped me to understand the HPLC apparatus; G. Owen, who obtained the mass spectra; and to many others who provided helpful advice. In addition, financial support from the Department of Chemistry is gratefully acknowledged.

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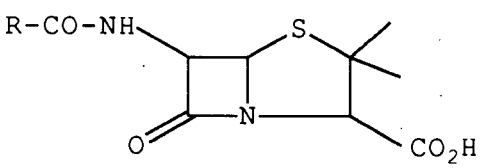
List of abbreviations

Boc	:	tert-butoxycarbonyl
Boc-ON	:	[2-(tert-Butoxycarbonyloxyimino)-2-phenylacetonitrile.
BMS	:	Borane dimethylsulfide
DBU	:	1,8-Diazabicyclo [5.4.0] undec-7-ene
DMAD	:	Dimethyl azodicarboxylate
LAH	:	Lithium aluminium hydride
LDA	:	Lithium diisopropylamide
MTPA	:	α-Methoxy-α-trifluoromethyl phenylacetyl
NBS	:	N-Bromosuccinimide
PBPs	:	Penicillin binding proteins
PPL	:	Porcine Pancreatic Lipase
TBDMS	:	tert-Butyldimethylsilyl group
TMS	:	Tetramethylsilane

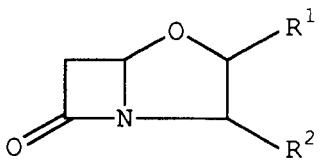
CHAPTER 1. INTRODUCTION

1.1 β - Lactam Antibiotics.

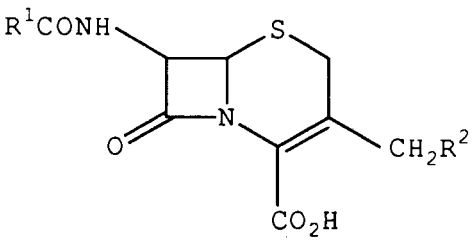
Penicillin was discovered by Alexander Fleming in 1929 (1), and the cephalosporins were characterized by Abraham and Newton in 1953 (2). These are the classical examples of β - lactam antibiotics. Subsequently, other β - lactam containing natural products were discovered, and the class currently includes: penicillins, cephalosporins, clavams (3), carbapenems (4,5), nocardicins (6) and monobactams (7). (Figure 1.1.)



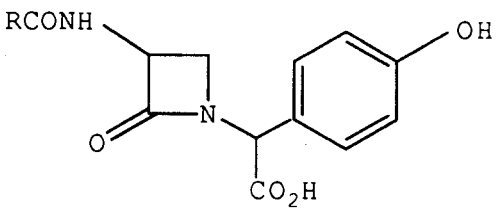
penicillin



clavam



cephalosporin



nocardicin

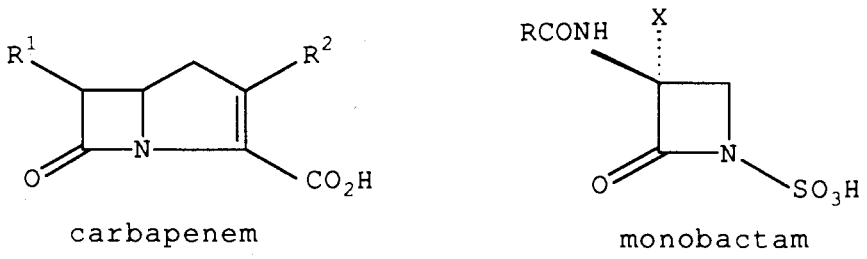
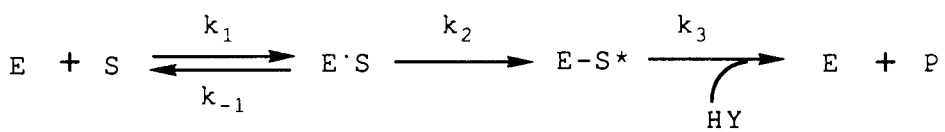


Figure 1.1. The β-lactam family of natural products

All of these compounds contain the β - lactam ring, a cyclic four - membered amide, and they function as antibacterial agents by inhibition of the final stages of bacterial cell wall biosynthesis (8).

1.2 Two Main Families of Enzymes.

There are two main families of enzymes which recognise β - lactam antibiotics. These are the penicillin binding proteins (PBPs) and the β - lactamases. The PBPs (9) are membrane - bound proteins with molecular weights from 40,000 - 91,000 daltons. Their active sites contain the sequence Ser - X - X - Lys (10), and the active site serine reacts chemically with the β - lactam carbonyl group (Equation 1.1) (Figure 1.2) (11).



Equation 1.1

In the first stage, the antibiotic (e.g penicillin) (S) "fits" reversibly (k_1,k_{-1}) to the serine enzyme (E), to form an enzyme-substrate complex (E·S). In the

second stage, the hydroxyl group of a serine residue, "reacts" (k_2) with the β -lactam group to form an acyl-enzyme (E·S*). In the final stage, the acyl-enzyme (E-OCOR) is hydrolysed to regenerate the enzyme and produce an inactive degradation product of the antibiotic.

The β-lactamases were discovered (12) almost as soon as the penicillin structure became known. Like the PBPs, most β - lactamases are active - site serine enzymes (13). Although both kinds of enzymes recognize and react with β - lactam compounds according to Equation 1.1 (14), their kinetic parameters differ in important ways (15) (Figure 1.2).

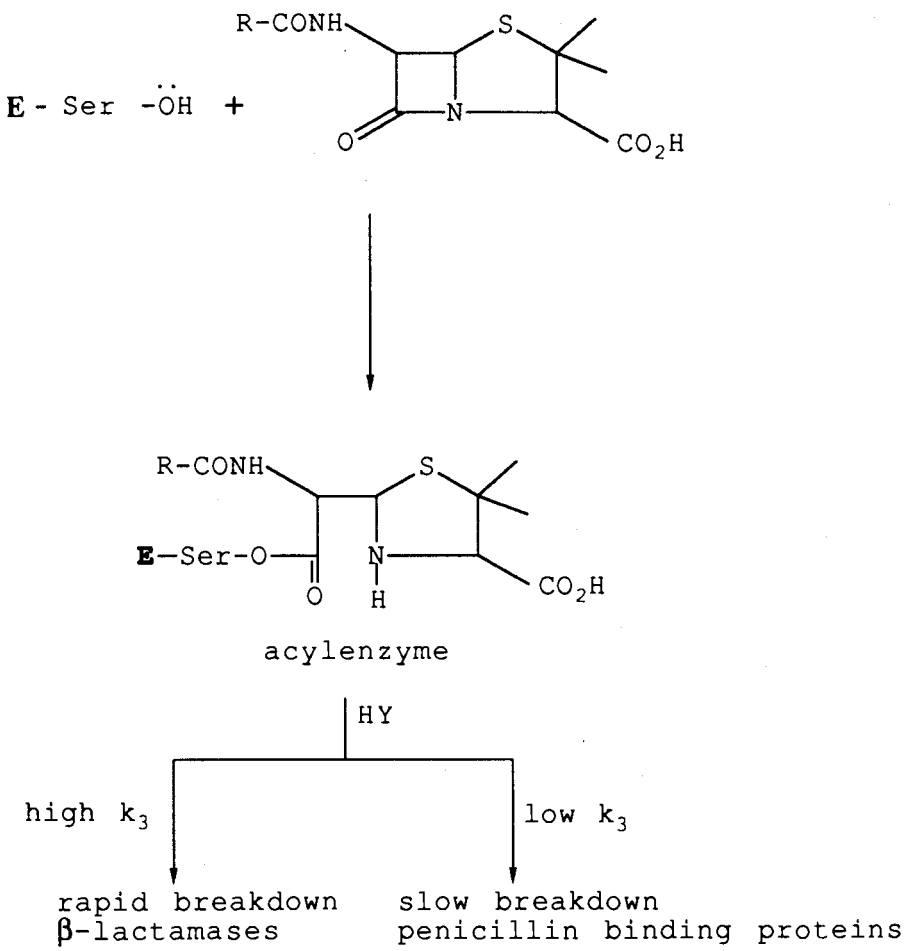


Figure 1.2. Reactions of penicillin with penicillin recognizing enzymes

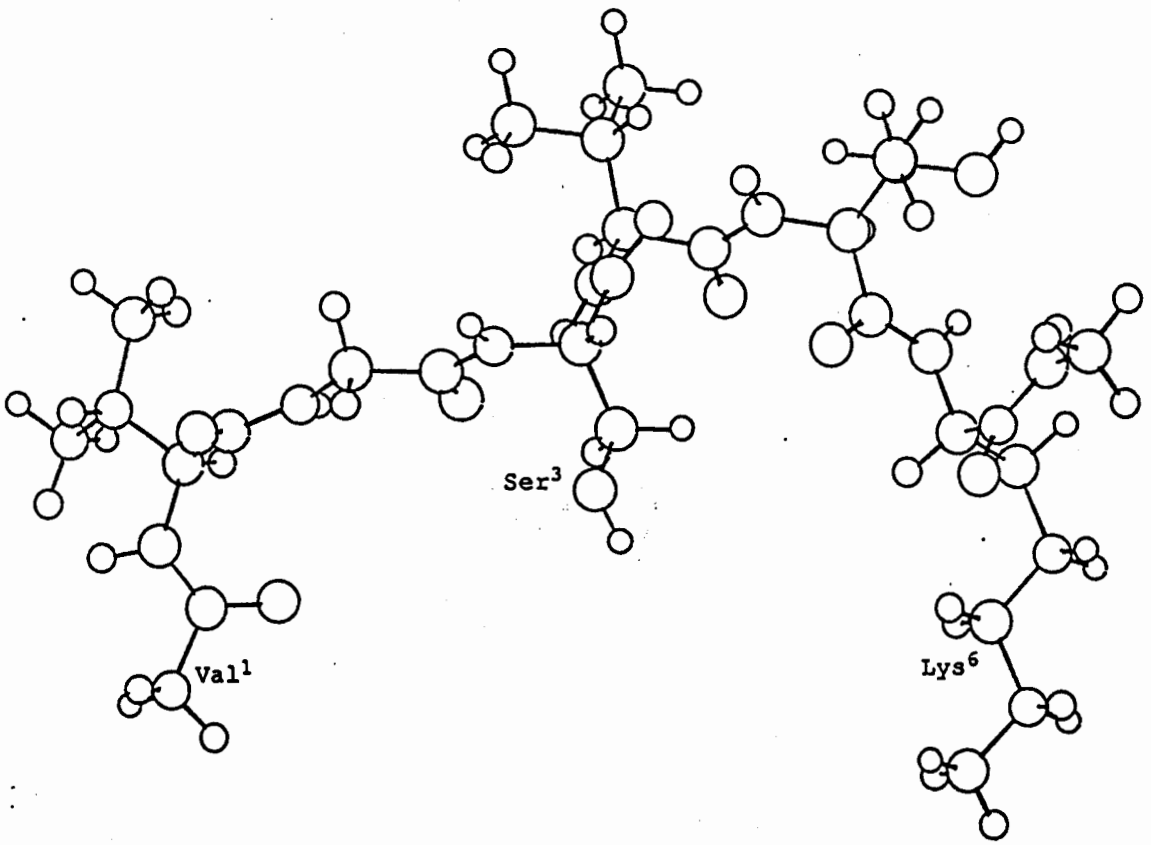
Table 1.1 shows how k_2 and k_3 determine whether the enzyme is inactivated (PBP) or the antibiotic is inactivated (β-lactamase) (16).

Table 1.1. Values of k_2 and k_3 for penicillin G

$k_2(s^{-1})$	$k_3(s^{-1})$	Enzyme
180	1.4×10^{-4}	PBP from *Streptomyces* R61
4090	3610	β-lactamase from *B. cereus*
173	96	β-lactamase from *S. aureus*
2800	1500	β-lactamase from *E. coli*

1.3 A Computer Model of The Penicillin Receptor

Wolfe and coworkers (17) analyzed 200,000 conformations of the hexapeptide **Ac-NH-Val^1-Gly^2-Ser^3-Val^4-Thr^5-Lys^6-NH-Me** (**P**), which contains the amino acids that surround the active site serine of the PBP of *Streptomyces* R61. This search led to the discovery of a low energy conformation in which the nitrogen atom of the terminal amino group of lysine is 8.5 Å from the serine hydroxyl oxygen (Figure 1.3).



(P)

Figure 1.3. The Low Energy Conformation of the Model (P)

Docking of penicillins and cephalosporins to this computer derived structure (18), using hydrogen bonds from the amide N-H of the antibiotic to the C=O of Val^1, and between the carboxyl group of the antibiotic and the terminal amino group of lysine, led to structures in which the hydroxyl group of serine was positioned on the convex face of the bicyclic ring system, poised for attack on the β-lactam ring. Figure 1.4 illustrates this for penicillin G.

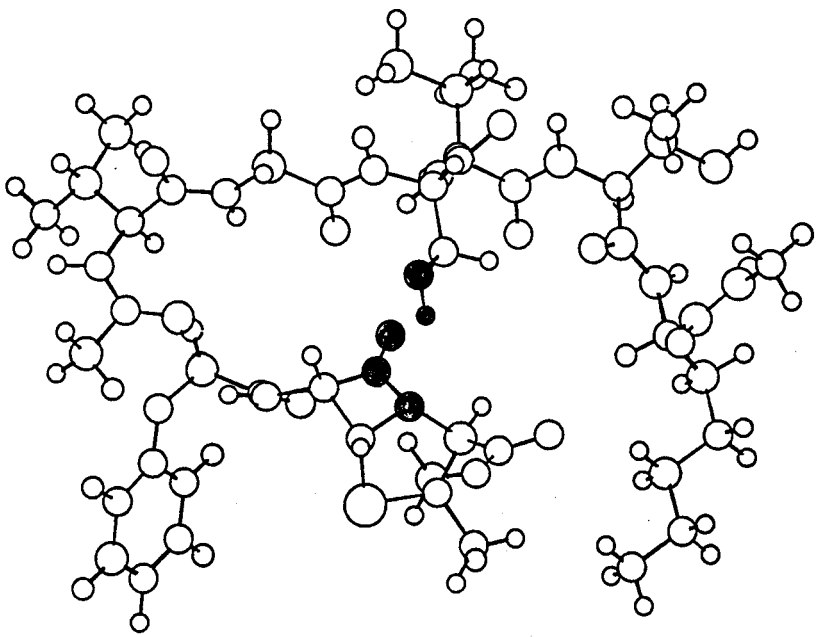


Figure 1.4. Docking of penicillin G to the model (**P**).
The serine O-H and the β-lactam N-C=O are highlighted.

Inspection of this structure revealed that the serine hydroxyl group exihibits a four-centred relationship with respect to the β-lactam group (Figure 1.5).

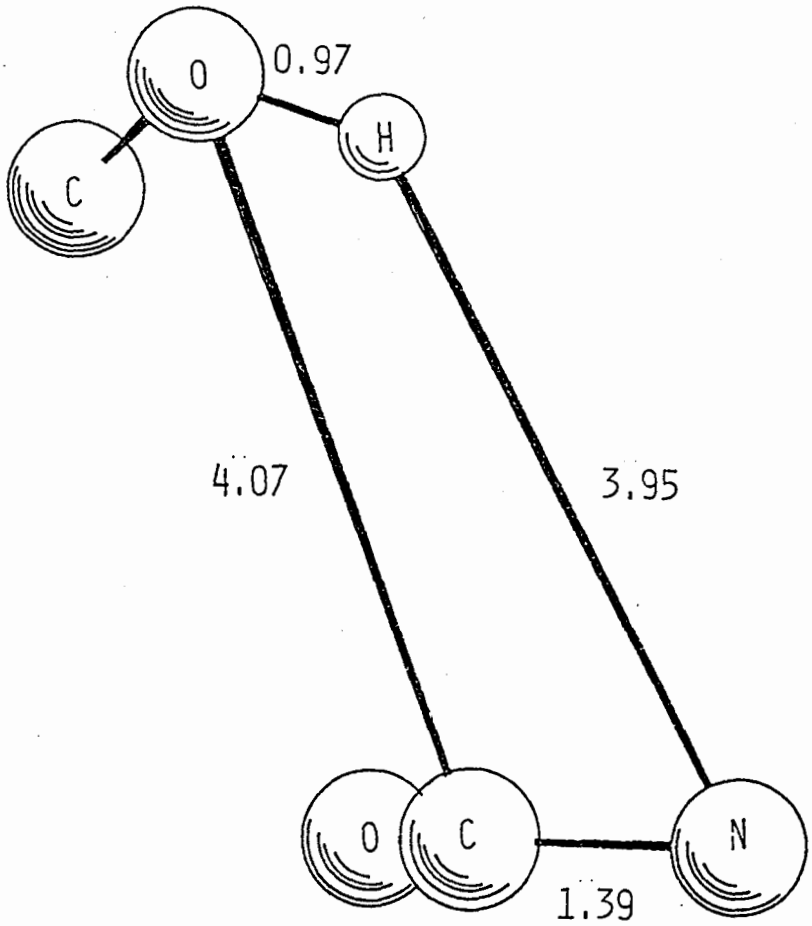
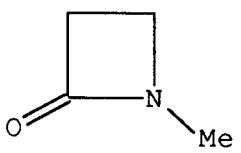
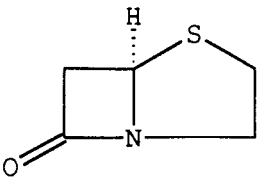


Figure 1.5 A close-up view of the C-O-H/N-C=O relationship in Figure 1.4

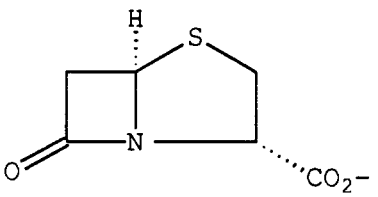
This structure suggested that, in the chemical reaction, C-O bond formation and proton transfer to nitrogen must be concerted with the cleavage of the β-lactam bond. This was confirmed by ab initio calculations of the neutral hydrolysis and alcoholysis of N-methylazetidinone and penam (18,19).



N-methylazetidinone



penam



3α-carboxypenam

Since some penicillin recognizing enzymes contain at least one water molecule in the region of the active-site serine, the effect of one water molecule on the chemical reaction was examined by computational studies, using N-methylazetidinone and 3α-carboxypenam as substrates (19). The reaction proceeds via a termolecular complex, whose C-O-H/N-C=O/H-O-H relationship is shown in Figure 1.6.

In addition, the water molecule has a pronounced catalytic effect, amounting to at least 10 kcal/mol. Inspection of Figure 1.5 and 1.6 suggests that the O-H/C(O)-N distances of Figure 1.5 allow a water molecule to be accommodated. The addition of this water molecule would then create the Ser-OH/H-O-H/N-C=O geometry which is necessary for the chemical reaction.

