CHEMOENZYMATIC SYNTHESES OF NOVEL ENANTIOPURE OXAZOLIDINES AND β-LACTAMS

by

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ABSTRACT

This thesis describes the preparation of several important enantiopure compounds. The "green chemistry" approach employed throughout the project combines simple traditional syntheses with biocatalysis to realize several transformations with high regio- and enantioselectivity.

Enantiopure 3-hydroxy-β-lactams are important building blocks for the synthesis of many bioactive compounds including paclitaxel side chain analogues. The poor aqueous solubility and growing drug resistance of paclitaxel significantly restricted its clinical applications and promoted a search for better analogues, particularly the analogues with modified C13 side chain since the modifications in the C13 side chain are relatively easy to make, and were shown to improve paclitaxel's performance. The novel β-lactams, with modified chains in position 4 (the precursors of C13 side chain) were obtained in a series of simple and efficient steps. The corresponding enantiopure products were obtained *via* baker's yeast reduction or lipase-mediated kinetic resolution.

Several wild type and mutated reductases overexpressed in *E. coli* enlarge the family of new enantioselective bioreductants. The enantio- and stereoselectivity of purified enzymes were evaluated against β-chloro-α-keto ester and α-keto-β-lactam substrates. Screening identified red yeast, *Sporobolomyces salmonicolor* (SSCR) and its mutants as the most highly enantioselective bioreagents for reduction of β-chloro-α-keto ester. The screening of individual reductases for α-keto-β-lactams demonstrated that none of the enzymes was selective towards these rigid substrates. Since most reductases, either from different microorganisms or from the site-directed mutagenesis, exhibit excellent enantioselectivity for the reduction of acyclic α-keto esters, the lack of enantioselectivity

in the reduction of β-lactams was rationalized in terms of the rigid and symmetrical structure of the latter compounds. The mutations of amino acids close to the active site in the SSCR mutants were expected to enhance the *S*-selectivity according to Prelog's rule for enzymatic reductions. In fact, screening of the mutants showed them to be highly enantioselective and provided a method for the preparation of enantiopure oxazolidines.

Oxazolidines are useful biologically active molecules and are widely used as important chiral auxiliaries and ligands for asymmetric syntheses. A series of novel oxazolidines were synthesized in high yields using the simple and clean reaction of DMSO/P_4O_{10} as formaldehyde equivalent. This method was extended to the preparation of optically pure oxazolidines and α-hydroxy-β-amino esters from the enantiopure β-chloro-α-hydroxy ester obtained *via* biotransformations with new reductases.

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LIST OF ABBREVIATIONS

Ac	acetyl
Ar	aryl(aromatic)
Å	Ångstrom
Bn	benzyl
t-Boc	*tert*-butyoxycarbonyl
CAN	$(NH_4)_2Ce(NO_3)_6$, ammonium cerium nitrate
CMCR	*Candida magnoliae*
COBE	ethyl 4-chloro-3-oxobutanoate
Conf.	configuration
Conv.	conversion
CSA	camphorsulfonic acid
L-(+)-DET	diethyl L-tartrate
DKR	dynamic kinetic resolution
δ	chemical shift
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
DQCB	dihydroquinidine 4-chlorobenzoate
d. r.	diastereomer ratio
E. coli	*Escherichia coli*
ee	enantiomeric excess
Equiv.	equivalent

Et	ethyl
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
FID	flame ionization detector
GC	gas chromatography
GDH	glucose dyhydrogenase
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
Hz	Herz
7-HSDH	7α-hydroxy-steroid dehydrogenase, *Bacteroides fragilis*
r.t.	room temperature
RT	retention time
IR	infrared spectroscopy
Kpi	potassium phosphate buffer
LB	Luria-Bertani
LDA	lithium diisopropylamide
Lit.	literature
Me	methyl
MTPA	α-methoxy-α-trifluoromethylphenylacetic acid
MS	molecular sieves
MW	molecular weight
NADH	nicotinamide adenine dinucleotide

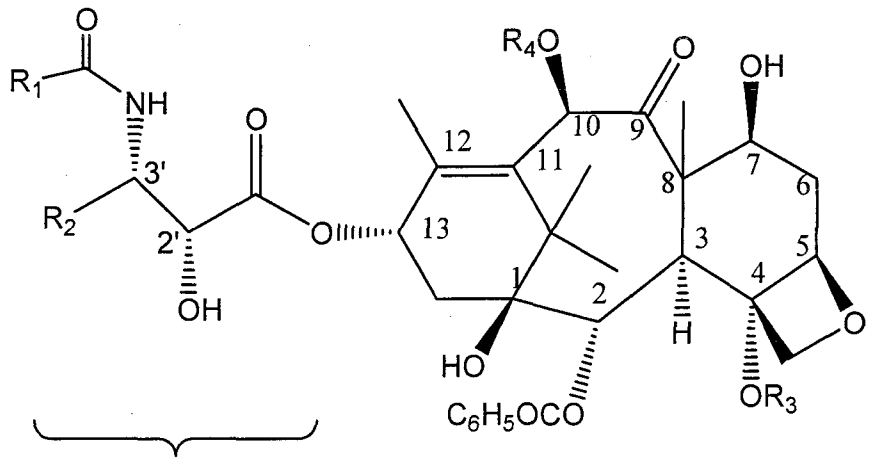
NADPH	nicotinamide adenine dinucleotide phosphate
NMR	nuclear magnetic resonance spectroscopy
NMMO	*N*-methyl-morpholine *N*-oxide
OD	Optical density
PFADH	*Archaeon Pyrococcus furiosus*
Ph	Phenyl
PMP	*p*-methoxylphenyl
i-Pr	*iso*-propyl
S.cerevisiae	*Saccharomyces cerevisiae*
SSCR	*Sporobolomyces salmonicolor*
TBDMS (TBS)	*tert*-butyldimethylsilyl
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy, free radical
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Tol.	toluene
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TsCl	*p*-toluenesulfonyl chloride
UV	ultraviolet spectroscopy
Vmax	the maximum enzyme velocity

CHAPTER I INTRODUCTION

1.1 Paclitaxel and its analogues

Paclitaxel (Taxol®) is one of the most powerful anticancer agents[1] and has been approved for the treatment of several cancers.[2] Because of its limited availability in nature, paclitaxel used in treatment is prepared by attaching a synthetic C-13 chain to a readily available natural product, 10-deacetylbaccatin III (baccatin III) obtained from the plants such as Taxus canadensis (**Figure 1-1**).[3, 4]

Although highly successful in cancer treatment, paclitaxel, like any drug, presents many problems. Its poor solubility in water, inability to cross the blood-brain barrier (BBB), lack of oral bioavailability,[5] and increasing paclitaxel-resistance, all of which were encountered in the treatment of many cancers, prompted the search for new paclitaxel analogues. In recent years, several analogues that compensate for some of paclitaxel's deficiencies have been identified.[6] For example, replacement of the benzoyl group with the Boc group on the nitrogen at position 3' of the C-13 side chain gave docetaxel (Taxotere®), which is more water soluble and highly active in the treatment of certain cancers.[7] Orally bioavailable analogue BMS-275183,[8] shows improved cytotoxicity and water solubility compared to paclitaxel,[9] and TX-67 can cross the BBB *in situ* (**Figure 1-1**).[10] In fact, many second-generation taxoids were synthesized in high yields from baccatin III or modified baccatin III coupled with two important chiral intermediates: (a) enantiopure phenyl glycidate **1-3**[11-13] and (b) enantiopure β-lactams **1-2**[14-17] (**Scheme 1-1**).

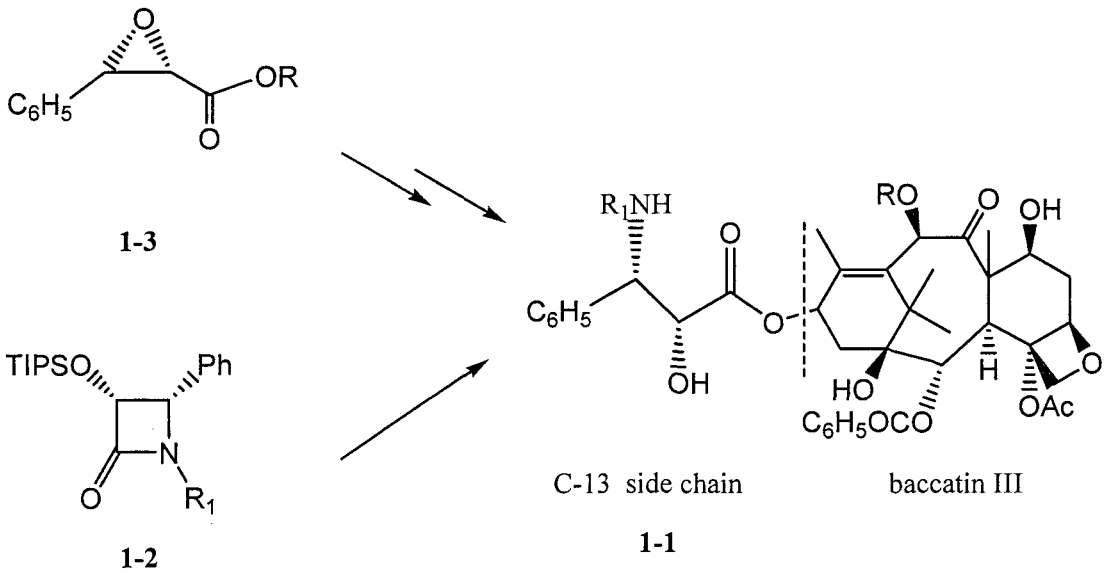


$R_1 = C_6H_5CO$, R_2 = Ph, R_3 = Ac, R_4 = Ac	Paclitaxel (Taxol®)
$R_1 = t$-BuO, R_2 = Ph, R_3 = Ac, R_4 = H	Docetaxel (Taxotere®)
R_1 = Ph, R_2 = Ph, R_3 = Ac, $R_4 = COCH_2CH_2CO_2H$	TX-67
$R_1 = t$- Bu, $R_2 = t$-Boc, $R_3 = CO_2Me$, R_4 = Ac	BMS-275183

Figure 1-1 Structures of paclitaxel and its analogues docetaxel, TX-67 and BMS-275183.

Enantiorich phenylglycidate **1-3** can be synthesized *via* asymmetric synthesis (**Schemes 1-2** and **1-3**)[11-12] or enzymatic resolution of **1-3** by *Mucor meihei* lipase[13] (**Scheme 1-4**). A suitably protected β-lactam **1-2**[18,19] can be prepared using chiral auxiliary groups during enolate condensation with an imine (**Scheme 1-5** and **Scheme 1-6**)[14,15] or by lipase resolution of 3-acetyl-β-lactams (**Scheme 1-7**).[16,17] Precursor β-lactams are readily attached to baccatin III through Holton's coupling protocol.[20-23] Both methodologies are discussed in the following sections.

Scheme 1-1 Alternative routes to coupling with baccatin III

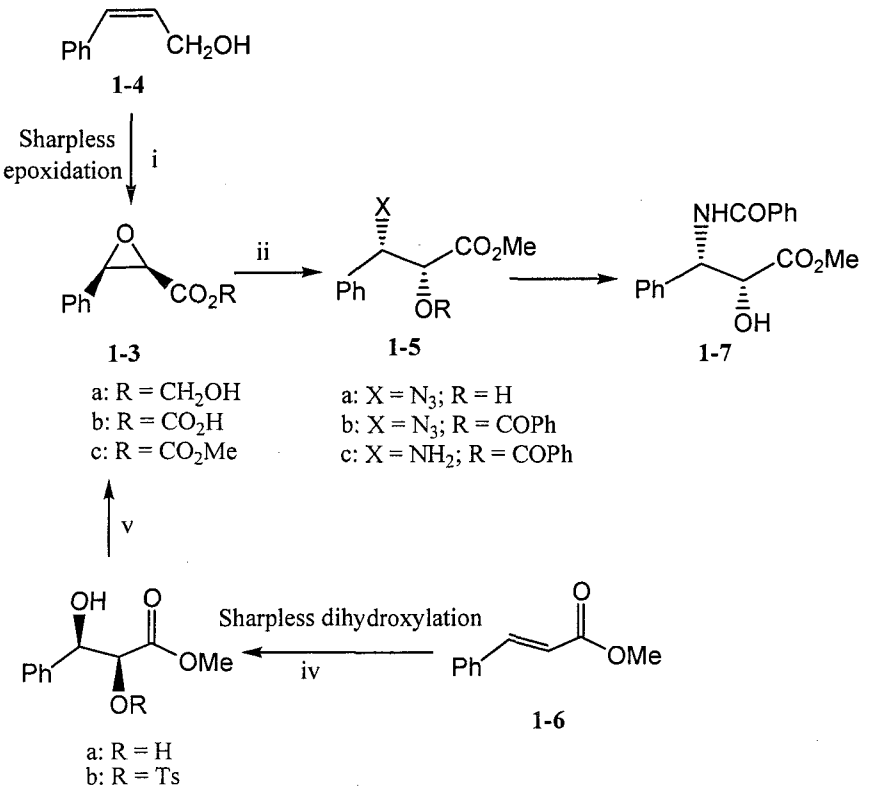


$R_1 = C_6H_5CO$ (Paclitaxel), R_2 = Ac
R_1 = *t*-BuOCO (Docetaxel), R_2 = H

1.1.1 Phenylglycidate routes toward the synthesis of the paclitaxel side chain

The epoxide functionality is a useful intermediate in the synthesis of the paclitaxel C-13 side chain. Greene and his group[11] were the first to report this phenylglycidate strategy. Sharpless epoxidation of *cis*-cinnamyl alcohol **1-4** followed by ruthenium trichloride-sodium periodate oxidation of the terminal alcohol to acid and subsequent esterification with diazomethane gave epoxy ester **1-3c.** Oxirane **1-3c** was opened with azidotrimethylsilane to give hydroxy azide **1-5a**. Azido benzoate **1-5b**, prepared from **1-5a**, was hydrogenated to produce the aminobenzoate **1-5c**, which rearranged *in situ* to give product **1-7** (**Scheme 1-2**). Greene *et al.* [3] reported an improved synthesis of the epoxide intermediate **1-3c** (82% ee) through Sharpless asymmetric dihydroxylation of the *trans*-methyl cinnamate **1-6,** also shown in **Scheme 1-2**.

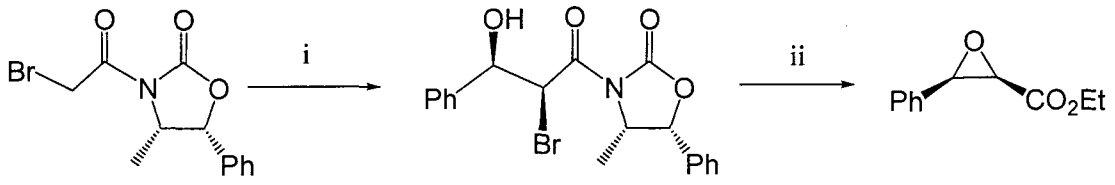
Scheme 1-2



Reagents: (i). (a). *t*-BuOOH, Ti(O*i*Pr)$_4$, L-(+)-DET (65%, 80% ee); (b). $RuCl_3$, $NaIO_4$, H^+; (c) CH_2N_2 (84%)
(ii).(a). Me_3SiN_3, $ZnCl_2$ (90%); (b). PhCOCl, Et_3N, DMAP (94%) (c). H_2 10%, Pd/C (89%)
(iv). (a). DQCB, NMMO, OsO_4 (cat.) 51%; (b). TsCl, Et_3N (88%)
(v). K_2CO_3, H_2O, DMF (91%)

Applying Evan's chiral auxiliary chemistry, Commerçon *et al.*[12] prepared bromoalcohol **1-9** through aldol condensation of benzaldehyde with bromoacetate **1-8**. The removal of the auxiliary group gave phenylglycidate **1-3** in 81% yield. Unfortunately, no enantiomeric excess was reported (**Scheme 1-3**).

Scheme 1-3

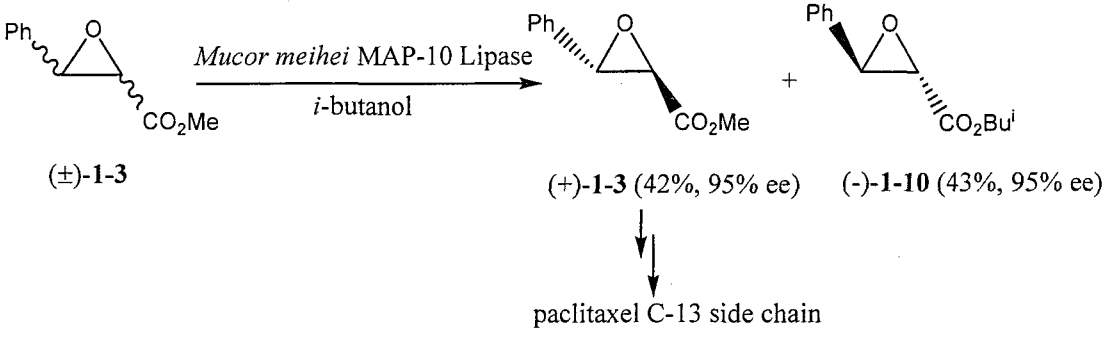


1-8 **1-9** **1-3**

Reagents: (i).(a). Et_3N, n-Bu_2BOTf; (b). PhCHO (58%)
(ii). EtOLi, THF (81%)

Another effective approach to the asymmetric synthesis of the paclitaxel side chain was reported by Chen *et al.*[13] who used lipase-mediated *trans*-esterification of methyl *trans*-phenylglycidate **1-3**. *Mucor meihei* MAP-10 lipase selectively hydrolyzed the (-)-methyl ester **1-3** and converted the acid to (-)-*i*-butyl ester **1-10** (95% ee) in the presence of *iso*-butanol. The unreacted (+)-methyl phenylgycidate **1-3** became an important enantiopure intermediate for making the final precursor of the paclitaxel side chain (**Scheme 1-4**).

Scheme 1-4



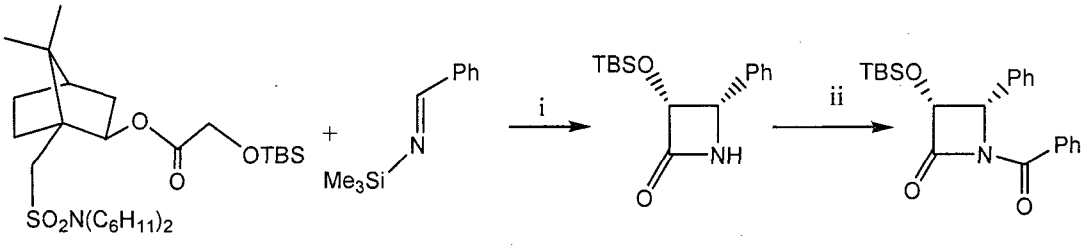
(±)-**1-3**

(+)-**1-3** (42%, 95% ee) (-)-**1-10** (43%, 95% ee)

1.1.2 Syntheses of paclitaxel C-13 side chain precursors: chiral β-lactams

Commercially, paclitaxel is prepared by acylation of 7-protected baccatin III with β-lactams (**Scheme 1-1**) which are prepared by the Staudinger reaction. In Holton's original approach, the racemic *cis* β-lactam was attached to baccatin III.[21] Highly successful asymmetric syntheses of suitably protected β-lactams were developed by Georg employing Oppolzer's chiral auxiliary **1-11**[15] (**Scheme 1-5**) and Ojima using (1*R*,2*S*)-2-phenyl cyclohexan-1-ol **1-13** (**Scheme 1-6**).[14] These two reactions involve additions of enolates derivatized with a chiral auxiliary to imines followed by lactamization. Other asymmetric syntheses of the β-lactam were developed using chiral imine precursors[24, 25] and oxazolidinone auxiliaries.[26]

Scheme 1-5. Georg's method.

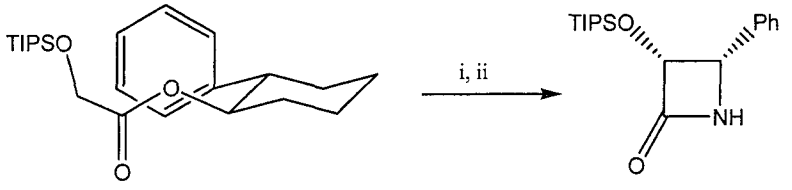


1-11 **1-12** **1-2**

Reagents: (i). LDA, THF (94%)

(ii). PhCOCl, CH_2Cl_2, Et_3N, DMAP (96% yield, 93-97% ee)

Scheme 1-6. Ojima's method.

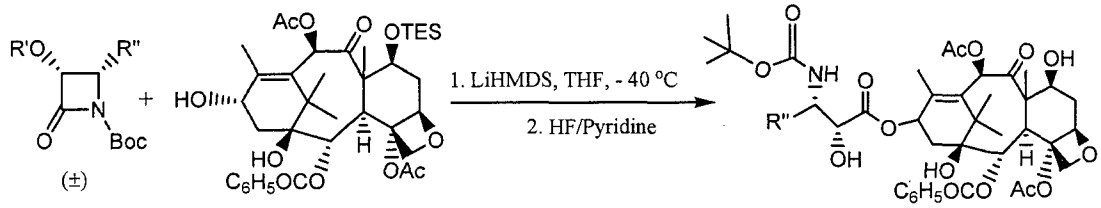


1-13 **1-12**

Reagents: (i). LDA (ii). PhCH=NTMS (85%yield, 96% ee)

Recently, Georg and her group[27] carried out a systematic study of the kinetic resolution of racemic *cis*-4-phenyl- and *cis*-4-*t*-butyl-3-hydroxy-β-lactam **1-2** with 7-*O*-triethylsilylbaccatin III . The product paclitaxel and butitaxel analogues were found to form with high diastereoselectivity (10:1 to 80:1) in favor of the natural 2'*R*, 3'*S* configuration (**Scheme 1-7**).

Scheme 1-7



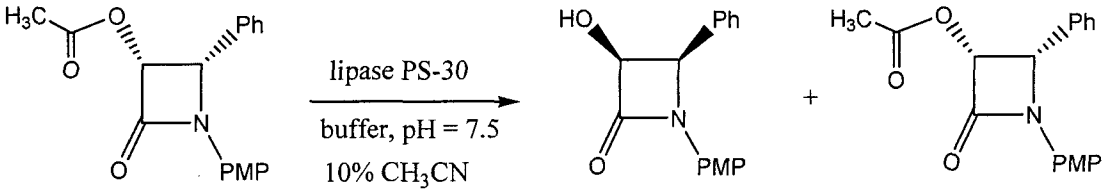
1-2
R' = TES, TBS, TIPS
R" = Ph, *t*-Bu

R" = Ph, *t*-Bu
10:1 to 80:1 d.r.

Enzyme-catalyzed resolution is another route to enantiopure β-lactams. Lipases are the most frequently used biocatalysts because they accept a wide spectrum of substrates and the reaction can be carried out in water or organic solvents.[28] These reactions require mild conditions, thus minimizing the problems associated with undesired side reactions such as isomerization, racemization, and rearrangements.[28] There have been several examples of lipase-catalyzed resolution of acetoxy β-lactams reported in the literature. The first enzyme-catalyzed kinetic resolution of racemic β-lactams was performed by Sih and his coworkers.[16] *Pseudomonas* lipase PS-30 was identified as the best catalyst among the several lipases studied.[16] Thus, lipase PS-30-catalyzed resolution of 3-acetoxy-β-lactam yielded the corresponding alcohol, (3*S*,4*R*) **1-14** in 98% ee and 46% yield (**Scheme 1-8**). This study showed that using 10% CH_3CN as

a co-solvent significantly improved the reaction rate and enantioselectivity of the hydrolysis. Later, Patel *et al.* [17] reported that lipase PS-30 immobilized on accurel polypropylene gave **1-14** with 96% ee and 48% yield.

Scheme 1-8



racemic *cis* **1-15**

(3*S*,4*R*) **1-14**
98% ee
46% yield

(3*R*,4*S*) **1-15**
99.5% ee
47% yield

It is important to remember that lipase resolution of racemic *cis* acetoxy-β-lactams can only give a maximum of 50% yield of the desired enantiomer. In order to achieve higher yields, enzymatic methods allowing dynamic kinetic resolution [29, 30] are necessary.

1.1.3 Structural modifications of paclitaxel

Structure-activity relationship studies,[31] have shown that the C-13 side chain and the baccatin ring system are both indispensable for bioactivity. In the side chain, the hydroxyl group at C-2' (**Figure 1-2**) is essential for activity. While the phenyl at C-3' can be replaced by other groups, the replacement of the 3'-N-benzoyl group with a *t*-butyloxycarbonyl (docetaxel) and other substituted benzoates provides analogues with equal or better biological activity as compared to paclitaxel. The natural stereochemistry at C-2' and C-3' (i.e. 2'*R*,3'*S*) is desirable for maximum activity (**Figure 1-2**).[32]

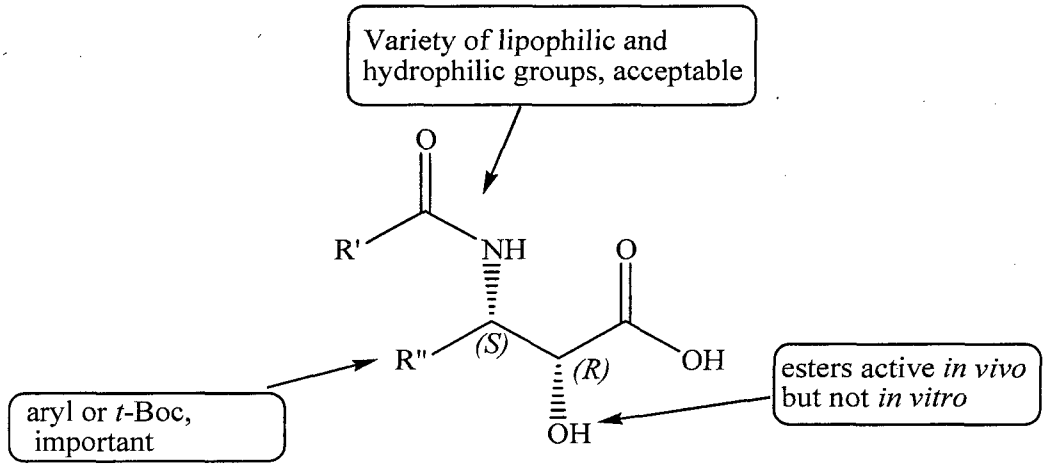


Figure 1-2 Influence of structural modifications on the cytotoxicity and improved water solubility of paclitaxel analogues.

Recently, successful modifications in baccatin III were reported by Soga and co-workers.[33] Four new analogues bearing a morpholine moiety on baccatin III **1-16a-d** showed stronger activity against several tumor cell lines expressing P-glycoprotein (PC-12, PC-6/VCR 29-9, and PC-6/VP1-1) than either paclitaxel or docetaxel (**Figure 1-3**).[34]

In view of the above results, it is important to develop new enantiopure paclitaxel side chains bearing polar functional groups such as a hydroxyl or morpholine group. The work describing the development of such novel paclitaxel side chains is the topic of Chapter **2**.

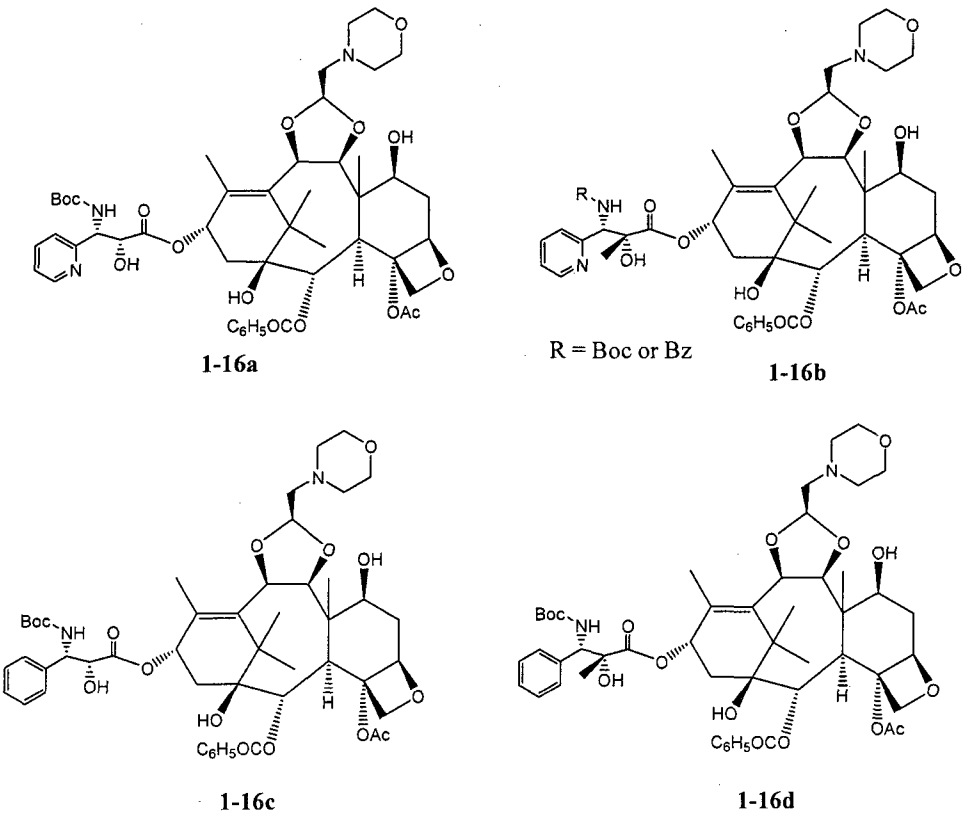


Figure 1-3 New taxane analogues bearing a morpholine moiety.

1.2 Reduction of α-ketoesters with reductases

Enzymatic or microbial transformations allow for the preparation of enantioenriched compounds in a "green" way because of the simplicity of the procedure and mildness of experimental conditions. Among enzymatic reactions, lipase-catalyzed asymmetric hydrolysis of esters and baker's yeast–mediated asymmetric reduction of prochiral ketones have been most extensively studied. Since the pioneering work of Neuberg at the beginning of the 20th century,[35] baker's yeast has remained the most popular whole-cell biocatalyst for asymmetric organic synthesis.[36,37] While baker's yeast reductions often proceed with high diastereo- and enantioselectivities, it is not always the

case. Since baker's yeast carries a large number of reducing enzymes, the low selectivities were believed to be the result of overlapping substrate specificities combined with differing enantio- and diastereoselectivities.[38] In other words, it was generally assumed that a mixture of products arises when a single substrate is accepted by multiple enzymes rather than a single enzyme with low selectivity. This assumption has inspired a search for methods to improve the selectivity of whole cell yeast-catalyzed reactions by altering the culture conditions during whole cell-mediated reductions (temperature, pH, *etc.*)[39] or use of inhibitors.[40] These methodologies work by selectively diminishing the catalytic activities of reductases that give unwanted products, but are rarely effective for a broad spectrum of substrates and give unpredictable results. Alternative approaches include the use of purified reductases from yeast or other organisms or gene knockout and overexpression technologies.[41] The concept for the design of the stereoselective yeast strain is illustrated in **Figure 1-4**.[42] Two basic genetic tools (gene overexpression and gene knockout) allow for the manipulation of the enzyme expression levels in yeast. In gene overexpression, the enzyme of interest is overproduced; while, in gene knockout, the competing enzyme is replaced with a nonfunctional variant.[42]

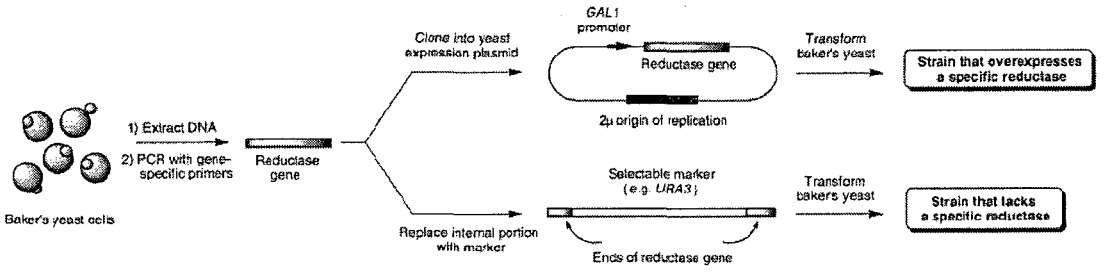
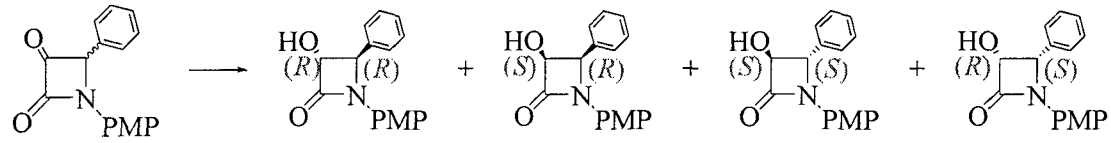


Figure 1-4 Two basic genetic tools (gene overexpression and gene knockout) allow for rational design of the engineered strain and improve the stereoselectivity of baker's yeast reduction.

The more efficient strategy, however, is to use reductases from microorganisms, such as the yeast cell *Saccharomyces cerevisiae*, cloned into a host cell such as *Escherichia coli* that possess fewer endogenous reductases. Much work in this field has been accomplished in recent years. For example, the nineteen reductases from *S. cerevisiae* were tested against 3-oxo-β-lactam as GST-purified enzymes and overexpressed in *E. coli*.[43] The results showed that only four enzymes were highly selective for the substrate tested (**Table 1-1**).

Table 1-1 Reduction of 3-oxo-β-lactam with selective GST-purified enzymes from baker's yeast. [43]

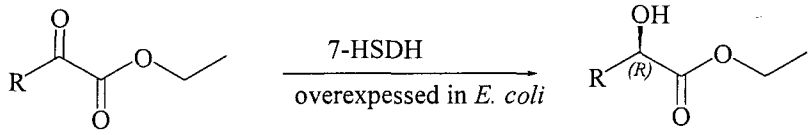


Yeast ORF	Conversion (%)	Product Composition (%)
YDL124W	68	*RR* (0), *SS* (10), *SR* (7), *RS* (83)
YCR107W	67	*RR* (0), *SS* (5), *SR* (89), *RS* (6)
YBR149W	73	*RR* (0), *SS* (4), *SR* (93), *RS* (3)
YJR096W	63	*RR* (0), *SS* (8), *SR* (15), *RS* (77)

Although much research has been focused on the enantioselective reductions of aryl ketones and β-ketoesters,[44] only a few studies on the enzymatic reduction of α-ketoesters have been reported.[45] It is especially evident in the case of biocatalytic reductions of aromatic α-ketoesters which have been shown to be less successful than their aliphatic counterparts. The enzymatic reduction of aromatic α-ketoesters with one chiral center has received scant attention, probably because of the difficulty in

preparation of the substrates and the instability of the products.[46,47] Recently, Hua *et al.*[48] have isolated and over-expressed a thermostable 7α-hydroxysteroid dehydrogenase (7-HSDH) from *Bacteroides fragilis* ATCC 25285 and examined its substrate specificity and stereoselectivity toward the reduction of various ketones including aromatic and aliphatic α-ketoesters. All α-ketoesters were reduced by 7-HSDH to (*S*)-enantiomers in >98% ee (**Table 1-2**).[49]

Table 1-2 Enantioselectivity of 7α-hydroxysteroid dehydrogenase toward various α-ketoesters.[49]



α-Ketoester (R=)	ee (%)	α-Ketoester (R=)	ee (%)
Phenyl	>99 (*S*)	4-Cyanophenyl	99 (*S*)
4-Flurophenyl	95 (*S*)	3,5-Difluorophenyl	98 (*S*)
4-Chlorophenyl	99 (*S*)	Isopropyl	99 (*S*)
4-Bromophenyl	99 (*S*)	Cyclohexanyl	>99 (*S*)
4-Methylphenyl	99 (*S*)	*t*-Butyl	>99 (*S*)

The use of engineered *E. coli* cells to express heterologous reductases is not restricted to those derived from *S. cerevisiae*. The heterologous reductases overexpressed in *E. coli* for screening in this project and the related references for overpression in *E. coli* and purification methods are listed in **Table 1-3**.

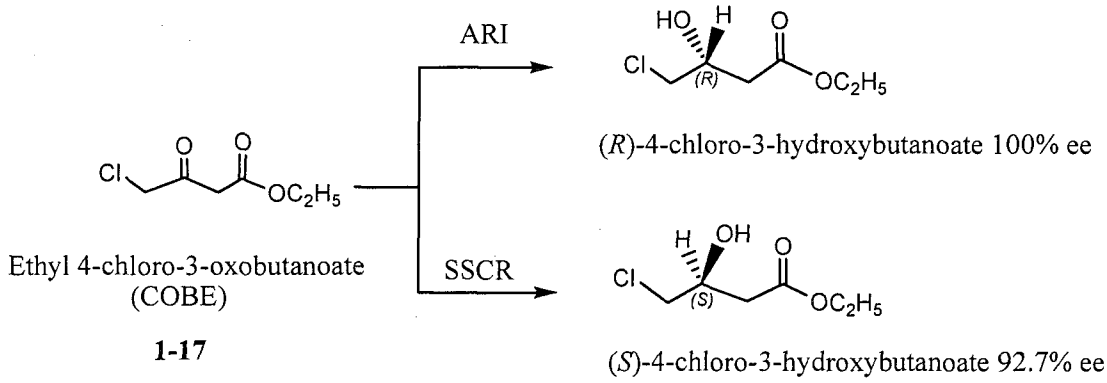
Table 1-3 Heterologous reductases overexpressed in *E. coli* with references for overexpression and purification methods.[50-56]

Gene name	Enzyme	Host	Reference
SSCR (wild type)	*Sporobolomyces salmonicolor* AKU4429	*E.coli BL21(DE3)*	[50], [51]
CMCR	*Candida magnoliae*	*E.coli Rosetta*2(DE3)pLysS	[52], [51]
7-HSDH	*Bacteroides fragilis* ATCC25285	*E.coli Rosetta2(DE3)pLysS*	[53], [51]
PFADH	Hyperthermophilic archaeon *Pyrococcus furiosus*	*E.coli BL21(DE3)*	[54], [51]
GRE2	*Saccharomyces cerevisiae EC100*	*E.coli Rosetta2(DE3)pLysS*	[55], [51]
YMR226c	*Saccharomyces cerevisiae EC100*	*E.coli Rosetta2(DE3)pLysS*	[56], [51]
SSCR N207V (Asn 207-Val)	*Sporobolomyces salmonicolor* (mutant)	*E.coli BL21(DE3)*	[50], [51]
SSCR Q245L (Gln 245-Lys)	*Sporobolomyces salmonicolor* (mutant)	*E.coli BL21(DE3)*	[50], [51]
SSCR K181R (Lys 181- Arg)	*Sporobolomyces salmonicolor* (mutant)	*E.coli BL21(DE3)*	[50], [51]
SSCR N207T (Asn 207-Tyr)	*Sporobolomyces salmonicolor* (mutant)	*E.coli BL21(DE3)*	[50], [51]
SSCR Q245P (Gln 245-Pro)	*Sporobolomyces salmonicolor* (mutant)	*E.coli BL21(DE3)*	[50], [51]
SSCR Q245H (Gln 245-His)	*Sporobolomyces salmonicolor* (mutant)	*E.coli BL21(DE3)*	[50], [51]
SSCR M242G (Met 242-Glu)	*Sporobolomyces salmonicolor* (mutant)	*E.coli BL21(DE3)*	[50], [51]

Note: (1) All enzymes use cofactor, NADPH except 7-HSDH and PFADH use NADH.
(2) All optimized pH is 6.5 except GRE2 and YMR226c use 6.5-7.0.

The purified reductases from different microorganisms, including *Candida magnoliae, Sporobolomyces salmonicolor* AKU4429, *Saccharomyces cerevisia, Bacteroides fragilis, Pyrococcus furiosus*, all belong to the short-chain dehydrogenese superfamily and share many similar properties, such as the requirement for a NAD(P)H cofactor.[57] All these enzymes have been purified and overexpressed in *E. coli* as a host vector.[51] *Sporobolomyces salmonicolor* AKU4429 (SSCR) introduced in this chapter, is also a member of the short-chain dehydrogenese family. Two enzymes, ARI and SSCR, isolated from *Sporobolomyces salmonicolor* AKU4429 by Kataoka and coworkers[58] were found to reduce 4-chloro-3-oxobutanoate (COBE) **1-17** to opposite enantiomers with very high enantioselectivity (**Scheme 1-9**). [50, 58]

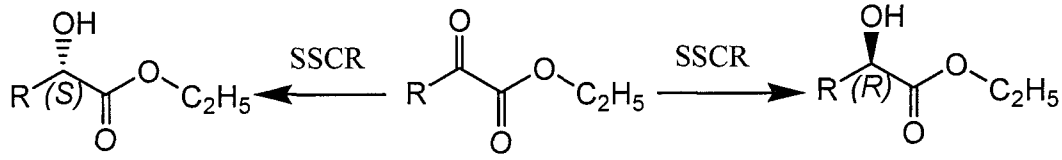
Scheme 1-9



Hua *et al.* reported that SSCR accepts a very broad range of substrates that include aliphatic and aromatic ketones, as well as α- and β-ketoesters.[59] The screening of a series of aromatic and aliphatic α-ketoesters showed that aliphatic α-ketoesters gave the (*R*)-configuration while aromatic compounds were reduced to (*S*)-alcohols. Concomitant substrate docking studies showed that the hydride transfer from NADPH

to the carbonyl groups of α-ketoesters occurs from different faces in the aromatic and the aliphatic compounds leading to the opposite enantiomers (**Table 1-4**).

Table 1-4 Reduction of α-ketoesters by SSCR.



R = aliphalic R = aromatic

R	ee (%)	Specific activity	Reference
Phenyl	99 (*S*)	6640	[59]
4-Fluorophenyl	74 (*S*)	5070	[59]
4-Chlorophenyl	63 (*S*)	6360	[59]
4-Bromophenyl	56 (*S*)	1400	[59]
4-Cyanophenyl	82 (*S*)	2280	[59]
3,5-Diflurophenyl	43 (*S*)	5730	[59]
4-Methylphenyl	88 (*S*)	1200	[59]
Isopropyl	99 (*R*)	17540	[59]
t-Butyl	99 (*R*)	5560	[59]

Note: The unit of specific activity : nmol min^{-1} mg^{-1}.

The available X-ray structures of SSCR and the SSCR/ NADPH complex, as well as the well-understood catalytic mechanism underlying the stereoselective reduction of SSCR,[60,61] allow the design of rational mutations of SSCR. Seven site-directed mutations of SSCR, shown in **Table 1-3,** were carried out by Hua and coworkers based on the stereoview of the interactions between SSCR and NADPH (**Figure 1-5**).[62] Some

exchanged amino acids and their positions can be viewed from the stereostructure in **Figure 1-5**. The substrate specificity and enantioselectivity of SSCR mutants were not fully evaluated at the beginning of this project. The initial screening of these mutants against α-ketoesters is discussed in Chapter **3**.

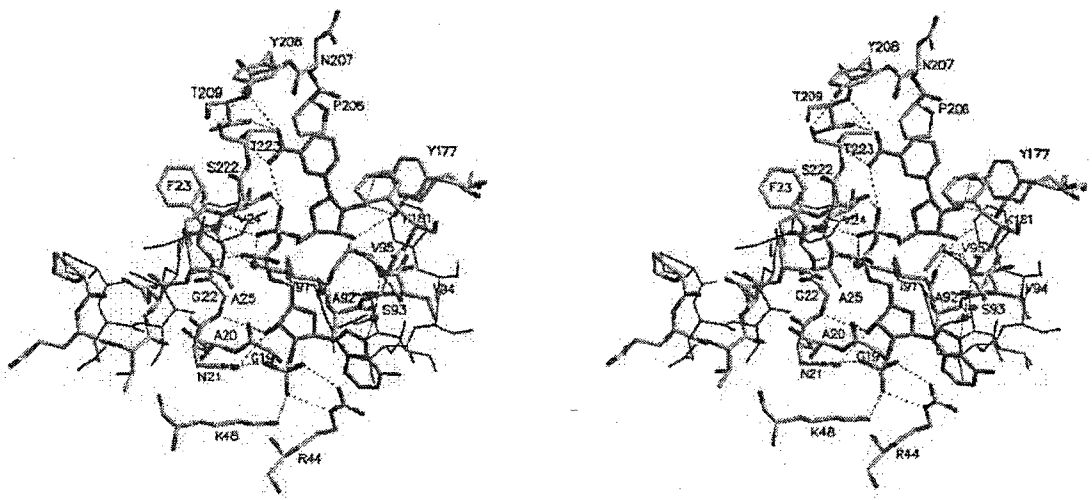
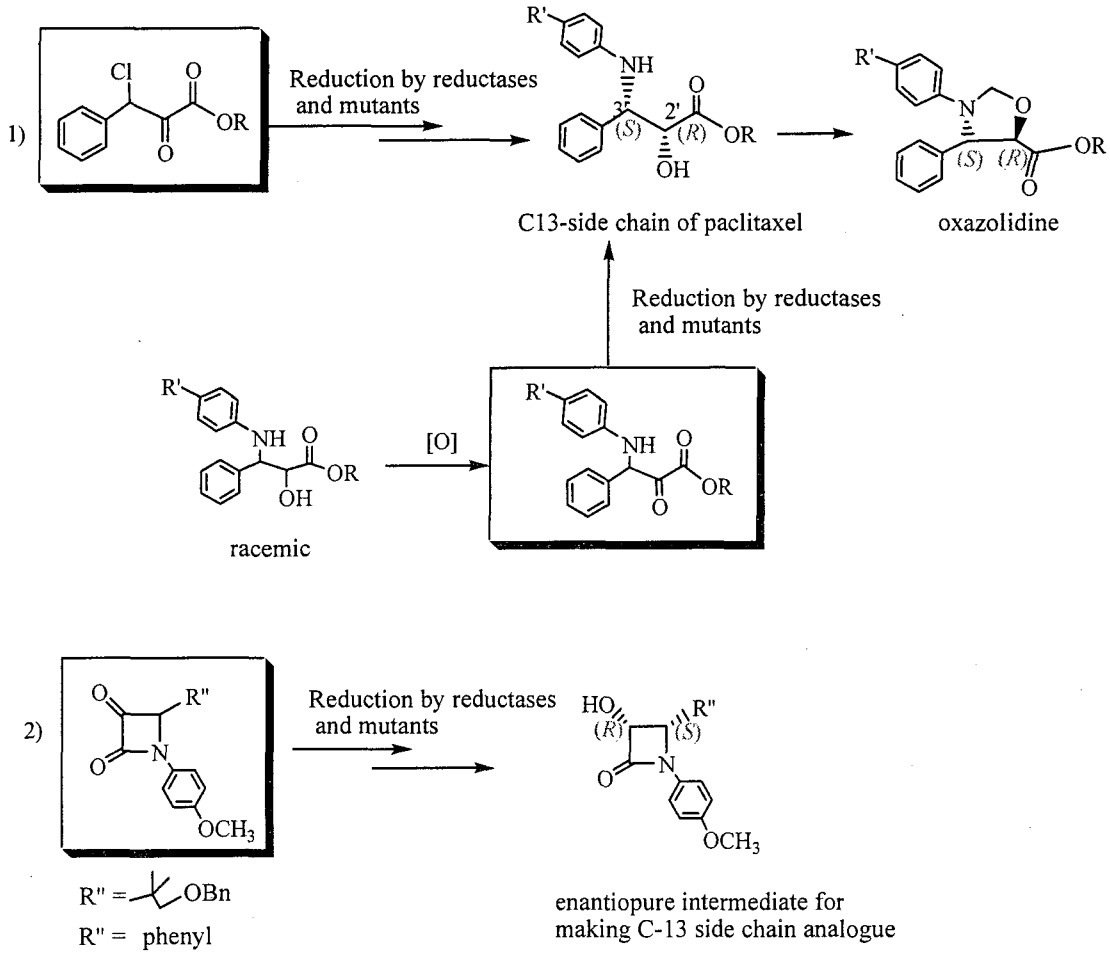


Figure 1-5 Stereoview of the interactions between SSCR (yellow carbon atoms) and NADPH (gray carbon atoms) illustrated by the programs MOLSCRIPT and Raster3D. The selected hydrogen bonds are shown with dotted lines. The structures of two mobile regions of unliganded SSCR are also shown in magenta carbon atoms. Mutated positions are N207, K181, Q245, M242 (Reprinted from reference [60]).

In an effort to prepare new enantiopure C-13 paclitaxel side chain analogues, several new substrates including β-chloro-α-ketoester and α-keto-β-lactams were selected for screening against the reductases listed in **Table 1-3** (**Scheme 1-10**). During the synthesis of one of these substrates, the unanticipated formation of oxazolidine opened the door to a new project. The identification of highly enantioselective reductases

(discussed in Chapter **3**) expanded this research project to the preparation of enantiopure oxazolidines, which is discussed in Chapter **4**.

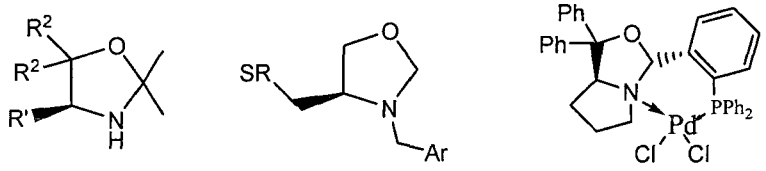
Scheme 1-10



1.3 Oxazolidine

Oxazolidines have received considerable attention as chiral ligands and are counted among the most efficient chiral auxiliaries.[63,64] For example, oxazolidine-based ligands in asymmetric catalytic transformations give up to 97% yield and 98% ee

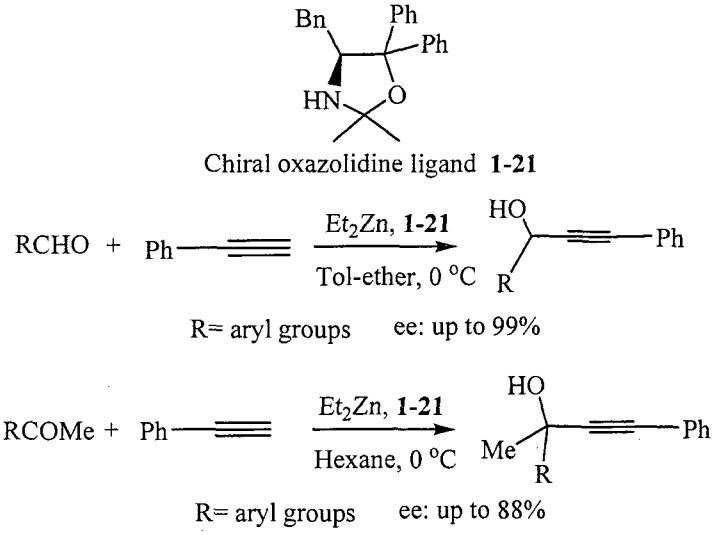
in Pd-catalyzed allylic alkylation reactions.[65] The types of oxazolidine ligands used as catalysts in asymmetric reactions are illustrated in **Figure 1-6**.[66]



1-18 **1-19** **1-20**

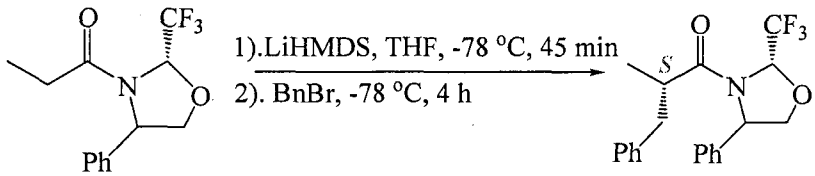
Figure 1-6 Examples of oxazolidine ligands used and structurally characterized metal complexes.

Scheme 1-11



Wang and his group[63] reported a new chiral oxazolidine ligand **1-21** synthesized from natural amino acids. Oxazolidine **1-21** promotes the asymmetric addition of diethylzinc to aromatic aldehydes with good yields and high enantioselectivity (**Scheme 1-11**). Similarly, Brigaud and co-workers[64] reported using chiral 2-trifluoromethyloxazolidine **1-22** as a chiral auxiliary for highly diastereoselective alkylation reactions of amide enolates (**Scheme 1-12**).

Scheme 1-12 Highly diastereoselective benzylation of N-propanoyl oxazolidine.

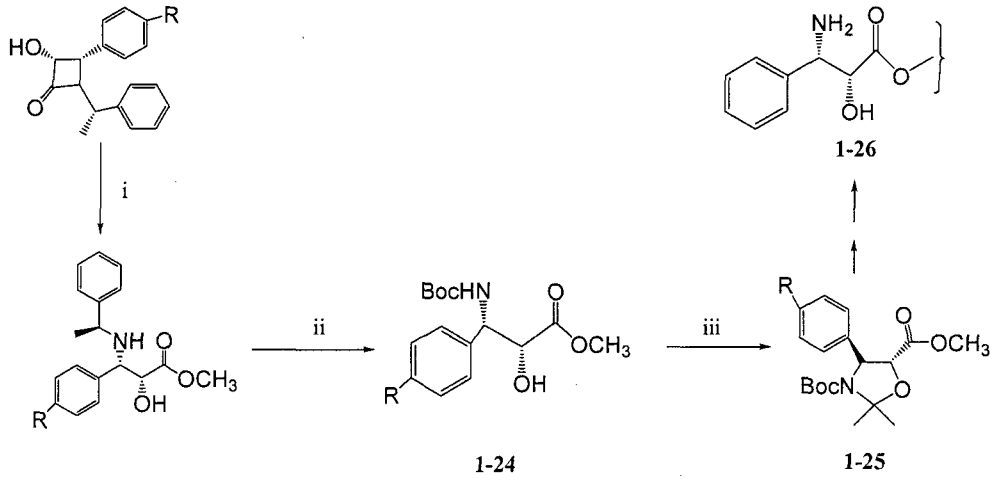


1-22 (*S*)-**1-23**

85% yield; d.e. > 99% (*S*)

Furthermore, oxazolidines have been used as protecting groups for aminoalcohols. For example, Commerçon and co-workers[20] reported using oxazolidine-type protection to attach baccatin III without epimerization, as shown in **Scheme 1-13**.

Scheme 1-13



1-24 **1-25** **1-26**

Reagents: (i). HCl gas, MeOH, 40 °C, 2.5 h.

(ii). (a).H_2 (345 psi), Pd/C (3%), MeOH/AcOH (3/1), 65 °C, 4 h.; (b) $(Boc)_2O$, CH_2Cl_2, Na_2CO_3, 20 °C, 72 h.

(iii). CH_2=C(OCH_3)CH_3, PPTS, toluene, 80 °C.

There have been also a number of studies involving conversion of 1,2-aminoalcohols to oxazolidines such as carbamates that were prepared as potential prodrugs.[67] As well as exhibiting chemical stability, the carbamate derivatives were shown to have favorable lipophilic properties because they are much weaker bases than

the parent β-amino alcohols thus leading to higher lipophilicity at physiological pH.[68] For instance, doxazolidine (Doxaz) **1-27b** is an oxazolidine derivative resulting from the reaction of the antitumor drug doxorubicin (Dox) **1-27a** with formaldehyde; another derivative doxoform (Doxf) **1-27c** is obtained by further coupling of two Doxaz molecules with formaldehyde (**Figure 1-7**).[67, 69] Doxaz **1-27b** is the active metabolite of Dox **1-27a** that cross-links DNA, leading to tumor cell death.[69, 70] Doxf **1-27c** is a very labile prodrug of Doxaz. Doxf and Doxaz are 10- to 10000-fold more active than Dox for growth inhibition of sensitive and resistant cancer cells.

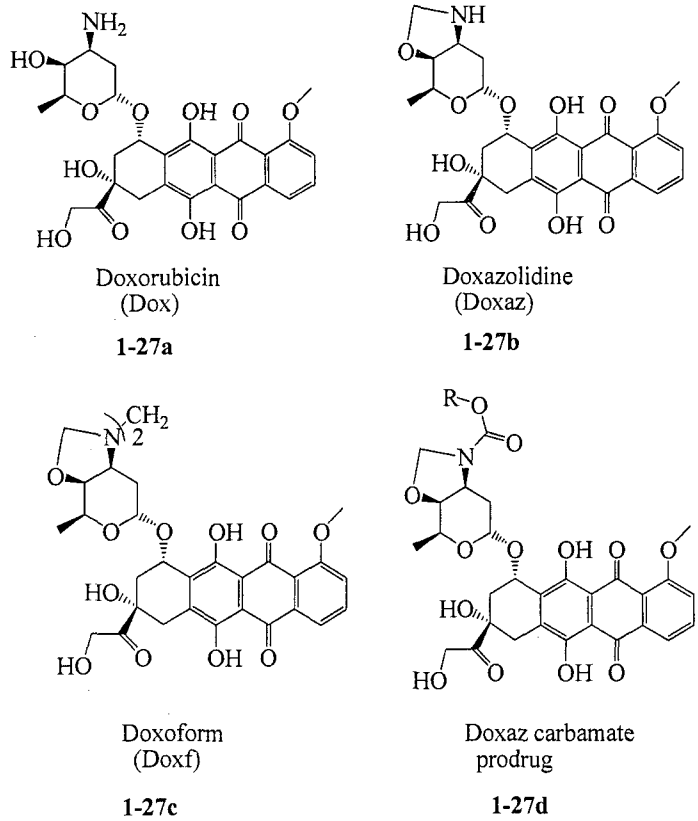
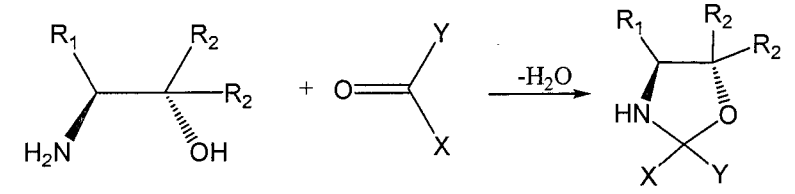


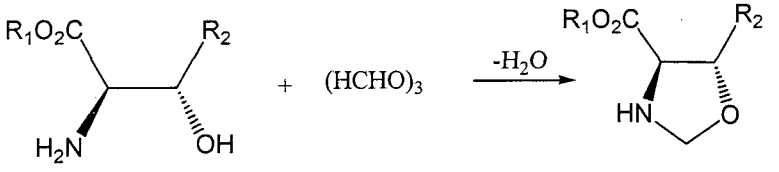
Figure 1-7 Clinical drug Dox (**1-27a**), Doxaz (**1-27b**), potent cytotoxins Doxf (**1-27c**) and proposed carbamate prodrug of Doxazolidine (**1-27d**).

