ENZYMES AS A REAGENT IN ORGANIC SYNTHESIS: AN EFFICIENT RESOLUTION OF KEY INTERMEDIATES OF PHARMACEUTICALLY IMPORTANT COMPOUNDS AND SYNTHESIS

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by

S. EASWAR

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NATIONAL CHEMICAL LABORATORY
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Dedicated to my parents….

CERTIFICATE

This is to certify that the work incorporated in the thesis entitled "***Enzymes as a Reagent in Organic Synthesis: An Efficient Resolution of Key Intermediates of Pharmaceutically Important Compounds and Synthesis***" which is being submitted to the ***University of Pune*** for the award of ***Doctor of Philosophy*** in ***Chemistry*** by ***Mr. S. Easwar*** was carried out by him under my supervision at the National Chemical Laboratory, Pune. Such material as has been obtained from other sources has been duly acknowledged in the thesis.

July 2006
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Candidate's Declaration

I hereby declare that the thesis entitled "***Enzymes as a Reagent in Organic Synthesis: An Efficient Resolution of Key Intermediates of Pharmaceutically Important Compounds and Synthesis***" submitted for the degree of ***Doctor of Philosophy*** in ***Chemistry*** to the ***University of Pune*** has not been submitted by me for a degree to any other University or Institution. This work was carried out at the Division of Organic Chemistry (Synthesis), National Chemical Laboratory, Pune, India.

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I am thankful to Wadia Sir and Dhavale Sir for their inspirational teaching and support during my research as well as my mentors at RKM-Vivekananda College whose ethics and discipline I shall look up to forever.

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My stay here was made livelier by GJ hostel, a hostel unique in ways more than one. It provided the perfect atmosphere to spend leisure time and I thoroughly enjoyed all the sports & cultural activities that abounded in the hostel. I owe a big “thanks” to all my friends who made GJH such a wonderful place to stay in.

The Tamil Sangam deserves a mention for the lively atmosphere and the support & care of the all the members, past & present. I enjoyed as well as admired the spontaneous wit and work culture of Kicha while Pasu was affable with his nonchalance and frankness. Selvakannan's sense of humour and ability to enliven proceedings as well as the "brutally" straightforward ways of Suresh were enjoyable and remain fresh in my memory to this day. They remain close to me for all the support that they have provided. Then there is Uncle, whom I always have and will look up to for his genial nature and care.

I also consider myself blessed in that I got to spend a major chunk of my time here with some truly special persons. I have a great mate in Bala from whom I learnt that a simple lifestyle can often be the most convenient. Amarish, Abhay & Mangesh treated me never as a stranger during my initial days in Pune and they remain three of my closest & most trustworthy friends. I enjoyed Thalai's subtlety and respected him for his no-nonsense approach while I admired Devaraj's silent and meticulous ways. Malli has a great presence of mind coupled with a natural helping tendency. We gelled extremely well with jolly-good conversations and heated arguments. It was a pleasure to share my room as well as spend time with Nagendra who was commitment and dedication personified. I hold him in awe, for he went out-of-the-way to help people. These were the guys that made "Sunday dinner" an occasion to eagerly look forward to every single time.

Kishor is an amazing cocktail of character and emotions who has an unbelievable zest for life with a great nature to boot. We forged a unique partnership involving many memorable experiences that I'll always cherish. Mangal has always been and will continue to be an inspiration with his counsel. We resonated on matters more than one that has made us share a special relationship. When it comes to Subbu and Jayanthi, I am lost for words to describe them as individuals and it should suffice to say that I am simply blessed to have them ever by my side; I've spent the best of times with them around. They have been great in putting up with a lot of my gibberish and form an indispensable part of my life. The care and emotional support of these people has been no less than that of my family and for all that they have done for me I don't want to thank them simply because I don't need to.

It goes without saying that my family has always been there for me, showering on me boundless affection and backing me in whatever I chose or did. The warmth and moral values of my parents have stood me in good stead throughout my life and I would always look up to them for strength no matter what I have to go through.

Finally, my acknowledgement would not be complete without thanking the Almighty, for the strength and determination to put my chin up when faced with hardships in life.

Easwar

Although human subtlety makes a variety of inventions by different means to the same end, it will never devise an invention more beautiful, more simple, or more direct than does Nature, because in Her inventions nothing is lacking, and nothing is superfluous.

- Leonardo DaVinci

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General Remarks

- All the solvents used were purified according to the literature procedures.
- Petroleum ether used in the experiments was of 60-80 °C boiling range.
- Column chromatographic separations were carried out by gradient elution with light petroleum ether-ethyl acetate mixture, unless otherwise mentioned and silica gel (60-120 mesh/100-200 mesh/230-400 mesh).
- TLC was performed on E-Merck pre-coated 60 F_{254} plates and the spots were rendered visible by exposing to UV light, iodine, phosphomolybdic acid (in ethanol), bromocresol green (in ethanol).
- IR spectra were recorded on Shimadzu FTIR instrument, for solid either as nujol mull or in chloroform solution (conc. 1 μM) and neat in case of liquid compounds.
- NMR spectra were recorded on Brucker ACF 200 (200 MHz for ^{1}H NMR and 50 MHz for ^{13}C NMR), MSL 300 (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) and DRX 500 (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) spectrometers. Chemical shifts (δ) reported are referred to internal reference tetramethyl silane.
- Mass spectra were recorded on Finnigan-Mat 1020C mass spectrometer and were obtained at an ionization potential of 70 eV.
- Microanalytical data were obtained using a Carlo-Erba CHNS-O EA 1108 Elemental Analyser. Elemental analyses observed for all the newly synthesized compounds were within the limits of accuracy (± 0.3%).
- All the melting points reported are uncorrected and were recorded using an electro-thermal melting point apparatus.
- All the compounds previously known in the literature were characterized by comparison of their R_f values on TLC, IR and NMR spectra as well as melting point (in case of solid) with authentic samples.
- All the new experiments were repeated two or more times.
- Starting materials were obtained from commercial sources or prepared using known procedures.
- ***Independent referencing and numbering of compounds, schemes, tables & figures have been employed for Section A & B of both Chapter I and Chapter II***

Abbreviations

AIBN	2,2′-Azobisisobutyronitrile
Aq.	Aqueous
BINOL	1,1′-Bi-(2-naphthol)
Bn	Benzyl
Boc	*t*-Butoxycarbonyl
Cat.	Catalytic
Conv.	Conversion
Cp	Cyclopentadienyl
CSA	10-Camphorsulfonic acid
DBP	Dibenzoyl peroxide
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	1,3-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	Diethyl azodicarboxylate
DEPT	Distortionless Enhancement by Polarization Transfer
DHP	Dihydropyran
DIBAL-H	Diisobutylaluminium hydride
DIPE	Diisopropyl ether
DMAP	4-(Dimethylamino)pyridine
DMF	Dimethylformamide
DMSO	Dimethyl sulphoxide
ee	Enantiomeric excess

equiv.	Equivalent(s)
GLC	Gas-liquid chromatography
h	Hour(s)
HMDS	Hexamethyldisilazane
HMPA	Hexamethylphosphoramide
HPLC	High Performance Liquid Chromatography
Hz	Hertz
IC	Inhibitory concentration
IPA	Isopropenyl acetate
IR	Infra Red
LAH	Lithium aluminum hydride
LDA	Lithium diisopropylamide
LiTMP	Lithium tetramethylpiperidide
m-CPBA	*m*-Chloroperbenzoic acid
min.	Minute(s)
mL	Millilitre(s)
mmol	Millimole(s)
Mp	Melting point
MS	Mass Spectrum
MsCl	Methanesulfonyl chloride
MTPA	α-Methoxy-α-trifluoromethylphenylacetic acid (Mosher's acid)
NBS	*N*-Bromosuccinimide
NMR	Nuclear Magnetic Resonance
ORTEP	Orthogonal Thermal Ellipsoid Plots
PCC	Pyridinium chlorochromate

PMB	*p*-Methoxybenzyl
PPA	Polyphosphoric acid
PPTS	Pyridinium *p*-toluenesulfonate
p-TSA	*p*-Toluenesulfonic acid
p-TsCl	*p*-Toluenesulfonyl chloride
Py	Pyridine
rac	Racemic
rt	Room temperature
TBAF	Tetrabutylammonium fluoride
TBDMS / TBS	*t*-Butyldimethylsilyl
TBME	*t*-Butylmethyl ether
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TIPS	Triisopropylsilyl
Tlc/TLC	Thin layer chromatography
TMEDA	Tetramethylethylenediamine
TMG	Tetramethyl guanidine
TMSCl	Trimethylchlorosilane
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TPP	Triphenylphosphine
VA	Vinyl acetate
VB	Vinyl butanoate

Lipase Abbreviations

Amano AK	Commercially available preparation of lipase from *Pseudomonas fluorescens*
Amano AY	Commercially available preparation of lipase from *Candida rugosa*
Amano PS	Commercially available preparation of lipase from *Burkholderia cepacia*
Amano PS-D	Lipase from *Pseudomonas cepacia* immobilized on diatomite
CAL – A	Lipase from *Candida antartica* (A)
CAL – B	Lipase from *Candida antartica* (B)
CCL	Lipase from *Candida cylindracea*
Chirazym	Commercially available preparation of CALB
CRL	Lipase from *Candida rugosa*
LIP – 300	Lipase from *Pseudomonas aeruginosa*
Lipase PS-C	Lipase from *Pseudomonas cepacia* immobilized on ceramic particles
Lipozyme	Lipase from *Mucor miehei*
Novozym 435	Commercially available immobilized CALB (Novo Nordisk)
PCL = PSL	Lipase from *Burkholderia cepacia*
PFL	Lipase from *Pseudomonas fluorescens*
PLE	Pig liver esterase
PPL	Porcine pancreatic lipase

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Abstract

The present dissertation is divided into two chapters. The first chapter presents a short overview of the applications of enzymes in organic synthesis followed by a description of our efforts in utilizing enzymes in the preparation of enantiomerically pure α-hydroxycyclopentanone, resolution of the key intermediate in the synthesis of talampanel, a drug candidate and alcoholysis of maleimides to the corresponding alkyl maleanilates. In the second chapter, concise accounts on alkylmethylmaleic anhydride natural products and homophthalic anhydride in organic synthesis precede a description of our studies towards the synthesis of aspergillus acids A-D, gusanlung D, nuevamine and charminarone.

Chapter One: Enzymes in Organic Synthesis and the Use of Lipases in the Preparation of Important Chiral Compounds and Intermediates

This chapter is divided into two sections. The first section presents a concise introduction on enzymes and the applications of lipases in synthetic organic chemistry whereas the second section summarizes our studies in using lipases for the resolution of key intermediates and other significant transformations. Subsequently, some selected spectra have been illustrated followed by the detailed experimental procedures and the analytical & spectral data for compounds of the second section.

Section A: Lipases in Organic Synthesis

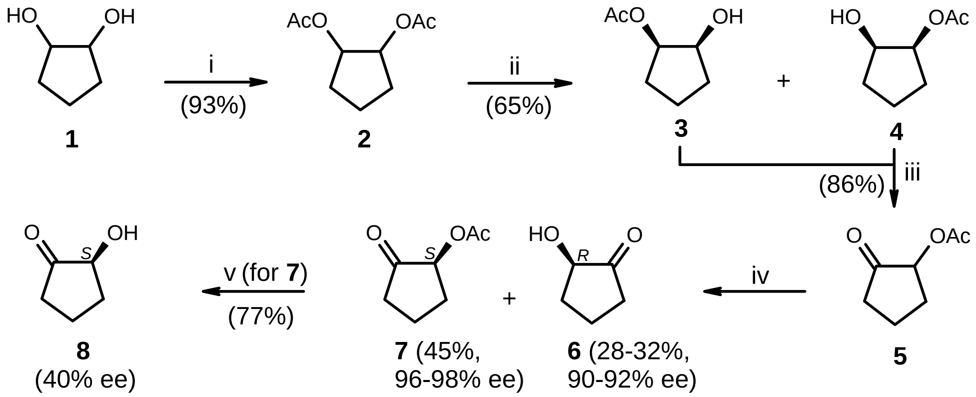
In recent years, enzymes have emerged as powerful tools in organic synthesis for bringing about kinetic resolution of racemates as they are extremely specific in their action and offer a high degree of chemo-, regio- and stereoselectivity, which is of huge importance in organic synthesis. Amongst all enzymes, lipases are the most popular given their tremendous versatility in applications. This section gives a brief introduction about enzymes in general, their classification and a concise account of the applications of lipases in synthetic organic chemistry.

<u>Section B</u>: The Applications of Lipases in Key Resolutions/Transformations

Section B describes our efforts in this field in three segments. The first segment presents an elegant enzymatic preparation of enantiomerically pure *α*-hydroxycyclopentanone while the second one details an efficient lipase-catalyzed resolution of the key intermediate in the synthesis of antiepileptic drug candidate, Talampanel. The third segment depicts an easy enzymatic access to methyl maleanilates from the corresponding maleimides.

<u>Section B</u>: I. Enantioselective Enzymatic Approach to (+)- and (–)-*α*-Hydroxy/acetoxycyclopentanones

α-Hydroxy/acetoxycyclopentanones are important chiral synthons whose utilities have been well proven in practice. To date there are five reports on the preparation of enantiomerically pure (*R*)-/(*S*)-2-hydroxycyclopentanones (**6**/**8**). This section describes an elegant chemoenzymatic access, starting from *cis*-diol **1**, to these unstable yet useful building blocks with 28-32% yield and 90-92% ee for (–)-(2*R*)-hydroxycyclopentanone (**6**) and 45% yield and 96-98% ee for (+)-(2*S*)-acetoxycyclopentanone (**7**) (Scheme 1).



Scheme 1 *Reagents, conditions and yields*: (i) Ac_2O, Py, rt, 48 h (93%); (ii) Petroleum ether/benzene (2:1), Amano PS, rt, 18 h, pH 7.0 (65%); (iii) $(COCl)_2$, DMSO, TEA, DCM, −60 °C, 15 min (86%); (iv) Petroleum ether/benzene (2:1), Amano PS, rt, 22 h, pH 6.5 (**6**, 28-32%; **7**, 45%); (v) Aq. K_2CO_3, MeOH, 0 °C, 4 h (77%).

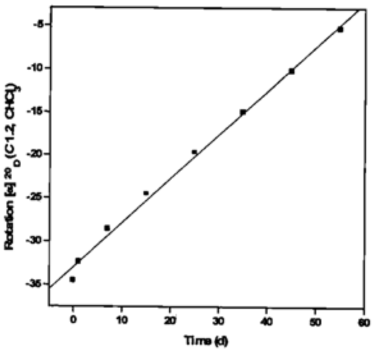
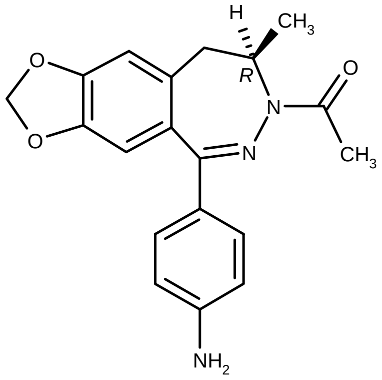


Figure 1

During these studies, we also noticed that (–)-**6** as neat or in chloroform solution at 0 ^{o}C slowly loses its optical purity and becomes completely racemic in nearly two months time, indicating that these optical isomers have two months half life span as determined by measuring the specific rotations with time interval (Figure 1).

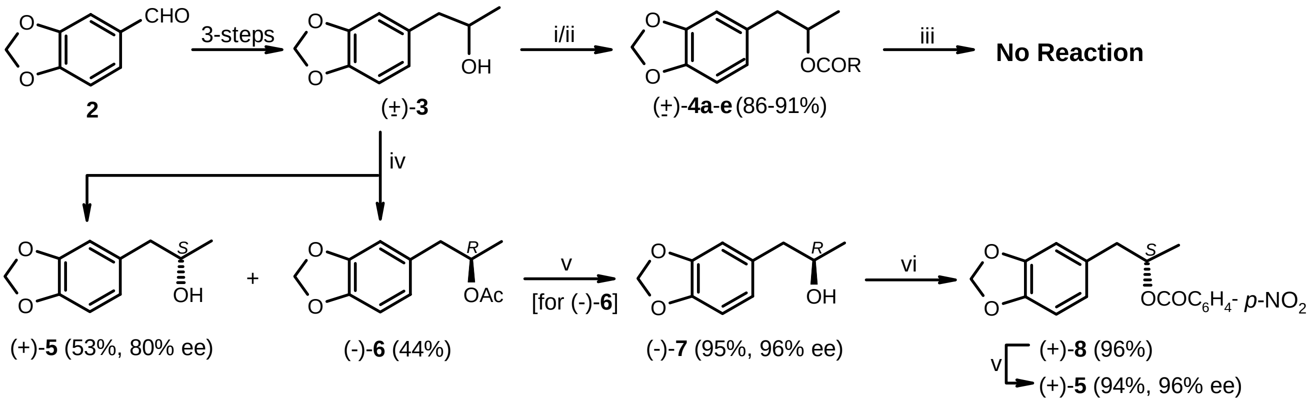
<u>Section B</u>: II. Amano PS Catalyzed Resolution of (±)-*α*-Methyl-1,3-benzodioxole-5-ethanol: Key Intermediate in the Synthesis of Antiepileptic Drug Candidate, Talampanel

(–)-Talampanel {7-acetyl-5-(4-aminophenyl)-8(*R*)-methyl-8,9-dihydro-7*H*-1,3-dioxolo[4,5-b][2,3]benzodiazepine, **1**} was recently discovered as an orally active novel compound with potential antiepileptic, neuroprotectant and skeletal muscle relaxant activities. Presently, (–)-talampanel (**1**) is in phase III of clinical studies having shown efficacy in the phase II studies in patients with severe epilepsy.



(–)-Talampanel (**1**)

The clinical potential of this compound has led to great interest in developing new syntheses. Two enantioselective syntheses and two other methods of obtaining the key intermediate alcohol (+)-(*S*)-*α*-methyl-1,3-benzodioxole-5-ethanol (**5**) have been reported. This section reports an efficient and enantioselective Amano PS catalyzed acylation of (±)-*α*-methyl-1,3-benzodioxole-5-ethanol (**3**) to obtain the potential chiral building block for the synthesis of (–)- Talampanel, the (+)-(*S*)-*α*-methyl-1,3-benzodioxole-5- ethanol (**5**) in 53% yield and 80% ee (Scheme 2). The enantiomerically pure opposite isomer (–)-**6** was also transformed to the desired (+)-**5** via Mitsunobu inversion in 38% overall yield (4-steps) with 96% ee. The conversion of (*S*)-alcohol **5** to (–)-talampanel (**1**) with 54% overall yield in 6-steps via exclusive generation of seven-membered 2,3-benzodiazepine skeleton is well established.

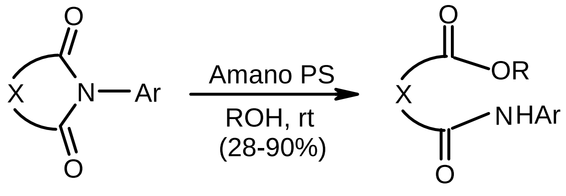


4a, R = Methyl; **4b**, R = Chloromethyl; **4c**, R = *p*-Anisyl; **4d**, R = *p*-Nitrophenyl; **4e**, R = Pentadecanyl

Scheme 2 *Reagents, conditions and yields*: (i) Ac_2O/py, rt, 24 h (91%); (ii) RCOOH, DCC, DMAP, DCM, rt, 8 h (86-90%); (iii) Petroleum ether/benzene (2:1), Amano PS/CCL/Amano AY, 50 mM Sodium phosphate buffer (pH 7.0), rt, 24 h; (iv) *n*-Hexane/benzene (2:1), Amano PS, vinyl acetate, 50 °C, 72 h [(+)-**5**, 53%; (–)-**6**, 44%]; (v) K_2CO_3/MeOH, 0 °C, 4 h (95/94%); (vi) DEAD, TPP, *p*-NO_2-C_6H_4COOH, THF, 0 °C, 6 h (96%).

Section B: III. Amano PS Catalyzed Methanolysis of Maleimides to Methyl Maleanilates

Alkyl maleanilates are important building blocks in the synthesis of several structurally interesting and biologically important heterocyclic systems. This section presents the first simple and efficient enzymatic method for the synthesis of methyl maleanilates in 80-90% yields from the corresponding cyclic imides.



R = Me, Et
X = CH = CH; CH = C(Me); CH_2-CH_2

We feel that the present protocol would be useful to perform in situ intramolecular cyclizations on suitably substituted alkyl maleanilates skeletons to generate 5/6/7-membered heterocyclic systems.

Section B: IV. Spectral Data

This section presents spectra of some selected compounds prepared in Section B I-III of Chapter One.

Section B: V. Experimental

This section provides the detailed experimental procedures, tabulated analytical and spectral data for all the compounds reported in Section B I-III of Chapter One.

Chapter Two: Studies towards the Synthesis of Anhydride Based Natural Products

This Chapter is divided into two sections. The first section portrays short accounts on alkylmethylmaleic anhydrides and applications of homophthalic anhydrides in organic synthesis while the second section describes our studies towards the synthesis of natural products and analogs based on three different cyclic anhydride precursors. Subsequently, some selected spectra have been illustrated followed by the detailed experimental procedures and the analytical & spectral data for compounds of the second section.

Section A: Maleic Anhydrides and Homophthalic Anhydrides in Organic Synthesis

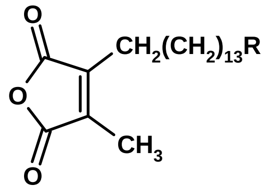
In the past decade, several structurally interesting compounds with dialkyl substituted maleic anhydride moieties have been isolated and synthesized in view of their promising bioactivities. Homophthalic anhydride has been an important synthon in organic chemistry for the synthesis of various compounds including bioactive natural products. This section provides an overview of substituted and unsubstituted maleic anhydride based natural products and natural product hybrids followed by a concise account of homophthalic anhydride and its applications in synthetic organic chemistry.

Section B: Studies towards the Synthesis of Natural Products Aspergillus Acids A-D, Gusanlung D, Nuevamine and Charminarone

This section portrays our studies towards the synthesis of cyclic anhydride based natural products in four parts. The first part depicts the first synthesis of four new secondary metabolites aspergillus acids A-D and an efficient enzymatic resolution to obtain two of them in their enantiomerically pure form. The second part details a formal synthesis of the protoberberine alkaloid gusanlung D as well as an elegant route to dehydrogusanlung D. The third section illustrates a remarkable serendipitous benzylic air oxidation that aided in the construction of the nuevamine alkaloid skeleton. The fourth part describes our ongoing studies towards the first total synthesis of the recently isolated natural product charminarone.

Section B: I. A Facile Synthesis and an Enzymatic Resolution of Naturally Occurring Remotely Functionalized Alkylmethylmaleic Anhydrides: Aspergillus Acids A-D

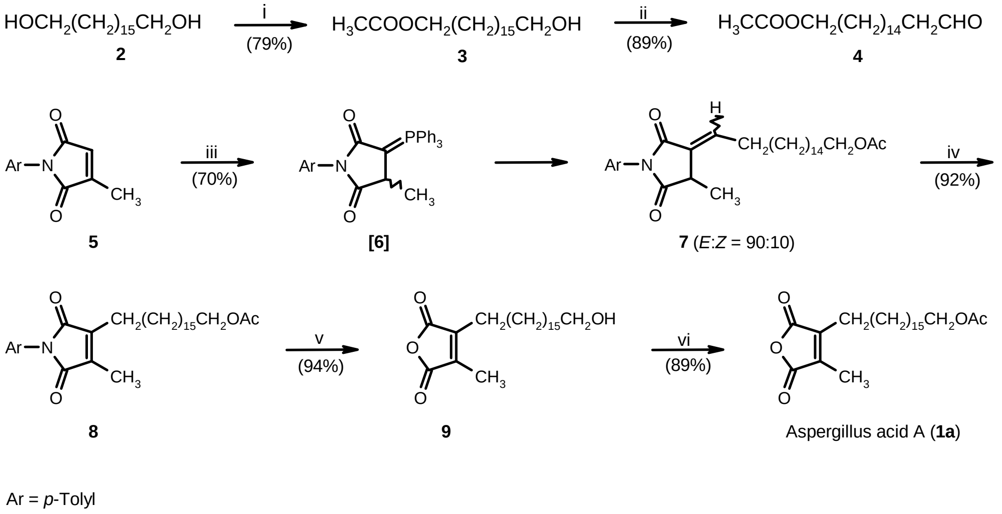
In 1979, Assante et al. isolated four new secondary metabolites, aspergillus acids A-D produced by the mould *Aspergillus wentti*. These natural products were established to be derivatives of citraconic anhydride with remotely functionalized long hydrocarbon chains and the chiral centre in **1c** & **1d** was assigned the *S*-configuration.



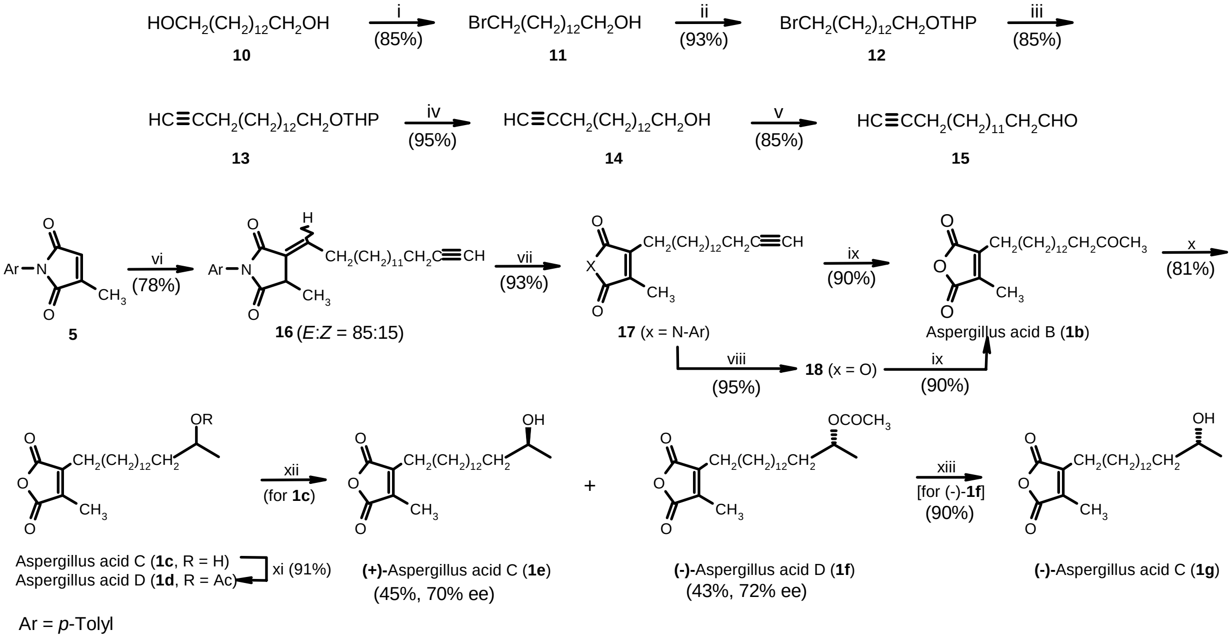
Aspergillus acid A [R = $(CH_2)_3OAc$, **1a**]
Aspergillus acid B [R = $COCH_3$, **1b**]
Aspergillus acid C [R = $CH(OH)CH_3$, **1c]**
Aspergillus acid D [R = $CH(OAc)CH_3$, **1d**]

Chaetomellic acid, which is structurally tetradecylmethylmaleic anhydride, also an alkylmethylmaleic anhydride, is presently in clinical use as a highly specific inhibitor of ras farnesyl-protein transferase. We feel that the remote functional groups in the hydrophobic long chain hydrocarbon units of these acids **1a-d** will anchor the binding process with target enzymes, resulting in a boost in activity.

This section describes the first synthesis of aspergillus acids (**1a-d**) starting from citraconimide (**5**) wherein we have utilized a terminal acetylinic unit to serve as a latent methyl ketone. This section also illustrates an efficient enzymatic resolution to obtain the acids **1c** and **1d** in their enantiomerically pure form. Thus, Wittig condensation of **5** with appropriate aldehydes **4** and **15** provided *exo*-imides **7** and **16** which on isomerization yielded *endo*-imides **8** and **17** respectively. Base catalyzed hydrolysis of **8** followed by acetylation yielded aspergillus acid A (**1a**) in 54% overall yield in 3-steps (Scheme 1). The maleimides **17** on treatment with acetic acid/H_2SO_4 directly furnished aspergillus acid B (**1b**), which on chemoselective reduction provided aspergillus acid C (**1c**), while the conversion of **1c** to aspergillus acid D (**1d**) was effected using Ac_2O. An elegant Amano PS catalyzed acylation of **1c** enabled us to obtain (+)-aspergillus acid C (**1e**) and (–)-aspergillus acid D (**1f**) in 45% yield/70% ee & 43% yield/72% ee respectively (Scheme 2).



Scheme 1 *Reagents, conditions and yields*: (i) Ac_2O (0.98 equiv.), Py, rt, 6 h (79%); (ii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, – 60 °C, 90 min (89%); (iii) Ph_3P, AcOH, **4**, reflux, 18 h (70%); (iv) Et_3N, THF, reflux, 48 h (92%); (v) (a) KOH (30% aq.), THF-MeOH (1:2), reflux, 12 h, (b) H^+/HCl (94%); (vi) Ac_2O, Py, rt, 8 h (89%).



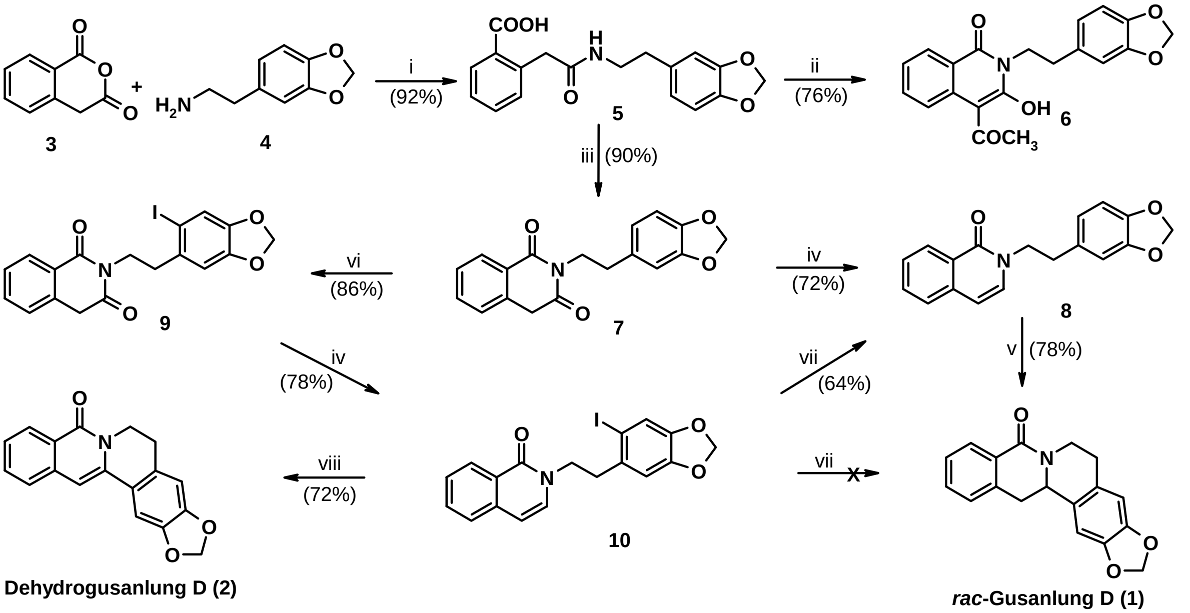
Scheme 2 *Reagents, conditions and yields*: (i) HBr (47% aq.), toluene, reflux, 96 h (85%); (ii) DHP, PPTS (cat.), CH_2Cl_2, rt, 4 h (93%); (iii) NaC≡CH, THF, HMPA, – 78 °C to rt, 40 h (85%); (iv) *p*-TSA, MeOH, rt, 2 h (95%); (v) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, – 60 °C, 90 min (85%); (vi) Ph_3P, AcOH, **15**, reflux, 24 h (78%); (vii) Et_3N, THF, reflux, 48 h (93%); (viii) (a) KOH (30% aq.), THF-MeOH (1:2), reflux, 12 h, (b) H^+/HCl (95%); (ix) 6 M H_2SO_4/AcOH (1:2), 100 °C, 8 h (90%); (x) (a) Aq. NaOH, THF, 50 ºC, 2 h, (b) $NaBH_4$, 0 ºC to rt, 3 h, (c) H^+/HCl (81%); (xi) Ac_2O, Py, rt, 12 h (91%); (xii) Amano PS, vinyl acetate, hexane benzene (2:1), 45 ºC, 72 h (**1e**, 45%; **1f**, 43%); (xiii) (a) Aq. NaOH, THF, 50 ºC, 4 h, (b) H^+/HCl (90%).

Overall yields for the aspergillus acid A (**1a**) in 3-steps was 54% whereas for the other three naturally occurring acids were 20 - 65%.

Section B: II. Studies towards the Synthesis of the Protoberberine Alkaloid Gusanlung D

Protoberberines form an important class of alkaloids comprising several tetracyclic bioactive compounds supplemented by a vast literature. Gusanlung D, which is structurally (*S*)-2,3-methylenedioxy-8-oxoberberine, is the first optically active natural protoberberine claimed to be unoxygenated at ring D (isolated in 1995). Three racemic and one asymmetric synthesis have been reported for gusanlung D, whereas, several synthetic routes are known for the construction of 8-oxoberberines and protoberberines.

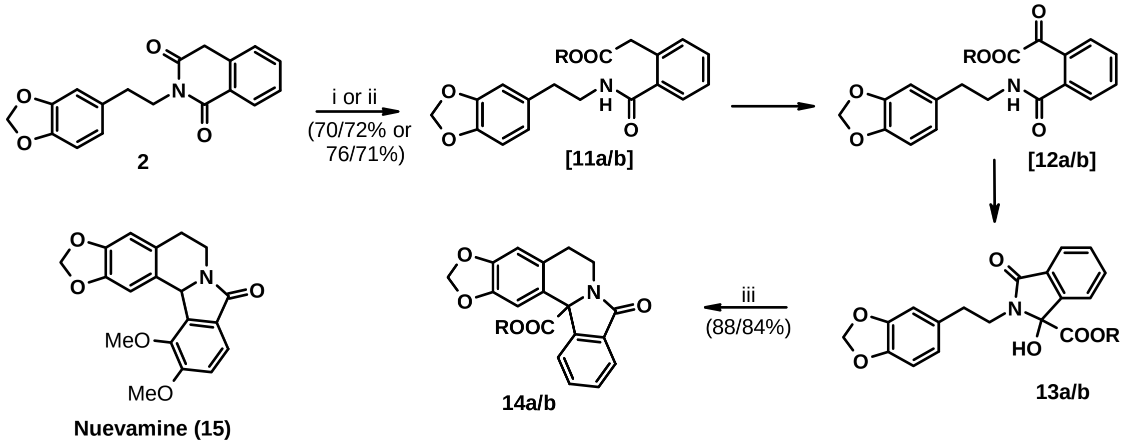
This section describes a formal synthesis of gusanlung D (**1**) starting from homophthalic anhydride (**3**), wherein the known intermediate olefin **8** was prepared. Also, an elegant construction of ring B, using of a Heck-type coupling has been illustrated to complete the synthesis of the dehydrogusanlung D (**2**). Starting from **3**, the overall yield of gusanlung D (**1**) in 4-steps was 47% and that of **2** in 5-steps was 40% (Scheme 1).



Scheme 1 *Reagents, conditions and yields*: i) Ether-THF (4:1), rt, 12 h (92%); (ii) Ac_2O, AcONa, 60 ˚C, 3 h (76%); (iii) HMDS, benzene, $ZnCl_2$, 2 h (90%); (iv) (a) $NaBH_4$, EtOH, 0 ˚C, 6 h, (b) H^+/HCl, 1 h (72%/78%); (v) Conc HCl, rt, 48 h (60%); (vi) I_2, CF_3COOAg, $CHCl_3$, rt, 8 h (86%); (vii) AIBN, Bu_3SnH, benzene, reflux, 4 h (63%); (viii) $Pd(OAc)_2$, TMG, AcONa, DMF, 110 ˚C, 20 h (72%).

Section B: III. Studies towards the Total Synthesis of Nuevamine

Nuevamine (**62**) occupies a very special place since it is the first isoindolo[1,2-*a*]isoquinolinone alkaloid known to occur in nature and can still be considered the lone representative of this class of isoquinoline alkaloids. Three synthetic approaches, the first of which revised & confirmed the structure of the natural product, of nuevamine are known in the literature. This section illustrates a remarkable serendipitous benzylic air-oxidation which aided in the construction of the nuevamine alkaloid framework. Thus, homophthalimide **7**, on treatment with $NaBH_4$ in MeOH, underwent a remarkable ring opening-benzylic air-oxidation-ring closure process to yield the hydroxy ester **13a** via the intermediate **11a** & **12a**. On treatment with acid, **13a** cyclized readily to yield a compound **14a** (structure was confirmed by single crystal X-ray analysis) possessing the structural skeleton of the alkaloid nuevamine (**15**) (Scheme 2). Application of this protocol to synthesize the actual natural product nuevamine and studies on the mechanistic aspects of the air-oxidation are in progress in our laboratory.



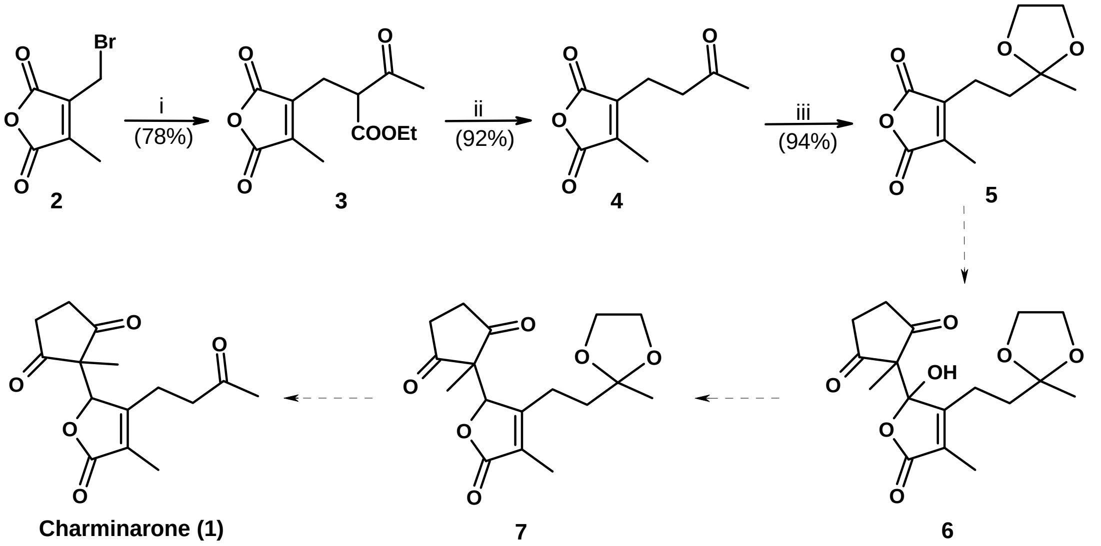
a, R = Me; **b**, R = Et

Scheme 2 *Reagents, conditions and yields*: (i) $NaBH_4$ (3 equiv.), ROH, rt, 20 h (70/72%); (ii) Et_3N (5 equiv.), ROH, rt, 12 h (76/71%); (iii) H_2SO_4 (cat.) /AcOH, rt, 2 h (88/84%).

Section B: IV. Studies towards the Total Synthesis of Charminarone

The tetracarbonyl system, Charminarone (**1**), which is the first *seco*-pseudoguaianolide reported from nature, was isolated very recently from the weed *Parthenium hysterophorus* Linn (Compositae). This obnoxious weed's extract is used as folk remedy against skin diseases, ulcerated sores, facial neuralgia, fever and anemia.

This section describes our ongoing efforts towards accomplishing the first total synthesis of this bioactive natural product **1** starting from (bromomethyl)methylmaleic anhydrdide (**2**). Reaction of ethylacetoacetate with **2** using NaH as a base provided us ester **3** which upon hydrolysis and in situ decarboxylation afforded the methyl ketone **4** in 72% yield over 2-steps. The carbonyl group was then protected using ethylene glycol to yield the ketal **5** in 94% yield. Efforts are in progress to bring about the regioselective addition of 2-methyl-1,3-cyclopentanedione to the desired carbonyl of anhydride **5** to afford the required charminarone precursor **6**, which could then be deoxygenated and subsequently deprotected to provide the desired natural product (Scheme 1). Work in this regard is in active progress in our laboratory.



Scheme 1 *Reagents, conditions and yields*: (i) Ethylacetoacetate, NaH, THF, rt, 8 h (78%); (ii) conc. HCl + H_2O (1:1), 12 h (92%); (iii) Ethylene glycol, benzene, reflux, 10 h (94%).

Section B: V. Spectral Data

This section presents spectra of some selected compounds prepared in Section B I-IV of Chapter Two.

Section B: VI. Experimental

This section provides the detailed experimental procedures, tabulated analytical and spectral data for all the compounds reported in Section B I-IV of Chapter Two.

Note: Compound numbers in the abstract are different from those in the thesis.

Chapter 1

Enzymes in Organic Synthesis and the Use of Lipases in the Preparation of Important Chiral Compounds and Intermediates

1A. Section A

Lipases in Organic Synthesis

This section features the following topics:

1A. 1 Introduction

1A.1.1 Chirality[1] and Medicine

In science, especially in the field of medicine and pharmaceuticals, the importance of chiral compounds cannot be over-emphasized. Chirality is not a pre-requisite for bioactivity but in bioactive molecules where a stereogenic centre is present, great differences have been observed in the activity of the enantiomers. This is a general phenomenon and applies to all substances, such as drugs, insecticides, herbicides, flavours and food additives. The overwhelming majority of naturally occurring drugs are chiral molecules, existing as single active enantiomer, whereas for many years it was common practice to market the synthetic drugs as racemates. Certain drugs such as antihypertensive agents, when administered as racemates, display no side effects arising from the distomer (inactive isomer). But in some other cases, the distomer may exhibit toxic side effects e.g., the (*S*)-isomer of ketamine has anesthetic and analgesic activity, whereas the (*R*)-isomer is a hallucinating agent. The most well known example is that of thalidomide (Figure 1), formerly used as a sedative (& immunomodulator) where the distomer, a teratogenic agent, caused fetal abnormalities.[2] The grave side-effects due to the distomers have now made administering a single pure isomer absolutely vital. In short, different pharmacodynamics and pharmacokinetics of the eutomer (active isomer) and the distomer in a racemic drug can lead to a variety of side-effects attributable to inactive isomers. Thus it becomes very important to synthesize and administer chiral molecules in the enantiomerically pure form, and asymmetric synthesis, kinetic resolution of racemates, preferential/diastereomeric crystallization have hence gained strategic importance. Before dwelling into the topic of this section, a brief outline of the means of preparation of optically pure compounds and methods for determining the optical purity is essential.

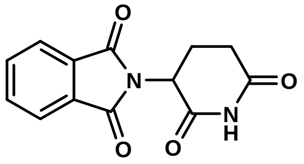


Figure 1 Thalidomide

1A.1.2 Routes to Enantiomerically Pure Compounds

In organic synthesis, apart from the vast chiral pool available in Nature, there are two approaches that can be employed to generate optically pure compounds, *viz* (i) asymmetric synthesis and (ii) resolution of racemates. Asymmetric synthesis involves the creation of a new chiral centre from a non-chiral or a prochiral carbon using a chiral reagent/auxiliary/catalyst, the latter two being more effective and widely used in modern organic preparations. Resolution of racemates may be effected either by diastereomeric recrystallization or kinetic resolution. In diastereomeric crystallization a solution of racemate is allowed to interact with a pure enantiomer (the resolving agent) thereby forming a mixture of diastereomers that can be separated by fractional crystallization. The most commonly used resolving agents are L-(+)-tartaric acid, D-(−)-camphorsulfonic acid and various alkaloid bases. A kinetic resolution depends on the fact that two enantiomers of a racemate react at different rates with a chiral reagent or catalyst, such as an enzyme. Herein, the chiral reagent/catalyst selectively recognizes the enantiomers and forms a fitting and a misfitting diastereomeric substrate-catalyst-transition-state complex that differ in transition state energy (ΔΔG#, Figure 2) and thus the enantiomers are resolved. Although stereoselective metal-catalyzed transformations can be employed to effect kinetic resolutions, biocatalysis has turned out to be the most advantageous method for this purpose and is being increasingly adopted in the industry as well.[3-5] The main reason for this tremendous surge can be attributed to the considerable advantages offered by enzymes.

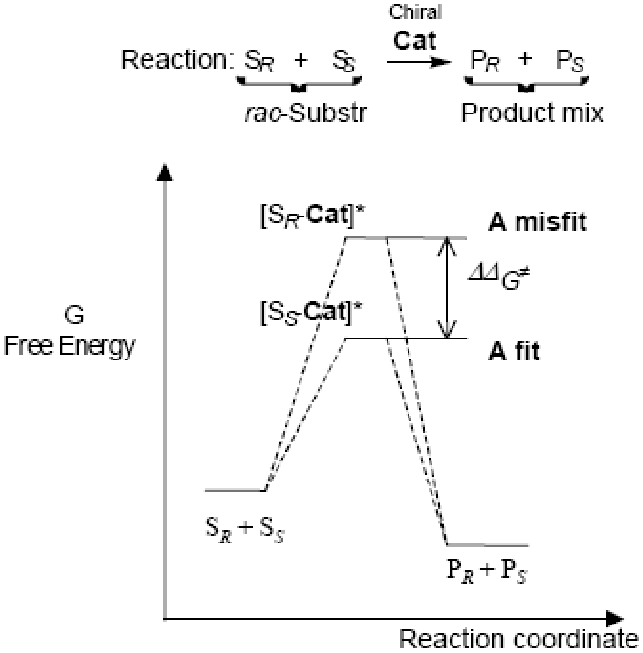


Figure 2 Reaction energy profile for the kinetic resolution of enantiomers: SR and SS represent the substrate; while PR and PS are the products. The two peaks in the reaction coordinate correspond to the transition states of the enantiomers

A cursory appraisal of the relative economics of asymmetric synthesis versus kinetic resolution would seem to indicate a clear preference for the former since it has a theoretical yield of 100% as compared to 50% for kinetic resolution. However, kinetic resolution has some advantages over asymmetric synthesis such as (i) experimentally simple processes, (ii) tuning of the enantiomeric excess by adjusting the degree of conversion. The major disadvantage of kinetic resolution is that they require at least one extra step for racemization of the unwanted isomer for recycling to increase the yield. This can be circumvented if spontaneous *in-situ* racemization of the substrate is carried out continuously, maintaining a 1:1 R/S ratio and thus allowing for a theoretical yield of 100%. This is called dynamic kinetic resolution[6] (Figure 3) and examples are illustrated later in the section.

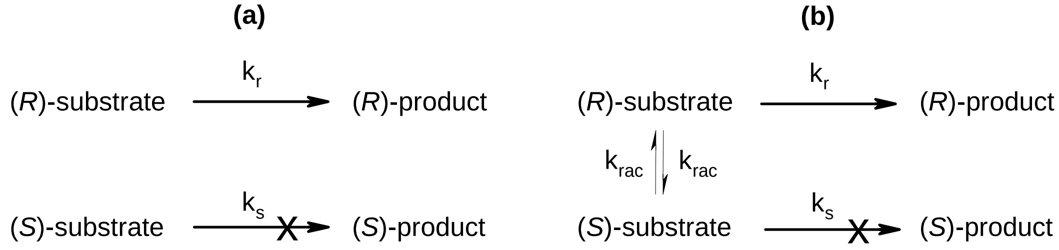


Figure 3 (a) Conventional kinetic resolution, (b) Dynamic kinetic resolution

1A.1.3 Enantiomeric Purity Determination

The importance of optical purity in the context of the field of pharmaceuticals has created a growing need for accurate unequivocal methods for the determination of optical purities.

In an enantiomerically enriched mixture, the enantiomeric excess (ee), as a percentage, is usually used to state the enantiomeric purity of a compound. Recently, Gawley has mentioned that enantiomeric ratio (er) is best descriptor of stereoselectivity, not enantiomeric excess (ee).[7] Various techniques are available for determining the ee:

(i) *Optical Rotation*

This classical and simple method commonly used in organic chemistry involves the determination of specific optical rotation of the compound. The optical purity of the substance is then determined from its optical rotation [α] and the optical rotation of the pure enantiomer $[\alpha]_o$ according to the equation:

$$\text{Optical Purity} = \frac{[\alpha]}{[\alpha]_o} \times 100$$

In practice, this may often lead to some confusion since the optical rotation is dependent upon various conditions of measurements such as solvents, temperature, purity etc. and ambiguity may exist if the compound is new or not well documented in literature.

(ii) *HPLC Methods*

As enantiomers have the same adsorption properties, they are not amenable to direct chromatographic separation on achiral adsorbents. However, this can be accomplished *via* the formation of diastereomers either by derivatization of sample with suitable chiral reagent[8] or formation of transient diastereomers via the interaction of enantiomers with chiral stationary phase/mobile phase additive.[9]

(iii) *GLC Methods*

For compounds that are readily vaporized without decomposition, gas chromatography on chiral stationary phase constitutes an accurate and reliable method for enantiomeric purity determination. The technique has inherent advantages of simplicity, speed, reproducibility and sensitivity.[10]

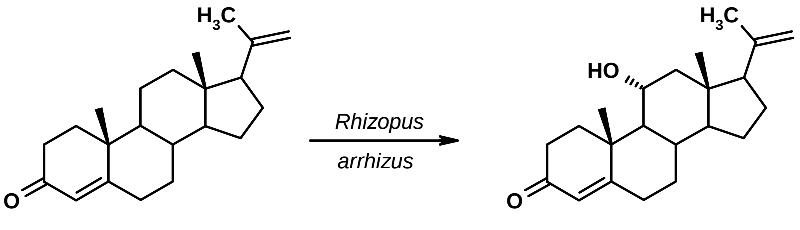
(iv) *NMR Methods*

NMR is a widely used technique for enantiopurity determination and one well-tested method involves conversion of mixture of enantiomers to a mixture of diastereomers by optically pure reagents eg. Mosher's reagent.[11] A closely related method employs the use of chiral Lanthanide shift reagents (LSRs),[12] having the property of shifting the NMR signals of substrates *via* diastereomeric complex formation.

1A.2 Enzymes as Catalysts: A Prologue

1A.2.1 Biotransformations

The term enzyme was coined by Kanne in 1876 and experimentation on enzymes began in 1897. An enzyme is born through repeated "trial & error" in Nature over an enormous length of time. Active sites of enzymes evolved to allow the enzymes to mediate biological reactions under ambient conditions and thus they serve as excellent biological "catalysts", forming a bridge between chemistry and biology. In biotransformations, enzymes are employed to consequent chemical changes on compounds that are not their natural substrates. This distinguishes biotransformation from biosynthesis, which involves action of biological systems in their normal habitat upon their natural substrates. In some biotransformations the synthetic substrate may resemble the natural substrates, while in some others it may be completely unrelated to the natural system. Whilst both may be used for synthetic purposes, the former can focus some light on structural and mechanistic features of biosynthesis. This type of study could be useful for the synthetic chemist in planning retrosynthetic approaches. In biotransformations, isolated enzyme systems or intact whole organisms may also be used. Many biotransformations are not only chemo- and regioselective but are also enantioselective allowing the production of chiral materials from racemic mixtures. The conditions for biotransformations are mild and in majority of cases do not require the protection of other functional groups. Furthermore the features governing their regioselectivity differ from those controlling the chemical specificity and indeed it is possible to obtain biotransformations at centers that are chemically non-reactive (e.g. in Scheme 1, compound **1** is transformed to **2** by hydroxylation at the chemically unreactive C-11 site). From a commercial point of view some biotransformations can be cheaper and more direct than their chemical analogues, whilst the transformations proceed under the conditions that are normally regarded as "environmentally friendly". There are many chemical reactions for which there are no equivalent biotransformation steps and the chemist should be versatile to use biotransformations in combination with the conventional chemical reagents in a synthesis. It is least surprising that biocatalysis has emerged as such an important tool in organic synthesis considering the advantages of using enzymes such as easy availability, mild and



Scheme 1

simple reaction conditions, good economy, low environmental impact and recyclability. A number of comprehensive reviews[13-27] and monographs[28-36] in recent literature only goes to reiterate the importance of biotransformations. These reviews provide an elegant overview on diverse aspects of biotransformations in organic synthesis.

1A.2.2 Specificity of Enzymes

Enzymes consist of proteins that are built up in Nature from twenty different amino acids. The catalytic ability of enzymes depends upon their three-dimensional architectures, which are basically determined by the L-amino acid sequence. The 3-D structure of an enzyme often reveals that it possesses an active site, where the reaction takes place. A rational explanation for the specificity of enzymes is the three-point attachment rule. In order for a substrate molecule to be held firmly in 3-D space, there must be at least three points of attachment of the substrate onto the active site. In a racemic substrate, only one isomer would possess a complementary structure, that is, the groups are correctly aligned and fit into the active site pockets, while the opposite isomer turns out to be a misfit and does not react.

1A.2.3 Classification of Enzymes

Enzymes are classified by rules prescribed by the commission on Enzymes of the International Union of Biochemistry, according to which each enzyme is designated by four numbers; the main class, the subclass, sub-subclass, and the serial numbering the sub-subclass. There are six main classes as shown:

1. **Oxidoreductases**: These enzymes mediate oxidation and reduction, including the insertion of oxygen to alkenes. This group also includes enzymes that are responsible for the addition or removal of hydrogen.
2. **Transferases**: These enzymes are involved in the transfer of one group, such as an acyl or a sugar unit from one substrate to another.
3. **Hydrolases**: This group includes the enzymes that mediate the hydrolysis or formation of amides, epoxides, esters and nitriles.
4. **Lyases**: These are group of enzymes that fragment larger molecules with the elimination of smaller units.

5. **Isomerases**: These enzymes are involved in epimerization, racemization and other isomerization reactions.
6. **Ligases**: This group includes the enzymes responsible for the formation of C-C, C-O, C-S and C-N bonds.

Units: The international unit (I.U.) of any enzyme activity is described as millimoles of substrate utilized per minute or millimoles of product formed per minute and the specific activity is defined as activity per milligram of enzyme.

1A.3 Lipases: Introduction

1A.3.1 Occurrence and Role

Hydrolases form the most important class of enzymes[32] and among them, lipases have been the most popular and widely used. Lipases are ubiquitous enzymes that are found in bacteria/fungi,[37-39] plants[40,41] and animals.[42,43] They are triglycerol ester hydrolases defined in class EC 3.1.1.3 by enzyme nomenclature. Usually lipases are just one member of a "hydrolytic enzyme cocktail" elaborated by an organism with the objective to sustain its growth. In general, cells produce lipases to hydrolyze the extracellular fats and lipases are specially structured to act at water/organic interface (they undergo an interfacial activation leading to a large increase in hydrolytic activity).[23,44] For this reason lipases appear to have optimum property among the enzymes to operate in organic solvents, in this case the interface is between the insoluble enzyme with its essential water of hydration and the organic solvent containing the acylating agent.

1A.3.2 Structure and Mechanism

The first two lipase structures were solved in 1990 by X-ray crystallography, which revealed a unique mechanism, unlike that of any other enzyme. Their three dimensional structures suggested that interfacial activation is due to the presence of an amphiphilic peptidic loop covering the active site of the enzyme in solution, just like a lid or a flap.[45a,b] From the X-ray structure of co-crystals between lipases and substrate analogue, there is a strong evidence that, upon contact with a lipid/water interface, the lid undergoes a conformational rearrangement which renders the activation site accessible to the substrate.[45c] The molecular machinery of lipases is much like that of the serine protease's.

The active site is generally characterized by the triad composed of the amino acids serine, histidine, and aspartic/glutamic acid, acyl-enzyme complexes being the crucial intermediates in all lipase-catalyzed reactions. The system operates through a charge-relay system *via* hydrogen bonds as shown in Figure **4**. The mechanism has been termed as the ping-pong bi-bi mechanism.[46] The structural shape of the protein creates hydrophilic or lipophilic pockets within the enzyme active site, which leads to chemo-, regio- and enantio/diastereoselectivity.

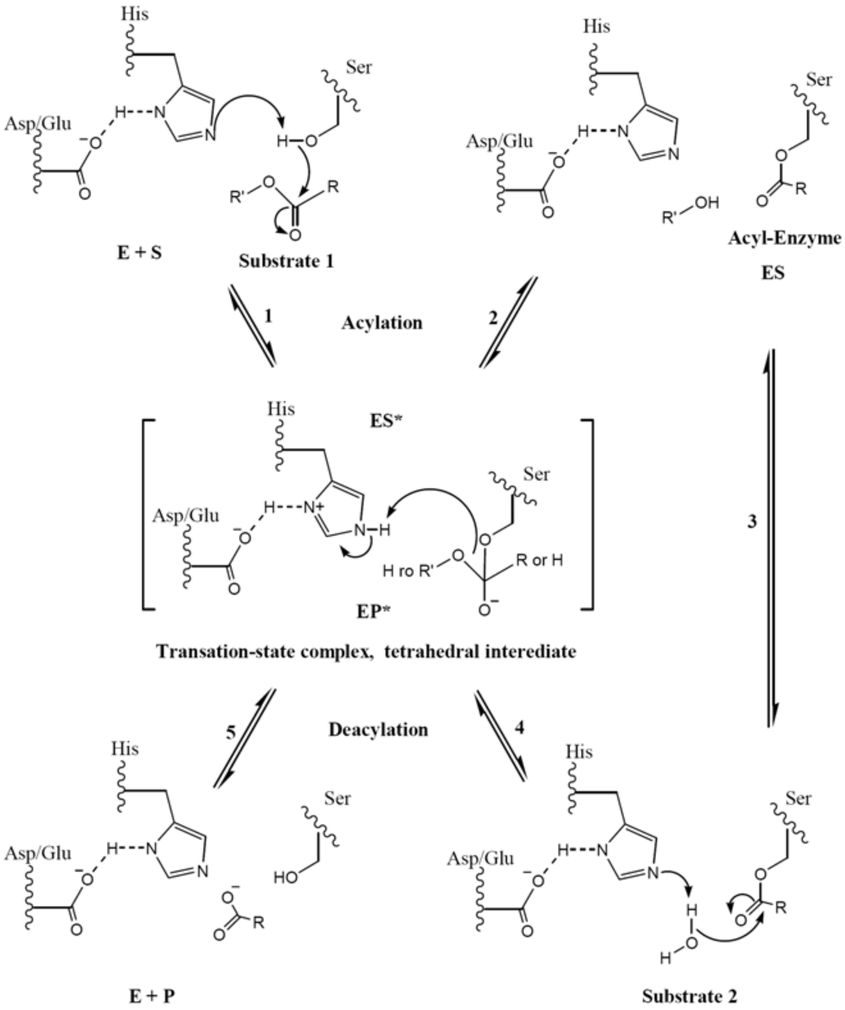


Figure 4 Catalytic mechanism of lipase action through procedure 1-5, where E is the enzyme, ES* & EP* are the substrate-enzyme and product-enzyme complexes respectively

(*Structure numbers in the above figure are from the original reference and do not correlate to the structure numbers in the present section*).

1A.3.3 Semiquantitative predictions for Lipase Selectivity

Following a thorough survey of the literature on chiral resolutions with lipases from *Candida rugosa* (CRL) and *Pseudomonas cepacia* (PCL), Kazlauskas et al. put forward tentative rules for the enantiopreference of these two enzymes based on the spatial requirements of the substituents on the reagent. These rules are often dubbed "Kazlauskas rules".[47] Experience has shown that the rules are highly predictive for lipase action on secondary alcohols, but less accurate for lipase-catalyzed transformations of primary alcohols and acids.

1A.4 Lipases as Biocatalysts in Organic Synthesis

1A.4.1 Foreword

Considering their specific and limited function in metabolism, one should expect lipases to be of limited interest for the organic chemist. However, chemists have discovered lipases to be one of the most versatile classes of biocatalysts in organic synthesis for a few simple reasons:

1. Owing to the large enzyme structural domains required for the acyl group binding and the unpronounced structural features of acyl chains, lipases can accommodate a wide variety of synthetic substrates, while still showing chemo-, regio, and/or stereoselectivity.
2. Lipases act at the water/lipid boundary, which exhibits high interfacial energy. To withstand the denaturing effect of the interface, lipases have evolved unusually stable structures that may survive even the effect of organic solvents.
3. The free energy of fat hydrolysis is close to 0 kJ mol-1.[48] As a result, thermodynamic equilibria are largely governed by the reactant concentrations, and lipase catalyzed ester hydrolysis in water can easily be reversed, in non-aqueous media, into ester synthesis or transesterification.
4. The acyl lipase formed in the first step of the enzymatic reaction can formally be considered as an acylating agent. The wide substrate specificity of this enzyme class allows acylation of nucleophiles other than those with hydroxyl groups, for example hydroperoxides, thiols and amines.

It must be mentioned here that the use of organic solvents for lipase-catalyzed reactions[49-51] has added a whole new perspective (in neat organic solvents enzymes retain the minimum amount of water which is necessary for their catalytic activity). This is because of the obvious advantages such as (i) increased substrate solubility and wider range of reactants, (ii) transformations of water-sensitive substrates, (iii) ease of operation and (iv) modified enzyme specificity. Moreover, the use of organic solvents is seen to enhance the enantioselectivity[52] and thermostability[53] of the enzymes, probably due to restricted conformational flexibility. Despite the advantages, enzymes nearly universally display low catalytic activities in non-aqueous conditions[54] compared to native aqueous solutions. One of the most influential parameters affecting enzymatic activity in aqueous solutions is pH. However, this does not operate in organic solvents since, in organic media, enzymes have a "pH memory": their catalytic activity reflects the pH of the last aqueous solution to which they were exposed.[49] Consequently enzymatic activity in organic solvents can be enhanced if enzymes are lypophilized from aqueous solutions of pH optimal for their catalysis. Various other techniques and approaches have been employed that have resulted in increasing enzymatic activities in organic solvents by up to three to four orders of magnitude.[54]

In a nutshell, the versatility and popularity of lipases could be attributed to their high catalytic efficiency on a broad range of substrates (they can accommodate substrates other than triglycerides such as aliphatic, aromatic, alicyclic and bicyclic esters including the esters based on organometallic sandwich compounds), combined with high regioselectivity and chiral recognition,[23] their high stability in organic solvents and at elevated temperatures,[23,49] the reversibility of their mode of action,[23,55] their non-toxic and environment friendly nature[18] and finally their low cost. In practice, lipases are very easy to handle. Hydrolysis is usually performed in a biphasic system consisting of an aqueous buffer and an organic solvent while esterification is effected in an organic solvent with an irreversible acyl donor[55] such as the enol ester vinyl acetate. The enzyme is conveniently removed by filtration during work-up.

It is not the intention of this review to comprehensively summarize all lipase-catalyzed transformations in the recent past simply because of the enormous literature on hand (excellent reviews on biocatalysis, as mentioned earlier, have appeared in recent years many of which have been exclusive lipase reports.[13,15,21,23,26] The reviews by S. M. Roberts,[19] especially, provide wonderful detailed descriptions). Lipases, as all the above literature would tell us, can be used for a plethora of reactions, simple acylation and deacylation, synthesis of amides and peptides, regioselective reactions on polyfunctional compounds, to mention only a few, and to encompass everything is beyond the scope of this section. Instead, only alcohol substrates have been taken up since those are the substrates that have been extensively studied for lipase catalysis. Within this realm, interesting examples that have had significant impact over the last three years have been chosen and illustrated. Some of the useful transformations have been presented in a tabular form providing the appropriate reference alongside. The section also looks to throw light on desymmetrization of prochiral & *meso*-substrates and dynamic kinetic resolution, wherein, the chemical yield of the resolution can be remarkably improved.

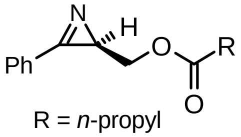
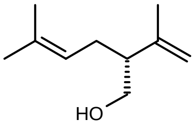
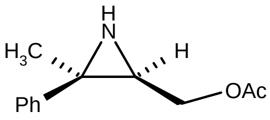
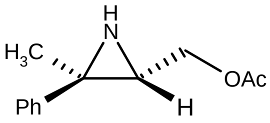
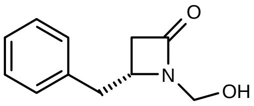
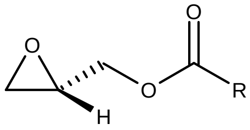
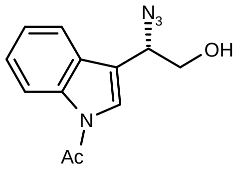
1A.4.2 Recent Applications of Lipase Catalysis in the Resolution of Alcohols

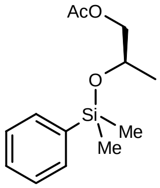
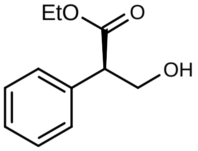
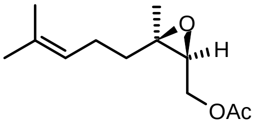
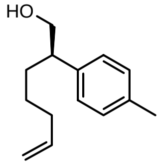
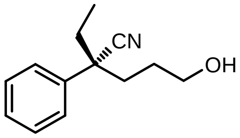
The most extensively studied substrates, as mentioned before, for lipase catalysis are alcohols. This can be attributed to the extremely good selectivities shown by lipases when acting upon alcohols as well as the vast and diverse utilities of chiral alcohol substrates in research and industry. The present section will portray some elegant reports of lipase catalysis in 1°, 2° & 3° alcohol substrates covering both enantioselective lipase-catalyzed hydrolysis as well as transesterification using acyl donors have been taken up. The “products” depicted in the tables in the following sections indicate the actual product formed from the recognized isomer in the lipase-catalyzed resolution. Entries wherein the non-recognized isomer of the substrate isomer is depicted have been indicated as “unreacted isomer” within brackets below the compound structure. Tables contain examples of both lipase-catalyzed hydrolysis as well as transesterification reactions.

1A.4.2.1 Primary alcohols

Homochiral primary alcohols are useful building blocks for the synthesis of a wide range of biologically active compounds. The kinetic resolution of racemates of primary alcohols by lipase-catalysis is more difficult to achieve due to lower enantioselectivities of lipases towards chiral primary alcohols. Lipase from *Pseudomonas cepacia* (PSL) has been most efficient, exhibiting high enantioselectivity towards a broad range of primary alcohols. Some primary alcohol substrates that have been successfully resolved using lipases are presented in Table 1. Illustrations of a handful of other appealing reports are presented subsequently.

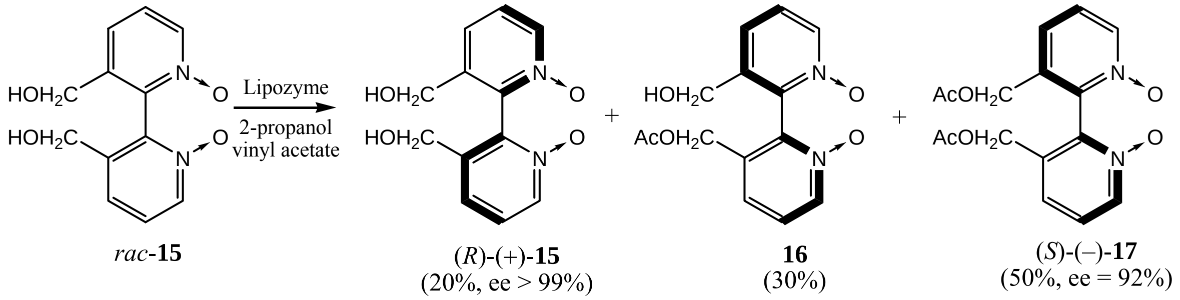
Table 1 Selected examples illustrating the lipase-catalyzed resolution of primary alcohols

Product	**Lipase**	**% Yield**	**%Ee**	**Reference**
 R = *n*-propyl **3**	PCL (on Toyonite)	35 (conv.)	97	56
 **4** (unreacted isomer)	*Hog pancreas*	85 (conv.)	90-96	57
 **5**	Lipase PS-C II	46 (conv.)	92	58
 **6**	Lipase PS-C II	30 (conv.)	96	58
 **7** (unreacted isomer)	Lipase PS	55 (conv.)	98	59
 R = *n*-propyl **8** (unreacted isomer)	CAL-B	64 (conv.)	90	60
 **9**	Lipase PS	42	83	61

 **10**	Lipase PS-D	51 (conv.)	99	62
 **11**	Lipase PS	46	94	63
 **12**	Lipase PS	16	~ 99	64
 **13**	Lipase PS	40	> 99	65
 **14** (unreacted isomer)	PCL	66 (conv.)	99	66

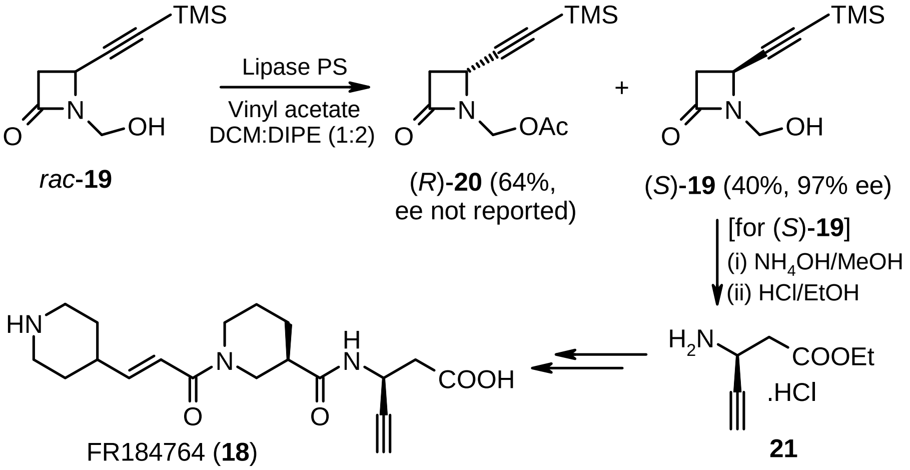
Apart from the above-displayed examples, there are some other interesting reports, as described below, wherein the primary alcohols resolved have served as intermediates for the preparation of important chiral compounds and natural products.

Chiral 2,2'-bipyridine-type ligands (bipys) have recently received considerable attention in the field of stereoselective organic synthesis and transition metal catalysis. Sanfilippo et al reported the first example of an enzymatic resolution of a bipyridine *N,N*-dioxide in the unusual medium of alcohol/vinyl acetate (1:4).[67] Lipase from *Mucor miehei* (immobilized lipase preparation, Lipozyme) was found to give good enantioselectivity in the resolution of atropisomeric (±)-3,3'-bis(hydroxymethyl)-2,2'-bipyridine *N,N*-dioxide (**15**) with an (a*S*)-enantiopreference in the axial recognition, and allowed to efficiently perform the preparation of both enantioforms with ee >98% (Scheme 2).



