SYNTHESIS OF BIOLOGICALLY ACTIVE NATURAL PRODUCTS AND PHARMACOLOGICALLY ACTIVE MOLECULES BY COMBINATORIAL CHEMISTRY

A THESIS
Submitted to the

SHIVAJI UNIVERSITY, KOLHAPUR
For the Degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY
Science Faculty

By

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Under the Guidance of

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Pune 411 008

SEPTEMBER 2001

DEDICATED To My Parents

For Their Warmth, Humor And Ethics

CERTIFICATE

This is to certify that the thesis entitled "**SYNTHESIS OF BIOLOGICALLY ACTIVE NATURAL PRODUCTS AND PHARMACOLOGICALLY ACTIVE MOLECULES BY COMBINATORIAL CHEMISTRY**" which is being submitted herewith for the award of the Degree of Philosophy in Chemistry of Shivaji University, Kolhapur is the result of the original research work completed by **Mr. Anil M. Deshpande** under my supervision and guidance at the National Chemical Laboratory, Pune and to the best of my knowledge and belief the work embodied in this thesis has not formed earlier the basis for the award of any Degree or similar title of this or any other University or examining body.

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Research Guide
Scientist, Division of Organic Chemistry (Synthesis)
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September 2001

DECLARATION

I hereby declare that the thesis entitled "**SYNTHESIS OF BIOLOGICALLY ACTIVE NATURAL PRODUCTS AND PHARMACOLOGICALLY ACTIVE MOLECULES BY COMBINATORIAL CHEMISTRY**" completed and written by me has not previously formed the basis for the award of any Degree or Diploma or other similar title of this or any other University or examining body.

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Division of Organic Chemistry (Synthesis)

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Pune 411 008.

September 2001

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GENERAL REMARKS

All the solvents used were purified according to the literature procedures. Petroleum ether used in the experiments is of 60-80 °C boiling range. 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). 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). The experimental procedures for preparation of starting materials has not been included in the experimental section and references have been cited at the appropriate places.

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 either Brucker ACF 200 (200 MHz for ^{1}H NMR and 50 MHz for ^{13}C NMR) or MSL 300 (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) spectrometers. Chemical shifts (δ) reported are referred to internal reference tetramethyl silane. Mass spectrums were recorded on Finning-Mat 1020C mass spectrometer and are 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. HPLC was performed on Hwlett Packard instrument using C-18 reverse phase column and diodearray UV detector. 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.

Biological evaluation of all combinatorial mixtures and individual compounds were carried at CytoMed, Inc. Cambridge, USA. All results are mean of duplicate and in most cases triplicate determination.

CONTENTS

LIST OF PUBLICATIONS

(1) Chemoselective carbon-carbon coupling of organocuprates with (bromomethyl)methylmaleic anhydride : Synthesis of Chaetomellic acid A
A. M. Deshpande, A. A. Natu and N .P. Argade

J. Org. Chem. **1998**, *63*, 9557.

(2) Synthesis and screening of a combinatorial library of naphthalene substituted chalcones : Inhibitors of leukotriene B_4

A. M. Deshpande, N. P. Argade, A. A. Natu and J. Eckman.

Bioorg. Med. Chem. **1999**, *7*, 1237.

(3) Chemo- and regioselective nucleophilic reactions of (bromomethyl) methylmaleic anhydride : Synthesis of α-quinoxalinyl- and α-benzothiazinyl acrylic acids

A. M. Deshpande**, A. A. Natu and N. P .Argade**

Heterocycles, **1999**, *51*, 2159.

(4) A facile synthesis of (±)-2,3-disubstituted maleic anhydride segment of Tautomycin

A. M. Deshpande, A. A. Natu and N. P. Argade

Synthesis, **2001**, 702.

(5) First synthesis of naturally occurring 2,3-Didehydrtelfairic Anhydride

A. M. Deshpande, A. A. Natu and N. P. Argade

Unpublished results

ABSTRACT

The thesis entitled "**Synthesis of biologically active natural products and pharmacologically active molecules by combinatorial chemistry**" is divided into three chapters. First chapter presents a concise summary of bioactive natural products based on dialkylmaleic anhydride scaffold; with structural diversity whereas the second chapter describes in detail our efforts towards towards the synthesis of some representative examples of this class of compounds. Third chapter describes synthesis and screening of mini combinatorial library of naphthalene substituted chalcones as potential leukotriene B_4 (LTB_4) inhibitors.

CHAPTER ONE: Concise Account on Chemistry of Dialkylsubstituted Maleic Anhydrides

Maleic anhydride is a multifunctional entity and hence has been extensively used for the construction of variety of heterocyclic structures in past century. Maleic anhydride and their derivatives are practically used in the synthesis of variety of key intermediates employed in the heavy and fine chemical industries and as such these compounds have been often used to model (i) compounds highlighting regiochemical dichotomy (ii) heterocyclic skeletons (iii) natural products and their precursors (iv) bioactive molecules (v) series of polymers with tailored material characteristics.

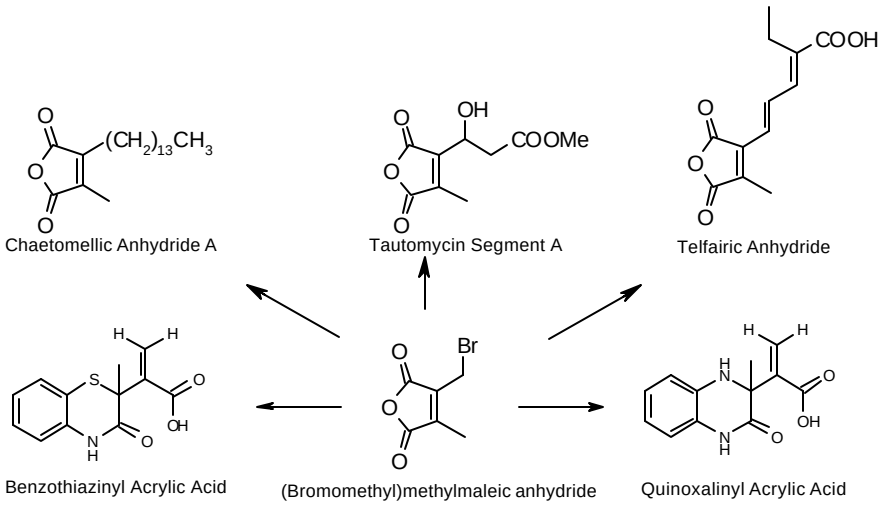
Recently several alkylmethylmaleic anhydrides, dialkylmaleic anhydrides and cyclic compounds containing two or more maleic anhydride moieties have been isolated as bioactive natural products. In recent literature several publications have appeared on synthesis of these bioactive natural products with special emphasis on their promising

bioactivities and presently some of these compounds are in human clinical trials. This chapter portrays a concise account on isolation, bioactivity and syntheses of these bioactive natural products.

CHAPTER TWO: Synthesis of Bioactive Natural Products and New Heterocycles

This chapter is divided into four sections. First three sections deal with the synthesis of bioactive natural products Chaetomellic anhydride A, Maleic anhydride segment of tautomycin and Telfairic anhydride respectively, whereas the fourth one describes the synthesis of ***a***-quinoxalinyl and ***a***-benzothiazinyl acrylic acids using same starting material i.e. (bromomethyl)methylmaleic anhydride which in turn was synthesized by NBS bromination of dimethylmaleic anhydride (**Fig 1**).

Fig. 1

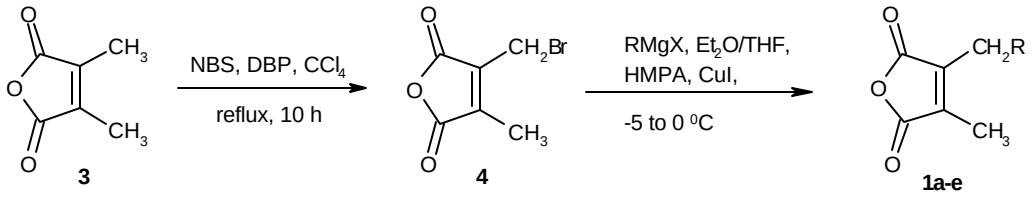


SECTION A: Chemoselective Carbon-Carbon Coupling of Organocuprates with (Bromomethyl)methylmaleic Anhydride: Syntyhesis of Chaetomellic Acid A

This chapter describes an easy two-step access to alkylmethylmaleic anhydrides (**1a-e**, **Scheme 1**) via copper (I) iodide induced, highly chemoselective, carbon-carbon

coupling of Grignard reagents with (bromomethyl)methylmaleic anhydride in 55-60% yields. The unsymmetrical anhydride **4** has five alternate sites for nucleophilic reactions and hence theoretically number of compounds can be obtained from the same nuclophile. However, chemoselective carbon-carbon coupling of organocuprates with preservation of anhydride moiety has been achieved for the first time to complete the synthesis of Chaetomellic anhydride A (**1e**), a ras farnesyl-protein transfersae inhibitor and its analogues (**1a** to **1d**).

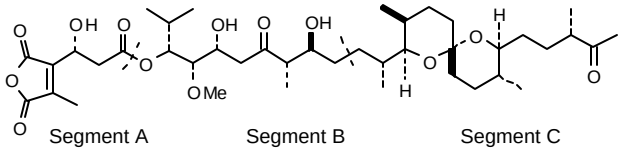
Scheme 1



1a R = CH_3, **1b** R = $(CH_2)_4CH_3$, **1c** R = $(CH_2)_7CH_3$, **1d** R = $(CH_2)_{11}CH_3$, **1e** R = $(CH_2)_{12}$ CH_3

<u>SECTION B</u>: A Facile Synthesis of a (±)-2,3-Disubstituted Maleic Anhydride Segment of Antifungal Antibiotic Tautomycin

The total synthesis of tautomycin involves synthesis of three segments A, B, and C followed by stepwise coupling of these building blocks. The greatest challenge in the



Tautomycin

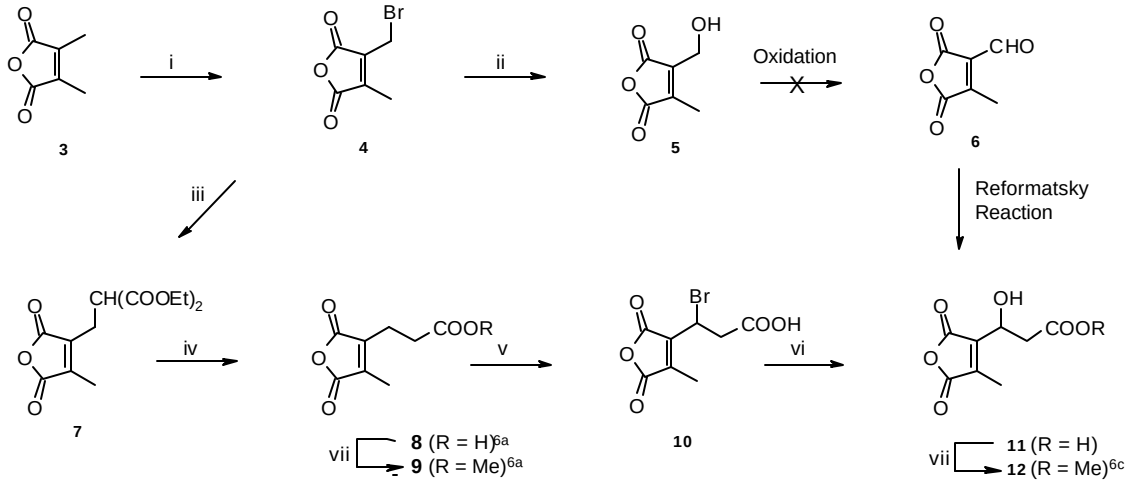
synthesis of tautomycin is in the construction of 2,3-disubstituted maleic anhydride

segment A, as it is highly oxygenated molecule with three carbonyl groups and one hydroxy group.

A convenient five-step synthesis for construction of segment A of tautomycin with 28% overall yield starting from dimethylmaleic anhydride is described. The chemoselective condensation of diethyl malonate with (bromomethyl)methylmaleic anhydride furnished the compound **7** in 74% yield, which on hydrolysis and decarboxylation furnished the corresponding mono acid in 94% yield. The regioselective NBS-bromination of monoacid **7** furnished bromo acid **10** in 74% yield,

which on hydrolysis with 1 N aqueous KOH followed by acidification and

Scheme 2



Reagents and conditions: (i) NBS, Benzoyl peroxide, CCl_4, reflux, 10 h, (60%); (ii) (a) 4 N aq. KOH, r.t., 5 h, (b) H^+/H_2SO_4 (86%); (iii) (a) Diethyl malonate, NaH, C_6H_6, r.t., 8 h, (b) H^+/HCl (74%); (iv) Con. HCl, reflux, 12 h, (94%); (v) NBS, Benzoyl peroxide, $CHCl_3$, reflux, 24 h, (74%); (vi) (a) 1N aq. KOH, r.t., 3 h, (b) H^+/HCl (91%); (vii) CH_2N_2, Et_2O, 0 °C, 3 h, (95%).

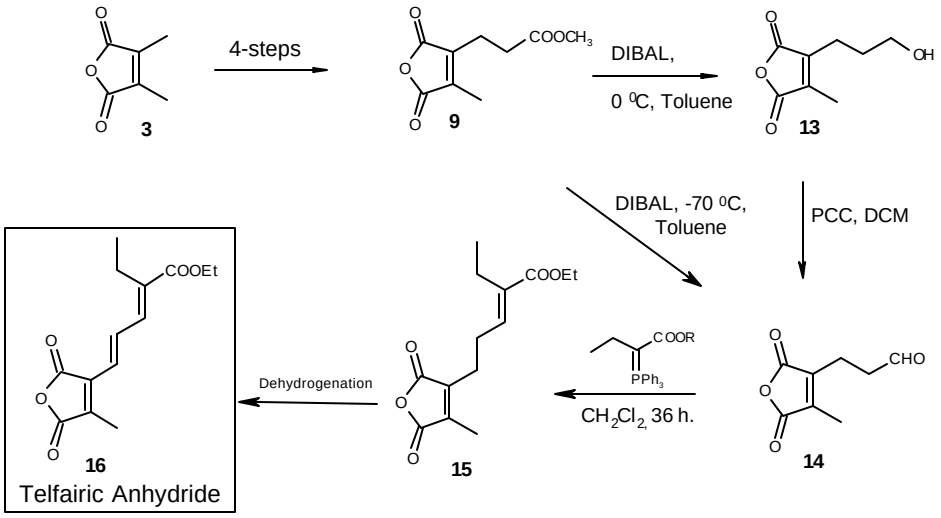
Subsequent esterification with diazomethane yielded the desired (±)-2,3-dialkyl

substituted maleic anhydride segment of tautomycin.

SECTION C: Synthesis of 2,3-Didehydrotelfairic Anhydride

This section describes the first synthetic approach towards the natural product telfairic anhydride, which has been recently isolated from fungus *Xylaria telfairii* in 1996. The synthetic strategy utilizes the ester **9** as a starting material. Chemoselective reduction of **9** furnished the alcohol **13**, which is converted into aldehyde **14** by oxidation. The aldehyde was also obtained directly by chemoselective reduction of ester using DIBAL

Scheme 3



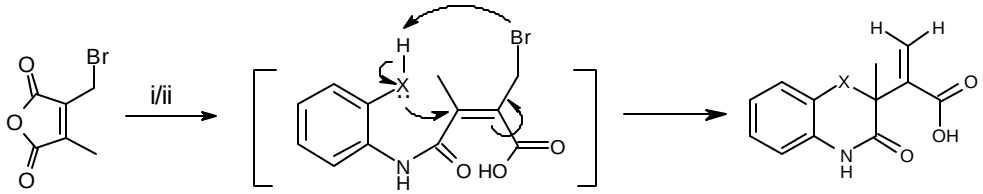
at –70 °C. The Wittig reaction of aldehyde with phosphorane obtained from methyl-2-bromobutyrate furnished the dihydro telfairic ester **15**. The studies to dehydrogenate ester **15** to yield the target molecule telfairic anhydride (**16**) are in progress.

SECTION D: Synthesis of α-Quinoxalinyl and α-Benzothiazinyl Acrylic Acids

The nucleophilic reactions of symmetrical and unsymmetrical cyclic anhydrides have been fully investigated as elegant strategy for the synthesis of several structurally interesting and biologically important heterocyclic systems. This section focuses on the nucleophilic reaction of unsymmetrical (bromomethyl)methylmaleic anhydride (**2**) with

suitably *ortho*-substituted aniline derivatives for designing the heterocyclic skeletons. The bromoanhydride **2** on reaction with *o*- phenylenediamine and *o*-aminothiophenol

Scheme 4



4

19 X = NH
20 X = S

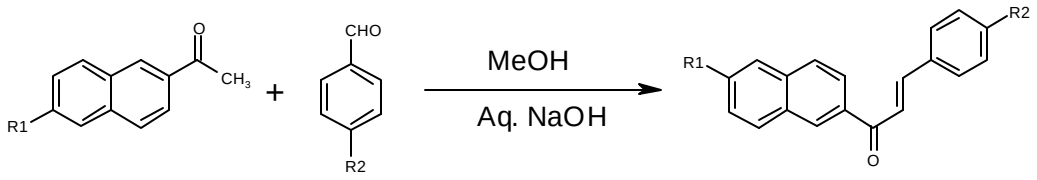
Reagents and conditions: (i) *o*-PDA, $CHCl_3$, -15 °C to rt, 3 h (ii)) *o*-ATP, $CHCl_3$, -15 °C to rt, 3 h.

underwent chemo- and regioselective ring opening and intramolecular Michael type addition followed by 1,4-elimination reaction to furnish kinetically controlled products *α*-quinoxalinyl and *α*-benzothiazinyl acrylic acids (**Scheme 4**) and such type of carbon-carbon double bond generation is note worthy and will be useful for synthesis of natural products.

<u>CHAPTER THREE</u>: **Synthesis and Screening of Naphthalene Substituted Chalcones as Leukotriene B_4 Inhibitors: A Combinatorial Approach**

There is growing interest in the drug design and development of 5-Lo inhibitors for their clinical application in the therapeutic treatment of various inflammatory diseases. The present work deals with the synthesis of mini combinatorial library of naphthalene substituted chalcones and its biological evaluation as LTB_4 inhibitor. The approach of two-dimensional deconvolution screening (positional scanning) is designed and the general synthetic strategy employed to prepare chalcone library was based on well-known Claisen-Schmidt condensation reaction (**Scheme 5**). The reaction conditions are

Scheme 5



K **A** **KA**

designed in such a way that they are easy to operate and will provide clean product (i.e. no byproduct) and can be adopted for combinatorial approach very efficiently to generate chemical diversity in one step. The product can be easily purified and obtained in quantitative yields with high degree of purity. The small combinatorial library of twenty chalcones was synthesized from 4 ketones and 5 aldehydes in two sets as nine combinatorial mixtures. The library mixtures were screened by Human Whole blood Cell Assay (HWBL) and the two dimensional deconvolution analysis of biological evaluation of combinatorial mixtures indicated the compound 1-(6-Butoxy-2-naphthyl)-3-(4-nitrophenyl)-prop-2-en-1-one as a lead candidate which showed 62% inhibition at 30 μM concentration with an IC_{50} of 18.5 μM.

In summary, the first chapter of this dissertation provides the literature over view on recently isolated bioactive natural products with maleic anhydride moieties. The second chapter covers in-detail our contribution to the subject with the synthesis of three natural products Chaetomellic anhydride A, Maleic anhydride segment of tautomycin and telfairic anhydride and two structurally interesting heterocycles. The third chapter presents the combinatorial approach to chalcones with generation of mini library aiming LTB_4 inhibitory activity, which provided a lead candidate with an IC_{50} of 18.5 μM.

ABBREVIATION

AA	Arachidonic acid
AIBN	α,α'-Azobisisobutyronitrile
Aq.	Aqueous
o-AP	*ortho*-Aminophenol
o-ATP	*ortho*-Aminothiophenol
ATP	Adenosine triphosphate
BHT	Butylated hydroxy toluene
BINAP	Binaphthyl
CMR	Carbon magnetic resonance
m-CPBA	*meta*-Chloroperbenzoic acid
Co	Cyclooxygenase
CysLTs	Cysetine Leukotrienes
DBP	Dibenzoyl peroxide
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	Dicyclohexyl carbodiimide
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	Diethylazido dicarboxylate
DIBAL	Diisobutylaluminum hydride
DMAP	4-(Dmethylamino)pyridine
DMF	Dimethylformamide
DMMA	Dimethylmaleic anhydride
DMSO	Dimethyl sulphoxide
EIA	Enzyme immuno assay
equiv	Equivalent

Fig.	Figure
FLAP	Five lipoxygenase protein
FPP	Farnesylpyrophosphate
FPTase	Farnesyl-protein transferase
h	Hour
HETE	Hydroxyecosatetraenoate
HMPA	Hexamethylphosphoramide
HPLC	High performance liquid chromatography
5-HPETE	5-Hydroperoxyecosatetraenoate
HTS	High throughput screening
HWBL	Human whole blood
Hz	Hertz
o-IBX	*ortho*-Iodoxybenzoic acid
IC	Inhibitory concentration
IR	Infra red
IUPHAR	International union of pharmacology
LAH	Lithium aluminum hydride
LDA	Lithium diisopropylamide
5-Lo	5-Lipoxygenase
LTs	Leukotrienes
μM	Micromolar
nM	Nanomolal
mmol	Milimolar
min.	Minute
mL	Milliliter
MOMCL	Methoxymethylchloride

MS	Mass spectrum
NBS	*N*-Bromosuccinimide
NDGA	Nordihydroguaiaretic acid
NSAIDs	Non steroidal anti-inflammatory drugs
PCC	Pyridinium chlorochromate
o-PDA	*ortho*-Phenylene diamine
PGs	Prostaglandins
PMR	Proton magnetic resonance
PPs	Protein phosphtase
PPA	Polyphosphonic acid
PPL	Pig pancreatic lipase
PPM	Parts per million
PPTS	Pyridinium *para*-toluene sulphonate
Py	Pyridine
RBL	Red blood leukocytes
rt	Room temperature
SAR	Structure activity relationship
SPOS	Solid phase organic synthesis
SPPS	Solid phase peptide synthesis
SRSA	Slow release substances of anaphylaxis
TEA	Triethylamine
THF	Tetrahydrofuran
tlc	Thin layer chromatography
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TPP	Triphenyl phosphine
TXAs	Thromboxanes
UV	Ultraviolet

CHAPTER ONE

CONCISE ACCOUNT ON CHEMISTRY OF

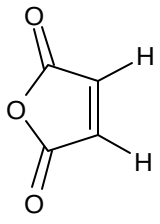
DIALKYLSUBSTITUTED

MALEIC ANHYDRIDES

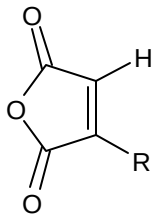
1.1 INTRODUCTION

1.1.1 Maleic Anhydride Derivatives and their Applications

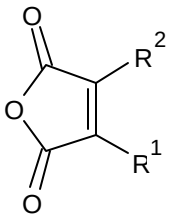
Maleic anhydride (2,5-furandione) was prepared for the first time in 1830 and became commercially available a century later in 1933 by the catalytic oxidation of benzene using vanadium pentoxide.[1] Maleic anhydride is a multifunctional entity and finds application in nearly every field of both laboratory and industrial chemistry. The greatest interest has been centered in the use of maleic anhydride as a building block in chemical synthesis. Its structure is ideally suited for synthetic purposes because of all sites available for reaction and highly balanced reactivity towards several nucleophiles. Vast array of nuclophilic 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 list of mono and disubstituted maleic anhydrides is very vast and a few of them are represented below.



1



2



3

$R/R^1/R^2$ = alkyl, benzyl, phenyl, aryl, hydroxy, alkoxy, halo and cyano.

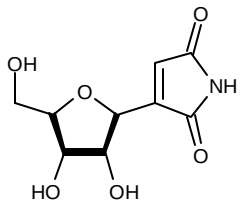
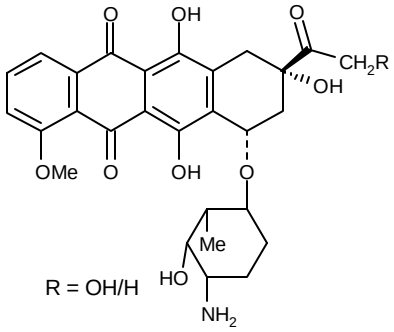
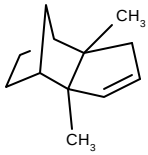
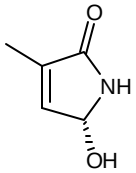
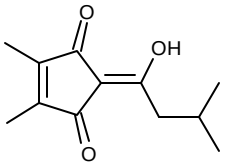
Maleic anhydride and their derivatives are used extensively in the synthesis of wide variety of key intermediates employed in the heavy and fine chemical industries[2] and as such these compounds have been often used to model

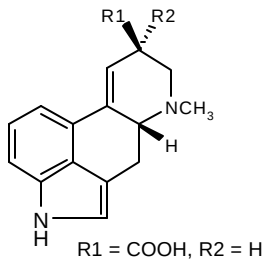
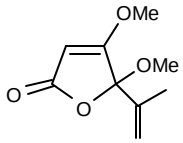
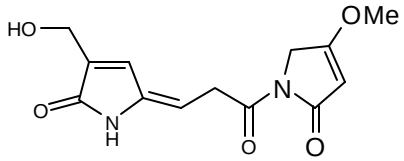
- Compounds highlighting regiochemical dichotomy,
- Heterocyclic skeletons,
- Natural products and their precursors,
- Bioactive molecules such as drugs, agrochemicals and
- Polymers with tailored material characteristics.

1.1.2 Synthetic Utility of Methyl and Dimethylmaleic Anhydride

The utilities of methyl and dimethylmaleic anhydrides have been well proved in laboratory as well as in industrial practice.[3] Methyl and dimethylmaleic anhydrides have been also used for the synthesis of important heterocyclic systems,[4] as a potential dienophile in Diels-Alder reaction.[5] Interestingly some of their derivatives possess herbicidal, fungicidal, insecticidal and defoliant activities.[6] A few representative examples of above-mentioned applications are enlisted in **Table 1**. The synthesis of antibiotic showdomycin and *epi*-showdomycin via the reaction of D-ribose with maleimide-triphenylphosphine (TPP) adduct and subsequent cyclisation using phenylselenenyl chloride followed by oxidative elimination using hydrogen peroxide is very elegant and practical[7] whereas the conversion of dimethylmaleic anhydride to antibiotic adriamycin and daunorubicin are of commercial interests.[8] The e*xo*-Diels Alder adduct of dimethylmaleic anhydride and cyclopentadiene has been used in the total synthesis of natural product (±)-Albene.[9] Jatrophan, an alkaloid isolated in 1973 by Cole *et al* has been synthesized[10] in three-steps from citraconic anhydride via highly regioselective reduction of corresponding citraconimide as a key step. Substituted maleic anhydrides have been used for the synthesis of pulvinic acid and pulvinone analogues via 2(5*H*)-one phosphonate derivatives by

Table 1: Important Synthetic Utility of Symmetrically and Unsymmetrically Substituted Maleic Anhydrides

Sr. No.	Compound Synthesized	Source and Activity	Characteriza -tion	Ref.
1	 Showdomycin	*Streptomyces showdoensis* Antibiotic	IR, PMR, CMR, Mass	7
2	 Adriamycin /Daunorubicin	*Streptomyces peucetius* Antibiotic, anticancer, immunomodulator and superoxide radical generation	IR, PMR, CMR, Mass	8
3	 (±)-Albene	*Petasites albus*	IR, PMR, CMR, Mass	9
4	 Jatropham	*Jatropha macrohiza* Antitumor	IR, PMR, CMR, Mass	10
6	 Claythrone	*Claythrix tetragona (from oil)*	IR, UV, PMR, mp of its Cu salt	12

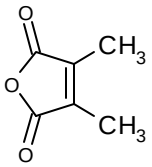
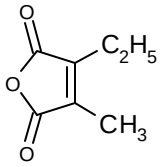
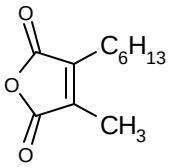
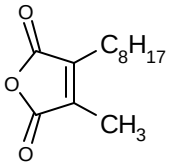
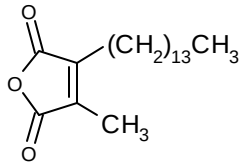
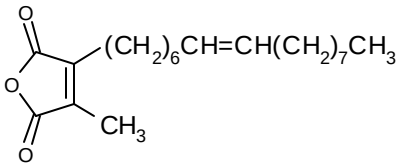
7	 Lysergic Acid	*Claviseps paspali* and *Claviseps purpurea* (ergot fungus) Hallucinogenic and anti-serotonin	IR, UV, PMR, Mass.	13
8	 Penicillic Acid	Lyngbya majuscula (marine blue-green algae) Not known	IR, PMR, CMR, Mass	14
9	 Pukelimide	*Lyngbya majuscula* (marine blue-green algae) Not known	IR, UV, PMR, Mass	15

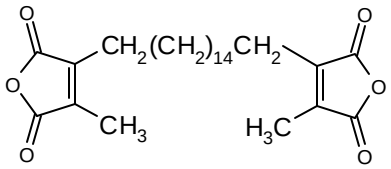
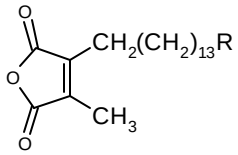
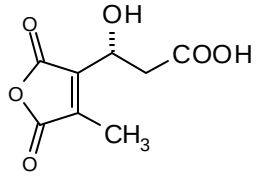
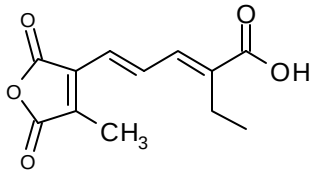
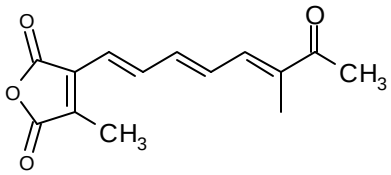
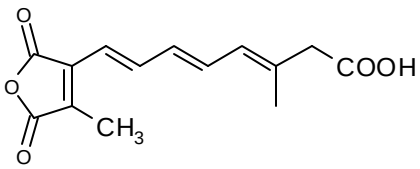
employing Wadsworth- Emmons olefination.[11] The use of dimethylmaleic anhydride in the synthesis of claythrone and related cyclopentene-1,3-dione through 4-ylidenebutenolide has been reported by Pattenden and co-workers.[12]

1.2 ALKYLMETHYLMALEIC ANHYDRIDE

Recently several structurally interesting and biologically important alkylmethylmaleic anhydrides have been isolated as bioactive natural products and are listed in **Table 2**. The structural features of these molecules reveal that nature may be making them from the combination of pyruvic acid and the respective long chain carboxylic acids. These target molecules received an immediate attention from several

Table 2: Recently Isolated Naturally Occurring Alkylmethylmaleic Anhydrides

Sr. No.	Compound Structure	Source and Bioactivity	Characterization	Ref.
1	 Dimethylmaleic Anhydride	Synthetic Derivative	PMR, CMR, Mass, C, H analysis	17
2	 2-Ethyl-3-methylmaleic Anhydride	*Paederia foetida L.* (from volatile oil) *Sambucus nigra L.* fruit	PMR, CMR, Mass, C, H analysis	18
3	 2-Hexyl-3-methylmaleic Anhydride	*Agropyrum repens* Rhizome Flavouring Agent	PMR, CMR, Mass, C, H analysis	19
4	 2-Octyl-3-methylmaleic Anhydride	*Pseudomonas cepacia* A-1419 Flavouring Agent	PMR, CMR, Mass, C, H analysis	20
5	 Chaetomellic Anhydride A	*Chaetomella acutiseta* Ras farnesyl-protein transferase inhibitor (Anticancer)	PMR, CMR, Mass, C, H analysis	21
6	 Chaetomellic Anhydride B	*Chaetomella acutiseta* Ras farnesyl-protein transferase inhibitor (Anticancer	PMR, CMR, Mass, C, H analysis	21

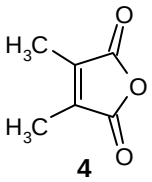
7	 Tyromycin A	*Tyromyces lacteus* Aminopeptidase inhibitor, potential cytostatic activity	IR, PMR, CMR, Mass,	22
8	 R = $COCH_3$ R = $CH(OCOCH_3)CH_3$ R = $CH(OH)CH_3$ R = $(CH_2)_3OCOCH_3$	*Aspergillus wentii* Not known	IR, PMR, CMR, Mass.	23
9	 Maleic anhydride segment of Tautomycin	*Streptomyces spirovertivillatus* Antifungal Antibiotic	IR, PMR, CMR, Mass.	24
10	 Telfairic Anhydride	*Xylaria telfairii* Not known	PMR, CMR, Mass, C, H analysis.	25
11	 Graphenone	Lichen mycobiont *Graphis scripta* Not known	IR, PMR, CMR, Mass	26
12	 Itaconitin	*Aspergillus itaconicus* Not known	IR, PMR, Mass	27

elegant schools of synthetic organic chemistry for the synthesis of natural product itself and its analogues for structure activity relationship studies. For example during past eight years nine synthesis of ras farnesyl-protein transferase inhibitor chaetomellic acid A (**5**) have been reported and some of the ras farnesyl protein-transferase inhibitors are currently in human clinical trials.[16] The complete details about synthetic efforts on all these natural products are summarized in following section.

1.2.1 Dimethylmaleic Anhydride

The simple and widely used derivative of alkylmethylmaleic anhydride is dimethylmaleic anhydride (DMMA, **4**). Several synthetic approaches (> 25) to **4** and other maleic

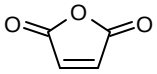
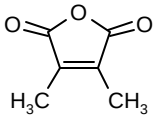
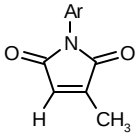
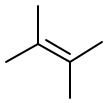
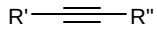
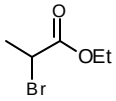
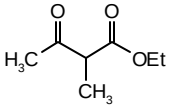
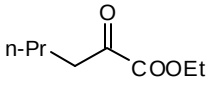
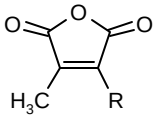
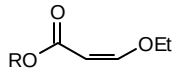
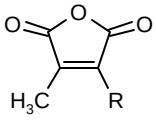
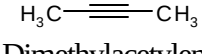
anhydrides are known in the literature and few of them with more than 50% yield and above are summarized in **Table 3**. One of the elegant, one pot synthesis of **4** starting with 2-aminopyridine and two equivalents of maleic anhydride via *in-situ* formation of nitrogen ylide with 75% yield



4

is reported by Bauman *et al*.[17] This methodology has been used several times during the work in our laboratory to obtain gram quantities of **4** in nearly 100% yield. Recently, a new three-step synthetic method with 75% overall yield for **4** has been developed in our laboratory starting with maleimide via methylmaleimide, using double Wittig reaction followed by alkaline hydrolysis.[28] The other approaches to **4** include (i) oxidation of 2-butene in presence of metal catalyst with 68% yield[29] (ii) oxidation of dimethyl acetylene in 2-steps with 59% overall yield[30] (iii) self condensation of α-bromopropionate with 67% yield.[31] Very high amount of utilities of dimethylmaleic anhydride (**4**) in organic and

Table 3: Known Approaches for the Synthesis of Dimethyl/alkylmethylmaleic Anhydrides

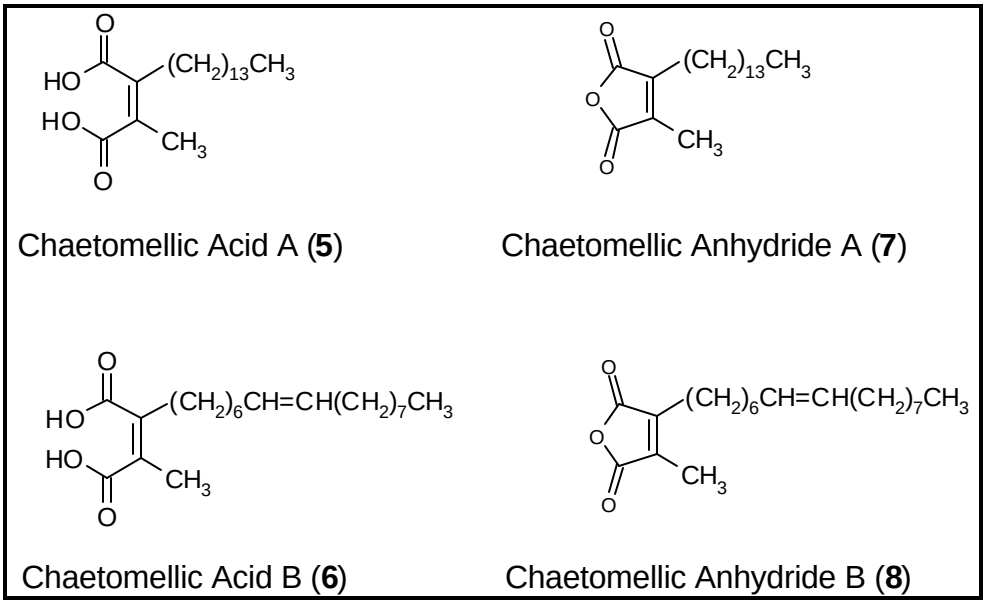
No.	Starting material	Reagents/ conditions	Product	Overall yield	Ref
1	 Maleicanhydride	2-Aminopyridine, AcOH, reflux	 (DMMA)	75%	17
2	 Methyl maleimide	i) PPh_3, AcOH, $(CH_2O)n$, reflux ii) Alkaline hydrolysis	DMMA	75%	28
3	 2,3-Dimethyl2-butene	Oxidised in air over V_2O_5:MoO_3:P_2O_5: TiO_2, 402 °C, 0.2 sec.	DMMA	68%	29
4	 Dimethylacetylene	i)(biPy)Ni$(CO)_2$/ THF ii) O_2, THF	DMMA	59%	30
5	 Ethyl 2-bromopropionate	Self coupling in Ca-Naphthalenide, Liq. NH_3	DMMA	67%	31
6	 Ethyl-2-methyl acetoacetate	i) $NaCN/H_2O$ ii) H_2SO_4	DMMA	__	32
7	 ***a***-Ketoester	NaH/1,2-dimethoxy ethane	 R = Me, Et	32% 56%	33
8	 1-Ethoxy-1-alkenyl esters	Pyrolysis of 1-Ethoxy-1alkenyl ester (ketoacid/DCM 1-ethoxy-1-alyne)	 R = Me, *n*-Bu	30% 44%	34
9	 Dimethylacetylene	Ir$(CO)_3$Br/THF,refl. Under CO or inert/ 4 N HNO_3	DMMA	__	35

bioorganic chemistry keep the scope open for the development of further new, elegant and practical routes to this molecule.

1.2.2 Tetradecylmethylmaleic Anhydride: Chaetomellic Anhydride A

The dicaboxylic acids chaetomellic acid A (**5**) and chaetomellic acid B (**6**) were isolated from fermentation extract of the coleomycete *Chaetomella acutiseta* (**Fig. 1**), by a group of scientists[21] at Merck, USA in 1993. The structural assignment of chaetomellic anhydride A (**7**) and chaetomellic anhydride B (**8**) has been done on the basis of analytical and spectral data. The position and geometry of double bond in chaetomellic acid B was established by

Fig. 1



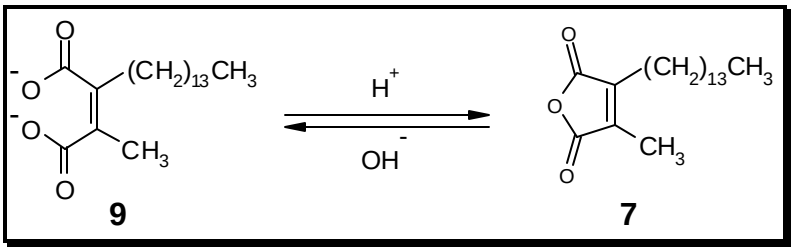
MS analysis of monoepoxide (prepared by reacting **8** with *meta*-chloroperbenzoic acid in dichloromethane). The biogenesis of these compounds[21] may be occurring through an aldol condensation of palmitate or *cis*-oleate with pyruvate followed by dehydration reaction pathway. Chaetomellic acid A and B have been identified as potent inhibitors of Ras

farnesyl-Protein transferase[21,36] (FPTase), an enzyme catalyzing a post-translational modification of Ras. Mutated form of *ras* oncogenes are found in about 25% of the human tumors[37] and are believed to play a key role in their growth. The *ras* genes encode 21 Kda proteins, called p21 or Ras. The normal and oncogenic activity of this protein is dependent on its ability to associate with the inner leaflet of plasma membrane that occurs as a result of post-translational modification. Out of several post-translational steps, farnesylation by farnesyl-protein transferase (FPTase) is first and obligatory step.[38] Genetic experiments have also shown that farnesylation is required for Ras cell transforming activity. FPTase may, therefore, represents a target for chemotherapeutic intervention of human tumors

having mutated *ras* genes. FPTase utilizes farnesylpyrophosphate (FPP) to modify the Cys residue at the C-terminus of Ras known as CaaX box (C, Cys; a, usually an aliphatic amino acid ; X, another amino acid). Analyses of the substrate requirements for FPTase have shown that the enzyme binds with selective isoprenoid pyrophosphates and CaaX tetrapeptides.[39] Potential peptide inhibitors [eg. CVLS (IC_{50} = 2 μM), CVIM (IC_{50} = 0.1 μM), CVFM (IC_{50} = 0.02 μM)] can be designed from the substrate. However, peptides are not desired compounds to be considered as therapeutic agents due to their rapid metabolism *in-vivo* and therefore the interest has developed for non-peptide inhibitors. During screening of the natural products, two novel dicarboxylic acids chaetomellic acid A (**5**) and chaetomellic acid B (**6**) were isolated and identified[21] as a potent FPTase inhibitors with IC_{50} value 55 and 185 nM respectively. These acids were characterized as alkyl *cis*-dicarboxylates and appear to mimic FPP at the active site of enzyme. Molecular modeling studies reveal that these acids structurally resemble with FPP. These classes of natural products have propensity to cyclise as shown in **Fig 2.** and all members of this family were

isolated in the anhydride form, however they actually exhibit their FPTase inhibitory activity in dianionic form as shown below in compound **9**. Chaetomellic acid A (**5**) is 3 times more potent than chaetomellic acid B (**6**) and became main attraction of synthetic

Fig. 2

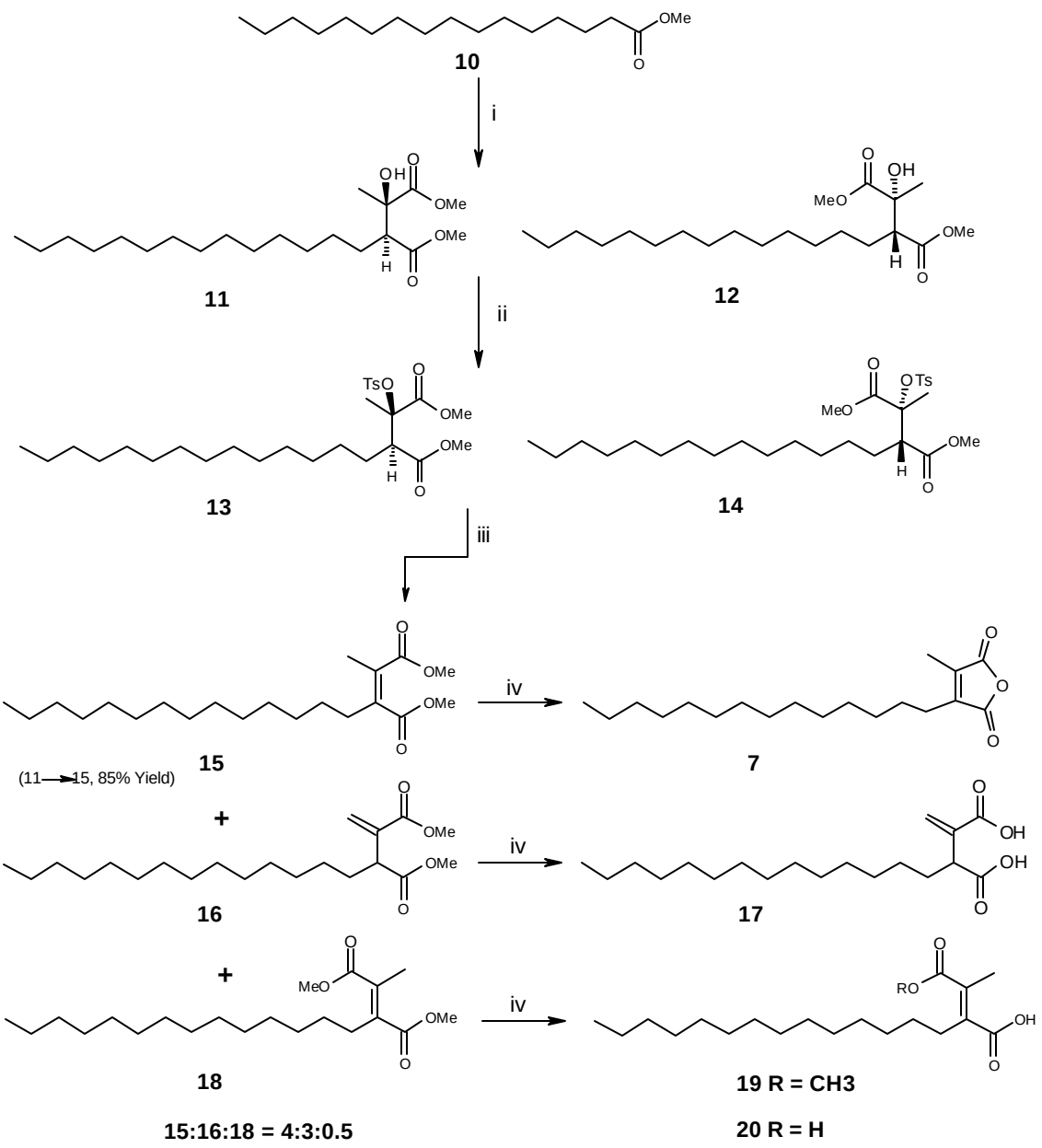


efforts because of its potent FPTase inhibitory activity for the treatment of cancer. After the isolation in 1993, in past eight years span nine syntheses have been accomplished including four from our group. The eighth approach will be discussed in the section A of the chapter two as a part of this dissertation. The chemistry of eight alternate syntheses of **7** is discussed briefly in the following schemes.[40-47]

First Synthesis

The four-step synthetic strategy by Singh[40] at Merck research laboratories is based on biogenetic type approach with 18% overall yield. It involves base catalyzed nonstereospecific aldol condensation of methyl palmitate (**10**) with methyl pyruvate, followed by tosylation, elimination and acid hydrolysis as shown in **Scheme 1**. Recently the authors have done several improvements in the reaction conditon to obtain them in good yield.[16]

Scheme 1

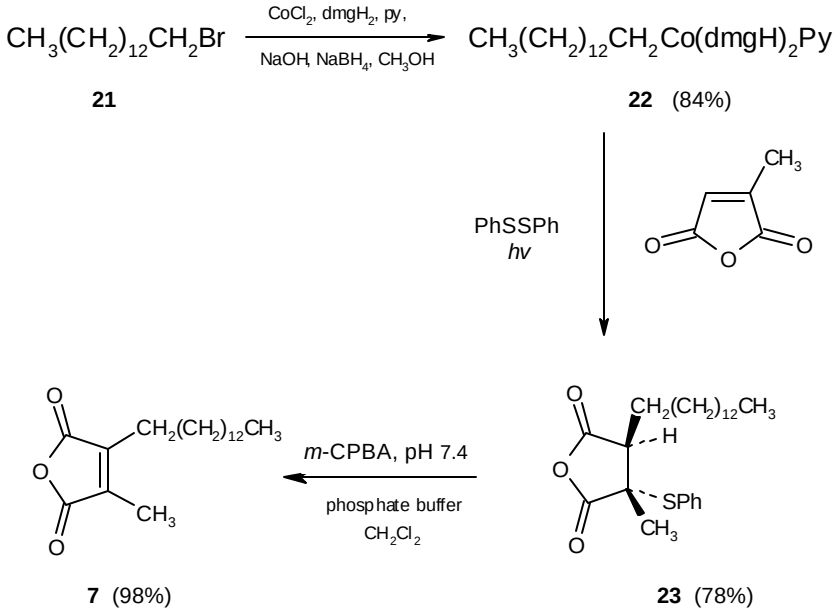


Scheme 1: (i) LDA, THF, -78 to -10 °C, methyl pyruvate; (ii) CH_2Cl_2, pyridine, 40 °C, 2,6-Di-*tert*-butyl-4-methylpyridine, *p*-toluenesulphonic anhydride; (iii) Toluene, DBU, reflux; (iv) a. NaOH-CH_3OH-THF-H_2O, 80 °C, b. 4 N HCl.