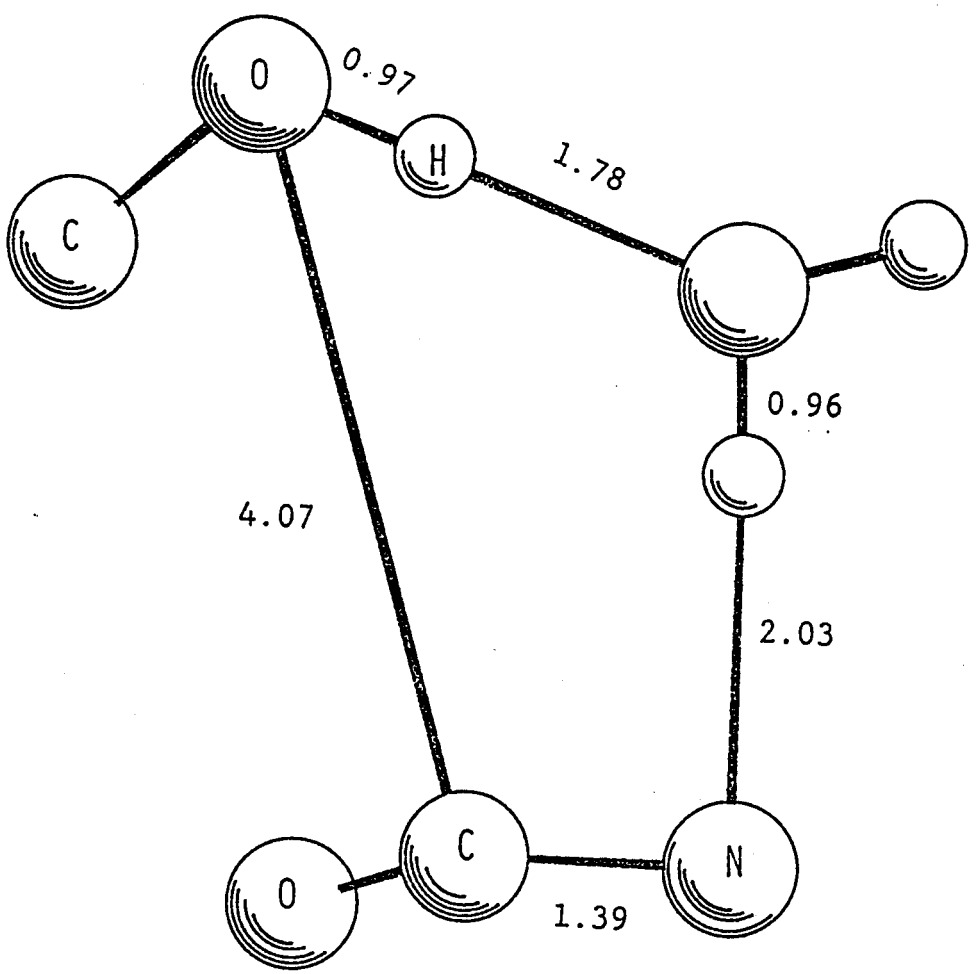


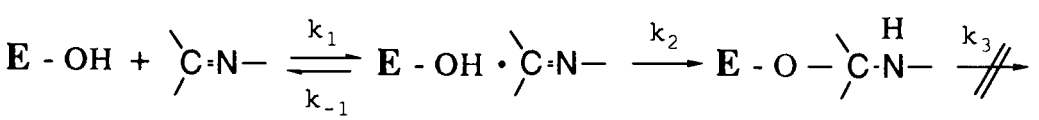
Figure 1.6 A close-up view of the C-O-H/N-C=O/H-O-H relationship

This implies that the enzyme has the ability to orient the reacting molecules on the proper reaction coordinate for the most favorable chemical reaction.

Additional studies were then undertaken to understand how the differing antibacterial activities exhibited by different β-lactam containing ring systems were determined by their different "fits" to the pencillin receptor (P) and

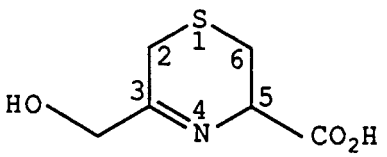
their different intrinsic "reactivities" towards alcoholysis via an N-protonated transition structure (18,20). Fits were compared by using the root mean square differences in the positions of the N-C=O and C-O-H atoms of different complexes, relative to their positions in penicillin. Reactivities were compared from the calculated activation energies for the neutral methanolysis of the β-lactam ring.

This kind of analysis eventually led to an attempt to design a molecule targeted to the penicillin receptor (18,21). The desired compound should be able to fit to the active site of a PBP and react with the serine hydroxyl group in the same manner as penicillin. This requires that the compound possess a carboxyl group or its bioisosteric equivalent (G1), to interact with the **Lys**^{6} amino group of **P**, and also a hydrogen bonding donor group X-H (G2), to interact with the **Val**^{1} carbonyl group of **P**. The distance between G1 and G2 could be determined from the corresponding distance in the crystal structure of penicillin G. In addition, the compound should contain a functional group (F) which can react with the hydroxyl group of serine in a four-centred geometry, possibly with catalysis by water, and with an activation energy comparable to that exhibited by a β-lactam (18,21). The computational search for this functional group (F) led to the selection of the imino group (C=N) as a possible candidate. This group can react with **E**-OH as shown in in Equation 1.2, and it is noteworthy that there is no k_3 step as in Equation 1.1.



Equation 1.2

This analysis led to compound A (2H-5,6-dihydro-1,4-thiazine) as a target for synthesis.



A

Figure 1.7 shows the interaction between compound A and the receptor model.

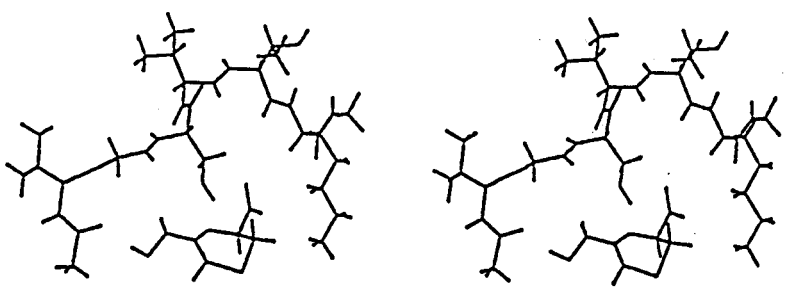
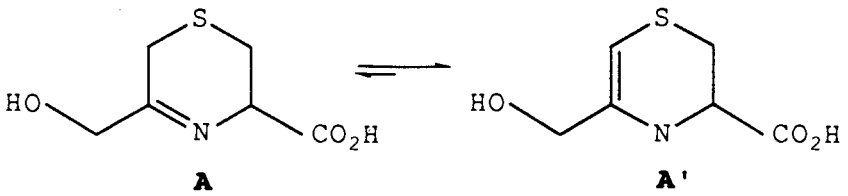


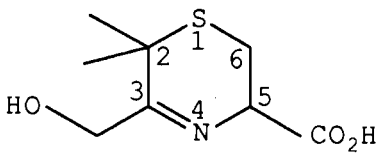
Figure 1.7. Stereoscopic view of the compound A, showing the S enantiomer docked to the model of the penicillin receptor

The dimensions appear to be correct for the proper positioning of the serine OH with respect to the C=N. Unfortunately compound A was found to exist entirely in the tautomeric structure A' (21).



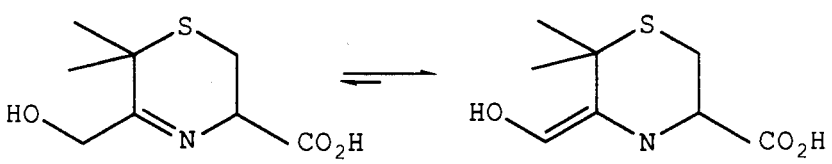
A **A'**

To prevent this, methyl groups were placed at C_2 yielding compound **B**.



B

However, structure (**B**) also exhibited imine $\rightleftharpoons$ enamine tautomerism (**B** $\rightleftharpoons$ **B'**) (21).



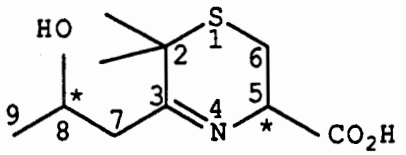
B **B'**

Additional modification of the target was necessary.

1.4 Structure (**1**), a Novel Antibacterial Agent

The six - membered ring of the thiazine seemed to be stable and was retained. The carboxyl group was also retained, supposedly to hydrogen bond to

the amino group of Lys^{6} of **P**. Finally, the hydroxyl group of compound **B** was repositioned in a hydroxypropyl side chain, leading to compound **1** (21).



1

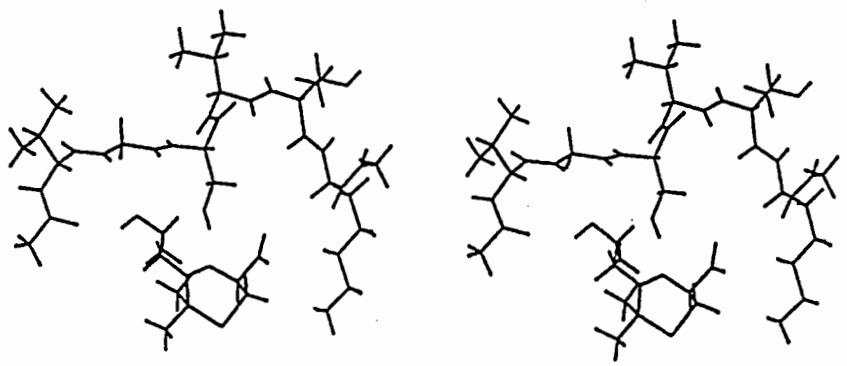


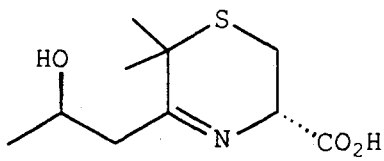
Figure 1.8. Stereoscopic view showing one of the conformations of **1a** docked to the penicillin receptor model (**P**)

Docking of one of the conformations of the (5S,8R) - isomer of compound **1** to the model of the penicillin receptor gave a structure which exhibited a reasonable fit between OH and C=N (Figure 1.8).

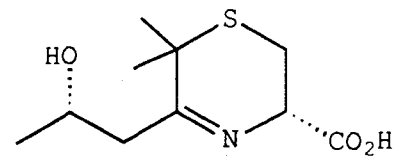
Synthetic and biological studies then revealed that this structure possesses antibacterial activity (18,21).

1.5 Research Proposal

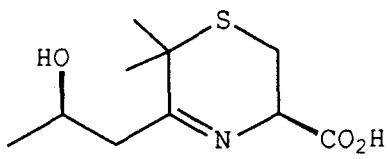
The objective of the present work was to prepare the four stereoisomers (**1a**, **1b**, **1c** and **1d**) of compound **1**, and evaluate their antibacterial activities.



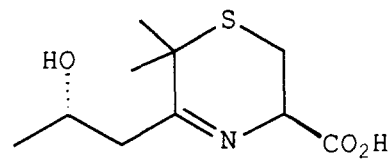
1a (5S,8R)-



1c (5S,8S)-



1b (5R,8R)-



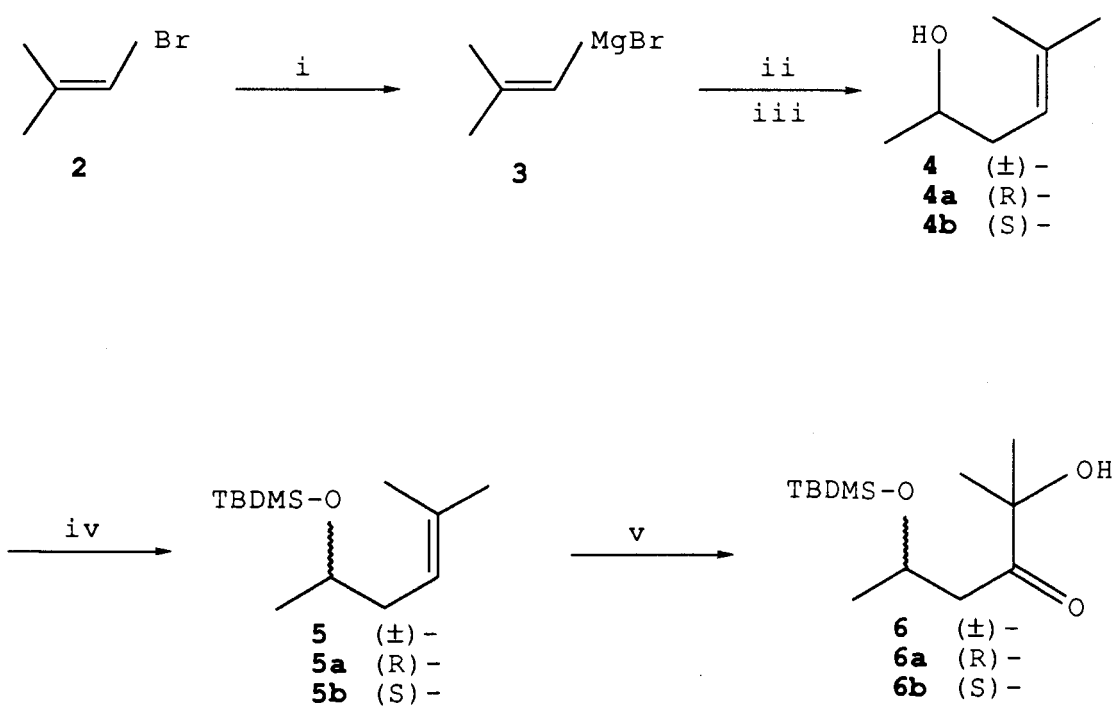
1d (5R,8S)-

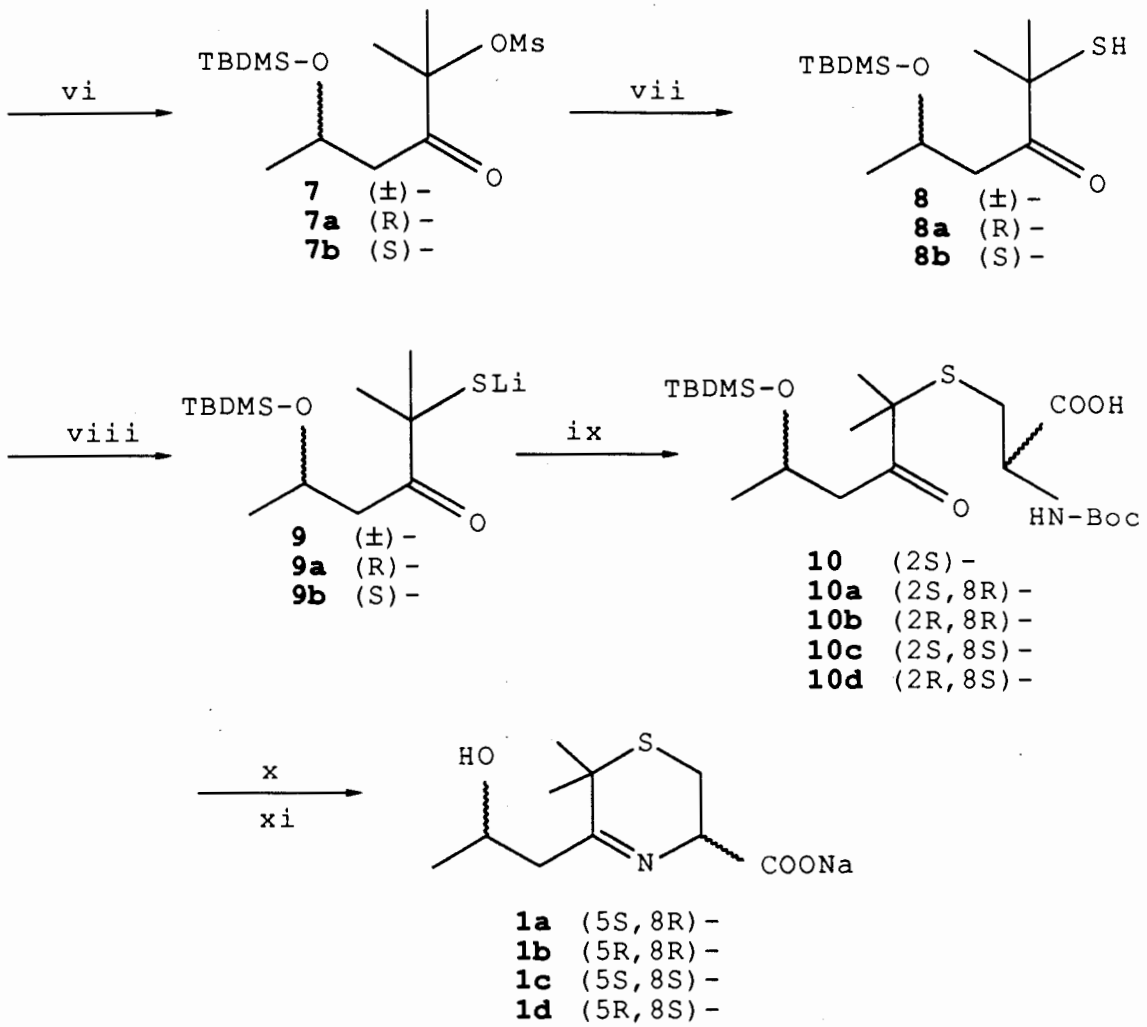
Chapter 2 . Syntheses and Bioassay.

2.1. A Summary for synthesis of the Stereoisomers of 2,2-Dimethyl-3-(2'-Hydroxypropyl)-5-Carboxy-Δ^3-1,4-Thiazine (1a, 1b, 1c and 1d).

Since compound **1** has two stereogenic centers, four stereoisomers are possible. The syntheses of these isomers are summarized in Scheme 1.

Scheme 1.





7 (±)-
7a (R)-
7b (S)-

8 (±)-
8a (R)-
8b (S)-

9 (±)-
9a (R)-
9b (S)-

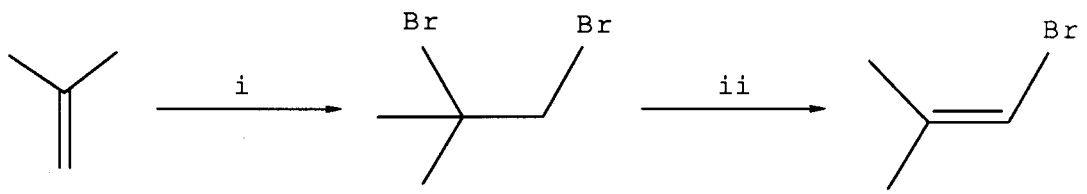
10 (2S)-
10a (2S,8R)-
10b (2R,8R)-
10c (2S,8S)-
10d (2R,8S)-

1a (5S,8R)-
1b (5R,8R)-
1c (5S,8S)-
1d (5R,8S)-

i. Mg, THF, reflux; ii. CuI, THF, -30°C; iii. racemic propylene oxide, THF, -30°C to r.t; a) (R)-propylene oxide (**18a**), THF, -30°C to r.t; b) (S)-propylene oxide (**18b**), THF, -30°C to r.t; iv. TBDMS-Cl, DBU, Et_3N, CH_2Cl_2; v. $KMnO_4$, AcOH, Acetone -H_2O; vi. CH_3SO_2Cl, Et_3N, CH_2Cl_2; vii. H_2S, Et_3N, CH_2Cl_2; viii. LDA, THF, -40°C; ix. a) and c) (D)-N-Boc-β-lactone (**24a**); b) and d) (L)-N-Boc-β-lactone (**24b**); x. HCOOH, CH_2Cl_2; xi. 5% $NaHCO_3$.

The olefin (**2**), 1-bromo-2-methylpropene, was prepared by a known procedure (22,23) (Scheme 2), involving the bromination of isobutylene (**11**) and dehydrobromination of the resulting 1,2-dibromo-2-methylpropane (**12**).

Scheme 2.



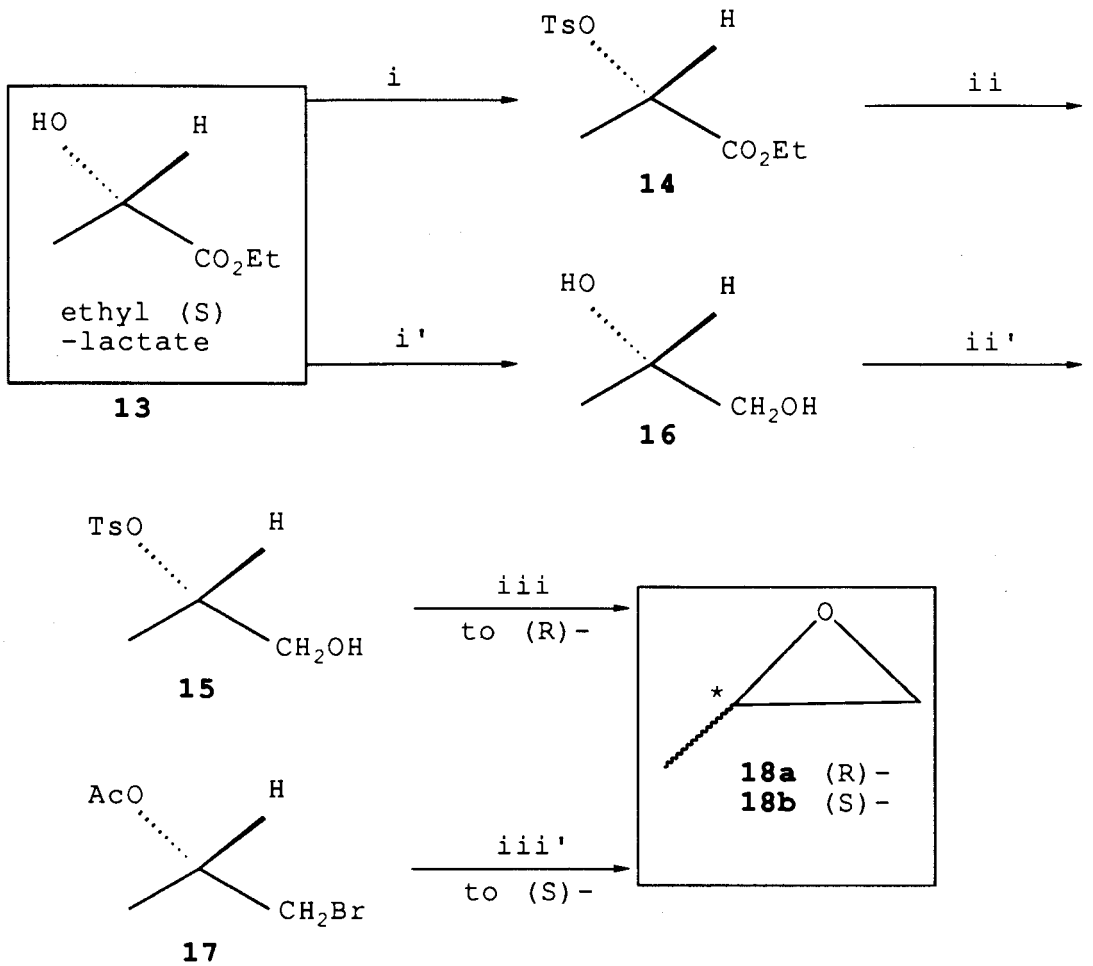
11 **12** **2**

i. Br_2, CH_2Cl_2, 69% ii. KOH, $HOCH_2CH_2OH$, 100°C, 80%.