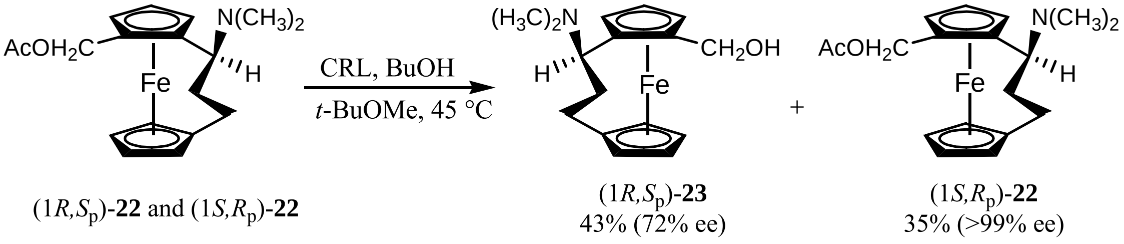
Scheme 2

Yamanaka et al. achieved an efficient synthesis of the orally active GpIIb/IIIa antagonist FR184764 (**18**), wherein, the key intermediate, an optically active ethynyl *β*-amino ester, was prepared utilizing a lipase catalyzed resolution (Scheme 3).[68]



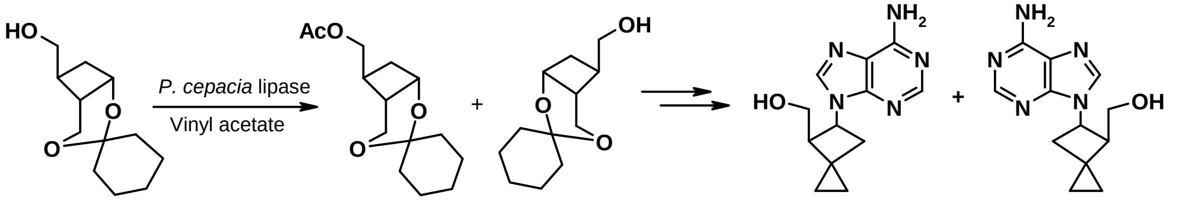
Scheme 3

A series of enantiopure 1,4-amino alcohols with a [3]ferrocenophane backbone were synthesized by Faux et al. employing a *Candida rugosa* lipase-catalyzed resolution as the key step (Scheme 4). The new ligands were used in the asymmetric ethylation of benzaldehyde by diethylzinc and gave good catalytic properties.[69]



Scheme 4

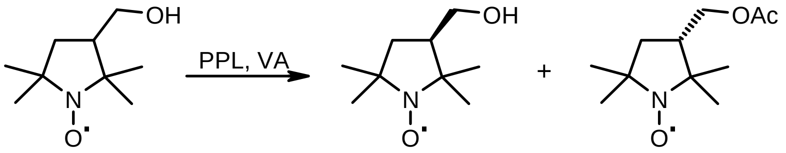
Four-membered ring containing nucleosides are interesting compounds that have been studied continuously in the recent past to explore their chemistry and more importantly, their antiviral activity. Chu et al. demonstrated an elegant *Pseudomonas cepacia* lipase-catalyzed resolution of racemic compound **24**, synthesized in 7-steps starting from diethoxyketene and diethyl fumarate, to give the acetate (+)-**25** and the alcohol (−)-**24** (Scheme 5). The enantiomerically enriched compounds were transformed to novel spiro[2.3]hexane carbocyclic nucleosides, *R*- & *S*-9-(6-hydroxymethylspiro[2.3]hexane)-4-adenine, [(*R*)-**26** & (*S*)-**26**], possessing 98% & >99% ee respectively.[70] Both the compounds exhibited moderate antiviral activity.



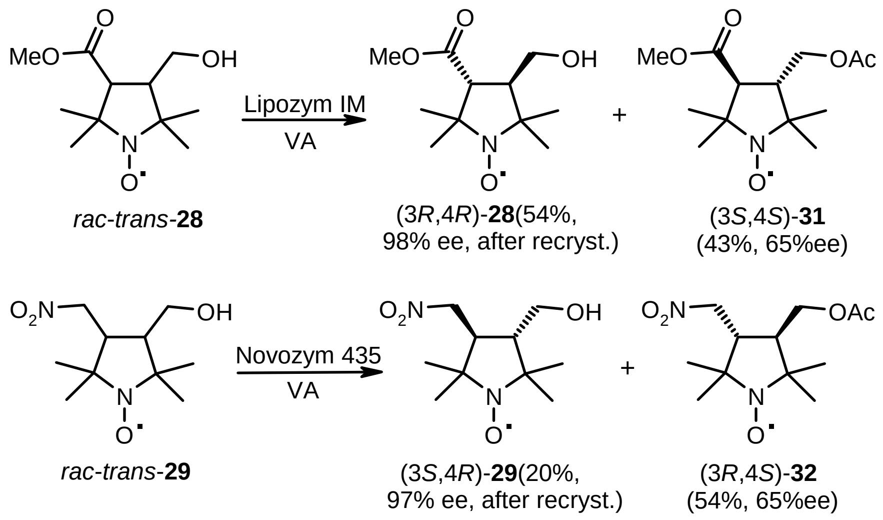
rac-**24** (+)-**25** (-)-**24** (*R*)-**26** (98% ee) (*S*)-**26** (>99% ee)

Scheme 5

An important role for nitroxide free radicals is spin labeling for exploration of protein structure dynamics and function. A new technique of site-directed spin labeling is based on the synthesis of cysteine point mutant peptides followed by labeling with the 1-oxyl-3-methanesulfonylthiomethyl-2,2,5,5-tetramethylpyrrolidine radical (sat-MTS-SL), containing a stereo centre. Balint et al. have reported the resolution of three target molecules (1-oxyl-3-hydroxymethyl-2,2,5,5-tetramethylpyrrolidine (**27**), 1-oxyl-3-hydroxymethyl-4-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (**28**) and 1-oxyl-3-hydroxymethyl-4-nitromethyl-2,2,5,5-tetramethylpyrrolidine (**29**) as starting compounds for spin labels (Scheme 6).[71]

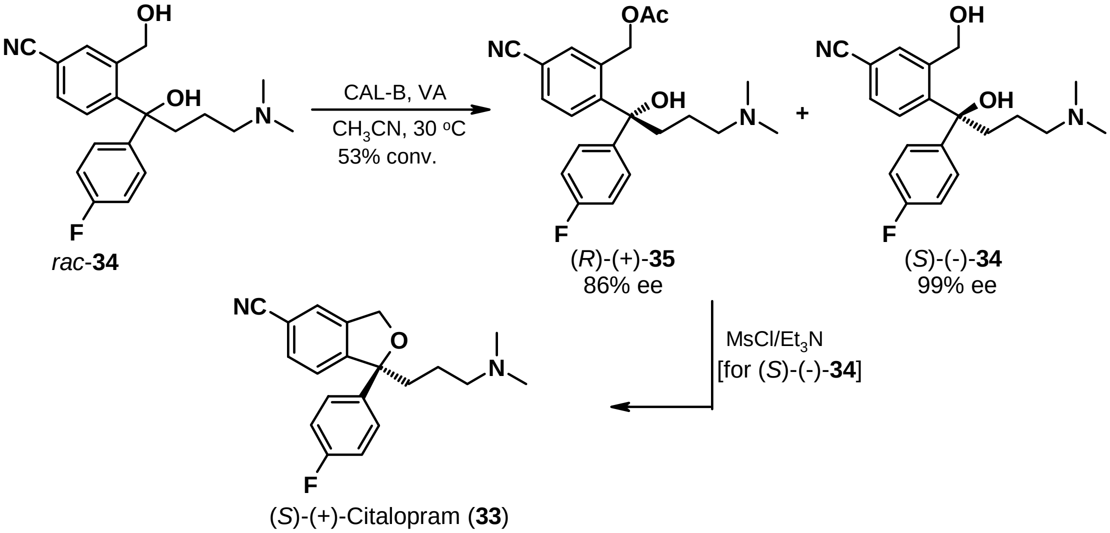


rac-**27** (*R*)-(+)-**27**(54%, 98% ee, after recryst.) (*S*)-(-)-**30** (34%, 65%ee)



Scheme 6

(*S*)-(+)-Citalopram (**33**) is a very selective inhibitor of serotonin (5-HT) reuptake that has proved to be an efficient antidepressant. Gotor et al. studied the enzymatic resolution of hydroxymethyl benzonitrile **34** (carrying a quaternary stereo centre), a useful intermediate in the synthesis of enantiomerically pure citalopram. They reported that *Candida antartica* lipase B (CAL-B) catalyzes the enzymatic acetylation of the primary benzylic alcohol with high enantioselectivity at the quaternary stereogenic centre placed four bonds away from the reaction site (Scheme 7).[72]



Scheme 7

Gotor et al have also carried out an exhaustive study of the resolution of 3-amino-3-phenyl propan-1-ol derivatives. They observed that the best conditions were actually when the primary alcohol itself was protected (as its silyl ether) and an enantioselective acylation of the amino group was brought about using CAL-A with ethylmethoxy acetate as the acyl donor.[73] The optically enriched intermediate was further elaborated to the natural product *S*-dapoxetine.

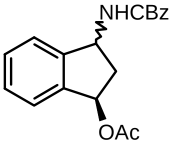
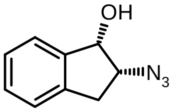
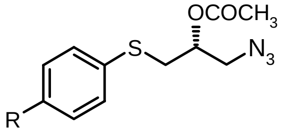
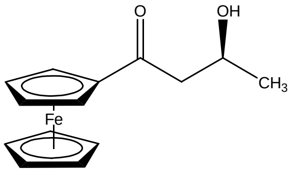
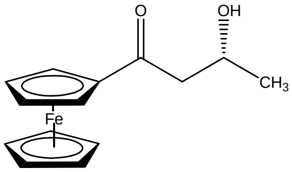
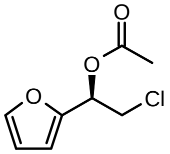
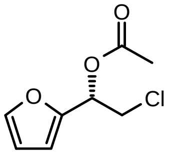
A few other natural products, the synthesis of which involved a lipase-mediated resolution as the key-step, are (*S*)-1,3-dihydroxy-3,7-dimethyl-6-octen-2-one, the male-produced aggregation pheromone of the Colorado potato beetle, the weakly antibacterial norsesquiterpene 5,6-dehydrosene digitalene and (*R*)-(+)-aminoglutethimide, an aromatase inhibitory drug (Table 1, entries 13-15).

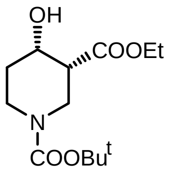
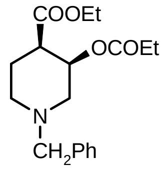
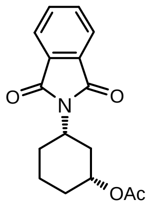
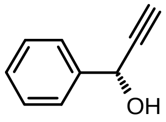
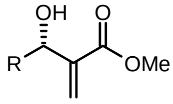
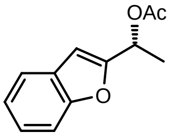
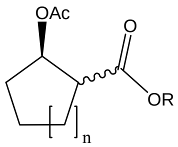
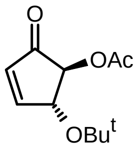
Another interesting application of lipases in the context of primary alcohols has been in the regioselective acylation reactions. Reports of regioselective transformations catalyzed by lipases can be found in the protection of α,α’-diols, α,ω-diols, including terpene diols,[74-77] 3(*R*)-(+)-bis[hydroxymethyl]-1,1-dimethoxycyclobutanone[78] and also in the synthesis of steroids.[79]

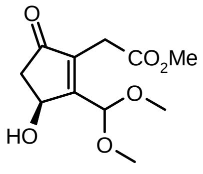
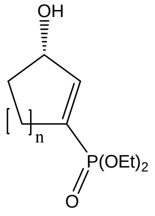
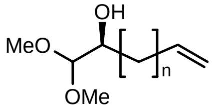
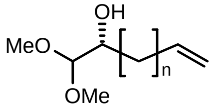
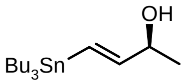
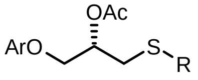
1A.4.2.2 Secondary Alcohols

Secondary alcohols are by far the most widely explored substrates in lipase-catalyzed resolutions. This is not only due to the importance of chiral secondary alcohols in organic synthesis (they constitute a class of industrially important aroma and flavour compounds) but also that lipases usually show much higher enantioselectivity in the case of secondary alcohols as against primary and tertiary ones. A gamut of examples with tremendous structural diversity can be found in the literature in just the last 2-3 years. A few reports have been presented below in a tabular format with the corresponding reference alongside (Table 2). Subsequently, another table presents some important natural products that have been synthesized employing lipase-catalyzed resolution of a secondary alcohol as the key step (Table 3). As in the section covering primary alcohols, brief descriptions of other interesting reports will follow Table 3.

Table 2 Selected examples of lipase-catalyzed resolution of secondary alcohols

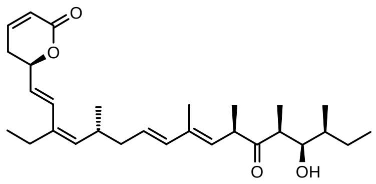
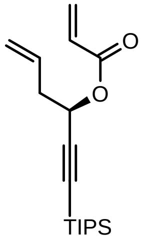
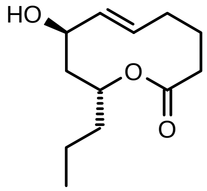
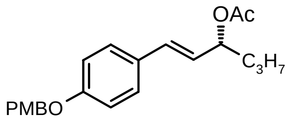
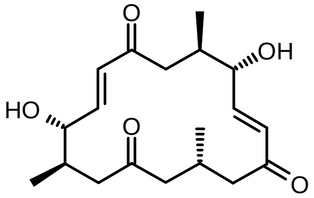
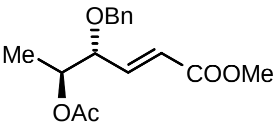
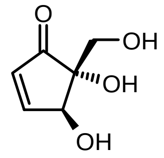
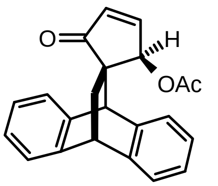
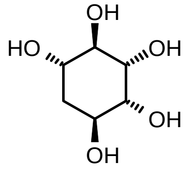
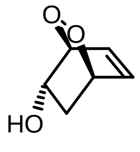
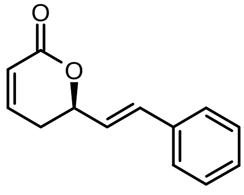
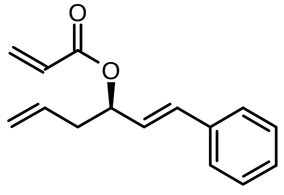
Product	Lipase used	% Yield	% Ee	Reference
 *syn & anti* **36**	CAL-B	49/48	~ 99	80
 **37**	Amano AK	33	99	81
 R = H; Cl; CH_3 **38**	CAL-B	35 - 42 (conv.)	92 - 96	82
 **39**	Lipozyme	-	> 99	83
 **40**	Novozym 435	-	> 99	83
 **41**	*C. antartica*	38	99	84
 **42**	*P. cepacia*	40	96	84

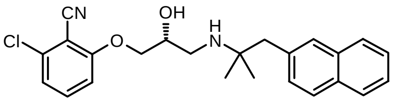
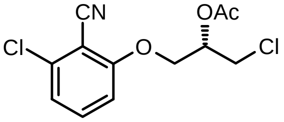
 **43**	Lipase AK	50 (conv.)	99	85
 **44**	CAL-A	27 (conv.)	96	85
 **45**	CAL-B	49 (conv.)	99	86
 **46**	Novozym 435	43	93	87
 R = Me; *n*-propyl **47** (unreacted isomer)	PSL (in poly(ethylene) oxide)	50/36 (conv.)	99/56	88
 **48**	Lipase AK	48	99	89
 *cis* & *trans* R = Me/Et; n = 1-3 **49**	CAL-B	50 (conv.)	> 99	90
 **50**	Lipase PS	50	95	91

 **51**	CAL-B	48	94	92
 n = 1,2,3 **52**	Amano PS/AK	47-48	> 99	93
 n = 0,1 **53**	CAL-B/PFL	50 (conv.)	93/92	94
 n = 0,1 **54**	CAL-B/LPL	50 (conv.)	94/98	94
 **55**	Lipozyme	48	99	95
 Ar = C_6H_5-, 4-CH_3-C_6H_4- R = Et, *n*-Bu, *tert*-Bu **56**	Amano AK	21-62 (conv.)	24-86	96

As mentioned earlier, synthesis of natural products in their enantiomerically pure form is of huge significance, to say the least. Lipase-catalyzed resolution of secondary alcohol intermediates have served as key steps in the total synthesis of several important natural products. In the case of complex natural products, such a resolution in the early phase of the synthesis not only provides an easy and economic access to the all-important chiral intermediate in a highly enantioselective fashion but also sets the stage for the introduction of contiguous chiral centres in a stereoselective fashion. A handful of such examples have been enlisted in Table 3 with the intermediate involved in the synthesis depicted alongside.

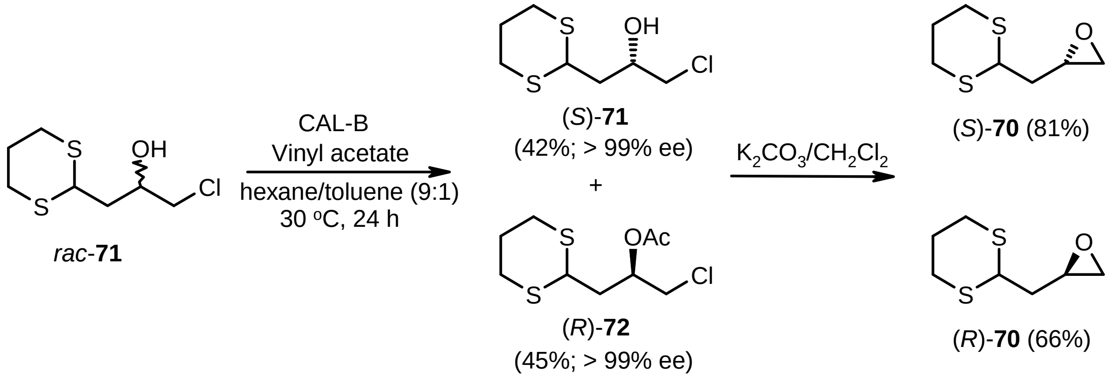
Table 3 Examples of natural product synthesized with the corresponding chiral intermediate prepared by lipase catalyzed resolution

Natural product	Intermediate (yield)	Lipase used	% Ee	Ref.
 (−)-Callystatin A (**57**)	 **58** (46%)	Lipase *P.* AK	> 95	97
 (+)-Herabrumin III (**59**)	 **60** (−)	CAL-B	98	98
 (+)-Macrosphelide (**61**)	 **61a** (48%)	Amano PS	>99	99
 (−)-Epipentenomycin (**62**)	 **63** (37%)	Amano PS-D	> 98	100
 (+)-*proto*-Quercitol (**64**)	 **65** (47%)	CCL	91	101
 (+)-Goniothalamin (**66**)	 **67** (38%)	CAL-B	93	102

 (+)-NPS-2143 (**68**)	 **69** (47%)	Lipase PS-D	99	103

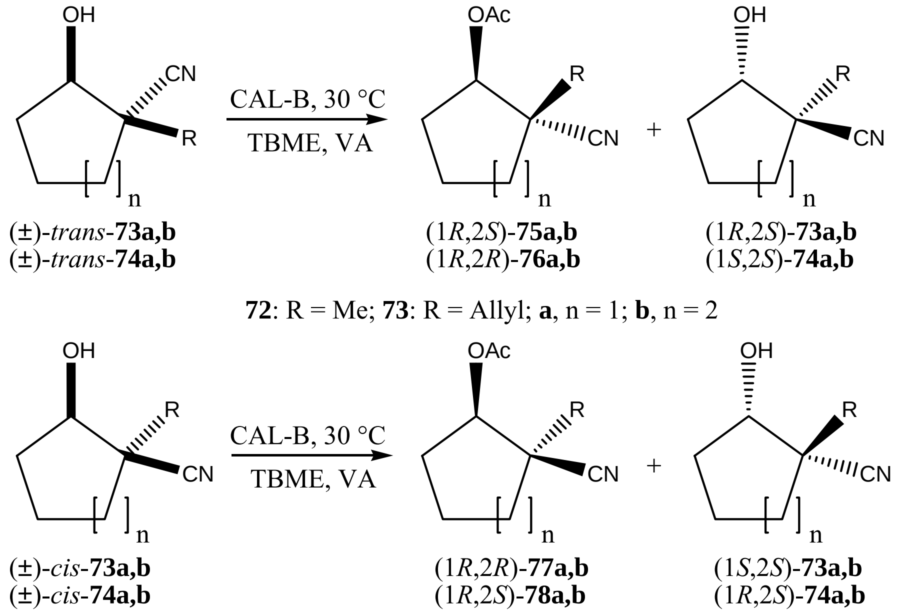
The diversity of the compounds that have been resolved by lipases, especially taking in to account their miscellaneous applications, is truly amazing. A few appealing reports have been illustrated below, revealing the variety in the substrates employed and their significance.

Enantiomers of 2-(1,3-dithian-2-ylmethyl)oxirane have been used to construct fragments of the cytotoxic macrolide Spongistatin,[104] the antiviral agent Hennoxazole A[105] and derivatives of maytansine.[106] An efficient chemoenzymatic synthesis of both enantiomers, (*R*)- & (*S*)-**70**, employing a *Candida antartica B* lipase-catalyzed resolution (Scheme 8) has been reported by Sundby et al.[107]



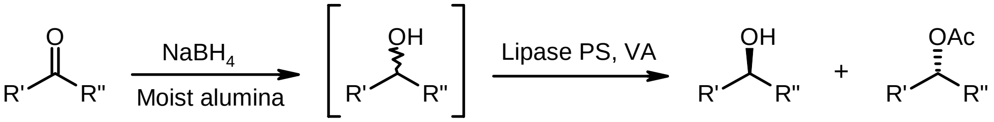
Scheme 8

The asymmetric construction of quaternary carbon centres represents a challenging task in organic synthesis. On the other hand, the importance of optically active *β*-hydroxy nitriles as suitable synthons for the preparation of *γ*-amino alcohols (like the antidepressant fluoxetine)[108] is steadily growing. Gotor et al., combining both the above targets have reported an elegant kinetic resolution of racemic *cis*- and *trans*-1-alkyl-2-hydroxycycloalkane nitriles **73** and **74** via enzymatic tranesterfication. Both five and six-membered substrates were studied and excellent enantioselectivities were observed with *Candida antartica B* lipase (Scheme 9).[109] The conversion was 50% in all cases because of the extremely good E-values (enantiomeric ratio) and the enantiomeric excess were ≥ 99% for all the stereoisomers.



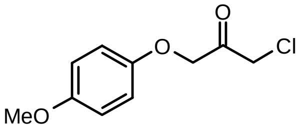
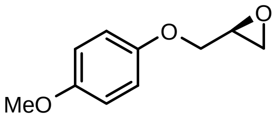
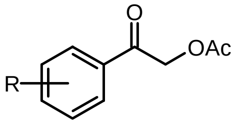
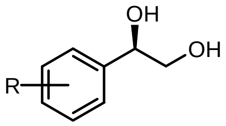
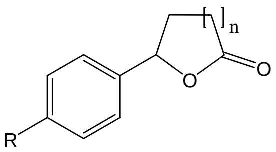
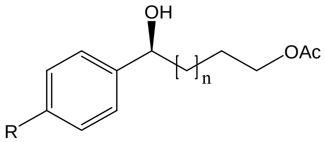
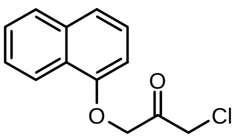
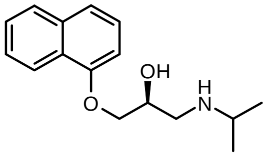
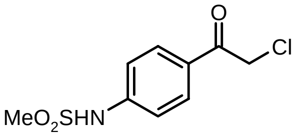
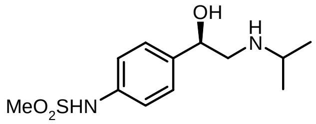
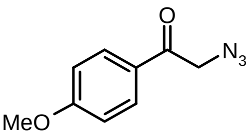
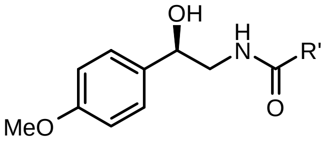
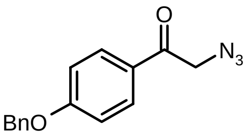
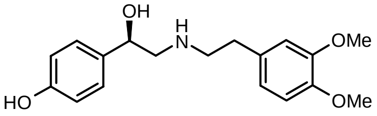
Scheme 9

Kamal et al. have carried out pioneering work in the field of lipase-catalyzed resolution of secondary alcohols. An attractive protocol developed by them was the one-pot synthesis of enantiomerically pure secondary alcohols from carbonyl compounds involving a tandem $NaBH_4$-reduction-lipase-catalyzed resolution.[110] A general illustration is provided in Scheme 10. Such integration of lipase catalysis into a reaction sequence can be rewarding in cases where stereoselective synthesis proves unsatisfactory. Indeed, Kamal and co-workers have applied this strategy for the synthesis of a variety of optically pure secondary alcohols with excellent enantioselectivities and also extrapolated a few of them to natural products. Some of the carbonyl substrates and the enantiopure end products (including natural products whose names are given) are illustrated in Table 4.

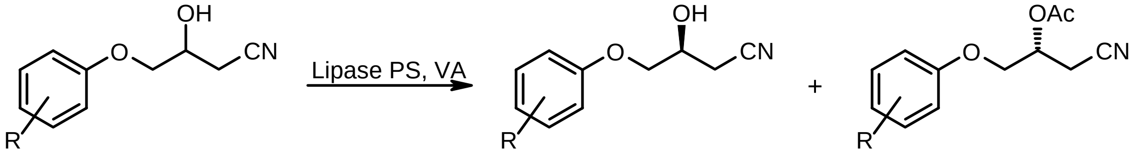


Scheme 10

Table 4 Applications of one-pot reduction-lipase-catalyzed resolution of carbonyl compounds

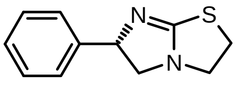
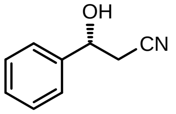
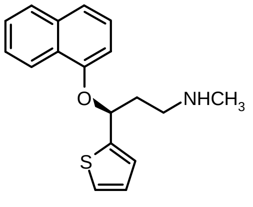
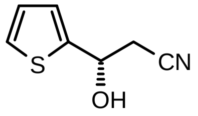
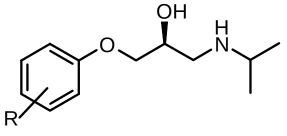
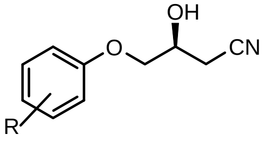
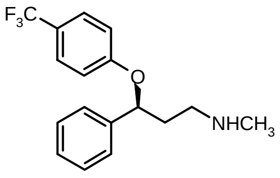
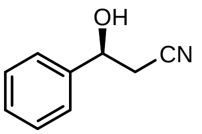
Carbonyl Substrate	End Product	Lipase used	Reference
 **79**	 **80** (99% ee)	Lipase PS-C	111
 R = H, *p*-Me, *p*-OMe **81**	 **82** (>99% ee)	Lipase PS-C	112
 R = H, OCH_3, Cl; n = 1,2 **83**	 R = H, OCH_3, Cl; n = 1,2 **84** (>99% ee)	Lipase PS-C	113
 **85**	 (−)-(*S*)-Propranolol (**86**, 96% ee)	Lipase PS-C	114
 **87**	 (−)-(*R*)-Sotalol (**88**, 90% ee)	Lipase PS-C	114
 **89**	 (*R*)-Tembamide (**90**): R’ = Ph (*R*)-Aegeline (**91**): R’ = -CH=CH-Ph (both in 98% ee)	Lipase PS-C	115
 **92**	 (*R*)-Denopamine (**93**, 99% ee)	Lipase PS-C	115

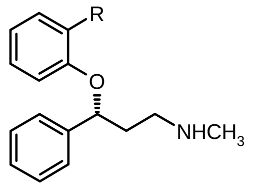
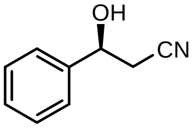
Kamal et al. have also shown continuing interest in the preparation of optically pure *β*-hydroxy nitriles[116] employing lipase-catalyzed resolution (Scheme 11) and their applications towards the preparation of biologically important compounds or intermediates. As in the case of the one-pot protocol, herein too, the strategy has been applied for the synthesis of some natural products (Table 5).



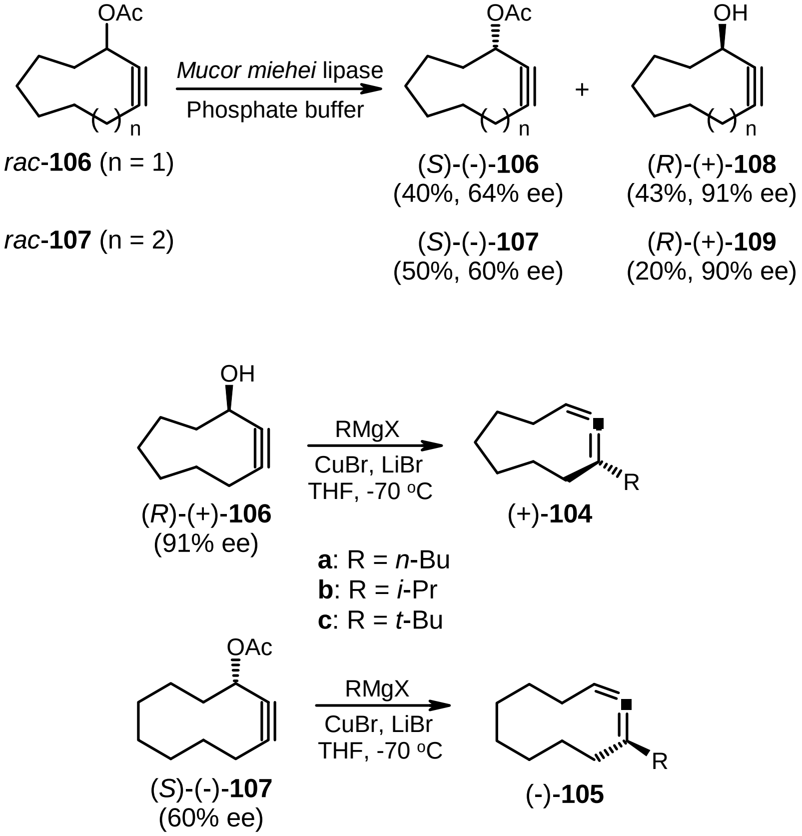
Scheme 11

Table 5 Examples of natural products with the corresponding optically pure *β*-hydroxy nitrile intermediates prepared by lipase-catalyzed resolution

Natural Product	Intermediate	Lipase used	Reference
 (−)-Levamisole (**94**)	 **95** (40%, >99% ee)	Lipase PS-C	117
 (+)-Duloxetine (**96**)	 **97** (42%, >99% ee)	Lipase PS-D	118
 R = 4-OCH_3, 2-(CH_2-CH=CH_2) *β*-Adrenergic blocking agents **98**	 R = 4-OCH_3, 2-(CH_2-CH=CH_2) **99** (43-45%, 90 - 99% ee)	Lipase PS-D	119
 (*S*)-Fluoxetine (**100**)	 **101** (46%, >99% ee)	Lipase PS-C	120

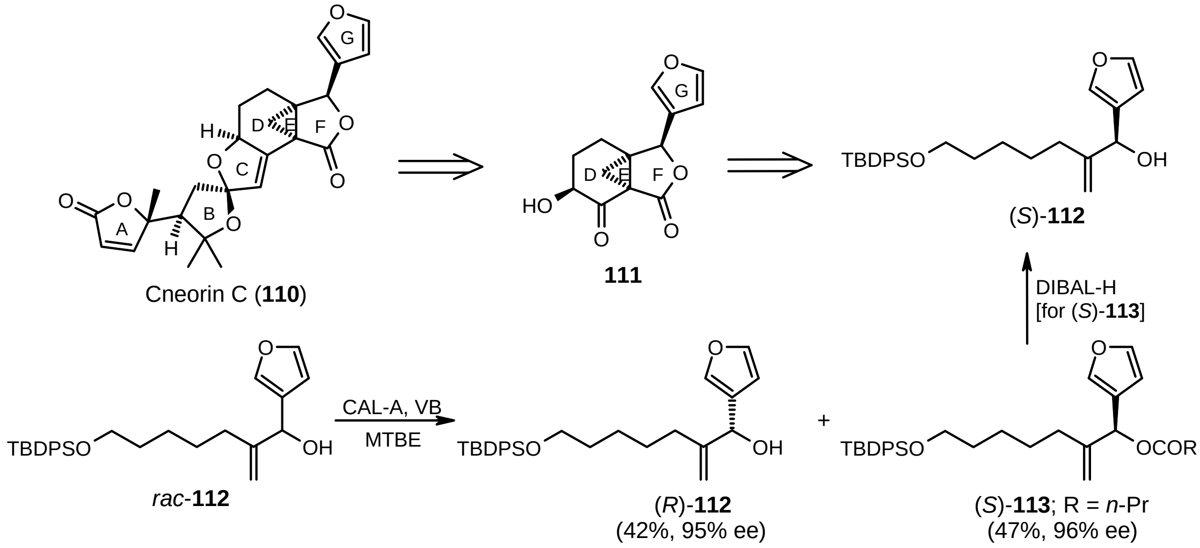
 R = CH_3: (*R*)-Tomoxetine (**102**) R = OCH_3: (*R*)-Nisoxetine (**103**)	 **101** (46%, >99% ee)	Lipase PS-C	120

Allenes are a class of compounds that exhibit axial chirality and preparation of optically pure allenes is a significant task in asymmetric synthesis. Krause et al. synthesized novel enantiomerically enriched nine- and ten-membered cyclic allenes (+)-**104a-c** & (−)-**105a-c** by the lipase catalyzed resolution of the propargylic acetates **106** & **107** and subsequent *anti*-stereoselective S_N2'-substitution with magnesium cuprates (Scheme 12). This is an interesting case of transfer of atom chirality in the acetylinic substrate to the axial chirality in the allene product. They also observed that the CD spectra of these allenes exhibit distinct features that are a function of the ring size and the alkyl substituent at the allene moiety.[121]



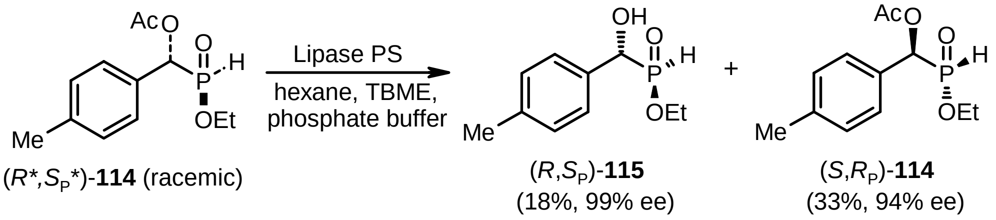
Scheme 12

Cneorin C (**110**) was originally isolated from the xerophytic shrub *Cneorum pulverulentum* native to the Canary Islands in the early 1970s. Despite its intriguing structure, no efforts toward its synthesis have been reported. In the course of their studies towards the synthesis of the DEFG ring system of the natural product, Koskinen and co-workers were faced with the task of preparing the furyl substituted allyl alcohol **112** enantioselectively. Interestingly, the authors reported that the best method for achieving the above was by using a lipase-catalyzed resolution, whereas the results with three other asymmetric protocols adopted for the same proved unsatisfactory.[122] The resolution of *rac*-**112** with CAL-A proved highly enantioselective (E > 300) yielding the ester (*S*)-**113** with 96% ee and the alcohol (*R*)-**112** with 95% ee (Scheme 13). Subsequent reduction of the ester (*S*)-**113** provided the requisite intermediate (*S*)-**112** for the total synthesis of cneorin.



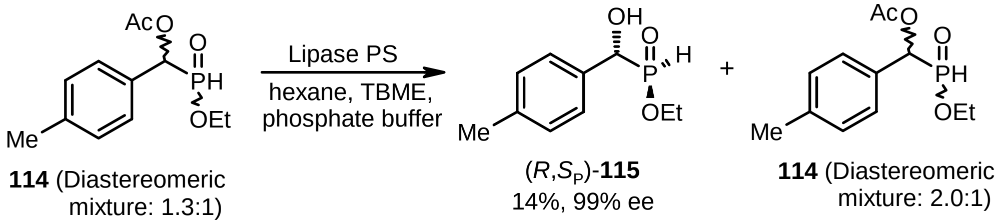
Scheme 13

α-Heteroatom substituted phosphinic acids are of much interest due to their utility both in the development of catalytic antibodies and pharmacologically active substances. Yamagishi et al. reported a new chiral synthesis of α-hydroxy-H-phosphinates having two chiral centres (one on phosphorous) via a lipase-catalyzed hydrolysis of the corresponding racemic acetate with excellent enantioselectivities (Scheme 14).[123]



Scheme 14

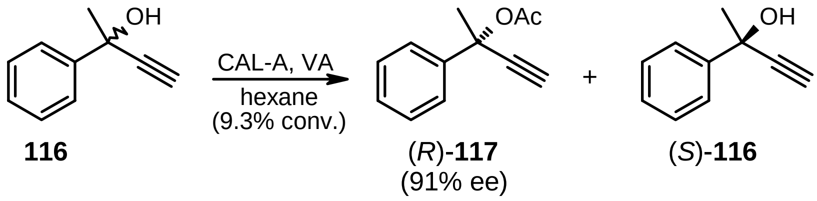
An appealing feature of this protocol was that a single stereoisomer with high enantiomeric purity was obtained even from a mixture of diastereomers, that is, from a total of 4-isomers (Scheme 15).



Scheme 15

1A.4.2.3 Tertiary alcohols

The kinetic resolution of tertiary alcohols using lipases is not that well documented in literature. This is probably due to the adverse steric interactions caused by the tertiary alcohols and consequently the difficulty associated with the accommodation of these substrates into the active site of the lipases. Bornscheuer and co-workers reported the enantioselective transesterification of the tertiary alcohol **116** using lipase A from *Candida antartica* (CAL-A).[124] This was the first example of a highly enantioselective enzyme-catalyzed resolution of a tertiary alcohol and the acetate (*R*)-**117** was obtained with 91% ee (Scheme 16). The ee of the unreacted substrate was not determined due to very low conversion.



Scheme 16

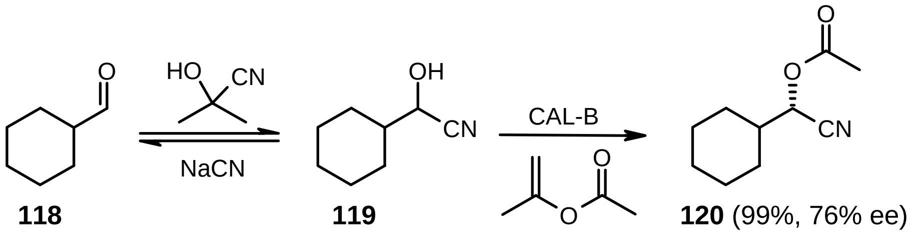
1A.4.3 Methods of Improving the Yield in Lipase Catalysis and Applications

The major drawback with kinetic resolution is that a maximum of 50% of the starting material can be used to give product. This has a huge impact in total synthesis of complex molecules involving several steps as it would drastically affect the overall yield. Yet, since kinetic resolution offers many other advantages chemists sought ways of circumventing this problem. Two excellent methods which have grown in popularity of late are (i) employing *meso* substrates ("the *meso* trick") or prochiral substrates, wherein all of the starting material can be utilized and (ii) racemizing the nonreacting enantiomer in kinetic resolution continuously (in situ) during the enzymatic resolution so that all of the racemic starting material can be used for transformation into one enantiomer. The former involves desymmetrization of compounds possessing a plane of symmetry whereas the latter is termed as dynamic kinetic resolution (DKR). A few examples of both these methods are illustrated below.

1A.4.3.1 Dynamic Kinetic Resolution

Dynamic Kinetic Resolution (DKR) is perhaps the methodology that has advanced most in recent years in improving enzyme catalysis.[125-128] Since enzymes offer a host of attractive advantages, the number of examples of chemoenzymatic DKR that combine the enzymatic resolution with an in situ racemization method has increased during the past few years. Classical racemization techniques include base-catalyzed (most common), Schiff's base mediated, thermal and even enzyme-catalyzed methods. A new concept that has emerged in recent years is the metal-catalyzed racemization, a technique that has provided a new dimension and added importance to DKR and its applications.

Amberlite is commonly used as the racemizing agent in DKRs but Hanefeld and co-workers observed that when it was used for the synthesis of aliphatic cyanohydrin acetates, only kinetic resolution took place. However, upon exchanging the base amberlite against NaCN, quantitative conversions with good enantioselectivity were obtained. The ee-values were slightly lower than what could be expected, most likely due to a base-catalyzed chemical acylation (Scheme 17).[129]

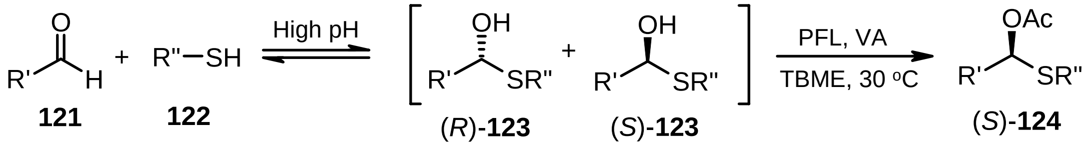


Scheme 17

The same group, nevertheless, successfully used amberlite in the DKR of aromatic cyanohydrin esters and obtained (*S*)-mandelonitrile acetate in 98% ee. Lipase immobilized on celite was used and the celite absorbed the water suppressing side reactions.[130] Kanerva et al. have also employed amberlite for effecting the DKR of phenothiazine-based cyanohydrin acetates.[131]

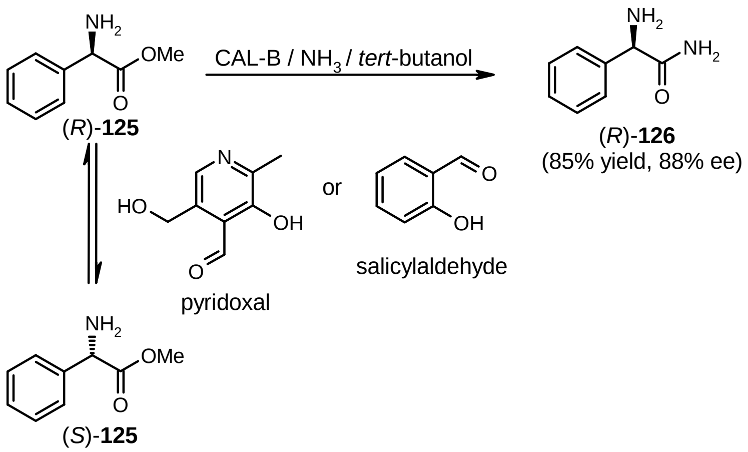
Base-catalyzed racemization can also be brought about by using triethyl amine as shown in the transformation of various 5-oxazolones into the corresponding enantiopure amino acid derivatives (using CAL-B).[132]

An efficient DKR process has been developed for the deracemization of chemically labile secondary alcohols such as hemithioacetals taking advantage of the propensity of these compounds to racemize via dissociation-recombination under mild conditions (Scheme 18).[133]



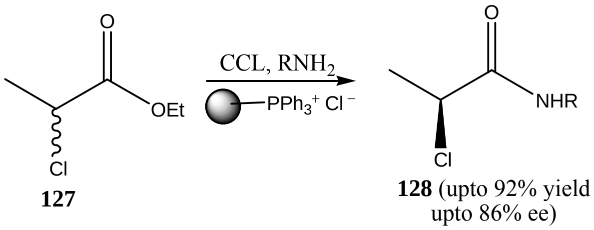
Scheme 18

An additional interesting example not involving an alcohol substrate that has been recently reported[134] is the DKR of phenylglycine esters where the substrate is racemized via Schiff base formation. Thus, in the CAL-B-catalyzed aminolysis of phenylglycine methyl ester, the unreactive enantiomer is in situ racemized via Schiff base formation with pyridoxal or salicylaldehyde under basic conditions (Scheme 19).



Scheme 19

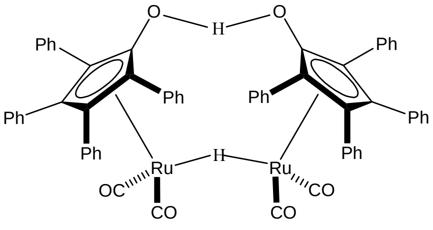
More recently a new chemoenzymatic DKR approach has been developed wherein the racemization is via nucleophilic displacement. This procedure has been used for the deracemization of α-halo esters by enzymatic hydrolysis and aminolysis. A recent example, portrayed by Kostic and co-workers, was the efficient DKR of ethyl 2-chloropropionate **127** achieved by the *Candida cylindracea* lipase-catalyzed aminolysis in the presence of supported triphenylphosphonium chloride (Scheme 20).[135]



Scheme 20

Transition Metal-Catalyzed Racemization

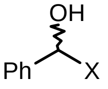
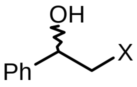
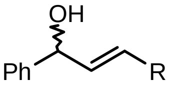
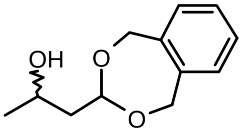
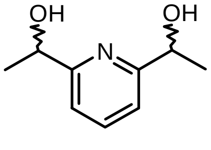
DKR has been elevated to new heights with the advent of racemization catalyzed by metals. Backvall and co-workers have been instrumental in the development of an efficient system for racemization by hydrogen transfer based on the use of a ruthenium catalyst [$Ru_2(CO)_4(\mu\text{-}H)(C_4Ph_4COHOCC_4Ph_4)$, **129**] and *p*-chlorophenyl acetate as acyl donor.[136] Thus an efficient DKR of a host of secondary alcohols (Table 7) was obtained by combining CAL-B with the above-mentioned acyl donor and ruthenium catalyst **129** (78 - 92% yield with >99% ee).[136,137] Important features of the above system are (i) no external base is required, (ii) the acyl donor is fully compatible with the ruthenium catalyst and (iii)



129

addition of the corresponding ketone is not required. The authors have also provided an insight into the mechanism for the racemization process.[138] Over the years there have also been modifications to improve the stability of the catalyst.[139]

Table 7 Efficient DKR of various substrates using the above Ru-catalyst

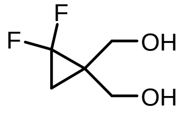
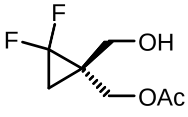
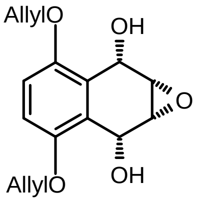
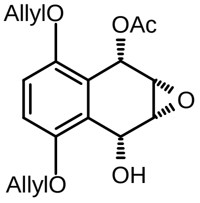
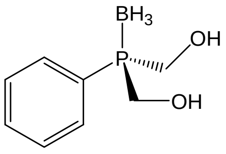
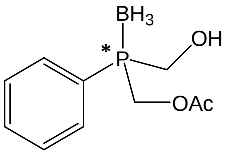
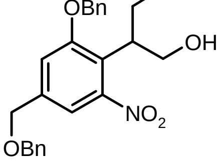
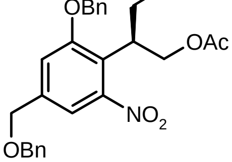
Substrate	Lipase used	% Yield	% Ee	Reference
 X = COOEt, PO(OEt)$_2$ **130**	*P. cepacia* / CAL-B	74 / 86	96% / >99 (*S*) / (*R*)	140a,b
 X = PO(OEt)$_2$, COOEt, Cl, CN, N$_3$, OTr **131**	*P. cepacia* / CAL-B	69 – 96	95 – 99 (*S*) / (*R*)	140b-g
 **132**	Lipase PS-C	84 – 92	95 - >99 (*R*)	140h
 **133**	CAL-B	90	96 (*R*)	140g
 **134** (*dl*/*meso* ~ 50/50)	CAL-B	78	99 (*R,R*) (*R,R*/*meso* : 100/0)	140i

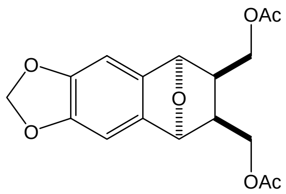
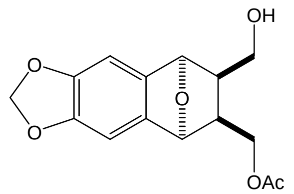
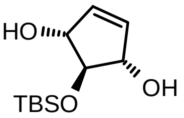
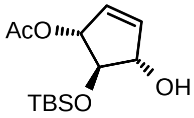
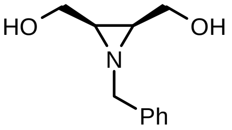
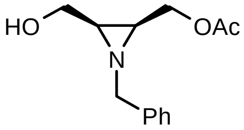
The other type of metal-catalyzed racemization, via (π-allyl)palladium complexes is also well-known, and the most common example of this method is the racemization of an allylic ester via Pd(0) catalysis. This isomerization can proceed via different mechanisms.[141] Trost and co-workers have taken advantage of the rapid equilibration between (π-allyl)palladium intermediates for the synthesis of enantiomerically pure lactones via a dynamic kinetic asymmetric transformation (DYKAT).[142]

1A.4.3.2 Enantiselective Enzymatic Desymmetrization

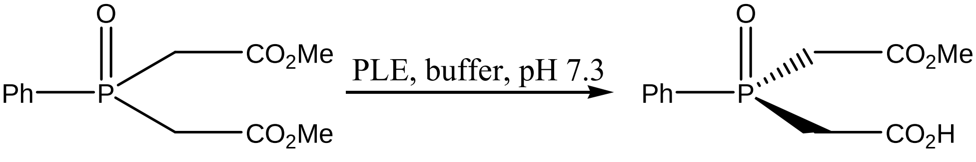
Desymmetrization of prochiral and *meso* substrates is another method that provides for a maximum yield of 100% and hence can be classified under asymmetric synthesis. For this reason, enzymatic desymmetrizations constitute a very interesting alternative to kinetic resolution for the preparation of optically active compounds, reflected by the increasing applications in recent literature.[14] A few examples useful from a synthetic point of view involving lipase-catalyzed desymmetrization are illustrated in this section. Some of the substrates have been presented in Table 8 while more interesting ones have been discussed subsequently.

Table 8 Enantioselective enzymatic desymmetrization of prochiral and *meso* substrates

Substrate	Product	Lipase used	% Yield	% Ee	Ref
 **135**	 **136**	Lipase PS	96	91	143
 **137**	 **138**	Novozym 435	93	98	144
 **139**	 **140**	CAL-B	-	Up to 98%	145
 **141**	 **142**	PSL	74	> 95	146

 **143**	 (1*R*,2*R*,3*S*,4*S*)-**144**	PPL	83	95	147
 **145**	 (–)-**146**	Amano AK	99	> 99	148
 **147**	 (2*S*,3*R*)-**148**	Amano PS	96	> 97	149

Recently, the first enzymatic desymmetrization of a prochiral phosphine oxide has been reported.[150] Kielbasinski et al. subjected bis(methoxycarbonylmethyl)phenylphosphine oxide (**149**) to hydrolysis in a phosphate buffer in the presence of several hydrolases, of which only pig liver esterase proved to be efficient (Scheme 21). The expected product, viz. the monoester of (*R*)-configuration [(*R*)-**150**], was isolated after acidification and purification by column chromatography in 92% yield and 72% *ee*.

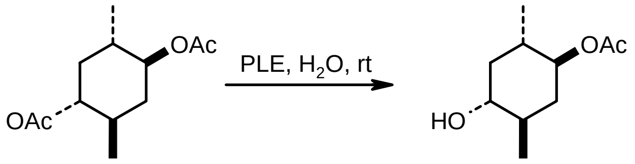


149

(*R*)-(+)-**150** (92%, 72% ee)

Scheme 21

Trauner and co-workers have reported one of the few examples of the desymmetrization of molecules not possessing a symmetry plane. Thus, the centrosymmetric *meso* 1,4-diacetoxy-2,5-dimethylcyclohexane (**151**), which belongs to the molecular point group C_i (= S_2) was successfully hydrolyzed using PLE as catalyst (Scheme 22). The monoester (+)-**152** was isolated enantiopure in excellent yield.[151]

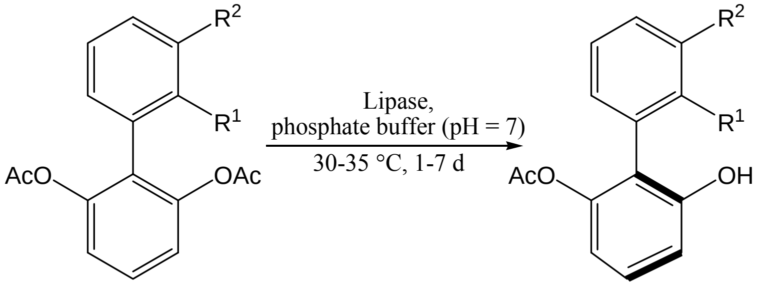


151
(*centro-symmetric*)

(+)-**152** (95%,
> 99.5% ee)

Scheme 22

Similarly, only a few enzymatic desymmetrizations of compounds with a chirality axis or plane have been reported. Matsumoto and co-workers have published the first application of an enzyme to desymmetrize axially chiral nonracemic biaryls (Scheme 23). This class of compounds is of current interest due to their importance as chiral ligands and auxiliaries in asymmetric synthesis. Furthermore, they also constitute a structural feature of many natural products. These authors have obtained biaryls **153a-d** in high ee and yields by desymmetrization of the corresponding σ-symmetric diacetates (**154a-d**) through lipase-catalyzed hydrolyses using lipases from *Candida antarctica* and *Pseudomonas cepacia*.[152]



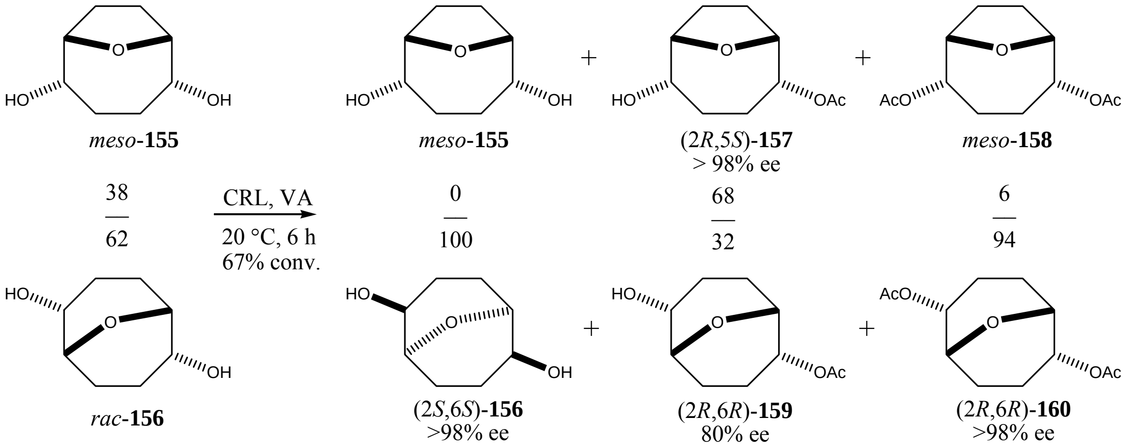
153a-d

154a-d

(+)-**154a**, $R^1 = R^2 = -(CH_4)-$ (93%, 99% ee)
PCL ⟹ (–)-**154b**, R^1 = Me, R^2 = H (86%, 99% ee)
(–)-**154c**, R^1 = Et, R^2 = H (67%, 96% ee)
CAL ⟹ (–)-**154d**, $R^1 = -CH_2OBn$, R^2 = H (68%, 99% ee)

Scheme 23

Hegemann et al. have recently published the first example of a lipase-catalyzed simultaneous separation of skeletal isomeric diols, with kinetic resolution of one and desymmetrization of the other constitutional isomer (Scheme 24).[153] *C. rugosa* lipase proved to be the most efficient enzyme for the transesterification of a 38:62 mixture of *meso*-**155** and *rac*-**156**. A 67% of degree of conversion was reached after 6 h of reaction, which implied that *meso*-**155** was transformed almost quantitatively, while the conversion of the racemate **156** stopped at 50% conversion. After separation of the enzyme, (-)-**156** was isolated enantiopure in 14% yield. Furthermore, (+)-**160** was also isolated enantiopure but was contaminated with 6% of *meso* **158**. Finally, a 68:32 mixture of enantiopure (+)-**157** and (+)-**159** (80% *ee*) was the third fraction obtained from the crude reaction.



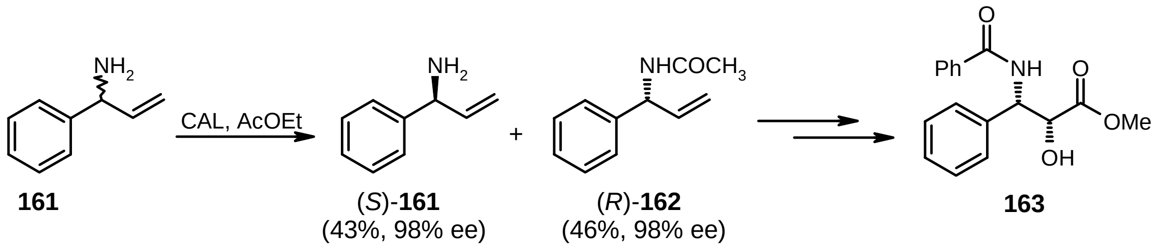
Scheme 25

Enantioconvergent synthesis is another method of enhancing yields in enzyme catalysis but detailed discussion on this is beyond the scope of this review. An interesting concept that has emerged recently and holds a lot of promise is Parallel Kinetic Resolution (PKR). In PKR, two different (although related) reactions of the racemate are catalyzed by two different catalysts, one being *R*-selective and the other *S*-selective. In this manner, the inherent dependence of the ee of the non-reacting substrate isomer on the degree of conversion can be circumvented. This was achieved in the PKR of alcohols using an *R*-selective lipase and *S*-selective phosphine based synthetic catalyst.[154]

1A.4.4 Miscellaneous Reports

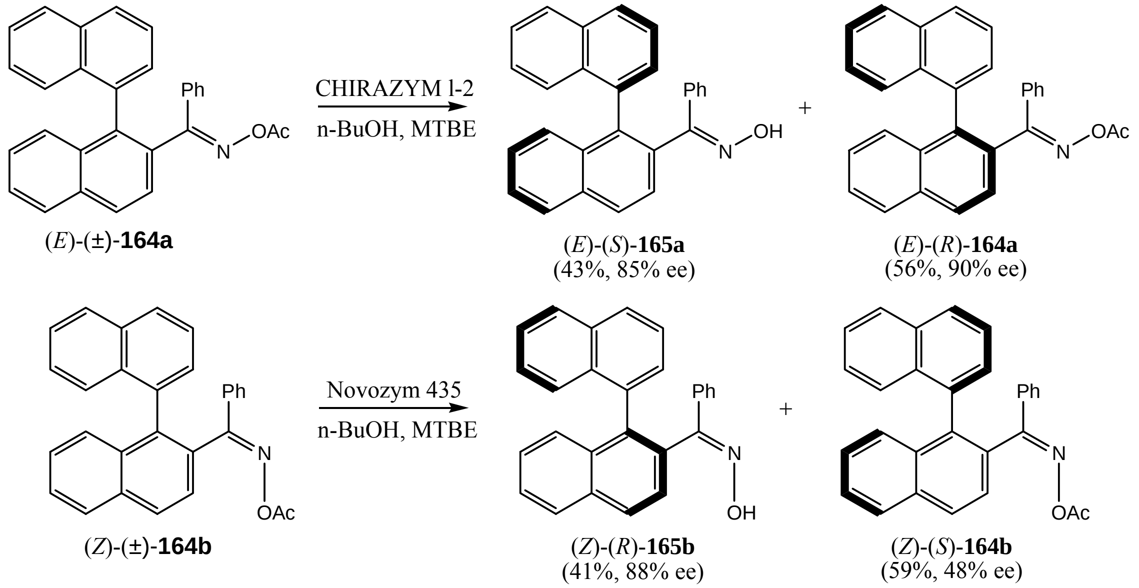
Apart the incredible number of reports concerning their deployment in the preparation of a wide range of enantiopure alcohols, lipases have been used in the preparation of chiral acids, lactones, amines, oximes, etc. They have also been used for regioselective protection/deprotection reactions, which carry a lot of importance in the field of carbohydrate chemistry. This section would cover a few examples involving diverse substrates and interesting observations in the field of lipase catalysis.

Chiral amines are important building blocks and auxiliaries for the stereoselective synthesis of biologically active compounds and natural products. Botta and co-workers prepared a variety of substituted 1-aryl-2-propenylamines of high enantiomeric purity via lipase-catalyzed resolution of the corresponding racemates (Scheme 26).[155] More importantly, they were successful in extrapolating the (*R*)-1-phenyl-2-propenylamine obtained from (*R*)-**162** into (2*R*,3*S*)-3-benzoylamino-2-hydroxy-3-phenylpropanoic acid methyl ester (**163**), the side chain of taxol.



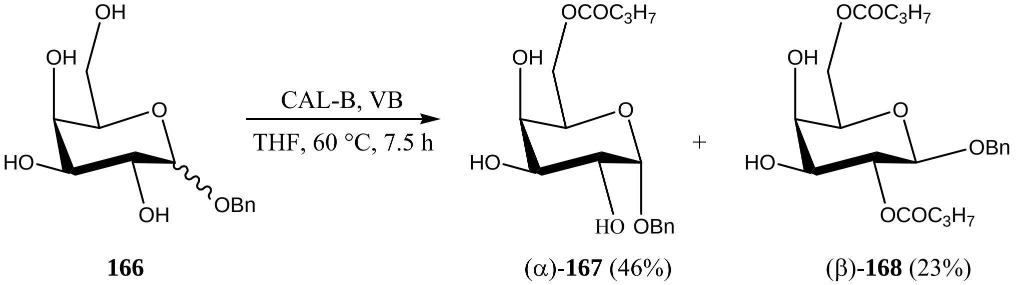
Scheme 26

Atropisomeric biphenyls are established as one of the most important classes of ligands in asymmetric synthesis. Aoyagi et al. carried out a successful resolution of both the isomeric oximes as *E*- and *Z*-isomers of *O*-acetyl binaphthyl ketoximes and obtained the corresponding optically active binaphthyl ketoximes. The lipase showed opposite axial selectivity for the *E*- and *Z*-isomers (Scheme 27).[156]



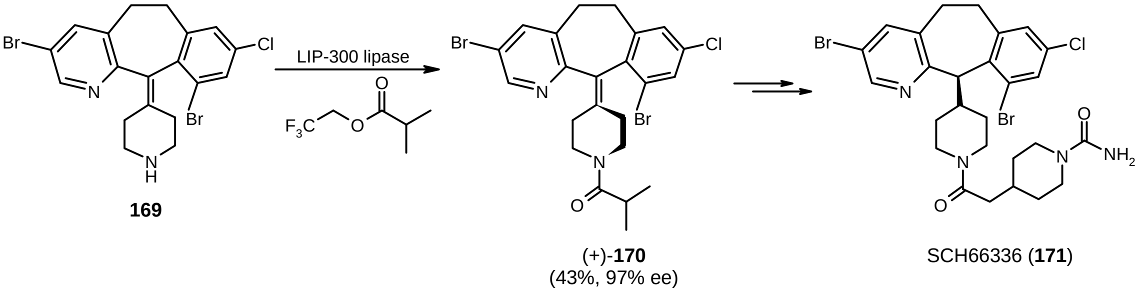
Scheme 27

In a study on regioselective acylation of pyranoses, Goncalves et al. unveiled enzymatic means for the resolution of α and β-anomers of galactose derivatives.[157] Both anomers were acylated in position 6, while the *β*-anomer reacted also on position 2 (Scheme 28).



Scheme 28

Morgan et al. have described the kinetic resolution of piperidine atropisomers leading to a chiral intermediate needed in the synthesis of the farnesyl protein transferase inhibitor SCH66336, an anti-cancer agent showing activity in the nanomolar range (Scheme 29).[158] Novel aspects include the fact that axial chirality is involved (which is transformed selectively into central chirality in a subsequent reduction step) and that lipase-catalyzed acylation occurs at a secondary amine function that is usually difficult and a rare process.



Scheme 29

1A.5 Conclusions and Perspectives

The use of enzymes in organic synthesis is widely accepted since the past 3-decades and the selected examples illustrated in this section demonstrate the broad applicability of lipases in terms of substrate structures and enantioselectivity. For their remarkable properties, enzymes were declared “Reagent of the Year” in 2000. As more and more synthetic research embraces the realization that enzymes are simply an alternative source of catalysis that may be as robust as others, sometimes more so, then their use will continue to flourish. Greater availability at a lower cost brought about by modern biotechnology will further assist in dispelling any existing prejudice against enzymes as practical catalysts. The advent of new catalysts for dynamic kinetic resolution and the use of prochiral and *meso* substrates (enantioselective enzymatic desymmetrization) have, as we have seen, taken the field to another level by remarkable improvement of the chemical yield. A synthetically useful new variant of dynamic kinetic resolution has been published that is based on racemizing reversible Michael reactions in combination with lipase-catalyzed hydrolysis.[159] Moreover, novel developments such as additive effects, solvent optimization, the use of ionic liquids and immobilization techniques offer additional exciting opportunities leading to further practical applications of lipases in synthetic organic chemistry. Protein engineering has an exciting potential of altering the enzymatic properties at will, eg., broadening substrate specificity, as well enhancing enzyme action in organic media. Rational design of new enzymatic catalysts to construct a protein with a designed catalytic activity selectivity from a designed sequence of amino acids is still very far from reality. Thus, the concept of artificial enzymes offers an attractive alternative though whether they will be able to match their natural counterparts is debatable. Supercritical biocatalysis[160] is another field of extensive research in the recent past though the advantages of replacing conventional organic solvents with supercritical fluids have not fully been demonstrated yet. More recently, modern molecular biology methods such as directed evolution[161] have given a further boost to the development of lipases for future applications. Considering their applications in the past, it can be therefore, said with assurance, that the impact that enzymes, lipases in particular, have had in organic synthesis has been enormous and their ever-increasing utilization is rather evident in the present scientific world. Recent developments only go to show that more path breaking methods and processes brought about by the catalysts of Nature lie in store for us in the future.

In our group, over the past few years, we have been successful in tapping the potential of enzymes in preparing important chiral intermediates and have also carried out studies relating to their selectivity pattern.[162] More recent work in our group utilizing lipases concerns a variety of applications[163] such as (i) preparation of enantiomerically pure 2-hydroxy/acetoxycyclopentanone, (ii) resolution of the key intermediate in the synthesis of the antiepileptic drug candidate talampanel and (iii) as a reagent in the preparation of alkyl maleanilates that could serve as precursors to interesting heterocycles. The following section (Section B) discusses in detail our efforts in the above-mentioned applications.

1A.6 References and Footnotes

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162. For a detailed account of earlier work in our group employing enzymes see Desai, S. B. *Ph. D. Thesis*, University of Pune, 2000.

163. We have also carried out an efficient lipase-catalyzed resolution of a natural product synthesized by us and this shall be discussed in Chapter II of this thesis.

1B. Section B

The Applications of Lipases in Key Resolutions/Transformations

This section features the following topics:

1B.1 Section B: I. Enantioselective Enzymatic Approach to (+)- and (−)-α-Hydroxy/acetoxycyclopentanones

1B.1.1 Background

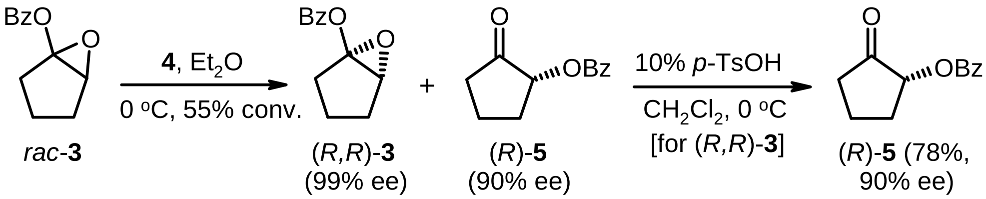
The utilities of (±)-2-hydroxy- and (±)-2-acetoxycyclopentanones (**1** & **2**) have been well proven in practice[1] and several methods for synthesis of them are known in the literature.[2] Nevertheless, it is their optically active forms that are far more important as chiral building blocks. These intriguing compounds have also attracted attention of organic chemists due to their inherent instability and delicate nature. There are not many reports on the preparation of enantiomerically pure (*R*)-/(*S*)-2-hydroxycyclopentanones [(−)- & (+)-**1** respectively]. The ones that have been documented involve a variety of methods such as enzymatic oxidation, enantioselective reduction, metal-catalyzed oxidation and so on. The more significant routes are discussed briefly below prior to elucidating our work in this context.

1B.1.1.1 Methods of Preparation of Enantiomerically Enriched 2-Hydroxycyclopentanones

To date there are only five reports on enantiopure access to 2-hydroxycyclopentanones (+)- & (−)-**1**. The earliest method involved an enzymatic oxidation of *cis*-cyclopentanediol using whole cells to obtain enantiomerically pure 2-hydroxycyclopentanone.[3] Another report[4] illustrates desymmetrization of *cis*-1,2-cyclopentanediol by stereoselective *Pseudomonas cepacia* lipase (PSL)-mediated transesterfication to obtain (1*S*)-acetoxy-(2*R*)-hydroxycyclopentane, a precursor to the desired (2*S*)-acetoxycyclopentanone [(+)-**2**], in 86% ee and 73% yield. However, the authors have reported that the monoacetate suffers an appreciable decrease in enantiomeric excess following work-up of the reaction mixture. Four other elegant approaches reported towards this end have been discussed in the following part.

[A] *Asymmetric Rearrangement*

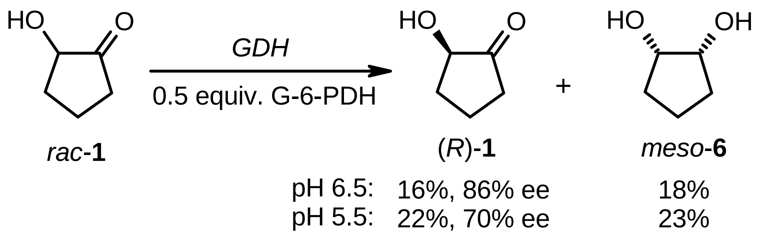
Enol ester epoxides can rearrange to α-acyloxy ketones under acidic conditions. Based on this, Shi and co-workers explored the feasibility of kinetically resolving a racemic enol ester epoxide using a chiral Lewis acid catalyst. During their studies, they found that a BINOL-Ti(O^{i}Pr)$_4$ system provided the best results with various substrates.[5] Thus, treatment of 1-benzyloxy-1,2-epoxycyclopentane (**3**) with 5 mol% of [(*R*)-BINOL]$_2$-Ti(O^{i}Pr)$_4$ (**4**) in Et$_2$O at 0 °C led to a 55 % conversion, providing (*R,R*)-**3** and (*R*)-2-benzoyloxycyclopentnaone (**5**) with 99% and 90% ee respectively (Scheme 1). The authors also observed that the above rearrangement proceeded with inversion of configuration (involving a 1,2-shift of the benzoyloxy group) and hence the enantiomerically enriched epoxide and 2-benzyloxy ketone obtained possess the same configuration at C$_2$. Moreover, the unreacted isomer could further be converted to the corresponding *α*-acyloxy ketone by using an achiral Lewis acid catalyst with retention of configuration thus resulting in a net complete conversion of a racemic enol ester epoxide into an enantiomerically enriched *α*-acyloxy ketone.



Scheme 1

[B] *Enantioselective Reduction*

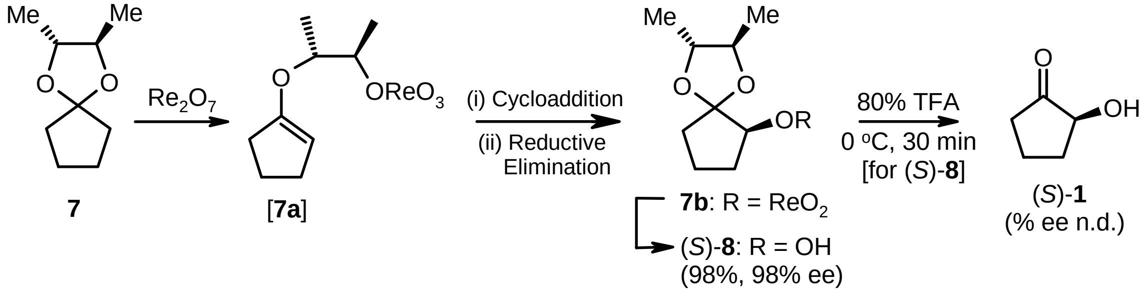
The NAD(P)(H)-dependent oxidoreductases are useful in the synthesis of chiral synthons. Whitesides and Lee have described the use of glyceroldehydrogenase (GDH, a nicotinamide cofactor-dependent oxidoreductase that catalyzes the interconversion of glycerol and dihydroxy acetone) for the preparation of chiral 1,2-diols and *α*-hydroxy ketones.[6] During the course of their studies they were successful in preparing (2*R*)-hydroxycyclopentanone [(–)-**1**] by the stereoselective reduction of the corresponding racemic *α*-hydroxy ketone (±)-**1** using GDH. Glucose-6-phosphate/glucose-6-phosphate dehydrogenase (G-6-PDH) or glucose/glucose dehydrogenase (GlcDH) were used for regeneration of the cofactor. The reduction of the compound of interest was carried out at two pH values, 5.5 & 6.5. Interestingly, the authors observed that the reaction at pH 5.5 produced (*R*)-**1** of 70% ee while the reaction at pH 6.5 yielded (*R*)-**1** of 86% ee, indicating the propensity for racemization and presumably its unstable nature (Scheme 2).