Second Synthesis

The three-step cobaloxime mediated synthesis with 64% overall yield is reported by Branchud *et al*[41] from university of Oregon (**Scheme 2**). The key step utilized here is a doubly chemoselective cross coupling of myristyl cobaloxime (**22**) with citraconic anhydride and diphenyl disulfide. The oxidation of the sulfide **23** to the sulfoxide with *meta*-chloroperbenzoic acid with an *in-situ syn*-elimination under the reaction conditions, provided the chaetomellic anhydride A. Facile elimination of the intermediate sulfoxide establishes the trans stereochemical relationship of the thiophenyl and myristyl substituents in **23**, which is a predicted stereochemistry based on steric effects. The synthetic strategy described here can easily be adapted for the synthesis of diverse chaetomellic acid A analogues.

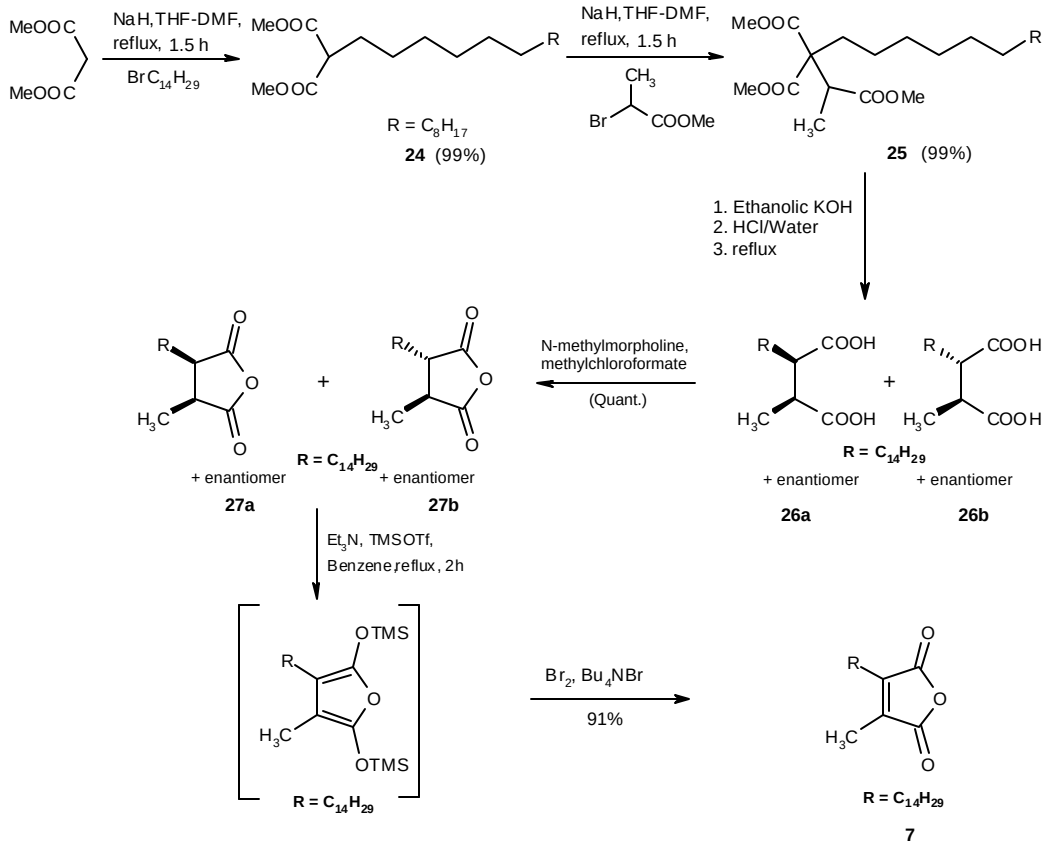
Scheme 2



Third Synthesis

The third five-step synthesis employing a novel succinate to maleate oxidation as a key step has been reported by Schauble and Kates[42] for the synthesis of **7** with 83% overall yield (**Scheme 3**). Malonic ester type syntheses were used to construct carbon skeleton of both chaetomellic anhydrides **7** and **8**. The standard base catalyzed condensation of dimethyl malonate with 1-bromotetradecane followed by condensation with methyl 2-bromopropionate offered triester, which on hydrolysis and decarboxylation furnished corresponding diastereomeric mixture of 2-tetradecyl-3-methyl succinic acids **26a** and **26b**. The diastereomeric mixture of succinic acids was converted to anhydride form using *N*-methylmorpholine and methyl chloroformate. The oxidative sequence for the conversion

Scheme 3

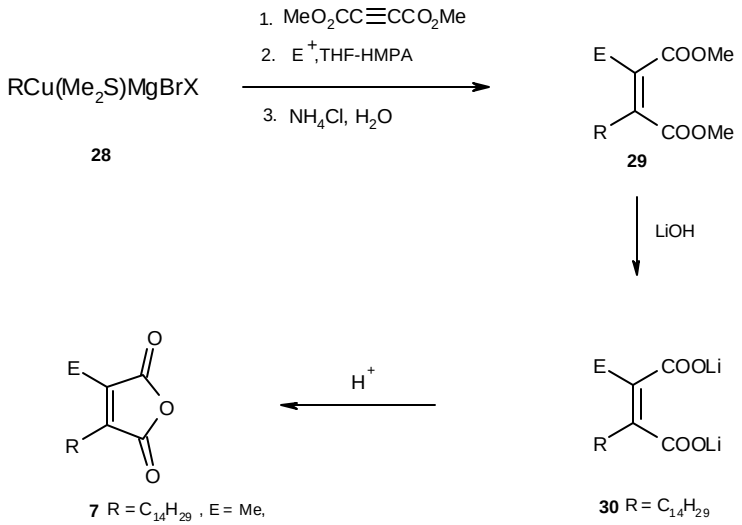


of succinic anhydrides to maleic anhydrides was carried out by reaction of the succinic anhydrides with Et_3N and TMSOTf in benzene under reflux to give the corresponding intermediate 3-tetradecyl-4-methyl-2,5-bis[(trimethylsilyl)oxy]furan. The *in-situ* subsequent desilylation using pure tetra-*n*-butylammonium bromide in methylene chloride, followed by addition of pure bromine at 0 °C provides chaetomellic anhydride A (**7**). The authors have skillfully extended the same strategy to celebrate the first synthesis of chaetomellic anhydride B (**8**).

Fourth Synthesis

This approach involves a facile two-step stereoselective synthesis by a group of Vederas and Poulter[43] using reaction of organocuprates and acetylene dicarboxylate with 78% overall yield (**Scheme 4**). The conjugate addition of organocuprates derived from Grignard reagents (tetradecylmagnesium chloride and $CuBr.Me_2S$) to dimethyl acetylenedicarboxylate in THF containing HMPA, was followed by capture of the resulting

Scheme4

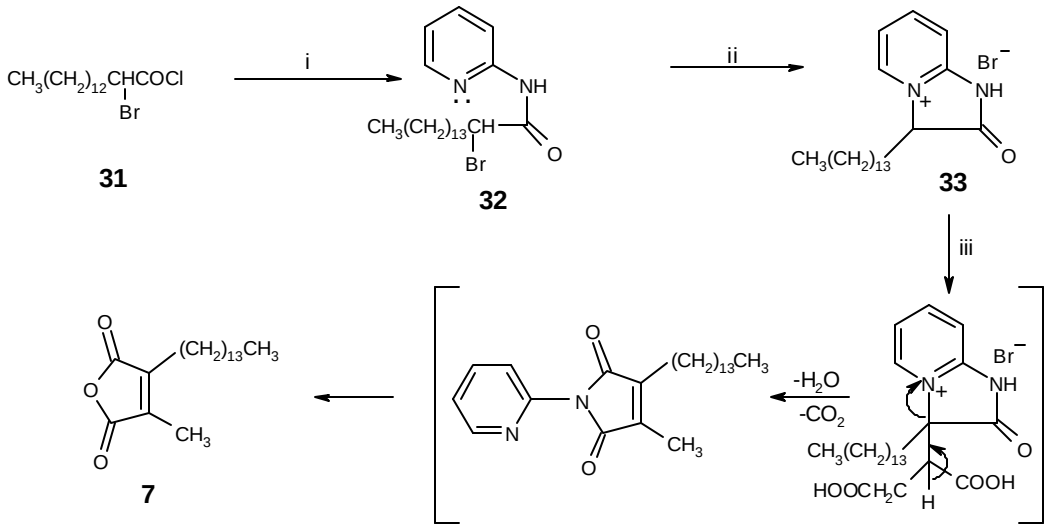


copper enolate with methyl iodide at lower temperature to give dimethyl *cis*-butenedioate derivative. Further hydrolysis with lithium hydroxide and acidification furnished the chaetomellic anhydride A (**7**). Similarly analogues wherein tetradecyl group was substituted with farnesyl/geranylgeranyl moieties were prepared and the farnesyl substituted compound exhibited 7-fold more potency than parent chaetomellic anhydride A.

Fifth Synthesis

A three-step approach for **7** has been reported[44] from our group with 62% overall yield (**Scheme 5**). The reaction of 2-bromopalmitoyl chloride (**31**) and 2-aminopyridine in presence of Et_3N furnished 2-bromopalmitamide derivative **32**, followed by intramolecular cyclisation to yield imidazopyridinium bromide **33**, which on condensation with maleic anhydride in the presence of NaOAc/AcOH gave chaetomellic anhydride A (**7**).

Scheme 5

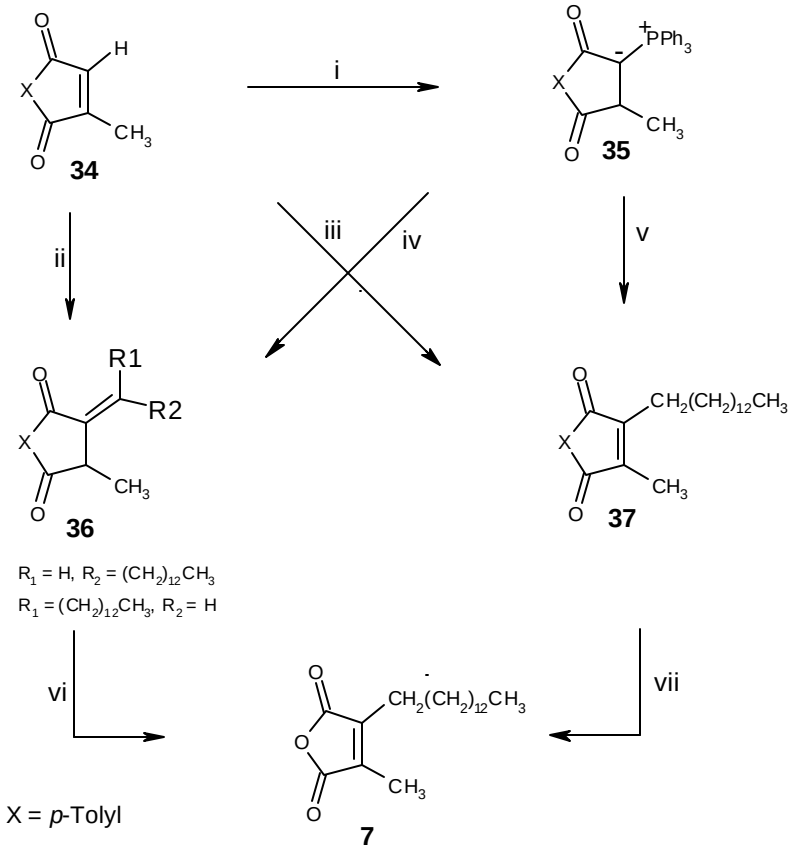


Reagents and conditions: (i) 2-Aminopyridine, TEA, Et_2O, rt; (ii) *t*-BuOH, reflux; (iii) maleic anhydride, NaOAc, AcOH, reflux.

Sixth Synthesis

A second approach from our laboratory involving a facile two-step synthesis of **7** via Wittig reaction with overall 89% yield has appeared recently[45] as a sixth synthesis of this hit molecule (**Scheme 6**). The first step in this strategy involves the formation of an ylide methyl-*N*-*p*-tolyl(triphenylphosphoranylidene)succinimide (**35**) obtained from citraconic anhydride which smoothly condensed with the tetradecanal in refluxing glacial

Scheme 6



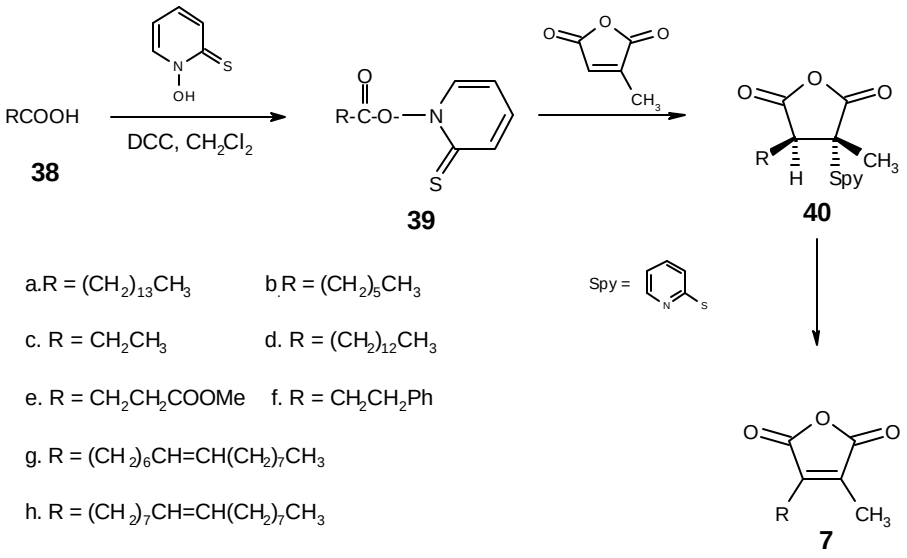
Reagents and conditions: (i) PPh_3, AcOH, reflux, 2 h; (ii) AcOH, $CH_3(CH_2)_{12}CHO$, reflux, 18 h; (iii) (a) condition ii, (b) Ä, 140-150 °C, 30 min. (iv) AcOH, $CH_3(CH_2)_{12}CHO$, reflux, 18 h. (v) (a) condition iv, (b) Ä, 140-150 °C, 30 min.; (vi) (a) CH_3ONa/CH_3OH, reflux, 2 h, (b) H^+/HCl; (vii) (a) $KOH/H_2O/CH_3OH/THF$, reflux, 2 h, (b) H^+/HCl.

acetic acid to yield mixture of geometric isomers **36** in 71% yield which on thermal isomerisation of double bond (exo to endo) in the same pot directly furnished maleimide derivative **37**. The alkaline hydrolysis of maleimide derivative followed by acidification furnished the chaetomellic anhydride A in 91% yield. The exo-isomers on hydrolysis and acidification also furnished the target molecule but in less yield as compared to maleimide derivative. Amongst the all existing synthesis this approach is most efficient and practical.

Seventh Synthesis

The one-step synthesis reported by Samadi *et al*[46] utilizes Barton radical decarboxylation of thiohydroxamic ester, in presence of citraconic anhydride, to obtain an intermediate addition product which, on ***b***-elimination afforded chaetomellic anhydride A, B and other analogues in 70% overall yield (**Scheme 7**).

Scheme 7



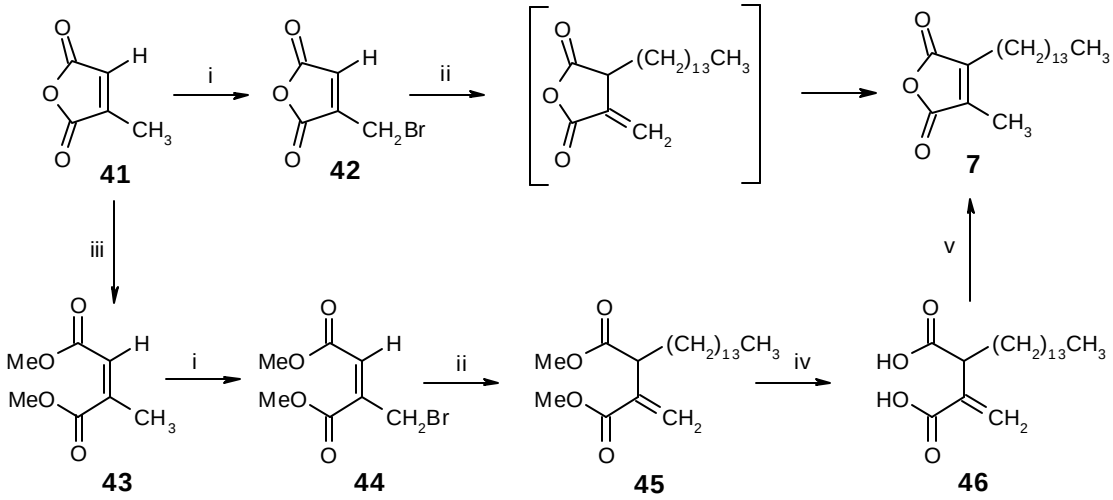
Eighth Synthesis

It will be discussed as apart of this dissertation in the section A of chapter two.

Ninth Synthesis

Recently one more four-step synthesis of chaetomellic anhydride A has been successfully completed with 56% overall yield from our laboratory (**Scheme 8**) by chemoselective carbon-carbon bond formation via Michael type addition of Grignard reagent to dimethyl-(bromomethyl)maleate (**44**) with elimination of allylic bromo atom (S_N2' reaction).[47] Dimethyl citraconate (**43**) on NBS bromination furnished bromoderivative **44** in 85% yield. Addition of Grignard reagent generated from 1-bromotetradecane to **44** at rt in presence of HMPA gave dimethylitaconate derivative (**45**)

Scheme 8



Reagents and conditions: (i) NBS, AIBN, CCl_4, 10 h. (ii) $CH_3(CH_2)_{13}MgBr$, HMPA, Et_2O, rt, 8 h. (iii) MeOH/ H_2SO_4 (9:1), 24 h. (iv) AcOH/HCl (6:4), 5 h. (v) Ac_2O, reflux,

in 70% yield. The hydrolysis of diester (**45**) in acetic acid: HCl (6:4) followed by refluxing the formed diacid **46** in acetic anhydride yielded the chaetomellic anhydride (**7**) in

quantitative yield. Both cyclisation followed by *gem*-disubstituted exocyclic to tetrasubstituted endocyclic carbon-carbon double bond isomerisation took place in one pot.

By now it is quite evident that very exciting novel chemistry has been reported in all above approaches towards the synthesis of **7,** each synthesis having its own advantages. The present work describes the eighth two-step approach for the synthesis of chaetomellic anhydride A (**7**) and its natural analogues employing chemoselective carbon-carbon coupling of organocuprates with (bromomethyl)methylmaleic anhydride with intact preservation of cyclic anhydride moiety in 55-60% overall yield.

A few secondary metabolites have been isolated[23] from the mycelium species *Aspergillus wentii* and characterized as a long chain derivatives of citraconic anhydride and may be called as remotely functionalized derivatives of chaetomellic anhydride A (entry **8**, **Table 2**). The structural elucidation has been done with the help of spectral data.[23] The biosynthesis of these metabolites may proceeds via condensation of C_{18} polyketide derived fatty acid unit with oxalacetic acid, followed by decarboxylation and dehydration. The synthesis of these derivatives has not been reported to date and it is proposed to synthesize some of them starting from naturally occurring Juniperic acid.

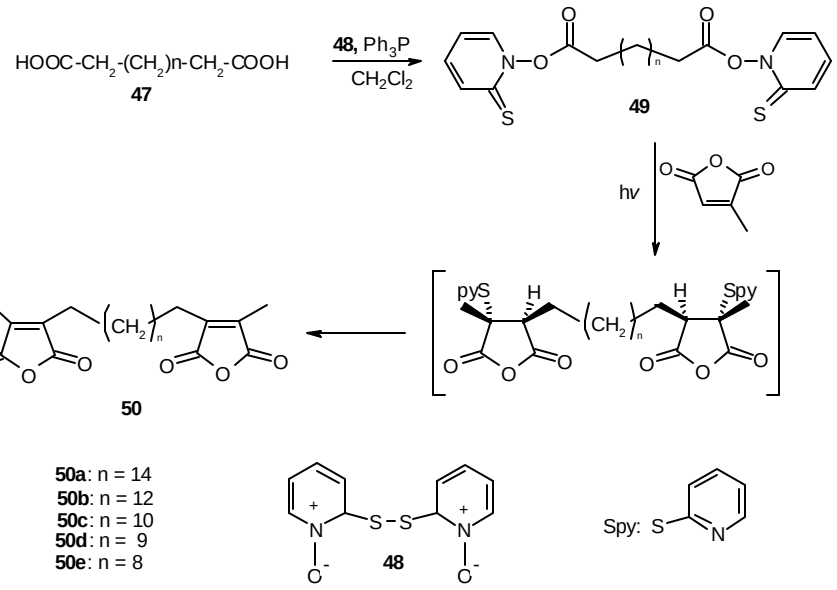
1.2.3 Tyromycin A

Tyromycin A (entry **7**, **Table 2**) has been recently isolated from mycelial cultures of basidiomycete *Tyromyces lacteus* (Fr.) Murr, and its structure was established as 1,16-bis[4-methyl-2,5-dioxo-3-furanyl]hexadecane by using spectral and analytical techniques and by transformation into corresponding tetramethyl ester and diimide derivatives.[22] Amongst the enzymes bound to the surfaces of mammalian cells, aminopeptidase have

been recognized as a potential target for immunomodulating drugs. Tyromycin A was found to inhibit the leucine and cysteine aminopeptidases bound to the outer surface of HeLa S3 cells and therefore may possess cytotostatic activity.[48] Two syntheses of this promising bioactive compound have been reported to date.

The first synthesis of tyromycin A and its analogues has been accomplished by Samadi *et al*[49] using double decarboxylative Barton-radical coupling reaction as a key step (**Scheme 9**). The dicarboxylic acids **47** were converted to their thiohydroxamic diesters **49**, using PPh_3/2,2'-dithiobis(pyridine *N*-oxide) coupling method. An i*n-situ* irradiation of the

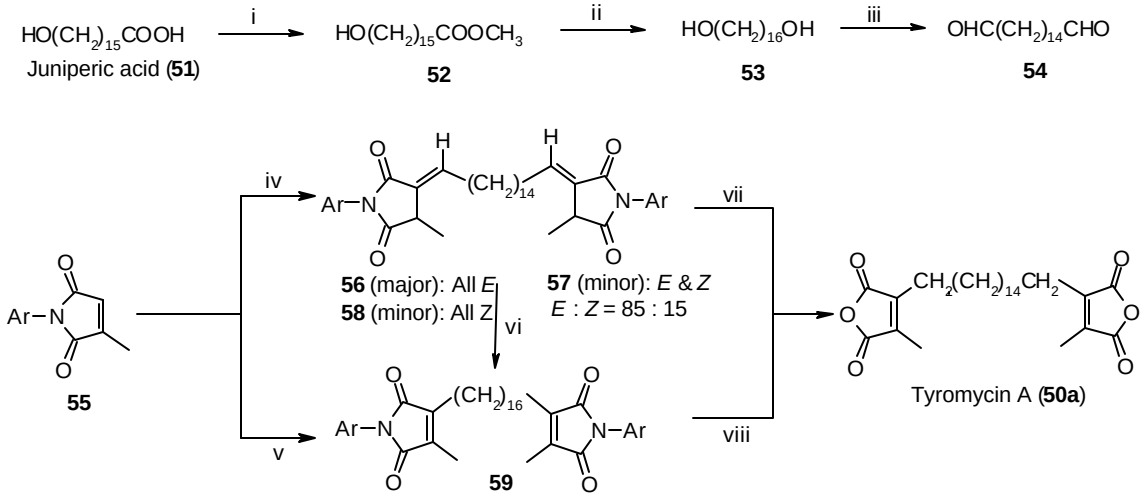
Scheme 9



thiohydroxamic diesters **49** in the presence of citraconic anhydride, with tungsten light (500 W), during 30 minutes furnished intermediate addition product which on facile elimination of pyridylthio group offered a rapid synthetic access to tyromycin A (**50a)** and its analogues (**50b-e**).

The second synthesis of tyromycin A has been recently reported[50] from our laboratory and utilizes citraconimide-TPP adduct coupling reaction with aliphatic dialdehyde to complete the practical two-step synthesis in 71% overall yield (**Scheme 10**). The reaction of dialdehyde (**54**) with an excess of citraconimide-TPP adduct in refluxing glacial acetic acid followed by removal of acetic acid in vacuo furnished a mixture of *bis*-condensed exo Wittig products **56** (*E*,*E* major), **57** (*E*,*Z* minor) and **58** (*Z*,*Z* minor) in 70% yield with an 85:15 ratio of *E*:*Z* geometry of the carbon-carbon double bond, whereas the removal of acetic acid under normal atmospheric pressure and heating the residue for 30 min. at 140-150 °C, the reaction directly furnished the endo bisimide **59** in 72% yield.

Scheme10



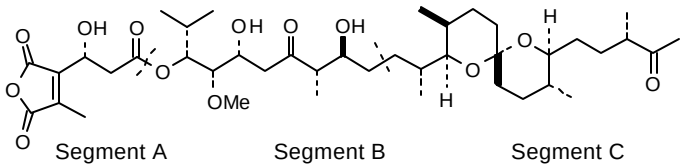
Ar = *p* -Tolyl

Reagents and conditions: (i) CH_2N_2, Et_2O, 0 ^oC, 2 h (95%). (ii) LAH, Et_2O, rt, 2 h (98%). (iii) PCC, CH_2Cl_2, rt, 10 h (77%). (iv) TPP, AcOH, **5**, reflux, 10 h (70%). (v) (a) TPP, AcOH, **5**, reflux, 10 h (b) △, 140-150 ^oC, 30 min. (72%). (vi) Tetralin, reflux, 1 h (98-100%). (vii) (a) CH_3ONa, CH_3OH, reflux, 2 h (b) H^+/HCl (60%). (viii) (a) KOH, H_2O, THF, CH_3OH, reflux, 2 h (b) H^+/HCl (98%).

The mixture of **56** + **57** + **58** in refluxing tetraline underwent *exo* to *endo* isomeristaion to yield bismaleimide derivative **59** in quantitative yield which on treatment with alkali followed by acidification furnished tyromycin A (**50a**). The mixture of *exo* isomers (**56, 57** and **58)** on treatment with sodium methoxide in methanol followed by acidification also gave tyromycin A (**50a)** in 60% yield.

1.2.4 2,3-Disubstituted Maleic Anhydride Segment of Tautomycin

Recently in 1987 Isono and co-workers[24] reported the isolation of tautomycin (**60**) from a strain of *Streptomyces spiroverticillatus* as a new antibiotic with strong antifungal activity against *Sclerotinia sclerotiorum*. The same group elucidated the structure of tautomycin on the basis of chemical degradation and spectroscopic evidence.[24] 2D INADEQUATE spectroscopy of tautomycin labeled with [1,2-^{13}C] acetate permitted the complete assignment of ^{13}C and ^{1}H signals and established the total structure. In 1993 the absolute configuration of **60** with 13 chiral centers was determined by the same research

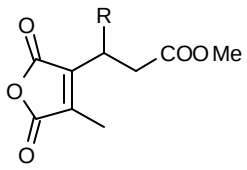


Tautomycin (60)

group,[51] using chemical transformation, spectroscopic data and conformational calculations. The absolute configuration by X-ray analysis was not possible because of its non-crystallinity. Apart from its antifungal activity the antibiotic was found to induce morphological changes (bleb formation) in human leukemia cells K562, which is correlated with protein phosphorylation and also inhibited spreading of human myeloid leukemia cells HL60.[52] Afterwards it was found that tautomycin is also a specific inhibitor of protein

phosphatases (PPs). One of the most pervasive controllers of signal transduction in eukaryotic cells is reversible phosphorylation of serine-, threonine- and tyrosine containing protein by PPs.[53] This molecular "on-off switch" is responsible for regulating such diverse and important processes as memory, cell growth, neurotransmission, glycogen metabolism, muscle contraction and many others.[53] The specific inhibitors of PPs became an useful tool for studying such intracellular events. Tautomycin was found to be potent and specific inhibitor of PP1 and PP2A with IC_{50} 22 and 32 nM respectively, whereas it inhibits PP2B at very high concentration (IC_{50} > 10 μM) and does not show any inhibition towards PP2C. The specific inhibition of particular PPs is physiologically important since four endogenous proteins, DARPP-32, inhibitor-1, inhibitor-2 and NIPP-1 act stringently to regulate PP1, and abnormally low levels of PP activity have been implicated in human cancer[54a] and Alzheimer's disease.[54b] Thus the tautomycin, a specific inhibitor of PP1 and PP2A has acquired a significant position in elucidating the physiological role of PPs.

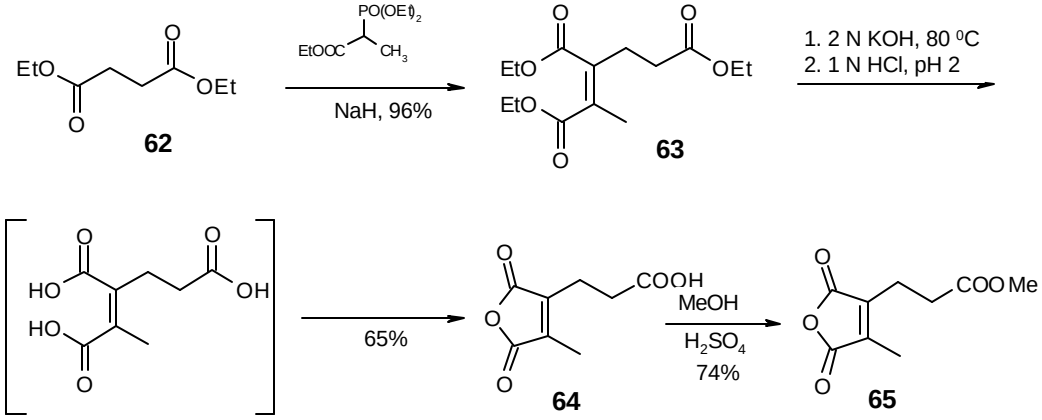
The important biological activity and interesting structural features attracted many chemists to put in their efforts for the total synthesis of tautomycin and is a challenging task of current interest. The reterosynthetic analysis of tautomycin afforded segment A, B and C as shown in structure **60**. Total synthesis of this molecule involves the synthesis of three segments followed by stepwise coupling of these building blocks.[55] To date five total synthesis of tautomycin have been accomplished by different groups.[56] Tautomycin has unique 2,3-disubstituted maleic anhydride ring at the left terminal of the molecule which is known as a segment A (**61**).[57] The segment A is highly oxygenated molecule with three carboxylic group and one hydroxy group. According to Chamberlin *et al*[58] the greatest challenge in the synthesis of tautomycin is in the construction of apparently simple-looking



61A R = OH, **61B** R = *α*-OH

2,3-disubstituted maleic anhydride segment A. The anhydride moiety of tautomycin shows an interesting chemical behavior in aqueous media, i.e. tautomycin exists in an equilibrium between anhydride and diacid.[57] Isobe and co-workers in earlier stage of their work, synthesized a model compound with 2,3-disubstituted maleic anhydride structure to confirm the chemical properties of the anhydride (**Scheme 11**).[59] Stereoselective Horner-

Scheme 11

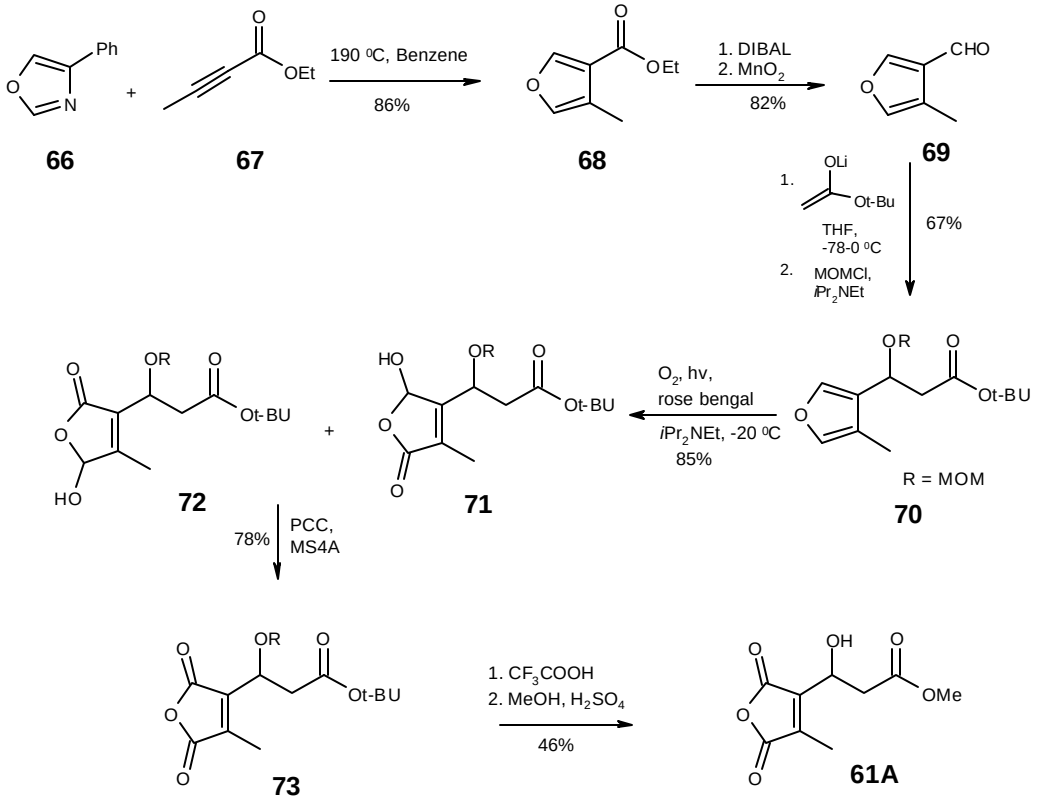


Emmons condensation of diethyl-2-ketoglutarate (**62**) with triethyl *α*-phosphonopropionate exclusively provided maleic ester (**63**) in 96% yield, which on alkaline hyrolysis followed by acidic workup afforded the 2,3-disubstituted maleic anhydride **64**.

To date, four multi-step synthesis of segment A have been accomplished using various elegant strategies. Isobe and co-workers successfully accomplished the first eight-step synthesis of segment A via aldol condensation as a key step with 15% overall yield

(**Scheme 12**)[59,60]. The 3,4-disubstituted furan (**68**) was synthesized by Diels-Alder addition of ethyl tetrolate (**67**) to 4-phenyloxazole (**66**) followed by retro-Diels-Alder reaction with elimination of benzonitrile. Reduction of **68** and subsequent oxidation gave the aldehyde

Scheme 12

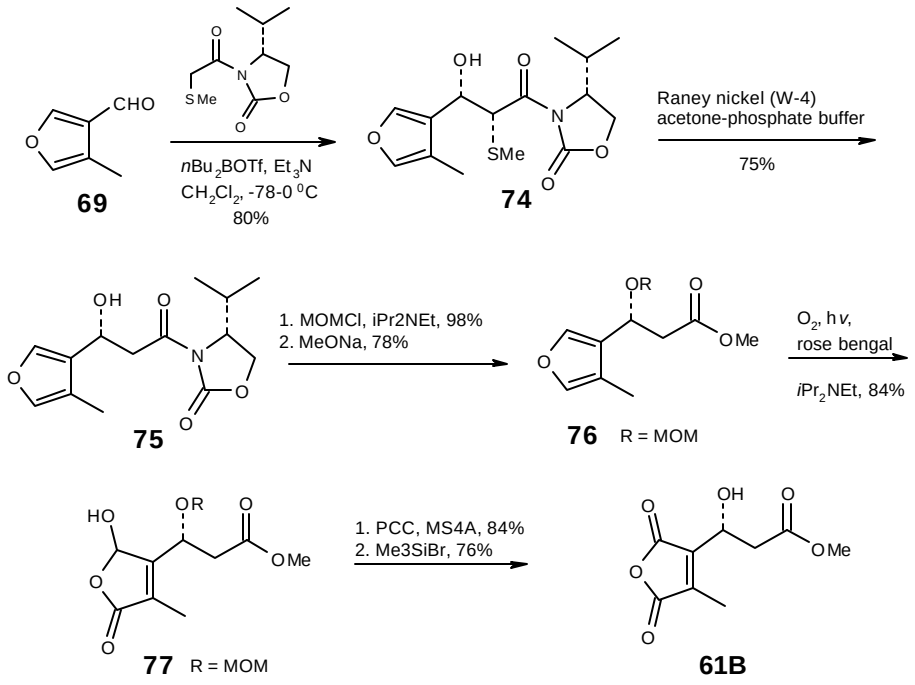


69, which is reacted with lithium enolate of *tert*-butylacetate to furnish the aldol product **70**. Finally the oxidation of furan moiety to maleic anhydride derivative furnished the target molecule, a segment A (**61A**) of tautomycin.

The same group has extended their approach for the synthesis of optically active segment A (**61B**) in a similar manner to their earlier synthesis employing asymmetric aldol reaction and photosensitized oxidation (**Scheme 13**).[59,61] Aldol reaction between boron enolate of chiral *N*-acetyloxazolidone (from D-valine) and aldehyde provided aldol adduct

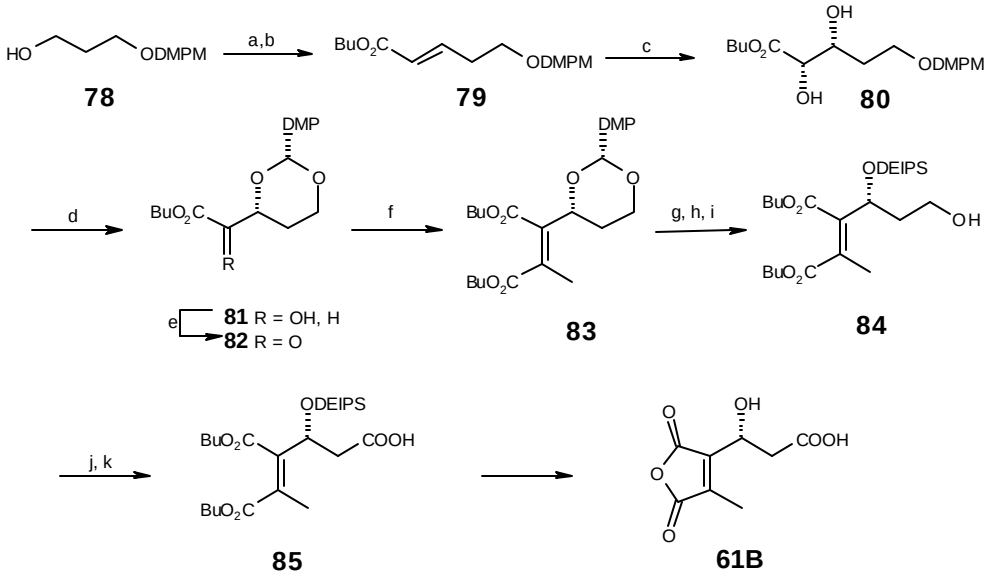
(**74**) in 80% yield. Desulfurization, protection of hydroxy group followed by methanolysis with sodium methoxide in methanol furnished methyl ester (**76**). The substituted furan derivative on subsequent oxidation gave the maleic anhydride segment of tautomycin **61B**.

Scheme 13



The second ten-step synthesis of segment A with 25% overall yield starting from 1,3-propanediol was reported by Oikawa and Ichihara (Scheme **14**).[62] Asymmetric dihydroxylation of the ***a,b***-unsaturated ester (**79**) obtained by Wittig reaction of aldehyde, which was in turn synthesized from monoprotected 1,3-propanediol is a key step utilized in this synthesis. The remaining reaction sequence utilized protection-deprotection chemistry and oxidation reactions to achieve the target molecule **61B** as shown in scheme

Scheme 14

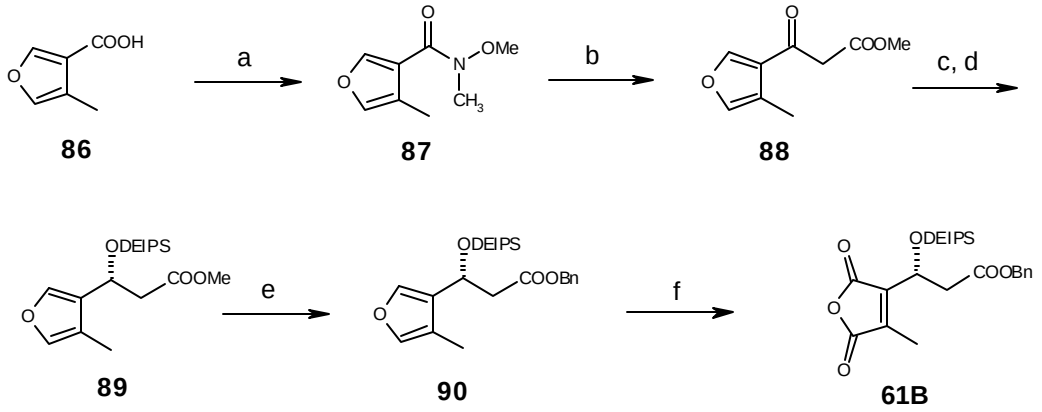


Reagents and conditions: (a) SO_3.Py, DMSO, Et_3N, CH_2Cl_2; (b) $^tBuO_2CCH{=}PPh_3$, CH_2Cl_2, 90%, (2 steps); (c) AD-mix-***b***, $MeSO_2NH_2$,) ^tBuOH-H_2O, 0 °C, 99%; (d) DDQ, MS3A, CH_2Cl_2, 5 °C, 66%; (e) Dess-Martin periodinane, Py, CH_2Cl_2; (f) $EtO_2CCH(CH_3)PO(OEt)_2$, ^tBuOK, THF, -60 to –20 °C (67%, 2 steps); (g) PPTS, MeOH, 98%; (h) DEIPSCl, Im, CH_2Cl_2, 89%; (i) AcOH-H_2O-THF (4:1:4), 95%; (j) Dess-Martin periodinane, Py, CH_2Cl_2, 87%; (k) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, ^tBuOH-H_2O, 91%

.

The third approach has been published from The University of Tokyo by Shibasaki *et al*[63] using an asymmetric reduction of ***b***-ketoester as a key step (**Scheme 15**). The starting furan carboxylic acid (**86**) was converted to amide, which was treated with litium enolate of methyl acetate to yield ***b***-ketoester **88**. Asymmetric hydrogenation of **88** has been achieved by two ways, one using Noyori's Ru-BINAP catalyst yielded alcohol with 86% ee whereas using BH_3-THF oxazaborolidine catalyst gave alcohol in 57% yield with 92% ee. Furan derivative 9**0** was finally oxidized photochemically to get maleic anhydride segment of tautomycin.

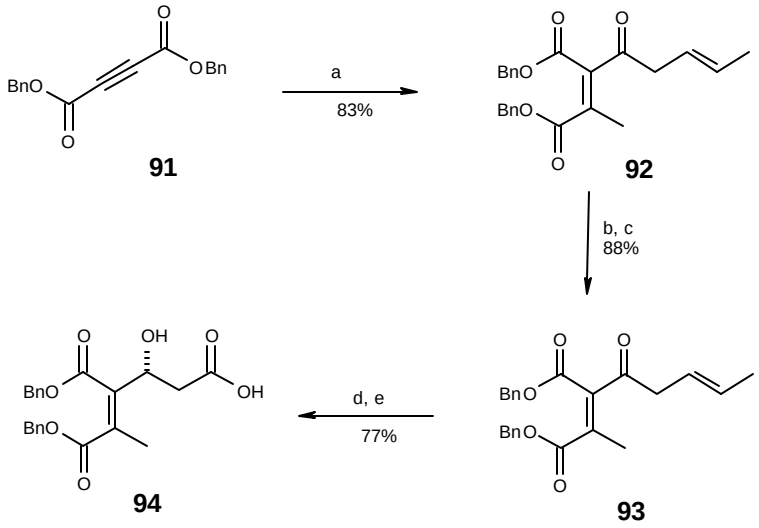
Scheme 15



Reagents and conditions: (a) $CH_3ONHCH_3.HCl$, Et_3N, DMF, 0 °C to rt 97%; (b) $AcOCH_3$, LDA, THF, -78 °C to 0 °C then HCl, 0 °C to rt, 58% (conv. 73%); (c) (i) H_2, 100 atmo., cat. (*S*)-BINAP-Ru(II), CH_3OH, 28 0 °C, 100%, 86% ee; (ii) (S) *, BH_3-THF, 0 °C, (conv. 67%), 92% ee; (d) DEIPSCl, imidazole, CH_2Cl_2, 0 °C to rt, 98%; (e) (i) LiOH, THF-H_2O (6:1), rt, (ii) BnOH, DCC, DMAP, THF, rt, 62% (2 steps); (f) (i) O_2, h*v*, rose bengal, i-Pr_2NEt, CH2Cl2, 0 °C; (ii) PCC, MS4A°, CH_2Cl_2, rt, 88% (2 steps); (g) (i) CH_3OH, Et_3N, 0 °C; (ii) CH_2Cl_2, 40 °C; (iii) DEIPSCl, imidazole, CH_2Cl_2, 0 °C to rt, 66% (3 steps); (h) H_2, Pd/C, rt, 87%.

The fourth elegant approach from Chamberlin and co-workers[58] describes five-step synthesis with 56% overall yield starting from acetylenedicarboxylic ester (**Scheme 16**). Addition of a mixed methyl cuprate to symmetrical acetylenedicarboxylic ester, followed by trapping of the intermediate with an electrophile 3-pentenoyl chloride gave the unstable enone (**92**) as a > 20:1 mixture of geometrical isomers in 83% yield. The reduction of ketone with (+)-DIP-Chloride furnished the desired (*R*) enantiomer of alcohol (**93**) in 88% yield with 80% ee. Ozonolytic cleavage of disubstituted alkene (**93**) and subsequent oxidation of the aldehyde to carboxylic acid provided maleic anhydride segment A of tautomycin **94** in 77% yield.