The racemic alcohol **4** was obtained by reaction of racemic propylene oxide with the Grignard reagent prepared from **2**, using cuprous iodide catalysis (24). The (R) and (S)- alcohols **4a** and **4b** were therefore accessible from the (R) and (S)-propylene oxides **18a** and **18b**.

These epoxides were synthesized in three steps from the same starting material, ethyl (S)- lactate (Scheme 3). To obtain (R)- propylene oxide (**18a**) (24), the lactic acid ester was tosylated, reduced with borane-dimethyl sulfide (24b), and treated with base. To obtain (S)-methyloxirane (**18b**) (25), the ester was reduced with lithium aluminium hydride and the diol was converted to the bromoacetate **17** with hydrogen bromide in acetic acid. Hydrolysis and ring closure were achieved with potassium pentoxide.

Scheme 3.

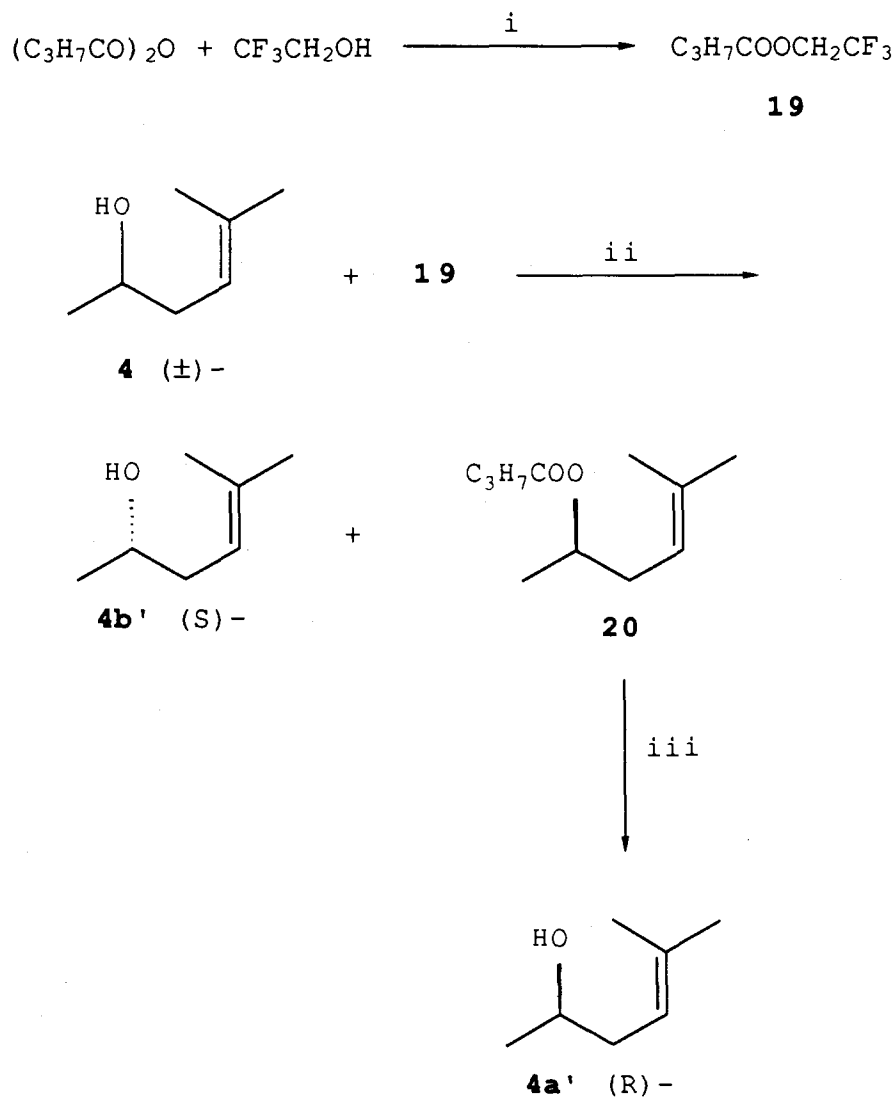


i. TsCl, pyridine, 0-5°C, 75%; ii. $BH_3.Me_2S$, THF, 97%; iii. 50% KOH, 55°C, 53%. i'. LAH, Et_2O, 77%; ii'. 48% HBr-AcOH, 79%; iii'. KOC_5H_{11}-HOC_5H_{11}, 55°C, 96%.

It has been observed (26) that hydrolytic enzymes not only retain their activity but often function more effectively when water is replaced by organic solvents. For example, Porcine Pancreatic Lipase (PPL) has been found to catalyse stereospecific esterification and transesterification in nearly anhydrous organic

solvents (27). This method was examined for the resolution of the racemic alcohol (**4**) (Scheme 4).

Scheme 4.



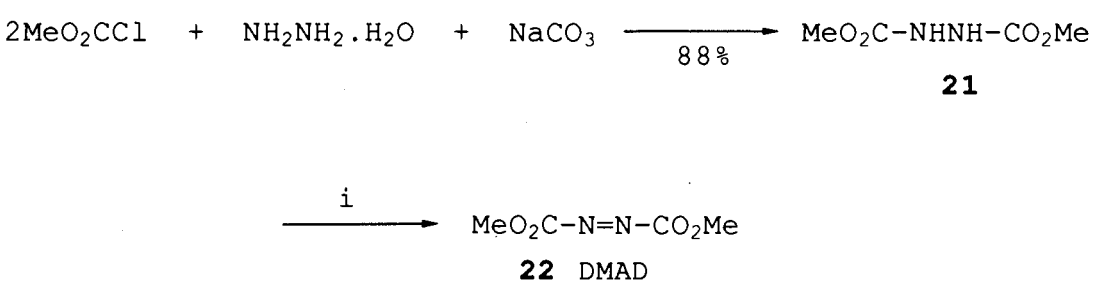
i. p-TsOH.H_2O, 90%; ii. PPL, Et_2O, 46% for (S)-**4b'**, 24% for (R)-**20**; iii. 1.0 M KOH/MeOH, 69%.

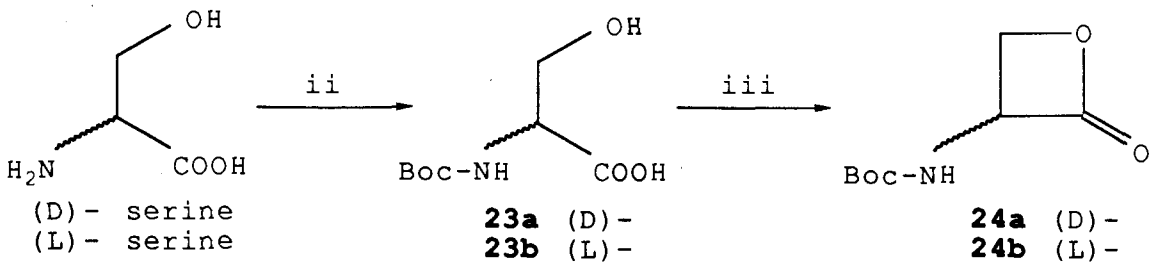
2,2,2-Trifluoroethyl butyrate (**19**) (28) was selected as the transesterification agent and prepared by a standard procedure (29). The enzymatic reaction was carried out at room temperature and was monitored by GC. It was found that the (R)-isomer of **4** reacted to form its ester. Hydrolysis of this ester afforded the (R)-alcohol (**4a'**). The chemical and optical purities of the alcohols **4a**, **4a'**, **4b** and **4b'** were then determined (see Discussion).

The hydroxyl groups of **4a**, **4a'** and **4b** were protected with the tert-butyldimethylsilyl group (30), and the olefins were oxidized to the α-ketols **6**, **6a** and **6b** with potassium permanganate in acidic acetone - water solution (31,32). The newly introduced hydroxyl group was mesylated (21) and reacted with hydrogen sulfide (21,33) to achieve the tranformation from OH to SH.

Concurrently, the N-Boc-β-lactones **24a** and **24b** were prepared by the procedure of Vederas (34) (Scheme 5). The dimethyl azodicarboxylate (DMAD) (**22**) required for this synthesis was prepared by a literature procedure (35). The (D) and (L)- serines were treated with Boc-ON to protect their amino groups, and cyclized to the N-Boc-β-lactones with DMAD and triphenylphosphine at low temperature.

Scheme 5.





(D)- serine
(L)- serine

23a (D)-
23b (L)-

24a (D)-
24b (L)-

i. NBS, CH_2Cl_2, 72%; ii. Boc-ON, Et_3N, Dioxane-H_2O, 77%; iii. DMAD, Ph_3P, -78°C, 58%.

Coupling of the appropriate combinations of (R) or (S)-N-Boc-β-lactone **24a** (D -) or **24b** (L -) to the lithium salts (R) or (S)-**9**, prepared by treating the mercaptans **8a** and **8b**, with lithium diisopropylamide (LDA) led to the compounds **10a**, **10b**, **10c** and **10d**.

Deprotection of **10a-10d** with 98% formic acid and cyclization in 5% sodium bicarbonate produced the final compounds **1a**, **1b**, **1c** and **1d** as their sodium salts.

2.2. **Details of the synthesis**

2.2.1 Synthesis and Characterization of Optically Active **4**.

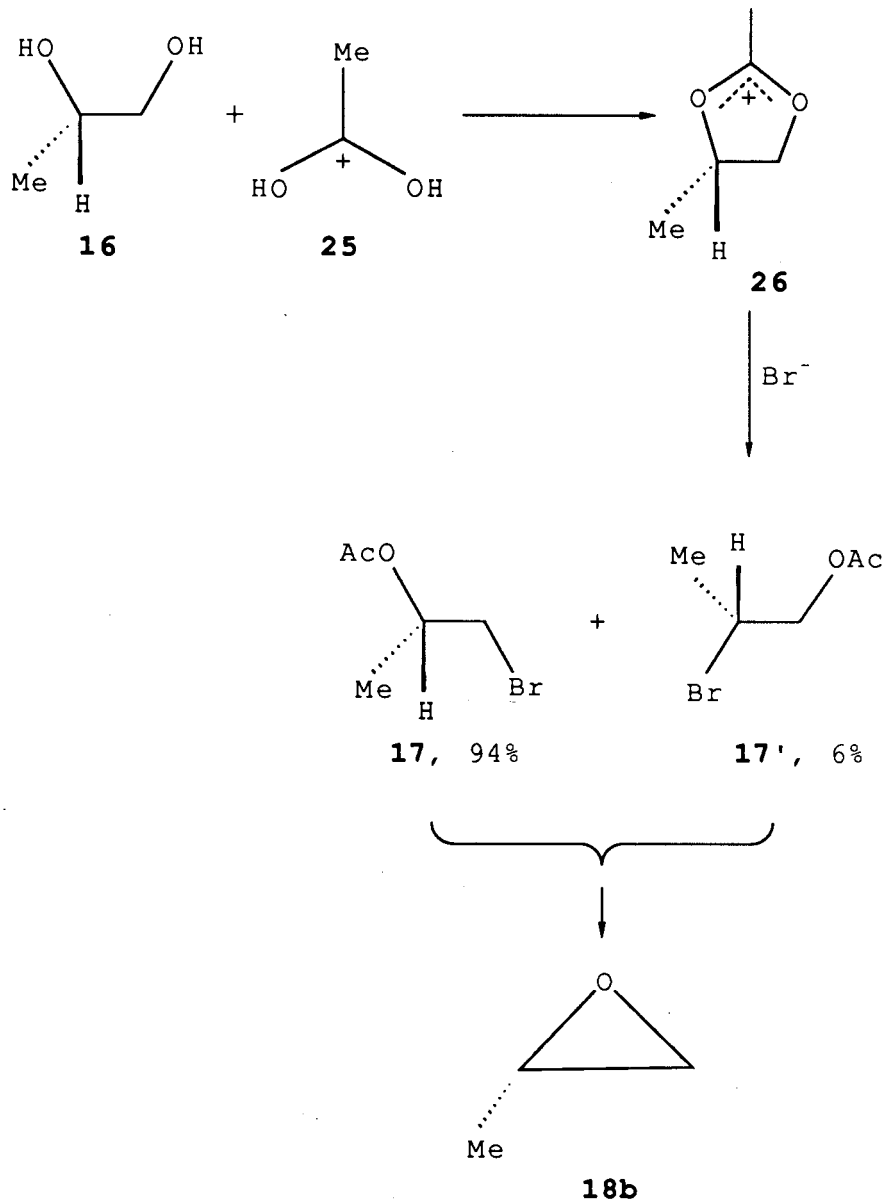
Oxiranes, especially enantiomerically pure oxiranes, are versatile building blocks for the preparation of a variety of optically pure natural products, pharmaceuticals and polymers (36a). There are numerous methods for the synthesis of these compounds (36b).

In the present work, the route to (R)-methyloxirane (**18a**) via **13** (Scheme 3) is unambiguous, and material having the same ee as the starting ethyl (S)-lactate could be expected. On the other hand, the reaction of (S)-propane-1,2-diol (**16**) with HBr/HOAc was regioselective (37). The GC analysis of **17**, isolated by distillation, showed that it consisted of 94% of 2-acetoxy-1-bromopropane (**17**) and 6% of 1-acetoxy-2-bromopropane (**17'**). Mechanistic studies by Golding et al (37) suggested that the reaction proceeds via the acetoxonium ion **26** (Scheme 6), which is opened preferentially at the primary centre.

However both **17** and **17'** lead to **18b** whose rotation was equal and opposite to that of **18a**.

Alkylmagnesium bromides generally react with asymmetrically substituted oxiranes at the less substituted carbon atom. Their effectiveness is improved, and the regioselectivity of attack by Grignard reagents is increased, in the presence of CuI (38). Therefore, both optically active methyloxiranes were reacted with vinylmagnesium bromide **3** in the presence of CuI to produce the enantiomers **4a** and **4b**.

Scheme 6.


HO
OH
Me
H
16
+
Me
HO
OH
25
O
O
Me
H
26
Br⁻
AcO
Me
H
Br
17, 94%
+
Me
H
OAc
Br
17', 6%
O
Me
18b

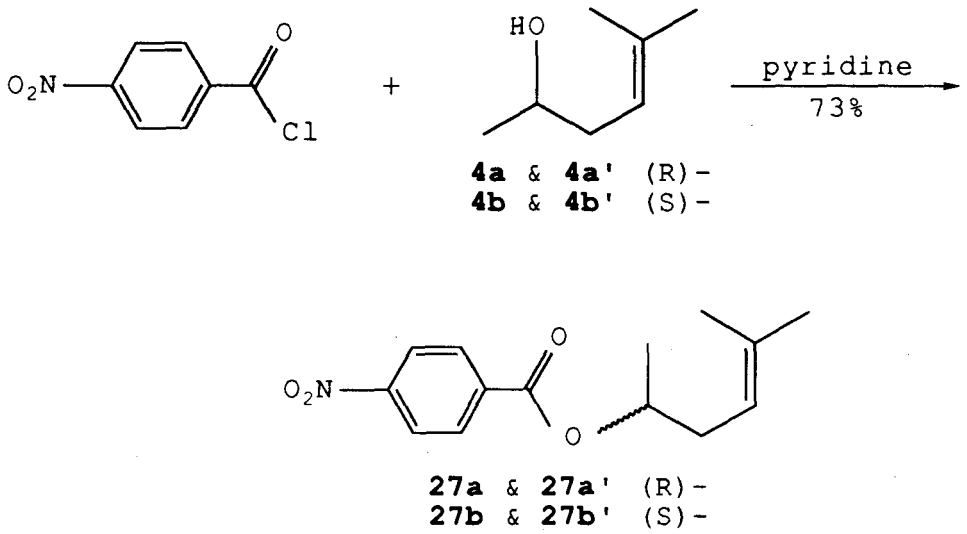
The four samples of **4** prepared in this work and the configurations and origins of these samples are shown in Table 2.1.

Table 2.1. Configurations of the alcohols **4a**, **4b**, **4a'** and **4b'** and their origins

Compound	Configuration	origins
4a	R	(R)-methyloxirane
4b	S	(S)-methyloxirane
4a'	R	Enzymatic resolution (hydrolysis of ester)
4b'	S	Enzymatic resolution (recovered alcohol)

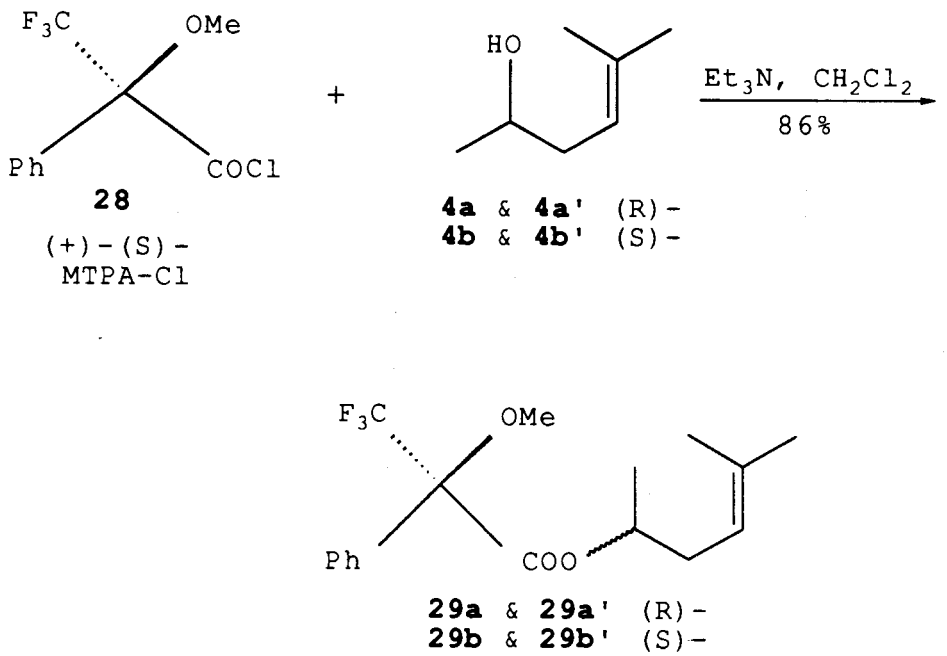
Because the alcohols **4** were relatively volatile (bp 65-70°/20 torr), full chemical characterization was performed on the crystalline p-nitrobenzoate derivatives prepared as shown in Scheme 7. These p-nitrobenzoate derivatives gave satisfactory IR, MS, NMR and microanalyses.

Scheme 7.



The chiral derivatization of a chiral alcohol is widely used to determine the enantiomeric purity of this alcohol (39). In particular, (+) or (-)-α-methoxy-α-trifluoromethyl phenylacetyl chloride (MTPA-Cl) (**28**) is often used for this purpose because the derivatization conditions are mild and the ester is formed in high yield (40). Accordingly, as a check of the enantiomeric purities of the alcohols **4**, each of these was converted to its Mosher ester (**29**) (41) (Scheme 8).

Scheme 8.



The enantiomeric excesses (ee) of **4a**, **4b**, **4a'** and **4b'** (or d.e of **29a**, **29b**, **29a'** and **29b'**) were calculated from the integration of the C_1 methyl doublets at 1.26-1.33 ppm in the high resolution ^1H NMR spectra. The chemically prepared enantiomers (**4a** and **4b**) were found to have enantiomeric purities (ee) of at least 97%. The (R)-enantiomer (**4a'**) of the alcohol prepared by enzymatic resolution was found to have 97% ee but the (S)-isomer (**4b'**) from the resolution had only 54% ee. These results are summarized in Table 2.2.