Not surprisingly, high interest in this class of molecules has led to the development of numerous strategies for their preparation. One of the most common routes involves the preparation of oxazolidines from the condensation of aminoalcohols with formaldehyde or acetone (**Scheme 1-14**).[71-73] Condensation of a β-aminoalcohol, including L-serine and L-cysteine methyl esters, with paraformaldehyde has been frequently reported in the literature despite the fact that the yields are rather low.[74-79] Much less work on the enantioselective preparation of oxazolidines has been reported.[80-83]

Scheme 1-14



1-28 **1-29**



1-30 **1-31**

Since oxazolidines derived from β-aminoalcohols are important chiral auxiliary groups frequently used in asymmetric synthesis, it is important to develop new methods for the preparation of these compounds in enantiopure form. The results of this investigation on the preparation of enantioselective oxazolidines are discussed in Chapter **4**.

In summary, the lipase-mediated syntheses of enantiopure novel β-lactams as new paclitaxel side chain analogues are discussed in Chapter **2**. The screening of α-ketoesters against new wild type and mutant reductases are discussed in Chapter **3**. The enantioselective reductases and the unexpected formation of oxazolidines leading to novel syntheses of enantiopure oxazolidines are discussed in Chapter **4**.

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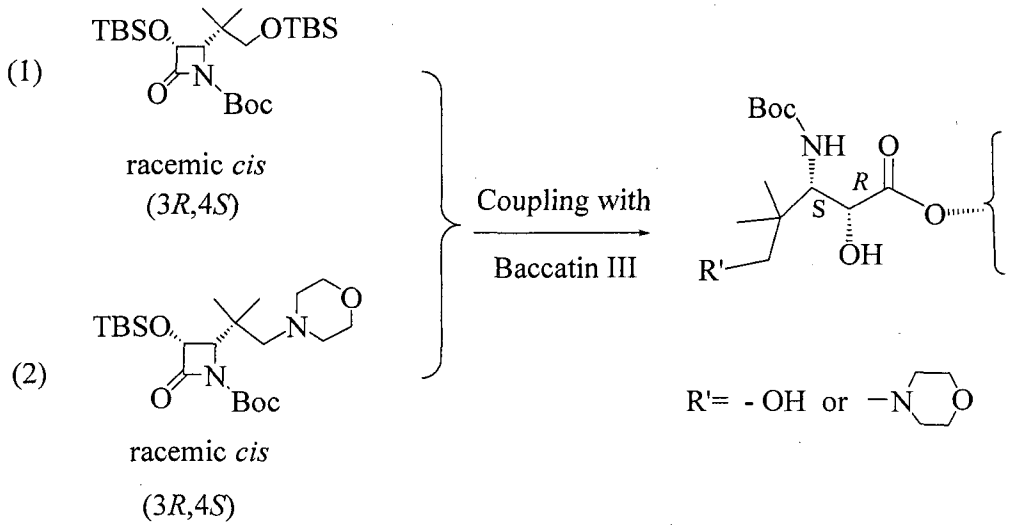
CHAPTER 2 CHEMOENZYMATIC SYNTHESIS OF NOVEL β-LACTAMS

2.1 Introduction

In recent years, β-lactams have been used for syntheses of α-hydroxy-β-amino acids which are present in many biologically active compounds such as paclitaxel, bestatin (inhibitor of aminopeptidases),[1] microginin,[2] and HIV-1 protease inhibitors.[3] The important anticancer activity of many of these compounds encouraged more research on structure modifications and synthesis of new analogues with more desirable physicochemical properties and enhanced potency.[4] It is well-known that paclitaxel's poor water solubility significantly hinders its oral administration and restricts injection applications.[5,6] Researchers continue to seek effective ways to increase its water-solubility; therefore, many techniques and approaches have been developed. These include addition of different cosolvents such as DMSO;[7] addition of cyclodextrin as a solubilizer through the formation of inclusion complexes [8,9] and use of water-soluble polymers.[10, 11]

It has been shown that introduction of a morpholine group into four new taxane analogues (non-side chains) provided greater pharmaceutical activity than paclitaxel and docetaxel, especially against the resistant cancer cell lines expressing P-glycoprotein (PC-12, PC-6/VCR 29-9, and PC-6/VP1-1).[12, 13] Taxane analogues with a morpholine or hydroxyl group in the C-13 side chain are therefore worth investigating. Many second-generation taxoids were synthesized from suitably modified baccatin and enantiopure (3*R*,4*S*)-β-lactam using Holton's coupling protocol (**Scheme 2-1**).[14]

Scheme 2-1 Coupling of β-lactam with baccatin III.



The well-documented Staudinger reaction[15] was used to prepare all target compounds in which cycloaddition readily proceeded between the nucleophilic imine and an electrophilic ketene that was generated *in situ*. The mechanism for this reaction is presented in **Figure 2-1**.

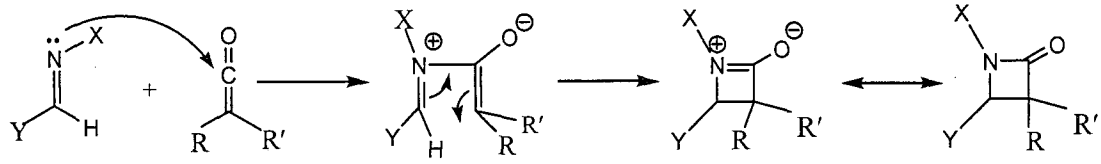


Figure 2-1 Mechanism of the Staudinger reaction.

In this project, new C-13 side chain analogues with improved water solubility were synthesized. A hydroxyl or morpholine polar group was introduced onto a β-lactam in such a way as to replace the native paclitaxel's 3' phenyl group. Protocols for the synthesis of racemic precursors were developed and optimized and the enantiopure

targets were prepared *via* lipase resolution. Synthesis of this compound is discussed in this chapter.

2.2 Results and Discussion

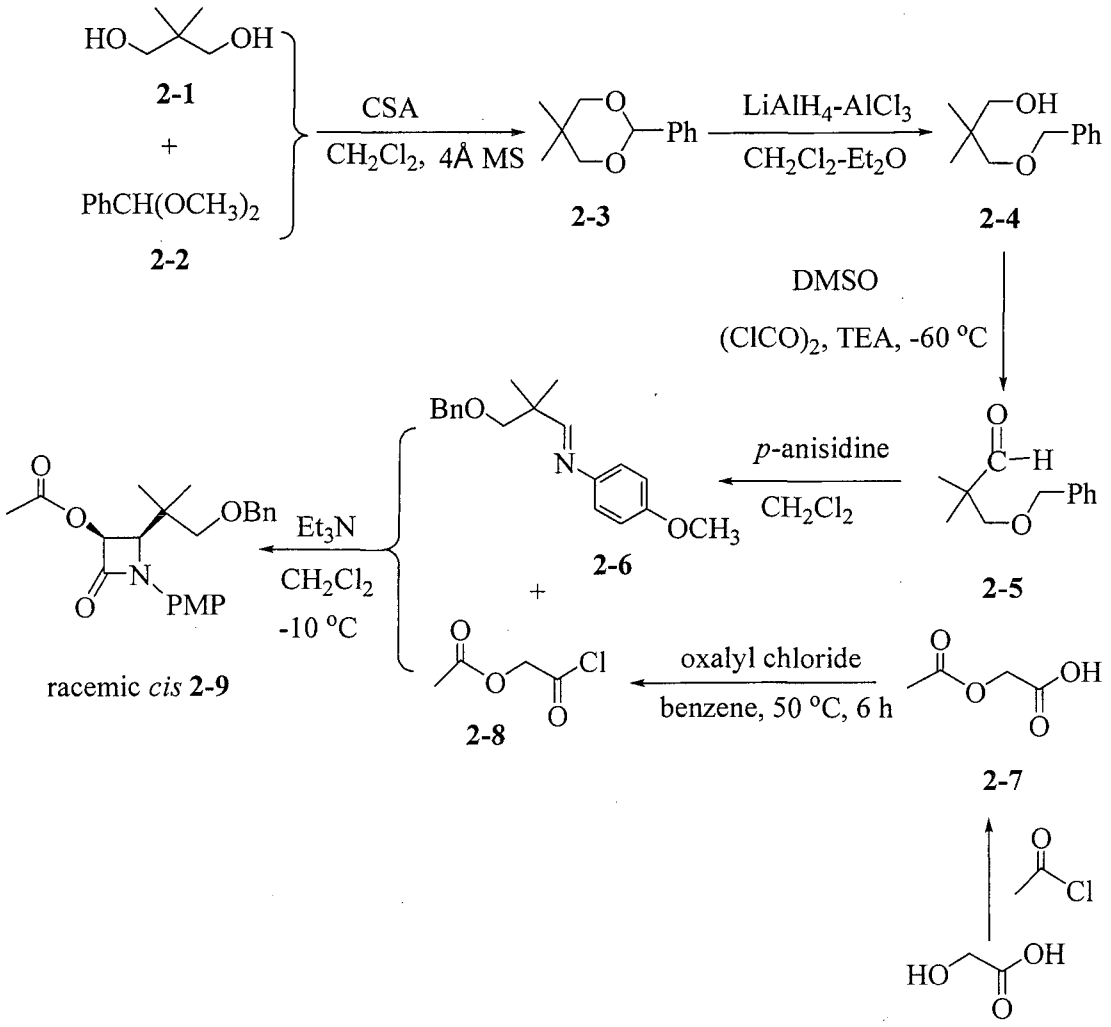
2.2.1 Synthesis of racemic *cis* acetoxy-β-lactam by the Staudinger reaction

Treatment of neopentyl glycol **2-1** with benzaldehyde dimethylacetal **2-2** in the presence of camphorsulfonic acid (CSA) and 4 Å molecular sieves, performed according to a protocol reported in the literature,[16] gave benzylidene acetal **2-3** in essentially quantitive yield (**Scheme 2-2**). Selective monobenzylation of glycols is an important protecting step in organic synthesis. It can be achieved *via* direct monoalkylation with benzyl chloride or *via* reductive cleavage of benzylidene acetals.[17] $LiAlH_4/AlCl_3$ efficiently cleaved the acetal **2-3** to 3-benzyloxy-2,2-dimethylpropan-1-ol **2-4** in 93% yield.[18] Subsequent Swern oxidation[19] of benzyloxy alcohol **2-4** yielded benzyloxyaldehyde **2-5** in 92% yield (**Scheme 2-2**; yields listed in **Table 2-1**). In this case, condensation of aldehyde **2-5** with *p*-anisidine generated exclusively *E* imine **2-6** as determined by the ^{1}H NMR of the crude product. The Staudinger reaction of imine **2-6** in the presence of acetoxyacetyl chloride **2-8**, gave racemic *cis* β-lactam **2-9** in 75% yield. The cycloaddition was completed in 3 hours and was free of by-products (**Scheme 2-2**).

Table 2-1 Yields of compounds from **2-3** to **2-9.**

Compound	**2-3**	**2-4**	**2-5**	**2-6**	**2-7**	**2-8**	**2-9**
Yield (%)	92	90	92	95	93	95	75

Scheme 2-2 Synthesis of racemic *cis*-3-acetoxy-β-lactam **2-9**.

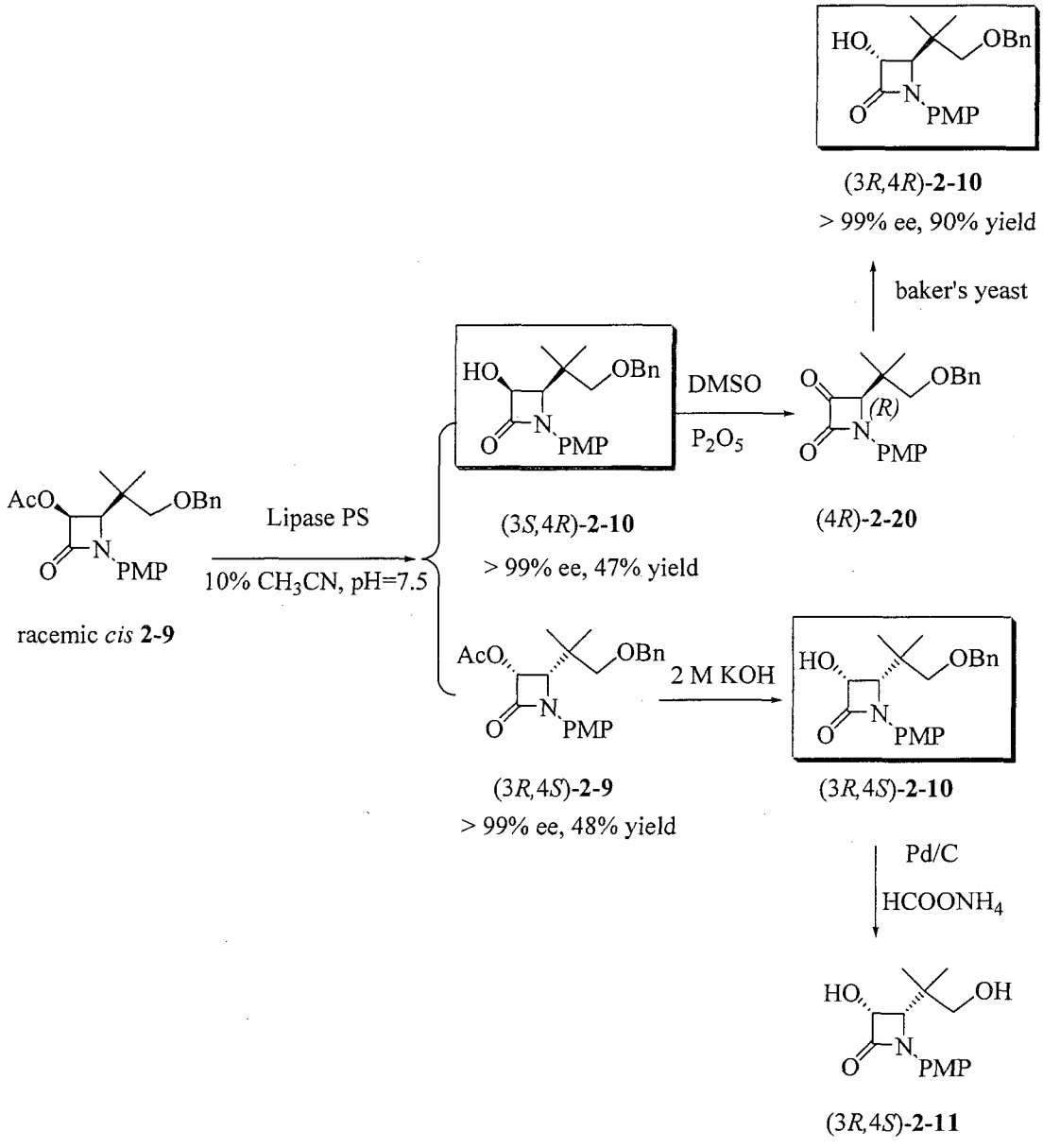


2.2.2 Preparation of (3*S*,4*R*)-2-10, (3*R*,4*S*)-2-10, and (3*R*,4*R*)-2-10 from lipase and baker's yeast resolution

Enantiopure **2-10** obtained *via* lipase resolution of racemic *cis* **2-9** is an important intermediate in the preparation of enantiopure C-13 side chain analogues substituted with a hydroxyl or morpholine group. Only *cis*-3-acetyl lactams are accepted and resolved by lipase PS. On the other hand, *trans*-3-hydroxy-4-*t*-butyl-β-lactam was obtained in the

yeast reduction of the 3-oxo-4-*t*-butyl-β-lactam.[20] To achieve enantiopure *trans*-3-hydroxy-β-lactam in high yield, 2-10 we decided that the combination of lipase PS resolution and baker's yeast reduction may be used to obtain enantiopure *trans*-β-lactam. The strategy is shown in Scheme **2-3**.

Scheme 2-3



With all chemical reactions optimized, lipase PS catalyzed kinetic resolution provided a route to both **2-10** enantiomers. Thus, (3*S*,4*R*)-**2-10** (99% ee) was prepared *via* kinetic resolution of racemic *cis* **2-9** since lipase only converts (3*S*,4*R*)-**2-9** to the hydrolyzed product (3*S*,4*R*)-**2-10** while remains (3*R*,4*S*)-**2-9** unreacted. Its antipode (3*R*,4*S*)-**2-10** was accessed by 2 M KOH hydrolysis of the unreacted (3*R*,4*S*)-**2-9** (99% ee). Oxidation of (3*S*,4*R*)-**2-10** gave (4*R*)-**2-20** which was reduced with baker's yeast to give (3*R*,4*R*)-**2-10** in 90% yield as shown in **Scheme 2-3** and **Table 2-2**.

Table 2-2 Properties of the three enantiomers of **2-10** *via* lipase and yeast resolution.

Substrate	Yield (%)	ee (%)	m.p. (°C)	$[\alpha]_D^{25}$
(3*S*,4*R*)-(-)-**2-10**	47	>99	133-134	-77.1
(3*R*,4*S*)-(+)-**2-10**	42	>99	138-140	+78.0
(3*R*,4*R*)-(+)-**2-10**	90	>99	95-96	+39.5

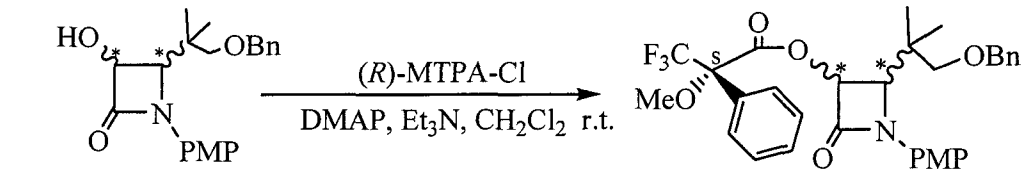
Note: (1). ee was determined by chiral HPLC analysis and Mosher's reagent.

(2). Enantiomers were assigned by ^{1}H NMR after derivatization with Mosher's reagent.

2.2.3 Absolute configuration of β-lactam 2-10

The absolute configuration of (3*S*,4*R*)-**2-10** was assigned from lipase resolution; its absolute configuration has been confirmed by X-ray crystallographic[21,22] and proton NMR analyses.[23] Enantiomeric excess of the three enantiomers of **2-10** was determined by derivitization with Mosher's reagent (*R*)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride ((*R*)-MTPA-Cl) [24] and chiral HPLC analysis. Three derivatized enantiopure species of **2-10** (**Scheme 2-4**) were >99% ee as shown by NMR spectra (^{1}H and ^{13}C NMR spectra are given in **Appendix III**).

Scheme 2-4



(3*S*,4*R*)-
(3*R*,4*S*)- } 3-hydroxy-β-lactam
(3*R*,4*R*)- **2-10**

derivative of 3-hydroxy-β-lactam by Mosher's reagent

The three compounds (3*R*,4*S*)-**2-10**, (3*S*,4*R*)-**2-10** and (3*R*,4*R*)-**2-10** could not be separated on a chiracel OD-H column (4.6 x 150 mm). However, they are resolved clearly on a chiral HPLC (*S*,*S*)-Whelk-O 1 column (25 cm x 4.6 mm, Regis Technologies Inc.) (**Figure 2-2**); each individual compound shows a single peak, indicating >99% enantiomeric excess for each compound.

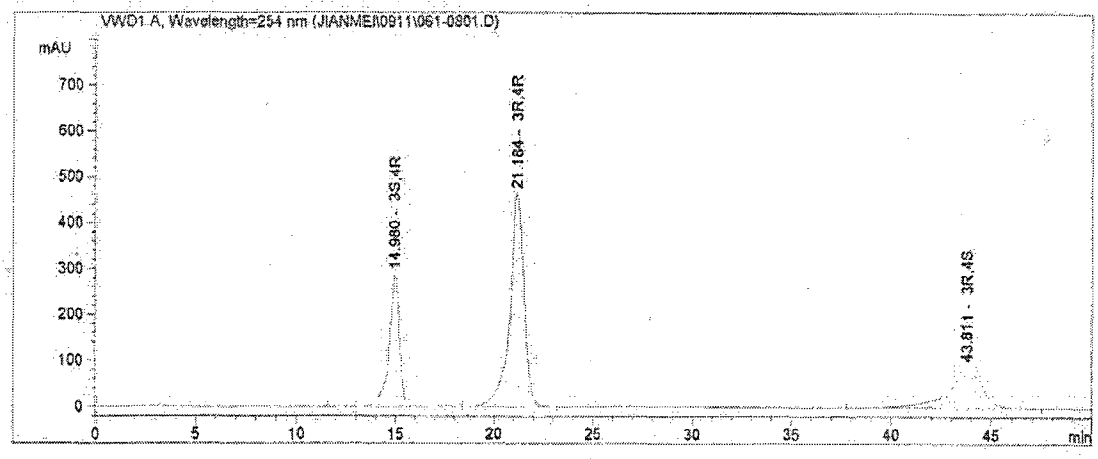


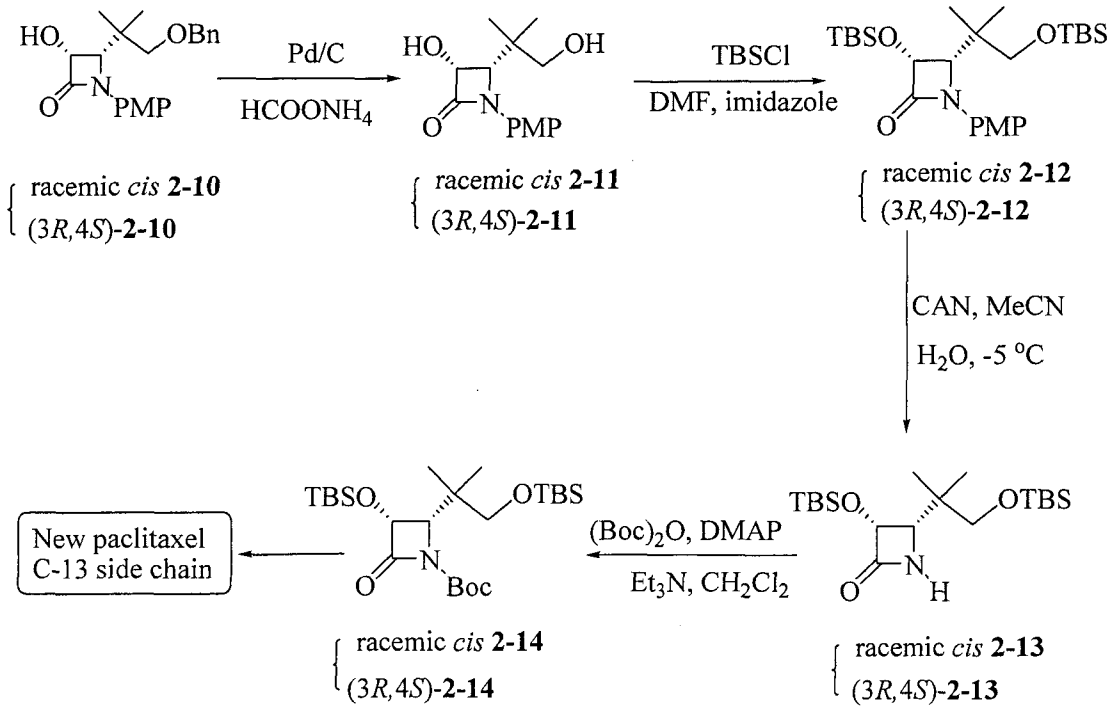
Figure 2-2 (3*R*,4*S*), (3*S*,4*R*) and (3*R*,4*R*)-**2-10** resolved on a chiral (*S*,*S*)-Whelk-O 1 column.

2.2.4 Synthesis of racemic *cis* and (3*R*,4*S*) β-lactam bearing a hydroxyl group

Both racemic *cis* and the enantiopure *cis* (3*R*,4*S*) β-lactams were synthesized because they are useful for preparing C-13 side chain analogues. Georg and her group[25] carried out a systematic study of the kinetic resolution of racemic *cis*-4-phenyl- and *cis*-4-*t*-butyl-3-hydroxy-β-lactam (obtained from our group) with 7-*O*-triethylsilylbaccatin III. The product paclitaxel and butitaxel analogues were found to form with high diastereoselectivity (10:1 to 80:1) in favor of the natural 2'*R*, 3'*S* configuration. To extend the research, novel racemic *cis* β-lactams as C-13 side chain analogues were synthesized to instigate a kinetic study and investigate the diastereoselectivity ratio.

The acetyl group of β-lactam **2-9** was hydrolyzed with 2 M potassium hydroxide in THF, and the resulting 3-hydroxy-β-lactam **2-10** was used in transformations leading to β-lactams derivatized with a hydroxyl or morpholine functional group as shown in **Scheme 2-5** and **Scheme 2-6**. The cleavage of the benzyl group, particularly in sensitive molecules such as β-lactams, was expected to be troublesome. In fact, the commonly used H_2-Pd/C hydrogenation[26] was ineffective at deprotecting (3*R*,4*S*)-**2-10**. Among several methods investigated, homogeneous hydrogenation with ammonium formate as the hydrogen source turned out to be fast and reliable[27] (**Scheme 2-5**). The same deprotection method was also effective with the *t*-butyldimethyl silyl (TBS) derivative (3*R*,4*S*)-**2-15**, making it suitable for the preparation of compound (3*R*,4*S*)-**2-16** (**Scheme 2-6**). 3-Hydroxy-β-lactam **2-10** was debenzylated to give β-lactam **2-11** in high yield.

Scheme 2-5



Hydroxy-β-lactam **2-11** was treated with two equivalents of *t*-butyldimethyl silyl (TBS) chloride to give the corresponding protected lactam **2-12**. In the following steps, the *p*-methoxyphenyl (PMP) group was oxididatively cleaved using cerium (IV) ammonium nitrate (CAN) to give NH-β-lactam **2-13**. Removal of the PMP group by oxidation with CAN was optimized in a series of small-scale reactions. The solvent and temperature were very important for the success of this reaction; in particular, it was critical that the water and acetonitrile ratio was strictly controlled to achieve a reasonably good yield. The optimized solvent ratio was determined to be acetonitrile : deionized water = 25 : 15 (based on 1 mmol of reaction) and the reaction had to be carried out in an ice-salt bath. For larger scale reactions the yields were always lower.

Protection of **2-13** with *di t*-butoxy dicarbonate (*t*-Boc) in the presence of 4-dimethylaminopyridine (DMAP) yielded **2-14** ready for coupling with baccatin III to make a new paclitaxel analogue.* The characteristics of all compounds discussed in **Scheme 2-5** are listed in **Table 2-3**.

Table 2-3 Enantiopure compounds **2-9** to **2-14.**

Comp.	(3*R*,4*S*)						(3*S*,4*R*)	
	2-9	**2-10**	**2-11**	**2-12**	**2-13**	**2-14**	**2-10**	**2-11**
m.p. (°C)	85-86	138-140	162-164	79-80	93-94	oil	133-134	155-157
$[\alpha]_D^{25}$	+ 51.0	+ 78.0	+ 70.2	+ 34.8	+ 47.1	+57	- 77.1	- 70.0
Yield %	44	90	50	72.3	74	93	47	52.5

2.2.5 Synthesis of racemic *cis* and (3*R*,4*S*) β-lactam bearing a morpholine group

To obtain a β-lactam substituted with a morpholine group, **2-18** was prepared in four steps from **2-10**. In the first step, the hydroxyl group in 3-hydroxy-β-lactam **2-10** was protected with TBSCl to give **2-15** in 96% yield. This was followed by debenzylation using 10% palladium on activated carbon with ammonium formate as the hydrogen source. Although this step needed more palladium and a longer reaction time, it proved equally effective in preparing debenzylated **2-16**. Swern oxidation ($DMSO/(COCl)_2$)[19] of compound **2-16** at -50 °C to -60 °C gave aldehyde **2-17** in high yield. The aldehyde **2-17** was then subjected to reductive amination with sodium triacetoxylborohydride ($NaBH(OAc)_3$)[28] in the presence of excess morpholine to give **2-18** in 53% yield (shown in **Scheme 2-6** and **Table 2-4**). Other reductive amination methods such as $NaBH_3CN$ or

* The products were tested at the University of Kansas, Laurence KS.

neat $Ti(OiPr)_4$ associated with $NaBH_3CN$ or $(NaBH(OAc)_3)$[29] gave lower yields as shown in **Table 2-5**. The reduction of the aldehyde **2-17** to alcohol **2-16** was a major side reaction under all conditions investigated; sodium triacetoxyborohydride protocol is the most effective in reductive amination and is superior to the commonly used toxic $NaBH_3CN$.[30]

Scheme 2-6

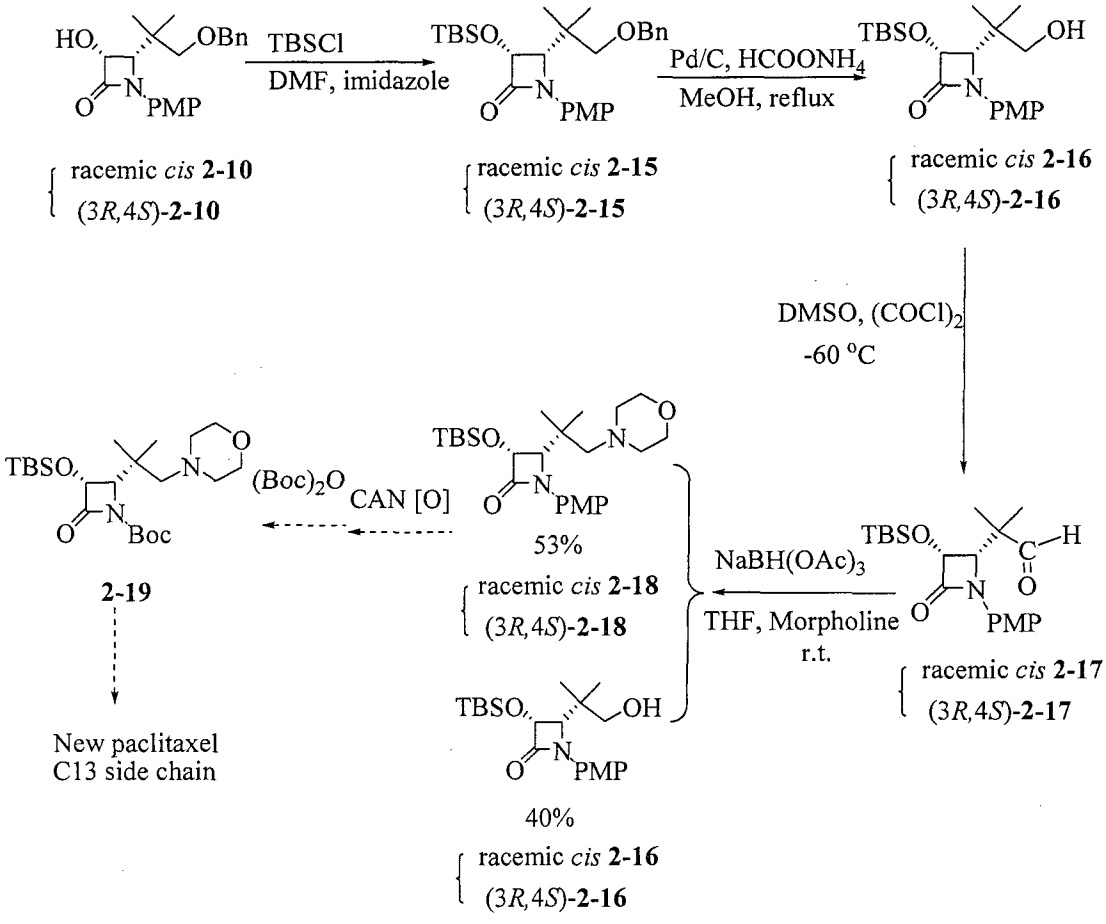


Table 2-4 Enantiopure compounds **2-15** to **2-18.**

Compound	(3*R*,4*S*)			
	2-15	**2-16**	**2-17**	**2-18**
m.p. (°C)	oil	103-104	110-111	90-91
$[\alpha]_D^{25}$	+ 52.1	+ 53.7	+ 56.4	+ 48.7
Yield (%)	97	57	83	53

Table 2-5 Methods of reductive amination.

Method	Reaction time (h)	Yield (%)
$NaBH_3CN$, AcOH, MeOH	3	30
$NaBH(OAc)_3$, THF	12	53
$Ti(OiPr)_4$ in $NaBH_3CN$	4	32
$Ti(OiPr)_4$ in $NaBH(OAc)_3$	4	45

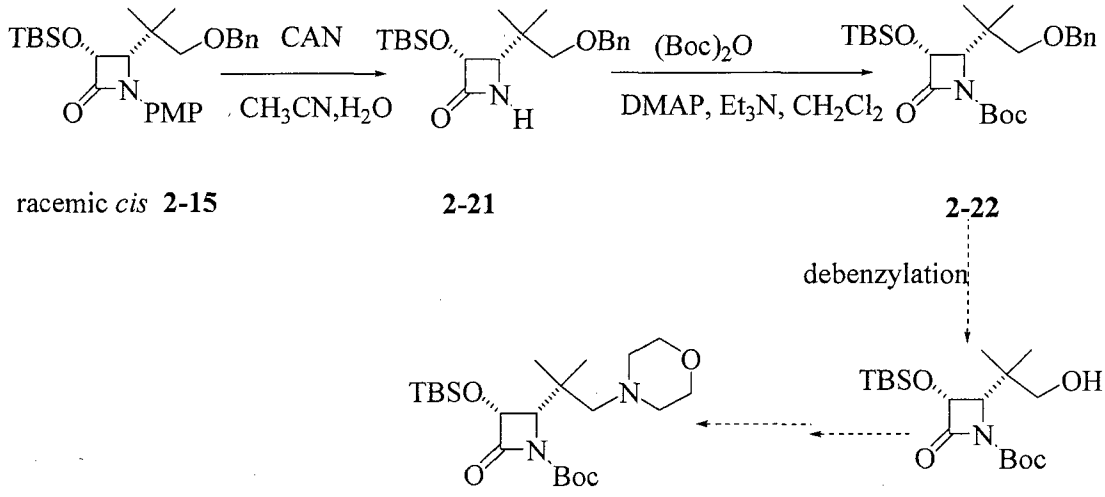
Note: Titanium (IV) isopropoxide was reacted with a mixture of the aldehyde and amine and then reduced by sodium cyanoborohydride in anhydrous ethanol.

The attempted removal of the PMP group was unsuccessful. Compound **2-18** on treatment with CAN gave multiple products, quinone imine derivatives being the most important.[31] Since the morpholine group did not appear in the ^1H NMR of the product mixture it must have been removed during this step. Thus, the PMP group has to be removed before morpholine is introduced into the β-lactam.

2.2.6 Other approaches for morpholino-β-lactam 2-19

Removal of the PMP in a β-lactam tends to be not only low yielding but also quite unpredictable. Therefore, it was not a great surprise when hydroxy-β-lactam **2-16** and aldehyde **2-17** were subjected to oxidation conditions, only decomposition mixtures were obtained. This confirms that sensitive functional groups like carbonyl or hydroxyl need to be protected before using cerium (IV) ammonium nitrate.

Scheme 2-7



Given these results, the functional group in position 4 of β-lactam must be protected before removal of the PMP. The projected, modified sequence of reactions is shown in **Scheme 2-7**. We know that racemic, benzyl protected *cis* **2-15** was oxidized with CAN to give the NH-β-lactam **2-21** in 52% yield and the following reaction with (t-Boc)$_2$O gave racemic *cis* **2-22** in 90% yield. Possibly, debenzylation, oxidation, and reductive amination performed on relatively readily available *cis* **2-22** would lead to an

acceptable yield of product **2-19**. Alternative approaches require several protection and deprotection steps and could not be carried out in the time frame of this M. Sc. thesis.

2.3 Conclusions

Novel β-lactams are useful chiral building blocks for the synthesis of paclitaxel analogues. Racemic *cis* and enantiopure β-lactams bearing hydroxyl groups were prepared *via* simple synthesis and lipase-catalyzed kinetic resolution. These building blocks were synthesized efficiently in high yields and sent for kinetic study upon attachment with baccatin III. Racemic *cis* and enantiopure β-lactams bearing the morpholine group were employed with a different synthetic route since sensitive functional groups such as hydroxyl, carbonyl, morpholine were very fragile to CAN oxidation and this route can be accomplished if time isn't limited. Two different enantiomers (3*R*,4*S*), (3*S*,4*R*) and one diastereomer (3*R*,4*R*)-**2-10** were obtained from the hydrolysis of lipase or baker's yeast in >99% ee. The enantiomeric excess was determined by chiral HPLC analysis, specific optical rotation and ^{1}H NMR after derivatization with Mosher's reagent. The method to preparation of three enantiopure forms of **2-10** can serve as a useful protocol for the preparation of other β-lactam enantiomers.

2.4 Experimental

2.4.1 Acetoxy glycolic acid 2-7.[32] Acetyl chloride (82 mL, 1.2 mol) and glycolic acid (35.1 g, 0.46 mol) were added to a flask with vigorous stirring at room temperature. After 30 min, TLC indicated complete conversion. Evaporation of the excess chloride under vacuum gave acetoxy glycolic acid (2-acetoxyacetic acid) **2-7** (50.5 g, 93% yield) as a pure white powder. m.p. 61-64 °C; IR ($CHCl_3$) γ_{max} /cm^{-1}: 3054 (broad peak), 1739, 1424, 1375, 1218,1079. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.15 (3H, s, CH_3CO), 4.62 (2H, s, OCH_2CO_2H), 11.1 (1H, CO_2H).

2.4.2 Carboxylic acid chloride (acetoxyacetyl chloride) 2-8. Oxalyl chloride $(COCl)_2$ (21.50 mL, 0.246 mol) was added to a solution of acid **2-7** (14.54 g, 0.123 mol) in benzene (100 mL). The reaction was heated and maintained at 50 °C for 6 hours, then cooled to room temperature and stirred overnight. Vacuum distillation removed the excess oxalyl chloride and the benzene solvent to give acid chloride **2-8** (14.9 g, 89% yield) as a colourless liquid. IR ($CHCl_3$) γ_{max} /cm^{-1}: 2999, 2947, 1811, 1758, 1407, 1374, 1221, 953. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.2 (3H, s, CH_3CO), 5.0 (2H, s, OCH_2COCl).

2.4.3 5,5-Dimethyl-2-phenyl-1,3-dioxane 2-3.[33] Camphorsulfonic acid (0.22 g, 0.95 mol) was added to a solution of neopentyl glycol **2-1** (8.32 g, 0.08 mol) and benzaldehyde dimethyl acetal **2-2** (12.8 mL, 0.09 mol) in methylene chloride (220 mL) containing activated 4Å molecular sieves (10 g). GC and TLC showed complete conversion after 30 minutes of reaction and the molecular sieves were filtered off and washed with methylene chloride (20 mL x 3). The filtrate was washed with 10% sodium bicarbonate and brine, dried over magnesium sulfate, filtered. Evaporation under vacuum afforded **2-3** (14.9 g, 99% yield) as colourless crystals. m.p. 30-31 °C; IR ($CHCl_3$) γ_{max}

/cm^{-1}: 3066, 3036, 2953, 2868, 1456, 1390, 1216, 1105, 1022; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80 (3H, s, C*H*$_3$), 1.30 (3H, s, C*H*$_3$), 3.65 (2H, d, *J* = 10.8 Hz, C*H*$_2$), 3.77 (2H, d, *J* = 11.0 Hz, C*H*$_2$), 5.39 (1H, s, C*H*), 7.37 (3H, dd, *J* = 6.7 Hz, *J* = 8.1 Hz, Ar*H*), 7.51 (2H, d, *J* = 6.7 Hz, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 21.9 (*C*H$_3$), 23.1 (*C*H$_3$), 30.2 (*C*Me$_2$), 77.7 (O*C*H$_2$), 101.8 (H*C*Ph), 126.1, 128.3, 128.7, 138.6; HRMS: $C_{12}H_{16}O_2$ (M^+), calc.: 192.11504, found: 192.11502.

2.4.4 3-(Benzyloxy)-2,2-dimethylpropan-1-ol 2-4.[33] Lithium aluminum hydride (1.98 g, 0.052 mol) was added to a solution of **2-3** (10.0 g, 0.052 mol) in 1:1 diethyl ether and methylene chloride (200 mL) cooled to –10 °C. Aluminum chloride (6.95 g, 0.052 mol) in 40 mL of diethyl ether was then added and the resulting mixture was stirred at –10 °C for 10 minutes. The reaction was allowed to warm to room temperature, then it was heated until reflux. Reflux was continued until GC showed complete conversion (4 h). After the reaction was cooled to –10 °C it was diluted with 50 mL of ethyl acetate. The reaction was hydrolyzed with water (150 mL) and extracted with ethyl acetate. The combined organic layers were washed sequentially with 10% sodium bicarbonate solution and brine, dried over magnesium sulfate, and filtered. Evaporation under vacuum gave **2-4** (9.35 g, 93% yield) as a colourless oil. IR ($CHCl_3$) γ_{max} /cm^{-1}: 3349, 2982, 2940, 2839, 1725, 1513, 1249, 1173; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (6H, s, (C*H*$_3$)$_2$), 2.68 (1H, s, O*H*), 3.32 (2H, s, C*H*$_2$), 3.45 (2H, s, C*H*$_2$), 4.51 (2H, s, OC*H*$_2$Ph), 7.32 (5H, m, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 21.8 (*C*H$_3$), 36.2 (*C*Me$_2$), 71.6 (*C*H$_2$OH), 73.5 (*C*H$_2$Ph), 79.3 (*C*H$_2$OCH$_2$Ph), 127.4, 127.6, 128.4, 138.2; HRMS: $C_{12}H_{18}O_2$ (M^+), calc.: 194.13068, found: 194.13067.

2.4.5 3-(Benzyloxy)-2,2-dimethylpropanal 2-5.[19] A solution of oxalyl chloride (4.9 g, 0.025 mol) in dry methylene chloride (63 mL) was placed in a flame-dried 250 mL three-neck round-bottom flask equipped with a thermometer and two pressure-equalizing dropping funnels containing dimethyl sulfoxide (3.6 mL, 0.051 mol) dissolved in methylene chloride (12 mL) and benzyl ether alcohol **2-4** (4.9 g, 0.025 mol) dissolved in methylene chloride (25 mL), respectively. The reaction mixture was cooled to –60 °C and the DMSO solution was added over a period of 5 minutes, followed by the alcohol solution (10 min). After stirring at –60 °C for 30 minutes, triethylamine (18 mL, 0.13 mol) was added and stirring was continued for an additional 45 min when GC showed complete conversion. After the reaction was warmed to room temperature, water (40 mL) was added and stirred for 10 minutes, followed by addition of 2 M HCl (30 mL). The solution was extracted with methylene chloride, washed with brine, dried over magnesium sulfate and filtered. Evaporation under vacuum gave **2-5** (6.0 g, 92% yield) as a colourless oil. IR ($CHCl_3$) γ_{max} /cm^{-1}: 3349, 2982, 2940, 2839, 1725, 1513, 1249, 1173; ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.09 (6H, s, (CH_3)$_2$), 3.45 (2H, s, OCH_2), 4.51 (2H, s, OCH_2Ph), 7.30 (5H, s, Ar*H*), 9.57 (1H, s, C*H*O); ^{13}C NMR (126 MHz, $CDCl_3$): δ 22.3 (CH_3), 43.4 (CMe_2), 73.4 (CH_2O), 76.5 (OCH_2Ph), 127.5, 127.6, 128.3, 138.0, 182.2; HRMS: $C_{12}H_{16}O_2$ (M^+), calc.: 192.1150, found: 192.1142.

2.4.6 (*E*)-*N*-(3-(Benzyloxy)-2,2-dimethylpropylidene)-4-methoxybenzenamine 2-6. To a 5% solution of the corresponding aldehyde **2-5** (4.239 g, 0.022 mol), in methylene chloride (18 mL) were added *p*-anisidine (2.883 g, 0.022 mol) and 4 Å molecular sieves (9 g). The resulting suspension was stirred at room temperature for 2 hours until TLC showed complete conversion. The molecular sieves were filtered and thoroughly washed

with methylene chloride. The combined organic solutions were concentrated under vacuum to give pure imine **2-6** (6.2 g, 95% yield) as a light yellow liquid. IR ($CHCl_3$) γ_{max} /cm^{-1}: 3031, 2961, 2930, 2858, 3708, 1731, 1648, 1504, 1454, 1244, 1101, 1034; ^1H NMR (400 MHz, $CDCl_3$): δ 1.15 (6H, s, $(CH_3)_2$), 3.51 (2H, s, OCH_2), 3.83 (3H, s, OCH_3), 4.56 (2H, s, CH_2Ph), 6.85 (2H, d, J = 8.9 Hz, ArH), 7.00 (2H, d, J = 8.8 Hz, ArH), 7.32 (5H, s, ArH), 7.89 (1H, s, N = CH).

2.4.7 *cis*-(±)-3-Acetoxy-4-(2-benzyloxy-1,1-dimethylethyl)-1-(4-methoxyphenyl)-azetidin-2-one 2-9. The crude imine **2-6** (10.8 g, 0.036 mol) and dry triethylamine (25 mL, 0.18 mol) in dry methylene chloride (100 mL) were cooled to -10 °C and treated under a nitrogen atmosphere with carboxylic acid chloride **2-8** (12.3 g, 0.09 mol) in dry methylene chloride (65 mL). After complete addition, the solution was warmed to room temperature and stirred for 6 hours. The reaction mixture was hydrolyzed with 2 M HCl, and extracted with methylene chloride. The combined organic layers were washed with saturated sodium carbonate solution, dried over magnesium sulfate, filtered, and evaporated to dryness. Crystallization from hexane and ethyl acetate gave racemic *cis* **2-9** (11.9 g, 75% yield) as white crystals. m.p. 78-79 °C; IR ($CHCl_3$) γ_{max} /cm^{-1}: 2961, 2934, 2871, 1758, 1513, 1373, 1221, 1112; ^1H NMR (400 MHz, $CDCl_3$): δ 1.03(3H, s, C*H*$_3$), 1.04 (3H, s, C*H*$_3$), 2.15 (3H, s, C*H*$_3$CO), 3.17 (2H, dd, J = 9.1 Hz, J = 9.0 Hz, CC*H*$_2$), 3.79 (3H, s, OC*H*$_3$), 4.39 (2H, q, J = 11.9 Hz, OC*H*$_2$Ph), 4.69 (1H, d, J = 5.5 Hz, NC*H*), 6.17 (1H, d, J = 5.5 Hz, C*H*O), 6.83 (2H, d, J = 9.0 Hz, Ar*H*), 7.34-7.38 (7H, m, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 20.39 (*C*H$_3$), 20.8 (*C*H$_3$), 21.5 (*C*H$_3$CO), 38.4 (*C*Me$_2$), 55.4 (O*C*H$_3$), 61.8 (NCH), 73.1 (O*C*H$_2$Ph), 73.4 (*C*HO), 77.6 (*C*CH$_2$O), 114.2, 121.2,

127.4, 127.7, 128.4, 130.3, 138.1, 157.1, 163.9, 169.1; HRMS: $C_{23}H_{27}NO_5$ (M^+), calc.: 397.18893, found: 397.18891.

(3*R*,4*S*)-(+)-3-Acetoxy-4-(2-benzyloxy-1,1-dimethylethyl)-1-(4-methoxyphenyl) azetidin-2-one 2-9. Colourless crystals. m.p. 85-86 °C; $[\alpha]_D^{25} = +51.0$ (c, 1.0, CH_2Cl_2).

2.4.8 General procedure for lipase resolution of racemic *cis* 3-acetoxy-β-lactams.

Amano PS lipase (2 g) was suspended in a 0.2 M potassium phosphate buffer (pH 7.5, 27 mL). 3-Acetoxy-β-lactam racemic *cis* **2-9** (2 g, 0.005 mol) in 10% acetonitrile (3 mL) was added to the reaction mixture. The reaction was vigorously stirred at room temperature for 72 hours until one of the isomers of the starting material reached more than 48% conversion (by chiral HPLC analysis). The mixture was extracted with ethyl acetate and the combined ethyl acetate layers were washed with brine and dried over magnesium sulfate. Removal of the solvent afforded a mixture of unreacted 3-acetoxy-β-lactam and hydrolyzed product 3-hydroxy-β-lactam. Separation by flash chromatography on a silica gel column gave enantiopure 3-hydroxy-β-lactam (3*S*,4*R*)-(-)-**2-10** (0.83 g, 47% yield) and the unreacted 3-acetoxy-β-lactam (3*R*,4*S*)-(+)-**2-9** (0.95 g 48% yield). Chemical hydrolysis of the latter gave (3*R*,4*S*)-(-)-**2-10** in 92% yield.

(3*S*,4*R*)-(-)-3-Hydroxy-4-(2-benzyloxy-1,1-dimethylethyl)-1-(4-methoxyphenyl)-azetidin-2-one 2-10. White crystals; m.p. 133-134 °C; $[\alpha]_D^{25} = -77.1$ (c, 1.0, CH_2Cl_2). IR ($CHCl_3$) γ_{max} /cm^{-1} 3344, 2943, 2876, 1726, 1512, 1243; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.97 (3H, s, CC*H*$_3$), 1.20 (3H, s, CC*H*$_3$), 3.10 (1H, d, J = 9.5 Hz, CC*H*$_2$), 3.48 (1H, d, J = 9.3 Hz, CC*H*$_2$), 3.78 (3H, s, OC*H*$_3$), 4.24 (1H, d, J = 5.5 Hz, NC*H*), 4.57 (2H, s, OC*H*$_2$Ph), 4.95 (1H, dd, J = 5.4 Hz, J = 11.5 Hz, OHC*H*), 5.40 (1H, d, J = 11.3 Hz, O*H*), 6.83 (2H, d, J = 9.0 Hz, Ar*H*), 7.19 (2H, d, J = 9.0 Hz, Ar*H*), 7.39 (5H, m, Ar*H*); ^{13}C

NMR (126 MHz, $CDCl_3$): δ 21.6 (CH_3), 27.3 (CH_3), 38.9 (CCH_3), 55.5 (OCH_3), 66.7 (NCH), 74.1 (HOCH), 74.6 (OCH_2Ph), 77.3 (CCH_2O), 114.2, 121.6, 128.4, 128.6, 128.7, 130.27, 136.2, 156.8, 168.4; HRMS: $C_{21}H_{25}NO_4$ (M^+), calc.: 355.1783, found: 355.1776.

(3*R*,4*S*)-(+)-3-Hydroxy-4-(2-benzyloxy-1,1-dimethylethyl)-1-(4-methoxyphenyl)-azetidin-2-one 2-10. Racemic *cis* or (3*R*,4*S*)-(+)-3-acetoxy-β-lactam **2-9** (2 g, 0.005 mol) was dissolved in tetrahydrofuran (40 mL) and cooled to 0 °C. The reaction mixture was slowly treated with 2 M KOH (20 mL) and stirred at 0 °C until TLC indicated complete conversion (2 h). The reaction was quenched with water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated. Crystallization from hexane and ethyl acetate yielded racemic *cis* or (3*R*,4*S*)-(+)-**2-10** (1.65 g, 92% yield) as colourless crystals. m.p.138-140 °C; $[\alpha]_D^{25}$ = +78.0 (c, 1.0, CH_2Cl_2). Spectra are identical to those of (3*S*,4*R*)-(-)-**2-10.**

2.4.9 (3*R*,4*S*)-(+)-3-Hydroxy-4-(2-hydroxy-1,1-dimethylethyl)-1-(4-methoxyphenyl)-azetidin-2-one 2-11. Ammonium formate (0.41 g, 0.006 mol) and palladium on activated carbon (1.2 g, 10 wt. %) were added to a solution of β-lactam (3*R*,4*S*)-(+)-**2-10** (1.1 g, 0.03 mol) in dry methanol (15 mL). The reaction was stirred under reflux for 20 minutes when TLC indicated complete conversion. The mixture was acidified with 2 M HCl to pH~3, and then extracted with ethyl acetate (40 mL x 3). The organic layers were combined and washed with brine, dried over magnesium sulfate, filtered, and evaporated to dryness to give the crude product. Crystallization with methylene chloride yielded (3*R*,4*S*)-(+)-**2-11** (0.398 g, 50% yield) as colourless crystals. m.p. 162-164 °C; $[\alpha]_D^{25}$ = + 70.2 (c, 1.0, CH_2Cl_2). IR ($CHCl_3$) γ_{max} /cm^{-1}: 3369, 2960, 2933, 1727, 1512, 1247, 1126, 1033; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.87 (3H, s, CH_3), 1.13 (3H, s, CH_3), 3.26 (1H, d, *J*

= 10.5 Hz, NC*H*), 3.72 (3H, s, OCH_3), 3.73 (1H, d, *J* = 10.2 Hz, CHO*H*), 4.23 (2H, d, *J* = 5.3 Hz, CH_2), 4.94 (1H, s, O*H*), 6.30 (1H, s, O*H*), 6.83 (2H, d, *J* = 10.0 Hz, Ar*H*), 7.23 (2H, d, *J* = 10.0 Hz, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 21.5 (*C*H_3), 26.5 (*C*H_3), 39.4 (*C*(Me)$_2$), 55.5 (O*C*H_3), 66.6 (N*C*H), 67.5 (OH*C*H), 76.5 (*C*H_2), 114.3, 122.4, 129.4, 157.3, 169.7 (*CO*); HRMS: $C_{14}H_{19}NO_4$ (M^+), calc.: 265.1001, found: 265.1006.

(3*S*,4*R*)-(-)-2-11: Colourless crystals. m.p. 155-157 °C; $[\alpha]_D^{25}$ = - 70.0 (c, 1.0, CH_2Cl_2).

Racemic *cis*-2-11: Colourless crystals. m.p. 133-134 °C.

2.4.10 (3*R*,4*S*)-(+)-3-(*t*-Butyldimethylsilanyloxy)-4-(2-benzyloxy-1,1-dimethylethyl)-1-(4-methoxyphenyl)azetidin-2-one 2-15. 3-Hydroxy-β-lactam (3*R*,4*S*)-(+)-**2-10** (1.1 g, 0.03 mol) was dissolved in dimethylformamide (2 mL). *t*-Butyldimethylsilyl chloride (0.73 g, 0.036 mol) and imidazole (2.5 equiv) were added. The mixture was stirred at 35 °C until TLC indicated complete conversion (3 h). The reaction was quenched with water and extracted with methylene chloride. The combined organic extracts were washed three times with water and brine, dried over magnesium sulfate. Filtration and concentration gave (3*R*,4*S*)-(+)-**2-15** (1.3 g, 97% yield) as a colourless oil. $[\alpha]_D^{25}$ = +52.1 (c, 1.2, CH_2Cl_2), IR ($CHCl_3$) γ_{max} /cm^{-1}: 2955, 2931, 2857, 1753, 1513, 1248, 1132; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.97 (3H, s, C*H*$_3$), 1.20 (3H, s, C*H*$_3$), 3.11 (1H, d, *J* = 9.3 Hz, OC*H*$_2$), 3.47 (1H, d, *J* = 9.3 Hz, CC*H*$_2$), 3.78 (3H, s, OC*H*$_3$), 4.35 (1H, d, *J* = 5.5 Hz, NC*H*), 4.57 (2H, s, OC*H*$_2$Ph), 4.96 (1H, dd, *J* = 5.4 Hz, *J* = 11.3 Hz, C*H*OH), 5.40 (1H, d, *J* = 11.3 Hz, OH), 6.83 (2H, d, *J* = 9.0 Hz, Ar*H*), 7.20 (2H, d, *J* = 9.0 Hz, Ar*H*), 7.39 (5H, m, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ -5.4 (Si*C*H$_3$), -4.6 (Si*C*H$_3$), 18.1 (Me$_3$*C*Si), 20.4 ((*C*H$_3$)$_2$C), 21.2 ((*C*H$_3$)$_2$C), 25.7 ((*C*H$_3$)$_3$CSi), 39.6 (*C*(CH$_3$)$_2$), 55.4 (O*C*H$_3$), 61.9 (N*C*H), 73.0 (HO*C*H), 76.1 (O*C*H$_2$Ph), 78.0 (C*C*H$_2$O), 114.0, 120.5, 127.3, 127.5, 128.3, 131.2, 138.4, 156.5, 167.4; HRMS: $C_{27}H_{39}NO_4Si$ (M^+), calc. for 469.2648, found: 469.2648.

Racemic *cis*-2-15: Colourless crystals. m.p.105-106 °C.

2.4.11 (3*R*,4*S*)-(+)-3-(*t*-Butyldimethylsilanyloxy)-4-(2-hydroxy-1,1-dimethylethyl)-1-(4-methoxyphenyl)azetidin-2-one 2-16. Ammonium formate (820 mg, 13 mmol) and palladium on activated carbon (1.5 g, 10 wt. %) were added to a solution of (3*R*,4*S*)-(+)-**2-15** (499 mg, 1 mmol) in dry methanol (20 mL). The reaction was stirred under reflux for 30 minutes when TLC indicated complete conversion. The mixture was acidified with 2 M HCl to pH~3, and then extracted with ethyl acetate (40 mL x 3). The organic layers were combined and washed with brine, dried over magnesium sulfate, filtered, and evaporated to dryness. Separation of the crude residue by flash column chromatography followed by crystallization with methylene chloride yielded (3*R*,4*S*)-(+)-**2-16** (230 mg, 57% yield) as colourless crystals. m.p. 103-104 °C; $[\alpha]_D^{25}$ = +53.7 (c, 1.0, CH_2Cl_2); IR ($CHCl_3$) γ_{max} /cm^{-1}: 3417, 2956, 2931, 2858, 1738, 1513, 1248, 836; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.97 (9H, s, $C(CH_3)_3$, 1.02 (3H, s, CH_3), 1.11 (3H, s, CH_3), 3.40(2H, q, OCH_2), 3.78 (1H, s, OCH_3), 4.39 (1H, d, J = 5.5 Hz, NCH), 5.02 (1H, d, J = 5.5 Hz), 6.86 (2H, d, J = 9.0 Hz, ArH), 7.37 (2H, d, J = 9.0 Hz, ArH), 7.39; ^{13}C NMR (126 MHz, $CDCl_3$): δ – 5.4 ($SiCH_3$), -4.6 ($SiCH_3$), 18.1 (Me_3CSi), 20.8 ($(CH_3)_2C$), 21.1 ($(CH_3)_2C$), 25.7 ($(CH_3)_3CSi$), 39.5 ($C(CH_3)_2$), 55.5 (OCH_3), 62.9 (NCH), 69.8 (HOCH), 114.2, 121.0, 131.0, 156.7, 167.2 (CON); HRMS: $C_{20}H_{33}NO_4Si$ (M^+), calc.: 379.21790, found: 379.21787.