Scheme 2

[C] *Metal-mediated Oxidation*

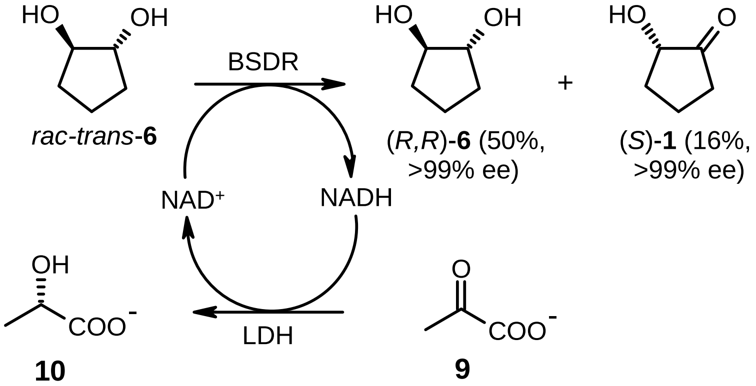
Kennedy and Tang demonstrated an elegant oxidation of homochiral ketals by rhenium(VII) oxide to obtain 2-hydroxy ketals with high diastereoselectivity.[7] The authors rationalized the stereocontrol of this novel oxidation by invoking a transition state that approximates the geometry of a metallaoxetane. They proposed that the first step of the reaction was the formation of a perrhenate ester **7a** of a 2-hydroxyethyl enol ether. This upon [2+2] cycloaddition followed by reductive elimination gave a rhenate ester **7b**, subsequent hydrolysis of which furnishes the observed products. Thus, the (*S*)-2-hydroxyketal **8** of the compound of our interest was obtained in 98% yield and 98% ee from the corresponding homochiral ketal **7** (Scheme 3).



Scheme 3

[D] *Enzymatic Oxidation*

The third significant report towards enantiopure **1** details an enzymatic oxidation of *anti*-1,2-cyclopentanediol (*rac-trans*-**6**) with *Bacillus stearothermophilus* diacetyl reductase (BSDR). The above enzyme is an efficient catalyst for the stereoselective reduction of prochiral α-diketones to (*S,S*)-vicinal diols and (*S*)-α-hydroxy ketones, in the presence of NADH as cofactor. Nevertheless, according to Fantin and co-workers, BSDR also proved to be a powerful catalyst in the oxidative sense and they have demonstrated the use of the same to successfully obtain vicinal diols and α-hydroxy ketones with excellent enantiomeric excess.[8] Thus the authors have, in the course of their studies, obtained (2*S*)-hydroxycyclopentanone [(+)-**1**] in 16% yield and 99% ee by the action of BSDR on *anti*-1,2-cyclopentanediol (*rac*-*trans*-**6**) (Scheme 4).



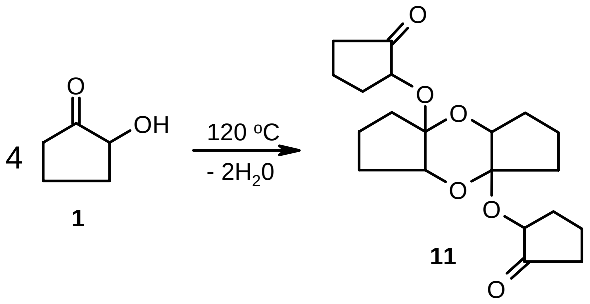
Scheme 4

Several diols (including acyclic diols) were studied for the reaction seen in the scheme above. The observation made was that the (*S*,*S*)-diol was selectively oxidized to the corresponding α-hydroxy ketone while the (*R*,*R*)-diol was recovered, also in remarkably high enantiomeric purity. The NADH produced during the oxidation process was conveniently transformed to NAD$^+$ in the presence of the inexpensive pyruvate **9** and lactic dehydrogenase (LDH).

An interesting observation made by the authors during these studies under the experimental conditions wherein the pH was maintained at 8.2, was a progressive disappearance of the formed α-hydroxy ketone. A possible explanation given by them was the formation of an enediol from the α-hydroxy ketone followed by further biotransformation.[9] This further goes to prove the unstable nature of α-hydroxycyclopentanones (+)- & (−)-**1**. However, the corresponding six-membered analogue, (2*R*)-hydroxycyclohexanone was stable and was recovered from the reaction mixture in almost quantitative yield.

1B.1.2 Rationale for Present Work

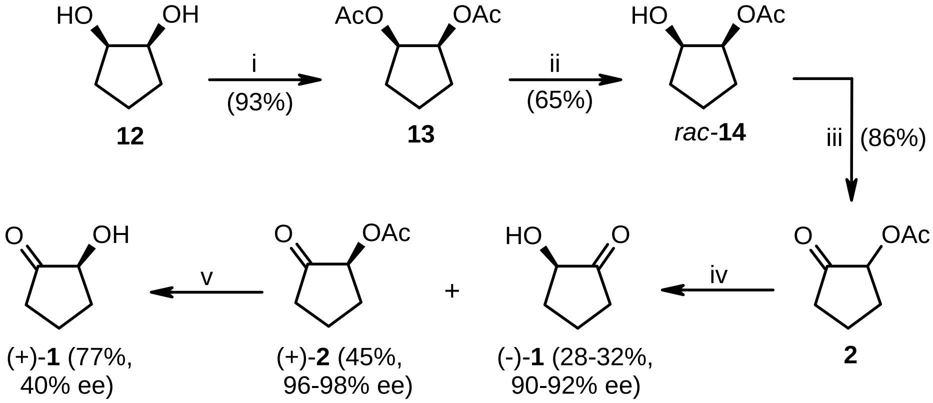
It is evident from the above reports that 2-hydroxycyclopentanones (+)- & (−)-**1** are not very stable compounds. They are sensitive to acids and bases, as well as to heat and organometallic reagents. It is noteworthy that (+)- & (−)-**1**, upon heating, are known to undergo self coupling reactions to form 1,4-dioxane derivatives (Scheme 5).[10,11] Similarly, in basic aqueous solutions they are known[6] to undergo a decomposition reaction with darkening to a black colour. Hence the provision of an unambiguous and facile route to (+)- & (−)-**1** and their derivatives is a challenging task of significant interest. We envisaged a simple enzymatic route to these unstable yet useful chiral building blocks and our studies on the preparation and stability of these intriguing compounds are presented in the following part.



Scheme 5

1B.1.3 Results and Discussion

Our strategy was based on the application of the “meso trick” for bringing about enantiomeric enrichment. Thus we envisaged a simple desymmetrization of *meso*-diacetate **13** for obtaining the enantiopure hydroxyactetate **14**. The *meso*-diacetate **13** was prepared easily from the *meso*-diol **12** (Scheme 6). However, to our surprise, the Amano PS-catalyzed biphasic hydrolysis of *meso*-diacetate **13** at pH 7.0 furnished in 65% yield hydroxyacetate **14** that was found to be optically inactive (the mixture of **13** and **14** did not show any rotation before column chromatographic separation),[12,13] indicating obviously the formation of a racemic compound. The formation of a racemic **14** could be a result of either non-selective random enzymatic hydrolysis of both the acetates or alternatively, the enzyme is actually enantioselective in its action, but the product mixture is formed by the instantaneous in situ intramolecular acyl migration in a cyclic-*cis*-vicinal hydroxy acetate system. At this stage, we were not in a position to confirm either and thus continued our efforts towards the preparation of enantiomerically pure 2-hydroxycyclopentanones (+)- & (−)-**1**. Oxidation of the racemic hydroxy acetate **14** employing Swern conditions provided us the racemic acetoxy ketone **2** in 86% yield. We intended to study the lipase-catalyzed resolution of **2** with two aims; *viz* (a) to draw conclusions about the enantioselectivity of the enzyme and (b) to develop a new enzymatic route to (*R*)- and (*S*)-2-acetoxy/hydroxycyclopentanones [(−)- & (+)-**1** respectively]. To our delight, the Amano PS-catalyzed biphasic hydrolysis afforded us a mixture of enantiomerically pure 2-hydroxycyclopentanone (−)-**1** and 2-acetoxycyclopentanone (+)-**2** (the reaction was carried out at pH 6.5 as hydrolysis at pH 7.0 provided (−)-**1** with a lower yield and ee). Silica-gel column chromatographic separation of the above mixture gave (2*R*)-hydroxycyclopentanone [(−)-**1**] in 28–32% yield and (2*S*)-acetoxycyclopentanone [(+)-**2**] in 45% yield.



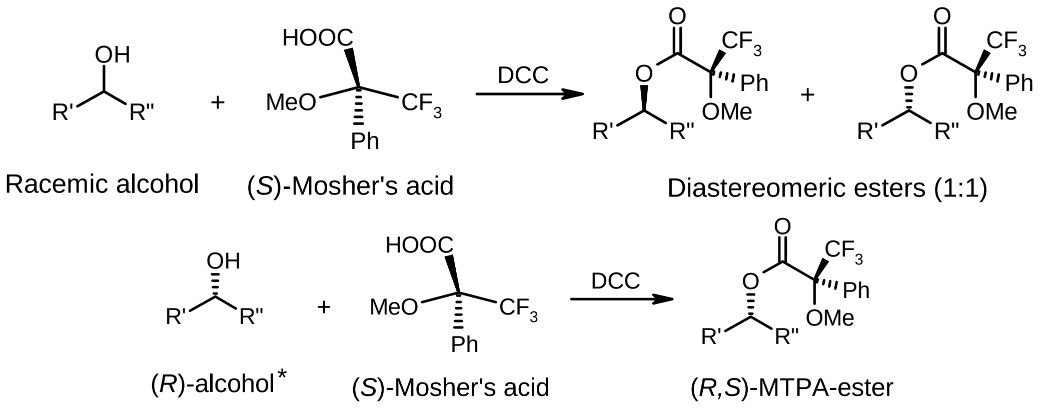
Scheme 6 *Reagents, conditions and yields*: (i) Ac_2O, Py, rt, 48 h (93%); (ii) Petroleum ether/benzene (2:1), Amano PS, rt, 18 h, pH 7.0 (65%); (iii) $(COCl)_2$, DMSO, TEA, DCM, − 60 °C, 15 min (86%); (iv) Petroleum ether/benzene (2:1), Amano PS, rt, 22 h, pH 6.5 [(−)-**1**, 28-32%; (+)-**2**, 45%]; (v) Aq. K_2CO_3, MeOH, 0 °C, 4 h (77%).

The ^{1}H NMR spectrum of a diastereomeric mixture of Mosher's esters obtained from (±)-**1** and (*R*)-Mosher's acid showed a very clean resolution of the $-OCH_3$ protons appearing at δ 3.58 and 3.64. Thus, the enantiomeric excess for (−)-**1** was deduced from the ratio of the integration values of the $-OCH_3$ proton peaks in the corresponding ^{1}H NMR spectrum of its Mosher's acid derivative (which showed a major singlet at δ 3.64 and a minor one at δ 3.58 for the $-OCH_3$ protons). The ratio revealed that (−)-**1** is formed with 90-92% ee. Also enantiomerically pure (−)-**1** on treatment with AcOH/DCC gave the acetyl derivative of (−)-**1** with 90-92% ee. A comparison of the specific rotation of (+)-**2** with the acetyl derivative of (−)-**1** revealed that acetoxy ketone (+)-**2** is obtained with 96-98% ee. However, surprisingly, we found a decrease in enantiomeric excess on preparing the corresponding hydroxy compound (+)-**1**. The base-induced hydrolysis of (+)-**2** to (+)-**1**

Principle of MTPA derivatization to obtain the enantiomeric excess:

A very efficient method of determining the ee of chiral secondary alcohols was designed by Mosher.[14] Diastereomeric esters are prepared from optically pure *S*- or *R*-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) and various chiral secondary alcohols. The ^{1}H NMR spectra of the esters prepared from the racemic alcohol is recorded and the proton/fluorine signals of the resulting diastereomers of such a racemate are assigned. The relative integral ratios of the signals corresponding to the two diastereomers would then be 1:1 (since the alcohol is racemic). Similarly, the enantiomerically enriched compound, whose ee is to be measured, is also derivatized to obtain diastereomeric esters (since the enantiomerically enriched compound would also contain a minor amount of the opposite isomer). The relative integral ratios of the diasteromers signals are calculated to obtain the enantiomeric excess of the enantiopure compound. The protons corresponding to the $-OCH_3$ group of Mosher's ester derivative generally show a clean resolution, appearing as two distinct singlets at different δ values. The ratio of their integration values is used to determine the ee in most cases. However, the major advantage of Mosher's acid lies in that ^{19}F NMR spactra of the derivatives can be recorded that would give us the ee values without ambiguity. Depiction of this concept is shown in the Figure 1 and as well in spectra of a few compounds prepared in this thesis. Mosher also derived empirically a correlation of configuration and NMR chemical shifts for the diastereomeric MTPA-esters that can be applied to assign the configuration of secondary alcohols.[14b]

$$\% \text{ ee} = \frac{\text{Isomer}_{\text{major}} - \text{Isomer}_{\text{minor}}}{\text{Isomer}_{\text{major}} + \text{Isomer}_{\text{minor}}}$$



* Order of priority: OH > R' > R"

Figure 1 Concept of MTPA-ester derivatization followed by MTPA-derivatisation of (+)-**1** indicated that it possesses only 40% ee (from the ratio of the –OCH_3 proton singlets, page 81). We are of the opinion that this decline in the ee could be due to base-induced racemization/1,2-carbonyl transposition during the hydrolysis. A similar base-induced carbonyl transposition was reported by Suarez and co-workers in the synthesis of (+)-8-deoxyvernolepin.[15] These results also clearly establish that the lipase Amano PS is actually very specific in its hydrolytic action on the *meso*-diacatate **13** and the racemic product **14** is formed because of in situ intramolecular acyl migrations. A nice study of selectivity paradigm and intramolecular acyl migrations in lipase-catalyzed hydrolysis of vicinal diols has been recently reported from our group.[16]

During these studies, we also noticed that (−)-**1**, either neat or in chloroform solution at 0 °C, slowly loses its enantiomeric purity and becomes completely racemic over a period of nearly two months, indicating that these isomers have a two months half life span as determined by measuring the specific rotations with time. A plot of specific rotation of (−)-**1** recorded at regular intervals of time is illustrated in Figure 2. At this stage, further studies were required to learn more about the mechanism of racemization, occurring apparently through an ene-diol formation. Towards this end, enantiopure (−)-**1** was subjected to D_2O treatment at room temperature for 10 days and ^1H NMR spectra were scanned after every 24 h. We observed that only the heteroatom proton (-OH) was exchanged, while the α-methine proton did not show any exchange with deuterium atom. This clearly revealed that only intramolecular prototropic shifts take place in the formation of enediol **15** and back to starting material. The isolation of monodeuterated (−)-**1** from D_2O and determination of the optical rotation indicated that the process of racemization of (−)-**1** was also relatively slow in D_2O, possibly because of extensive intermolecular hydrogen bonding. Following the above studies, we felt that determination of the ee of (−)-**1** as early as possible after the reaction would serve us well in determining more precisely the ee with which (−)-**1** is

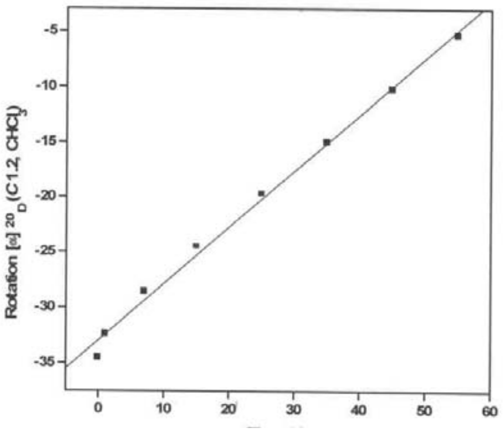
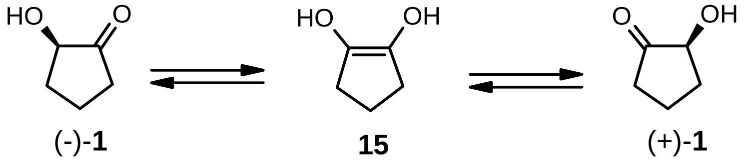


Figure 2 Plot of rotation against time for (−)-**1**

formed. Thus, upon performing the lipase-mediated hydrolysis of racemic **2**, the reaction mixture containing (−)-**1** and (+)-**2** (immediately after the work-up procedure) was directly treated with (*R*)-Mosher's acid and DCC to form a mixture of (+)-**2** and the MTPA-ester derivative of (−)-**1**. Subsequently, silica-gel column chromatographic separation of the above two compounds was done to obtain the pure MTPA-ester derivative of (−)-**1**. The ^{1}H NMR spectrum of the thus obtained derivative revealed (from the ratio of the $-OCH_3$ proton singlets) that (−)-**1** actually possesses an ee of 96-98% (page 81). These observations clearly suggest that intramolecular hydrogen bonding in (+)-/(−)-**1** enhances the keto-enol tautomerism and the 2-hydroxycyclopentanones (−)- & (+)-**1** are in equilibrium with enediol **15** (Scheme 7). This is slowly transformed to the racemic mixture through keto-enol tautomerism. Also, the (2*S*)-acetoxycyclopentanone [(+)-**2**] was fairly stable and did not show any decline in enantiomeric purity over time (evidently, here, there is no intramolecular hydrogen bonding to facilitate the keto-enol tautomerism; also, the formed tautomer would experience intramolecular hydrogen bonding involving a 7-membered ring).



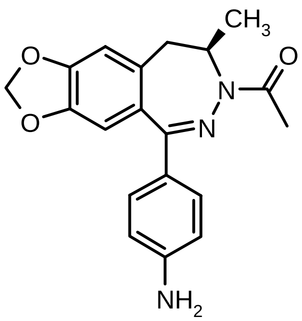
Scheme 7

These results clearly assert the labile nature of 2-hydroxycyclopentanones (+)- and (−)-**1**. They also show that the lipase Amano PS is very specific in its action on both the *meso*-diacetate **13** as well as on the acetoxy ketone *rac*-**2**. Thus, we have demonstrated a facile and unambiguous new practical chemoenzymatic approach to (2*R*)-hydroxycyclopentanone [(−)-**1**] with 28-32% yield and 90-92% ee, and (2*S*)-acetoxycyclopentanone [(+)-**2**] with 45% yield and 96-98% ee. Considering the unstable nature of 2-hydroxycyclopentanone, we are of the opinion that chemoenzymatic methods such as the one presented are best suited for the preparation of such compounds under mild conditions and with excellent ee. The acetoxy derivative (+)-**2**, prepared in very high enantiomeric purity, is quite stable and could also serve as important chiral building blocks in place of their unstable hydroxy counterparts.

1B.2 Section B: II. Amano PS Catalyzed Resolution of (±)-α-Methyl-1,3-benzodioxole-5-ethanol: Key Intermediate in the Synthesis of Antiepileptic Drug Candidate, Talampanel

1B.2.1 Background

A group of scientists from the Hungarian Institute of Drug Research have recently discovered an orally active novel compound 7-acetyl-5-(4-aminophenyl)-8(*R*)-methyl-8,9-dihydro-7*H*-1,3-dioxolo[4,5-*b*][2,3]-benzodiazepine [talampanel, GYKI-53773, IDR-53773, LY-300164, (–)-**16**] that attracted considerable attention due to its potential *antiepileptic*, *neuroprotectant* and *skeletal muscle relaxant* activities.[17-19] *In vitro*, talampanel exhibited selective noncompetitive AMPA antagonism and *in vivo*, it displayed broad-spectrum anticonvulsant and muscle relaxant effects in mice as well as providing neuroprotection from ischemic brain damage. The exciting promise shown by talampanel led to human trials and phase II trials of the drug (−)-talampanel (**16**) in patients with severe epilepsy not responsive to other drugs have shown efficacy. Phase III studies are in progress to confirm and expand these results. Hence, considering its properties and the recent results, talampanel has been identified as a future drug.[20,21]



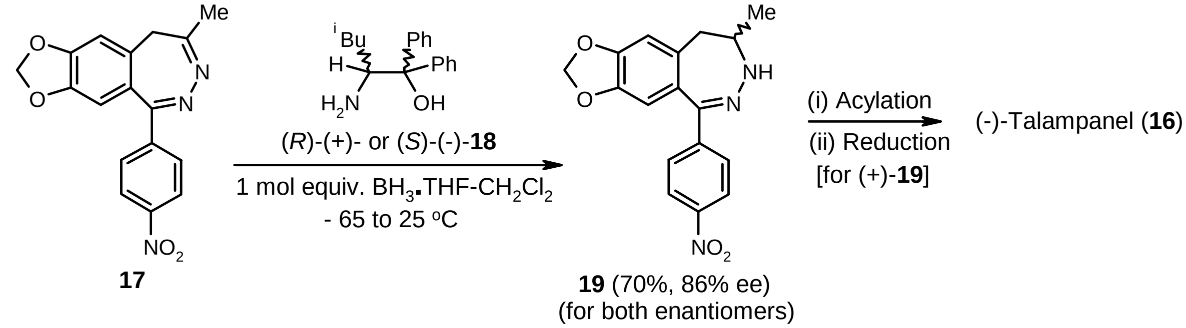
Talampanel [(−)-**16**]
CAS: 161832-65-1

1B.2.1.1 Synthetic Approaches Towards Talampanel

The clinical potential of this new compound (−)-**16** has led to great interest in developing new syntheses. Before discussing our results, the reported synthetic approaches towards (−)-**16** are illustrated in brief in the following part.

[A] *Ling's approach*

Ling et al. have completed an enantioselective synthesis of (−)-**16** via an asymmetric reduction of the carbon-nitrogen double bond of the 2,3-benzodiazepine **17** using a borane-homochiral amino alcohol complex.[22] The complex, formed from equivalent amounts of optically active 2-substituted-1,1-diphenyl-2-amino alcohols and borane (applied in THF solution or as the methyl sulfide complex), reduced 2,3-benzodiazepine **17** with good chemo-, regio- and enantioselectivity. The most significant optical induction was obtained with (*S*)-(−)- or (*R*)-(+)-2-amino-4-methyl-1,1-diphenylpentane-1-ol (**18**, prepared from L- & D-leucine) providing both enantiomers (depending upon the reagent isomer used) of nitro compound **19** in 86% ee and 70 % yield. The enantiomerically enriched (+)-**19** was transformed in two simple steps comprising acylation and reduction to (−)-talampanel (**16**) (Scheme 8).



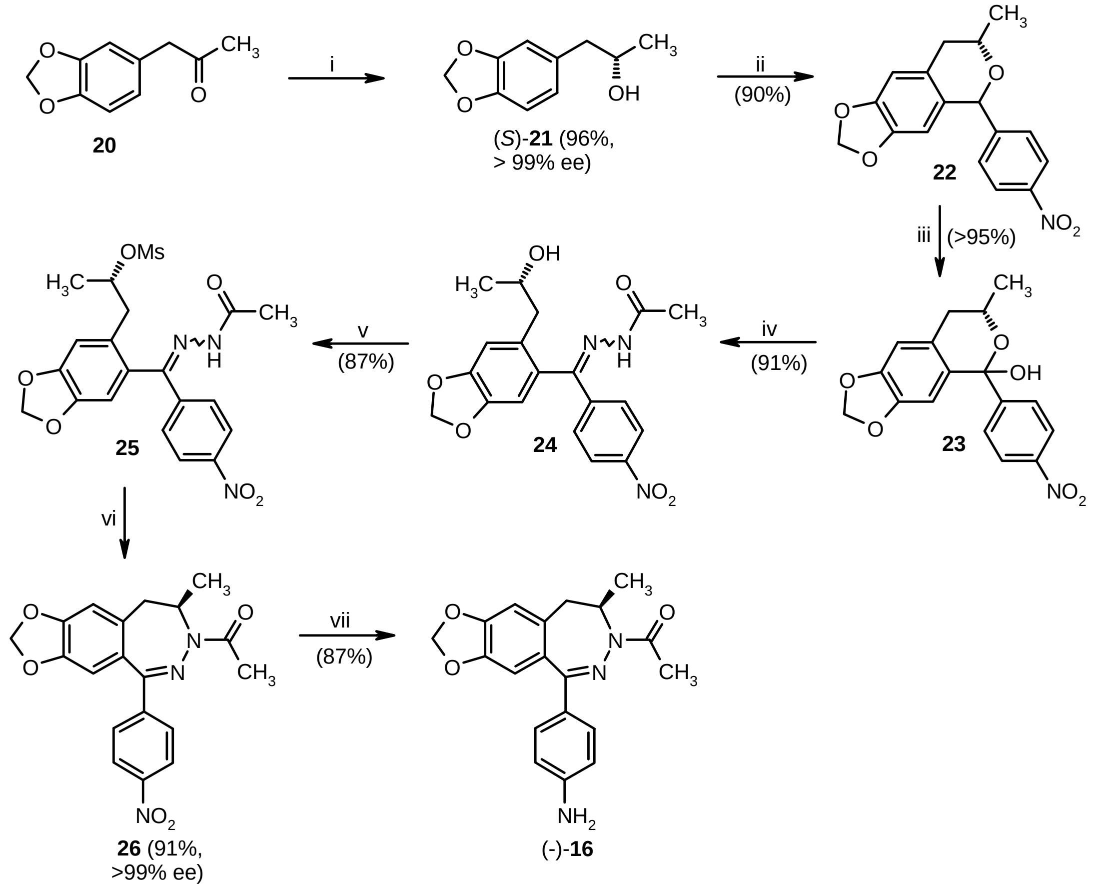
Scheme 8

[B] *Anderson's approach*

The stereoselective enzymatic reduction of 3,4-methylenedioxyphenylacetone (**20**) to the corresponding (+)-(*S*)-(α)-methyl-1,3-benzodioxole-5-ethanol (**21**) is the first step of a stereoselective route designed by Anderson et al. for the synthesis of (−)-talampanel (**16**).[21] An efficient large scale enantioselective reduction of 3,4-methylenedioxyphenyl acetone was accomplished using an NAD(P)H-dependent oxidoreductase from *Zygosaccharomyces rouxii*.[23] The system involved the use of polymeric hydrophobic resins both to supply substrate to the enzyme and remove the product from the reaction mixture as it is formed. In this manner, product toxicity to the yeast and copious emulsions encountered during product isolation could be avoided. High conversion to the desired alcohol (+)-**21** was accomplished with excellent stereocontrol.

The synthesis of (−)-talampanel (**16**) from enantiomerically enriched alcohol (+)-**21** was realized in 6-steps and 54% overall yield, a novel air-oxidation being the key-step in the sequence. Reaction of (+)-**21** with 4-nitrobenzaldehyde led to the convergent introduction of the remaining carbon constituents and generation of an isomeric mixture of optically pure **22**. The authors took advantage of the propensity of isochromans to undergo autoxidation and thus obtained the hemiketal **23** by an air-oxidation at C-1 of **22** in the presence of a base. Reaction of **23** with acyl hydrazide yielded the open-chain hydrazone

24, which on mesylation and subsequent cyclization generated the seven-membered 2,3-benzodiazapine skeleton **26**. The synthesis was then completed by reduction of the nitro group to deliver the natural product (Scheme 9).



Scheme 9 *Reactions, conditions and yields*: (i) *Z. rouxii*, XAD-7 resin (96%); (ii) *p*-NO_2-C_6H_4-CHO, HCl, toluene (90%); (iii) 50% NaOH, air, DMSO/DMF (>95%); (iv) H_2NNHAc, EtOH, HCl (cat.) (91%); (v) CH_3SO_2Cl, Et_3N, CH_2Cl_2 (87%); (vi) *t*-BuOLi, THF, (91%); (vii) Pd/C, H_2, EtOH (87%).

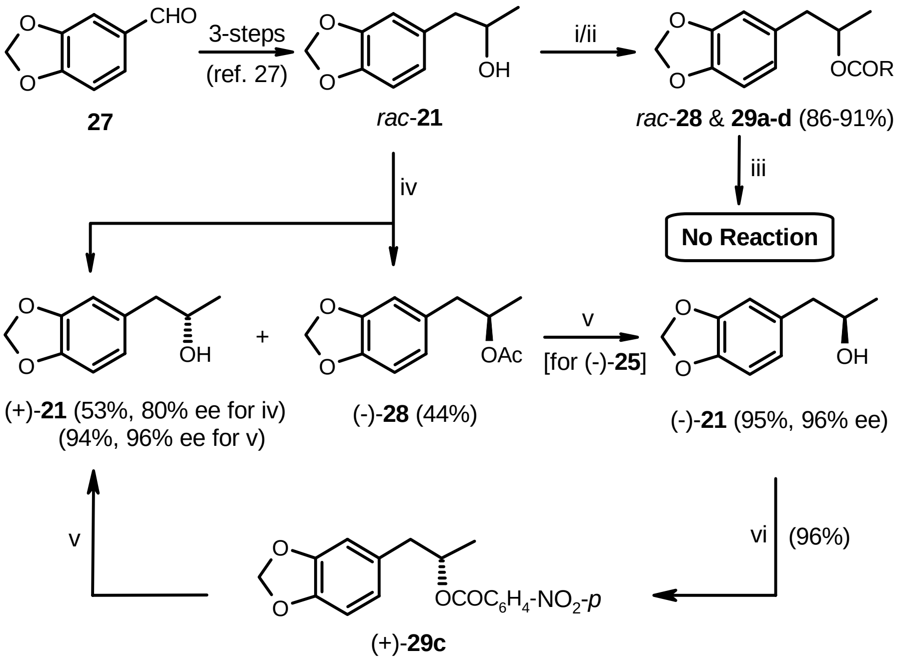
1B.2.2 Rationale for Present Work

Chemical methods for the enantioselective reduction of methyl benzyl ketones require the stoichiometric use of costly reagents.[24] Selectivity and conversion of the corresponding ketone to (+)-**21** with Baker's yeast and other common microorganisms were found to be unsatisfactory.[21,25] Copper(I)-catalyzed reaction of (*S*)-propylene oxide with the corresponding aryl Grignard reagent is known to produce the desired alcohol (+)-**21** in 91% yield. However, this method is not economical as the enantiomerically pure epoxide starting material is expensive.[21] Biotransformations, as shown by Anderson et al., are often more efficient than chemical methods and in continuation of our earlier studies on the preparation of important chiral compounds using lipase-catalyzed resolutions, we planned to prepare the desired intermediate (+)-**21** by enantioselective enzyme-catalyzed acylation of (±)-**21** and hydrolysis of acyl derivatives of (±)-**21**.[26]

1B.2.3 Results and Discussion

The (±)-α-methyl-1,3-benzodioxole-5-ethanol (**21**) was obtained in 3-steps from piperonal (**27**) using a known procedure involving nitroethane condensation, reductive hydrolysis and sodium borohydride reduction.[27] We prepared a systematic plan to study the lipase-catalyzed enantioselective hydrolysis of acyl derivatives of (±)-**21** as well as the lipase-catalyzed enantioselective acylation of (±)-**21**. At the outset, we synthesized the acetyl as well as chloroacetyl, *p*-methoxybenzoyl, *p*-nitrobenzoyl and palmitoyl derivatives **28** & **29a-d** from (±)-**21** for screening lipase-catalyzed hydrolysis with the lipases Amano PS, CCL and Amano AY (Scheme 10). Unfortunately, the substrates **28** & **29a-d** were not recognized by these lipases and all attempts at their biphasic enantioselective enzymatic hydrolysis met with failure. Interestingly and to our joy, the Amano PS-catalyzed acylation of (±)-**21** using vinyl acetate (5 equiv.) as an acyl donor at 25 °C was successful and resulted in a 12% yield of (−)-**28** in 48 h (the stereochemical assignment was done on the basis of comparison with literature[21] information). In our attempts to improve the conversion of the enzymatic resolution, we noticed during our studies that the reaction is time, temperature and vinyl acetate concentration dependent. Several experiments were carried out to optimize the yield of both (+)-**21** and (−)-**28** so as to achieve the best possible enantiomeric purity (Table 1). The most favourable outcome resulted when the reaction was performed using 5 equiv. of vinyl acetate at 50°C over a time period of 72 h, affording (−)-**28** with 96% ee in 46% yield and (+)-**21** with 80% ee in 54% yield (Table 1, entry 5). The percentage conversion was deduced by recording the ^{1}H NMR spectrum of the reaction mixture prior to column chromatographic separation of the products (after the work-up procedure). The α-proton to the hydroxyl group (substrate) appeared cleanly as a sextet at δ 3.96 whereas the α-proton to the acetate group (product) appeared as a sextet at δ 5.05. The percentage conversion was thus calculated from the ratio of their integration values. We also observed that under the present set of reaction conditions, the enzyme

Amano PS exhibited highest activity at 50 °C and showed a decline in its activity at 55 and 60 °C (Table 1, entries 5–8). Also, the conversion was not satisfactory when the reaction was carried out over 24/48 h or when the vinyl acetate concentration was increased (Table 1, entries 2-4). The formed hydroxy compound (+)-**21** and the acetyl derivative (−)-**28** (under optimized conditions) were easily separated by silica-gel column chromatography. Methanolysis of the acetyl derivative (−)-**28** in the presence of K_2CO_3 as a catalyst so provided the alcohol (−)-**21** in 95% yield.



28, R = Methyl; **29a**, R = Chloromethyl; **29b**, R = *p*-Anisyl; **29c**, R = *p*-Nitrophenyl; **29d**, R = Pentadecanyl

Scheme 10 *Reagents, conditions and yields*: (i) Ac_2O/py, rt, 24 h (91%); (ii) RCOOH, DCC, DMAP, DCM, rt, 8 h (86-90%); (iii) Petroleum ether/benzene (2:1), Amano PS/CCL/Amano AY, 50 mM Sodium phosphate buffer (pH 7.0), rt, 24 h; (iv) *n*-Hexane/benzene (2:1), Amano PS, vinyl acetate, 50 °C, 72 h [(−)-**28**, 44%; (+)-**21**, 53%]; (v) K_2CO_3/MeOH, 0 °C, 4 h (94%/95%); (vi) DEAD, TPP, *p*-NO_2-C_6H_4COOH, THF, 0 °C, 6 h (96%) .

The ^1H NMR spectrum of a diastereomeric mixture of Mosher's esters obtained from (±)-**21** and (*R*)-Mosher's acid showed a very clean resolution of the $-OCH_3$ and the 1,3-benzodioxole (-O-CH_2-O-) methylene protons. The ^1H NMR spectrum of Mosher's esters of (+)- & (−)-**21** revealed that (+)-**21** possesses 80% ee[28] while (−)-**21** is formed with 96% ee. A comparison of the ^1H NMR spectra of MTPA-esters of enantiomerically enriched (+)- & (−)-**21** (page 82) clearly showed us that the $-OCH_3$ proton singlet corresponding to

(+)-**21** appeared at δ 3.51 while that corresponding to (−)-**21** appeared at δ 3.42. Also, in the ^{1}H NMR spectrum of Mosher's ester of enantiomerically enriched (+)-**21**, the 1,3-benzodioxole (-O-CH$_2$-O-) methylene protons appeared as singlets at δ 5.92 and 5.93, corresponding to the major and minor isomers of the parent compound respectively. However, the ^{1}H NMR spectrum of Mosher's ester of enantiomerically enriched (−)-**21** did not show any splitting for the 1,3-benzodioxole (-O-CH$_2$-O-) methylene protons (presumably because the peak arising from the minor isomer got completely submerged under the major peak). Hence, the ee for both (+)- & (−)-**21** was deduced from the ratio of the integration values of the –OCH$_3$ proton peaks in the above ^{1}H NMR spectra individually. The ee values for other entries in the table were determined by measuring the specific rotation and comparison with the standard values available (please see Table 1 footnote).

Table 1 Amano PS Catalyzed Enantioselective Acylation of (±)-α-Methyl-1,3-benzodioxole-5-ethanol (**21**)

S. No.	Temp (°C)	Time (h)	VA (equiv.)	% Conv. (by ^{1}H NMR)	(+)-**21**[a] $[\alpha]^{20}_{D}$	(−)-**28**[a] $[\alpha]^{20}_{D}$	(+)-**21** % ee	(−)-**28** % ee
1	25	48	5	12	+3.1	−5.3	10[b]	95[b]
2	50	24	5	35	+11.6	−5.4	34[b]	96[b]
3	50	48	5	44	+26.1	−5.3	76[c]	95[d]
4	50	60	10	40	+19.3	−5.3	56[b]	95[b]
5	**50**	**72**	**5**	**46**	**+27.6**	**−5.4**	**80**[c]	**96**[d,e]
6	50	96	5	46	+27.5	−5.3	80[c]	96[d]
7	55	48	5	33	+10.7	−5.3	31[b]	95[b]
8	60	48	5	25	+6.9	−5.3	20[b]	95[b]

[a] Specific rotations were determined in CHCl$_3$ (c = 1 in all cases).

[b] Ee determined from the specific rotation value.

[c] Ee determined by MTPA-ester preparation with (*R*)-Mosher's acid.

[d] Ee determined by conversion of -OAc to -OH followed by MTPA-derivatisation with (*R*)-Mosher's acid.

[e] *E* value: 125.

Thus, in the above resolution, the lipase had actually recognized the opposite isomer, providing us the desired intermediate for the synthesis of talampanel, (+)-**21**, in only 80% ee. Hence, although we were successful in obtaining enantiomerically enriched compounds, we needed to improve upon the optical purity of the desired isomer. Towards this end, we carried out a Mitsunobu inversion[30] of alcohol (−)-**21** using DEAD, TPP and *p*-nitrobenzoic acid so as to obtain (+)-**29c** in 96% yield. Subsequent alcoholysis of (+)-**29c** (using similar conditions as for the acetate alcoholysis mentioned above) provided the desired alcohol (+)-**21** in 94% yield and 96% ee (from the ^{1}H NMR spectrum of the MTPA-ester).

The conversion of the enantiomerically pure (*S*)-alcohol **21** to (−)-talampanel (**16**) is a well established protocol (discussed above in Anderson's approach).[21,23] Thus we have demonstrated an elegant practical enantioselective Amano PS-catalyzed acylation of (±)-α-methyl-1,3-benzodioxole-5-ethanol (*rac*-**21**) to obtain the potential chiral building block (*S*)-α-methyl-1,3- benzodioxole-5-ethanol [(+)-**21**] for promising antiepileptic future drug (−)-talampanel (**16**) in 53% yield and 80% ee. The higher enantiomerically enriched antipode, (*R*)-(−)-**28** was also transformed to the desired isomer (+)-**21** via Mitsunobu inversion in 38% overall yield (four steps) with 96% ee. Such enzymatic resolutions at an early stage of the synthesis are always of interest from a practical point of view to both academic and industrial chemists, particularly more so when the end-product is as important and exciting as the drug candidate talampanel.

1B.3 Section B: III. Amano PS Catalyzed Methanolysis of Maleimides to Methyl Maleanilates

1B.3.1 Background

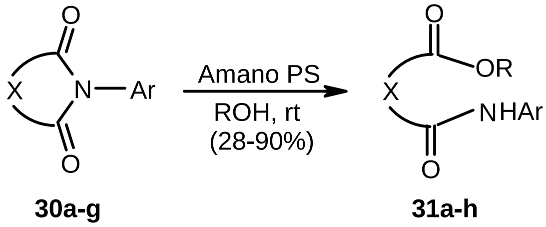
Preparation of esters is to be treated not just as a common reaction in synthetic field but as an important reaction as well since esters form a part of almost any synthetic endeavour. Thus development of new methods for the preparation of esters carries a lot of significance. Alkyl maleanilates are a class of esters that have shown potential as important building blocks in the synthesis of several structurally interesting and biologically important natural and unnatural heterocyclic systems.[31] Alkyl maleanilates are generally prepared by the treatment of corresponding anilic acids with (i) alkanol/HCl(gas),[32] (ii) alkanol/H_2SO_4,[33] (iii) alkanol/triethylamine,[34] (iv) dialkyl sulphite,[35] (v) alkanol/$SOCl_2$,[36] (vi) alkanol/P_2O_5,[37] (vii) alkanol/PCl_5,[38] (viii) CH_2N_2[39] and (ix) NaOH/alkyl iodide.[40] In all these acid/base catalyzed esterification reactions, however, there is a possibility of amide bond cleavage, both in the starting material and the formed product; while if diazomethane is employed, then there is a likelihood that the excess diazomethane present could add to the ester formed in a Michael fashion yielding the corresponding cyclopropane derivative. Moreover, these methods would have to involve protection/deprotection protocols in case the substrate anilic acid happens to carry acid/base sensitive functional groups.

1B.3.2 Rationale for Present Work

Development of a new simple, efficient and environment friendly method free of the above-mentioned drawbacks would serve synthetic chemists well. Enzymes, as we have seen in Section A, have been employed as catalysts extensively for resolution of racemates because of their ability to bring about highly chemo-, regio- and stereoselective transformations. These attributes could work wonders if chemists explore more extensively the possibility of using enzymes as reagents to bring about other transformations in synthetic organic chemistry. In this context, this section describes the first simple enzyme-catalyzed preparation of alkyl maleanilates **31a-h** starting from corresponding cyclic imides **30a-g**.

1B.3.3 Results and Discussion

The desired imides **30a-h** were prepared following the well-established 2-step protocol starting from the corresponding anhydride.[41] The anhydrides upon reacting with the required aromatic amine in ether at room temperature yielded the corresponding anilic acids, which on dehydration under thermodynamically controlled conditions (Ac_2O/NaOAc, Δ, 70 ^{o}C, 2 hr) gave the corresponding imides **30a-h** in nearly quantitative yields.



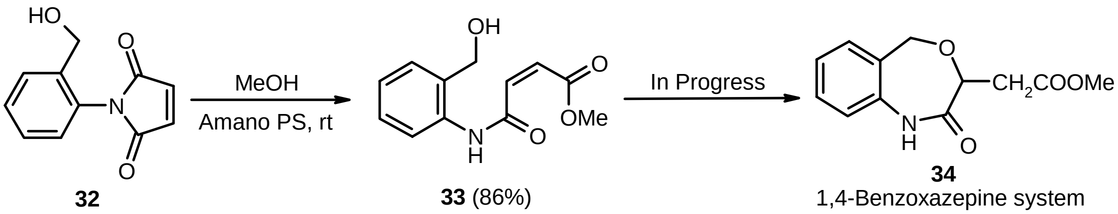
Scheme 11 Variations in "X", "Ar" and "R" have been illustrated in Table 2. Please see Table 2 for details including individual yields for each variation.

The maleimides **30a-f** in methanol underwent a smooth alcoholysis in the presence of catalytic amount of Amano PS (10% by weight) at room temperature to yield the corresponding alkyl maleanilates **31a-f** in more than 80% yields (Scheme 11, Table 2, entries 1-6). All theses reactions were monitored by TLC and it was found that the reaction of **30a** was the fastest among all the imides studied proceeding to completion in 6 h time (Table 2, entry 1). The methanolysis of substituted maleimides **30b-f** were relatively slow and took 18 to 24 h time for major completion although the yields were good for all the above reactions, ranging between 81 and 88% (Table 2, entries 2-6). On the other hand, the reaction of maleimide **30d** with ethanol and succinimide **30g** with methanol were very sluggish and the esters **31g** & **31h** were obtained only in 28% & 31% yields, respectively in 100 h reaction time. This indicates that the reaction is more efficient for methanolysis and works better with maleimides as against succinimides. The phthalimide **30h** did not undergo the enzyme-catalyzed methanolysis to yield **31i** under neutral reaction conditions. In our opinion, it was not surprising that the conditions employed failed to work on *N*-arylphthalimide **30h**. It is known that the *N*-arylphthalimides are thermodynamically more stable than the corresponding phthalanilic esters and the condition to obtain the esters of phthalanilic acids is still elusive.[37]

Table 2 Alcoholysis of various maleimides yielding the corresponding alkyl maleanilates

S. No.	Substrate	X	Ar	ROH	Time (h)	Product	Yield (%)
1	**30a**	-CH=CH-	Phenyl	MeOH	6	**31a**	92
2	**30b**	-CH=CH-	*o*-Tolyl	MeOH	24	**31b**	81
3	**30c**	-CH=CH-	*m*-Tolyl	MeOH	18	**31c**	85
4	**30d**	-CH=CH-	*p*-Tolyl	MeOH	21	**31d**	88
5	**30e**	-CH=CH-	*p*-Anisyl	MeOH	24	**31e**	87
6	**30f**	-CH=CH-	*p*-Chloro-phenyl	MeOH	24	**31f**	83
7	**30d**	-CH=CH-	*p*-Tolyl	EtOH	100	**31g**	28
8	**30g**	-CH_2-CH_2-	*p*-Tolyl	MeOH	100	**31h**	31
9	**30h**	-*o*-C_6H_4-	*p*-Tolyl	MeOH	48	**31i**	-

We envisaged the application of this strategy for the preparation of seven-membered heterocycles such as the 1,4-benzoxazepine system. Towards this end, *o*-aminobenzyl alcohol was converted to the corresponding *N*-arylmaleimide **32** using the standard conditions. Methanolysis of this imide employing Amano PS under the conditions reported in this section afforded the corresponding alkyl maleanilate **33**. Efforts are in progress to transform this ester to the desired heterocycle **34** via an intramolecular Michael-type addition (Scheme 12).



Scheme 12

In summary, we have demonstrated the first simple and efficient enzymatic method for synthesis of methyl maleanilates in 80-90% yields. The present method may also be useful for alcoholysis of several other imides, isoimides and desymmetrization of *meso*-cyclic anhydride derivatives.[42] Using the present conditions, we are also looking at the prospect of an enantioselective methanolysis of imides carrying a chiral centre (on the *N*-substituent), which would in turn afford us enantiomerically enriched imides and the corresponding enantiomerically enriched methyl maleamates.

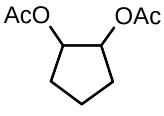
1B.4 Summary

The importance and tremendous utilities of lipases have already been detailed in Section A of this chapter. We, in our group, have also put in our efforts to utilize these remarkable natural catalysts for further important applications. Thus, as seen in Section B, we have designed an elegant chemoenzymatic route to the unstable yet potentially useful chiral building blocks, 2-hydroxy/acetoxycylopentanones with excellent ee. We have also carried out a lipase-mediated resolution of (±)-α-methyl-1,3-benzodioxole-5-ethanol, the key intermediate in the synthesis of the remarkable antiepileptic drug candidate, talampanel, with very good yield and ee. Moreover, our efforts have also focused on tapping the potential of lipases as reagents in organic synthesis. In this context, transformation of maleimides to the corresponding methyl maleanilates, which could serve as intermediates for some interesting heterocyclic compounds, has been effected using the lipase Amano PS (we are also currently working on lipase-catalyzed deprotection of silyl ethers). Thus, in summary, we have successfully employed lipases for the resolution of important chiral compounds and intermediates. Further exploration of lipases as reagents in organic synthesis is our target and efforts are in progress in this regard. There has been an immense increase in the applications of enzymes, in general, worldwide in both academia and industry. This promising trend assures a very bright future to enzymology.

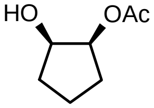
1B.5 Experimental Section

Commercially available cyclopentanediol, acetic anhydride, oxalyl chloride, DCC, DMAP, DEAD, TPP and (*R*)-Mosher's acid were used. Amano PS-800 U from Amano Pharmaceuticals, Japan was used. The activity of the lipase powder used is expressed in terms of units, 1 unit corresponding to micromoles of butyric acid liberated (estimation by GC) from glyceryl tributyrate per minute per milligram of enzyme powder.[43]

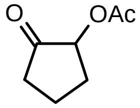
***meso*-1,2-Diacetoxycyclopentane (13).** To a stirred solution of *meso*-cyclopentanediol (**12**) (2.04 g, 20 mmol) in pyridine (10 mL) was added acetic anhydride (8 mL) and the reaction mixture was kept in the dark for 24 h at rt. The reaction mixture was poured into water and extracted with ethyl acetate (15 mL x 5). The combined organic layer was washed with $CuSO_4$ solution, water, brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica-gel column chromatographic purification of the residue using 5 to 10% ethyl acetate:petroleum ether mixture as an eluant gave **13** (3.5 g, 93% yield).

 **13** **$C_9H_{14}O_4$ (186)**	Thick oil. **IR** (Neat) ν_{max} 1742 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.50-2.10 (m, 6H), 2.04 (s, 6H), 5.00-5.25 (m, 2H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 18.8, 20.6, 27.9, 73.8, 170.0. **Ms** (*m*/*e*) 143, 126, 101, 83, 67, 57.

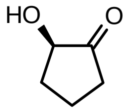
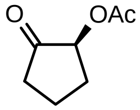
(±)-*cis*-1-Acetoxy-2-hydroxycyclopentane (14). A solution of diacetate **13** (2.79 g, 15 mmol) in petroleum ether:benzene (2:1) mixture (60 mL) was added to a suspension of Amano PS lipase (350 mg) in aq. sodium phosphate (0.01 M, 20 mL) at pH 7.0. The reaction mixture was stirred at 25 °C for 18 h. The reaction mixture was filtered through celite and the aqueous layer was extracted with ethyl acetate (15 mL x 5). The combined organic layer was washed with water, brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica-gel column chromatographic separation of residue using 10 to 15% ethyl acetate:petroleum ether mixture as an eluant gave **13** (840 mg, 30% yield) and (±)-**14** (1.4 g, 65% yield) respectively (increased reaction time resulted in the formation of **12**).

 **(±)-14** **$C_7H_{12}O_3$ (144)**	Thick oil. **IR** (Neat) ν_{max} 3429, 1722 cm^{-1}. **^{1}H NMR** (CDCl$_3$, 200 MHz) δ 1.40-2.05 (m, 6H), 2.08 (s, 3H), 2.25 (bs, 1H), 4.15 (q, J = 5 Hz, 1H), 4.87-5.03 (m, 1H). **^{13}C NMR** (CDCl$_3$, 50 MHz) δ 19.0, 20.8, 27.7, 30.4, 72.5, 76.4, 171.0. **Ms** (m/e) 101, 84, 73, 67, 57, 55.

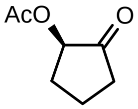
(±)-2-Acetoxycyclopentanone (2). To a solution of oxalyl chloride (0.74 mL, 8.4 mmol) in dry CH_2Cl_2 (5 mL) under argon at – 60 ºC was added a solution of DMSO (0.8 mL, 11 mmol) in dry CH_2Cl_2 (5 mL) in a dropwise fashion over a period of 5 min. Hydroxyacetate (±)-**14** (1.0 g, 7 mmol) in dry CH_2Cl_2 was charged dropwise over a period of 5 min and the reaction mixture was stirred at – 60 ºC for 15 min. Et_3N (4.9 mL, 35 mmol) was added and the reaction mixture was further stirred at rt for 5 min. Water (10 mL) was added to the reaction mixture and the aqueous layer was extracted with CH_2Cl_2 (5 x 15 mL). The combined organic layer was washed with water, brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica-gel column chromatographic purification of the residue provided (±)-**2** (855 mg, 86% yield).

 **(±)-2** **$C_7H_{10}O_3$ (142)**	Thick oil. **IR** (Neat) ν_{max} 1753, 1744 cm^{-1}. **^{1}H NMR** (CDCl$_3$, 200 MHz) δ 1.70-2.00 (m, 2H), 2.13 (s, 3H), 2.20-2.50 (m, 4H), 5.07 (t, J = 9 Hz, 1H). **^{13}C NMR** (CDCl$_3$, 50 MHz) δ 17.0, 20.5, 28.2, 34.6, 75.5, 169.9, 212.2. **Ms** (m/e) 142, 99, 86, 71.

Amano PS catalyzed hydrolysis of (±)-2. A solution of (±)-2-acetoxyketone **2** (710 mg, 5 mmol) in petroleum ether:benzene (2:1) mixture (30 mL) was added to a suspension of Amano PS lipase (250 mg) in aq. sodium phosphate (0.01 M, 10 mL) at pH 6.5. The reaction mixture was stirred at 25 °C for 22 h. The reaction mixture was filtered through celite and the aqueous layer was extracted with ethyl acetate (15 mL x 5). The combined organic layer was washed with water, brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica-gel column chromatographic separation using 10 to 15% ethyl acetate:petroleum ether mixture as an eluant gave (+)-**2** (318 mg, 45% yield) and (–)-**1** (150 mg, 32% yield) respectively.

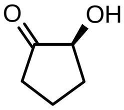
 **(–)-1** **$C_5H_8O_2$ (100)**	Thick oil. $[\alpha]^{20}_{D}$ = – 38.4 (*c* 1.2, $CHCl_3$). **IR** (Neat) ν_{max} 3404, 1746 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.60-1.95 (m, 2H), 1.95-2.60 (m, 4H), 2.92 (bs, 1H, D_2O exchangeable), 4.09 (t, *J* = 10 Hz, 1H); **^{1}H NMR** (D_2O, 200 MHz) δ 1.50-2.50 (m, 6H), 4.19 (t, *J* = 10 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 16.4, 30.7, 33.9, 75.7, 159.8. **Ms** (*m*/*e*) 100, 84, 72, 57, 55.
 **(+)-2** **$C_7H_{10}O_3$ (142)**	Thick oil. $[\alpha]^{20}_{D}$ = + 61.0 (*c* 2.0, $CHCl_3$). Analytical and spectral data obtained were identical with (±)-**2**.

Acetyl derivative of (–)-1. To a solution of acetic acid (10 mg), (–)-2-hydroxyketone **1** (10 mg, 0.1 mmol) and DMAP (cat.) in dry CH_2Cl_2 (3 mL) was added a solution of DCC (15 mg) in dry CH_2Cl_2 (2 mL) at 0 ºC. The reaction mixture was allowed to warm to rt and stirred for 8 h. The urea formed was filtered off and the organic layer was concentrated in vacuo. Silica-gel column chromatographic purification of the residue using 10% ethyl acetate:petroleum ether mixture as an eluant gave the acetyl derivative of (–)-**1** in quantitative yield.

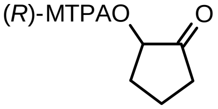
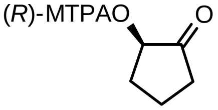
 **(–)-2** **$C_7H_{10}O_3$ (142)**	Thick oil. $[\alpha]^{20}_{D}$ = – 54.9 (*c* 1.0, $CHCl_3$). Analytical and spectral data obtained were identical with (±)-**2**.

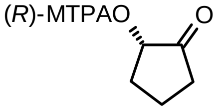
(2*S*)-Hydroxycyclopentanone [(+)-1]. To a solution of acetoxyketone (+)-**2** (71 mg, 0.5 mmol) in MeOH (15 mL) and H_2O (15 mL) was added K_2CO_3 (70 mg, 0.6 mmol) and the reaction mixture was stirred at 0 °C for 4 h. The reaction mixture was diluted with water

(30 mL) and the aqueous solution was extracted with CH_2Cl_2 (15 mL x 5). The combined organic layer was washed with water, brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica-gel column chromatographic purification of the residue using 15% ethyl acetate:petroleum ether mixture as an eluant gave (+)-**1** (39 mg, 77% yield).

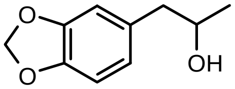
 **(+)-1** **$C_5H_8O_2$ (100)**	Thick oil. $[\alpha]^{20}_D$ = + 20.3 (*c* 1.2, $CHCl_3$). Analytical and spectral data obtained were identical with (–)-**1**.

General procedure for MTPA-Ester preparation. To a solution of (*R*)-Mosher's acid (23 mg), 2-hydroxyketone (–)-**1** (10 mg, 0.1 mmol) and DMAP (cat.) in dry CH_2Cl_2 (3 mL) was added a solution of DCC (15 mg) in dry CH_2Cl_2 (2 mL) at 0 ºC. The reaction mixture was allowed to warm to rt and stirred for 8 h. The urea formed was filtered off and the organic layer was concentrated in vacuo. Silica-gel column chromatographic purification of the residue using 10% ethyl acetate:petroleum ether mixture as an eluant gave the MTPA-Ester in quantitative yield.

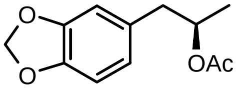
 **MTPA-Ester of (±)-2-hydroxycyclopentanone** **$C_{15}H_{15}F_3O_4$ (316)**	**^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.70-2.60 (m, 12H), 3.58 (s, 3H), 3.64 (s, 3H), 5.25 (t, *J* = 8 Hz, 1H), 5.35 (t, *J* = 8 Hz, 1H), 7.30-7.75 (m, 10H). **Ms** (*m*/*e*) 316, 286, 216, 189, 158, 139, 119, 105, 91, 77, 69, 55.
 **MTPA-Ester of (–)-(2*R*)-hydroxycyclopentanone** **$C_{15}H_{15}F_3O_4$ (316)**	**^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.75-2.50 (m, 6H), 3.64 (s, 3H), 5.35 (t, *J* = 8 Hz, 1H), 7.30-7.70 (m, 5H). **Ms** (*m*/*e*) 316, 286, 216, 189, 158, 139, 119, 105, 91, 77, 69, 55.

 **MTPA-Ester of (+)-(2*S*)-hydroxycyclopentanone** **$C_{15}H_{15}F_3O_4$ (316)**	**^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.80-2.60 (m, 6H), 3.57 (s, 3H), 5.25 (t, J = 8 Hz, 1H), 7.35-7.70 (m, 5H). **Ms** (*m*/*e*) 316, 286, 216, 189, 158, 139, 119, 105, 91, 77, 69, 55.

(±)-α-Methyl-1,3-benzodioxole-5-ethanol (**21).** It was prepared in three steps from piperonal using known literature procedures.[27]

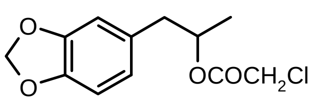
 **(±)-21** **$C_{10}H_{12}O_3$ (180)**	Analytical and spectral data obtained were identical with (+)-**21**.

(±)-α-Methyl-1,3-benzodioxole-5-ethyl acetate (**28).** To a stirred solution of alcohol (±)-**21** (900 mg, 5 mmol) in pyridine (10 mL) was added acetic anhydride (5 mL) and the reaction mixture was kept in the dark at room temperature for 24 h. The reaction mixture was poured into water and extracted with ethyl acetate (15 mL x 5). The combined organic layer was washed with aq. $CuSO_4$ solution, water and brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether and ethyl acetate (9:1) gave (±)-**28** (1.01 g, 91% yield).

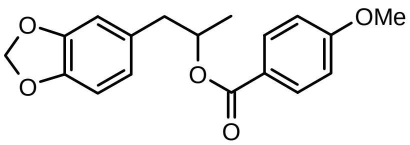
 **(±)-28** **$C_{12}H_{14}O_4$ (222)**	Analytical and spectral data obtained were identical with (−)-**28**.

General procedure for the preparation of esters (±)-29a-d. To a solution/slurry of carboxylic acid (1.1 mmol), alcohol (±)-**21** (180 mg, 1 mmol) and catalytic amount of DMAP in dry CH_2Cl_2 (10 mL) was added a solution of DCC (1.1 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 8 h. The urea formed was filtered off and the organic layer was concentrated in vacuo. The residue was chromatographed over silica gel using petroleum ether and ethyl acetate (9.5:0.5) to give the desired esters (±)-**29a-d** in 86-90% yield.

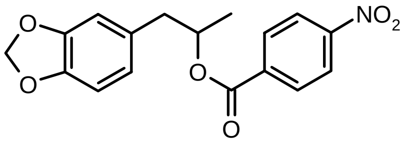
(±)-α-Methyl-1,3-benzodioxole-5-ethyl chloro acetate 29a (226 mg, 88% yield).

 **(±)-29a** **$C_{12}H_{13}ClO_4$ (256.5)**	Oil. **IR** (Neat) ν_{max} 1751, 1740, 1502, 1491, 1443, 1250, 1188, 1040 cm^{-1}. **^{1}H NMR** (CDCl$_3$, 200 MHz) δ 1.26 (d, J = 6 Hz, 3H), 2.71 (dd, J = 14 and 6 Hz, 1H), 2.87 (dd, J = 14 and 6 Hz, 1H), 4.01 (s, 2H), 5.13 (sextet, J = 6 Hz, 1H), 5.93 (s, 2H), 6.63 (dd, J = 8 and 2 Hz, 1H), 6.69 (s, 1H), 6.74 (d, J = 8 Hz, 1H). **Anal. Calcd for $C_{12}H_{13}ClO_4$**: C, 56.15; H, 5.07. Found: C, 56.23; H, 5.11.

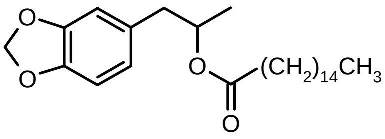
(±)-α-Methyl-1,3-benzodioxole-5-ethyl *p*-methoxybenzoate 29b (270 mg, 86% yield).

 **(±)-29b** **$C_{18}H_{18}O_5$ (314)**	Thick oil. **IR** (Neat) ν_{max} 1709, 1607, 1502, 1256, 1038 cm^{-1}. **^{1}H NMR** (CDCl$_3$, 200 MHz) δ 1.32 (d, J = 6 Hz, 3H), 2.79 (dd, J = 14 and 6 Hz, 1H), 2.98 (dd, J = 14 and 6 Hz, 1H), 3.86 (s, 3H), 5.27 (sextet, J = 6 Hz, 1H), 5.91 (s, 2H), 6.60-6.80 (m, 3H), 6.91 (d, J = 8 Hz, 2H), 7.98 (d, J = 8 Hz, 2H). **Anal. Calcd for $C_{18}H_{18}O_5$**: C, 68.78; H, 5.73. Found: C, 68.88; H, 5.91.

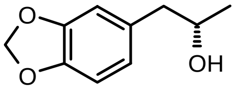
(±)-α-Methyl-1,3-benzodioxole-5-ethyl *p*-nitrobenzoate 29c (289 mg, 88% yield).

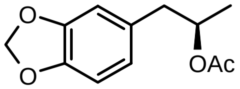
 **(±)-29c** **$C_{17}H_{15}NO_6$ (329)**	Analytical and spectral data obtained were identical with (+)-**29c**.

(±)-α-Methyl-1,3-benzodioxole-5-ethyl hexadecanoate 29d (376 mg, 90% yield).

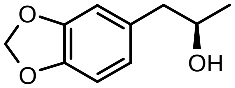
 **(±)-29d** **$C_{26}H_{42}O_4$ (418)**	Thick oil. **IR** (Neat) ν_{max} 1732, 1504, 1491, 1443, 1248, 1042 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 0.88 (t, *J* = 8 Hz, 3H), 1.20 (d, *J* = 6 Hz, 3H), 1.25 (bs, 24H), 1.45-1.65 (m, 2H), 2.24 (t, *J* = 8 Hz, 2H), 2.66 (dd, *J* = 14 and 6 Hz, 1H), 2.83 (dd, *J* = 14 and 8 Hz, 1H), 5.06 (sextet, *J* = 6 Hz, 1H), 5.92 (s, 2H), 6.63 (dd, *J* = 6 and 2 Hz, 1H), 6.69 (s, 1H), 6.73 (d, *J* = 6 Hz, 1H). **Anal. Calcd for $C_{26}H_{42}O_4$**: C, 74.64; H, 10.05. Found: C, 74.51; H, 10.13.

Amano PS catalyzed acylation of (±)-21. A solution of alcohol (±)-**21** (900 mg, 5 mmol) in *n*-hexane/benzene (2:1) (25 mL) was added to Amano PS lipase (300 mg) followed by vinyl acetate (2.3 mL, 25 mmol). The reaction mixture was stirred at 50 °C for 72 h and then allowed to reach room temperature. The enzyme was filtered off, washed with ethyl acetate and the organic layer was concentrated in vacuo. The residue was chromatographed over silica gel using petroleum ether and ethyl acetate (9:1) to obtain (+)-**21** (474 mg, 53% yield) and (−)-**28** (498 mg, 44% yield), respectively.

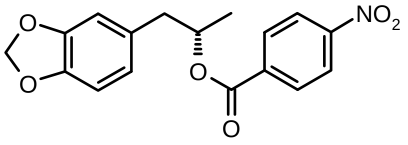
 **(+)-21** **$C_{10}H_{12}O_3$ (180)**	Oil. $[\alpha]^{20}_D$ = + 27.6° (*c* 1.0, $CHCl_3$). **IR** (Neat) ν_{max} 3398, 1609, 1502, 1490, 1443, 1248, 1040 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.23 (d, *J* = 6 Hz, 3H), 1.63 (bs, 1H), 2.59 (dd, *J* = 14 and 8 Hz, 1H), 2.72 (dd, *J* = 14 and 4 Hz, 1H), 3.96 (sextet, *J* = 6 Hz, 1H), 5.93 (s, 2H), 6.66 (d, *J* = 8 Hz, 1H), 6.70 (s, 1H), 6.76 (d, *J* = 8 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 22.3, 45.0, 68.5, 100.5, 107.9, 109.4, 122.0, 132.2, 145.7, 147.3. **MS** (*m*/e) 180, 148, 135, 121, 106, 91, 77, 63, 51, 45. **Anal. Calcd** for $C_{10}H_{12}O_3$: C, 66.67; H, 6.67. Found: C, 66.52; H, 6.55.

 **(–)-28** **$C_{12}H_{14}O_4$ (222)**	Oil. $[\alpha]^{20}_D = -5.4°$ (*c* 1.0, $CHCl_3$). **IR** (Neat) ν_{max} 1736, 1609, 1505, 1491, 1443, 1373, 1246, 1042 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.21 (d, *J* = 6 Hz, 3H), 2.01 (s, 3H), 2.66 (dd, *J* = 14 and 6 Hz, 1H), 2.84 (dd, *J* = 14 and 6 Hz, 1H), 5.05 (sextet, *J* = 6 Hz, 1H), 5.93 (s, 2H), 6.64 (d, *J* = 8 Hz, 1H), 6.69 (s, 1H), 6.74 (d, *J* = 8 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 19.1, 21.0, 41.7, 71.3, 100.6, 107.9, 109.5, 122.1, 131.1, 146.0, 147.4, 170.3. **MS** (*m/*e) 222, 162, 147, 135, 121, 104, 91, 77, 69, 63. **Anal. Calcd for $C_{12}H_{14}O_4$**: C, 64.86; H, 6.31. Found: C, 65.02; H, 6.49.

(*R*)-α-Methyl-1,3-benzodioxole-5-ethanol [(–)-21]. To a solution of acetate (–)-**28** (444 mg, 2 mmol) in dry methanol (10 mL) was added anhydrous K_2CO_3 (5 mg) at 0 °C with stirring. The reaction mixture was further stirred at 0 °C for 4 h and subsequently filtered through celite. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether and ethyl acetate (9:1) yielded (–)-**21** (340 mg, 95% yield).

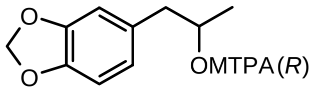
 **(–)-21** **$C_{10}H_{12}O_3$ (180)**	Thick oil. $[\alpha]^{20}_D = -34.2°$ (*c* 1.0, $CHCl_3$). Analytical and spectral data obtained were identical with (+)-**21**.

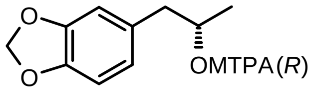
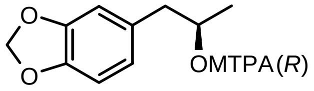
Mitsunobu inversion of (–)-21. A solution of DEAD (192 mg, 1.1 mmol) in dry THF (5 mL) was added dropwise to a solution of TPP (288 mg, 1.1 mmol), *p*-nitrobenzoic acid (184 mg, 1.1 mmol) and alcohol (–)-**21** (180 mg, 1 mmol) in dry THF (10 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 8 h. Concentration of reaction mixture in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether and ethyl acetate (9:1) gave (+)-**29c** (316 mg, 96% yield).

 **(+)-29c** **$C_{17}H_{15}NO_6$ (329)**	**Mp** 57-59 °C. $[\alpha]^{20}_{D} = + 103.1°$ (c 1.0, $CHCl_3$). **IR** (Nujol) ν_{max} 1720, 1607, 1526, 1493, 1443, 1281 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.38 (d, J = 6 Hz, 3H), 2.84 (dd, J = 14 and 6 Hz, 1H), 3.00 (dd, J = 14 and 6 Hz, 1H), 5.34 (sextet, J = 6 Hz, 1H), 5.92 (s, 2H), 6.60-6.80 (m, 3H), 8.17 (d, J = 8 Hz, 2H), 8.29 (d, J = 8 Hz, 2H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 19.4, 41.9, 73.5, 100.9, 108.2, 109.7, 122.4, 123.5, 130.6, 130.8, 136.0, 146.3, 147.7, 150.4, 164.1. **Anal. Calcd for $C_{17}H_{15}NO_6$**: C, 62.00; H, 4.56; N, 4.26. Found: C, 62.11; H, 4.60; N, 4.11.

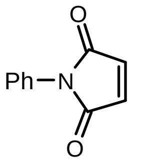
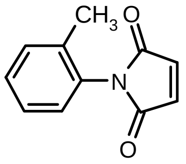
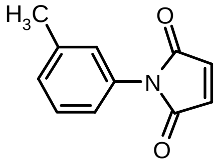
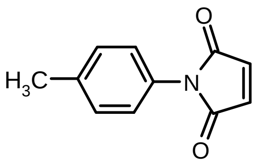
(*S*)-α-Methyl-1,3-benzodioxole-5-ethanol [(+)-21]. (+)-**29c** On repetition of above procedure for conversion of (–)-**28** to (–)-**21** gave (+)-**21** in 94% yield. $[\alpha]^{20}_{D} = + 34.0°$ (c 1.0, $CHCl_3$).

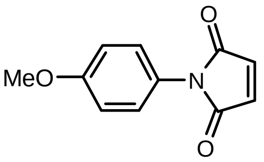
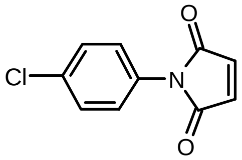
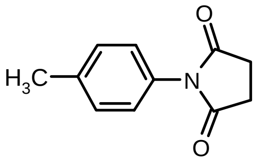
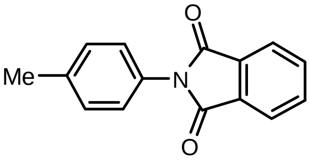
General procedure for MTPA-Ester preparation. To a solution of (*R*)-Mosher's acid (26 mg, 0.11 mmol), alcohol (±)- or (+)- or (–)-**21** (18 mg, 0.1 mmol) and DMAP (cat.) in dry CH_2Cl_2 (3 mL) was added a solution of DCC (23 mg, 0.11 mmol) in dry CH_2Cl_2 (2 mL) at 0 °C. The reaction mixture was allowed to warm to rt and stirred for 8 h. The formed urea was filtered off and the organic layer was concentrated in vacuo. Silica-gel column chromatographic purification of the residue using petroleum ether and ethyl acetate (9.5:0.5) gave the MTPA-ester in quantitative yield.

 **MTPA-Ester of (±)-α-methyl-1,3-benzodioxole-5-ethanol** **$C_{20}H_{19}F_3O_5$ (396)**	Thick oil. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.31 (d, J = 6 Hz, 3H), 1.34 (d, J = 6 Hz, 3H), 2.60-3.00 (m, 4H), 3.42 (s, 3H), 3.50 (s, 3H), 5.20-5.45 (m, 2H), 5.91 (s, 2H), 5.93 (s, 2H), 6.45-6.80 (m, 6H), 7.20-7.50 (m, 10H).

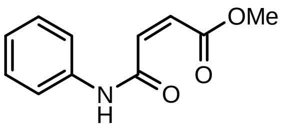
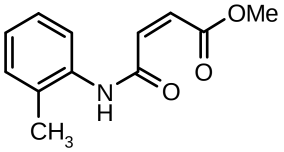
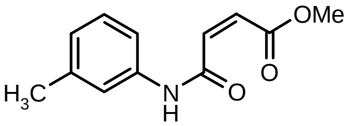
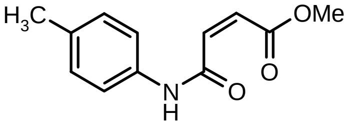
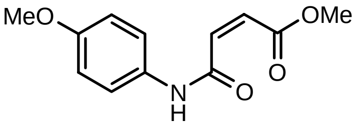
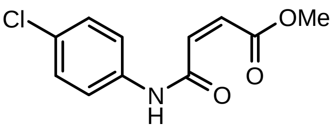
 **MTPA-Ester of (+)-(*S*)-α-methyl-1,3-benzodioxole-5-ethanol** **$C_{20}H_{19}F_3O_5$ (396)**	Thick oil. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.30 (d, *J* = 6 Hz, 0.30H), 1.34 (d, *J* = 8 Hz, 2.70H), 2.60-2.95 (m, 2H), 3.42 (s, 0.30H), 3.51 (s, 2.70H), 5.34 (sextet, *J* = 6 Hz, 1H), 5.92 (s, 1.80H), 5.93 (s, 0.20H), 6.45-6.80 (m, 3H), 7.25-7.55 (m, 5H).
 **MTPA-Ester of (–)-(*R*)-α-methyl-1,3-benzodioxole-5-ethanol** **$C_{20}H_{19}F_3O_5$ (396)**	Thick oil. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.31 (d, *J* = 6 Hz, 3H), 2.70-3.00 (m, 2H), 3.42 (s, 2.94H), 3.51 (s, 0.06H), 5.32 (sextet, *J* = 6 Hz, 1H), 5.93 (s, 2H), 6.60-6.80 (m, 3H), 7.15-7.50 (m, 5H).