Table 2.2 . Data for **4a**, **4b**, **4a'** and **4b'** and their p-nitrobenzoates

Alcohol[a]		p-nitrobenzoate[a]		Mosher Ester
	$[\alpha]_D^{21.0}$	m.p.	$[\alpha]_D^{21.0}$	d.e[b]
4a	-11.7	47-8°C	-32.0	98%
4b	+11.8	47-8°C	+31.9	97%
4a'	-11.9	47-8°C	-31.5	97%
4b'	+9.2	44-7°C	+18.2	54%

Note : [a] Rotations were determined in methylene chloride solution.

[b] d.e were calculated from NMR spectra.

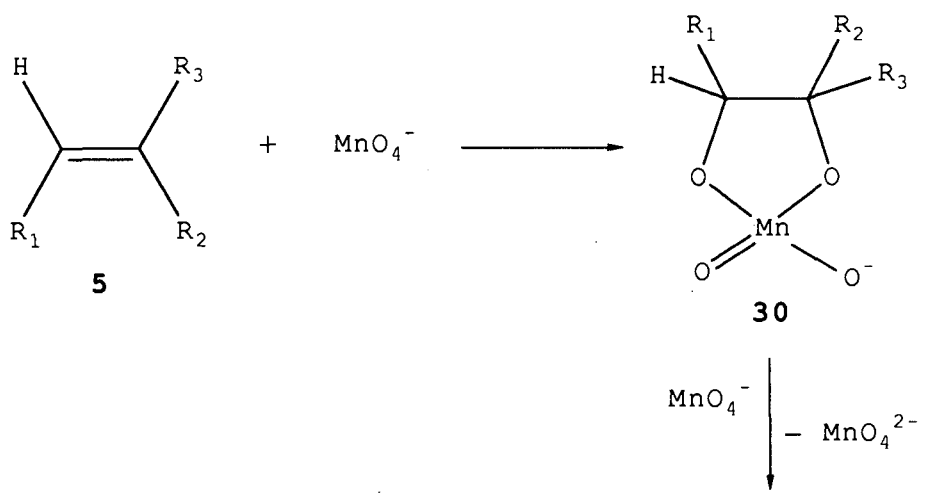
Comparing the chemical synthesis to the enzymatic resolution, the chemical approach seems to have the advantage of providing chiral compounds with high enantiomeric purity (no less than 97% for both enantiomers), but a pure chiral starting material and several synthetic steps are necessary. The enzymatic resolution has the advantage of fewer steps, fewer reagents, and more convenient and milder reaction conditions. However only one enantiomer (**4a'**) could meet our requirements. Consequently, the 'chiral pool' seems to be superior for the preparation of this alcohol.

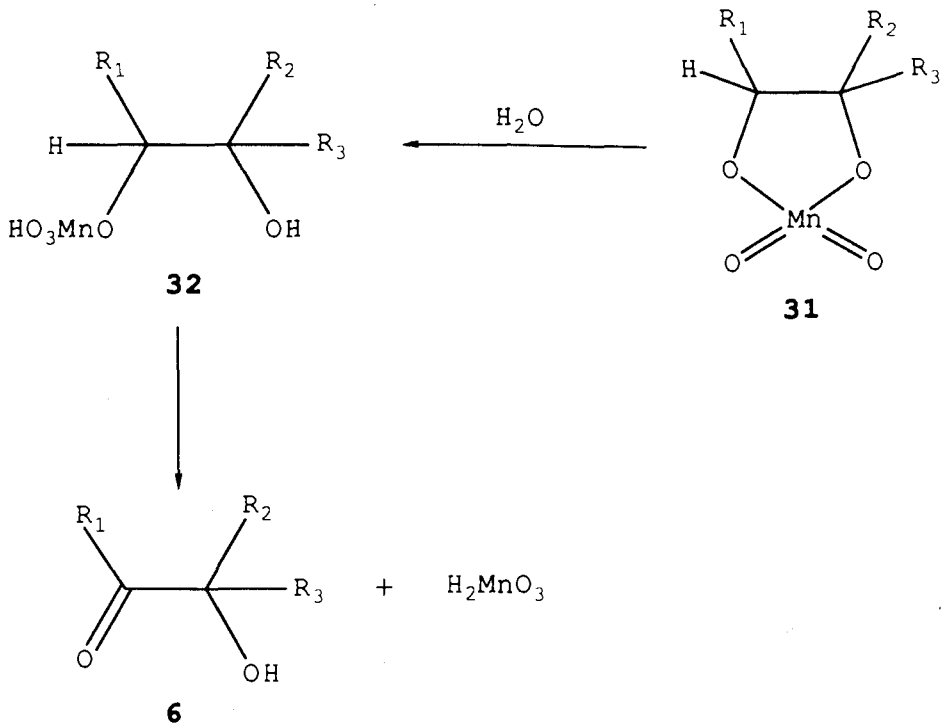
2.2.2. Conversion of **4** to the protected ketol **6**.

The t-butyldimethylsilyl protecting group is more stable to hydrolysis than trimethylsilyl or dimethylisopropylsilyl, but is still readily cleaved by a variety of acidic or fluoride reagents (42). The t-butyldimethylsilyl protecting group is also resistant to many oxidizing agents and to basic conditions.

A number of routes to α-ketols (43) have been developed, including acyloin condensation of esters and oxidation of silyl enol esters, α-glycols, epoxides, and ketones. A different approach (31) involves the one-step oxidation of an olefin. α-Ketols can be prepared from non-terminal alkenes by oxidation with permanganate in aqueous acetone containing a small amount of acetic acid (2-5%), added to neutralize hydroxide ions formed during the reaction (44). The reaction proceeds by oxidative decomposition of a cyclic manganate ester, followed by hydrolysis of the resulting Mn (IV) ester (Scheme 9).

Scheme 9.





2.2.3 Conversion of **6** to **8**; Introduction of Sulfur

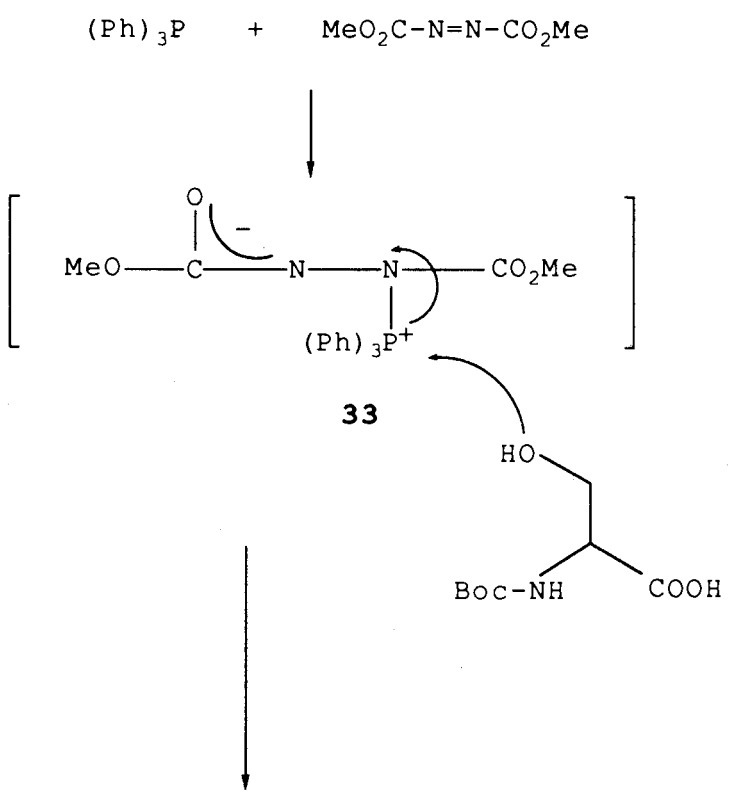
Although the α-ketol is a tertiary alcohol, the conversion of OH to SH was not as difficult as expected. Activation of the hydroxyl group was achieved via mesylation. Although the mesylates (**7**, **7a** and **7b**) were unstable, and decomposed within 2 days when stored at 0^{o} under nitrogen, they reacted smoothly with hydrogen sulfide at low temperature.

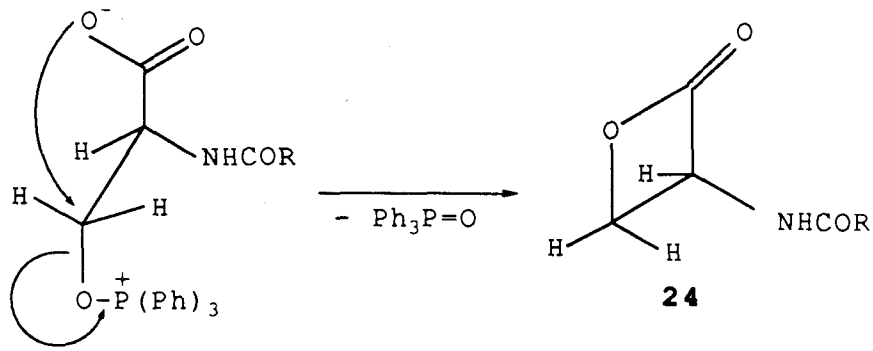
2.2.4 Serine β-Lactone **24**

The preparation and reactions of serine β-lactone have been investigated by Vederas and his colleagues (45). Serine β-lactone became an important chiral synthon because (i) both enantiomers of the amino acid serine are available in high optical purity at relatively low cost, (ii) chiral N-protected serine β-lactones are

readily formed under modified Mitsunobu conditions and (iii) the readily accessible N-protected serine β-lactones behave as chiral electrophilic alanine cation equivalents which react with many different carbon, nitrogen, oxygen, sulfur and halogen nucleophiles by ring opening with alkyl-oxygen cleavage. The formation of the N-protected serine β-lactone proceeds according to the mechanism (46,47,48) shown as Scheme 10.

Scheme 10.





34 24

2.2.5 Coupling to β-Lactones

In the work of Vederas, dimethylformamide was used for ring opening of β-lactones with nucleophiles (44). In the present work it was found that this reaction is more satisfactorily carried out in anhydrous tetrahydrofuran.

2.2.6 Deprotection

Kinetic studies, monitored by ^{1}Hmr, of the reaction of formic acid with **8**, indicated that less than 40 min were needed to cleave the t-butyldimethylsilyl protecting group, and up to 4 h were necessary to remove the t-butoxycarbonyl group completely. 1,3-Propanedithiol was added to act as a scavenger of the t-butyl cation (49,50).

2.2.7 Cyclization

Cyclization under weakly basic conditions produced the final product as a mixture of the sodium salt of compound **1**, sodium formate and excess sodium bicarbonate. The inorganic salts were removed, and the final product was purified by reverse - phase high performance liquid chromatography (HPLC). The gradient programme summarized in Table 2.3 and 2.4 afforded good separation.

Table 2.3. Gradient program for the analytical column

Time (min)	Flow Rate (mL/min)	H_2O (Percent)	ACN (Percent)	Curve
Initial	1.5	100	0	*
3	1.5	100	0	6[a]
15	1.5	50	50	6
18	1.5	0	100	6
20	1.5	0	100	6
25	1.5	100	0	6
30	1.5	100	0	6

[a] number 6 means linear change of gradient.

Table 2.4. Gradient program for the preparative column

Time (min)	Flow Rate (mL/min)	H_2O (Percent)	ACN (Percent)	Curve
Initial	10.	100	0	*
3	10.	100	0	6[a]
15	10.	50	50	6
18	10.	0	100	6
20	10.	0	100	6
25	10.	100	0	6
30	10.	100	0	6

[a] number 6 means linear change of gradient.

The gradient change employed in both Tables is illustrated by the diagram shown in Figure 2.1.

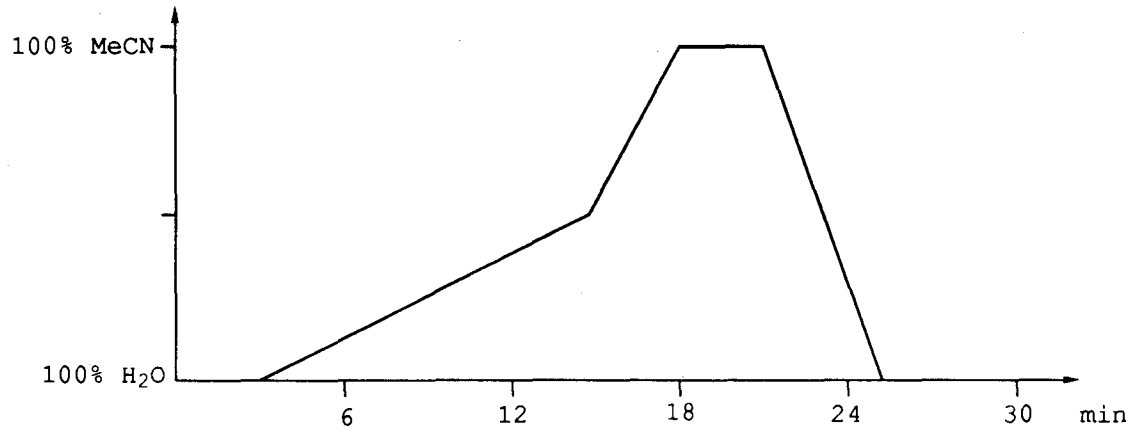


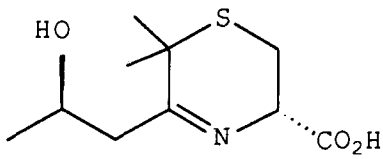
Figure 2.1. Gradient changing curves.

Peaks were detected by UV absorption at 254 nm.

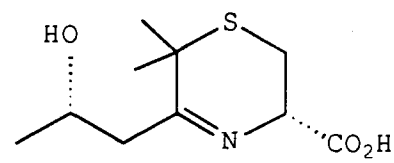
Compound **1** is not very stable, and none of the isomers could be crystallized. Even though the major peak could be collected without difficulty on the HPLC apparatus, analytical HPLC showed additional peaks within one day (Figure 2.2). Bioassays were, therefore, carried out immediately after the isolation of **1a-1d** on the preparative HPLC apparatus.

2.2.8 Bioassay (51)

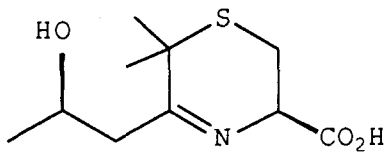
As seen in Figure 2.3, compound **1a** and **1c** exhibit inhibition of penicillin sensitive *S. aureus*.



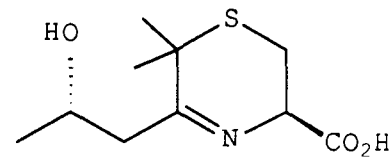
1a (5R,8S)-



1c (5S,8S)-



1b (5R,8R)-



1d (5S,8R)-

Compounds **1b** and **1d** are much less active. Interestingly, **1a** and **1c** have the same activity. This suggests that the antibacterial activity of **1** depends on the stereochemistry of the carboxyl group at C_5 and not on the stereochemistry of the hydroxyl group in the side chain.

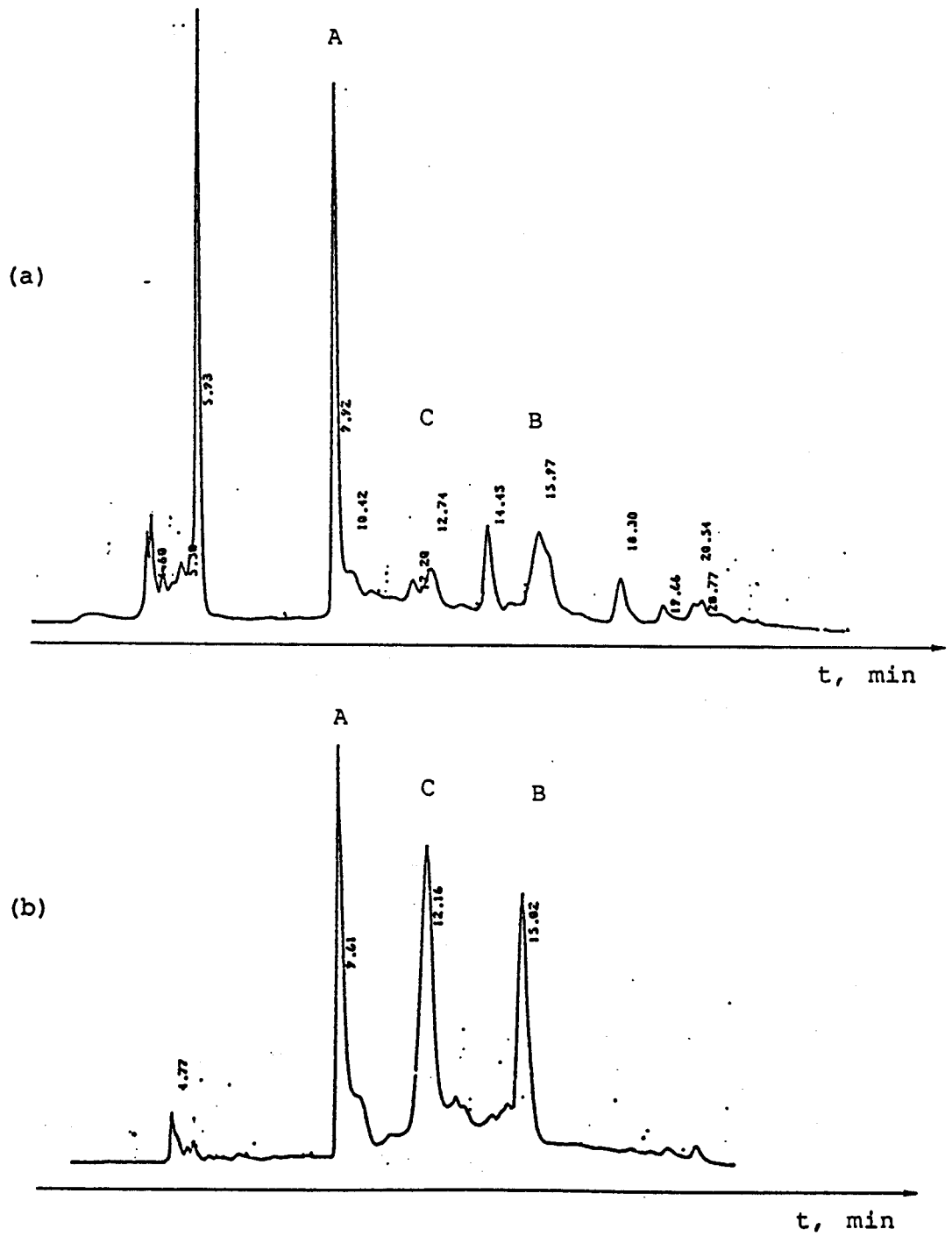


Figure 2.2. The chromatogram of **1b**. Peak A represents the desired product. (a) before separation; (b) purified **1b**, one day after separation.