Racemic *cis*-2-16: Colourless crystals. m.p. 101-102 °C.

2.4.12 (3*R*,4*S*)-(+)-3-(*t*-Butyldimethylsilanyloxy)-4-(1-formyl-1,1-dimethylmethyl)-1-(4-methoxyphenyl)azetidin-2-one 2-17. [19] A solution of oxalyl chloride (0.134 mL, 1.74 mmol) in dry methylene chloride (12 mL) was placed in a 50 mL three-neck round-

bottom flask equipped with two dropping funnels containing DMSO (0.265 mL, 3.76 mmol) dissolved in methylene chloride (4 mL) and (3*R*,4*S*)-(+)-**2-16** (660 mg, 1.88 mmol) dissolved in methylene chloride (8 mL), respectively. When the reaction mixture was cooled to –60 °C, the DMSO solution was added to the mixture, stirred for 5 minutes; then, the alcohol solution was added over a period of 10 minutes. After stirring at –60 °C for one hour, triethylamine (1.368 mL, 9.88 mmol) was added and stirred for an additional 4 hours at room temperature. When TLC indicated complete conversion, water (10 mL) was added and stirred for 10 min, followed by addition of saturated ammonium chloride solution (10 mL). The solution was extracted with methylene chloride, washed with brine, dried over magnesium sulfate, and filtered. Evaporation under vacuum gave (3*R*,4*S*)-(+)-**2-17** (600 mg, 83 % yield) as colourless crystals. m.p.110-111 °C; $[\alpha]_D^{25}$ = +56.4 (c, 1.05, CH_2Cl_2); IR ($CHCl_3$) γ_{max} /cm^{-1}: 2955, 2932, 2857, 1756, 1724, 1513, 1466, 1384, 1249, 1180, 1129, 894, 837, 783; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.15 (3H, s, CH_3Si), 0.22 (3H, s, CH_3Si), 0.92 (9H, s, $(CH_3)_3$ CSi), 1.21 (3H, s, CH_3C), 1.24 (3H, s, CH_3C), 3.78 (3H, s, OCH_3), 4.55 (1H, d, *J* = 5.5 Hz, NC*H*), 5.02 (1H, d, *J* = 5.5 Hz, C*H*OH), 6.85 (2H, d, *J* = 8.6 Hz, Ar*H*), 7.27 (2H, d, *J* = 8.6 Hz, Ar*H*), 9.64 (1H, s, C*H*O); ^{13}C NMR (126 MHz, $CDCl_3$): δ –5.5 ($SiCH_3$), -4.8 ($SiCH_3$), 18.1 (Me_3CSi), 19.3 (CH_3C), 22.1 (CH_3C), 25.6 (($CH_3)_3CSi$), 48.0 ($C(CH_3)_2$), 55.4 (OCH_3), 64.1 (NCH), 76.2 (OCH), 114.4, 114.4, 121.1, 122.5, 130.3, 156.9 (*C*-Ph), 166.3 (*C*O), 204.3 (*C*HO); HRMS: $C_{21}H_{33}NO_4Si$ (M^+), calc.: 391.21787, found: 391.21824.

Racemic *cis*-2-17: Colourless crystals. m.p.107-107.5 °C.

2.4.13 (3*R*,4*S*)-(+)-3-(*t*-Butyldimethylsilanyloxy)-4-(2-morpholin-1,1-dimethylethyl)-1-(4-methoxyphenyl)azetidin-2-one 2-18.[28] Aldehyde (3*R*,4*S*)-(+)-**2-17** (541 mg, 1.43

mmol) was dissolved in MeOH (4 mL); to this solution were added morpholine (0.36 mL, 4.2 mmol), AcOH (0.075 mL, 1.43 mmol), and $NaBH(OAc)_3$ (417 mg, 2 mmol). With ice cooling, the mixture was stirred at room temperature for 3 hours. The reaction mixture was quenched with saturated $NaHCO_3$ solution (20 mL) and extracted with EtOAc (20 mL x 3). The combined organic layer was washed with brine, and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel with hexane and ethyl acetate (4 : 1) to give (3*R*,4*S*)-(+)-**2-18** (340 mg, 53% yield) as a colourless solid. m.p. 90-91 °C; $[\alpha]_D^{25}$ = + 48.7 (c, 1.5, CH_2Cl_2); IR ($CHCl_3$) γ_{max} /cm^{-1}: 2956, 2931, 2857, 1749, 1512, 1378, 1247, 1131, 1036, 888, 836, 782; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.16 (3H, s, SiC*H*$_3$), 0.25 (3H, s, SiC*H*$_3$), 0.95 (6H, s, (C*H*$_3$)$_3$CSi), 1.01 (3H, s, C*H*$_3$CC), 1.04 (3H, s, C*H*$_3$CC), 2.21 (1H, d, *J* = 13.8 Hz, OC*H*$_2$Ph), 2.56 (1H, d, *J* = 13.8 Hz, OC*H*$_2$Ph), 2.43-2.48 (4H, m, C*H*$_2$NC*H*$_2$), 3.65-3.66 (4H, m, C*H*$_2$OC*H*$_2$), 3.77 (3H, s, OC*H*$_3$), 4.27 (1H, d, *J* = 5.5 Hz, NC*H*), 4.96 (1H, d, *J* = 5.5 Hz, OC*H*), 6.83 (2H, d, *J* = 8.8 Hz, Ar*H*), 7.28 (2H, d, *J* = 8.8 Hz, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ –5.4 (SiCH$_3$), -4.5 (SiCH$_3$), 18.1 (Me$_3$CSi), 22.4 ((CH$_3$)$_2$C), 25.2 ((CH$_3$)$_2$C), 25.7 ((CH$_3$)$_3$CSi), 40.3 (CMe$_2$), 55.4 (OCH$_3$), 56.2 (CH$_2$NCH$_2$), 65.7 (NCH), 76.5 (HOCH), 65.0 (CCH$_2$O), 67.3 (CH$_2$OCH$_2$), 114.0, 121.7, 130.9, 156.6 (*C*-Ph), 167.4 (*C*O); HRMS: $C_{24}H_{40}N_2O_4Si$ (M^+), calc.: 448.27572, found: 448.27602.

Racemic *cis*-**2-18**: Colourless solid; m.p. 91-92 °C.

2.4.14 (3*R*,4*S*)-(+)-3-(*t*-Butyldimethylsilanyloxy)-4-(2-(*t*-butyldimethylsilanyloxy)-1,1-dimethylethyl)-1-(4-methoxyphenyl)azetidin-2-one 2-12. Hydroxy-β-lactam (3*R*,4*S*)-(+)-**2-11** (1.06 g, 0.004 mol, 1.0 equiv) was dissolved in dimethylformamide (2 mL). *t*-Butyldimethylsilyl chloride (1.32 g, 0.009 mol) and imidazole (4 equiv) were

added. The mixture was stirred at 35 °C until TLC indicated complete conversion (4 h). The reaction was quenched with water and extracted with methylene chloride. The combined organic extracts were washed three times with water and brine, dried over magnesium sulfate. Filtration and concentration gave (3*R*,4*S*)-(+)-**2-12** (1.4 g, 72% yield) as colourless crystals; m.p. 82-83 °C; $[\alpha]_D^{25}$ = + 34.8 (c, 1.0, CH_2Cl_2). IR ($CHCl_3$) γ_{max} /cm^{-1}: 2955, 2931, 2857, 1753, 1513, 1248, 1132; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.17 (6H, s, C*H*$_3$Si), 0.24 (6H, s, C*H*$_3$Si), 0.95 (18H, s, (C*H*$_3$)$_3$C), 3.15 (1H, d, *J* = 10.1 Hz, C*H*OBn), 3.32 (1H, d, *J* = 10.1 Hz, C*H*OBn), 3.77 (3H, s, OC*H*$_3$), 4.42 (1H, d, *J* = 5.5 Hz, NC*H*), 4.98 (1H, d, *J* = 5.5 Hz, C*HO*), 6.80 (2H, d, *J* = 8.9 Hz, Ar*H*), 7.30 (2H, d, *J* = 8.9 Hz, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ –5.5 (CH_3Si), -5.4 (*C*H$_3$Si), -5.4 (*C*H$_3$Si), -4.6 (*C*H$_3$Si), 18.1 (Me$_3$*C*), 18.3 (Me$_3$*C*), 19.5 (*C*H$_3$C), 20.8 (*C*H$_3$C), 25.7 ((CH$_3$)$_3$C), 25.9 ((*C*H$_3$)$_3$C), 39.3 (*C*(Me)$_2$), 55.4 (OC*H*$_3$), 61.3 (NC*H*), 76.0 (O*CH*), 70.5 (*C*H$_2$), 113.9, 120.3, 131.5, 156.4 (*C*-Ph), 167.5 (*CO*). HRMS: $C_{26}H_{47}NO_4Si_2$ (M^+), calc.: 493.30435, found: 493.30518.

Racemic *cis*-2-12: Colourless crystal. m.p. 79-80 °C.

2.4.15 (3*R*,4*S*)-(+)-3-(*t*-Butyldimethylsilanyloxy)-4-(2-(*t*-butyldimethylsilanyloxy)-1,1-dimethylethyl)azetidin-2-one 2-13. A solution of β-lactam (3*R*,4*S*)-(+)-**2-12** (262 mg, 0.531 mmol) in acetonitrile (25 mL) was cooled to -10 °C. CAN (1.016 g, 1.858 mmol) (3.5 equiv) in distilled water (14 mL) was added dropwise to the solution over the period of one hour. The reaction mixture was diluted with distilled water (10 mL) and stirred at -10 °C for 20 minutes. Then, the mixture was extracted with ethyl acetate (three times), and the combined organic layers were washed with 5% sodium bisulfite solution, 10% sodium carbonate solution, 5% sodium bisulfite solution, and brine. The yellow

organic layers were dried over magnesium sulfate, filtered, and concentrated under vacuum. Purification of the crude products by flash chromatography on silica gel with hexane and ethyl acetate (7 : 1) as eluting solvent gave the N-H-lactam (3*R*,4*S*)-(+)-**2-13** (152.3 mg, 74 % yield) as colourless crystals. m.p. 97-98 °C; $[\alpha]_D^{25}$ = + 47.1 (c, 0.25, CH_2Cl_2); IR ($CHCl_3$) γ_{max} /cm^{-1}: 3232, 2955, 2930, 2858, 1763, 1472, 1254, 1190, 196, 890, 837, 729, 668; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.14 (6H, s, C*H*$_3$Si), 0.19 (6H, s, C*H*$_3$Si), 0.87 (9H, s, (C*H*$_3$)$_3$C), 0.92 (9H, s, (C*H*$_3$)$_3$C), 1.02 ((C*H*$_3$)$_2$C), 3.26 (1H, d, *J* = 9.6 Hz, (C*H*)$_2$O), 3.40 (1H, d, *J* = 9.6 Hz, (C*H*)$_2$O), 3.56 (1H, d, *J* = 5.0 Hz, NC*H*), 4.87 (1H, d, *J* = 5.0 Hz, C*HO*), 5.88 (N*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ -5.6 ((*C*H$_3$)$_2$Si), -5.5 (*C*H$_3$Si), -4.6 (*C*H$_3$Si), 18.0 (Me$_3$*C*Si), 18.3 (Me$_3$*C*Si), 19.3 (*C*H$_3$C), 19.9 (*C*H$_3$C), 25.7 ((*C*H$_3$)$_3$C), 25.9 ((*C*H$_3$)$_3$C), 37.3 (*C*(Me)$_2$), 60.5 (NCH), 78.3 (OCH), 73.2 (*C*H$_2$), 169.5 (*CO*); HRMS: $C_{19}H_{41}NO_3Si_2$ (M^+), calc.: 387.26251, found: 387.26272.

Racemic *cis*-2-13: Colourless crystals. m.p. 93-94 °C.

2.4.16 (3*R*,4*S*)-(+)-3-(*t*-Butyldimethylsilanyloxy)-4-(2-(*t*-butyldimethylsilanyloxy)-1,1-dimethylethyl)-1-(*t*-butoxycarbonyl)azetidin-2-one 2-14. Triethylamine (4 equiv) was added dropwise to a stirred solution of N-H-β-lactam (3*R*,4*S*)-(+)-**2-13** (775 mg, 2 mmol), di-*t*-butyl-dicarbonate (808 mg, 4 mmol) (2 equiv), and DMAP (0.3 equiv) in 15 mL of dry methylene chloride at room temperature. After the addition of amine, the reaction mixture was monitored by TLC until complete conversion was indicated. The reaction was quenched with saturated aqueous NH_4Cl solution, and extracted with ethyl acetate. The combined organic layers were washed with saturated aqueous NH_4Cl and brine solution, dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum. The crude material was purified by flash chromatography on silica gel (hexane: EtOAc =

8 : 1) to afford (3*R*, 4*S*)-(+)-**2-14** (917 mg, 94% yield) as a colourless oil. $[\alpha]_D^{25} = +57.1$(c, 1.01, CH_2Cl_2); IR ($CHCl_3$) γ_{max} /cm^{-1}: 2956, 2931, 2858, 1808, 1729, 1472,1318, 1256, 1156, 1095, 838, 780; ^{1}H NMR (500 MHz, $CDCl_3$): δ -0.17 (3H, s, C*H*$_3$Si), -0.18 (6H, s, C*H*$_3$Si), -0.05 (3H, s, C*H*$_3$Si), 0.70 (9H, s, ((C*H*$_3$)$_3$CSi), 0.72 (9H, s, ((C*H*$_3$)$_3$CSi), 0.82 (1H, s, C*H*$_3$C), 0.85 (1H, s, C*H*$_3$C), 1.31 (9H, s, (C*H*$_3$)$_3$CO), 3.20 (1H, d, *J* = 9.5 Hz, (C*H*)$_2$O), 3.41 (1H, d, *J* = 9.5 Hz, (C*H*)$_2$O), 3.93 (1H, d, *J* = 6.6 Hz, NC*H*), 4.72 (1H, d, *J* = 6.6 Hz, C*HO*); ^{13}C NMR (126 MHz, $CDCl_3$): δ -5.5 ((*C*H$_3$)$_2$Si), -5.5 (*C*H$_3$Si), -4.7 (*C*H$_3$Si), 18.0 (Me$_3$*C*Si), 18.3 (Me$_3$*C*Si), 20.1 (*C*H$_3$C), 22.7 (*C*H$_3$C), 25.6 ((*C*H$_3$)$_3$CSi), 25.9 ((*C*H$_3$)$_3$CSi), 28.0 ((*C*H$_3$)$_3$CO), 39.4 (*C*(Me)$_2$), 63.2 (N*C*H), 76.1 (O*C*H), 83.0 (Me$_3$*C*O), 69.6 (*C*H$_2$), 149.1 (COO), 167.8 (*CO*); HRMS: $C_{24}H_{49}NO_5Si_2$ (M^+), calc.: 487.31491, found: 487.31496.

2.4.17 (4*R*)-4-(2-Benzyloxy-1,1-dimethylethyl)-1-(4-methoxyphenyl)azetidin-2,3-dione 2-20. Phosphorus pentoxide (568 mg, 1.5 equiv) was added to dry DMSO (15 mL) and stirred at room temperature for 10 minutes. The starting material (3*S*,4*R*)-**2-10** (710 mg, 2 mmol) dissolved in 6 mL of DMSO, was added dropwise. The resulting mixture was stirred at room temperature, until TLC indicated complete conversion (24 h). The reaction was quenched with cooled saturated $NaHCO_3$ solution and extracted with ethyl acetate (25 mL x 3). The combined organic layers were washed with water (20 mL x 3) to remove excess DMSO, washed with brine, dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by flash chromatography on silica gel and crystallized from hexane and ethyl acetate (8 : 1) to give (4*R*)-**2-20** (310 mg, 55 % yield) as yellow crystals. mp: 137-138 °C; IR ($CHCl_3$) γ_{max} /cm^{-1}: 2962, 2930, 2874, 1813, 1759, 1512, 1464, 1251, 1113, 1030, 978, 830, 739, 604; ^{1}H NMR (500 MHz, $CDCl_3$): δ

1.10 (3H, s, CH_3), 1.14 (3H, s, CH_3), 3.19 (1H, s, OCH_2Ph), 3.20 (1H, s, OCH_2Ph), 3.86 (3H, s, OCH_3), 4.49 (1H, s, CH_2OBn), 4.51 (1H, s, CH_2OBn), 4.78 (1H, s, NC*H*), 6.92 (2H, d, J = 9.2 Hz, Ar*H*), 7.45 (2H, d, J = 9.2 Hz, Ar*H*), 7.30-7.43 (5H, m, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 21.3(*C*H_3), 23.71 (*C*H_3), 39.4 (*C*Me$_2$), 55.6 (O*C*H_3), 73.5 (O*C*H_2Ph), 75.8 (N*C*H), 76.9 (C*C*H_2O), 114.4, 121.1, 127.8, 128.5, 129.9, 137.6, 158.0 (*C*-Ph), 161.4 (CON), 194.4 (*C*OC); HRMS: $C_{20}H_{21}NO_4$ (M^+), calc.: 339.14706, found: 339.14711.

2.4.18 (3*R*,4*R*)-(+)-3-Hydroxy-4-(2-benzyloxy-1,1-dimethylethyl)-1-(4-methoxy-phenyl)azetidin-2-one 2-10. Dry baker's yeast (7 g) was added to a solution of sucrose (26 g) in sterilized water (250 mL) contained in a 1L flask with 500 mL working volume. The mixture was stirred vigorously at 30 °C for 30 minutes in order to activate the yeast. (4*R*)-**2-20** (300 mg, 0.89 mmol), finely ground with 300 mg of β-cyclodextrin, was added to a fermenting yeast and the reaction was monitored by TLC. When the reaction reached 100% conversion (48 h), the reaction was stopped. The reaction mixture was saturated with sodium chloride and centrifuged at 3000 x g for 10 minutes in order to remove yeast cells. The cell pellet was washed with ethyl acetate. The supernatant liquid was extracted continuously with ethyl acetate for 24 hours and the combined extracts were washed with brine and dried over anhydrous $MgSO_4$. After removing the solvent under reduced pressure, the crude residue was purified by flash chromatography to yield optically pure *trans*-3-hydroxy-β-lactam (3*R*,4*R*)-**2-10** (270 mg, 90% yield) as colourless crystals. m.p. 95-96 °C; $[\alpha]_D^{25}$ = +39.5 (c, 0.25, CH_2Cl_2); IR ($CHCl_3$) γ_{max}/cm^{-1} 3350, 2960, 2930, 2870, 1750, 1510, 1250; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, s, CH_3), 1.02 (3H, s, CH_3), 3.10 (1H, s, CH_2OBn), 3.11 (1H, s, CH_2OBn), 3.75 (3H, s, OCH_3), 4.13 (1H, s, NC*H*),

4.80 (1H, s, C*H*OH), 4.40 (1H, s, OC*H*$_2$Ph), 4.42 (1H, s, OC*H*$_2$Ph), 6.75 (2H, d, *J* = 8.5 Hz, Ar*H*), 7.16 (2H, d, *J* = 8.5 Hz, Ar*H*), 7.24-7.39 (5H, m, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 21.6 (*C*H$_3$), 27.3 (*C*Me$_2$), 5.5 (O*C*H$_3$), 66.9 (N*C*H), 74.0 (HO*C*H), 74.6 (O*C*H$_2$Ph), 77.2 (C*C*H$_2$O), 114.2, 121.6, 128.3, 128.6, 128.6, 130.3, 136.2, 156.8, 168.4; HRMS: $C_{21}H_{25}NO_4$ (M^+), calc.: 355.17857, found: 355.17838.

2.4.19 *cis*-(±)-3-(*t*-Butyldimethylsilanyloxy)-4-(2-benzyloxy-1,1-dimethylethyl)-azetidin-2-one 2-21. A solution of β-lactam **2-15** (249 mg, 0.531 mmol) in acetonitrile (25 mL) was cooled to -10 °C. CAN (1.016 g, 1.858 mmol) (3.5 equiv) in distilled water (14 mL) was added dropwise to the solution over the period of one hour. The reaction mixture was diluted with distilled water (10 mL) and stirred at –10 °C for 20 minutes. Then, the mixture was extracted with ethyl acetate (three times), and the combined organic layers were washed with 5% sodium bisulfite solution, 10% sodium carbonate solution, 5% sodium bisulfite solution, and brine. The organic yellow layers were dried over magnesium sulfate, filtered, and concentrated under vacuum. Purification of the crude products by flash column chromatography on silica gel with hexane and ethyl acetate (7 : 1) as eluting solvent gave the N-H-lactam **2-21** (270 mg, 52% yield) as a beige oil. IR ($CHCl_3$) γ_{max} /cm^{-1}: 3210, 2955, 2930, 2857, 1761, 1471, 1361, 1254, 1191, 1099, 894, 838, 781, 697. ^{1}H NMR (500 MHz, $CDCl_3$): δ -0.05 (3H, s, SiC*H*$_3$), -0.03 (3H, s, SiC*H*$_3$), 0.73 (9H, s, (C*H*$_3$)$_3$C), 0.79 (3H, s, C*H*$_3$C), 0.92 (3H, s, C*H*$_3$C), 2.97 (1H, d, *J* = 8.5 Hz, C*H*$_2$OBn), 3.10 (1H, d, *J* = 8.5 Hz, C*H*$_2$OBn), 4.24 (1H, s, OC*H*$_2$Ph), 4.26 (1H, s, OC*H*$_2$Ph), 3.39 (1H, d, *J* = 4.9 Hz, NC*H*), 4.68 (1H, d, *J* = 4.9 Hz, OC*H*), 5.6 (1H, s, N*H*), 7.06-7.17 (5H, m, Ar*H*).

2.4.20 *cis*-(±)-3-(*t*-Butyldimethylsilanyloxy)-4-(2-benzyloxy-1,1-dimethylethyl)-1-(*t*-butyloxycarbonyl)azetidin-2-one 2-22. Triethylamine (4 equiv) was added dropwise to a stirred solution of N-H-β-lactam **2-21** (1 equiv), di-*t*-butyl-dicarbonate (2 equiv), and DMAP (0.3 equiv) in 10 mL of dry methylene chloride at room temperature. After the addition of amine, the reaction mixture was monitored by TLC until complete conversion was indicated. The reaction was quenched with saturated aqueous NH_4Cl solution, and extracted with ethyl acetate. The combined organic layers were washed with saturated aqueous NH_4Cl and brine solution, dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum. The crude material was purified by flash chromatography on silica gel (hexane : EtOAc = 8 : 1) to afford N-*Boc*-lactam **2-22** as a beige oil. IR ($CHCl_3$) γ_{max} /cm^{-1}: 2956, 2931, 2858, 1808, 1729, 1472, 1254, 1170, 838, 670; ^{1}H NMR (500 MHz, $CDCl_3$): δ -0.06 (3H, s, SiC*H*$_3$), -0.08 (3H, s, SiC*H*$_3$), 0.71 (9H, s, (C*H*$_3$)$_3$CSi), 0.91 (6H, s, (C*H*$_3$)$_2$C), 1.29 (9H, s, (C*H*$_3$)$_3$CO), 3.14 (1H, d, *J* = 8.7 Hz, C*H*$_2$OBn), 3.23 (1H, d, *J* = 8.7 Hz, C*H*$_2$OBn), 4.24 (1H, s, OC*H*$_2$Ph), 4.27 (2H, s, OC*H*$_2$Ph), 4.01 (1H, d, *J* = 6.6 Hz, NC*H*), 4.70 (1H, d, *J* = 6.6 Hz, OC*H*), 7.06-7.11 (5H, m, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ -5.6 ((*C*H$_3$)$_2$Si), -0.7 (*C*H$_3$Si), -0.5 (*C*H$_3$Si), 22.8 (Me$_3$*C*Si), 25.6 (*C*H$_3$C), 27.1 (*C*H$_3$C), 30.4 ((*C*H$_3$)$_3$CSi), 32.8 ((*C*H$_3$)$_3$CO), 43.3 (*C*(Me)$_2$), 68.0 (NC*H*), 70.0 (OC*H*), 77.9 (OCH$_2$Ph), 80.8 (O*C*(Me)$_3$), 87.8 (*C*OBn), 143.6-132.1 (*C*-Ph), 152.2 (*C*OO), 169.5 (*CO*).

2.4.21 General procedure for preparation of (*S*)-MTPA-derivatives of 2-10 [34,35]

A solution of (*R*)-(-)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride (22.73 mg, 0.108 mmol) in CH_2Cl_2 (0.3 mL) was added dropwise to a solution of (3*R*,4*S*); (3*R*,4*R*); (3*S*,4*S*)-**2-10** (32 mg, 0.09 mmol), Et_3N (41 μL, 0.294 mmol), and DMAP (3 equiv) in CH_2Cl_2 (0.9 mL) at 0 °C. After stirring overnight, the reaction was diluted with

Et_2O (6 mL) and then poured into an aqueous saturated $NaHCO_3$ solution (10 mL). The phases were separated, and the aqueous phase was extracted with Et_2O (10 mL x 2). The combined organic fractions were dried over $MgSO_4$ and concentrated under vacuum. The resulting crude product was purified by column chromatography (SiO_2, hexane : EtOAc = 6 : 1) providing the derivatized product (47 mg , 83% yield).

3-(*S*)-(-)-MTPA-(3*R*,4*S*)-(+)-2-10. Colourless oil. IR ($CHCl_3$) γ_{max} /cm^{-1}: 2980, 2958, 2853, 1766, 1513, 1245, 1453, 1387, 1245, 1173, 1107, 1032, 982, 830, 754, 717, 521; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.78 (3H, s, C*H*$_3$), 0.91 (3H, s, C*H*$_3$), 2.91 (1H, d, *J* = 9.1 Hz, C*H*$_2$OBn), 3.04 (1H, d, *J* = 9.1 Hz, C*H*$_2$OBn), 3.44 (3H, s, OC*H*$_3$), 3.75 (3H, s, OC*H*$_3$), 4.68 (1H, d, *J* = 5.5 Hz, NC*H*), 6.18 (1H, d, *J* = 5.5 Hz, C*H*OH), 4.27 (1H, d, *J* = 11.5 Hz, OC*H*$_2$Ph), 4.31 (1H, d, *J* = 11.5 Hz, OC*H*$_2$Ph), 6.73 (2H, d, *J* = 11.1 Hz, Ar*H*), 7.18-7.51 (12H, m, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 21.6 (*C*H$_3$), 27.3 (*C*Me$_2$), 5.4 (O*C*H$_3$), 66.9 (N*C*H), 74.0 (HO*C*H), 74.6 (O*C*H$_2$Ph), 77.2 (C*C*H$_2$O), 114.2, 121.6, 128.3, 128.5, 128.7, 130.3, 136.2, 156.8, 168.

3-(*S*)-(-)-MTPA-(3*S*,4*R*)-(+)-2-10. Colourless oil. IR ($CHCl_3$) γ_{max} /cm^{-1}: 2980, 2958, 2853, 1766, 1513, 1245, 1453, 1387, 1245, 1173, 1107, 1032, 982, 830, 754, 717, 521; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.60 (3H, s, C*H*$_3$), 0.72 (3H, s, C*H*$_3$), 2.74 (1H, d, *J* = 9.2 Hz, C*H*$_2$OBn), 2.93 (1H, d, *J* = 9.2 Hz, C*H*$_2$OBn), 3.63 (3H, s, OC*H*$_3$), 3.70 (3H, s, OC*H*$_3$), 4.16 (1H, d, *J* = 12.2 Hz, OC*H*$_2$Ph), 4.23 (1H, d, *J* = 12.2 Hz, OC*H*$_2$Ph), 4.61 (1H, d, *J* = 6.1 Hz, NC*H*), 6.22 (1H, d, *J* = 5.5 Hz, C*H*OH), 6.73 (2H, d, *J* = 9.1 Hz, Ar*H*), 7.17-7.51 (12H, m, Ar*H*).

3-(*S*)-(-)-MTPA-(3*R*,4*R*)-(+)-2-10. Colourless oil. IR ($CHCl_3$) γ_{max} /cm^{-1}: 2980, 2958, 2853, 1766, 1513, 1245, 1453, 1387, 1245, 1173, 1107, 1032, 982, 830, 754, 717, 521;

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.90 (3H, s, CH_3), 0.95 (3H, s, CH_3), 3.04 (1H, d, J = 9.1 Hz, CH_2OBn), 3.10 (1H, d, J = 9.1 Hz, CH_2OBn), 3.46 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 5.94 (1H, s, NC*H*), 4.31 (1H, d, J = 5.5 Hz, C*H*OH), 4.33 (1H, s, OCH_2Ph), 4.36 (1H, s, OCH_2Ph), 6.73 (2H, d, J = 9.2 Hz, Ar*H*), 7.13 (2H, d, J = 9.2 Hz, Ar*H*), 7.51 (10H, m, Ar*H*).

2.5 References

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CHAPTER 3 EVALUATION OF SEVERAL MICROBIAL REDUCTASES AS ENANTIOSELECTIVE REDUCING AGENTS FOR α-KETOESTERS

3.1 Introduction

The development of methods for the synthesis of enantiopure compounds by microbial transformations has become an important goal in bioorganic chemistry.[1] Microbial transformations with high stereoselectivities have been applied to asymmetric syntheses in order to circumvent the disadvantages of conventional organic synthetic processes.[1] Reduction of a carbonyl group to a homochiral alcohol can be achieved efficiently by a variety of microorganisms. Baker's yeast (*Saccharomyces cerevisiae*) is by far the most commonly used[2]; other frequently used microorganisms include *Thermoanerobic brockii,*[3] *Lactobacillus kefir,*[4] *Pseudomonas sp.*,[5] and *Candida magnoliae*.[6] During the last decade, reductases overexpressed in host organisms have become important bioreagents for the reduction of carbonyl compounds.[7]

It is interesting to note that many, but certainly not all, microbial reductions of prochiral ketones follow Prelog's *Re*-face attack rule [8] to give *S*-alcohols as shown in **Figure 3-1**.[9] Prelog's rule depends on the relative size of the two groups (R_S and R_L) attached to the carbonyl since they can be recognized by reductases. [10]

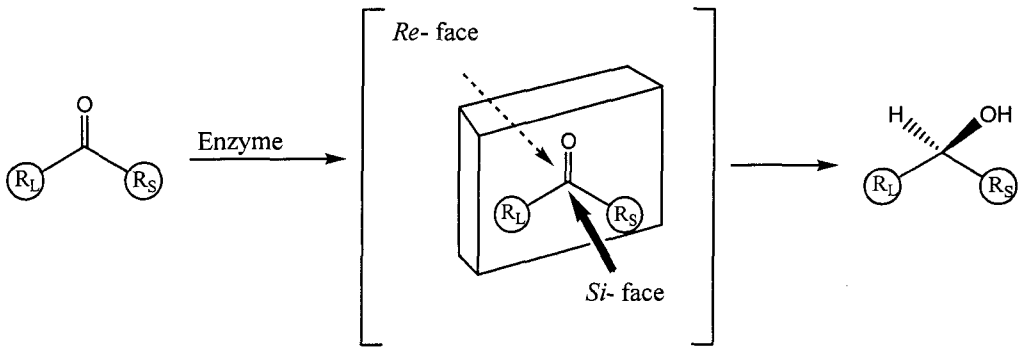


Figure 3-1 (*S*)-Alcohol obtained following Prelog's rule.

Yeast genome encodes a large number of reductases, several of which can accept a given substrate but not necessarily with the same enantioselectivities, thus leading to product alcohols with reduced enantioselectivity. However, the problem of competing reductases is minimized when a desired reductase is overexpressed in other simple and easy-to-handle hosts.[7] The common host *Escherichia coli* (*E. coli*) is often used since it is well suited for genetic manipulations and can be used in large-scale transformations. It has been shown that individual reductases from different microorganisms overexpressed in *E. coli* lead to improved reductions of many ketoesters.[9-11] The mutated reductases overexpressed in *E. coli* further enlarge the family of new enantioselective bioreductants. The advantage of the overexpression systems, in general, is that they produce the required cofactor(s) as well as a large quantity of a target enzyme.

Often, the preliminary evaluation of new enzymes is carried out with purified enzymes to establish unambiguously stereo- and enantioselectivity. The screening with purified reductases requires the addition of commercially available but expensive cofactor NAD(P)H. In this project, several purified carbonyl reductases from various

organisms were evaluated.† To reduce the cost, the glucose dehydrogenase (GDH)/NAD(P)H-recycling system was used (**Figure 3-2**) to provide *in situ* continuous regeneration of the NAD(P)H cofactor.

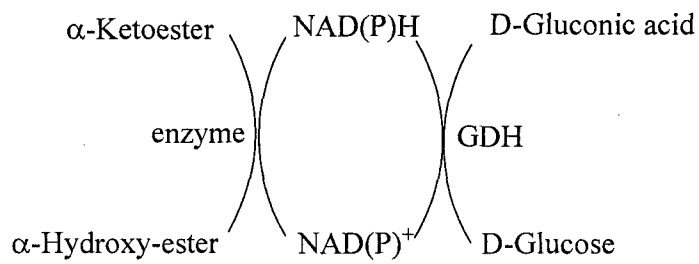


Figure 3-2 NAD(P)H-regeneration system for the reduction of α-ketoesters.

The wild type enzymes used in this project came from several microorganisms described in Chapter **1**. All mutants were the products of rational design by site-directed mutagenesis of enzymes from a single organism red yeast *Sporobolomyces salmonicolor* (SSCR). In site-directed mutagenesis, the information in the genetic material is changed by modifying a particular codon in the DNA molecule. Ideally, the modifications should be carried out within or close to the active site, providing that the active site is known. This reprogrammed DNA molecule will then direct the synthesis of a protein with an exchanged amino acid close to the active site.[12] Since both the active site and the catalytic mechanism of wild type SSCR are known, all SSCR mutants were prepared *via* site-directed mutagenesis. Hua and coworkers used a modeling program [10] to design amino acid exchanges close to the hydrophobic pocket of the active site that would modify the enzyme in such a way as to accommodate larger molecules. The exchanged amino acids in the mutants are listed in **Table 1-3**.

† The protocols of gene expression and purification for the carbonyl reductases are described in **Appendix I**. Various organisms were listed in **Table 1-3**.

As was discussed before (Chapter **2**) the paclitaxel C-13 side-chain and its analogues can be derived from enantiopure 3-hydroxy-β-lactams or from linear β-amino-α-hydroxyesters.[13] In either case, the desired enantioselectivity can be introduced during bioreduction of their α-ketoesters. Since enantioselectivity of the wild type and mutant reductases (**Table 1-3**) towards α-ketoesters with pre-existing chiral center has not been established before, four α-ketoesters shown in **Figure 3-3** were chosen as substrates for screening. Among them, α-keto esters **3-3** and **3-7** are potential precursors of the enantiopure paclitaxel C-13 side chain. The remaining two are precursors of paclitaxel C-13 side chain analogues. The proposed route to the enantiopure (2*R*,3*S*) C-13 paclitaxel side chain is outlined in **Scheme 3-1**. Unfortunately, we were unable to prepare substrate **4-3**‡ and only compounds **3-3, 3-7** and **2-20** were used in screening against the purified enzymes listed in **Table 1-3**.

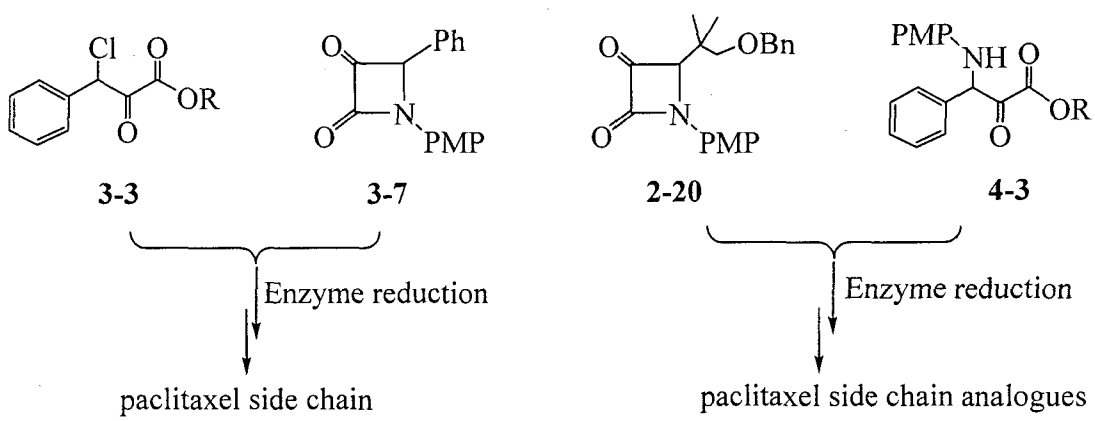
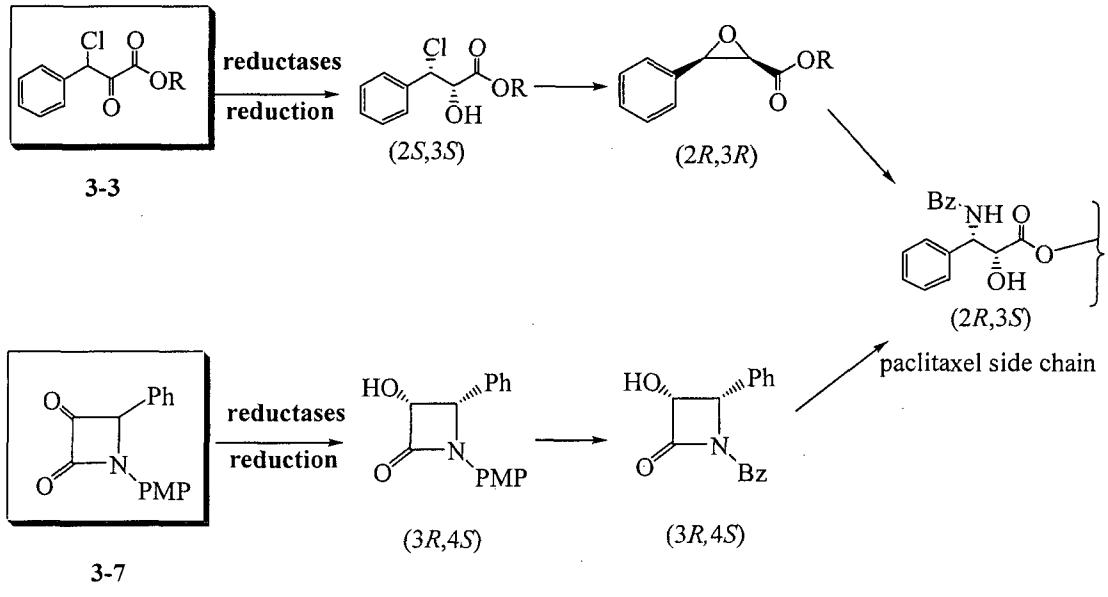


Figure 3-3 Four α-ketoesters attempted for screening.

‡ The details are discussed in Chapter 4.

Scheme 3-1 The route to the enantiopure (2*R*,3*S*) C-13 paclitaxel side chain from the α-ketoesters **3-3** and **3-7**.



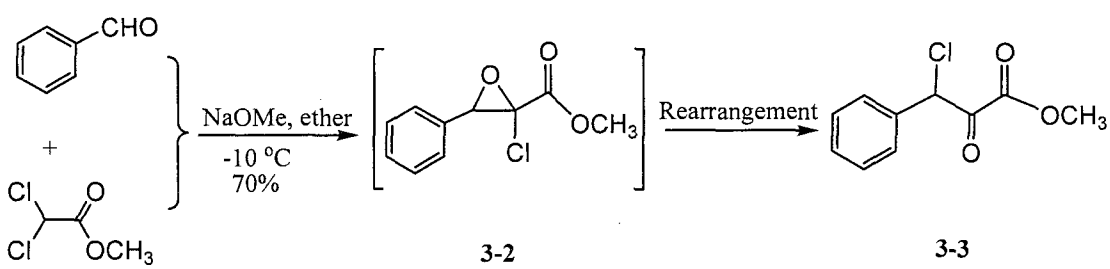
Thus, in order to identify the most enantioselective enzymes for each substrate, β-chloro-α-keto ester **3-3** and β-lactams **3-7** and **2-20** were screened against reductases from six microorganisms and seven SSCR mutant reductases (shown in **Table 1-3**).

3.2 Results and Discussion

3.2.1 Substrates for screening of reductases overexpressed in *E. coli.*

The syntheses of β-lactams **2-20** and **3-7** and the assignment of absolute configurations of their reduction products were discussed in Chapter **2**. The 3-chloro-2-ketoester **3-3** was synthesized by base-catalyzed condensation of benzaldehyde with dichloroacetate **3-1**. This type of Darzens condesation[14] has been frequently used to obtain *trans*-glycidic esters. In this case, *trans*-glycidic ester **3-2** was not isolated but underwent spontaneous rearrangement to the more stable chloro ketone **3-3** as shown in **Scheme 3-2**. Attempts to isolate the product by vacuum distillation (b.p. 105 °C/ 5 mmHg[15]) gave a mixture of **3-3** and unreacted aldehyde (b.p. 106 °C /5 mmHg). Subsequent purification by column chromatography allowed easy separation of **3-3** from the unreacted aldehyde. The product **3-3** was isolated as a yellow oil which became a semi-solid below -10 °C.

Scheme 3-2



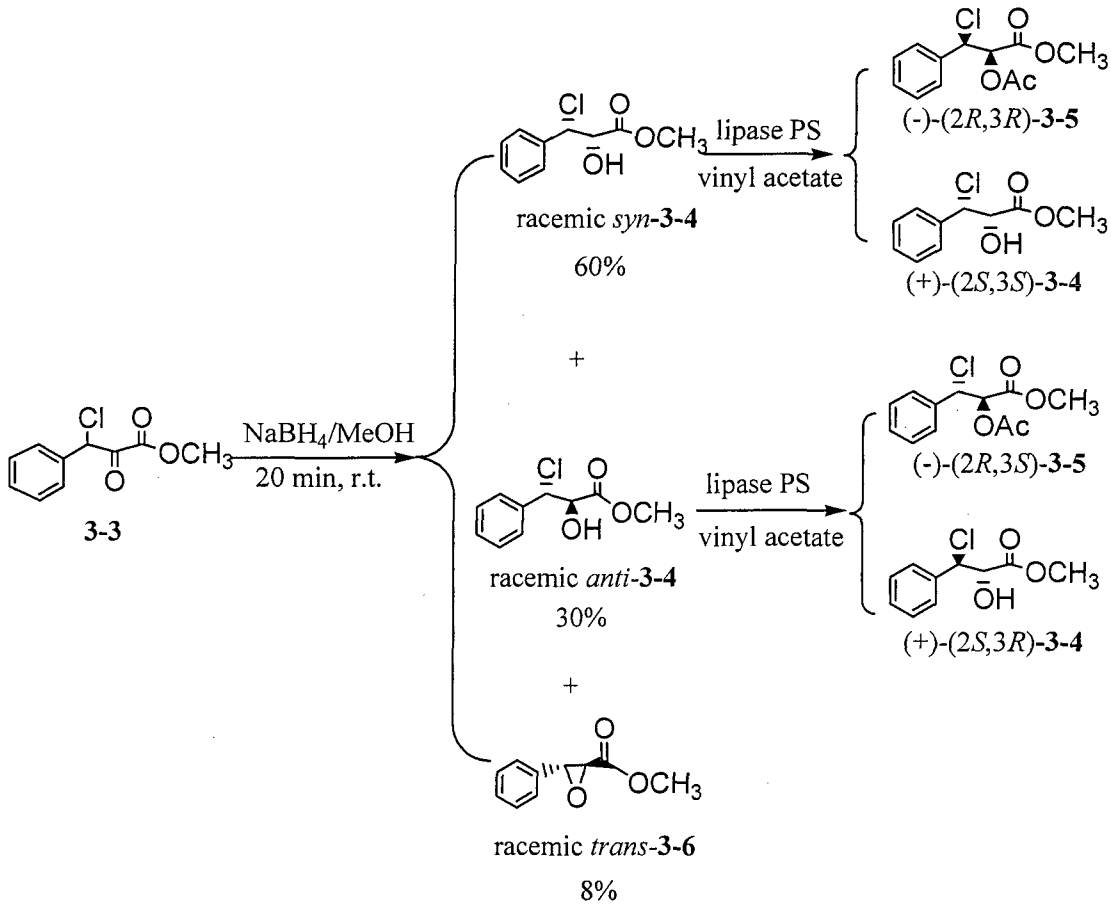
3-1 **3-2** **3-3**

3.2.2 Assignment of absolute configuration by lipase resolution

The chloro-ketoester **3-3** was rapidly (20 min) reduced with sodium borohydride to yield the *syn*- and *anti*-alcohols **3-4** in 60% and 30% yield respectively (**Scheme 3-3**).

The amount of $NaBH_4$ (whether 1.5 or 4 equiv) or the length of time of reaction did not influence the ratio of the *syn* and *anti* products; therefore, the minimum (1.5 equiv.) amount of $NaBH_4$ was used in the optimized reaction. In addition to two alcohols, 8% of *trans*-epoxide **3-6** was isolated from the reaction mixture. The formation of **3-6** was caused by the spontaneous ring closure of the *anti*-**3-4** which accounted for the lower yield of *anti*-**3-4** product.

Scheme 3-3



The absolute configuration of the alcohol was deduced from the kinetic resolution of racemic *syn*-and *anti*-**3-4** by lipase PS mediated transesterification in diisopropyl ether [15] (**Scheme 3-3**). The racemic *syn*- and *anti*-**3-4** diastereomers were separated on silica

column monitored by GC and in the following step lipase converted (2*R*,3*R*)-**3-4** and (2*R*,3*S*)-**3-4** to the acetylesters (2*R*,3*R*)-**3-5** and (2*R*,3*S*)-**3-5** leaving enantiomers (2*S*,3*S*)-**3-4** and (2*S*,3*R*)-**3-4** as alcohols. The four enantiomers are fully resolved on chiral phase GC. The reaction was monitored by chiral GC and was stopped when (2*R*)-**3-4** was totally consumed (spectra shown in **Appendix III**). The retention times for all isomers are shown in **Table 3-1**. The optical rotation and the assignment of individual enantiomers are in accordance with the literature data. [15]

Table 3-1 Retention times of stereoisomers on chiral phase GC.

Racemic *syn*-**3-4**	(2*S*,3*S*)-(+)-**3-4**	(2*R*,3*R*)-(-)-**3-4**	(2*R*,3*R*)-(-)-**3-5**
RT (min)	64.80	64.22	71.01
Racemic *anti*-**3-4**	(2*S*,3*R*)-(+)-**3-4**	(2*R*,3*S*)-(-)-**3-4**	(2*R*,3*S*)-(-)-**3-5**
RT (min)	65.92	67.69	68.72

3.2.3 Reduction with baker's yeast

Baker's yeast reduces a wide spectrum of substrates. Prior to testing the reductases overexpressed in *E.coli,* verification of whether whole yeast accepts substrate **3-3** and whether the reduction is diastereo- and/or enantioselective was required. Following the standard protocol, the reaction, monitored by GC, reached total conversion in 24 hours. The values of conversion versus time are shown in **Table 3-2** and plotted in **Figure 3-4**.

Table 3-2 Conversions (%) obtained in yeast-catalyzed reduction of **3-3**.

Time (h)	**3-3** (%)	Racemic *syn*-**3-4** (%)	Racemic *anti*-**3-4** (%)	Total conv. (%)
0	100	0	0	0
1.5	65.65	5.09	29.26	34.35
3.5	40.66	8.94	50.40	59.34
5.5	30.09	10.25	59.66	69.91
7.5	24.73	11.32	63.95	75.27
20	4.24	14.10	81.66	95.76

Note: DB-1301 non chiral GC column (15 cm x 0.53 mm x 1.0 μm). Program:100 °C for 4 min, 10 °C min^{-1} to 80 °C, 180 °C for 10 min.

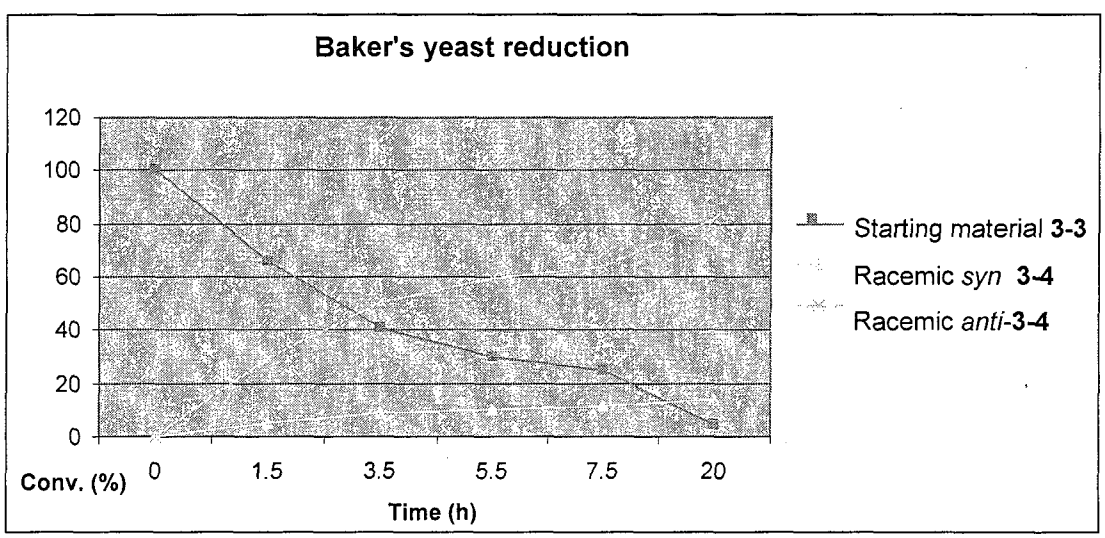
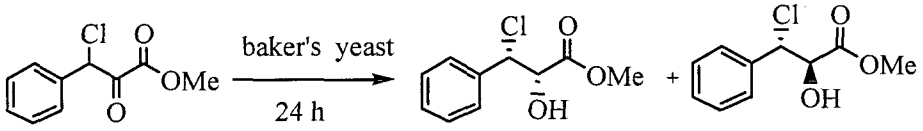


Figure 3-4 Conversion of β-chloro-α-ketoester **3-3** in baker's yeast reduction.

Figure 3-4 indicates that for longer reaction times, more racemic *anti*-**3-4** is obtained. On the other hand, the minor *syn* product **3-4** was produced only during the

initial 1.5 hours and no significant increase was observed after longer reaction times. Chiral GC spectra indicated, after the starting material was consumed in 24 hours that the products consisted of (2*S*,3*S*)-**3-4** in 98% ee and racemic *anti*-**3-4** in a ratio of 15 : 85 (syn : anti) (**Scheme 3-5 and Figure 3-5**).

Scheme 3-5



3-3 (2*S*,3*S*)-(+)-**3-4** racemic *anti*-**3-4**

15 : 85

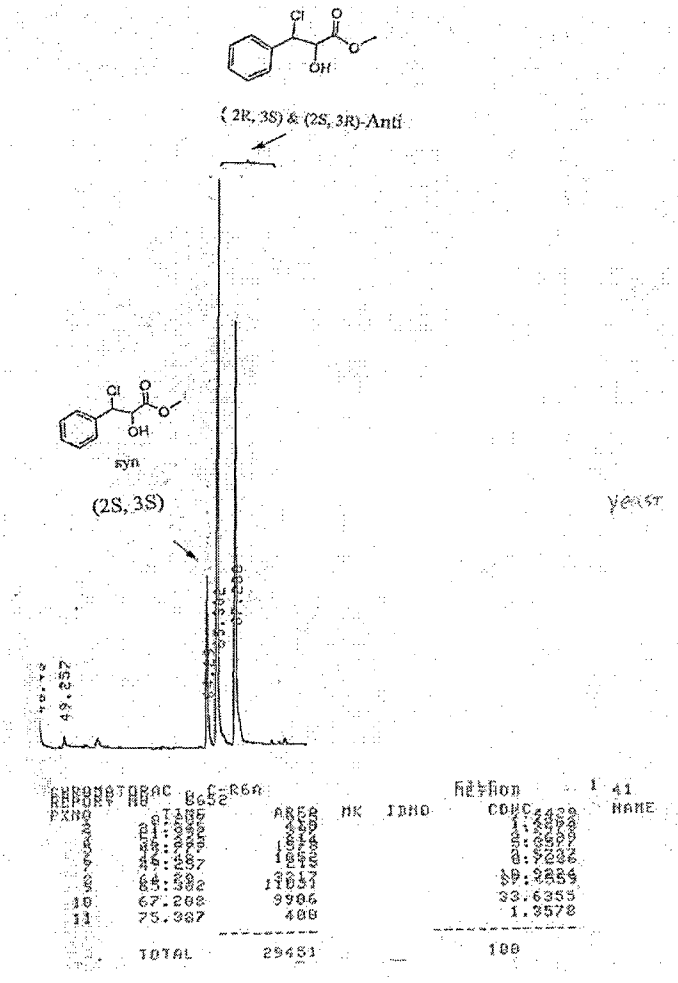


Figure 3-5 Chiral GC spectra of baker's yeast reduction.

Although (2*S*,3*S*)-**3-4** was enantiorich, the major *anti* product was racemic; Clearly baker's yeast was not sufficiently selective, perhaps because of the presence of several enzymes with opposite stereoselectivity. Interestingly, the reduction of **3-3** by $NaBH_4$ gave quite different ratios of diastereomers with *syn* being the major product (**Table 3-3**).

Table 3-3 Comparison between chemical reduction and yeast reduction.

Method	Conv. (%)	Time	Diastereoisomeric ratio (*syn*: *anti*)
Baker's yeast	>98	24 hours	15 : 85
$NaBH_4$	>98	10 minutes	65 : 35

3.2.4 Screening result of compound 3-4 with isolated reductases and mutants

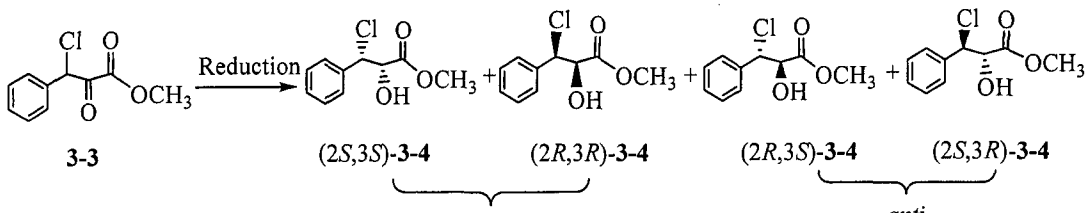
The screening experiments were carried out on a 1 mL scale at room temperature with the exception of the thermophilic PFADH enzyme which was performed at 37 °C. All enzymes used in this screening were pure as indicated by protein gel. As described in the experimental section, the cofactor regeneration system and NAD(P)H were dissolved in KPi buffer which contained substrate dissolved in biograde DMSO. The pH was adjusted depending on the enzyme used (shown in **Table 1-3**). The reaction was stopped after 12 hours, and analyzed by chiral phase GC. The absolute configuration was assigned by comparison of the retention times to authentic samples from lipase resolution. Specific activity of each enzyme was determined by spectrophotometrically using SpectraMax M2 microplate reader (Molecular Devices) and by measuring the

oxidation of NAD(P)H at 340 nm ($\varepsilon = 6.22\ mM^{-1}cm^{-1}$) every 9 seconds during the first 3 minutes of reaction in the presence of the substrate and enzyme in the potassium phosphate buffer. Details are presented in experimental section.

The results of the screening of the purified enzymes are summarized in **Table 3-4**. Enzymes CMCR, 7-HSDH, PFADH, GRE2, YMR226c gave side-reactions yielding more than 80% of by-products which appeared within one hour (**Table 3-5**).[§] Because of the side-reaction, the yield of alcohol **3-4** was low. On the other hand, the reactions catalyzed by SSCR (wild type) and SSCR mutants proceeded with a high degree of enantioselectivity in all cases to give (2*S*,3*S*)-hydroxy-ester **3-4** as a major product. The site-directed mutagenesis did not change the enantiomeric excess, it was at least 98% ee in all cases. All mutants except for SSCRQ245P, however, gave much higher diastereomeric ratios in favor of the *syn* (*SS*) products than the wild type SSCR.

[§] This phenomenon also happened previously with the twelve yeast reductases in the *E.coli* whole cell.

Table 3-4 Screening results of the biocatalytic reduction of compound **3-3**.



3-3 (2*S*,3*S*)-**3-4** (2*R*,3*R*)-**3-4** (2*R*,3*S*)-**3-4** (2*S*,3*R*)-**3-4**

syn *anti*

Enzyme	Product composition (%) *RR, SS, SR, RS*	*Syn*-**3-4** (ee %)	*Anti*-**3-4** (ee %)	Specific activity
SSCR (wild type)	1, 51, 39, 9	99 (2*S*,3*S*)	61 (2*S*,3*R*)	29169
SSCRN207V	1, 79, 17, 3	99 (2*S*,3*S*)	70 (2*S*,3*R*)	51793
SSCRQ245L	1, 78, 15, 6	98 (2*S*,3*S*)	43 (2*S*,3*R*)	29773
SSCRK181R	0, 76, 19, 3	98 (2*S*,3*S*)	67 (2*S*,3*R*)	43377
SSCRN207T	0, 70, 27, 3	99 (2*S*,3*S*)	80 (2*S*,3*R*)	25113
SSCRQ245P	0, 38, 43, 19	99 (2*S*,3*S*)	37 (2*S*,3*R*)	52817
SSCRQ245H	0, 41, 6, 3	98 (2*S*,3*S*)	31 (2*S*,3*R*)	17484
SSCRM242G	0, 80, 16, 6	99 (2*S*,3*S*)	47 (2*S*,3*R*)	41109
CMCR	80, 10, 8, 2	77 (2*R*,3*R*)	56 (2*S*,3*R*)	8998
7-HSDH	24, 4, 70, 2	71 (2*R*,3*R*)	94 (2*S*,3*R*)	2983
PFADH	42, 56, 1, 2	14 (2*S*,3*S*)	22 (2*R*,3*S*)	19086
GRE2	51, 38, 5, 6	16 (2*R*,3*R*)	1 (2*R*,3*S*)	45153
YMR226c	41, 51, 4, 4	10 (2*S*,3*S*)	7(2*R*,3*S*)	72338

Note: (1). Chiral GC, 100 °C for 10 min, 5 °C min^{-1} to 180 °C, 180 °C for 10 min.

(2). The unit of specific activity: nmol min^{-1} mg^{-1}.

Table 3-5 Conversion and side-product percentages of **3-4**.