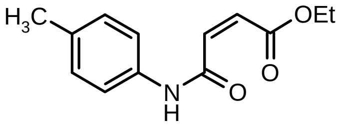
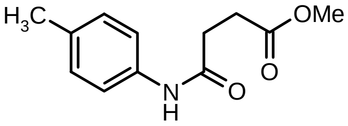
Analytical and Spectral Data for Imides 30a-h.[41]

 **30a** **$C_{10}H_7NO_2$ (173)**	**Mp** 87 ^{o}C. **IR** (Nujol) ν_{max} 1770, 1709, 1595 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 6.83 (s, 2H), 7.20-7.70 (m, 5H).
 **30b** **$C_{11}H_9NO_2$ (187)**	**Mp** 80-81 ^{o}C. **IR** (Nujol) ν_{max} 1770, 1709, 1600 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.16 (s, 3H), 6.87 (s, 2H), 7.05-7.20 (m, 1H), 7.20-7.45 (m, 3H).
 **30c** **$C_{11}H_9NO_2$ (187)**	Thick oil. **IR** (Neat) ν_{max} 1770, 1708, 1608 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.40 (s, 3H), 6.84 (s, 2H), 7.05-7.25 (m, 3H), 7.36 (t, *J* = 6 Hz, 1H).
 **30d** **$C_{11}H_9NO_2$ (187)**	**Mp** 158 ^{o}C. **IR** (Nujol) ν_{max} 1770, 1709, 1605 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.40 (s, 3H), 6.85 (s, 2H), 7.20 (d, *J* = 8 Hz, 2H), 7.30 (d, *J* = 8 Hz, 2H).

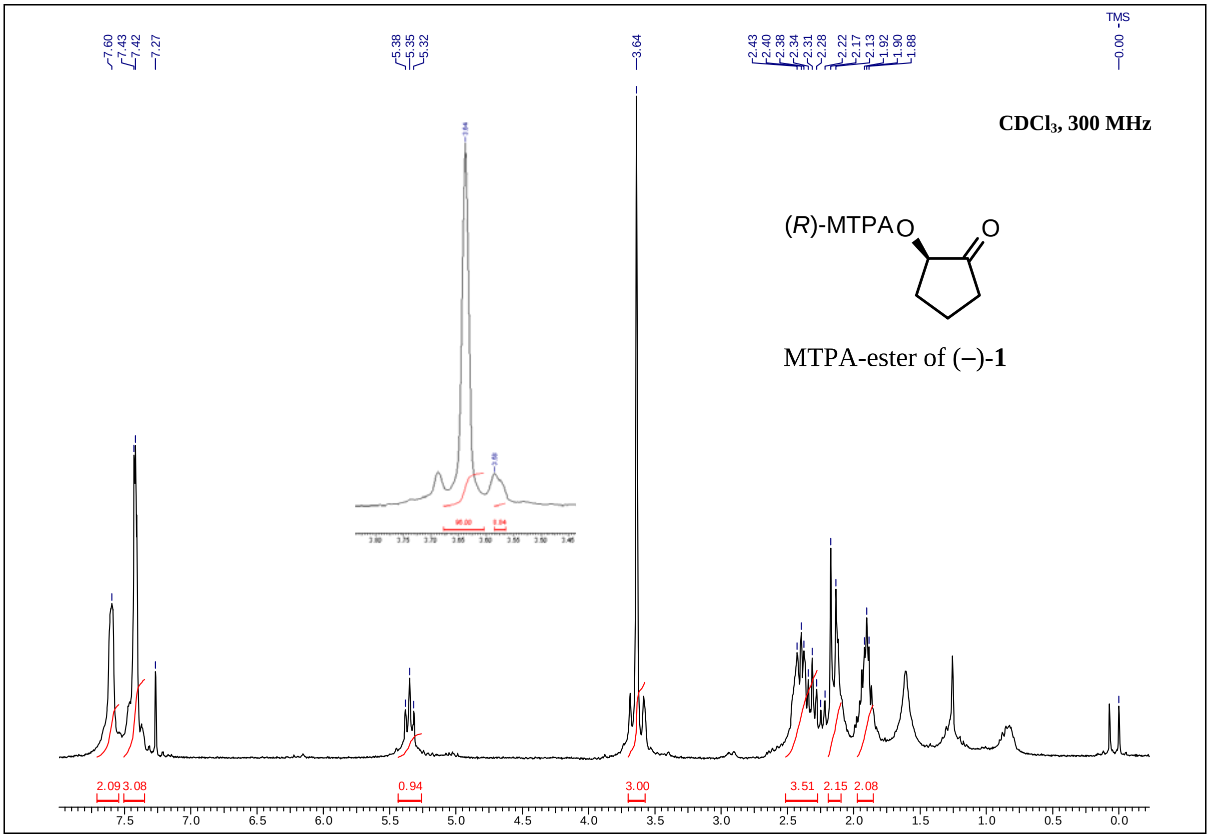
 **30e** **$C_{11}H_9NO_3$ (203)**	**Mp** 152-54 °C. **IR** (Nujol) ν_{max} 1769, 1709, 1607 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 3.83 (s, 3H), 6.83 (s, 2H), 6.98 (d, J = 8 Hz, 2H), 7.24 (d, J = 8 Hz, 2H).
 **30f** **$C_{10}H_6ClNO_2$ (207.5)**	**Mp** 118-20 °C. **IR** (Nujol) ν_{max} 1775, 1713, 1595 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 6.87 (s, 2H), 7.32 (d, J = 8 Hz, 2H), 7.45 (d, J = 8 Hz, 2H).
 **30g** **$C_{11}H_{11}NO_2$ (189)**	**Mp** 158-60 °C. **IR** (Nujol) ν_{max} 1770, 1705, 1600 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.38 (s, 3H), 2.87 (s, 4H), 7.15 (d, J = 6 Hz, 2H), 7.28 (d, J = 6 Hz, 2H).
 **30h** **$C_{15}H_{11}NO_2$ (237)**	**Mp** 210-12 °C. **IR** (Nujol) ν_{max} 1745, 1710, 1600 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.41 (s, 3H), 7.31 (apparent singlet, 4H), 7.70-8.10 (m, 4H).

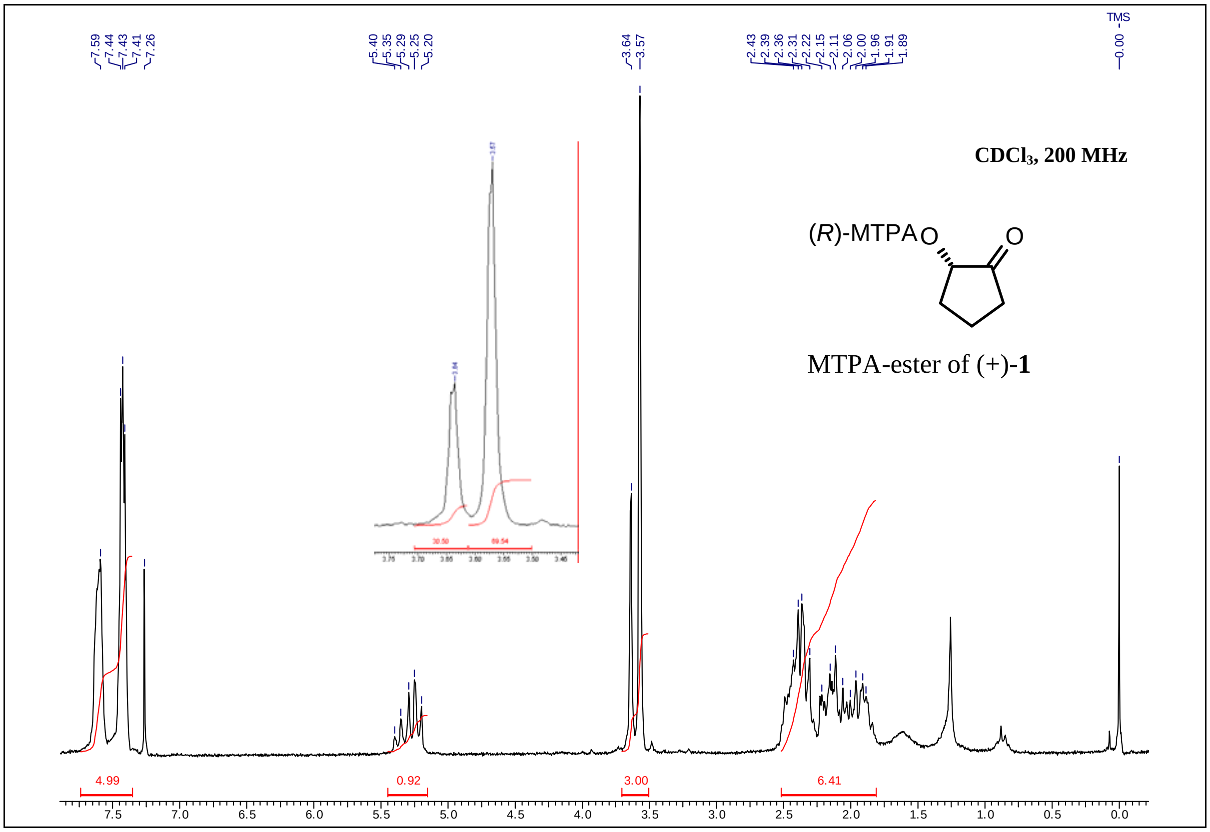
General procedure for methanolysis of maleimides. To a stirred solution of maleimide (**30a-f**, 500 mg) in methanol (25 mL) was added Amano PS (50 mg) and the reaction mixture was stirred at rt (6 to 24 hr). The reaction mixture was filtered through a sintered glass funnel, washed with methanol and concentrated *in vacuo*. Silica gel column chromatographic purification of the residue [elution with petroleum ether - ethyl acetate (85:15) mixture] furnished pure esters **31a-f** in 80 to 90% yields. The esters **31g** and **31h** were also prepared similarly (in lower yields) starting from the corresponding anhydride employing the appropriate alcohol. The isolated enzyme was dried at rt and used for second conversion without any noticeable loss of activity.

 **31a** **$C_{11}H_{11}NO_3$ (205)**	**Mp** 76-77 °C. **IR** (Nujol) ν_{max} 3252, 1732, 1668, 1632, 1597 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 3.85 (s, 3H), 6.22 (d, J = 12 Hz, 1H), 6.45 (d, J = 12 Hz, 1H), 7.13 (t, J = 8 Hz, 1H), 7.35 (t, J = 8 Hz, 2H), 7.67 (d, J = 8 Hz, 2H), 10.85 (bs, 1H).
 **31b** **$C_{12}H_{13}NO_3$ (219)**	**Mp** 97-99 °C. **IR** (Nujol) ν_{max} 3217, 1730, 1668, 1641, 1600 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.35 (s, 3H), 3.83 (s, 3H), 6.24 (d, J = 10 Hz, 1H), 6.49 (d, J = 10 Hz, 1H), 7.05-7.40 (m, 3H), 7.94 (d, J = 6 Hz, 1H), 10.20 (bs, 1H).
 **31c** **$C_{12}H_{13}NO_3$ (219)**	Thick oil. **IR** (Neat) ν_{max} 3304, 1732, 1666, 1614, 1595 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.35 (s, 3H), 3.84 (s, 3H), 6.20 (d, J = 12 Hz, 1H), 6.43 (d, J = 12 Hz, 1H), 6.95 (d, J = 8 Hz, 1H), 7.22 (t, J = 8 Hz, 1H), 7.40-7.60 (m, 2H), 10.70 (bs, 1H).
 **31d** **$C_{12}H_{13}NO_3$ (219)**	**Mp** 108-09 °C. **IR** (Nujol) ν_{max} 3283, 3250, 1726, 1670, 1636, 1607 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.33 (s, 3H), 3.84 (s, 3H), 6.21 (d, J = 12 Hz, 1H), 6.44 (d, J = 12 Hz, 1H), 7.14 (d, J = 8 Hz, 2H), 7.55 (d, J = 8 Hz, 2H), 10.73 (bs, 1H).
 **31e** **$C_{12}H_{13}NO_4$ (235)**	**Mp** 58-60 °C. **IR** (Nujol) ν_{max} 3300, 1724, 1661, 1632, 1605 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 3.80 (s, 3H), 3.84 (s, 3H), 6.20 (d, J = 12 Hz, 1H), 6.44 (d, J = 12 Hz, 1H), 6.88 (d, J = 8 Hz, 2H), 7.58 (d, J = 8 Hz, 2H), 10.68 (bs, 1H).
 **31f** **$C_{11}H_{10}ClNO_3$ (239.5)**	**Mp** 98-100 °C. **IR** (Nujol) ν_{max} 3281, 3246, 1722, 1666, 1643, 1607 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 3.86 (s, 3H), 6.24 (d, J = 12 Hz, 1H), 6.44 (d, J = 12 Hz, 1H), 7.30 (d, J = 8 Hz, 2H), 7.63 (d, J = 8 Hz, 2H), 11.09 (bs, 1H).

 **31g** **$C_{13}H_{15}NO_3$ (233)**	Thick oil. **IR** (Neat) v_{max} 3306, 1726, 1666, 1635, 1607 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.33 (t, *J* = 8 Hz, 3H), 2.32 (s, 3H), 4.29 (q, *J* = 8 Hz, 2H), 6.19 (d, *J* = 12 Hz, 1H), 6.42 (d, *J* = 12 Hz, 1H), 7.14 (d, *J* = 8 Hz, 2H), 7.55 (d, *J* = 8 Hz, 2H), 10.84 (bs, 1H).
 **31h** **$C_{12}H_{15}NO_3$ (221)**	**Mp** 103-04 °C. **IR** (Nujol) v_{max} 3356, 1724, 1707, 1684, 1599 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.30 (s, 3H), 2.55-2.90 (m, 4H), 3.70 (s, 3H), 7.10 (d, *J* = 8 Hz, 2H), 7.38 (d, *J* = 8 Hz, 2H), 7.64 (bs, 1H).

1B.6 Spectra Section


TMS
7.60
7.43
7.42
7.27
5.38
5.35
5.32
3.64
2.43
2.40
2.38
2.34
2.31
2.28
2.22
2.17
2.13
1.92
1.90
1.88
0.00
CDCl₃, 300 MHz
(R)-MTPA
O
O
MTPA-ester of (–)-1
3.64
3.59
2.09
3.08
0.94
3.00
3.51
2.15
2.08
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


TMS
7.59
7.44
7.43
7.41
7.26
5.40
5.35
5.29
5.25
5.20
3.64
3.57
2.43
2.39
2.36
2.31
2.22
2.15
2.11
2.06
2.00
1.96
1.91
1.89
0.00
CDCl₃, 200 MHz
(R)-MTPA
O
O
MTPA-ester of (+)-1
3.57
3.64
30.50
69.54
4.99
0.92
3.00
6.41
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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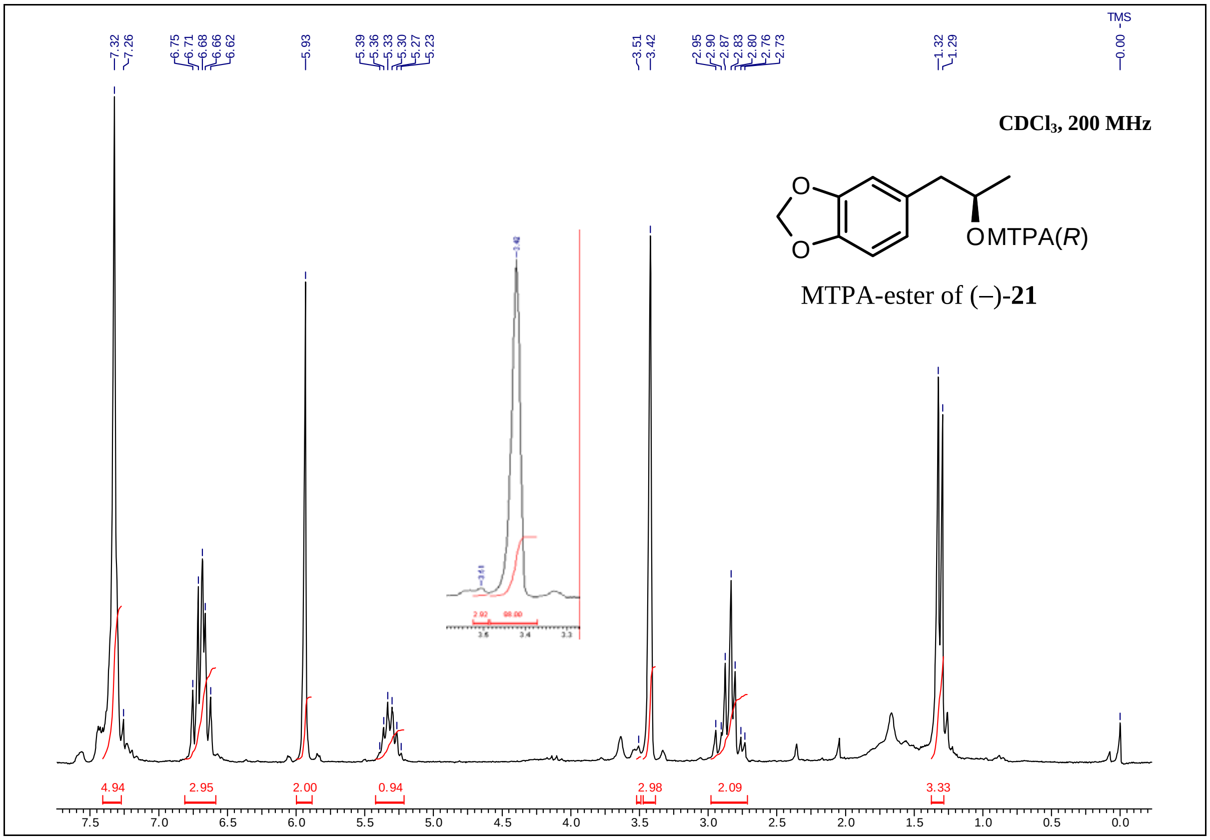
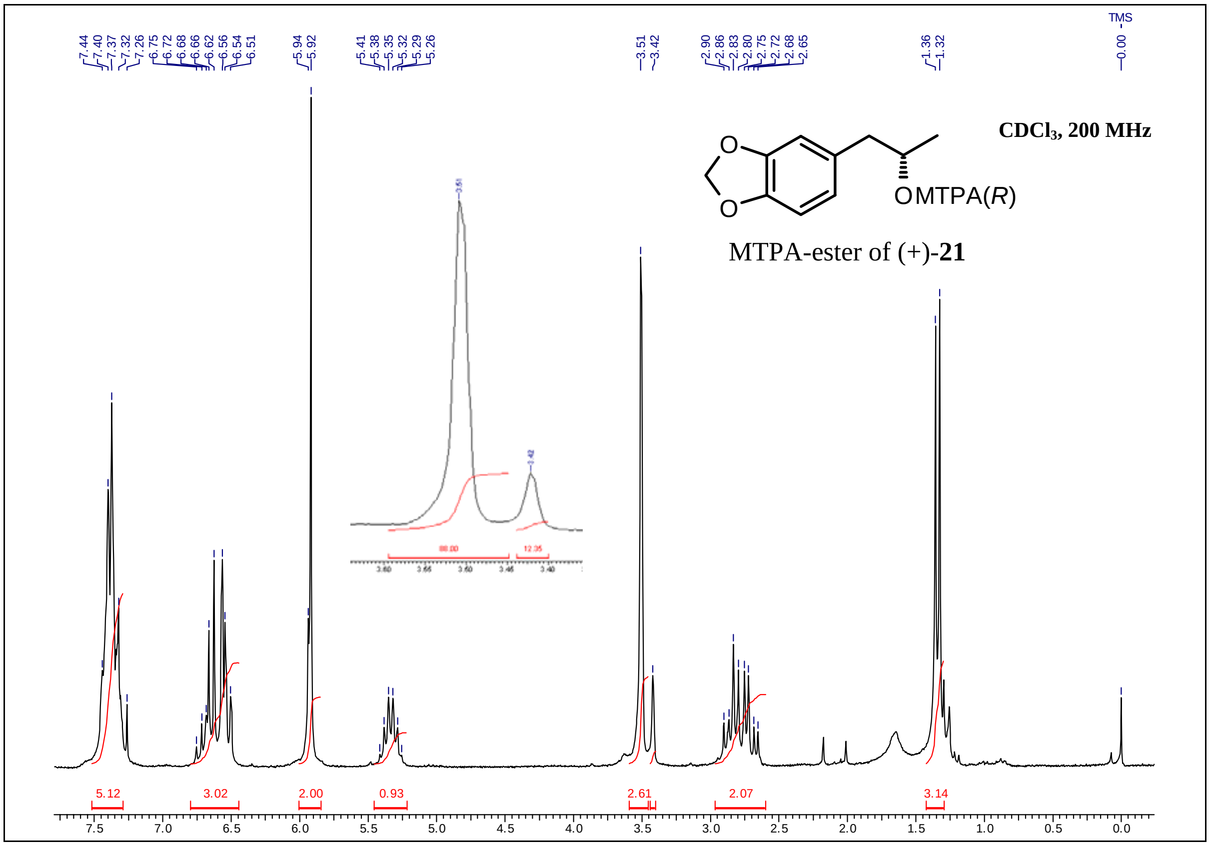

MTPA-ester of (−)-**21**


MTPA-ester of (+)-**21**

1B.7 References and Footnotes

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Chapter 2

Studies towards the Synthesis of Anhydride Based Natural Products

2A. Section A

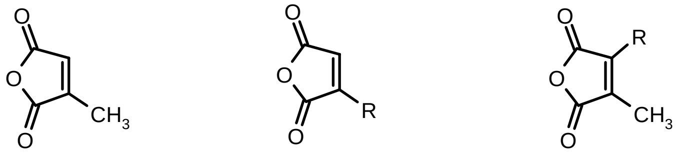
Maleic Anhydrides and Homophthalic Anhydrides in Organic Synthesis

This section features the following topics:

2A.1 Section A: I. Maleic Anhydride and Derivatives: An Overview

2A.1.1 Introduction & Monoalkylmaleic Anhydrides

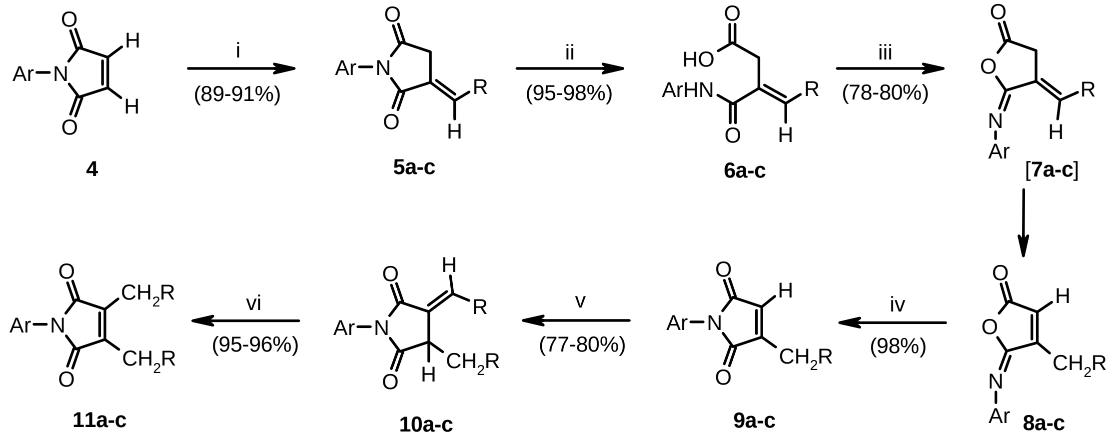
Maleic anhydride (2,5-furandione) was prepared for the first time two centuries ago and became commercially available a century later by the catalytic oxidation of benzene using vanadium pentoxide.[1] It is a versatile synthon wherein all the sites are amenable for a variety of reactions and possesses exceptional selectivity in reactions towards several nucleophiles. The vast array of nucleophilic reactions undergone by maleic anhydrides confer a high synthetic potential on them.[2] In the past century, several symmetrically and unsymmetrically substituted maleic anhydride derivatives have been prepared, the simplest of them being methylmaleic anhydride or citraconic anhydride (**1**). Although the utilities of methylmaleic anhydride (**1**) have been well proven in laboratory as well as in industrial practice,[3] only three synthetic approaches towards methylmaleic anhydride are known in the literature: (i) starting from citric acid by double dehydrative decarboxylation and isomerization,[4] (ii) from ethyl acetoacetate via cyanohydrin formation followed by dehydrative cyclization[5] and (iii) by the gas phase oxidation of isoprene.[6] In the case of higher monoalkylmaleic anhydrides **2** only a few approaches are known for their synthesis. The first approach involves the conjugate addition of organocuprates to dimethyl acetylenedicarboxylate as a key reaction.[7] Recently, two synthetic approaches have been reported for compound **2** by palladium-catalyzed dicarbonylation of terminal acetylenes.[8,9]



1 **2** R = alkyl **3**

Figure 1 Monoalkylsubstituted and methylalkylmaleic anhydrides

A simple and efficient access to monoalkyl and dialkylmaleimides was recently developed in our laboratory involving a novel contrathermodynamic rearrangement of alkylidenesuccinimides to alkylmaleimides via the corresponding isoimides.[10] The sequence consisted of two Wittig coupling reactions, taking advantage for the first time of kinetically controlled isoimides as intermediates to enforce the difficult migration of exocyclic carbon–carbon double bonds to the endocyclic position (Scheme 1). This strategy holds a lot of potential to design complex disubstituted maleic anhydride based natural products such as byssochlamic acid (in this context, it must be mentioned that the Wittig condensation of maleimide-TPP adducts with aldehydes has been used extensively in our lab to generate a wide array of natural products).

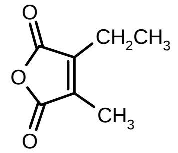


Ar = *p*-Tolyl; **a**, R = $-CH_2(CH_2)_3CH_3$; **b**, R = $-CH_2(CH_2)_7CH_3$; **c**, R = $-CH_2(CH_2)_{11}CH_3$

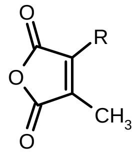
Scheme 1 *Reagents, conditions and yields*: (i) PPh_3, THF, RCHO, reflux, 10 h (89-91%); (ii) Aq. 2 N LiOH, THF, 0 ^{o}C to rt, 5 h, (95-98%); (iii) Cyanuric chloride, NEt_3, DCM, 0 ^{o}C to rt, 8 h (78-80%); (iv) AcOH, reflux, 5 h (98%); (v) PPh_3, AcOH, RCHO, reflux,18 h (77-80%); (vi) NEt_3 + THF (1:1), reflux, 48 h (95-96%).

2A.1.2 Dialkylsubstituted Maleic Anhydrides

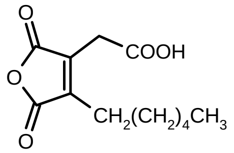
Literature reveals that dialkylsubstituted maleic anhydrides have been far more significant than their monoalkyl counterparts although the latter could serve as potential building blocks in natural product synthesis. Among the dialkylsubstituted maleic anhydrides, the simplest and most widely used is dimethylmaleic anhydride (DMMA). More than 20 synthetic approaches to dimethylmaleic anhydride using a variety of strategies are known in literature.[11] The list of approaches to dimethylmaleic anhydride with an overall yield of greater than 60% includes (i) oxidation of but-2-ene in the presence of a metal catalyst with 68% yield[12] (ii) self condensation of ethyl α-bromopropionates with 67% yield[13] (iii) oxidation of but-2-yne in 2-steps with 63% overall yield[14] (iv) one-pot approach employing maleic anhydride and 2-aminopyridine with 75% yield[15] and (v) an elegant approach using Wittig reaction of methylmaleimide-TPP adduct with paraformaldehyde.[11] Recently, several structurally interesting and biologically important dialkylsubstituted maleic anhydrides have been isolated as bioactive natural products and some of them are listed in Figure 2. The structural features of these molecules reveal that they might have been designed in nature by the combination of pyruvic acid and the respective long chain carboxylic acids (except **14** & **15**). These target molecules received immediate attention from several elegant schools of synthetic organic chemistry for the synthesis of the natural product itself and also of its analogues for structure activity relationship studies. A look at the figure shows that almost all the molecules depicted are actually methylalkyl maleic anhydride based structures, which establishes them as an important class of compounds.



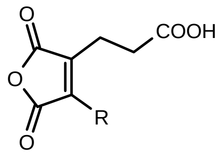
2-Ethyl-3-methylmaleic anhydride (**12**) (flavouring agent)[16]



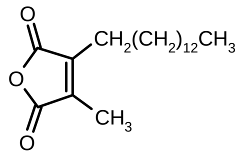
R = C_6H_{13}, C_8H_{17}
2-Alkyl-3-methylmaleic anhydrides (**13a**,**b**) (flavouring agents)[17]



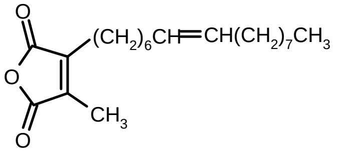
2-Carboxymethyl-3-hexylmaleic anhydride (**14**) (flavouring agent)[18]



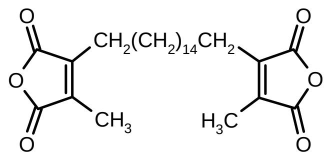
R = C_6H_{13}, C_8H_{17}
2-(2-Carboxyethyl-3-alkylmaleic anhydrides (**15a**,**b**) (flavouring agents)[19]



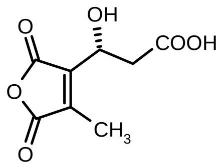
Chaetomellic acid A anhydride (**16**) (ras farnesyl-protein transferase inhibitor)[20]



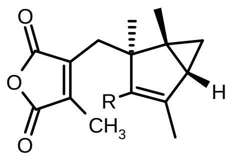
Chaetomellic acid B anhydride (**17**) (ras farnesyl-protein transferase inhibitor)[20]



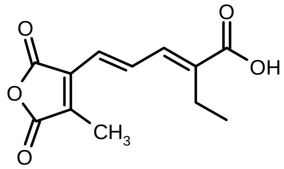
Tyromycin A (**18**) (aminopeptidase inhibitor, potential cytostatic activity)[21]



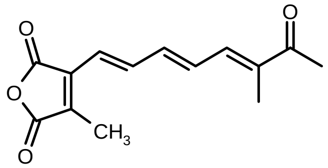
Maleic anhydride segment of tautomycin [(*S*)-**19**] (antifungal, antibiotic)[22]



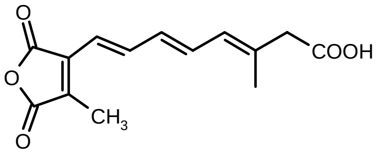
Lindenanolide E (R = CHO) (**20**) (HIV-1 integrase inhibitor)[23]



Telfairic anhydride (**21**) (activity unknown)[24]



Graphenone (**22**) (activity unknown)[25]



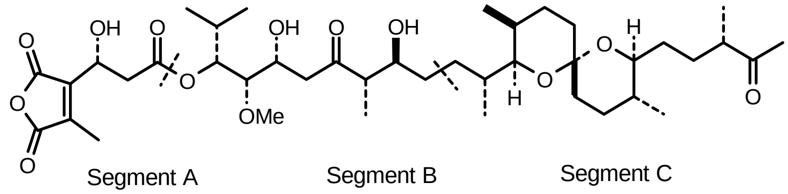
Itaconitin (**23**) (activity unknown)[26]

Figure 2 Naturally occurring dialkylsubstituted maleic anhydrides

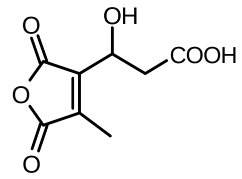
2-Carboxymethyl-3-hexylmaleic anhydride (**14**) has been isolated[18] as a novel metabolite of the *Aspergillus* FH-X-213 from an apple and has been reported to show a weak *in vitro* activity against grampositive bacteria.[18] In 1994, Soda et al. reported the biotransformation of stearic acid with a microbial strain isolated from soil, *Pseudomonas* *cepacica* A-1419, to produce two new maleic anhydride derivatives 2-(*β*-carboxyethyl)-3-hexylmaleic anhydride (**15a**) and 2-(*β*-carboxyethyl)-3-octylmaleic anhydride (**15b**).[19] The biological role of the above two natural products have not been examined. The first general synthetic route to these diverse dialkylsubstituted maleic anhydride analogs was demonstrated by Baldwin et al. using a versatile copper mediated tandem vicinal difunctionalization of dimethyl acetylenedicarboxylate.[27] More recently, a successful approach to all the above three natural products was designed in our group using a S_N2' Grignard coupling strategy developed in our laboratory.[28] This strategy will be illustrated in more detail later in this section.

Tyromycin A (**18**) has been recently isolated from mycelial cultures of basidomycete *Tyromyces lacteus* (Fr.) Murr.[21] Tyromycin A (**18**) is the first bioactive natural product with two citraconic anhydride units, which suggests a biosynthesis by condensation of an activated eicosanedioic acid with two molecules of oxaloacetate. Till date only two syntheses of tyromycin A are reported. The first synthesis involves the well-known decarboxylative Barton-radical coupling reaction.[29] The second synthesis of tyromycin A was reported from our laboratory employing a one-pot double Wittig reaction involving methylmaleimide-TPP adduct and aliphatic dialdehyde as a key step.[30]

Isono and co-workers[22] reported the isolation and structural elucidation of tautomycin (**24**) from a strain of *Streptomyces spiroverticillatus* as a new antibiotic with strong antifungal activity against *Sclerotinia sclerotiorum*. The broad retrosynthetic analysis of tautomycin afforded segment A, B, and C as shown in structure **24** (Figure 3).



Tautomycin (24)



rac-**19**

Figure 3

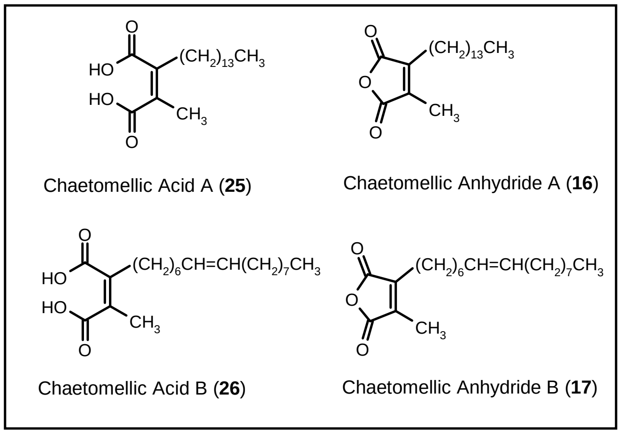
Segment A of tautomycin is a highly oxygenated molecule with three carboxylic groups and one hydroxy group. According to Chamberlin et al.[31] the greatest challenge in the synthesis of tautomycin lies in the construction of the simple looking 2,3-disubstituted maleic anhydride segment A **19**. To date, five multi-step synthesis of segment A **19** have been accomplished.[31-35] The most recent of the five reported approaches has been developed in our lab using a simple strategy to provide *rac*-**19**.[35]

A new methylmaleic anhydride metabolite has been isolated in 1996 by Edward and his co-workers[24] from the culture medium of the fungus *Xylaria telfairii* Berk and was identified as telfairic anhydride (**21**). Two other structurally similar natural products, graphenone (**22**)[25] and itaconitin (**23**)[26] have been isolated from the cultures of spore derived mycobionts of the lichens *Graphis scripta* and from the species *Aspergillus itaconicus* & *Aspergillus gorakhpurensis* respectively. A biological role for such a structure does not appear to have been established yet. Very recently, lindenanolide E (**20**)[23] has been isolated from the species *Lindera chunii* Merr possessing HIV-1 integrase inhibiting activity. Till date, no synthesis has been reported for telfairic anhydride (**21**), graphenone (**22**), itaconitin (**23**) and lindenanolide E (**20**).

2A.1.2.1 Chaetomellic Acid A Anhydride

The most important among all methylalkylsubstituted maleic anhydrides is undoubtedly chaetomellic acid A anhydride (2-tetradecyl-3-methylmaleic anhydride, **16**) because of its remarkable biological activity. The dicarboxylic acids chaetomellic acid A (**25**) and chaetomellic acid B (**26**) were isolated from fermentation extract of the coleomycete *Chaetomella acutiseta* (Figure 4), by a group of scientists[20] at Merck, USA in 1993. The structural assignment of chaetomellic anhydride A (**16**) and chaetomellic anhydride B (**17**) has been done on the basis of analytical and spectral data. The position and geometry of the double bond in chaetomellic acid B was established by MS analysis of monoepoxide (prepared by reacting **17** with *meta*-chloroperbenzoic acid in dichloromethane). The biogenesis of these compounds[20] may be occurring through an aldol condensation of palmitate or *cis*-oleate with pyruvate followed by dehydration pathway.

Chaetomellic acid A and B have been identified as potent inhibitors of Ras farnesyl-protein transferase[20,36] (FPTase), an enzyme catalyzing a post-translational modification of Ras. Mutated form of Ras oncogens are found in about 25% of the human tumors[37] and are believed to play a key role in their growth. Increased interest towards non-peptide inhibitors led to the identification of chaetomellic acid A (**25**) and chaetomellic acid B (**26**) as potent FPTase inhibitors with IC_{50} value 55 and 185 nM respectively.

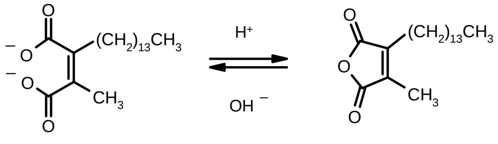

Chaetomellic Acid A (**25**)

Chaetomellic Anhydride A (**16**)

Chaetomellic Acid B (**26**)

Chaetomellic Anhydride B (**17**)

Figure 4



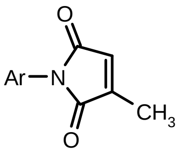
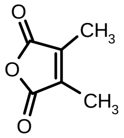
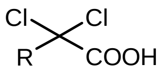
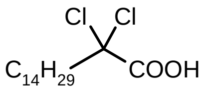
27 **16**

Figure 5

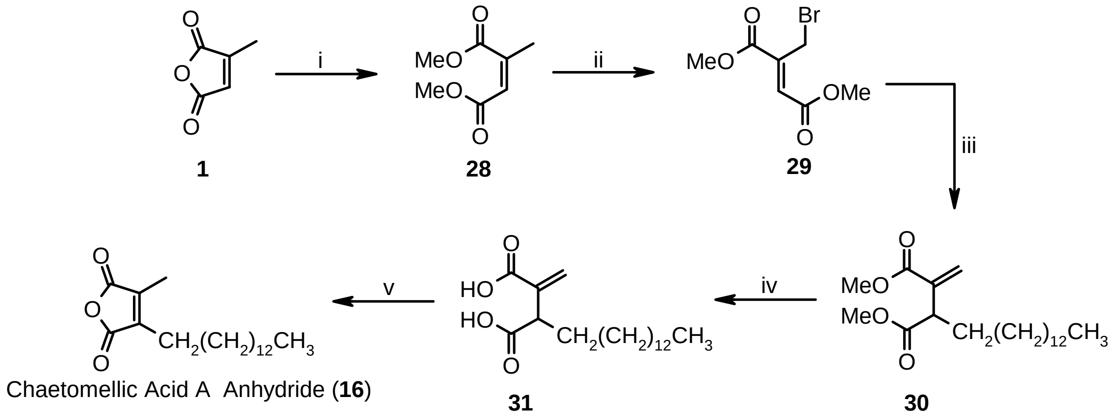
These classes of natural products have a propensity to cyclize as shown in Figure 5 and all members of this family were isolated in the anhydride form. However, they actually exhibit their FPTase inhibitory activity in the dianionic form as shown above in compound **27**. Chaetomellic acid A (**25**) is three times more potent than chaetomellic acid B (**26**) and hence became the main attraction of synthetic efforts. After its isolation in 1993, the past eleven years have seen eleven syntheses[38-47,28] of **16** and two synthesis[40,44] of **17** being accomplished including four from our group. The chemistry of ten syntheses of **16** (and two of **17**) is summarized in Table 1, whereas another recent synthesis of **16** from our laboratory has been discussed subsequently.

Table 1 Reported syntheses of chaetomellic anhydride A (**16**) and chaetomellic anhydride B (**17**)

No.	Starting Compound	Reagents and Conditions	Product	Overall Yield	Ref.
1	$CH_3(CH_2)_{14}COOMe$ Methyl palmitate	(i) Methyl pyruvate, LDA, THF, − 78 °C to − 10 °C; (ii) 2,6-Di-^{t}butyl-4-methyl pyridine, *p*-tolunesulphonic anhydride, pyridine, DCM; (iii) DBU, toluene, reflux; (iv) (a) NaOH-CH_3OH-THF-H_2O; (b) 4 N HCl.	**16**	18%	38
2	$CH_3(CH_2)_{12}CH_2Br$ Tetradecyl bromide	(i) $CoCl_2$, dmgH_2, pyridine, $NaBH_4$, NaOH, CH_3OH; (ii) PhSSPh, *hv*; (iii) *m*-CPBA, pH 7.4 phosphate buffer, DCM.	**16**	64%	39

3	$MeOOCCH_2COOMe$ Dimethyl malonate	(i) NaH, $C_{14}H_{29}Br$ or $C_{16}H_{29}Br$, THF-DMF, reflux, 1.5 h; (ii) $BrCH(CH_3)COOMe$, NaH, THF-DMF, reflux, 1.5 h; (iii) (a) Ethanolic KOH, (b) H^+/H_2O, reflux; (iv) *N*-methylmorpholine, methylchloroformate; (v) Et_3N, TMSOTf, C_6H_6, reflux, 2 h; (vi) Br_2, Bu_4NBr.	**16** or **17**	83% for **6** & 80% for **7**	40
4	$MeOOC{-}C{\equiv}C{-}COOMe$ Dimethyl acetylenedicarboxylate	(i) (a) $C_{14}H_{29}Cu(Me_2S)MgBr$; (b) MeI, THF-HMPA, (c) Aq. NH_4Cl; (ii) (a) $LiOH/H_2O$, (b) H^+/HCl.	**16**	78%	41
5	$CH_3(CH_2)_{12}CH(Br)COCl$ 2-Bromopalmitoyl chloride	(i) 2-Aminopyridine, Et_3N, Et_2O, rt; (ii) ^{t}BuOH, reflux; (iii) Maleic anhydride, NaOAc, reflux, 5 h.	**16**	62%	42
6	 Ar = *p*-Tolyl *N*-*p*-Tolyl methylmaleimide	(i) (a) PPh_3, AcOH, reflux, 2 h, (b) $CH_3(CH_2)_{12}CHO$, reflux, 18 h; (c) Δ, 140-150 °C, 0.5 h; (ii) (a) $KOH/H_2O/CH_3OH/THF$, reflux, 2 h, (b) H^+/HCl.	**16**	89%	43
7	RCOOH R = $C_{14}H_{29}$ Pentadecanoic acid R = $C_{16}H_{31}$ (*Z*)-8-Heptadecanoic acid	(i) (a) DCC, 2-mercaptopyridine *N*-oxide, DCM, 2 h, (b) citraconic anhydride, *hv* (500 W), 10-15 °C, 0.5 h.	**16** or **17**	70% for **6** & 60% for **7**	44
8	 Dimethylmaleic anhydride	(i) NBS, DBP, CCl_4, reflux, 10 h; (ii) $CH_3(CH_2)_{13}MgX$, Et_2O/THF, HMPA, CuI, – 5 to 0 °C.	**16**	36%	45
9	 R = $CH_2(CH_2)_{12}CH_3$ 2,2-Dichloropalmitic acid	(i) (a) $(COCl)_2$, CH_2Cl_2, DMF, 23 °C, 2 h, (b) $BnN(H)CH_2CH{=}CHCl$, py, 23 °C, 1 h (89%); (ii) CuCl/TMEDA, MeCN-CH_2Cl_2, 60 °C, 20 h (99%); (iii) (a) Na, MeOH-Et_2O, 25 °C, 20 h, (b) H^+/H_2O (89%); (iv) (a) KOH, MeOH-THF, reflux, 2 h, (b) H^+/H_2O (70%).	**16**	55%	46
10		(i) (a) $(COCl)_2$, CH_2Cl_2, DMF 23 °C, 2 h, (b) 2-(3-chloro-2-propenylamino)pyridine, py, 23 °C, 1 h (94%); (ii) CuCl/TMEDA, MeCN, argon, 60 °C, 20 h (93%); (iii) (a) Na, CH_3OH/ether, 25 °C, 20 h, (b) H_2SO_4 (2 N), 110 °C, 2 h (78%).	**16**	68%	47

A recent synthesis of **16** was demonstrated by our group using a novel S_N2' Grignard coupling strategy. In this approach a freshly prepared Grignard reagent from tetradecyl bromide, in the presence of HMPA reacted in a highly chemo- and regioselective S_N2' fashion with bromodiester **29** (which has five alternate sites available for nucleophilic reaction), that is, Michael addition followed by elimination of allylic bromo atom to give the net S_N2' product **30** in 60% yield. Such type of migration of trisubstituted to *gem*-disubstituted carbon-carbon double bond with the loss of conjugation with one of the ester carbonyl is note-worthy. The synthesis of chaetomellic acid A anhydride (**16**) was then completed as shown in Scheme 2 (38% overall yield).[28a]

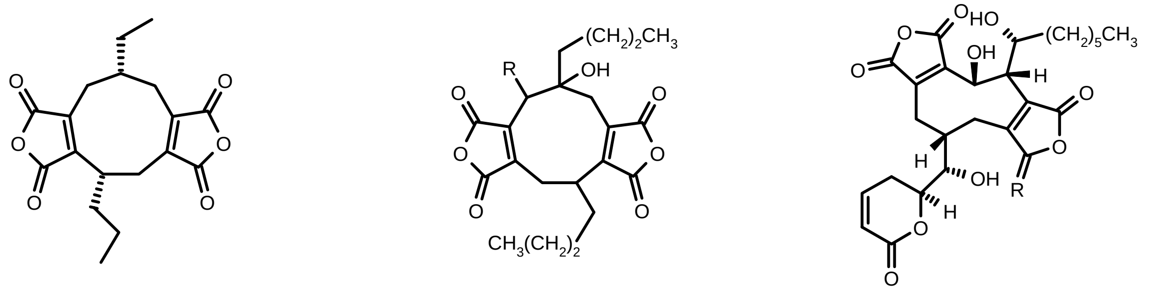


Scheme 2 *Reagents, conditions and yields*: (i) CH_3OH, H^+/H_2SO_4, reflux, 12 h (75%); (ii) NBS, AIBN, CCl_4, reflux, 12 h (85%); (iii) $C_{14}H_{29}MgBr$, Et_2O, HMPA, rt, 8 h (60%); (iv) AcOH + HCl (7:3), reflux, 2 h (98%); (v) Ac_2O, reflux, 2 h (~100%).

The same strategy formed the basis for the synthesis of other molecules such as 1,7(*Z*)-nonadecadiene-2,3-dicarboxylic acid, isochaetomellic anhydride B, 2-carboxymethyl-3-hexylmaleic anhydride, 2-(*β*-carboxyethyl)-3-hexylmaleic anhydride and 2-(*β*-carboxyethyl)-3-octylmaleic anhydride from our group.[28b,c] The present strategy also provides a general approach for the synthesis of compounds containing exo-type or exocyclic carbon-carbon double bonds.

2A.1.3 Complex Dialkylsubstituted Maleic Anhydrides

A more complex dialkylsubstituted class of maleic anhydrides known in the literature is the nonadride class of natural products. Natural products in this small structural class possess a nine membered ring as the core unit.[48] Two five-membered anhydrides or an anhydride and a lactol are fused to the core. A few of them are listed in Figure 6. Apart from the molecules depicted in the figure, other members of this family include glaucanic and glauconic acids (which were the first members of this class to be discovered), cornexistin, scytalidin, etc.



Byssoclamic Acid (**32**)

Scytalidin (**33**) R = H
Castaneolide (**34**) R = OH

Rubratoxin A (**35**) R = H & OH
Rubratoxin B (**36**) R = O

(+)-CP-225,917 (phomoidride A, **37**)

(-)-CP-263,114 (phomoidride B, **38**)

Figure 6 Naturally occurring complex dialkylsubstituted maleic anhydrides

Byssochlamic acid (**32**)[49] was isolated from ascomycete *Byssochlamys fulva* while both scytalidin (**33**) and castaneolide (**34**) were isolated[50] from *Macrophoma castaneicola*. Biological activity of these natural products has yet to be established. Hepatotoxic rubratoxin A (**35**) and rubratoxin B (**36**) have been isolated[51] from the species *Penicillium rubrum* and the latest examples of this structural family, phomoidrides A (**37**) and B (**38**) (CP-225,917 and CP-263,114), have been isolated by a research group at Pfizer from an unidentified fungus which also produces zaragozic acid.[52] These nonadrides attracted the attention of the researchers due to their powerful inhibition of Ras farnesyl protein transferase.[52] The CP-molecules exemplify architectures of unprecedented molecular connectivities and complexities and possess intriguing biological activity.

The intriguing structural features and promising biological activity of the nonadrides inspired several synthetic chemists to embark on a journey toward the total synthesis of the natural products falling in this rather unique league. Much of the attention, not surprisingly, has been captured by the phomoidrides, which with their fascinating yet complex structures and potential activity prove to be an alluring yet challenging target. Amongst the other nonadrides, only byssochlamic acid and phomoidrides have been successfully synthesized while not much attention has been focused on the other members of the nonadride family. Only two synthesis of byssochlamic acid are known in the literature[53] whereas the Herculean efforts of the synthetic community toward the total syntheses of phomoidrides A and B have culminated in four completed total syntheses and at least eighteen advanced synthetic approaches have been developed in recent years. Recently, the chemistry of phomoidrides A and B has been comprehensively reviewed by John L. Wood and coworkers.[54]

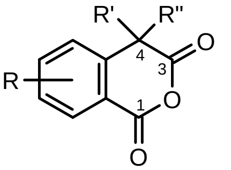
2A.1.4 Summary

The advantages offered by cyclic anhydrides to the synthetic organic chemist can never be overestimated. Maleic anhydride and its derivatives especially have been useful in the synthesis of bioactive natural products, heterocycles, drug & drug intermediates and a variety of polymers with tailored material characteristics. Methylalkylsubstituted maleic anhydrides, among all members of this class, have been the most significant due to their presence in a number of natural products with remarkable biological activity. Many natural products in this class have been synthesized, the most important being chaetomellic anhydride A, which is a promising anticancer agent. The fascinating structural features and remarkable activity of the nonadrides has spurred a lot of activity in the synthetic community towards their total synthesis that would pave the way for breakthroughs in medicinal chemistry. Thus, the chemistry of substituted maleic anhydrides is very valuable from both basic and applied standpoint. It is, therefore, imperative to keep our efforts persistent in the synthesis of newer and more potent substituted maleic anhydride natural products. In this context, as a part of the present dissertation, we have designed the first synthesis of four new naturally occurring secondary metabolites that are remotely functionalized methylalkylsubstituted maleic anhydrides. These have been named as aspergillus acid A-D (isolated from *Aspergillus wentii*) and our synthetic strategy towards these natural products will be discussed in detail in Section B of this chapter.

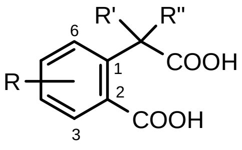
2A.2 Section A: II. Homophthalic Anhydrides and their Applications

2A.2.1 Introduction

Homophthalic anhydride (HPA) has been known for more than a century and is widely used in organic synthesis. According to IUPAC nomenclature, the homophthalic anhydrides **39** are named as 1*H*-2-benzopyran-1,3(4*H*)-diones. Points of substitution in the benzene nucleus are usually indicated by the numbering system for the respective 2-carboxy phenylacetic (homophthalic) acids **40** and substitution at the methylene position is called α-substitution (Figure 7).



39



40

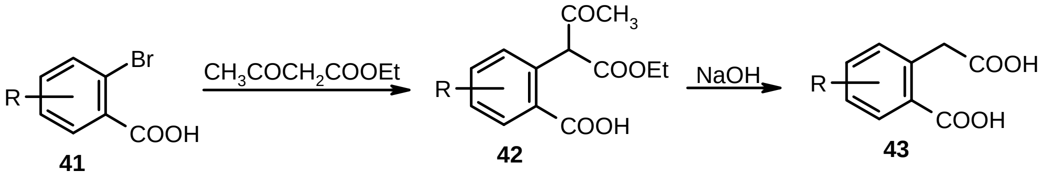
Figure 7

Lately, interest in the homophthalic anhydrides chemistry has grown, thanks mainly to their successful use as starting materials in the synthesis of a variety of heterocyclic compounds. The recent surge in the use of homophthalic anhydrides for diverse applications in organic synthesis is supported by the increase in the number of literature reports over the past few years. A comprehensive review on homophthalic anhydrides and their applications in the synthesis of heterocyclic compounds was published by Stanoeva et al in 1984.[55] This section summarizes the various reports over the last twenty years utilizing homophthalic anhydrides/acids in synthetic organic chemistry. It is well known that total synthesis of natural products occupies a keystone position in organic chemistry and hence an attempt has been made to focus more on natural product synthesis reports involving homophthalic anhydrides/acids in the synthetic scheme towards the target molecule. A short overview of synthesis of homophthalic anhydrides has been presented preceding their applications in organic synthesis, however, no pretension of completeness is claimed.

2A.2.2 Synthesis of Homophthalic Anhydrides

Synthesis of homophthalic anhydrides consists of synthesizing the homophthalic acids, which are converted to the respective anhydrides by heating with acetyl chloride or acetic anhydride.[56] Various methods are known in literature for the synthesis of homophthalic acids. They can be synthesized by hydrogen peroxide oxidation of 1,2-indanediones[57] or 1-indanone-2-glyoxylates,[58] or by the Beckmann rearrangement of 2-nitrosol-1-indanones followed by hydrolysis of the resulting acetonitriles.[59-61]

Homophthalic acids are also obtained in high yields by the direct arylation of *β*-dicarbonyl compounds with an ortho-halobenzoic acid **41** in the presence of a strong base and a copper(I) halide, followed by retro-Claisen condensation of the intermediate *α*-aryl-*β*-dicarbonyl compound **42** (Scheme 3).[62-64]



R = H, 3,4-$(OCH_3)_2$, 3,4-OCH_2O, 4-OCH_3, 5-CH_3

Scheme 3

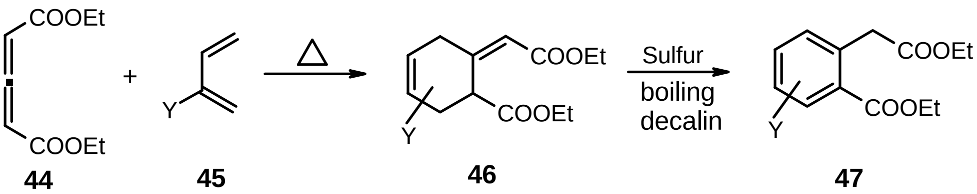
Guyot et al. reported that arylation of malonic ester with bromobenzenes in the presence of a strong base also gives homophthalic acids.[65]

More recently, homophthalic and *α*-methylhomophthalic acids (Cushman et al.)[66,67] and homophthalic anhydride (Larock et al.)[68] were synthesized by metallization of the aromatic nucleus of appropriate starting compounds with butyllithium or thallium(II) trifluoroacetate, followed by carboxylation or carbonylation, respectively.

Another reported method for the preparation of homophthalic acids was by the deprotonation of the methyl group of ortho-toluic acids and carboxylation of the resulting dianion.[69]

A nice and efficient two-step procedure for the preparation of homophthalic acid is by the treatment of phthalide with potassium cyanide at 180 °C to obtain the ring-opened 2-carboxybenzyl nitrile followed by hydrolysis of the nitrile using sulfuric acid.[70]

Kozikowski et al. portrayed an elegant preparation of homophthalic acids from nonaromatic compounds by employing a Diels-Alder reaction.[71] Thus, Diels-Alder reaction of allene **44** with various substituted butadienes **45** yielded the cycloadducts **46** in high yields, which were then easily transformed to the desired compounds by a sulfur-mediated dehydrogenation (Scheme 4).



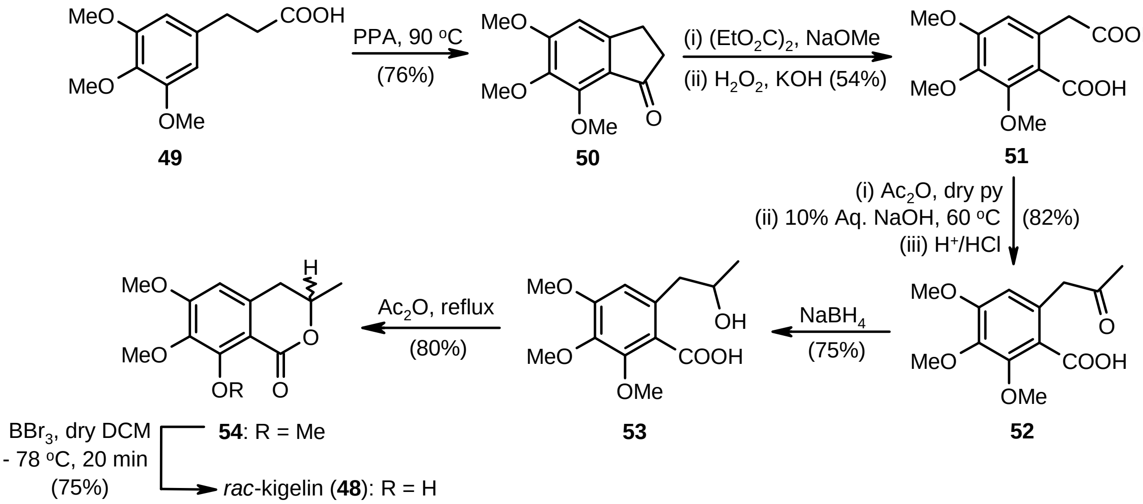
Scheme 4

2A.2.3 Applications of Homophthalic Anhydrides in Organic Synthesis

Prior to discussing their applications, it is necessary to reason out why homophthalic anhydrides are such useful synthons in organic chemistry. A look at their structure clearly reveals the difference between the two carbonyl groups arising due to their position with respect to the benzene ring. Evidently, this results in different reactivities and thus, nucleophiles react in a highly regioselective fashion with homophthalic anhydrides attacking the non-conjugated carbonyl group. Also, the α-methylene protons are highly acidic and hence a number of α-substituted derivatives can be easily generated in high yields. These attributes have rendered homophthalic anhydrides/acids as useful synthons in organic chemistry and they have therefore been employed in different capacities to generate a great number of heterocyclic compounds. Quite a few biologically active natural products have also been synthesized wherein homophthalic anhydrides/acids are involved in the construction of key intermediates. The importance of total synthesis of natural products, as mentioned before, can never be overemphasized and hence such reports will occupy the focus of attention in the following part.

2A.2.3.1 Total Synthesis of (±)-Kigelin (Saeed et al.)

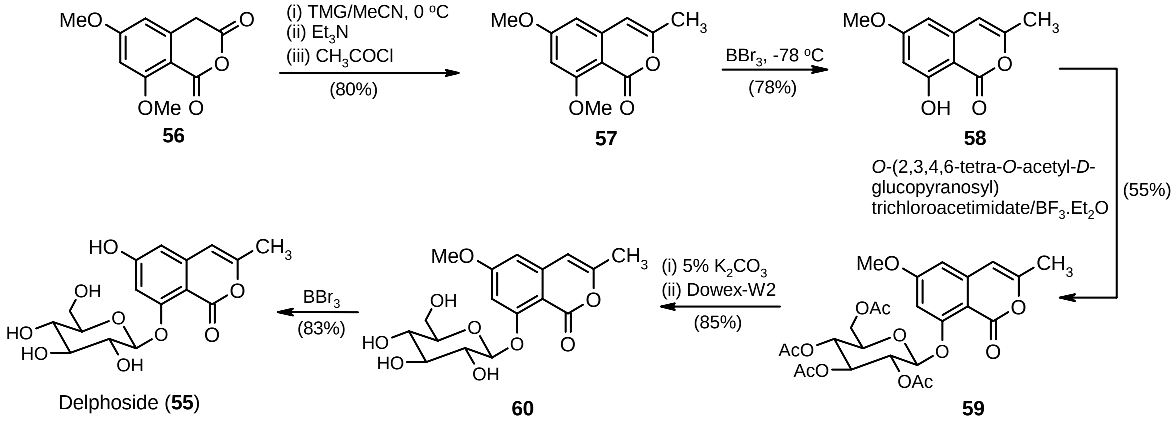
Kigelia pinnata (also known as the 'sausage tree') is cultivated in many parts of India as an ornamental and roadside tree and in Africa where it finds a variety of medicinal uses. Govindachari *et al.* isolated kigelin (**48**) as the major constituent of plant from the root of *Kigelia pinnata*. Kigelin is an active ingredient of cosmetics and skin lotions and is likely to be responsible for skin healing properties, thus making it an attractive synthetic target. A simple synthesis of racemic 8-hydroxy-6,7-dimethoxy-3-methyl-3,4-dihydroisocoumarin [(±)-kigelin, **48**] was reported by Saeed et al.[72] using 3,4,5-trimethoxyhomophthalic acid (**51**) as the key starting material (Scheme 5). The acid **51** was treated with acetic anhydride in dry pyridine to yield the α-acylated compound, which was subsequently decarboxylated in the same pot to afford 2,3,4-trimethoxy-6-(2-oxopropyl)benzoic acid (**52**). Reduction of the latter followed by cyclodehydration yielded 6,7,8-trimethoxy-3-methyl-3,4-dihydroisocoumarin (**54**), which on regioselective demethylation furnished (±)-kigelin (**48**). The key acid **51** was in turn prepared in 2-steps from the commercially available 3-(3,4,5-trimethoxyphenyl)propionic acid (**49**).



Scheme 5

2A.2.3.2 Total Synthesis of Delphoside (Saeed)

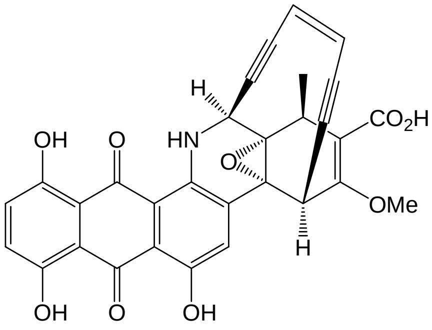
Saeed also carried out a short synthesis of another isocoumarin, 3-methyl-6-hydroxy-8-*O-β-D*-glucopyranosyloxy isocoumarin (delphoside, **55**), isolated from *Delphinium spp.* The synthesis started with 3,5-dimethoxyhomophthalic anhydride (**56**), which upon treatment with acetyl chloride in the presence of TMG and triethylamine afforded the 6,8-dimethoxy-3-methylisocoumarin (**57**). Regioselective demethylation of the latter furnished 8-hydroxy-6-methoxy-3-methylisocoumarin (**58**). Glycosylation with *O*-(2,3,4,6-tetra-*O*-acetyl-*D*-glucopyranosyl)trichloroacetimidate in the presence of boron trifluoride followed by deacetylation afforded 3-methyl-6-methoxy-8-*O-β-D*-glucopyranosyloxyisocoumarin (**60**) that was finally demethylated to yield delphoside (**55**) (Scheme 6).[73] The author also proposed a plausible pathway for the formation of isocouamrin **57** from anhydride **56** wherein the formation of the *α*-acyl derivative takes place initially. This *α*-acyl derivative, in the presence of a base, generates the oxy-anion, which forms a tricyclic intermediate via an attack on the conjugated carbonyl. Rearrangement of this tricyclic intermediate to a bicyclic one and subsequent expulsion of CO_2 delivers the isocoumarin **57**.



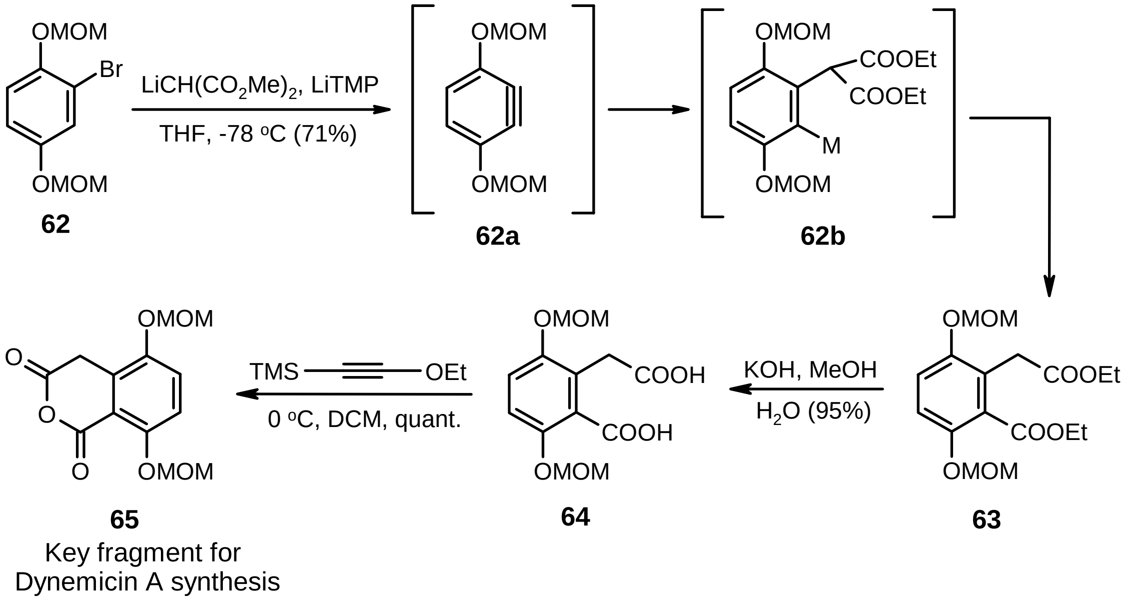
Scheme 6

2A.2.3.3 Total Synthesis of Dynemicin (Danishefsky & co-workers)

The condensation of bromo arenes with malonate anions to produce homophthalate esters is a very interesting reaction originally reported by Guyot et al.[65] Yet, it was Danishefsky's group that optimized the conditions of the above reaction to obtain the desired esters in good yields and applied the same for the synthesis of natural products. The above reaction proceeds as shown in Scheme 7 via the intermediacy of a benzyne **62a** that is intercepted by the malonate anion giving rise to adduct **62b**. Subsequent rearrangement of this adduct **62b** provides the desired homophthalate ester **63**. Danishefsky's optimization proved quite valuable to the general synthesis of highly substituted homophthalic esters and related compounds from easily accessible bromo arenes. As mentioned above, Danishefsky's group itself applied this methodology to prepare the key intermediate **65** in the synthesis of dynemicin A (**61**) (Scheme 7),[74] which was the newest member of the enediyne family of antibiotics when isolated in 1989 (this metabolite demonstrated high levels of in vitro antitumor activity comparable to those registered for two other enediynes calicheamycin and esperamicin).



Dynemicin A (**61**)

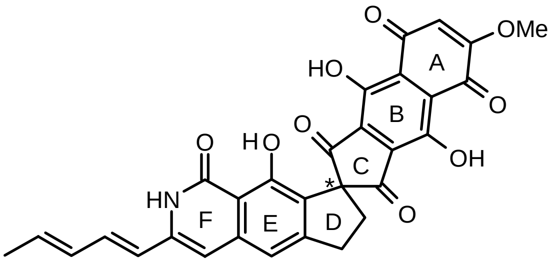


Scheme 7

The above methodology optimized by Danishefsky was utilized in synthesis of other important natural products such as fredericamycin A and angiogenesis inhibitor NM-3 also as illustrated below.

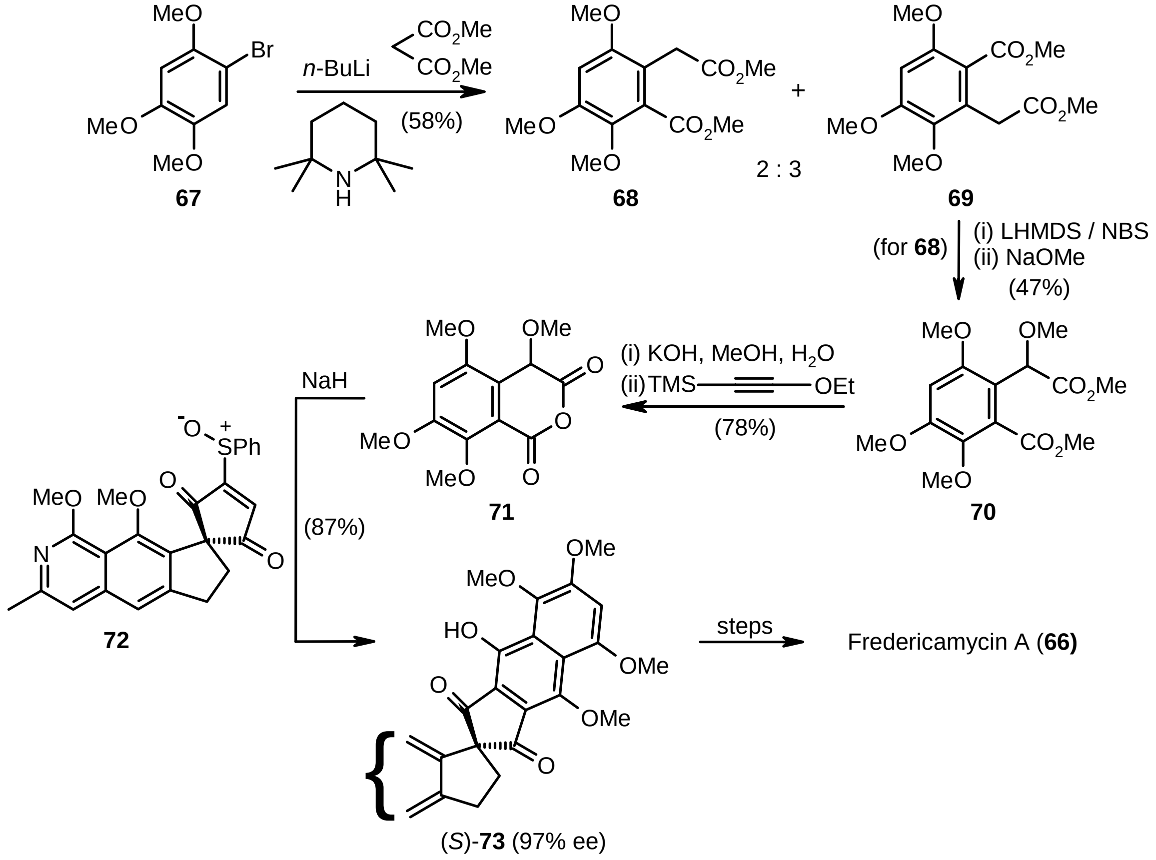
2A.2.3.4 Total Synthesis of Fredericamycin A (Kita et al.)

Fredericamycin A (**66**), isolated from *Streptomyces griseus* in 1961, exhibits potent anti-tumor activity against several tumor models (in vivo). Recent studies revealed that **66** inhibits both topoisomerases I & II. In addition to its promising biological profile, its unique structural features have attracted many synthetic organic chemists. Kita and co-workers employed the method reported by Danishefsky with some modification in the construction of the AB-ring unit of the natural product.[75] Thus reaction of bromoarene **67** with dimethyl malonate, *n*-BuLi and tetramethylpiperidine afforded a regioisomeric mixture (2:3) of the homophthalates **68** and **69** (through a nonregioselective addition of the lithiomalonate to the benzyne intermediate). Each regioisomer was readily separated by SiO_2 column



Fredericamycin A (**66**)
* Configuration is *S*

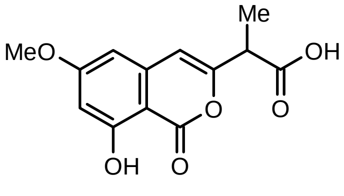
chromatography to give pure **68** and **69** (Scheme 8). The desired isomer **68** was subjected to sequential bromination and methanolysis to give the corresponding α-methoxy ester **70**. Alkaline hydrolysis of this diester **70** followed by a very efficient dehydration of the resulting dicarboxylic acid with trimethylsilyl(ethoxy)acetylene afforded the corresponding anhydride **71**. The anhydride **71**, on condensation with the other fragment **72** provided the key intermediate **73** (in 97% ee) in the synthetic scheme designed towards Fredericamycin A (**66**). Such a [4 + 2] cycloaddition of homophthalic anhydrides with α-sulfinyl substituted enones is a strategy that has been developed by the authors themselves and has been discussed later in this section.