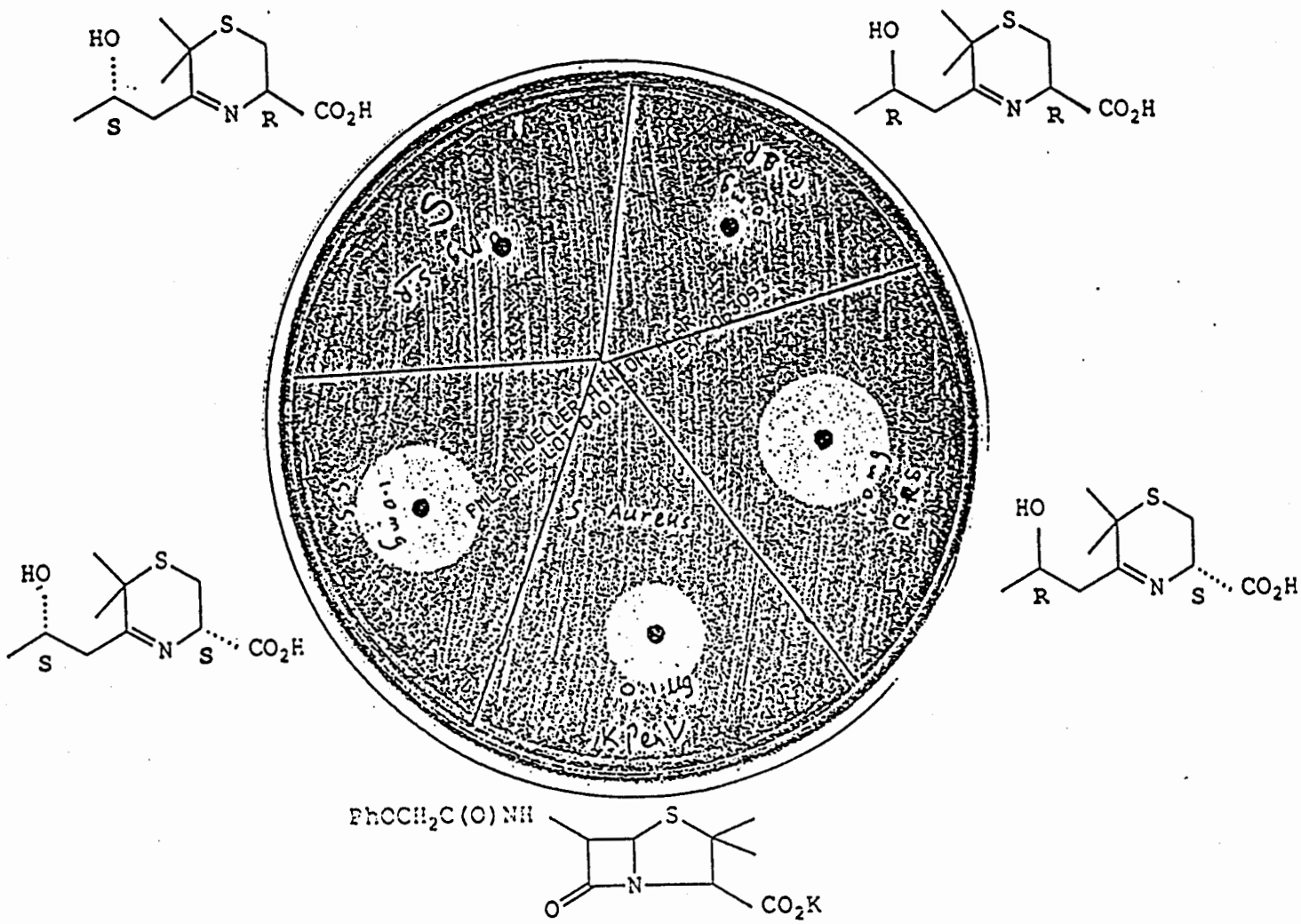


Figure 2.3. Bioassay of potassium penicillin V (0.1 ug) **1a**, **1b**, **1c** and **1d** (1.0 mg) versus penicillin sensitive *S. aureus*.

Chapter 3. Conclusions

The four isomers **1a**, **1b**, **1c**, and **1d** have been prepared by an 11 step synthetic route. The (5**S**,8**R**)- and (5**S**,8**S**)-isomers (**1a** and **1c**) demonstrated antibacterial activity against penicillin sensitive *S. aureus* and (5**R**,8**R**)- and (5**R**,8**S**)-isomers (**1b** and **1d**) were much less active.

The different antibacterial activities of **1a-1d** are consistent with their different extents of fit to the model of the penicillin receptor (**P**) (52).

CHAPTER 4 . EXPERIMENTAL

General

Solvents were dried by standard procedures and distilled before use. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Model WM-400 Spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS). The coupling signals are presented as s(singlet), d(doublet), t(triplet), q(quartet), dd(doublet of doublets), m(multiplet), etc. ^{1}Hmr data are reported in the following abbreviated form: chemical shift, number of protons (xH), peak multiplicity, coupling constants, identification. ^{13}Cmr chemical shifts are reported as δ values in ppm relative to TMS. Melting points (mp) were determined on a Fisher-Johns melting point apparatus, and are uncorrected. Infrared spectra (IR) were recorded on a Perkin-Elmer 599B Spectrophotometer (neat film or 0.2% KBr pellet or 0.2% solution). Gas chromatography-mass spectral (GC-MS) data were obtained on a Hewlett-Packard 5985 GC/MS/DS system equipped with a DB-1 capillary column, and operating at 70 eV, in electron impact (EI) or chemical ionization (CI) mode. Elemental analyses were carried out by Mr. M. K. Yang on a Carlo Erba Model-1106 Elemental Analyzer. Gas chromatographic (GC) analyses were performed on a Hewlett Packard Model 5890A Chromatograph equipped with a F.I.D. and a DB-1 capillary column (length: 15m, film thickness: 0.25μm, id: 0.25μm). Retention times (R_f) are reported in minutes (min). Optical rotations were determined using a Rudolph Automatic Polarimeter Model Autopol®II. The cell length was 10 cm. Concentrations are reported in g/100 mL of solvent. Analytical thin layer chromatography (tlc) was performed on precoated Merck silica gel 60 F-254 plates with aluminium backing. Preparative layer chromatography (plc) was performed on Silica gel 60 F-254 precoated 20 x 20 cm plates (layer thickness 2 mm) or, alternatively, glass plate were coated with a slurry of EM Science silica

gel for thin layer chromatography (layer thickness 1.0 mm). Spots were observed under short wave ultraviolet light, or were visualized with iodine vapour, ninhydrin or with 1% ceric sulfate solution. Flash chromatography was carried out using 230-400 mesh silica gel (Merck). The high performance liquid chromatography (HPLC) system consisted of a Waters 600E system controller, Waters 486 Tunable Absorbance Detector and Waters 746 Data Module. A Waters RCM8x10 cartridge was used for analytical work (Radial Pak column, 8x10mm, reverse-phase, I.D 8mm, C_{18}, 10μm particle size). A Waters RCM25x10 cartridge was used for preparative work (Bondapak, 25x10mm, C_{18}, 125Å, 15-20μm particle size). The acetonitrile and methanol solvents for HPLC analysis were HPLC grade. Water for HPLC was collected through a Millipore purifier and degassed before use. Antibacterial activities were determinated by the agar diffustion procedure. The bacterial plates were provided by Dr. John Smith of Vancouver General Hospital.

1,2-Dibromo-2-methylpropane (12) (22)

A solution of bromine (150 mL, 465.3 g, 2.9 mole) in methylene chloride (500 mL) was cooled in an ice bath. Isobutylene was introduced into this stirred mixture at a rate which maintained a gentle reflux. When the colour of bromine had disappeared the solvent was removed and the residue was distilled to give 433.4 g (69%) of 1,2-dibromo-2-methylpropane, bp 46-8°/20 torr. ^{1}Hmr($CDCl_3$, δ): 1.87(6H, s, $2CH_3$), 4.86(2H, s, CH_2).

1-Bromo-2-methylpropene (2) (23)

1,2-Dibromo-2-methyl-propane (84.0 g, 0.39 mole) was added dropwise at 100° over 45 min to a solution of potassium hydroxide (30 g, 0.53 mole) in ethylene glycol (100 mL) in an apparatus set up for distillation. The ethanediol and vinyl bromide were collected by distillation as the dibromide was added. The reaction was terminated when the

temperature had reached 160° in the pot and 90° at the distillation head. Methylene chloride was added to the distillate. The lower phase was separated, dried over anhydrous calcium chloride and distilled to give 41.8 g (80%) of colourless 1-bromo-2-methylpropene, bp 90-2°. ^{1}Hmr($CDCl_3$, δ): 1.78(6H, s, 2C*H*$_3$), 5.87(1H, m, BrC*H*=).

Ethyl-2-p-toluenesulphonyl-S-(-)-lactate (14) (24a)

A solution of ethyl S-(-)-lactate (20 g, 0.17 mole) in pyridine (200 mL) was cooled in an ice-bath, and p-toluenesulphonyl chloride (40 g, 0.21 mole) was added in three portions with stirring over 30 min. The pale yellow solution was maintained at -5° for 20 h, and then poured onto ice-water (300 mL) and extracted with chloroform (4 x 200 mL). The combined chloroform extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure to give 33.4 g (75%) of an oil. This was used for the next reaction without further purification. $[\alpha]_D^{21.0}$ = -33.4°(c 2.5, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ): 1.22(3H, t, 7.1Hz, -CH_2CH_3), 1.53(3H, d, 6.7Hz, OCHC*H*$_3$), 2.46(3H, s, PhC*H*$_3$), 4.13(2H, q, 7.1Hz, O*CH*$_2$CH$_3$), 4.96(1H, q, 6.7Hz, C*H*CH$_3$), 7.36(2H, q), 7.84(2H, q). IR(film): 2987.2, 2359.7, 1755.0, 1598.2, 1448.7, 1369.6, 1100.8, 1082.4, 817.6, 665.4 cm^{-1}. MS(m/e, CI): 273 (M^++1), 199 (M^+-CO_2Et), 155 (CH_3-C_6H_4-SO_2).

(R)-(+)-Methyloxirane (18a) (24a,b)

A solution of ethyl-2-p-toluenesulphonyl-S-(-)-lactate (26.0 g, 95.5 mmoles) in tetrahydrofuran (20 mL) was added dropwise at room temperature during 30 min to 8.7 M borane-dimethylsulfide (9.5 ml, 82.6 mmoles) in tetrahydrofuran (50 mL). When the addition was complete, the reaction mixture was refluxed for 1.5 h, cooled to room temperature, and water (15 mL) was added dropwise with stirring, followed by anhydrous potassium carbonate (4.5 g). The aqueous phase was extracted with ether (3 x 20 mL), and the combined organic extracts were washed with saturated sodium chloride (2 x 20 mL),

dried over anhydrous magnesium sulfate and evaporated to yield 21.3 g (97%) of 2-p-toluenesulphonyloxy-1-propanol. This oil was added dropwise at 55° to 50% potassium hydroxide. The R-(+)-methyloxirane which distilled out was collected in a dry ice-acetone trap and redistilled to give 2.85 g (53.0%) of the product. $[\alpha]_D^{23.0}$ = +7.32(c 2.5, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ): 1.29(3H, d, 5.2Hz, C*H*$_3$), 2.40(1H, dd, 5.0, 2.7Hz, C*H*$_2$), 2.72(1H, dd, 5.0, 5.0Hz, C*H*$_2$), 2.95(1H, m, C*H*). IR(film): 2994.5, 1407.0, 1265.2, 1022.9, 949.8, 828.9 cm^{-1}.

(S)-(+)-Propane-1,2-diol (16) (25)

A solution of ethyl L-(-)-lactate (33 g, 0.28 mole) in ether (150 mL) was added dropwise to a stirred solution of lithium aluminium hydride (10.8 g, 0.284 mole) in ether (200 mL), at such a rate as to maintain gentle reflux. After the addition was complete, stirring was continued for 3 h. Water (25 mL, 1.39 mole) was added cautiously and stirring was continued for a further 1.5 h. The mixture was filtered and the white solid was washed with ether (2 x 20 mL) and dichloromethane (2 x 20 mL). The combined organic phase was dried over anhydrous magnesium sulfate and concentrated to give a portion of the crude product (3 g). The solid was treated with aqueous sulfuric acid (1.0 M) until the milky suspension was just acidic (pH 6-6.5), and this suspension was subjected to continuous extraction with dichloromethane (ca. 500 mL) for 6 days. The dichloromethane layer was dried over anhydrous magnesium sulfate and concentrated. The crude products were combined and distilled to give 16.4 g (77%) of (S)-(+)-propane-1,2-diol, bp 59-62° (0.8 torr), as a colourless liquid. $[\alpha]_D^{21.0}$ = +20.6°(c 3.4, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ): 1.09(3H, d, 7.2Hz, CHC*H*$_3$), 3.34(1H, dd, 11.0, 9.2Hz, C*H*$_2$OH), 3.55(1H, dd, 11.2, 9.2Hz, C*H*$_2$OH), 3.86(1H, m, C*H*CH$_3$), 3.90(2H, br, OH). IR(film): 3346.3, 2970.9, 2932.8, 1460.3, 1377.8, 1137.9, 1045.8 cm^{-1}. MS(m/e, CI): 77 (M^++1), 59 (M^+-H_2O). Anal. Calcd for $C_3H_8O_2$: C, 47.35; H, 10.60; Found : C, 47.01; H, 10.76.

(S)-(-)-2-Acetoxy-1-bromopropane (17) (25)

A 45% w/v solution of hydrogen bromide in acetic acid (70 mL, 0.41 mole) was added during 5 min with cooling at 0 - 5° to (S)-(+)-propane-1,2-diol (10.67 g, 0.14 mole). The solution was stirred at room temperature for 45 min and water (200 mL) was then added. The reaction mixture was neutralized immediatedly by addition of solid sodium carbonate, and extracted with ether (3 x1 50 mL). The combined organic phase was dried over anhydrous magnesium sulfate and concentrated. Distillation yielded 20.0.g (79%) of (S)-(-)-2-acetoxy-1-bromopropane, bp 66-75° (17 torr), as a colourless liquid. $[\alpha]_D^{21.0}$ = -13.7°(c 2.8, CH_2Cl_2); ^{1}Hmr($CDCl_3$, δ): 1.33(3H, d, 6.0Hz, OCHC*H*$_3$), 2.05(3H, s, CC*H*$_3$), 3.44(2H, m, C*H*$_2$Br), 5.06(1H, m, OC*H*CH$_3$). IR(film): 3313.3, 2981.4, 1721.2, 1374.9, 1234.3, 1191.9, 1033.3 cm^{-1}. MS(m/e, CI): 182 (M^++1), 121 (M^+-CH_3COOH). Anal. Calcd for $C_5H_8O_2Br$: C, 33.17; H, 5.01; Found : C, 33.38; H, 5.20.

(S)-(-)-Methyloxirane (18b) (25)

A mixture of the acetoxybromopropane (9.81 g, 54.2 mmoles) and 1-pentanol (20 mL) was stirred at room temperature and a solution of potassium pentoxide in 1-pentanol (1.2 M, 42.66 mL, 52 mmoles) was added during 20 min. When the addition was complete, the flask was warmed in an oil bath maintained at 130-145° and the product, (S)-(-)-methyloxirane, 3.01 g (96%), was collected by distillation, bp 34-35°. $[\alpha]_D^{20.0}$ = -7.57(c 1.58, CH_2Cl_2); ^{1}Hmr($CDCl_3$, δ): 1.29(3H, d, 5.2Hz, CHC*H*$_3$), 2.42(1H, dd, 5.0, 2.7Hz, C*H*$_2$), 2.72(1H, dd, 5.0, 5.0Hz, C*H*$_2$), 2.95(1H, m, C*H*CH$_3$). IR(film): 2994.8, 1407.2, 1265.3, 1023.1, 949.8, 828.8 cm^{-1}.

(R)-(-)-5-Methyl-4-hexen-2-ol (4a) (24a)

A mixture of tetrahydrofuran (30 mL) and magnesium turnings (2.0 g, 82 mmoles) was stirred under nitrogen. A small crystal of iodine and 0.2 mL of 1,2-dibromoethane

were added and the mixture was refluxed until the iodine colour disappeared. Then 1-bromo-2-methyl-propene (5.58 g, 4.2 mL, 41 mmoles) in tetrahydrofuran (10 mL) was added dropwise over 30 min. The reaction mixture was refluxed for 3.0 h, cooled to room temperature, and transferred via canulla to a dried flask. The Grignard solution was cooled to -30°, dry cuprous iodide (0.16 g, 8 mmoles) was added in one portion and the mixture was stirred for 15 min. A solution of (R)-(+)-methyloxirane (2.0 g, 2.4 mL, 34.4 mmoles) in tetrahydrofuran (20 mL) was added dropwise during 1 h with vigorous stirring. When the addition was complete, the mixture was warmed to room temperature, stirred for 4 h, and then poured onto rapidly stirred saturated ammonium chloride (150 mL). The organic layer was separated and the blue aqueous phase was extracted with ether (3 x 20 mL). The combined organic phase were washed with saturated sodium chloride (2 x 50 mL), dried over anhydrous magnesium sulfate, evaporated and distilled to give 3.12 g (79%) of a colourless oil. bp 65-70°/20 torr. $[\alpha]_D^{21.0}$ = -11.74°(c 3.15, CH_2Cl_2); ^{1}Hmr($CDCl_3$, δ): 1.18(3H, d, 6.7Hz, CHC*H*$_3$), 1.63(1H, s, OH), 1.63(3H, s, CC*H*$_3$), 1.72(3H, s, CC*H*$_3$), 2.16(2H, dd, 7.5, 7.5Hz, CHC*H*$_2$CH), 3.79(1H, m, C*H*OH), 5.15(1H, m, C*H*=C). IR(film): 3362.0, 2969.5, 2926.8, 1716.0, 1455.8, 1375.9, 1121.8, 1078.3 cm^{-1}. MS(m/e, CI): 114 (M^++1), 97 (M^+-H_2O).

(S)-(+)-5-Methyl-4-hexen-2-ol (4b) (24a)

This compound was prepared from (S)-(-)-methyloxirane in 80% yield. $[\alpha]_D^{21.0}$ = +11.75° (c 1.6, CH_2Cl_2); ^{1}Hmr($CDCl_3$, δ): 1.18(3H, d, 6.7Hz, CHC*H*$_3$), 1.63(1H, s, OH), 1.63(3H, s, CC*H*$_3$), 1.72(3H, s, CC*H*$_3$), 2.16(2H, dd, 7.5, 7.5Hz, CHC*H*$_2$CH), 3.79(1H, m, C*H*OH), 5.15(1H, m, C*H*=C). IR(film): 3362.0, 2969.5, 2926.8, 1716.0, 1455.8, 1375.9, 1121.8, 1078.3 cm^{-1}. MS(m/e, CI): 114 (M^++1), 97 (M^+-H_2O).

(±)-5-Methyl-4-hexen-2-ol (4) (24a)

This compound was prepared from (±)-methyloxirane in 80% yield. ^{1}Hmr($CDCl_3$, δ): 1.18(3H, d, 6.7Hz, $CHCH_3$), 1.63(1H, s, OH), 1.63(3H, s, CCH_3), 1.72(3H, s, CCH_3), 2.16(2H, dd, 7.5, 7.5Hz, $CHCH_2CH$), 3.79(1H, m, CHOH), 5.15(1H, m, CH=C). IR(film): 3362.0, 2969.5, 2926.8, 1716.0, 1455.8, 1375.9, 1121.8, 1078.3 cm^{-1}. MS(m/e, CI): 114 (M^++1), 97 (M^+-H_2O).

Porcine Pancreatic Lipase Catalyzed Production of the (R) and (S)-5-Methyl-4-Hexen-2-ols from (±)-5-Methyl-4-Hexen-2-ol

Trifluoroethyl butyrate (19) (29)

A mixture of trifluoroethanol (20.6 g, 15.0 mL, 206 mmoles), butyric anhydride (31.9 g, 23.0 mL, 202 mmoles) and p-toluenesulfonic acid monohydrate (0.3 g) was stirred and heated to reflux under nitrogen. When the temperature of the vapour reached 105°, the condenser was replaced with a distillation head and rapid distillation was performed. Fractions boiling to 130° were collected. The crude distillate was redistilled through a 20 cm Vigreux column to give a central fraction, bp 105-115°, 33.2 g (90%), as a colourless liquid. ^{1}Hmr ($CDCl_3$, δ): 1.00(3H, t, 7.2Hz, $CH_2CH_2CH_3$), 1.71(2H, m, $CH_2CH_2CH_3$), 2.43(2H, t, 7.0Hz, $CH_2CH_2CH_3$), 4.49(2H, q, 8.3Hz, CH_2CF_3). IR(film): 2972.4, 2881.3, 1762.4, 1713.0, 1413.7, 1282.9, 1169.9, 1102.5 cm^{-1}. MS(m/e): 170 (M^+), 155 (M^+-CH_3).

(S)-(+)-5-Methyl-4-hexen-2-ol (4b') and (R)-(-)-5-Methyl-4-hexen-2-ol (4a') (27a)

A solution of racemic 5-methyl-4-hexen-2-ol (10.66 g, 93.3 mmoles), trifluoroethyl butyrate (18.8 g, 110 mmoles), dry ether (55 mL) and Porcine Pancreatic Lipase (E.C. 3.1.1.3, Sigma crude preparation lipase, type 2, #L-3126, 8.2 g) was stirred in a 250 mL Erlenmeyer flask protected from moisture. The progress of the reaction was

monitored by GC. When GC showed that 50% of the alcohol had been esterified the reaction was terminated by filtration. The filtrate was concentrated and the residue was distilled to give (S)-(+)-5-methyl-4-hexen-2-ol, 4.87 g (46%), bp 65-75°/17 torr, and the ester of (R)-5-methyl-4-hexen-2-ol, 4.07 g (24%), bp 80-95°/17 torr. The ester (4.07 g, 21.7 mmoles), in 1.0 M methanolic potassium hydroxide (50 mL, 50 mmoles), was refluxed for 50 min. The solvent was then removed and the residue was triturated with ether (3 x 20 mL). The extract was washed with saturated sodium chloride (2 x 20 mL), dried over anhydrous magnesium sulfate and evaporated. The residue was distilled to give 1.5 g (69%) of (R)-(-)-5-methyl-4-hexen-2-ol, bp 60-70°/15 torr.