Enzyme	Product composition (%) *RR, SS, SR, RS*	GC Conv. (%)	d.r.	Side-product (%)
SSCR (wild type)	1, 51, 39, 9	98	49/51	0
SSCRN207V	1, 79, 17, 3	98	80/20	0
SSCRQ245L	1, 78, 15, 6	98	79/21	0
SSCRK181R	0, 76, 19, 3	96	77/23	1.2
SSCRN207T	0, 70, 27, 3	96	70/30	0
SSCRQ245P	0, 38, 43, 19	96	38/62	0
SSCRQ245H	0, 41, 6, 3	96	81/19	0
SSCRM242G	0, 80, 16, 6	96	78/22	0
CMCR	80, 10, 8, 2	94	90/10	80
7-HSDH	24, 4, 70, 2	98	28/72	82
PFADH	42, 56, 1, 2	98	97/3	88
GRE2	51, 38, 5, 6	98	89/11	85
YMR226c	41, 51, 4, 4	98	92/8	84

Note: (1). All reactions were stopped after 12 hours.

(2). d.r.= *syn/anti* ratio.

In biocatalysis, the major application of enzymes in enantioselective organic synthesis is the kinetic resolution (KR) of racemates.[16] A major drawback of KR, however, is that the yield is limited to a maximum of 50%. In some cases, dynamic kinetic resolution (DKR) can be developed in which the non-reacting enantiomer is racemized *in situ* during the desymmetrization reaction. The optimal DKR can give the desired product in a 100% yield and 100% enantiomeric excess.[17] The success of

enzymatic DKR is limited by pH. Since the pH window for biocatalysis is rather narrow, it is not surprising that successful applications were found only for chiral centers which may be racemized *in situ* under weakly alkaline or acidic conditions also observed in this project.[18] Under the optimized pH condition, acid/base-catalyzed enol(ate) formation was expected to facilitate DKR.

Reductions of chloroketone **3-3** generate the second asymmetric center. However, because β-chloro-α-ketoester can undergo rapid keto-enol equilibration under the reaction conditions, dynamic kinetic resolution (K_R > K*rac* > K*s*) takes place as shown in **Figure 3-6**. The observed selectivity results from rapid equilibrium established between the two enantiomers of the oxoester. From the improved diastereomeric ratio, it may be concluded that the SSCR mutants' k_R reaction rate is faster than that of the wild type SSCR under the same pH condition. The improved yield of (2*S*,3*S*)-**3-4** product in the reductions with SSCR mutants is probably the result of the mutant enzymes' active site reconstruction that lowered the transition state energy leading to the *SS* product (because of better fit hence better stabilization of the transition state) rather than changes in the reaction conditions such as enhanced solubility or increase of enzyme concentration.

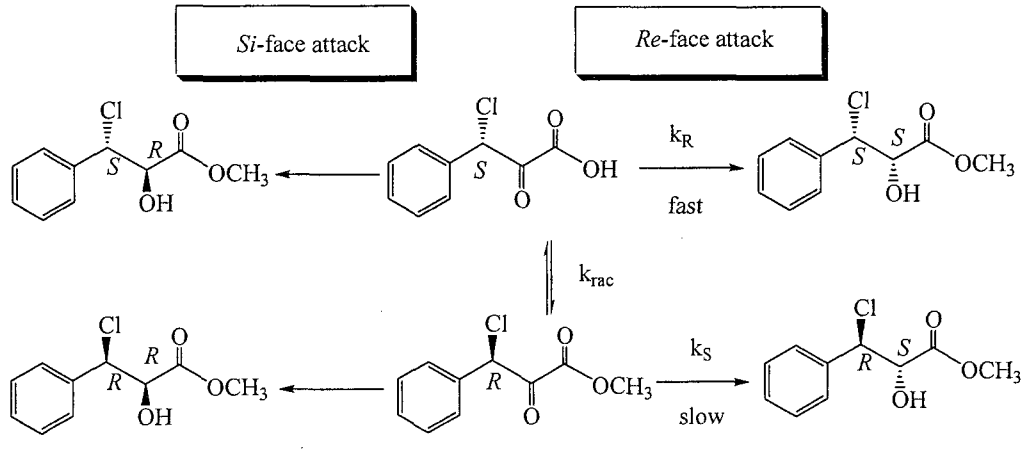


Figure 3-6 Correlation between starting material and products in resolution.

The reductions with the SSCR mutants producing (2*S*,3*S*)-**3-4** (99% ee) showed that the reactions followed Prelog's rule, i.e. the *Re*-face attack was preferred and gave the major product. The SSCR X-ray structure and docking studies[10] on wild type SSCR and its mutants assumed that the best conformation for β-chloro-α-ketoester facilitated π-π interactions in the hydrophobic pocket of the active site, which favors the (2*S*,3*S*)-product. The modeling studies helped in the interpretation of the results. The improved enantiomeric excess of the *anti*-product in the reaction catalyzed by mutant N207V** (80% ee compared to 60% ee for the wild type SSCR) may be because of the fact that the neighboring hydrophobic pocket was enlarged to better accommodate the phenyl group, leading to increased selectivity. Other SSCR mutants actually blocked this pocket and limited the phenyl ring movement, which apparently decreased enantioselectivity.

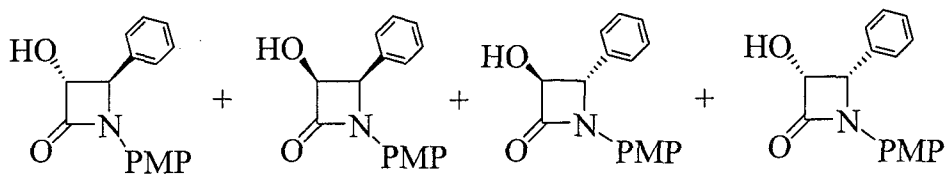
3.2.5 Screening results for 3-oxo-β-lactams 2-20 and 3-7

3-Oxo-β-lactams **2-20** and **3-7** were screened with purified reductases and mutants overexpressed in *E. coli*. Unfortunately, substrate **2-20** was not accepted by any of these enzymes under a variety of tested transformation conditions, such as increased enzyme concentration, addition of β-cyclodextrin, longer reaction times. This may be because β-lactam **2-20** with a large substituent in position 4 does not fit into the relatively small active site of these enzymes. This rationalization is supported by the fact that even substrate **3-7**, with just a phenyl group in position 4, was transformed slowly and maximum conversion of only 10% was achieved after 48 hours of reaction. The results of the screening with these purified reductases overexpressed in *E.coli* are displayed in

** asparagine was changed into threonine at position 207.

Table 3-6. The four enantiomers of the reduction (**Figure 3-7**) were cleanly separable on a chiral phase HPLC column as shown in **Figure 3-8**.

Table 3-6 Screening substrate **3-7** with reductases and mutants.



(3*R*,4*R*)-**3-8** (3*S*,4*R*)-**3-8** (3*S*,4*S*)-**3-8** (3*R*,4*S*)-**3-8**

Enzyme	Product composition (%) *SS, RR, SR, RS*	*trans*-**3-8** ee (%)	*cis*-**3-8** ee (%)	Conv. (%)
SSCR(wild type)	57, 7, 32, 4	78 (3*S*,4*S*)	79 (3S,4R)	9.8
SSCRN207V	60, 8, 27, 5	76 (3*S*,4*S*)	71 (3*S*,4*R*)	11.2
SSCRQ245L	39, 11, 41, 8	55 (3*S*,4*S*)	66 (3*S*,4*R*)	7.4
SSCRK181R	61, 8, 29, 2	76 (3*S*,4*S*)	85 (3*S*,4*R*)	10.6
SSCRN207T	45, 9, 31, 14	67 (3*S*,4*S*)	37 (3*S*,4*R*)	9.1
SSCRQ245P	46, 10, 33, 10	65 (3*S*,4*S*)	53 (3*S*,4*R*)	9.3
SSCRQ245H	54, 11, 36,	70 (3*S*,4*S*)	88 (3*S*,4*R*)	9.2
SSCRM242G	51, 11, 36, 9	66 (3*S*,4*S*)	61 (3*S*,4*R*)	9.4
CMCR	60, 13, 25, 2	65 (3*S*,4*S*)	82 (3*S*,4*R*)	6.2
7-HSDH	20, 9, 48, 22	37 (3*S*,4*S*)	36 (3*S*,4*R*)	4.5
PFADH	48, 47, 3, 2	0	30 (3*S*,4*R*)	5.1
GRE2	54, 13, 23, 9	61 (3*S*,4*S*)	43 (3*S*,4*R*)	8.9
YMR226c	63, 13, 21, 2	65 (3*S*,4*S*)	80 (3*S*,4*R*)	7.9

Note: The reaction was initiated with 1.5 mg of enzyme, 3 mg of GDH, 12 mg of glucose over 24 hours, then 1.5 mg of enzyme, 3 mg of GDH, 12 mg of glucose were added during a second 24 hours (total 48 hours).

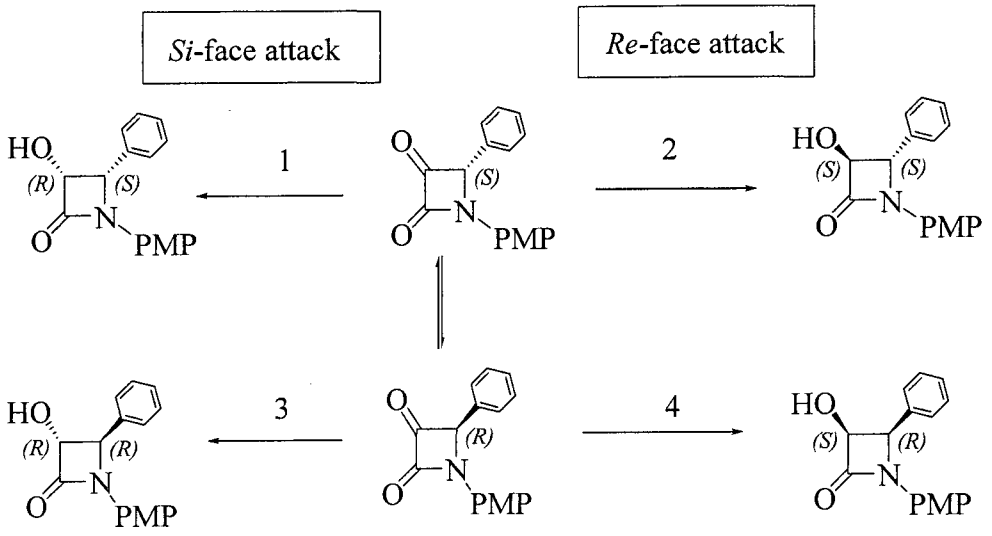


Figure 3-7 Reduction and product distribution in the screening test.

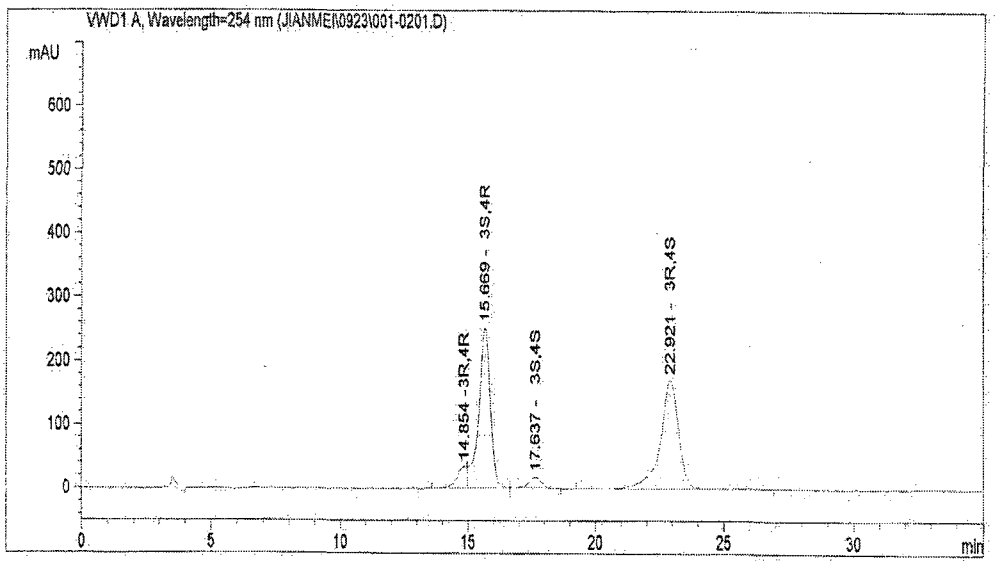


Figure 3-8 Separation of four enantiomers of **3-8** on a chiral (*S*,*S*)-Whelk-O 1 column.

Screening of 3-oxo-β-lactam **3-7** was carried out with the set of reductases listed in **Table 1-3**. The regeneration system and reaction conditions were the same as described for compound **3-4**. The samples were analyzed after 12, 24, and 48 hours by

chiral phase HPLC (Agilent HPLC 1100). It is interesting to note that in this case, as well, all enzymes favored *Re*-face attack and gave (3*S*)-Prelog products (**Figure 3-8**). Poor substrate acceptance, coupled with the low solubility of β-lactams in aqueous media, are likely responsible for the low conversions and limited the usefulness of these reactions. The apparent lack of enantioselectivity may be related to the rigid and symmetrical structure of compound **3-7**. Since low enantioselectivities in reductions of this substrate were also observed in the transformations performed with several aldoketo reductases, the rigidity and symmetry of β-lactams may be a general problem.[19] The in-depth study, including modeling, of the few reductases that are selective *vis a vis* these compounds may suggest active-site modifications that would allow engineering of better reductases for β-lactams.

3.3 Conclusions

Screening of reductases from six microorganisms and seven SSCR mutant reductases against β-chloro-α-ketoester **3-3** identified several highly enantioselective enzymes. The formation of by-products, observed with other reductases, combined with the difficult syntheses of β-substituted-α-ketoesters may explain the reason why these substrates have been seldom investigated. The SSCR mutants showed an improved selectivity over that of the wild type SSCR. The availability of enantioselective reductases for β-chloro-α-ketoester **3-3** facilitated the syntheses of the enantiopure paclitaxel side chain and oxazolidine (discussed in Chapter **4**). Screening of the same reductases against β-lactams **2-20** and **3-7** indicated that none of these enzymes was a suitable bioreductant.

3.4 Experimental

3.4.1 Activity assay of the carbonyl reductase

The activity of the carbonyl reductases from SSCR and other microorganisms[10] toward the reduction of α-ketoesters was determined spectrophotometrically by measuring the oxidation of NAD(P)H at 340 nm (ε = 6.22 mM^{-1} cm^{-1}) in the presence of excess α-ketoesters. The activity was measured at room temperature in a 96-well plate, in which each well contained α-ketoester (6.25 mM), NAD(P)H (0.25 mM) in potassium phosphate buffer (100 mM, pH = 6.5-7, 180 μL). The reaction was initiated by the addition of purified enzyme (20 μL solution containing 2-40 μg of enzyme). The specific activity is defined as the number of micromoles of NAD(P)H converted in 1 min by 1 mg of enzyme (μmol min^{-1} mg^{-1}). Calculation is based on $V_{max} = \Delta A/\Delta t = \Delta c/\Delta t * \varepsilon * l$ (Beer's law).

3.4.2 Enantioselectivity of the enzymatic (SSCR) reduction of α-ketoesters 3-3, 2-20, and 3-7

The enantioselectivity of the enzymatic reduction of the α-ketoesters **3-3**, **3-7** and **2-20** catalyzed by the carbonyl reductase from SSCR and other microorganisms[10] was studied using an NAD(P)H recycling system. The general procedure was as follows: D-glucose (4 mg), D-glucose dehydrogenase (0.5 mg), NAD(P)H (0.5 mg), carbonyl reductase (SSCR or others, 0.5 mg) and a solution of the α-ketoester in DMSO (50 μL, 0.25 M) were mixed in a potassium phosphate buffer (1 mL, 100 mM, pH 6.5 or 7.0) and the mixture was shaken overnight at room temperature. The mixture was extracted with methyl *t*-butyl ether (1 mL). The organic extract was dried over anhydrous sodium sulfate

and was subjected to chiral GC analysis to determine the enantiomeric excess. The absolute configurations of the product alcohols were identified by comparing the chiral GC data with materials obtained *via* lipase resolution.[18] Details of the chiral GC analysis are summarized in Table **3-4** and Appendix III.

3.4.3 General procedure for biotransformations with baker's yeast

Dry baker's yeast (2 g) was added to a solution of sucrose (8 g) in sterile water (100 mL) contained in a 250 mL Erlenmeyer flask. The mixture was stirred at 30 °C for 30 minutes to activate the yeast. The substrate methyl 3-chloro-2-oxo-3-phenylpropanoate **3-3** (1 g) was added to initiate the reaction. The conversion was monitored by GC and chiral phase GC and was shown to be completed in 24 hours. Analytical samples were collected after 1.5, 3.5, 5.5, 7.5 and 24 hours and prepared by mixing 300 μL of the reaction mixture with 300 μL of ethyl acetate . After vortex mixing for 1 min, the sample was spun in a microcentrifuge for 1 minute, then 200 μL of the organic layer was collected and dried over magnesium sulfate. 1 μL of sample was used for GC analysis.

3.4.4 Methyl 3-chloro-2-oxo-3-phenylpropanoate 3-3.[15] Methyl dichloroacetate (43.20 g, 0.302 mol) and benzaldehyde (32.0 g, 0.302 mol) were added dropwise into NaOMe (16.308 g, 0.302 mol) in diethyl ether (400 mL) at -10 °C under argon. After stirring for 6 hours, the reaction mixture was warmed to room temperature and stirred overnight. The mixture was then quenched with 5% HCl solution, and extracted twice with diethyl ether. The combined organic layers was washed with brine and dried over $MgSO_4$. After evaporation, the crude product was purified by flash chromatography with hexane and ethyl acetate (5:1) and gave **3-3** (58.40 g, 90% yield) as a yellow oil. IR

($CHCl_3$) ν_{max}/cm^{-1}: 3062, 2955, 1737 (very strong), 1454, 1245, 1062, 701; ^1H NMR (400 MHz, $CDCl_3$): δ 3.84 (3H, s, C*H*$_3$), 6.18 (1H, s, C*H*), 7.37-7.42 (5H, s, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 53.4 (OCH_3), 61.9 (*C*HCl), 129.7 (Ar-*C* para), 128.9 (Ar-*C* meta), 129.1 (Ar-*C* ortho), 132.8 (CAr-*C*), 160.4 (*C*OOCH$_3$), 184.6 (*CO*).

3.4.5 Reduction of 3-3 by $NaBH_4$

To a stirred solution of β-chloro-α-ketoester **3-3** (401.2 mg, 2 mmol) in MeOH (30 mL) was added $NaBH_4$ (249 mg, 3 mmol, 1.5 equiv.) in three portions every 2 minutes at 0 °C. Stirring was continued and the reaction was monitored by GC until complete conversion (20 min). Then the mixture was poured into brine and extracted with EtOAc (30 mL x 3). The combined organic layer was washed with brine, dried over $MgSO_4$, evaporated. The residue was purified by column chromatography with hexane and EtOAc (6:1) to give racemic **3-5** (28.5 mg, 8% yield) as a yellow oil; racemic *syn*-**3-4** (257 mg, 60% yield) as white crystals; racemic *anti*-**3-4** (128 mg, 30% yield) as yellow crystals.

Methyl (±)-*syn*-3-chloro-2-hydroxy-3-phenylpropanoate 3-4. White crystals. m.p: 77-78 °C. Spectrum was identical to an authentic sample.[20] IR ($CHCl_3$) ν_{max}/cm^{-1}: 3484, 2954, 2920, 2850, 1742, 1452, 1263, 1214, 1117, 994, 904, 698; ^1H NMR (400 MHz, $CDCl_3$): δ 3.26 (1H, s, O*H*), 3.91(3H, s, OC*H*$_3$), 4.58 (1H, d, *J* = 2.4 Hz, C*H*OH), 5.36 (1H, d, *J* = 2.4 Hz, ClC*H*), 7.42-7.57 (5H, m, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 53.2 (OCH_3), 63.7 (*C*HOH), 74.6 (*C*HCl), 128.8 (Ar-*C* para), 127.9 (Ar-*C* meta), 128.5 (Ar-*C* ortho), 137.6 (CAr-*C*), 171.7 (*C*O). HRMS: for $C_{10}H_{11}ClO_3$ (M^+): calc. 214.03967; found: 214.03974.

Methyl (±)-*anti*-3-chloro-2-hydroxy-3-phenylpropanoate 3-4. Yellow crystal. mp: 81-81.5 °C; IR($CHCl_3$) γ_{max}/cm $^{-1}$: 3456, 3062, 3032, 2954, 1742, 1494, 1453, 1282, 1214, 1153; 1116, 699; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.1 (1H, s, O*H*), 3.78 (3H, s, OCH_3), 4.70 (1H, d, *J* = 4.2 Hz, C*H*OH), 5.26 (1H, d, *J* = 4.2 Hz, ClC*H*), 7.40-7.44 (5H, m, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 52.7 (O*C*H$_3$), 62.9 (*C*HOH), 75.3 (*C*HCl) , 128.0 (Ar-*C* para), 128.4 (Ar-*C* meta), 128.9 (Ar-*C* ortho), 135.9 (CAr-*C*), 171.1 (*C*O). HRMS: for $C_{10}H_{11}ClO_3$ (M^+): calc. 214.03967; found : 214.03974.

Racemic *trans*-methyl 3-phenyloxirane-2-carboxylate 3-5. Yellow oil. IR ($CHCl_3$) γ_{max}/cm $^{-1}$: 3646, 3485, 3035, 2955, 1747, 1441; 1294, 1210, 1022, 896, 760, 697, 600, 517; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.86 (3H, s, CH_3), 3.55 (1H, s, OC*H*CO), 4.14 (1H, s, OC*H*C); 7.33 (5H, m, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 52.6 (O*C*H$_3$), 56.7 (O*C*HCO), 58.0 (Ar*C*HC), 129.0 (Ar-*C* para), 128.7 (Ar-*C* meta), 125.8 (Ar-*C* ortho), 134.4 (CAr-*C*), 168.6 (*C*O).

3.4.6 General procedure for lipase catalyzed kinetic resolution of *syn*- and *anti*-methyl 3-chloro-2-hydroxy-3-phenylpropanoate 3-4

Racemic *syn*-**3-4** or *anti*-**3-4** (28 mg, 0.13 mmol) was added into lipase PS (Amano, 56 mg) and vinyl acetate (33.6 mg, 0.39 mmol) in diisopropyl ether (4 mL) and stirred for 24 hours at room temperature.[15] The reaction was monitored by GC until 48% conversion of the starting material. The reaction mixture was filtered and quenched with water, and extracted with ethyl acetate. 1 μL of sample was used for chiral GC analysis.

3.5 References

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CHAPTER 4 CHEMOENZYMATIC SYNTHESIS OF NEW ENANTIOPURE OXAZOLIDINES

4.1 Introduction

In recent years, oxazolidine derivatives of optically pure β-amino alcohols such as (-)-ephedrine [1,2] have received considerable attention because of their wide occurence in biologically active molecules and applications as chiral auxiliaries and ligands for asymmetric synthesis.[3] Oxazolidines are useful as prodrugs for β-amino alcohols because the resulting "masked" amines do not ionize and hence are more compatible with organic and lipophilic media.[1,2] For example, it has been shown that at pH values around 7, an oxazolidine can penetrate a biological membrane (faster than a β-amino alcohol) from water.[4] In addition, parent drugs can be easily regenerated from prodrug oxazolidines *via* hydrolysis.[4-6] For the latter reason, oxazolidines are used also as protecting groups for amino alcohols in asymmetric syntheses.[7]

Chiral oxazolidines are usually prepared by the reaction of carbonyl compounds (mainly aldehydes) with chiral β-amino alcohols (**Figure 4-1**).[8] Control over the chemical stability of the oxazolidine systems can be enforced by the choice of different aldehyde moieties.[9] Thus, facile and high yielding syntheses of oxazolidines, especially enantiopure 2-oxazolidines (**Figure 4-2**), continue to be developed by many research groups.[10]

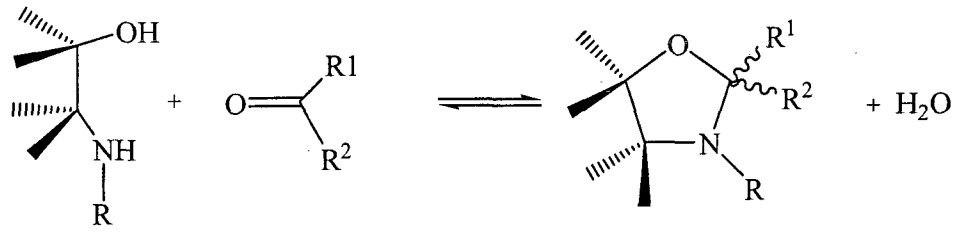
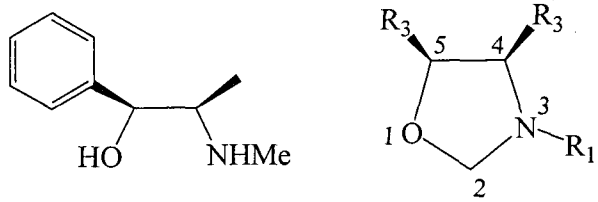


Figure 4-1 Oxazolidines obtained from an amino-alcohol and a carbonyl compound.



Ephedrine 2-Oxazolidine

Figure 4-2 Ephedrine and oxazolidines are useful chiral auxiliaries.

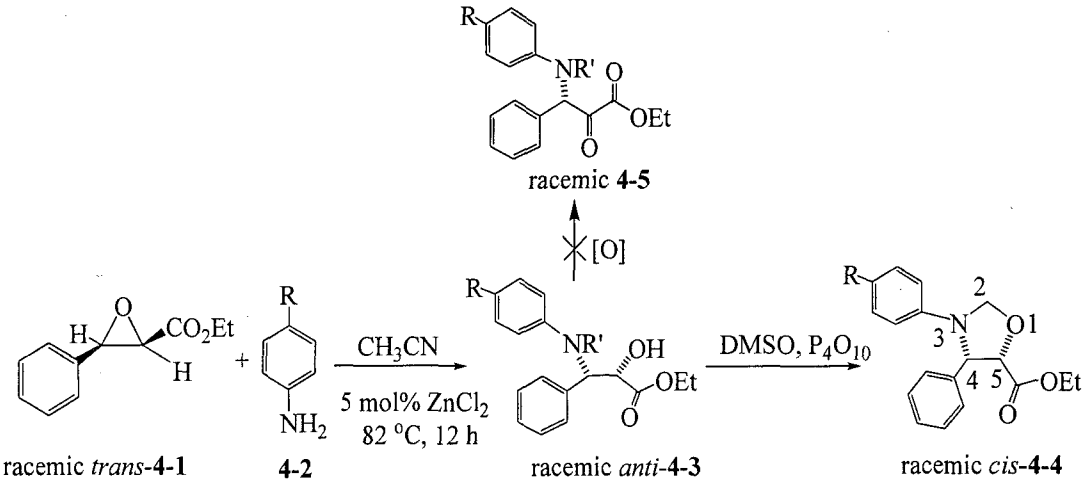
The goal of the present work is to provide a new strategy for chemoenzymatic asymmetric synthesis of enantiopure 2-oxazolidines and α-hydroxy-β-amino esters. Sharpless osmium-catalyzed aminohydroxylation of olefins[11] and the Mannich-type reaction[12] provide a powerful entry to highly enantioenriched α-hydroxy-β-amino esters or α,β-epoxy esters. Sharpless asymmetric epoxidation (AE) usually fails to give more than 80% ee for the *cis*-α,β-epoxy ester from a *Z*-allylic alcohol.[13] *trans*-α,β-Epoxy esters, on the other hand, are easily obtained with >90% ee from Sharpless AE of *E* allylic alcohols and subsequent oxidation and esterification.[14] The *cis*-epoxy esters can be prepared *via* enzymatic reduction of β-ketoesters.[15, 16] In this work, it will be shown that enantiopure *cis*-α, β-epoxy ester can be obtained *via* enzymatic reduction of α-ketoester and can served as a precursor in the syntheses of enantiopure α-hydroxy-β-amino esters and oxazolidines.

4.2 Results and Discussion

4.2.1 Synthesis of *cis*-oxazolidine 4-4 from racemic *trans*-epoxide 4-1

At the beginning of this project, oxazolidines were not the target of our synthesis; our target was β-amino-α-ketoester **4-5b**, a substrate for enzymatic reductions, which was to be prepared *via* a straightforward three-step synthesis as shown in **Scheme 4-1**.

Scheme 4-1



a R = H
b R = OCH_3 R' = H; *t*-Boc
c R = Cl
d R = NO_2

This route was appealing, since the precursor, racemic *trans*-epoxide **4-1**, was commercially available, and its readily accessible enantiopure forms[17,18] allowed access to both enantiomers of **4-5b**. The synthesis started well. The zinc chloride-catalyzed[19] ring opening of racemic *trans*-ethyl 3-phenyl glycidate **4-1**[20] with *p*-anisidine **4-2b** gave readily separable *anti*-β-amino alcohol **4-3b** and a small amount of α-amino alcohol (9:1 confirmed by GC-MS) in excellent yield (**Scheme 4-1**). The following step, however, did

not proceed along the projected route since all attempts to oxidize alcohol **4-3b** (Jones' reagent,[21] PCC, TEMPO/NaOCl,[22] and Dess Martin reagent [23]) to **4-5b** under a variety of conditions gave inseparable mixtures of many compounds. Swern oxidation, [24] on the other hand, has been shown to be a favorable method for the oxidation of a few *t*-Boc protected primary and secondary β-amino alcohols.[25, 26, 28] The oxidation of the Boc-protected (R' = *t*-Boc) alcohol **4-3b** with P_4O_{10} in DMSO, however, was not successful and no product (Boc-protected) **4-5b** could be detected. We decided to attempt the same reaction with an unprotected **4-3b** (R' = H). Although DMSO in hydrochloric acid had been used as formaldehyde replacement (one-carbon source) in the synthesis of Tröger base,[27] we hoped that the P_4O_{10} in DMSO combination might favor oxidation, even in the presence of an unprotected secondary amine.[28] The P_4O_{10} in DMSO reaction with an unprotected **4-3b** (R' = H) gave a single crystalline compound (85% yield), which was identified by ^1H and ^{13}C NMR as 3-(methoxyphenyl)-4-phenyl-oxazolidine-5-carboxylic acid ethyl ester **4-4b**. The structure of **4-4b** was confirmed by X-ray crystallographic analysis (**Figure 4-3**).[29]

To investigate the generality of oxazolidine formation, the reaction sequence was repeated with aniline **4-2a**, *p*-chloroaniline **4-2c**, and *p*-nitroaniline **4-2d**. Only *p*-chloroaniline **4-2c** was successful; *p*-nitroaniline **4-2d** did not react with the oxirane **4-1**, while aniline gave **4-3a** in good yield but failed to form oxazolidine **4-4a**. The results and yields of products are summarized in **Table 4-1**.

Table 4-1 Isolated yields of compounds **4-3** and **4-4**.

	4-2	**4-3**		**4-4**	
Entry		m.p. (°C)	Yield (%)	m.p. (°C)	Yield (%)
a	Aniline	57.0-58.0	89	NR	
b	*p*-Anisidine	74.8-75.0	91	78.0-78.5	85
c	*p*-Chloroaniline	96.0-96.5	93	105.0-105.5	90
d	*p*-Nitroaniline	NR		-	

Note: NR = no reaction.

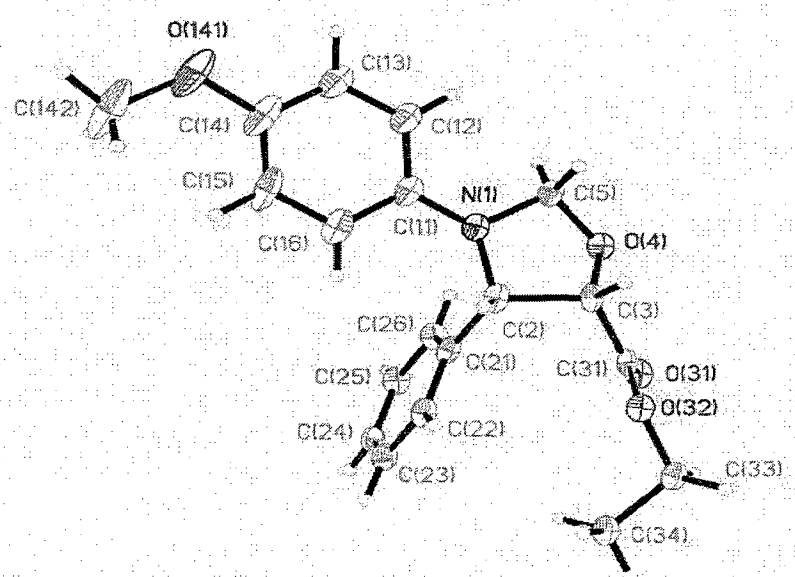
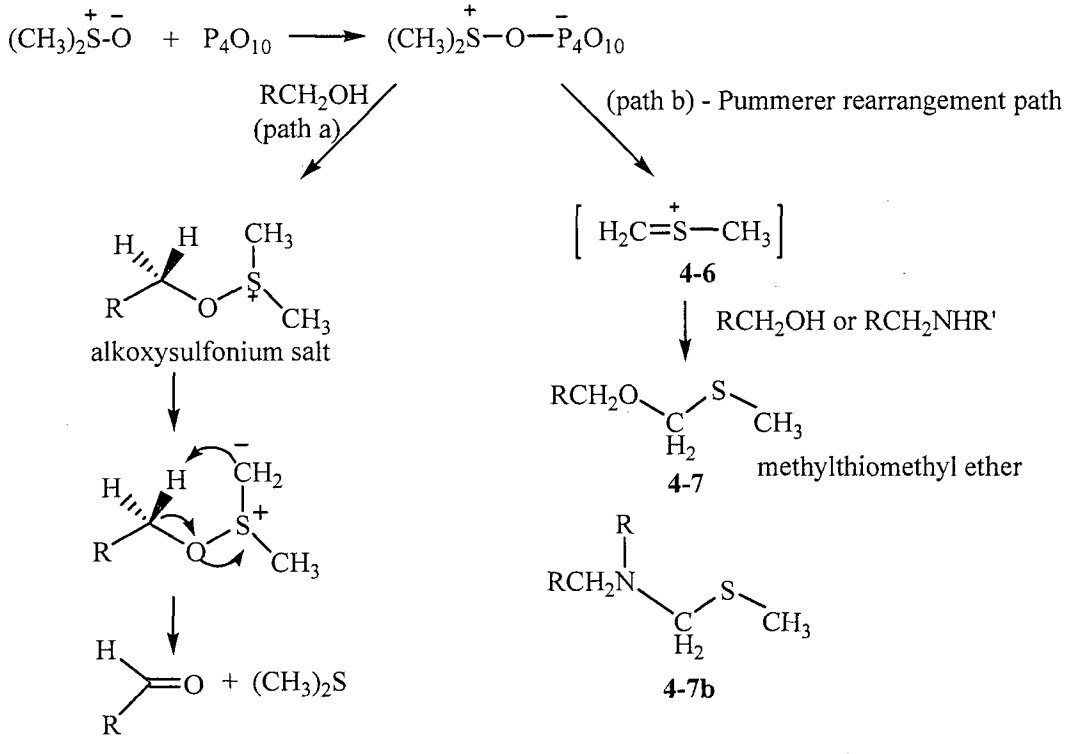


Figure 4-3 X-Ray crystal structure of ethyl 3-(methoxyphenyl)-4-phenyl-1,3-oxazolidine-5-carboxylate **4-4b**.[29]

4.2.2 Proposed mechanism of oxazolidine formation

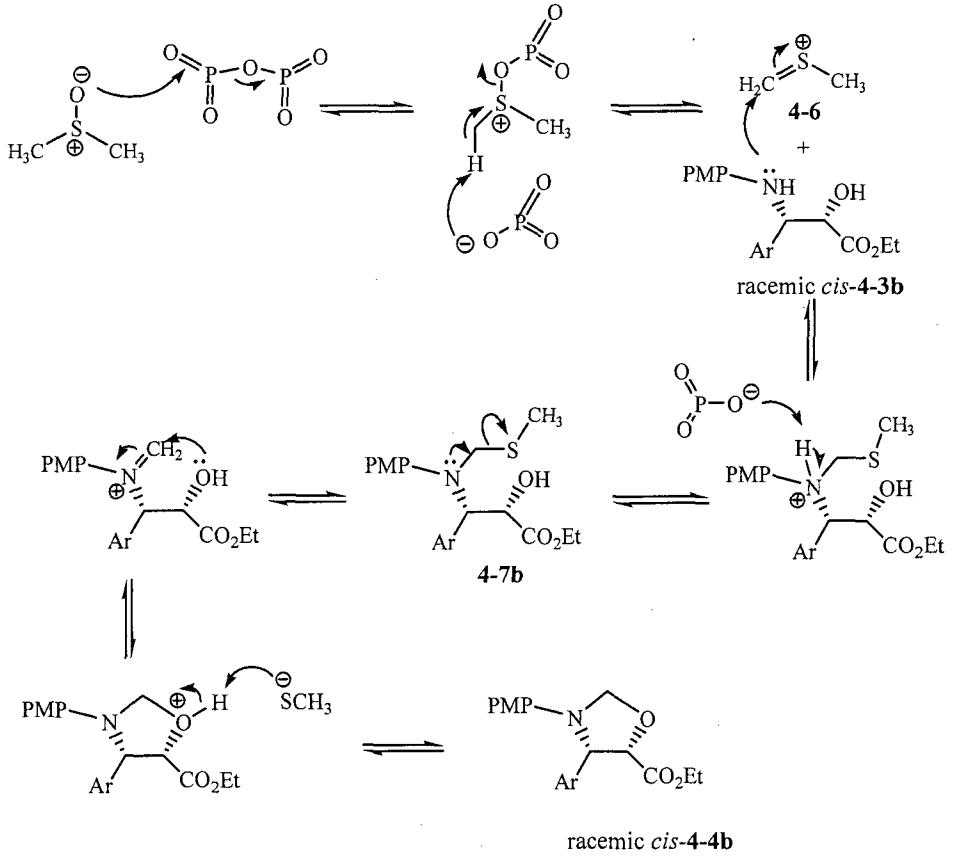
The formation of the oxazolidine **4-4b** may be rationalized as follows. It is generally accepted that in the oxidation of alcohols, DMSO is activated by a reaction with an electrophile (here, P_4O_{10}), and that subsequent nucleophilic attack of an alcohol on the activated sulfoxonium intermediate leads to the formation of the alkoxysulfonium salt, which breaks down under basic conditions to give the carbonyl compound and dimethyl sulfide[30] (**Scheme 4-2**). It is also known that problems can arise when the formation of methylthiomethyl ether from the alcohol becomes an important competitive reaction.[31,32] Sulfonium ions, such as **4-6**, are recognized as intermediates in Pummerer rearrangements.[30]

Scheme 4-2



In the case of the attempted oxidation of the alcohol **4-3b**, the formation of the alkoxysulfonium salt is probably very slow and the reaction preferentially follows path b (**Scheme 4-2**). The competing formation of oxazolidine **4-4b** (or **4-4c**) can be envisaged as outlined in **Scheme 4-3**. The unprotected, strongly nucleophilic, β-amino group in compound **4-3b** (or **4-3c**) competes with the α-alcohol for sulfonium ion **4-6** but does not stop at the Pummerer rearrangement product since ether **4-7b** possesses not only a potential leaving group (CH_3S^-) but also a neighboring electron-rich hydroxyl group. The proposed mechanism is supported by the fact that C-2 of the oxazolidine ring originates from DMSO, as was established when the signal for this carbon atom at 83.43 ppm was enhanced (5x) when the reaction was performed in ^{13}C enriched DMSO.

Scheme 4-3



The importance of the electron-rich amine group is confirmed by the fact that alcohol **4-3c** was readily converted to **4-4c** in excellent yield, but no oxazolidine **4-4a** could be detected in the cyclization of **4-3a**. The latter reaction gave a mixture of unidentifiable products. The reduced capacity to donate electrons, coupled with the vulnerability to oxidation of the non-substituted aniline likely contributed to the failure of this reaction. A poor electron donor, *p*-nitroaniline **4-2d**, did not react with epoxide **4-1** even under forced conditions (heating at 90 °C for four days) as shown in **Table 4-1**.

Other characteristics which have an important bearing on the success of this reaction are the reversibility and the instability of the oxazolidine heterocycles towards hydrolysis, which often precludes their purification by chromatography. When run in dry, distilled DMSO, oxazolidines **4-4b** and **4-4c** crystallized from the reaction mixture in excellent yields. On the other hand, when the purification and drying of DMSO was not possible, as was the case in the reaction performed with ^{13}C-DMSO, the product did not crystallize spontaneously. During purification by flash chromatography, the proportion of ^{13}C-labelled oxazolidine **4-4b** in the mixture rapidly decreased (accompanied by the formation of several other unidentified products), resulting in a low yield of (still) impure **4-4b** (**Figure 4-4**).

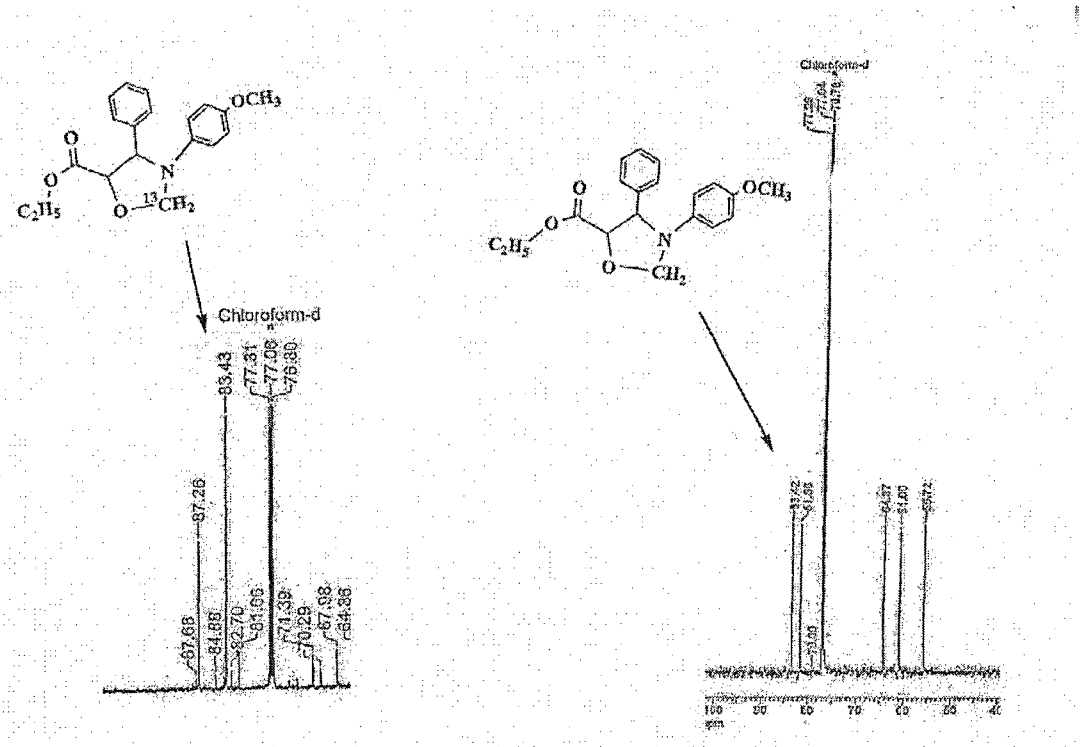


Figure 4-4 ^{13}C-labelled oxazolidine in ^{13}C-NMR.

4.2.3 Syntheses of enantiopure oxazolidines *via* enzymatic chemical reaction

Having optimized all steps leading to the formation of racemic *cis* oxazolidines, we turned to the synthesis of enantiopure *trans* products. β-Chloro-α-ketoester **4-8**, prepared according to the literature protocol,[33] was reduced using several carbonyl reductases from various microorganisms *Bacterodies fragilis,*[34] *Pyrococcus furiosus,*[35] *Candida magnoliae,*[36] and *Sporobolomyces salmonicolor* (SSCR).[37,38] The carbonyl reductase from SSCR which gave (2*S*,3*S*)-**4-9** with >99% ee has been discussed in Chapter **3**. The enantiomeric excess of (2*S*,3*S*)-**4-9** was determined by chiral phase GC and the absolute configuration was assigned by comparing the retention time of a known

sample from lipase resolution.[39] Overall formation of (2*S*)-α-hydroxyl ester **4-9** is consistent with the enzyme-substrate docking studies of Hua and co-workers.[38]

Ring closure of (2*S*,3*S*)-(+)-**4-9** gave (2*R*,3*R*)-(+)-methyl glycidate ester **4-10**. This epoxide is very sensitive to water and several protocols (NaOMe/MeOH,[34] K_2CO_3/DMF,[40] K_2CO_3/MeOH[41]) were investigated to ensure a good yield. The K_2CO_3/MeOH method gave the best yield, providing that K_2CO_3 was added gradually. The product (2*R*,3*R*)-(+)-**4-10** was isolated as a colourless oil, in 85% yield. The *cis* stereochemistry and absolute configuration were confirmed by ^{1}H NMR and optical rotation.[42] *

The availability, *via* enzymatic reductions, of enantiopure glycidates **4-10** provides access to the corresponding enantiopure β-amino alcohols and oxazolidines, compounds not previously reported in the literature. The asymmetric aminolysis (Lewis acid catalyst $ZnCl_2$ at 82 °C) of (2*R*,3*R*)-(+)-**4-10** with amines **4-2b** and **4-2c** gave the products (2*R*,3*S*)-(+)-**4-11b** and **4-11c** respectively (**Scheme 4-4**).

Both amino alcohols (3*R*,3*S*)-(+)-**4-11b** and **4-11c** reacted with dry DMSO in the presence of P_4O_{10} at room temperature to give (4*S*,5*R*)-(+)-**4-12b** and **4-12c** in excellent yields. Their melting points and optical rotations are listed in **Table 4-2**.

---

* The $J_{2,3}$ value of 4.6 Hz is consistent with *cis* configuration; $[\alpha]_D^{25}$ +10.8, c 1.03, CH_2Cl_2; (lit.[42] $J_{2,3}$ = 4.7 Hz, $[\alpha]_D^{25}$ +11, c 4.4, $CHCl_3$).

Scheme 4-4

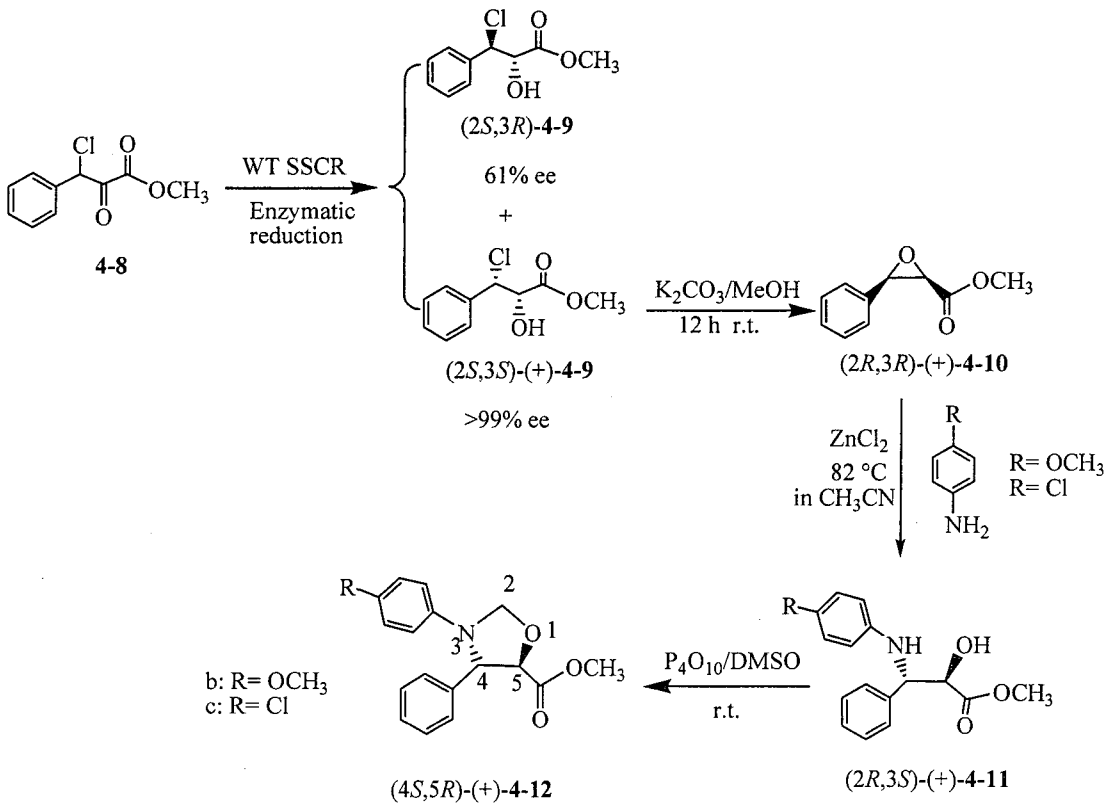


Table 4-2 Melting points, optical rotations and isolated yields for **4-11** and **4-12.**

Entry		(2*R*,3*S*)-(+)-**4-11**			(4*S*,5*R*)-(+)-**4-12**		
		m.p. (°C)	$[\alpha]_D^{25}$	Yield (%)	m.p. (°C)	$[\alpha]_D^{25}$	Yield (%)
1	*p*-anisidine (b)	76-77	+ 10.3	90	90-90.5	+ 48.7	86
2	*p*-chloroaniline (c)	89-89.5	+ 7.86	92	93-93.5	+ 36.3	95

The *syn*-configurations of (2*R*,3*S*)-(+)-**4-11b** and **4-11c** were deduced from their ^{1}H-NMR spectra. The $J_{2,3}$ values of 2 Hz are considerably smaller than 3.2 Hz reported for *anti* isomers.[43] The following DMSO/P_4O_{10} ring closure gave both oxazolidine products in high yields and without a decrease in enantiopurity. The analysis by chiral

HPLC showed a single peak (compared to two well-resolved peaks observed in the racemic products).

4.3 Conclusions

Oxazolidines can be obtained in a simple and clean reaction using DMSO as the cosolvent and P_4O_{10} as the catalyst. This method allows the preparation of substituted oxazolidines from epoxide precursors, providing that the amines used to open the epoxide ring are good electron donors. An important aspect of this protocol is that it can be adapted for the synthesis of enantiopure oxazolidines, since enantiopure epoxides are available through the enzymatic reductions of α-chloro-β-ketoesters.[18] The success of this methodology encourages future exploration of related reactions.

4.4 Experimental

4.4.1 Racemic *trans*-ethyl phenylglycidate 4-1.[33,39] Commercially available starting material ethyl 3-phenylglycidate contains 14% cis, 85% trans, and 1% unidentified impurity as established in a SPB-5 column of GC-MS. The commercial product was purified by florosil column in hexane and ethyl acetate (10:1) and gave 80% pure trans-glycidate **4-1** as a colourless oil; IR ($CHCl_3$) γ max/cm^{-1}: 2983, 1748, 1459, 1290, 1202, 1026, 894, 759, 697; ^1H NMR (500 MHz, $CDCl_3$) δ: 1.22 (3H, t, J = 7.3 Hz, OCH_2CH_3), 3.40 (1H, d, J = 1.8 Hz, CHCO), 4.18 (2H, m, OCH_2CH_3), 3.99 (1H, d, J = 1.8 Hz, CHPh), 7.18-7.30 (5H, m, ArH); ^{13}C NMR (126 MHz, $CDCl_3$): δ 14.1 (OCH_2CH_3), 56.7 (CHPh), 57.8(CHCO), 61.7 (OCH_2CH_3), 125.8 (Ar-C para), 128.6 (Ar-C meta), 128.9 (Ar-C ortho), 135.0 (Ar-C), 168.1 (CO); EI-MS, m/z (%): 135 (100) $[M-57]^+$, 118 $[M-74]^+$, 107 (79) $[M-85]^+$. HRMS: for $C11H_{12}O_3$: calc. C, 68.73; H, 6.29, found: C, 68.65; H, 6.32.

4.4.2 General procedure for enzymatic SSCR reduction

Purified reductase from SSCR[37] (60 mg), D-glucose dehydrogenase (GDH) (60 mg), NADPH (60 mg) and glucose (2 g) were dissolved in 200 mL of 100 mM potassium phosphate buffer (pH = 6.5) and then 1 g of **4-8** dissolved in 10 mL DMSO was added. The reaction mixture was stirred at room temperature overnight, extracted with ethyl acetate (200 mL x 3), dried over sodium sulfate. The reaction mixture was filtered, concentrated and the residual crude mixture was purified by column chromatography (silica gel Merk 60) with hexane and EtOAc (6:1) to give (2*S*,3*S*)-**4-9**

(0.41 g, 41% yield, >99% ee) and (2*S*,3*R*)-**4-9** (0.50 g, 50% yield, 61% ee) as colourless oils.

(2*S*,3*S*)-(+)-Methyl-3-chloro-2-hydroxy-3-phenyl-propanoate 4-9. Colourless oil, >99% ee. The ee was determined by GC analysis using a CP-Chirasil-Dex CB chiral capillary column (25 m x 0.25 mm); RT = 19.4 min. Only one diastereomer was observed by ^{1}H and ^{13}C NMR and GC analysis: $[\alpha]_D^{25} = +46$ (c, 1.07, CH_2Cl_2) (lit.[39] $[\alpha]_D^{25}$ +47, c 1.4, $CHCl_3$). IR ($CHCl_3$) γ_{max} /cm^{-1}: 3484, 2954, 2920, 2850, 17428, 1453, 1263, 1214, 1118, 995, 905, 699; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.36 (1H, d, *J* = 7.6 Hz, CHO*H*), 3.88(3H, s, OCH_3), 4.58 (1H, dd, *J* = 7.4 Hz, *J* = 2.2 Hz C*H*OH), 5.35 (H, d, *J* = 2.2 Hz ClC*H*), 7.42-7.57 (5H, m, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 53.2 (O*C*H_3), 63.7 (*C*HOH), 74.7 (*C*HCl), 128.8 (Ar-*C* para), 127.9 (Ar-*C* meta), 128.5 (Ar-*C* ortho), 137.5 (CAr-*C*), 171.7 (*C*O). HRMS: for $C_{10}H_{11}ClO_3$ (M^+): calc. 214.03967; found: 214.03974.

(2*S*,3*R*)-Methyl-3-chloro-2-hydroxy-3-phenyl-propanoate 4-9. Colourless oil, 61% ee. The ee was determined by GC analysis using a CP-Chirasil-Dex CB chiral capillary column (25 m x 0.25 mm); RT = 20.9 min; IR ($CHCl_3$) γ_{max} /cm^{-1}: 3456, 3062, 3032, 2954, 1742, 1494, 1453, 1282, 1214, 1153; 1116, 699; ^{1}H NMR (400MHz, $CDCl_3$): δ 3.1 (1H, s, O*H*), 3.71 (3H, s, OCH_3), 4.60 (1H, d, *J* = 4.2 Hz, C*H*OH), 5.26 (1H, d, *J* = 4.2 Hz, ClC*H*), 7.32-7.39 (5H, m, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 52.7 (O*C*H_3), 62.9 (*C*HOH), 75.4 (*C*HCl), 128.0 (Ar-*C* para), 128.4 (Ar-*C* meta), 128.9 (Ar-*C* ortho), 135.9 (CAr-*C*), 171.1 (*C*O). HRMS: for $C_{10}H_{11}ClO_3$ (M^+): calc. 214.03967; found: 214.03974.

4.4.3 (2*R*,3*R*)-(+)-Methyl 3-phenylglycidate 4-10. To a solution of (2*S*,3*S*)-chlorohydrin **4-9** (1 g, 4.6 mmol) in 30 mL of methanol, K_2CO_3 (0.76 g, 5.52 mmol, 1.2 equiv) was added gradually and then the mixture was stirred at room temperature until the starting

material had reacted (12 h). The reaction mixture was quenched with NH_4Cl solution (25 mL)and extracted with ethyl acetate (25 mL x 3). The combined organic layers were washed with brine, dried over sodium sulfate, evaporated. The crude residue was purified by flash chromatography and eluted with 10% dry ether in hexane to give (2*R*,3*R*)-(+)-**4-10** (0.82 g, 85% yield) as a colourless oil.$[\alpha]_D^{25}$ +10.8 (c 1.03, CH_2Cl_2) (lit.[39] $[\alpha]_D^{25}$ +13, c 1.1, $CHCl_3$; lit.[42] $[\alpha]_D^{25}$ +11, c 4.4, $CHCl_3$). IR ($CHCl_3$) γ_{max} /cm^{-1}: 3080, 3060, 2980, 2950, 1750, 1435, 1210; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.53 (3H, s, O*CH*$_3$), 3.83 (1H, d, *J* = 4.7 Hz, O*CH*), 4.25 (1H, d, *J* = 4.7 Hz, O*CH*), 7.26-7.41 (5H, m, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 52.0 (*OCH*$_3$), 55.8 (CO*C*HO), 57.5 (PhC*C*HO), 126.6 (Ar-*C* para), 128.1 (Ar-*C* meta), 128.5 (Ar-*C* ortho), 132.8 (CAr-*C*), 167.0 (*C*O).

4.4.4 General procedure for epoxide ring opening

A mixture of ethyl (or methyl) 3-phenyl glycidate (5 mmol, 1 equiv) and *p*-substituted aniline (5 mmol, 1 equiv) was dissolved in acetonitrile (20 mL). Dry $ZnCl_2$ (34 mg, 0.25 mmol) (1.25 mmol %) was added and the resulting mixture was stirred under nitrogen atmosphere for 12-28 hours at 82 °C. Removal of the solvent gave a residue, which was extracted with ethyl acetate (50 mL) and washed with a saturated solution of sodium bicarbonate (20 mL), water and brine. After drying over anhydrous magnesium sulfate and removal of the solvent, the residue was purified by chromatography over ultra pure silica gel using hexane and ethyl acetate (2:1) to give the product.