Scheme 16



Reagents and conditions: (a) MeCuLiCN, Et_2O, -78 °C, then 3-pentenoyl chloride, -78 to 0 °C; (b) (-)-DIP-Chloride, THF, -20 °C, 3 days, 0 °C, 4 h; (c) TESCl, TEA, CH_2Cl_2, 0 °C to rt; (d) O_3, CH_2Cl_2, -78 °C, PPh_3, -78 °C to rt (e) $NaClO_2$, 2-Methyl-2-butene, *t*-BuOH/H_2O, 0 °C.

The fifth approach will be discussed as a part of this dissertation in the section B of chapter two.

1.2.5 Telfairic Anhydride and Related Compounds

The new methylmaleic anhydride metabolite has been isolated in 1996 by Edward and his co-workers[25] from the culture medium of the fungus *Xylaria telfairii* Berk and was named as 2,3-didehydrotelfairic anhydride (entry **10**, **Table 2**). The structural assignment of telfairic anhydride has been done on the basis of analytical and spectral data.[25] The biosynthesis of telfairic anhydride presumably originates from a process involving a condensation of an acetate malonate-derived acid with oxaloacetate. To date a biological role for such structure does not appear to have been established. The unique structure of 2,3-didehydrotelfairic anhydride attracted us to put in our synthetic efforts to achieve the

first synthesis of this natural product. Our efforts are documented as a part of this dissertation and discussed in the section C of the chapter two.

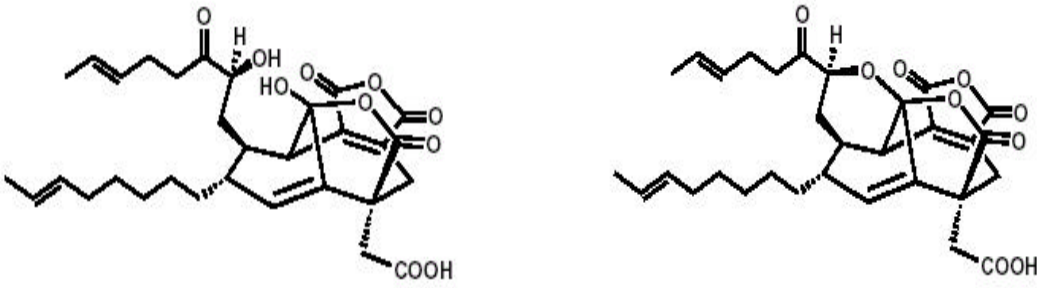
The other related methylmaleic anhydrides graphenone[26] (entry **11**, **Table 2**) and itaconitin[27] (entry **12**, **Table 2**) have been reported in the literature. Graphenone, a new pigment has been isolated from the cultures of spore derived mycobionts of the lichens *Graphis scripta* and the structure was established using spectral and X-ray diffraction analysis. Tetraenoic anhydride, itaconitin has been isolated as a secondary fungal metabolite from the species *Aspergillus itaconicus* and *Aspergillus gorakhpurensis* . The parent acids related to both of these anhydrides are currently unknown as natural products.[25]

1.3 NONADRIDE AND DIALKYL/DIARYL SUBSTITUTED DERIVATIVES OF MALEIC ANHYDRIDE

The natural products known collectively as nonadrides comprises a small structural class in which the core unit is nine membered carbocyclic ring.[64] Many cyclic compounds containing two maleic anhydride moieties (mainly nonadrides) have been isolated and characterized as bioactive natural products more prominent amongst them are Glaucanic acid (entry **1**, **Table 4**), byssochlamic acid (entry **2**, **Table 4**), heveadride (entry **4**, **Table 4**), CP molecules (**Fig. 3**), and so on as listed in **Table 4**. Two five membered anhydrides or anhydride and a lactol are fused to the core, which also bears a *n*-alkyl chain and in some cases one or more hydroxy substituents. Glaucanic and glauconic acids were the first members of this class to be discovered,[65] and soon after a "symmetrical" variant,

byssochlamic acid[66] was isolated by Raistrick from the ascomycete *Byssochlamys fulva*. Subsequently two hepatotoxic substances, rubratoxins A and B were obtained from extracts of fungus *Penicillium rubrum*[67] and more recently the nonadrides scytalidin,[68] heveadride[69] and castaneiolide[70] also have been isolated. The latest example of this structural family are CP-225,917 (phomoidride A) and CP-263,114 (phomoidride B) two metabolites isolated by a research group at Pfizer from an unidentified fungus which also produces zaragozic acid.[71] Nonadrides attracted the attention of the researchers due to the powerful inhibition

Fig. 3 CP Molecules

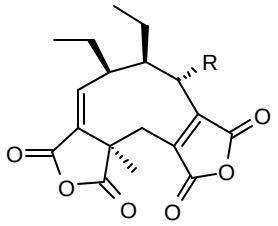
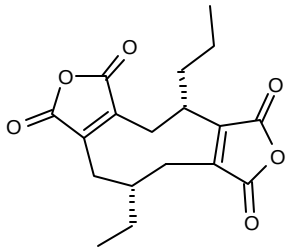
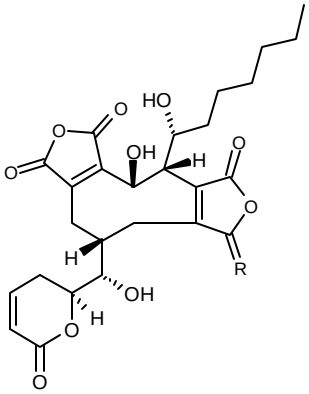
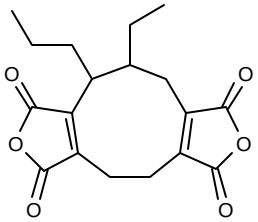


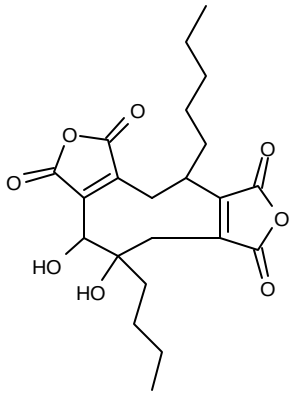
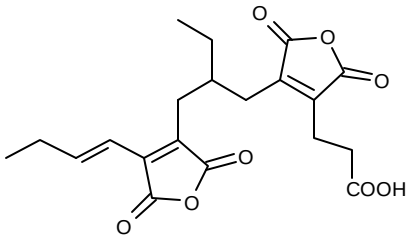
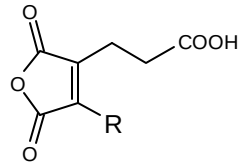
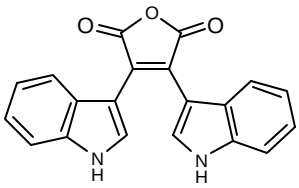
(+)-CP-225,917 (phomoidride A) **(-)-CP-263,114 (phomoidride B)**

of ras farnesyl transferase by CP molecules[72] has made these nonadrides the object of much interest.[73] CP molecules exemplify architectures of unprecedented molecular connectivities and complexities and possess intriguing biological activity. Recently two new alkenoic acids bearing two or three maleic anhydride moieties in the linear acid chain have been isolated from fungus *Cordyceps pseudomilitaris* and named as cordyanhydride A and B.[74]

The first published synthesis of nonadride family was that of byssochlamic acid by Stork *et al*[75] in 1972. The pioneering accomplishment, i.e. creation of the nine membered

Table 4: Naturally Occurring Nonadride and Dialkyl/Diaryl Derivatives of Maleic Anhydride

Sr. No.	Compound Synthesized	Source and Activity	Characterization	Ref.
1	 Glaucanic Acid R = H Glauconic Acid R = OH	*Penicillium purpurogenum* Activity Unknown	IR, PMR, CMR, Mass.	65
2	 Byssochlamic acid	*Byssochlamys fulva* Activity Unknown	IR, PMR, CMR, Mass.	66
3	 Rubratoxin A R = H, OH Rubratoxin B R = O	*Penicillium rubrum* Hepatotoxic activity	IR, PMR, CMR, Mass.	67
4	 Heveadride	*Helminthosporium heveae* Activity Unknown	IR, PMR, CMR, Mass and UV absorption spectra	69

5	 Castaneiolide	*Macrophoma castaneicola* Activity Unknown	IR, PMR, CMR, Mass.	70
6	 Cordyanhydride A or FR222398	*Cordyceps pseudomilitaris* Activity Unknown	IR, PMR, CMR, Mass.	74
7	 R = CH=CH-C_2H_5, C_6H_{13}, C_8H_{17}	*Psudomans cepacia* A-1419 *Paecilomyces Variotii* Activity Unknown	IR, PMR, CMR, Mass.	82
8	 Bisindolyl maleic anhydride	Starting material in the synthesis of indolocarbazole alkaloid	IR, PMR, Mass.	83, 85

ring of the target molecule via Beckman fragmentation as a key step. The second report on the synthesis of racemic byssochlamic acid was published by White *et al*[76] in 1992 using [2+2] photoaddition-cycloreversion strategy. Recently the authors have extended the same strategy to achieve the asymmetric synthesis of natural (+)-byssochlamic acid as well as its

enantiomer (-)-byssochlamic acid.[77] The synthesis of isoglaucanic acid via 6π + 4π cyclodimerisation of 2-[(*E*)-1-pentenyl]-3-methylmaleic anhydride was proposed by Sutherland[78] and completed by Baldwin *et al*[79] in 1999. Recently Nicolaou and co-workers from Scripps Research Institute has successfully completed[80] the first total synthesis of highly complex CP molecules through a very high intellectual and well planned rationale synthetic efforts overcoming countless obstacles with discovery of novel cascade reactions, new synthetic technologies and unprecedented synthetic tactics. The synthetic strategy involves (i) intramolecular Diels-Alder reaction as a key step to generate the core skeleton of the molecule, (ii) Stereoselective fastening of the upper side chain by using substrate-directed dithiane chemistry and (iii) installment of anhydride moiety on the periphery of the bicyclic CP skeleton by employing an unprecedented seven-step cascade reaction sequence. The same group has also reported[81] the asymmetric synthesis of these molecules employing the reaction sequence described in their earlier synthesis using key step of substrate-based control of diastereoselectivity of intramolecular cycloaddition specifically by introducing bulcky chiral moiety in the substrate capable of influencing facial selectivity of intramolecular Diels-Alder reaction.

Few unsymmetrically substituted maleic anhydrides[74,82] (entry **6** and **7**, **Table 5**) have been isolated as natural products. Based on the literature study to date there is no report directly focusing on the synthesis of symmetrical and unsymmetrical substituted long chain dialkylmaleic anhydrides. The diarylmaleic anhydrides are vital intermediates in the synthesis of few alkaloids and another natural products possessing various biological activities.[83] Recently Beccalli *et al*[84] have reported a new three-step access to diarylmaleic

anhydride from 3-aryl-2-hydroxybut-2-enedioate, whereas previous synthesis was reported using Perkin condensation.[85]

1.4 SUMMARY

Maleic anhydride and its derivatives have been used as a potential starting material for the elegant design of several biologically active heterocycles, natural products, drugs, drug intermediates and variety of polymers. Nature also offers a diverse menu of methylalkylmaleic anhydrides and complex organic molecules containing two or more maleic anhydride moieties with well-established and new promising bioactivities. Huge amount of synthetic efforts have been put in to cast these useful maleic anhydride skeletons in highly efficient fashion, employing variety of new elegant strategies and efforts are also in progress to invent still newer methods. It is noteworthy that natural/synthetic genesis, is unknown for the higher alkylsubstituted and symmetrically/unsymmetrically dialkylsubstituted maleic anhydrides/maleic acids. Newer methods to synthesize these systems are still awaited and will certainly contribute substantially to several application parts of these structures and their chemistry.

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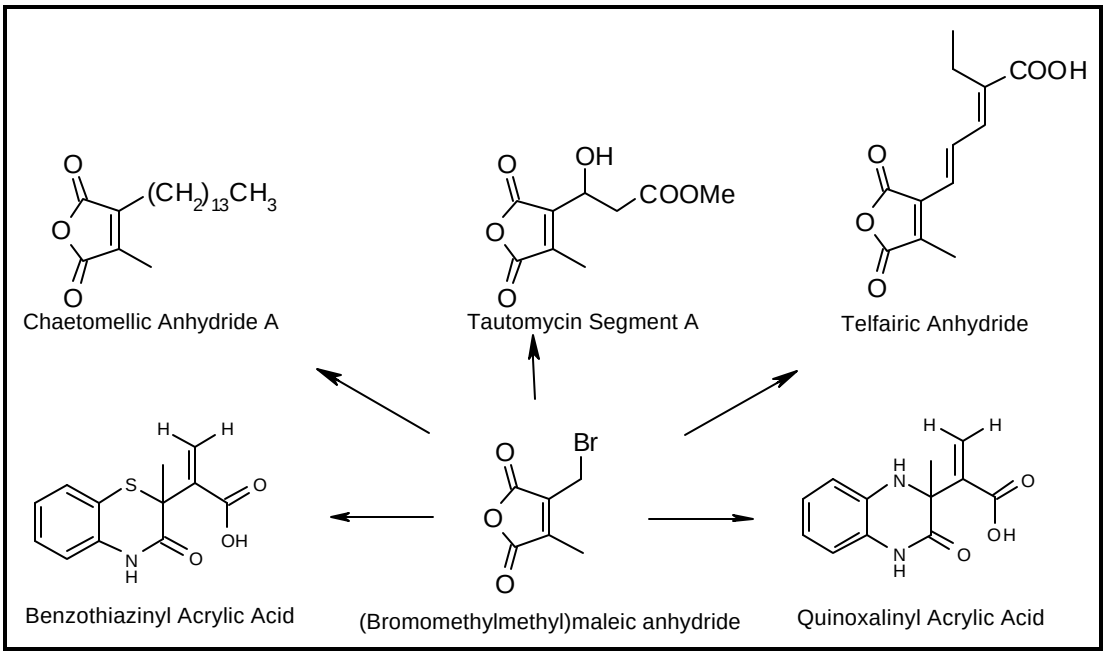
CHAPTER TWO

SYNTHESIS OF BIOACTIVE NATURAL PRODUCTS

AND NEW HETEROCYCLES

This chapter is divided into four sections (A to D). First three sections (A to C) deal with the synthesis of bioactive natural products chaetomellic anhydride A, maleic anhydride segment of tautomycin and 2,3-didehydrotelfairic anhydride respectively, where as the fourth one (section D) describes the synthesis of ***a***-quinoxalinyl and ***a***-benzothiazinyl acrylic acids starting from (bromomethyl)methylmaleic anhydride, which was obtained by NBS bromination of dimethylmaleic anhydride (**Fig 1**).

Fig. 1

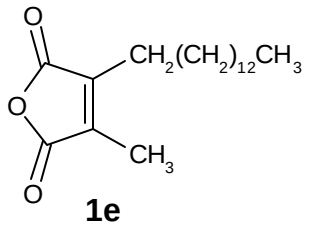


SECTION A

CHEMOSELECTIVE CARBON-CARBON COUPLING OF ORGANOCUPRATES WITH (BROMOMETHYL)METHYLMALEIC ANHYDRIDE: SYNTHESIS OF CHAETOMELLIC ACID A

2.1.1 BACKGROUND

Chaetomellic anhydride A and B have been recently isolated[1] from *Chaetomella acutiseta*, and their dianionic forms are potent and highly specific inhibitors of ras farnesyl-protein transferase. The provision of facile synthetic approaches to this bioactive natural product, chaetomellic acid A anhydride (tetradecylmethylmaleic anhydride,) is a task of current interest. After isolation of this bioactive compound in 1993 to date nine alternate syntheses of **1e,** five from abroad and four from our group have been accomplished using variety of elegant strategies[2-10] (discussed in earlier chapter). In recent years, a number of 2-alkyl-3-methyl substituted maleic anhydrides[11] have been isolated as natural products such as 2-ethyl-3- methylmaleic anhydride,[11b] 2-hexyl-3-methylmaleic anhydride,[11c] 2-octyl-3-methylmaleic anhydride,[11c] chaetomellic acid C[11a] and some of them exhibit specific biological activities. In an attempt to have an easy access to this type of substituted maleic anhydrides, we planned a facile two-step approach to *n*-alkylmethylmaleic anhydrides via chemoselective carbon-carbon coupling of Grignard reagents with (bromomethyl)methylmaleic anhydride (**22**).



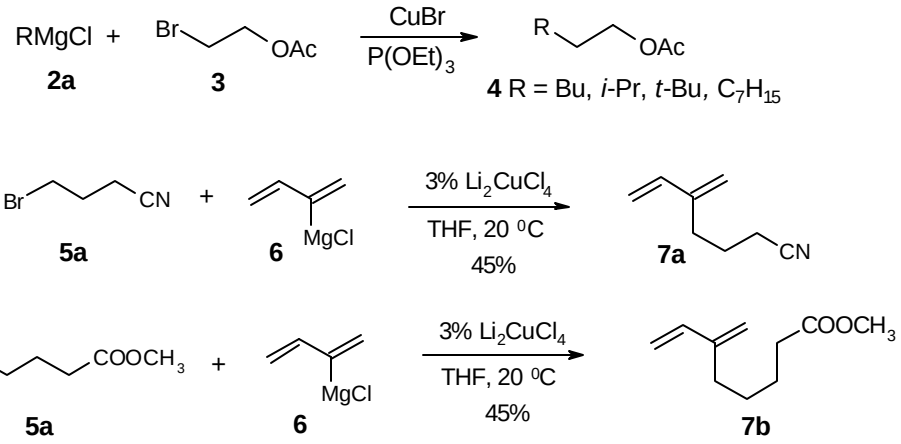
1e

Chaetomellic Anhydride A (**1e**)

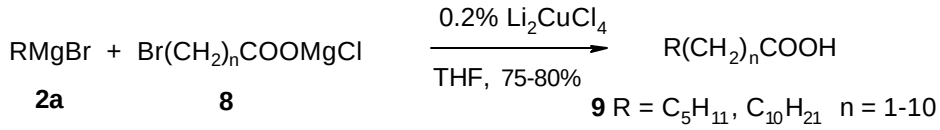
Chemoselective Grignard reactions, with preservation of certain functionalities have been reported in the literature (**Scheme 1**). Normant *et al*[12] reported cross coupling of Grignard reagent with 2-acetyl bromoethane (**3**) by preserving acetate group where as Numomoto *et al*[13] have successfully carried out the cross coupling reaction of 1,3-butadiene-2-ylmagnesium chloride (**6**) and substituted halide keeping the cyano and ester groups intact as shown below (**Scheme 1a**). Baer *et al*[14] have reported the copper catalyzed

reaction of Grignard reagents with chloromagnesium salt of ω-bromoacids **8** to prepare variety of alkylcarboxylic acids with preservation of carboxylic acid functionality (**Scheme 1b**).

Scheme 1a

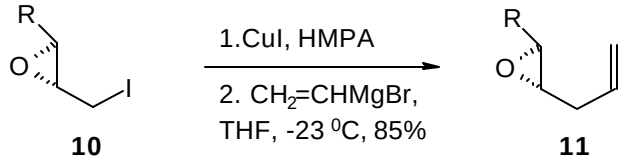


Scheme 1b



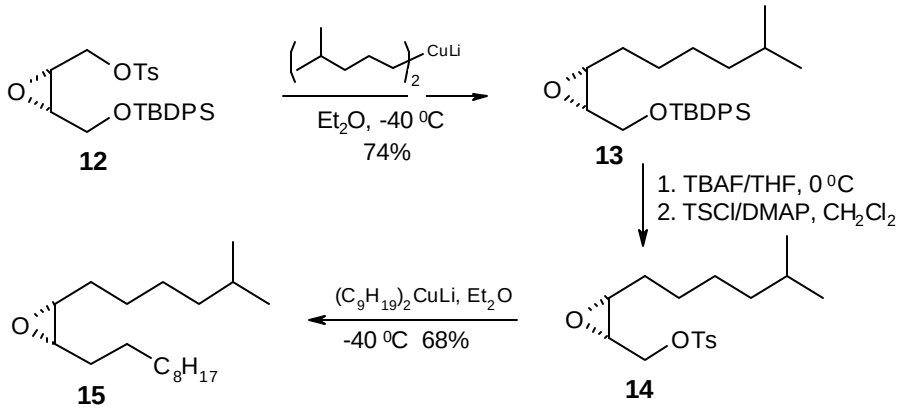
Grignard substitution reaction with intact preservation of epoxide functionality has been reported for the first time by Nicolaou *et al*[15] wherein the slow addition of allylmagnesium bromide to a mixture of substrate **10**, in the presence of catalytic amount of CuI (0.1 equiv) and HMPA (4 equiv) in THF at –23 °C gave exclusively the substitution product, homoallylic epoxide **11** in 85% yield (**scheme 1c**).

Scheme 1c



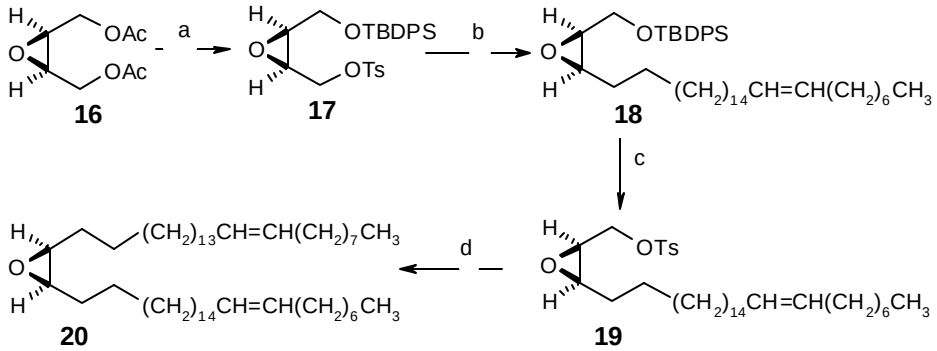
Mori and co-workers[16] have successfully completed the synthesis of pheromones with preserving the epoxide by using dialkyllithiumcuprate (**Scheme 1d**). Another

Scheme 1d



report[17] from Mori's group has focused on the synthesis of nymph recognition pheromone utilizes Grignard coupling reaction as a key step with an intact preservation of epoxide moiety (**Scheme 1e**). The enantiomerically pure epoxy building block **17** has been used as a starting material that in turn was synthesized from *meso*-diacetate **16** by asymmetric hydrolysis catalyzed by pig pancreatic lipases (PPL) and several other steps. Incorporation of carbon chain on **17** to get target molecule **20** was executed by copper

Scheme 1e



Reagents and conditions: (a) PPL and several other steps;
(b) (*Z*)-Me$(CH_2)_6$CH=CH$(CH_2)_{15}$MgBr, Et_2O, HMPA, CuI; (c) (i) $(Bu)_4NF$, THF, (ii) TsCl, TEA, DMAP, CH_2Cl_2; (d) (*Z*)-Me$(CH_2)_7$CH=CH$(CH_2)_{13}$MgBr, Et_2O, HMPA, CuI.

catalyzed Grignard reaction in diethylether in the presence of HMPA. They also synthesized the antipode by altering the sequence of two Grignard coupling reactions.

2.1.2 PRESENT WORK

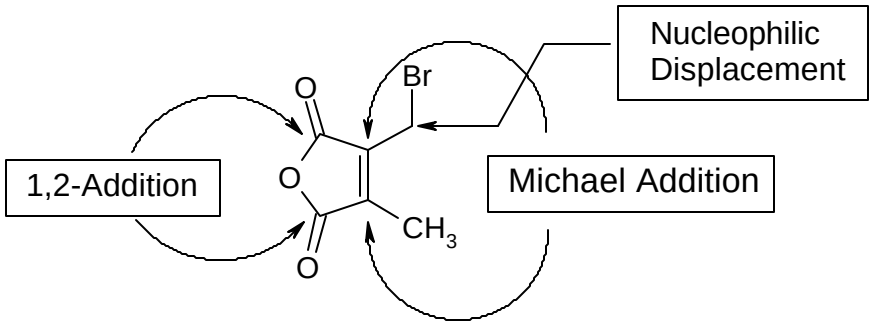
In the present work our idea was to synthesize the (bromomethyl)methylmaleic anhydride from the readily available dimethylmaleic anhydride and utilize this allylic halide as a substrate for the chemoselective nucleophilic displacement reaction (S_N2) using the Grignard reagent in a short two-step approach for the synthesis of 2-alkyl-3-methylmaleic anhydrides (**1a-e**) including ras farnesyl-protein transferase inhibitor chaetomellic anhydride A (**1e**) (**Scheme 2**). The unsymmetrical anhydride has five alternate sites (as shown in **Fig. 2**) available for nucleophilic reactions, viz

(i) two carbonyl carbons for 1,2-addition (with or without ring opening),

(ii) two sites for Michael addition and

(iii) allylic halide for nucleophilic displacement reaction.

Fig. 2



In general, a Grignard reagent is not likely to furnish coupling product in synthetically useful yields and can be significantly improved when these reaction are carried out in the presence of catalytic amount of copper(I) salts and other transition metals (eg. Ni, Pd, Fe). The copper-catalyzed substitutions of organic halides and related

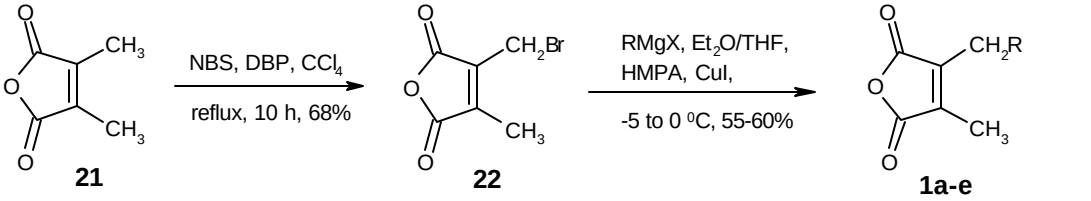
substrates with Grignard reactions are standard synthetic transformation in organic synthesis and have been reviewed extensively.[18,19] Chemoselective carbon-carbon coupling of organocuprates and bromoanhydride **3** by nucleophilic displacement reaction with preservation of maleic anhydride moiety has not been reported in the literature to date[219] and will be highly useful in synthesis of natural products bearing such type of anhydride skeletons.

2.1.3 RESULTS AND DISCUSSION

Reaction of dimethylmaleic anhydride (**21**) with *N*-bromosuccinimide (NBS, 2 eqiv) using catalytic amount of dibenzoyl peroxide (DBP) in carbon tetrachloride under gentle reflux for 10 h gave (bromomethyl)methylmaleic anhydride (**22**). Reaction conditions were optimized to get the major amount of required product (bromomethyl)methylmaleic anhydride (68%), accompanied by di(bromomethyl)maleic anhydride (2%) and starting material (30%) without formation of any other brominated products such as *gem*-dibromo (^{1}H NMR). Purification by vacuum distillation of this mixture using Kugelrohr apparatus yielded 60% of **22** with 98% purity (^{1}H NMR). The reaction of (bromomethyl)methylmaleic anhydride (**22**) with excess (5 equiv) of freshly prepared Grignard reagents derived from methyl iodide, *n*-pentyl iodide, *n*-octyl bromide, *n*-dodecyl bromide and *n*-tridecyl bromide, in the presence of catalytic amount of CuI(I) in ether and HMPA (Hexamethylphosphoramide) as a solvent system at –5 to 0 °C furnished exclusively the corresponding anhydrides **1a-e** in 55 to 60% yields via chemoselective nucleophilic displacement of allylic bromo atom. The same results were obtained when the

THF was used in place of diethyl ether. Optimization of the coupling reaction was carried out by changing reaction temperature, mode of addition, molar

Scheme 2



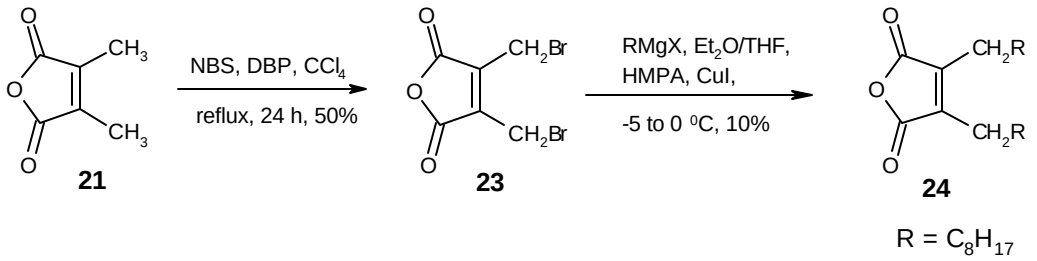
1a R = CH_3, **1b** R = $(CH_2)_4CH_3$,
1c R = $(CH_2)_7CH_3$, **1d** R = $(CH_2)_{11}CH_3$,
1e R = $(CH2)_{12}$ CH_3

amount of Grignard reagents, CuI and HMPA. The experiments using CuI in the absence of HMPA as well as using HMPA without CuI failed to give the desired coupling products. The observed chemoselectivity could be ascribed to the controlled reactivity of cuprates generated from Grignard reagents and CuI in the presence of HMPA[15,16] and will be useful for the tailor-made synthesis of wide range of alkylmethylmaleic anhydride derivatives.

After the successful synthesis of chaetomellic anhydride A and its natural analogues we planned to extrapolate the same strategy for the synthesis of symmetrically substituted dialkylmaleic anhydride derivatives via di(bromomethyl)maleic anhydride (**Scheme 3**). Dimethylmaleic anhydride, NBS (4 equiv) and catalytic amount of DBP in CCl_4 were refluxed for 24 h to furnish di(bromomethyl)maleic anhydride (**23**) in 50% yield (^{1}H NMR). The NBS and DBP were added in two lots (initially 2 equiv of NBS and after 12 h another 2 equiv) so as to convert the starting material in maximum amount to the dibromoanhydride. Purification by Kugelrhor distillation gave solid **23** with 98% purity and recrystalisation of the product in benzene/petroleum ether (1:4) furnished analytically pure di(bromomethyl)maleic anhydride (**23**). The reaction of **23** with Grignard reagent derived

from *n*-bromooctane using the same reaction conditions used in above coupling reaction gave the symmetrically disubstituted maleic anhydride **24** but only in 10-11% yield.

Scheme 3



In our hands several attempts to increase the yield by varying the reaction conditions met with failure. An attempt using Li_2CuCl_4 as catalyst[20] for the coupling reaction also failed to give the desired product. The work is in progress to achieve the coupling products employing organolithium reagents and successful completion of this reaction will be useful to provide an easy access for the synthesis of dialkyl substituted maleic anhydride derivatives and will also find application for the synthesis of natural products from nonadride class of compounds such as byssochlamic acid.[21]

In summary, we have demonstrated for the first time a simple chemoselective carbon-carbon coupling reaction of (bromomethyl)methylmaleic anhydride **22** and organocuprates for the synthesis of alkylmethylmaleic anhydrides **1a-e** in 55-60% yields with preservation of substituted maleic anhydride moiety. However, reaction of organocuprates with di(bromomethyl)maleic anhydride **23** furnished symmetrically disubstituted maleic anhydride **24** only in 10-11% yield.

SECTION B

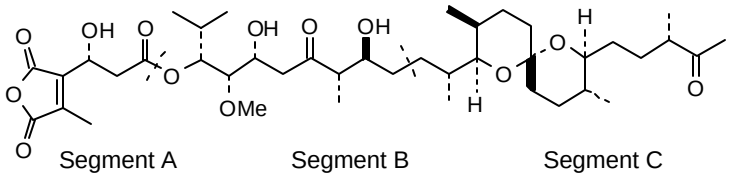
A FACILE SYNTHESIS OF

(±)-2,3-DISUBSTITUTED MALEIC ANHYDRIDE SEGMENT

OF ANTIFUNGAL ANTIBIOTIC TAUTOMYCIN

2.2.1 BACKGROUND

Tautomycin (**25**) was isolated in 1987 by Isono and co-workers from *Streptomyces spiroverticillatus* as new antibiotic with strong antifungal activity against *Sclerotinia sclerotiorm* and inhibitory activity to protein phosphatase of type 1 and 2A.[22] In 1993 the same group determined the absolute configuration of **25** with 13-chiral centres by using chemical transformations and spectroscopic data.[23] The tautomycin (**25**)

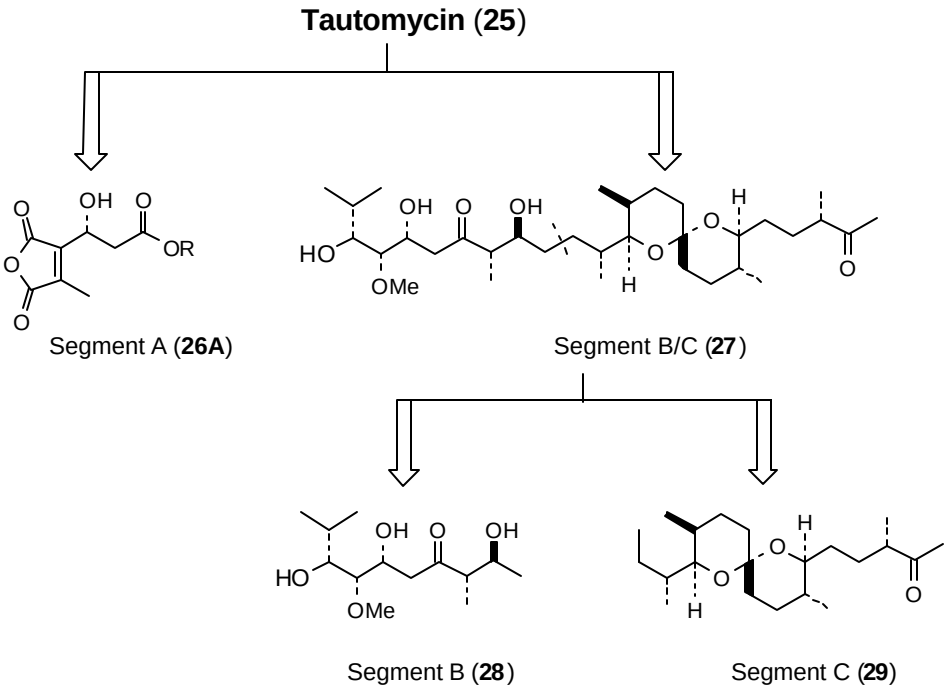


Tautomycin (25)

has attracted an attention of many synthetic organic chemists because of its interesting structural architect and novel biological activity. Retrosynthetic analysis of tautomycin[24] divided the target molecule into three subunits as shown in scheme **4**. Disconnection of an ester bond afforded segment A (left wing), segment B (middle wing) and segment C (right wing) (**Scheme 4**). The total synthesis of **25** involves synthesis of three segments A, B and C followed by stepwise coupling of these potential building blocks.[25] To date four total syntheses[25-28] and one recent formal total synthesis[29] of this molecule have been reported apart from the related synthesis of tautomycin subunits such as 2,3-disubstituted maleic anhydride segment A and polyketide portion of tautomycin, segment B and C.[30] According to Chamberlin *et al*[26] the greatest challenge in the synthesis of tautomycin (**25**) is in the construction of simple looking 2,3-disubstituted maleic anhydride segment A. Segment A is highly functionalised molecule with three carboxylic groups and one hydroxy group. This *b*-hydroxy ester is highly prone to dehydration utilising acidic *a*-hydrogen atom to give

a,b-unsaturated ester and hence the synthesis of this segment requires a special care in choosing reagents/reaction conditions. To date four-multistep synthesis of segment A (**26**) have been accomplished starting from suitably 3,4-

Scheme 4



disubstituted furans,[25a,27,31-33] 1,3-propanediol,[34] and symmetrical acetylenedicarboxylic ester,[26] using various synthetic strategies which have been already discussed in the first chapter [section **1.2.3**].

Recently we have successfully completed[9] the synthesis of naturally occurring alkylmethylmaleic anhydrides including farnesyl-protein transferase inhibitor chaetomellic anhydride A starting from (bromomethyl)methylmaleic anhydride which in turn was synthesised from dimethylmaleic anhydride as discussed in the earlier section of this

chapter. We have also reported the synthesis of fulgenic acid[35] starting from (bromomethyl)methylmaleic anhydride via (hydroxymethyl)methylmaleic anhydride (scheme discussed in section D of this chapter). With our notional interest towards the synthesis of highly oxygenated 2,3-disubstituted maleic anhydride segment A of tautomycin, we planned two different synthetic strategies starting with (bromomethyl)methylmaleic anhydride and are discussed in detail in the present section. An appealing feature of our approach is a short route synthesis employing anhydride to anhydride conversion without involving any protection-deprotection chemistry (a present need of time).[36]

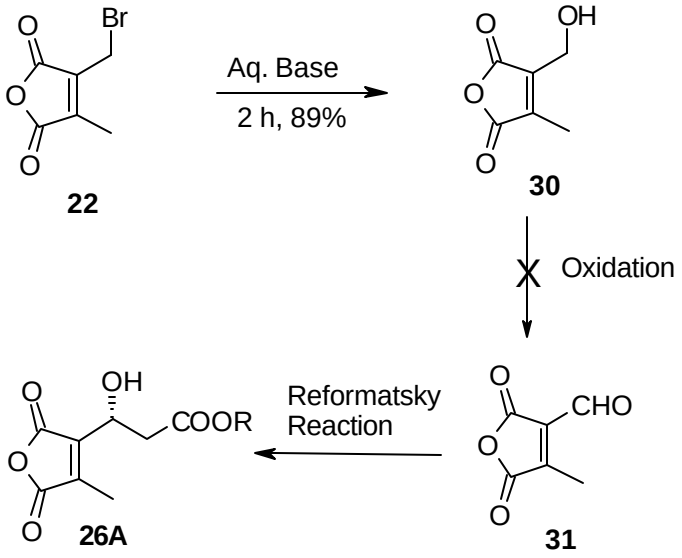
2.2.2 PRESENT WORK: RESULTS AND DISCUSSION

Our first synthetic strategy to reach the target molecule 2,3-disubstituted maleic anhydride segment A of tautomycin was through a key step of chemoselective and (or) enantioselective Reformatsky reaction (**Scheme 5**). Recently Weissjohann *et al*[37] introduced chromium(II) mediated Reformatsky reaction as a highly useful alternative to the conventional heterogenous method with many advantages such as excellent chemoselectivity towards aldehydes compared to zinc ester enolate coupled with very high enantioselectivity/diastereoselectivity. The starting material (bromomethyl)methylmaleic anhydride (**22**) on alkaline hydrolysis was converted to (hydroxymethyl)methylmaleic anhydride (**30**) in 89% yield. The next step was to convert allylic alcohol to aldehyde to get the 2-formyl-3-methylmaleic anhydride **(31)** in hands for Reformatsky reaction. We were disappointed to find out that all our attempts to convert (hydroxymethyl)methylmaleic anhydride (**30**) to **31** using variety of oxidizing reagents [DMSO/$(COCl)_2$, PCC, MnO_2]

met with failure under various reaction conditions and only the decomposed or polymeric materials were obtained.

Since all the starting material **30** was getting consumed during the course of reaction, probably the formed **31** may be undergoing further oxidation to corresponding carboxylic acid, which in turn might be decraboxylating to yield methylmaleic anhydride or its

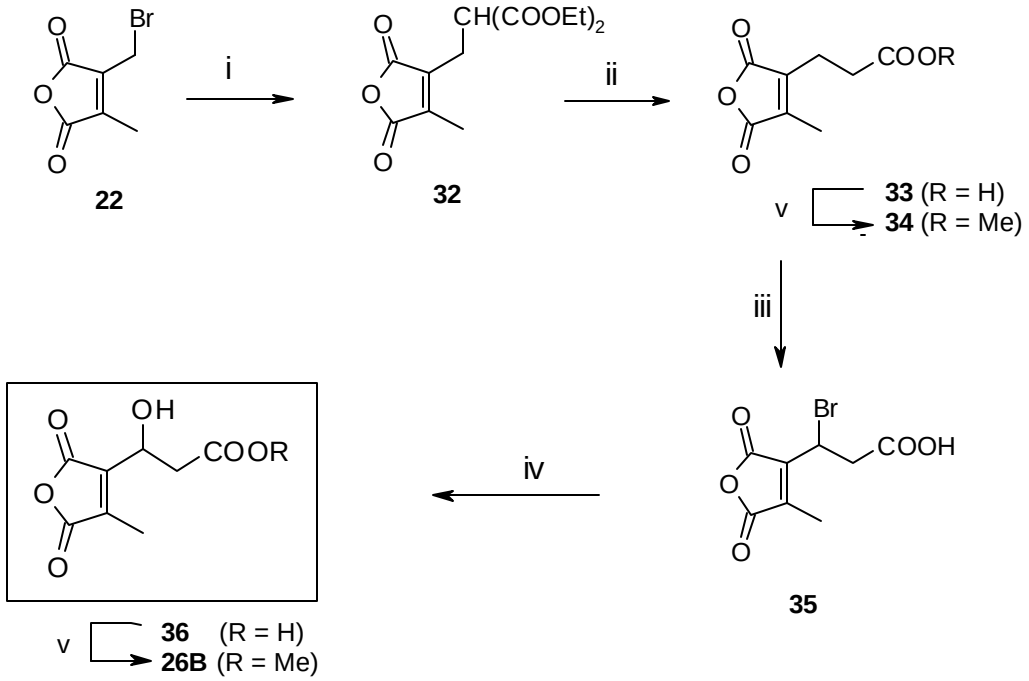
Scheme 5



decomposed polymeric products. Hence we could not continue with this approach and diverted our attention to an alternate strategy to reach the target molecule.

In our second strategy we planned for chemoselective condensation of diethyl malonate with (bromomethyl)methylmaleic anhydride (**22**) followed by regioselective NBS-bromination as a key step to accomplish the synthesis of (±)-2,3-disubstituted maleic anhydride segment A (**26B**) of tautomycin (**Scheme 6**).

Scheme 6



Reagents and conditions:

i. (a) Diethyl malonate, NaH, C_6H_6, rt, 8 h, (b) H^+/HCl (74%);

ii. Con. HCl, reflux, 12 h, (94%);

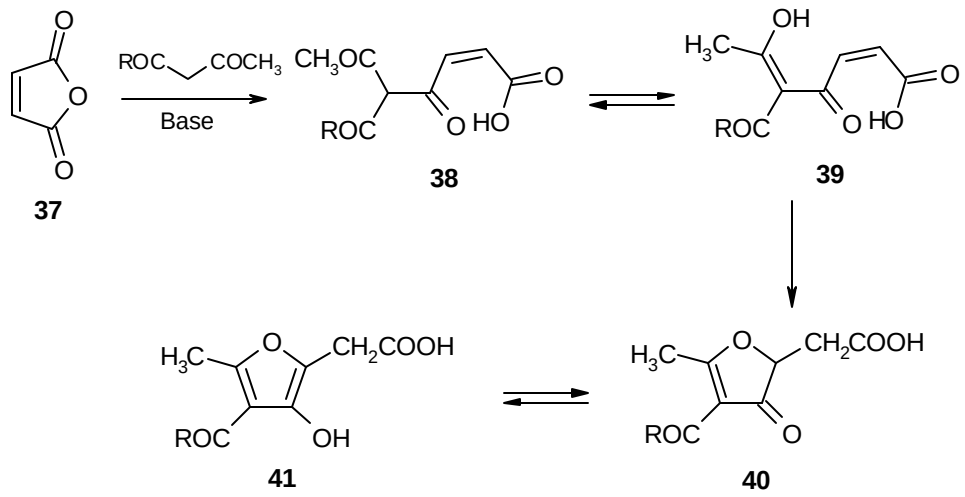
iii. NBS, Benzoyl peroxide, $CHCl_3$, reflux, 24 h, (74%);

iv. (a) 1N aq. KOH, rt, 3 h, (b) H^+/HCl (91%);

v. CH_2N_2, Et_2O, 0 °C, 3 h, (95%).

The literature report[38] reveals that the reactions of cyclic anhydride especially maleic anhydride (**37**) with carbon nucleophiles such as acetyl acetone and ethyl acetoacetate resulted in the ring opened product as an intermediate which then rearrangs to give the substituted furan derivatives **40 and 41** (**Scheme 7**).

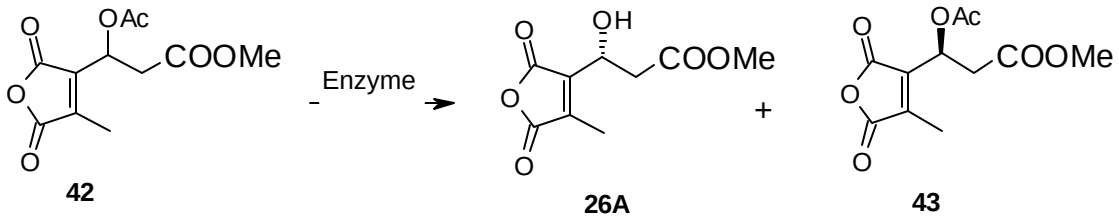
Shceme 7



In case of our substrate, (bromomethyl)methylmaleic anhydride (**22**), we envisioned the high reactivity of allylic halide along with high stability of disubstituted maleic anhydrides which can easily serve our purpose to get chemoselectively condensed product without any problem of Michael addition as well as forming of ring opened products. Indeed, diethyl malonate in benzene solution using NaH as base, chemoselectively displaced the allylic bromo atom in anhydride **22** to furnish the desired diester **32** with 74% yield. The diester **32** on refluxing with concentrated hydrochloric acid underwent a hydrolysis followed by *in-situ* decarboxylation of the *gem*-dicarboxylic acid formed to yield monoacid **33** in 94% yield. The monoacid on further reaction with diazomethane in ether gave the corresponding ester **34** in quantitative yield. The spectral data of monoacid as well as ester were in complete agreement with the reported data.[33] The next step was to achieve the regioselective bromination of ester, as there are two alternate sites for allylic bromination reaction. The reaction of ester **34** with NBS (1.25 eqiv) in refluxing CCl_4 was not

regioselective and yielded the mixture of brominated products. The ^{1}H NMR spectrum of crude product revealed that only 65% bromination takes place at the desired allylic methylene carbon, while 35% on allylic methyl carbon. It was practically very difficult to separate the two brominated products. We decided to use an alternative substrate for the allylic bromination reaction. The bromination of monoacid (**33**) in carbon tetrachloride failed to give the product in good yield due to its poor solubility in solvent even at refluxing temperature. The NBS-bromination reaction of acid **33** with excess of NBS (5 equiv) in refluxing chloroform was possible using benzoyl peroxide as a catalyst in nearly 100% regioselective fashion and gave the desired bromoacid **35** in 74% yield. The bromoacid **35** on treatment with 1 N aq. KOH at 0 °C followed by acidification with dilute hydrochloric acid in presence of diethylether smoothly yielded the desired (±)-2,3-dialkylsubstituted maleic anhydride segment **36** of tautomycin, which was further characterized as its methyl ester **26B**. According to our previous experience on biotransformations,[39,40] we feel that the enzymatic resolution of (±)-**26B** to obtain the naturally occurring (*R*)-isomer appears plausible (**scheme 8**).

Scheme 8



42 **26A** **43**

In summary, we have demonstrated[41] a facile 5-step synthesis of (±)-2,3-disubstituted maleic anhydride segment **26B** of tautomycin with 28% overall yield, directly starting from dimethylmaleic anhydride (**22**) without using any protection deprotection chemistry.