For **(S)-(+)-5-methyl-4-hexen-2-ol.(4b')**

$[\alpha]D^{21.0}$ = +9.19°(c 1.35, CH_2Cl_2); ^{1}Hmr($CDCl_3$, δ): 1.18(3H, d, 6.7Hz, CHC*H*$_3$), 1.63(1H, s, OH), 1.63(3H, s, CC*H*$_3$), 1.72(3H, s, CC*H*$_3$), 2.16(2H, dd, 7.5, 7.5Hz, CHC*H*$_2$CH), 3.79(1H, m, C*H*OH), 5.15(1H, m, C*H*=C). IR(film): 3362.0, 2969.5, 2926.8, 1716.0, 1455.8, 1375.9, 1121.8, 1078.3 cm^{-1}. MS(m/e, CI): 114 (M^++1), 97 (M^+-H_2O).

For **(R)-(-)-5-methyl-4-hexane-2-ol (4a')**

$[\alpha]D^{21.0}$ = -11.95°(c 1.26, CH_2Cl_2); ^{1}Hmr($CDCl_3$, δ): 1.18(3H, d, 6.7Hz, CHC*H*$_3$), 1.63(1H, s, OH), 1.63(3H, s, CC*H*$_3$), 1.72(3H, s, CC*H*$_3$), 2.16(2H, dd, 7.5, 7.5Hz, CHC*H*$_2$CH), 3.79(1H, m, C*H*OH), 5.15(1H, m, C*H*=C). IR(film): 3362.0, 2969.5, 2926.8, 1716.0, 1455.8, 1375.9, 1121.8, 1078.3 cm^{-1}. MS(m/e, CI): 114 (M^++1), 97 (M^+-H_2O).

(R)-(-)-5-Methyl-4-hexen-2-ol p-nitrobenzoate (27a) (24a)

p-Nitrobenzoyl chloride (80 mg, 0.47 mmole) was added to a solution of (R)-(-)-5-methyl-4-hexen-2-ol (50 mg, 0.44 mmole, from epoxide) in pyridine (2 mL). The solution

was stirred at room temperature for 3 h, and then poured into water (5 mL). The aqueous layer was extracted with ether (2 x 5 mL), and this extract was washed successively with 5% aqueous sodium bicarbonate (2 x 5 mL), saturated sodium chloride (2 x 5 mL), dried over anhydrous magnesium sulfate and evaporated to a syrup This was purified by flash chromatography on silica gel (ethyl acetate/hexane, 10%, 50 mL) to give 80.1 mg (73%) of material, mp 47.0-48.0° after recrystallization from methanol. $[\alpha]_D^{21.0}$ = -32.0°(c 1.03, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ): 1.36(3H, d, 6.3Hz, CHC*H*$_3$), 1.63(3H, s, CC*H*$_3$), 1.70(3H, s, CC*H*$_3$), 2.34(1H, m, CHC*H*$_2$CH), 2.45(1H, m, CHC*H*$_2$CH), 5.16(1H, m, OC*H*CH$_3$), 5.16(1H, m, C*H*=C), 8.19(2H, m),.8.28(2H, m). IR(film): 3113.8, 2976.1, 1714.8, 1607.7, 1528.6, 1450.6, 1353.5, 1274.4, 1105.8, 1056.1 cm^{-1}. MS(m/e, CI): 264 (M^++1). Anal. Calcd for $C_{14}H_{17}O_4N$: C, 63.86; H, 6.51; N, 5.32; Found : C, 64.08; H, 6.58; N, 5.27.

(S)-(+)-5-Methyl-4-hexen-2-ol p-nitrobenzoate (27b) (24a)

This compound was prepared from (S)-(+)-5-methyl-4-hexen-2-ol (50 mg, 0.44 mmole, from epoxide) in the same manner as (R)-(-)-5-methyl-4-hexen-2-ol p-nitrobenzoate. mp 47.0-48.0°. $[\alpha]_D^{21.0}$ = +31.90°(c 0.94, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ): 1.36(3H, d, 6.3Hz, CHC*H*$_3$), 1.63(3H, s, CC*H*$_3$), 1.70(3H, s, CC*H*$_3$), 2.34(1H, m, CHC*H*$_2$CH), 2.45(1H, m, CHC*H*$_2$CH), 5.16(1H, m, OC*H*CH$_3$), 5.16(1H, m, C*H*=C), 8.19(2H, m), 8.28(2H, m). IR(film): 3113.8, 2976.1, 1714.8, 1607.7, 1528.6, 1450.6, 1353.5, 1274.4, 1105.8, 1056.1 cm^{-1}. MS(m/e, CI): 264 (M^++1). Anal. Calcd for $C_{14}H_{17}O_4N$: C, 63.86; H, 6.51; N, 5.32; Found : C, 64.06; H, 6.54; N, 5.19.

(R)-(-)-5-Methyl-4-hexen-2-ol p-nitrobenzoate (27a') (24a)

This compound was prepared from (R)-(-)-5-methyl-4-hexen-2-ol (50 mg, 0.44 mmole, from the enzymatic resolution). mp 47.0-49.0°. $[\alpha]_D^{21.0}$ = -31.5°(c 1.30, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ): 1.36(3H, d, 6.3Hz, CHC*H*$_3$), 1.63(3H, s, CC*H*$_3$),

1.70(3H, s, CCH_3), 2.34(1H, m, CHCH_2CH), 2.45(1H, m, CHCH_2CH), 5.16(1H, m, OCHCH3), 5.16(1H, m, CH=C), 8.19(2H, m), 8.28(2H, m). IR(film): 3113.8, 2976.1, 1714.8, 1607.7, 1528.6, 1450.6, 1353.5, 1274.4, 1105.8, 1056.1 cm^{-1}. MS(m/e, CI): 264 (M^++1). Anal. Calcd for $C_{14}H_{17}O_4N$: C, 63.86; H, 6.51; N, 5.32; Found : C, 64.06; H, 6.54; N, 5.19.

(S)-(+)-5-Methyl-4-hexen-2-ol p-nitrobenzoate (27b') (24a)

This compound was prepared from (S)-(+)-5-methyl-4-hexen-2-ol (50 mg, 0.44 mmole, from the enzymatic resolution). mp 44.0-47.0°. $[\alpha]_D^{21.0}$ = +18.24°(c 1.65, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ): 1.36(3H, d, 6.3Hz, CHCH_3), 1.63(3H, s, CCH_3), 1.70(3H, s, CCH_3), 2.34(1H, m, CHCH_2CH), 2.45(1H, m, CHCH_2CH), 5.16(1H, m, OCHCH3), 5.16(1H, m, CH=C), 8.19(2H, m), 8.28(2H, m). IR(film): 3113.8, 2976.1, 1714.8, 1607.7, 1528.6, 1450.6, 1353.5, 1274.4, 1105.8, 1056.1 cm^{-1}. MS(m/e, CI): 264 (M^++1). Anal. Calcd for $C_{14}H_{17}O_4N$: C, 63.86; H, 6.51; N, 5.32; Found : C, 64.06; H, 6.54; N, 5.19.

Preparation of the Mosher esters of (R) and (S)-5-methyl-4-hexen-2-ol (41).

(R)-5-Methyl-4-hexen-2-ol, (S)-α-Methoxy-α-(trifluoromethyl)-phenylacetate (29a)

A mixture of 4-dimethylaminopyridine (DMAP, 18 mg, 0.15 mmole) and triethylamine (100 μL), in methylene chloride (0.5 mL), was treated with (R)-(-)-5-methyl-4-hexen-2-ol (17 mg, 0.15 mmole, from epoxide), and 30 μL of neat (S)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA chloride, 40.5 mg, 0.16 mmole) was added. This solution was stirred at room temperature for 2 h and the reaction was then quenched by addition of 3 drops of water. The mixture was passed through a short plug of

silica gel (1.5 cm, 20% ethyl actate in hexane as eluent) to yield 40.3 mg (82%) of oily product.

^{1}Hmr ($CDCl_3$, δ): 1.258(3H, d, 6.3Hz, $CHCH_3$), 1.48(3H, s, CCH_3), 1.76(3H, s, CCH_3), 2.17(1H, m, $CHCH_2CH$), 2.42(1H, m, $CHCH_2CH$), 3.55(3H, s, OCH_3), 3.55(1H, m, $CHCH_3$), 5.10(1H, m, C*H*=C), 7.43(3H, m) 7.53(2H, m). d.e. 98%

(S)-5-Methyl-4-hexen-2-ol, (S)-α-Methoxy-α-(trifluoromethyl)-phenylacetate (29b)

This compound was prepared from (S)-(+)-5-methyl-4-hexen-2-ol (17 mg, 0.15 mmole, from epoxide). 41.2 mg of oily product were collected (86%). ^{1}Hmr ($CDCl_3$, δ): 1.326(3H, d, 6.3Hz, $CHCH_3$), 1.53(3H, s, CCH_3), 1.63(3H, s, CCH_3), 2.20(1H, m, $CHCH_2CH$), 2.33(1H, m, $CHCH_2CH$), 3.55(3H, s, OCH_3), 3.56(1H, m, $CHCH_3$), 5.13(1H, m, C*H*=C), 7.43(3H, m), 7.53(2H, m). d.e. 97.2%

(R)-5-Methyl-4-hexen-2-ol, (S)-α-Methoxy-α-(trifluoromethyl)-phenylacetate (29a')

This compound was prepared from (R)-(-)-5-methyl-4-hexen-2-ol (17 mg, 0.15 mmole, from enzymatic resolution). 41.2 mg of oily product were collected (86%). ^{1}Hmr ($CDCl_3$, δ): 1.258(3H, d, 6.3Hz, $CHCH_3$), 1.48(3H, s, CCH_3), 1.76(3H, s, CCH_3), 2.17(1H, m, $CHCH_2CH$), 2.42(1H, m, $CHCH_2CH$), 3.55(3H, s, OCH_3), 3.55(1H, m, $CHCH_3$), 5.10(1H, m, C*H*=C), 7.43(3H, m) 7.53(2H, m). d.e. 97%

(S)-5-Methyl-4-hexen-2-ol, (S)-α-Methoxy-α-(trifluoromethyl)-phenylacetate (29b')

This compound was prepared from (S)-(+)-5-methyl-4-hexen-2-ol (17 mg, 0.15 mmole, from enzymatic resolution). 41.2 mg of oily product were collected (86%). ^{1}Hmr ($CDCl_3$, δ): 1.326(3H, d, 6.3Hz, $CHCH_3$), 1.53(3H, s, CCH_3), 1.63(3H, s, CCH_3),

2.20(1H, m, CHC*H*2CH), 2.33(1H, m, CHC*H*2CH), 3.55(3H, s, OC*H*3), 3.56(1H, m, C*H*CH3), 5.13(1H, m, C*H*=C), 7.43(3H, m), 7.53(2H, m). d.e. 54%

(±)-5-Methyl-4-hexen-2-ol, (S)-α-Methoxy-α-(trifluoromethyl)-phenylacetate (29)

This compound was prepared from (±)-5-methyl-4-hexen-2-ol (17 mg, 0.15 mmole, from epoxide). 41.2 mg of oily product was collected (86%). ^{1}Hmr ($CDCl_3$, δ): 1.258(1.5H, d, 6.3Hz, CHC*H*3), 1.326(1.5H, d, 6.3Hz, CHC*H*3), 1.48(1.5H, s, CC*H*3), 1.53(1.5H, s, CC*H*3), 1.63(1.5H, s, CC*H*3), 1.76(1.5H, s, CC*H*3), 2.17(0.5H, m, CHCH*H*CH), 2.20(0.5H, m, CHCH*H*CH), 2.33(0.5H, m, CHC*H*HCH), 2.42(0.5H, m, CHCH*H*CH), 3.55(3H, s, OC*H*3), 3.56(1H, m, C*H*CH3), 5.10(0.5H, m, C*H*=C), 5.13(0.5H, m, C*H*=C), 7.43(3H, m), 7.53(2H, m).The spectrum shows 1:1 mixture of epimers.

(R)-(-)-2-Tert-butyldimethylsiloxy-5-methyl-4-hexene(5a) (30)

A solution of (R)-(-)-5-methyl-4-hexen-2-ol (2.721 g, 23.83 mmoles, $[\alpha]_D^{21.0}$ = -11.95°), DBU (0.7 mL, 713 mg, 4.7 mmoles), and triethylamine (3.4 mL, 2.45 g, 24.2 mmoles) in methylene chloride (150 mL) was cooled in an ice-water bath, and t-butyldimethylsilyl chloride (5.75 g, 38.1 mmoles) was added in three portions during 10 min. The reaction mixture was stirred overnight while being warmed to room temperature, and then poured into 150 mL of water. The aqueous phase was extracted with ether (3 x 30 mL) and the combined organic layers were washed with 0.5 N hydrochloric acid (30 mL), saturated sodium bisulfate (2 x 30 mL), saturated sodium chloride (2 x 30 mL), dried over anhydrous magnesium sulfate and concentrated. The residue was distilled to give 3.42 g (62%) of colourless liquid, bp 61-65°/0.1 torr. $[\alpha]_D^{23.5}$ = -5.04°(c 1.19, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ): 0.033(3H, s, SiC*H*3), 0.050(3H, s, SiC*H*3), 0.88(9H, s, SiC(C*H*3)3), 1.11(3H, d, 6.1Hz, CHC*H*3), 1.692(3H, s, CC*H*3), 1.694(3H, s, CC*H*3), 2.06(1H, m,

$CHCH_2CH$), 2.16(1H, m, $CHCH_2CH$), 3.77(1H, m, $CHCH_3$), 5.13(1H, m, CH=C). IR(film): 2940, 2915, 2842, 1652, 1464, 1455, 1370, 1250, 1130, 1089, 840, 776 cm^{-1}.

(S)-(+)-2-Tert-butyldimethylsiloxy-5-methyl-4-hexene(5b) (30)

This compound was prepared from (S)-(+)-5-methyl-4-hexen-2-ol ($[\alpha]_D^{21.0}$ = +11.75°) and purified by distillation. (65%), bp 62-66°/0.1 torr. $[\alpha]_D^{21.5}$ = +5.04°(c 1.80, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ): 0.033(3H, s, $SiCH_3$), 0.050(3H, s, $SiCH_3$), 0.88(9H, s, $SiC(CH_3)_3$), 1.11(3H, d, 6.1Hz, $CHCH_3$), 1.692(3H, s, CCH_3), 1.694(3H, s, CCH_3), 2.06(1H, m, $CHCH_2CH$), 2.16(1H, m, $CHCH_2CH$), 3.77(1H, m, $CHCH_3$), 5.13(1H, m, CH=C). IR(film): 2940, 2915, 2842, 1652, 1464, 1455, 1370, 1250, 1130, 1089, 840, 776 cm^{-1}.

(±)-2-Tert-butyldimethylsiloxy-5-methyl-4-hexene (5) (30)

This compound was obtained from (±)-5-methyl-4-hexen-2-ol in 64% yield, bp 62-66°/0.1 torr. ^{1}Hmr ($CDCl_3$, δ): 0.033(3H, s, $SiCH_3$), 0.050(3H, s, $SiCH_3$), 0.88(9H, s, $SiC(CH_3)_3$), 1.11(3H, d, 6.1Hz, $CHCH_3$), 1.692(3H, s, CCH_3), 1.694(3H, s, CCH_3), 2.06(1H, m, $CHCH_2CH$), 2.16(1H, m, $CHCH_2CH$), 3.77(1H, m, $CHCH_3$), 5.13(1H, m, CH=C). IR(film): 2940, 2915, 2842, 1652, 1464, 1455, 1370, 1250, 1130, 1089, 840, 776 cm^{-1}.

(R)-(-)-5-Tert-butyldimethylsiloxy-2-hydroxy-2-methyl-3-hexanone (6a) (31,32)

A mixture of (R)-(-)-2-tert-butyldimethylsiloxy-5-methyl-4-hexene (5.85 g, 25.4 mmoles, $[\alpha]_D^{23.5}$ = -5.04°), reagent grade acetone (115 mL), water (43 mL) and acetic acid (4.75 mL) was stirred at room temperature, and a solution of potassium permanganate (6.0 g, 38.0 mmoles) in water (25 mL) and acetone (93 mL) was added quickly. The reaction mixture was stirred for 1.5 h and then poured onto saturated sodium bisulfite (500

mL). The aqueous phase was extracted with ether (4 x 60 mL), and the combined organic extracts were washed successively with saturated sodium bicarbonate (2 x 50 mL), saturated sodium chloride (2 x 60 mL), dried over anhydrous magnesium sulfate and concentrated. The residue was purified by flash chromatography on silica gel (5% and 14% ethyl acetate in hexane) to yield 5.85 g (88.4%) of a colourless liquid. $[\alpha]_D^{21.0}$ = -32.05°(c 1.56, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ): 0.017(3H, s, SiC*H*$_3$), 0.063(3H, s, SiC*H*$_3$), 0.840(9H, s, SiC(C*H*$_3$)$_3$), 1.179(3H, d, 6.1Hz, CHC*H*$_3$), 1.328(3H, s, CC*H*$_3$), 1.339(3H, s, CC*H*$_3$), 2.474(1H, dd, 16.0, 4.8Hz, CHC*H*$_2$CO), 2.834(1H, dd, 16.0, 7.6Hz, CHC*H*$_2$CO), 4.39(1H, m, C*H*CH$_3$). IR(film): 3480.3, 2957.8, 2857.5, 1711.7, 1472.2, 1375.5, 1255.7, 1136.4, 1078.8, 1004.5, 836.2 cm^{-1}. MS(m/e, Cl): 261 (M^++1), 243 (M^+-H_2O), 159 [$CH_3CH^+O(CH_3)_2C(CH_3)_3$]. Anal. Calcd for $C_{13}H_{28}O_3Si$: C, 59.95; H, 10.84; Found : C, 59.78; H, 10.99.

(S)-(+)-5-Tert-butyldimethylsiloxy-2-hydroxy-2-methyl-3-hexanone (6b) (31,32)

This compound was prepared from (S)-(+)-2-tert-butyldimethylsiloxy-5-methyl-4-hexene ($[\alpha]_D^{21.5}$ = +5.04°) in 88% yield. $[\alpha]_D^{23.0}$ = +32.2°(c, 1.42, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ): 0.017(3H, s, SiC*H*$_3$), 0.063(3H, s, SiC*H*$_3$), 0.840(9H, s, SiC(C*H*$_3$)$_3$), 1.179(3H, d, 6.1Hz, CHC*H*$_3$), 1.328(3H, s, CC*H*$_3$), 1.339(3H, s, CC*H*$_3$), 2.474(1H, dd, 16.0, 4.8Hz, CHC*H*$_2$CO), 2.834(1H, dd, 16.0, 7.6Hz, CHC*H*$_2$CO), 4.39(1H, m, C*H*CH$_3$). IR(film): 3480.3, 2957.8, 2857.5, 1711.7, 1472.2, 1375.5, 1255.7, 1136.4, 1078.8, 1004.5, 836.2 cm^{-1}. MS(m/e, CI): 261 (M^++1), 243 (M^+-H_2O), 159 [$CH_3CH^+O(CH_3)_2C(CH_3)_3$]. Anal. Calcd for $C_{13}H_{28}O_3Si$: C, 59.95; H, 10.84; Found : C, 60.09; H, 10.70.

(±)-5-Tert-butyldimethylsiloxy-2-hydroxy-2-methyl-3-hexanone (6) (31,32)

This compound was prepared from (±)-2-tert-butyldimethylsiloxy-5-methyl-4-hexene in 88% yield. ^{1}Hmr ($CDCl_3$, δ): 0.017(3H, s, SiC*H*$_3$), 0.063(3H, s, SiC*H*$_3$), 0.840(9H, s, SiC(C*H*$_3$)$_3$), 1.179(3H, d, 6.1Hz, CHC*H*$_3$), 1.328(3H, s, CC*H*$_3$), 1.339(3H, s, CC*H*$_3$), 2.474(1H, dd, 16.0, 4.8Hz, CHC*H*$_2$CO), 2.834(1H, dd, 16.0, 7.6Hz, CHC*H*$_2$CO), 4.39(1H, m, C*H*CH$_3$). IR(film): 3480.3, 2957.8, 2857.5, 1711.7, 1472.2, 1375.5, 1255.7, 1136.4, 1078.8, 1004.5, 836.2 cm^{-1}. MS(m/e, CI): 261 (M^++1), 243 (M^+-H_2O), 159 [$CH_3CH^+O(CH_3)_2C(CH_3)_3$]. Anal. Calcd for $C_{13}H_{28}O_3Si$: C, 59.95; H, 10.84; Found : C, 60.15; H, 10.77.