Scheme 8

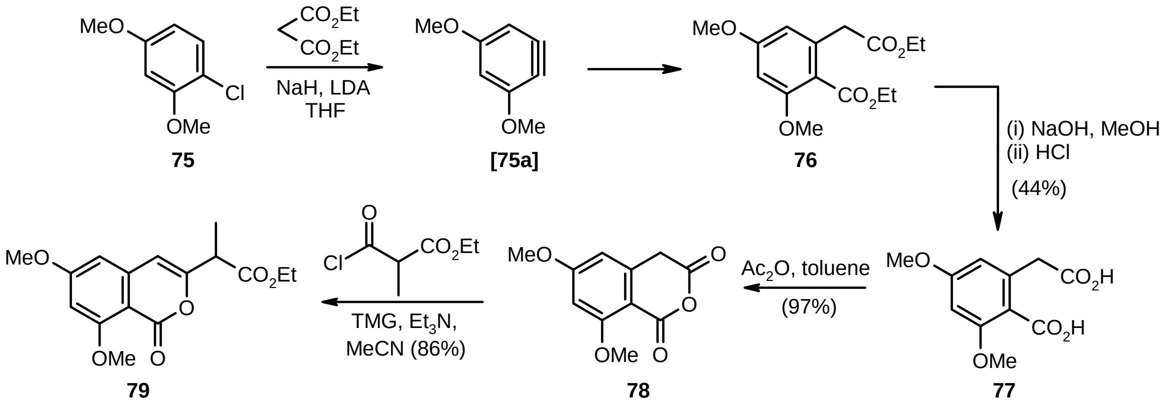
2A.2.3.5 Formal Synthesis of Angiogenesis Inhibitor NM-3 (Bauta et al.)

Danishefsky's optimized strategy for preparing important highly substituted homophthalic anhydride intermediates was also employed by Bauta et al. in the formal synthesis of angiogenesis inhibitor NM-3 (**74**), an isocoumarin natural product analogue.



NM-3 (**74**)

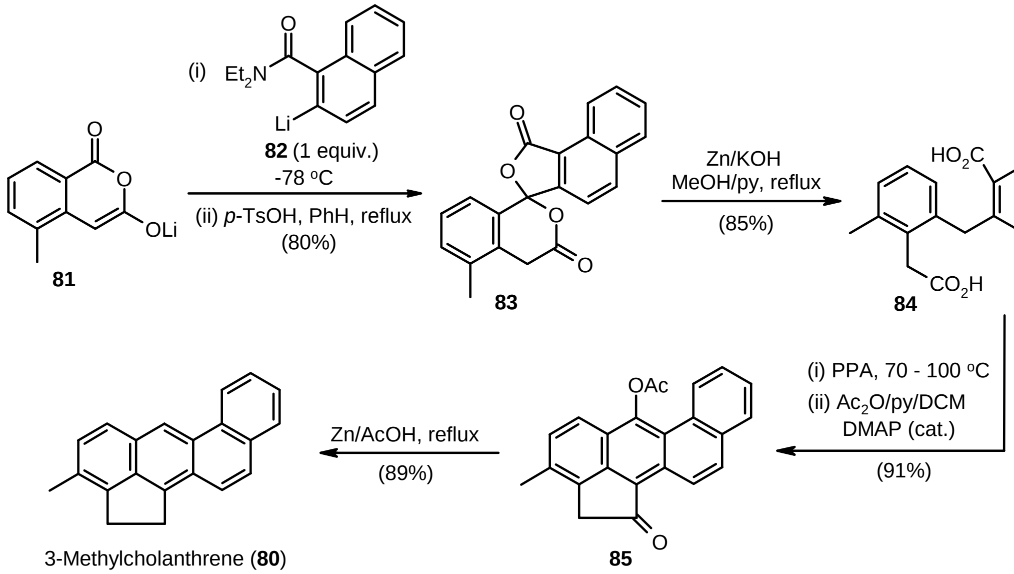
The authors reasoned that, for achieving the desired regiochemistry to prepare the key anhydride, an aryne of type **75a** would be useful as it would react with diethyl malonate anion to afford **76** preferentially owing primarily to the ortho-stabilization of the aryl anion by the methoxy group (Scheme 9).[76] Thus the reaction of halobenzene **75** with sodium diethylmalonate in THF provided the desired diester **76** as the major product, hydrolysis and dehydrative cyclization of which afforded the key intermediate anhydride **78**. The second significant step of the synthetic scheme was the reaction of the anhydride **78** with ethyl 2-methylmalonate, under basic conditions, to afford key isocoumarin **79**. The preparation of **79** constitutes a formal synthesis of NM-3.[77]



Scheme 9

2A.2.3.6 Total Synthesis of 3-Methylcholanthrene (Koreeda & co-workers)

3-Methylcholanthrene (**80**) occupies a unique position among a large number of carcinogenic and/or mutagenic polycyclic aromatic hydrocarbons due to the highly intricate mode of metabolic activation necessary for the expression of its biological activity. Koreeda and co-workers designed a five-step synthesis of 3-methylcholanthrene starting from 5-methylhomophthalic anhydride and *N,N*-diethyl-1-naphthamide in 55% overall yield.[78] Treatment of a solution of the preformed lithium enolate of 5-methylhomophthalic anhydride (**81**) with an equimolar solution of 2-lithio-1-naphthamide (**82**) followed by acid hydrolysis, provided cleanly the spirobislactone **83** in 80% overall yield. In addition, the synthesis also featured a unique, highly selective double Friedel-Crafts cyclization of the aryl diacid **84** with PPA to give rise, after acetylation, to the keto acetate **85**. The completion of the synthesis of the natural product was accomplished by the exhaustive deoxygenation of **85** with activated zinc in acetic acid (Scheme 10).

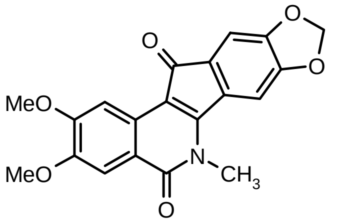


Scheme 10

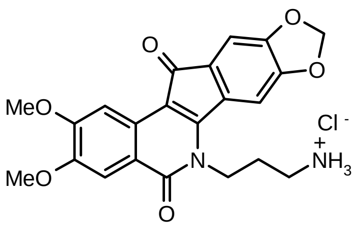
Thus the authors have synthesized the target molecule from 5-methylhomophthalic anhydride and *N,N*-diethyl-1-naphthamide in five steps and in 55% overall yield, featuring in situ protection of the former by deprotonation with *s*-BuLi prior to the addition of 2-lithio-1-naphthamide and a highly selective double Friedel-Craft cyclization of the aryl diacid promoted by the use of PPA.

2A.2.3.7 Design & Synthesis of Indeneisoquinoline Topoisomerase I Inhibitors (Cushman & co-workers)

The indenoisoquinolines are a class of noncamptothecin topoisomerase I inhibitors that display significant cytotoxicity in human cancer cell cultures. They offer a number of potential advantages over the camptothecins, including greater chemical stability, formation of more persistent cleavage complexes, and induction of a unique pattern of DNA cleavage sites. The discovery of the topoisomerase I inhibitory activity of the indenoisoquinoline **86** (NSC 314622) has naturally generated interest in its potential clinical utility as an anticancer agent. In a search for more potent and useful indenoisoquinolines based on the lead structure **86**, a series of analogues were synthesized in which the *N*-methyl group was replaced by various substituents. One of the most cytotoxic topoisomerase I inhibitors proved to be compound **87**, having a 3-aminopropyl substituent on the indenoisoquinoline nitrogen. Compound **87** was found to be more cytotoxic than **86** in human cancer cell cultures by a factor of 125, and it was also more potent as a topo-isomerase I inhibitor though the reasons for the enhanced potency of the 3-aminopropyl analogue **87** remain to be established.

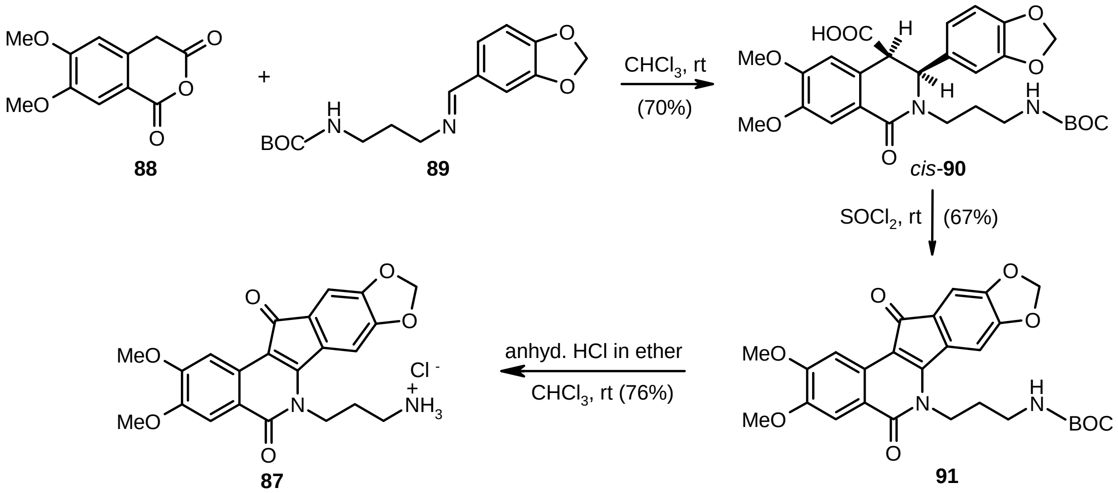


NSC 314622 (**86**)



87

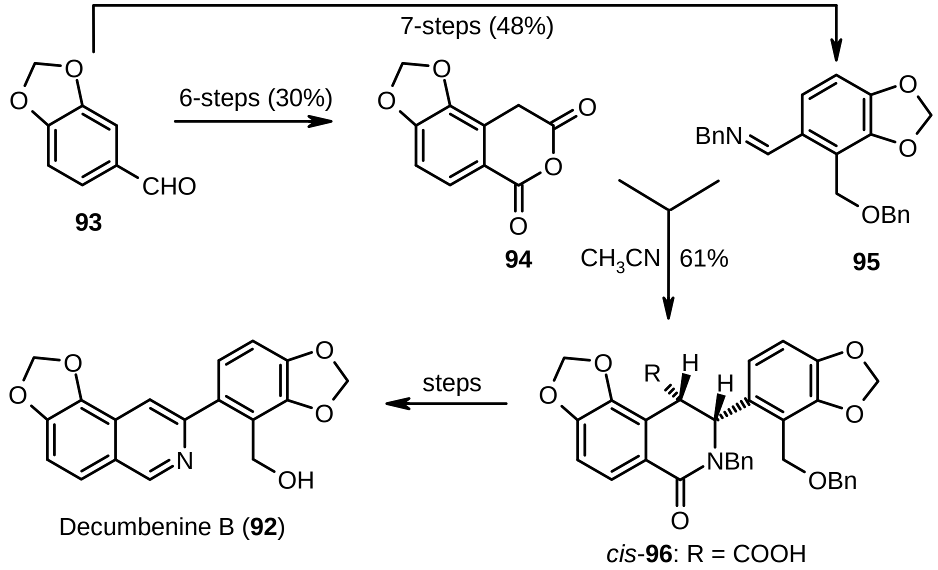
Cushman et al. designed a synthetic sequence for the preparation of the indeneisoquinolene in which the key reaction was the condensation of a Schiff base with a suitably substituted homophthalic anhydride.[79a] Thus, the reaction of 4,5-dimethoxyhomophthalic anhydride (**88**) with Schiff bases **89** afforded the expected racemic *cis*-3-aryl-4-carboxy-1-isoquinolone **90** in moderate yield, which on treatment with thionyl chloride afforded the corresponding indeno[1,2-*c*]isoquinolone **91**. Finally, the hydrochloride salt **87** was obtained by subjection of the protected compound **91** to anhydrous hydrochloric acid (Scheme 11). Such a condensation of homophthalic anhydrides with Schiff bases has been discussed in detail later in the section, whereas the conversion of compounds of the type **90** to indeno[1,2-*c*]isoquinolines such as **91** using thionyl chloride is a useful strategy developed by the authors themselves.[79b]



Scheme 11

2A.2.3.8 Total Synthesis of Decumbenine B (Xu & co-workers)

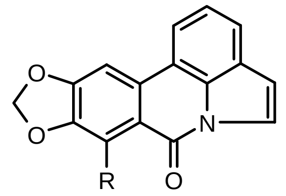
Decumbenine (**92**) is a 3-aryl isoquinoline alkaloid isolated from *Corydalis decumbens* (tubers of this plant have been used in Chinese folk medicine for treatment of hypertension, hemiplegia, rheumatoid arthritis and sciatic neuralgia). Xu and co-workers reported the first total synthesis of this alkaloid using the successful Cushman approach as seen above (and also conducted a preliminary study of its inhibition of spontaneous intestinal contraction).[80] The key step in the synthesis was the condensation of the homophthalic anhydride **94** with the Schiff base **95**, both of which were prepared starting from piperonal in 6 & 7 steps (in 30% & 48% overall yield) respectively. The condensation of the two key fragments proceeded to give the *cis*-isoquinolinone **96** (Scheme 12). The authors elaborated the key intermediate **96** to complete the total synthesis of the natural product via a pathway involving decarboxylation, carbonyl reduction, debenzylation and finally dehydrogenation of the tetrahydroquinoline.



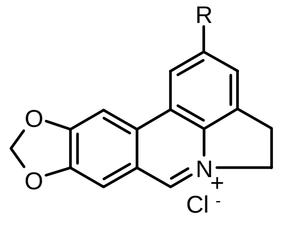
Scheme 12

2A.2.3.9 Synthesis of Antitumor Lycorines (Castedo & co-workers)

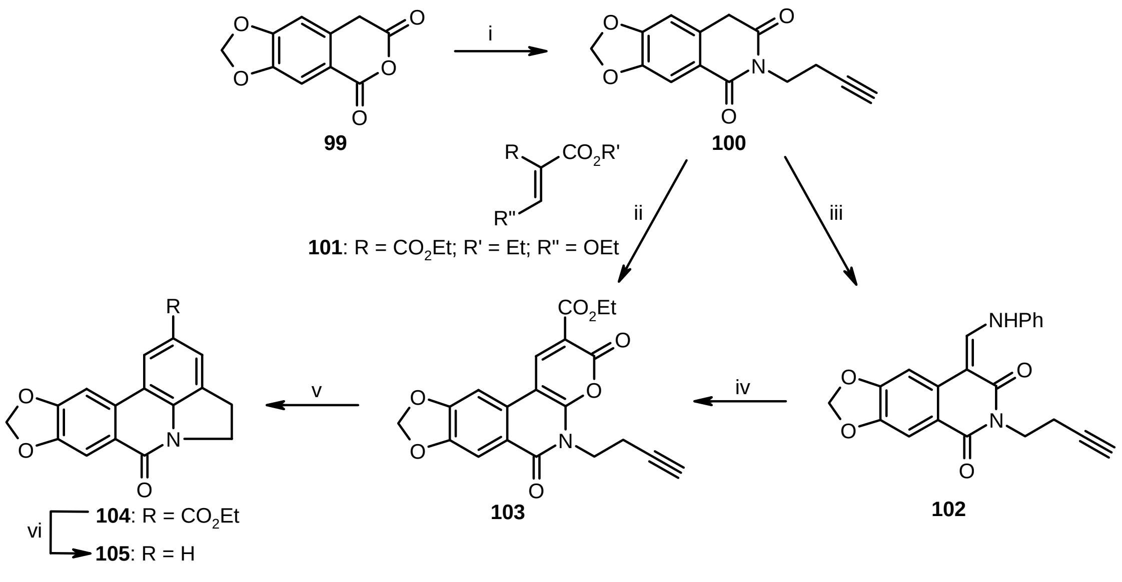
Lycorine alkaloids such as hippadine (**97a**), anhydrolycorinium chloride (**98a**), ungeremine (**97b**) and kalbretorine (**98b**) are active against several types of tumor. Castedo and co-workers reported a short, efficient method for the synthesis of these pharmacologically important lycorines based on an intramolecular Diels-Alder reaction between an α-pyrone and an alkyne, followed by loss of CO_2 in a retro Diels-Alder reaction.[81] The cyclization precursors were obtained in good yields in two or three steps from the corresponding homophthalic anhydride. Thus, treatment of 4,5-(methylenedioxy) homophthalic anhydride (**99**) with a solution of 3-butyn-1-amine followed by heating of the resulting salt, afforded imide **100** in 78% yield. Transformation of imide **100** into pyrone **103** was accomplished by two routes as shown in Scheme 13. The key step in the synthetic scheme was heating a solution of **103** in nitrobenzene at 210 °C, which brought about both intramolecular cycloaddition between pyrone and alkyne, and subsequent loss of CO_2 through a retro-Diels-Alder reaction, affording **104** in 83% yield. Synthesis of the naturally occurring alkaloid anhydrolycorin-7-one (**105**) was completed by hydrolysis of the ethyl ester followed by decarboxylation. The transformation of **105** into hippadine (**97a**) and anhydrolycorinium chloride (**98a**) has already been reported thus completing a formal synthesis of these two biologically active lycorines.



Hippadine (**97a**): R = H
Ungeremine (**97b**): R = OH



Anhydrolycorinium chloride (**98a**): R = H
Kalbretorine (**98b**): R = OH

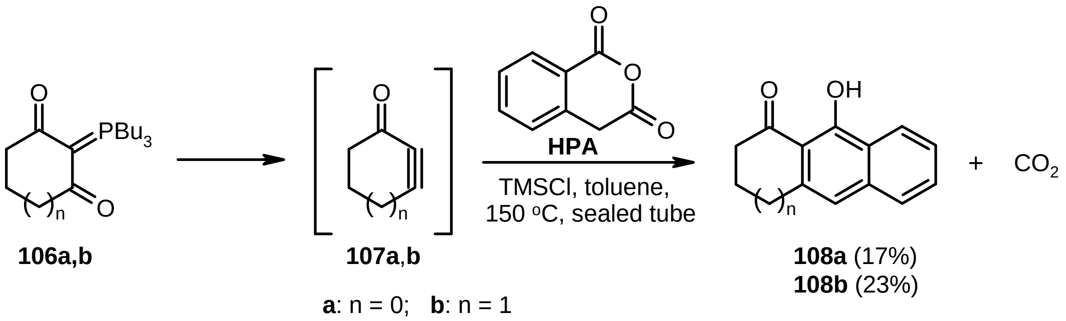


Scheme 13 *Reagents, conditions and yields*: (i) (a) 3-Butyl-1-amine, DCM, rt, (b) Δ (79%); (ii) Δ (60%); (iii) $HC(OMe)_3$, $PhNH_2$, AcOH (89%); (iv) $NCCH_2CO_2Et$, KO*t*-Bu, DMF (76%); (v) Nitrobenzene, Δ (83%); (vi) (a) KOH, (b) Cu, quinoline, Δ (81%).

Total synthesis of other natural products involving homophthalic anhydrides as intermediates or intermediates generated from homophthalic anhydride precursors include tetracenomycin D, saintopin analogues, 6-*O*-mehtylreticulol, etc.

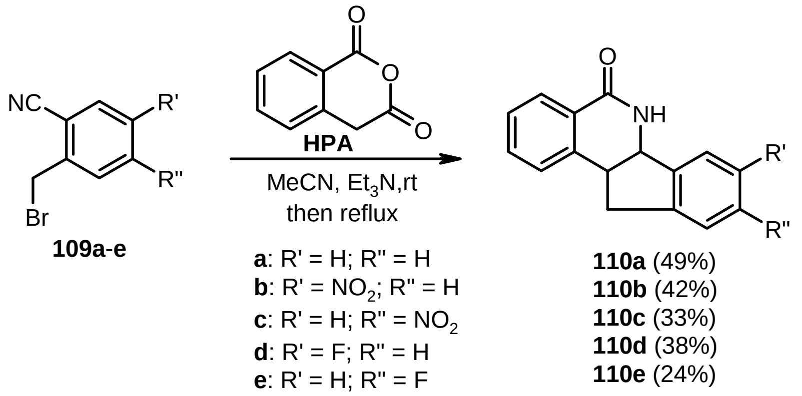
Thus, the utility and significance of homophthalic anhydrides in natural product synthesis have been amply demonstrated. That apart, due to their tremendous versatility, homophthalic anhydrides find application as synthons in several other aspects in organic synthesis. A few important reports have been illustrated subsequently reiterating the importance of homophthalic anhydrides.

The existence of short-lived cycloalkynes such as cyclopentynes and cyclohexynes has been demonstrated by several methods but the corresponding conjugated cycloalkynones of type **107** are not known. Masui and co-workers reported for the first time trapping of cycloalkynones **107a**,**b** generated by intramolecular Wittig reaction of *α,α*'-dioxocycloalkylidenetributylphosphoranes using homophthalic anhydride.[82] Thus, when a mixture of **106a**,**b** (1 mmol), HPA (diene equiva1ent) (3.3 mmol), and Me_3SiC1 (3 mmol) in toluene was heated at 150 °C for 40 h in a sealed tube, the Diels-Alder adducts **108a**,**b** were obtained in yields of 17 and 23% respectively (Scheme 14), establishing the intermediacy of highly reactive cycloalkenes **107a**,**b**.

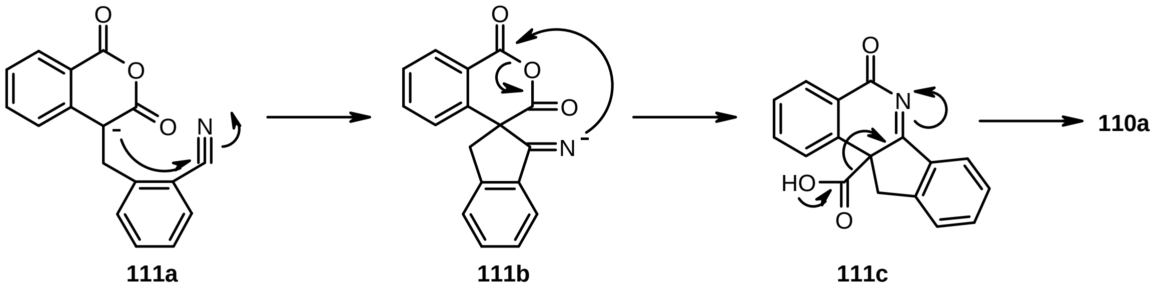


Scheme 14

Jagtap et al. envisaged indeno[1,2-*c*]isoquinolinones as attractive targets for synthesis and biological evaluation due to their structural similarity with the known inhibitors of poly(ADP-ribose)polymerase-1 such as isoquinolin-1(2*H*)-one. A facile and convenient synthesis of several derivatives of such compounds **110a-e** was carried out by the authors using a base promoted reaction of HPA with substituted 2-(bromomethyl)benzonitrile **109a-e** (Scheme 15).[83] The authors also proposed a mechanism for the formation of the tetracyclic compounds **110a-e** through the initial formation of the inisolable **111a** as illustrated in Scheme 16 (only the intermediate structures are depicted).

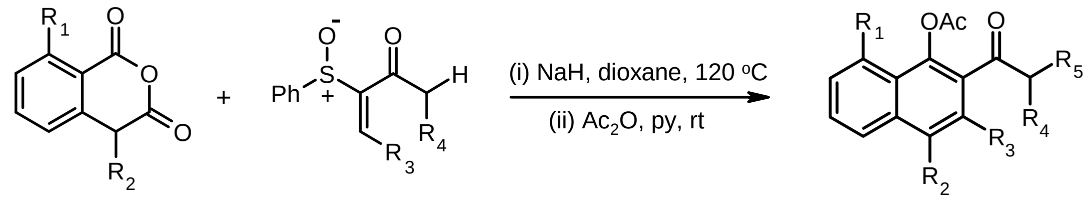


Scheme 15



Scheme 16

The *peri*-hydroxy aromatic framework is present in important biologically active natural products such as fredericamycin A, granaticin, olivin, the anthracyclines and many other polycyclic antibiotics. Thus considerable attention has been paid towards developing various methods for the construction of this framework in efforts towards the target molecules. The best method to date has been the strong base-induced [4 + 2] cycloaddition reaction of homophthalic anhydrides with dienophiles developed by Kita and co-workers in 1982.[84] Recently, the same authors designed a strategy for the effective use of enolizable enones in the above reaction (earlier this was a hurdle since, under basic conditions, the use of enolizable enones retarded the progress of the reaction). They reported that introduction of an electron sulfinyl group at the *α*-position of the enone moiety increased the dienophilicity by a great deal and also, the sulfinyl function acted as a good leaving group thus providing directly the *peri*-hydroxy aromatic compounds. Thus various *peri*-hydroxy aromatic compounds were prepared by the strong base-induced [4 + 2] cycloaddition reaction of homophthalic anhydrides **112a**,**b** with *α*-sulfinyl substituted enones **113a-c** (Scheme 17).[85] The authors completed the synthesis of the natural products fredericamycin A (as seen earlier) and SS-228 R applying the above strategy.



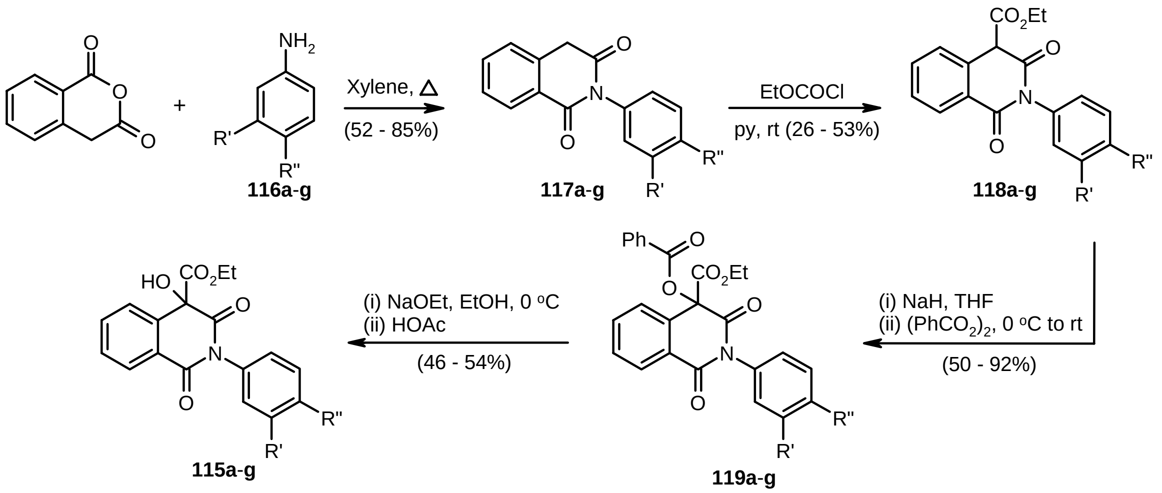
112a,b **113a-c** **114a-f**

Scheme 17 (Table 2 provides the details in the variation of the "R" groups and also the yields in each case).

Table 2 NaH induced cycloaddition of homophthalic anhydrides **112a**,**b** with sulfinyl substituted enones **113a-c**

S. No.	HPA			Dienophile			Product 114 (% yield)
	112	R_1	R_2	113	R_3	R_4	
1	**a**	H	H	**a**	$-CH_2-$		**a** (62)
2	**b**	H	OMe	**a**	$-CH_2-$		**b** (59)
3	**a**	H	H	**b**	$-(CH_2)_2-$		**c** (70)
4	**b**	H	OMe	**b**	$-(CH_2)_2-$		**d** (62)
5	**a**	H	H	**c**	Ph	H	**e** (49)
6	**b**	H	OMe	**c**	Ph	H	**f** (41)

A series of 10 novel auxin transport inhibitors, ethyl 2-aryl-4-hydroxy-1,3(2*H*,4*H*)-dioxoisoquinoline-4-carboxylates **115** possessing different *N*-phenyl substituents have been synthesized by Semple et al. employing a convenient four-step protocol starting from HPA. The synthetic scheme involved condensation of HPA with various anilines **116a-g** to provide substituted *N*-arylhomophthalimides **117a-g**. C-4 Ethoxy carbonylation of imides **117a-g** in the presence of a base afforded the α-substituted products **118a-g**, which were transformed to the target compounds **115a-g** in 2-steps (since attempts by the authors at installing the α-hydroxy group using different strategies led to a complex mixture of products) as shown in Scheme 18.[86]

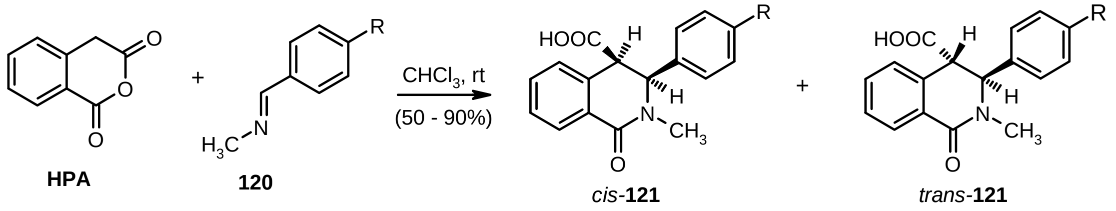


Scheme 18 (Table 3 provides the details in the variation of the “R” groups and also the yields in each case).

Table 3 Substituents and yields of intermediates **117a-g**, **118a-g** & **119a-g** and products **115a-g**

Entry	**R’**	**R”**	**% Yield**			
			117	**118**	**119**	**115**
a	H	H	85	51	89	54
b	H	CO_2Et	80	53	92	45
c	H	Cl	68	43	92	51
d	H	Me	78	49	72	49
e	Me	H	52	26	50	46
f	Me	*i*-Pr	80	64	85	61
g	H	*i*-Pr	80	45	78	38

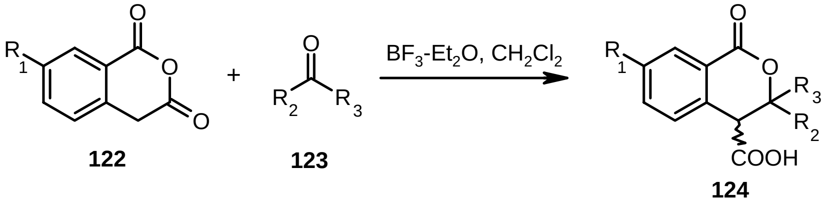
One of the most important reactions that has been the subject of extensive studies and has also had a significant impact in organic synthesis is the condensation of homophthalic anhydrides with Schiff bases yielding isoquinolonic acids (Scheme 19). The original discovery was the reaction of benzylidenemethylamine with succinic anhydride carried out by Cushman et al. to form substituted 2-pyrrolidones[87a] and was later extended by the same authors to glutaric and homophthalic anhydrides.[87b] The latter reaction has been utilized in the synthesis of various natural products including a variety of protoberberine, benzophenanthridine and B-secoprotoberberine alkaloids as well as indeneisoquinolines possessing antitumor activity (as seen earlier). Since the reaction involves the generation of two chiral centres, the products are therefore capable of existing as *cis* & *trans* diastereomers. Cushman et al. have carried out extensive studies on these aspects and a mechanistic interpretation has been reported.[87c] When para-substituted Schiff bases were used, electron donating substituents were found to favour the formation of the *cis* diastereomers while electron withdrawing substituents resulted in the production of greater amounts of *trans* diastereomers. In addition, bulky substituents on the nitrogen atom of the Schiff base resulted in the exclusive formation of *cis* diastereomers. The above-mentioned report[87c] by the authors provides a plausible interpretation for their findings. Scheme 19 portrays a general representation of this useful reaction.



Scheme 19

A variety of polyfunctional imines such as chlorimines[88] and 1-aza-1,3-dienes[89] have been used as the Schiff base component in the above reaction. There have also been numerous reports concerning the preparation of different isoquinolonic acids employing the above reaction under a variety of conditions to bring about *cis*/*trans* selectivity. An ionic liquid promoted (wherein the ionic liquid acted as reaction medium as well as a promoter) three-component coupling reaction involving homophthalic anhydride, an amine and an aldehyde was reported by Yadav et al. to produce *cis*-isoquinolonic acids.[90] More recently, Azizian et al. employed alum, which is inexpensive and non-toxic, as a reusable catalyst for a similar three-component reaction to generate *cis*-isoquinolonic acids.[91] Ytterbium triflate[92] has also been used as a catalyst for *cis*-selectivity whereas the preparation of *cis*-isoquinolonic acids containing boronate esters was also recently reported.[93] The diastereoselectivity in the above reaction was found to be dramatically (favouring the *trans* diastereomers) increased by the employment of Lewis acids such as $AlMe_3$[94] and MgI_2. Another Lewis acid, BF_3, and trimethyl orthoformate have been employed for the preparation of *trans*- isoquinolonic acids.[95,96]

The cycloaddition of homophthalic anhydrides with carbon-oxygen double bonds, that is, with aldehydes and ketones has not been as extensively explored as the reaction with carbon-nitrogen seen above. It was Gesquire et al. that brought out a nice methodology for the preparation of 3,4-dihydroisocoumarin-4-carboxylic acid derivatives based on the reaction of homophthalic anhydrides with aldehydes and ketones.[97] The authors used a $BF_3.Et_2O$ complex (which they had previously used for inducing the condensation with imines also) for effecting the above reaction, achieving good to excellent yields in the case of aldehydes under mild conditions (Scheme 20, Table 4). The reaction was then extended to some aliphatic ketones which afforded products in lower yields. According to their optimised method, the reactions of homophthalic anhydrides with aldehydes were carried out by stirring the reactants in dichloromethane at 25-35 °C for 5-14 hours. The protocol developed by the authors is especially useful in case where base catalysts need to be avoided. Moreover, this reaction gives access to three centers of chemical diversity around the heterocyclic scaffold, which can be further increased due to the presence of the carboxylic function, leading to derivatives such as amides or esters.



Scheme 20

Table 4 Reaction of homophthalic anhydrides **122** with various carbonyl compounds **123** and individual yields of **124**

Entry	**R_1**	**R_2**	**R_3**	**Time (h)**	**% Yield**
a	H	C_6H_5	H	5	81
b	H	2,4-$Cl_2C_6H_3$	H	7	71
c	NO_2	C_6H_5	H	14	90
d	NO_2	3-$NO_2C_6H_4$	H	14	65
e	H	CH_3	CH_3	14	50
f	H	CH_2CH_3	CH_3	14	22
g	NO_2	CH_3	CH_3	14	50

A similar study was also carried out by Palamareva and co-workers using DMAP to catalyze the reaction. Herein, the authors observed that the reaction was highly stereoselective towards the *trans*-cycloadducts.[98]

2A.2.4 Summary

Cyclic anhydrides, as already mentioned, offer remarkable advantages to the family of synthetic organic chemists. Homophthalic anhydrides, amongst them, have assumed greater significance due to the added reaction sites available on them for the chemist to explore. Homophthalic anhydrides present the additional advantage of two carbonyl groups residing in different environments giving rise to highly regioselective chemistry, which is of huge importance in organic synthesis. They also bear an extremely active methylene group through which an array of α-substituted derivatives can be generated. It is this tremendous versatility in structure and reactivity that has endeared the homophthalic anhydrides to the synthetic chemist and it is least surprising that they have been utilized in diverse applications over the years. These include, as we have seen earlier, the total synthesis of several structurally intriguing and biologically important natural products, wherein the homophthalic anhydrides have played a pivotal role either as key intermediates or in the generation of such intermediates in the synthetic scheme. That apart, they have also been utilized in other diverse aspects and have been the subject of various noteworthy methodologies that have had great impact in organic synthesis. We, in our group, have made considerable efforts in using these versatile synthons for the synthesis of bioactive natural products. In this context, our synthetic studies towards the protoberberine alkaloid Gusanlund D and dehydrogusanlung D starting from homophthalic anhydride have been presented in the following section (Section B of this chapter). We have also been successful in the construction of the nuevamine alkaloid framework, also starting from homophthalic anhydride, through a novel synthetic route and these studies have been described subsequently.

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2B. Section B

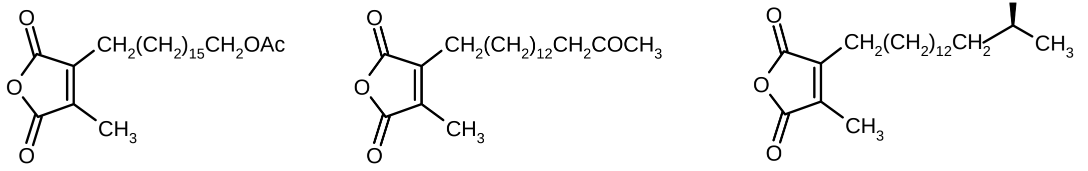
Studies towards the Synthesis of Natural Products Aspergillus Acids A-D, Gusanlung D, Nuevamine and Charminarone

This section features the following topics:

2B.1 Section B: I. A Facile Synthesis and an Enzymatic Resolution of Naturally Occurring Remotely Functionalized Alkylmethylmaleic Anhydrides from *Aspergillus wentii*: Aspergillus Acids A-D

2B.1.1 Background

In the past decade, several structurally interesting compounds with dialkyl substituted maleic anhydride moieties have been isolated as bioactive natural products and synthesized in view of their promising bioactivities;[1-3] of these, alkylmethylmaleic anhydrides, as we have seen in the previous section, are the more popular especially because of the large number of natural products wherein the alkylmethylmaleic anhydride moiety forms the core and also due to the interesting biological properties exhibited by these natural products. One can surmise that nature might be designing these alkylmethylmaleic anhydrides employing the condensation between pyruvic acid and other respective carboxylic acids. A specific example is that of chaetomellic acid A, which has been isolated from *Chaetomella acutiseta*[4] (genesis from pyruvic acid and palmitic acid).[5] Structure activity relationship studies have revealed that the hydrophilic dianion part of chaetomellic acid A binds with the phosphate group in the enzyme and the hydrophobic tetradecyl group coils with the farnesyl chain thus deactivating the enzyme. Hence, such compounds highlighting regiochemical dichotomy are known to be potent and highly specific inhibitors of ras farnesyl-protein transferase (Ras-FPTase)[4,6] and presently chaetomellic acid A is of commercial interest as a bioactive natural product.[7] Assante et al., in 1979, isolated[8] four new secondary metabolites aspergillus acids A-D (Figure 1) produced by the mould *Aspergillus wentii* when grown on yeast-glucose medium; these natural products were also established to be derivatives of citraconic anhydride with



Aspergillus acid A (**1a**) Aspergillus acid B (**1b**) R = H: (*S*)-Aspergillus acid C (**1c**)
R = OAc: (*S*)-Aspergillus acid D (**1d**)

Figure 1

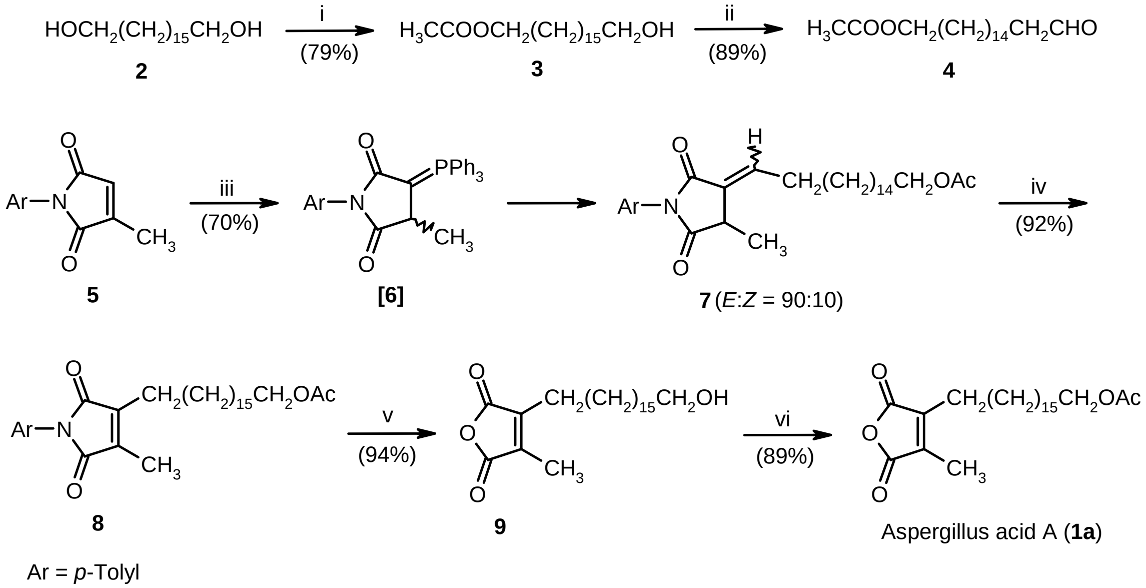
remotely functionalized long hydrocarbon chains. On the basis of Horeau method, the chiral centre in acids **1c** and **1d** has been assigned the *S*-configuration.[8]

2B.1.2 Rationale for Present Work

To date, the synthesis of these achiral/chiral natural products **1a-d** has not been reported. We were of the opinion that the remote functional groups in the hydrophobic long chain hydrocarbon units of these acids **1a-d** would anchor the binding process with target enzymes, thus resulting in a boost in activity.[6,9] The tremendous clinical potential of such compounds, as mentioned above, makes them attractive synthetic targets and the provision of a facile synthetic route to these molecules is an imperative task of current interest. In the past decade, we have in our group designed several bioactive natural and unnatural compounds using cyclic anhydrides as potential precursors.[10] We have also accomplished some important enzymatic resolutions,[11] some of which have formed a part of this dissertation and have been described in Section B of Chapter I. Thus, we launched a scheme for the synthesis of all the four secondary metabolites and also planned for an enzymatic resolution to enantiomerically enrich two of the four natural products in search of better activities with realistic supply. In the following part, the first synthesis of these naturally occurring acids **1a-d** comprising an elegant introduction of the remote functional groups and a lipase-catalyzed resolution of acid **1c** have been described.

2B.1.3 Results and Discussion

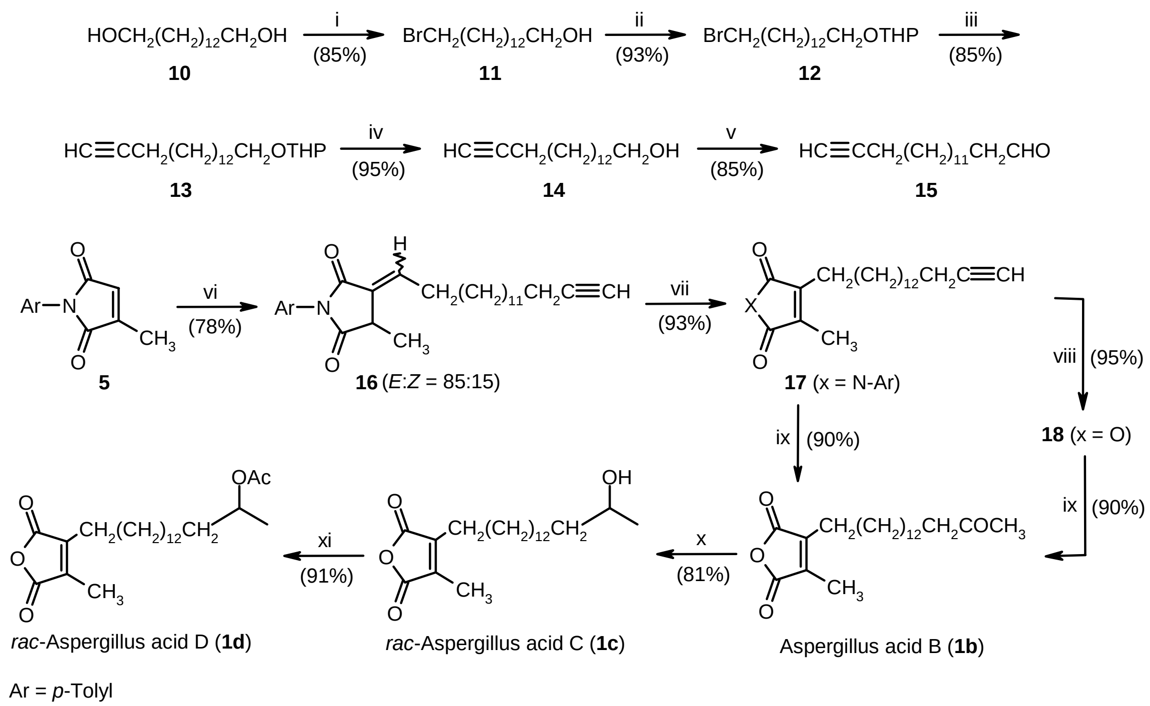
Aspergillus acid A (**1a**) is 2-(17-acetoxyheptadecyl)-3-methylmaleic anhydride while aspergillus acids B-D (**1b-d**) contain a 16-carbon chain with carbonyl, hydroxyl and acetyl groups at carbon number-15 respectively. In 1997, we have developed elegant reaction conditions to couple citraconimide-triphenylphosphine adduct with aliphatic aldehydes leading to alkylmethyl substituted maleic anhydrides.[6c,12] We envisaged that this coupling reaction would be useful for the preparation of these remotely functionalized anhydrides **1a-d**. With this in mind, towards the synthesis of aspergillus acid A (**1a**), we prepared the appropriate 17-acetoxyhexadecanal (**4**) in 2-steps starting from 1,17-heptadecanediol (**2**) via monoacetylation and Swern oxidation pathway with 70% overall yield (Scheme 1). The triphenylphosphine induced Wittig olefination of citraconimide **5** with acetoxyaldehyde **4** in refluxing acetic acid gave the corresponding *exo*-alkylidene succinimide **7** (E:Z = 90:10, by ^{1}H NMR) in 70% yield. The trisubstituted exocyclic to tetrasubstituted endocyclic carbon-carbon double bond migration using triethylamine as a base furnished the desired maleimide **8** in 92% yield. Base catalyzed hydrolysis of maleimide **8** furnished the 2-(17-hydroxytetradecyl)-3-methylmaleic anhydride (**9**) in 94% yield. Acetic anhydride mediated acylation of anhydride **9** gave the naturally occurring aspergillus acid A (**1a**) in 89% yield. The analytical and spectral data obtained for acid **1a** were in complete agreement with the reported data[8] and the overall yield of **1a**, for the four steps was 54%.



Scheme 1 *Reagents, conditions and yields*: (i) Ac_2O (0.98 equiv.), py, rt, 6 h (79%); (ii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, – 60 °C, 90 min (89%); (iii) Ph_3P, AcOH, 4, reflux, 18 h (70%); (iv) Et_3N, THF, reflux, 48 h (92%); (v) (a) KOH (30% aq.), THF-MeOH (1:2), reflux, 12 h, (b) H^+/HCl (94%); (vi) Ac_2O, py, rt, 8 h (89%).

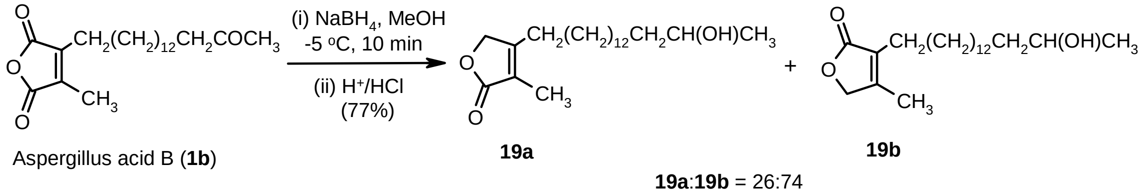
The successful synthesis of **1a** proved that our present synthetic route would allow the preparation of the chiral acids **1c** and **1d** via acid **1b**. At this stage we had two options for the synthesis of aspergillus acids B-D, (i) start with a suitable ω-substituted 16-carbon aldehyde keeping the desired functional group latent, perform the selective remote functionalization at the end and then attempt a new enzymatic resolution, or (ii) directly design and start with a protected enantiomerically pure remotely functionalized appropriate16-carbon aldehyde. We reasoned and chose the former, as the latter would involve (i) stoichiometric use of costly reagents for the enantioselective reduction of alkyl methyl ketone,[13,14] (ii) appropriate protection-deprotection of secondary alcohol moiety and (iii) possibility of loss of enantiomeric purity during the course of total synthesis.

In our efforts to functionalize the remote carbon in accordance with the established structures of the target molecules, we envisaged a Markonikov hydration of a terminal acetylene as the key step in the synthesis of these mould metabolites **1b-d**. In this context, the synthesis started with 1,14-tetradecane diol (**10**) which was transformed in 2-steps to the *ω*-bromo-*O*-THP protected alcohol **12** via the corresponding bromohydrin in 79% overall yield. The bromo compound **12** was then converted using standard functional group interconversions to the desired aldehyde **15** in 3-steps (69%) with the incorporation of an acetylene unit at the terminal position of the chain (Scheme 2). The overall yield of aldehyde **15** in 5-steps was 54%. Wittig condensation of aldehyde **15** with the imide/TPP adduct generated in situ from citraconimide **5** proceeded smoothly in refluxing acetic acid to yield the *exo*-imide **16** (*E*:*Z* = 85:15, by ^{1}H NMR) in 78% yield with the carbon-carbon triple bond intact. Subsequently, triethylamine efficiently catalyzed the isomerization of the exocyclic trisubstituted carbon-carbon double bond in *exo*-alkylidene succinimide **16** to afford the tetrasubstituted endocyclic maleimide **17** in 93% yield. To complete the synthesis of the second metabolite in the series, we were now left with two important tasks, converting the maleimide **17** to the anhydride and exposing the latent methyl ketone present as the terminal acetylene. Both the above steps were achieved in one pot with 90% yield upon the treatment of imide **17** with a mixture of acetic acid and 6 M sulfuric acid (2:1) at 100 °C. Under these conditions, the imide was hydrolyzed to the anilic acid that subsequently ring-closed to the anhydride; simultaneous addition of a water molecule to the carbon-carbon triple bond in a Markonikov fashion led to the generation of an alkyl methyl ketone, thus furnishing the natural product **1b**. We were also able to successfully isolate the intermediate acetylinic anhydride **18** by carrying out base catalyzed hydrolysis of the corresponding maleimide **17**. The anhydride **18** on acid catalyzed hydration gave the desired aspergillus acid B (**1b**) in 90% yield. The overall yield of aspergillus acid B in three steps was 65%.



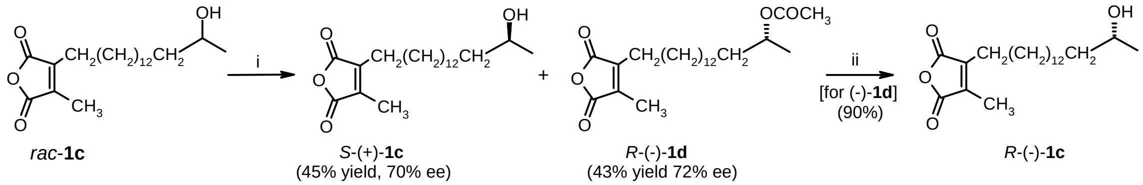
Scheme 2 *Reagents, conditions and yields*: (i) HBr (47% aq.), toluene, reflux, 96 h (85%); (ii) DHP, PPTS (cat.), CH_2Cl_2, rt, 4 h (93%); (iii) NaC≡CH, THF, HMPA, – 78 °C to rt, 40 h (85%); (iv) *p*-TSA, MeOH, rt, 2 h (95%); (v) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, – 60 °C, 90 min (85%); (vi) Ph_3P, AcOH, **15**, reflux, 24 h (78%); (vii) Et_3N, THF, reflux, 48 h (93%); (viii) (a) KOH (30% aq.), THF-MeOH (1:2), reflux, 12 h, (b) H^+/HCl (95%); (ix) 6 M H_2SO_4-AcOH (1:2), 100 °C, 8 h (90%); (x) (a) Aq. NaOH, THF, 50 ºC, 2 h, (b) $NaBH_4$, 0 ºC to rt, 3 h, (c) H^+/HCl (81%); (xi) Ac_2O, py, rt, 12 h (91%).

A chemoselective reduction of the ketone carbonyl in **1b** was then necessary to obtain the corresponding hydroxyl compound **1c**. In our hands, sodium borohydride reduction of **1b** in organic solvents under a variety of conditions failed to provide us with the required chemoselectivity. We always observed the reduction of anhydride carbonyl as well as the ketone moiety and obtained the mixture of corresponding butyrolactones **19a/b** (26:74) with the remote hydroxyl functional group (Scheme 3). The structural assignment of regioisomers **19a** and **19b** was done on the basis of ^{1}H NMR signals for the vinylic methyl group. As expected, the β-methyl group signal in the major isomer **19b** appeared at δ 2.02, while the α-methyl group in the minor isomer **19a** appeared at δ 1.83. Finally, the desired chemoselectivity was obtained by carrying out a sodium borohydride reduction of the corresponding di-sodium salt of **1b** followed by quenching the reaction with dilute HCl to afford, exclusively, the racemic natural product **1c** in 81% yield. We believe that the hydroxy anhydride **1c** will be a potential precursor for the synthesis of macrocyclic lactone skeleton via intramolecular lactonization pathway. Acetylation of hydroxy anhydride **1c** with acetic anhydride-pyridine furnished the fourth metabolite in the series **1d** with 91% yield. The overall yield of aspergillus acid C in four steps was 53% and that of aspergillus acid D in five steps was 48%. The analytical and spectral data obtained for the three natural products **1b-d** were in complete agreement with the reported data.[8] (See pages 177-182 for ^{1}H & ^{13}C NMR spectra of aspergillus acids A-D (**1a-d**).



Scheme 3

The conversion of methyl alkyl ketones to enantiomerically pure secondary alcohols using Baker's yeast and other microorganisms are known to be unsatisfactory[14,15] and enzymatic resolution is a better option in such cases. We felt that the presence of a remote anhydride moiety in aspergillus acid C would improve the recognition and binding of the substrate acids **1c**/**1d** during resolution and hence we systematically studied both the enzyme catalyzed hydrolysis of (±)-**1d** and the enzyme catalyzed acylation of (±)-**1c** using vinyl acetate as an acyl donor. The biphasic Amano PS catalyzed hydrolysis of (±)-**1d** proceeded rather slowly and after a prolonged reaction time (seven days), we observed only 8-10% formation of hydrolyzed product (by ^{1}H NMR). Following our earlier observation in the case of resolution of the key intermediate in the synthesis of talampanel (Chapter I, Section B), we performed the Amano PS-catalyzed acylation of (±)-**1c** in hexane-benzene mixture (2:1) and observed a very clean acylation at 45 °C to obtain the acids *S*-(+)-**1c** (45%) and *R*-(–)-**1d** (43%), after separation by column chromatography (stereochemical assignments were based on the optical rotation of the known compounds).[8] The base catalyzed hydrolysis of (*R*)-aspergillus acid D [(–)-**1d**] furnished (*R*)-aspergillus acid C [(–)-**1c**] in 90% yield (Scheme 4). The ^{1}H NMR spectra of MTPA derivatives[16] of both aspergillus acids (+)- & (–)-**1c** (page 183) showed a very clean separation of the two diastereomeric –OMe group singlet signals (appearing at δ 3.55 & 3.57), revealing that the acids (+)- & (–)-**1c** possess 70% ee and 72% ee respectively (the ee values were calculated from the ratio of the integration values for the two –OMe group singlet signals as described in Chapter 1). To the best of our knowledge[17] this is the first enzymatic resolution of a secondary alcohol coupled with a maleic anhydride moiety.



Scheme 4 *Reagents, conditions and yields*: (i) Amano PS, vinyl acetate, hexane-benzene (2:1), 45 ºC, 72 h [(+)-**1c**, 45%; (–)-**1d**, 43%]; (ii) (a) Aq. NaOH, THF, 50 ºC, 4 h, (b) H^+/HCl (90%).

In summary, a facile route for the synthesis of four new secondary metabolites **1a-d** has been demonstrated in three to five steps with very good overall yields. In the present synthesis, a terminal acetylene has been employed to bring about an elegant means of remote functionalization; the chemoselective sodium borohydride reduction and an Amano PS-catalyzed enzymatic resolution are both noteworthy. The Amano PS-catalyzed enzymatic resolution of (±)-**1c** gave the *S*-aspergillus acid C [(+)-**1c**] with 45% yield & 70% ee and *R*-aspergillus acid D [(–)-**1d**] with 43% yield & 72% ee. The biological importance of natural products structurally resembling the aspergillus acids (such as chaetomellic acid A) has been discussed earlier and we feel that the present approach is general in nature and will be useful to design several analogues of these metabolites for structure activity relationship studies.

2B.2 Section B: II. Studies towards the Synthesis of the Protoberberine Alkaloid Gusanlung D

2B.2.1 Background

Protoberberines are a large class of naturally occurring alkaloids characterized by a tetracyclic ring skeleton with an isoquinoline core (Figure 2).[18] These molecules display a wide range of substitution patterns surrounding the ring skeleton particularly in the A- and D-rings (these are usually alkoxy groups such as methoxy or methylene dioxy). In many of these alkaloids, a stereogenic carbon atom is present at C-13a.

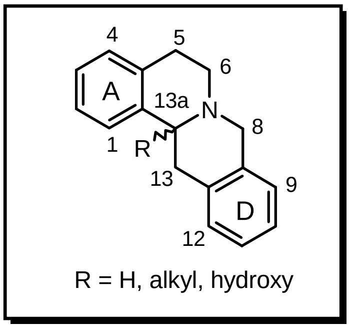
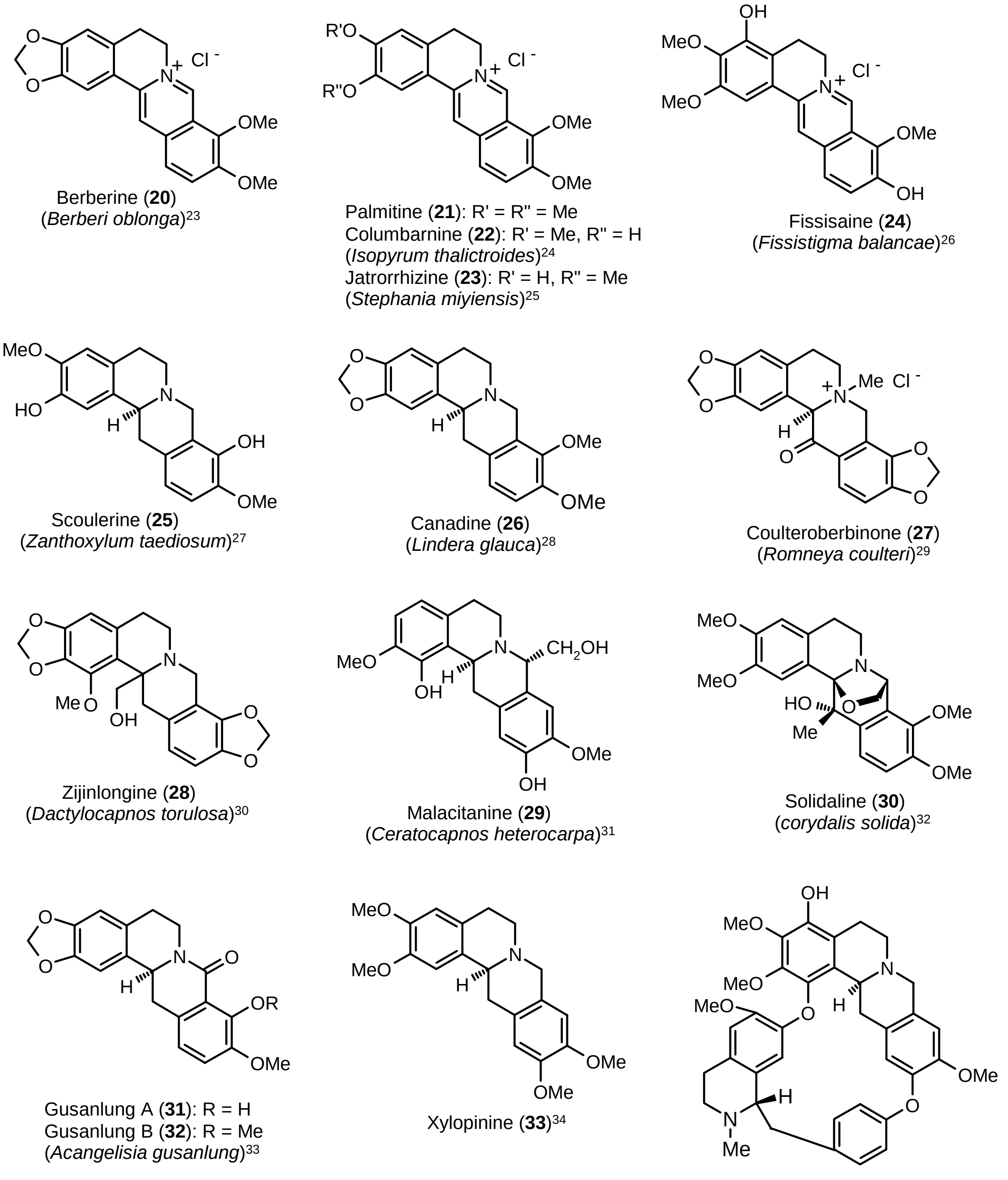


Figure 2 Protoberberine alkaloid skeleton

The various biological properties that have been attributed to this class of alkaloids include antimicrobial, antileukemic, antitumor and antiinflammatory activities. In addition, the protoberberines play key roles as precursors in the biosynthesis of many isoquinoline alkaloids such as rhoeadine, secoberbine, and benzo[*c*]phenanthridine alkaloids.[19] These attributes render them as important compounds in the field of medicine and also make them attractive targets for synthetic organic chemists. Thus, considerable efforts have been expended in the study of these molecules for both their synthetic[20] and their biological[21] significance. The number of protoberberine alkaloids known is enormous and so too is the variety and diversity in the species from which they have been isolated (only a few of them have been illustrated in Figure 3). In fact, there are many cases wherein a particular alkaloid has been isolated from several different species. The number is constantly on the rise as newer and structurally interesting protoberberine alkaloids, such as longiberine (**34**, Figure 3), are being discovered on a regular basis. The importance of this family of alkaloids is further emphasized by the annual comprehensive reviews that keep a track and provide information about all the recently isolated isoquinoline alkaloids (these reviews also provide information about already known alkaloids that are isolated again from a new species).[22]



Berberine (**20**)
(*Berberi oblonga*)[23]

Palmitine (**21**): R' = R'' = Me
Columbarnine (**22**): R' = Me, R'' = H
(*Isopyrum thalictroides*)[24]
Jatrorrhizine (**23**): R' = H, R'' = Me
(*Stephania miyiensis*)[25]

Fissisaine (**24**)
(*Fissistigma balancae*)[26]

Scoulerine (**25**)
(*Zanthoxylum taediosum*)[27]

Canadine (**26**)
(*Lindera glauca*)[28]

Coulteroberbinone (**27**)
(*Romneya coulteri*)[29]

Zijinlongine (**28**)
(*Dactylocapnos torulosa*)[30]

Malacitanine (**29**)
(*Ceratocapnos heterocarpa*)[31]

Solidaline (**30**)
(*corydalis solida*)[32]

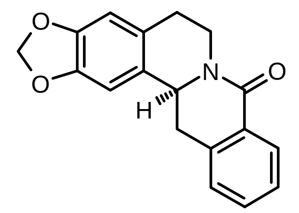
Gusanlung A (**31**): R = H
Gusanlung B (**32**): R = Me
(*Acangelisia gusanlung*)[33]

Xylopinine (**33**)[34]

Longiberine (**34**)
(*Thalictrum longistylium)*[35]

Figure 3 (please note that the species name provided may not be the only species from which that particular alkaloid has been isolated).

Acangelisia gusanlung H. S. Lo is a small shrub distributed in Hainan Island of southern China (its whole stem is used in folk medicine). Recently, Zhang et al.[36] isolated another protoberberine alkaloid, Gusanlung D from the stem of the above shrub [the same authors had earlier isolated two other protoberberines Gusanlung A & B (**31** & **32** respectively, see Figure 3) from the same species]. The structure of Gusanlung D was elucidated as (*S*)-(−)-2,3-methylenedioxy-8-oxoberbine (**35**) on the basis of analytical & spectral data analysis. This was a very interesting outcome since the assignment of the above structure established Gusanlung D as the first natural protoberberine alkaloid unoxygenated at ring D.[37]



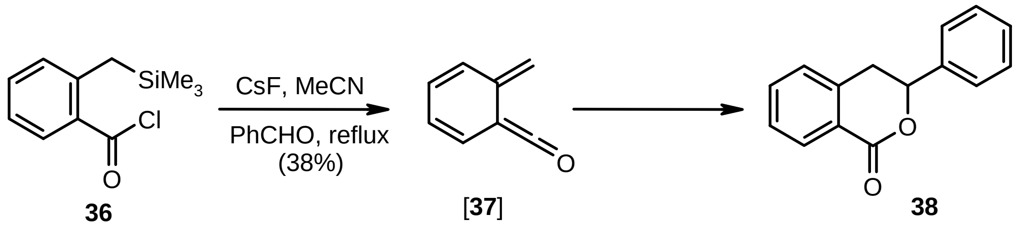
Gusanlung D [(−)-**35**]

2B.2.1.1 Synthetic Approaches Towards Gusanlung D

The structural features of Gusanlung D [(−)-**35**] attracted organic chemists towards designing a route for the synthesis of this novel protoberberine alkaloid. To date there are three racemic syntheses[38-40] and one asymmetric synthesis[37] of Gusanlung D [(−)-**35**] known in the literature. Prior to the description of our studies towards the synthesis of this interesting alkaloid, two important racemic synthesis reported and the only known asymmetric synthesis have been discussed briefly below.

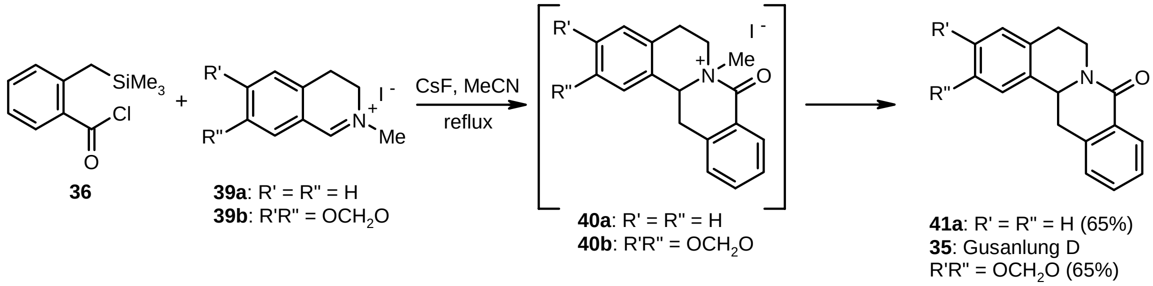
[A] *Kessar's approach*[38]

Kessar et al. developed an elegant method for the facile generation and trapping of α-oxo-*o*-quinodimethanes. They found that fluoride promoted desilylation of *o*-[(trimethylsilyl)methyl]benzoyl derivatives can directly lead to adducts by trapping the generated ketene intermediate appropriately (the ketene intermediate is formed upon desilylation via a 1,4-elimination). Thus, reaction of the acid chloride **36** with anhydrous CsF (in MeCN under reflux) in the presence of benzaldehyde afforded 3-phenyl-3,4-dihydroisocoumarin **38** via the ketene intermediate **37** (Scheme 5). Several other isocoumarins were similarly prepared by using different aldehydes as dienophiles. Tetralone skeletons were also generated using dimethyl or diethyl fumarate as the trapping agents.



Scheme 5

A significant extension of the above methodology was provided by the authors wherein they explored the use of 3,4-dihydroisoquinolinium salts as dienophiles in the above reaction for the construction of the protoberberine skeleton. Thus, the same acid chloride **36**, upon treatment with CsF (in refluxing MeCN) in the presence of iminium salt **39a**, furnished the 8-oxo berbine **41a** via the intermediacy of the adduct **40**. Similarly, when iminium salt **39b** was used, the racemic natural product Gusanlung D was obtained in 65% yield (Scheme 6). Other derivatives were also prepared starting from different substituted acid chlorides **36** and iminium salts **39**. Amongst those, two derivatives yielded adducts that underwent LAH induced reduction of the carbonyl functionality to afford two other protoberberine alkaloids (±)-tetrahydropalmatine and (±)-canadine.

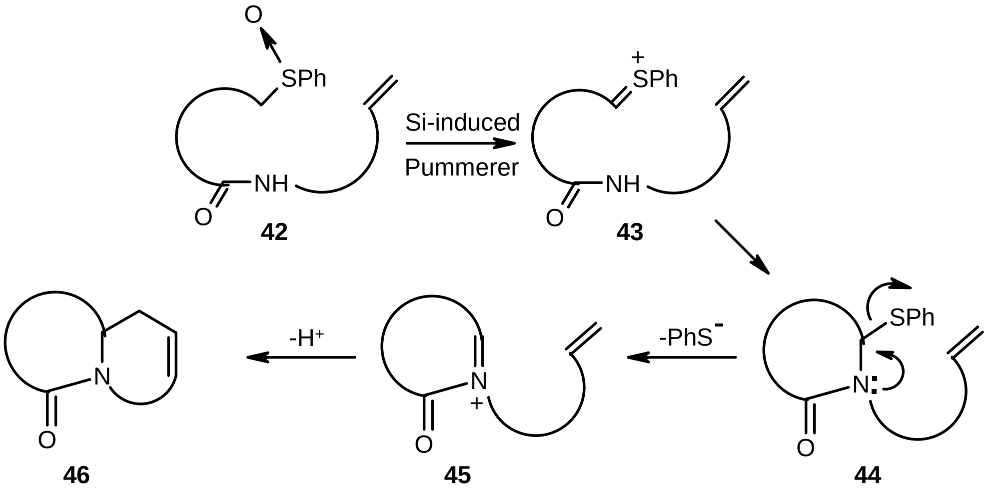


Scheme 6

[B] *Padwa's approach*[39]

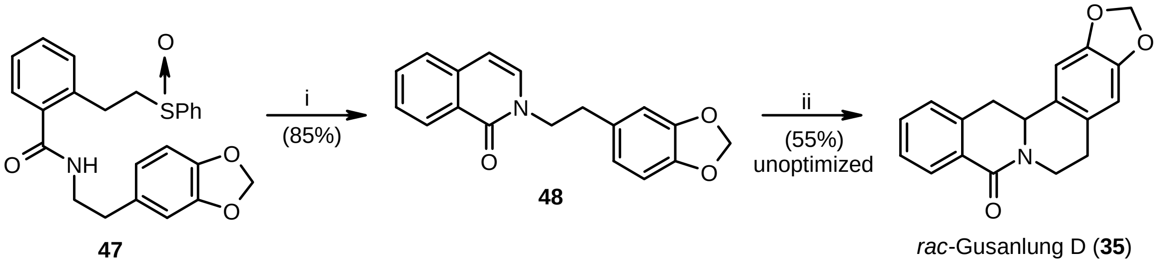
Padwa et al. explored the possibility of employing a silicon-induced Pummerer reaction of several amido sulfoxides for the construction of various azabicyclic ring systems by way of a thionium/*N*-acyliminium ion cascade. The authors envisaged that amido sulfoxides possessing tethered π-bonds as represented by **42** would, upon a Pummerer reaction, generate the thionium ion of type **43**, which would readily react with the neighbouring amido nitrogen to provide the 2-thiophenyl substituted lactam **44**.

Subsequent elimination of the thiophenyl group would lead ultimately to the azabicyclic lactam system **46** via cyclization of a transient *N*-acyliminium ion as in **45** (Scheme 7). Padwa's group was successful in realizing the synthesis of several indolizidine alkaloids employing the above strategy.



Scheme 7

Padwa et al.'s interest in establishing amidosulfoxides as useful building blocks for heterocyclic systems prompted them to use the above cascade sequence for the synthesis of the protoberberine alkaloid Gusanlung D (**35**). Thus, amidosulfoxide **47** was subjected to the optimized Pummerer reaction protocol using TBDMS(OMe)=CH_2 and ZnI_2. However, this reaction did not lead directly to the formation of the natural product as expected; instead the eneamide **48** was obtained in 85% yield. To complete the synthesis of Gusanlung D (**35**), the authors exposed **48** to acidic conditions to obtain the protoberberine alkaloid **35** in 55% yield via the intramolecular cyclization pathway (Scheme 8).