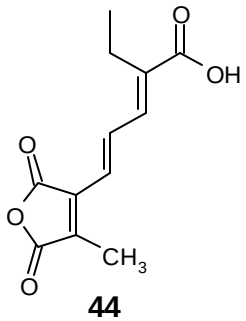
SECTION C

SYNTHESIS OF

2,3-DIDEHYDROTELFAIRIC ANHYDRIDE

2.3.1 BACKGROUND

A new methylmaleic anhydride metabolite was isolated[42] in 1996 by Edwards group from the culture medium of the fungus *Xylaria telfairii* Berk and named as 2,3-didehydrotelfairic anhydride ([1'*E*,3'*E*)-2-(4-carboxyhexa-1,3-dienyl)-3-methylmaleic anhydride, **44**). The structure of telfairic anhydride was determined on the basis of analytical and spectral data.[42] The biological role for natural product **44** has not been established but the activity exhibited by several other alkylmethylmaleic anhydrides[43] in this series reveal that the synthesis and biological screening of this natural product **44** will be useful. To date there is no report on the synthesis of this recently isolated natural product. In pusuit of our ongoing program towards the synthesis of natural products having 2-alkyl-3-methylmaleic anhydride[9] as structural skeleton or as a part structure,[41] the interesting structure of 2,3-didehydrotelfairic anhydride caught our attention. We planned to put in synthetic efforts to achieve the first total synthesis of this natural product for structural confirmation and biological evaluation.



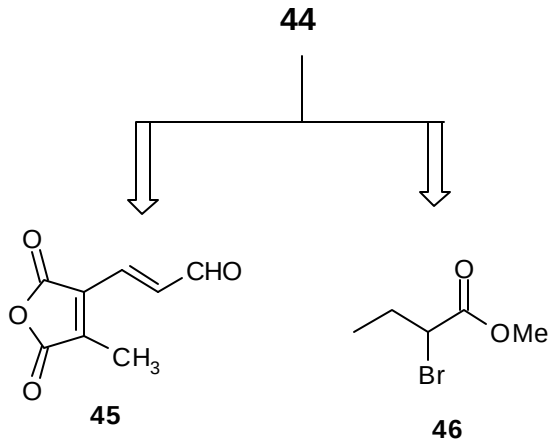
44

2,3-Didehydrotelfairic anhydride

2.3.2 PRESENT WORK: RESULTS AND DISCUSSION

The retrosynthetic analysis of target molecule **44** furnished two potential building blocks ***a,b***-unsaturated aldehyde (**45**) and methyl 2-bromobutyrate (**46**) (**Scheme 9**). A chemoselective Wittig reaction of this substrate may easily furnish the target molecule, 2,3-didehydrotelfairic anhydride (**44**).

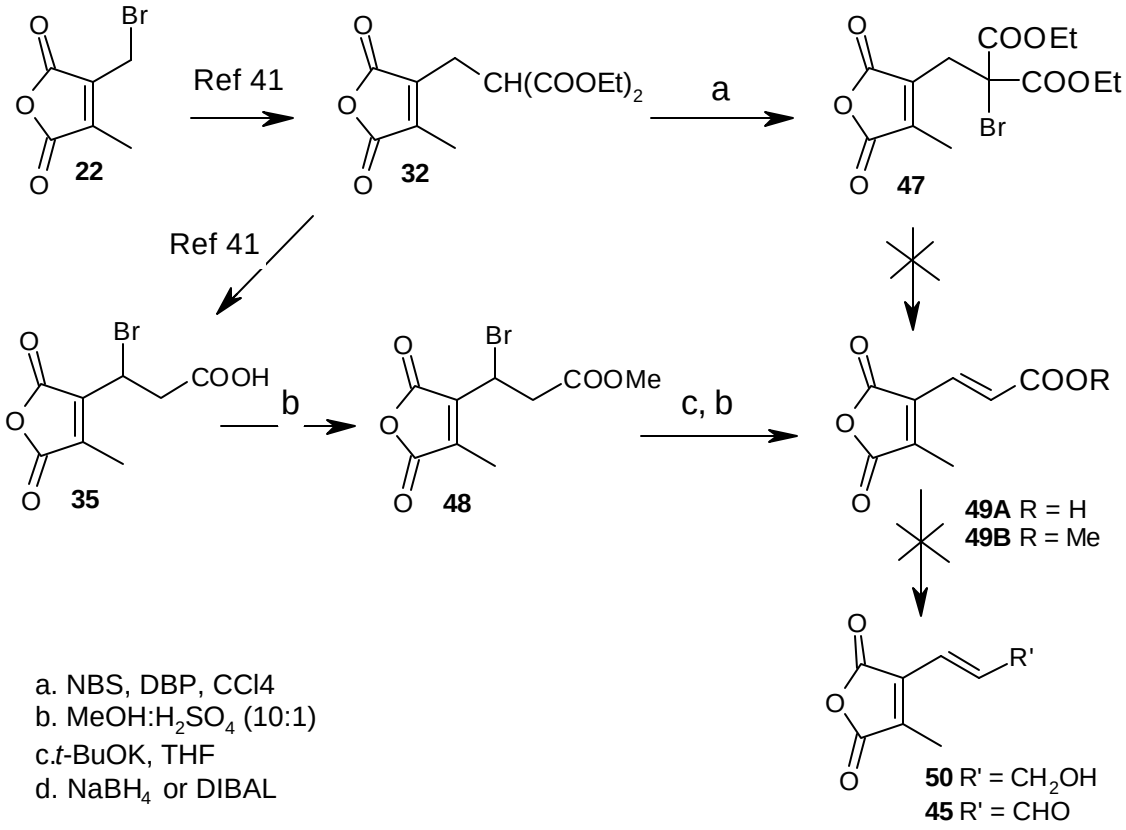
Scheme 9



The first building block, unsaturated aldehyde **45** was planned to obtain from (bromomethyl)methylmaleic anhydride (**Scheme 10**). Starting from bromoanhydride **22** the diester **32** was obtained [discussed in earlier section] and which was subjected to chemoselective bromination at ***a*** position of diester functionality. The bromination using NBS (1.25 equiv) in the presence of catalytic amount of DBP furnished the product **47** in 65% yield. All attempts to dehydrobrominate the ***a***-bromodiester **47** using various bases such as triethylamine, sodium hydride, potassium *tert*-butoxide and even aq. potassium hydroxide followed by decarboxylation of the formed *gem*-diacid under acidic condition failed to give an expected ***a,b***-unsaturated acid **50A**. We changed our route and planned to reach the target aldehyde **45** via ***b***-bromoacid derivative of maleic anhydride **35** obtained from diester **32** as discussed in scheme **6** of section B in this chapter. The bromoacid **35** was reluctant to undergo dehydrobromination and hence we decided to synthesize its methyl ester to complete dehydrobromination. Esterification using $MeOH/H_2SO_4$ at room temperature smoothly furnished ***b***-bromoester **48**, which easily underwent

dehydrobromination using potassium *tert*-butoxide as a base to give ***a,b***-unsaturated acid **49A** and which was again converted to its methyl ester **49B** by using $MeOH/H_2SO_4$.

Scheme 10

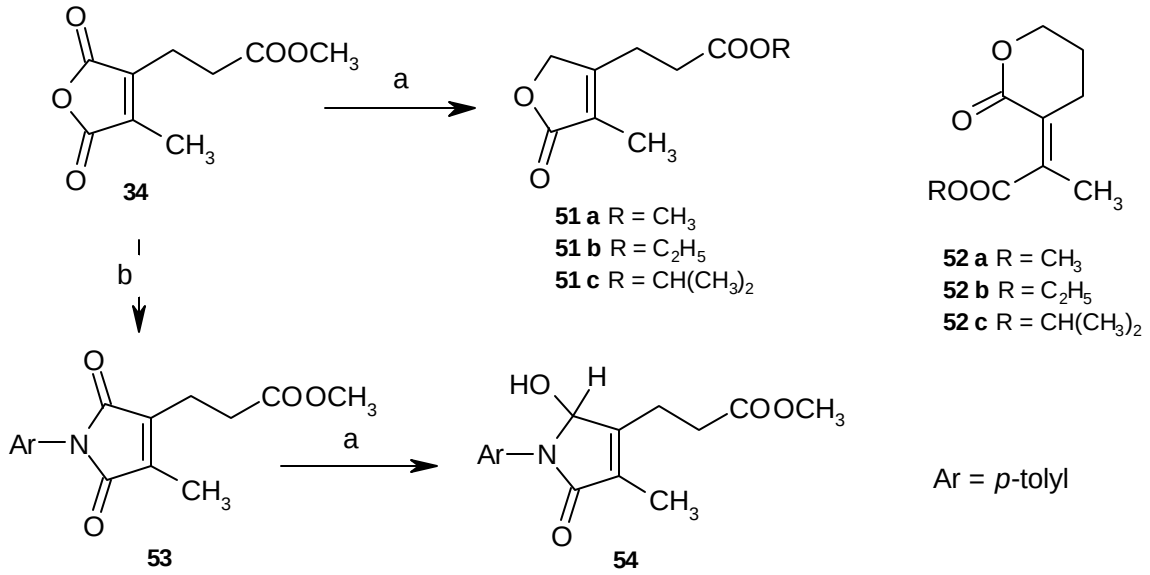


We systematically studied the reduction of ***a,b***-unsaturated ester **49B** to get ***a,b***-unsaturated aldehyde **45**. There were two options to reach aldehyde either through chemoselective reduction of ester to get an allylic alcohol followed by oxidation using MnO_2 or chemoselective reduction of ester to aldehyde **45** using DIBAL at lower temperature. All our attempts towards the reduction of ***a,b***-unsaturated ester (**49B**) using above-mentioned reagents resulted in the formation of complex mixture of products with loss of chemoselectivity. The observed loss of chemoselectivity could be because of

comparable reactivity of ***a,b***-unsaturated ester and conjugated carbonyls from maleic anhydride moiety.

In view of unsatisfactory results in chemoselective reduction of ***a,b***-unsaturated ester, we decided to reach a target building block by other pathway. Recently a very novel synthetic methodology has been published by Nicolaou's group[44] to get ***a,b***-unsaturated aldehyde from saturated alcohol using *o*-iodoxybenzoic acid (*o*-IBX) as an oxidizing agent under very mild condition with highly efficient conversion. Applying this strategy we wanted to procure ***a,b***-unsaturated aldehyde **45** starting from corresponding saturated alcohol. Hence we aimed to synthesize the alcohol starting from ester **34** using $NaBH_4$ to yield an expected alcohol (**Scheme 11**). Our attempts to chemoselectively reduce ester **34** using $NaBH_4$ in methanol resulted in the formation of new product other

Scheme 11



Reagents and conditions: (a) $NaBH_4$, MeOH (EtOH or *iso*-Propanol), rt, 4h. (b) *p*-Toluidine, MeOH, reflux, 5h.

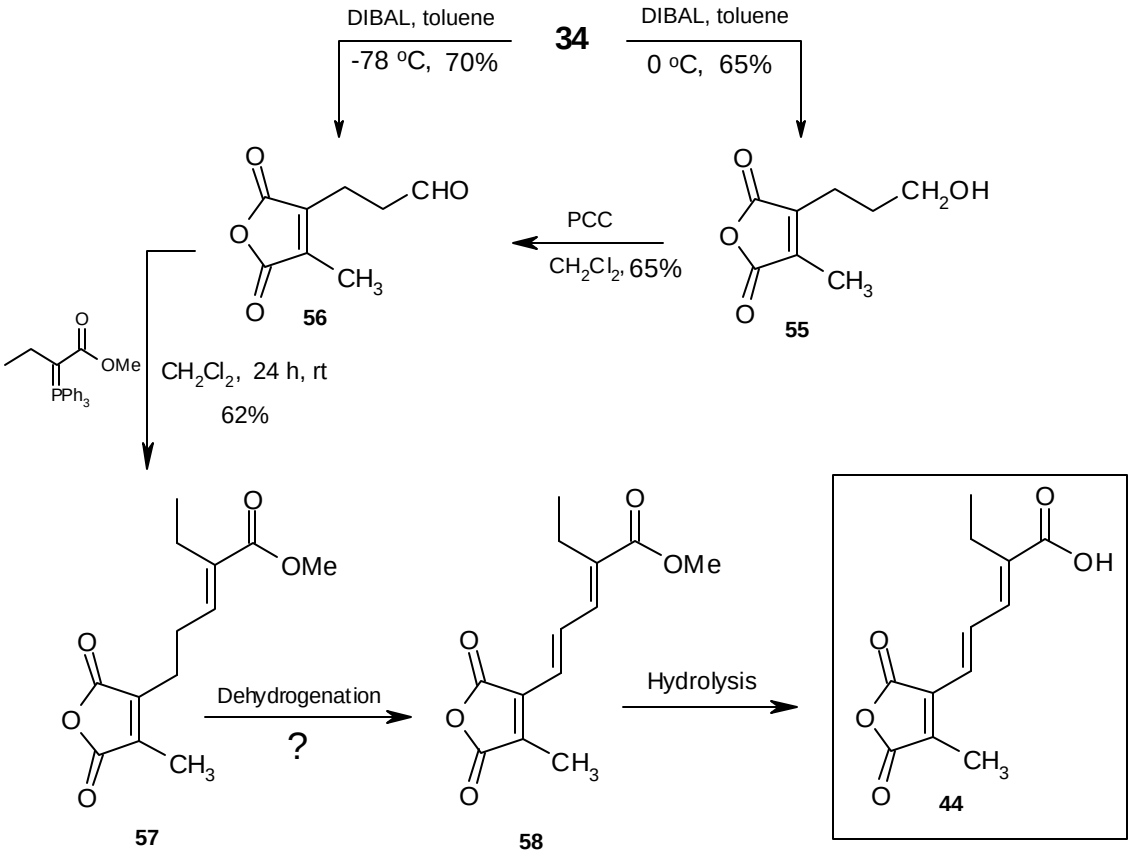
than the expected alcohol and was characterized as substituted *g*-butyrolactone (**51a**). Repetition of same experiment using ethanol and *iso*-propanol as solvent furnished *g*-butyrolactone derivatives (**51b** and **51c**). The formation of compounds **51a-c** was also supported by literature reports[45] and from this as well as with the help of our specral data we ruled out the possibility of formation of *d*-valerolactone derivatives (**52a-c**). At this stage we felt that converting ester **34** to imide could solve the problem and $NaBH_4$ may reduce chemoselectively the ester functionality. Refluxing the ester **34** with *p*-toluidine in methanol furnished the imide **53** in 80% yield. Reduction of imide using $NaBH_4$ did not serve our purpose and reduced the carbonyl group of cyclic imide to yield **54**.

Our next alternative for the chemoselective reduction of ester was to use very specific and mild reagent like DIBAL. The reduction of ester **34** with DIBAL (2 eqiv) at 0 °C successfully furnished the desired alcohol (**55**) in 60% yield (**Scheme 12**). After getting alcohol in hands the next step was the oxidation of alcohol using *o*-IBX (*ortho*-Iodoxybenzoicacid) to obtain *a,b*-unsaturated aldehyde **45**. Oxidation of alcohol **55** using 4 eqivalent of *o*-IBX in toluene/DMSO (3:1) resulted in the formation of monoacid **33** (**Scheme 6**, section B of this chapter) instead of giving aldehyde **45**. All attempts to reach the *a,b*-unsaturated aldehyde met with failure so we decided to make a little change in our scheme. We felt that the use of saturated aldehyde in chemoselective Wittig reaction[46] and dehydrogenation of resulting product would also provide a way to reach the target molecule (**Scheme 12**). The saturated aldehyde can be synthesized in two ways either by oxidation of alcohol or reduction of ester **34** at lower temperature. The PCC oxidation of alcohol **55** in methylene dichloride smoothly furnished the desired aldehyde **56** in 65% yield. In second

approach reduction of ester **34** using 1.5 equivalent of DIBAL at −78 °C in toluene gave aldehyde **56** in 70% yield.

Our next plan was to synthesize the another building block, methyl 2-bromobutyrate (**46**)[47] and use this halide to form Wittig salt. The Wittig salt was prepared as mentioned in the literature[48] with little modification to increase the yield. Gentle reflux

Scheme 12



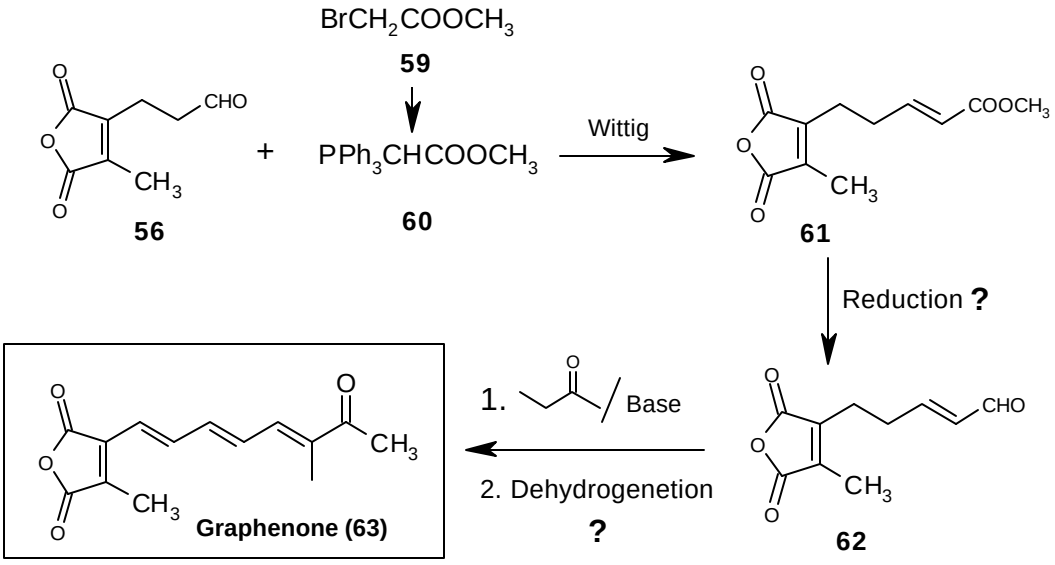
of methyl 2-bromobutyrate and triphenylphosphine (TPP, 1.2 eqiv) in benzene at 70-75 °C for 12 h and leaving the reaction mixture at room temperature for further 12 h resulted in the formation of salt in 55% yield. The salt was used to generate a stable ylide, methyl 2-(triphenylphosphoranylidene)butyrate using KOH as a base and this priorly generated ylide

was used for coupling with an aldehyde **56**. The ylide was dissolved in methylene dichloride and to this was added the solution of aldehyde **56** in methylene dichloride at 0 °C and the reaction mixture was stirred further for 24 h at room temperature to obtain dihydrotelfairic anhydride (**57**) in 62 % yield. The next crucial step to get the target molecule was dehydrogenation reaction. There are different conventional reagents to introduce the unsaturation in the system.[49] At this stage the dehydrogenation in our system appeared very easy, as the newly generated carbon-carbon double bond will form with an extension of conjugation. The first choice of reagent for dehydrogenation was obviously the DDQ. Refluxing the substrate **57** in presence of DDQ in benzene for 12 h and even after using stoichiometric quantities of DDQ and extended reaction time did not show any formation of product. Number of experiments using various reagents such as selenium dioxide, mercuric acetate, palladium on charcoal, palladium chloride and PPh_3-DEAD met with failure and we were unable to get the dehydrogenated product. The option of conventional way of allylic bromination followed by dehydrobromination to introduce unsaturation was kept aside as there are four different sites available for allylic bromination and the site of methylene carbon from ethyl group was not desired where as the bromination at other two methylene sites could serve our purpose. Use of NBS/DBP resulted in mixture of products. Several experiments with different reaction conditions (use of AIBN instead of DBP) were not fruitful. Bromination was not selective and it was occurring at desired site along with formation of other undesired brominated products (^{1}H NMR). The results were not consistent and we felt that hydrolyzing ester to acid will be helpful to get the desired compound but this attempt also failed. Still the experiments are in progress to successfully complete the dehydrogenation step and converting the anhydride to

imide and also some other options such as metal sulphur, *t*-BuOCl with triethylamine[50] and selenenyl derivative[51] may prove fruitful to get the target molecule 2,3-didehydrotelfairic anhydride in hands. To start the present synthesis with suitably substituted furan derivative will demand lot of steps to synthesize starting material.

After completing the synthesis of 2,3-didehydrotelfairic anhydride the same strategy can be extended to complete the first total synthesis of Graphenone, a new natural product recently isolated from the cultures of spore derived mycobionts of the lichens *Graphis scripta*.[52] Its structure was established using spectral and X-ray diffraction analysis. Our synthetic plan for Graphenone is highlighted in **Scheme 13**. Witting reaction of aldehyde **56** and methyl bromoacetate would easily furnish the compound **61**. Chemoselective reduction of ester functionality in **61** to obtain aldehyde **62** though seems to be difficult at this stage but may be possible via imide. The last step will be base catalyzed chemoselective condensation of ethylmetyl ketone with aldehyde **62** followed by dehydration should give the target molecule Graphenone (**63**).

Scheme 13



In conclusion, we have synthesized dihydrotelfairic anhydride in five-steps starting from (bromomethyl)methylmaleic anhydride with 29% overall yield using chemoselective reduction of ester as a key step. We are hopeful that the reasoned and planned efforts will help us in meeting with the success on dehydrogenation reaction to complete the first total synthesis of the target molecule 2,3-didehydrotelfairic anhydride. An extension of same strategy will provide a way to the first total synthesis of Graphenone.

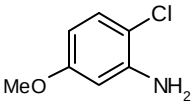
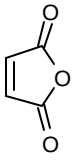
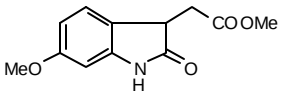
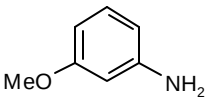
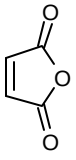
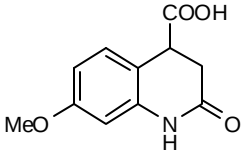
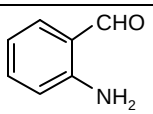
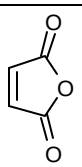
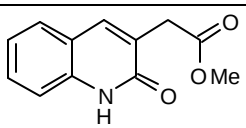
SECTION D

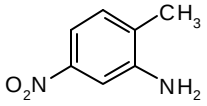
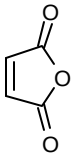
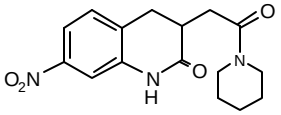
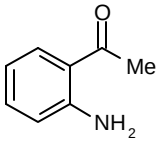
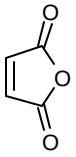
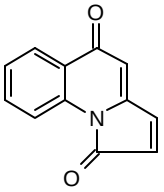
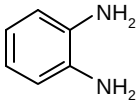
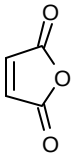
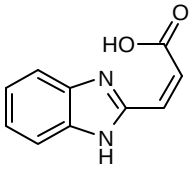
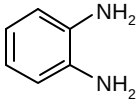
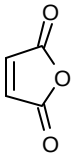
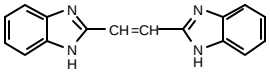
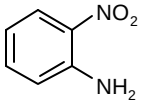
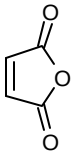
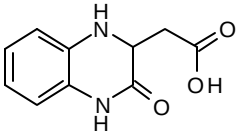
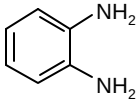
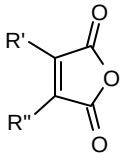
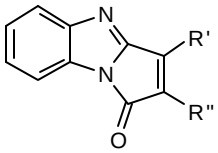
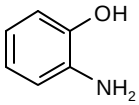
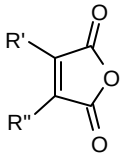
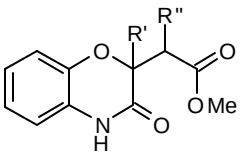
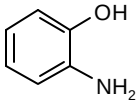
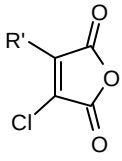
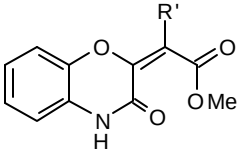
SYNTHESIS OF *α*-QUINOXALINYLACRYLIC AND *α*-BENZOTHIAZINYLACRYLIC ACIDS

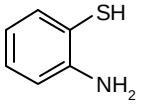
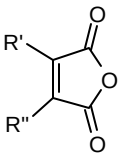
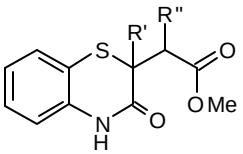
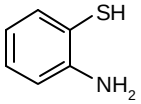
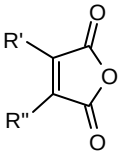
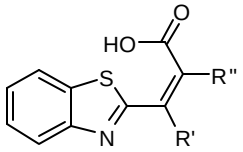
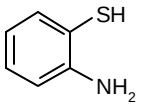
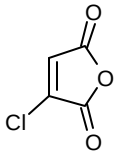
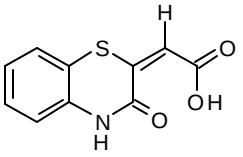
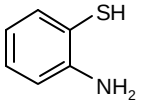
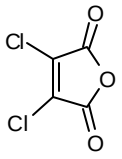
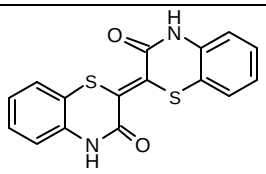
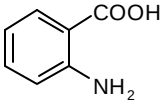
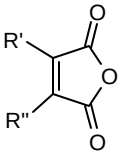
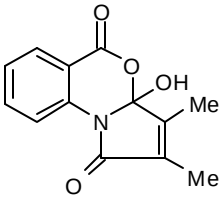
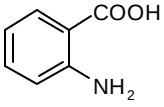
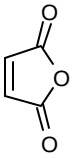
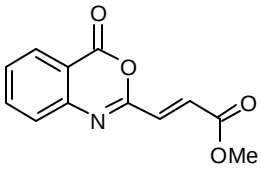
2.4.1 BACKGROUND

Ring closure reactions of suitably *ortho*-substituted maleanilic acid have been elegantly employed for the synthesis of structurally interesting and biologically important heterocycles via intramolecular Michael addition, condensation and dehydrative cyclisation reactions with pivotal role of an *ortho* substituents.[53] The multifunctional maleanilic acids are obtained from the reactions of suitably *ortho*-substituted aniline derivatives (*ortho*-substituents = H, Cl, CH_3, CHO, COMe, COOH, NH_2, NO_2, OH, SH) and variety of symmetrical and unsymmetrical maleic anhydrides. The intramolecular cyclisation reactions of these anilic acids have been systematically studied with the generation of carbon-carbon, oxygen-carbon, nitrogen-carbon and sulfur-carbon bond.[53] The representative examples of above mentioned class of reactions are summarized in the following table.

Table 1: Heterocycles Derived from Suitably *ortho*-Substituted Aniline Derivatives and Maleic Anhydrides

Sr. No.	Aniline Derivative	Maleic anhydride	Reaction Condition (% Yield)	Product	Ref.
1			1. Et_2O, rt 2. MeOH/H^+ 3. $Ni(PPh_3)_4$, DMF 50-60 °C, 5 h (61%)		54
2			1. Et_2O, rt 2. *hn*, MeOH, 5 h (38%)		55
3			1. Et_2O, rt 2. MeOH/H^+ 3. PPh_3, EtOH, reflux, 4 h. (60%)		56

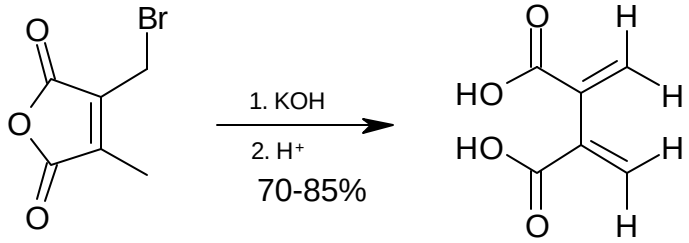
4			1. Et_2O, rt 2. MeOH/H^+ 3. piperdineacetate 50-60 °C, 0.5 h (60%)		57
5			Xylene, excess TEA, 120-140 °C (90%)		58
6			Aq. HCl, reflux (51%)		59
7			1. Et_2O, rt 2. *o*-PDA, PPA 220-230 °C (70%)		60
8			1. $AlCl_3$,80 –100 °C 2. P_2O_5, MeOH, reflux. 3. Ni/ H_2 , EtOH (82%)		61
9		 R' = CH_3, Ph, Me, R' = Ph, Me	1.EtOH, reflux 2. AcOH, reflux (95%)		62
10		 R'/R" = H, Me	TEA, MeOH, reflux (82%)		63, 64
11		 R' = H, Cl	1. Acetone, rt 2. TEA, MeOH, reflux (89%)		65

12		 R' = H, Me R" = H, Me, Ph	1. Et_2O, rt (R'/R'' = H) (98%) 2. AcOH, reflux (R'/R'' = Me) (90%) 3. PhCl, reflux (R' = H, R'' = Ph) (87%)		61b, 63b, 65, 66
13		 R' = H, Ph R" = OH, OMe	1.Acetone, reflux or 2. Pyridine, reflux (75%)		67
14			Acetone, rt (60%)		65
15			Acetic acid, reflux (60%)		68
16		 R' = H, Ph R" = H, Me	H_2O, reflux, 3 h. (95%)		69
17			1. Et_2O, rt, 0.5 h 2. Aq. MeOH. 3. Ac_2O, NaOAc, rt, 4 h. (75%)		70

The various examples mentioned in the above table reveal that the nucleophilic reactions of symmetrical and unsymmetrical cyclic anhydrides have provided several interesting and important heterocyclic systems.[53] It has been well established that the

dimethylmaleic anhydride on reaction with primary amine yields the corresponding imide where as on reaction with *o*-phenylenediamine (*o*-PDA), first the corresponding imide and then pyrrolobenzimidazole (entry **9**, **Table 1**), while on reaction with *o*-aminothiophenol (*o*-ATP) yields benzothiazinylpropionic acid (entry **12**, **Table 1**) via ring opening and intramolecular Michael addition reaction. Recently we have synthesized (bromomethyl)methylmaleic anhydride (**22**), which has five alternate sites for nucleophilic reactions as discussed in section A of this chapter. We planned for systematic study of chemo- and regioselective nucleophilic reactions of this unsymmetrical anhydride more specifically with suitably *ortho*-substituted aniline derivatives to design new heterocycles (**Scheme 15**). Apart from the present work to date only one reaction of (bromomethyl)methylmaleic anhydride (**22**) is known in literature wherein the bromoanhydride has been elegantly used for the most concise synthesis of fulgenic acid via base induced 1,4-dehydrobromination (**Scheme 14**).

Scheme 14



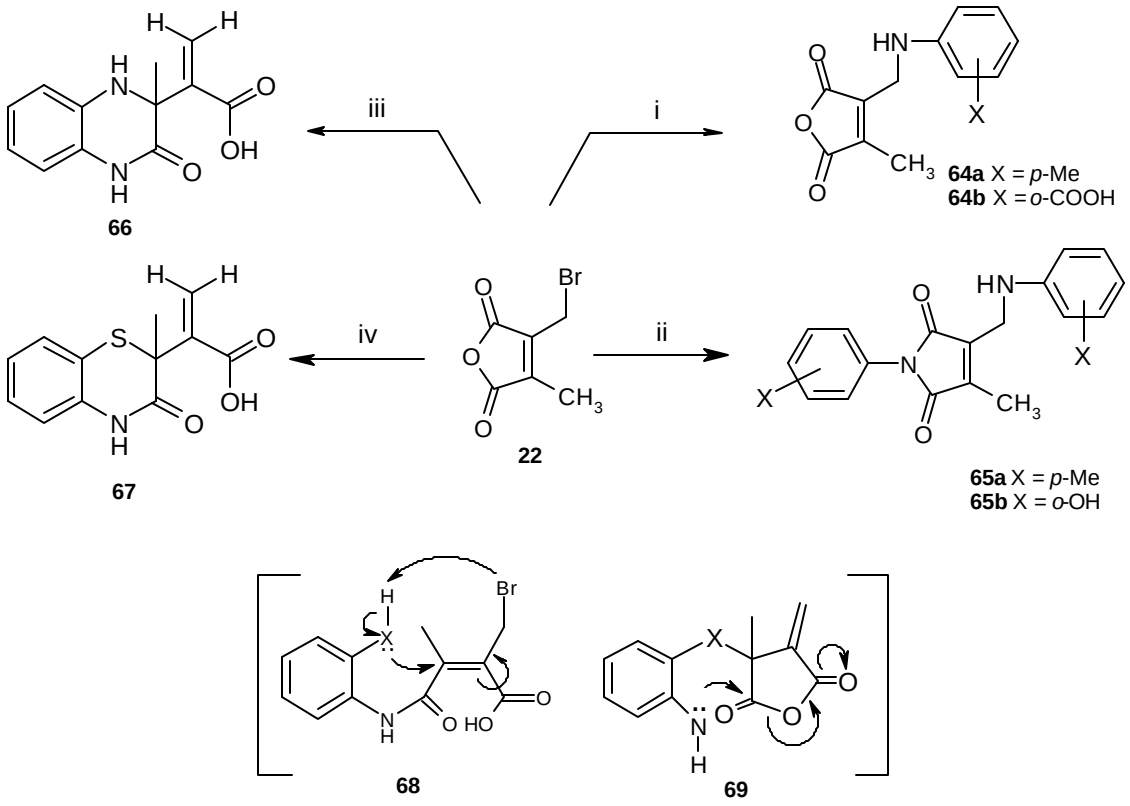
22 **64**

2.4.2 PRESENT WORK: RESULTS AND DISCUSSION

The reaction of bromoanhydride **22** with two equivalents of *p*-toluidine in chloroform underwent highly chemoselective fashion at room temperature to yield exclusively the (*p*-toludinylmethyl)methylmaleic anhydride **64a** with 69% yield (**Scheme 15**). The excess of *p*-toluidine was required in the reaction to trap the formed hydrobromic

acid (HBr) because the use of any other base like TEA was acting itself as nucleophile giving complex mixture of products. The anhydride **65a** on further reaction with *p*-toluidine furnished the imide (**64b**), which was also obtained by the direct reaction of **22** with excess of *p*-toluidine (4 equiv) at room temperature in chloroform. At this stage we planned to study the reactions of *o*-aminophenol (*o*-AP), *o*-PDA and *o*-ATP with bromoanhydride **22**, aiming for biologically important heterocyclic systems such as oxazepine, diazepine and thiazepine derivatives via nucleophilic displacement of allylic

Scheme 15



Reagents and conditions:

(i) *p*-Toluidine, $CHCl_3$, rt, 2 h (for **64a**)/ anthranilic acid, $CHCl_3$, rt, 2 h (for **64b**);

(ii) *o*-AP, $CHCl_3$, reflux, 2 h (for **65b**);

(iii) *o*-PDA, $CHCl_3$, -15 °C to rt, 3h (86%);

(iv) *o*-ATP, $CHCl_3$, -15 °C to rt, 3h, (90%).

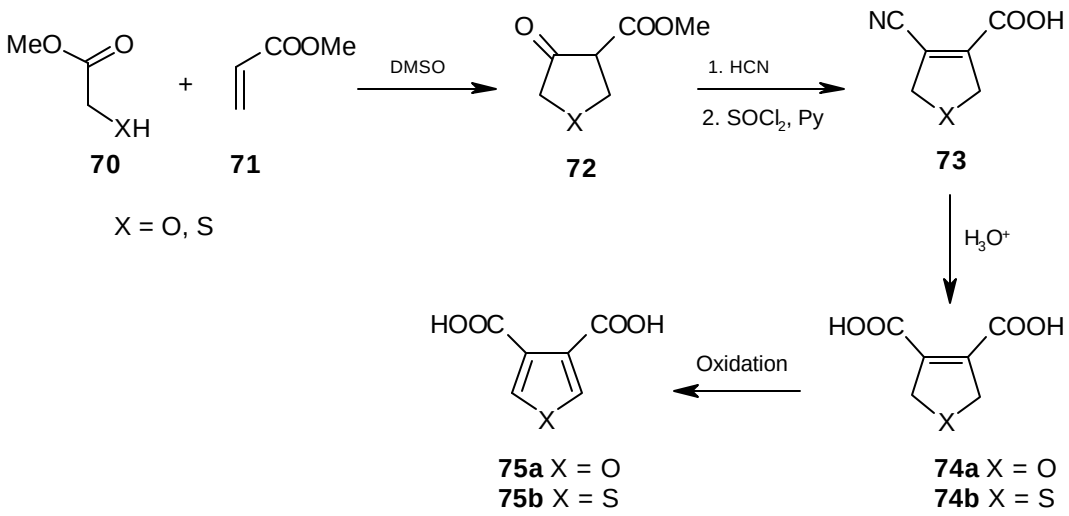
bromoatom followed by intramolecular ring opening. In contrast, the reaction of bromoanhydride **22** with *o*-PDA and *o*-ATP at –15 °C resulted in the formation of new products other than expected one, which have been fully characterized, with the help of spectral data. The IR spectrum represented the peculiar C=O stretching peaks of acrylic acid i.e. α,β-unsaturated acid at 1684 cm^{-1}. The ^1H NMR spectrum showed two singlet in the range of δ 5.50 to 6.50 for both the compounds indicating the presence of methylene group and presence of proton from lactam functionality where as the study of ^{13}C NMR and DEPT revealed the presence of two carbonyl carbons and two vinylic carbons from terminal methylene group. The mass spectrum fragmentation fully supported the assigned structures for eg. the peaks resulted due to acrylate fragmentation (CH_2=C-COOH = 71) were observed in both the compounds. Thus the spectroscopic data unambiguously confirmed the structures of assigned compounds as ***α***-quinoxalinylacrylic acid (**66**) and ***α***-benzothiazinylacrylic acid (**67**) respectively (**Scheme 15**). The formation of products might have taken place via either of the two intermediate structures **68** or **69** (**Scheme 15**). The possibility of product formation via the intermediate **69** obtained by first Michael type addition and then undergoing the regioselective ring opening has been ruled out, as bromo anhydride **22** did not react with thiophenol under identical set of reaction conditions. Both reactions underwent highly chemo- and regioselective ring opening at unhindered carbonyl to form the inisolable intermediate acid **68**, followed by intramolecular Michael type addition and 1,4-elimination (-HBr) reactions[71] to yield exclusively the corresponding kinetically controlled products ***α***-quinoxalinylacrylic acid (**66**) and ***α***-benzothiazinylacrylic acid (**67**). At room temperature these reactions loose their selectivities and furnished

complex mixture of products. Structural analogues of these compounds have been reported to possess 5-lipoxygenase inhibitory activity.[64] Many bioactive natural products such as secocrispiolide,[72] linderanolide,[73] mitomycin K,[74] rhopaloic acid[75] and 1,7(*Z*)-nondecadiene-2,3-dicarboxylic acid[76] possess exocyclic methylene group or this type of carbon-carbon double bond and this methodology to generate such type of carbon-carbon double bond will be amply useful in near future.

Anthranilic acid and *o*-AP on reaction with **22** furnished respectively the thermodynamically controlled products **64b** and **65b** in 63% and 50% yield due to weaker tendency of a –COOH and phenolic –OH towards Michael addition. In our hands the reactions of ethanolamine, ethylenediamine and thioethanolamine with anhydride **22** always ended up with formation of polymeric gums.

The next plan of our work describes the synthesize of furan dicarboxylic acid and thiophene dicarboxylic acid utilising 2,3-di(bromomethyl)maleic anhydride (**24**) as a starting material. The literature study revealed that only one, five-step method for the synthesis of these compounds is known[77] (**Scheme 16)**. The condensation of methyl

Scheme 16



70 **71** **72** **73**

X = O, S

75a X = O
75b X = S

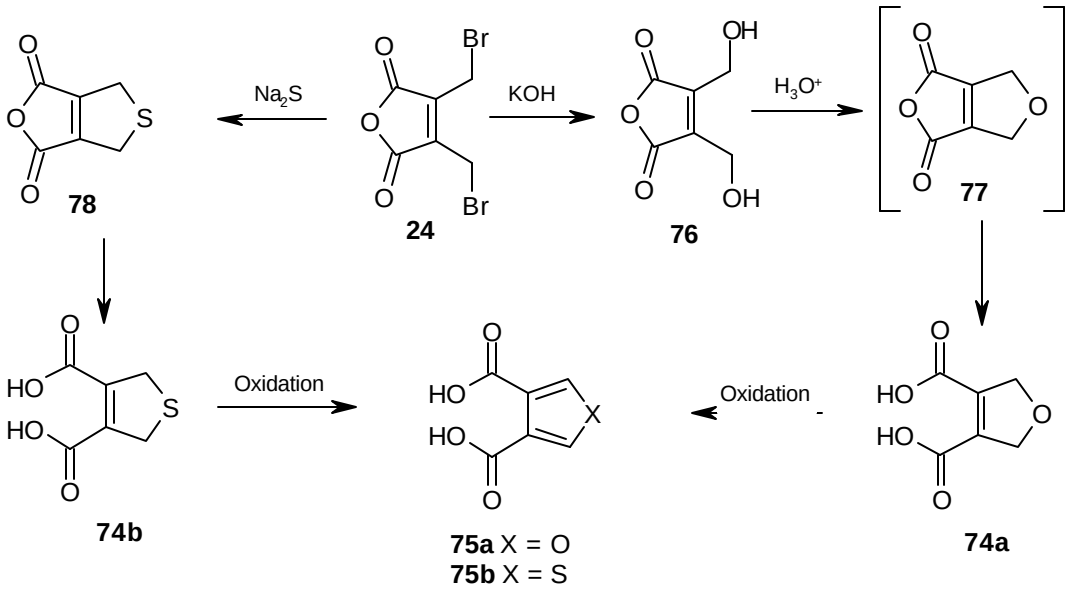
74a X = O
74b X = S

glycolate (**70**) and methyl acrylate (**71**) provides the tetrahydrofuranone derivative **72**. The reaction of hydrogen cyanide with furanone **72** was shown to yield the cynohydrin

which on dehydration followed by acid hydrolysis provided them 2,5-dihydro-3,4-furandicarboxylic acid (**74**, X = O). The oxidation[78] of **74** with hydrogen peroxide in acetic acid or iodosobenzene in dioxane furnished the furan 3,4-dicaboxylic acid (**75a**). The same strategy has been employed to obtain thiophene 3,4-dicarboxylic (**75b**) acid using ethyl thioglycolate as one of the starting material.

Our synthetic strategy is depicted in **Scheme 17**. The di(bromomethyl)maleic anhydride (**24**) on reaction with aqueous KOH furnished the dihydroxy compound **76** which on dehydration gave the 2,5-dihydrofuran dicarboxylicacid (**74a**). The oxidation as per the literature method or by DDQ will easily give the furan dicarboxylic acid (**75a**).

Scheme 17



Our studies on synthesis of thiophene dicarboxylic acid (**75b**) are in progress wherein the treatment of soft nucleophile sodium sulfide with di(bromomethyl)maleic anhydride (**24**) may give the 2,5-dihydrothiophene-3,4-dicarboxylic acid (**74b**). The compound **74b** is important starting material for the synthesis bioactive natural product cantharidine a potent vesicant used in the removal of benign epithelial growth.[79] Further oxidation under the similar set of reaction conditions used for furan will easily lead to thiophene dicaroxylic acid (**75b**).

In summary, the (bromomethyl)methylmaleic anhydride (**22**) reacts with *o*-PDA and *o*-ATP in remarkably chemo- and regioselective fashion to yield the corresponding kinetically controlled products ***a***-quinoxalinylacrylic acid (**66**) and ***a***-benzothiazinylacrylic acid (**67**) respectively in very good yields.[80] A short route synthesis of furan dicarboxylic acid starting from di(bromomethyl)maleic anhydride (**24**) has been achieved where as the synthesis of thiophene dicarboxylic acid is in progress.

2.5 CONCLUSIONS: We prepared (bromomethyl)methylmaleic anhydride and 2,3-dibromomethylmaleic anhydride via NBS bromination of dimethylmaleic anhydride and in the present dissertation we have demonstrated the synthetic utilities of these potential starting materials for the first time in the field of synthetic organic chemistry. The chemoselective carbon-carbon coupling of organocuprates with (bromomethyl)methylmaleic anhydride furnished ras farnesyl-protein transferase inhibitor chaetomellic anhydride A and its analogues in 55-60% yields. Starting from (bromomethyl)methylmaleic anhydride we synthesized (±)-maleic anhydride segment of antifungal antibiotics tautomycin in five-steps (28% overall yield) without using any protection-deprotection chemistry. Starting from (bromomethyl)methylmaleic anhydride we have completed five-step synthesis of dihydrotelfairic anhydride via chemoselective Wittig reaction with 29% over all yield and our efforts to dehydrogenate the same to naturally occurring telfairic anhydride are in active progress. In the last section we have studied chemo- and regioselective nucleophilic reactions of (bromomethyl)methylmaleic anhydride with suitably substituted aniline derivatives to design new heterocycles *α*-quinoxalinylacrylic and *α*-benzothiazinylacrylic acids with eliminative generation of new carbon-carbon double bond and the present strategy will be amply useful to synthesize several natural products with this kind of double bond.

2,3-Dibromomethylmaleic anhydride (**23**) has been successfully used to obtain 2,5-dihydro-3,4-furandicarboxylic acid. Our studies to obtain symmetrically dialkylsubstituted maleic anhydrides starting from **23** are also in progress.

In the present studies towards the synthesis of bioactive natural product we successfully used the multifunctional (bromomethyl)methylmaleic anhydride as a potential starting material without using any protection-deprotection chemistry.

The present studies on maleic anhydride chemistry have provided us a clean and clear impression that these multifunctional maleic anhydride moieties have been used in the past century by practically all type of chemists as potential starting materials. They undergo variety of reactions and the chemo-, regio-, and enantioselectivities observed with these multifunctional molecules are remarkable. The development of new solid support synthesis/combinatorial chemistry techniques will now provide lot of new useful applications of these molecules in near future. In short, maleic anhydride and their derivatives have very rich history and very bright present to their credit in the field of chemistry and highly useful future is assured.

EXPERIMENTAL PART

OF SECTION A to D

3-(Bromomethyl)-4-methyl-2,5-furandione (22): A mixture of dimethylmaleic anhydride (5.04 g, 40 mmol), *N*-bromosuccinimide (14.24 g, 80 mmol) and catalytic amount of dibenzoyl peroxide (200 mg, 0.83 mmol) in carbon tetrachloride (300 mL) was gently refluxed for 5 h in a 500 mL round bottom flask. The reaction mixture was allowed to cool at room temperature, a second portion of dibenzoyl peroxide (200 mg, 0.83 mmol) was added and again the refluxing was continued 5 h longer. The mixture was left overnight at room temperature and then filtered. The residue was washed with CCl_4 (25 mL X 2); the combined organic layer was washed with water (100 mL X 2) and brine (100 mL) and then dried over Na_2SO_4 and concentrated in vacuo to furnish thick yellow oil, which was purified by chromatography on silica gel column [elution with petroleum ether/ethyl acetate (8:2)] to obtain a crude product (7.0 g) and then further purified by distillation using Kugelrhor apparatus. The first fraction (2.5 g) was a mixture of **21** and **22** while a second fraction obtained at 120-125 °C (2 mm) was the anhydride **22**, 4.2 g, (60% yield, 98% purity by ^{1}H NMR).