Methyl hydrazodicarboxylate (21) (35a)

A solution of hydrazine hydrate (10.0 g, 0.2 mole) in 95% alcohol (50 mL) was cooled to 10°, and methyl chloroformate (30.91 mL, 37.8 g, 0.4 mole) was added dropwise with stirring at a rate sufficient to maintain the temperature between 15° and 20°. After half of the methyl chloroformate had been added, a solution of sodium carbonate (21.2 g, 0.2 mole) in water (80 mL) was added dropwise simultaneously with the remaining methyl chloroformate at a rate sufficient to maintain the temperature below 20°. After the addition of the reactants was complete, the mixture was stirred at room temperature for 1 h. The precipitate was collected, washed with 10 mL of ice water and air dried. The filtrate was concentrated to 70 mL under reduced pressure, and cooled in an ice-water bath. The second crop was collected, washed with 10 mL of ice-water and air dried. The combined crude methyl hydrazodicarboxylate was dried in vacuo at 60° and then stirred with warm acetone (2 x 80 mL) to produce 26.0 g of methyl hydrazodicarboxylate as a white solid, yield 88%. ^{1}Hmr($CDCl_3$, δ): 3.76(6H, s, C*H*$_3$), 6.48(2H, br, N*H*). MS(m/e, CI): 149 (M^++1).

Dimethylazodicarboxylate (DMAD) (22) (35b)

Methyl hydrazodicarboxylate (6.4 g, 43.2 mmoles) was added at room temperature to a solution of N-bromosuccinimide (12 g, 67 mmoles) and pyridine (5.3 mL, 67 mmoles) in methylene chloride (250 mL). The mixture was shaken occusionally for 30 min, and then washed with water (4 x 250 mL), dried over anhydrous sodium sulfate and concentrated. The residue was distilled to yield 4.5 g (72%) of an orange liquid, bp 45-50°/0.8 torr. ^{1}Hmr ($CDCl_3$, δ): 4.10(6H, s, C*H*$_3$). IR(film): 3061.2, 2960.9, 1782.3, 1724.5, 1583.7, 1454.5, 1286.6, 1244.2, 1105.3 cm^{-1}. MS(m/e, CI): 147 (M^++1).

N-(Tert-butoxycarbonyl)-D-serine (23a) (34)

A mixture of D-serine (3.8 g, 36.2 mmoles), dioxane (30 mL), water (30 mL) and triethylamine (8.0 mL, 57.1 mmoles) was stirred at room temperature and Boc-ON (9.8 g, 39.8 mmoles) was added in one portion. The resulting solution was stirred at 25° for 4 h, and then concentrated under reduced pressure to a volume of 30 mL. Water (150 mL) was added, and the solution was washed with ether (2 x 20 mL). The aqueous phase was acidified to pH 4 with 10% potassium bisulfate, and extracted with ethyl acetate (4 x 50 mL). The combined ethyl acetate extracts were washed with saturated sodium chloride (2 x 60 mL), dried over anhydrous magnesium sulfate and concentrated to give 5.282 g of a pale yellow syrup. ^{1}Hmr ($CDCl_3$, δ): 1.47(9H, s, C(C*H*$_3$)$_3$), 3.94(2H, m, C*H*$_2$), 4.41(1H, m, C*H*), 5.78(1H, m, N*H*). IR(film): 3333.9, 2980.6, 1716.2, 1514.8, 1454.2, 1370.2, 1246.5, 1163.5, 1047.9 cm^{-1}. MS(m/e, CI): 206 (M^++1),

N-(Tert-butoxycarbonyl)-L-serine (23b) (34)

This compound was prepared in 77% yield from L-serine. ^{1}Hmr ($CDCl_3$, δ): 1.47(9H, s, C(C*H*$_3$)$_3$), 3.94(2H, m, C*H*$_2$), 4.41(1H, m, C*H*), 5.78(1H, m, N*H*). IR(film): 3333.9, 2980.6, 1716.2, 1514.8, 1454.2, 1370.2, 1246.5, 1163.5, 1047.9 cm^{-1}. MS(m/e, CI): 206(M^++1),

N-(Tert-butoxycarbonyl)-D-serine-β-lactone (24a) (34)

A solution of triphenylphosphine (2.0 g, 7.65 mmoles) in anhydrous tetrahydrofuran (30 mL) was stirred under nitrogen, cooled to -78°, and dimethylazodicarboxylate (DMAD, 0.9 mL, 1.19 g, 8.2 mmoles) was added dropwise during 10 min. The mixture was stirred for an additional 10 min, and a solution of dry N-(tert-butoxycarbonyl)-D-serine (1.20 g, 5.85 mmoles) in tetrahydrofuran (30 mL) was then added dropwise during 25 min. Stirring was continued at -78° for 30 min and for 3 h at room temperature. The solvent was then removed and the residue was chromatographed on silica gel (35% ethyl acetate in hexane) to give 0.638 g (58%) of a white solid. mp. 117.5-118.5°. $[\alpha]_D^{21.4}$ = +10.91°(c 1.10, CH_2Cl_2); ^{1}Hmr (d_6-acetone, δ): 1.40(9H, s, $OC(CH_3)_3$), 4.404(2H, m, CH_2), 4.454(1H, m, CH), 5.214(1H, m, NH). IR(KBr pellet): 3360, 3032, 2978, 1844, 1680, 1534, 1370, 1290 cm^{-1}. MS(m/e, CI): 188 (M^++1), 132 (M^+-$C(CH_3)_3$). Anal. Calcd for $C_8H_{13}O_4N$: C, 51.33; H, 6.99; N, 7.48; Found : C, 51.60; H, 6.96; N, 7.53.

N-(Tert-butoxycarbonyl)-L-serine-β-lactone (24b) (34)

This material was prepared from N-(tert-butoxycarbonyl)-L-serine in 57% yield. mp. 117.5-118.5°. $[\alpha]_D^{21.4}$ = -10.81°(c, 0.93, CH_2Cl_2); ^{1}Hmr (d_6-acetone, δ): 1.40(9H, s, $OC(CH_3)_3$), 4.404(2H, m, CH_2), 4.454(1H, m, CH), 5.214(1H, m, NH). IR(KBr pellet): 3360, 3032, 2978, 1844, 1680, 1534, 1370, 1290 cm^{-1}. MS(m/e, CI): 188 (M^++1), 132 (M^+-$C(CH_3)_3$). Anal. Calcd for $C_8H_{13}O_4N$: C, 51.33; H, 6.99; N, 7.48; Found : C, 51.59; H, 7.05; N, 7.62.

(±)-5-Tert-butyldimethylsiloxy-2-methyl-2-methanesulfonyl-oxy-3-hexanone (7) (21)

A mixture of (±)-5-tert-butyldimethylsiloxy-2-hydroxy-2-methyl-3-hexanone (1.10 g, 4.2 mmoles) and triethylamine (1.0 mL, 7.1 mmoles) in methylene chloride (150 mL)

was stirred in an ice-water bath, and methanesulfonyl chloride (0.5 mL, 6.3 mmoles) in methylene chloride (10 mL) was added dropwise. The reaction mixture was stirred at 0-10° for 5 h and poured onto water (150 mL). The organic layer was separated and the aqueous layer was extracted with methylene chloride (3 x 50 mL). The combined organic extracts were washed with 0.5 N hydrochloric acid (20 mL), saturated sodium bicarbonate (2 x 60 mL), saturated sodium chloride (2 x 60 mL), dried over anhydrous magnesium sulfate and concentrated. The residue was flash chromatographed on silica gel (20% ethyl acetate in hexane) to yield 1.12 g (79%) of a colourless liquid. ^{1}Hmr ($CDCl_3$, δ): 0.016(3H, s, $SiCH_3$), 0.069(3H, s, $SiCH_3$), 0.840(9H, s, $SiC(CH_3)_3$), 1.184(3H, d, 6.1Hz, $CHCH_3$), 1.633(3H, s, CCH_3), 1.675(3H, s, CCH_3), 2.589(1H, dd, 17.3, 4.6Hz, $CHCH_2CO$), 2.941(1H, dd, 17.3, 7.5Hz, $CHCH_2CH$), 3.113(3H, s, OCH_3), 4.40(1H, m, $CHCH_3$). IR(film): 2957.5, 2856.8, 2360.1, 1706.8, 1471.9, 1373.9, 1255.5, 1138.3, 1079.8, 1006.1 cm^{-1}.

(R)-5-Tert-butyldimethylsiloxy-2-methyl-2-methanesulfonyloxy-3-hexanone (7a) (21)

This compound was prepared in 78% yield from (R)-(-)-5-tert-butyldimethylsiloxy-2-hydroxy-2-methyl-3-hexanone ($[\alpha]_D^{21.0}$ = -32.1°). $[\alpha]_D^{21.0}$ = -10.1°(c 3.16, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ): 0.016(3H, s, $SiCH_3$), 0.069(3H, s, $SiCH_3$), 0.840(9H, s, $SiC(CH_3)_3$), 1.184(3H, d, 6.1Hz, $CHCH_3$), 1.633(3H, s, CCH_3), 1.675(3H, s, CCH_3), 2.589(1H, dd, 17.3, 4.6Hz, $CHCH_2CO$), 2.941(1H, dd, 17.3, 7.5Hz, $CHCH_2CH$), 3.113(3H, s, OCH_3), 4.40(1H, m, $CHCH_3$). IR(film): 2957.5, 2856.8, 2360.1, 1706.8, 1471.9, 1373.9, 1255.5, 1138.3, 1079.8, 1006.1 cm^{-1}.

(S)-5-Tert-butyldimethylsiloxy-2-methyl-2-methanesulfonyloxy-3-hexanone (7b) (21)

This compound was prepared in 78% yield from (S)-(+)-5-tert-butyldimethylsiloxy-2-hydroxy-2-methyl-3-hexanone ($[\alpha]D^{23.0}$ = +32.3°). $[\alpha]D^{21.0}$ = +10.6°(c 1.22, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ): 0.016(3H, s, SiC*H*$_3$), 0.069(3H, s, SiC*H*$_3$), 0.840(9H, s, SiC(C*H*$_3$)$_3$), 1.184(3H, d, 6.1Hz, CHC*H*$_3$), 1.633(3H, s, CC*H*$_3$), 1.675(3H, s, CC*H*$_3$), 2.589(1H, dd, 17.3, 4.6Hz, CHC*H*$_2$CO), 2.941(1H, dd, 17.3, 7.5Hz, CHC*H*$_2$CH), 3.113(3H, s, OC*H*$_3$), 4.40(1H, m, C*H*CH$_3$). IR(film): 2957.5, 2856.8, 2360.1, 1706.8, 1471.9, 1373.9, 1255.5, 1138.3, 1079.8, 1006.1 cm^{-1}.

(±)-5-Tert-butyldimethylsiloxy-2-methyl-2-mercapto-3-hexanone (8) (21,33)

Triethylamine (4.75 mL) was added to methylene chloride (60 mL). This solution was stirred, cooled to -15° and gaseous hydrogen sulfide was passed in during 20 min. A solution of (±)-5-tert-butyldimethylsiloxy-2-methyl-2-methanesulfonyloxy-3-hexanone (3.93 g, 11.6 mmoles) in methylene chloride (20 mL) was then added dropwise with stirring during 30 min. The mixture was stirred at -15° - 0° for 5 h with continous introduction of hydrogen sulfide, and was then poured into water (100 mL). The organic layer was separated and the aqueous layer was extracted with methylene chloride (3 x 50 mL). The combined organic layers were washed with 0.5 N hydrochloric acid (20 mL), water (2 x 50 ml), saturated sodium sulfate (2 x 60 mL), dried over anhydrous magnesium sulfate, and concentrated. Chromatography on silica gel (10% ethyl acetate in hexane) yielded 2.58 g (80%) of a colourless liquid. ^{1}Hmr ($CDCl_3$, δ): 0.011(3H, s, SiC*H*$_3$), 0.056(3H, s, SiC*H*$_3$), 0.844(9H, s, SiC(C*H*$_3$)$_3$), 1.18(3H, d, 6.0Hz, CHC*H*$_3$), 1.484(3H, s, CC*H*$_3$), 1.521(3H, s, CC*H*$_3$), 1.98(1H, s, S*H*), 2.626(1H, dd, 16.0, 5.2Hz, CHC*H*HCO), 2.980(1H, dd, 16.0, 7.2Hz, CHC*H*HCO), 4.36(1H, m, C*H*CH$_3$). IR(film): 2929.7, 2856.9, 1707.0, 1461.8, 1374.0, 1255.4, 1137.9, 1079.6, 1005.8 cm^{-1}. MS(m/e): 277 (M^+), 245 (M^+-SH). Anal. Calcd for $C_{13}H_{28}O_2SSi$: C, 56.47; H, 10.21; Found : C, 56.25; H, 10.07.

(R)-5-Tert-butyldimethylsiloxy-2-methyl-2-mercapto-3-hexanone (8a) (21,33)

This compound was prepared from (R)-(-)-5-tert-butyldimethylsiloxy-2-methyl-2-methanesulfonyloxy-3-hexanone ($[\alpha]_D^{21.0}$ = -10.1°) in 78% yield. $[\alpha]_D^{21.0}$ = -33.5°(c 1.01, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ): 0.011(3H, s, SiC*H*$_3$), 0.056(3H, s, SiC*H*$_3$), 0.844(9H, s, SiC(C*H*$_3$)$_3$), 1.18(3H, d, 6.0Hz, CHC*H*$_3$), 1.484(3H, s, CC*H*$_3$), 1.521(3H, s, CC*H*$_3$), 1.98(1H, s, S*H*), 2.626(1H, dd, 16.0, 5.2Hz, CHC*H*HCO), 2.980(1H, dd, 16.0, 7.2Hz, CHC*H*HCO), 4.36(1H, m, C*H*CH$_3$). IR(film): 2929.7, 2856.9, 1707.0, 1461.8, 1374.0, 1255.4, 1137.9, 1079.6, 1005.8 cm^{-1}. MS(m/e): 277 (M^+), 245 (M^+-SH). Anal. Calcd for $C_{13}H_{28}O_2SSi$: C, 56.47; H, 10.21; Found : C, 56.34; H, 10.28.

(S)-5-Tert-butyldimethylsiloxy-2-methyl-2-mercapto-3-hexanone (8b) (21,33)

This compound was prepared from (S)-(+)-5-tert-butyldimethylsiloxy-2-methyl-2-methanesulfonyloxy-3-hexanone ($[\alpha]_D^{21.0}$ = +10.6°) in 78% yield. $[\alpha]_D^{21.0}$ = +33.3°(c 1.02, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ): 0.011(3H, s, SiC*H*$_3$), 0.056(3H, s, SiC*H*$_3$), 0.844(9H, s, SiC(C*H*$_3$)$_3$), 1.18(3H, d, 6.0Hz, CHC*H*$_3$), 1.484(3H, s, CC*H*$_3$), 1.521(3H, s, CC*H*$_3$), 1.98(1H, s, S*H*), 2.626(1H, dd, 16.0, 5.2Hz, CHC*H*HCO), 2.980(1H, dd, 16.0, 7.2Hz, CHC*H*HCO), 4.36(1H, m, C*H*CH$_3$). IR(film): 2929.7, 2856.9, 1707.0, 1461.8, 1374.0, 1255.4, 1137.9, 1079.6, 1005.8 cm^{-1}. MS(m/e): 277 (M^+), 245 (M^+-SH). Anal. Calcd for $C_{13}H_{28}O_2SSi$: C, 56.47; H, 10.21; Found : C, 56.52; H, 10.02.

(2S)-2-(Tert-butoxycarbonylamino)-4-thia-5,5-dimethyl-6-oxo-8-(tert-butyldimethylsiloxy)-nonanoic acid (10) (45)

A solution of (±)-5-tert-butyldimethylsiloxy-2-methyl-2-mercapto-3-hexanone (147 mg, 0.53 mmole) in dry tetrahydrofuran (5 mL) was cooled to -40°, stirred, and lithium diisopropylamide [1.0 N, 530 μL, 0.53 mmole, freshly prepared by adding 1.0 mL of n-butyllithium (2.5 M in ether) to 1.5 mL diisopropyl amine in tetrahydrofuran solution (v/v=1/2) at -40°] was added dropwise. When the addition was complete, N-(tert-butoxycarbonyl)-D-serine-β-lactone (68.8 mg, 0.37 mmole, $[\alpha]_D^{21.4}$ = +10.91°) in tetrahydrofuran (2 x 5 mL) was added dropwise. The reaction mixture was stirred for 6 h at -40 to -5°, diluted with methylene chloride (50 mL) and washed with 0.5 N hydrochloric acid (10 mL). The organic layer was separated and the aqueous layer was extracted with methylene chloride (3 x 15 mL). The combined organic extracts were washed with water (2 x 15 mL), saturated sodium chloride (2 x 20 mL), dried over anhydrous magnesium sulfate and concentrated. The residue was chromatographed on silica gel (15% ethyl acetate in methylene chloride, containing 1% acetic acid) to yield 60.5 mg of a pale yellow oil. This material was azeotroped with toluene to yield 52.4 mg (34%) of the product. $[\alpha]_D^{26.0}$ = -9.4°(c 0.42, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ) : 0.01(3H, s, SiC*H*$_3$), 0.06(3H, s, SiC*H*$_3$), 0.89(9H, s, SiC(C*H*$_3$)$_3$), 1.18(3H, d, 6.0Hz, CHC*H*$_3$), 1.44(15H, s, OC(C*H*$_3$)$_3$ & 2 -CC*H*$_3$), 2.60(1H, dd, 5.4Hz, 16Hz, CHC*H*HCO), 2.81(2H, m, SC*H*$_2$CH), 2.96(1H, dd, 7.3Hz, 16Hz, CHC*H*HCO), 4.33(1H, m, SCH$_2$C*H*), 4.49(1H, m, OC*H*CH$_3$), 5.25(1H, m, N*H*). IR(film): 3429.7, 2968.6, 2872.2, 1751.5, 1712.9, 1500.7, 1569.5, 1161.2 cm^{-1}. MS(m/e, CI): 464 (M^++1), 446 (M^+-H_2O). Anal. Calcd for $C_{21}H_{41}O_6SSi$: C, 54.39; H, 8.91; N, 3.02; Found : C, 54.16; H, 8.72; N, 2.96.

(2R,8R)-2-(Tert-butoxycarbonylamino)-4-thia-5,5-dimethyl-6-oxo-8-(tert-butyldimethylsiloxy)-nonanoic acid (10b) (45)

A solution of (R)-5-tert-butyldimethylsiloxy-2-methyl-2-mercapto-3-hexanone (322.1 mg, 1.165 mmoles, $[\alpha]_D^{21.0}$ = -33.5°) in dry tetrahydrofuran (15 mL) was cooled to -40°, stirred, and lithium diisopropylamide (1.0 N, 1200 μL, 1.2 mmoles) was added

dropwise. Then N-(tert-butoxycarbonyl)-L-serine-β-lactone (223.8 mg, 1.19 mmoles, $[\alpha]_D^{21.4}$ = -10.81°) in dry tetrahydrofuran (2 x 10 mL) was added dropwise. The reaction mixture was stirred for 6 h at -40 to -5°, and then diluted with methylene chloride (100 mL), and washed with 0.5 N hydrochloric acid (20 mL). The organic layer was separated and the aqueous layer was extracted with methylene chloride (3 x 25 mL). The combined organic layers were washed with water (2 x 25 mL), saturated sodium chloride (2 x 30 mL), dried over anhydrous magnesium sulfate and concentrated. The residue was purified by flash chromatography on silica gel (9% methanol in methylene chloride) and then by preparative layer chromatography (silica gel, thickness 1.0 mm, size 20 x 20 cm) to give 84.3 mg (16%) of (2R,8R)-2-(tert-butoxycarbonylamino)-4-thia-5,5-dimethyl-6-oxo-8-(tert- butyldimethylsiloxy)-nonanoic acid as a pale yellow syrup. $[\alpha]_D^{23.5}$ = -26.9°(c 0.45, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ) : 0.005(3H, s, SiC*H*$_3$), 0.055(3H, s, SiC*H*$_3$), 0.862(9H, s, SiC(C*H*$_3$)$_3$), 1.165(3H, d, 6.0Hz, CHC*H*$_3$), 1.459(15H, s, OC(C*H*$_3$)$_3$ & 2 -CC*H*$_3$), 2.598(1H, dd, 5.4Hz, 16Hz, CHC*H*HCO), 2.76(2H, m, SC*H*$_2$CH), 2.949(1H, dd, 7.3Hz, 16Hz, CHC*H*HCO), 4.157(1H, m, SCH$_2$C*H*). 4.327(1H, m, OC*H*CH$_3$), 5.523(1H, m, N*H*). IR(film): 3429.7, 2974.4, 2858.7, 1755.3, 1712.9, 1498.8, 1369.5, 1263.5, 1163.2 cm^{-1}. MS(m/e, CI): 464 (M^++1), 446 (M^+-H_2O). Anal. Calcd for $C_{21}H_{41}O_6SSi$: C, 54.39; H, 8.91; N, 3.02; Found : C, 54.13; H, 9.00; N, 2.89 .