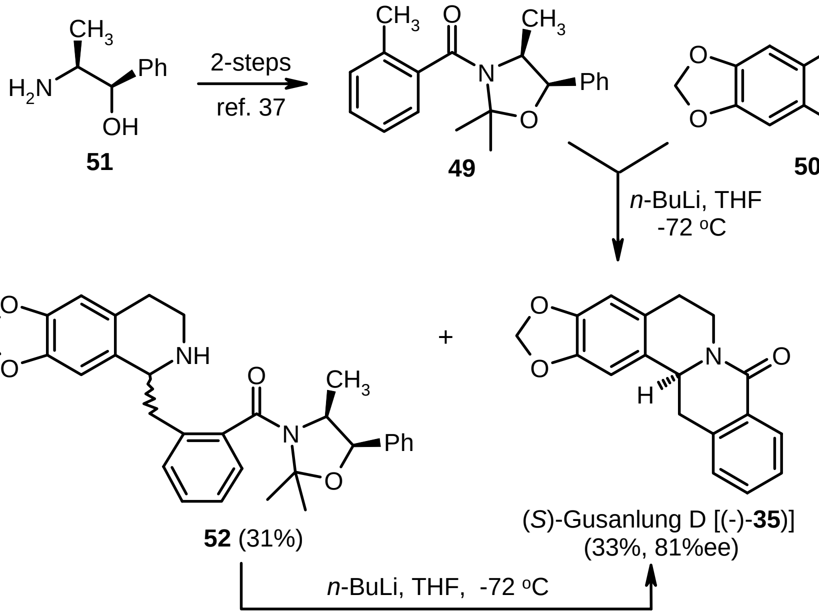


Scheme 8 *Reagents, conditions and yields*: (i) TBDMSOC(OMe)=CH_2, ZnI_2 (85%); (ii) H^+ (55%, unoptimized).

[C] *Chrzanowska's asymmetric synthesis*[37]

Chrzanowska et al. reported the only asymmetric synthesis of Gusanlung D known till date. The key step of the synthesis, in which a new stereogenic centre at C-13a was created, involved the addition of laterally metallated chiral *o*-toluamides to cyclic imines (the authors had earlier reported a study on stereoselective syntheses of isoquinoline alkaloids based on the addition of carbon nucleophiles to imines).[41] The authors envisaged the metallation to proceed with high regiochemical control[42] and coupled with the simultaneous cyclization, this feature would offer a direct approach to the protoberberine skeleton.

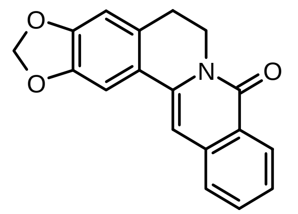
Thus, for the asymmetric synthesis of Gusanlung D, the authors planned for a coupling between the *o*-toluamide **49** and 6,7-methylenedioxy-3,4-dihydroisoqunoline (**50**). The requisite *o*-toluamide **49** was prepared in two steps from (1*R*,2*S*)-2-amino-1-phenylpropanol (**51**) via the reaction of **51** with *o*-toluoyl chloride followed by protection of the NH and OH functional groups of the resultant amide as an oxazolidine. The thus prepared amide **49** was treated with *n*-BuLi (in THF at −72 °C) to generate the benzylic carbanion, which upon reaction with the other coupling fragment **50** yielded directly (*S*)-gusanlung D [(−)-**35**] in 33% yield and 81% ee. Additionally, the secondary amine **52** was isolated in 31% yield as a diastereomeric mixture (Scheme 9).



Scheme 9

The secondary amine **52** was cyclized to the desired 8-oxoberbine **35** by addition of *n*-butyllithium to the THF solution of **52** at −72 °C, and then allowing the reaction mixture to reach ambient temperature. Pure gusanlung D (**35**), with the same absolute configuration at C-13a, was isolated with 98% ee (Scheme 9). The total yield of the natural product was thus raised to 64%.

However, the authors reported major discrepancies in the analytical & spectral data for the compound synthesized by them as against the data reported for the isolated natural product.[36] The opinion of the authors was that the discrepancy could have arisen due to a possible contamination of the isolated natural product with a considerable amount of the dehydrolactam **53**[43] during isolation as a result of an in situ air-oxidation. Nevertheless, a comparison of the physical and NMR data published for isolated gusanlung D with those published for compound **53**[43] and with the data for synthetic **35** obtained by the authors did not lead to any constructive explanation of the differences in physical & spectral data for isolated gusanlung D and the synthesized compound.



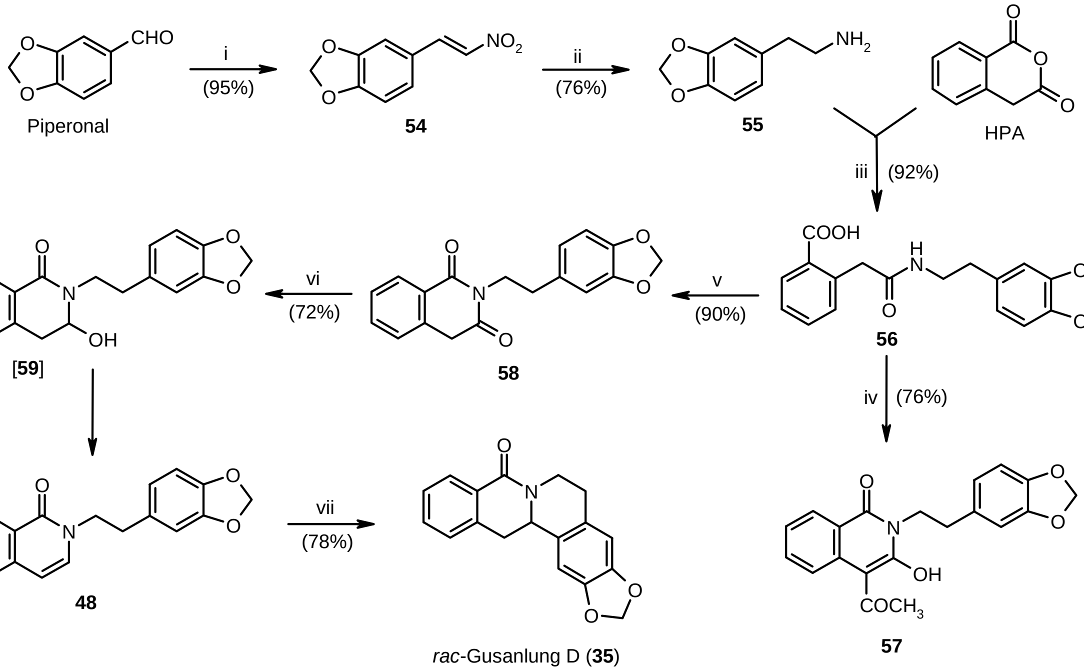
Dehydrogusanlung D (**53**)

2B.2.2 Rationale for Present Work

Over the past decade, we in our group have focused our research primarily on the chemistry of cyclic anhydrides and extensive efforts have been put in towards the synthesis of cyclic anhydride based natural products (as seen in the previous section) as well as other natural products using cyclic anhydride precursors. The protoberberine alkaloid skeleton presents a nice opportunity for employing homophthalic anhydride (HPA), hitherto unexplored in our group, as starting material for its construction.[44] We were attracted towards Gusanlung D not just because we envisaged its synthesis starting from homophthalic anhydride but also because of the ambiguity in the structure of the alkaloid as reported by earlier syntheses. Synthesis of this protoberberine would enable the comparison of our data with the earlier reported ones and with the data reported for the isolated natural product. Thus, with an aim getting some insight into the actual structure of the natural product, we set about the synthesis of Gusanlung D starting from HPA as described in the following part.

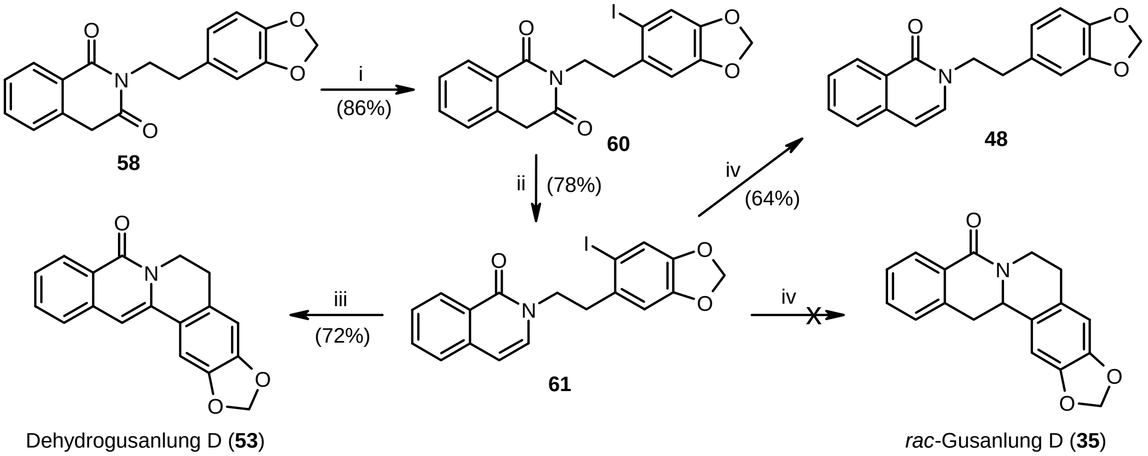
2B.2.3 Results and Discussion

The regioselective ring opening of HPA at the more reactive non-conjugated carbonyl with homopiperonyl amine (**55**) (prepared in two steps from piperonal via nitromethane condensation and subsequent LAH reduction) in an ether-THF mixture at room temperature exclusively furnished the *N*-homopiperonyl homophthalamic acid (**56**) in 92% yield (Scheme 10). An attempted preparation of the corresponding homophthalimide **58** using an acetic anhydride-sodium acetate induced dehydration of **56** resulted instead in the formation of a homophthalimide bearing an α-acyl substitution (formed due to the highly acidic nature of the α-methylene protons of such systems). A singlet signal at δ 11.05 (and the corresponding absence of a methylene proton signal) in the ^{1}H NMR spectrum of the above product clearly revealed that the product existed exclusively as the enol **57** (stabilized by conjugation of the double bond with the carbonyl group and the phenyl ring as well as by intramolecular hydrogen bonding). Ultimately, the acid **56** on treatment with hexamethyldisilazane (HMDS)-$ZnCl_2$[45] gave the desired *N*-homopiperonyl homophthalimide **58** in 90% yield. We envisaged a regioselective reduction of the non-conjugated carbonyl in imide **58** to provide the corresponding hydroxylactam **59** which, upon intramolecular cyclization would provide in our hands the desired racemic protoberberine (±)-**35**. Also, a successful lipase-catalyzed resolution of the hydroxy lactam **59** would provide access to both natural and unnatural stereoisomers of the natural product (although there existed the possibility that we would still end up with a racemic product in case of a complete participation of the neighbouring nitrogen in displacing the hydroxyl group prior to intramolecular cyclization). Interestingly, when we carried out the sodium borohydride reduction of **58** using the conditions reported by Speckamp et al.,[46] the expected hydroxylactam **59** formed (by regioselective reduction) as an inisolable intermediate furnishing directly the *N*-homopiperonylisoquinolone **48** via an in situ dehydration (confirmed by the absence of a signal corresponding to an α-methylene proton and the appearance of two doublets at δ 6.39 & 6.81 corresponding to the olefinic protons in the ^{1}H NMR spectrum of **48**). The formation of **48** constitutes a formal synthesis of Gusanlung D [(±)-**35**] since **48** is a known precursor that can be easily transformed to the protoberberine as shown by Padwa (see Padwa's approach above).[39] Since we were very much interested in comparing and verifying the spectral and analytical data of the synthesized protoberberine with those reported earlier and the data reported for the isolated natural product, we employed Padwa's conditions to convert the enamide **48** to Gusanlung D [(±)-**35**] in 78% yield.



Scheme 10 *Reagents, conditions and yields*: (i) Nitromethane, AcOH, NH_4OAc, 6 h, reflux (95%); (ii) $LiAlH_4$, THF, reflux, 6 h (76%); (iii) Ether-THF (4:1), rt, 12 h (92%); (iv) Ac_2O, AcONa, 60 °C, 3 h (76%); (v) HMDS, benzene, $ZnCl_2$, 2 h (90%); (vi) (a) $NaBH_4$, EtOH, 0 °C, 5 h, (b) H^+/HCl (72%); (vii) Conc. HCl, rt, 48 h (78%).

However, the analytical and spectral data for the compound synthesized by us did not match with those reported for the isolated natural product but were in full agreement with those reported[37] by Chrzanowska et al (see Table 1). Chrzanowska et al. had opined that the discrepancy could have arisen due to a possible contamination of the isolated natural product with a considerable amount of the dehydrogusanlung D (**53**)[43] during isolation. Therefore, we planned for a synthesis of dehydrolactam **53** via a route that had the potential to deliver Gusanlung D [(±)-**35**] as well. Towards this end, a very selective iodination of the imide **58** using iodine-silvertrifluoro acetate afforded the *N*-(*o*-iodohomopiperonyl) homophthalimide **60** in 86% yield (Scheme 11). As expected, the $NaBH_4$ reduction of **60** using Speckamp conditions as before, furnished the *N*-(*o*-iodohomopiperonyl) isoquinolone **61** in 78% yield. This compound **61** could potentially serve as a precursor to both gusanlung D [(±)-**35**] through an intramolecular radical cyclization as well as dehydrogusanlung D (**53**) via an intramolecular Heck coupling reaction.[47,48] Unfortunately our attempts at intramolecular radical cyclization of **61** using the standard conditions (AIBN-Bu_3SnH) failed to deliver the protoberberine (±)-**35** [we ended up instead with the reduced (dehalogenated) product **48** in 64% yield]. We were, nevertheless, successful in carrying out a $Pd(OAc)_2$-TMG induced[49] intramolecular Heck coupling of iodoisoquinolone **61** and obtained the desired dehydrogusanlung D (**53**) in 72% yield. The analytical and spectral data for **53** were in complete agreement with the reported data.[43] [See pages 184-186 for ^1H & ^{13}C NMR spectra of (±)-**35** and **53**].



Scheme 11 *Reagents, conditions and yields*: (i) I_2, CF_3COOAg, $CHCl_3$, rt, 8 h (86%); (ii) (a) $NaBH_4$, EtOH, 0 °C, 7 h, (b) H^+/HCl, rt, 12 h (78%); (iii) $Pd(OAc)_2$, TMG, AcONa, DMF, 110 °C, 20 h (72%); (iv) AIBN, Bu_3SnH, benzene, reflux, 4 h (64%).

However, a comparison of the analytical and spectral data of **53** with those of gusanlung D [(±)-**35**] and those reported for the isolated for the natural product did not lead to any constructive explanation for the possibility that the isolated alkaloid could have been contaminated with a considerable amount of **53**. Therefore, ambiguity still exists concerning the actual structure of the natural product and is an interesting problem that remains to be clarified. In our opinion, there is a possibility that the isolated natural product could have an alternate structure as shown below in Figure 4. Herein, one can note that ring D is actually oxygenated contrary to the claims that gusanlung D is the first naturally occurring protoberberine alkaloid unoxygenated at ring D. Moreover, a δ value of 7.35 in the ^1H NMR data of isolated gusanlung D (see Table 1) suggests a *peri*-interaction between the D-ring aromatic proton (at C-9) and the carbonyl group. We are currently working towards the synthesis of this compound so as to verify our hypothesis.

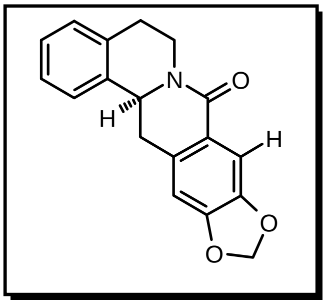


Figure 4 Possible alternate structure for the isolated natural product gusanlung D

Table 1 Comparison of the ^{1}H NMR spectral data for isolated, Chrzanowska et al. and present dissertation gusanlung D.

Proton No.[a]	**^{1}H NMR Spectral Data**[b] **δ value (Splitting, J value in Hz)**		
	Gusanlung D (Isolated)[36]	**Gusanlung D (Chrzanowska et al.)**[37]	**Gusanlung D (Present Dissertation)**
1	7.35 (s)	6.71 (s)	6.72 (s)
4	6.80 (s)	6.67 (s)	6.67 (s)
5	2.70 – 3.40 (m)	2.70 – 2.80 (m) 2.82 – 3.02 (m)	2.70 – 2.80 (m) 2.85 – 3.05 (m)
6	2.70 – 3.40 (m) 4.80 (m)	2.82 – 3.02 (m) 4.93 – 4.99 (m)	2.85 – 3.05 (m) 4.90 – 5.00 (m)
9	8.07 (d, 8.0)	8.13 (d, 7.4)	8.13 (d, 8)
10	7.29 – 7.41 (m)	7.34 – 7.40 (m)	7.39 (t, 8)
11	7.29 – 7.41 (m)	7.41 – 7.49 (m)	7.46 (t, 8)
12	7.29 – 7.41 (m)	7.24 (d, 7.4)	7.24 (d, 8)
13	2.70 – 3.40 (m)	2.82 – 3.02 (m) 3.18 (dd, 15.6, 3.7)	2.85 – 3.05 (m) 3.18 (dd, 16, 4)
13a	3.95 (m)	4.83 (dd, 13.3, 3.7)	4.83 (dd, 13, 4)
OCH_2O	6.06, 6.20 (2s)	5.96 (s)	5.96 (s)

[a] Proton No. indicates the proton present at the corresponding carbon whose numbering pattern is same as shown in Figure 2.

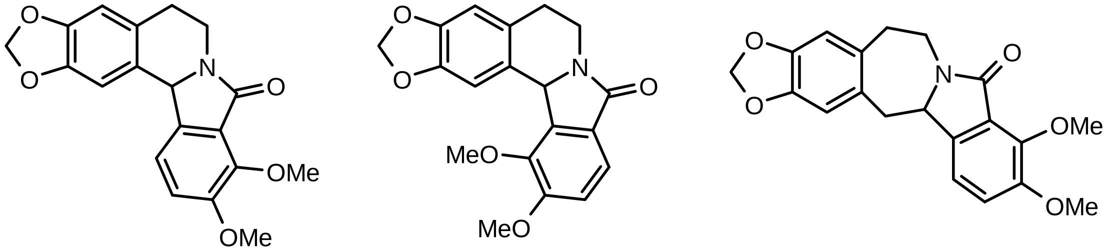
[b] ^{1}H NMR spectra recorded for the isolated gusanlung D and by Chrzanowska et al. were in $CDCl_3$ on a 300 MHz instrument, whereas ^{1}H NMR spectra recorded by us was in $CDCl_3$ on a 500 MHz instrument.

In summary, we have completed a simple formal synthesis of the claimed protoberberine alkaloid gusanlung D [(±)-**35**] starting from HPA in four steps and 47% overall yield. We have also designed an access to dehydrogusanlung D (**53**) employing an elegant intramolecular Heck cyclization as the key reaction in five steps and 40% overall yield. We feel that strategy is quite general in nature and would be useful for the generation of several natural and unnatural protoberberines. We wish to apply the same protocol for the synthesis of the alternate structure of the natural product shown in Figure 4 from appropriate starting materials in order to compare its spectral and analytical data with those of the isolated natural product and possibly establish unambiguously the correct structure of gusanlung D.

2B.3 Section B: III. Studies towards the Total Synthesis of Nuevamine

2B.3.1 Background

Members of the plant family *Berberidaceae* are known to produce an array of unusual isoquinoline alkaloids. Their study is facilitated by the fact that relatively large amounts of these plants can be collected. This therefore makes conceivable the study of minor alkaloids present, that is, those available only in small amounts, which often afford an insight into the catabolic pathways for the principal alkaloids. In a noteworthy example, Valencia et al. isolated two new alkaloids, nuevamine (**62**) and lennoxamine (**63**), as racemates from *Berberis darwinii* Hook, gathered in southern Chile, in the vicinity of Cuidad Osorno (Figure 5).[50]



Erroneously assigned structure of nuevamine

Nuevamine (**62**)

Lennoxamine (**63**)

Figure 5

Nuevamine (**62**) occupies a very special place since it is the first isoindolo[1,2-*a*]isoquinolinone alkaloid known to occur in nature and can still be considered the lone representative of this class of isoquinoline alkaloids. Initially, the structure of this natural product was erroneously assigned but later revision led unambiguously to structure **62**.[51] Isoindolo[1,2-*a*]isoquinolinones are an interesting class of tetracyclic lactams due to the already known and potential biological activities of many of their derivatives.[52] It was least surprising therefore that nuevamine (**62**), being an eminent member of the isoindoloisoquinolone alkaloid family, attracted the synthetic organic chemistry fraternity and several synthetic approaches were designed towards this class of tetracyclic lactams. However, most of the methods developed were found inadequate for the synthesis of the natural product. The many synthetic approaches known towards building up the above skeleton, followed by two important strategies which led to the completion of the total synthesis of nuevamine, are discussed briefly in the following part.

2B.3.1.1 Synthetic Approaches towards Isoindolo[1,2-a]isoquinolinones

The synthetic approaches towards isoindoloisoquinolinones **64** can be broadly classified into two categories, which differ in the nature of the carbon-carbon formed in the last step (Figure 6 shows retrosynthetically the two different paths A & B adopted for the construction of this skeleton). Thus, generation of the carbon-carbon bond (a) (Figure 6, Path A) has been achieved by an intramolecular amido arylation reaction involving a transient *N*-acyliminium species obtained by treatment of α-hydroxy (X = OH)[53] and α-alkoxy (X = OMe)[54] lactamic precursors **65** with a wide variety of Lewis acids (construction of ring C). An original structural modification recently developed by Katritzky led to the generation of the *N*-acyliminium cation by loss of a benzotriazolyl anion upon treatment of **65** (X = benzotriazolyl) with $TiCl_4$.[55] An alternative method for the formation of carbon-carbon bond (a) has been designed by Sotomayor & co-workers[56] involving an anionic cyclization mechanism (Parham cyclization) applied to *N*-[(iodoaryl)ethyl]-imides **67**. Reduction of the cyclization product **68** provided a nuevamine-type compound **69** in excellent yield (Scheme 12). This skillful approach also allowed intermolecular α-amidoalkylation of **68**, giving access to a wide variety of C-12b substituted nuevamine-type alkaloids.

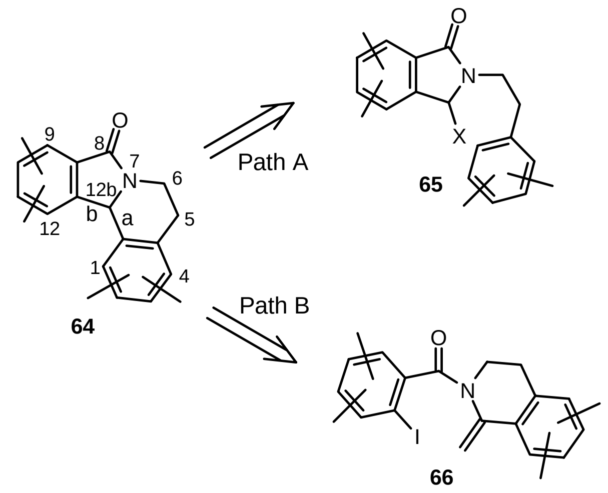
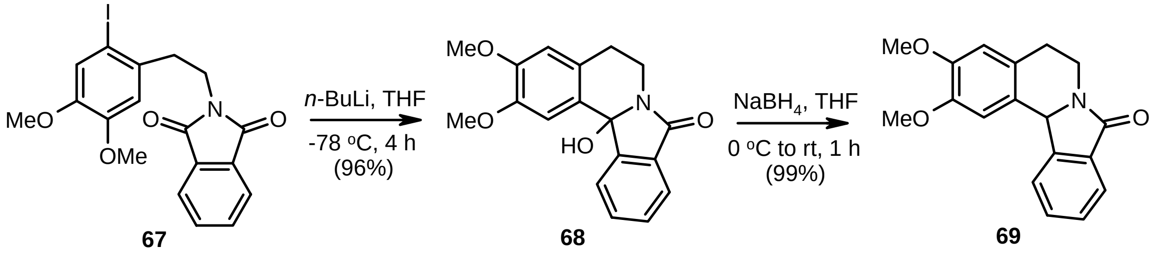


Figure 6 Retrosynthetic disconnections of the (a) and (b) carbon-carbon bonds of the isoindoloisoquinolinone framework.



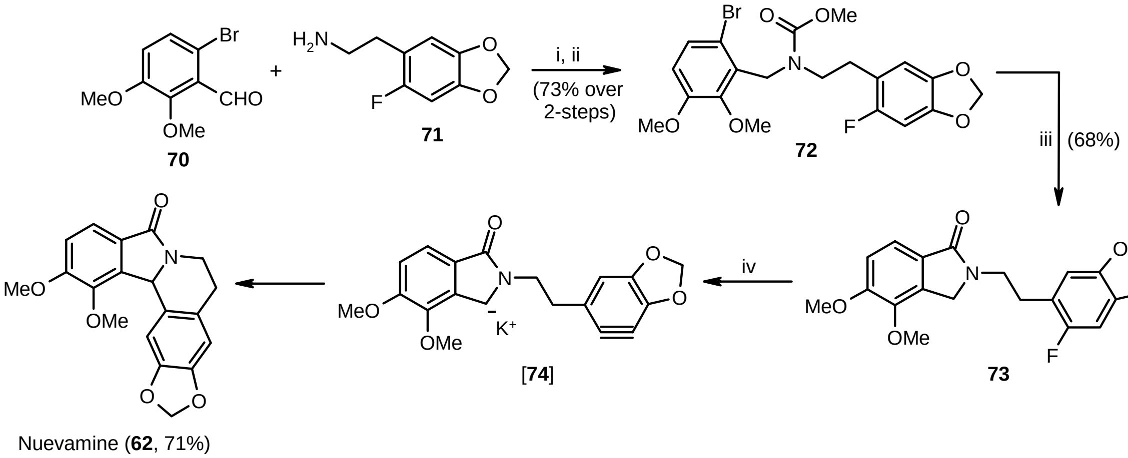
Scheme 12

The isoindolinone nucleus has also been accessed by formation of carbon-carbon bond (b) (Figure 6, Path B) through intramolecular Heck cyclization[57] of aromatic enamides **66** performed in the presence of a hydride source which favored the regiocontrolled formation of the five-membered product (construction of ring B). Other procedures involving the cyclization of β-phenylethylaminophthalide[58] or a combination of the Pictet-Spangler reaction with palladium-catalyzed carbonylation applied to (2-iodobenzylidene)-phenethylamine derivatives[59] have also been used occasionally for the assembling of the isoindoloisoquinolinone framework.

However, although the above methods were extremely useful for assembling the isoindoloisoquinolone framework, they fell short on different accounts when it came to the synthesis of the unique natural product nuevamine (**62**). Thus, the α-substituted lactamic precursors **65** are generally obtained from the corresponding imides (X = O) but the lack of regioselectivity of this chemical transformation requires the absolute necessity of having bare models or symmetrically substituted isoindolinone parent compounds.[53-55] The Parham cyclization process is also fraught with difficulties associated with the regioselectivity of the aromatic metallation/cyclization sequence applied to unsymmetrically substituted imides **67**.[56] Finally, all models structurally related to **62** which have been elaborated by intramolecular Heck cyclizations are inevitably alkylated at the C-12b position.[57] Consequently, all these structural requirements and synthetic limitations preclude the synthesis of the poly-, differentially and unsymmetrically substituted alkaloid nuevamine (**62**), so that the elegant synthetic method reported by Castedo et al.,[51] which revised & confirmed the structure of the natural product and the two elegant strategies recently developed by Couture & co-workers[60,61] are the sole total syntheses to date of this natural product. Couture's syntheses are discussed briefly below preceding a detailed description of our efforts towards the synthesis of this novel alkaloid.

[A] *Parham procedure/Aryne-mediated cyclization*[60]

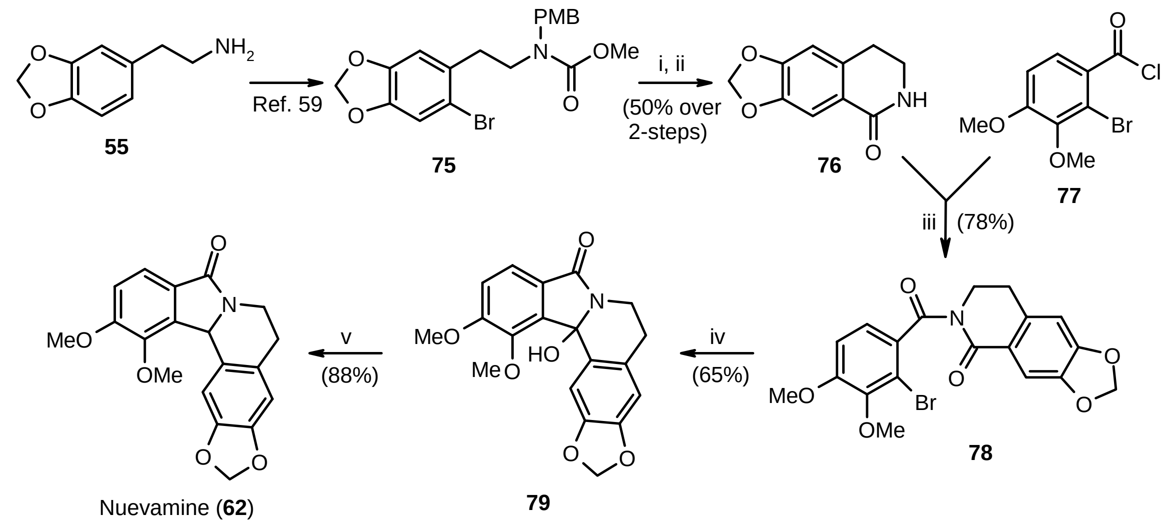
In the first total synthesis of nuevamine from their group, Couture et al. identified a novel and flexible approach, wherein, the isoindolinone template was assembled in only two key synthetic steps from easily available precursors. The two steps involved a tactical combination of the Parham procedure and an aryne-mediated cyclization. Thus, the key intermediate lactam **73** was obtained by a Parham-type cyclization induced by aromatic lithiation of the halogenodiarylalkylamine **72** (prepared in 2-steps from **70**, Scheme 13). The key strategy was the choice of the halides as metal-halogen exchange occurs preferentially with bromides over fluorides (for the first key step) whereas aryl fluorides are good for *ortho*-metallation and aryne formation (second key step). Thus, the aryl fluoride **73**, on treatment with KHMDS (in conditions as shown in Scheme 13) generated the aryne intermediate **74**; an immediate intramolecular attack of the benzylic carbanion on the aryne took place to deliver the natural product in 71% yield. The total synthesis of nuevamine (**62**) emphasizes the versatility and regiospecificity of this novel approach.



Scheme 13 *Reagents, conditions and yields*: (i) (a) $PhCH_3$, *p*-TSA, reflux, 3 h, (b) $NaBH_4$, MeOH, rt, 1 h (94%); (ii) ClCOOMe, CH_2Cl_2, Et_3N, 0 °C to rt, 3 h (78%); (iii) *t*-BuLi, THF, – 100 °C to rt, 30 min (68%); (iv) KHMDS (2 equiv.), THF, – 78 °C to rt, 2 h (71%).

[B] *Double Parham cyclization*[61]

Couture & co-workers brought out another efficient methodology for the synthesis of the isoindolinone template based on two Parham cyclizations allowing the formation of both the five- and six-membered nitrogenated rings. Thus, sequential application of the Parham protocol with carbamate and diacylamine precursors lead to regiospecific construction of the isoindolo[1,2-*a*]isoquinolinone framework. The authors further illustrated the synthetic potential of this method by completing the total synthesis of nuevamine (**62**) as shown in Scheme 14. Thus the first Parham cyclization was performed on the carbamate **75** (prepared in 3-steps from **55**) providing lactam **76** after deprotection. The second Parham protocol was applied to the diacylamine **78** affording the 12b-hydroxy isoindolo[1,2-*a*]isoquinolinone **79**, which was subsequently reduced using triethylsilylhydride to deliver the natural product nuevamine (**62**).



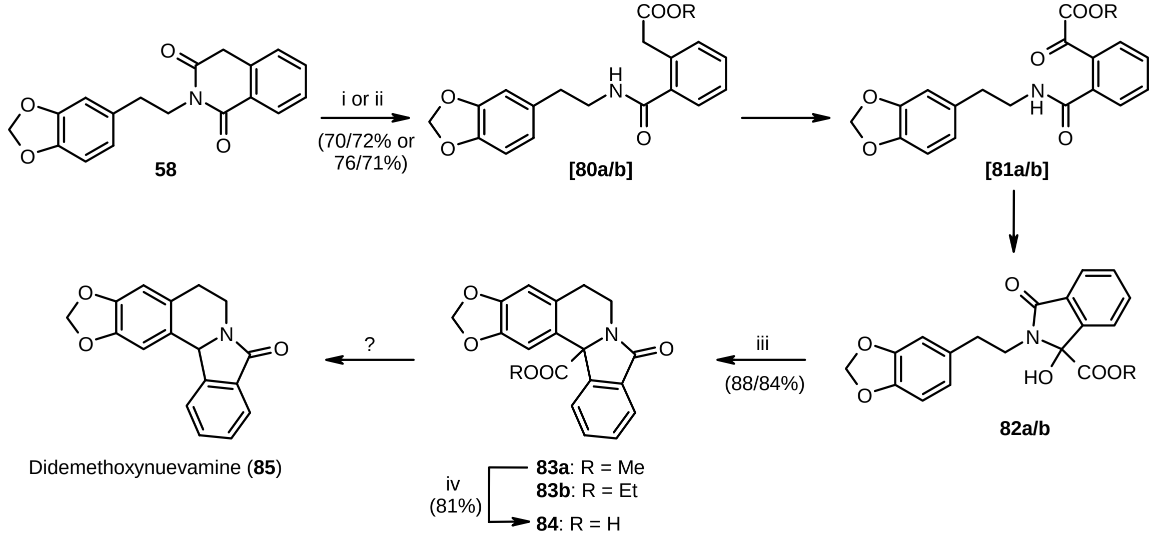
Scheme 14 *Reagents, conditions and yields*: (i) *t*-BuLi, THF, – 100 °C to rt, 30 min (68%); (ii) TFA, anisole, reflux, 24 h (87%); (iii) **76**, THF, *n*-BuLi, – 78 °C to rt, 30 min, then **77** in THF, – 78 °C to rt, 2 h (78%); (iv)) *t*-BuLi, THF, – 100 °C to rt, 30 min (65%); (v) Et_3SiH (2 equiv.), TFA (1 equiv.), CH_2Cl_2, rt, 2 h (88%).

2B.3.2 Rationale for Present Work

A serendipitous benzylic air-oxidation, discussed in more detail subsequently, provided in our hands a compound that could be easily elaborated to the isoindolo[1,2-*a*]isoquinolinone framework. Thus, considering the importance and unique structural features associated with the only such naturally occurring alkaloid nuevamine (**62**), we then focused our attention towards the synthesis of didemethoxynuevamine starting from homophthalic anhydride. Our efforts towards this end are described in the following part.

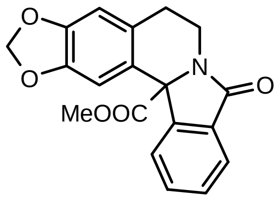
2B.3.3 Results and Discussion

During the course of our initial studies towards the synthesis of the protoberberine alkaloid gusanlung D, a regioselective reduction of the non-conjugated carbonyl group of the homophthalimide was the requisite at the time so as to obtain a hydroxy lactam (subsequent dehydrative intramolecular ring-closure of which would provide gusanlung D, see Section 2B.2.3 of this chapter). Towards this end, homophthalimide **58** was treated with sodium borohydride in methanol at room temperature. Interestingly, in the above reaction, we observed the formation of a product in 70% yield, the ^{1}H NMR spectrum of which revealed, to our surprise, the absence of both the benzylic protons from the homophthalimide moiety of the starting material **58**. A new singlet signal was also observed at δ 3.72 corresponding to 3-protons and moreover, analysis of the ^{13}C (& DEPT) clearly indicated the presence of an additional carbon as well as a new quarternary centre at δ 88.3 (page 187 & 188). Careful scrutiny of all the analytical and spectral data put together pointed to the formation of an isoindole skeleton **82a** with a net oxidative ring contraction (Scheme 15). Since we did not observe any formation of our originally desired reduced product, we presume that a catalytic amount of $NaBH_4$ was converted into sodium methoxide in situ and thus, we propose that a very fast regioselective methanolysis (catalyzed by the sodium methoxide) occurred giving rise to the intermediate ester **80a**. This homophthalamic ester **80a**, proposedly, underwent a facile air-oxidation at the benzylic carbon (α to the ester moiety) to yield another intermediate, α-keto ester **81a**, subsequent intramolecular ring closure of which delivered the observed product, α-hydroxy ester **82a**. Our hypothesis was justified when we carried out the reaction of imide **58** in methanol and ethanol using triethyl amine as the base catalyst and obtained the same α-hydroxy ester skeletons **82a** & **82b** in 76 & 71% yields respectively. However, as expected, *t*-butanol showed a great reluctance for the alcoholysis under the same reaction conditions and we recovered the starting imide **58** even after 24 hours reaction time. This observation helped us in tentatively ruling out the possibility of an air-oxidation of the imide **58** preceding the alcoholysis and ring closure in the formation of **82a/b**.



Scheme 15 *Reagents, conditions and yields*: (i) $NaBH_4$ (3 equiv.), ROH, rt, 20 h (70/72%); (ii) Et_3N (5 equiv.), ROH, rt, 12 h (76/71%); (iii) H_2SO_4 (cat.) /AcOH, rt, 2 h (88/84%); (iv) LiOH, THF-H_2O (4:1), rt, 18 h (81%).

Thus, a serendipitous novel benzylic air-oxidation had provided in our hands a product that could be easily elaborated to the C-12b substituted isoindoloisoquinolinone scaffold. Our aim was achieved when both the compounds **82a** & **82b**, on treatment with catalytic amount of sulfuric acid in acetic acid at room temperature, underwent an expeditious intramolecular dehydrative ring closure to yield the nuevamine skeletons **83a** & **83b** with an angular carbalkoxy function in 88 & 84% yields respectively. The ^{1}H NMR of the methyl ester of **83a** (page 188) revealed the presence of only two aromatic protons (as singlet signals at δ6.61 & δ7.44) correponding to the homopiperonyl part while the DEPT spectrum (page 189) indicated the complete saturation of one aromatic carbon atom, confirming clearly the formation of the desired isoindolo[1,2-*a*]isoquinolinone skeleton. Finally, the structure of **83a** was established unambiguously by single-crystal X-ray crystallographic analysis. The ORTEP diagram lucidly depicts the isoindoloisoquinolinone skeleton bearing the carbmethoxy functionality at the C-12b (denoted as C11B in Figure 7) position. Hydrolysis of the methyl ester **83a** followed by a decarboxylation was then required to provide an access to didemethoxynuevamine **85**. Towards this, **83a** was subjected LiOH-induced hydrolysis to afford the desired acid **84** in 81% yield. We are currently working on a suitable condition for the decarboxylation of the angular acid moiety of **84** so as to obtain didemthoxynuevamine (**85**).



83a

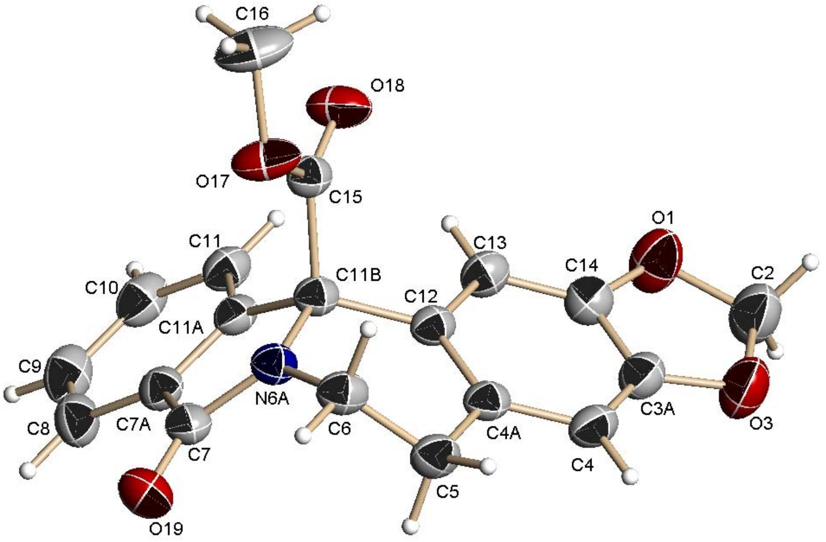
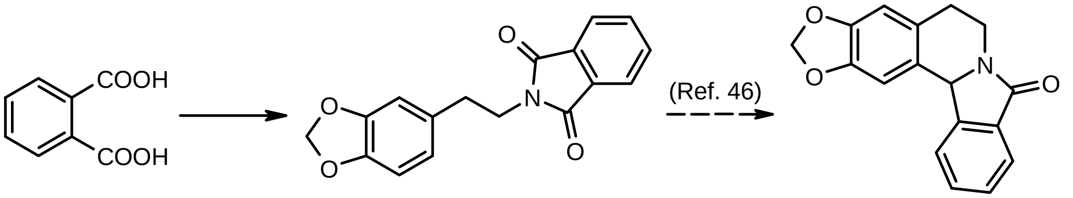


Figure 7 ORTEP diagram of **83a** (please note that the atom numbering pattern here is different from the standard numbering pattern for isoindoloisoquinolinones used in text).

We struck upon another interesting aspect during our studies towards didemethoxynuevamine (**85**) as discussed below. We envisage a much simpler access to this particular compound in only two steps starting from phthalic anhydride/acid as shown in Scheme 16. Thus, subjecting imide **86**, prepared from phthalic acid (PA), to the Speckamp conditions (seen earlier in Section 2B.3.1.1) could potentially furnish the didemethoxynuevamine in one pot.[46] However, as in the case of Sotomayor's methodology (see synthetic approaches to isoindolo[1,2-*a*]isoquinolinones above), this 2-step strategy would also be fraught with difficulties related to regiochemical control when it comes to the synthesis of the actual unique natural product nuevamine (**62**).



PA

86

Didemethoxynuevamine (**85**)

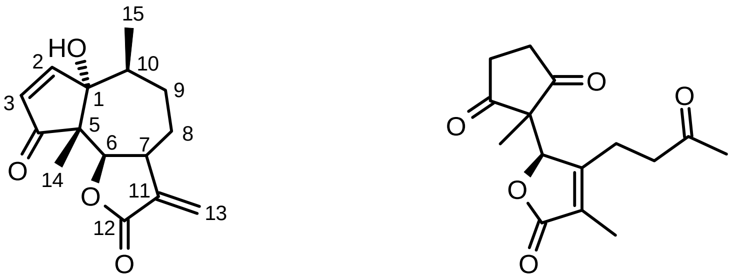
Scheme 16

In this context, the benzylic air-oxidation method is highly noteworthy, since it would exercise the desired regiocontrol in the synthetic route towards the total synthesis of the only naturally occurring isoindolo[1,2-*a*]isoquinolinone alkaloid. In summary, the present serendipitous facile air-oxidation helped us design a concise and efficient access to the nuevamine alkaloid skeleton. The corresponding methy ester of phenyl acetic acid and dimethyl homophthalate did not undergo any such oxidation which makes us speculate that an *N*-oxide could be involved in the mechanism leading to the formation of the observed product. Application of this protocol to synthesize the actual natural product nuevamine and further studies on the detailed mechanistic aspects of the air-oxidation are in progress. We are of the opinion that this strategy would also be useful to exclusively design several congeners for structure activity relationship studies.

2B.4 Section B: IV. Studies towards the Total Synthesis of Charminarone

2B.4.1 Background

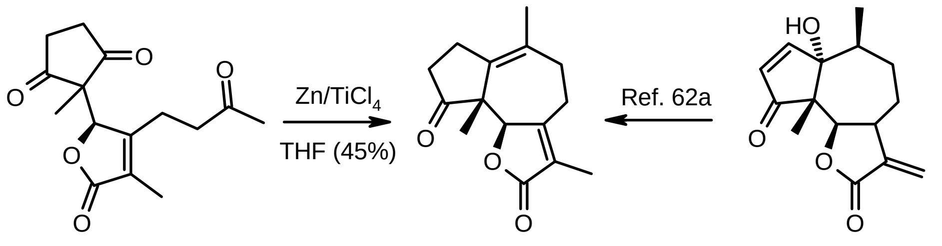
Parthenium hysterophorus Linn (Compositae) is an obnoxious weed whose extract is used as folk remedy against skin diseases, ulcerated sores, facial neuralgia, fever and anemia. Its allelopathic effects on plants have also been reported. The plant is a rich source of chemically complex sesquiterpenoids such as parthenin, hysterin, coronopilin, etc,[62a-h] parthenin (**87**) being the major constituent. Parthenin, isolated by Herz et al. in 1962[62a] exhibits significant medicinal activity including anticancer property.[63a,b] Das & co-workers, during their investigation on the plant's constituents, recently isolated[64] a novel sesquiterpenoid, charminarone (**88**) which is the first *seco*-pseudoguaianolide reported from nature (Figure 8).



Parthenin (**87**) Charminarone (**88**)

Figure 8

The structure of charminarone (**88**) was established from its ^{1}H NMR spectrum, which was directly compared to that of parthenin (**87**), as 1,10-*seco*-dihydroisoparthenin-1,10-dione. The authors confirmed the structure as well as the stereochemistry of **88** from its transformation by a reductive coupling reaction using $Zn/TiCl_4$ (Scheme 17) to the known compound anhydrodihydroisoparthenin (**89**). The compound **89** had been previously synthesized from parthenin (**87**) via a regioselective carbon-carbon double bond reduction (accompanied by the isomerization of the other carbon-carbon double bond) and subsequent dehydration,[62a] thus establishing a chemical co-relation between the latter and charminarone (**88**). To our knowledge, no synthesis of this structurally intriguing natural product charminarone (**88**) has been reported to date in the literature.



Charminarone (**88**) **89** Parthenin (**87**)

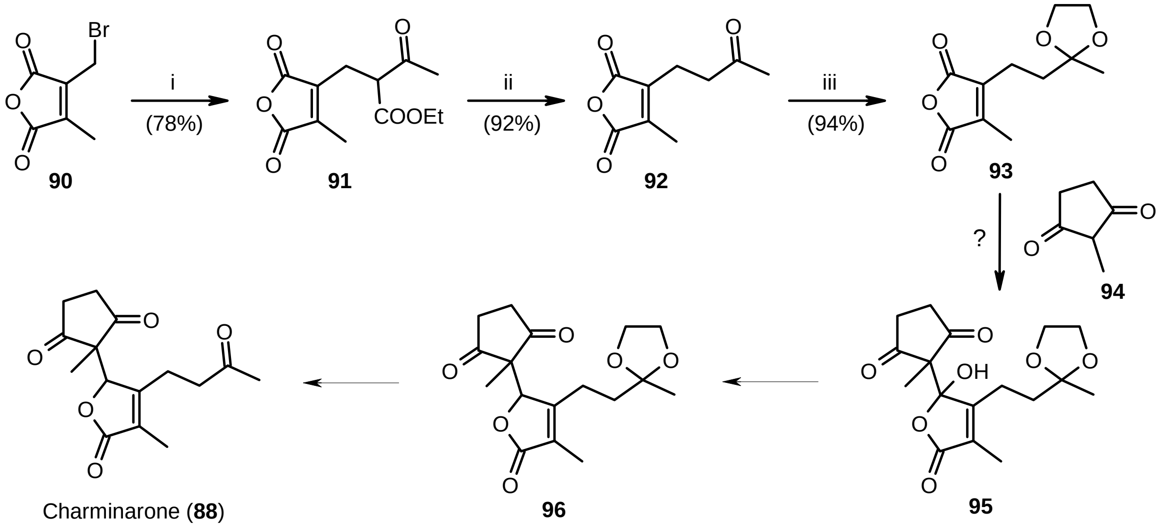
Scheme 17

2B.4.2 Rationale for Present Work

The unique structural features of charminarone (**88**) and the impact that the sesquiterpenoids isolated from the parent plant species have had in the field of biology and medicine attracted our attention. Our interest in employing cyclic anhydrides for the total synthesis of recently isolated bioactive natural products has already been amply demonstrated. Thus, in keeping with the trend, we launched a systematic plan towards the first total synthesis of this unique natural product. Our ongoing efforts towards this goal have been detailed in the following part.

2B.4.3 Results and Discussion

We envisaged a nucleophilic addition of the commercially available 2-methyl-1,3-cyclopentanedione to a maleic anhydride fragment decorated with the desired substituents as the key (and penultimate) step in the total synthesis of the sesquiterpene **88**. Thus, towards the synthesis of the desired fragment for the key step, (bromomethyl)methylmaleic anhydride (**90**, obtained[6d] by NBS bromination of dimethylmaleic anhydride) was reacted with ethyl acetoacetate in THF using sodium hydride as the base to provide the β-keto ester **91** in 78% yield. The β-keto ester **91** upon treatment with concentrated hydrochloric acid under refluxing conditions underwent a hydrolysis followed by an in situ decarboxylation to yield the ketone **92** in 92% yield. Protection of the ketone carbonyl of **92** as the 1,3-dioxolane using ethylene glycol and *p*-TSA proceeded smoothly to afford (in 94% yield) the required precursor **93** for the key nucleophilic addition reaction (Scheme 18). We are in search of a suitable reaction condition to bring about the regioselective addition of 2-methyl-1,3-cyclopentanedione (**94**) to the desired carbonyl of anhydride **93** to afford the required charminarone precursor **95**, which we presume would subsequently deliver the natural product upon reductive deoxygenation and subsequent deprotection. A successful synthesis would also pave the way for a possible enzymatic resolution of the racemic lactone **88**, which would give an access to the enantiomerically pure natural product.



Scheme 18 *Reagents, conditions and yields*: (i) Ethylacetoacetate, NaH, THF, rt, 8 h (78%); (ii) HCl (conc.) + H_2O (1:1), 12 h (92%); (iii) Ethylene glycol, *p*-TSA (cat.), benzene, reflux, 10 h (94%).

In summary, we have designed the synthesis of a suitably decorated maleic anhydride scaffold **93** in three steps and 67% overall yield. In our opinion, **93** has the potential for providing the first and simple access to the unique sesquiterpene charminarone (**88**) via a key regioselective addition reaction. We also surmise that the synthetic route can be further extrapolated to the other natural product parthenin (**87**) via charminarone (**88**).

2B.5 Conclusions and Perspectives

In the present chapter, we have described our studies towards the total synthesis of some significant natural products based on cyclic anhydride precursors. We have presented a facile synthesis of four new secondary metabolites, aspergillus acids A-D employing the condensation of maleimide-TPP adduct with aldehydes, a strategy developed in our laboratory and used for the total synthesis of several bioactive natural products. Herein, an elegant means of remote functionalization and a chemoselective sodium borohydride reduction have also been demonstrated to complete the synthesis of the four natural products in three to six steps with 20-65% overall yields. Also noteworthy in the present synthesis is the efficient lipase-catalyzed resolution that delivered aspergillus acids C & D in their enantiomerically enriched forms.

We have also utilized homophthalic anhydride, hitherto unexplored by our group, during our studies towards the synthesis of two naturally occurring alkaloids. The first study illustrates the completion of a formal synthesis of the proposed structure of the protoberberine alkaloid gusanlung D in four steps with 47% overall yield. A successful synthesis of dehydrogusanlung D, in five steps and 40% overall yield, has also been portrayed employing an elegant intramolecular Heck reaction for bringing about the key cyclization. In the present synthetic route, we had also aimed for a possible lipase-catalyzed resolution that had the potential to deliver both the enantiomers of the natural product, but unfortunately, we were unable to isolate the requisite intermediate alcohol. Discrepancies in the analytical & spectral data of the isolated and synthetic versions of this novel protoberberine have instigated a need for revision of its structure; we are currently working on this problem towards establishing unambiguously the correct structure of gusanlung D.

Another significant contribution in the synthesis of naturally occurring alkaloids resulted from a serendipitous benzylic air-oxidation. We have designed, aided by this novel air-oxidation, an access to the nuevamine alkaloid (which is the only naturally occurring isoindolo[1,2-*a*]isoquinolinone alkaloid till date) framework. This protocol, in our opinion, would be instrumental in providing the necessary regiochemical control that proved elusive in some of the earlier attempts towards the synthesis of the unique natural product nuevamine. Application of the present strategy for the total synthesis of the actual natural product is in progress currently in our laboratory.

A three step synthesis (with an overall yield of 67%) of a suitably decorated maleic anhydride scaffold was also accomplished towards the synthesis of the first *seco*-pseudoguaianolide reported in nature, charminarone. This disubstituted maleic anhydride fragment could potentially be elaborated to the above recently isolated natural product through a key regioselective nucleophilic addition.

A large number of cyclic anhydrides are known in the literature and we in our group have been successful in using a few of them for designing concise synthesis of several bioactive natural (30 plus) and unnatural (25 plus) products using a variety of new synthetic strategies, without much of protection-deprotection chemistry. Our group has also brought out some useful methodologies in maleic anhydride chemistry that could be influential in the synthesis of complex maleic anhydride based natural products such as byssochlamic acid. In the present chapter too, cyclic anhydrides have presented us an opening for carrying out studies towards the synthesis of some important (related to potential biological activity) anhydride based natural products as well as naturally occurring alkaloids (possessing intriguing structural features) based on cyclic anhydrides as starting materials. Both substituted maleic anhydrides and homophthalic anhydride are highly useful holding different advantages; the utilities of the former need not be reiterated while the latter, with its structure, has the power to exercise regiochemical control, which if explored further, could lead to the construction of more complex systems. The studies also provided us a nice opportunity for learning a lot of new chemistry not just from our work but also from the vast literature in this field. We also feel that approaches that we have developed are quite general in nature and would be useful in designing several analogs of the natural products for structure activity relationship studies.

A look at the literature reveals that the five- and six-membered cyclic anhydrides are the more common and widely used ones whereas few four- and seven-membered cyclic anhydrides are known. The design of macrocyclic anhydrides, in this context, could open up a new field for synthetic organic chemists to explore.

Thus, overall, in the present dissertation, we have seen the utility of lipases for the enantiopure synthesis of important chiral compounds and also as reagents in organic *synthesis. In the synthesis of α-hydroxycyclopentanone seen in the present dissertation, enzymes are the real reagents of choice as chemical approaches do not provide satisfactory results. This result, coupled with their use in the preparation of alkyl maleanilates, lends further substantiation to the potential of enzymes as reagents in organic synthesis. We have also seen the use of cyclic anhydrides in the construction of natural products and natural product skeletons. Both the above disciplines have enjoyed a glorious past and hold a great deal of significance in the present day world of chemistry (& medicine). It can be said with assurance that both these interesting disciplines will spread their wings wider over the field of organic and pharmaceutical chemistry in the future. In addition, in our opinion, a combination of the two, that is cyclic anhydrides and enzymes as reagents, could serve as a launching pad for the generation of several useful enantiopure heterocyclic scaffolds in the years to come.*

2B.6 Experimental Section

Stereochemical assignments are based on the optical rotation of the known compounds. Commercially available 1,14-tetradecanediol, oxalyl chloride, PPh_3, dihydropyran, Ac_2O, sodium acetylide, DCC, (*R*)-Mosher's acid, piperonal, homophthalic anhydride, nitromethane, HMDS, silvertrifluoro acetate, $Pd(OAc)_2$, TMG, Bu_3SnH, LiOH, ethyl acetoacetate and ethylene glycol were used. Amano PS-800 U from Amano Pharmaceuticals, Japan was used. The activity of the lipase powder used is expressed in terms of units, 1 unit corresponding to micromoles of butyric acid liberated (estimation by GC) from glyceryl tributyrate per minute per milligram of enzyme powder.[65]

17-Acetoxyheptadecan-1-ol (3). Acetic anhydride (0.92 mL, 9.80 mmol) was added to a solution of 1,17-heptadecanediol (**2**)[66] (2.72 g, 10 mmol) in anhydrous pyridine (10 mL) with stirring and cooling in an ice bath. The reaction mixture was allowed to warm to room temperature and stirred for a further 6 h and then it was poured into ice-water (30 mL) and extracted with ethyl acetate (30 mL × 3). The combined organic layer was washed with 5% aq. $CuSO_4$ solution, water & brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether/ethyl acetate mixture (8:2) as an eluent gave **3** as a white solid (2.48 g, 79% yield).

$H_3CCOOCH_2(CH_2)_{15}CH_2OH$ **3** **$C_{19}H_{38}O_3$ (314)**	**Mp** 60-61 ºC. **IR** ($CHCl_3$) ν_{max} 3447, 1726 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.26 (bs, 26H), 1.50-1.70 (m, 5H), 2.05(s, 3H), 3.64 (t, *J* = 6 Hz, 2H), 4.05 (t, *J* = 6 Hz, 2H).

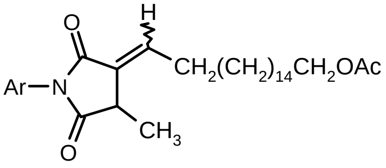
17-Acetoxyheptadecan-1-aldehyde (4). A solution of oxalyl chloride (0.88 mL, 10.00 mmol) in dry CH_2Cl_2 (5 mL) was placed in a two-necked RB flask and kept under argon at – 60 ºC. A solution of DMSO (1.42 mL, 20 mmol) in dry CH_2Cl_2 (5 mL) was added to it dropwise over a period of 5 min. Alcohol **3** (1.57 g, 5 mmol) in dry CH_2Cl_2 (5 mL) was charged dropwise over a period of 5 min and stirred at – 60 ºC for 90 min. Triethylamine (4.17 mL, 30 mmol) was added and the reaction mixture was allowed to warm to room temperature and stirred for a further 45 min. The reaction was quenched with water (20 mL) and the aqueous layer was extracted with CH_2Cl_2 (20 mL × 3). The combined organic layer was washed with water & brine and dried over Na_2SO_4. Concentration of organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether/ethyl acetate mixture (9:1) as an eluent afforded **4** (1.39 g, 89% yield).

$H_3CCOOCH_2(CH_2)_{14}CH_2CHO$ **4** **$C_{19}H_{36}O_3$ (312)**	Thick oil. **IR** ($CHCl_3$) ν_{max} 2716, 1740, 1729 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.28 (bs, 24H), 1.50-1.75 (m, 4H), 2.05 (s, 3H), 2.43 (dt, J = 8 & 2 Hz, 2H), 4.05 (t, J = 6 Hz, 2H), 9.77 (s, 1H).

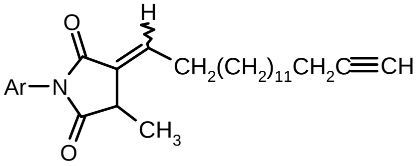
Hexadec-15-yne-1-aldehyde (15). It was prepared from **14** (1.50 g, 6.30 mmol) using the same procedure as described for **4** above (1.26 g, 85% yield).

$HC{\equiv}CCH_2(CH_2)_{11}CH_2CHO$ **15** **$C_{16}H_{28}O$ (236)**	Thick oil. **IR** (Neat) ν_{max} 3310, 2718, 2116, 1726 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.28 (bs, 20H), 1.45-1.80 (m, 2H), 1.94 (bs, 1H), 2.19 (t, J = 7 Hz, 2H), 2.43 (t, J = 7 Hz, 2H), 9.77 (s, 1H).

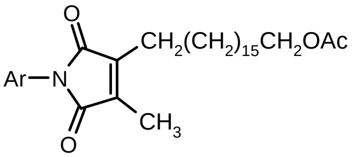
(±)-2-Heptadec-(17-acetoxy)ylidene-3-methyl-*N*-(*p*-tolyl)succinimide (7). A mixture of citraconimide **5** (603 mg, 3 mmol) and TPP (0.95 g, 3.60 mmol) in glacial acetic acid (15 mL) was stirred at room temperature for 1 h. Aldehyde **4** (1.31 g, 4.20 mmol) in glacial acetic acid (7 mL) was added to the reaction mixture and refluxed with stirring for 18 h. Acetic acid was distilled off in vacuo at 50 ^{o}C and the residue was dissolved in ethyl acetate (30 mL). The organic layer was washed successively with water, aq. $NaHCO_3$ & brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue (eluent: petroleum ether/ethyl acetate mixture, 9:1) gave **7** as a yellow solid (1.04 g, 70% yield).

 **7** (Ar = *p*-tolyl) **$C_{31}H_{47}NO_4$ (497)**	**Mp** 60-61 °C. **IR** ($CHCl_3$) ν_{max} 1771, 1713, 1672 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.26 (bs, 24H), 1.52 (d, *J* = 8 Hz, 3H), 1.50-1.70 (m, 4H), 2.05 (s, 3H), 2.20-2.40 (m, 2H), 2.38 (s, 3H), 3.46 (q, *J* = 8 Hz, 1H), 4.05 (t, *J* = 6 Hz, 2H), 6.25 (dt, *J* = 8 & 2 Hz, 0.1H), 6.92 (dt, *J* = 8 & 2 Hz, 0.9H), 7.15-7.30 (m, 4H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 16.4, 20.9, 21.1, 25.8, 28.5, 29.1, 29.4 - 29.5 (12 × CH_2), 37.4, 64.5, 126.1, 129.3, 129.6, 130.5, 138.3, 140.5, 168.8, 171.1, 177.3; The proton bearing olefinic carbon from the corresponding (*Z*)-isomer appeared at δ 145.0. **Anal. Calcd** for $C_{31}H_{47}NO_4$: C, 74.81; H, 9.52; N, 2.81. Found: C, 74.93; H, 9.33; N, 2.86.

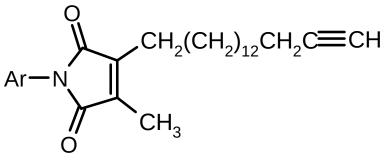
(±)-2-Hexadec-(15-yne)ylidene-3-methyl-*N*-(*p*-tolyl)succinimide (16). It was prepared from **5** (603 mg, 3 mmol) using the same procedure as described for **7** above (985 mg, 78% yield).

 **16** (Ar = *p*-tolyl) **$C_{28}H_{39}NO_2$ (421)**	Thick oil. **IR** ($CHCl_3$) ν_{max} 3306, 2116, 1771, 1713, 1672 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.29 (bs, 18H), 1.45-1.65 (m, 4H), 1.52 (d, *J* = 6 Hz, 3H), 1.94 (t, *J* = 2 Hz, 1H), 2.18 (dt, *J* = 7 & 2 Hz, 2H), 2.25-2.35 (m, 2H), 2.38 (s, 3H), 3.45 (q, *J* = 6 Hz, 1H), 6.23 (dt, *J* = 8 & 2 Hz, 0.15H), 6.91 (dt, *J* = 8 & 2 Hz, 0.85H), 7.10-7.30 (m, 4H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 16.4, 18.3, 21.1, 28.4 - 29.4 (12 × CH_2), 37.5, 68.0, 84.7, 126.2, 129.3, 129.6, 130.5, 138.4, 140.5, 168.8, 177.3; The proton bearing olefinic carbon from the corresponding (*Z*)-isomer appeared at δ 144.4. **Anal. Calcd** for $C_{28}H_{39}NO_2$: C, 79.76; H, 9.32; N, 3.32. Found: C, 79.81; H, 9.36; N, 3.27.

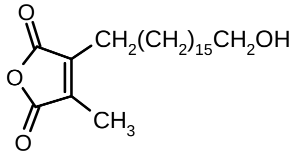
2-Heptadec-(17-acetoxy)yl-3-methyl-*N*-(*p*-tolyl)maleimide (8). A solution of **7** (994 mg, 2 mmol) in THF (3 mL) and triethylamine (3 mL) was refluxed for 48 h with stirring. The reaction mixture was then allowed to reach room temperature and concentrated in vacuo. Silica gel column chromatographic purification of the residue (eluent: petroleum ether/ethyl acetate mixture, 9:1) gave **8** as a white solid (915 mg, 92% yield).

 **8** (Ar = *p*-tolyl) **$C_{31}H_{47}NO_4$ (497)**	**Mp** 70-71 ºC. **IR** ($CHCl_3$) ν_{max} 1736, 1707 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.26 (bs, 26H), 1.50-1.70 (m, 4H), 2.05 (s, 6H), 2.37 (s, 3H), 2.46 (t, *J* = 6 Hz, 2H), 4.05 (t, *J* = 6 Hz, 2H), 7.15-7.30 (m, 4H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 8.7, 20.8, 20.9, 23.7, 25.8, 28.1, 28.5, 29.1, 29.2, 29.4, 29.5 (9 × CH_2), 64.5, 125.5, 129.3, 129.4, 137.0, 137.1, 141.2, 170.7, 170.9, 171.0. **Anal. Calcd** for $C_{31}H_{47}NO_4$: C, 74.81; H, 9.52; N, 2.81. Found: C, 74.65; H, 9.41; N, 2.92.

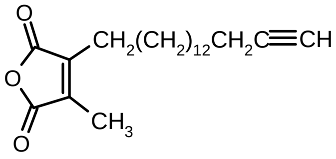
2-Hexadec-(15-yne)yl-3-methyl-*N*-(*p*-tolyl)maleimide (17). It was prepared from **16** (945 mg, 2.25 mmol) using the same procedure as described for **8** above (880 mg, 93% yield).

 **17** (Ar = *p*-tolyl) **$C_{28}H_{39}NO_2$ (421)**	**Mp** 71-72 ºC. **IR** ($CHCl_3$) ν_{max} 3308, 2116, 1709 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.27 (bs, 20H), 1.40-1.70 (m, 4H), 1.94 (t, *J* = 2 Hz, 1H), 2.05 (s, 3H), 2.18 (dt, *J* = 7 & 2 Hz, 2H), 2.37 (s, 3H), 2.46 (t, *J* = 7 Hz, 2H), 7.23 (bs, 4H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 8.9, 18.4, 21.1, 23.8, 28.2 - 29.6 (12 × CH_2), 68.0, 84.8, 125.7, 129.3, 129.6, 137.1, 137.4, 141.3, 170.9, 171.2. **Anal. Calcd** for $C_{28}H_{39}NO_2$: C, 79.76; H, 9.32; N, 3.32. Found: C, 79.62; H, 9.25; N, 3.20.

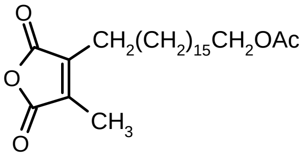
2-Heptadec-(17-hydroxy)yl-3-methylmaleic anhydride (9). To a solution of imide **8** (745 mg, 1.50 mmol) in a THF-methanol mixture (1:2, 12 mL) was added 30% aqueous KOH solution (10 mL) and the reaction mixture was refluxed for 12 h with stirring. The reaction mixture was concentrated in vacuo, the residue was acidified with dilute HCl and extracted with diethyl ether (50 mL × 3). The combined organic layer was washed with water & brine and dried over Na_2SO_4. Concentration of organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether/ethyl acetate mixture (9:1) furnished pure **9** as a white solid (516 mg, 94% yield).

 **9** **$C_{22}H_{38}O_4$ (366)**	**Mp** 63 ºC. **IR** ($CHCl_3$) ν_{max} 3404, 1765, 1707 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.25 (bs, 26H), 1.50-1.70 (m, 5H), 2.07 (s, 3H), 2.45 (t, J = 6 Hz, 2H), 3.64 (t, J = 4 Hz, 2H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 9.4, 24.3, 25.7, 27.5, 29.1, 29.3 - 29.5 (11 × CH_2), 32.7, 62.9, 140.4, 144.7, 165.8, 166.2. **Anal. Calcd** for $C_{22}H_{38}O_4$: C, 72.09; H, 10.45. Found: C, 71.97; H, 10.36.

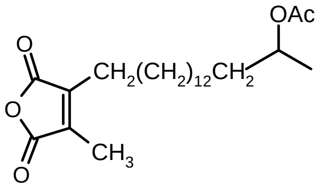
2-Hexadec-(15-yne)yl-3-methylmaleic anhydride (18). It was prepared from **17** (210 mg, 0.50 mmol) using the same procedure as described for **9** above (157 mg, 95% yield).

 **18** **$C_{21}H_{32}O_3$ (332)**	Thick oil. **IR** ($CHCl_3$) ν_{max} 3314, 2116, 1765, 1713 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.26 (bs, 20H), 1.45-1.65 (m, 4H), 1.94 (t, J = 2 Hz, 1H), 2.07 (s, 3H), 2.19 (dt, J = 6 & 2 Hz, 2H), 2.46 (t, J = 6 Hz, 2H). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 9.5, 18.4, 24.4, 27.6, 28.5, 28.7, 29.1, 29.2, 29.4 - 29.5 (6 × CH_2), 29.7, 68.0, 84.8, 140.4, 144.8, 165.9, 166.3. **Anal. Calcd** for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 76.01; H, 9.63.

2-Heptadec-(17-acetoxy)yl-3-methylmaleic anhydride (Aspergillus acid A, 1a). To a stirred solution of alcohol **9** (366 mg, 1 mmol) in pyridine (4 mL) was added acetic anhydride (0.47 mL, 5 mmol) and the reaction mixture was kept in the dark at room temperature for 8 h. The reaction mixture was poured into water and extracted with ethyl acetate (15 mL × 3). The combined organic layer was washed with aq. $CuSO_4$ solution, water & brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether/ethyl acetate mixture (9:1) as an eluent gave **1a** as a white solid (363 mg, 89% yield).

 **Aspergillus acid A (1a)** **$C_{24}H_{40}O_5$ (408)**	**Mp** 43-44 ºC (lit.[8] mp 45-46 ºC). **IR** ($CHCl_3$) ν_{max} 1857, 1774, 1726, 1472, 1250 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.26 (bs, 26H), 1.45-1.70 (m, 4H), 2.05 (s, 3H), 2.07 (s, 3H), 2.45 (t, J = 6 Hz, 2H), 4.05 (t, J = 6 Hz, 2H). **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 9.4, 20.9, 24.4, 25.9, 27.5, 28.6, 29.2 - 29.6 (12 × CH_2), 64.6, 140.4, 144.7, 165.8, 166.2, 171.1. **Anal. Calcd** for $C_{24}H_{40}O_5$: C, 70.55; H, 9.87. Found: C, 70.61; H, 10.00.

(±)-2-Hexadec-(15-acetoxy)yl-3-methylmaleic anhydride (Aspergillus acid D, 1d). It was prepared from (±)-**1c** (106 mg, 0.30 mmol) using the same procedure as described for **1a** above (108 mg, 91% yield).

 ***rac*-Aspergillus acid D** **[(±)-1d]** **$C_{23}H_{38}O_5$ (394)**	Thick oil. **IR** ($CHCl_3$) ν_{max} 1767, 1732 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.20 (d, J = 8 Hz, 3H), 1.26 (bs, 22H), 1.40-1.70 (m, 4H), 2.03 (s, 3H), 2.07 (s, 3H), 2.46 (t, J = 7 Hz, 2H), 4.89 (sextet, J = 6 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 9.4, 19.8, 21.2, 24.3, 25.3, 27.5, 29.1, 29.3 - 29.4 (9 × CH_2), 35.8, 70.9, 140.4, 144.6, 165.8, 166.2, 170.7. **Anal. Calcd** for $C_{23}H_{38}O_5$: C, 70.02; H, 9.71. Found: C, 70.09; H, 9.82.

14-Bromotetradecan-1-ol (11). To a mixture of 1,14-tetradecanediol (**10**, 3.45 g, 15 mmol) and toluene (60 mL) was added concentrated HBr (2 mL, 48% aqueous solution, 17.65 mmol). The heterogeneous mixture was stirred and heated at reflux for 48 h. Second lot of HBr (0.75 mL, 6.62 mmol) was added and the reaction mixture was heated at reflux for a further 48 h. TLC of reaction mixture showed complete consumption of starting diol. The reaction mixture was allowed to cool to room temperature and the phases were separated. The organic layer was diluted with ethyl acetate, washed with 1 M NaOH & brine and dried over Na_2SO_4. Concentration of organic layer in vacuo afforded a thick oil, which on silica gel column chromatographic purification using petroleum ether/ethyl acetate mixture (8:2) as an eluent provided **11** as a white solid (3.74 g, 85% yield).

$BrCH_2(CH_2)_{12}CH_2OH$ **11** **$C_{14}H_{29}BrO$ (293)**	**Mp** 52-54 ºC. **IR** ($CHCl_3$) ν_{max} 3393 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.26 (bs, 20H), 1.50-1.70 (m, 3H), 1.86 (quintet, J = 7 Hz, 2H), 3.42 (t, J = 7 Hz, 2H), 3.65 (t, J = 7 Hz, 2H).

14-Bromo-1-(tetrahydropyranyloxy)tetradecane (12). To a solution of **11** (3.30 g, 11.26 mmol) in dry dichloromethane (20 mL) were added dihydropyran (1.54 mL, 16.90 mmol) and pyridinium *p*-toluenesulfonate (28 mg, 0.11 mmol). The reaction mixture was stirred at room temperature for 4 h. After dilution with additional dichloromethane (15 mL) the reaction mixture was washed with a satd. aqueous $NaHCO_3$ solution and brine. The organic layer was dried with Na_2SO_4 and concentrated in vacuo. Silica gel column chromatographic purification of the residue (eluent: petroleum ether/ethyl acetate mixture, 9:1) provided **12** (3.95 g, 93% yield).