(±)-*anti*-Ethyl 2-hydroxy- 3-phenyl-3-(phenylamino) propanoate 4-3a. White powder, 89% yield. m. p.: 57-58 °C; IR ($CHCl_3$) γ_{max} /cm^{-1}: 3404, 3054, 2982, 2934, 1736, 1298, 1603, 1504, 1214, 1106, 1026, 868, 750, 694, 561, 509; ^{1}H NMR (500 MHz, $CDCl_3$): δ

1.31 (3H, t, J = 7.3 Hz, CH_3), 2.10 (1H, s, O*H*), 2.91 (1H, N*H*), 4.22 (3H, m, J = 7.1 Hz, CH_2), 4.71 (1H, d, J = 3.7 Hz, NHC*H*), 4.92 (1H, d, J = 3.7 Hz, C*H*OH), 6.67 (2H, d, J = 7.9 Hz, Ar*H-N*), 6.73 (1H, d, J = 7.4 Hz, Ar*H-N*), 7.14 (2H, d, J = 7.9 Hz, Ar*H-N*), 7.34 (5H, m, Ar*H*). ^{13}C NMR (126 MHz, $CDCl_3$): δ 14.1 (OCH_2CH_3), 59.6 (*C*HNH), 62.0 (OCH_2CH_3), 73.6 (*C*HCO), 113.9 (NHAr-*C* ortho), 118.1 (NHAr-*C* para), 127.6 (Ar-*C* ortho), 128.5 (Ar-*C* meta), 129.2 (NHAr-*C* meta), 137.2 (Ar-*C*-CH), 146.2 (Ar-*C*-NH), 172.1 (*C*O); HRMS: for $C_{17}O_3NH_{19}$ (M^+): calc. 285.13635; found: 285.13651.

(±)-*anti*-Ethyl 3-(4-methoxyphenylamino)-2-hydroxy-3-phenylpropanoate 4-3b. Yellow crystals, 91% yield. m. p.: 74.8-75 °C; IR ($CHCl_3$) γ_{max} /cm^{-1}: 3285, 2979, 2936, 2471, 1737, 1511, 1258, 1217, 1028, 701; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.23 (3H, t, J = 7.3 Hz, CH_3), 2.91 (2H, d, J = 7.6 Hz, N*H*), 3.68 (3H, s, OCH_3), 4.16 (3H, m, J = 7 Hz, CH_2 and O*H*), 4.63 (1H, d, J = 3.4 Hz, NHC*H*), 4.78 (1H, d, J = 3.4 Hz, C*H*OH), 6.70 (2H, d, J = 6 Hz, Ar*H*), 6.58 (2H, d, J = 6 Hz, Ar*H*), 7.25 (5H, m, Ar*H*). ^{13}C NMR (126 MHz, $CDCl_3$): δ 14.1 (OCH_2CH_3), 55.7 (OCH_3), 60.6 (*C*HNH), 73.5 (*C*HCO), 61.9 (OCH_2CH_3), 114.8 (NHAr-*C* ortho), 115.5 (NHAr-*C* meta), 127.6 (Ar-*C* ortho), 128.4 (Ar-*C* meta), 136.1 (Ar-*C*-NH), 140.5 (Ar-*C*-CH), 152.5 (Ar-*C*-OCH_3), 172.3 (*C*O); EI-MS, m/z : M^+: 315, 211 $(M-103)^+$, 103 $(M-211)^+$, 77, 89. HRMS: for $C_{18}O_4NH_{21}$ (M^+): calc. 315.14706; found: 315.14686.

(±)-*anti*-Ethyl 3-(4-chlorophenylamino)-2-hydroxy-3-phenylpropanoate 4-3c. Yellow crystals, 93% yield; m. p.: 96-96.5 °C; IR ($CHCl_3$) γ_{max} /cm^{-1}: 3473, 3400, 3029, 2981, 2932, 1936, 1600, 1453, 1245, 1210, 1095, 1024, 816, 720, 700; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.25 (3H, t, J = 6.7 Hz, CH_3), 2.92 (1H, d, J = 7.3 Hz, N*H*), 4.15 (3H, m, J = 7.3 Hz ,CH_2 and O*H*), 4.63 (1H, d, J = 3.7 Hz, NHC*H*), 4.78 (1H, d, J = 3.7 Hz, C*H*OH),

6.51 (2H, d, J = 8.6 Hz, Ar*H*), 6.70 (2H, d, J = 8.6 Hz, Ar*H*), 7.25 (5H, m, Ar*H*). ^{13}C NMR (126 MHz, $CDCl_3$): δ 14.1 (OCH_2CH_3), 59.6 (*C*HNH), 62.1 (OCH_2CH_3), 73.4 (*C*HCO), 115.0 (NHAr-*C* ortho), 122.6 (NHAr-*C* para), 127.4 (Ar-*C* ortho), 128.5 (Ar-*C* meta), 129.0 (NHAr-*C* meta), 136.6 (Ar-*C*-CH), 144.7 (Ar-*C*-NH), 171.8 (*C*O); HRMS: for $C_{17}H_{18}ClO_3N$ (M^+): calc. 319.09649; found: 319.09753.

(2*R*,3*S*)-(+)-Methyl 3-(4-methoxyphenylamino)-2-hydroxy-3-phenylpropanoate 4-11b. Yellow crystals, 90% yield. $[\alpha]_D^{25}$ +10.3, (c, 0.99, CH_2Cl_2); >99% ee; m. p.: 76-77 °C; IR ($CHCl_3$) γ_{max} /cm^{-1}: 3285, 2979, 2936, 2471, 1737, 1511, 1258, 1217, 1028, 701, ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.69 (3H, s, C*H*$_3$), 3.74 (3H, s, OC*H*$_3$), 4.47 (1H, d, J = 2.4 Hz, NHC*H*), 4.84 (1H, d, J = 2.4 Hz, C*H*OH), 6.50 (2H, d, J = 8.8 Hz, Ar*H*), 6.63 (2H, d, J = 8.8 Hz, Ar*H*), 7.25-7.36 (5H, m, Ar*H*). ^{13}C NMR (126 MHz, $CDCl_3$): δ 52.3 (OCH_3), 55.7 (OCH_3), 60.0 (*C*HNH), 74.7 (*C*HCO), 114.7 (NHAr-*C* ortho), 115.4 (NHAr-*C* meta), 127.0 (Ar-*C* ortho), 128.6 (Ar-*C* meta), 136.1 (Ar-*C*-NH), 140.4 (Ar-*C*-CH), 152.3 (Ar-*C*-OCH_3), 173.3 (*C*O); HRMS: for $C_{17}O_4NH_{19}$ (M^+): calc. 301.13141; found: 301.13142.

(2*R*,3*S*)-(+)-Methyl 3-(4-chlorophenylamino)-2-hydroxy-3-phenylpropanoate 4-11c. Colourless crystals, 92% yield. $[\alpha]_D^{25}$ +7.86 (c, 1.36, CH_2Cl_2); >98% ee; m. p.: 89-89.5 °C; IR ($CHCl_3$) γ_{max} /cm^{-1}: 3473, 3400, 3029, 2981, 2932, 1936, 1600, 1453, 1245, 1210, 1095, 1024, 816, 720, 700; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.6 (3H, s, C*H*$_3$), 3.14 (1H, O*H*), 4.49 (1H, d, J = 2.0 Hz, NHC*H*), 4.79 (1H, d, J = 2.0 Hz, C*H*OH), 6.45 (2H, d, J = 8.6 Hz, Ar*H*), 7.05 (2H, d, J = 8.6 Hz, Ar*H*), 7.24-7.33 (5H, m, Ar*H*). ^{13}C NMR (126 MHz, $CDCl_3$): δ 53.1 (OCH_3), 59.0 (*C*HNH), 74.5 (*C*HCOH), 115.0 (NHAr-*C* ortho), 122.6 (NHAr-*C* para), 127.8 (Ar-*C* ortho), 128.7 (Ar-*C* meta), 129.0 (NHAr-*C* meta),

138.8 (Ar-*C*-CH), 144.7 (Ar-*C*-NH), 173.2 (*C*O); HRMS: for $C_{16}H_{16}ClO_3N$ (M^+): calc. 305.08187; found: 305.08065.

4.4.5 General procedure for formation of oxazolidines 4-4 and 4-12

Phosphorus pentoxide (568 mg, 2 mmol, calculated with P_4O_{10}, MW = 284) was added to dry DMSO (3 mL) and ultrasonicated for 10 minutes. Compound **4-3** or **4-11** (1 mmol) in DMSO (2 mL) was added and the resulting mixture was stirred at room temperature until TLC indicated complete conversion (24 h). The reaction mixture was quenched with cooled saturated sodium bicarbonate solution (20 mL) followed by a small amount of water. The mixture was extracted with ethyl acetate (30 Ml x 3). The combined organic layers were washed with water (40 mL x 3) to remove unreacted DMSO, then washed with brine, dried over magnesium sulfate, filtered, concentrated, separated by flash chromatography on silica gel and then crystallized from hexane and ethyl acetate to give colourless crystals.

(±)-*cis*-Ethyl 3-(4-methoxyphenyl)-4-phenyl-1,3-oxazolidine-5-carboxylate 4-4b. Yellow crystals, 85% yield. m. p: 78-78.5 °C; IR ($CHCl_3$) γ_{max} /cm^{-1}: 2980, 2930, 2836, 1746, 1514, 1244, 1200, 1038; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.94 (3H, t, *J* = 7.3 Hz, C*H*$_3$), 3.88 (2H, dd, *J* = 7.3 Hz, C*H*$_2$), 3.74 (3H, s, OC*H*$_3$), 5.59 (1H, d, *J* = 1.9 Hz, NC*H*O), 5.01 (1H, d, *J* = 1.9 Hz, NC*H*O), 4.94 (1H, d, *J* = 7.3 Hz, NC*H*), 4.90 (1H, d, *J* = 7.3 Hz, CO*H*), 6.74 (2H, d, *J* = 6.9 Hz, Ar*H*), 6.42 (2H, d, *J* = 6.9 Hz, Ar*H*), 7.29 (5H, m, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 13.7 (OCH_2CH_3), 55.7 (OCH_3), 61.0 (OCH_2CH_3), 64.4 (NCHC), 83.4 (NCH_2O), 81.7 (OCHC), 115.0 (NAr-*C* ortho), 114.1 (NAr-*C* meta), 152.5 (NAr-*C* para), 127.6 (Ar-*C* para), 128.4 (Ar-*C* meta), 128.2 (Ar-*C* ortho), 138.8 (Ar-*C*-CH), 168.1 (CO); GC-MS (SPB-5 column)-MS. RT = 10.65 min.

EI-MS, m/z : M^+ 327, 73, 77, 86, 91,105, 118, 122. HRMS: for $C_{19}O_4NH_{21}$ (M^+): calc. 327.14706; found: 327.14743.

(±)-*cis*-Ethyl 3-(4-chlorophenyl)-4-phenyl-1,3-oxazolidine-5-carboxylate 4-4c. Colourless crystals, 90% yield. m. p: 105-105.5 °C; IR ($CHCl_3$) γ_{max}/cm $^{-1}$: 2982, 2902, 2836, 1760, 1744, 1599, 1493, 1469, 1341, 1201, 1097, 1041, 809, 737, 699, 504; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.90 (3H, t, *J* = 7.1 Hz, C*H*$_3$), 3.70 (1H, m, *J* = 3.6 Hz, C*H*$_2$), 3.86 (1H, m, *J* = 3.6 Hz, C*H*$_2$), 4.92 (1H, d, *J* = 7.8 Hz, OC*H*), 4.96 (1H, d, *J* = 7.8 Hz, NC*H*), 5.04 (1H, d, *J* = 1.8 Hz, NC*H*O), 5.56 (1H, d, *J* = 1.8 Hz, NC*H*O), 6.35 (2H, d, *J* = 8.7 Hz, Ar*H*), 6.74 (2H, d, *J* = 8.7 Hz, Ar*H*), 7.29 (5H, m, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 13.7 (OCH$_2$*C*H$_3$), 61.2 (O*C*H$_2$CH$_3$), 63.6 (N*C*HC), 82.6 (N*C*H$_2$O), 81.6 (O*C*HC), 113.8 (NAr-*C* ortho), 123.1 (Ar-*C* para), 127.5 (Ar-*C* meta), 128.6 (Ar-*C* ortho), 129.2 (NAr-*C* meta), 137.1 (Ar-*C*-CH), 142.5 (NAr-*C* para), 167.6 (*C*O); HRMS: for $C_{18}O_3NClH_{18}$ (M^+): calc. 331.09829; found: 331.09753.

(4*S*,5*R*)-(+)-Methyl 3-(4-methoxyphenyl)-4-phenyl-1,3-oxazolidine-5-carboxylate 4-12b. Colourless crystals, 86% yield; $[\alpha]_D^{25}$ +48.7 (c, 1.02, CH_2Cl_2); >99% ee; m. p: 90-90.5 °C; IR ($CHCl_3$) γ_{max}/cm^{-1}: 2982, 2902, 2836, 1760, 1744, 1599, 1493, 1469, 1341, 1201, 1097, 1041, 809, 737, 699, 504; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.68 (3H, s, C*H*$_3$), 3.79 (3H, s, OC*H*$_3$), 4.59 (1H, d, *J* = 3.0 Hz, OC*H*), 4.85 (1H, d, *J* = 3.0 Hz, NC*H*), 5.24 (1H, d, *J* = 1.6 Hz, NC*H*O), 5.38 (1H, d, *J* = 1.6 Hz, NC*H*O), 6.35 (2H, d, *J* = 8.8 Hz, Ar*H*), 7.10 (2H, d, *J* = 8.8 Hz, Ar*H*), 7.29 (5H, m, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 51.8 (O*C*H$_3$), 54.22 (O*C*H$_3$), 64.7 (N*C*HC), 82.8 (N*C*H$_2$O), 84.2 (O*C*HC), 114.1 (NAr-*C* ortho), 123.2 (Ar-*C* para), 126.2 (Ar-*C* meta), 128.7 (Ar-*C* ortho), 129.2 (NAr-*C* meta),

140.2 (Ar-*C*-CH), 142.6 (NAr-*C* para), 167.6 (*C*O); HRMS: calc. for $C_{18}O_4NH_{19}$ (M^+): 313.13141; found: 313.13145.

(4*S*,5*R*)-(+)-Methyl 3-(4-chlorophenyl)-4-phenyl-1,3-oxazolidine-5-carboxylate 4-12c. Colourless crystals, 95% yield; $[\alpha]_D^{25}$ +36.3 (c, 0.55, CH_2Cl_2); >98% ee; m. p: 93-93.5 °C; IR ($CHCl_3$) γ_{max} /cm^{-1}: 2982, 2902, 2836, 1760, 1744, 1599, 1493, 1469, 1341, 1201, 1097, 1041, 809, 737, 699, 504; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.81 (3H, s, C*H*$_3$), 4.63 (1H, s, OC*H*), 4.88 (1H, s, NC*H*), 5.24 (1H, s, NC*H*O), 5.38 (1H, s, NC*H*O), 6.35 (2H, d, *J* = 7.1 Hz, Ar*H*), 7.10 (2H, d, *J* = 7.1 Hz, Ar*H*), 7.25-7.36 (5H, m, Ar*H*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 52.6 (OCH_3), 64.6 (N*C*HC), 82.7 (N*C*H$_2$O), 84.1 (O*C*HC), 114.0 (NAr-*C* ortho) , 123.2 (Ar-*C* para), 126.1 (Ar-*C* meta), 128.1 (Ar-*C* ortho), 129.1 (NAr-*C* meta), 140.1 (Ar-*C*-CH), 142.4 (NAr-*C* para), 170.9 (*C*O); HRMS: calc. for $C_{17}O_3NClH_{16}$ (M^+): 317.08188; found: 317.08177.

(^{13}C) Ethyl 3-(4-methoxyphenyl)-4-phenyl-1, 3-oxazolidine-5-carboxylate (^{13}C)-4-4b. Phosphorus pentoxide (568 mg, 2 mmol calculated with P_4O_{10}, M W = 284) was added to dry DMSO (2 mL) containing C^{13}-DMSO (5 %) and ultrasonicated for 10 minutes. Compound 2-1 (315 mg, 1 mmol) in DMSO (3 mL) containing C^{13}-DMSO (5%) was added and the resulting mixture was stirred at room temperature until TLC indicated complete conversion (24 h). The reaction was quenched with cooled saturated sodium bicarbonate solution followed by a small amount of water. The mixture was extracted with ethyl acetate (30 mL x 3). The combined organic layers were washed with water (40 mL x 3) to remove unreacted DMSO, then washed with brine, dried over magnesium sulfate, filtered, concentrated, and separated by flash chromatography on silica gel with

hexane and ethyl acetate (6:1) to give a yellow product which was highly enriched but not pure (^{13}C)-**4**-**4b**.

4.5 References

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1.456 (6) and 1.463 (6) Å, while the N-C11 is shorter (1.395 (6) Å. In the oxazolidine ring, the C-O bonds are 1.400 (6) and 1.434 (5) Å, while the C-C bond is 1.553 (6) Å. For the ester group, the C=O bond (ave. 1.201 (5) Å) is shorter than the C-O bond (ave. 1.340 (5) Å) as expected. The H atoms on C4 and C5 are *cis* to each other. The molecules are stabilized in the crystal only by van der Waals interactions.

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CHAPTER 5 CONCLUSIONS AND FUTURE WORK

5.1 Conclusions

Three projects have been successfully carried out and are outlined in this thesis: (1) Synthesis of novel substituted β-lactams; (2) Evaluation and application of new reductases in synthesis of important, enantiopure synthetic intermediates for organic synthesis; (3) Development of new methodology for the preparation of oxazolidines.

New β-lactams synthetic building blocks for new paclitaxel analogues were synthesized and fully characterized. The introduction of polar groups such as a hydroxyl group or morpholine group at C4 of the β-lactams ring makes them useful not only as paclitaxel analogue precursors but also as general β-lactams synthons (a term proposed by Ojima) for synthetically and medicinally important compounds.

The use of acetoxyacetyl chloride as ketene precursor in the Staudinger reaction was found to be the easiest way to achieve high *cis* selectivity while Lipase PS-mediated hydrolysis made enantiopure (3*R*,4*S*)-β-lactams possible. Both racemic *cis* and (3*R*,4*S*)-β-lactams with protected hydroxyl groups, the paclitaxel C-13 side chain precursors ready for in coupling with 10-deacetylbaccatin III have been submitted for further studies and evaluation by our coworkers at the University of Kansas.

Thirteen heterologous reductases overexpressed in *E. coli* were evaluated for their enantioselectivity in the reductions of 3-oxo-β-lactams. The results show that these reductases (belonging to the family of short chain dehydrogenases) have a limited ability to accept rigid and large compounds and show little selectivity possibly because of relative symmetry of β-lactam molecules.

The screening of β-chloro-α-ketoester with the corresponding purified enzymes showed that the reductases and mutants from red yeast *Sporobolomyces salmonicolor* reduced this substrate in good yields with excellent enantiomeric excess. The dynamic kinetic resolution observed in these transformations is more important in the reactions with mutants than in the reactions with the wild type enzyme. Other reductases used in the study produced numerous by-products.

DMSO/P_4O_{10} oxidation of aryl substituted β-amino-β-hydroxy esters was shown to give oxazolidines in high yield. This reaction offers a practical and feasible method to synthesize substituted oxazolidines providing that the amino group is sufficiently nucleophilic to facilitate formation of the oxazolidine. Based on the promising screening results on β-chloro-α-ketoester (Chapter **3**), scale-up of the wild type *Sporobolomyces salmonicolor* reduction reaction allowed for the preparation of enantiopure alcohol esters (>99% ee). Further manipulation at these alcohols, such as epoxide formation, ring opening, oxazolidine formation were undertaken and ultimately led to the successful synthesis of (4*S*,5*R*)-oxazolidines.

Overall these studies established optimized protocols for the chemoenzymatic syntheses of (2*R*,3*S*)-β-aminoalcohols, (4*S*,5*R*)-oxazolidines and (3*R*,4*S*)-β-lactams bearing polar functional groups. Further investigation of these new compounds is interesting because of their application important in drug delivery, prodrug protection, and use as chiral auxiliaries.

5.2 Future work

Interdisciplinary projects described in my thesis not only provide some interesting and useful results but also open the door to further investigations.

The poor screening results obtained in reductions of 3-oxo-β-lactams coupled with the importance of securing an access to these very important compounds in enantiopure form suggested that the search for effective reductases for 3-oxo-β-lactams through screening and enzyme engineering is an important goal in bioorganic chemistry.

The high proportion of by-products observed in the reduction of β-chloro-α-ketoester by many reductases can be an interesting future project that would explore whether these enzymes facilitate other, potentially useful, reactions.

The results of reductions with wild type SSCR and its mutants are baseline studies that will require modeling and directed mutations to engineered mutants with improved enantioselectivity. These studies have an excellent chance to be highly successful since SSCR's gene sequence and X-ray structure are well-established.

The work on the synthesis of β-lactams bearing a morpholine group has shown that PMP needs to be removed by CAN before the sensitive morpholine group is introduced. Differing from the previous synthetic route, the following two steps leading to the morpholine-substituted β-lactam appear to be likely to succeed.

The new synthesis of oxazolidine leads into a broad research field. A variety of available amino alcohols and amino alcohol esters may be suitable candidates. The latter compounds may be accessed in enantiopure form from amino acid pool.

APPENDIX I General experimental conditions

(1). General instrumentation

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution at room temperature on a 400 MHz or 500 MHz Bruker spectrometer and chemical shifts are reported in ppm using Me_4Si as internal standard. *J* values are expressed in Herz. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. IR spectra were recorded as thin films on NaCl plates on a Mattson Satellite FT-IR spectrometer. GC-EI-MS measurements were performed on a SPB-5 GC column of Agilent 5890 series II GC-MS instrument. Chiral HPLC analysis was performed on an Agilent 1100 series high-performance liquid chromatography system with (*S*,*S*)-Whelk-O 1 column (25 cm x 4.6 mm, Regis Technologies Inc.) or Chiracel OD-H column (4.6 x 150 mm) using hexane and *iso*-propanol (90 : 10) as the mobile phase. The UV detector used was set at 254 nm. Chiral GC analysis was performed on an Agilent 5890 series II plus gas chromatography equipped with autosampler, EPC, split/splitless injector, FID detector and CP-Chirasil-Dex CB chiral capillary column (25 m x 0.25 mm). Analysis of racemic compounds was performed on an Agilent 5890 instrument employing a DB-1301 (15 m x 0.53 mm x 1.0 μm) column from J & W Scientific. High resolution mass spectra were obtained on a Kratos MS50TC mass spectrometer. Optical rotations were measured on a Perkin-Elmer 241 Polarimeter operating at room temperature with energy source Na 589. Thin layer chromatography was performed on Sigma-Aldrich 0.2 mm aluminum-backed silica gel plates with UV indicator or $KMnO_4$ dipping reagent. Flash chromatography was performed on 230 to 400 mesh silica gel (Silicycle). The X-ray crystallographic data were

collected using a Bruker platform or Enraf-Nonius CAD-4 diffractomer, operating with CuKα radiation. Vaccum evaporation was performed using a Bűchi Rotavapor R-200.

Chiral GC program: Initial temperature 120 °C for 10 minutes, then increased 5 °C per minute until final temperature 180 °C, then kept at 180 °C for 10 minutes, total run time 32 minutes.

Chiral HPLC program: Mobile phase: Hexane and *iso*-proanol (87 : 13).

Flow rate: 0.9 ml/min. ambient temperature.

λ max : UV 254 nm.

(2). Treatment of chemicals and solvents

All chemicals were purchased from Fisher Scientific or Sigma-Aldrich Co. and were used as received except where noted. DMSO was prepared by distillation over calcium hydride and stored over molecular sieves (4 Å). All purified enzymes were provided by Dr. Hua Lin of Chemistry Department of Southern Methodist University (Dallas, US). Lipases were generous gifts from Amano Enzyme USA Co. Ltd. Commercial baker's yeast was obtained from a local grocery chain.

APPENDIX II Protocols of gene expression and purification

(1). The protocol of gene expression and purification of the carbonyl reductase from Sporobolomyces salmonicolor (SSCR)

The carbonyl reductase gene from *Sporobolomyces salmonicolor* was cloned by gene assembly techniques.[50] Twelve oligonucleotides ranging from 100 to 120 nucleotides were designed on the basis of the nucleotide sequence of the *Sporobolomyces salmonicolor* carbonyl reductase gene. The *Nco* I and *Bam* HI sites were franked to the open reading frame for easy cloning into the expression vector pet 15b (Novagen). Plasmid DNA containing the SSCR gene was transformed into *E.coli* BL21(DE3) strain. Overnight culture was diluted with fresh LB medium containing ampicillin (100 $\mu g\ mL^{-1}$) and incubated at 37 °C until the optical density reached 0.6 at 595 nm. The expression was induced by addition of IPTG to 0.1 mM and the culture was incubated at 30 °C for another 6 h. Cells were harvested by centrifugation at 4100 rpm at 4 °C for 30 min. The cell pellet was resuspended in potassium phosphate buffer (100 mM, pH 7.4) and the cells were disrupted by an Emulsion Flex-C5 Homogenizer. The cell-free extract was mixed with an equal volume of PEI solution (0.25% polyethyleneimine MW 40K-60K, 6% NaCl, 100 mM borax, pH 7.4) to remove lipids.[51] The supernatant was precipitated with 50% ammonium sulfate. The resulting precipitate was collected after centrifugation and dissolved in potassium phosphate buffer (10 mM, pH 7.4) containing 0.1 mM dithiothreitol. The lysate was desalted by gel filtration into potassium phosphate buffer (10 mM, pH 7.4, 0.1 mM dithiothreitol) and lyophilized to afford the SSCR enzyme as a white powder with a protein content of 83% as measured with the Bradford assay.

(2). The protocol of gene expression and purification of the carbonyl reductase from *Candida magnoliae* (CMCR).

The carbonyl reductase gene from *Candida magnoliae* (Genbank Accession No. AB036927) was cloned by gene assembly techniques.[53] ††Ten oligonucleotides ranging from 100-120 nucleotides were designed on the basis of the nucleotide sequence of the *C. magonoliae* carbonyl reductase gene. The open reading frame is composed of 850 nucleotides (284 amino acid residues). The *Nco* I and *Bam* HI sites were franked to the open reading frame for easy cloning into expression vector Pet15b (Novagen). The plasmid DNA containing the CMCR gene was transformed into the *E.coli* Rosetta2(DE3)pLysS strain. Overnight the culture was diluted with fresh LB medium containing ampicillin (100 μg/mL) and chloramphenicol (34 μg/mL) and incubated at 37 °C until the optical density reached 0.6 at 595 nm. The expression was induced by addition of IPTG to 0.5 mM and the culture was incubated at 30 °C for another 6 hours. Cells were harvested by centrifugation at 4100 rpm at 4 °C for 30 minutes. The cell pellet was resuspended in a100 mM potassium phosphate buffer (pH 6.5) and the cells were disrupted by an EmulsiFlex-C5 Homogenizer. The cell-free extract was mixed with an equal volume of PEI solution (0.25% polyethyleneimine MW 40K-60K, 6% NaCl, 100 mM borax, pH 7.4) to remove lipids.[51] The supernatant was precipitated with 25% ammonium sulfate and the precipitate was discarded. The remaining supernatant was precipitated with 55% ammonium sulfate. The resulting precipitate was collected after centrifugation and dissolved in potassium phosphate buffer (10 mM, pH 7.0, 2 mM 2-mercaptoethanol). The lysate was desalted by gel filtration into potassium phosphate

†† All references were listed in Chapter 1.

buffer (10 mM, pH 7.0, 2 mM 2-mercaptoethanol) and lyophilized to yield the CMCR enzyme as a white powder with a protein content of 86% as measured with the Bradford assay. The expression vector pet15b without the CMCR gene was also expressed in Rosetta2(DE3)pLysS. The cell-free extract was purified by the same procedure and used as a control in the activity assay.

(3). The protocol of gene expression and purification of the carbonyl reductase from 7α-hydroxy-steroid dehydrogenase (7α-HSDH).

The carbonyl reductase gene from *Bacteroides fragilis* ATCC 25285 (Genbank Accession No.) was cloned by gene assembly techniques.[53] Plasmid pBPC-1 (from James P. Coleman) containing the 7-HSDH gene from *B. fragilis* ATCC 25285 was used as a template for PCR amplification. The PCR fragment was cloned into the pTXB1 expression vector at the *Nde* I and *Bam* HI sites to give JS2.2. The cloned 7-HSDH gene was confirmed by DNA sequencing. The plasmid JS2.2 was transformed into Rosetta2(DE3)pLysS for expression. Overnight the culture (20 mL) was diluted into 1 L of LB media containing 100 μg/mL of ampicillin and 34 μg/mL of chloramphenicol and propagated until OD_{595} reached 0.6-1.0 at 37 °C. The cells were then induced with 0.1 mM IPTG and continued growing at 30 °C for 5 hours. The cells were harvested and lysed in 10 mM potassium phosphate buffer (pH 7.0) *via* Homogenizer. The cell-free extract was heat-treated in a water-bath for 30 min at 55-60 °C and centrifuged at 20,000 g for 30 minutes. The heat-treated lysate was then mixed with an equal volume of PEI solution (0.25% polyethyleneimine MW 40K-60K, 6% NaCl, 100 mM borax, pH 7.4) to remove lipids.[51] The PEI-treated supernatant was precipitated with 45% ammonium sulfate. The resulting precipitate was collected after centrifugation and dissolved in a

potassium phosphate buffer (10 mM, pH 7.0). The lysate was dialysed by gel filtration into a potassium phosphate buffer (10 mM, pH 7.0), and then lyophilized as a powder.

(4). The protocol of gene expression and purification of the carbonyl reductase *adh*D (PFADH) from hyperthermophilic archaeon *pyrococcus furiosus.*

The carbonyl reductase gene *adh*D from hyperthermophilic archaeon *pyrococcus furiosus* (Genbank Accession No. Ae010289, region from nucleotides 7356 to 8192; National Center for Biotechnology information) was cloned by gene assembly techniques.[54] The *Nco* I and *Bam* HI sites were franked to the open reading frame for easy cloning into the expression vector Pet24d (Novagen). Plasmid DNA containing *adh*D gene was transformed into *E.coli* BL21(DE3) strain. Overnight the culture was diluted with fresh LB medium containing kanamycin and spectinomycin (both 50 μg mL^{-1}) and incubated at 37 °C until optical density reached 0.6 at 595 nm. The expression was induced by addition of IPTG to 0.1 mM and the culture was incubated at 37 °C for another 18 hours. Cells were harvested by centrifugation at 4100 rpm for 30 min. The cell pellet was resuspended in 20 mM Tris-HCl buffer (pH 7.5) and the cells were disrupted by Emulsi Flex-C5 Homogenizer. The cell-free extract was mixed with an equal volume of PEI solution (0.25% polyethyleneimine MW 40K-60K, 6% NaCl, 100 mM borax, pH 7.4) to remove lipids.[51] The supernatant was heated for 30 minutes at 80 °C and was precipitated with 50% ammonium sulfate.

The resulting precipitate was collected after centrifugation and dissolved in 20 mM Tris-HCl buffer (pH 7.8). The lysate was desalted by gel filtration into 20 mM Tris-HCl buffer (pH 7.8) and lyophilized to afford the PFADH or *adh*D enzyme as a white powder.

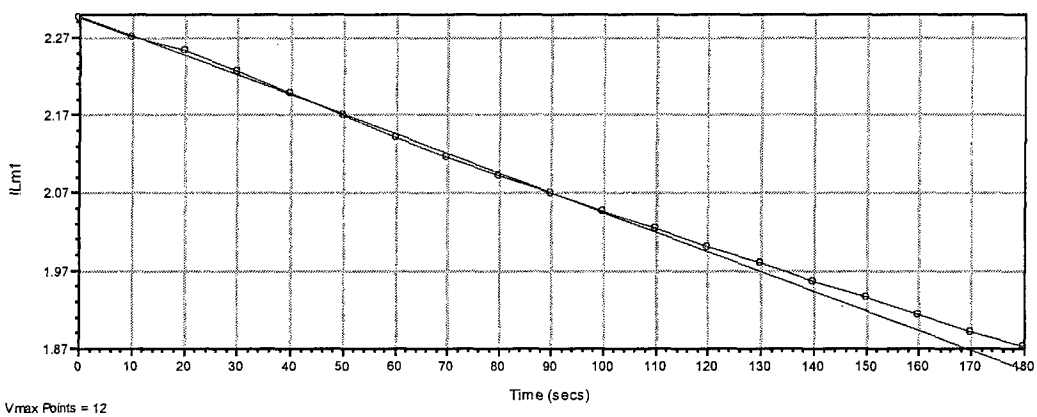
APPENDIX III Representatives of specific activity of enzymes

Specific activity of each enzyme was assayed by using a SpectraMax M2 microplate reader (Molecular Devices) and by measuring the oxidation of NAD(P)H at 340 nm (ε = 6.22 $mM^{-1}cm^{-1}$) every 9 seconds in the first 3 minutes of the reaction in the presence of substrate and enzyme in potassium phosphate buffer. The unit of specific activity was $nmol^{-1}min^{-1}mg^{-1}$ ($V_{max} = \Delta A/\Delta t = \Delta c/\Delta t * \varepsilon * l$)

(1). Enzyme: SSCRN207T (100 μL)

Concentration: 7.8 mg/2000 mL

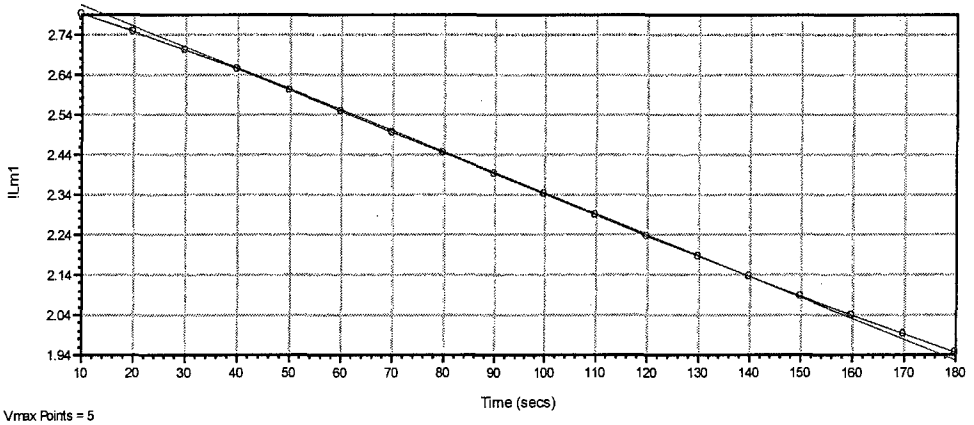
Substrate: β-chloro-α-ketoester **3-3**



(2). Enzyme: SSCRN207V (100 μL)

Concentration: 11.7 mg /3000 mL

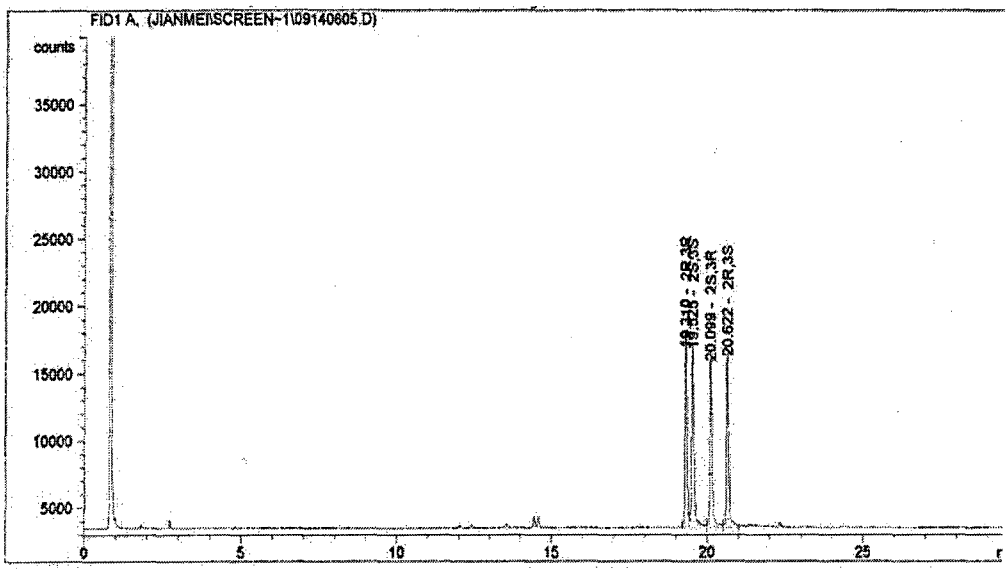
Substrate: β-chloro-α-ketoester **3-3**



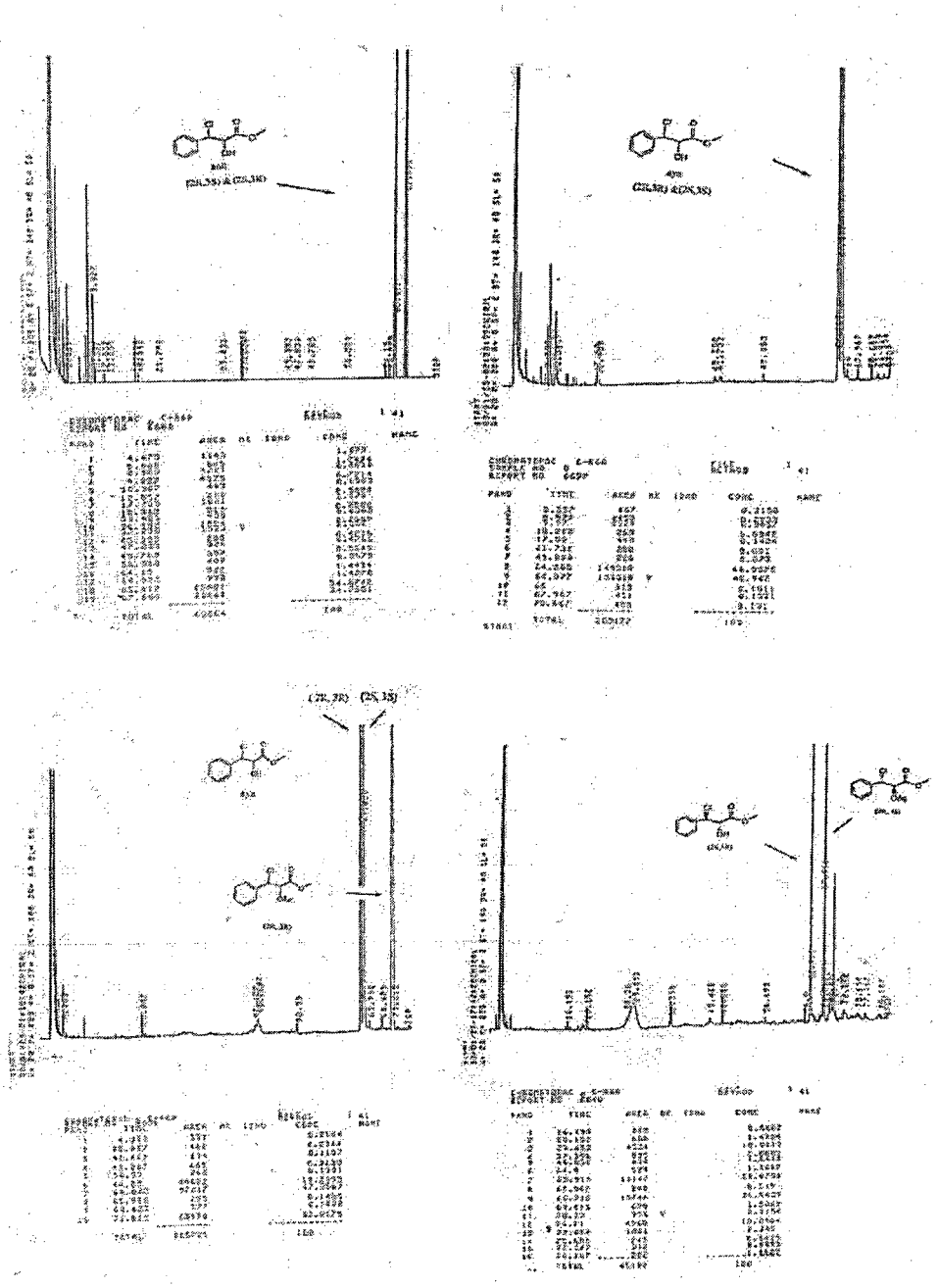
Vmax Points = 5
Well o H3
Vmax -314.1
R^2 1.000

APPENDIX IV GC, HPLC spectra

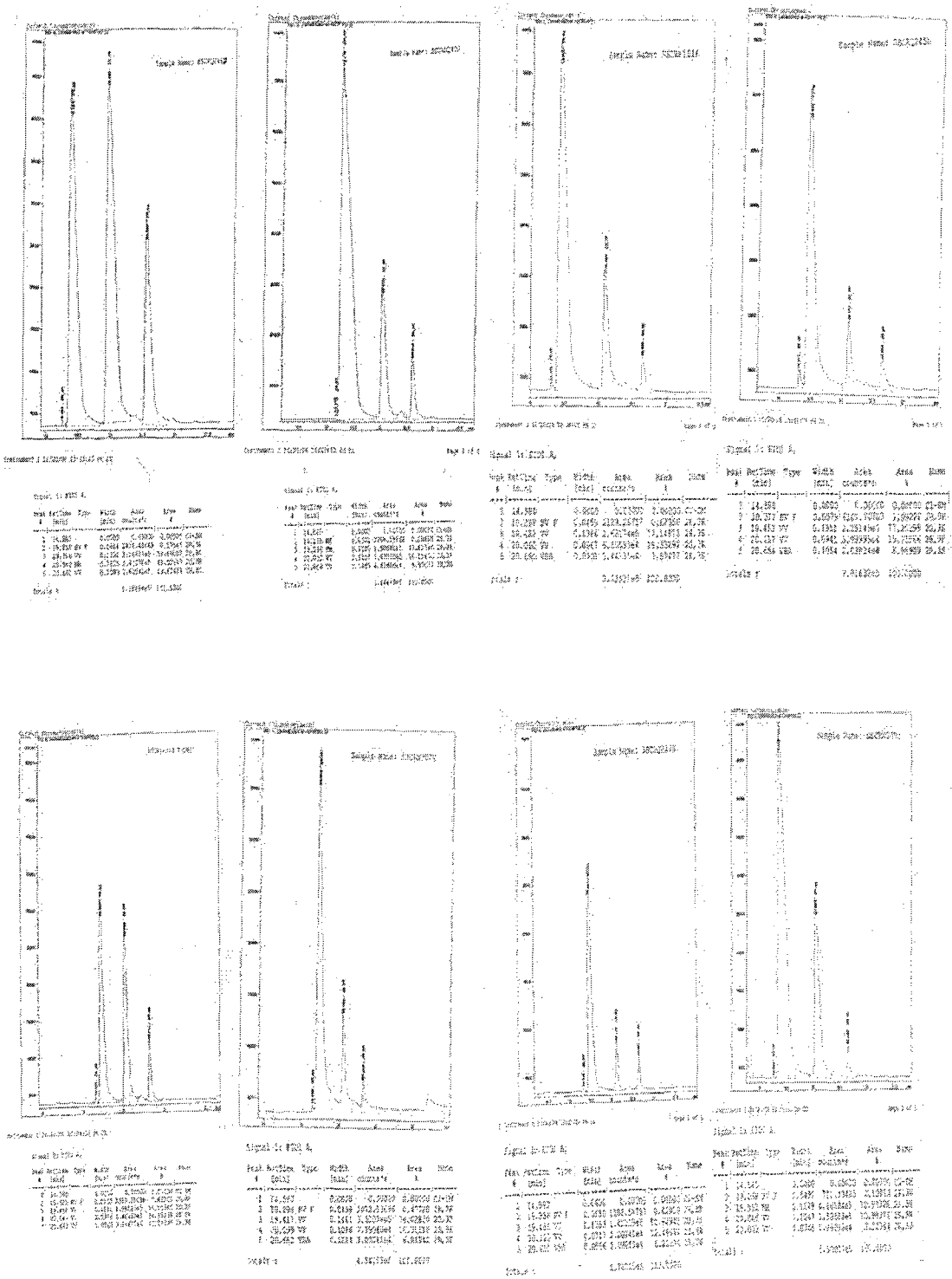
(1). Chiral GC spectra for four diastereoisomers of 3-4 from $NaBH_4$ reduction.

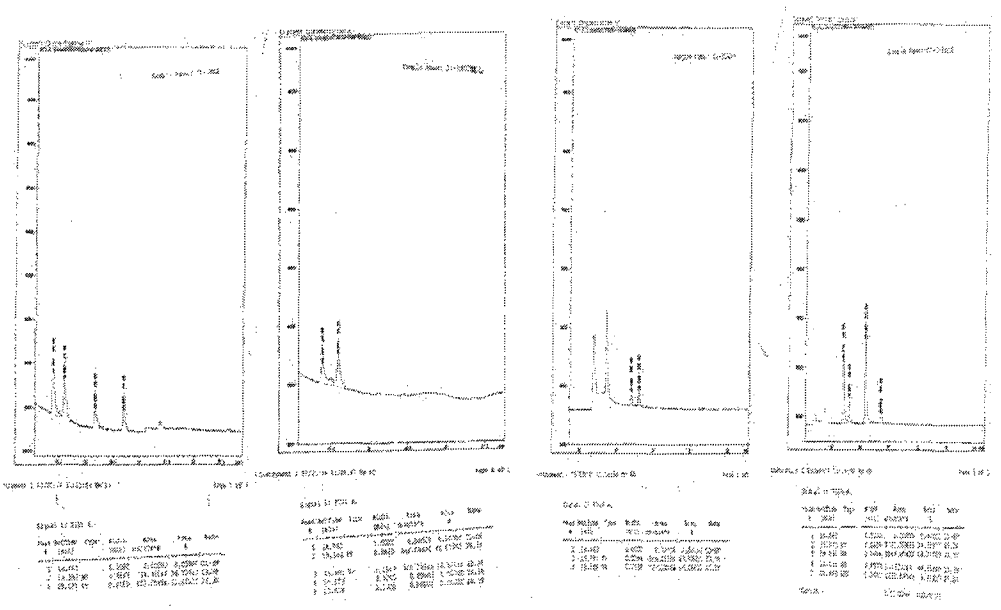


(2). *syn*-and *anti*-3-4 were resolved by Lipase PS to yield acetylated ester respectively with chiral GC analysis.

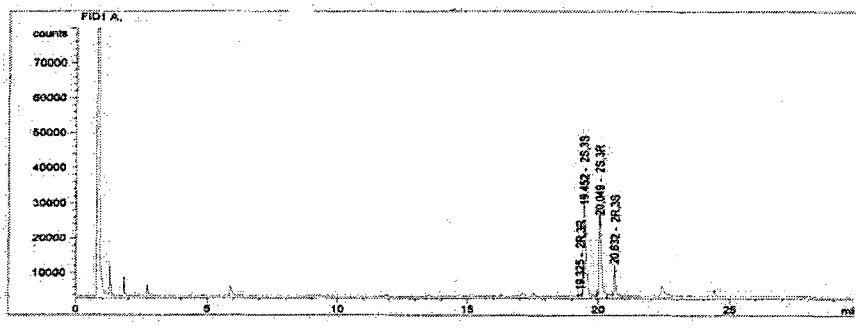


(3). Chiral GC spectra of screening results from α-ketoester 3-3 reduction.





(4). Chiral GC trace of SSCR (wild type) reduction of β-chloro-α-ketoester 3-3.



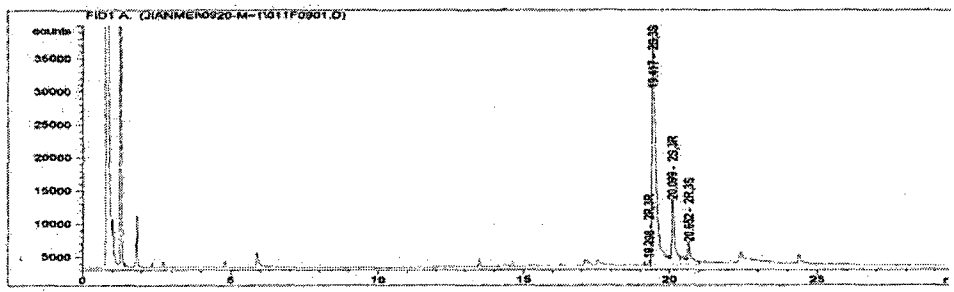
Area Percent Report

Sorted By : Signal
Calib. Data Modified : 10/13/06 11:12:39 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area counts*s	Area %	Name
1	14.580		0.0000	0.00000	0.00000	Cl-SM
2	19.325	PV F	0.0502	2389.21094	0.62220	2R,3R
3	19.452	VV	0.1181	1.94123e5	50.55345	2S,3S
4	20.049	VV	0.0976	1.48035e5	38.55139	2S,3R
5	20.632	VV	0.0764	3.94477e4	10.27297	2R,3S

(5). SSCR mutant (N207V) reduction (representative of mutants) on β-chloro-α-ketoester 3-3.



Area Percent Report

Sorted By : Signal
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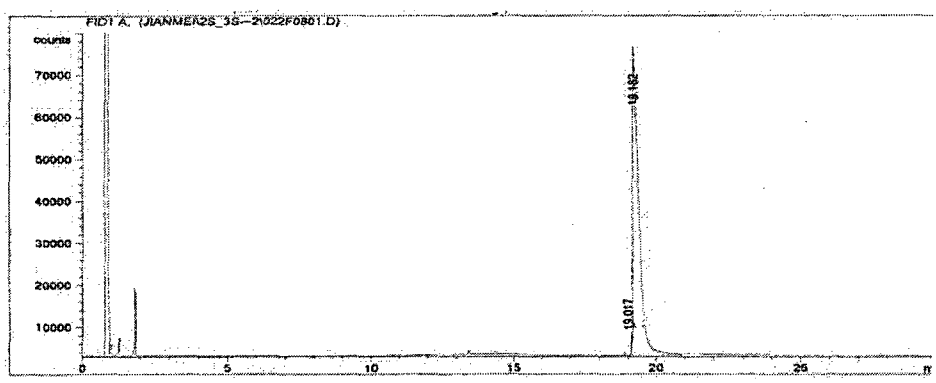
Dilution : 1.0000

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area counts*s	Area %	Name
1	14.580		0.0000	0.00000	0.00000	C1-SM
2	19.298	BV F	0.0498	2072.13696	0.47728	2R,3R
3	19.417	VV	0.1461	3.30078e5	76.02820	2S,3S
4	20.099	VV	0.1086	7.19960e4	16.58310	2S,3R
5	20.652	VBA	0.1284	3.00061e4	6.91142	2R,3S
Totals :				4.34153e5	100.0000	

(6). Chiral GC analysis of isolated (2*S*,3*S*)-(+)-4-9 from enzymatic reduction.



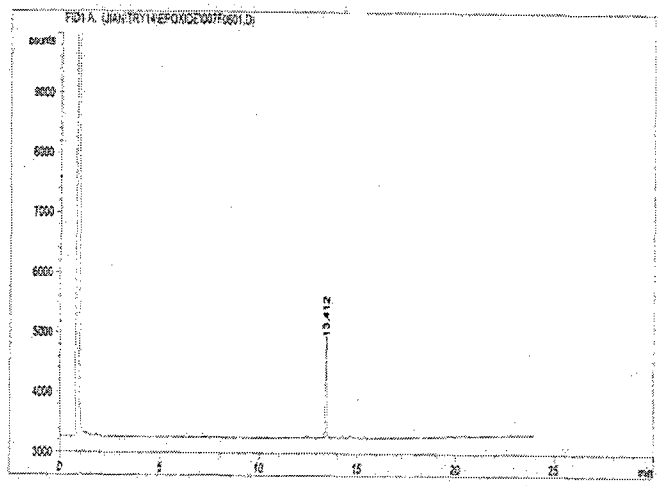
Area Percent Report

Sorted By : Signal
Calib. Data Modified : 10/21/06 11:09:43 AM
Multiplier : 1.0000
Dilution : 1.0000

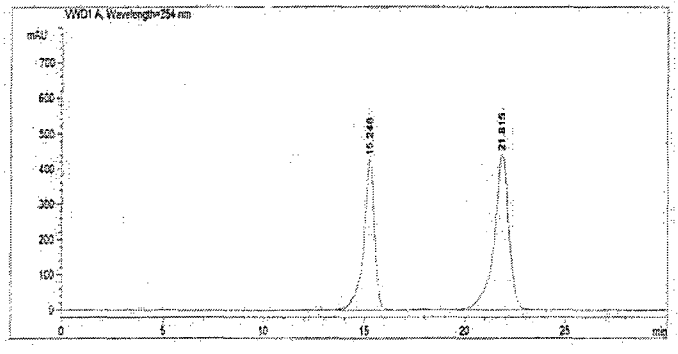
Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area counts*s	Area %	Name
1	14.580		0.0000	0.00000	0.00000	C1-SM
2	19.017	MF	0.0305	9529.43945	0.90457	2R,3R
3	19.162	FM	0.2370	1.04395e6	99.09543	2S,3S
4	20.103		0.0000	0.00000	0.00000	2S,3R

(7). Chiral GC trace of (2*R*,3*R*)-(+)- methyl 3-phenylglycidate 4-10.



(8). Chiral HPLC trace of racemic 4-12c.

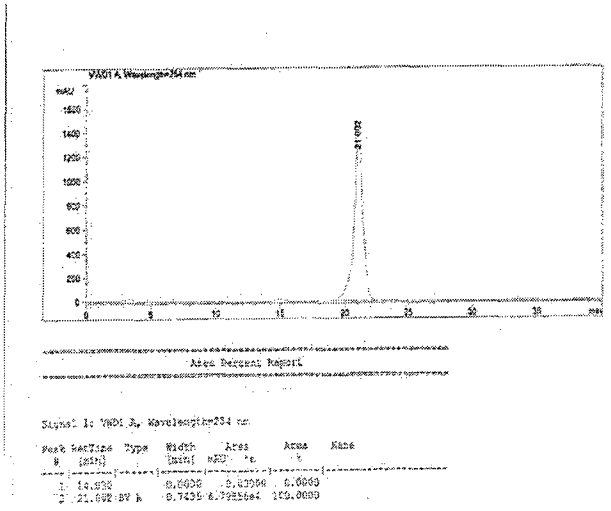


Area Percent Report

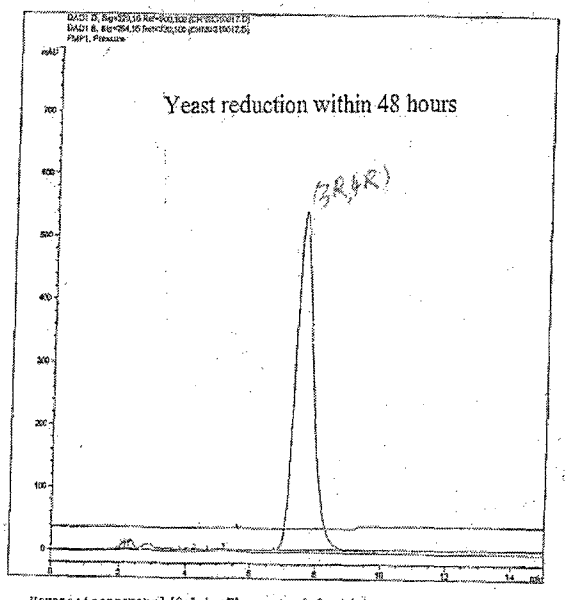
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Area %	Name
1	15.248	BV R	0.5497	1.59931e4	40.9756	
2	21.815	BB	0.7742	2.30377e4	59.0244	

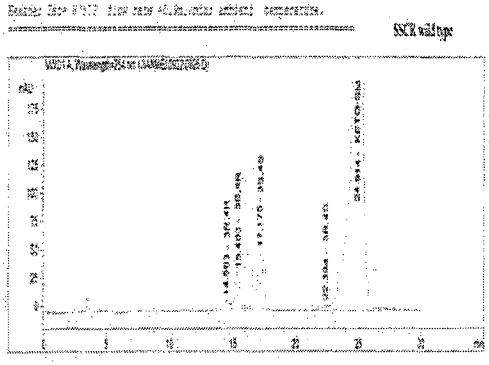
(9). Chiral HPLC trace of (4*S*,5*R*)-(+)-4-12c.

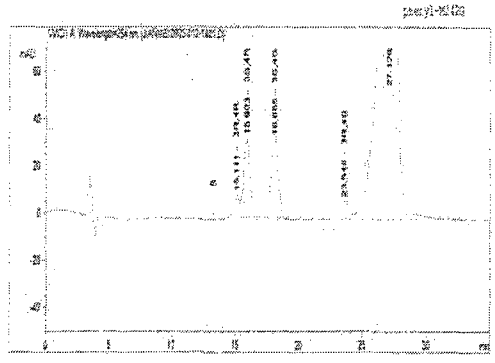


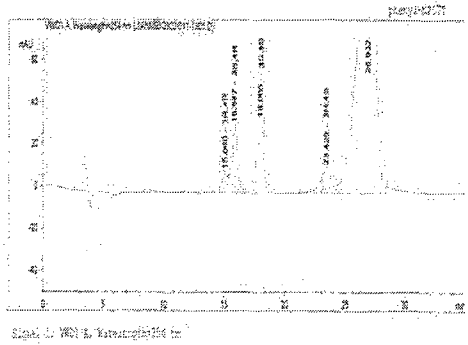
(10). Chiral HPLC trace of (3*R*,4*R*)-2-10 in chiracel OD-H column.

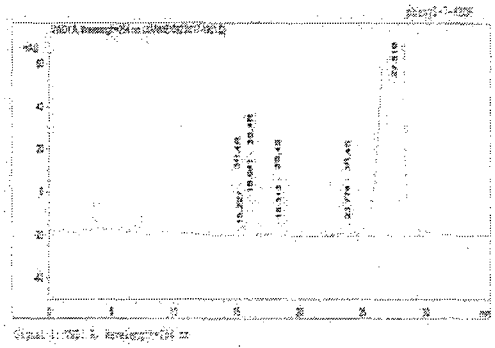


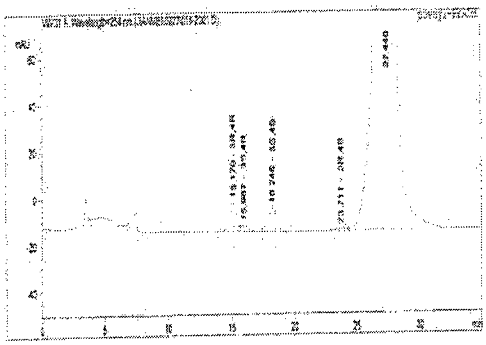
(11). Chiral HPLC analysis of screening results on 3-oxo-β-lactam 3-7.