(2R,8S)-2-(Tert-butoxycarbonylamino)-4-thia-5,5-dimethyl-6-oxo-8-(tert-butyldimethylsiloxy)-nonanoic acid (10a) (45)

This material was prepared from (S)-5-tert-butyldimethylsiloxy-2-methyl-2-mercapto-3-hexanone (0.230 g, 0.832 mmole, $[\alpha]_D^{21.0}$ = +33.3°) and N-(tert-butoxycarbonyl)-L-serine-β-lactone (0.197 g, 1.0 mmole, $[\alpha]_D^{21.4}$ = -10.81°). 51.4 mg (11%). $[\alpha]_D^{23.5}$ = -33.0°(c 0.46, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ) : 0.007(3H, s, SiC*H*$_3$), 0.047(3H, s, SiC*H*$_3$), 0.846(9H, s, SiC(C*H*$_3$)$_3$), 1.161(3H, d, 6.0Hz, CHC*H*$_3$),

1.442(15H, s, OC(CH_3)$_3$.& 2 -CCH_3), 2.563(1H, dd, 5.4Hz, 16.3Hz, CHC*H*HCO), 2.77(2H, m, SCH_2CH), 2.905(1H, dd, 7.3Hz, 16.3Hz, CHC*H*HCO), 4.332(1H, m, SCH_2C*H*). 4.489(1H, m, OC*H*CH_3), 5.250(1H, m, N*H*). IR(film): 3427.7, 2974.4, 2858.7, 1714.8, 1500.7, 1369.5, 1161.2, 1064.9 cm^{-1}. MS(m/e, CI): 464 (M^++1), 446 (M^+-H_2O). Anal. Calcd for $C_{21}H_{41}O_6SSi$: C, 54.39; H, 8.91; N, 3.02; Found : C, 54.59; H, 8.77; N, 3.22.

(2S,8R)-2-(Tert-butoxycarbonylamino)-4-thia-5,5-dimethyl-6-oxo-8-(tert-butyldimethylsiloxy)-nonanoic acid (10d) (45)

This compound was prepared from (R)-5-tert-butyldimethylsiloxy-2-methyl-2-mercapto-3-hexanone (0.245 g, 0.9 mmole, $[\alpha]_D^{21.0}$ = -33.5°) and N-(tert-butoxycarbonyl)-D-serine-β-lactone (0.198 g, 1.05 mmoles, $[\alpha]_D^{21.4}$ = +10.91°). 94.0 mg (23%). $[\alpha]_D^{23.5}$ = +33.9°(c 1.12, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ) : 0.003(3H, s, SiCH_3), 0.057(3H, s, SiCH_3), 0.843(9H, s, SiC(CH_3)$_3$), 1.162(3H, d, 6.0Hz, CHCH_3), 1.419(15H, s, OC(CH_3)$_3$.& 2 -CCH_3), 2.604(1H, dd, 5.4Hz, 16.3Hz, CHC*H*HCO), 2.81(2H, m, SCH_2CH), 2.947(1H, dd, 7.3Hz, 16.3Hz, CHC*H*HCO), 4.110(1H, m, SCH_2C*H*). 4.325(1H, m, OC*H*CH_3), 5.592(1H, m, N*H*). IR(film): 3429.7, 2972.5, 2856.8, 1757.3, 1712.9, 1500.7, 1369.5, 1163.1 cm^{-1}. MS(m/e, CI): 464 (M^++1), 446 (M^+-H_2O). Anal. Calcd for $C_{21}H_{41}O_6SSi$: C, 54.39; H, 8.91; N, 3.02; Found : C, 54.40; H, 8.80; N, 2.97.

(2S,8S)-2-(Tert-butoxycarbonylamino)-4-thia-5,5-dimethyl-6-oxo-8-(tert-butyldimethylsiloxy)-nonanoic acid (10c) (45)

This material was prepared from (S)-5-tert-butyldimethylsiloxy-2-methyl-2-mercapto-3-hexanone (0.245 g, 0.9 mmole, $[\alpha]_D^{21.0}$ = +33.3°) and N-(tert-butoxycarbonyl)-D-serine-β-lactone (0.200 g, 1.07 mmoles, $[\alpha]_D^{21.4}$ = +10.91°). 60.0 mg (15%). $[\alpha]_D^{23.5}$ = +27.2°(c 0.81, CH_2Cl_2); ^{1}Hmr ($CDCl_3$, δ) : 0.006(3H, s,

SiC*H*3), 0.056(3H, s, SiC*H*3), 0.843(9H, s, SiC(C*H*3)3), 1.163(3H, d, 6.0Hz, CHC*H*3), 1.442(15H, s, OC(C*H*3)3 & 2 -CC*H*3), 2.602(1H, dd, 5.4Hz, 16.3Hz, CHC*H*HCO), 2.78(2H, m, SC*H*2CH), 2.968(1H, dd, 7.3Hz, 16.3Hz, CHC*H*HCO), 4.173(1H, m, SCH2C*H*). 4.312(1H, m, OC*H*CH3), 5.490(1H, m, N*H*). IR(film): 3429.7, 2968.6, 2872.2, 1751.5, 1712.9, 1500.7, 1369.5, 1161.2 cm^{-1}. MS(m/e, CI): 464 (M^++1), 446 (M^+-H_2O). Anal. Calcd for $C_{21}H_{41}O_6SSi$: C, 54.39; H, 8.91; N, 3.02; Found : C, 54.47; H, 8.75; N, 3.12.

(5S,8R)-2,2-Dimethyl-3-(2'-hydroxypropyl)-5-carboxy-Δ^3-1,4-thiazine (1a)

(2S,8R)-2-(Tert-butoxycarbonylamino)-4-thia-5,5-dimethyl-6-oxo-8-(tert-butyldimethylsiloxy)-nonanoic acid (20.0 mg, 0.043 mmole, $[\alpha]_D^{23.5}$ = +33.9°) was dissolved in 0.5 mL of methylene chloride and 1.0 mL of formic acid and 3 drops of 1,3-propanedithiol were added. The reaction mixture was stirred at room temperature for 10 h. Most of the formic acid and solvent were then removed by lyophilization and the residue was diluted with ether (5 mL) and water (5 mL). The ether layer was extracted with water (2 x 5 mL) and the combined aqueous extracts were brought to pH 8 with 5% sodium bicarbonate. Lyophilization of this solvent yielded a white solid (20 mg). This was purified by HPLC, The HPLC conditions were:

wavelength of UV detector : 254 nm ; sensitivity : 1.0 AUS

injection volume: 1.0-1.5 mL ; concentration : 5.0 mg/mL

Collection of the fraction with retention time 9.79 min yielded 2.5 mg (25%) of purified product. $[\alpha]_D^{24.0}$ = -75°(c 0.080, 50% acetone/H_2O); ^{1}Hmr (D_2O, δ) : 1.16(3H, d, 6.2Hz, CHC*H*3), 1.45(6H, s, 2 -C*H*3), 2.75(1H, m, CHC*H*HC=N), 2.82(2H, m, SC*H*2CH), 2.93(1H, m, CHC*H*HC=N), 4.23(1H, m, SCH2C*H*). 4.52(1H, m, C*H*CH2C=N). IR(film): 3421.7, 2972.4, 2364.5 1751.5, 1686.1, 1636.7, 1388.2, 1074.3 cm^{-1}. MS(m/e): 231 (M^+).

(5R,8R)-2,2-Dimethyl-3-(2'-hydroxypropyl)-5-carboxy-Δ^3-1,4-thiazine (1b)

This material was prepared from (2R,8R)-2-(tert-butoxycarbonylamino)-4-thia-5,5-dimethyl-6-oxo-8-(tert-butyldimethylsil-oxy)-nonanoic acid (20.0 mg, 0.043 mmole, $[\alpha]_D^{23.5}$ = -26.9°). The crude product was obtained a white solid (21.0 mg), and purified by HPLC. Collection of the fraction with retention time 9.87 min yielded 3.1 mg (31%) of material. $[\alpha]_D^{24.0}$ = -155°(c 0.045, 50% acetone/H_2O); ^{1}Hmr (D_2O, δ) : 1.18(3H, d, 6.2Hz, CHC*H*$_3$), 1.39(6H, s, 2 -C*H*$_3$), 2.68(1H, m, CHC*H*HC=N), 2.84(2H, m, SC*H*$_2$CH), 2.97(1H, m, CHC*H*HC=N), 4.24(1H, m, SCH$_2$C*H*). 4.53(1H, m, C*H*CH$_2$C=N). IR(film): 3421.7, 2972.4, 2364.5 1751.5, 1686.1, 1636.7, 1388.2, 1074.3 cm^{-1}. MS(m/e): 231(M^+).

(5S,8S)-2,2-Dimethyl-3-(2'-hydroxypropyl)-5-carboxy-Δ^3-1,4-thiazine (1c)

This material was prepared from (2S,8S)-2-(tert-butoxycarbonyl-amino)-4-thia-5,5-dimethyl-6-oxo-8-(tert-butyldimethylsiloxy)-nonanoic acid (20.0 mg, 0.043 mmole, $[\alpha]_D^{23.5}$ = +27.2°). The crude product 19.8 mg, was purified by HPLC to yield 2.4 mg (24%) of material. $[\alpha]_D^{24.0}$ = +155°(c 0.055, 50% acetone/H_2O); ^{1}Hmr (D_2O, δ) : 1.19(3H, d, 6.2Hz, CHC*H*$_3$), 1.456(6H, s, 2 -C*H*$_3$), 2.68(1H, m, CHC*H*HC=N), 2.84(2H, m, SC*H*$_2$CH), 2.97(1H, m, CHC*H*HC=N), 4.22(1H, m, SCH$_2$C*H*). 4.32(1H, m, C*H*CH$_2$C=N). IR(film): 3421.7, 2972.4, 2364.5 1751.5, 1686.1, 1636.7, 1388.2, 1074.3 cm^{-1}. MS(m/e): 231 (M^+).

(5R,8S)-2,2-Dimethyl-3-(2'-hydroxypropyl)-5-carboxy-Δ^3-1,4-thiazine (1d)

This material was prepared from (2R,8S)-2-(tert-butoxycarbonylamino)-4-thia-5,5-dimethyl-6-oxo-8-(tert-butyldimethylsil-oxy)-nonanoic acid (20.0 mg, 0.043 mmole, $[\alpha]_D^{23.5}$ = -33.0°). The crude product was obtained a white solid (20.5 mg), and purified by HPLC. 3.0 mg (30%) of product. $[\alpha]_D^{24.0}$ = +73°(c 0.060, 50% acetone/H_2O); ^{1}Hmr (D_2O, δ) : 1.18(3H, d, 6.2Hz, CHC*H*$_3$), 1.45(6H, s, 2 -C*H*$_3$), 2.68(1H, m, CHC*H*HC=N), 2.84(2H, m, SC*H*$_2$CH), 2.97(1H, m, CHC*H*HC=N), 4.22(1H, m, SCH$_2$C*H*). 4.53(1H, m, C*H*CH$_2$C=N). IR(film): 3421.7, 2972.4, 2364.5 1751.5, 1686.1, 1636.7, 1388.2, 1074.3 cm^{-1}. MS(m/e): 231 (M^+).

Bioassay of 1a-1b

Bioassays were carried out using 4.0 mm filter paper discs loaded with known weights of material and placed on each bacterial plate seeded with a sensitive strain of *S. aureus*. The plate was incubated at 37° overnight (51).

References

[1] A. Fleming. Br. J. Exp. Pathol. **10**, 226 (1929)

[2] G. G. F. Newton and E. P. Abraham. Biochem. J. **58**, 103 (1954)

[3] C. Breading and M. Cole. Antimicrob. Agents. Chemother. **11**, 852 (1977); T. T. Howarth and A. G. Brown. J. Chem. Soc. Commun. 266 (1976)

[4] D. F. Corbett, A. J. Eglington and T. T. Howarth. J. Chem. Soc., Chem. Commun. 953 (1977); A. G. Brown, D. F. Corbett, A. J. Eglinton and T. T. Howarth. ibid. 523 (1977);

[5] G. Albers-Schonberg, B. H. Arison, O. D. Hensens, J. Hirshfield, K. Hoogsteen, E. A. Kaczka, R. E. Rhodes, J. S. Kahan, F. M. Kahan, R. W. Ratcliffe, E. Walton, L. J. Ruswinkle, R. B. Morin and B. G. Christensen. J. Amer. Chem. Soc. **100**, 649 (1978)

[6] H. Aoki, H. Sakai, M. Kohsaka, T. Konami, J. Hosoda, Y. Kubochi, E. Iguchi and H. Imanaka. J. Antibiot. **29**, 492 (1976)

[7] I. Phillips, A. King, K. Shannon and C. Warren. J. Antimicrob. Chemother. **11**, 1 (1983)

[8] P. M. Blumberg and J. L. Strominger. Bacteriol, Rev. **38**, 291 (1974)

[9] J. L. Strominger and D. J. Waxman. Ann. Rev. Biochem. **52**, 825 (1983).

[10] R. A. Nicholas, J. L. Strominger, H. Suzuki and Y. Hirota. J. Bacteriol. **104**, 456 (1985); J. -M. Ghuysen, J. -M. Frere, M. Leyh-Bouille, M. Nguyen-Disteche and J. Coyette. Biochem. J. **235**, 159 (1986)

[11] A. M. Hadonou, J. M. Wilkin, L. Varetto, B. Joris, J. Lamotte-Brasseur, D. Klein, C. Duez, J. M. Ghuysen and J. M. Frere. Eur. J. Biochem. **207**, 97 (1992)

[12] E. P. Abraham and E. Chain. Nature. **146**, 837 (1940)

[13] O. Herzberg and J. Moult. Current Opinion in Structural Biology, **1**, 946 (1991)

[14] J. A. Kelly, O. Dideberg, P. Charlier, J. P. Wery, M. Libert, P. C. Moews, J. R. Knox, C. Duez, Cl. Fraipont, B. Joris, J, Dusart, J. -M. Ghuysen and J. -M. Frere. Science, **231**, 1429 (1986).

[15] J. -M. Ghuysen. Annu. Rev. Microbiol. **45**, 37 (1991).

[16] H. Christensen, M. T. Martin and S. G. Waley. Biochem. J. **266**, 853 (1990).

[17] S. Wolfe, K. Yang and M. Khalil. Can. J. Chem. **66**, 2733 (1988).

[18] S. Wolfe. Can. J. Chem. In press.

[19] S. Wolfe, C.-K. Kim and K. Yang. Can. J. Chem. Submitted for publication.

[20] S. Wolfe and T. Hoz. Can. J. Chem. Submitted for publication.

[21] S. Wolfe, H. Jin, C.-K. Kim and E. McEachern. Can. J. Chem. Submitted for publication.

[22] G. Hilgetag and A. Martini. "Preparative Organic Chemistry", John Wiley & Sons Inc. 1972, New York, pp 821.

[23] E. A. Braude and E.A. Evans. J. Chem. Soc. 3328 (1955).

[24] a) B. D. Johnston and K. N. Slessor. Can. J. Chem. **57**, 733 (1979).
b) H. C. Brown, Y. M. Choi and S. Narasimhan. J. Org. Chem. **47**, 3153 (1982).

[25] M. K. Ellis and T. Golding. Org. Synth. **63**, 140 (1985).

[26] P. E. Sonnet. Chemtech. 94 (1988).

[27] G. Kirchner, M. P. Scollar and A. M. Klibanov. J. Amer. Chem. Soc. **107**, 7072 (1985); T. M. Stokes and A. C. Oehlschlager. Tetrahedron Lett. **28**, 2091 (1987); B. Morgan, A. C. Oehlschlager and T. M. Stokes. Tetrahedron. **47**, 1611 (1991);

[28] L. T. Kanerva, J. Vihanto, E. Pajunen and E. K. Euranto. Acta. Chem. Scand. **44**, 489 (1990).

[29] C. F. H. Allen and F. W. Spangler "Organic Syntheses"; Coll. Vol. III. E. C. Horning, Ed.; Wiley; New York, 1955, pp 203-204.

[30] T. Kolasa and M. J. Miller. J. Org. Chem. **55**, 1711 (1990).

[31] S. Wolfe, C. F. Ingold and R. U. Lemieux. J. Am. Chem. Soc. **103**, 938 (1981).

[32] N. S. Srinivasan and D. G. Lee. Synthesis. 520 (1979).

[33] A. K. Bose, V. Sudarsanam, B. Anjaneyulu and M. S. Manhas. Tetrahedron, **25**, 1194 (1969).

[34] L. D. Arnold, R. G. May and J. C. Vederas. J. Amer. Chem. Soc. **110**, 2237 (1985).

[35] a) J. C. Kauer. "Organic Syntheses"; Coll. Vol. IV, Wiley; New York, 1963, pp 411-415.

b) D. Mackay and D. D. McIntyre. Can. J. Chem. **62**, 355 (1984).

[36] a) A. S. Rao, S. K. Pakinikar and J. G. Kirtane. Tetrahedron. **39**, 2323 (1983), and references cited therein.

b) C. -S. Chen and Y. -C. Liu. Tetra. Lett. **30**, 7165 (1989), and references cited therein.

[37] B. T. Golding, D. R. Hall and S. Sakrikar. J. Chem. Soc. Perkin I, 1214 (1973).

[38] C. Huynh, F. D. Boumechal and G. Liustrumelle. Tetra. Lett. **22**, 1503 (1981).

[39] a) J. A. Dale and H. S. Mosher. J. Amer. Chem. Soc. **95**, 512 (1973).

b) G. R. Sullivan, J. A. Dale and H. S. Mosher. J. Org. Chem. **38**, 2143 (1973).

[40] D. Parker. Chem. Rev. **91**, 1441 (1991).

[41] Y. Gao, R. M. Hanson, J. M. Klunder, S. Y. Ko, H. Masamune and K. B. Sharpless. J. Am. Chem. Soc. **109**, 5765 (1987).

[42] S. Hanessian and P. Lavallee. Can. J. Chem. **53**, 2975 (1975); E. J. Corey and A. Venkateswarlu. J. Am. Chem. Soc. **94**, 6190 (1972).

[43] Y. Sakata, Y. Katayama and Y. Ishii. Chem. Lett. 671 (1992) and references cited therein.

[44] D. G. Lee and V. S. Cheng. J. Org. Chem. **43**, 1532 (1978).

[45] L. D. Arnold, J. C. G. Drover and J. C. Vederas. J. Amer. Chem. Soc. **109**, 4649 (1987).

[46] S. E. Ramer, R. N. Moore and J. C. Vederas. Can. J. Chem. **64**, 706 (1986).

[47] O. Mitsunobu. Synthesis. 1 (1981).

[48] E. Grochowski, B. D. Hilton, R. J. Kupper and C. J. Michejda. J. Am. Chem. Soc. **104**, 6876 (1982).

[49] R. L. Noble, D. Yamashiro and C. H. Li. J. Am. Chem. Soc. **98**, 2324 (1976).

[50] B. F. Lundt, N. L. Johansen, A. Volund and J. Markussen. Int. J. Peptide Protein Res. **12**, 258 (1978).

[51] E. P. Abraham, E. Chain, C. M. Fletcher, H. W. Florey, A. G. Gardener, N. G. Heatley and M. A. Jennings. Lancet. **2**, 177 (1941).

[52] S. Wolfe, C. Zhang, B. D. Johnston and C.-K. Kim. Can. J. Chem. Submitted for publication.