$BrCH_2(CH_2)_{12}CH_2OTHP$ **12** **$C_{19}H_{37}BrO_2$ (377)**	Thick oil. **IR** ($CHCl_3$) ν_{max} 1462, 1215 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.27 (bs, 20H), 1.45-1.75 (m, 6H), 1.75-2.00 (m, 4H), 3.41 (t, J = 7 Hz, 2H), 3.30-3.60 (m, 2H), 3.65-3.95 (m, 2H), 4.58 (t, J = 2 Hz, 1H).

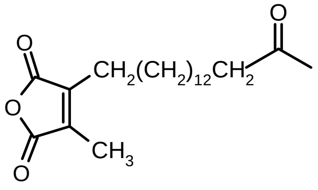
1-Tetrahydropyranyloxypentadec-15-yne (13). A mixture of sodium acetylide slurry in xylene (18%, 0.84 g, 17.50 mmol) and THF (20 mL) under argon was cooled to – 78 ºC, and a solution of **12** (3.30 g, 8.75 mmol) in HMPA (10 mL) and THF (4 mL) was slowly injected into the reaction mixture with stirring. The reaction mixture was allowed to warm to room temperature and stirred for a further 40 h. The reaction was slowly quenched with a satd. $NaHCO_3$ solution and a 5% NH_4OAc solution was added to dissolve the white precipitate formed. The reaction mixture was extracted with ethyl acetate (30 mL × 3) and the combined organic extract was washed with water & brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue (eluent: petroleum ether/ethyl acetate mixture, 9:1) gave **13** as a thick oil (2.40 g, 85% yield).

$HC\equiv CCH_2(CH_2)_{12}CH_2OTHP$ **13** **$C_{21}H_{38}O_2$ (322)**	Thick oil. **IR** ($CHCl_3$) ν_{max} 3310, 2118, 1466, 1454 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.27 (bs, 20H), 1.40-1.65 (m, 8H), 1.94 (t, J = 2 Hz, 1H), 2.18 (dt, J = 7 & 2 Hz, 2H), 3.30-3.60 (m, 3H), 3.65-3.95 (m, 3H), 4.58 (t, J = 2 Hz, 1H).

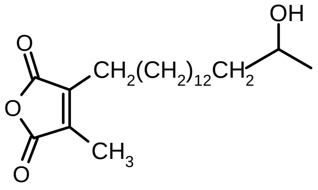
Hexadec-15-yn-1-ol (14). To a solution of **13** (2.19 g, 6.80 mmol) in methanol (20 mL) was added *p*-toluenesulfonic acid monohydrate (129 mg, 0.68 mmol) and the reaction mixture was stirred for 2 h at room temperature. Sodium bicarbonate (500 mg) was added and the reaction mixture was stirred for a further 30 min. The reaction mixture was filtered through celite and the filtrate was concentrated in vacuo. The residue was diluted with water (20 mL) and the aqueous phase was extracted with ethyl acetate (30 mL × 3). The combined organic extracts were washed with water, a satd. $NaHCO_3$ solution & brine and dried over Na_2SO_4. The organic layer was concentrated in vacuo and column chromatographic purification of the residue over silica gel (eluent: petroleum ether/ethyl acetate mixture, 8:2) gave **14** as a white solid (1.54 g, 95% yield).

$HC\equiv CCH_2(CH_2)_{12}CH_2OH$ **14** **$C_{16}H_{30}O$ (238)**	**Mp** 35 ºC. **IR** ($CHCl_3$) ν_{max} 3381, 3310, 2116, 1464, 1433 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.28 (bs, 20H), 1.45-1.75 (m, 5H), 1.95 (t, J = 2 Hz, 1H), 2.18 (dt, J = 7 & 2 Hz, 2H), 3.64 (t, J = 6 Hz, 2H).

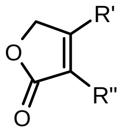
2-Hexadec-(15-oxo)yl-3-methylmaleic anhydride (Aspergillus acid B, 1b). A solution of **17** (632 mg, 1.50 mmol) in a mixture of acetic acid (6 mL) and 6 M sulphuric acid (3 mL) was heated at 100 °C for 8 h with stirring. The reaction mixture was then allowed to reach room temperature and concentrated in vacuo and the residue was diluted with water and extracted with ethyl acetate (30 mL × 3). The combined organic extract was washed successively with water & brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo and silica gel column chromatographic purification of the residue (eluent: petroleum ether/ethyl acetate mixture, 8:2) gave **1b** as a white solid (473 mg, 90% yield).

 **Aspergillus acid B (1b)** **$C_{21}H_{34}O_4$ (350)**	**Mp** 43-44 ºC (lit.[8] mp 43 ºC). **IR** ($CHCl_3$) ν_{max} 1765, 1713 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.26 (bs, 20H), 1.45-1.65 (m, 4H), 2.07 (s, 3H), 2.14 (s, 3H), 2.42 (t, *J* = 8 Hz, 2H), 2.45 (t, *J* = 8 Hz, 2H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 9.5, 23.8, 24.4, 27.6, 29.1 - 29.4 (10 × CH_2), 29.8, 43.8, 140.4, 144.7, 165.9, 166.3, 209.4. **Anal. Calcd** for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78. Found: C, 71.81; H, 9.90.

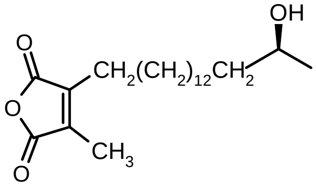
(±)-2-Hexadec-(15-hydroxy)yl-3-methylmaleic anhydride (Aspergillus acid C, 1c). A solution of **1b** (350 mg, 1 mmol) in a 1:1 mixture of aq. NaOH (30%, 5 mL) and THF (5 mL) was stirred for 2 h at 50 ºC. The reaction mixture was cooled to 0 ºC and then $NaBH_4$ (76 mg, 2 mmol) was added in one lot. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. The reaction mixture was concentrated in vacuo and then acidified by slow addition of dilute HCl. It was extracted with ethyl acetate (30 mL × 3). The combined organic extracts were washed with water & brine and dried over Na_2SO_4. Concentration of organic layer in vacuo and silica gel column chromatographic purification of the residue (eluent: petroleum ether/ethyl acetate mixture, 8:2) gave (±)-**1c** as a white solid (285 mg, 81% yield).

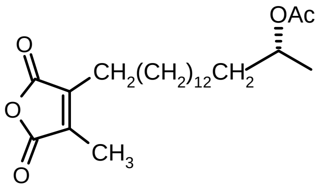
 ***rac*-Aspergillus acid C** **[(±)-1c]** **$C_{21}H_{36}O_4$ (352)**	**Mp** 51-52 ºC (lit.[8] mp 50 ºC). **IR** ($CHCl_3$) ν_{max} 3398, 1765 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.18 (d, *J* = 8 Hz, 3H), 1.26 (bs, 22H), 1.45-1.65 (m, 4H), 2.07 (s, 3H), 2.45 (t, *J* = 7 Hz, 2H), 3.80 (sextet, *J* = 6 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 9.0, 23.0, 24.0, 25.4, 27.1, 28.8, 29.2 - 29.3 (9 × CH_2), 38.9, 67.5, 140.2, 144.3, 165.5, 165.9. **Anal. Calcd** for $C_{21}H_{36}O_4$: C, 71.55; H, 10.29. Found: C, 71.62; H, 10.36.

4-(15'-Hydroxyhexadecyl)-3-methyl-2(5*H*)-furanone (19a) and 3-(15'-hydroxyhexadecyl)-4-methyl-2(5*H*)-furanone (19b). Anhydride **1b** (35 mg, 0.10 mmol) was dissolved in MeOH (4 mL). $NaBH_4$ (10 mg, 0.26 mmol) was added to the above solution at – 5 °C and stirred for 10 min. The reaction was then quenched by slow addition of dilute HCl and extracted with ethyl acetate (15 mL × 3). The combined organic extract was washed successively with water & brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether/ethyl acetate mixture (8:2) as an eluent gave **19b** and **19a** (**19a**:**19b** = 26:74).

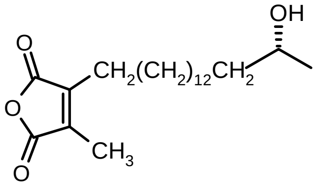
 **19a**: R' = $CH_2(CH_2)_{12}CH_2CH(OH)CH_3$; R'' = CH_3 **19b**: R' = CH_3; R'' = $CH_2(CH_2)_{12}CH_2CH(OH)CH_3$ **19a + 19b** **$C_{21}H_{38}O_3$ (338)**	**19a** (7 mg, 21% yield). **Mp** 72-73 ºC (lit.[8] mp 72-74 ºC). **IR** (nujol): ν_{max} 3500, 1765 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.19 (d, J = 6 Hz, 3H), 1.30 (bs, 22H), 1.40-1.60 (m, 4H), 1.83 (s, 3H), 2.41 (t, J = 8 Hz, 2H), 3.80 (sextet, J = 6 Hz, 1H), 4.66 (s, 2H). **Anal. Calcd** for $C_{21}H_{38}O_3$: C, 74.51; H, 11.31. Found: C, 74.39; H, 11.22. **19b** (19 mg, 56% yield). **Mp** 53-54 ºC (lit.[8] mp 53-55 ºC). **IR** (nujol) ν_{max} 3472, 1734 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.19 (d, J = 6 Hz, 3H), 1.30 (bs, 22H), 1.40-1.60 (m, 4H), 2.02 (s, 3H), 2.25 (t, J = 7 Hz, 2H), 3.80 (sextet, J = 6 Hz, 1H), 4.62 (s, 2H). **Anal. Calcd** for $C_{21}H_{38}O_3$: C, 74.51; H, 11.31. Found: C, 74.62; H, 11.29.

Amano PS catalysed acylation of (±)-1c. A solution of alcohol (±)-**1c** (176 mg, 0.50 mmol) in *n*-hexane/benzene (2:1) (9 mL) was added to Amano PS lipase (100 mg) followed by vinyl acetate (0.23 mL, 2.50 mmol). The reaction mixture was stirred at 45 °C for 72 h and then allowed to cool to room temperature. The enzyme was filtered off, washed with ethyl acetate and the organic layer was concentrated in vacuo. The residue was chromatographed over silica gel using petroleum ether/ethyl acetate mixture (9:1) to obtain (+)-**1c** (79 mg, 45% yield) and (−)-**1d** (85 mg, 43% yield).

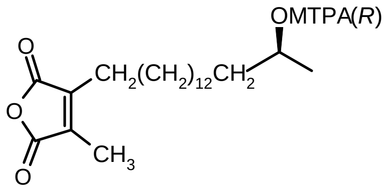
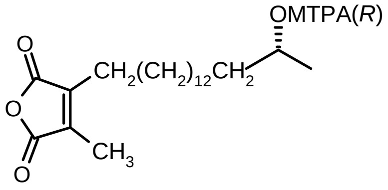
 **(*S*)-Aspergillus acid C [(+)-1c]** **$C_{21}H_{36}O_4$ (352)**	**Mp** 52 ºC. $[\alpha]^{20}_{D}$ = + 1.3° (c 1.0, $CHCl_3$) [lit.[8] α = + 1.83° (c 0.38, $CHCl_3$)]. Analytical and spectral data obtained were identical with (±)-**1c**.

 **(*R*)-Aspergillus acid D [(–)-1d]** **$C_{23}H_{38}O_5$ (394)**	$[\alpha]^{20}_{D}$ = – 2.0° (*c* 1.0, $CHCl_3$) [lit.[8] for the other enantiomer α = + 2.70° (*c* 0.22, $CHCl_3$)]. Analytical and spectral data obtained were identical with (±)-**1d**.

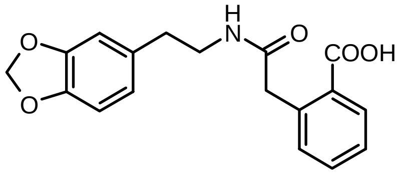
(*R*)-2-Hexadec-(15-hydroxy)yl-3-methylmaleic anhydride [(–)-1c]. A solution of acetate (–)-**1d** (60 mg, 0.15 mmol) in a 1:1 mixture of aq. NaOH (30%, 3 mL) and THF (3 mL) was stirred for 4 h at 50 ºC. The reaction mixture was cooled to room temperature, concentrated in vacuo and acidified by addition of dilute HCl. It was then extracted with ethyl acteate (15 mL × 3) and the combined organic extract was washed successively with water & brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether/ethyl acetate mixture (9:1) yielded (–)-**1c** as a thick oil (48 mg, 90% yield).

 **(*R*)-Aspergillus acid C [(–)-1c]** **$C_{21}H_{36}O_4$ (352)**	$[\alpha]^{20}_{D}$ = – 1.4° (*c* 1.0, $CHCl_3$). Analytical and spectral data obtained were identical with (±)-**1c**.

General procedure for MTPA-Ester preparation. To a solution of (*R*)-Mosher's acid (15 mg, 0.06 mmol), alcohol (+)- or (–)-**1c** (18 mg, 0.05 mmol) and DMAP (cat.) in dry CH_2Cl_2 (3 mL) was added a solution of DCC (12 mg, 0.06 mmol) in dry CH_2Cl_2 (2 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 8 h. The formed urea was filtered off and the organic layer was concentrated in vacuo. Silica gel column chromatographic purification of the residue (60-120 mesh) using petroleum ether/ethyl acetate mixture (9.5:0.5) gave the MTPA-ester as a thick oil in quantitative yield.

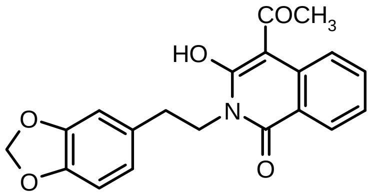
 **MTPA-Ester of (+)-(*S*)-2-Hexadec-(15-hydroxy)yl-3-methylmaleic anhydride** **$C_{31}H_{43}F_3O_6$ (568)**	**^{1}H NMR** ($CDCl_3$, 500 MHz) δ 1.15-1.35 (m, 25H), 1.55-1.70 (m, 4H), 2.07 (s, 3H), 2.45 (t, *J* = 10 Hz, 2H), 3.55 (s, 0.45H), 3.57 (s, 2.55H), 5.16 (sextet, *J* = 5 Hz, 1H), 7.35-7.43 (m, 3H), 7.50-7.55 (m, 2H).
 **MTPA-Ester of (–)-(*R*)-2-Hexadec-(15-hydroxy)yl-3-methylmaleic anhydride** **$C_{31}H_{43}F_3O_6$ (568)**	**^{1}H NMR** ($CDCl_3$, 500 MHz) δ 1.20-1.35 (m, 25H), 1.50-1.63 (m, 4H), 2.07 (s, 3H), 2.45 (t, *J* = 10 Hz, 2H), 3.55 (s, 2.58H), 3.57 (s, 0.42H), 5.14 (sextet, *J* = 5 Hz, 1H), 7.35-7.43 (m, 3H), 7.50-7.55 (m, 2H).

2-[2-(2-(Benzo[*d*][1,3]dioxol-5-yl)ethylamino)-2-oxoethyl]benzoic acid (56). To a stirred solution of homophthalic anhydride (**HPA**, 2.43 g, 15 mmol) in a 4:1 ether-THF mixture (15 mL) was added a solution of homopiperonyl amine (**55**, 2.48 g, 15 mmol) in ether (15 mL) with constant stirring in a drop wise fashion over a period of 5 min. The reaction mixture was stirred for 2 h at room temperature and the precipitated product was filtered, washed with ether and vacuum-dried to obtain the homophthalamic acid **56** (4.52 g, 92% yield).

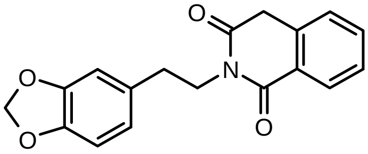
 **56** **$C_{18}H_{17}NO_5$ (327)**	**Mp** 130-134 ºC. **IR** ($CHCl_3$) ν_{max} 3346, 3269, 1694, 1579, 1500, 1246 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.58 (t, *J* = 6 Hz, 2H), 3.35 (q, *J* = 6 Hz, 2H), 3.81 (s, 2H), 5.86 (s, 2H), 6.26 (bs, 2H), 6.35-6.65 (m, 3H), 7.30-7.50 (m, 3H), 7.94 (d, *J* = 8 Hz, 1H). **Anal. Calcd** for $C_{18}H_{17}NO_5$: C, 66.05; H, 5.23; N, 4.28. Found: C, 65.96; H, 5.11; N, 4.35.

4-Acetyl-2-(2-benzo[1,3]dioxol-5-yl-ethyl)-3-hydroxy-2*H*-isoquinolin-1-one (57). Ac_2O (5 mL) and fused NaOAc (20 mg, cat.) were added to the homophthalamic acid **56** (490 mg, 1.50 mmol) and the reaction mixture was heated in a water bath at 60-65 ºC for 2 h.

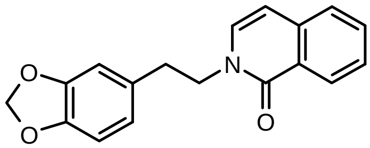
The reaction mixture was allowed to reach room temperature and poured into ice-cold water. The formed precipitate was filtered, washed with excess of water and vacuum-dried to provide **57** (400 mg, 76% yield).

 **57** **$C_{20}H_{17}NO_5$ (351)**	**Mp** 112-114 ºC. **IR** ($CHCl_3$) ν_{max} 3311, 1739, 1648, 1535, 1486, 1246 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.57 (s, 3H), 2.88 (t, J = 7 Hz, 2H), 3.73 (apparent q, J = 7 Hz, 2H), 5.92 (s, 2H), 6.60-6.80 (m, 3H), 7.10-7.25 (m, 1H), 7.50-7.70 (m, 2H), 8.16 (d, J = 8 Hz, 1H), 11.05 (bs, 1H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 31.0, 35.5, 42.7, 92.4, 100.8, 108.2, 108.9, 114.8, 121.6, 123.3, 123.4, 130.2, 131.5, 134.8, 138.7, 146.2, 147.7, 158.6, 160.9, 194.2. **Anal. Calcd** for $C_{20}H_{17}NO_5$: C, 68.37; H, 4.88; N, 3.99. Found: C, 68.57; H, 4.93; N, 4.05.

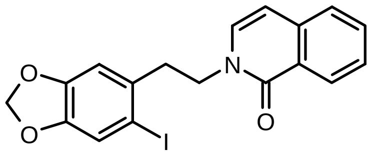
2-(2-Benzo[1,3]dioxol-5-yl-ethyl)-4*H*-isoquinoline-1,3-dione (58). To a suspension of acid **56** (3.92 g, 12 mmol) in dry benzene (30 mL) was added $ZnCl_2$ (1.64 g, 12 mmol) and the mixture was heated at 80 ºC. To this was added a solution of HMDS (3.75 mL, 18 mmol) in dry benzene (15 mL) slowly over a period of 20 min. The reaction was refluxed for an additional 2 h. The reaction mixture was then cooled to room temperature and poured into 0.5 N HCl (30 mL). The aqueous phase was extracted with ethyl acetate (30 mL × 3). The combined organic layer was washed with aq. $NaHCO_3$ & brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether/ethyl acetate mixture (8:2) as an eluent gave **58** (3.33 g, 90% yield).

 **58** **$C_{18}H_{15}NO_4$ (309)**	**Mp** 158 ºC. **IR** ($CHCl_3$) ν_{max} 1709, 1662, 1607, 1352, 1246 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.75-2.90 (m, 2H), 4.03 (s, 2H), 4.05-4.25 (m, 2H), 5.92 (s, 2H), 6.65-6.85 (m, 3H), 7.28 (d, J = 8 Hz, 1H), 7.45 (t, J = 8 Hz, 1H), 7.60 (dt, J = 8 & 2 Hz, 1H), 8.22 (d, J = 8 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 33.7, 36.3, 41.6, 100.7, 108.1, 109.3, 121.7, 125.2, 127.0, 127.6, 129.0, 132.2, 133.5, 134.0, 146.0, 147.5, 164.6, 169.7. **Anal. Calcd** for $C_{18}H_{15}NO_4$: C, 69.89; H, 4.89; N, 4.53. Found: C, 70.02; H, 4.81; N, 4.59.

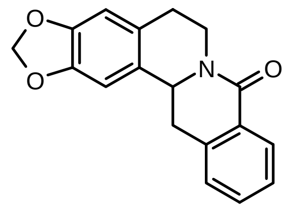
2-(2-Benzo[1,3]dioxol-5-yl-ethyl)-2*H*-isoquinolin-1-one (48). To a solution of the imide **58** (309 mg, 1 mmol) in EtOH (10 mL) at 0 °C was added an excess of NaBH$_4$ (304 mg, 8 mmol) with stirring. The reaction mixture was stirred for a further 6 h at 0 °C with addition of 2-3 drops of 2 N HCl in EtOH at intervals of 20 min. The excess of NaBH$_4$ was then destroyed at 0 °C by addition of 2 N HCl in EtOH until pH = 3. The reaction mixture was allowed to warm to room temperature and stirred for a further 2 h. The EtOH was distilled off under reduced pressure and the residue was diluted with water (20 mL) and extracted with ethyl acetate (25 mL × 3). The combined organic layer was washed with water, NaHCO$_3$ & brine and dried over Na$_2$SO$_4$. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether/ethyl acetate mixture (8:2) as an eluent gave **48** (211 mg, 72% yield).

 **48** **$C_{18}H_{15}NO_3$ (293)**	**Mp** 80-82 ºC. **IR** ($CHCl_3$) ν_{max} 1749, 1649, 1626, 1597, 1504, 1489, 1248, 1215 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 3.00 (t, J = 8 Hz, 2H), 4.16 (t, J = 8 Hz, 2H), 5.93 (s, 2H), 6.39 (d, J = 8 Hz, 1H), 6.60-6.75 (m, 3H), 6.81 (d, J = 8 Hz, 1H), 7.40-7.55 (m, 2H), 7.55-7.70 (m, 1H), 8.45 (d, J = 8 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 34.9, 51.6, 100.8, 105.6, 108.3, 109.2, 121.8, 125.8, 126.1, 126.6, 127.6, 131.9 (2 carbons), 132.0, 137.0, 146.2, 147.7, 161.9. **Anal. Calcd** for $C_{18}H_{15}NO_3$: C, 73.71; H, 5.15; N, 4.78. Found: C, 73.59; H, 5.22; N, 4.39.

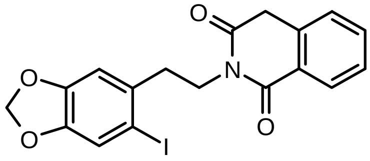
2-[2-(6-Iodo-benzo[1,3]dioxol-5-yl)-ethyl]-2*H*-isoquinolin-1-one (61). It was prepared from **60** (1.09 g, 2.51 mmol) using the same procedure as described for **48** above (819 mg, 78% yield).

 **61** **$C_{18}H_{14}INO_3$ (419)**	**Mp** 168-170 ºC. **IR** ($CHCl_3$) ν_{max} 1666, 1626, 1461, 1377, 1227 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 3.10-3.20 (m, 2H), 4.10-4.20 (m, 2H), 5.94 (s, 2H), 6.44 (d, J = 8 Hz, 1H), 6.75 (s, 1H), 6.94 (d, J = 8 Hz, 1H), 7.24 (s, 1H), 7.40-7.70 (m, 3H), 8.45 (d, J = 8 Hz, 1H). **Anal. Calcd** for $C_{18}H_{14}INO_3$: C, 51.57; H, 3.37; N, 3.34. Found: C, 51.42; H, 3.48; N, 3.29.

5,6,13,13a-Tetrahydro-[1,3]dioxolo[4,5-*g*]isoquino[3,2-*a*]isoquinolin-8-one [*rac*-Gusanlung D, (±)-35]. A solution containing lactam **48** (147 mg, 0.50 mmol) and concentrated HCl (10 mL) was stirred at rt for 48 h. The reaction mixture was poured over ice and extracted with CH_2Cl_2. The combined organic layer was washed with a saturated solution of $NaHCO_3$ and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was subjected to silica gel chromatography using petroleum ether/ethyl acetate mixture (8:2) as an eluent to give gusanlung D [(±)-**35**, 115 mg, 78% yield].

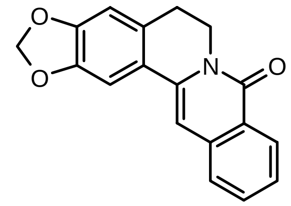
 ***rac*-Gusanlung D [(±)-35]** **$C_{18}H_{15}NO_3$ (293)**	**Mp** 194-196 ºC [lit.[36] mp 250-251 ºC (natural), lit.[37] mp 195-197 ºC (synthetic)]. **IR** ($CHCl_3$) ν_{max} 1647, 1451 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 2.70-2.80 (m, 1H), 2.85-3.05 (m, 3H), 3.18 (dd, J = 16 & 4 Hz, 1H), 4.83 (dd, J = 13 & 4 Hz, 1H), 4.90-5.00 (m, 1H), 5.96 (s, 2H), 6.67 (s, 1H), 6.72 (s, 1H), 7.24 (d, J = 8 Hz, 1H), 7.39 (t, J = 8 Hz, 1H), 7.46 (t, J = 8 Hz, 1H), 8.13 (d, J = 8 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 29.7, 38.1, 38.8, 55.3, 101.1, 105.9, 108.7, 126.9, 127.3, 128.5, 128.6, 128.9, 129.1, 131.8, 137.2, 146.6, 146.8, 164.6. **Anal. Calcd** for $C_{18}H_{15}NO_3$: C, 73.71; H, 5.15; N, 4.78. Found: C, 73.56; H, 5.28; N, 4.81.

2-[2-(6-Iodo-benzo[1,3]dioxol-5-yl)-ethyl]-4*H*-isoquinoline-1,3-dione (60). To a solution of imide **58** (943 mg, 3.05 mmol) and silver trifluoroacetate (674 mg, 3.05 mmol) in dry chloroform (15 mL) was added iodine (775 mg, 3.05 mmol) in small portions with stirring over a period of 15 min. The reaction mixture was stirred for a further 4 h at room temperature. The reaction mixture was filtered, washed with dichloromethane (25 mL) and the filtrate was washed with sodium thiosulphate, water & brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel chromatographic column purification of the residue yielded the iodo imide **60** (1.14 g, 86% yield).

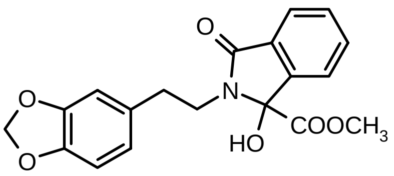
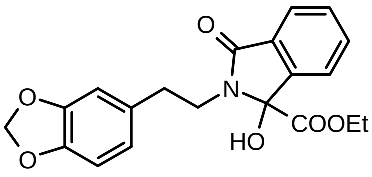
 **60** **$C_{18}H_{14}INO_4$ (435)**	**Mp** 217-219 ºC. **IR** ($CHCl_3$) ν_{max} 1701, 1668, 1499, 1474, 1215 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.90-3.10 (m, 2H), 4.04 (s, 2H), 4.10-4.25 (m, 2H), 5.95 (s, 2H), 6.85 (s, 1H), 7.22 (s, 1H), 7.25-7.35 (m, 1H), 7.45 (t, J = 8 Hz, 1H), 7.60 (dt, J = 8 & 2 Hz, 1H), 8.22 (dd, J = 8 & 2 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 36.4, 38.6, 40.2, 87.7, 101.5, 109.7, 118.6, 125.3, 127.1, 127.7, 129.1, 133.6, 134.1, 135.0, 147.2, 148.5, 164.7, 169.8. **Anal. Calcd** for $C_{18}H_{14}INO_4$: C, 49.68; H, 3.24; N, 3.22. Found: C, 49.53; H, 3.15; N, 3.10.

Attempted synthesis of 5,6,13,13a-Tetrahydro-[1,3]dioxolo[4,5-*g*]isoquino[3,2-*a*]isoquinolin-8-one [*rac*-Gusanlung D, (±)-35]. A solution of Bu_3SnH (0.20 mL, 0.75 mmol) and AIBN (16 mg, 0.10 mmol) in toluene (12 mL) was added to a refluxing solution of the iodo compound **61** (210 mg, 0.50 mmol) in toluene (24 mL) over a period of 3 h. The reaction mixture was refluxed for a further 3 h. The solvent was distilled off under reduced pressure and the crude residue was purified by flash column chromatography to afford back the olefinic compound **48** (94 mg, 64% yield).

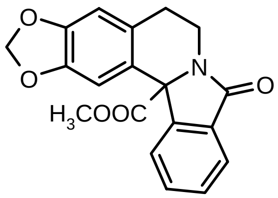
5,6-Dihydro-[1,3]dioxolo[4,5-*g*]isoquino[3,2-*a*]isoquinolin-8-one (Dehydrogusanlung D, 53). To an argon-flushed flask containing the iodo compound **61** (335 mg, 0.80 mmol) and NaOAc (98.5 mg, 1.20 mmol) was added a solution of $Pd(OAc)_2$ (0.5 mg, 0.25 mol%) and tetramethyl guanidine (1 mg, 1 mol%) in dimethylacetamide (10 mL) using syringe and the reaction mixture was stirred at 120 °C for 20 h. After cooling to room temperature, the reaction mixture was poured into water (20 mL) and extracted with ethyl acetate (25 mL × 3). The combined organic layer was washed with water, $NaHCO_3$ & brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether/ethyl acetate mixture (8:2) as an eluent furnished **53** (168 mg, 72% yield).

 **Dehydrogusanlung D (53)** **$C_{18}H_{13}NO_3$ (291)**	**Mp** 181-183 ºC (lit.[43] mp 182-183 ºC). **IR** ($CHCl_3$) ν_{max} 1715, 1645, 1616, 1504, 1485, 1261, 1215 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 2.91 (t, J = 6 Hz, 2H), 4.34 (t, J = 6 Hz, 2H), 6.01 (s, 2H), 6.71 (s, 1H), 6.83 (s, 1H), 7.25 (s, 1H), 7.43 (t, J = 8 Hz, 1H), 7.53 (d, J = 8 Hz, 1H), 7.62 (t, J = 8 Hz, 1H), 8.41 (d, J = 8 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 28.5, 39.7, 101.5, 101.9, 105.0, 107.9, 123.7, 124.6, 126.0, 126.3, 127.9, 130.3, 132.3, 136.6, 137.4, 147.4, 148.7, 162.1. **Anal. Calcd** for $C_{18}H_{13}NO_3$: C, 74.22; H, 4.50; N, 4.81. Found: C, 74.31; H, 4.47; N, 4.96.

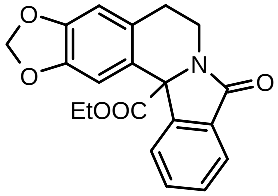
2-(2-Benzo[1,3]dioxol-5-yl-ethyl)-1-hydroxy-3-oxo-2,3-dihydro-1*H*-isoindole-1-carboxylic acid, methyl ester (82a)/ethyl ester (82b). *Method A*: To a solution of imide **58** (309 mg, 1 mmol) in MeOH/EtOH (10 mL) was added $NaBH_4$ (95 mg, 2.50 mmol) and the reaction mixture was stirred for 12 h at room temperature. Water (20 mL) was added to the reaction mixture and it was extracted with ethyl acetate (25 mL × 3). The combined organic layer was washed with water & brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether/ethyl acetate mixture (8.5:1.5) as an eluent furnished **82a** (249 mg, 70% yield)/**82b** (266 mg, 72% yield). *Method B*: To a solution of imide **58** (989 mg, 3.20 mmol) in MeOH/EtOH (10 mL) was added Et_3N (0.50 mL) and the reaction mixture was stirred for 12 h at room temperature. Water (20 mL) was added to the reaction mixture and it was extracted with ethyl acetate (25 mL × 3). The combined organic layer was washed with water & brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether/ethyl acetate mixture (8.5:1.5) as an eluent furnished **82a** (863 mg, 76% yield)/**82b** (838 mg, 71% yield).

 **82a** **$C_{19}H_{17}NO_6$ (355)**	**Mp** 162-164 ºC. **IR** ($CHCl_3$) ν_{max} 3431, 1749, 1707, 1502, 1491, 1439, 1215 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.75-3.05 (m, 2H), 3.20-3.45 (m, 1H), 3.65-3.85 (m, 1H), 3.72 (s, 3H), 4.58 (bs, 1H), 5.92 (s, 2H), 6.65-6.80 (m, 3H), 7.40-7.50 (m, 1H), 7.50-7.65 (m, 2H), 7.75-7.90 (m, 1H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 34.6, 41.9, 54.1, 88.3, 100.8, 108.3, 109.3, 121.7 (2 carbons), 123.6, 130.4, 131.5, 132.5, 132.6, 143.2, 146.1, 147.6, 167.9, 171.5. **Anal. Calcd** for $C_{19}H_{17}NO_6$: C, 64.22; H, 4.82; N, 3.94. Found: C, 64.13; H, 5.00; N, 4.06.
 **82b** **$C_{20}H_{19}NO_6$ (369)**	**Mp** 108-109 ºC. **IR** ($CHCl_3$) ν_{max} 3331, 1744, 1697, 1504, 1491, 1470, 1445, 1250, 1215 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.14 (t, J = 7 Hz, 3H), 2.75-3.05 (m, 2H), 3.20-3.40 (m, 1H), 3.65-3.90 (m, 1H), 4.05-4.30 (m, 2H), 4.79 (bs, 1H), 5.92 (s, 2H), 6.65-6.80 (m, 3H), 7.40-7.50 (m, 1H), 7.50-7.60 (m, 2H), 7.75-7.85 (m, 1H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 13.8, 34.6, 41.8, 63.5, 88.4, 100.8, 108.2, 109.2, 121.6 (2 carbons), 123.5, 130.3, 131.5, 132.4, 132.6, 143.4, 146.0, 147.6, 168.0, 170.8. **Anal. Calcd** for $C_{20}H_{19}NO_6$: C, 65.03; H, 5.18; N, 3.79. Found: C, 64.87; H, 5.09; N, 3.88.

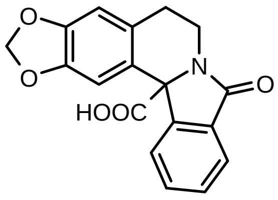
7-Oxo-5,6-dihydro-7*H*-1,3-dioxa-6a-aza-indeno[5,6-*c*]fluorene-11b-carboxylic acid, methyl ester (83a). A solution of **82a** (888 mg, 2.50 mmol) in a mixture of acetic acid (6 mL) and 6 M sulphuric acid (3 mL) was stirred for 2 h at room temperature. The reaction mixture was diluted with water and extracted with ethyl acetate (25 mL × 3). The combined organic layer was washed successively with water, $NaHCO_3$ & brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether/ethyl acetate mixture (8:2) as an eluent gave **83a** (742 mg, 88% yield).

 **83a** **$C_{19}H_{15}NO_5$ (337)**	**Mp** 185-187 ºC. **IR** ($CHCl_3$) ν_{max} 1736, 1697, 1614, 1504, 1487, 1391, 1246, 1231 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.65-3.10 (m, 2H), 3.60-3.80 (m, 1H), 3.74 (s, 3H), 4.35-4.55 (m, 1H), 5.93 (d, J = 12 Hz, 1H), 5.94 (d, J = 12 Hz, 1H), 6.61 (s, 1H), 7.44 (s, 1H), 7.53 (dt, J = 8 & 2 Hz, 1H), 7.65 (dt, J = 8 & 2 Hz, 1H), 7.86 (d, J = 8 Hz, 1H), 8.04 (d, J = 8 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 28.7, 36.9, 53.4, 69.3, 101.2, 107.3, 108.8, 123.9, 124.2, 126.3, 128.5, 129.4, 131.7, 132.0, 143.4, 146.4, 147.6, 167.5, 170.1. **Anal. Calcd** for $C_{19}H_{15}NO_5$: C, 67.65; H, 4.48; N, 4.15. Found: C, 67.75; H, 4.63; N, 4.08.

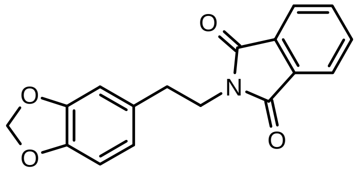
7-Oxo-5,6-dihydro-7*H*-1,3-dioxa-6a-aza-indeno[5,6-*c*]fluorene-11b-carboxylic acid, ethyl ester (83b). Repetition of the above procedure with **82b** (738 mg, 2 mmol) provided **83b** (590 mg, 84% yield).

 **83b** **$C_{20}H_{17}NO_5$ (351)**	Gummy solid. **IR** ($CHCl_3$) ν_{max} 1740, 1697, 1612, 1504, 1487, 1391, 1248 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.24 (t, J = 7 Hz, 3H), 2.65-3.10 (m, 2H), 3.60-3.80 (m, 1H), 4.00-4.30 (m, 2H), 4.35-4.55 (m, 1H), 5.92 (d, J = 12 Hz, 1H), 5.93 (d, J = 12 Hz, 1H), 6.60 (s, 1H), 7.44 (s, 1H), 7.53 (dt, J = 8 & 2 Hz, 1H), 7.65 (dt, J = 8 & 2 Hz, 1H), 7.85 (d, J = 8 Hz, 1H), 8.05 (d, J = 8 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 13.9, 28.6, 36.8, 62.5, 69.3, 101.2, 107.3, 108.7, 123.8, 124.1, 126.4, 128.4, 129.3, 131.7, 131.9, 143.5, 146.3, 147.5, 167.5, 169.4. **Anal. Calcd** for $C_{20}H_{17}NO_5$: C, 68.37; H, 4.88; N, 3.99. Found: C, 68.30; H, 4.65; N, 4.04.

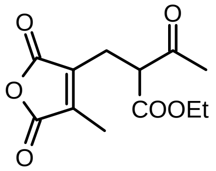
7-Oxo-5,6-dihydro-7*H*-1,3-dioxa-6a-aza-indeno[5,6-*c*]fluorene-11b-carboxylic acid (84). A solution of lithium hydroxide (126 mg, 3 mmol) in water (2 mL) was added to a solution of **83a** (506 mg, 1.50 mmol) in tetrahydrofuran (8 mL) at room temperature and the reaction mixture was stirred for 18 h. The reaction mixture was then concentrated in vacuo, the residue was diluted with water and extracted with dichloromethane (20 mL × 5). The combined organic layer was washed with brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether/ethyl acetate mixture (9:1) as an eluent gave **84** (392 mg, 81% yield).

 **84** **$C_{18}H_{13}NO_5$ (323)**	**Mp** 176-178 ºC. **IR** ($CHCl_3$) ν_{max} 1710, 1676, 1458, 1375, 1242, 1226 cm^{-1}. **^{1}H NMR** ($CDCl_3$ + DMSO-d_6, 400 MHz) δ 2.65-3.05 (m, 2H), 3.35-3.60 (m, 1H), 4.20-4.40 (m, 1H), 5.91 (d, *J* = 18 Hz, 1H), 5.92 (d, *J* = 18 Hz, 1H), 6.57 (s, 1H), 7.40 (s, 1H), 7.47 (t, *J* = 8 Hz, 1H), 7.62 (t, *J* = 8 Hz, 1H), 7.74 (d, *J* = 8 Hz, 1H), 7.97 (d, *J* = 8 Hz, 1H). **^{13}C NMR** ($CDCl_3$ + DMSO-d_6, 50 MHz) δ 28.7, 34.0, 85.4, 100.3, 106.7, 107.7, 122.3, 122.6, 122.8, 127.8, 128.5, 129.4, 130.1, 131.5, 145.6, 146.6, 147.8, 166.2. **Anal. Calcd** for $C_{18}H_{13}NO_5$: C, 66.87; H, 4.05; N, 4.33. Found: C, 66.69; H, 3.87; N, 4.22.

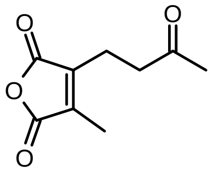
2-(2-(benzo[*d*][1,3]dioxol-5-yl)ethyl)isoindoline-1,3-dione (86). A mixture of phthalic acid (**PA**, 1.66 g, 10 mmol) and homopiperonyl amine (**55**, 1.65 g, 10 mmol) were heated at 160 °C for 3 h. The crude residue was purified by silica gel column chromatography using petroleum ether/ethyl acetate mixture (8.5:1.5) as an eluent to furnish pure **86** (1.98 g, 67% yield).

 **86** **$C_{17}H_{13}NO_4$ (295)**	**Mp** 140-142 ºC. **IR** (Nujol) ν_{max} 1771, 1705, 1464, 1377 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.80-3.00 (m, 2H), 3.75-3.95 (m, 2H), 5.92 (s, 2H), 6.60-6.80 (m, 3H), 7.60-7.75 (m, 2H), 7.75-7.95 (m, 2H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 34.2, 39.3, 100.7, 108.1, 109.1, 121.6, 123.1, 131.6, 131.9, 133.8, 146.1, 147.6, 168.0. **Anal. Calcd** for $C_{17}H_{13}NO_4$: C, 69.15; H, 4.44; N, 4.74. Found: C, 68.99; H, 4.56; N, 4.67.

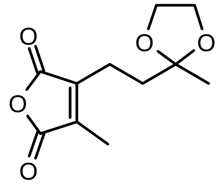
2-(2-Carbethoxy-3-oxo)butyl-3-methymaleic anhydride (91). To a slurry of sodium hydride (288 mg, 12 mmol) in THF (10 mL) was added ethyl acetoacetate (1.53 mL, 12 mmol) in a dropwise fashion at room temperature and stirred for 15 min. Bromoanhydride **90** (2.05 g, 10 mmol) in THF (20 mL) was added to the reaction mixture at room temperature and stirred for a further 8 h. The reaction mixture was acidified with dilute HCl and extracted with ethyl acetate (20 mL × 3). The combined organic layer was washed with water & brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether/ethyl acetate mixture (8.5:1.5) as an eluent gave **91** (1.78 g, 78% yield).

 **91** **$C_{12}H_{14}O_6$ (254)**	Thick oil. **IR** (Neat) ν_{max} 1859, 1827, 1774, 1719, 1447, 1371 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.29 (t, J = 8 Hz, 3H), 2.14 (s, 3H), 2.33 (s, 3H), 2.94 (d, J = 8 Hz, 2H), 4.07 (t, J = 8 Hz, 1H), 4.22 (q, J = 8 Hz, 2H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 9.5, 13.8, 22.6, 29.3, 55.8, 62.0, 140.4, 143.2, 165.4, 165.5, 167.8, 200.6. **Anal. Calcd** for $C_{12}H_{14}O_6$: C, 56.69; H, 5.55. Found: C, 56.56; H, 5.49.

2-(3-Oxo)butyl-3-methymaleic anhydride (92). The β-keto ester **91** (1.52 g, 6 mmol) in conc. HCl plus water (1:1, 20 mL) was refluxed with stirring for 12 h. The reaction mixture was allowed to reach room temperature and then saturated by the addition of solid NaCl. The filtered aqueous layer was extracted with ethyl acetate (20 mL × 5), washed with brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether/ethyl acetate mixture (8:2) as an eluent gave **92** (1.00 g, 92% yield).

 **92** **$C_9H_{10}O_4$ (182)**	Thick oil. **IR** (Neat) ν_{max} 1852, 1823, 1767, 1715, 1674 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.14 (s, 3H), 2.17 (s, 3H), 2.69 (t, J = 6 Hz, 2H), 2.86 (t, J = 6 Hz, 2H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 9.4, 18.4, 29.4, 39.6, 141.6, 142.7, 165.6, 165.8, 206.0. **Anal. Calcd** for $C_9H_{10}O_4$: C, 59.34; H, 5.53. Found: C, 59.50; H, 5.44.

2-(1,3-Dioxolan-3-yl)butyl-3-methylmaleic anhydride (93). To a stirred solution of ketone **92** (910 mg, 5 mmol) and ethylene glycol (0.42 mL, 7.50 mmol) in dry benzene (15 mL) was added *p*-toluenesulfonic acid monohydrate (95 mg, 0.50 mmol) and the reaction mixture was heated under reflux for 10 h using Dean and Stark apparatus containing 3 Å molecular sieves. The reaction mixture was concentrated under vacuum and the residue was dissolved in ethyl acetate (100 mL). The organic layer was washed with aq. $NaHCO_3$ & brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue using petroleum ether/ethyl acetate mixture (8.5:1.5) as an eluent afforded the ketal **93** (1.13 g, 94% yield).

 **93** **$C_{11}H_{14}O_5$ (226)**	Thick oil. **IR** (Neat) ν_{max} 1765, 1715, 1674 cm^{-1}. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.33 (s, 3H), 1.99 (t, J = 8 Hz, 2H) 2.08 (s, 3H), 2.57 (t, J = 8 Hz, 2H), 3.85-4.00 (m, 4H). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 8.7, 18.4, 23.0, 35.1, 64.0 (2 carbons), 108.3, 139.5, 144.1, 165.3, 165.8. **Anal. Calcd** for $C_{11}H_{14}O_5$: C, 58.40; H, 6.24. Found: C, 58.32; H, 6.12.

X-ray Crystallographic Studies of 83a

Table 1 Crystal Data and Structure Refinement

Empirical formula	$C_{19}H_{15}NO_5$
Formula weight	337.32
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, C2/c
Unit cell dimensions	a = 15.4222(11) Å b = 14.4208(10) Å β = 117.425(1) deg c = 15.7175(11) Å
Volume	3102.7(4) $Å^3$
Z, Calculated density	8, 1.444 Mg/m^3
Crystal size	0.51 × 0.32 × 0.26
θ range for data collection	2.05 to 25.00
Reflections collected / unique	7775 / 2745 [R(int) = 0.0173]
Completeness to θ = 25.00	100.0%
Goodness-of-fit on F^2	1.033
Final R indices [I>2sigma(I)]	R1 = 0.0392, wR2 = 0.0975
R indices (all data)	R1 = 0.0486, wR2 = 0.1038

Table 2 Bond lengths [Å] and angles [deg] for **83a**

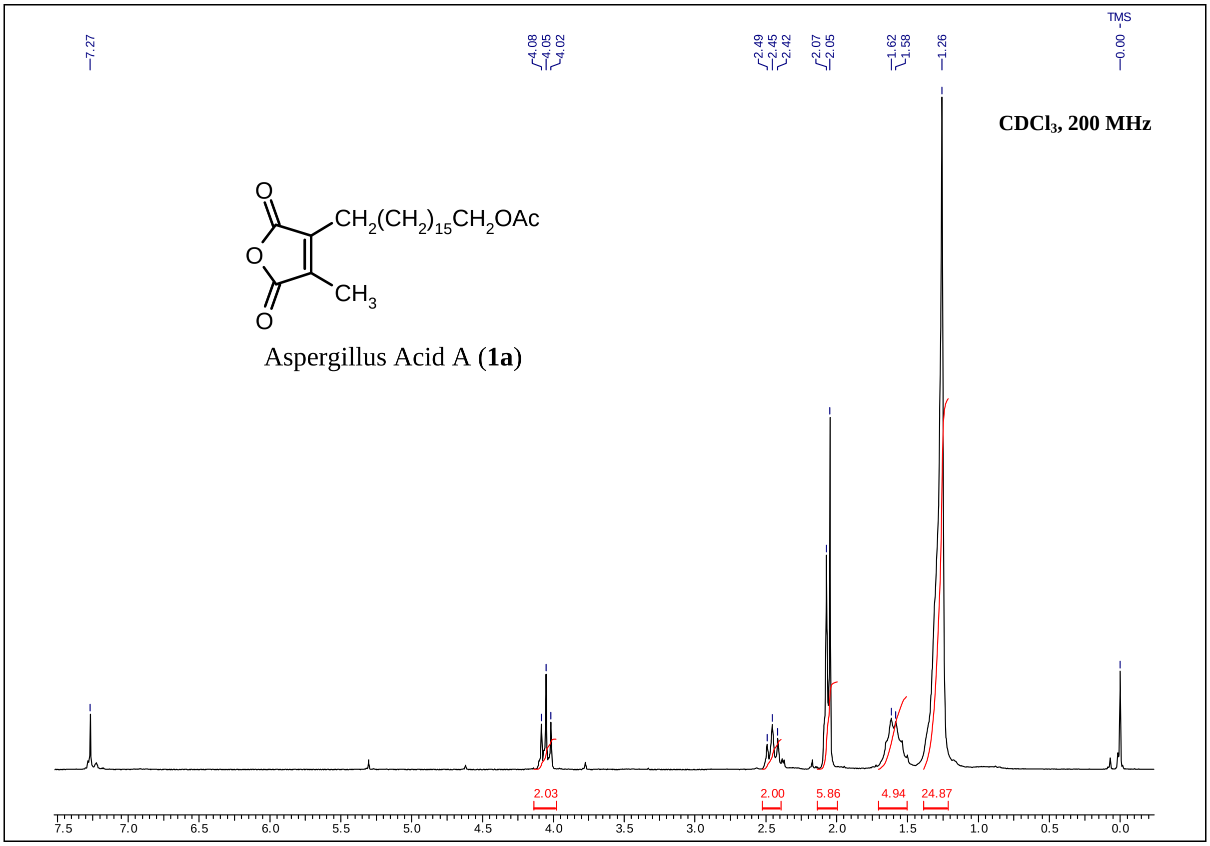
O(1)-C(14)	1.375(2)	C(6)-C(5)-C(4A)	111.57(13)
O(1)-C(2)	1.424(2)	C(6)-C(5)-H(5A)	109.3
C(2)-O(3)	1.416(2)	C(4A)-C(5)-H(5A)	109.3
C(2)-H(2A)	0.9700	C(6)-C(5)-H(5B)	109.3
C(2)-H(2B)	0.9700	C(4A)-C(5)-H(5B)	109.3
C(3A)-C(4)	1.360(2)	H(5A)-C(5)-H(5B)	108.0
C(3A)-O(3)	1.373(2)	N(6A)-C(6)-C(5)	108.05 (13)
C(3A)-C(14)	1.376(2)	N(6A)-C(6)-H(6A)	110.1
C(4)-C(4A)	1.401(2)	C(5)-C(6)-H(6A)	110.1
C(4)-H(4)	0.9300	N(6A)-C(6)-H(6B)	110.1
C(4A)-C(12)	1.397(2)	C(5)-C(6)-H(6B)	110.1
C(4A)-C(5)	1.507(2)	H(6A)-C(6)-H(6B)	108.4
C(5)-C(6)	1.506(2)	C(7)-N(6A)-C(6)	122.85(13)
C(5)-H(5A)	0.9700	C(7)-N(6A)-C(11B)	114.06(13)
C(5)-H(5B)	0.9700	C(6)-N(6A)-C(11B)	119.06(13)
C(6)-N(6A)	1.4537(19)	O(19)-C(7)-N(6A)	125.58(16)
C(6)-H(6A)	0.9700	O(19)-C(7)-C(7A)	128.48(15)
C(6)-H(6B)	0.9700	N(6A)-C(7)-C(7A)	105.93(14)
N(6A)-C(7)	1.362(2)	C(11A)-C(7A)-C(8)	121.50(16)
N(6A)-C(11B)	1.4569(19)	C(11A)-C(7A)-C(7)	109.43(14)
C(7)-O(19)	1.221(2)	C(8)-C(7A)-C(7)	129.07(16)
C(7)-C(7A)	1.478(2)	C(9)-C(8)-C(7A)	118.17(18)
C(7A)-C(11A)	1.381(2)	C(9)-C(8)-H(8)	120.9
C(7A)-C(8)	1.386(2)	C(7A)-C(8)-H(8)	120.9
C(8)-C(9)	1.379(3)	C(8)-C(9)-C(10)	120.35(17)
C(8)-H(8)	0.9300	C(8)-C(9)-H(9)	119.8
C(9)-C(10)	1.384(3)	C(10)-C(9)-H(9)	119.8
C(9)-H(9)	0.9300	C(11)-C(10)-C(9)	121.62(17)
C(10)-C(11)	1.384(2)	C(11)-C(10)-H(10)	119.2
C(10)-H(10)	0.9300	C(9)-C(10)-H(10)	119.2
C(11)-C(11A)	1.385(2)	C(10)-C(11)-C(11A)	117.94(17)
C(11)-H(11)	0.9300	C(10)-C(11)-H(11)	121.0
C(11A)-C(11B)	1.530(2)	C(11A)-C(11)-H(11)	121.0
C(11B)-C(12)	1.537(2)	C(7A)-C(11A)-C(11)	120.40(15)
C(11B)-C(15)	1.545(2)	C(7A)-C(11A)-C(11B)	108.76(13)
C(12)-C(13)	1.407(2)	C(11)-C(11A)-C(11B)	130.80(15)
C(13)-C(14)	1.363(2)	N(6A)-C(11B)-C(11A)	101.51(12)
C(13)-H(13)	0.9300	N(6A)-C(11B)-C(12)	110.86(12)
C(15)-O(18)	1.1954(19)	C(11A)-C(11B)-C(12)	115.93(12)
C(15)-O(17)	1.307(2)	N(6A)-C(11B)-C(15)	111.96(12)
C(16)-O(17)	1.456(2)	C(11A)-C(11B)-C(15)	106.67(12)
C(16)-H(16A)	0.9600	C(12)-C(11B)-C(15)	109.68(12)
C(16)-H(16B)	0.9600	C(4A)-C(12)-C(13)	120.36(15)
C(16)-H(16C)	0.9600	C(4A)-C(12)-C(11B)	120.38(14)
C(14)-O(1)-C(2)	105.52(14)	C(13)-C(12)-C(11B)	119.25(13)
O(3)-C(2)-O(1)	108.89(15)	C(14)-C(13)-C(12)	117.77(15)
O(3)-C(2)-H(2A)	109.9	C(14)-C(13)-H(13)	121.1
O(1)-C(2)-H(2A)	109.9	C(12)-C(13)-H(13)	121.1
O(3)-C(2)-H(2B)	109.9	C(13)-C(14)-O(1)	128.21(16)
O(1)-C(2)-H(2B)	109.9	C(13)-C(14)-C(3A)	121.96(16)
H(2A)-C(2)-H(2B)	108.3	O(1)-C(14)-C(3A)	109.83(15)
C(4)-C(3A)-O(3)	128.56(16)	O(18)-C(15)-O(17)	124.02(15)

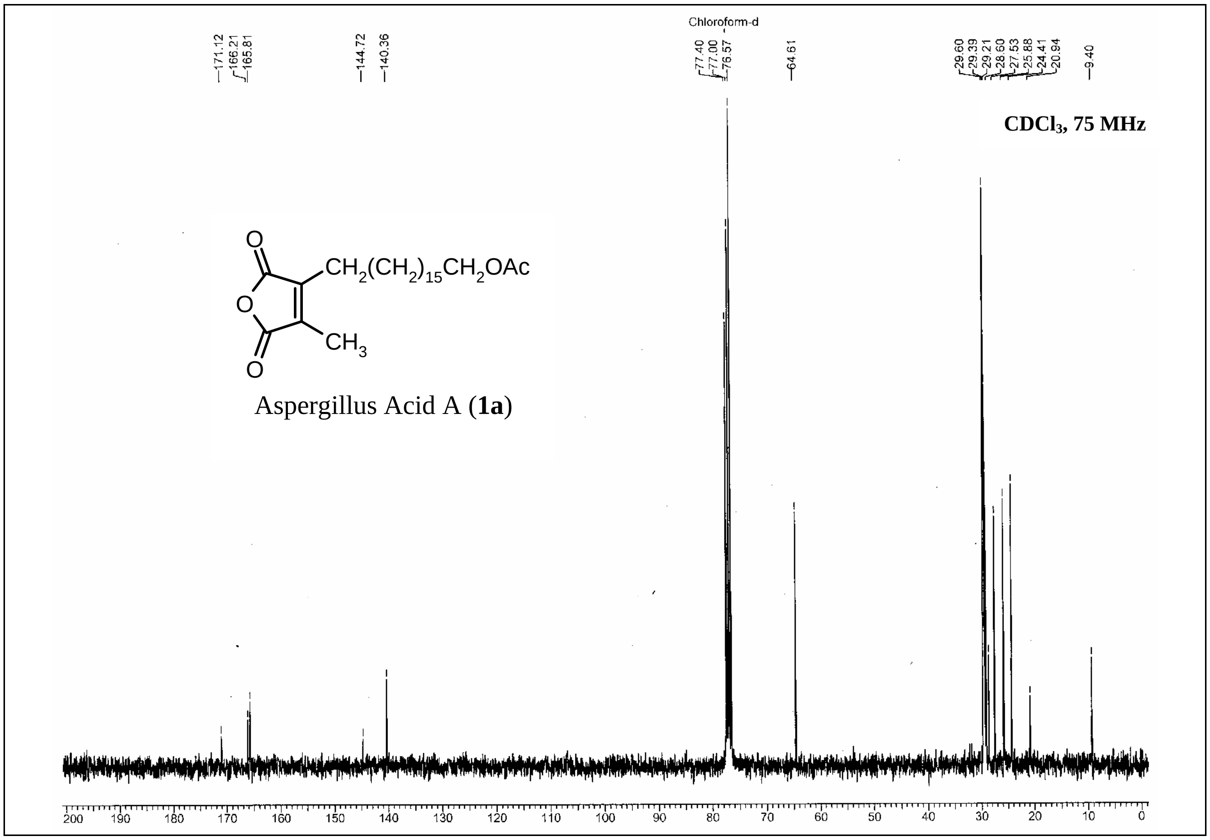
C(4)-C(3A)-C(14)	121.48(16)	O(18)-C(15)-C(11B)	122.78(15)
O(3)-C(3A)-C(14)	109.96(16)	O(17)-C(15)-C(11B)	113.18(13)
C(3A)-O(3)-C(2)	105.75(14)	O(17)-C(16)-H(16A)	109.5
C(3A)-C(4)-C(4A)	118.52(15)	O(17)-C(16)-H(16B)	109.5
C(3A)-C(4)-H(4)	120.7	H(16A)-C(16)-H(16B)	109.5
C(4A)-C(4)-H(4)	120.7	O(17)-C(16)-H(16C)	109.5
C(12)-C(4A)-C(4)	119.91(15)	H(16A)-C(16)-H(16C)	109.5
C(12)-C(4A)-C(5)	122.37(15)	H(16B)-C(16)-H(16C)	109.5
C(4)-C(4A)-C(5)	117.73(14)	C(15)-O(17)-C(16)	116.60(14)

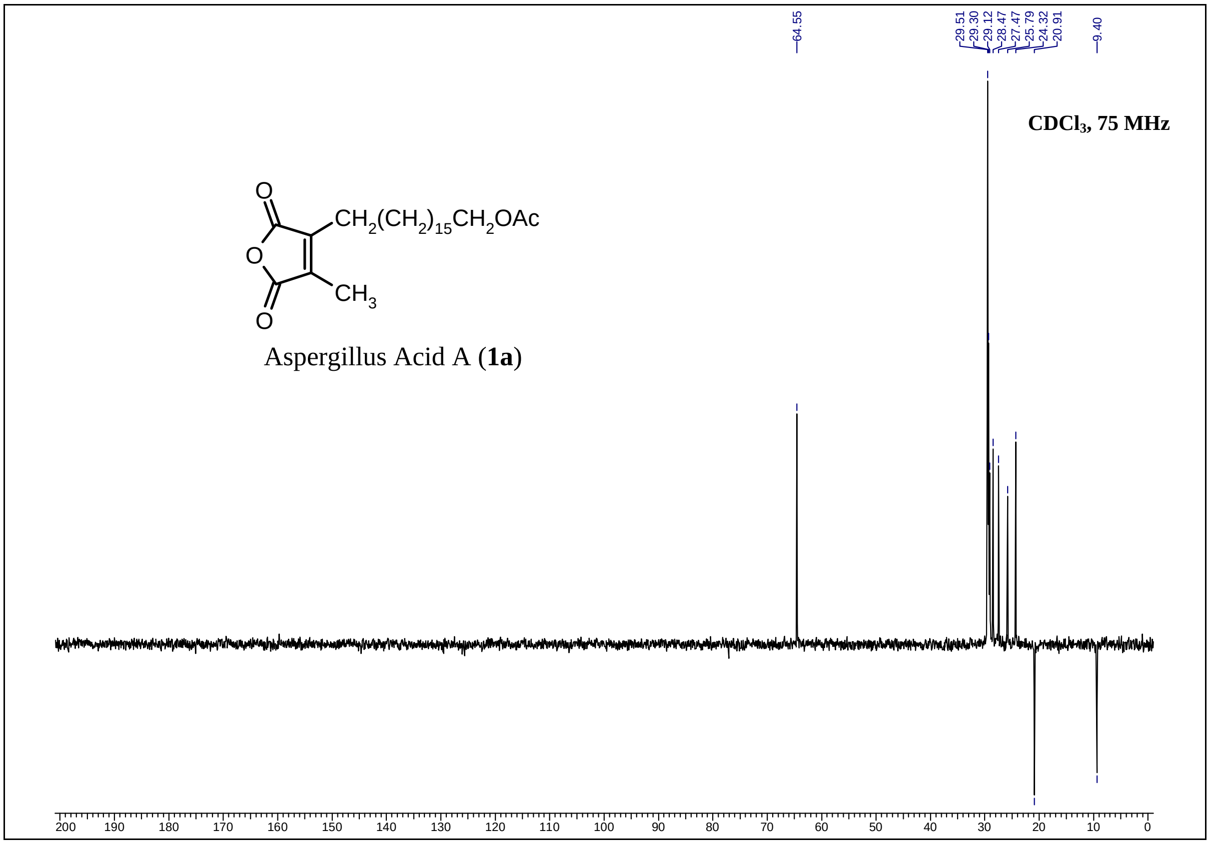
Table 3 Torsion angles [deg] for **83a**

C(14)-O(1)-C(2)-O(3)	1.9(2)	C(7)-N(6A)-C(11B)-C(15)	118.31(14)
C(4)-C(3A)-O(3)-C(2)	-177.88(19)	C(6)-N(6A)-C(11B)-C(15)	-83.60(16)
C(14)-C(3A)-O(3)-C(2)	1.8(2)	C(7A)-C(11A)-C(11B)-N(6A)	-1.77(15)
O(1)-C(2)-O(3)-C(3A)	-2.3(2)	C(11)-C(11A)-C(11B)-N(6A)	175.79(15)
O(3)-C(3A)-C(4)-C(4A)	-179.98(17)	C(7A)-C(11A)-C(11B)-C(12)	118.45(14)
C(14)-C(3A)-C(4)-C(4A)	0.3(3)	C(11)-C(11A)-C(11B)-C(12)	-64.0(2)
C(3A)-C(4)-C(4A)-C(12)	0.5(2)	C(7A)-C(11A)-C(11B)-C(15)	-119.11(14)
C(3A)-C(4)-C(4A)-C(5)	-178.99(15)	C(11)-C(11A)-C(11B)-C(15)	58.4(2)
C(12)-C(4A)-C(5)-C(6)	-22.7(2)	C(4)-C(4A)-C(12)-C(13)	-0.8(2)
C(4)-C(4A)-C(5)-C(6)	156.83(15)	C(5)-C(4A)-C(12)-C(13)	178.70(15)
C(4A)-C(5)-C(6)-N(6A)	49.74(18)	C(4)-C(4A)-C(12)-C(11B)	-179.32(14)
C(5)-C(6)-N(6A)-C(7)	93.61(18)	C(5)-C(4A)-C(12)-C(11B)	0.2(2)
C(5)-C(6)-N(6A)-C(11B)	-62.46(17)	N(6A)-C(11B)-C(12)-C(4A)	-6.44(19)
C(6)-N(6A)-C(7)-O(19)	15.9(2)	C(11A)-C(11B)-C(12)-C(4A)	-121.45(15)
C(11B)-N(6A)-C(7)-O(19)	173.09(15)	C(15)-C(11B)-C(12)-C(4A)	117.70(15)
C(6)-N(6A)-C(7)-C(7A)	-163.05(13)	N(6A)-C(11B)-C(12)-C(13)	175.01(13)
C(11B)-N(6A)-C(7)-C(7A)	-5.90(17)	C(11A)-C(11B)-C(12)-C(13)	60.00(19)
O(19)-C(7)-C(7A)-C(11A)	-174.48(16)	C(15)-C(11B)-C(12)-C(13)	-60.84(17)
N(6A)-C(7)-C(7A)-C(11A)	4.47(17)	C(4A)-C(12)-C(13)-C(14)	0.2(2)
O(19)-C(7)-C(7A)-C(8)	4.6(3)	C(11B)-C(12)-C(13)-C(14)	178.75(14)
N(6A)-C(7)-C(7A)-C(8)	-176.47(16)	C(12)-C(13)-C(14)-O(1)	-179.34(17)
C(11A)-C(7A)-C(8)-C(9)	-1.2(3)	C(12)-C(13)-C(14)-C(3A)	0.7(3)
C(7)-C(7A)-C(8)-C(9)	179.80(16)	C(2)-O(1)-C(14)-C(13)	179.3(2)
C(7A)-C(8)-C(9)-C(10)	0.3(3)	C(2)-O(1)-C(14)-C(3A)	-0.8(2)
C(8)-C(9)-C(10)-C(11)	0.3(3)	C(4)-C(3A)-C(14)-C(13)	-1.0(3)
C(9)-C(10)-C(11)-C(11A)	-0.1(3)	O(3)-C(3A)-C(14)-C(13)	179.30(16)
C(8)-C(7A)-C(11A)-C(11)	1.5(2)	C(4)-C(3A)-C(14)-O(1)	179.05(16)
C(7)-C(7A)-C(11A)-C(11)	-179.38(13)	O(3)-C(3A)-C(14)-O(1)	-0.7(2)
C(8)-C(7A)-C(11A)-C(11B)	179.33(14)	N(6A)-C(11B)-C(15)-O(18)	-179.44(15)
C(7)-C(7A)-C(11A)-C(11B)	-1.53(17)	C(11A)-C(11B)-C(15)-O(18)	-69.2(2)
C(10)-C(11)-C(11A)-C(7A)	-0.8(2)	C(12)-C(11B)-C(15)-O(18)	57.1(2)
C(10)-C(11)-C(11A)-C(11B)	-178.08(15)	N(6A)-C(11B)-C(15)-O(17)	-0.68(18)
C(7)-N(6A)-C(11B)-C(11A)	4.87(16)	C(11A)-C(11B)-C(15)-O(17)	109.53(15)
C(6)-N(6A)-C(11B)-C(11A)	162.95(12)	C(12)-C(11B)-C(15)-O(17)	-124.19(14)
C(7)-N(6A)-C(11B)-C(12)	-118.85(14)	O(18)-C(15)-O(17)-C(16)	0.4(3)
C(6)-N(6A)-C(11B)-C(12)	39.23(18)	C(11B)-C(15)-O(17)-C(16)	-178.35(16)

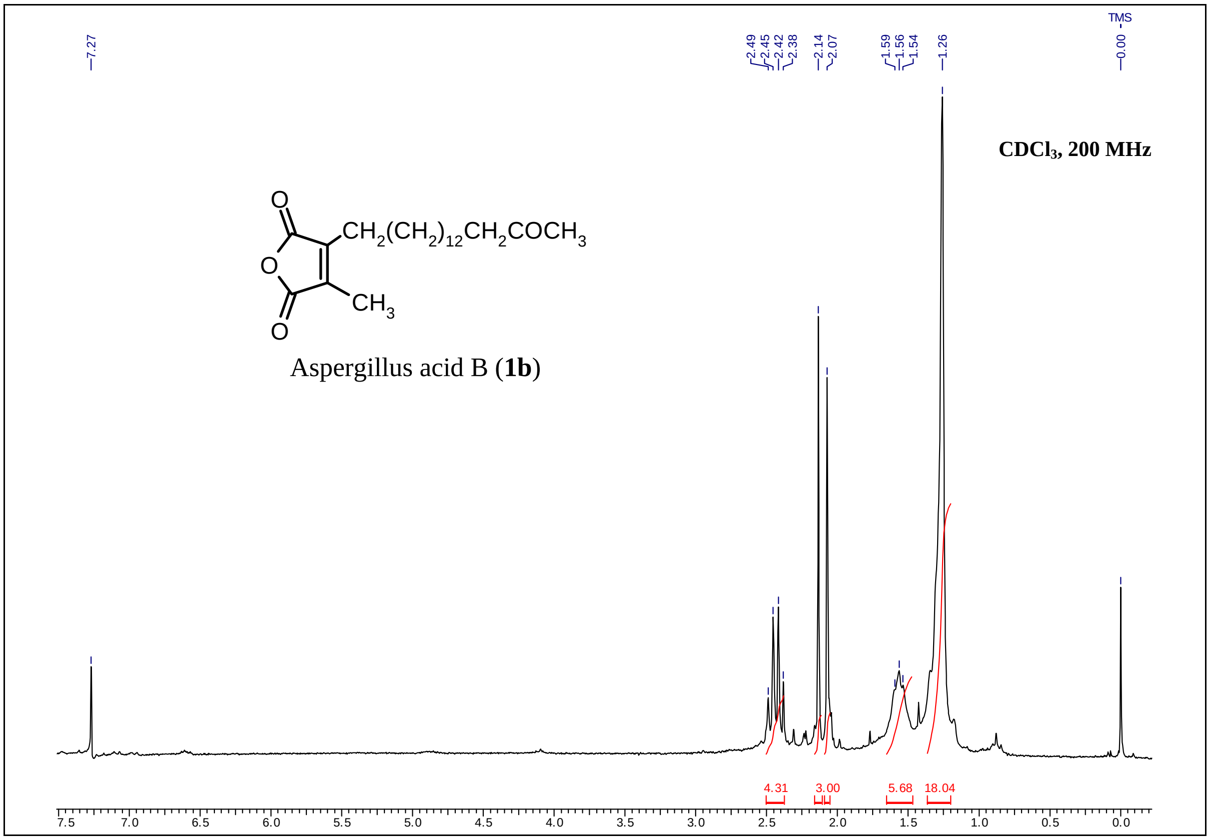
2B.7 Spectra Section


7.27
4.08
4.05
4.02
2.49
2.45
2.42
2.07
2.05
1.62
1.58
1.26
TMS
0.00
CDCl$_3$, 200 MHz
O
CH$_2$(CH$_2$)$_{15}$CH$_2$OAc
O
CH$_3$
O
Aspergillus Acid A (**1a**)
2.03
2.00
5.86
4.94
24.87
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

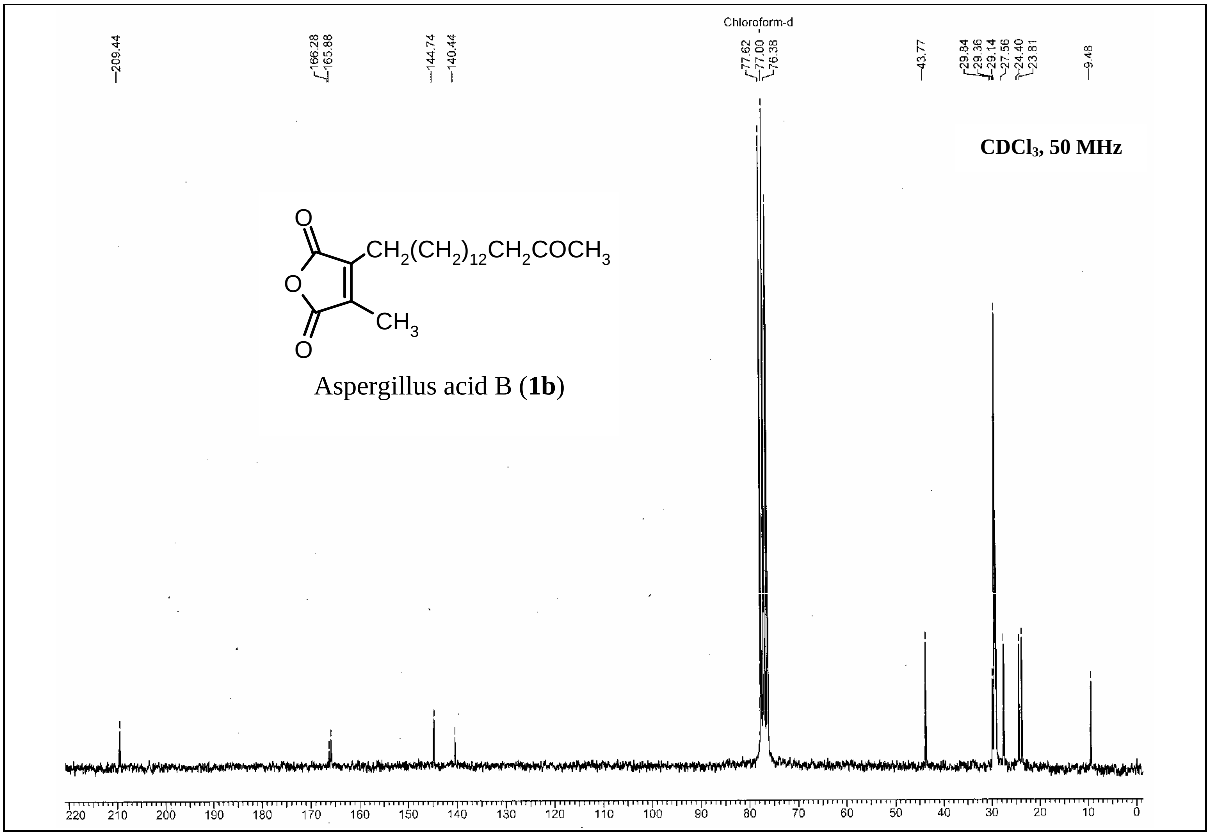

171.12
166.21
165.81
144.72
140.36
Chloroform-d
77.40
77.00
76.57
64.61
29.60
29.39
29.21
28.60
27.53
25.88
24.41
20.94
9.40
CDCl$_3$, 75 MHz
O
CH$_2$(CH$_2$)$_{15}$CH$_2$OAc
O
CH$_3$
O
Aspergillus Acid A (**1a**)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