General procedure for the synthesis of alkylmethylmaleic anhydrides (1a-e): A freshly prepared solution of Grignard reagent (10 mmol) in ether (15 mL) was added dropwise to the solution of (bromomethyl)methylmaleic anhydride **22** (410 mg, 2 mmol) and CuI (38 mg, 0.2 mmol) in ether (10 mL) and HMPA (4 mL) under argon at -5 to 0 °C over 15 to 20 minutes under stirring. The reaction mixture was allowed to reach room temperature and further stirred for 8 h. It was diluted with ether (15 mL) and acidified with 4 N H_2SO_4 (10 mL) and the aqueous layer was further extracted with ether (15 mL X 3). The combined organic layer was washed with water (20 mL X 2), brine and dried over Na_2SO_4. Concentration of organic layer in vacuo followed by silica gel column chromatographic

purification of the crude product using petroleum ether/ethyl acetate (9:1) as eluent furnished pure alkylmethylmaleic anhydrides **1a-e** in 55-60% yields. These reactions were also carried out under identical conditions in THF without any loss of yield.

The products obtained can also be purified by chemical treatment. The crude products were basified with 5% aqueous NaOH solution and the aqueous layer was washed with ether (10 mL X 3). Subsequent acidification of the aqueous layer, followed by ether extraction (15 mL X 3), washing of extract with water (15 mL), brine (15 mL), drying over Na_2SO_4 and concentration in vacuo furnished pure product with similar yield.

3,4-Dibromomethyl-2,5-furandione (23): A mixture of dimethylmaleic anhydride (5.04 g, 40 mmol), *N*-bromosuccinimide (14.24 g, 80 mmol) and catalytic amount of dibenzoyl peroxide (200 mg, 0.83 mmol) in carbon tetrachloride (300 mL) was gently refluxed for 12 h in a 500 mL round bottom flask. The reaction mixture was allowed to cool to room temperature and a second portion of *N*-bromosuccinimide (14.24 g, 80 mmol) and dibenzoyl peroxide (200 mg, 0.83 mmol) was added and again the refluxing was continued 12 h longer. The mixture was left overnight at room temperature and then filtered. The residue was washed with CCl_4 (25 mL X 2); the combined organic layer was washed with water (100 mL X 2), brine (100 mL) and then dried over Na_2SO_4 and concentrated in vacuo to furnish thick yellow oil, which was purified by chromatography on silica gel column [elution with petroleum ether/ethyl acetate (8:2)] to obtain a crude product (9.5 g) and then further purified by distillation using Kugelrhor apparatus. The first fraction (4.0 g) was a mixture of small amount of **21** and major portion of **22** while a second fraction obtained at 145-150 °C (2 mm) was the dibromoanhydride **23**, 5.2 g, (46% yield, 98% purity by ^1H

NMR). Recrystalisation from mixture of benzene and petroleum ether (1:3) gave analytically pure sample of **23**, mp 84-86 °C.

3,4-Ditridecyl-2,5-furandione (24): A freshly prepared solution of Grignard reagent of bromododecane (10 mmol) in ether (15 mL) was added dropwise to the solution of 2,3-dibromomethylmaleic anhydride **23** (284 mg, 1 mmol) and CuI (19 mg, 0.1 mmol) in ether (10 mL) and HMPA (4 mL) under argon at -5 to 0 °C over 15 to 20 minutes under stirring. The reaction mixture was allowed to reach room temperature and further stirred for 8 h. It was diluted with ether (15 mL) and acidified with 4 N H_2SO_4 (10 mL) and the aqueous layer was further extracted with ether (15 mLX 3). The combined organic layer was washed with water (20 mL X 2), brine and dried over Na_2SO_4. Concentration of organic layer in vacuo followed by silica gel column chromatographic purification of the crude product using petroleulm ether/ethyl acetate (9:1) as eluent furnished pure ditridecylmaleic anhydride **24**, 47 mg (10% yield). The product obtained can also be purified by chemical treatment as per the procedure mentioned in the synthesis of alkylmethylmaleic anhydride.

3-Hydroxymethyl-4-methyl-2,5-furandione (30): To an ice-cold solution of 4 N aq. KOH (10 mL) was added, (bromomethyl)methylmaleic anhydride (**22**, 2.05 gm, 10 mmol) and the mixture was stirred at room temperature for 5 h. The mixture was slowly acidified with 6 N H_2SO_4 (10 mL), and saturated with solid NaCl and stirred at room temperature for 30 min. The aqueous layer was extracted with ethyl acetate (4 X 20 mL), the organic layer was washed with brine (20 mL) 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 (3:1) gave pure **30** 1.22 gm (86% yield), mp 54-56 °C.

3-[2,2-Bis(ethoxycarbonyl)ethyl]-4-methyl-2,5-furandione (32): To the slurry of sodium hydride (480 mg, 20 mmol) in benzene (50 mL) was added diethyl malonate (3.20 g, 20 mmol) in a dropwise fashion at rt and the reaction mixture was stirred for 5 min. The solution of bromoanhydride (**22**, 2.05 g, 10 mmol) in benzene (20 mL) was added to the reaction mixture at rt and further stirred for 8 h. The reaction mixture was acidified with dil. HCl and extracted with ethyl acetate (30 mL X 3). The organic layer was washed with water (30 mL), brine (30 mL) and dried over Na_2SO_4. Concentration of organic layer in vacuo followed by silica gel column chromatographic purification of residue using petroleum ether and ethyl acetate mixture (4:1) furnished pure **32** (thick oil), 1.95 g (74% yield).

3-(2-Carboxy)ethyl-4-methyl-2,5-furandione (33): The diester (**32,** 2.84 g, 10 mmol) in con. HCl plus water (30 mL, 1:1) was refluxed with stirring for 12 h. The reaction mixture was allowed to reach rt and then saturated by adding solid NaCl. The filtered aqueous layer was extracted with ethyl acetate (20 mL X 5) and washed with brine (25 mL), dried over Na_2SO_4 and concentration in vacuo furnished pure acid **33**, 1.73 g (94% yield). It was recrystallized from dichloromethane/petroleum ether mixture (1:2) to obtain analytically pure sample of **33**, mp 97-98 °C (Lit.[6a] mp 96-97 °C).

3-(2-Methoxycarbonyl)ethyl-4-methyl-2,5-furandione (34): A solution of **33** (184 mg, 1 mmol) in ether (20 mL) was treated with a solution of diazomethane in ether at 0 °C until the starting material was completely consumed (3 h). Excess of diazomethane was quenched with acetic acid and the reaction mixture was concentrated in vacuo. The silica gel column chromatographic purification of the reaction mixture using petroleum ether and ethyl acetate mixture (4:1) gave pure **34** (thick oil), 188 mg (95% yield).

3-(1-Bromo-2-carboxy)ethyl-4-methyl-2,5-furandione (35): A mixture of acid **33** (552 mg, 3 mmol), NBS (2.67 g, 15 mmol), and a catalytic amount of dibenzoyl peroxide (30 mg) in chloroform (40 mL) was gently refluxed for 24 h. The reaction mixture was allowed to cool to room temperature and concentrated in vacuo. The residue was purified by silica gel column chromatography using petroleum ether and ethyl acetate mixture (1:3) to obtain pure bromo acid **35** (waxy solid), 584 mg (74% yield).

3-(1-Hydroxy-2-carboxy)ethyl-4-methyl-2,5-furandione (36): To an ice-cold solution of 1 N aq. KOH (10 mL) was added bromoacid (**35**, 526 mg, 2 mmol) and the mixture was stirred at 0 °C for 3 h. The reaction mixture was slowly acidified with 3 N HCl and saturated with solid NaCl. The filtered aq. layer was extracted with ethyl acetate (20 mL X 4) and the organic layer was washed with brine (20 mL) and dried over Na_2SO_4. Concentration of organic layer in vacuo furnished hydroxy acid **36**, 360 mg (90% yield), mp 132-133 °C.

3-(1-Hydroxy-2-methoxycabonyl)ethyl-4-methyl-2,5-furandione (26B): A solution of **36** (200 mg, 1 mmol) in ether (20 mL) was treated with a solution of diazomethane in ether at 0 °C until the starting material was completely consumed (3 h). Excess of diazomethane was quenched with acetic acid and the reaction mixture was concentrated in vacuo. The silica gel column chromatographic purification of the reaction mixture using petroleum ether and ethyl acetate mixture (7:3) gave pure **26B** (thick oil), 203 mg, (95% yield).

3-[2,2-Bis(ethoxycarbonyl)-2-bromoethyl]-4-methyl-2,5-furandione (47): A mixture of diester **32** (852 mg, 3 mmol), NBS (588 mg, 3.3 mmol), and a catalytic amount of dibenzoyl peroxide (30 mg) in carbon tetrachloride (40 mL) was gently refluxed for 10 h. The reaction mixture was allowed to cool to room temperature, then filtered and

concentrated in vacuo. The residue was purified by silica gel column chromatography using petroleum ether and ethyl acetate mixture (3:1) to obtain pure bromo compound **47** (waxy solid), 763 mg (64% yield).

3-(1-Bromo-2-methoxycarbonyl)ethyl-4-methyl-2,5-furandione (48): A solution of **35** (528, 2mmol) in methanol and H_2SO_4 (9.5:0.5, 10 mL) was stirred at rt for 4 h under nitrogen. The solvent was removed by evaporation under vacuo. The residue was diluted with water and extracted with ethyl acetate (10 mL X 2). The combined organic layer was washed with water (10 mL), brine and dried over Na_2SO_4. Concentration in vacuo followed by silica gel column chromatographic purification of the crude product using petroleulm ether/ethyl acetate (7:3) as eluent furnished pure bromoester **48**, 389 mg (70 %yield).

3-[(*E*)-2-Carboxyethylene]-4-methyl-2,5-furandione (49A): To a solution of **48** (556 mg, 2 mmol) in THF (20 mL) was added potassium *tert*-butoxide (280 mg, 2.5 mmol) at 0 °C. The reaction mixture was allowed to reach room temperature and then stirred for 2 h at room temperature under argon atmosphere. The solvent was evaporated in vacuo and ethyl acetate was added to the residue, acidified with 2 N HCl, diluted with water and extracted with ethyl acetate (30 mL X 2). The combined organic layer was washed with water (20 mL), brine (20 mL) and dried over Na_2SO_4. Concentration in vacuo followed by silica gel column chromatographic purification of the crude product using petroleulm ether/ethyl acetate (4:6) as eluent furnished pure ***a,b***-unsaturated acid **49A**, 266 mg (73 %yield).

3-[(*E*)-2-Carbmethoxyethylene]-4-methyl-2,5-furandione (49B): A solution of **49A** (364 mg, 2 mmol) in methanol and H_2SO_4 (9.5:0.5, 10 mL) was stirred at rt for 4 h under nitrogen. The reaction mixture was concentrated in vacuo. The residue was diluted with water and extracted with ethyl acetate (10 mL X 2). The combined organic layer was

washed with water (10 mL), brine and dried over Na_2SO_4. Concentration of organic layer in vacuo followed by silica gel column chromatographic purification of the crude product using petroleum ether/ethyl acetate (7:3) as eluent furnished pure ***a,b***-unsaturated ester **49B**, 274 mg (70 % yield).

General procedure for the synthesis *g*-butyrolactone (51a-c): To a solution of ester **34** (396 mg, 2 mmol) in methanol (or ethanol or *iso*-propanol) was added $NaBH_4$ (152 mg, 5 mmol) and the reaction mixture was stirred at room temperature for 3 h. Methanol was removed under vacuo, the residue was diluted with water, acidified with 2 N HCl and extracted with ethyl acetate (2 X 25 mL). The combined organic layer was washed with water (20 mL), brine (20 mL) and dried over Na_2SO_4. Concentration in vacuo followed by silica gel column chromatographic purification of the crude product using petroleum ether/ethyl acetate (7:3) as eluent furnished pure *g*-butyrolactones (**51a-c**) in 65-70% yield.

3-(2-Methoxycarbonyl)ethyl-4-methyl-*N*-*p*-tolylmaleimide (53): To a stirred solution of **34** (396 mg, 2 mmol) in methanol (20 mL) was added *p*-toluidine (214 mg, 2 mmol) and the reaction mixture was refluxed for 5 h and allowed to cool to room temperature. Methanol was removed under vacuo and crude product was purified by column chromatography using petroleum ether and ethyl acetate (7:3) to furnish pue compound **53**, 481 mg (80% yield).

The compound **53** on reaction with $NaBH_4$ in a similar way to that of **34** gave the compound **54** in 60% yield.

3-(3-Hydroxypropyl)-4-methyl-2,5-furandione (55): To a solution of ester **34** (990 mg, 5 mmol) in toluene (15 mL) at 0 °C was added 2-molar DIBAL solution in toluene (5 mL, 10 mmol) dropwise over 5 minutes and continued to stirr at the same temperature for 4 h. The

reaction was quenched with 2 N HCl, diluted with water and extracted with ethyl acetate (3 X 30 mL) and the organic layer was washed with water (30 mL), brine (30 mL) and dried over Na_2SO_4. Concentration of organic layer in vacuo followed by silica gel column chromatographic purification of residue using petroleum ether and ethyl acetate mixture (4:6) furnished pure **55**, 510 mg (60% yield).

3-(3-Formylethyl)-4-methyl-2,5-furandione (56): Procedure A: To a stirred slurry of PCC (862 mg, 4 mmol) in dichloromethane (15 mL) at 0 °C was slowly added a solution of alcohol **55** (340 mg, 2 mmol) in dichloromethane (10 mL). Reaction mixture was further stirred for 6 h at room temperature. The reaction mixture was diluted with anhydrous ether (20 mL) and stirred vigorously for 10 minutes. The supernatant solution from the reaction mixture was passed through celite plus silica bed and concentrated in vacuo. The silica gel column chromatographic purification of residue using petroleum ether and ethyl acetate (7:3) furnished pure **56**, 218 mg (65% yield).

Procedure B: To a stirred solution of ester **34** (990 mg, 5 mmol) in toluene (15 mL) at -78 °C was added 2-molar DIBAL solution in toluene (3.75 mL, 7.5 mmol) in a dropwise fashion over a period of 5 minutes. The reaction was continued to stirr at the same temperature for 1.5 h and then quenched with 2 N HCl, diluted with water and extracted with ethyl acetate (3 X 30 mL) and the combined organic layer was washed with water (25 mL), brine (25 mL) and dried over Na_2SO_4. Concentration of organic layer in vacuo followed by silica gel column chromatographic purification of residue using petroleum ether and ethyl acetate mixture (7:3) furnished pure **56**, 588 mg (70% yield).

3-[3(*E*)-(4-Methoxycarbonyl)hexenyl]-4-methyl-2,5-furandione (57): To a solution of an ylide, 2-(triphenylphosphoranylidene)butyrate (1.086 gm, 3 mmol) in dichloromethane (10

mL) at 0 °C under argon atmosphere was added a solution of aldehyde **56** (336 mg, 2 mmol) in dichloromethane (8 mL) The solution was allowed to come to room temperature and then stirred for 24 h. The solvent was evaporated in vacuo. The silica gel column chromatographic purification of residue using petroleum ether and ethyl acetate (8:2) furnished pure **57**, 313 mg (62% yield).

3-(*p*-Toluidinylmethyl)-4-methyl-2,5-furandione (64a): To a stirred solution of *p*-toluidine (470 mg, 4.4 mmol) in chloroform (10 mL) was added a solution of anhydride **22** (410 mg, 2 mmol) in chloroform (10 mL) and reaction mixture was stirred at room temperature for 2 h. The reaction mixture was filtered to remove the salt, residue was washed with chloroform (10 mL), and chloroform layer was concentrated in vacuo. The obtained residue in on column chromatographic purification using petroleum ether and ethyl acetate (4:1) gave pure product **64a**, 320 mg, (69 % yield).

Similarly the reaction of anhydride **22** (410 mg, 2 mmol) with anthranilic acid (548 mg, 4 mmol) in Et_2O (20 mL) furnished pure **64b**, 330 mg (63% yield).

In similar way the reaction of anhydride **22** (410 mg, 2 mmol) with excess of *p*-toluidine (642 mg, 6 mmol) and anhydride **65a** (462 mg, 2 mmol) with of *p*- toluidine (429 mg, 4 mmol) in chloroform (20 mL) at room temperature for 3 h furnished the imide **65a** 190 mg, (59% yield).

***o*-Hydroxy-*N*-phenyl-2-(*o*-hydroxyanilinomethyl)-3-methylmaleimide (65b):** To a solution of *o*-aminophenol (764 mg, 7 mmol) in chloroform (25 mL) was added slowly the solution of **22** (410 mg, 2 mmol) in chloroform (10 mL) and the reaction was refluxed for 7 h. The reaction mixture was filtered and the residue was washed with chloroform and organic layer was concentrated in vacuo to furnish **65b** 325 mg, (50% yield).

***α*-(2-Methyl-2,3-dihydro-3-oxo-1,4-Quinoxalinyl-2-yl)acrylic acid (66):** To a stirred solution of *o*-phenylenediamine (432 mg, 4 mmol) in chloroform (10 mL) at -15 0 °C was added a solution of **22** (410 mg, 2 mmol) in chloroform (10 mL) and the reaction mixture was allowed to reach room temperature for 3 h. The reaction mixture was filtered, the residue was washed with chloroform and the organic layer was concentrated in vacuo. Silica gel column chromatographic purification of the residue with petroleum ether, ethyl acetate and methanol (12:7:1) as an eluent system furnished pure **66**, 400 mg (86% yield).

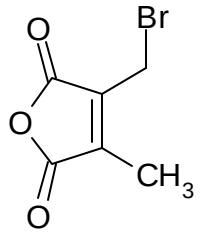
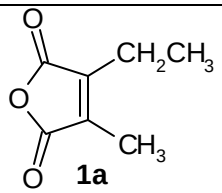
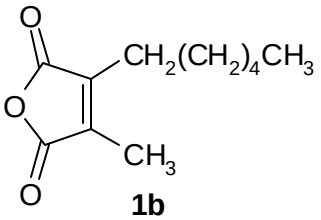
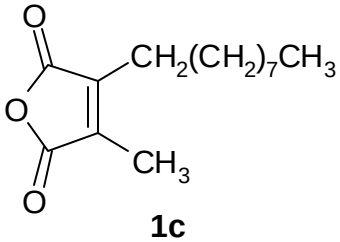
a-(2-Methyl-2,3-dihydro-3-oxo-1,4-benzothiazin-2-yl)acrylic acid (67) was prepared in a similar way using *o*-ATP in 90% yield.

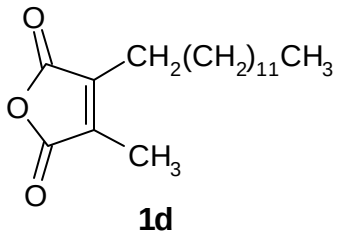
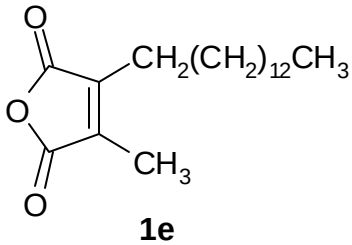
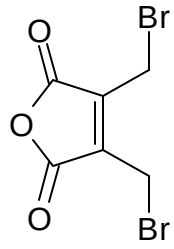
3,4-Dihydroxymethyl-2,5-furandione (76): To an ice-cold solution of 4 N aq. KOH (10 mL) was added, di(bromomethyl)maleic anhydride (**23**, 568 mg, 2 mmol) and the mixture was stirred at room temperature for 5 h. The mixture was slowly acidified with 6 N H_2SO_4 (10 mL), and saturated with solid NaCl and stirred at room temperature for 30 min. The aqueous layer was extracted with ethyl acetate (4 X 20 mL), the organic layer was washed with brine (20 mL) and dried over Na_2SO_4. Concentration of the organic layer in vacuo gave pure **76**, 250 mg (79% yield).

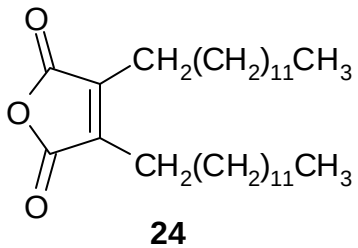
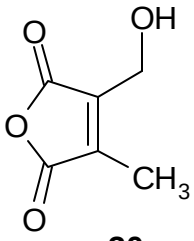
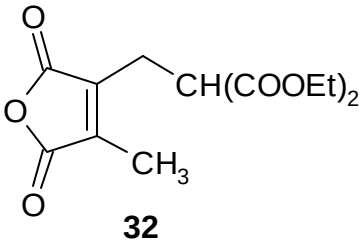
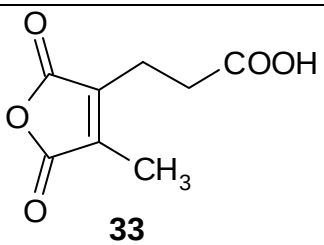
2,5-Dihydrofuran-3,4-dicarboxylic acid (74a): A solution of dihydroxy compound **76** (158 mg, 1 mmol) in con. HCl plus water (8 mL, 1:1) was refluxed with stirring for 5 h. The solvent was removed under vacuo and the residue was dissolved in ethyl acetate, washed with brine (10 mL) and dried over Na_2SO_4. The concentration of organic layer in vacuo gave pure **74a**, 100 mg (72% yield).

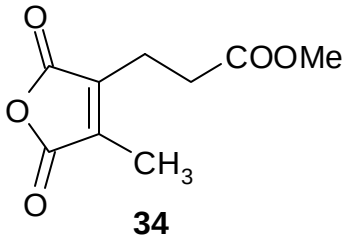
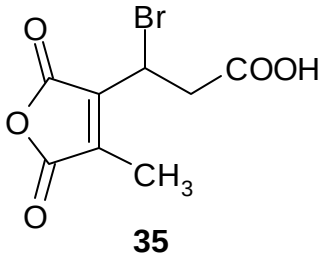
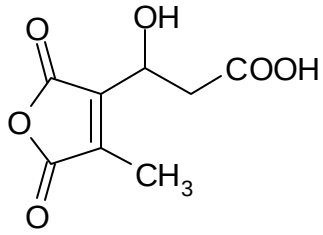
TABULATED SPECRAL DATA FOR COMPOUNDS

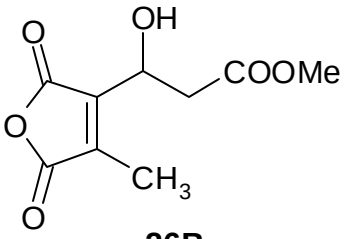
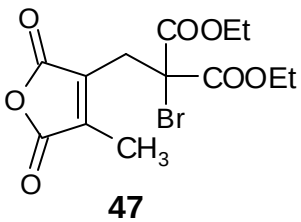
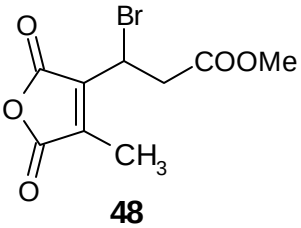
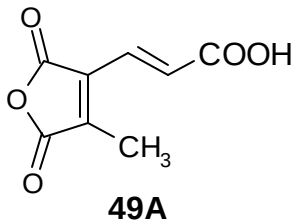
IN SECTION A TO D

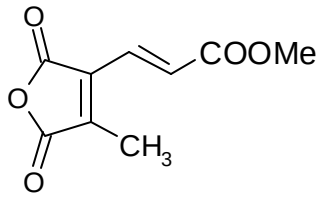
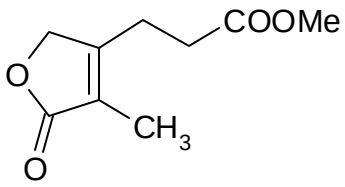
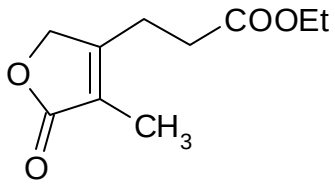
Sr. No.	Structure (Text Number)	PMR (d), CMR (d), and Mass spectral data, IR (cm^{-1})
1	 **22**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.18 (s, 3H), 4.20 (s, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 10.2, 16.2, 139.4, 144.0, 163.9, 165.2. MS (*m/e*) 206, 204, 125, 80, 53. IR (neat) ν_{max} 1775, 1765 cm^{-1}.
2	 **1a**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.22 (t, *J* = 8 Hz, 3H), 2.09 (s, 3H), 2.52 (q, *J* = 8 Hz, 2H). IR (neat) ν_{max} 1775, 1765, 1660 cm^{-1}.
3	 **1b**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.90 (m, 3H), 1.15-1.45 (m, 6H), 1.45-1.70 (m, 2H), 2.08 (s, 3H), 2.46 (t, *J* = 7 Hz, 2H). MS (*m/e*) 196, 168, 139, 126, 98, 81, 70, 55. IR (neat) ν_{max} 1765, 1655 cm^{-1}.
4	 **1c**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.90 (t, *J* = 6 Hz, 3H), 1.15-1.48 (bs, 12H), 1.48-1.70 (m, 2H), 2.10 (s, 3H), 2.48 (t, *J* = 8 Hz, 2H). MS (*m/e*) 238, 210, 193, 178, 153, 140, 126, 98, 81, 55. IR (neat) ν_{max} 1765, 1670 cm^{-1}.

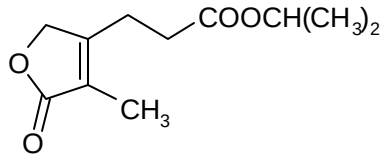
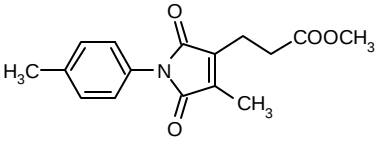
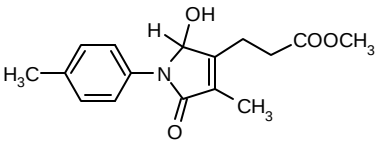
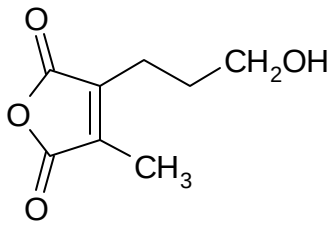
5	 **1d**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.88 (t, *J* = 7 Hz, 3H), 1.10-1.45 (bs, 20H), 1.45-1.70 (m, 2H), 2.08 (s, 3H), 2.48 (t, *J* = 7 Hz, 2H). MS (*m/e*) 294, 276, 249, 221, 192, 163, 150, 140, 126, 98, 55. IR (neat) ν_{max} 1770, 1675 cm^{-1}.
6	 **1e**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.88 (t, *J* = 7 Hz, 3H), 1.15-1.45 (bs, 22 H), 1.46-1.69 (m, 2H), 2.07 (s, 3H), 2.45 (t, *J* = 7 Hz, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 9.6, 14.3, 22.9, 24.6, 27.7, 29-31 (9 CH_2), 32.1, 140.6, 144.9, 166.0, 166.4. MS (*m/e*) 308, 290, 191, 150, 126, 91, 81, 69; IR (neat) ν_{max} 1770, 1680 cm^{-1}.
7	 **23**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.28 (s, 4H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 15.1, 141.1, 162.7. MS (*m/e*) 284, 266, 252, 235, 217, 203, 175, 159, 131, 124, 95, 80. IR (Nujol) ν_{max} 1784, 1664 cm^{-1}.

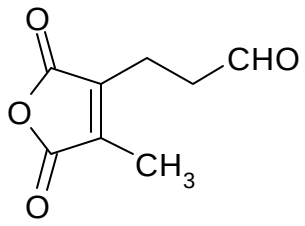
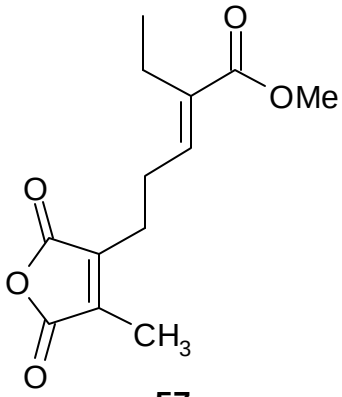
8	 **24**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.89 (t, *J* = 8 Hz, 6H), 1.27 (bs, 40H), 1.57(m, 4H), 2.44 (t, *J* = 7 Hz, 4H). MS (*m/e*) 462, 444, 437, 389, 331, 294, 265, 239, 211, 197, 168, 135, 123, 111, 83, 71, 57.
9	 **30**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.22 (s, 3H), 2.40 (bs, 1H), 4.63 (d, *J* = 2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 9.9, 55.2, 141.3, 143.4, 165.7, 166.3. MS (*m/e*) 142, 124, 113, 98, 85, 69, 55. IR (neat) ν_{max} 3255, 1800, 1760 cm^{-1}.
9	 **32**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.25 (t, *J* = 8 Hz, 6H), 2.11 (s, 3H), 3.01 (d, *J* = 6 Hz, 2H), 3.84 (t, *J* = 6 Hz, 1H), 4.19 (q, *J* = 8 Hz, 4H). MS (*m/e*) 284, 238, 212, 192, 165, 138, 125, 110, 93, 82, 55. IR (neat) ν_{max} 1826, 1744, 1732 cm^{-1}.
10	 **33**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.13 (s, 3H), 2.78 (s, 4H). MS (*m/e*)184, 166, 138, 110, 97, 93, 83, 66, 55. IR (Nujol) ν_{max} 1826, 1767, 1715 cm^{-1}.

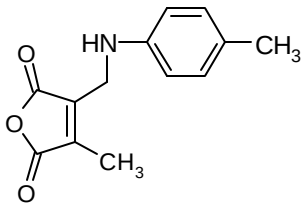
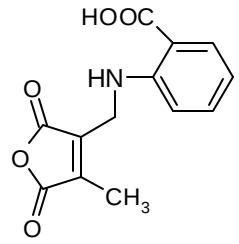
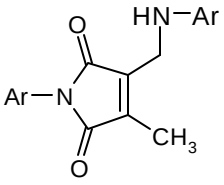
11	 **34**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.13 (s, 3H), 2.60-2.85 (m, 4H), 3.68 (s, 3H). MS (*m/e*) 198, 180, 166, 138, 126, 110, 97, 93, 83, 66, 55. IR (neat) ν_{max} 1828, 1771, 1738 cm^{-1}.
12	 **35**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.20 (s, 3H), 3.40 (dd, *J* = 12 and 4 Hz, 1H), 3.58 (dd, *J* = 12 and 6 Hz, 1H); 5.15 (t, *J* = 6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 9.8, 31.7, 39.9, 140.3, 142.6, 162.9, 164.7, 174.1. MS (*m/e*) 264, 262, 246, 244, 218, 216, 183, 165, 139, 111, 93, 82, 64, 55. IR (neat) ν_{max} 1828, 1778, 1722 cm^{-1}.
13	 **36**	^{1}H NMR (Acetone-d_6, 200 MHz) δ 2.22 (s, 3H), 2.85 (d, *J* = 6 Hz, 2H), 4.10 (br s, 1H), 5.17 (t, *J* = 6 Hz, 1H). MS (*m/e*) 200, 182, 164, 139, 154, 141, 136, 123, 113, 95, 85, 67. IR (Nujol) ν_{max} 3340, 1863, 1778, 1728 cm^{-1}.

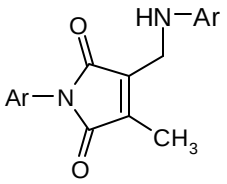
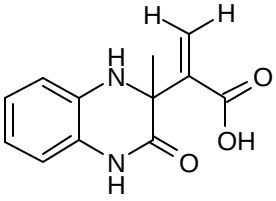
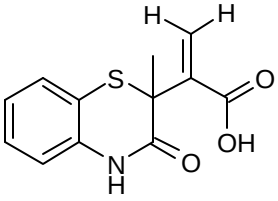
14	 **26B**	^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.26 (s, 3H), 2.87 (m, 2H), 3.30 (bs, 1H), 3.74 (s, 3H), 5.13 (t, *J* = 6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.0, 39.2, 52.3, 63.9, 141.6, 143.1, 164.7, 165.5, 171.7. MS (*m/e*) 214, 196, 183, 165, 154, 141, 136, 123, 113, 103, 95, 74, 67. IR (neat) ν_{max} 3493, 1842, 1763, 1732 cm^{-1}.
15	 **47**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.32 (t, *J* = 8 Hz, 6H), 2.22 (s, 3H), 3.53 (s, 2H), 4.33 (q, *J* = 6 Hz, 4H).
16	 **48**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.19 (s, 3H), 3.41 (dd, *J* = 12 and 4 Hz, 2H), 3.68 (s, 3H), 5.15 (t, *J* = 6 Hz, 1H).
17	 **49A**	^{1}H NMR (Acetone-d_6, 200 MHz) δ 2.32 (d, *J* = 2 Hz, 3H), 7.02 (d, *J* = 16 Hz, 1H), 7.50 (d, *J* = 16 Hz, 1H). MS (*m/e*) 182, 164, 154, 137, 120, 110, 93, 82, 65, 54.

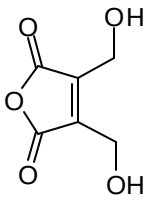
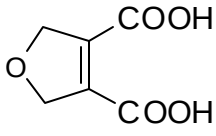
18	 **49B**	^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.27 (s, 3H), 3.34 (s, 3H), 7.16 (d, *J* = 16 Hz, 1H), 7.43 (d, *J* = 16 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 9.9, 52.2, 128.4, 130.1, 134.7, 143.7, 163.2, 165.0, 165.8. MS (*m/e*) 196, 165, 152, 137, 124, 109, 93, 81, 63, 55. IR (neat) ν_{max} 1869, 1821, 1778,1713, 1614 cm^{-1}
19	 **51a**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.05 (s, 3H), 2.56 (bs, 4H), 3.64 (s, 3H), 4.62 (s, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.1, 18.1, 31.4, 51.5, 72.6, 125.5, 157.9, 172.9, 174.4. MS (*m/e*) 184, 166, 152, 138, 124, 110, 95, 85, 79, 67, 59, 55. IR (Nujol) ν_{max} 1751, 1730, 1678 $cm^{-1.}$
20	 **51b**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.24 (t, *J* = 8 Hz, 3H), 2.07 (s, 3H), 2.58 (bs, 4H), 4.11 (q, *J* = 8 Hz, 2H), 4.63 (s, 2H). MS (*m/e*) 198, 180, 164, 152, 124, 110, 95, 79, 67, 55. IR (Nujol) ν_{max} 1748, 1728, 1675 cm^{-1}.

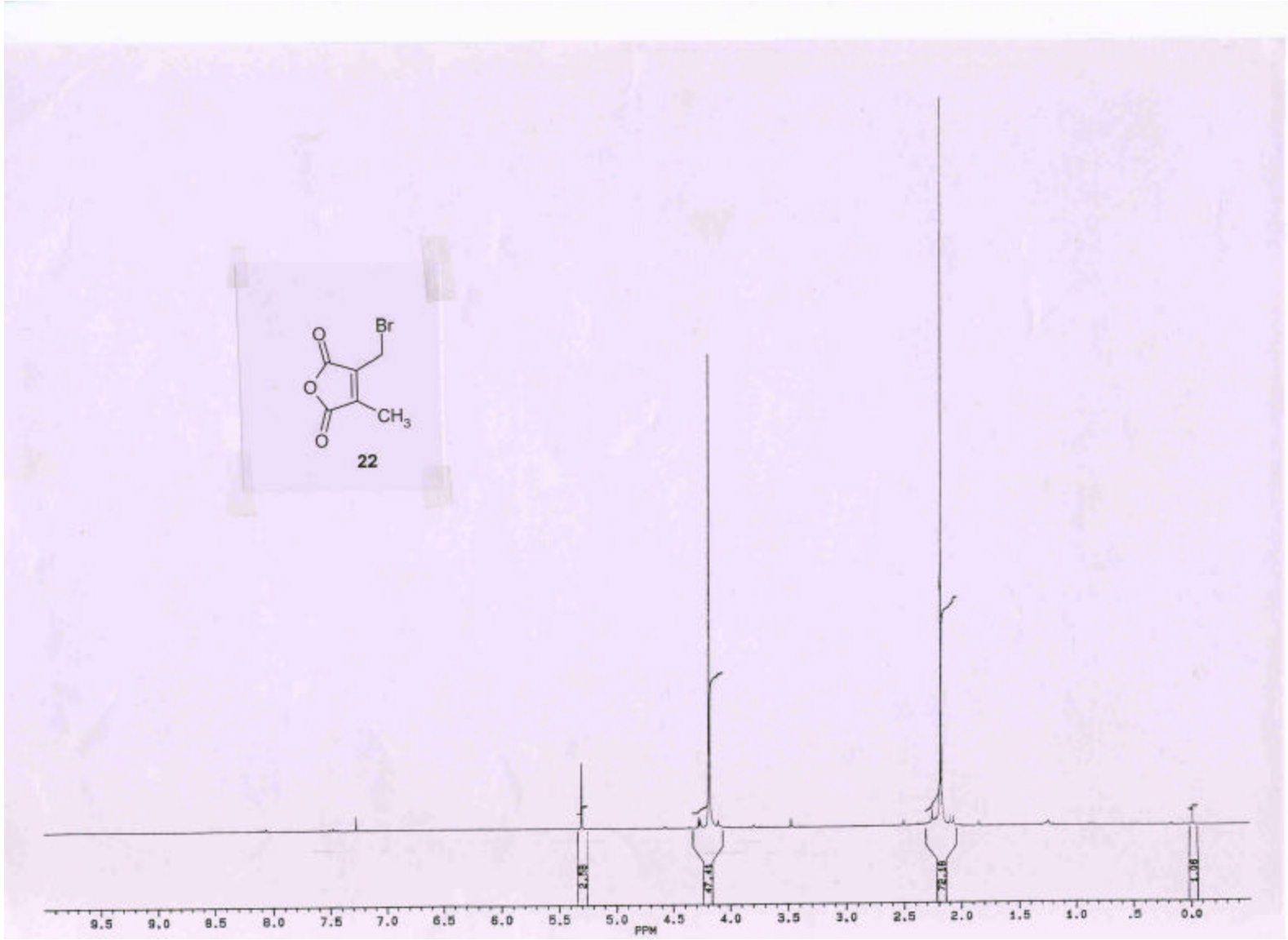
21	 **51c**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.20 (d, *J* = 6 Hz, 6H), 2.05 (s, 3H), 2.54 (bs, 4H), 4.61 (s, 2H), 4.95 (septet, *J* = 6 Hz, 1H). MS (*m/e*) 212, 170, 152, 124, 110, 97, 79, 67, 55. IR (neat) ν_{max} 1759, 1728, 1681 cm^{-1}.
22	 **53**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.10 (s, 3H), 2.37 (s, 3H), 2.69 (t, *J* = 6 Hz, 2H), 2.79 (t, *J* = 6 Hz, 2H), 3.70 (s, 3H), 7.21 (d, *J* = 8 Hz, 2H), 7.50 (d, *J* = 8 Hz, 2H). MS (*m/e*) 301, 287, 254, 226, 198, 171, 131, 103, 90, 76, 66. IR (Nujol) ν_{max} 2359, 1738, 1709 cm^{-1}.
23	 **54**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.02 (s, 3H), 2.32 (s, 3H), 2.40-2.60 (m, 4H), 3.63 (s, 3H), 5.56 (d, *J* = 10 Hz, 1H), 7.14 (d, *J* = 8 Hz, 2H), 7.52 (d, *J* = 8 Hz, 2H).
24	 **55**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.84 (quintet, *J* = 8 Hz, 2H), 2.09 (s, 3H), 2.44 (bs, 1H), 2.59 (t, *J* = 8 Hz, 2H), 3.67 (t, *J* = 6 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 9.3, 20.9, 30.0, 61.4, 140.9, 144.2. 166.1 (2 carbon of carbonyl).

		MS (*m/e*) 170, 152, 140, 126, 110, 97, 81, 67, 55. IR (neat) ν_{max} 3445, 1825, 1771, 1678 cm^{-1}.
25	 **56**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.15 (s, 3H), 2.73 (t, *J* = 9 Hz, 2H), 2.91 (t, *J* = 9 Hz, 2H), 9.79 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 9.6, 17.1, 40.5, 142.0, 144.2, 165.6, 165.7, 199.0. MS (*m/e*) 168, 152, 140, 126, 110, 97, 81, 67, 55. IR (neat) ν_{max} 1823, 1763, 1724 cm^{-1}.
26	 **57**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.99 (t, *J* = 9 Hz, 3H), 2.09 (s, 3H), 2.29 (q, *J* = 6 Hz, 2H), 2.45-2.55 (m, 2H), 2.55-2.67 (m, 2H), 3.75 (s, 3H), 6.61 (t, *J* = 9 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 9.6, 13.9, 20.1, 23.8, 26.1, 51.8, 136.2, 137.9, 141.6, 142.9, 165.6, 165.9, 167.7. MS (*m/e*) 252, 234, 220, 205, 192, 176, 163, 147, 126, 105, 76, 67, 55. IR (neat) ν_{max} 1825, 1765, 1713, 1647 cm^{-1}.

27	 **64a**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.15 (s, 3H), 2.20 (s, 3H), 2.90-3.35 (bs, 1H), 4.22 (s, 2H), 6.55 (d, *J* = 8 Hz, 2H), 7.02 (d, *J* = 8 Hz, 2H). MS (*m/e*) 231, 202, 188, 158, 144, 120, 106, 91, 77, 65. IR (Nujol) ν_{max} 3387, 1832, 1754, 1615 cm^{-1}.
28	 **64b**	^{1}H NMR ($CDCl_3$ + DMSO-d_6, 200 MHz) δ 1.90 (s, 3H), 4.18 (d, *J* = 6 Hz, 2H,), 6.38 (dd, *J* = 8 and 2 Hz, 1H), 6.50 (dt, *J* = 8 and 2 Hz, 1H), 7.17 (dt, *J* = 8 and 2 Hz, 1H), 7.78 (dd, *J* = 8 and 2 Hz, 1H), 8.15 (t, *J* = 6 Hz, 1H). MS (*m/e*) 261, 243, 214, 197, 170, 142, 132, 116, 92, 78, 66, 53. IR (Nujol) ν_{max} 3382, 1857, 1765, 1656 cm^{-1}.
29	 **65a** Ar = *p*-Tolyl	^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.40-1.75 (bs, 1H), 2.15 (s, 3H), 2.25 (s, 3H), 2.38 (s, 3H), 4.23 (s, 2H), 6.60 (d, *J* = 9 Hz, 2H), 7.05 (d, *J* = 9 Hz, 2H), 7.18 (d, *J* = 6 Hz, 2H), 7.25 (d, *J* = 6 Hz, 2H). MS (*m/e*) 320, 277, 214, 186, 158, 144, 120, 106, 91, 77, 65. IR (Nujol) ν_{max} 3386, 1769, 1704, 1619 cm^{-1}.

30	 Ar = *o*-Hydroxyphenyl **65b**	^{1}H NMR ($CDCl_3$ + DMSO-d_6, 200 MHz) δ 2.05 (s, 3H), 4.17 (s, 2H), 6.50-7.35 (m, 8H). MS (*m/e*) 324, 306, 281, 264, 231, 215, 199, 188, 170, 160, 144, 120, 108, 81, 65, 52. IR (Nujol) ν_{max} 3380, 3169, 1796, 1691, 1620 cm^{-1}.
31	 **66**	^{1}H NMR ($CDCl_3$ + DMSO-d_6, 200 MHz) δ 1.65 (s, 3H), 5.10-5.25 (br s, 1H), 5.55 (s, 1H), 6.10 (s, 1H), 6.50-6.75 (m, 4H), 9.55-9.75 (s, 1H). ^{13}C NMR ($CDCl_3$+ DMSO-d_6, 50 MHz) δ 22.5, 59.0, 112.8, 113.7, 117.6, 121.7, 124.1, 125.0, 132.0, 139.9, 166.0, 166.7. MS (*m/e*) 232, 217, 199, 171, 161, 143, 133, 118, 105, 92, 77, 64. IR (Nujol) ν_{max} 3352, 3190, 1711, 1662, 1613 cm^{-1}.
32	 **67**	^{1}H NMR ($CDCl_3$ + DMSO-d_6, 200 MHz) δ 1.70 (s, 3H), 5.57 (s, 1H), 6.13 (s, 1H), 6.80-7.15 (m, 4H), 10.20 (s, 1H). ^{13}C NMR ($CDCl_3$ + DMSO-d_6, 75 MHz) δ 22.5, 48.0, 116.5, 119.1, 122.8, 126.2, 126.5, 126.8, 136.2, 139.1, 166.3, 167.9. MS (*m/e*) 249, 231, 203, 175, 160, 151, 123, 109, 96, 80, 69, 53.

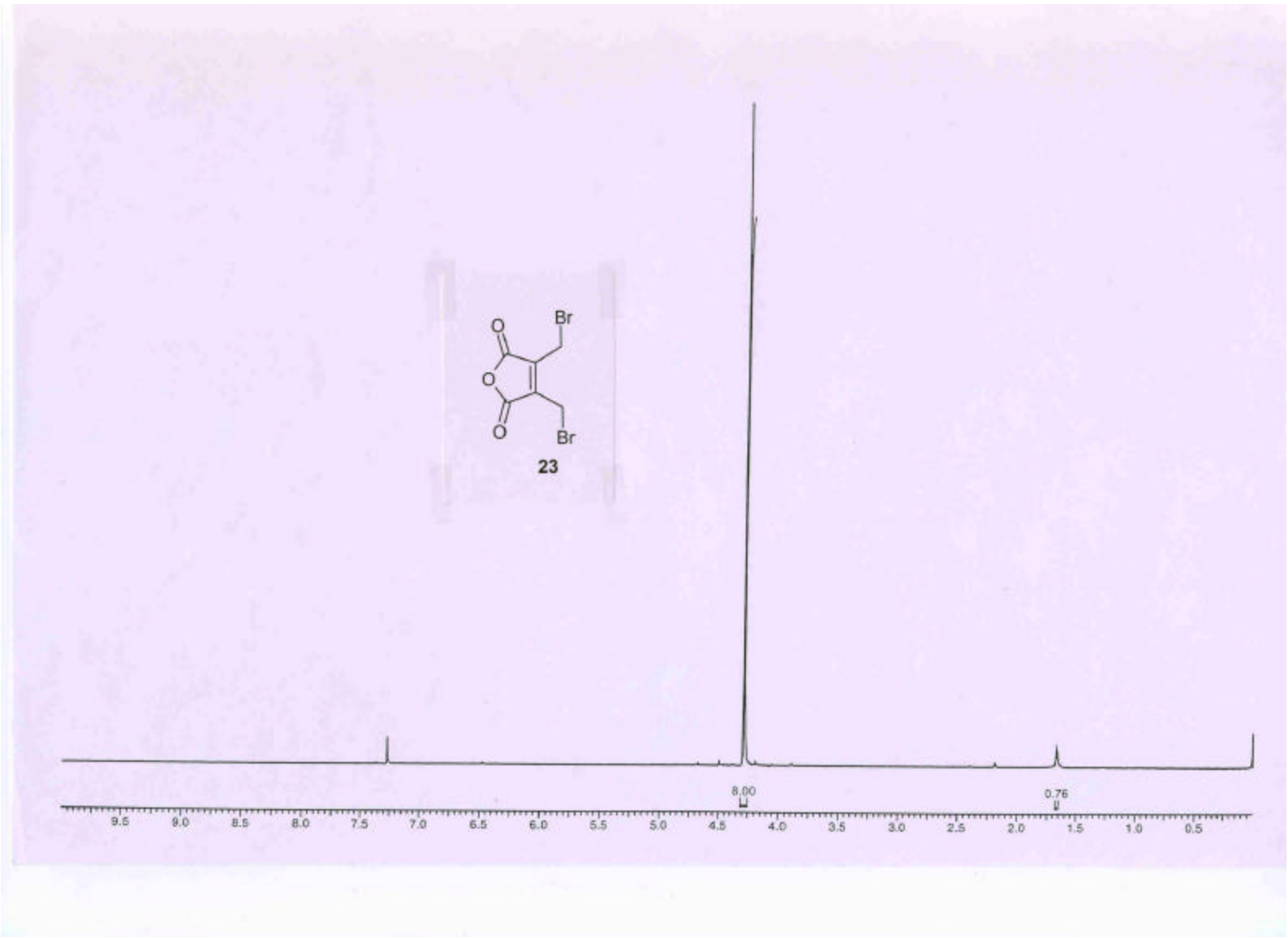
		IR (Nujol): ν_{max} 3162, 1684, 1635, 1563 cm^{-1}.
33	 **76**	^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.96 (s, 4H), 6.50 (bs, 2H) ^{13}C NMR (Acetone-d_6, 200 MHz) δ 77.7, 139.9, 164.4. MS (*m/e*) 140, 113, 96, 84, 69, 55.
34	 **74a**	^{1}H NMR (DMSO-d_6, 200 MHz) δ 4.75 (s, 4H). ^{13}C NMR (DMSO-d_6, 50 MHz) δ 77.3, 139.2, 164.2. MS (*m/e*) 158, 140, 112, 96, 84, 69, 66, 55.