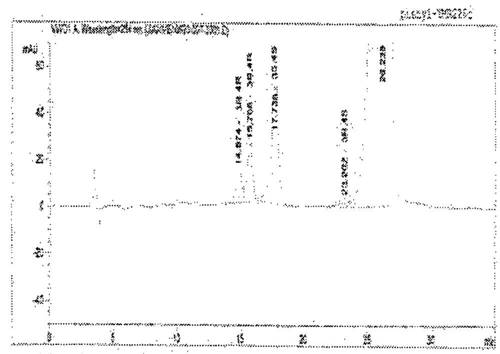






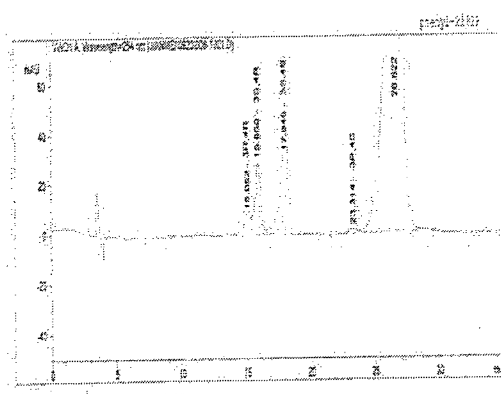
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [illegible]	Area %	Name
1	15.170	BV	0.4746	287.73462	0.6922	38.4R
2	15.907	VB	0.4452	78.58159	0.1703	38.4R
3	18.246	VB	0.5365	414.61359	0.9992	35.4S
4	23.711	BV	0.9460	64.22106	0.1460	28.4S
5	27.440	BV	0.8386	4.36523e4	97.9234	?
6	29.769		0.0000	0.00000	0.0000	[illegible]



Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [illegible]	Area %	Name
1	14.874	BV	0.4817	479.33356	1.0833	38.4R
2	15.708	VB	0.4588	712.05972	1.6596	38.4R
3	17.730	BB	0.5552	2276.21422	5.0215	35.4S
4	23.202	VV X	0.6291	83.72782	0.1935	38.4S
5	26.228	VV R	0.7861	4.17402e4	93.2774	?
6	29.767		0.0000	0.00000	0.0000	[illegible]

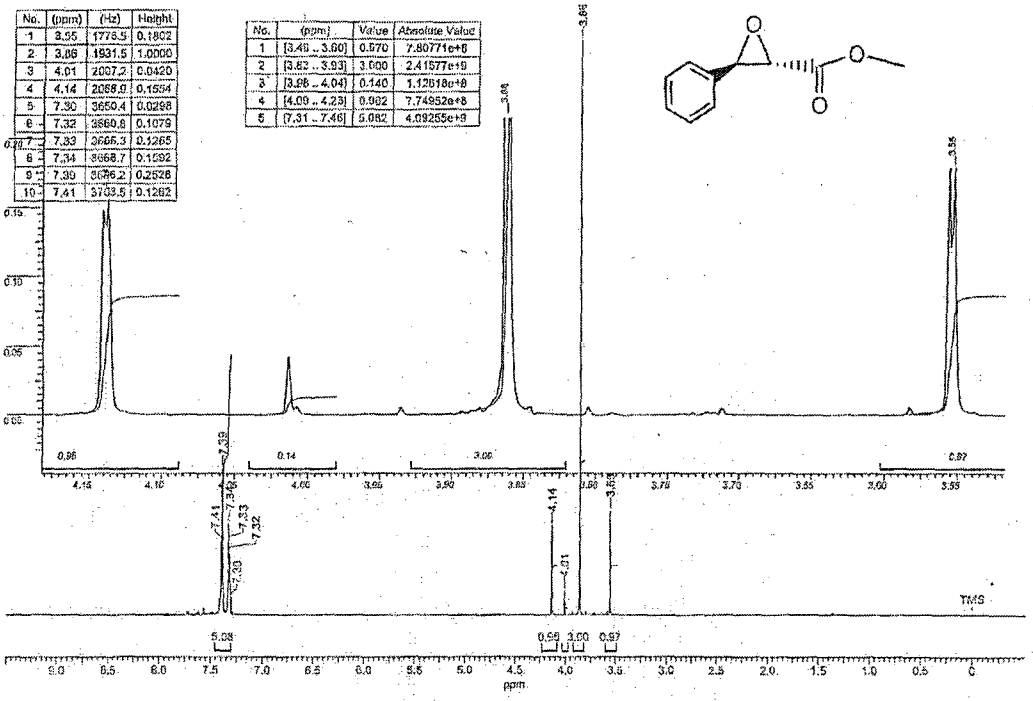


Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [illegible]	Area %	Name
1	15.082	BV	0.4785	274.54745	0.6671	38.4R
2	15.860	VV	0.3869	942.15764	1.0577	38.4R
3	17.846	VB	0.5812	1965.34284	0.4840	35.4S
4	23.314	BV	0.7657	106.42984	0.2249	38.4S
5	26.622	BB	0.8930	2.79410e4	96.3192	?
6	29.757		0.0000	0.00000	0.0000	[illegible]

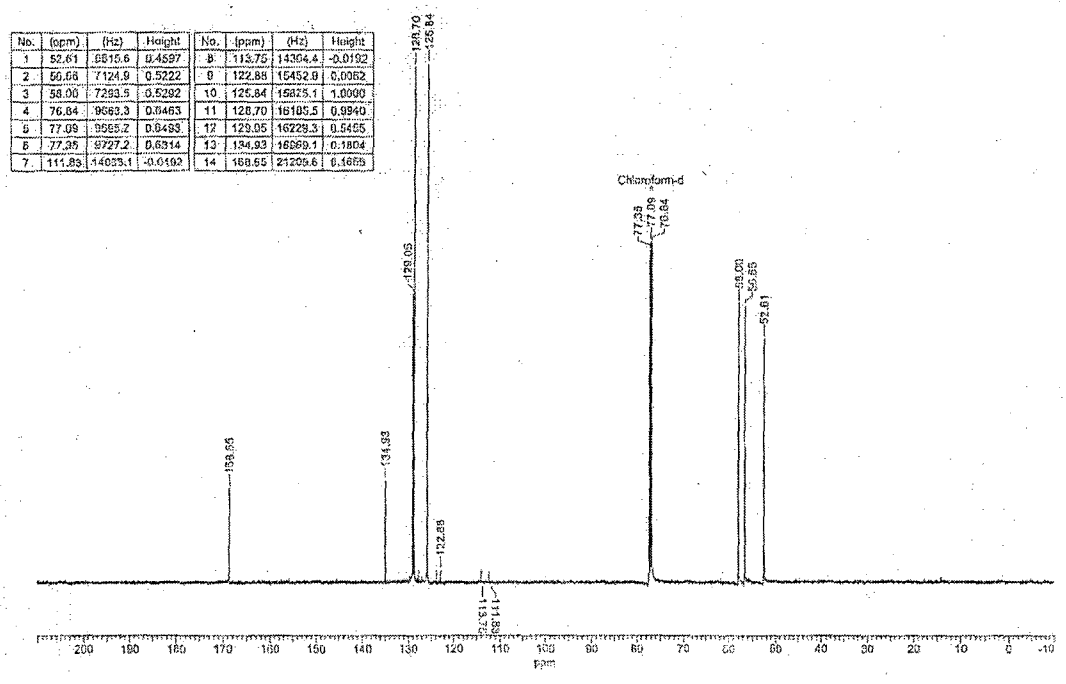
APPENDIX V NMR spectra

(1). *trans*-(±)-Methyl 3-phenyloxirane-2-carboxylate 3-6



No.	(ppm)	(Hz)	Height
1	3.55	1776.5	0.1802
2	3.86	1931.5	1.0000
3	4.01	2007.2	0.0420
4	4.14	2068.0	0.1554
5	7.30	3650.4	0.0298
6	7.32	3660.8	0.1079
7	7.33	3666.3	0.1265
8	7.34	3668.7	0.1592
9	7.39	3696.2	0.2926
10	7.41	3703.5	0.1262

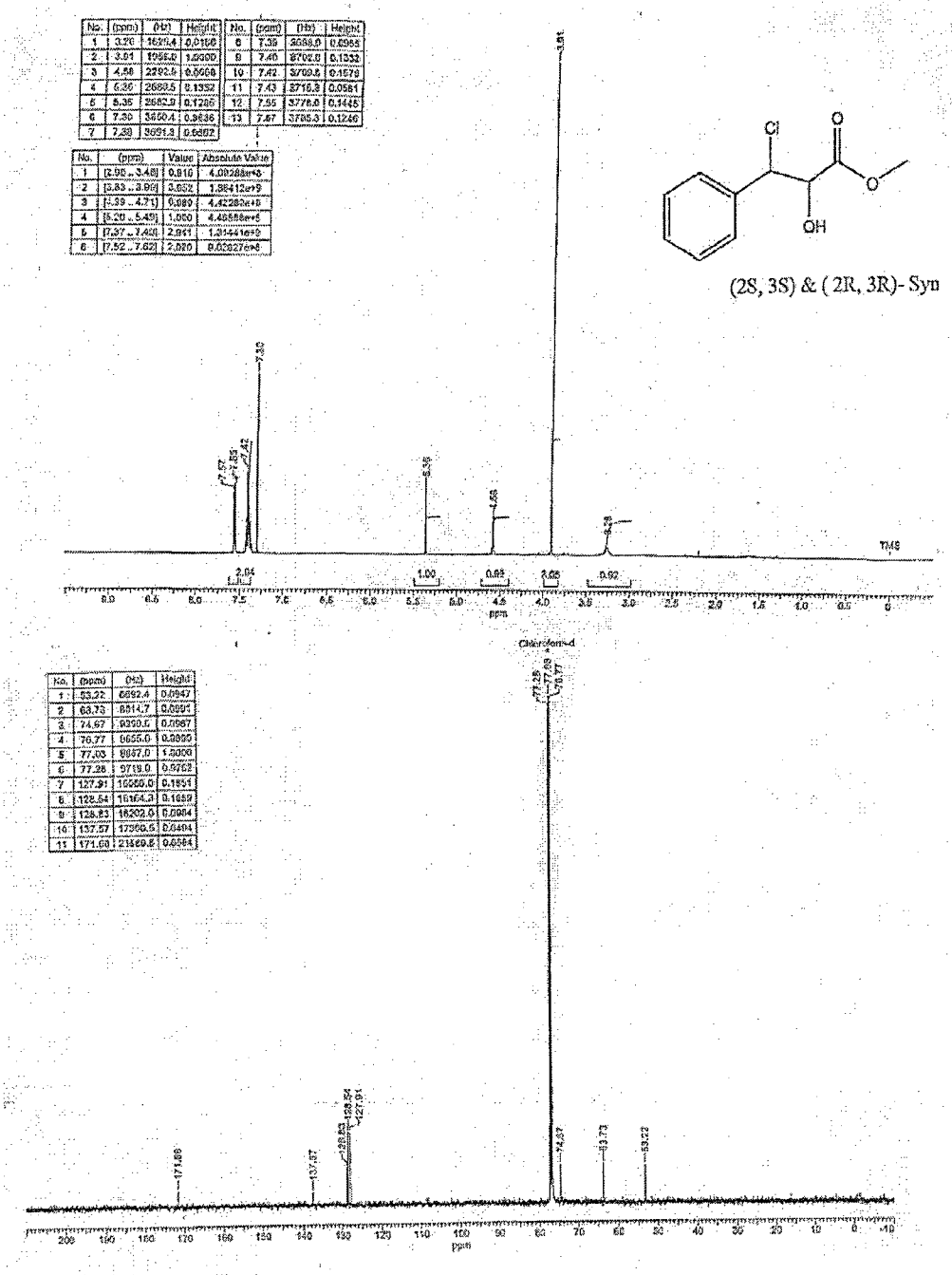
No.	(ppm)	Value	Absolute Value
1	[3.46 .. 3.90]	0.970	7.80771e+8
2	[3.82 .. 3.93]	3.000	2.41577e+9
3	[3.96 .. 4.04]	0.140	1.12618e+8
4	[4.09 .. 4.23]	0.962	7.74952e+8
5	[7.31 .. 7.46]	5.082	4.09255e+9



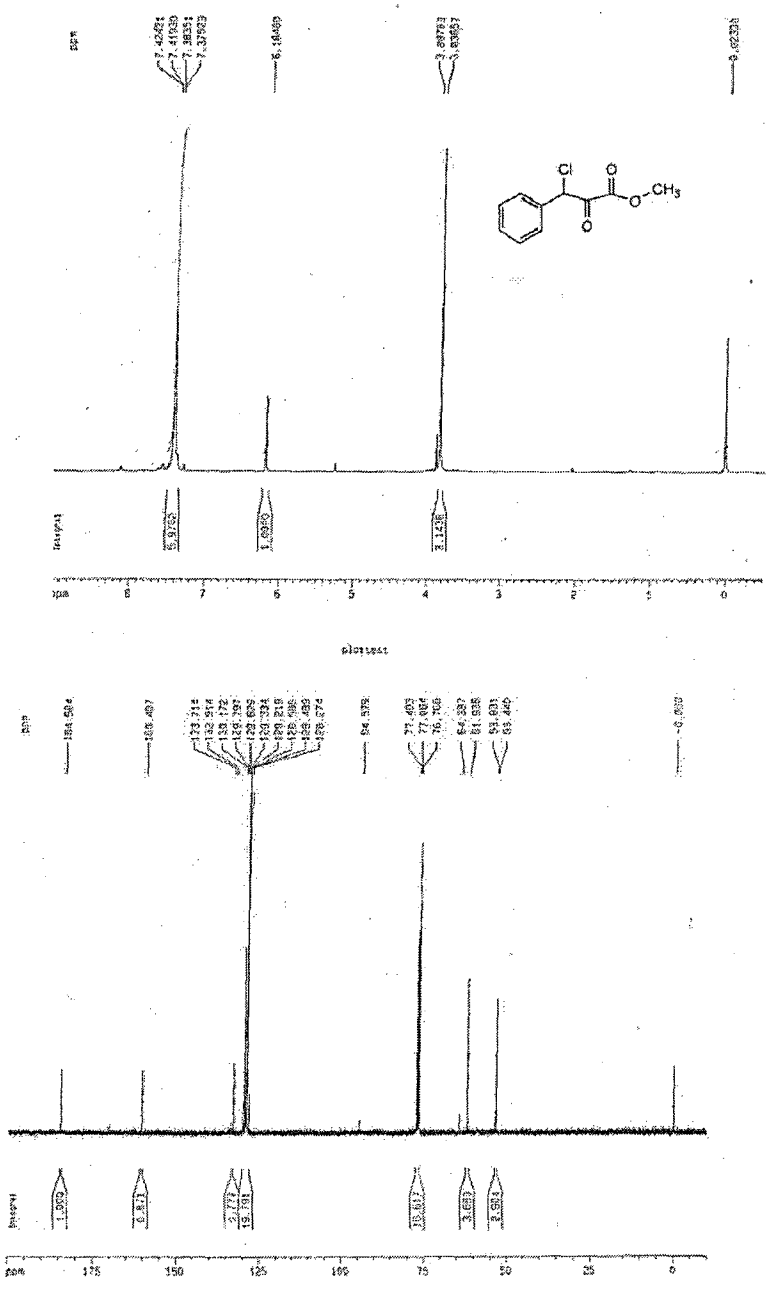
No.	(ppm)	(Hz)	Height
1	52.61	6615.6	0.4597
2	55.66	7124.9	0.5222
3	58.00	7293.5	0.5292
4	76.84	9663.3	0.6463
5	77.09	9695.2	0.6483
6	77.35	9727.2	0.6314
7	111.83	14063.1	-0.0192

No.	(ppm)	(Hz)	Height
8	113.75	14304.4	-0.0192
9	122.88	15452.9	0.0062
10	125.84	15825.1	1.0000
11	128.70	16185.5	0.9940
12	129.05	16229.3	0.5455
13	134.93	16969.1	0.1804
14	158.65	21209.6	0.1665

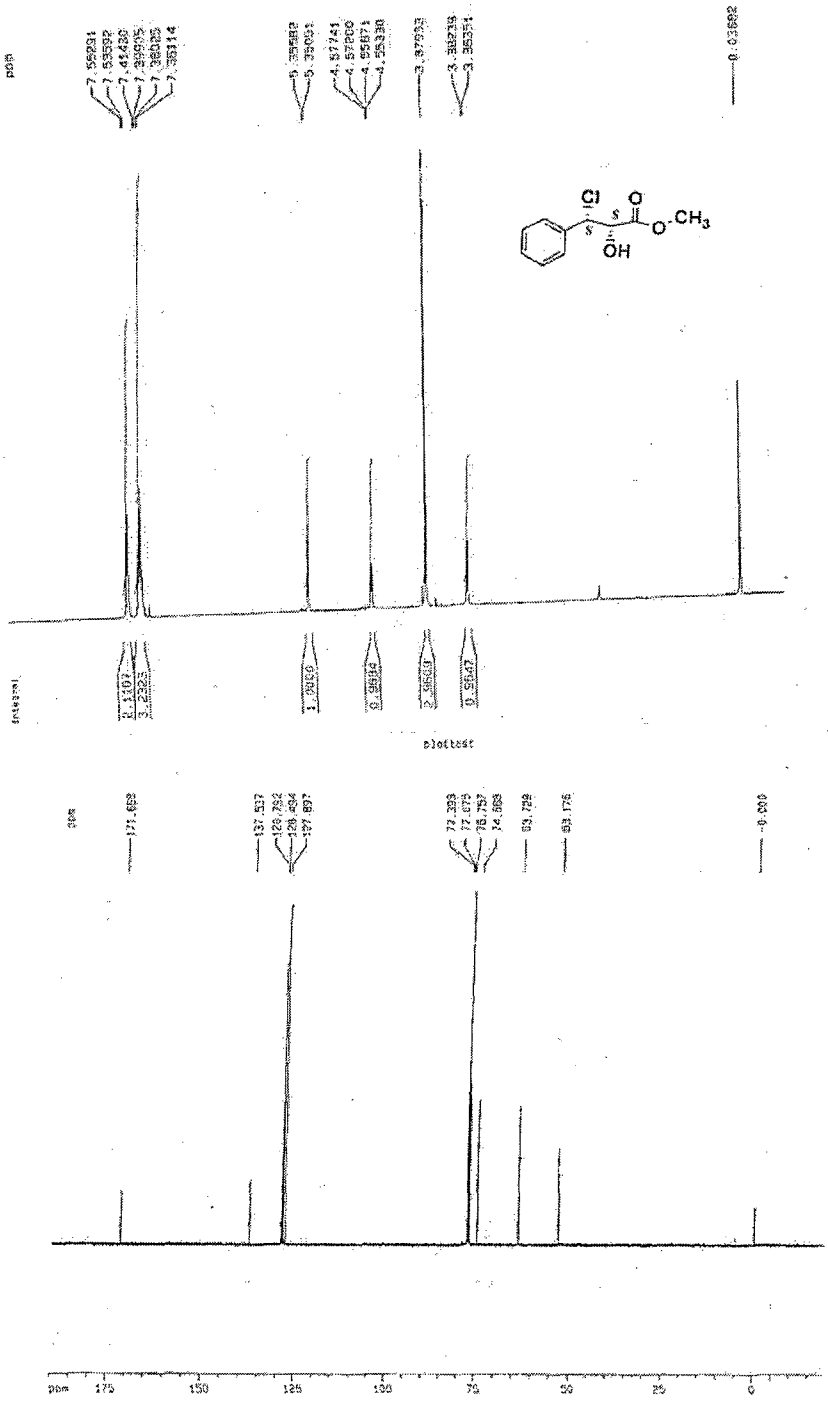
(2). *syn*-(±)-Methyl 3-chloro-2-hydroxy-3-phenyl-propanoate 3-4



(3). Methyl 3-chloro-2-oxo-3-phenylpropanoate 3-3 (or 4-8)



(4). (2*S*,3*S*)-(+)-Methyl-3-chloro-2-hydroxy-3-phenyl-propanoate 4-9

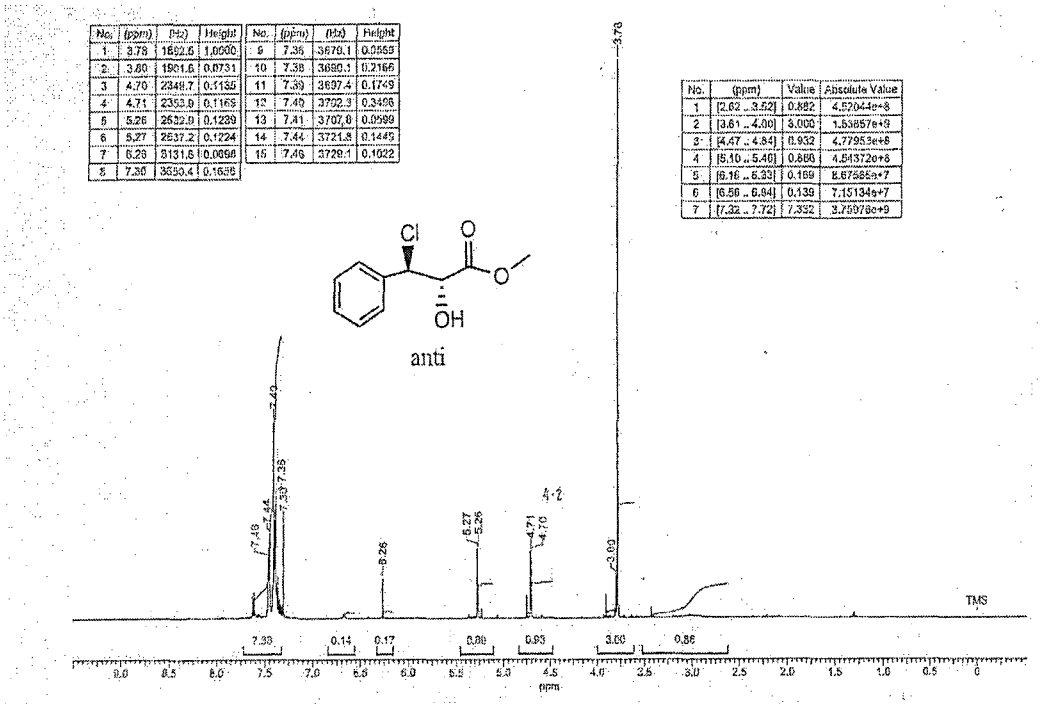


(5). (2*S*,3*R*)-(+)-Methyl-3-chloro-2-hydroxy-3-phenyl-propanoate 4-9

No.	(ppm)	(Hz)	Height
1	3.78	1892.6	1.0000
2	3.80	1901.6	0.0731
3	4.70	2348.7	0.1135
4	4.71	2353.9	0.1169
5	5.26	2632.9	0.1239
6	5.27	2637.2	0.1224
7	6.29	3131.6	0.0096
8	7.36	3680.4	0.1636

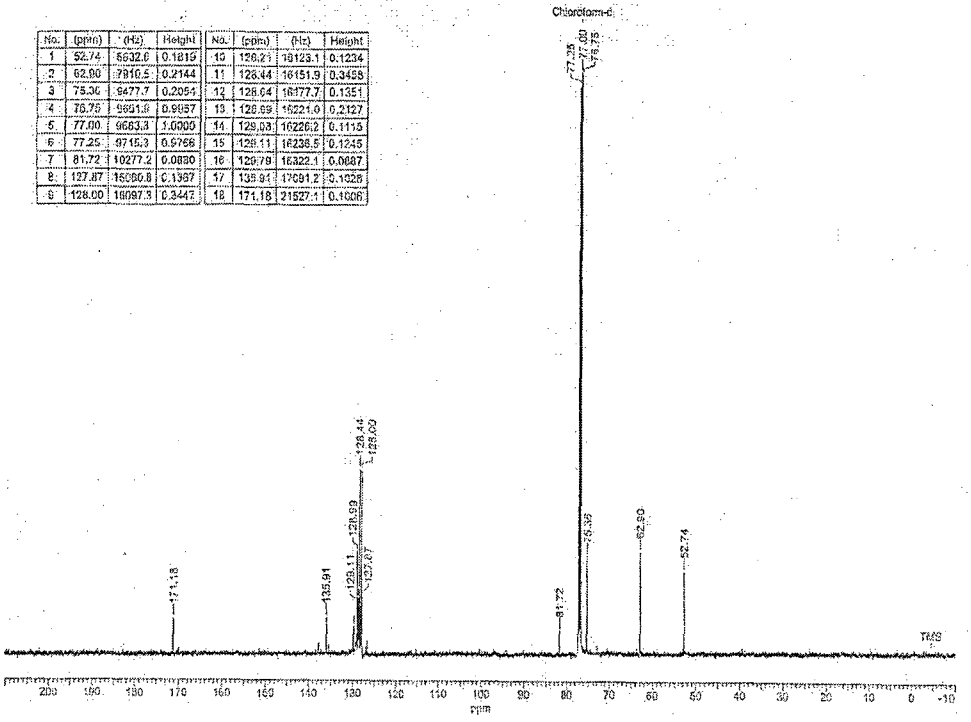
No.	(ppm)	(Hz)	Height
9	7.38	3679.1	0.0959
10	7.38	3680.1	0.2166
11	7.39	3697.4	0.1749
12	7.40	3702.3	0.3426
13	7.41	3707.9	0.0599
14	7.44	3721.8	0.1445
15	7.46	3729.1	0.1022

No.	(ppm)	Value	Absolute Value
1	[2.62 .. 3.52]	0.882	4.52044e+8
2	[3.61 .. 4.00]	3.000	1.53857e+9
3	[4.47 .. 4.84]	0.932	4.77953e+8
4	[5.10 .. 5.46]	0.886	4.54372e+8
5	[6.16 .. 6.33]	0.169	8.67585e+7
6	[6.56 .. 6.84]	0.139	7.15134e+7
7	[7.32 .. 7.72]	7.392	3.79078e+9



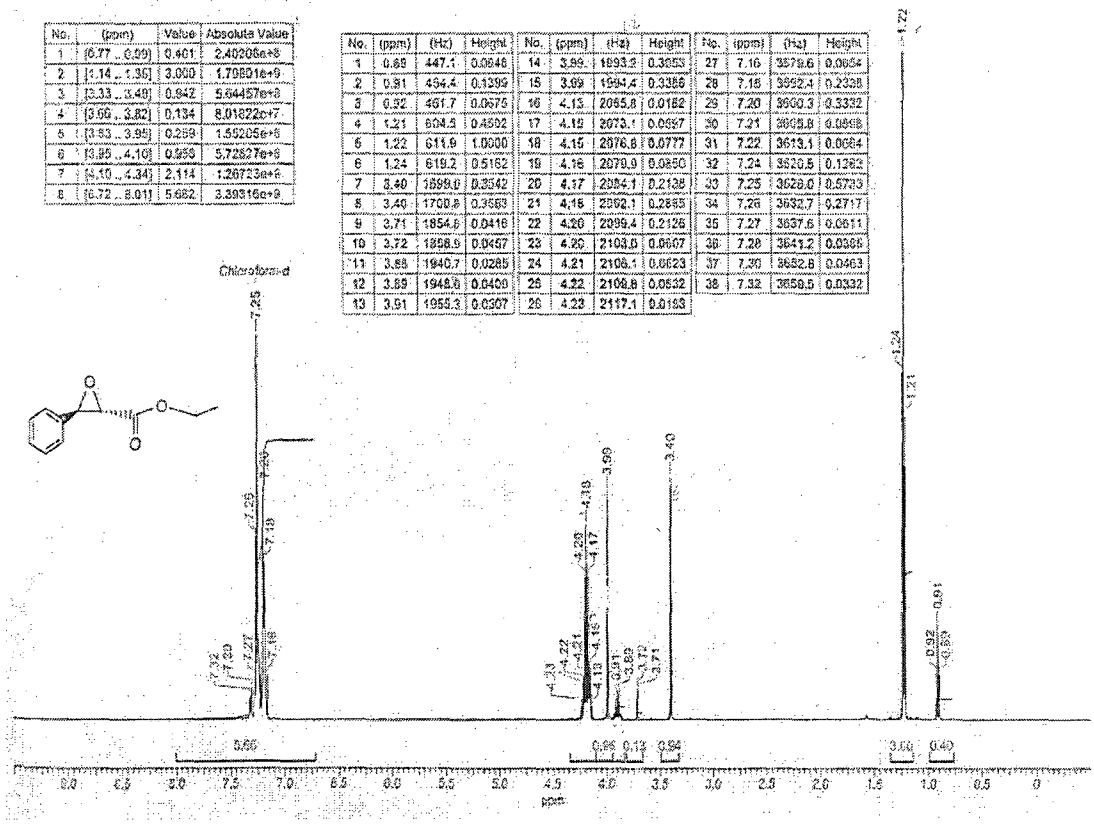
No.	(ppm)	(Hz)	Height
1	52.74	5532.6	0.1819
2	62.90	7910.5	0.2144
3	75.36	9477.7	0.2054
4	76.75	9901.8	0.9057
5	77.00	9663.3	1.0000
6	77.25	9715.3	0.9756
7	81.72	10277.2	0.0830
8	127.87	16080.8	0.1367
9	128.00	16097.3	0.3447

No.	(ppm)	(Hz)	Height
10	128.21	16123.1	0.1234
11	128.44	16151.9	0.3458
12	128.64	16177.7	0.1351
13	128.69	16221.0	0.2127
14	129.03	16226.2	0.1115
15	129.11	16238.5	0.1245
16	129.79	16322.1	0.0887
17	139.91	17091.2	0.1028
18	171.18	21527.1	0.1006



(6). (2*R*,3*R*)-(+)-Methyl 3-phenylglycidate 4-10

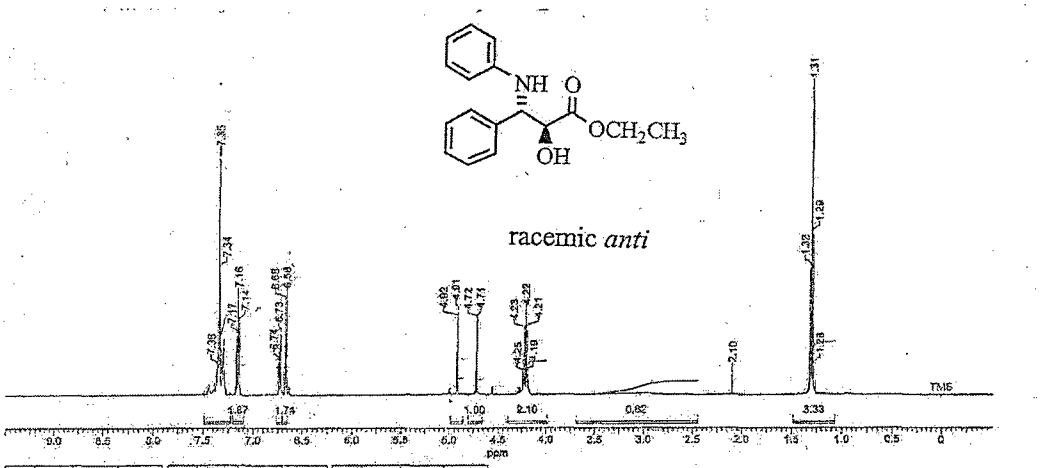
(7). *trans*-(±)-Ethyl 3-phenylglycidate 4-1



No.	(ppm)	Value	Absolute Value
1	[0.77 .. 0.99]	0.401	2.402056e+8
2	[1.14 .. 1.36]	3.000	1.798016e+9
3	[3.33 .. 3.48]	0.942	5.644457e+8
4	[3.66 .. 3.82]	0.134	8.018222e+7
5	[3.83 .. 3.95]	0.259	1.552056e+8
6	[3.95 .. 4.10]	0.953	5.712827e+8
7	[4.10 .. 4.34]	2.114	1.267123e+9
8	[6.72 .. 8.01]	5.662	3.393116e+9

No.	(ppm)	(Hz)	Height
1	0.88	447.1	0.0646
2	0.91	454.4	0.1299
3	0.92	461.7	0.0675
4	1.21	604.5	0.4502
5	1.22	611.9	1.0000
6	1.24	619.2	0.5162
7	3.40	1699.0	0.3542
8	3.40	1700.8	0.3563
9	3.71	1854.6	0.0418
10	3.72	1858.9	0.0457
11	3.88	1940.7	0.0285
12	3.89	1948.6	0.0409
13	3.91	1956.3	0.0507
14	3.99	1993.2	0.3053
15	3.99	1994.4	0.3356
16	4.15	2065.8	0.0162
17	4.16	2073.1	0.0557
18	4.15	2076.8	0.0777
19	4.16	2079.9	0.0850
20	4.17	2084.1	0.2128
21	4.15	2092.1	0.2855
22	4.20	2099.4	0.2126
23	4.20	2103.0	0.0607
24	4.21	2106.1	0.0623
25	4.22	2108.8	0.0532
26	4.23	2117.1	0.0193
27	7.16	3579.6	0.0684
28	7.18	3592.4	0.2328
29	7.20	3600.3	0.3332
30	7.21	3605.8	0.0898
31	7.22	3613.1	0.0684
32	7.24	3620.5	0.1282
33	7.25	3626.0	0.5793
34	7.26	3632.7	0.2717
35	7.27	3637.6	0.0611
36	7.28	3641.2	0.0389
37	7.30	3652.8	0.0463
38	7.32	3659.5	0.0332

(8). *anti*-(±)-Ethyl 2-hydroxy-3-phenyl-3-(phenylamino) propanoate 4-3a

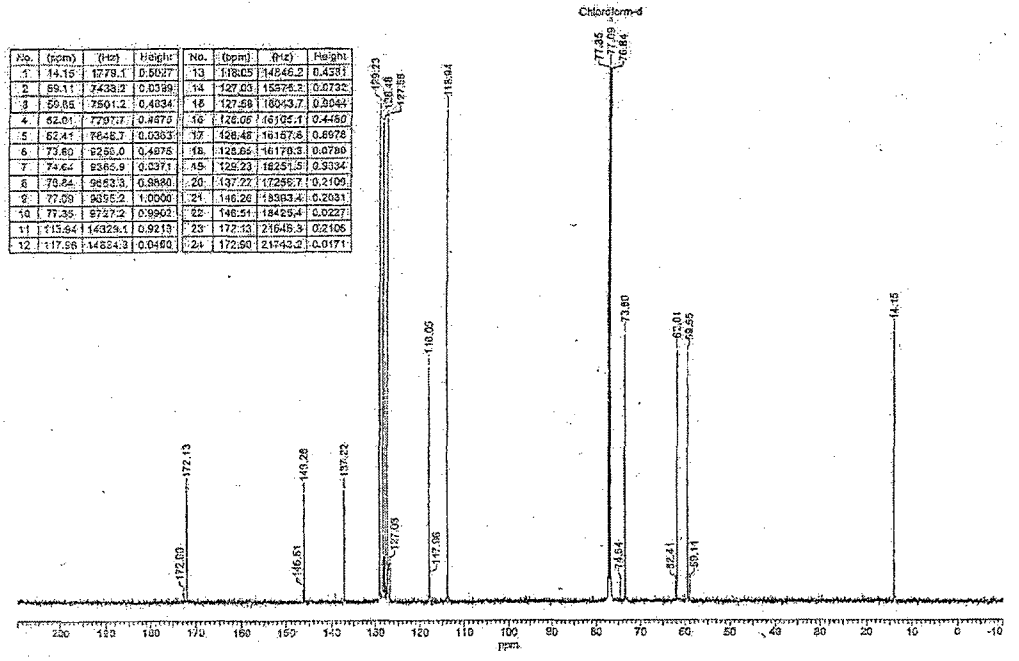


No.	(ppm)	(Hz)	Height
1	1.28	640.6	0.0913
2	1.29	646.7	0.3912
3	1.31	654.0	1.0000
4	1.32	660.7	0.4164
5	2.10	1048.3	0.0606
6	4.19	2090.7	0.0665
7	4.20	2099.4	0.0594
8	4.21	2103.1	0.2115
9	4.22	2109.2	0.2568
10	4.23	2116.5	0.2056

No.	(ppm)	(Hz)	Height
11	4.25	2123.2	0.0814
12	4.74	2367.6	0.2458
13	4.72	2581.3	0.2429
14	4.81	2457.1	0.2735
15	4.92	2460.8	0.2489
16	6.69	3331.2	0.3134
17	6.69	3339.1	0.3084
18	6.71	3357.9	0.0963
19	6.73	3364.7	0.1591
20	6.74	3372.1	0.1076

No.	(ppm)	(Hz)	Height
21	7.14	3572.3	0.2875
22	7.16	3580.8	0.3131
23	7.17	3586.1	0.1936
24	7.29	3648.0	0.1100
25	7.30	3650.4	0.1557
26	7.31	3654.1	0.1264
27	7.32	3660.8	0.4194
28	7.34	3668.7	0.4131
29	7.35	3676.4	0.7773
30	7.36	3681.5	0.0697

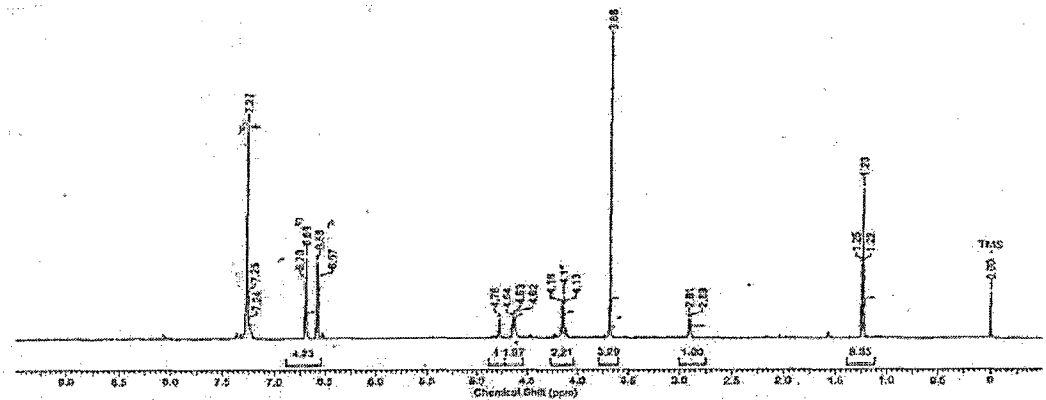
No.	(ppm)	Value	Absolute Value
1	[1.07 .. 1.40]	3.378	1.39401e+9
2	[2.45 .. 3.69]	0.824	3.45215e+8
3	[3.99 .. 4.40]	2.103	8.81198e+8
4	[4.56 .. 4.80]	1.000	4.19098e+8
5	[4.86 .. 4.99]	1.057	4.42797e+8
6	[6.65 .. 6.76]	1.738	7.28598e+8
7	[6.76 .. 6.76]	0.938	3.93016e+8
8	[7.10 .. 7.21]	1.870	7.83541e+8
9	[7.23 .. 7.46]	5.082	2.13004e+9



No.	(ppm)	(Hz)	Height
1	14.15	1779.1	0.5027
2	59.11	7433.2	0.0399
3	59.55	7501.2	0.4834
4	62.01	7797.7	0.4879
5	62.41	7848.7	0.0383
6	73.60	9256.0	0.4875
7	74.64	9386.9	0.0371
8	76.84	9663.3	0.9880
9	77.09	9695.2	1.0000
10	77.35	9727.2	0.9902
11	115.94	14329.1	0.9219
12	117.56	14634.3	0.0469

No.	(ppm)	(Hz)	Height
13	118.05	14846.2	0.4381
14	127.03	15976.3	0.0732
15	127.58	16043.7	0.2044
16	128.06	16105.1	0.4460
17	128.48	16157.6	0.8978
18	128.66	16179.3	0.0786
19	129.23	16251.5	0.9334
20	137.22	17256.7	0.2109
21	146.26	18393.4	0.2031
22	146.51	18425.4	0.0227
23	172.13	21648.3	0.2106
24	172.60	21743.2	0.0171

(9). *anti*-(±)-Ethyl 3-(4-methoxyphenylamino)-2-hydroxy-3-phenylpropanoate 4-3b



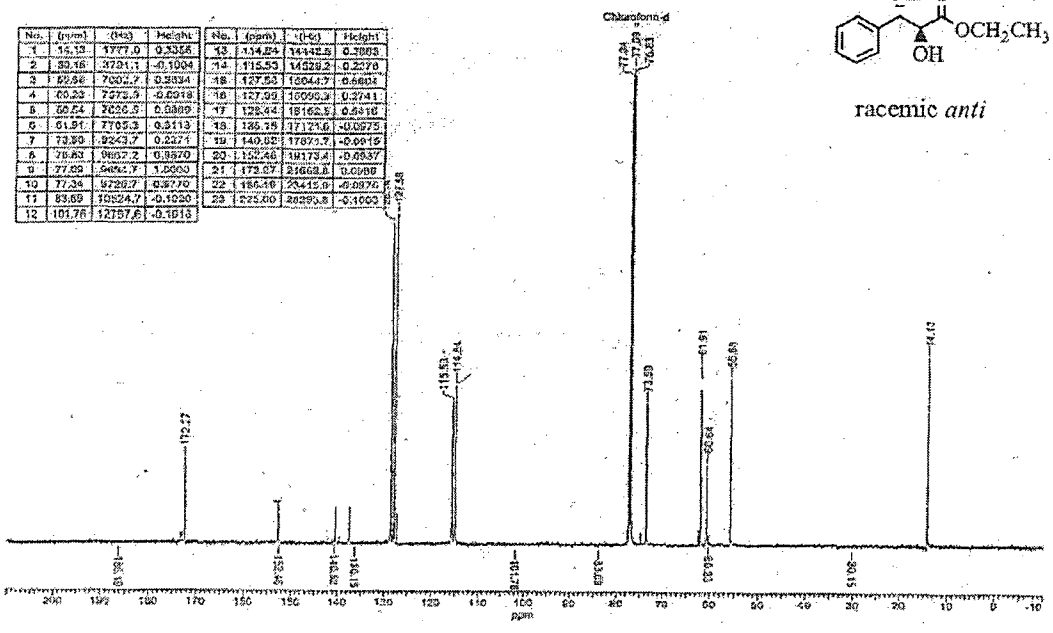
No.	(ppm)	(Hz)	Height
1	0.00	0.0	0.1536
2	1.22	818.4	0.2466
3	1.23	817.4	0.5026
4	1.25	824.7	0.2452
5	2.59	1447.5	0.0606
6	2.91	1476.1	0.0616
7	3.68	1541.9	1.0000
8	4.12	2008.4	0.1051
9	4.13	2073.1	0.1412
10	4.16	2080.1	0.1002
11	4.62	2312.7	0.0731

No.	(ppm)	(Hz)	Height
12	4.63	2316.0	0.0738
13	4.64	2318.7	0.0618
14	4.64	2323.1	0.0521
15	4.75	2306.7	0.0537
16	4.78	2091.1	0.0565
17	6.67	3264.4	0.1058
18	6.67	3266.5	0.0984
19	6.68	3291.1	0.0839
20	6.68	3292.5	0.2359
21	6.69	3343.5	0.2742
22	6.69	3348.0	0.2705

No.	(ppm)	(Hz)	Height
23	6.70	3350.0	0.0724
24	6.70	3352.8	0.1529
25	7.24	3610.5	0.0570
26	7.24	3621.0	0.0548
27	7.25	3623.8	0.0639
28	7.25	3626.2	0.1490
29	7.26	3628.7	0.0631
30	7.27	3633.8	0.7211
31	7.27	3637.5	0.2136

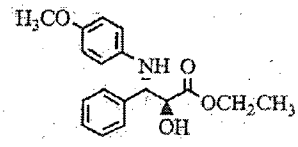
No.	Annotation	(ppm)
1	TMS	0.00

No.	(ppm)	Value	Absolute Value
1	[1.11 .. 1.99]	3.351	3.10814e+9
2	[2.76 .. 3.00]	1.000	9.27468e+8
3	[3.60 .. 3.76]	3.202	3.03317e+9
4	[4.05 .. 4.27]	2.208	2.04778e+9
5	[4.54 .. 4.72]	1.921	1.62830e+9
6	[4.72 .. 4.88]	1.106	1.02585e+9
7	[6.54 .. 6.88]	4.222	3.91478e+9



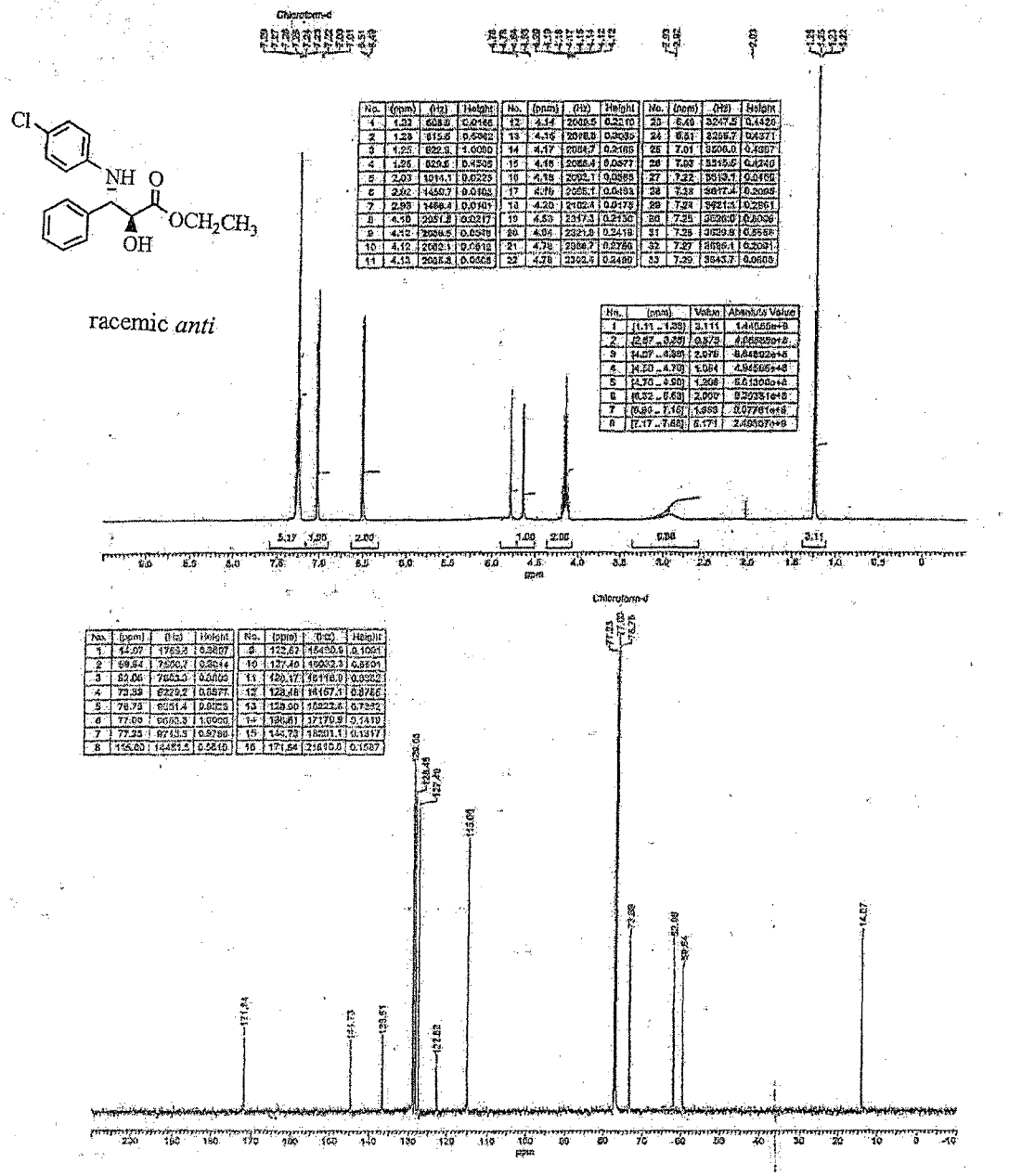
No.	(ppm)	(Hz)	Height
1	14.13	1777.0	0.3356
2	60.16	3791.1	-0.1004
3	60.64	7602.7	0.9934
4	60.83	7275.9	-0.6018
5	60.84	7626.5	0.0869
6	61.91	7705.3	0.3113
7	73.59	9243.7	0.2271
8	76.83	9657.2	0.9570
9	77.09	9692.7	1.0000
10	77.34	9726.7	0.9770
11	83.69	10524.7	-0.1020
12	101.76	12787.6	-0.1013

No.	(ppm)	(Hz)	Height
13	114.84	14442.5	0.3883
14	115.53	14529.2	0.2278
15	127.53	16044.7	0.6604
16	127.99	16096.3	0.2741
17	128.64	16162.5	0.6618
18	135.35	17121.6	-0.0975
19	140.02	17671.7	-0.0919
20	152.46	19173.4	-0.0937
21	172.27	21668.8	0.0000
22	196.16	25415.0	-0.0976
23	225.00	28290.8	-0.1000

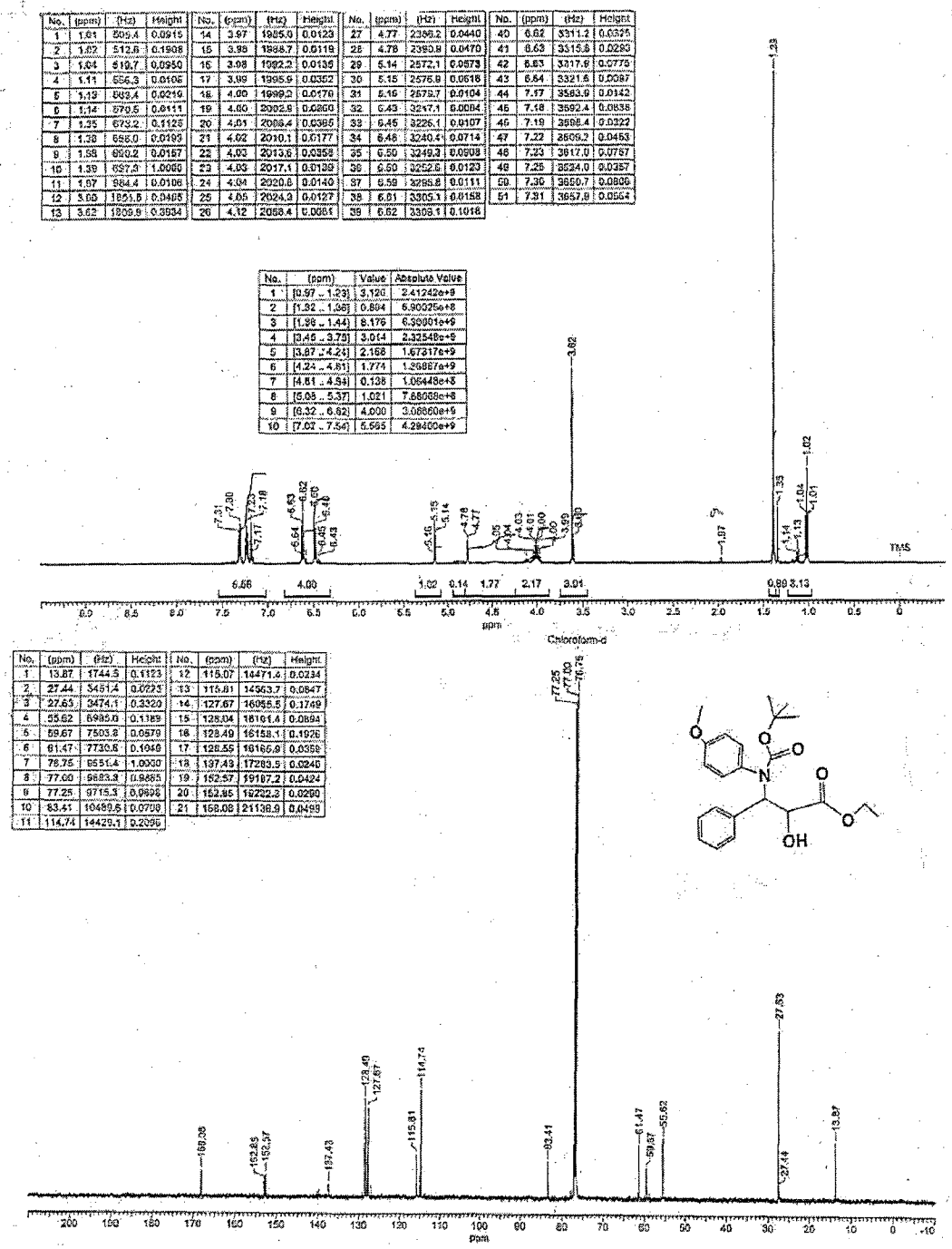


racemic *anti*

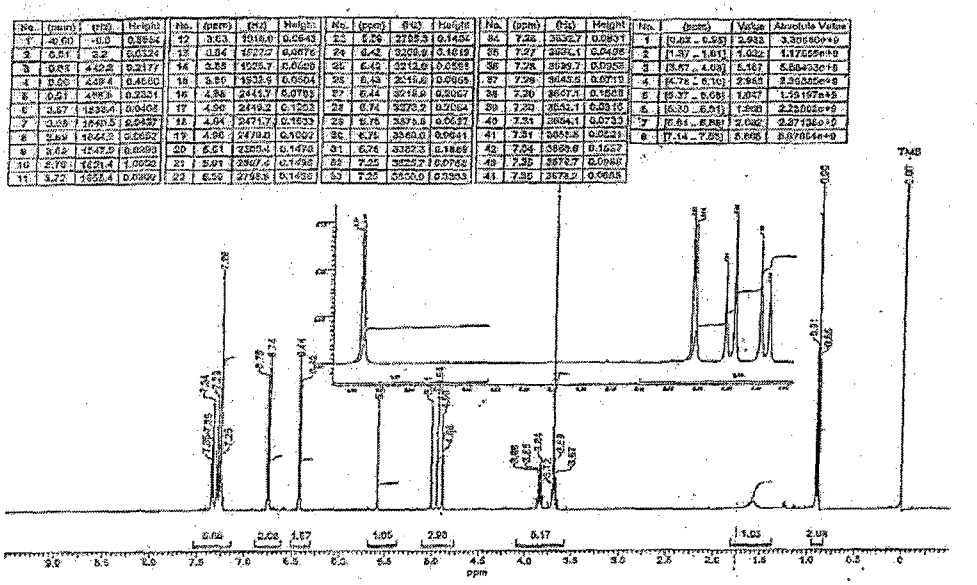
(10). *anti*-(±)-Ethyl 3-(4-chlorophenylamino)-2-hydroxy-3-phenylpropanoate 4-3c

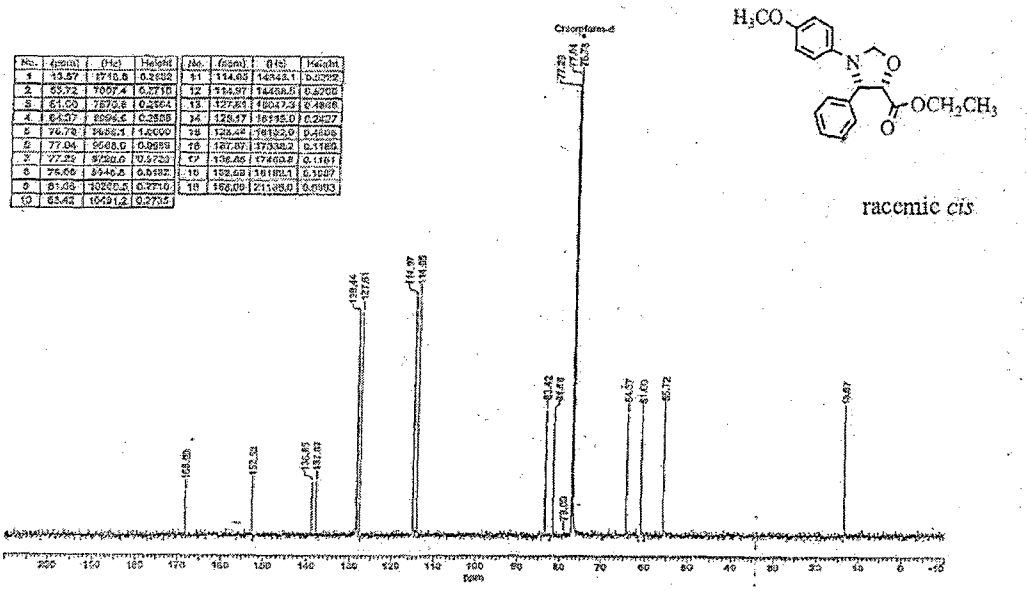


(11). *anti*-(±)-*t*-Boc protected 4-3b

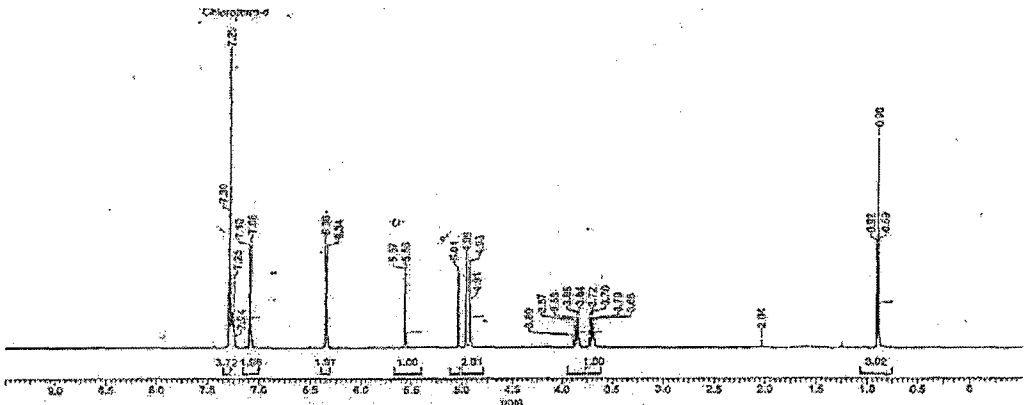


(12). *cis*-(±)-Ethyl 3-(4-methoxyphenyl)-4-phenyl-1,3-oxazolidine-5-carboxylate 4-4b





(13). *cis*-(±)-Ethyl 3-(4-chlorophenyl)-4-phenyl-1,3-oxazolidine-5-carboxylate 4-4c