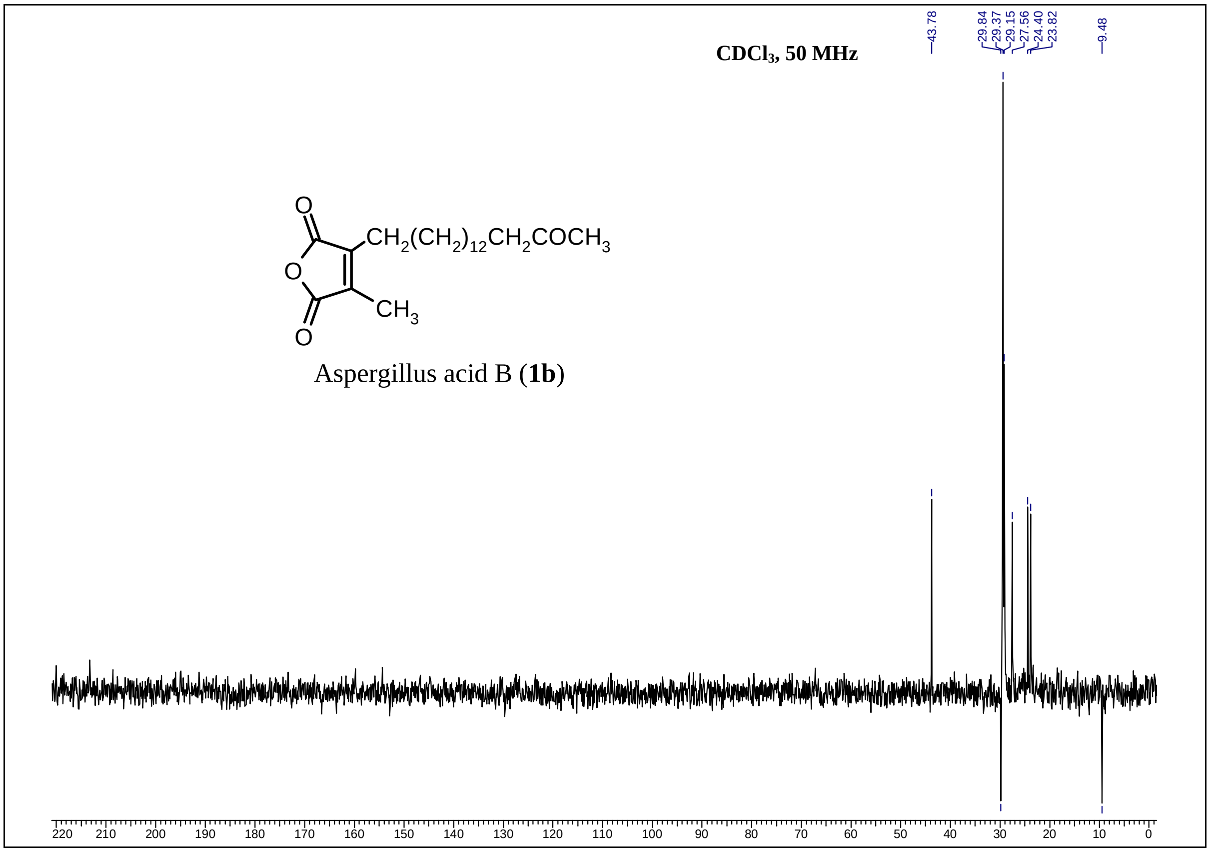


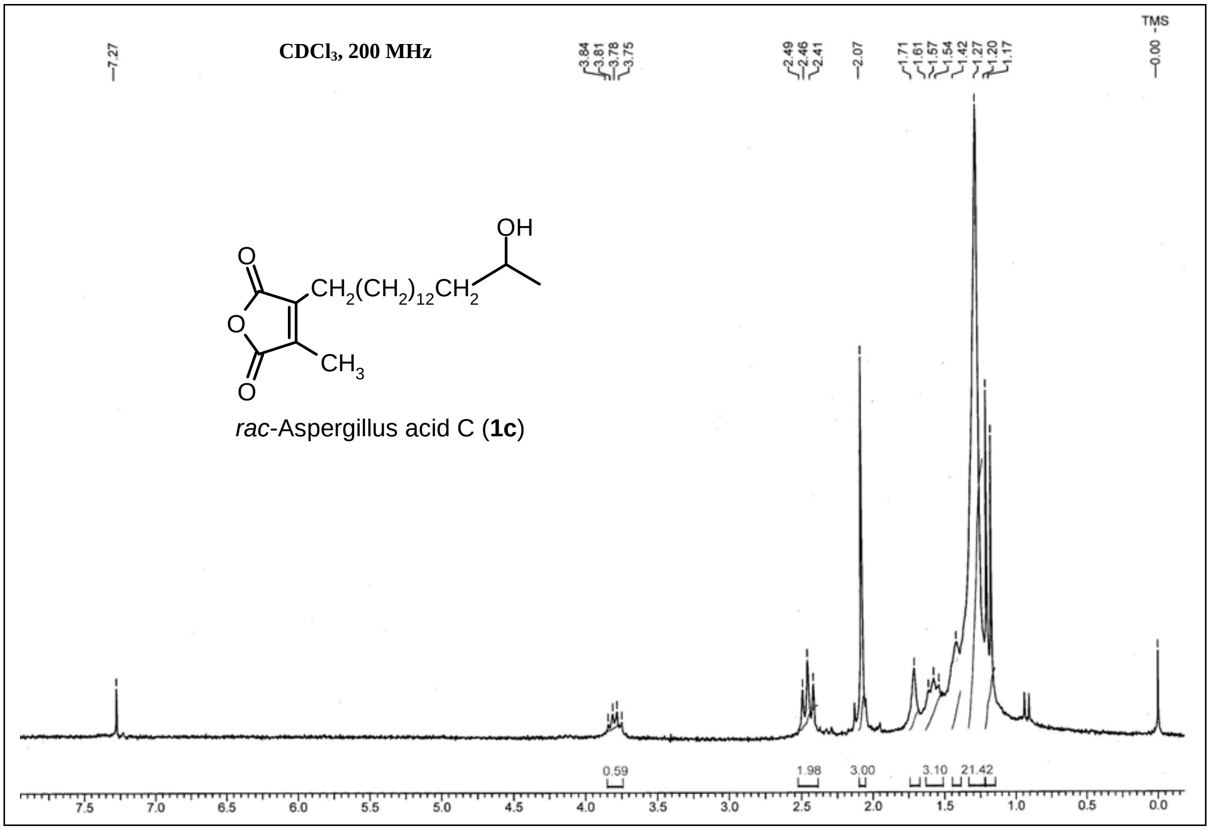
Aspergillus Acid A (**1a**)



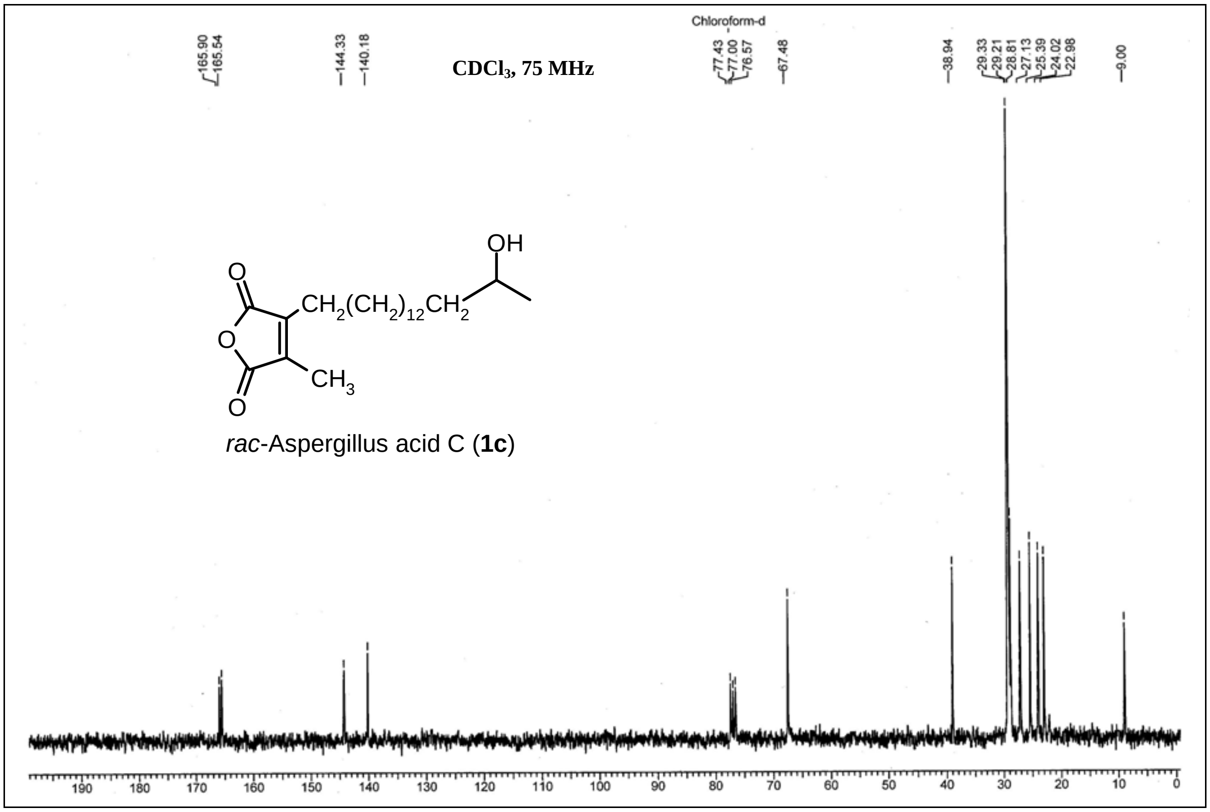
Aspergillus acid B (**1b**)


Chloroform-d
209.44
166.28
165.88
144.74
140.44
77.62
77.00
76.38
43.77
29.84
29.36
29.14
27.56
24.40
23.81
9.48
CDCl₃, 50 MHz
O
O
O
CH2(CH2)12CH2COCH3
CH3
Aspergillus acid B (1b)
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

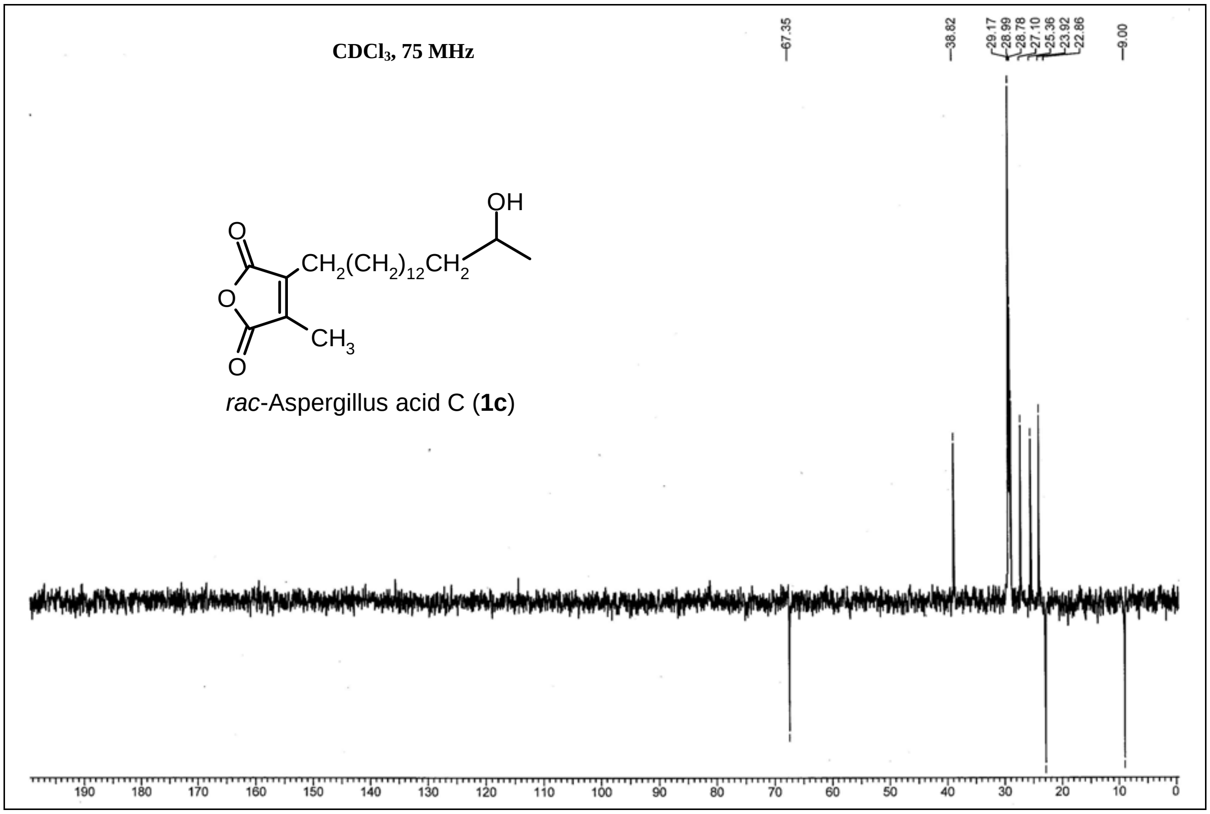

43.78
29.84
29.37
29.15
27.56
24.40
23.82
9.48
CDCl₃, 50 MHz
O
O
O
CH2(CH2)12CH2COCH3
CH3
Aspergillus acid B (1b)
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0



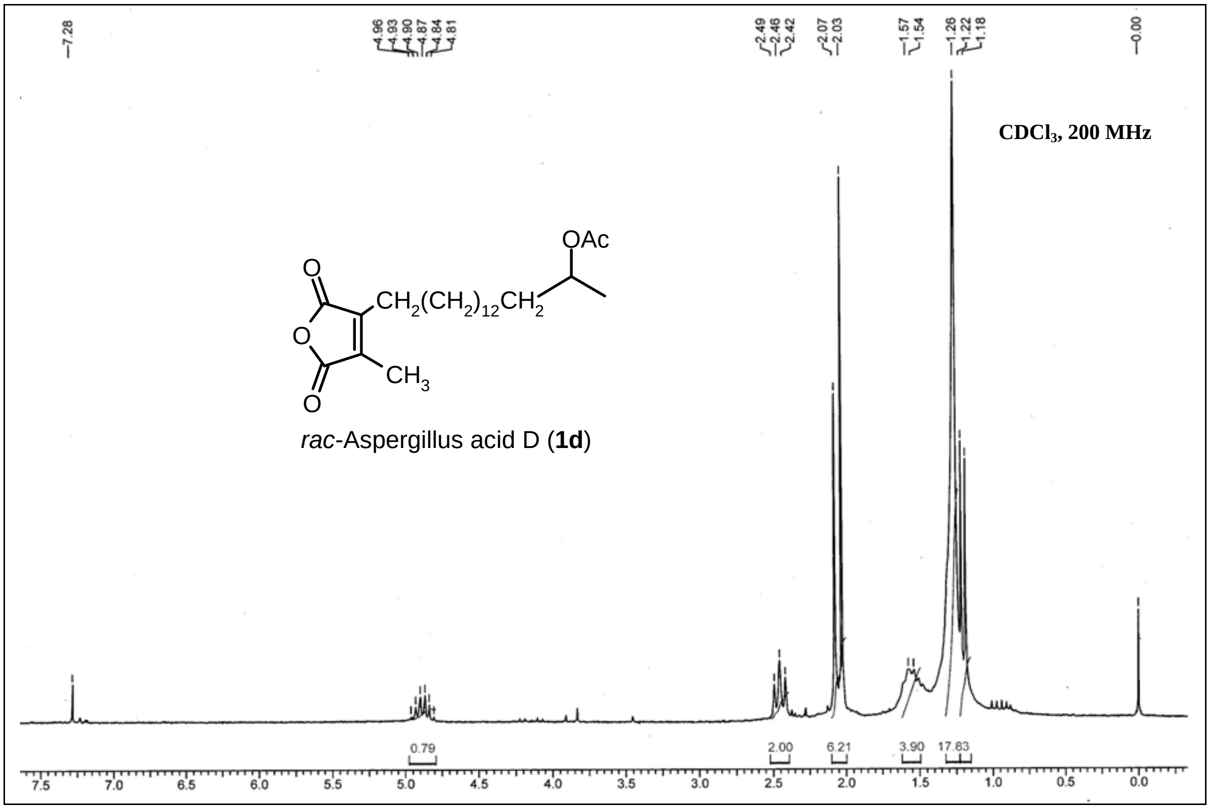
rac-Aspergillus acid C (**1c**)



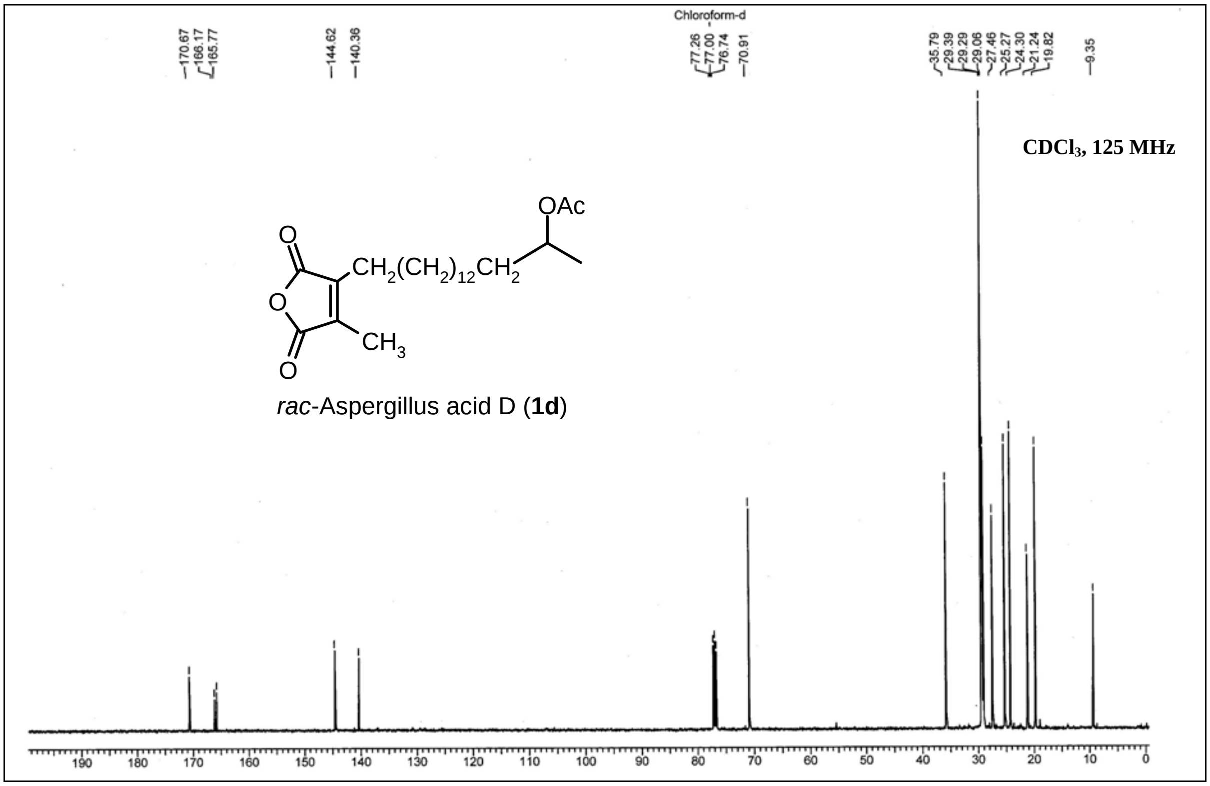
rac-Aspergillus acid C (**1c**)



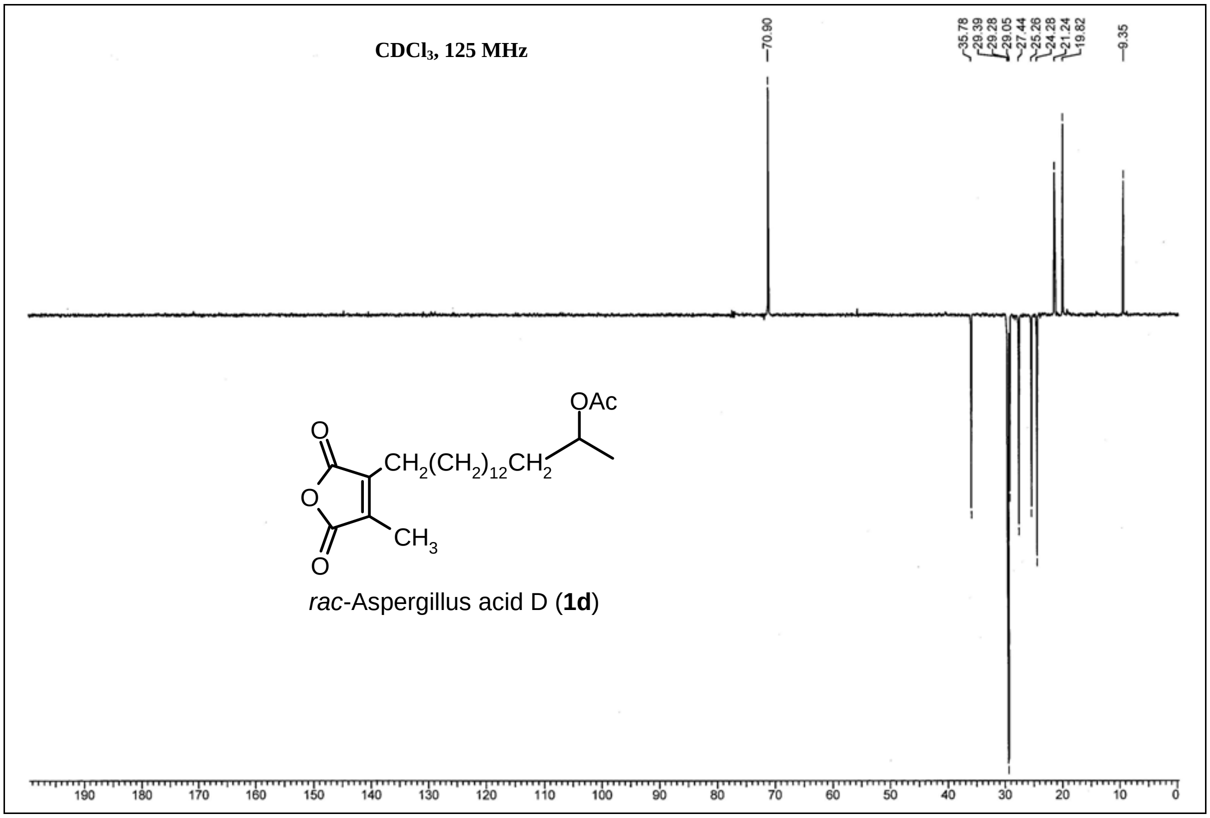
rac-Aspergillus acid C (**1c**)



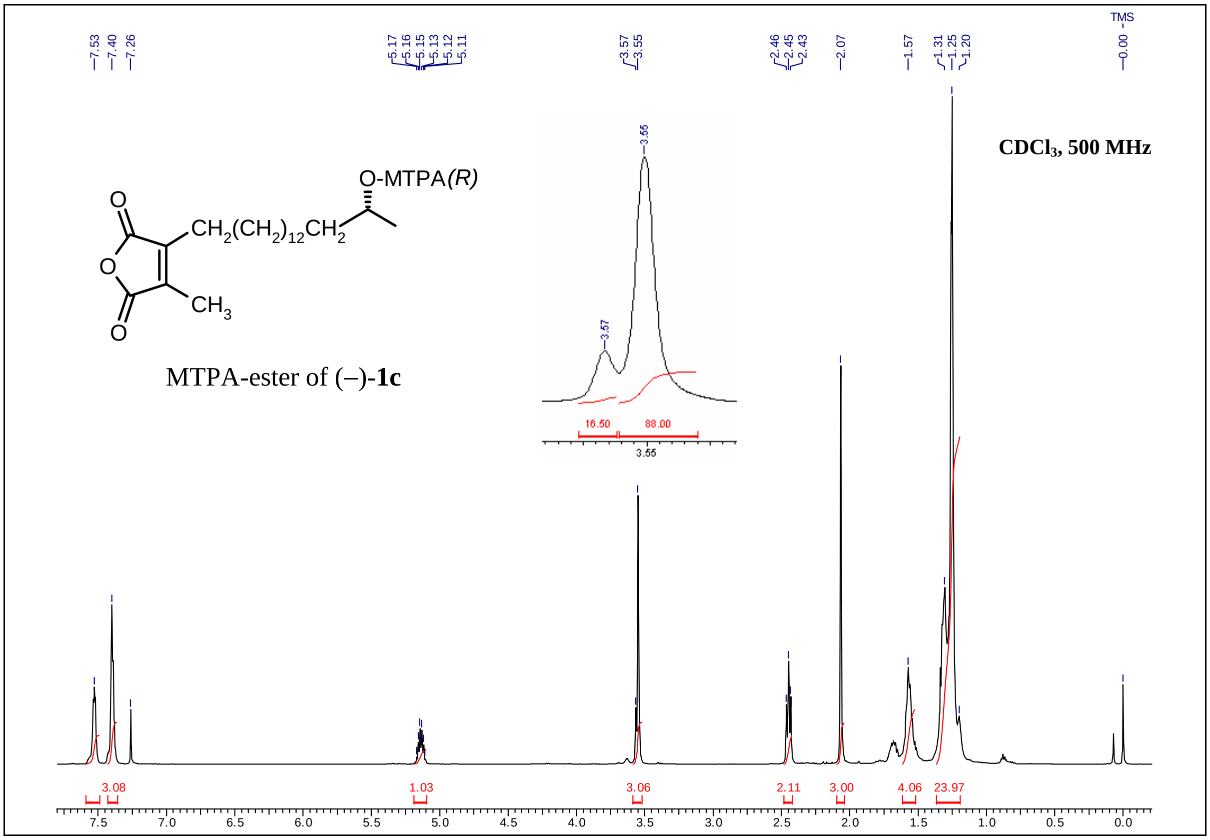
rac-Aspergillus acid D (**1d**)

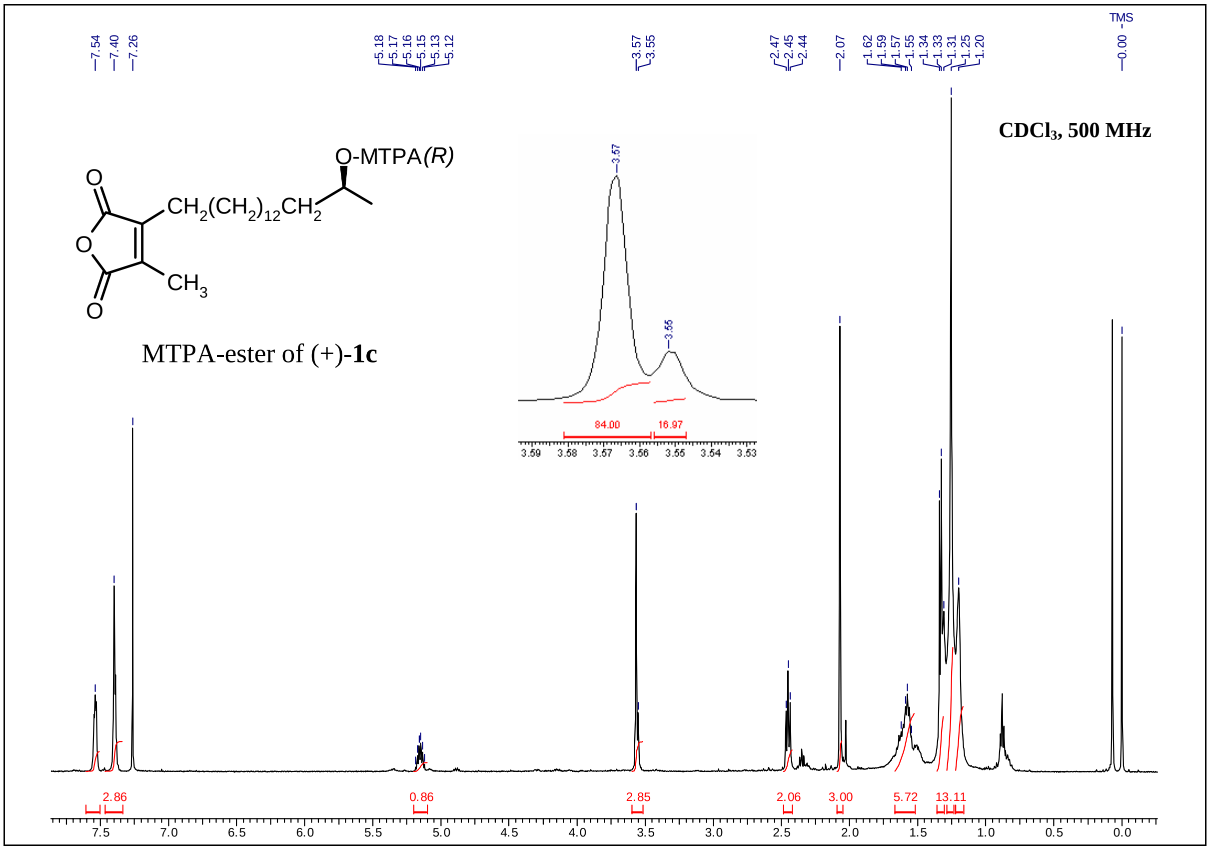


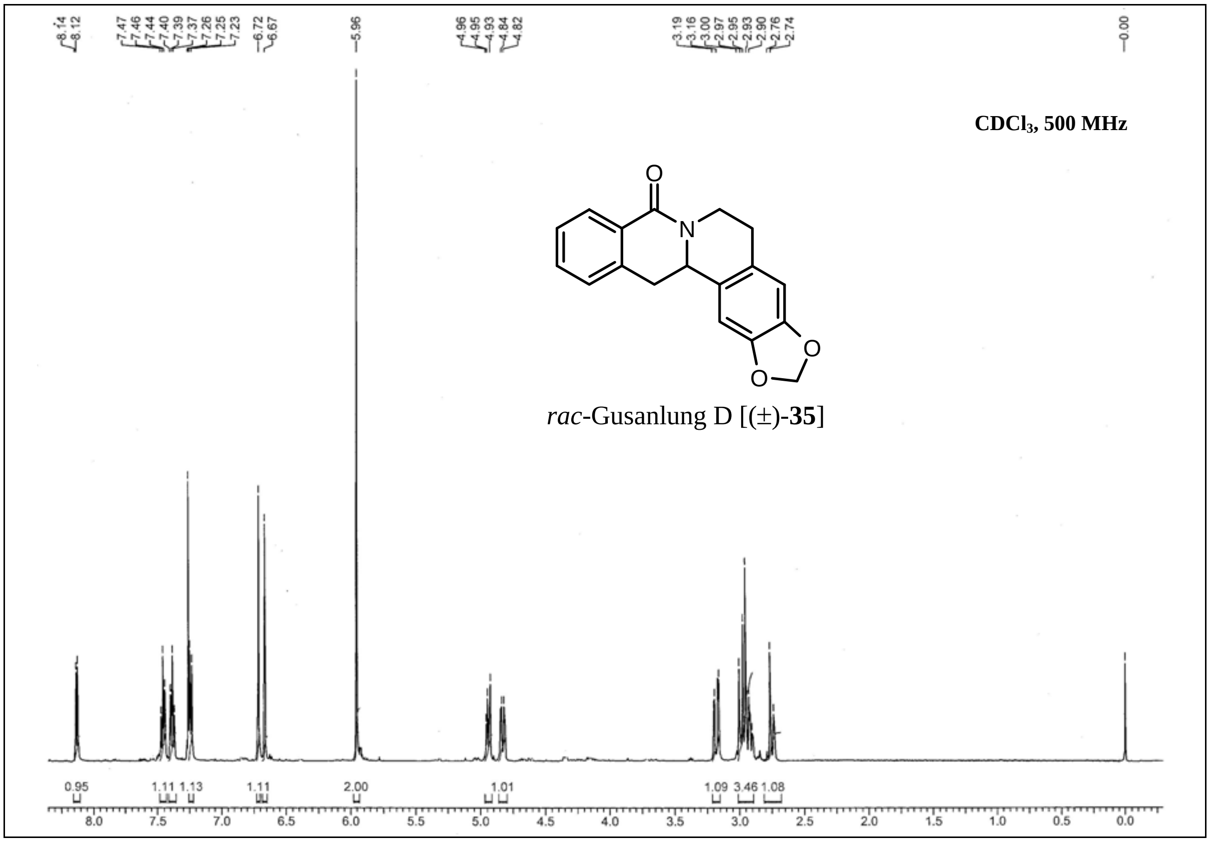
rac-Aspergillus acid D (**1d**)



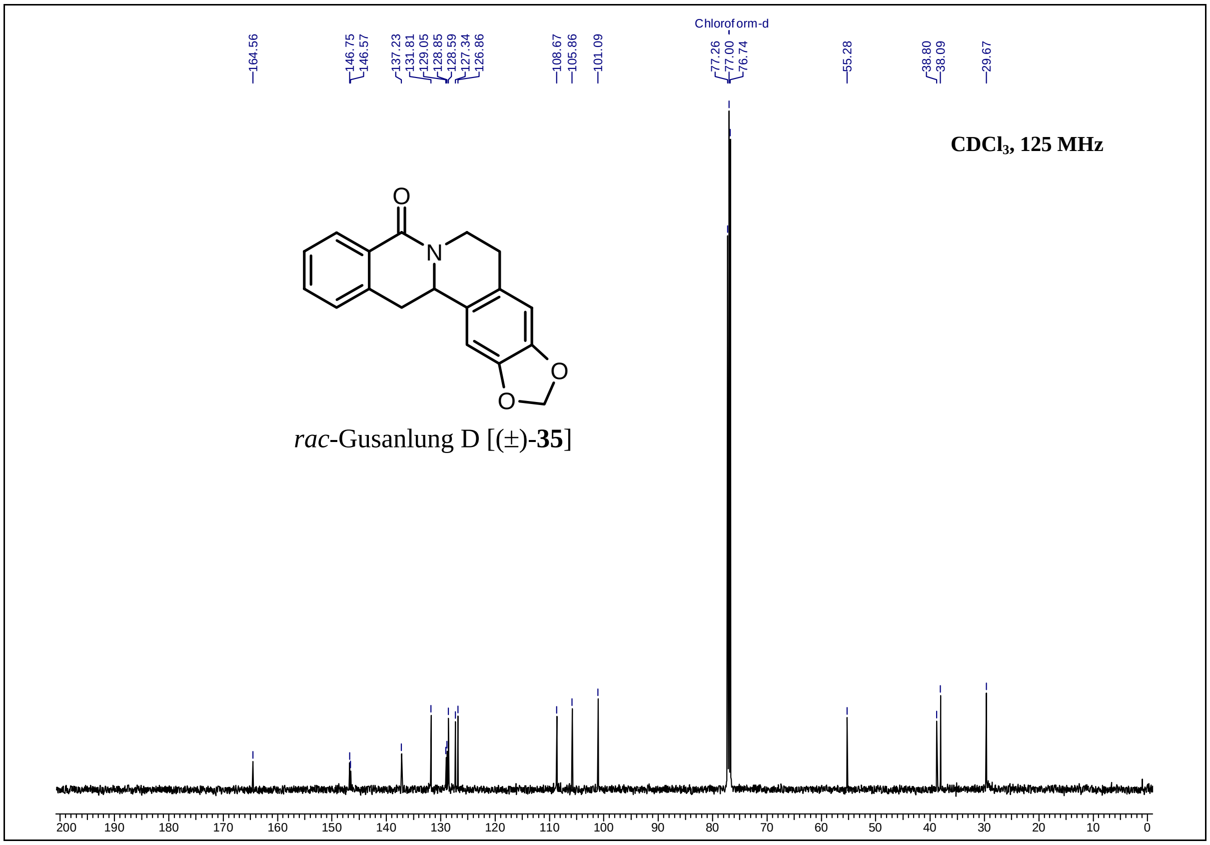
rac-Aspergillus acid D (**1d**)


7.53
7.40
7.26
5.17
5.16
5.15
5.13
5.12
5.11
3.57
3.55
2.46
2.45
2.43
2.07
1.57
1.31
1.25
1.20
TMS
0.00
CDCl3, 500 MHz
O-MTPA(R)
CH2(CH2)12CH2
CH3
O
O
O
MTPA-ester of (–)-1c
16.50
88.00
3.55
3.08
1.03
3.06
2.11
3.00
4.06
23.97
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

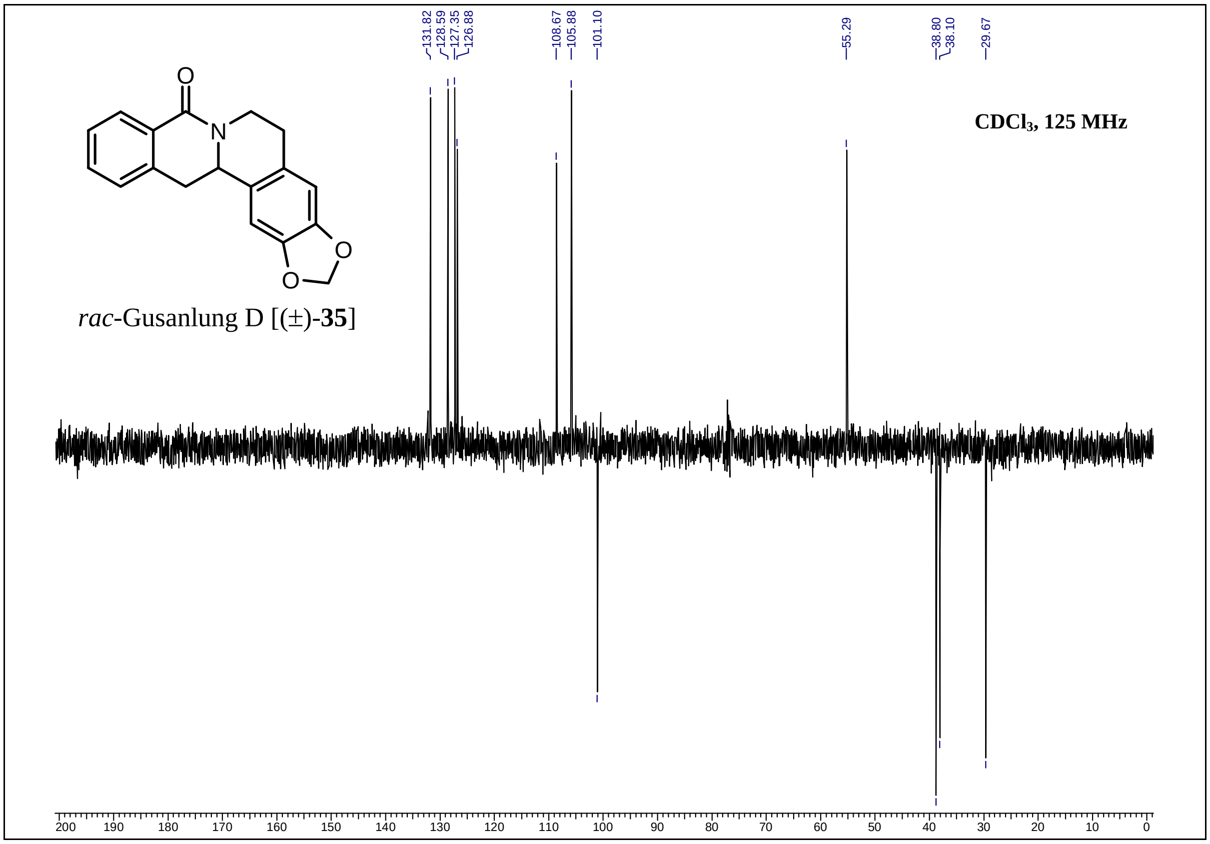

7.54
7.40
7.26
5.18
5.17
5.16
5.15
5.13
5.12
3.57
3.55
2.47
2.45
2.44
2.07
1.62
1.59
1.57
1.55
1.34
1.33
1.31
1.25
1.20
TMS
0.00
CDCl3, 500 MHz
O-MTPA(R)
CH2(CH2)12CH2
CH3
O
O
O
MTPA-ester of (+)-1c
84.00
16.97
3.59
3.58
3.57
3.56
3.55
3.54
3.53
2.86
0.86
2.85
2.06
3.00
5.72
13.11
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

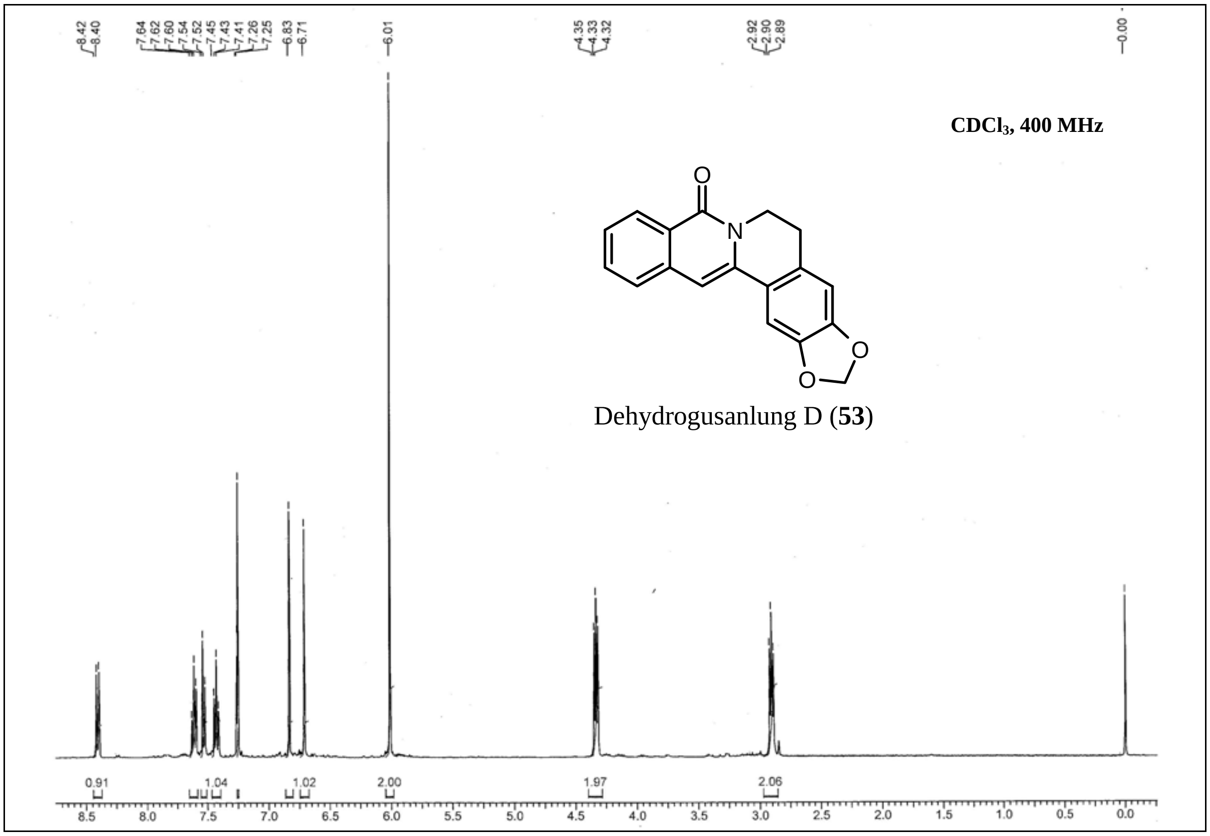


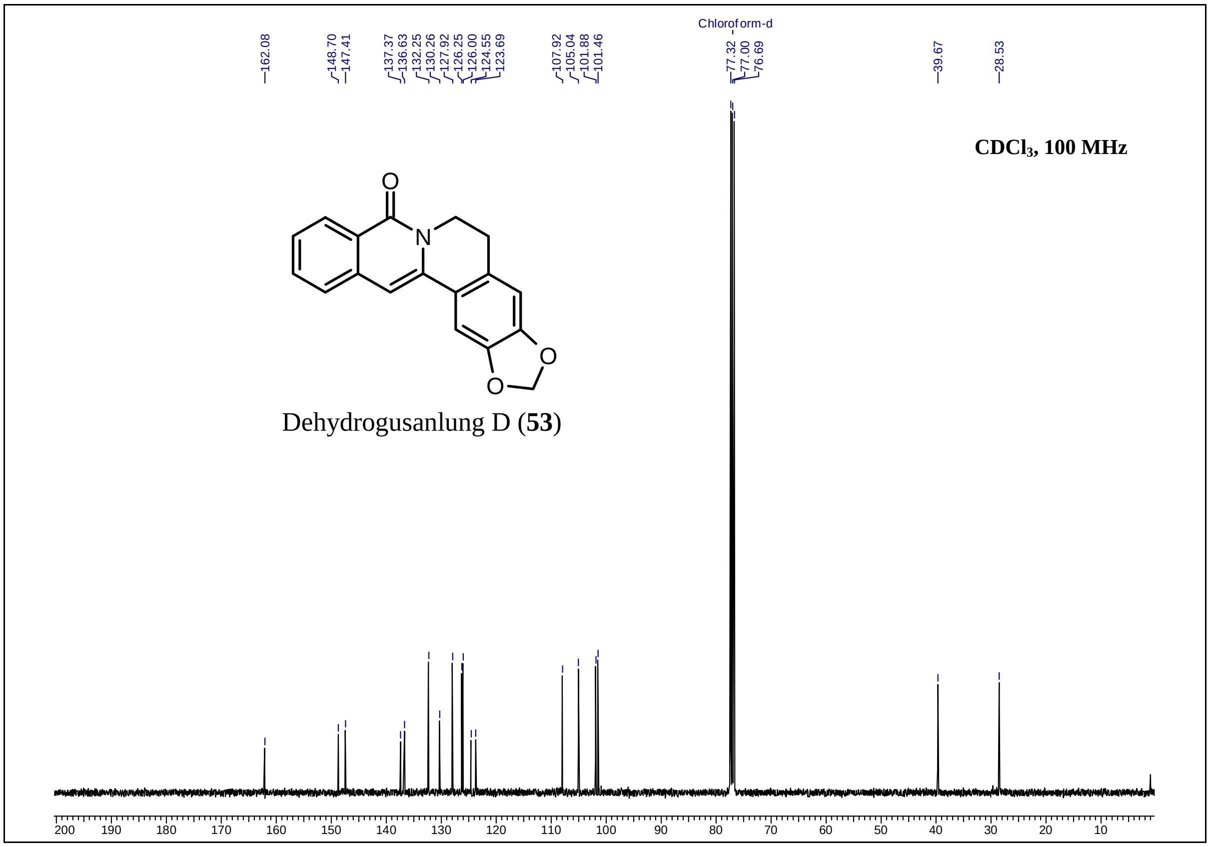
rac-Gusanlung D [(±)-**35**]

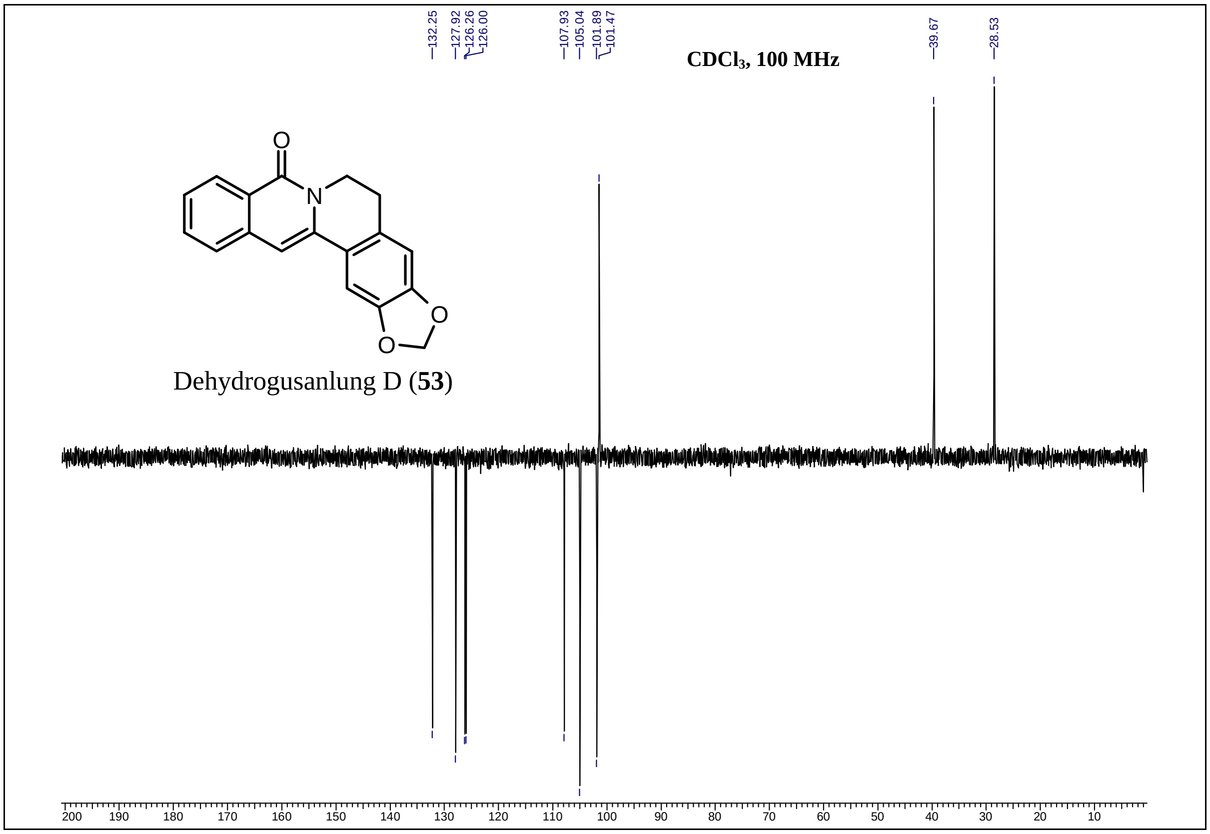


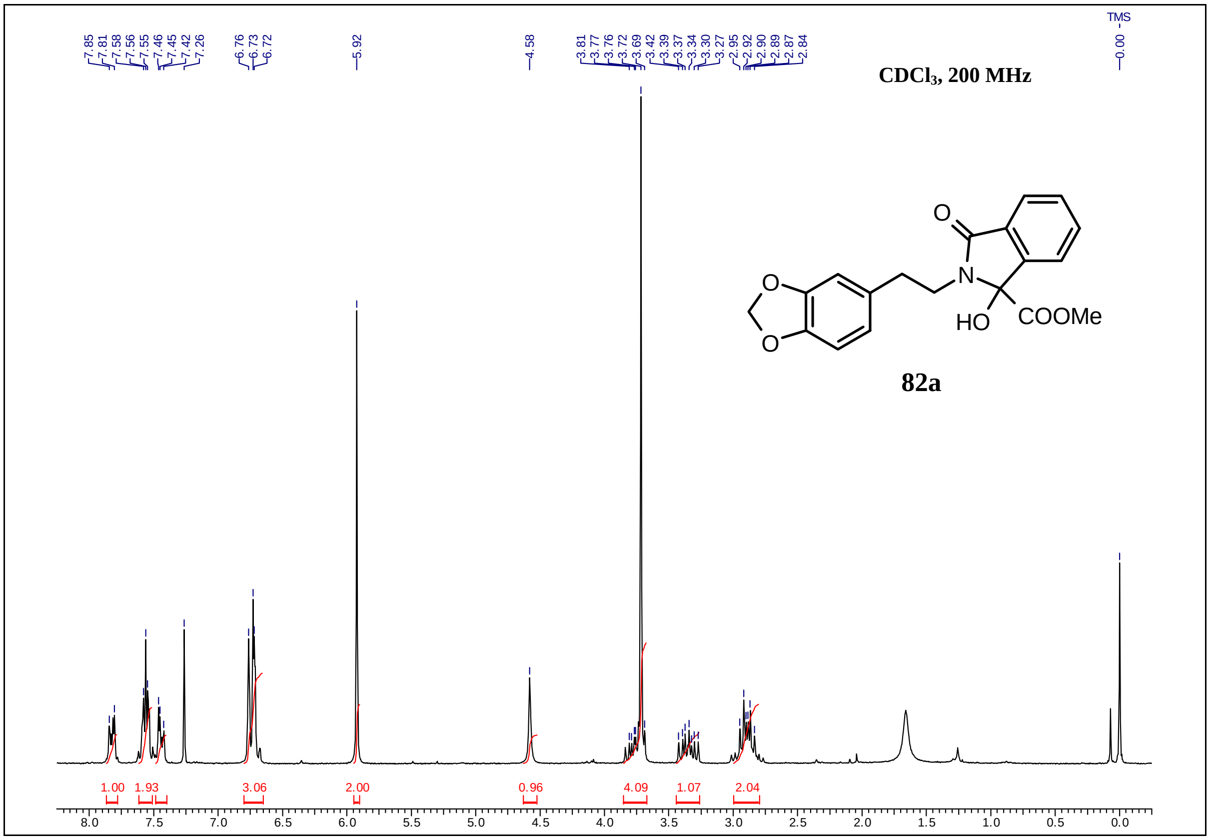
rac-Gusanlung D [(±)-**35**]


rac-Gusanlung D [(±)-**35**]

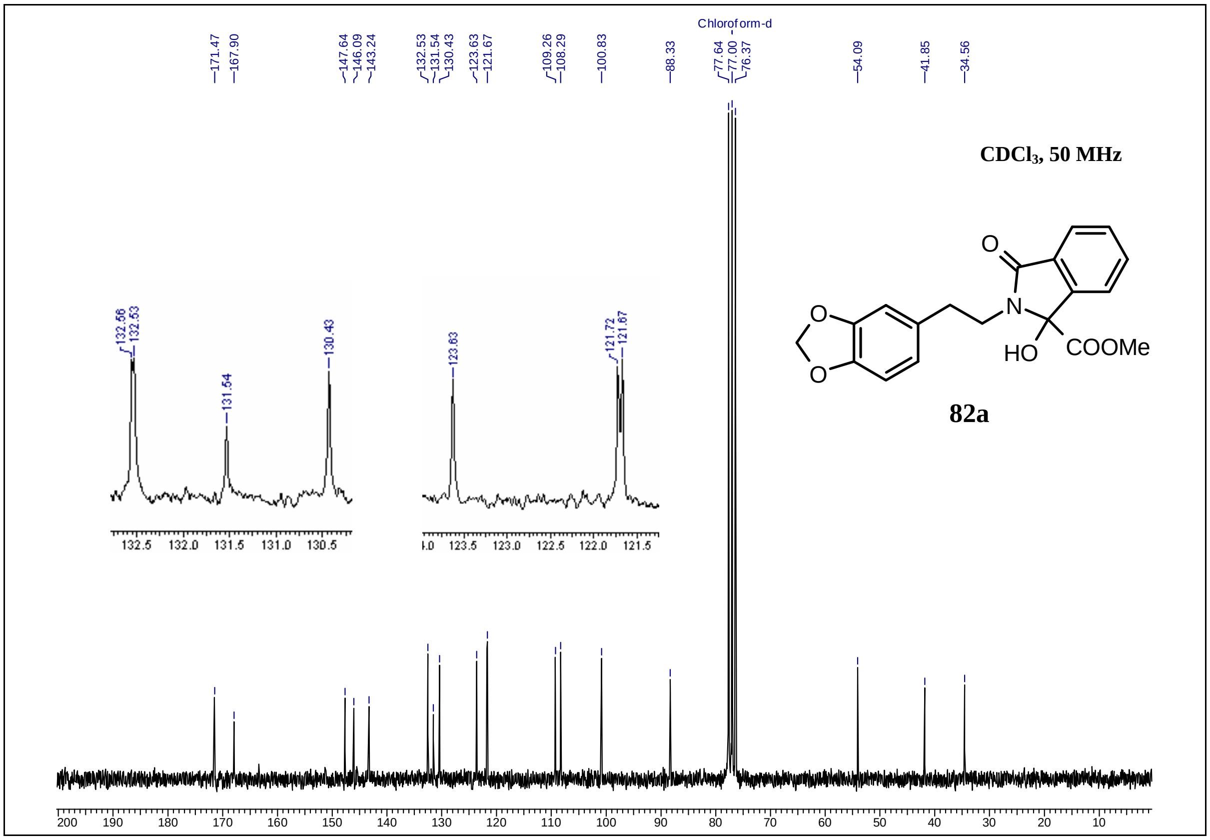

Dehydrogusanlung D (**53**)


162.08
148.70
147.41
137.37
136.63
132.25
130.26
127.92
126.25
126.00
124.55
123.69
107.92
105.04
101.88
101.46
Chloroform-d
77.32
77.00
76.69
39.67
28.53
CDCl$_3$, 100 MHz
O
N
O
O
Dehydrogusanlung D (**53**)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

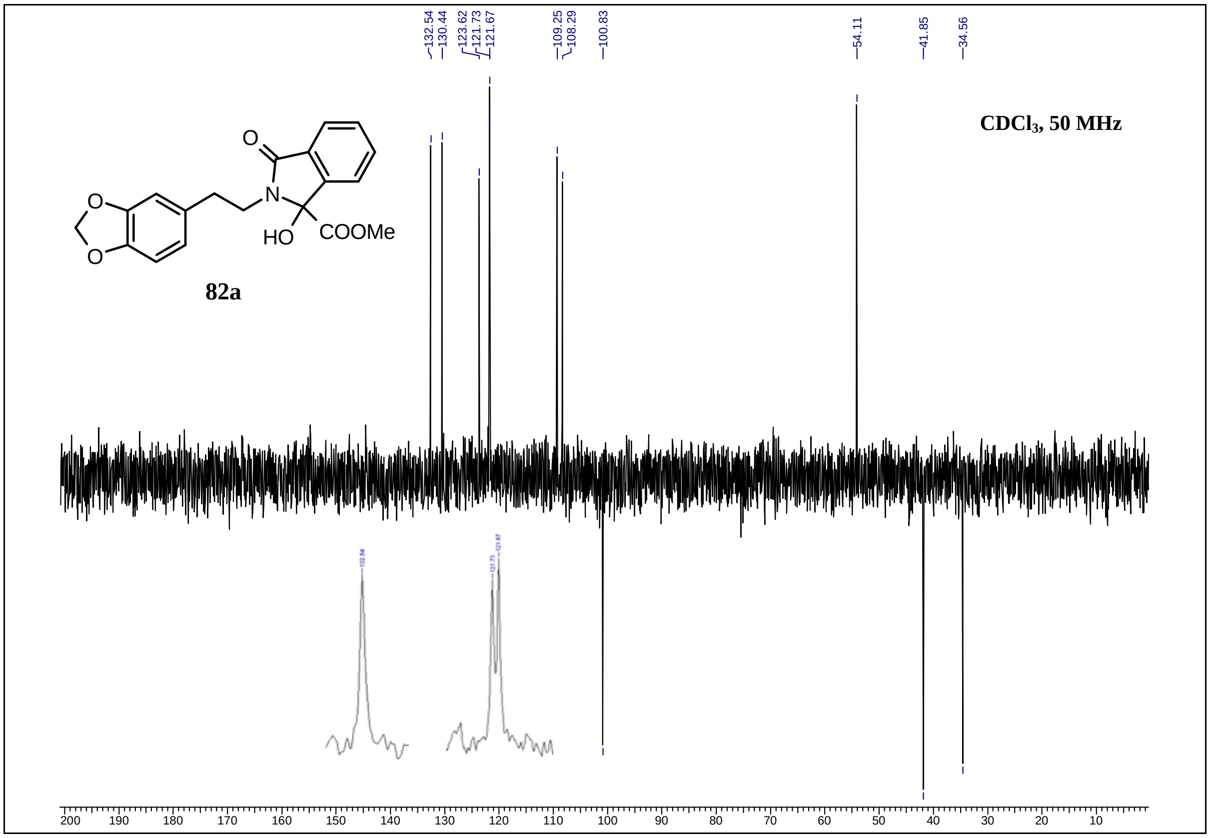

132.25
127.92
126.26
126.00
107.93
105.04
101.89
101.47
39.67
28.53
CDCl$_3$, 100 MHz
O
N
O
O
Dehydrogusanlung D (**53**)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

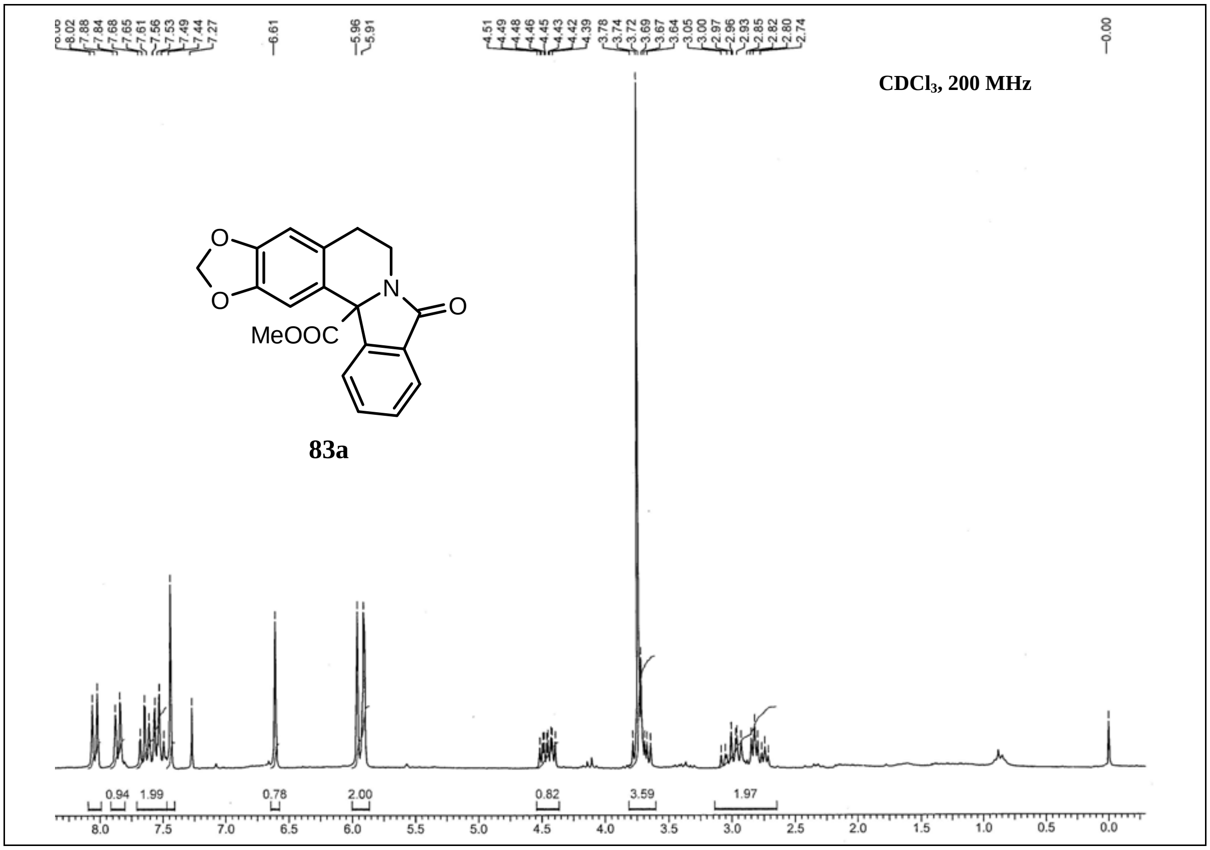


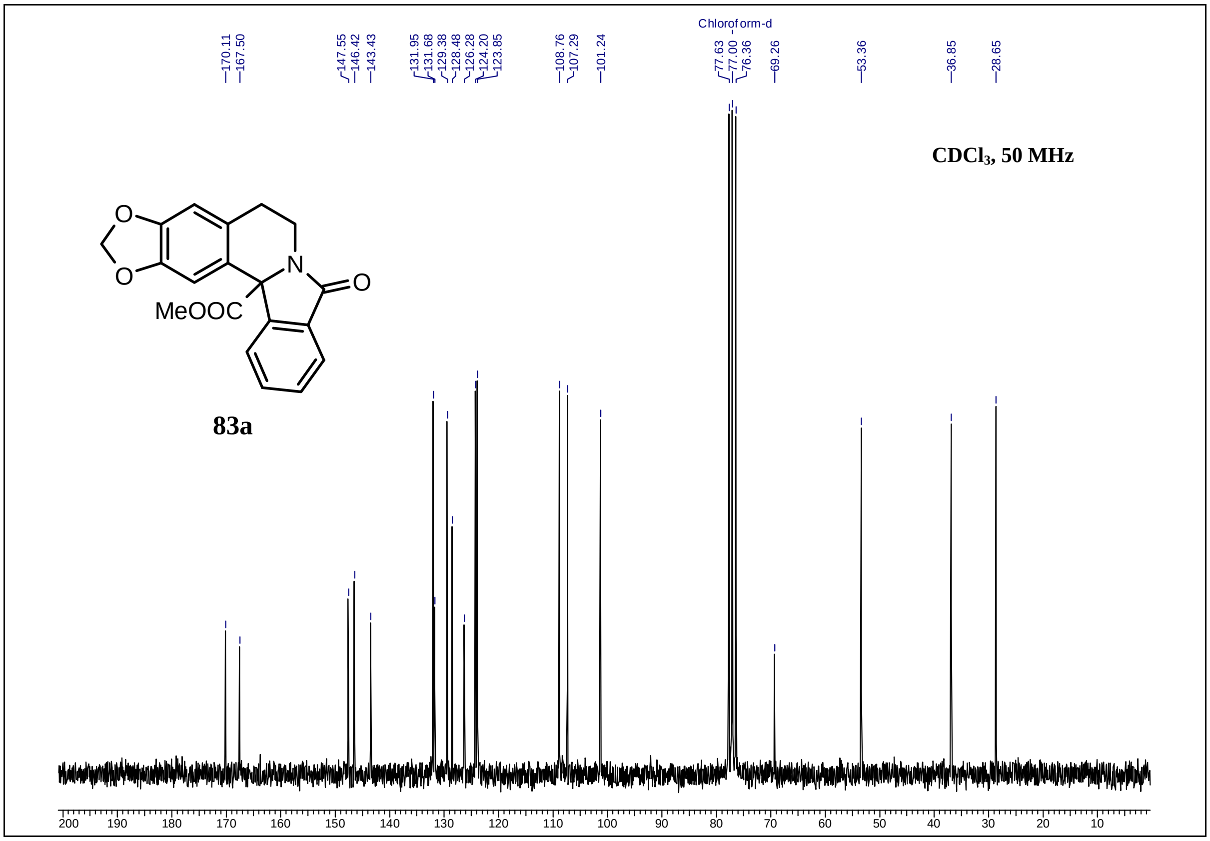
7.85
7.81
7.58
7.56
7.55
7.46
7.45
7.42
7.26
6.76
6.73
6.72
5.92
4.58
3.81
3.77
3.76
3.72
3.69
3.42
3.39
3.37
3.34
3.30
3.27
2.95
2.92
2.90
2.89
2.87
2.84
TMS
0.00
CDCl3, 200 MHz
O
N
O
O
HO
COOMe
82a
1.00
1.93
3.06
2.00
0.96
4.09
1.07
2.04
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

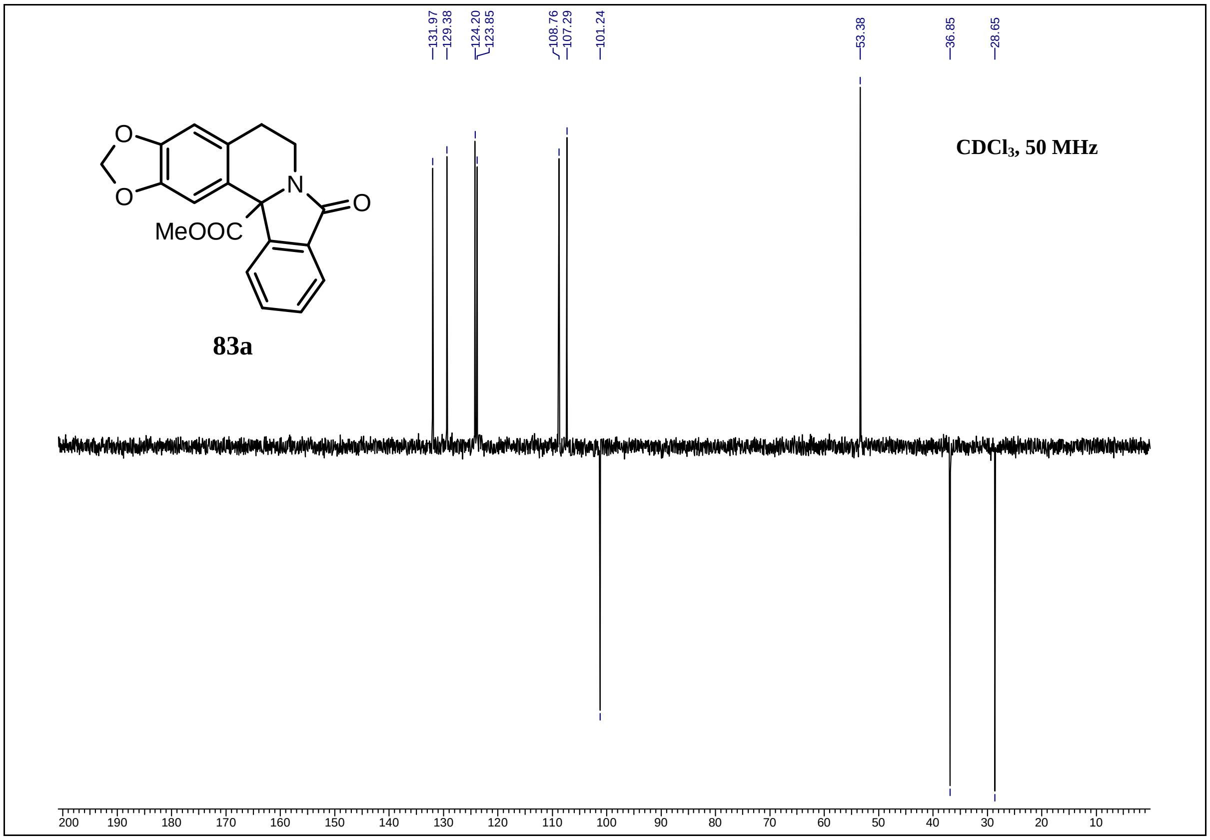


Chloroform-d
171.47
167.90
147.64
146.09
143.24
132.53
131.54
130.43
123.63
121.67
109.26
108.29
100.83
88.33
77.64
77.00
76.37
54.09
41.85
34.56
CDCl3, 50 MHz
132.56
132.53
131.54
130.43
132.5
132.0
131.5
131.0
130.5
123.63
121.72
121.67
123.5
123.0
122.5
122.0
121.5
O
N
O
O
HO
COOMe
82a
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10


132.54
130.44
123.62
121.73
121.67
109.25
108.29
100.83
54.11
41.85
34.56
CDCl₃, 50 MHz
O
N
O
O
HO
COOMe
82a
132.54
121.73
121.67
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10


8.02
7.88
7.84
7.68
7.65
7.61
7.56
7.53
7.49
7.44
7.27
6.61
5.96
5.91
4.51
4.49
4.48
4.46
4.45
4.43
4.42
4.39
3.78
3.74
3.72
3.69
3.67
3.64
3.05
3.00
2.97
2.96
2.93
2.85
2.82
2.80
2.74
0.00
CDCl₃, 200 MHz
O
O
N
O
MeOOC
83a
0.94
1.99
0.78
2.00
0.82
3.59
1.97
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


170.11
167.50
147.55
146.42
143.43
131.95
131.68
129.38
128.48
126.28
124.20
123.85
108.76
107.29
101.24
Chloroform-d
77.63
77.00
76.36
69.26
53.36
36.85
28.65
CDCl3, 50 MHz
O
O
N
O
MeOOC
83a
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10


131.97
129.38
124.20
123.85
108.76
107.29
101.24
53.38
36.85
28.65
CDCl3, 50 MHz
O
O
N
O
MeOOC
83a
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

2B.8 References

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Erratum