Br
O
O
O
CH3
22
2.53
37.41
78.18
1.00
9.5
9.0
8.5
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
.5
0.0
PPM

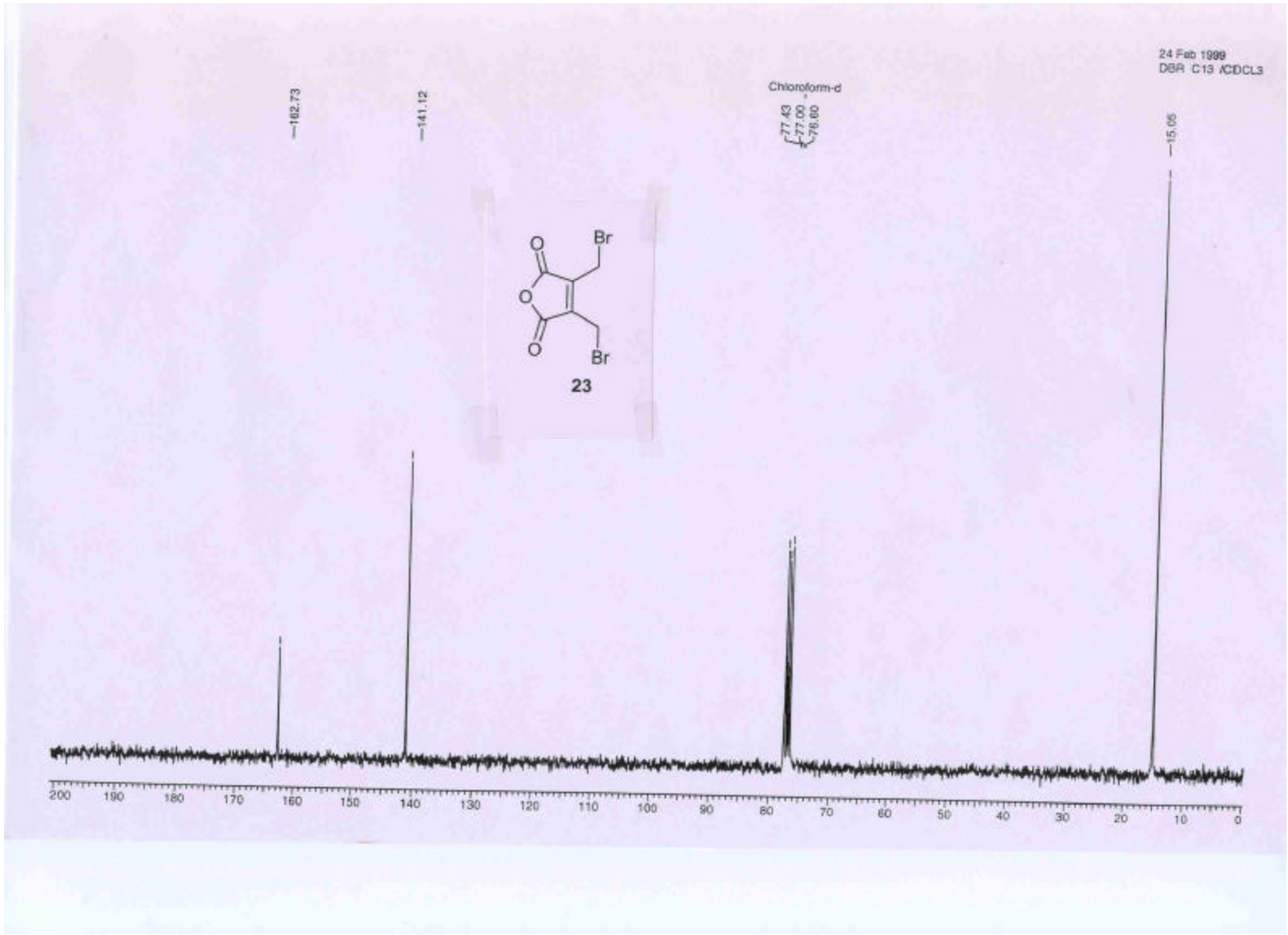
SD-MBR/C13/ CDCL3/ **

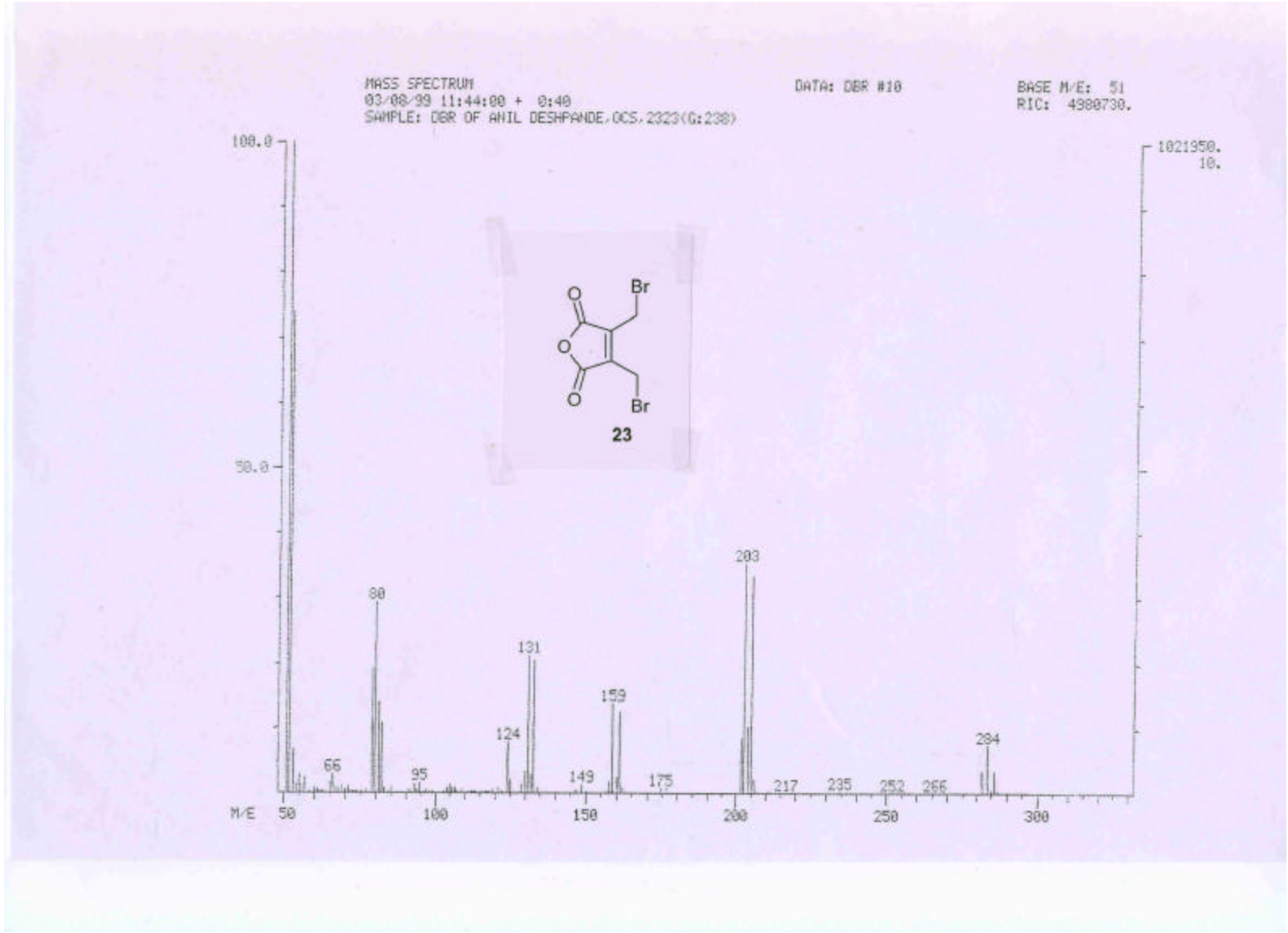
22

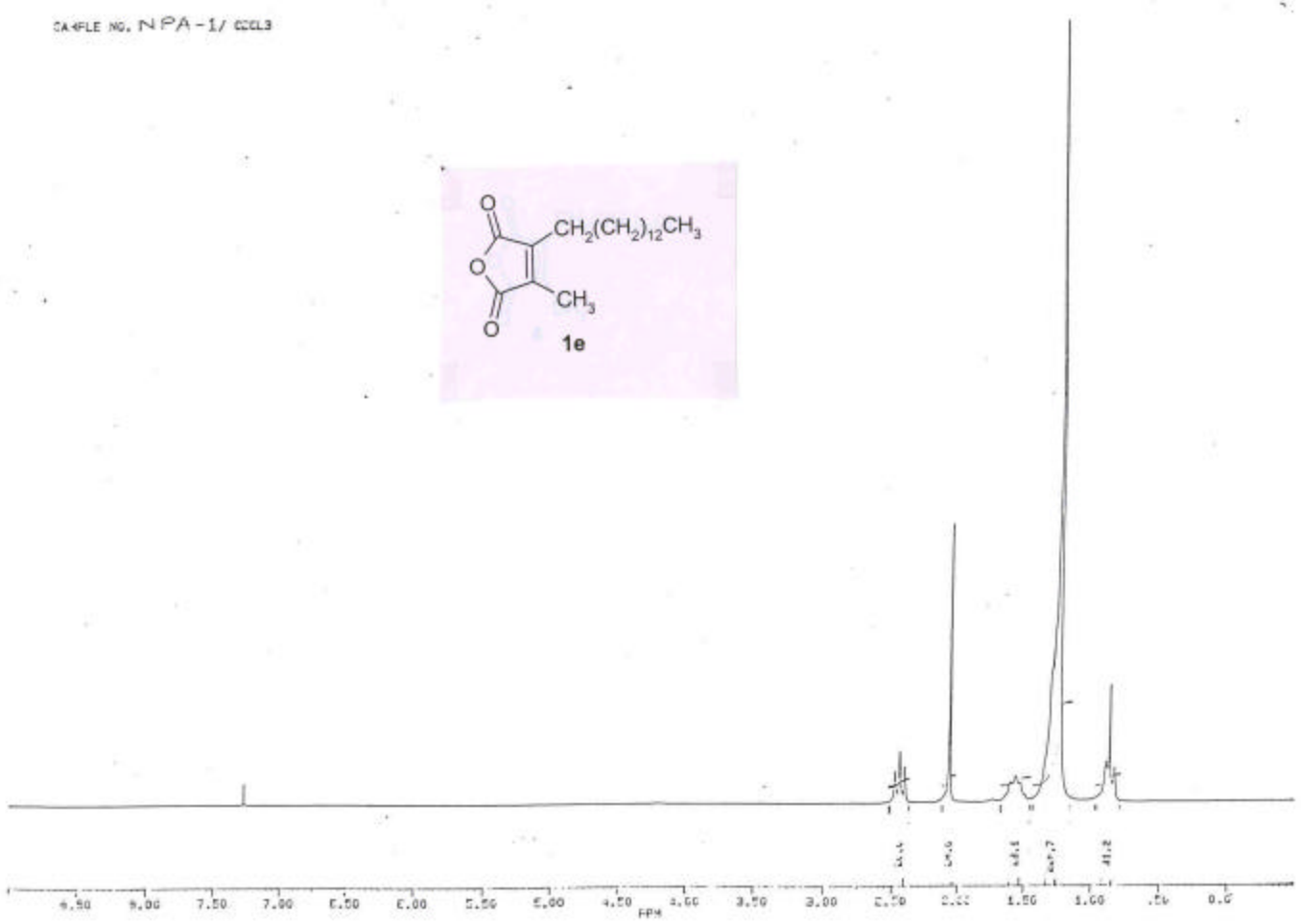

MASS SPECTRUM
01/05/97 13:39:00 + 1:41
SAMPLE: MBR OF ANIL DESHPANDE,OCS,2323(F:30)
DATA: MBR #25
BASE M/E: 125
RIC: 2908150.
100.0
50.0
M/E
125
80
53
205
Br
O
O
O
CH3
22

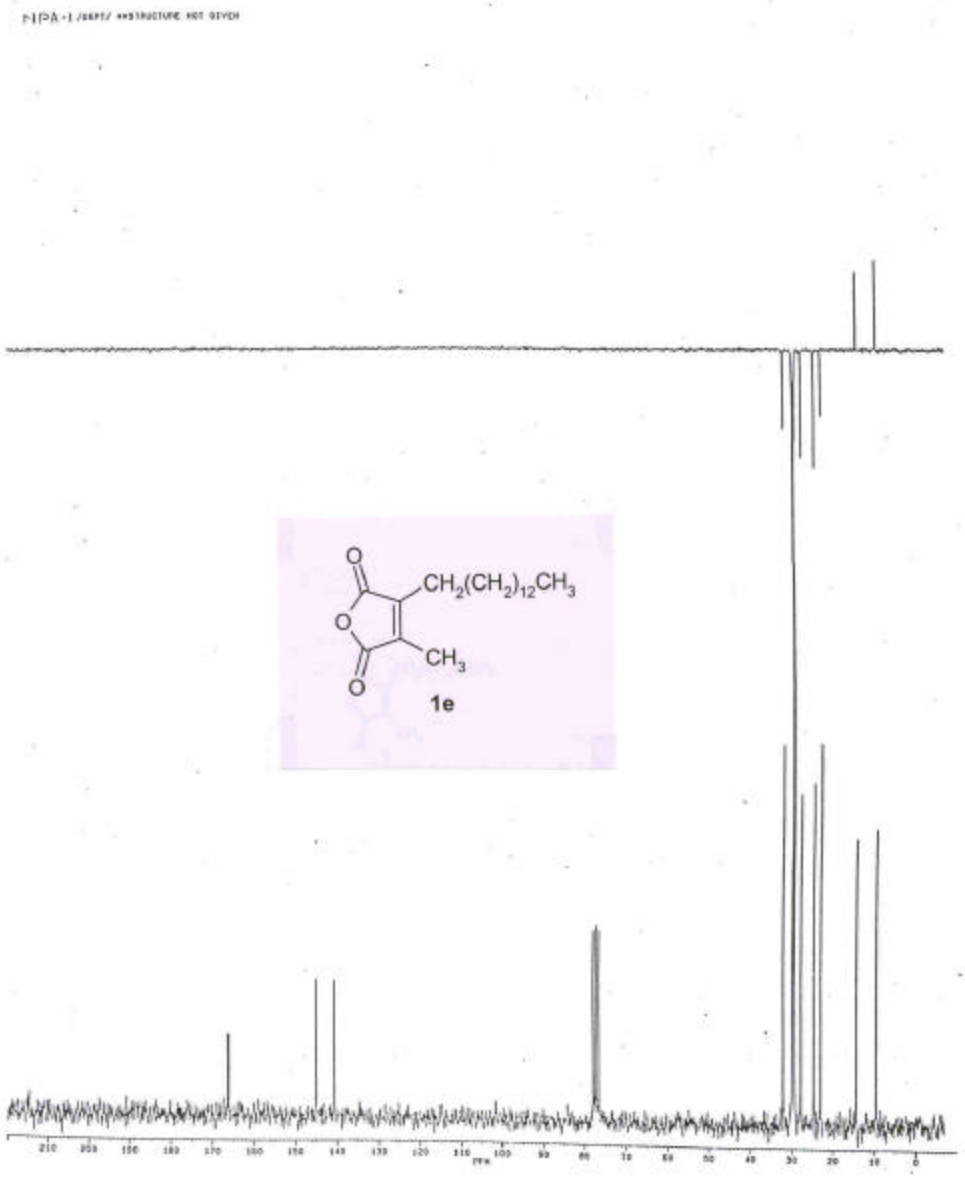

O
O
O
Br
Br
8.00
0.76
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

23

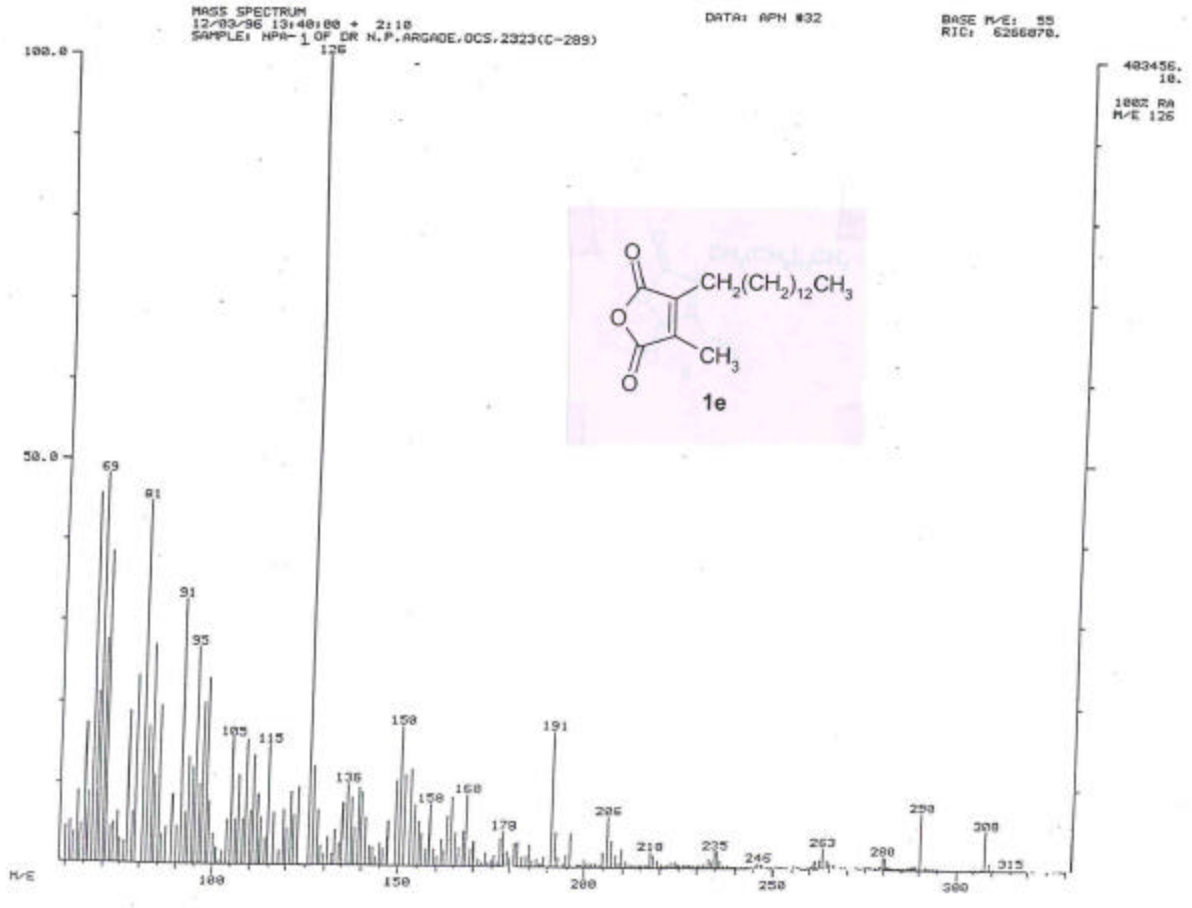

24 Feb 1999
DBR C13 /CDCL3
162.73
141.12
Chloroform-d
77.43
77.00
76.60
15.05
Br
Br
O
O
O
23
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

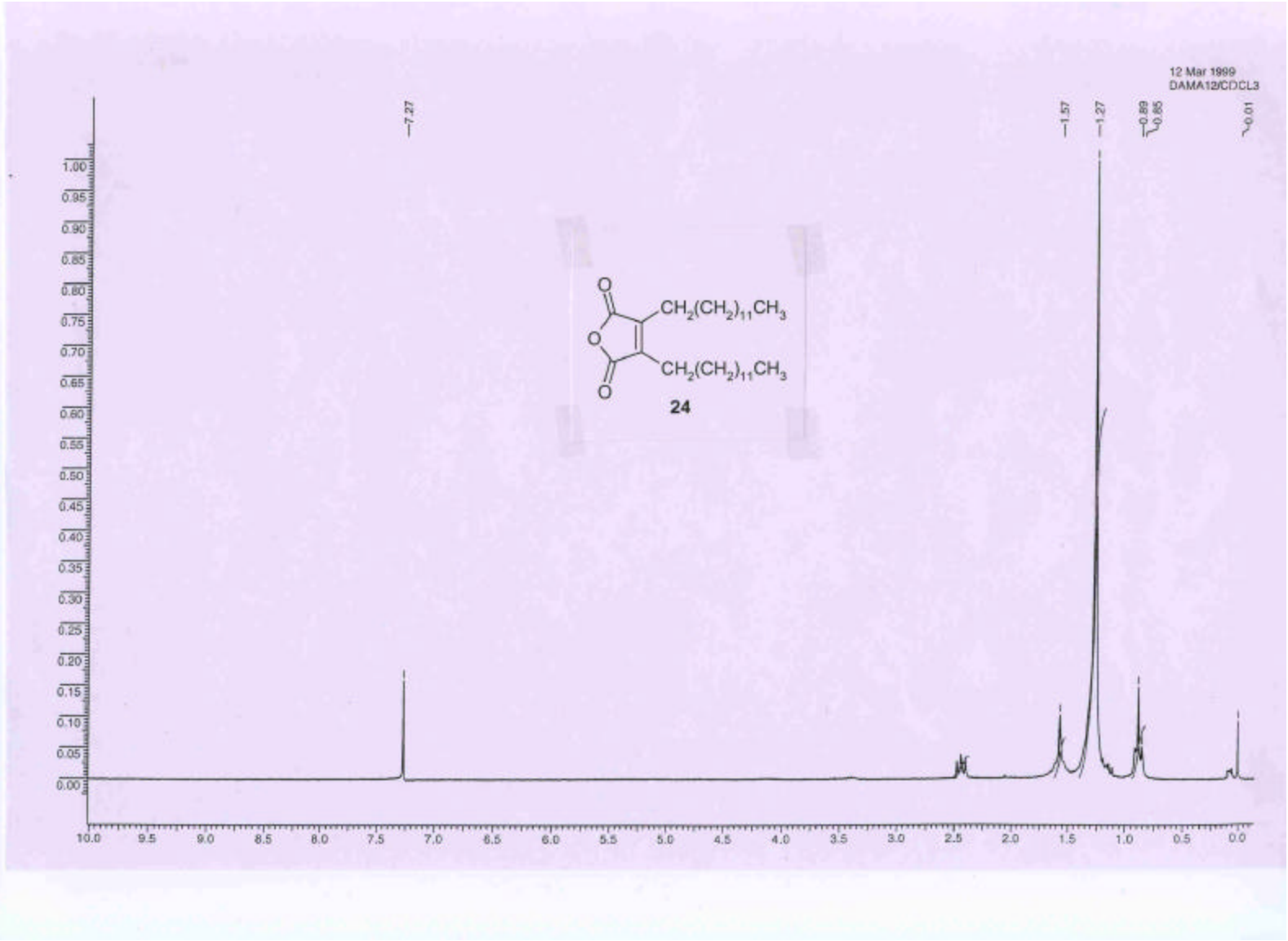

MASS SPECTRUM
03/08/99 11:44:00 + 0:40
SAMPLE: DBR OF ANIL DESHPANDE,OCS,2323(G:238)
DATA: DBR #10
BASE M/E: 51
RIC: 4980730.
100.0
50.0
1021950.
10.
Br
Br
23
M/E
50
100
150
200
250
300
66
80
95
124
131
149
159
175
203
217
235
252
266
284

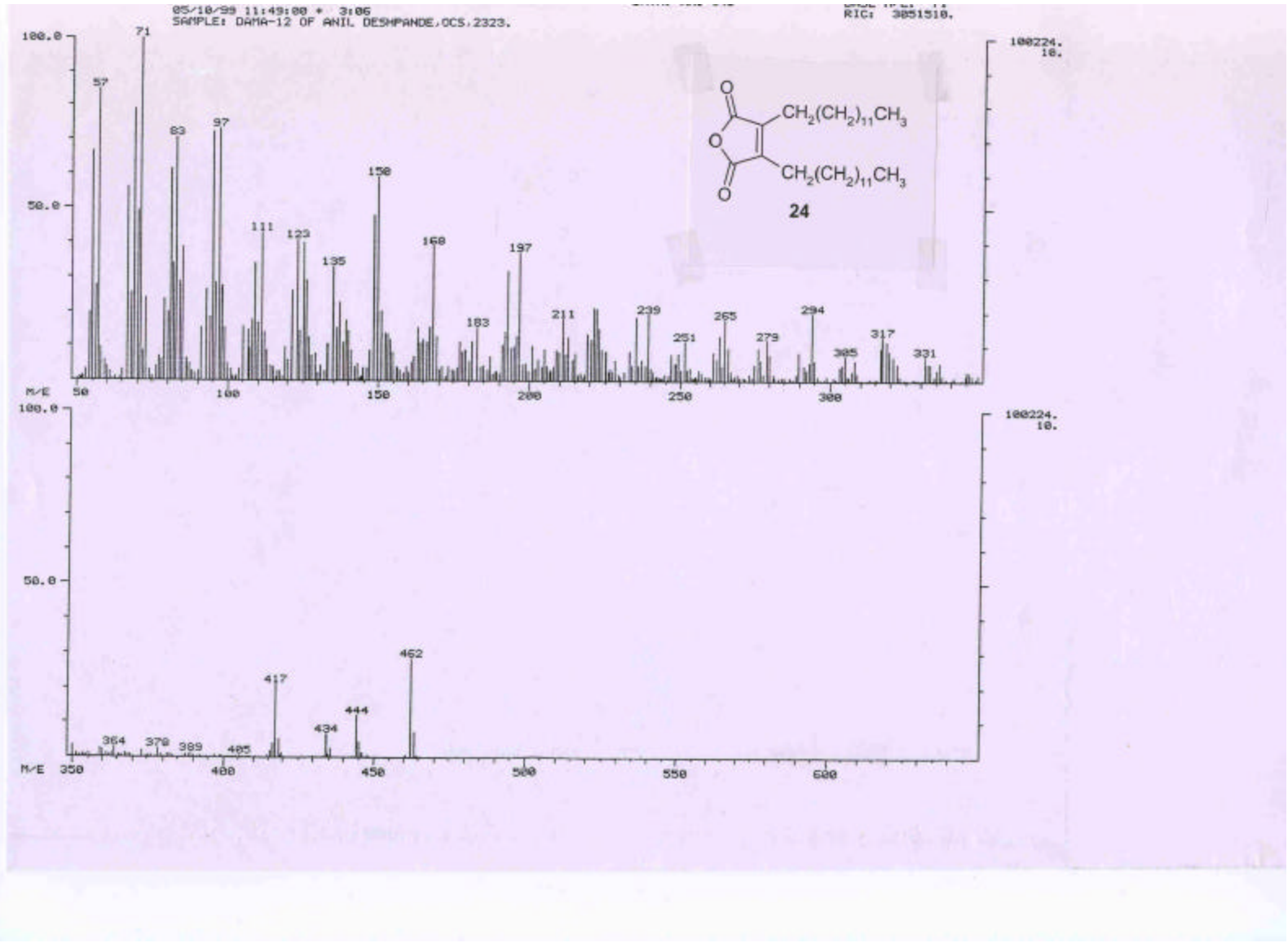

SAMPLE NO. NPA-1/ CDCL3
O
O
O
CH2(CH2)12CH3
CH3
1e
FPM

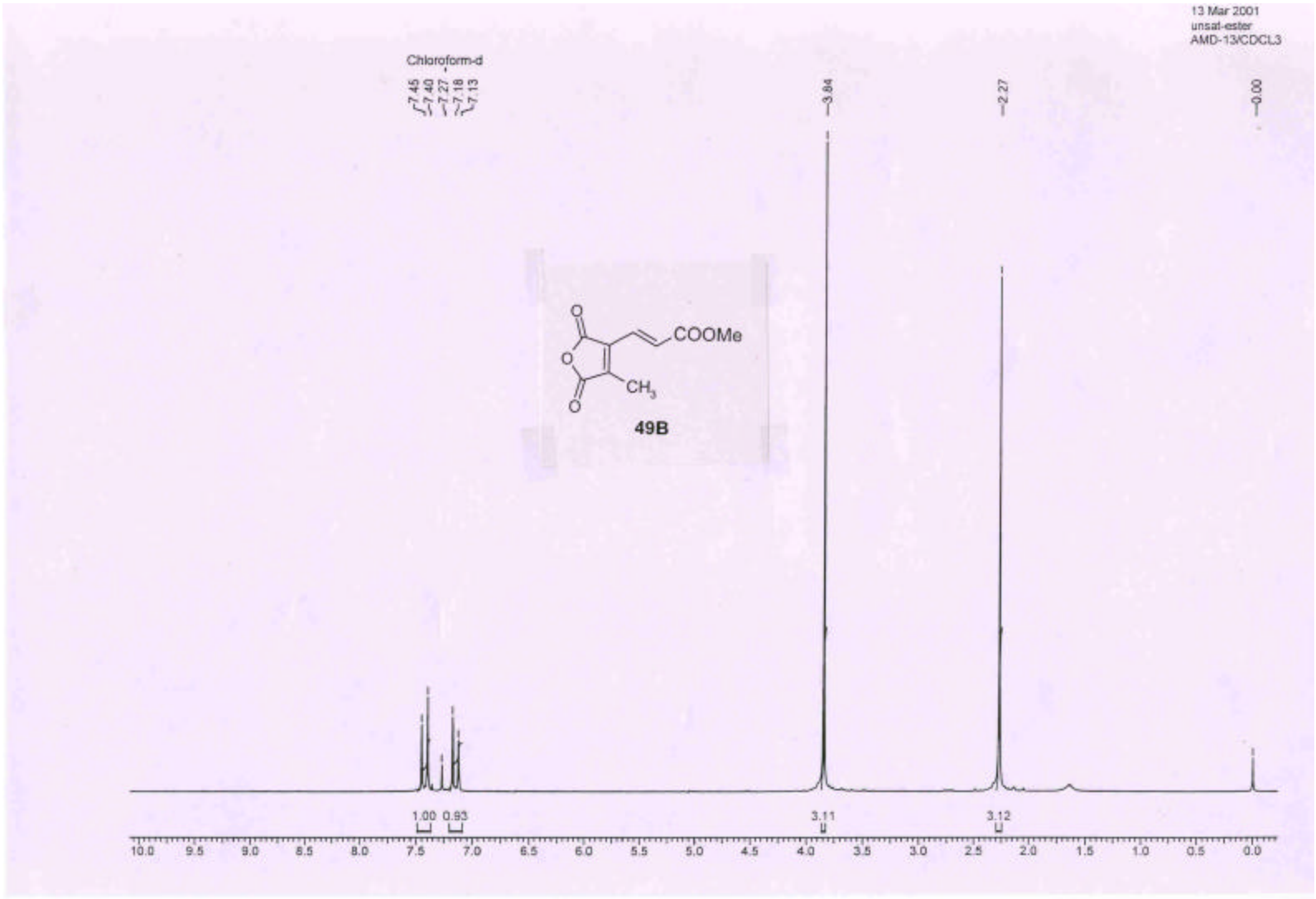


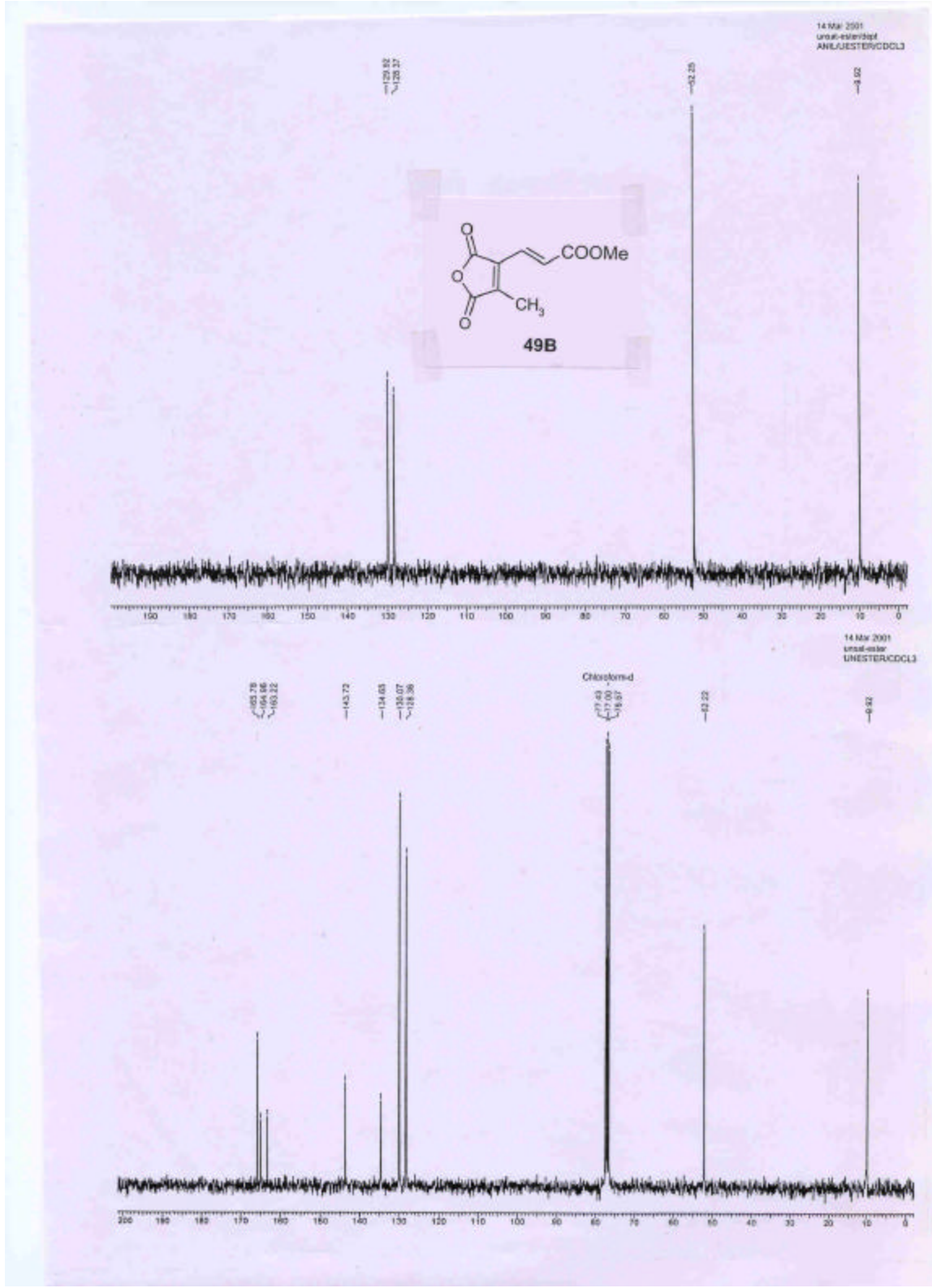
1e

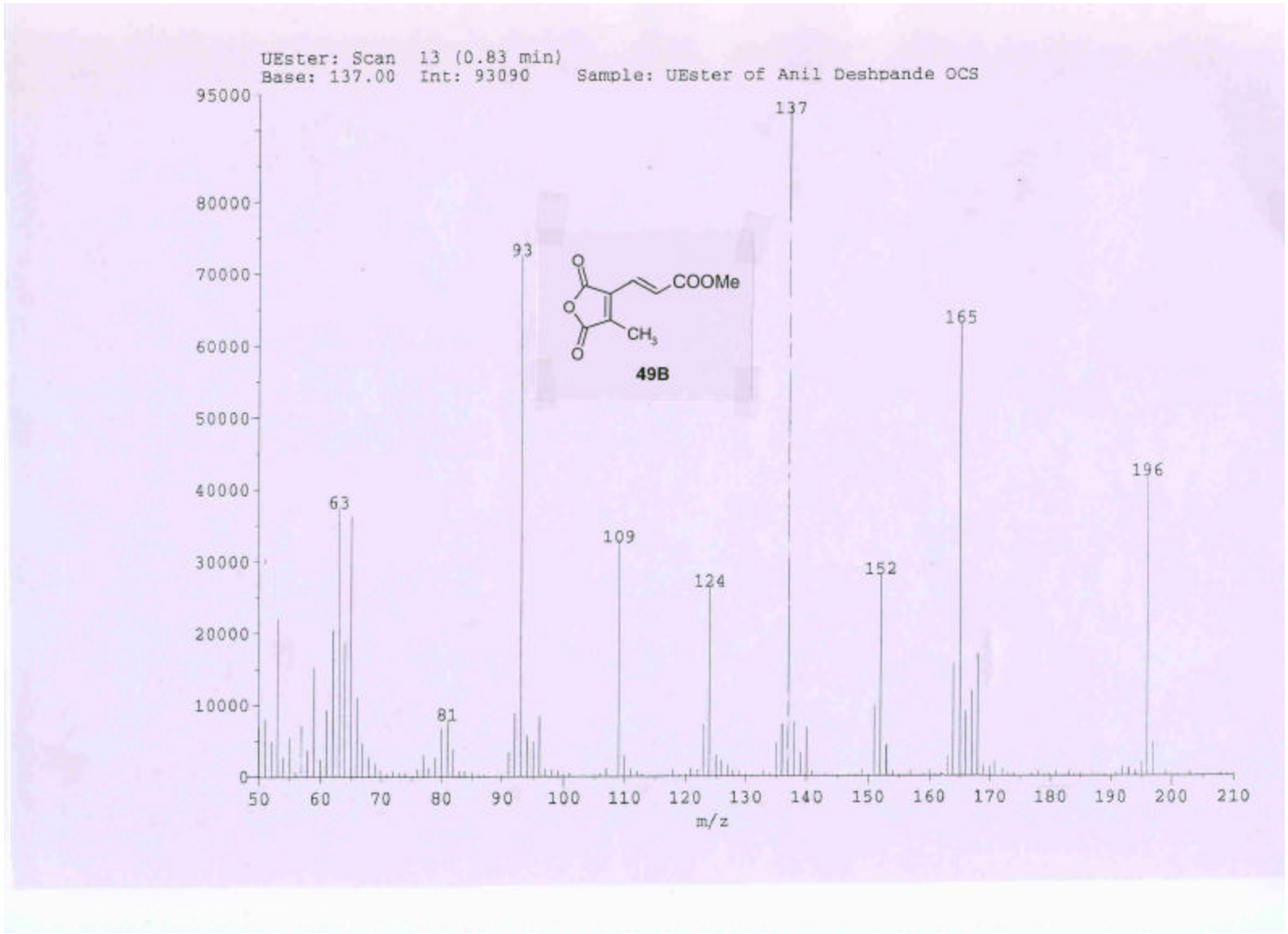

MASS SPECTRUM
12/03/96 13:40:00 + 2:10
SAMPLE: NPA-1 OF DR N.P.ARGADE,OCS.2323(C-289)
DATA: APH #32
BASE M/E: 55
RIC: 6266870.
100.0
50.0
126
69
81
91
95
105
115
135
150
158
160
178
191
205
210
225
245
263
280
250
300
315
M/E
100
150
200
250
300
403456.
10.
100% RA
M/E 126
O
O
O
CH2(CH2)12CH3
CH3
1e

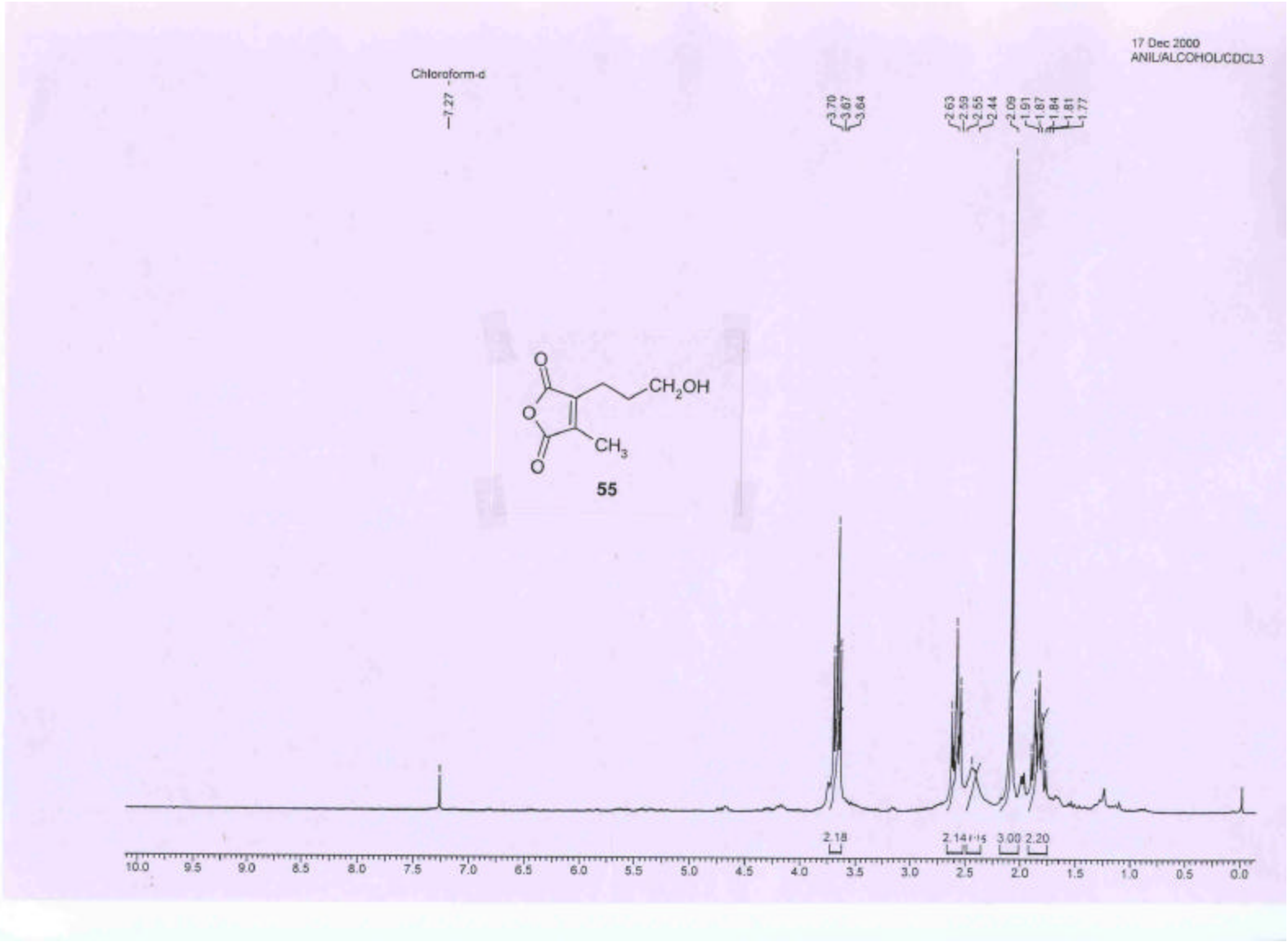

12 Mar 1999
DAMA12/CDCL3
7.27
1.57
1.27
0.89
0.85
0.01
CH2(CH2)11CH3
CH2(CH2)11CH3
24

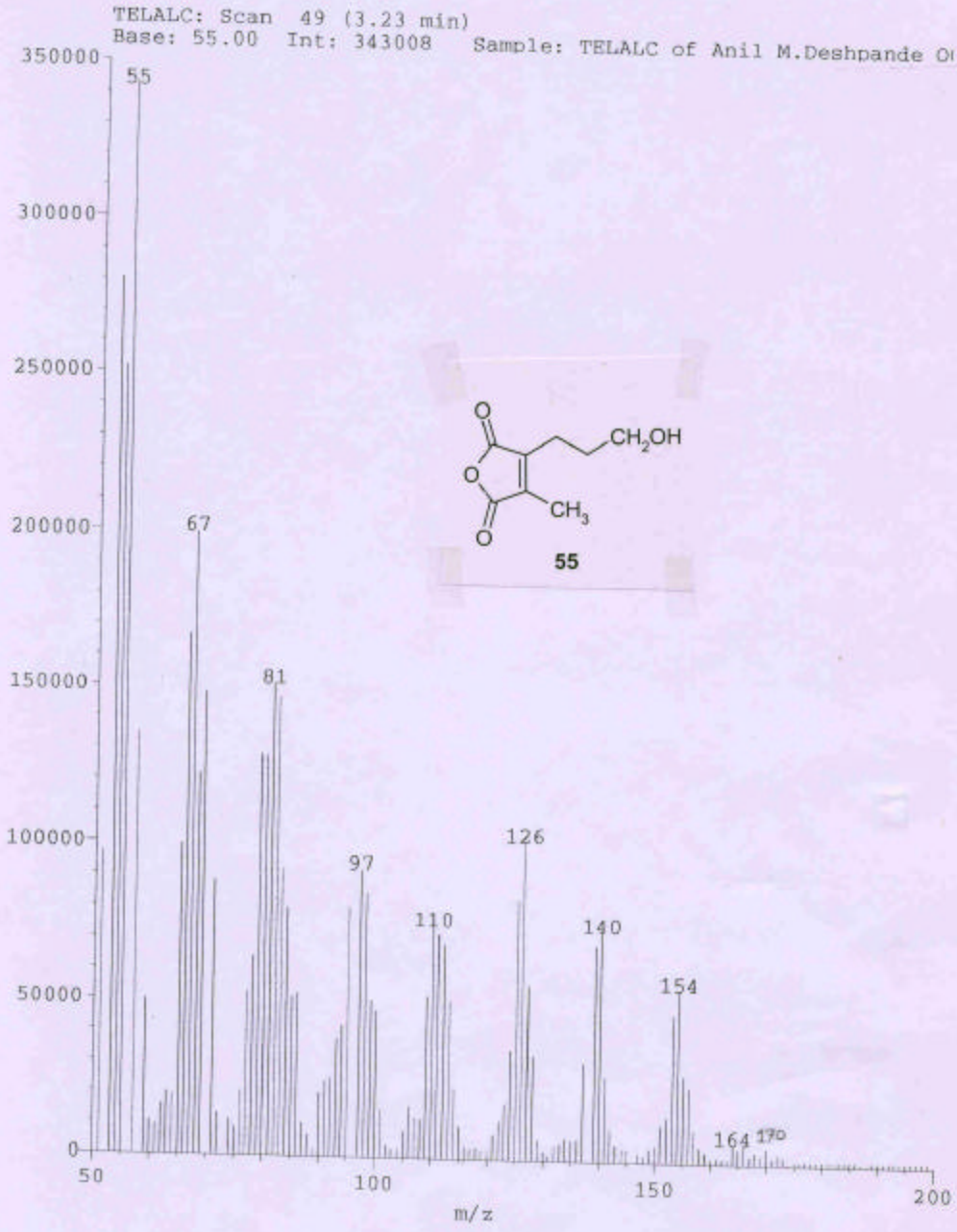

05/10/99 11:49:00 * 3:06
SAMPLE: DAMA-12 OF ANIL DESHPANDE,OCS,2323.
RIC: 3851510.
100224.
10.
100.0
50.0
57
71
83
97
111
123
135
150
168
183
197
211
239
251
265
279
294
305
317
331
M/E
50
100
150
200
250
300
CH2(CH2)11CH3
CH2(CH2)11CH3
24
462
417
444
434
364
379
389
405
350
400
450
500
550
600