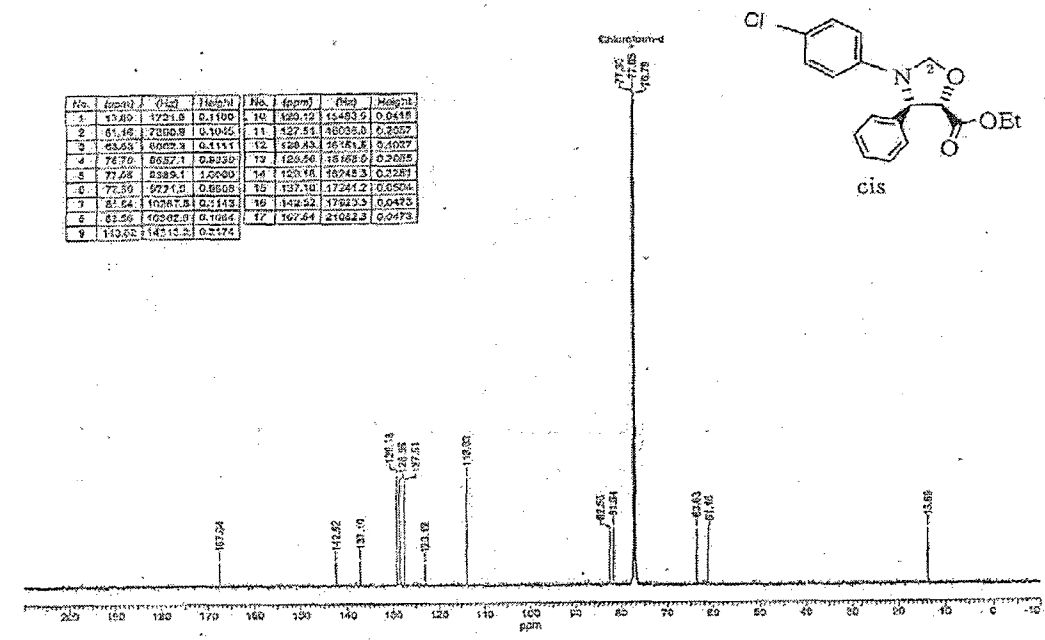


No.	(ppm)	Diff.	Height
1	0.00	413.7	0.1603
2	0.80	450.6	0.7251
3	0.92	450.0	0.2267
4	2.04	1018.9	0.3241
5	3.67	1838.0	0.8262
6	3.85	1842.0	0.6783
7	3.89	1948.7	0.0405
8	3.70	1849.3	0.0908
9	3.70	1852.8	0.1033
10	3.71	1855.4	0.0352
11	3.72	1860.0	0.0898
12	3.73	1867.2	0.0316
13	3.83	1913.7	0.0349

No.	(ppm)	(Hz)	Height
14	3.85	1918.8	0.1052
15	3.88	1953.3	0.0343
16	3.95	1977.0	0.1033
17	3.96	1980.5	0.0810
18	3.97	1984.0	0.0360
19	3.97	1987.9	0.0799
20	3.99	1994.0	0.0246
21	4.01	2486.3	0.1830
22	4.03	2463.8	0.2841
23	4.90	2473.4	0.2933
24	4.97	2486.2	0.1544
25	5.04	2519.7	0.2580
26	5.04	2521.8	0.2822

No.	(ppm)	(Hz)	Height
27	6.68	3781.8	0.2575
28	6.67	3783.3	0.2972
29	6.36	3171.4	0.5578
30	6.36	3180.3	0.5818
31	7.26	3540.8	0.9631
32	7.30	3549.5	0.8384
33	7.34	3628.0	0.2253
34	7.35	3629.9	0.2310
35	7.38	3636.3	0.0941
36	7.27	3634.2	0.1230
37	7.27	3637.7	0.1356
38	7.29	3645.5	1.0000
39	7.30	3649.7	0.4002

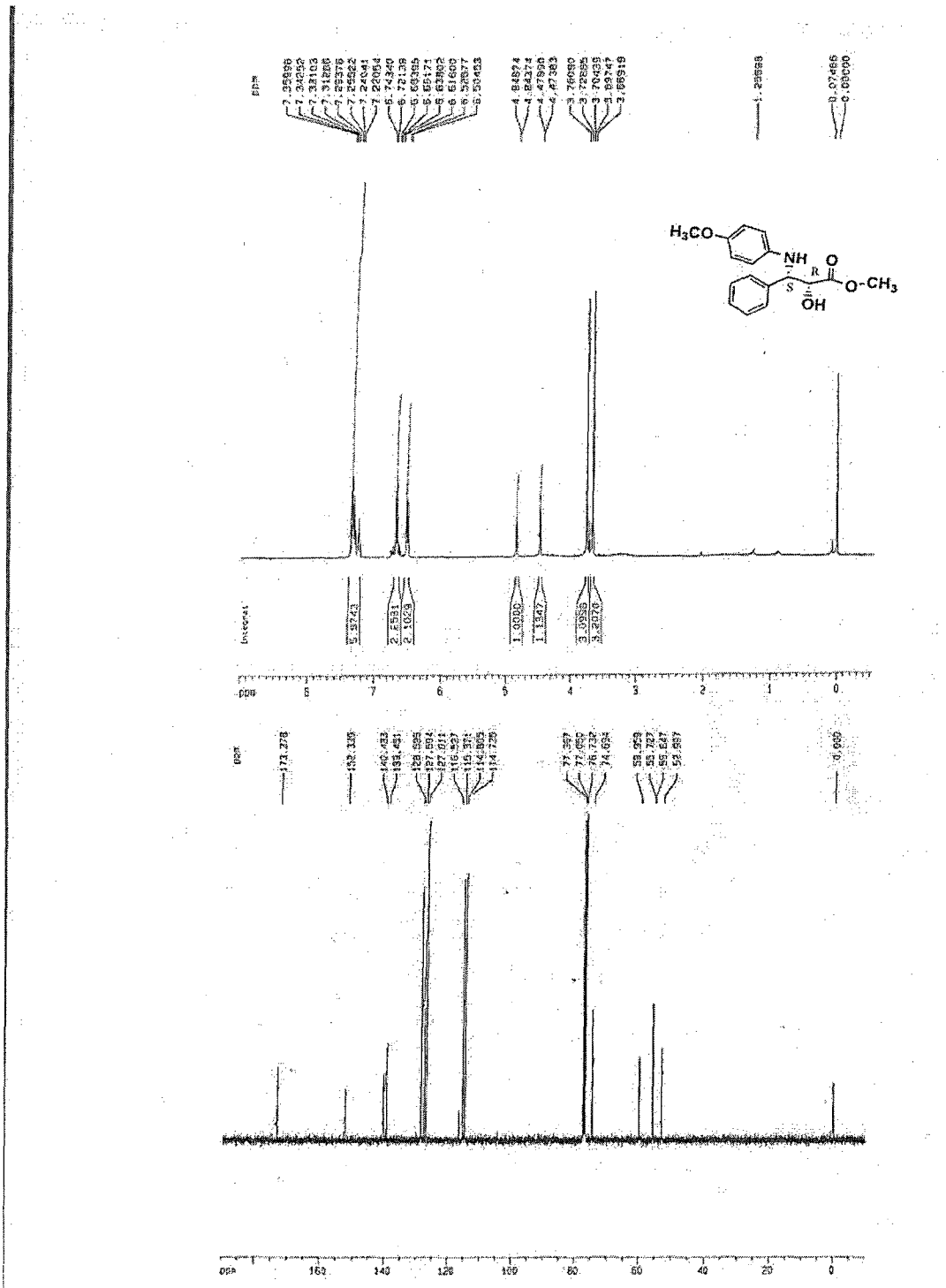
No.	(ppm)	Value	Absolute Value
1	0.75 - 1.07	3.015	1.036194e+10
2	0.91 - 0.74	1.000	3.443273e+9
3	6.77 - 8.04	0.985	3.391192e+9
4	4.79 - 5.00	2.010	6.921009e+9
5	6.02 - 6.13	0.976	3.360980e+9
6	6.40 - 6.67	1.004	3.456604e+9
7	6.31 - 6.39	1.074	3.700360e+9
8	7.00 - 7.16	1.990	6.851610e+9
9	7.25 - 7.35	5.721	1.969916e+10



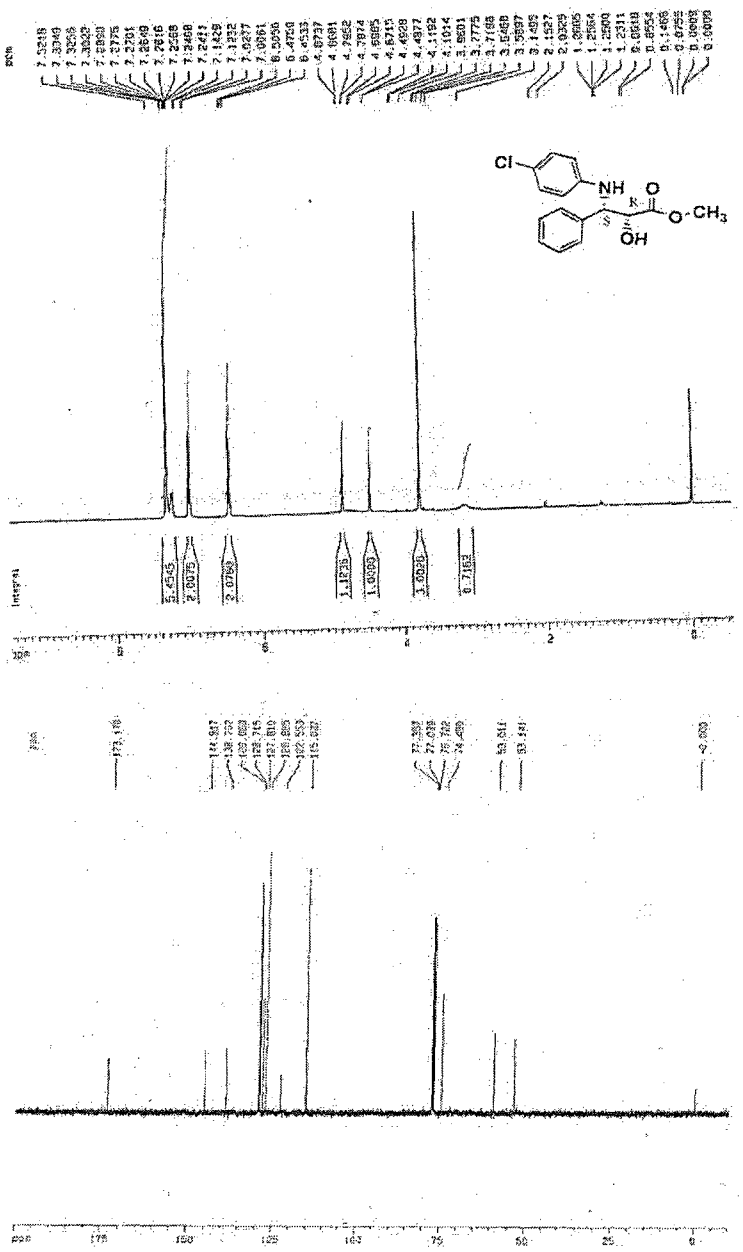
No.	(ppm)	(Hz)	Height
1	13.80	1721.8	0.1100
2	61.16	7350.8	0.1045
3	63.63	6662.3	0.1111
4	76.70	9557.1	0.9230
5	77.05	9389.1	1.0000
6	77.30	9271.0	0.9508
7	81.54	10287.8	0.1143
8	82.56	10362.0	0.1084
9	110.02	14215.3	0.2174

No.	(ppm)	(Hz)	Height
10	120.12	15483.0	0.0418
11	127.51	16038.0	0.2357
12	128.33	16761.8	0.1027
13	129.56	16168.0	0.2085
14	129.16	16248.3	0.2281
15	137.10	17241.2	0.0504
16	142.52	17923.3	0.0473
17	167.64	21082.3	0.0473

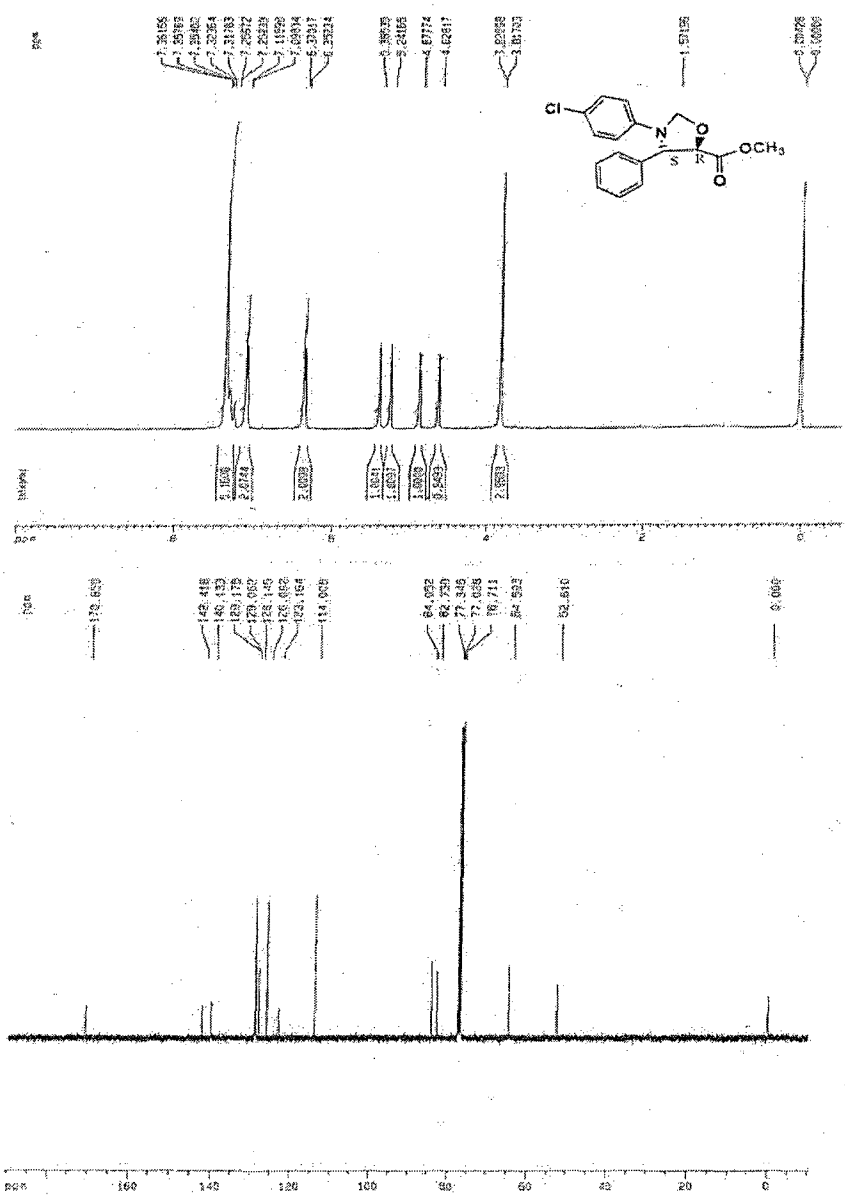
(14). (2*R*,3*S*)-(+)-Methyl 3-(4-methoxyphenylamino)-2-hydroxy-3-phenylpropanoate 4-11b



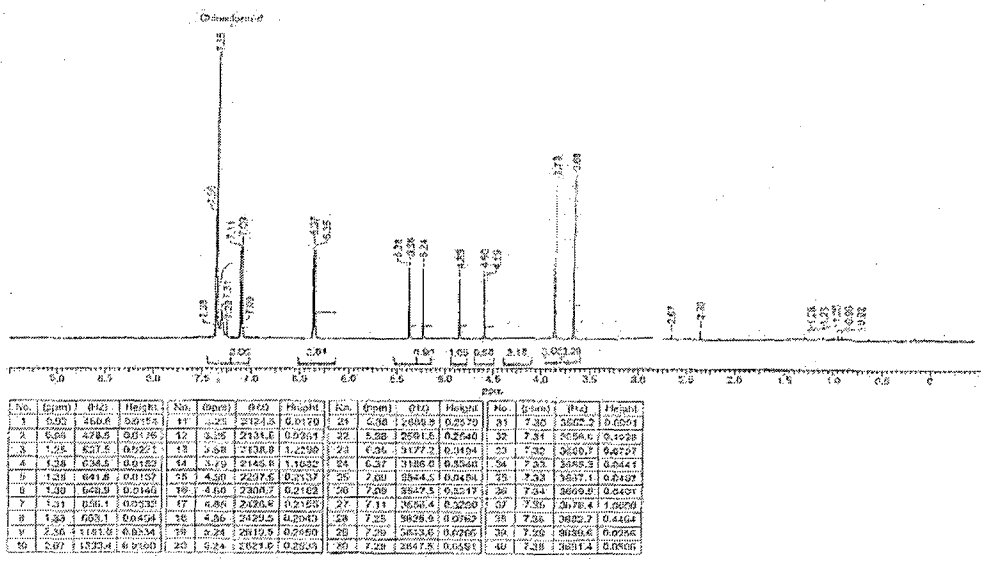
(15). (2*R*,3*S*)-(+)-Methyl 3-(4-chlorophenylamino)-2-hydroxy-3-phenylpropanoate 4-11c

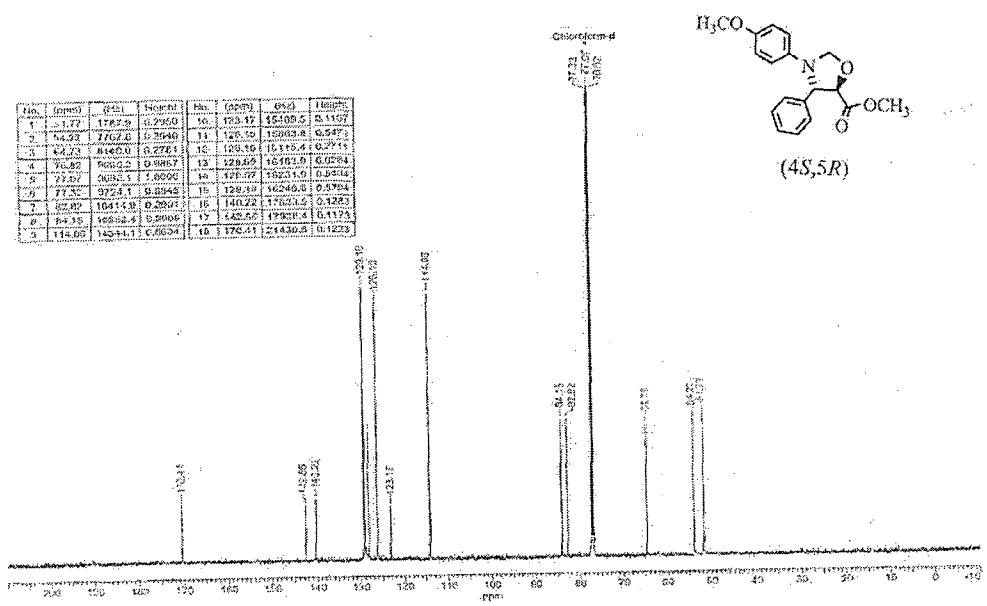


(16). (2*R*,3*S*)–(+)-Methyl 3-(4-chlorophenyl)-4-phenyl-1,3-oxazolidine-5-carboxylate 4-12c



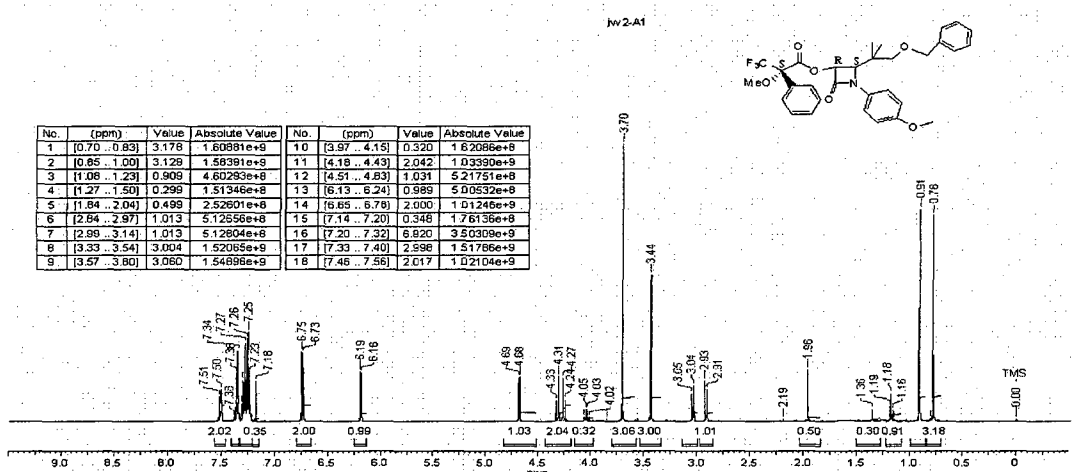
(17). (4*S*,5*R*)-(+)-Methyl 3-(4-methoxyphenyl)-4-phenyl-1,3-oxazolidine-5-carboxylate 4-12b





(4*S*,5*R*)

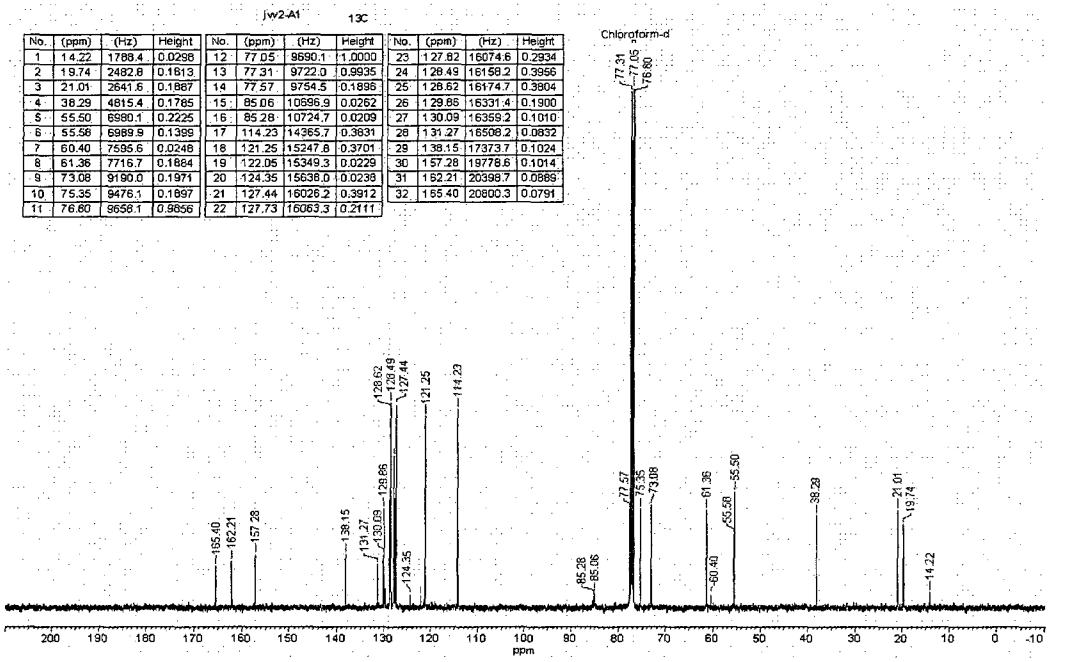
(18). 3-(*S*)-(-)-MTPA-(3*R*,4*S*)-(+)-2-10 from lipase PS resolution



No.	(ppm)	Value	Absolute Value
1	[0.70 .. 0.83]	3.178	1.60881e+9
2	[0.85 .. 1.00]	3.129	1.58391e+9
3	[1.08 .. 1.23]	0.909	4.60293e+8
4	[1.27 .. 1.50]	0.299	1.51346e+8
5	[1.84 .. 2.04]	0.499	2.52601e+8
6	[2.84 .. 2.97]	1.013	5.12556e+8
7	[2.99 .. 3.14]	1.013	5.12604e+8
8	[3.33 .. 3.54]	3.004	1.52065e+9
9	[3.57 .. 3.80]	3.060	1.54896e+9

No.	(ppm)	Value	Absolute Value
10	[3.97 .. 4.15]	0.320	1.62086e+8
11	[4.18 .. 4.43]	2.042	1.03390e+9
12	[4.51 .. 4.83]	1.031	5.21751e+8
13	[6.13 .. 6.24]	0.989	5.00532e+8
14	[6.65 .. 6.78]	2.000	1.01246e+9
15	[7.14 .. 7.20]	0.348	1.76136e+8
16	[7.20 .. 7.32]	6.920	3.50309e+9
17	[7.33 .. 7.40]	2.998	1.51786e+9
18	[7.46 .. 7.56]	2.017	1.02104e+9

No.	(ppm)	(Hz)	Height
1	0.00	0.0	0.0072
2	0.78	390.6	0.7428
3	0.80	401.0	0.0077
4	0.91	456.6	0.7640
5	1.15	574.4	0.0051
6	1.16	581.7	0.0408
7	1.18	589.0	0.0983
8	1.19	596.4	0.0539
9	1.36	678.1	0.0416
10	1.95	980.9	0.1836
11	2.19	1097.5	0.0056
12	2.91	1455.8	0.1122
13	2.93	1464.9	0.1424
14	3.04	1518.0	0.1269
15	3.05	1527.2	0.0980
16	3.44	1720.1	0.5247
17	3.70	1852.5	1.0000
18	3.85	1923.3	0.0055
19	4.02	2010.5	0.0126
20	4.03	2017.9	0.0417
21	4.05	2025.3	0.0403
22	4.06	2032.6	0.0114
23	4.24	2122.3	0.0750
24	4.27	2133.9	0.1557
25	4.31	2153.5	0.1602
26	4.35	2165.0	0.0735
27	4.68	2339.0	0.1561
28	4.69	2344.5	0.1559
29	6.18	3092.2	0.1721
30	6.19	3097.7	0.1784
31	6.73	3365.1	0.2439
32	6.75	3374.2	0.2466
33	7.18	3588.5	0.1423
34	7.22	3610.5	0.0276
35	7.23	3618.4	0.1691
36	7.25	3625.7	0.3173
37	7.26	3628.8	0.2946
38	7.27	3637.9	0.2798
39	7.29	3647.1	0.1466
40	7.31	3654.4	0.0612
41	7.34	3672.7	0.2536
42	7.35	3675.2	0.2532
43	7.36	3680.0	0.1689
44	7.38	3689.2	0.0135
45	7.50	3750.8	0.1129
46	7.51	3754.5	0.1054
47	7.51	3758.2	0.0866

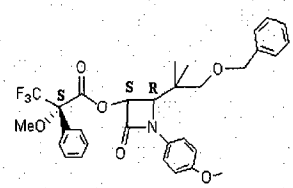


No.	(ppm)	(Hz)	Height
1	14.22	1788.4	0.0298
2	19.74	2482.8	0.1613
3	21.01	2641.6	0.1887
4	38.29	4815.4	0.1785
5	55.50	6980.1	0.2225
6	55.58	6989.9	0.1399
7	60.40	7595.6	0.0248
8	61.36	7716.7	0.1884
9	73.08	9190.0	0.1971
10	75.35	9476.1	0.1897
11	76.80	9656.1	0.9856
12	77.05	9690.1	1.0000
13	77.31	9722.0	0.9935
14	77.57	9754.5	0.1896
15	85.06	10696.9	0.0262
16	85.28	10724.7	0.0209
17	114.23	14365.7	0.3831
18	121.25	15247.8	0.3701
19	122.05	15349.3	0.0229
20	124.35	15638.0	0.0238
21	127.44	16026.2	0.3912
22	127.73	16063.3	0.2111
23	127.82	16074.6	0.2934
24	128.49	16158.2	0.3956
25	128.62	16174.7	0.3804
26	129.86	16331.4	0.1900
27	130.09	16359.2	0.1010
28	131.27	16506.2	0.0832
29	138.15	17373.7	0.1024
30	157.28	19778.6	0.1014
31	162.21	20398.7	0.0869
32	165.40	20800.3	0.0791

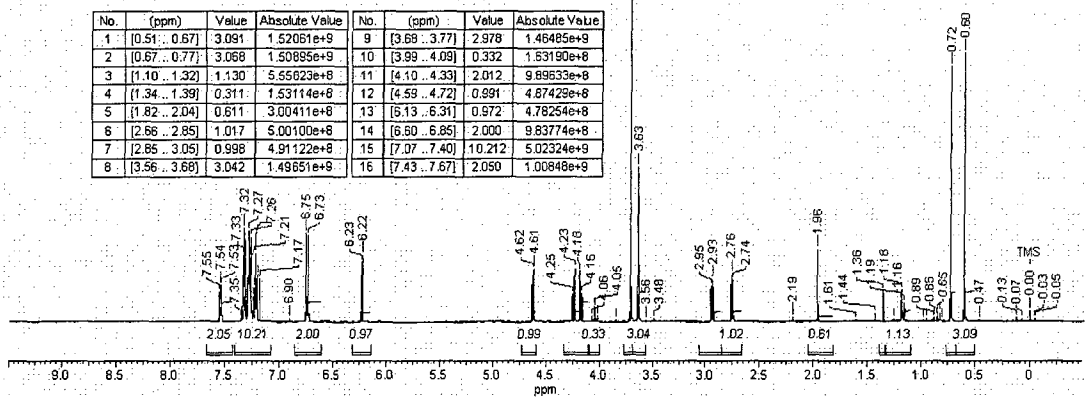
(19). 3-(*S*)-(-)-MTPA-(3*S*,4*R*)-(+)-2-10 from lipase PS resolution

jw2-A2

No.	(ppm)	(Hz)	Height	No.	(ppm)	(Hz)	Height	No.	(ppm)	(Hz)	Height	No.	(ppm)	(Hz)	Height
1	-0.05	-22.6	0.0074	18	1.16	581.7	0.0526	35	3.70	1851.3	1.0000	52	7.17	3587.9	0.1202
2	-0.03	-17.1	0.0069	19	1.18	586.4	0.0884	36	3.84	1922.1	0.0052	53	7.20	3600.1	0.1282
3	0.00	0.0	0.0281	20	1.18	591.5	0.0443	37	4.02	2010.6	0.0152	54	7.21	3606.8	0.1884
4	0.07	37.2	0.0073	21	1.19	595.7	0.0595	38	4.03	2017.9	0.0380	55	7.22	3612.3	0.0930
5	0.13	64.7	0.0101	22	1.26	628.7	0.0072	39	4.05	2024.7	0.0437	56	7.24	3619.6	0.0887
6	0.47	233.8	0.0052	23	1.36	678.1	0.0854	40	4.06	2032.0	0.0149	57	7.26	3628.8	0.2346
7	0.60	297.9	0.7514	24	1.44	717.8	0.0211	41	4.16	2078.4	0.0782	58	7.27	3633.6	0.1780
8	0.72	362.0	0.7134	25	1.61	803.3	0.0053	42	4.18	2090.6	0.1408	59	7.27	3637.9	0.2543
9	0.81	402.9	0.0055	26	1.96	980.3	0.2001	43	4.23	2114.4	0.1473	60	7.28	3641.0	0.1948
10	0.83	414.5	0.0234	27	2.19	1097.5	0.0121	44	4.25	2126.0	0.0762	61	7.29	3648.3	0.0583
11	0.85	426.1	0.0257	28	2.74	1371.5	0.1148	45	4.61	2306.7	0.1455	62	7.32	3658.7	0.2604
12	0.86	429.1	0.0132	29	2.76	1380.7	0.1337	46	4.62	2312.8	0.1425	63	7.32	3661.1	0.2476
13	0.88	441.9	0.0109	30	2.93	1464.9	0.1180	47	6.22	3109.3	0.1838	64	7.33	3665.4	0.1841
14	0.89	447.4	0.0076	31	2.95	1474.1	0.0989	48	6.23	3114.8	0.1659	65	7.35	3673.9	0.0094
15	0.96	476.5	0.0065	32	3.48	1742.0	0.0075	49	6.73	3365.1	0.2398	66	7.53	3766.7	0.1024
16	0.98	490.8	0.0055	33	3.56	1778.1	0.0052	50	6.75	3374.2	0.2452	67	7.54	3771.0	0.1027
17	1.15	573.8	0.0052	34	3.63	1815.3	0.4315	51	6.90	3452.4	0.0075	68	7.55	3774.6	0.0828



No.	(ppm)	Value	Absolute Value	No.	(ppm)	Value	Absolute Value
1	[0.51 .. 0.67]	3.091	1.52061e+9	9	[3.68 .. 3.77]	2.978	1.46485e+9
2	[0.67 .. 0.77]	3.068	1.50895e+9	10	[3.99 .. 4.09]	0.332	1.63190e+8
3	[1.10 .. 1.32]	1.130	5.55623e+8	11	[4.10 .. 4.33]	2.012	9.89633e+8
4	[1.34 .. 1.39]	0.311	1.53114e+8	12	[4.59 .. 4.72]	0.991	4.87429e+8
5	[1.82 .. 2.04]	0.611	3.00411e+8	13	[5.13 .. 6.31]	0.972	4.78254e+8
6	[2.66 .. 2.85]	1.017	5.00100e+8	14	[6.60 .. 6.85]	2.000	9.83774e+8
7	[2.85 .. 3.05]	0.998	4.91122e+8	15	[7.07 .. 7.40]	10.212	5.02324e+9
8	[3.56 .. 3.68]	3.042	1.49651e+9	16	[7.43 .. 7.67]	2.050	1.00848e+9



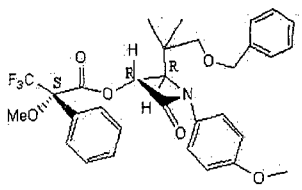
(20). 3-(*S*)-(-)-MTPA-(3*R*,4*R*)-(+)-2-10 from baker's yeast reduction of (4*R*)-oxo-β-Lactam

No.	(ppm)	(Hz)	Height
1	0.90	448.0	0.6673
2	0.95	475.5	0.6244
3	1.02	509.7	0.0062
4	1.16	579.9	0.0455
5	1.17	587.2	0.0968
6	1.18	590.9	0.0252
7	1.19	594.5	0.0479
8	1.35	676.9	0.0110
9	1.96	979.1	0.1683
10	3.04	1519.3	0.0827
11	3.06	1528.4	0.1462

No.	(ppm)	(Hz)	Height
12	3.10	1548.6	0.1536
13	3.11	1557.7	0.0864
14	3.46	1729.2	0.4745
15	3.55	1775.0	0.0051
16	3.70	1848.3	1.0000
17	3.72	1859.9	0.0067
18	3.84	1919.1	0.0051
19	4.02	2009.4	0.0126
20	4.03	2016.1	0.0379
21	4.05	2023.4	0.0408
22	4.06	2030.8	0.0128

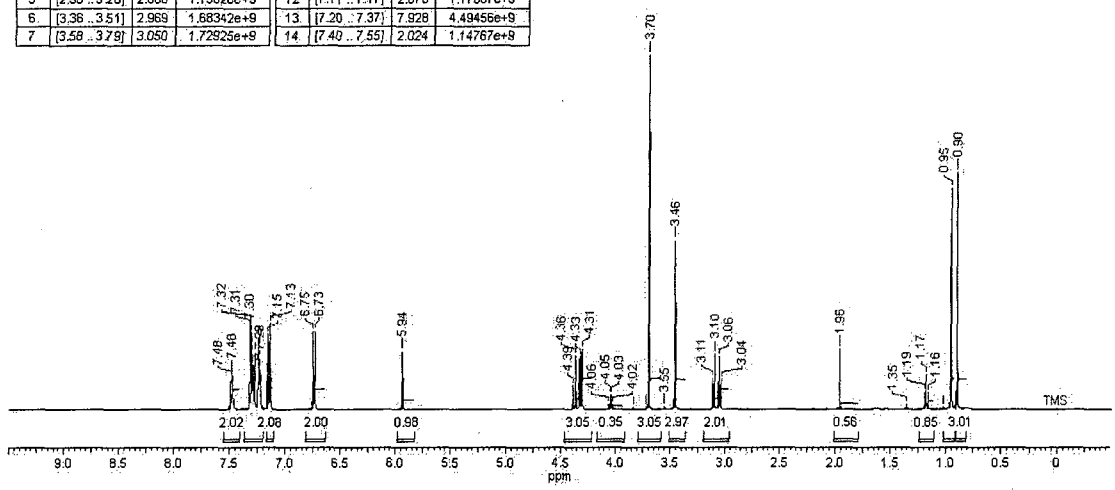
No.	(ppm)	(Hz)	Height
23	4.31	2154.7	0.1682
24	4.33	2164.4	0.1467
25	4.36	2182.8	0.1495
26	4.39	2195.0	0.0647
27	5.94	2968.9	0.1621
28	6.73	3367.5	0.2188
29	6.75	3376.7	0.2175
30	7.13	3567.7	0.2417
31	7.15	3576.9	0.2102
32	7.17	3586.0	0.0971
33	7.23	3614.1	0.1116

No.	(ppm)	(Hz)	Height
34	7.24	3621.4	0.1996
35	7.28	3638.5	0.1586
36	7.29	3645.2	0.1250
37	7.30	3652.0	0.2140
38	7.31	3655.6	0.2300
39	7.32	3658.7	0.2440
40	7.47	3735.6	0.0934
41	7.48	3739.2	0.0994
42	7.48	3742.9	0.0821



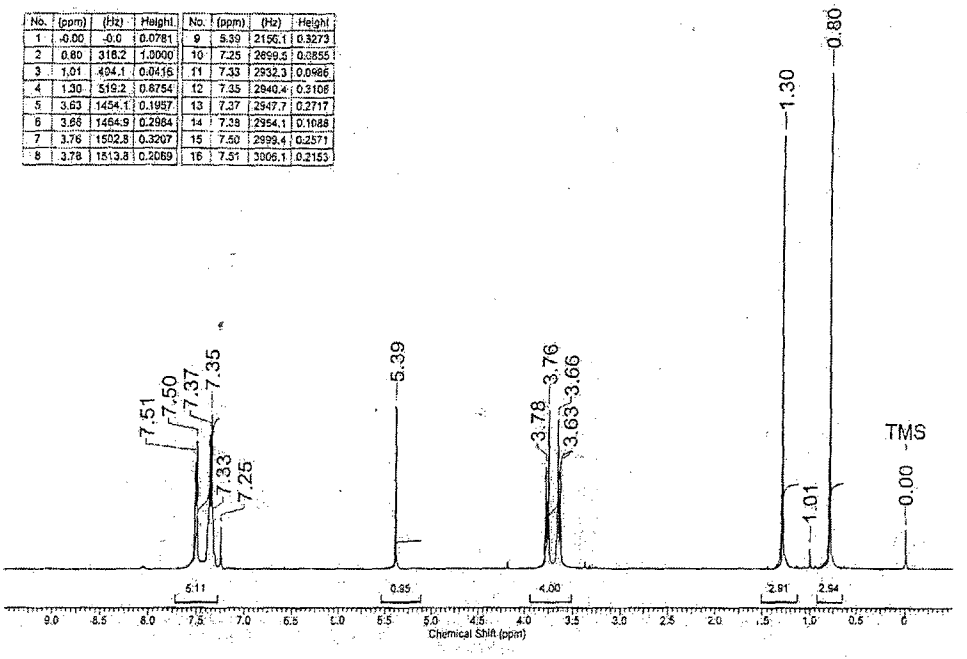
No.	(ppm)	Value	Absolute Value
1	[0.81 .. 0.91]	3.005	1.70369e+9
2	[0.92 .. 1.02]	3.069	1.73976e+9
3	[1.11 .. 1.24]	0.848	4.80836e+8
4	[1.80 .. 2.01]	0.560	3.17203e+8
5	[2.96 .. 3.20]	2.008	1.13828e+9
6	[3.36 .. 3.51]	2.969	1.68342e+9
7	[3.58 .. 3.79]	3.050	1.72925e+9

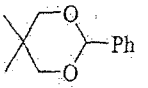
No.	(ppm)	Value	Absolute Value
8	[3.92 .. 4.16]	0.345	1.95799e+8
9	[4.22 .. 4.47]	3.046	1.72657e+9
10	[5.82 .. 5.98]	0.984	5.57994e+8
11	[6.64 .. 6.81]	1.999	1.13351e+9
12	[7.11 .. 7.17]	2.076	1.17667e+9
13	[7.20 .. 7.37]	7.928	4.49456e+9
14	[7.40 .. 7.55]	2.024	1.14767e+9



(21). 5,5-Dimethyl-2-phenyl-1,3-dioxane 2-3

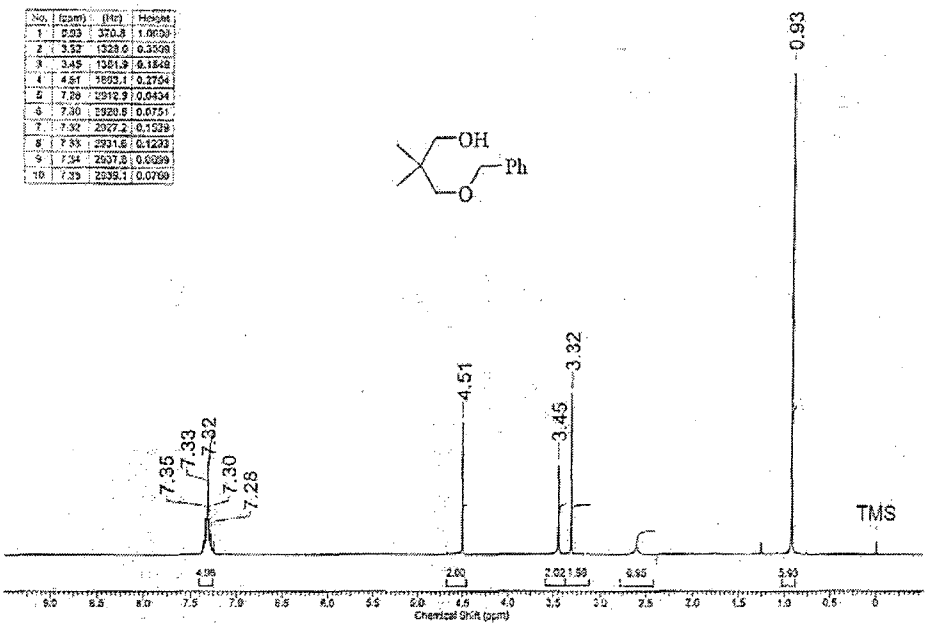
No.	(ppm)	(Hz)	Height	No.	(ppm)	(Hz)	Height
1	-0.00	-0.0	0.0781	9	5.39	2156.1	0.3273
2	0.80	316.2	1.0000	10	7.25	2899.5	0.0855
3	1.01	404.1	0.0416	11	7.33	2932.3	0.0986
4	1.30	519.2	0.6754	12	7.35	2940.4	0.3108
5	3.63	1454.1	0.1957	13	7.37	2947.7	0.2717
6	3.66	1464.9	0.2984	14	7.39	2954.1	0.1688
7	3.76	1502.8	0.3207	15	7.50	2999.4	0.2571
8	3.78	1513.8	0.2069	16	7.51	3006.1	0.2153

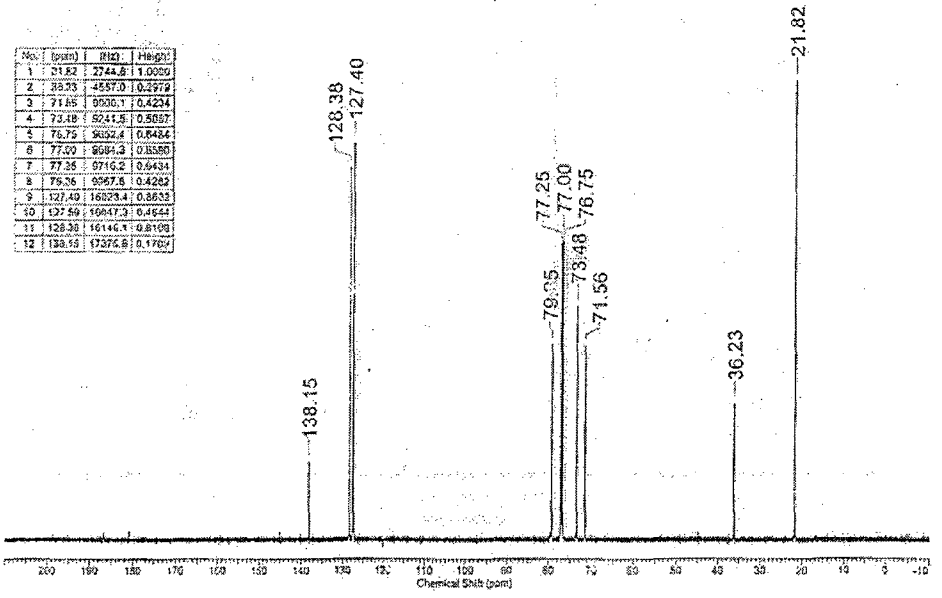




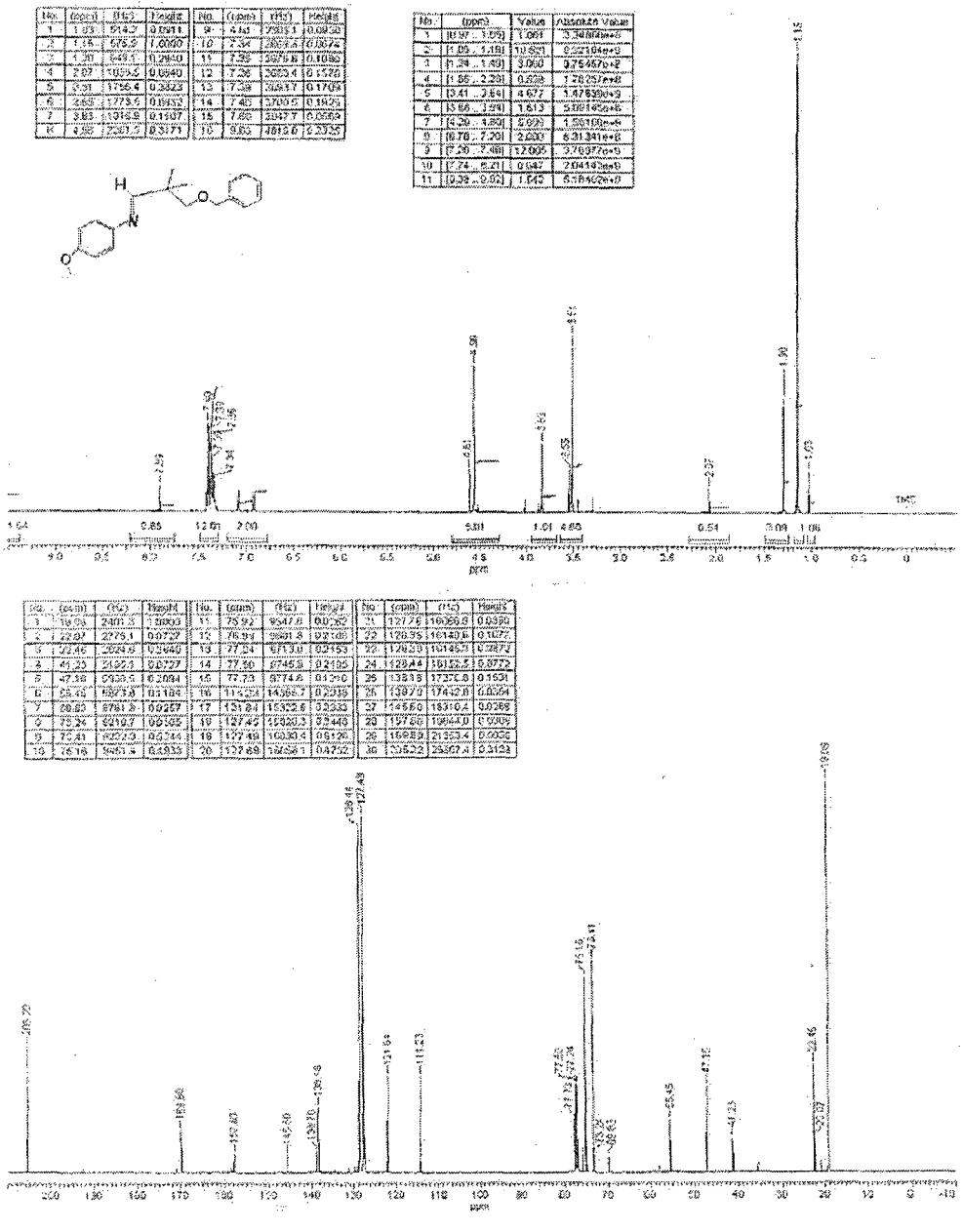
No.	(ppm)	(Hz)	Height
1	21.91	2756.6	0.2206
2	23.08	2902.3	0.2304
3	30.24	3803.8	0.110[illegible]
4	76.77	9655.2	0.9847
5	77.02	9687.1	1.0000
6	77.28	9719.0	0.9914
7	77.71	9773.9	0.4690
8	101.82	12805.8	0.2057
9	125.16	15866.6	0.3904
10	128.29	16134.9	0.3860
11	128.86	16205.7	0.2141
12	138.57	17427.5	0.0777

(22). 3-(Benzyloxy)-2,2-dimethylpropan-1-ol 2-4

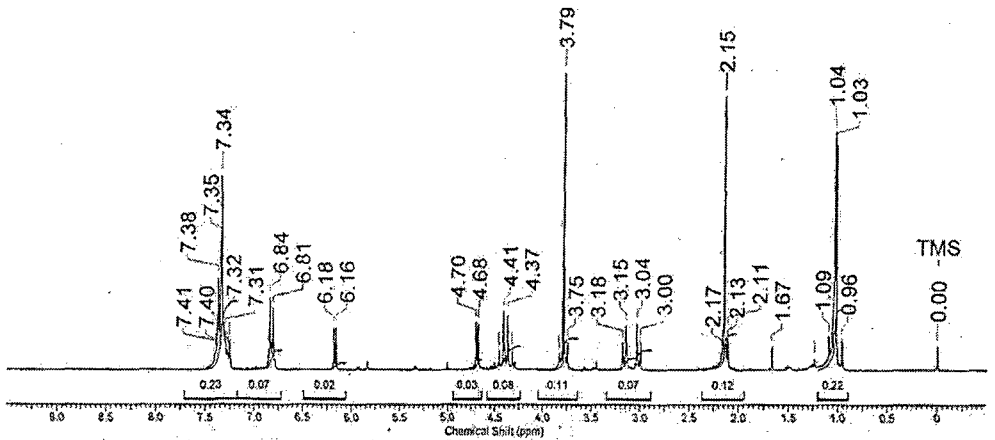




(23). (*E*)-N-(3-(benzyloxy)-2,2-dimethylpropylidene)-4-methoxybenzenamine 2-6



(24). *cis*-(±)-3-Acetoxy-4-(2-benzyloxy-1,1-dimethylethyl-1-(4-methoxyphenyl)azetidin-2-one 2-9

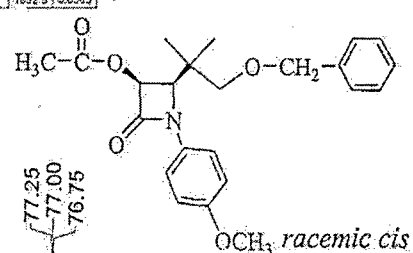


No.	(ppm)	(Hz)	Height
1	0.00	0.0	0.0800
2	0.96	240.2	0.1022
3	1.03	256.5	0.7598
4	1.04	260.5	0.8049
5	1.09	272.7	0.1133
6	1.24	309.5	0.0377
7	1.67	418.1	0.0775
8	2.11	528.3	0.1391
9	2.13	533.3	0.0991
10	2.15	537.3	0.9213
11	2.17	543.7	0.3402

No.	(ppm)	(Hz)	Height
12	3.00	750.6	0.0693
13	3.04	759.6	0.1552
14	3.15	787.4	0.1487
15	3.18	796.5	0.0917
16	3.75	937.5	0.0939
17	3.79	947.8	1.0000
18	3.83	958.0	0.0561
19	4.32	1081.4	0.0630
20	4.37	1093.9	0.1662
21	4.41	1103.9	0.1953
22	4.46	1115.8	0.0558

No.	(ppm)	(Hz)	Height
23	4.68	1171.0	0.1532
24	4.70	1176.5	0.1569
25	6.15	1541.5	0.1426
26	6.18	1547.0	0.1386
27	6.81	1702.6	0.2663
28	6.82	1704.8	0.0950
29	6.84	1711.6	0.2387
30	6.86	1714.8	0.0701
31	7.25	1813.4	0.0450
32	7.26	1816.0	0.0941
33	7.27	1817.8	0.0463

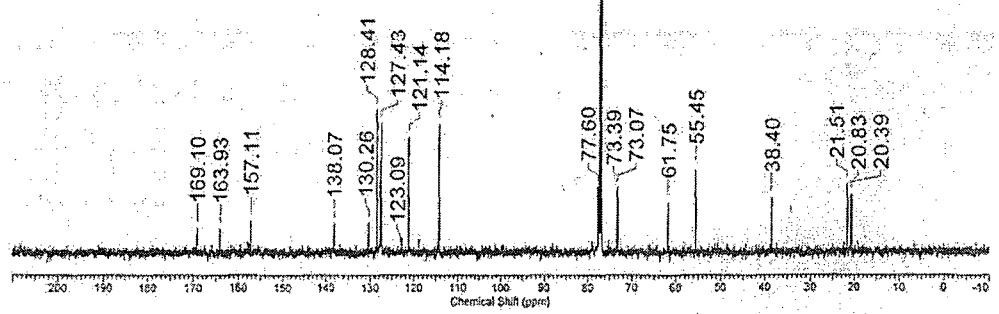
No.	(ppm)	(Hz)	Height
34	7.28	1820.3	0.0457
35	7.29	1822.4	0.0648
36	7.31	1827.8	0.1265
37	7.32	1832.0	0.1607
38	7.34	1835.6	0.6515
39	7.35	1838.9	0.4320
40	7.37	1843.5	0.2183
41	7.38	1845.6	0.3074
42	7.40	1850.9	0.0568
43	7.41	1852.5	0.0565



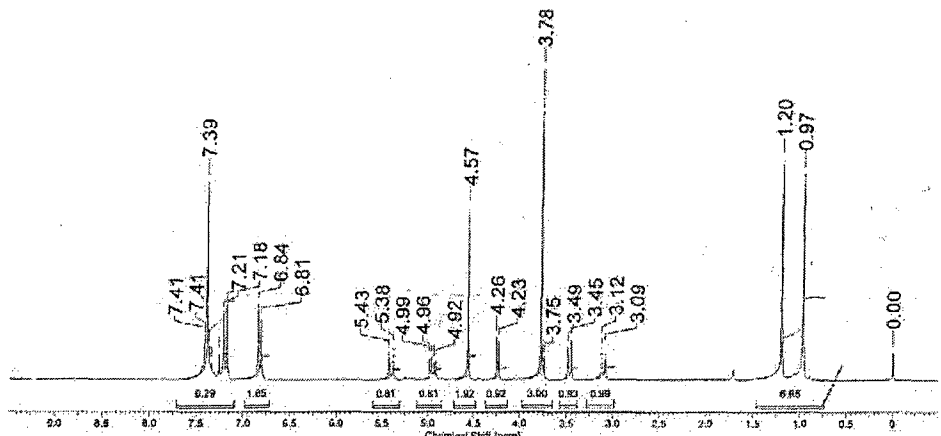
racemic cis

No.	(ppm)	(Hz)	Height
1	20.39	2564.3	0.1137
2	20.83	2619.2	0.1122
3	21.51	2705.0	0.1342
4	38.40	4829.3	0.1076
5	55.45	6974.6	0.1536
6	61.75	7765.4	0.0984
7	73.07	9189.6	0.1282
8	73.39	9230.5	0.1225
9	76.75	9652.4	0.9637
10	77.00	9684.3	1.0000
11	77.25	9716.2	0.9902
12	77.60	9760.1	0.1296

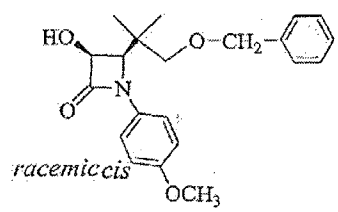
No.	(ppm)	(Hz)	Height
13	114.18	14359.8	0.2533
14	121.14	15235.5	0.2285
15	123.09	15480.6	0.0076
16	127.43	16026.4	0.2559
17	127.66	16055.3	0.1447
18	128.41	16150.1	0.2624
19	130.26	16382.4	0.0595
20	138.07	17364.8	0.0565
21	157.11	19759.4	0.0622
22	163.93	20616.1	0.0487
23	169.10	21268.4	0.0484



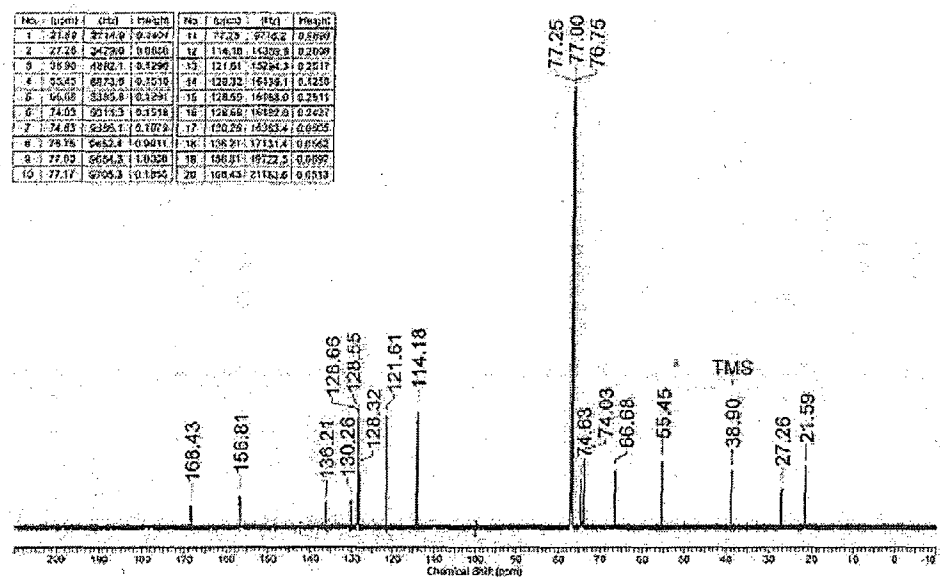
(25). (3*R*,4*S*)-(+)-3-Hydroxy-4-(2-benzyloxy-1,1-dimethylethyl)-1-(4-methoxyphenyl)azetidin-2-one 2-10



No.	(ppm)	(Hz)	Height	No.	(ppm)	(Hz)	Height	No.	(ppm)	(Hz)	Height
1	0.00	0.0	0.0888	13	4.92	1230.2	0.0580	25	7.20	1801.8	0.0858
2	0.97	243.8	0.6836	14	4.94	1235.7	0.0663	26	7.21	1803.9	0.2106
3	1.20	300.0	0.7060	15	4.96	1241.6	0.0722	27	7.26	1816.3	0.6629
4	3.09	771.8	0.1087	16	4.99	1247.0	0.0893	28	7.34	1838.8	0.0513
5	3.12	781.1	0.1335	17	5.38	1346.6	0.1098	29	7.36	1840.7	0.0520
6	3.45	863.7	0.1276	18	5.43	1357.9	0.0899	30	7.37	1843.5	0.1046
7	3.49	873.0	0.1038	19	6.81	1703.1	0.1933	31	7.38	1845.1	0.1509
8	3.75	937.8	0.0888	20	6.82	1705.2	0.0843	32	7.39	1848.5	0.6372
9	3.78	944.7	1.0000	21	6.84	1710.0	0.0892	33	7.41	1852.6	0.1458
10	4.23	1059.0	0.1253	22	6.84	1712.1	0.2411	34	7.41	1853.8	0.1319
11	4.26	1064.5	0.1357	23	7.18	1794.9	0.2416				
12	4.57	1142.8	0.5602	24	7.18	1797.1	0.0857				