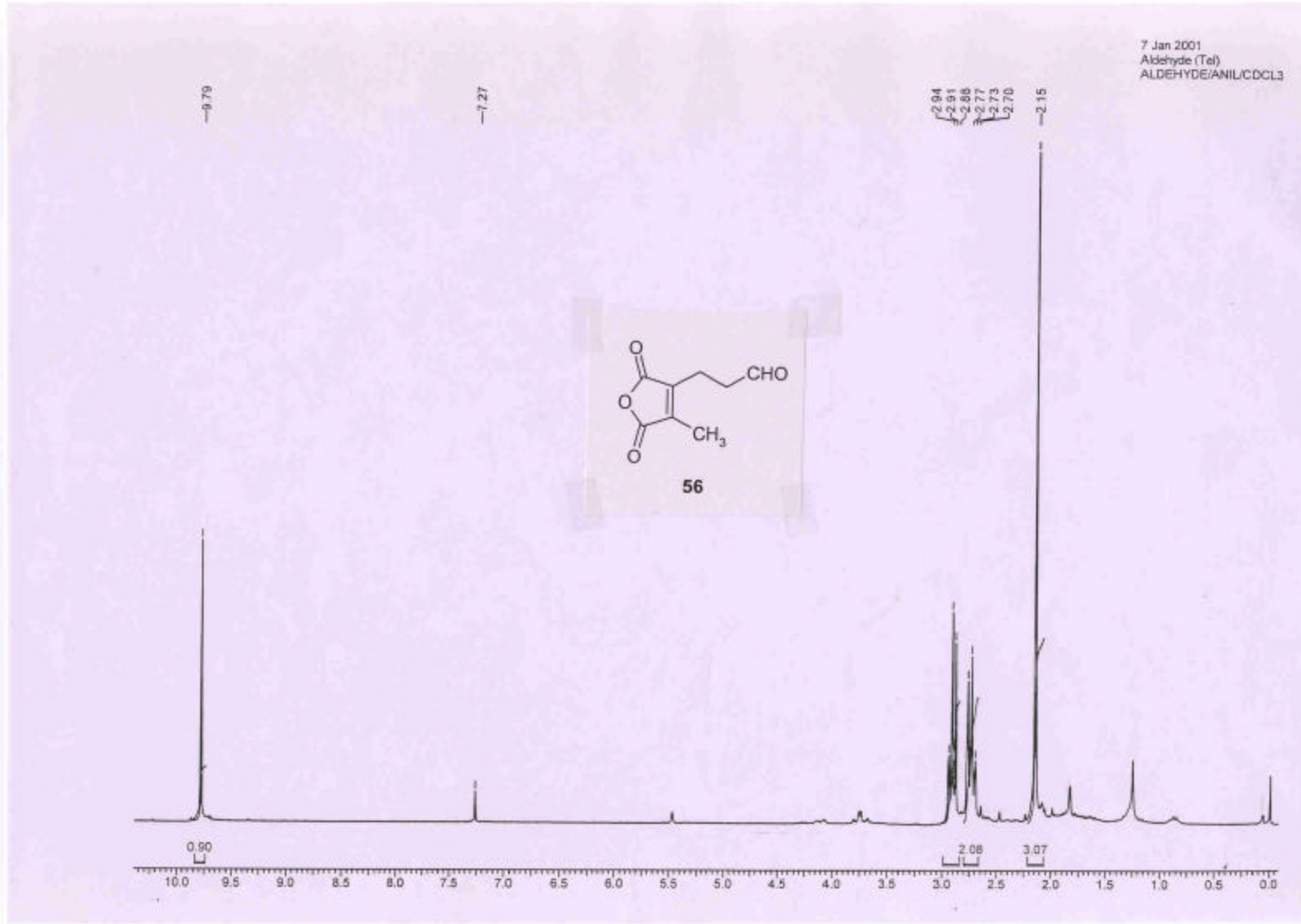

13 Mar 2001
unsat-ester
AMD-13/CDCL3
Chloroform-d
7.45
7.40
7.27
7.18
7.13
3.84
2.27
0.00
COOMe
CH3
49B
1.00
0.93
3.11
3.12
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

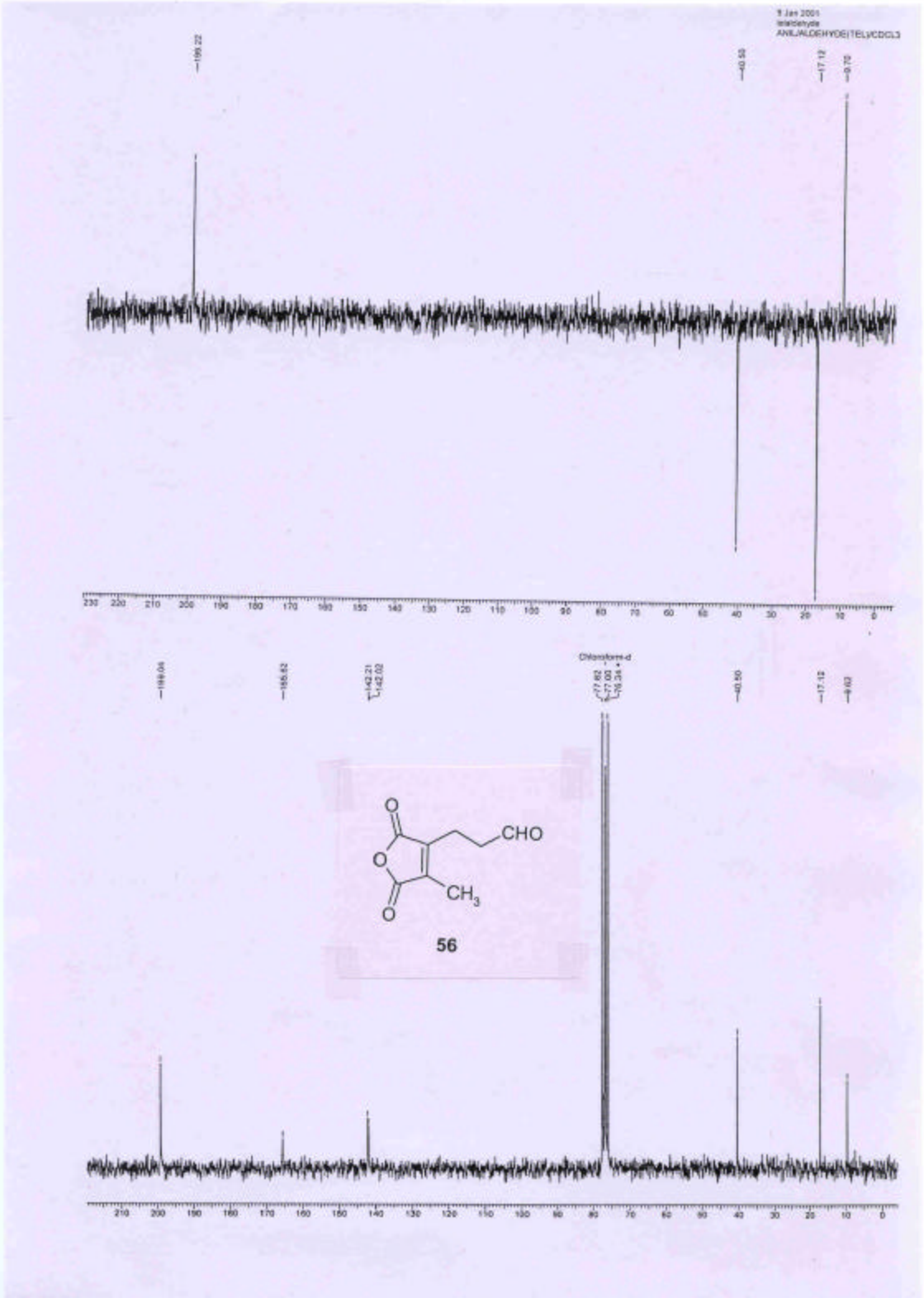

14 Mar 2001
ANILJUESTER/CDCL3
129.92
128.37
52.25
9.92
COOMe
CH3
49B
14 Mar 2001
UNESTER/CDCL3
165.78
164.66
163.22
143.72
134.63
130.07
128.36
Chloroform-d
77.43
77.00
76.57
52.22
9.92


UEster: Scan 13 (0.83 min)
Base: 137.00 Int: 93090 Sample: UEster of Anil Deshpande OCS
95000
80000
70000
60000
50000
40000
30000
20000
10000
0
137
93
165
196
63
109
124
152
81
COOMe
CH3
49B
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210
m/z

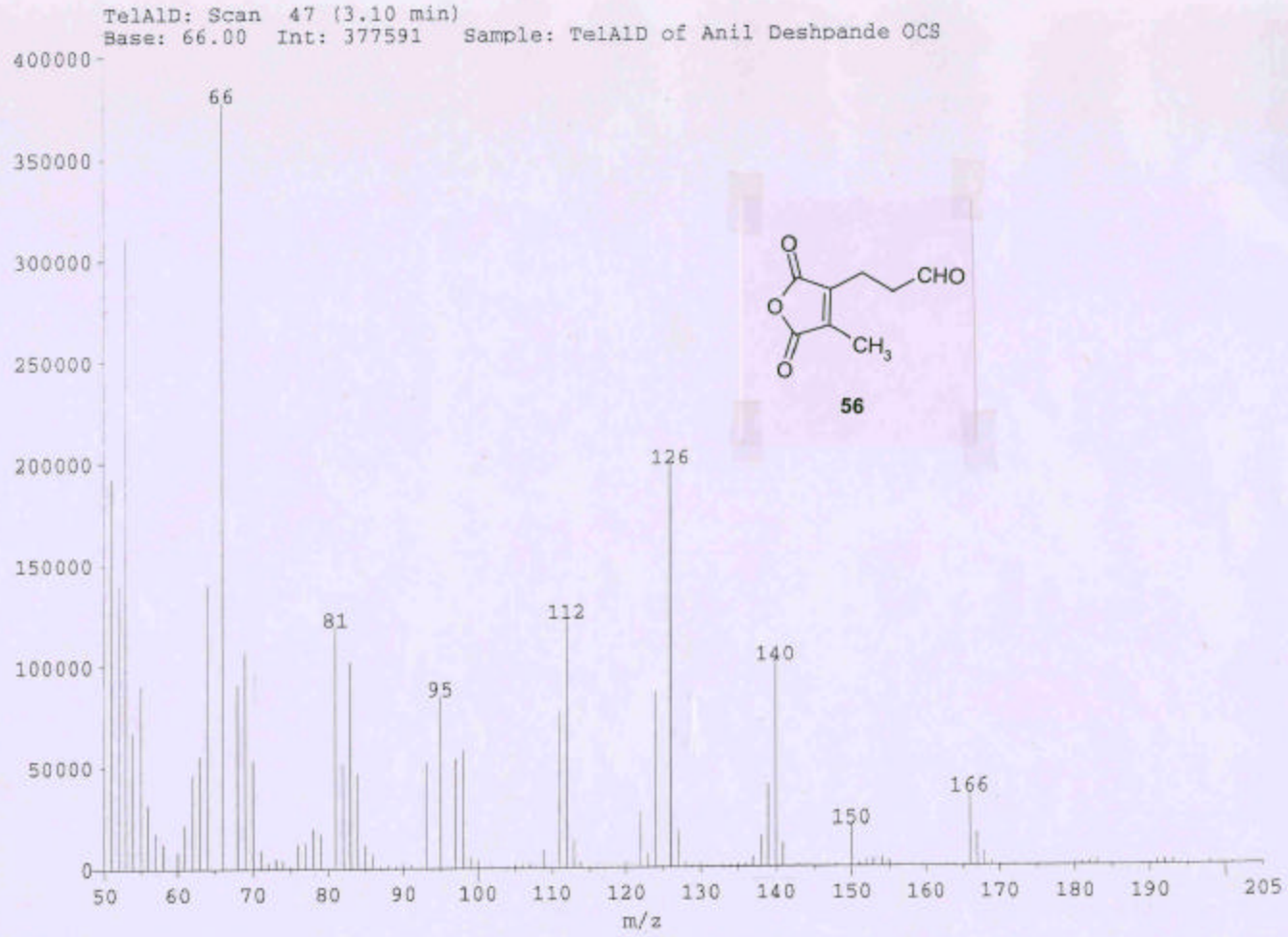

17 Dec 2000
ANIL/ALCOHOL/CDCL3
Chloroform-d
7.27
3.70
3.67
3.64
2.63
2.59
2.55
2.44
2.09
1.91
1.87
1.84
1.81
1.77
CH2OH
CH3
O
O
O
55
2.18
2.14
3.00
2.20
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

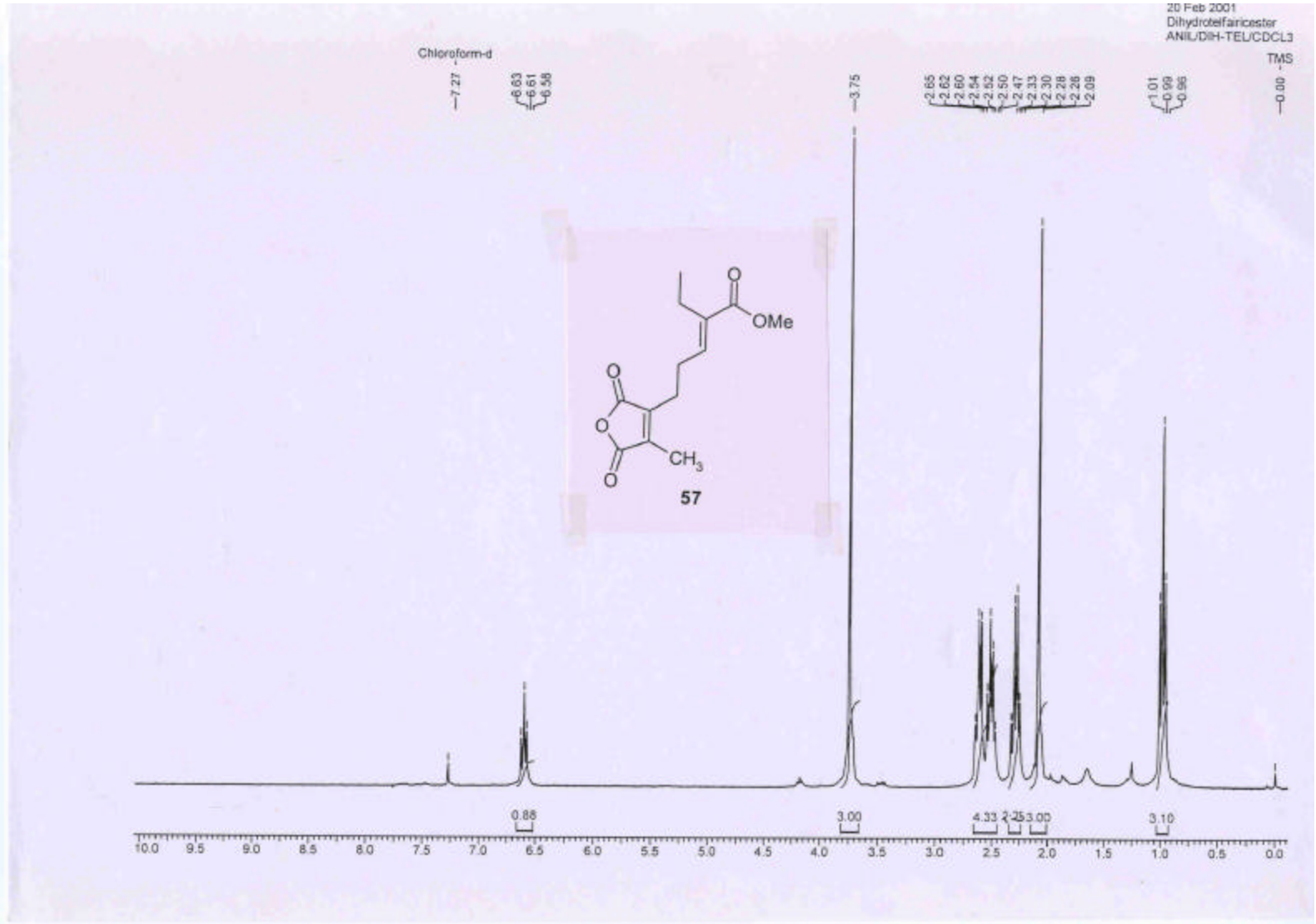

TELALC: Scan 49 (3.23 min)
Base: 55.00 Int: 343008 Sample: TELALC of Anil M.Deshpande O
350000
300000
250000
200000
150000
100000
50000
0
55
67
81
97
110
126
140
154
164
170
50
100
150
200
m/z
O
O
O
CH2OH
CH3
55

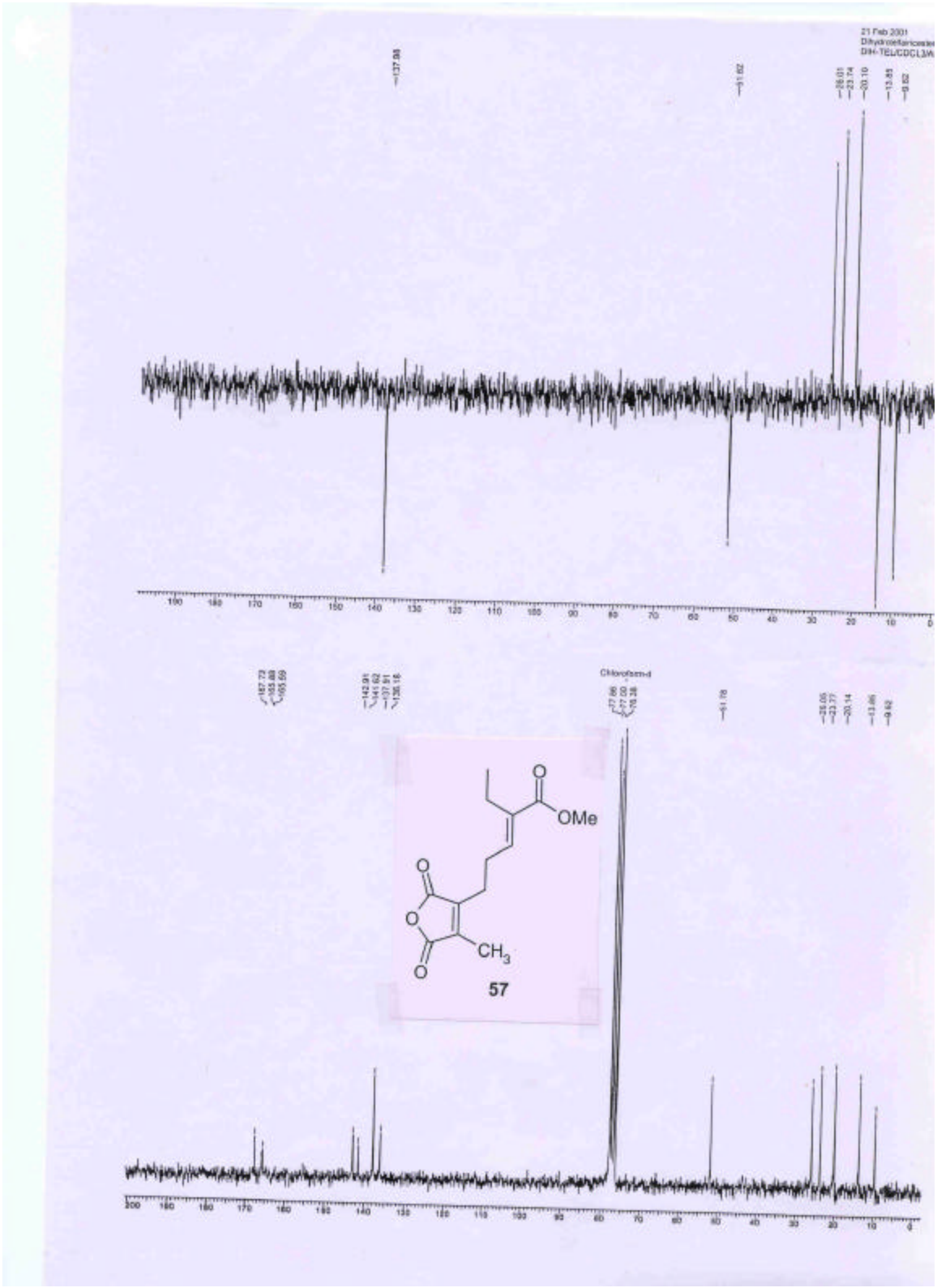

7 Jan 2001
Aldehyde (Tel)
ALDEHYDE/ANIL/CDCL3
9.79
7.27
2.94
2.91
2.88
2.77
2.73
2.70
2.15
CHO
CH3
O
O
O
56
0.90
2.08
3.07
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

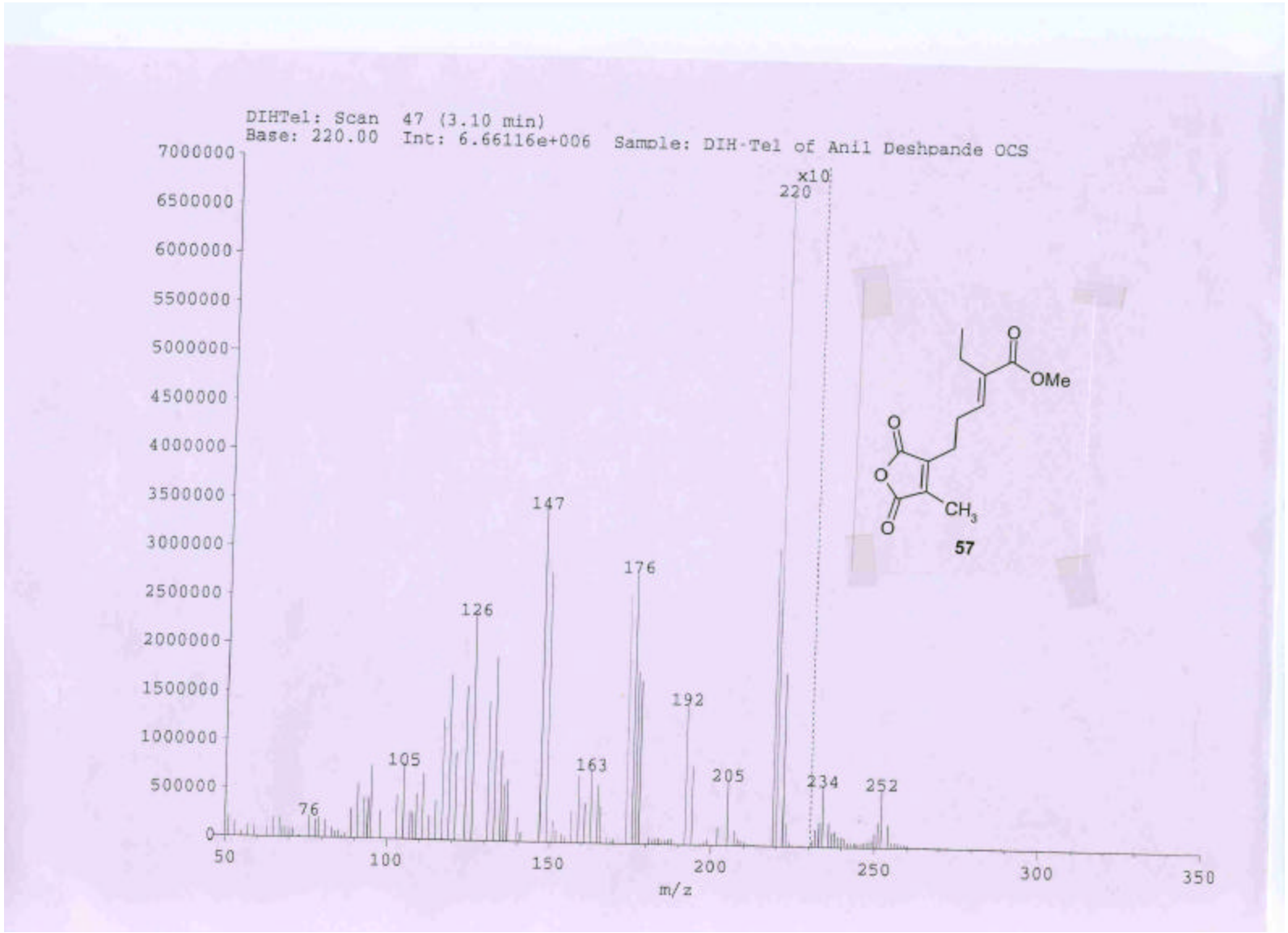

9 Jan 2001
telaldehyde
ANEJALDEHYDE(TEL)/CDCL3
199.22
40.59
17.12
9.70
Chloroform-d
77.82
77.00
76.34
40.50
17.12
9.63
CHO
O
O
O
CH3

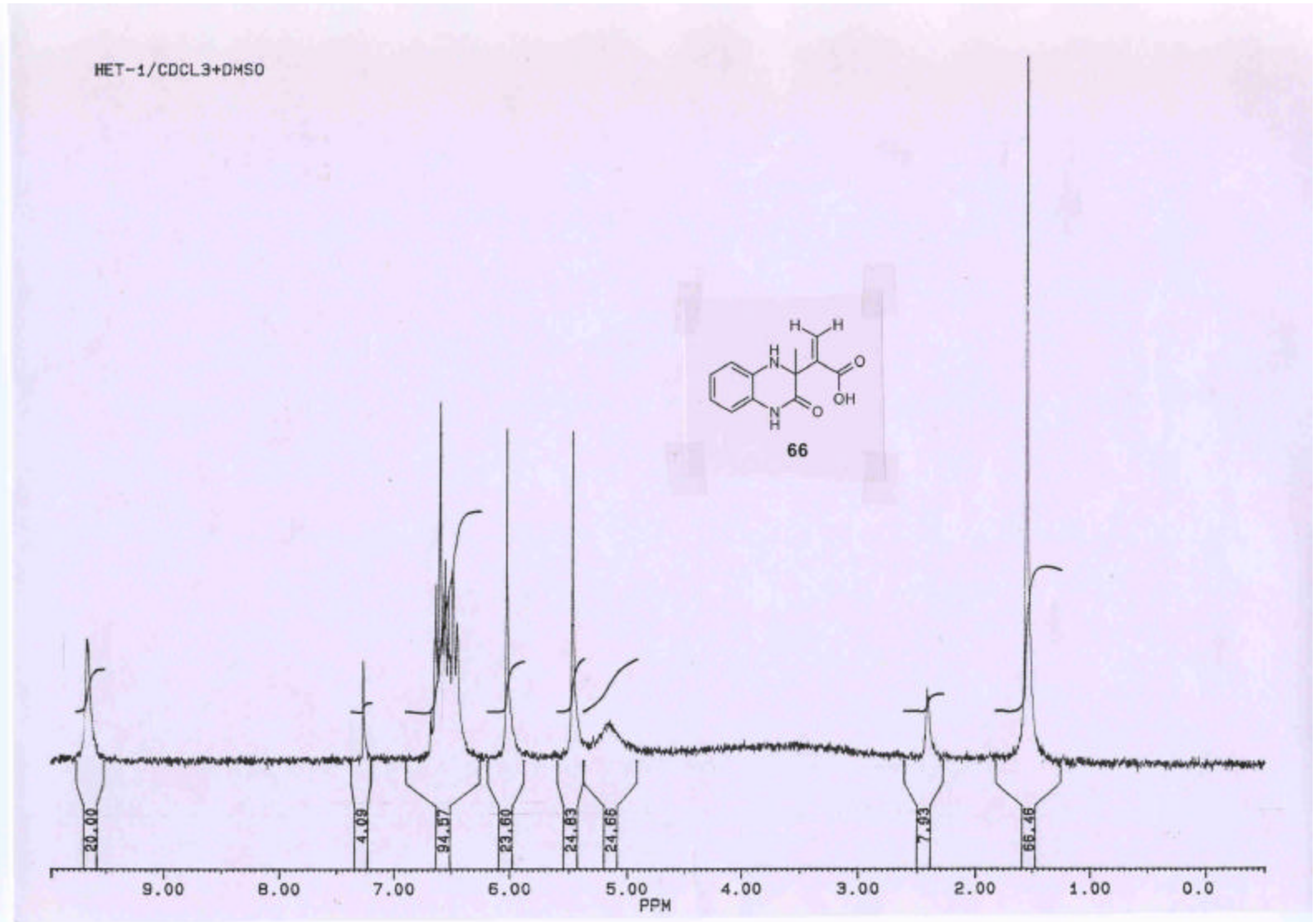
56

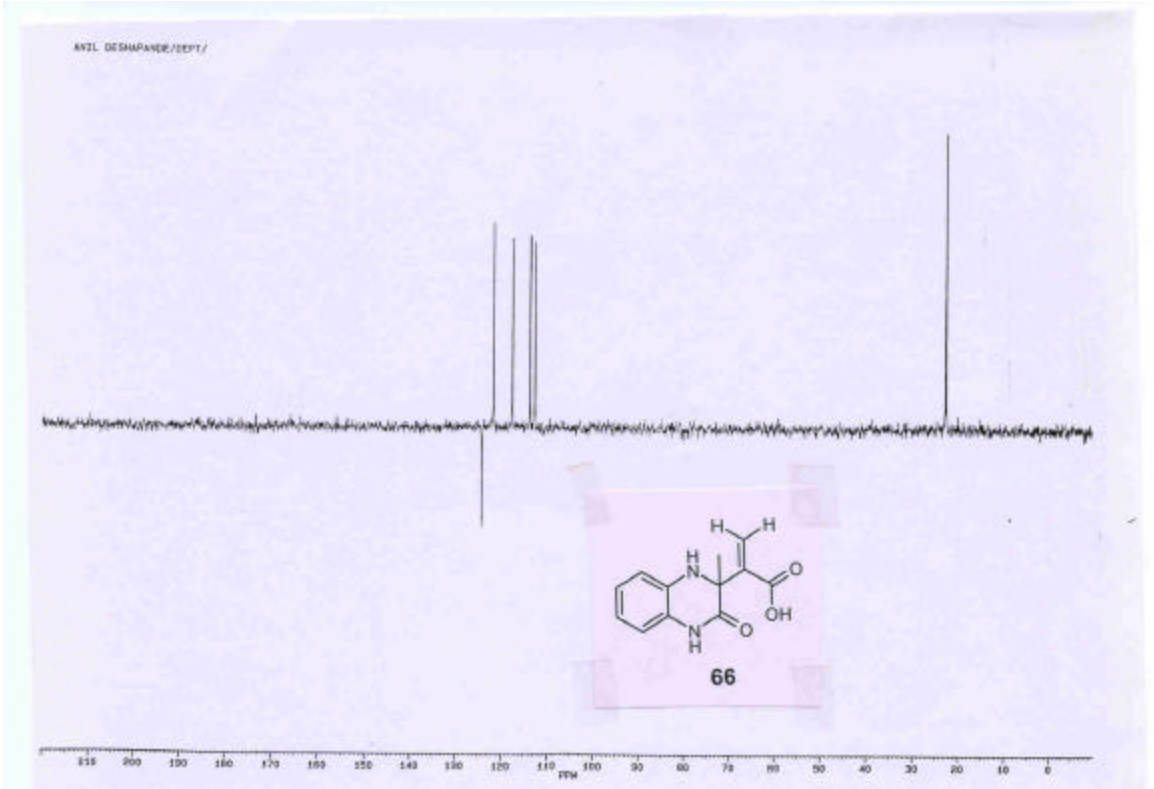

TelAlD: Scan 47 (3.10 min)
Base: 66.00 Int: 377591 Sample: TelAlD of Anil Deshpande OCS
400000
350000
300000
250000
200000
150000
100000
50000
0
66
81
95
112
126
140
150
166
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 205
m/z
O
O
O
CHO
CH3
56

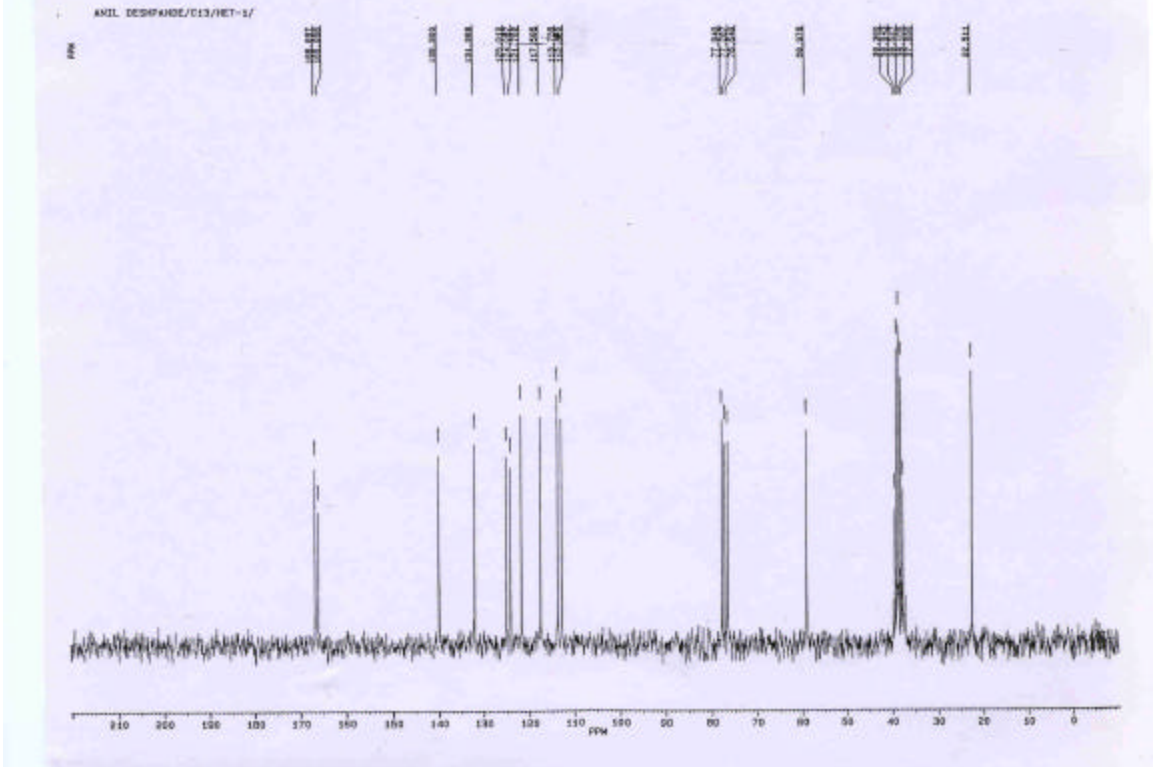

20 Feb 2001
Dihydrotelfairicester
ANIL/DIH-TEL/CDCL3
Chloroform-d
TMS
7.27
6.63
6.61
6.58
3.75
2.65
2.62
2.60
2.54
2.52
2.50
2.47
2.33
2.30
2.28
2.26
2.09
1.01
0.99
0.96
0.00
OMe
CH3
57
0.88
3.00
4.33
2.25
3.00
3.10

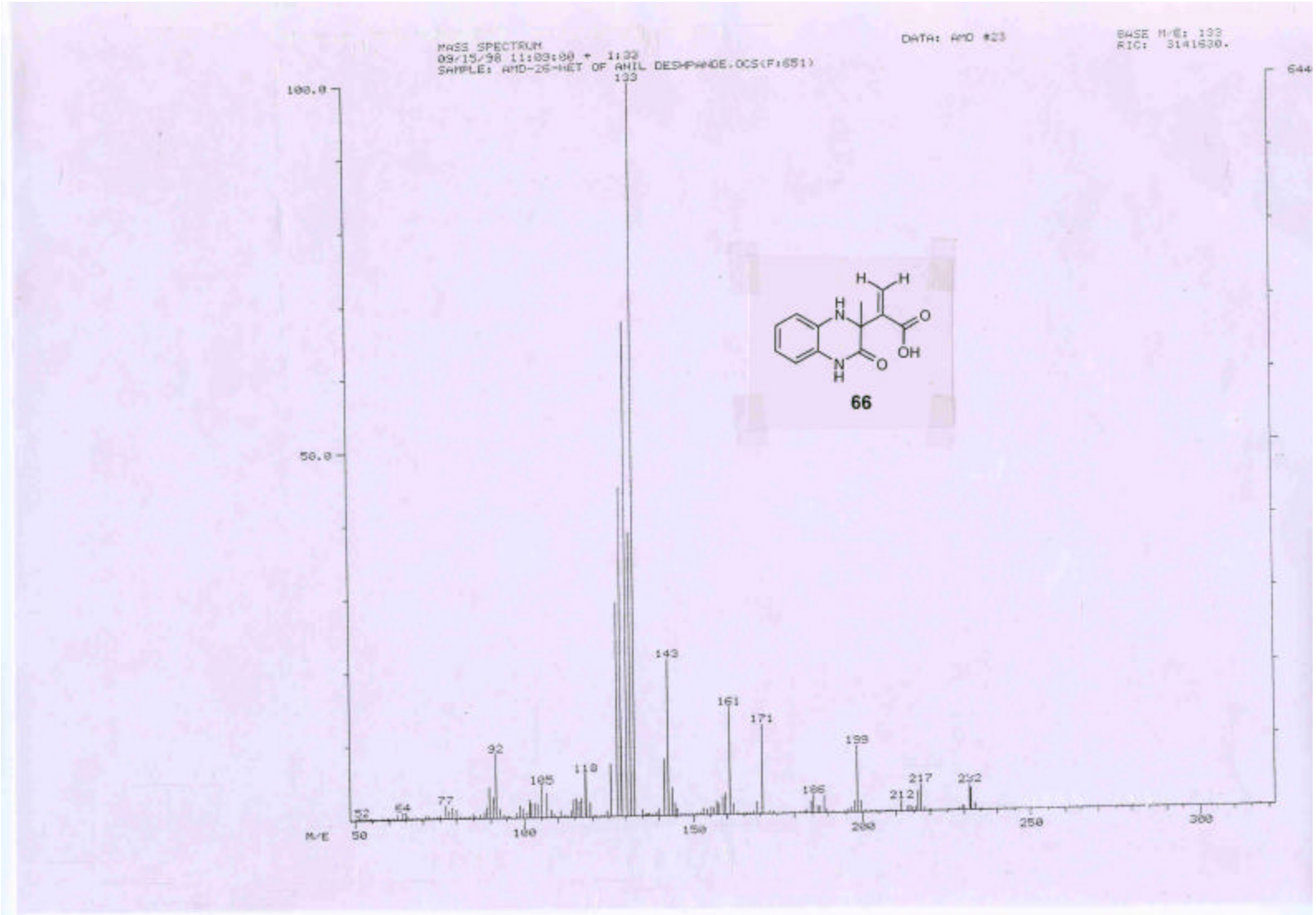

OMe
O
O
O
CH3
57


DIHTel: Scan 47 (3.10 min)
Base: 220.00 Int: 6.66116e+006 Sample: DIH-Tel of Anil Deshpande OCS
x10
220
147
176
126
192
105
163
205
234
252
76
OMe
CH3
57
m/z


HET-1/CDCL3+DMSO
H
H
H
N
O
OH
N
O
66
20.00
4.09
94.57
23.60
24.63
24.66
7.93
66.48
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM


ANIL DESHAPANDE/DEPT/
H
H
N
H
O
OH
N
H
O
66
PPM


ANIL DESHPANDE/C13/MET-1/
PPM

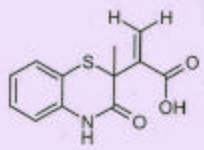

MASS SPECTRUM
09/15/98 11:03:00 * 1:32
SAMPLE: AMD-26-MET OF AHIL DESMPHNDE.OCS(F:651)
DATA: AMD #23
BASE M/E: 133
RIC: 3141630.
100.0
50.0
644
133
143
161
171
199
92
105
119
186
212
217
242
32
64
77
M/E
50
100
150
200
250
300
H
H
N
H
O
OH
N
H
O

66

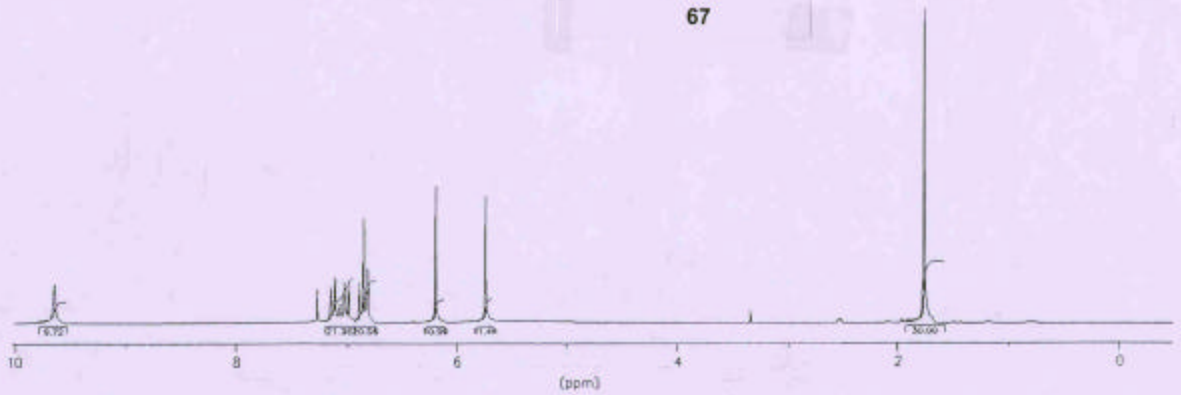
None

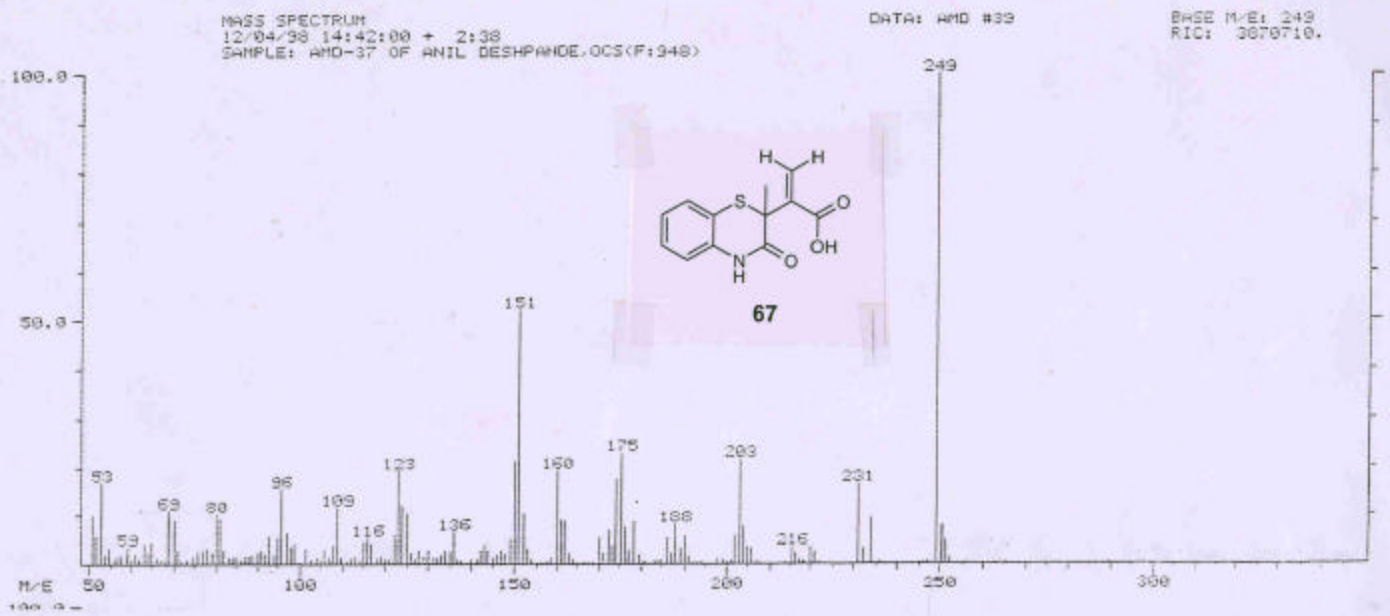
File: D:\NMR\AGS\ANIL\001001.1R

Date: 18.12.1998 Time: 9:19



67




MASS SPECTRUM
12/04/98 14:42:00 + 2:38
SAMPLE: AMD-37 OF ANIL DESHPANDE.OCS(F:948)
DATA: AMD #39
BASE M/E: 249
RIC: 3070710.
100.0
50.0
M/E
50
100
150
200
250
300
249
151
53
59
69
80
96
109
116
123
136
160
175
188
203
215
231
H
H
S
O
OH
N
H
O
67

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CHAPTER 3

SYNTHESIS AND SCREENING OF NAPHTHALENE SUBSTITUTED CHALCONES AS LEUKOTRIENE B_4 INHIBITORS: A COMBINATORIAL APPROACH

INTRODUCTION

3.1 A COMBINATORIAL APPROACH

Recent advances in the new drug discovery such as the accessibility of multiple targets for e.g. ~70 targets in case of malaria and high throughput screening (HTS) has necessitated the availability of large number of organic compounds with structural diversity. Conventional "synthesis-purification-testing" approach for the preparation of organic compounds will not be able to cope up with this increasing demand in time and thus will not be able to cater to the exponentially increasing need of futuristic drug industry. Combinatorial chemistry is one of the attempts to solve this problem to some extent. In the beginning it was apparently developing as a science but today it has developed to such an extent that it has boiled down to a operational technique practiced routinely in the research laboratories and industry for the variety of organic synthetic reactions and process optimization as is evident from the plethora of publications, books, monographs and even a special journal.[1] However one must bear in mind that it is not an alternative to the novel hardcore organic synthesis but may play a complimentary role for the expediting the process in order to suit the rapidly growing modern drug discovery program.

Combinatorial chemistry is a modern technique for the synthesis of a very large number of structurally diverse molecules for biological evaluation in a time and resource effective manner. One strategy at the heart of combinatorial chemistry is the concept of combining of readily available reactive chemical building blocks with potentially all possible combinations and permutations to generate the diversity. The Nobel laureate, R. B. Merrifield,[2] first popularized this idea, wherein the solid phase peptide synthesis (SPPS) concept was broadened to incorporate the synthesis of multiple compounds at a time. It

took a decade to move from SPPS to solid-phase organic synthesis (SPOS), another decade to move to libraries of peptides[3] and oligonucleotides[4] and yet another decade i. e. in 1990, to achieve organic molecules for e.g. heterocyclic compounds[5] that are truly useful for the drug discovery program. Finding a novel lead and its optimization is a challenging process in drug discovery program. Synthesis of compounds using combinatorial library[6] concept has made significant impact on drug discovery process and currently is of enormous interest within both the synthetic and medicinal chemist communities.