No.	(ppm)	(Hz)	Height	No.	(ppm)	(Hz)	Height
1	27.59	2214.9	0.2407	11	77.25	9716.2	0.5899
2	27.26	2472.9	0.0808	12	114.18	14359.8	0.2009
3	38.90	4882.1	0.4296	13	121.61	15294.3	0.2217
4	55.45	6973.9	0.1518	14	128.32	16136.1	0.1259
5	66.68	8385.8	0.1291	15	128.55	16163.0	0.2511
6	74.03	9311.3	0.1518	16	128.66	16182.8	0.2437
7	74.63	9386.1	0.1079	17	130.26	16383.4	0.0905
8	76.75	9652.4	0.0911	18	136.21	17131.4	0.0562
9	77.00	9684.3	1.0000	19	156.81	19722.5	0.0899
10	77.17	9705.3	0.1852	20	168.43	21183.6	0.0519

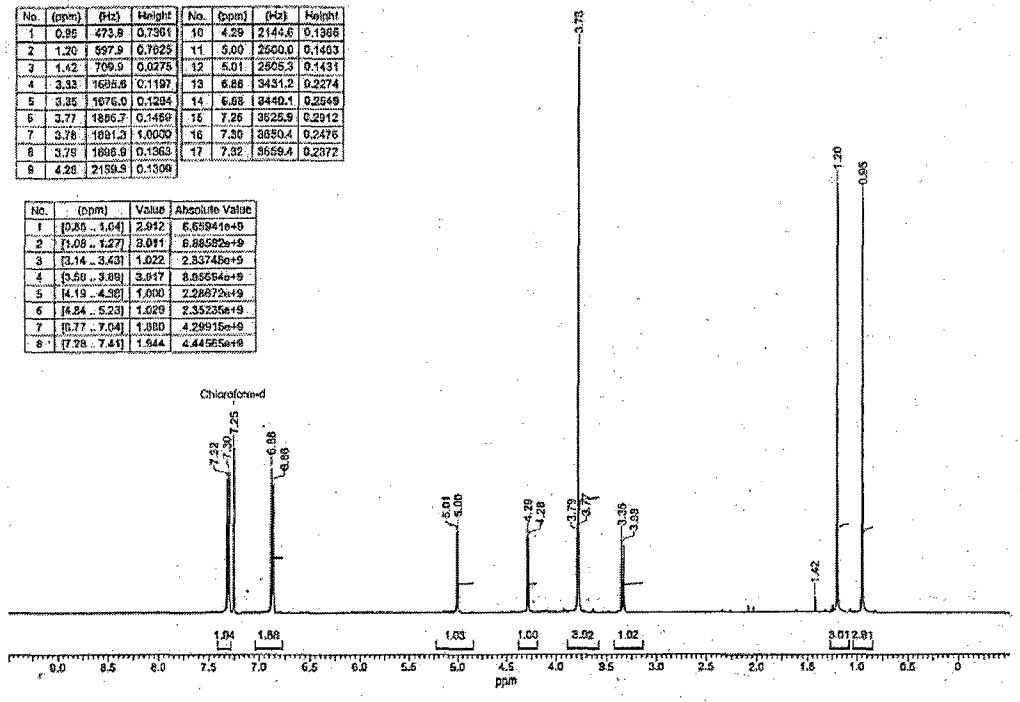


(26). (3*R*,4*S*)-(+)-3-Hydroxy-4-(2-hydroxy-1,1-dimethylethyl)-1-(4-methoxyphenyl)azetidin-2-one 2-11

No.	(ppm)	(Hz)	Height
1	0.95	473.9	0.7361
2	1.20	597.9	0.7825
3	1.42	709.9	0.0275
4	3.33	1686.6	0.1197
5	3.35	1676.0	0.1294
6	3.77	1886.7	0.1459
7	3.78	1891.3	1.0000
8	3.79	1898.9	0.1363
9	4.28	2139.9	0.1309

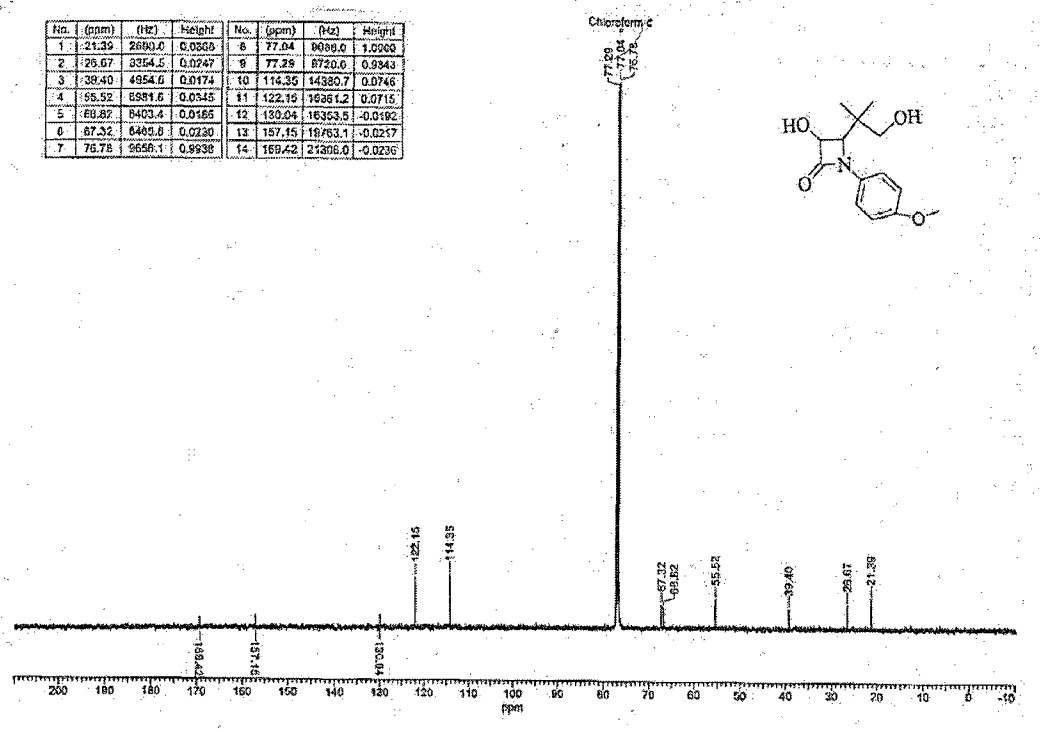
No.	(ppm)	(Hz)	Height
10	4.29	2144.6	0.1366
11	5.00	2500.0	0.1403
12	5.01	2505.3	0.1431
13	6.88	3431.2	0.2274
14	6.88	3440.1	0.2549
15	7.25	3625.9	0.2912
16	7.30	3650.4	0.2476
17	7.32	3659.4	0.2872

No.	(ppm)	Value	Absolute Value
1	[0.85 .. 1.04]	2.942	6.659941e+9
2	[1.08 .. 1.27]	3.911	8.88582e+9
3	[3.14 .. 3.43]	1.022	2.33748e+9
4	[3.50 .. 3.89]	3.917	8.85694e+9
5	[4.19 .. 4.36]	1.000	2.28672e+9
6	[4.84 .. 5.23]	1.029	2.35235e+9
7	[6.77 .. 7.04]	1.880	4.29915e+9
8	[7.28 .. 7.41]	1.544	4.44565e+8

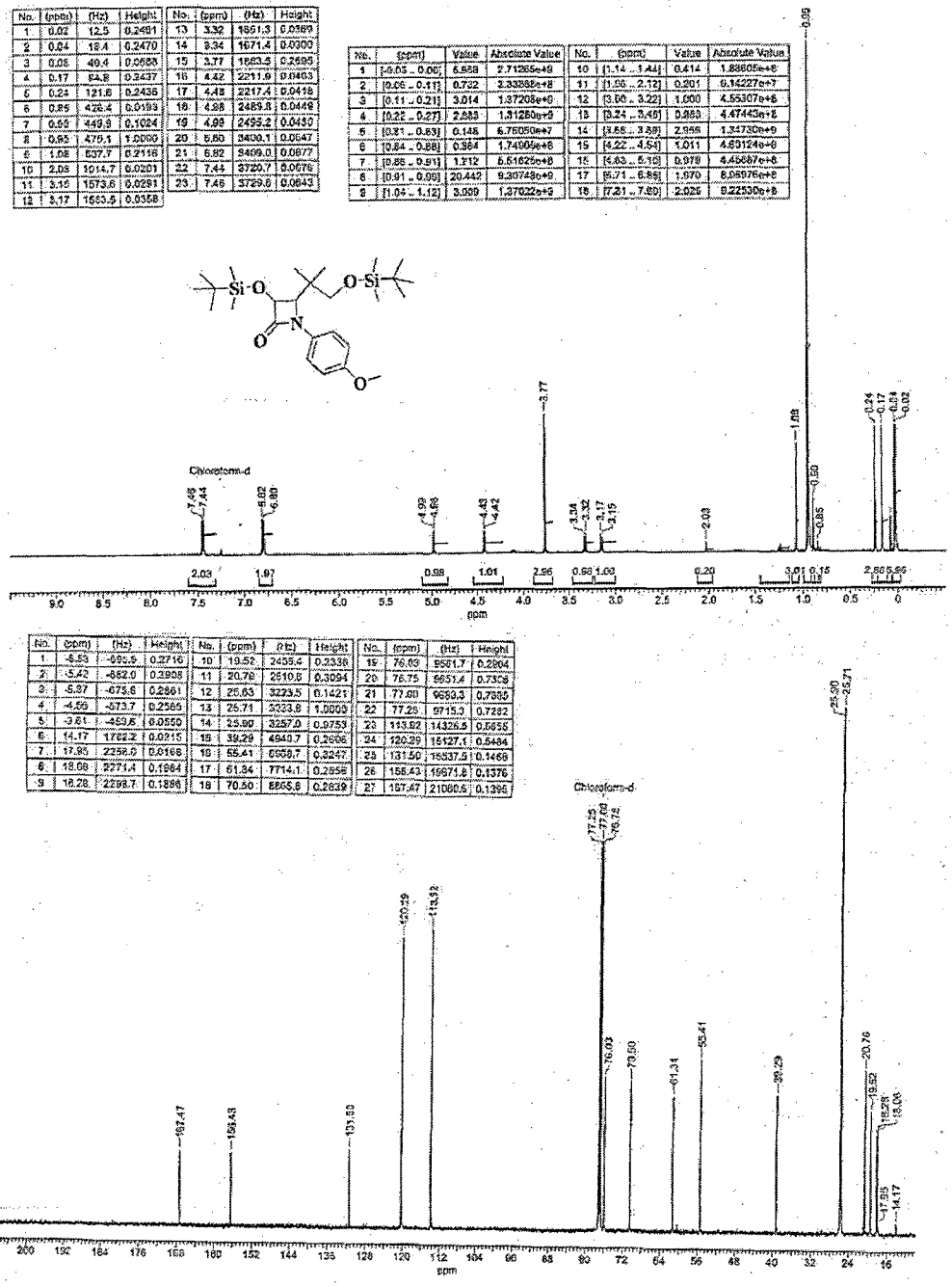


No.	(ppm)	(Hz)	Height
1	21.39	2690.0	0.0358
2	26.07	3354.5	0.0247
3	39.40	4954.6	0.0174
4	55.52	6981.6	0.0345
5	65.82	8403.4	0.0185
6	67.32	8466.8	0.0230
7	76.78	9656.1	0.9938

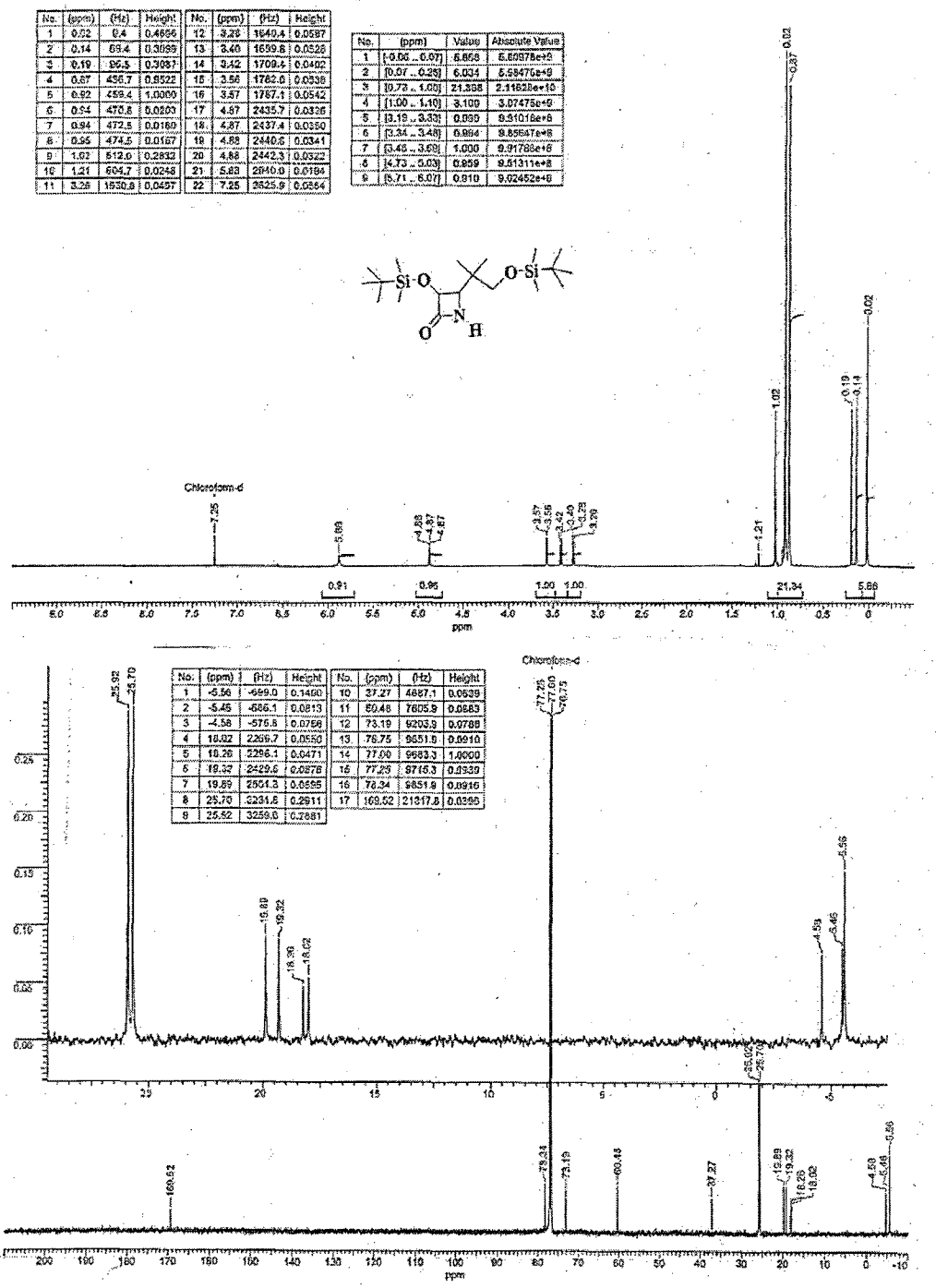
No.	(ppm)	(Hz)	Height
8	77.04	9686.0	1.0000
9	77.29	9720.0	0.9843
10	114.35	14380.7	0.0746
11	122.15	15361.2	0.0715
12	130.04	16353.5	-0.0192
13	157.15	19763.1	-0.0217
14	159.42	21306.0	-0.0236



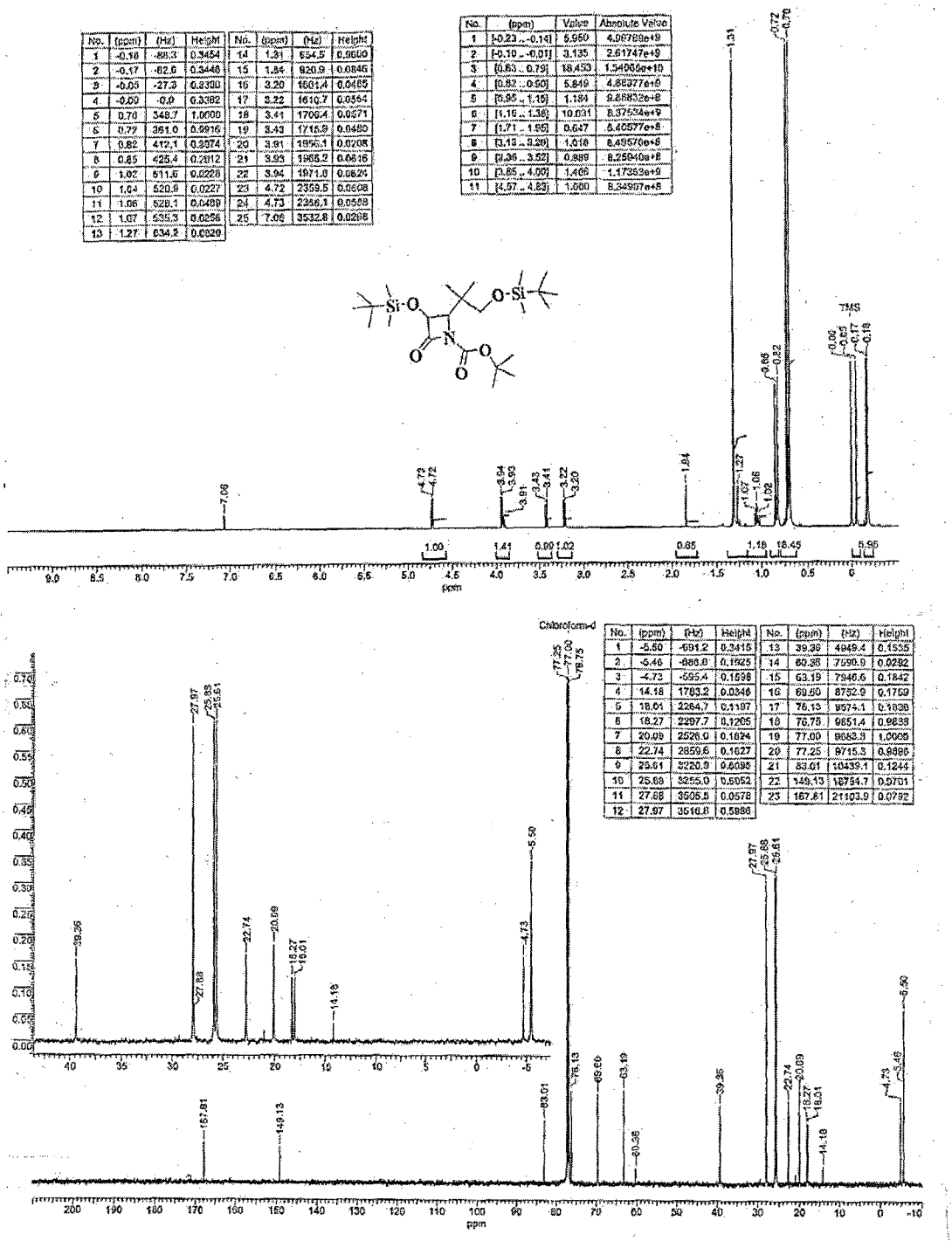
(27). (3*R*,4*S*)-(+)-3-(*t*-Butyldimethylsilanyloxy)-4-(2-(*t*-butyldimethylsilanyloxy)-1,1-dimethylethyl)-1-(4-methoxyphenyl)azetidin-2-one 2-12



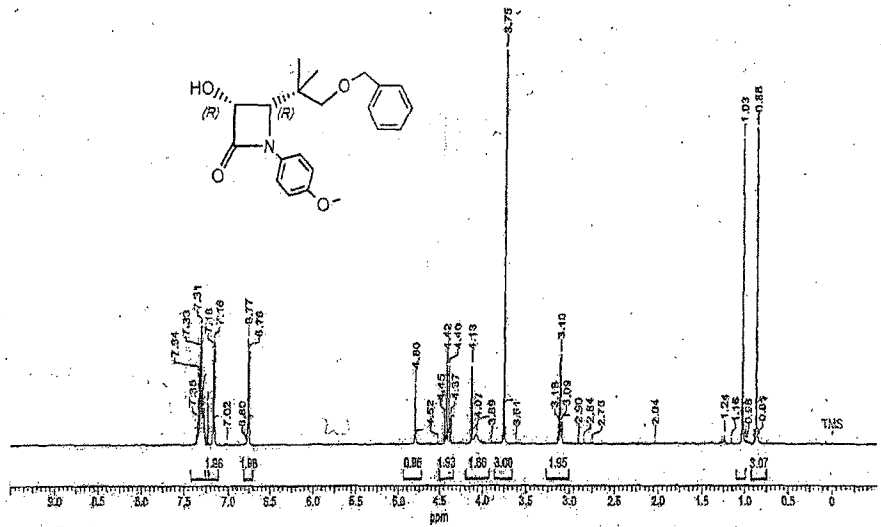
(28). (3*R*,4*S*)-(+)-3-(*t*-Butyldimethylsilanyloxy)-4-(2-(*t*-butyldimethylsilanyloxy)-1,1-dimethylethyl)azetidin-2-one 2-13



(29). (3*R*,4*S*)-(+)-3-(*t*-Butyldimethylsilanyloxy)-4-(2-(*t*-butyldimethylsilanyloxy)-1,1-dimethylethyl)-1-(*t*-butoxycarbonyl)azetidin-2-one 2-14



(30). (3*R*,4*R*)-(+)-3-Hydroxy-4-(2-benzyloxy-1,1-dimethylethyl)-1-(4-methoxyphenyl) azetidin-2-one 2-10

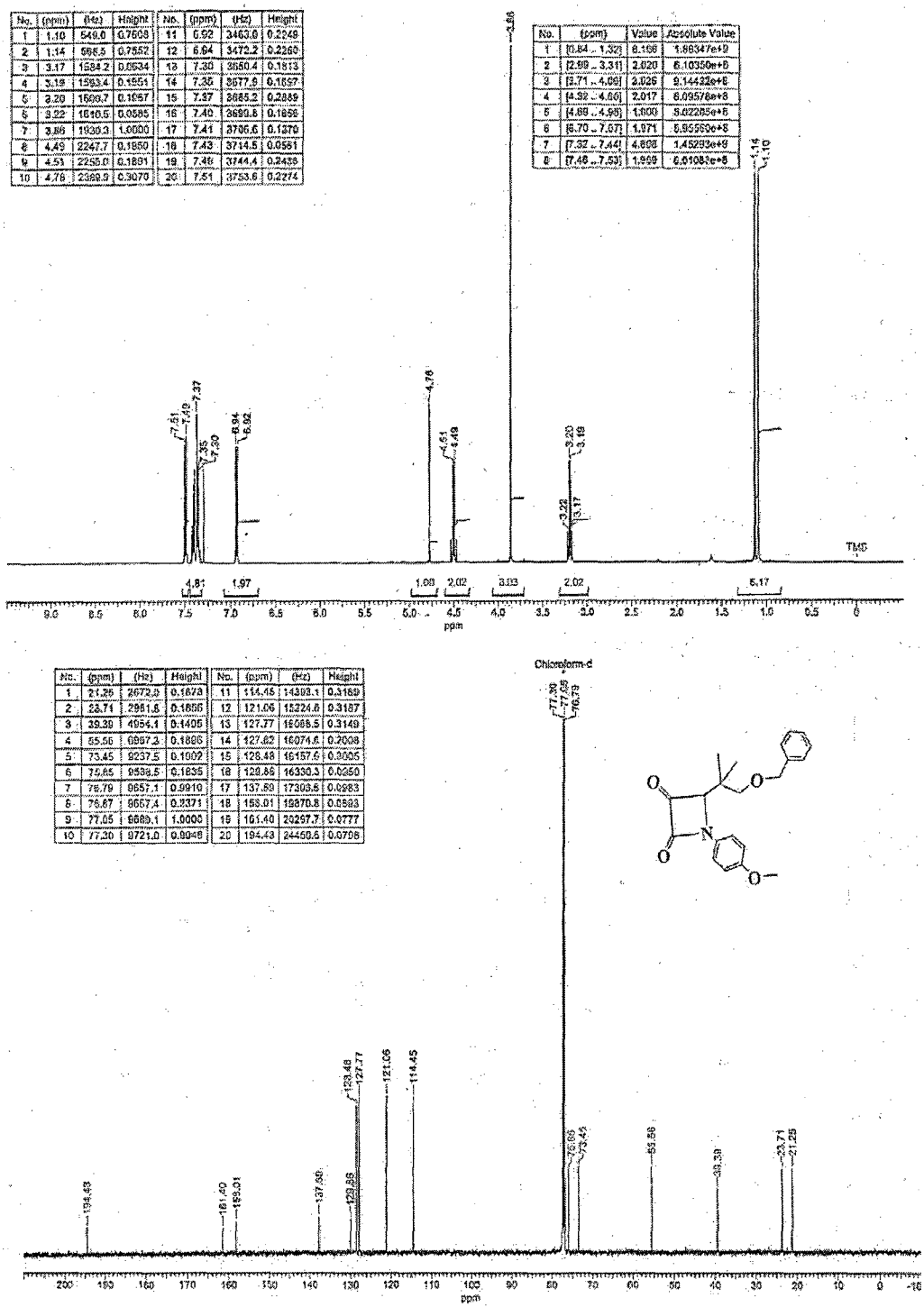


No.	(ppm)	(Hz)	Height
1	0.85	424.2	0.0162
2	0.88	437.6	0.8054
3	0.89	452.3	0.0083
4	0.93	468.3	0.0089
5	1.00	499.9	0.0088
6	1.03	517.0	0.7775
7	1.15	578.3	0.0129
8	1.24	618.5	0.0217
9	2.04	1018.7	0.0093
10	2.75	1376.4	0.0052
11	2.84	1419.8	0.0124
12	2.90	1452.1	0.0126
13	3.09	1543.1	0.0563
14	3.10	1552.2	0.2260
15	3.11	1556.3	0.2173
16	3.13	1565.8	0.0516
17	3.61	1804.2	0.0052
18	3.75	1876.9	1.0000
19	3.89	1947.8	0.0051
20	4.07	2035.0	0.0253
21	4.13	2063.7	0.1966
22	4.37	2187.3	0.0723
23	4.40	2199.2	0.1980
24	4.42	2211.4	0.1988
25	4.45	2223.6	0.0711
26	4.52	2262.7	0.0095
27	4.80	2403.1	0.1516
28	6.75	3377.8	0.2353
29	6.77	3386.4	0.2064
30	6.89	3398.6	0.0058
31	7.02	3509.1	0.0054
32	7.16	3580.5	0.2641
33	7.18	3589.1	0.2426
34	7.24	3619.5	0.1063
35	7.27	3633.6	0.0320
36	7.28	3640.4	0.0859
37	7.30	3650.1	0.1353
38	7.31	3657.5	0.3040
39	7.33	3663.8	0.2375
40	7.34	3670.9	0.1333
41	7.35	3676.2	0.0604

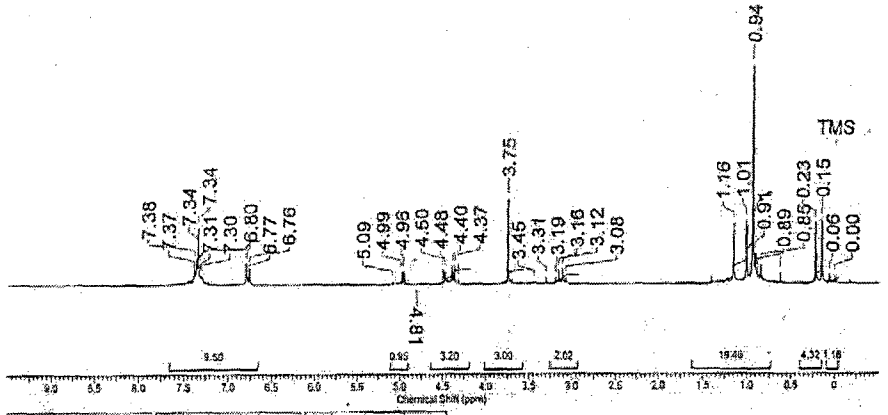
No.	(ppm)	Value	Absolute Value
1	[0.75 .. 0.92]	3.087	2.31947e+9
2	[0.99 .. 1.10]	2.882	2.17947e+9
3	[3.00 .. 3.26]	1.946	1.47203e+9
4	[3.85 .. 3.88]	3.000	2.26883e+9
5	[3.92 .. 4.18]	1.857	1.40465e+9
6	[4.34 .. 4.50]	1.926	1.45957e+9
7	[4.72 .. 4.94]	0.964	7.28912e+8
8	[6.70 .. 6.81]	1.960	1.48208e+9
9	[7.11 .. 7.23]	1.955	1.47868e+9
10	[7.22 .. 7.26]	0.264	1.99483e+8
11	[7.26 .. 7.42]	4.786	3.61945e+9

(31). (4*R*)-4-(2-Benzyloxy-1,1-dimethylethyl)-1-(4-methoxyphenyl)azetidin-2,3-dione

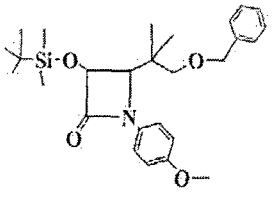
2-20

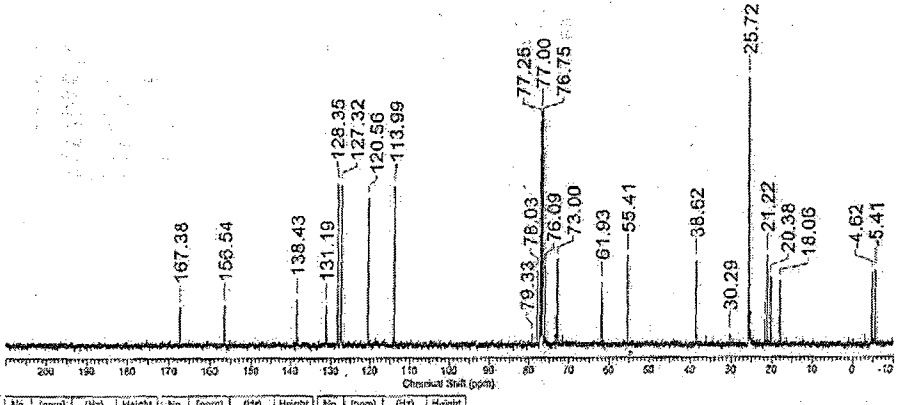


(32). (3*R*,4*S*)-(+)-3-(*t*-Butyldimethylsilanyloxy)-4-(2-benzyloxy-1,1-dimethylethyl)-1-(4-methoxyphenyl)azetidin-2-one 2-15



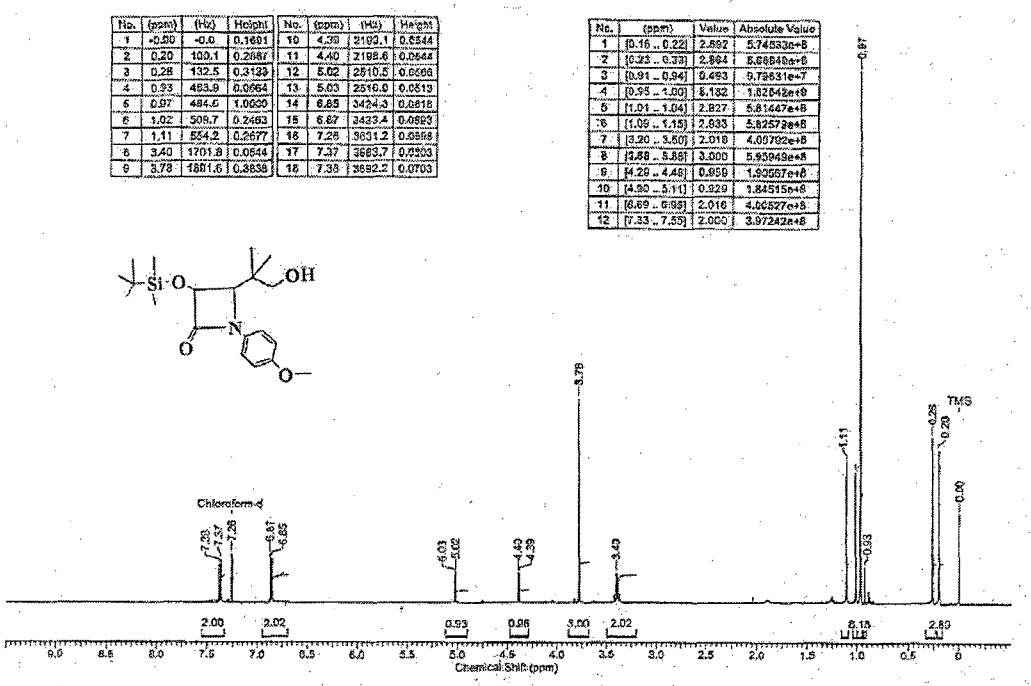
No.	(ppm)	(Hz)	Height	No.	(ppm)	(Hz)	Height	No.	(ppm)	(Hz)	Height
1	0.00	0.0	0.0394	14	3.08	770.4	0.0306	27	4.81	1202.6	-0.0003
2	0.06	15.6	0.0395	15	3.12	779.4	0.0589	28	4.96	1241.5	0.0012
3	0.15	39.1	0.2925	16	3.16	789.0	0.0635	29	4.99	1247.0	0.0570
4	0.23	56.9	0.2870	17	3.19	798.5	0.0272	30	5.09	1272.8	0.0103
5	0.89	157.8	0.0478	18	3.31	828.9	0.0290	31	6.76	1692.1	0.0801
6	0.85	212.4	0.0843	19	3.45	863.4	0.0973	32	6.77	1694.2	0.0397
7	0.89	214.1	0.0432	20	3.75	939.1	0.3936	33	6.80	1701.1	0.0994
8	0.89	229.5	0.0301	21	4.33	1082.3	0.0198	34	7.30	1827.0	0.0398
9	0.89	233.4	0.0632	22	4.37	1094.2	0.0615	35	7.31	1829.3	0.0444
10	0.91	228.3	0.1410	23	4.40	1100.8	0.0358	36	7.34	1835.1	0.2880
11	0.94	235.5	1.0000	24	4.48	1119.7	0.0611	37	7.34	1837.2	0.1699
12	1.01	253.5	0.2919	25	4.49	1123.5	0.0397	38	7.37	1844.3	0.0577
13	1.16	290.2	0.2510	26	4.60	1126.2	0.0680	39	7.38	1846.2	0.1099





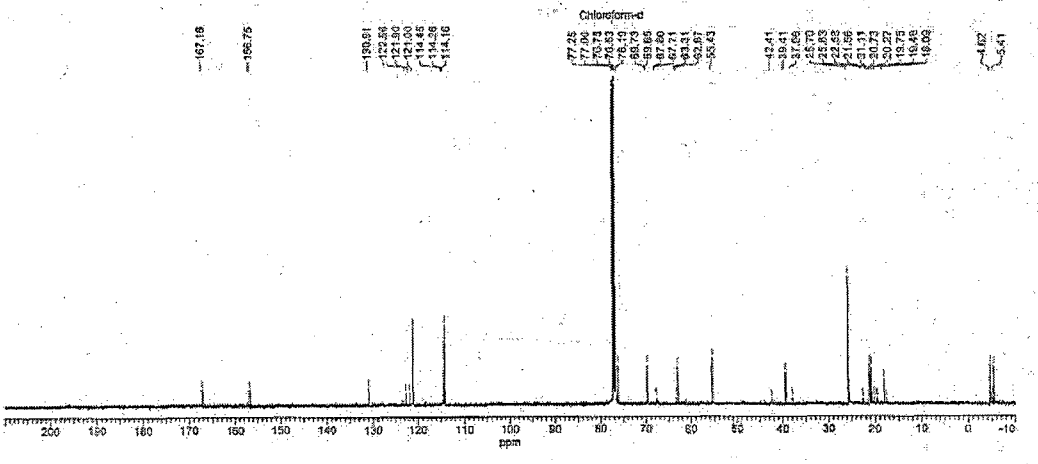
No.	(ppm)	(Hz)	Height	No.	(ppm)	(Hz)	Height	No.	(ppm)	(Hz)	Height
1	-5.41	-681.0	0.2763	12	61.93	7788.4	0.2359	23	127.32	16013.4	0.6034
2	-4.62	-581.3	0.2000	13	73.00	9180.7	0.3190	24	127.40	16023.4	0.0902
3	18.06	2271.1	0.1345	14	73.49	9242.5	0.0386	25	127.51	16037.4	0.7083
4	20.38	2563.3	0.2592	15	76.09	9569.8	0.2943	26	127.60	16048.3	0.0502
5	21.22	2668.8	0.3329	16	76.75	9652.4	0.8435	27	128.16	16119.1	0.0735
6	21.94	2746.8	0.0919	17	77.00	9684.3	0.0731	28	128.35	16142.1	0.0653
7	25.25	3175.7	0.0542	18	77.25	9716.2	0.6366	29	131.19	16500.1	0.1489
8	25.72	3234.5	1.0000	19	79.03	9814.0	0.2778	30	138.43	17410.7	0.1659
9	30.29	3810.0	0.0370	20	79.33	9977.5	0.0369	31	156.54	19685.8	0.1472
10	38.62	4857.2	0.3084	21	113.99	14339.9	0.5968	32	167.38	21052.0	0.1423
11	55.41	6968.9	0.3303	22	120.56	15163.7	0.5549				

(33). (3*R*,4*S*)-(+)-3-(*t*-Butyldimethylsilanyloxy)-4-(2-hydroxy-1,1-dimethylethyl)-1-(4-methoxyphenyl)azetidin-2-one 2-16



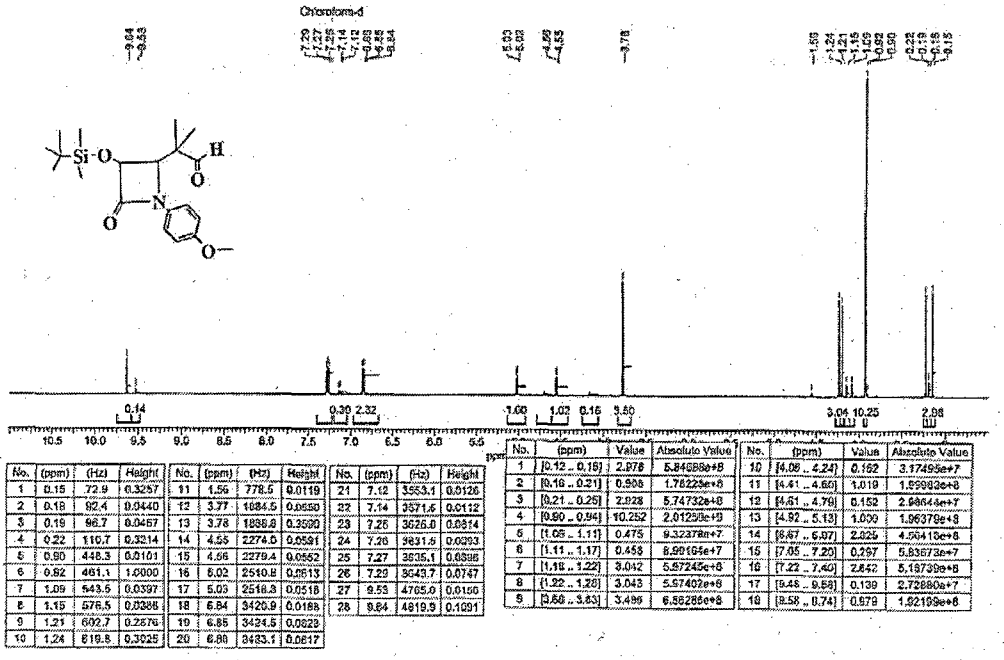
No.	(ppm)	(Hz)	Height
1	-0.00	-0.0	0.1691
2	0.20	100.1	0.2087
3	0.28	132.5	0.3129
4	0.93	463.9	0.0664
5	0.97	484.6	1.0000
6	1.02	509.7	0.2463
7	1.11	554.2	0.2677
8	3.40	1701.8	0.0544
9	3.78	1891.6	0.3838
10	4.39	2193.1	0.0544
11	4.40	2198.6	0.0644
12	5.02	2510.5	0.0566
13	5.03	2516.0	0.0519
14	6.85	3424.3	0.0618
15	6.87	3433.4	0.0693
16	7.28	3631.2	0.0566
17	7.37	3683.7	0.0503
18	7.38	3692.2	0.0703

No.	(ppm)	Value	Absolute Value
1	[0.16 .. 0.22]	2.592	5.745336e+8
2	[0.23 .. 0.33]	2.864	5.68848e+8
3	[0.91 .. 0.94]	0.493	9.726631e+7
4	[0.95 .. 1.00]	5.132	1.02542e+9
5	[1.01 .. 1.04]	2.827	5.61447e+8
6	[1.09 .. 1.15]	2.633	5.82573e+8
7	[3.20 .. 3.50]	2.018	4.00792e+8
8	[3.68 .. 5.86]	3.000	5.95949e+8
9	[4.29 .. 4.48]	0.959	1.90567e+8
10	[4.90 .. 5.11]	0.929	1.84515e+8
11	[6.69 .. 6.95]	2.016	4.00527e+8
12	[7.33 .. 7.55]	2.000	3.97242e+8



No.	(ppm)	(Hz)	Height
1	-5.41	-650.4	0.1249
2	-5.11	-542.3	0.0338
3	-4.62	-560.4	0.1806
4	17.53	2217.3	0.0274
5	18.05	2269.8	0.0471
6	18.09	2275.0	0.0666
7	19.48	2450.3	0.0290
8	19.75	2483.3	0.0295
9	20.27	2548.7	0.0310
10	20.73	2607.0	0.1246
11	21.11	2654.9	0.1927
12	21.56	2711.6	0.0261
13	22.58	2839.5	0.0287
14	25.63	3223.0	0.1641
15	25.70	3231.8	0.4034
16	37.06	4776.7	0.0291
17	39.41	4955.7	0.1091
18	42.41	5333.0	0.0271
19	55.43	6971.3	0.1502
20	62.87	7906.9	0.1253
21	63.31	7961.6	0.0310
22	67.71	8514.7	0.0299
23	67.80	8526.6	0.0352
24	69.65	8758.5	0.0822
25	69.73	8769.4	0.1294
26	76.19	9581.8	0.1297
27	76.29	9594.2	0.0802
28	76.53	9624.6	0.0366
29	76.75	9651.4	1.0000
30	77.00	9683.3	0.9989
31	77.25	9715.3	0.9842
32	77.39	9731.8	0.0404
33	114.16	14356.9	0.2560
34	114.36	14382.6	0.0525
35	114.45	14393.5	0.0507
36	114.60	14409.2	0.0414
37	121.00	15216.8	0.2449
38	121.80	15320.7	0.0449
39	122.58	15415.1	0.0444
40	130.91	16462.8	0.0536
41	156.75	19713.1	0.0571
42	167.18	21021.9	0.0581

(34). (3*R*,4*S*)-(+)-3-(*t*-Butyldimethylsilanyloxy)-4-(2-formyl-1,1-dimethylethyl)-1-(4-methoxyphenyl)azetidin-2-one 2-17



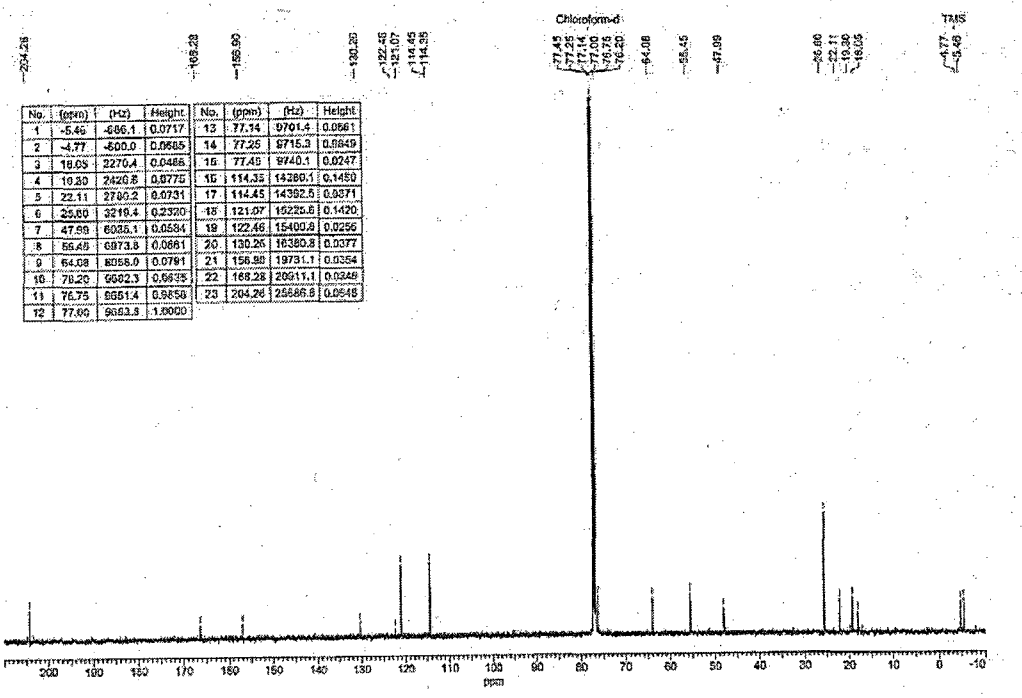
No.	(ppm)	(Hz)	Height
1	0.15	72.9	0.3257
2	0.18	92.4	0.0440
3	0.19	96.7	0.0457
4	0.22	110.7	0.3214
5	0.90	448.3	0.0101
6	0.92	461.1	1.0000
7	1.09	543.5	0.0397
8	1.15	576.5	0.0386
9	1.21	602.7	0.2676
10	1.24	619.5	0.3025

No.	(ppm)	(Hz)	Height
11	1.56	778.5	0.0119
12	3.77	1884.5	0.0650
13	3.78	1886.8	0.3500
14	4.55	2274.5	0.0591
15	4.56	2279.4	0.0552
16	5.02	2510.8	0.0613
17	5.03	2516.3	0.0518
18	6.84	3420.9	0.0188
19	6.85	3424.5	0.0829
20	6.86	3433.1	0.0617

No.	(ppm)	(Hz)	Height
21	7.12	3553.1	0.0126
22	7.14	3571.6	0.0112
23	7.25	3626.0	0.0514
24	7.26	3631.6	0.0293
25	7.27	3635.1	0.0596
26	7.29	3643.7	0.0747
27	9.53	4765.0	0.0156
28	9.84	4919.9	0.1091

No.	(ppm)	Value	Absolute Value
1	[0.12 .. 0.19]	2.978	5.84088e+8
2	[0.19 .. 0.21]	0.908	1.78223e+8
3	[0.21 .. 0.25]	2.928	5.74732e+8
4	[0.90 .. 0.94]	10.252	2.01259e+9
5	[1.05 .. 1.11]	0.475	9.32378e+7
6	[1.11 .. 1.17]	0.453	8.89164e+7
7	[1.19 .. 1.22]	3.042	5.97245e+8
8	[1.22 .. 1.26]	3.043	5.97402e+8
9	[3.50 .. 3.83]	3.499	6.86286e+8

No.	(ppm)	Value	Absolute Value
10	[4.06 .. 4.24]	0.162	3.17495e+7
11	[4.41 .. 4.60]	1.019	1.99962e+8
12	[4.61 .. 4.79]	0.152	2.98644e+7
13	[4.92 .. 5.13]	1.000	1.96379e+8
14	[6.67 .. 6.87]	2.025	4.00415e+8
15	[7.05 .. 7.20]	0.297	5.83673e+7
16	[7.22 .. 7.40]	2.842	5.57939e+8
17	[9.45 .. 9.69]	0.139	2.72880e+7
18	[9.58 .. 9.74]	0.679	1.32199e+8



No.	(ppm)	(Hz)	Height
1	-5.46	-686.1	0.0717
2	-4.77	-600.0	0.0685
3	18.05	2270.4	0.0486
4	19.30	2428.8	0.0775
5	22.11	2780.2	0.0731
6	25.60	3219.4	0.2320
7	47.09	6035.1	0.0584
8	55.45	6973.8	0.0661
9	64.08	8058.0	0.0791
10	76.20	9582.3	0.6635
11	76.75	9651.4	0.9858
12	77.00	9683.8	1.0000

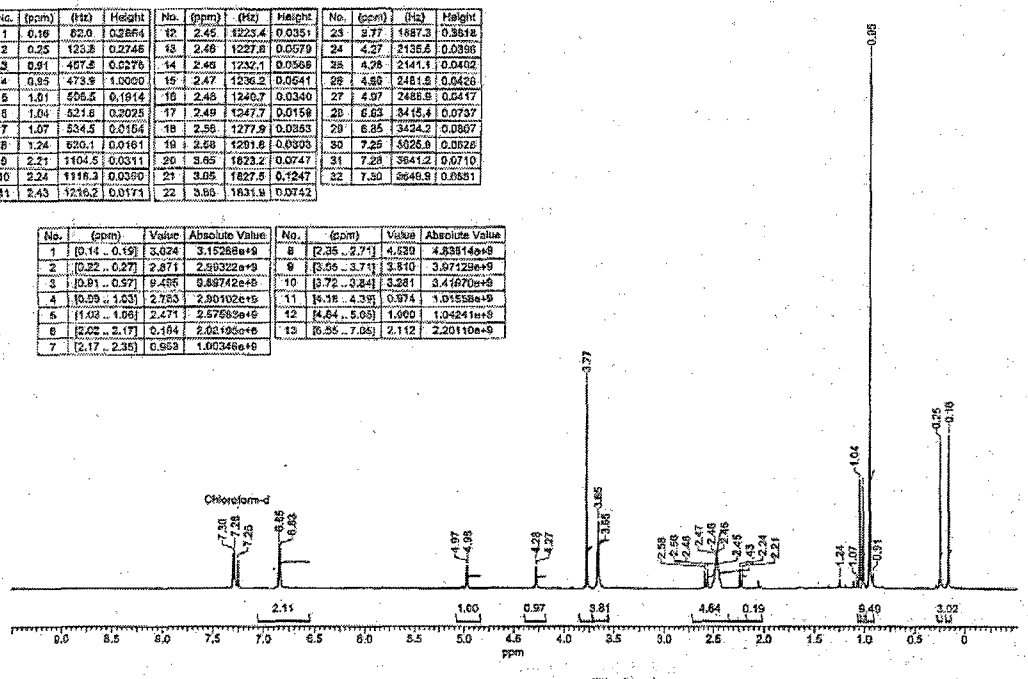
No.	(ppm)	(Hz)	Height
13	77.14	9701.4	0.0561
14	77.25	9715.3	0.0849
15	77.45	9740.1	0.0247
16	114.35	14380.1	0.1450
17	114.45	14392.5	0.0871
18	121.07	15225.6	0.1420
19	122.46	15400.9	0.0256
20	130.26	16380.8	0.0377
21	156.99	19751.1	0.0354
22	168.28	20911.1	0.0349
23	204.26	25686.8	0.0548

(35). (3*R*,4*S*)-(+)-3-(*t*-Butyldimethylsilanyloxy)-4-(2-morpholin-1,1-dimethylethyl)-1-(4-methoxyphenyl)azetidin-2-one 2-18

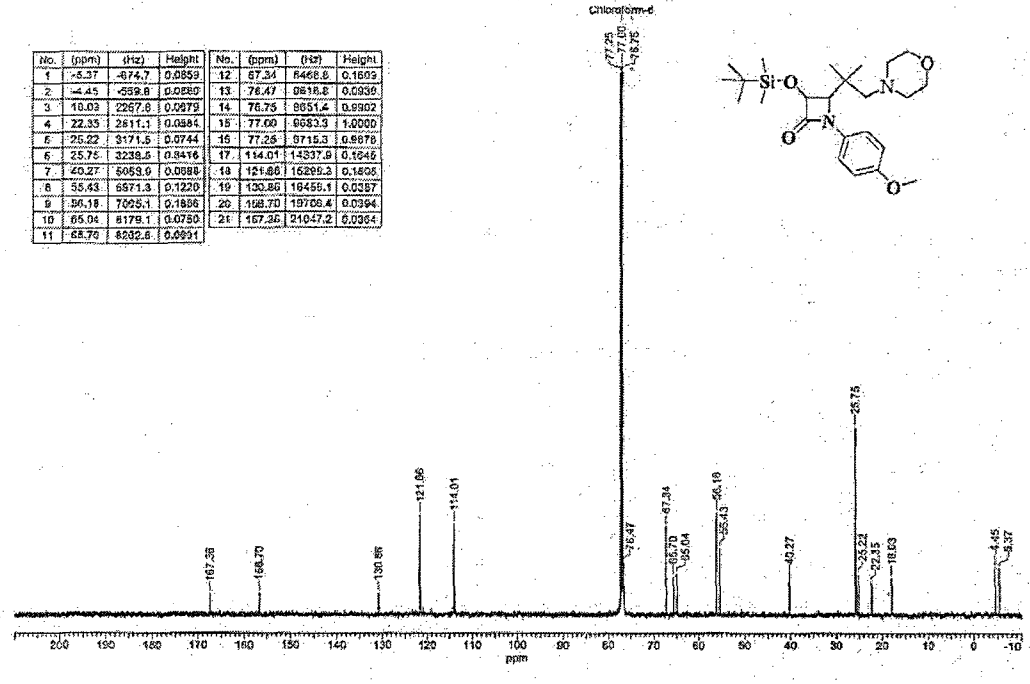
No.	(ppm)	(Hz)	Height	No.	(ppm)	(Hz)	Height	No.	(ppm)	(Hz)	Height
1	0.19	82.0	0.2864	12	2.45	1223.4	0.0351	23	3.77	1887.3	0.3518
2	0.25	123.8	0.2745	13	2.46	1227.8	0.0579	24	4.27	2135.5	0.0396
3	0.91	457.5	0.0276	14	2.48	1232.1	0.0366	25	4.28	2141.1	0.0402
4	0.95	473.9	1.0000	15	2.47	1236.2	0.0541	26	4.58	2481.5	0.0426
5	1.01	506.5	0.1914	16	2.48	1240.7	0.0340	27	4.97	2488.9	0.0417
6	1.04	521.6	0.2025	17	2.49	1247.7	0.0158	28	6.83	3415.4	0.0737
7	1.07	534.5	0.0164	18	2.56	1277.9	0.0353	29	6.85	3424.2	0.0807
8	1.24	620.1	0.0161	19	2.58	1291.6	0.0303	30	7.25	3625.9	0.0526
9	2.21	1104.5	0.0311	20	3.65	1823.2	0.0747	31	7.28	3641.2	0.0710
10	2.24	1118.3	0.0360	21	3.85	1927.5	0.1247	32	7.30	3649.9	0.0681
11	2.43	1216.2	0.0171	22	3.86	1931.9	0.0742				

No.	(ppm)	Value	Absolute Value
1	[0.14 .. 0.19]	3.024	3.15288e+9
2	[0.22 .. 0.27]	2.871	2.99322e+9
3	[0.91 .. 0.97]	9.466	9.89742e+9
4	[0.99 .. 1.03]	2.783	2.90102e+9
5	[1.02 .. 1.06]	2.471	2.57663e+9
6	[2.02 .. 2.17]	0.184	2.02196e+8
7	[2.17 .. 2.35]	0.968	1.00346e+9

No.	(ppm)	Value	Absolute Value
8	[2.35 .. 2.71]	4.539	4.83514e+9
9	[3.55 .. 3.71]	3.810	3.97129e+9
10	[3.72 .. 3.84]	3.281	3.41970e+9
11	[4.18 .. 4.39]	0.974	1.01558e+9
12	[4.64 .. 5.05]	1.000	1.04241e+9
13	[6.85 .. 7.05]	2.112	2.20110e+9



No.	(ppm)	(Hz)	Height	No.	(ppm)	(Hz)	Height
1	-5.37	-674.7	0.0859	12	67.34	8466.8	0.1609
2	-4.45	-559.8	0.0880	13	76.47	9618.8	0.0936
3	18.03	2267.8	0.0979	14	76.75	9651.4	0.9902
4	22.35	2811.1	0.0584	15	77.00	9683.3	1.0000
5	25.22	3171.5	0.0744	16	77.26	9715.3	0.9878
6	25.75	3238.5	0.8416	17	114.01	14337.9	0.1645
7	40.27	5059.9	0.0888	18	121.86	15299.3	0.1805
8	55.43	6971.3	0.1220	19	130.86	16455.1	0.0357
9	56.19	7065.1	0.1656	20	158.70	19706.4	0.0394
10	65.04	8179.1	0.0750	21	167.36	21047.2	0.0364
11	68.70	8632.6	0.0991				



Vita

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II. EDUCATION

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* B. Sc. *Beijing University of Traditional Medicine*, Beijing, China 1990-1994

III. EMPLOYMENT HISTORY

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Chatham biotech Co. Ltd. & Atlantic Forestry Centre (N.R.C.) Fredericton, N.B. Canada

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Team Academy of Pharmaceutical Science Beijing, China

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IV. PUBLICATIONS AND CONFERENCE PRESENTATIONS

(1). Jianmei Wang, F. D. Rochon, Y. Yang, L. Hua, M. M. Kayser. Synthesis of oxazolidine using DMSO/P_4O_{10} as formaldehyde equivalent. *Tetrahedron: Asymmetry* **2007**. (Accepted for publication)

(2). Jianmei Wang, F. D. Rochon, M. M. Kayser. Chemoenzymatic Synthesis of Enantiopure Oxazolidine. *Canadian Institute of Chemistry* May 26-30, **2007**.

Submitted:

(1).Y. Yang, Jianmei Wang, M. M. Kayser. Approaches to the synthesis of enantiopure α-hydroxy-β-lactams with functionalized side chains. **2007**.

(2). H. Ge, C. Schneider, Jianmei Wang, M. M. Kayser, J. K. Huff, R. H. Himes, G. Georg. Synthesis and biological evaluation of 3'-debenzoyl-3'-boc-3'-hydroxy-*tert*-butylpaclitaxel analogue through a highly efficient kinetic resolution of racemic 4-*tert*-butyldimethysiloxy-*tert*-butyl-β-lactam with 7-O-triethylsilylbaccatin III. **2007**.