Broadly the chemistry adopted in synthesizing the combinatorial libraries fall in to two classes (i) solid phase supported methodologies (ii) solution phase chemistry. The main advantage of the solution phase synthesis is its wide acceptability for the variety of substrates, reagents and practically limitless reaction conditions.[7,8] However the purification after each step in the multistep synthesis and removal of excess reagents/ solvents are the stumbling blocks in the development of this otherwise simple methodology. Therefore in the last few years significant efforts have been made in adopting organic reactions to work well on the solid phase.[9] Although it suffers from two major drawbacks i.e. additional steps of attaching and detaching the molecule from the solid support and the limitations on the choice of reaction conditions due to the inherent structural chemical properties of the solid phase, with easy removal of excess reagents/solvents, no purification at each step and amongst all the ease of automation, the solid phase synthesis has really scored over the solution one. It is really the last factor i.e. automization has revised the very concept of manual synthesis. Today one can synthesize thousands of structurally diverse compounds in a week time without any personal attention

throughout the course of the reaction as well as workup, thus saving money and human resourses.

Though these two methods have their inherent advantages and disadvantages one has to born in mind that almost in all cases the reaction is carried out and optimized first in solution phase and then it is extended to solid phase. Thus the solution and solid phase methods are sequentially used and the final choice is made depending on the desired structural diversity and essential reaction conditions.

Similarly two approaches for the construction and testing of compound libraries are generally used (i) the preparation and testing of compounds as mixtures or pools, which necessarily involves decoding or tagging protocol to determine the structure of the active component, (ii) the preparation and testing of compounds as discrete entities (parallel synthesis). Mixtures of compounds are easy to prepare for screening than individual compounds. Indeed, number of pharmaceutical companies routinely create artificial libraries by mixing the pure compounds in their drug discovery screening program as a mean of increasing assay throughput and controlling cost. However it suffers from major drawback that extended time and human resources are required for the purification, identification and characterization of the lead compound, which has to be resynthesized again for further testing. As against this, parallel synthesis[8] involves individual compound synthesis with structural diversity in an array of assembly. Here characterization is much easier because of the predetermined format of the reactants involved. Critical choice amongst these two methods depends on the end use of compounds. The former method is more suitable for the biological screening minimizing the number of entities to be tested,

whereas the later is widely used for the process optimization. However this is just a thumb rule and the selection of this methodology is generally left to the researchers.

There are basically two methods used for the identification of the active compounds in combinatorial chemistry. First one is tagging technique either chemical or readiofrequency, involves linking an identifier to each molecule present in the library. Deconvolution tool is a second method of analysis wherein library is portioned into a series of sub-libraries that are organized in such a way as to allow identification of an active component without any tagging. These deconvolution methods are subdivided into three major group (i) Iterative deconvolution (ii) Positional scanning (iii) Orthogonal libraries.

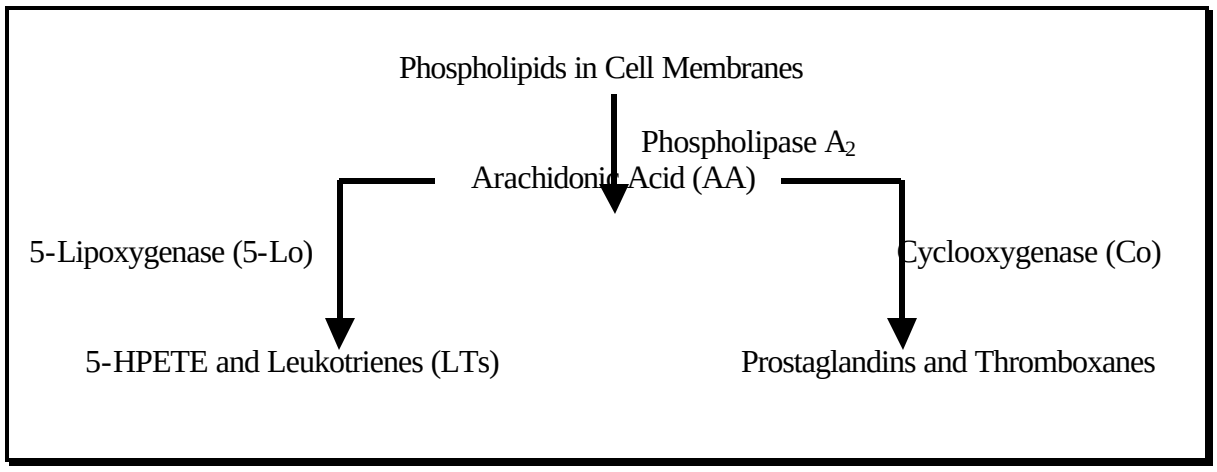
It is really a difficult task to select appropriate combination of particular methodologies i.e. solid or solution phase, mixture or as individual compound for the synthesis of libraries. It mainly depends on the structural diversity of library, nature of the reagents, reaction conditions to be employed, characterization/deconvolution methods, suitability of bioassay and above all the basic reactivity of the individual components. Scientific wisdom coupled with judicious manipulation of these variable factors finally leads to the successful creation of the meaningful libraries. Therefore taking into consideration the above factors, it was planned to synthesize a solution phase combinatorial library of naphthalene substituted chalcones and its biological testing as 5-lipoxygenase (5-Lo) inhibitors, which plays a vital role in the treatment of various inflammatory conditions.

3.2 ANTI-INFLAMMATORY AGENTS

Current approaches for controlling the lipid mediators [i.e. prostaglandins (PGs) and leukotrienes (LTs)] of inflammation (**Fig**. **1**) include steroids and non-steroidal anti-inflammatory drugs (NSAIDs). Steroids are broadly effective for controlling the

inflammation because they non-selectively block all inflammatory pathways and as a result they suffer from the side effects such as immunosuppression, muscle and skin atrophy and growth retardation. NSAIDs in clinical use mainly block the cyclooxygenase (Co) pathway in arachidonic acid (AA) cascade (**Fig**. 1) inhibiting the synthesis of inflammatory mediators such as prostaglandins (PGs), thromboxanes (TXAs) and prostacyclins. However, these NSAIDs have no utility in the treatment of many inflammatory conditions like asthama, dermatitis and also give rise to severe side effects such as ulcer, gastric distress, edema due to sodium and calcium retention and other general side effect like nausea, vomiting etc. Another inflammatory process in the AA cascade known as lipoxygenase (Lo) pathway has been well established and presents an exciting target in the

Fig. 1: Arachidonic Acid Cascade



treatment of many inflammatory conditions. In last two decades there was an intensive search[10] in the area of LTs biosynthesis, its mechanism of action, biological role, and LTs receptors (discussed briefly in the coming sections). It is becoming increasingly evident that LTs, the metabolic products of 5-Lo pathway, play a key role as the major mediators of inflammatory reactions and hypersensitivity in humans.[11] LTs elicit a variety of biological

responses such as smooth muscle constriction,[12a,12b] increased vascular permeability[12c] and leukocyte chemotaxis[12d] and have a pathophysiological role in variety of inflammatory diseases[13] such as asthama, rheumatoid arthritis, allergic rhenitis, inflammatory bowel disease, psoriasis and ulcerative colitis.

3.2.1 Arachidonic Acid Cascade

Arachidonic acid (AA), a polyunsaturated twenty-carbon chain fatty acid, plays a central role in the biological control system. AA is transformed into potent oxygenated mediators with far ranging effects. Usually in the biological system, concentration of free AA is very low and it is present as an esterified form of phospholipids of cell membrane or as an ester linkage of other complex lipids. Immunological and non-immunological stimuli can release AA from membrane phospholipids by activating enzyme phospholipase A_2. Free AA is then metabolized by two enzymes i.e. cyclooxygenase (Co) and lipoxygenase (Lo) to the oxygenated derivatives (**Fig. 1**) as prostaglandins (PGs), prostacyclins, thromboxanes (TXs) and leukotrienes (LTs), collectively called as ecosanoids.[14]

PGs and TXAs are identified and characterized as inflammatory mediators synthesized from AA by enzyme Co. The second major pathway of AA metabolism has been realized and understood in which AA is converted into the pro-inflammatory mediators LTs. The name leukotriene has been derived from the original discovery of these substances in polymorphonuclear leukocytes (i.e. white blood cells) and presence of a conjugated triene system as a common structural feature. Various members of this family are designated alphabetically as LTA_4, LTB_4, LTC_4 and several others in a similar fashion (see **Scheme 1**).

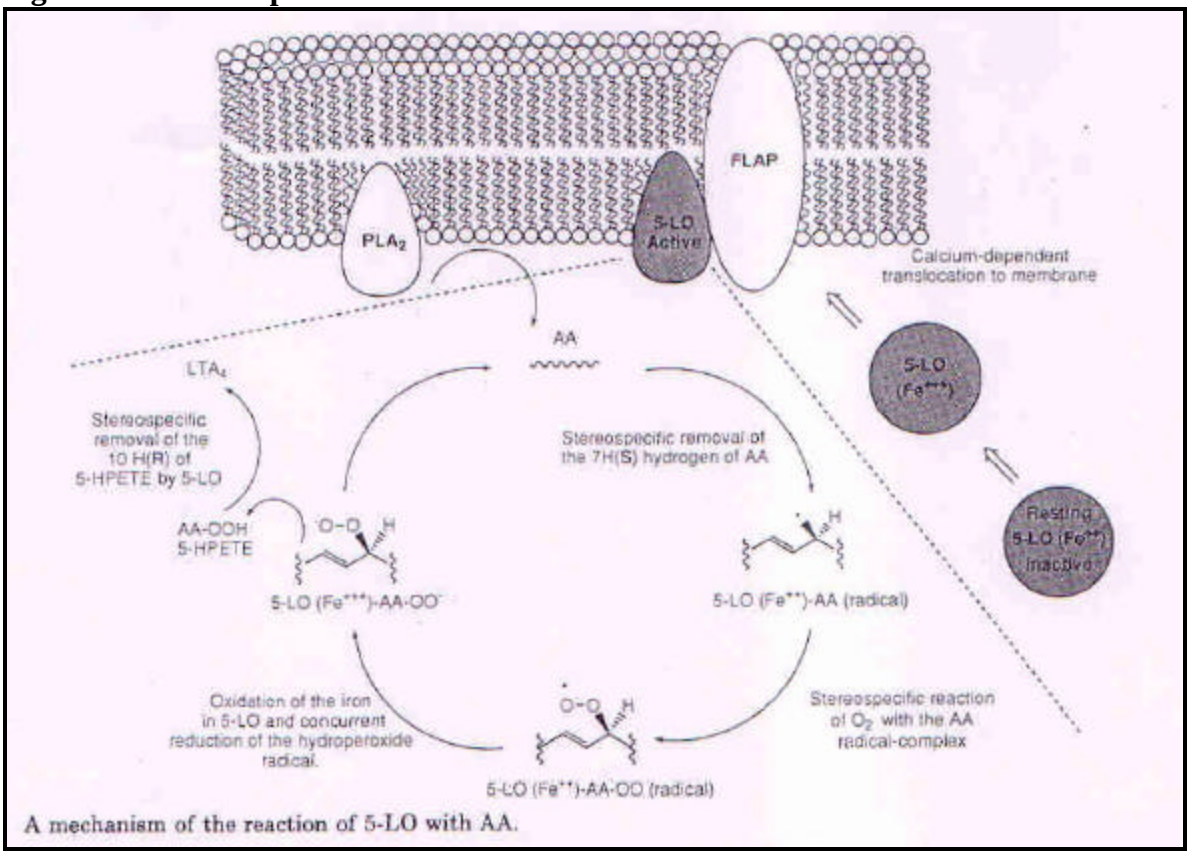
3.2.2 Mechanism of Action of 5-Lo and Biosynthesis of Leukotrienes (LTs)

More recently studies on the metabolism of AA in leukocytes lead to the recognition of biosynthetic pathway[10,11a] of Lo (**scheme 1**). The first step in the generation of LTs is catalyzed by calcium and ATP dependent enzyme 5-Lipooxygenase (5-Lo).[10]

5-Lo, is a 78 K da protein or enzyme found mainly in the cells of myeloid origin where it normally resides in the cytosol. The characterization and kinetic studies with 5-Lo is often difficult, because of self-inactivation and complex cofactor requirement. The enzyme 5-Lo is fairly unstable, possessing half-life of 45 min. at 37 °C. The structure determination has not been accomplished for 5-Lo but it is known to contain non-heme iron at its active site.[15] In 1989, for the first time reviews on the properties of 5-Lo as a general class[16a] and in mammalians in particular[16b] were published followed by the detailed discussion on the mechanisms of 5-Lo.[17] Musser *et al*[11a] have summarized a large amount of data on 5-Lo and presented its plausible mechanism of action as shown in **Fig. 2**. The 5-Lo enzyme is postulated to be in dormant ferrous state (Fe^{2+}), which is found in the cytosol and spatially removed by AA, the substrate found in cell membrane. Upon activation by hydroperoxides, adenosine triphosphate (ATP) and calcium ion (Ca^{2+}), the 5-Lo enzyme is converted to the active ferric (Fe^{3+}) form and this form translocates, possibly due to a change from the hydrophilic conformation into hydrophobic conformation. After translocation of the enzyme to cell membrane, where it docks with transmembrane protein called as five lipoxygenase activating protein (FLAP). Once docked to the cell membrane, it acts on the substrate AA which is then oxidized in a stereoselective fashion via free radical process. First there is removal of 7H (*S*) hydrogen of AA, which is then followed by the reaction of oxygen (O_2) with the AA complex. 5-Lo catalyses the insertion of oxygen at

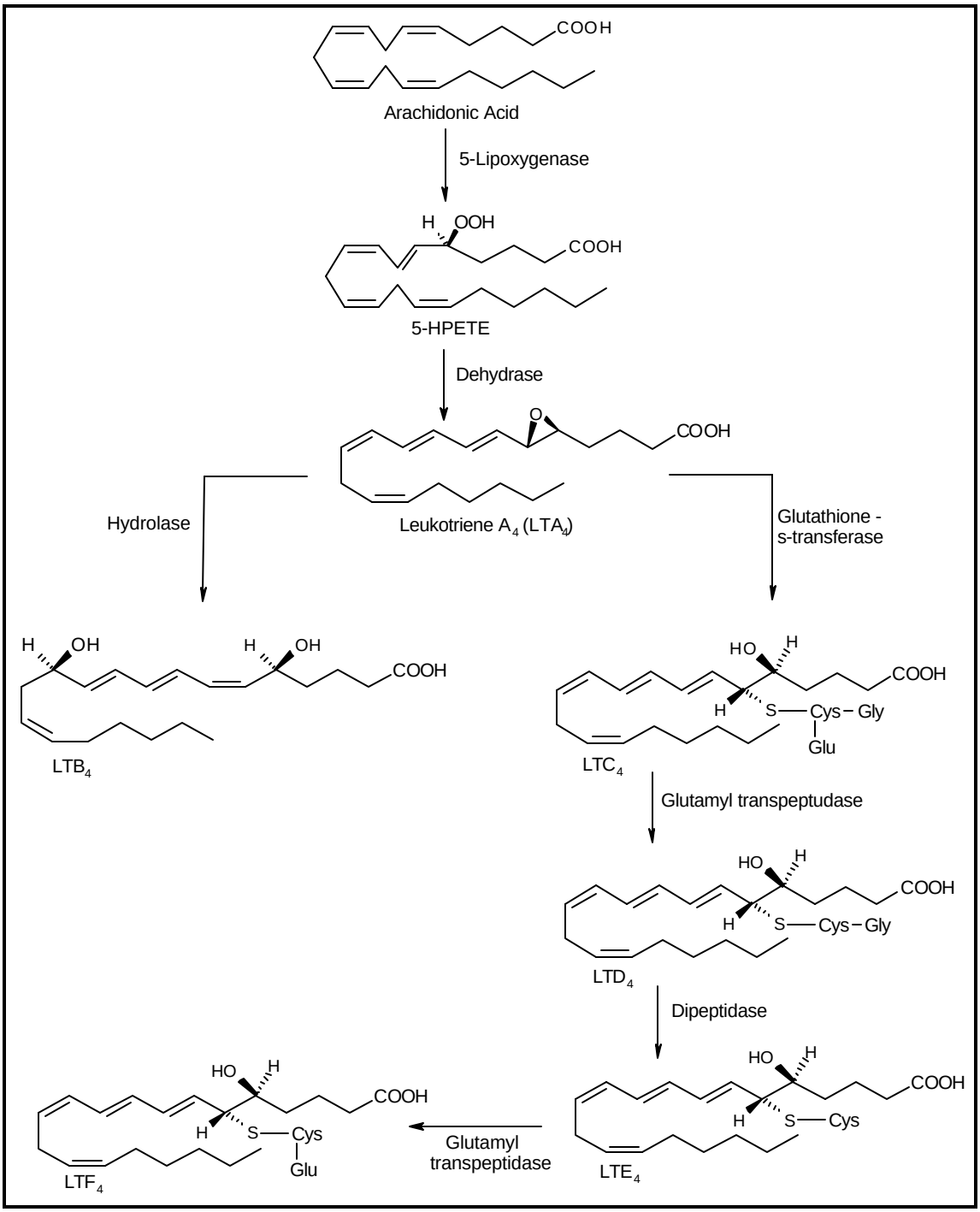
the fifth carbon of AA resulting in the formation of 5-hydroperoxyecosatetraenoic acid (5-HPETE). Subsequently 5-HPETE undergoes dehydration under the influence of enzyme 5-Lo to give unstable epoxide called LTA_4. This step is a rate limiting or determining step in the synthesis of LTs. The unstable leukotrienes LTA_4 is then converted to either LTB_4 (by hydration of LTA_4) under the influence of enzyme LTB_4 hydrolase or to LTC_4 (by the addition of glutathione to LTA_4) in the presence of enzyme LTC_4 synthase. LTC_4 is actively transported out of the cell and rapidly metabolizes to LTD_4 and LTE_4 by successive elimination of a *g*-glutamyl residue and glycine. LTC_4, LTD_4 and LTE_4 are

Fig 2: Schematic Representation of Mechanism of Action of 5-Lo with AA



A mechanism of the reaction of 5-LO with AA.

Scheme 1: Biosynthetic Pathway of Lipoxygenase (Lo)



collectively referred as cysteinyl-leukotrienes (CysLTs) because of their chemical nature. Finally LTE_4 is either excreted in urine or metabolized to less inactive metabolites

including LTF_4. The above description reveals that the ability to synthesis leukotrienes depends on the enzymatic capability to cleave AA from its phosphorylated ester and the 5-Lo enzyme to utilize the substrate AA to synthesize LTA_4.

3.2.3 Physiological Role and Receptors of Leukotrienes

LTs are potent bioactive agents and exert various types of responses in human physiological systems.[10] LTB_4 causes dramatic adhesion of leukocytes to endothelium[12a] and release of toxic oxygen products, lysosomal enzymes and cytokines from proinflammatory cells. It is a mediator in the migration of leukocyte from blood to the area of inflammation, indicating that it is potent chemotactic agent. Similarly it also activates neutrophil. In the cell LTB_4 causes aggregation, degranulation, superoxide generation and mobilisation of membrane associated calcium. The level of LTB_4 is significantly elevated in the patients with atopic eczema, osteoarthritis and rheumatoid arthritis. All these facts made LTB_4 to consider as a major potential mediator of inflammation.

The cysteine LTs (LTC_4, LTD_4 and LTE_4) also called as slow reacting substances of anaphylaxis (SRSA), promote bronchoconstriction and mucus hypersecretion in several species including human.[21] In addition to contractile responses in lungs, cysteine LTs have been shown to contract coronary artery, distal and mesenteric pulmonary artery and vasoconstrictors and negative inotropic effect on the cardiac contraction.[18] Early studies of LTs were focused on their functional responses and their rank order of potency as agonists for various responses. These studies have revealed that responses of LTB_4, and hence possibility of its receptor were distinguishable from CysLTs. There was also an indication that there may be subtypes of receptor for the CysLTs. An IUPHAR (International union of

pharmacology) has formulated recommendations for nomenclature of LT receptors[11b], which have been summarized in **Table 1**.

Table 1: Classification of Leukotriene (LT) Receptor

	Leukotriene receptor type		
	BLT receptor	**$CysLT_1$**	**$CysLT_2$**
Previously known as	LTB_4 receptor	LTD_4 receptor	LTC_4 receptor
Order of potency of agonists	LTB_4 > 12(R)-HETE (LTC_4 and LTD_4 are inactive)	$LTC_4 = LTD_4 > LTE_4$ (in some tissue LTE_4 is partial agonist)	$LTC_4 > LTD_4 > LTE_4$ (in some tissue LTE_4 is partial agonist)

3.2.4 5-Lipoxygenase (5-Lo) a Target for Drug Development

As a first key enzyme in the biosynthesis of LTs, 5-Lo represents an exciting target for therapeutic intervention.[19] The unraveling of biosynthetic pathway and pathological role of LTs in human disease has lead to the development of leukotriene inhibitors. Selective inhibition of this enzyme provides definitive means to limit the effect of LTs. The inhibition of 5-Lo may lead to the discovery of new drugs as an alternative to conventional anti-inflammatory agents having severe side effects. The realization of therapeutically useful 5-Lo inhibitors with satisfactory oral bioavailability, duration of action and minimal toxicity has proven to be quite challenging task as measured by the extent of research and development progressing in this area. To date no detailed structural information is available for the enzyme 5-Lo. In the absence of this, inhibitor design involves intuitive medicinal chemistry guided by biological evaluation (*in-vitroin*/*in-vivo*) in various leukotriene

inhibition assays. At least following four mechanisms can be considered for 5-Lo inhibition.[11a]

(1) Antioxidant/or free radical scavenger

(2) Iron chelation

(3) The inhibition of 5-Lo translocation

(4) Substrate mimics

All inhibitors reported till date act by either single or by the combination of the above mechanisms.

3.3 SYNTHETIC 5-LO INHIBITORS

The contributory role of LTs in the human disorders has prompted the search of the drugs to prevent the deleterious effects of these substances either by inhibiting their synthesis through 5-Lo inhibition or synthesizing antagonists of LTs. Numerous 5-Lo inhibitors have been reported in the scientific and patented literature since the discovery of this enzyme. The present status has been reviewed by J. H. Musser,[11a] D. Steinhilber[20] and D. G. Batt.[21] The compounds reported till date are described below and have been classified based on the structural and mechanistic lines. This organization is somewhat arbitrary mainly due to the diversity of structures for which 5-Lo inhibitory activity is reported.

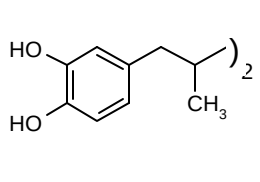
3.3.1 Substrate and Product Analogues

In the earlier development of 5-Lo inhibitors the research efforts have been centered on the rationally designed substrate and product analogues. General approaches specifically from the Corey's group at the Harvard University, include preparation of acetylenic,[22a]

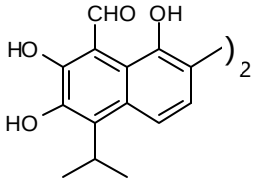
allenic,[22b] methylated,[22c] cyclised and thia analogues of AA and cyclopropyl analogues of LTB_4. 5-HPETE and LTA_4.

3.3.2 Phenolic Compounds

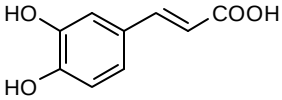
Vast array of phenolic compounds have been explored, as Lo inhibitors.[21] The activity of phenolic compounds against 5-Lo is not surprising, as phenols are well known as a reducing agents. Lipophilic character is a very common feature of most of these inhibitors. These compounds reduce active site iron from ferric (Fe^{+++}) to ferrous (Fe^{++}), one electron oxidation of these compounds yielded detectable free radicals. A large number of catechol containing compounds are reported as 5-Lo inhibitors, many of them are either natural products (nordihydroguaiaretic acid (NDGA), gossypol, caffeic acid etc.) or synthetic analogues. Few reports appeared in the literature dealing with 5-Lo inhibition by



NDGA



Gossypol



Caffeic acid

flavonoid[23] with phenolic hydroxy moiety, quercetin is the most studied compound from this class. Chalcone[24a-d] containing catechol and phenolic hydroxy functionality is the another important class of compounds reported as lipoxygenase inhibitors. Butylated hydroxy toluene (BHT) is another widely used lipophilic antioxidant. In the past decade number of BHT-analogues have been evaluated as Lo and Co inhibitors. Naphthol derivatives[25] have been shown to exhibit promising 5-Lo inhibitory activity. A group at Du Pont discovered that simple 2-substituted 1-naphthol derivatives were potent 5-Lo inhibitor as well as topical anti-inflammatory agents. Amino substituted naphthoquinones[26] and

heterocyclic variants have been disclosed in the patent literature as 5-Lo inhibitors whereas *ortho*-naphthoquinone derivatives from Ciba-Geigy have been recently reported as inhibitors of LTs.

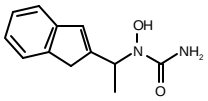
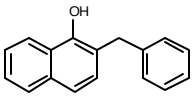
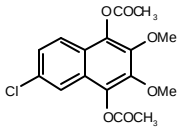
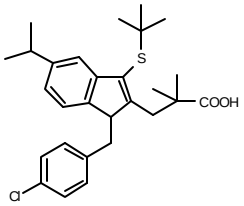
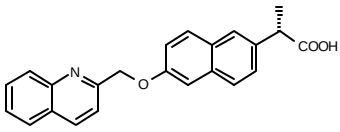
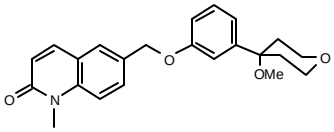
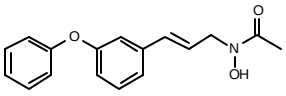
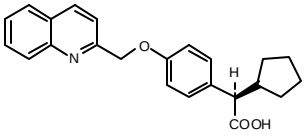
3.3.3 Heterocyclic Compounds

Heterocyclic compounds are well known in the literature for their broad spectrum of biological activities. To date exhaustive study on various series of heterocyclic analogues such as benzimidazole, hydroxythiazole, styrylpyrazole, 1,4-Benzodioxan and indazolinone possessing 5-Lo inhibitory activity has been reported in the literature.[21] Few of them have been proved to be potent and promising as a lead candidate for the further optimization.

3.3.4 Hydroxamic Acid and Related Compounds

In 1984, Corey and co-workers inferred that iron is a critical part of the active site of enzyme 5-Lo. The ability of hydroxamic acid to chelate iron provided the rational for designing the potent Lo inhibitors.[27] Corey[28] and Kredesky[29] reported hydroxamate analogues of AA, which are *in-vitro* potent inhibitors of 5-Lo and are unlikely to be of therapeutic value due to their rapid metabolism *in-vivo*. This concept prompted a number of research groups to explore the hydroxamic acids with more stable lipophilic residues. A series of aralkylhydroxamic acids[30] from Bristol-Myers are based on 9-phenylnonanohydroxamic acid, which is known to inhibit the production of 5-HPETE. Hydroxamic acids have been extensively investigated at Abbott laboratories,[31] where binding site hypothesis was based on the examination of many simple *w*-aralkylhydroxamic acids and hence several series of conjugated hydroxamic acids were explored. Recently Brooks and co-workers from the same pharma company synthesized and evaluated

Table 2: 5-Lo Inhibitors in Clinical Trials

Sr. No.	Name	Structure	Reference
1	Zileuton		34
2	Dup-654		24
3	Lonaplene (RS 43179)		35
4	MK-886		36
5	WY-50,295		11
6	ZD-2138		37
7	BW A4C		38
8	Bay X1005		39

hydroxamic acid derivatives of common NSAIDs[32] and a series of *N*-hydroxyurea[33] as 5-Lo inhibitors.

As a result of this intense drug discovery in the last decade, several inhibitors of 5-Lo have been identified and entered in the clinical evaluation, some of them with promising activity have been listed in **Table 2**.

In short, an effective modulator of the AA cascade for the treatment of asthama and other inflammatory diseases may require 5-Lo inhibitory activity. A wide variety of compounds have been reported as 5-Lo inhibitors. The majority of them appear to be lipophilic reducing agents, including phenol, partially saturated aromatic, compounds containing heteroatoms and heteroatom bonds, hydroxamates and hydroxyureas. However, very few 5-Lo inhibitors progressed to clinical trials mainly due to the insufficient oral bioavailability or toxicity. There is an extensive search going on to get the potent 5-Lo inhibitors with minimal side effects. As more is learned about the enzymology and cellular control mechanism of LTs biosynthesis, exciting new approaches to the therapeutic control of inflammatory diseases, which address this pathway, will certainly be developed and explored. In coming years 5-Lo inhibitors will become a major class of clinically used therapeutic agents for treating various inflammatory conditions mentioned above for which currently no drug devoid of side effect is available.

PRESENT WORK

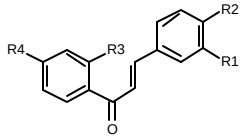
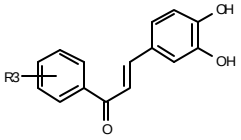
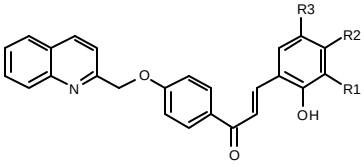
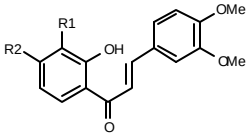
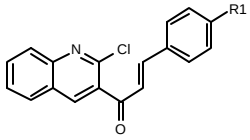
Generation of new chemical lead with specific pharmacological activity is always desired process in medicinal chemistry. As described in the earlier sections there is a growing interest in the drug design and development of 5-Lo inhibitors for the clinical application in the therapeutic treatment of various inflammatory diseases. The search for the better NSAIDs coupled with the rapidly growing combinatorial approach in mind it was planned to design a mini combinatorial library of substituted naphthalene chalcones and their biological evaluation as 5-Lo inhibitors.

3.4 Rational in Designing the Naphthalene Substituted Chalcones

Chalcones, an open chain analogue of flavones are one of the important chemical class of compounds with various biological activities.[40] In 1980 and subsequently some chalcone derivatives have been reported as anti-inflammatory and anti-allergic agents[41] (**Table 3**). Nakadata *et al*[24f] have reported the effect of hydroxy chalcones on the inhibition of 12-Lo and Co of mouse epidermis. The extension of this work is carried out by Sogawa *et al*,[24e] wherein they studied a novel series of 3,4-dihydroxychalcones as potent 5-Lo and Co inhibitors has been studied. The work of both the groups indicated that the chalcones with 3,4-dihydroxycinnamoyl moiety, an active pharmacophore, strongly inhibited the lipid peroxidation. In 1997, Zwaagstra *et al*[24b] have studied synthesis and structure activity relationship (SAR) of quinolinyl substituted carboxylated chalcones as a novel series of $CysLT_1$ (LTD_4) receptor antagonists. Alcaraz and co-workers[24a,24c] have reported 2'-hydroxychalcones and 2-chloroquinolinyl chalcones as inhibitors of inflammatory mediators. Hydroxy chalcones are rapidly and extensively metabolized after systemic administration is one of the major drawbacks in the development of orally active 5-Lo

inhibitors. These findings have directed us to design a series of chalcones without phenolic hydroxy group.

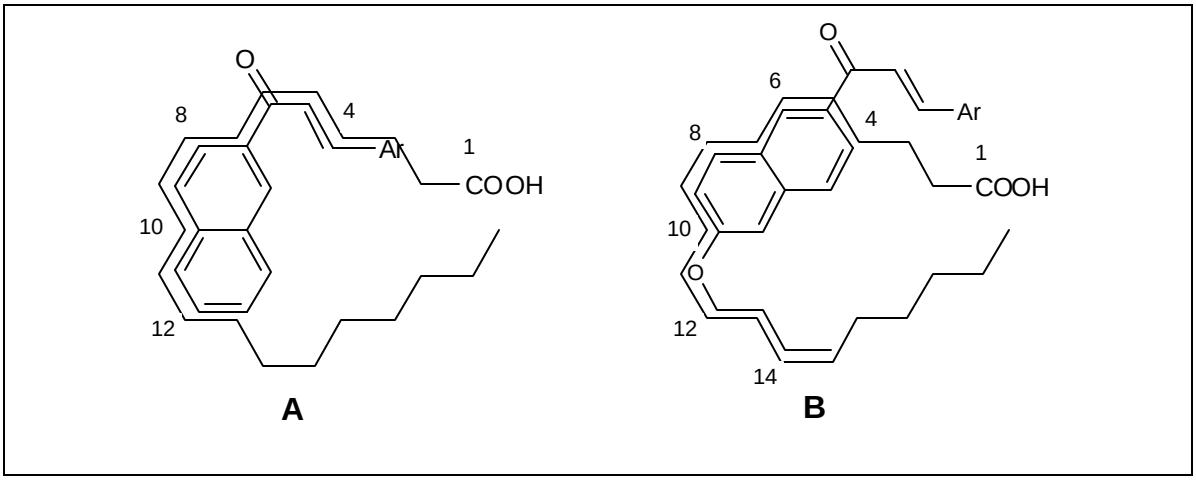
Table 3: Chalcone Derivatives as an Antiinflammatory Agents

Sr.No.	General Structure of Chalcone	Activity	Reference
1	 R1/R2/R3/R4 = H,OH	Co and Lo inhibitors	24f
2	 R3 = Cl, CH_3, OCH_3, NO_2, CF_3, $N(CH_3)_2$	Co and Lo inhibitors	24e
3	 R1 = H/COOH, R2 = H/ COOH, CN, R3 = H, Cl, Br, COOH, OH	$CysLT_1$ (LTD_4) receptor antagonist	24b
4	 R1/R2 = Me, OMe	LTB_4 inhibitors	24c
5	 R1 = OMe, Me, F, CF3	5-Lo inhibitor	24a

The major problem in designing the 5-Lo inhibitors is that there is no experimental evidence available on the conformation of arachidonic acid when bound to 5-Lo and hence one has to mainly depend on the intuitive thinking for the conceptualization of the design of

the molecule. The rational in designing the naphthalene substituted chalcones mainly came from the hypothetical conformation of AA demonstrated by Summers *et al*,[31] who have proposed a partial hypothetical conformation of AA based on the knowledge of Lo reaction. The hypothesis has been supported by their studies on hydroxamate series of ***w***-phenylalkyl derivatives, in which the compounds containing naphthyl groups are generally more potent than the corresponding phenyl derivatives. Following the rules proposed by Summer *et al*[31] the naphthyl ring precisely fits in the arachidonic acid conformation. Combining these two themes, designing of naphthyl substituted chalcones was conceptualized utilizing the hypothetical conformation. Simple graphical representation of these fits is shown below **Fig. 3**. The unsubstituted naphthyl moiety (**A** in **Fig. 3**) might align with C_4-C_{13} portion of

Fig 3: Graphical Representation of Hypothetical Conformation of Arachidonate



archidonate or it was thought that the naphthalene derivative with 6-alkoxy functionality (**B** in **Fig. 3**) may have an additional advantage i.e. naphthyl ring as well as alkoxy chain may align with C_4-C_{10} and C_{11}-C_{15} fragment respectively giving rise to better fit. The another important factor for the selection of naphthalene substituted chalcones is the preference of

enzyme for the more lipophilic substrates.[31] It has been shown that the naphthalene substituted at ***b***-position are more active than those substituted at ***a***-position. Phenolic hydroxyl groups have been categorically avoided because of their known toxicity mentioned earlier. By taking into account of above mentioned facts i.e. Summers model, preference for ***b*** substitution, lipophilicity and enzyme preference, a program to synthesize a new series of 6-alkoxynaphthalene substituted chalcones (devoid of phenolic hydroxy groups) using combinatorial approach, aiming for the potent 5-Lo inhibitory activity with minimal toxicity was launched.

As this work was in progress two reports dealing with the synthesis of combinatorial libraries of chalcones have been appeared in the literature. Marzinzik and co-workers,[42] a group from Novartis Pharma, have demonstrated an access to various heterocycles from ***a,b***-unsaturated ketones on solid support, starting from aldehyde grafted on polystyrene support. The Wittig and Claisen-Schmidt reaction conditions were adopted efficiently to prepare ***a,b***-unsaturated ketone on solid phase. Powers *et al*[43] have reported automated parallel synthesis of chalcone based screening library. Broad range of compounds with structural diversity has been created by using variety of condensation and cyclisation reactions. Very recently Katritzky and co-workers[44] from the University of Florida, disclosed the new protocol for the preparation of resin bound chalcone through a modified Mitsunobu reaction. This resin bound chalcone, a commonly used building block, has been utilized for the synthesis of 2-dialkylamino- and 2-alkylamino-4,6-diarylpyridine and 2,4,6-trisubstituted pyrimidine.

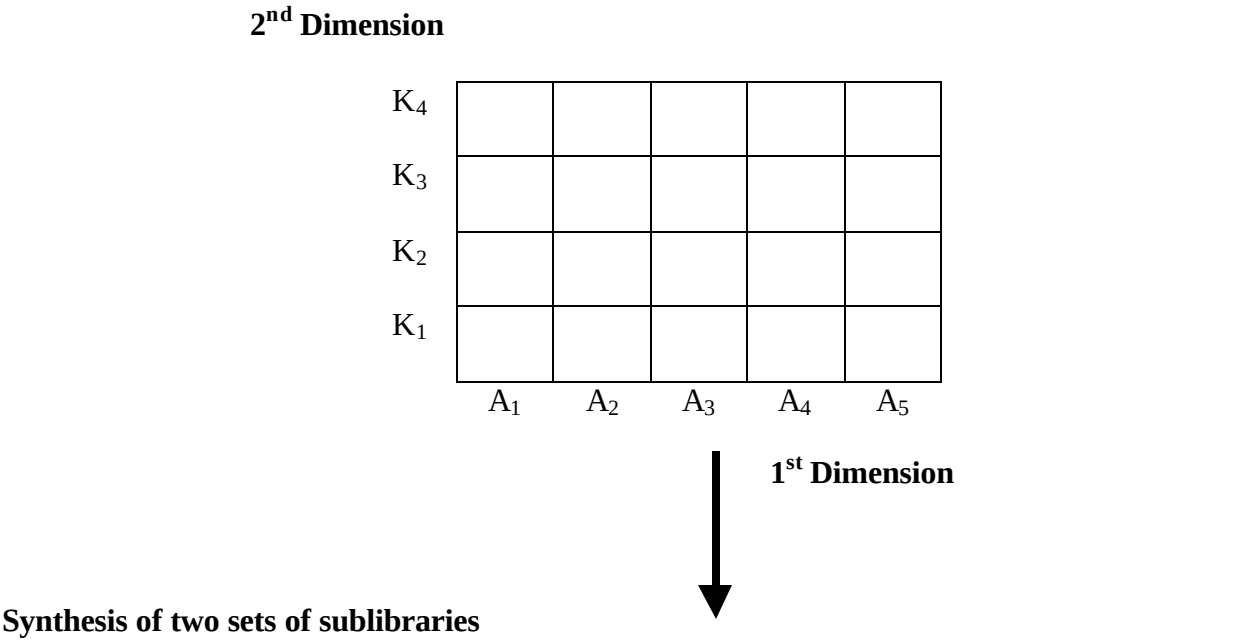
3.5 SYNTHESIS AND BIOLOGICAL EVALUATION OF COMBINATORIAL LIBRARY OF NAPHTHALENE SUBSTITUTED CHALCONES

The combinatorial library of chalcones by solution phase chemistry has been employed for the lead generation of LTB_4 inhibitor. The approach of two dimensional deconvolution screening (positional scanning) is designed on parallel line of previously reported solution phase combinatorial library by Glaxo[7a] and Pirrung.[7b] A simpler positional scan is shown as a matrix representation (**fig. 4**). In this case library is constructed in two combinatorial steps (N = 2) from 2 sets, each of 4 and 5 building blocks. The resulting library is represented as a two dimensional (2D) matrix with 20 cells. Positional scanning of this library involves the division of the matrix into rows and columns. In each of the first four sublibraries, a single **K** building block is reacted with a mixture of the five **A** building blocks. In another set of five sublibraries, a single **A** building block is reacted with the mixture of a four **K** building blocks. Here every compound was prepared twice in the mixtures of different composition. Testing all of the mixtures (nine) allows identification of likely active compounds without a need to resynthesise every compound individually.

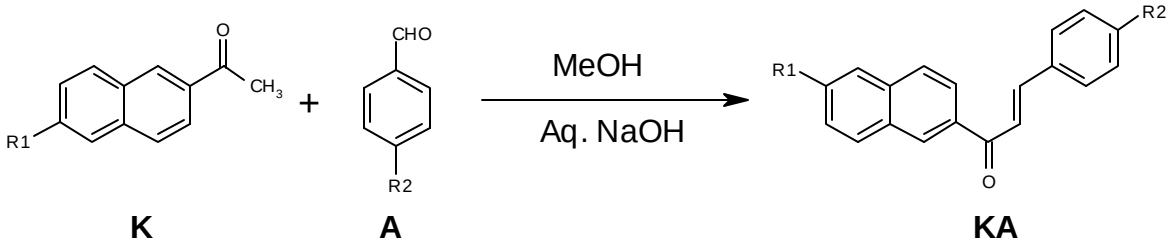
3.5.1 Chemistry

The general synthetic strategy employed to prepare chalcone library was based on the well-known Claisen-Schmidt condensation[45] reaction (**Scheme 2**). A particular advantage of base catalyzed Claisen-Schmidt reaction is that it tolerates variety of functional groups giving rise to large structural diversity i.e. a large choice for the easily accessible carbonyl building block designated as '**K'** is available for the reaction with commercially available aldehydes designated as '**A'** (**Scheme 2**). Under the stipulated reaction conditions, which

Fig. 4: Two Dimensional Matrix Representation:



Scheme 2



K **A** **KA**

are operationally simple and did not give rise to any byproducts, moreover it can be easily and efficiently adopted for the combinatorial synthesis to generate chemical diversity in one step. The products are obtained in quantitative yields (within experimental limitations) and if required can be easily purified to achieve high degree of purity. The required ketones (**K_1** to **K_4**) were synthesized employing Friedel-Crafts acylation of corresponding 6-alkoxynaphthalene, according to the literature procedure.[46]

3.5.2 Library Generation

The library of naphthalene substituted chalcones was constructed by carrying out all possible reactions between ketones and aldehydes. In order to ensure the creditability of reaction a model condensation reactions of few individual components was carried out in order to optimize the reaction conditions. The protocol utilized has been summarized in **Fig. 5**. The small combinatorial library of 20 chalcones (**Fig 6**) was synthesized from 4 ketones (**K_1-K_4**) and 5 aldehydes (**A_1-A_5**) in two sets as 9 combinatorial mixtures (**K_1A_{1-5}---- K_4A_{1-5}** and **A_1-K_{1-4}----- A_5-K_{1-4}**). In the first set each pure ketone (**K_1** to **K_4**) was reacted with stochiometric amount of equimolar mixture of aldehydes (**A_1-A_5**), where as in another set each pure aldehyde (**A_1 to A_5**) was reacted with stoichiometric amount of equimolar mixture of ketones (**K_1-K_4**). Although the reactivities of aldehydes and ketones as well as the reaction rates are different, the reaction conditions employed for the synthesis of combinatorial library were optimized for the quantitative conversion of mixtures of aldehydes and ketones into chalcones. The completion of reactions were monitored by tlc. The individual compounds of the two combinatorial mixtures, one from each set [**K_2A_{1-5}** from set one and **A_3K_{1-4}** from set two] were synthesized and model equimolecular mixtures (authentic mixtures) were prepared. This was essential to establish that the mixtures were

prepared have nearly equal amounts of the individual components. The HPLC analysis of both mixtures (i.e. combinatorial as well as model) revealed that the concentration of anticipated components in the combinatorial mixtures are almost in the same proportion as that of authentic model mixture.

Fig 5: A Protocol for Library Synthesis

Reactants	**Ketone** $K_1, K_2,---K_4$	**Aldehyde** $A_1, A_2,---A_5$
General representation of reaction	**K + A = KA**	
Generation of Library Mixtures	**Set one**	**Set two**
	$K_1 + A_{1-5} = K_1A_{1-5}$ $K_2 + A_{1-5} = K_2A_{1-5}$ $K_3 + A_{1-5} = K_3A_{1-5}$ $K_4 + A_{1-5} = K_4A_{1-5}$	$A_1 + K_{1-4} = A_1K_{1-4}$ $A_2 + K_{1-4} = A_2K_{1-4}$ $A_3 + K_{1-4} = A_3K_{1-4}$ $A_4 + K_{1-4} = A_4K_{1-4}$ $A_5 + K_{1-4} = A_5K_{1-4}$

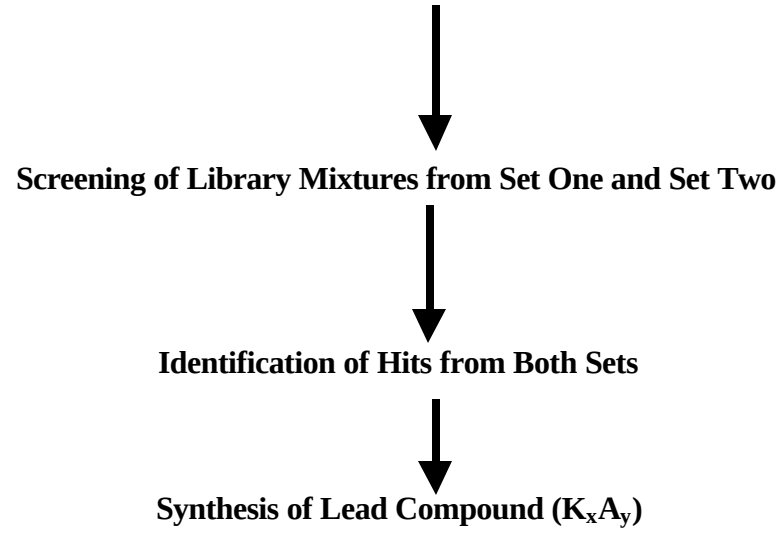
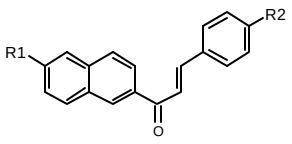
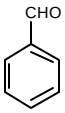
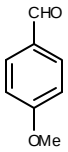
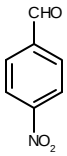
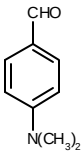
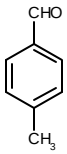
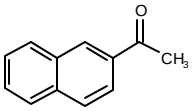
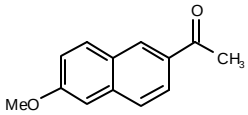
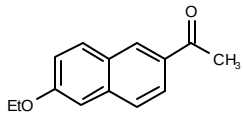
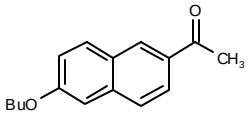


Fig. 6: A Combinatorial Library of Naphthalene Substituted Chalcones

 **KA**	 **A_1**	 **A_2**	 **A_3**	 **A_4**	 **A_5**
K 1 	**K_1A_1**	**K_1A_2**	**K_1A_3**	**K_1A_4**	**K_1A_5**
K 2 	**K_2A_1**	**K_2A_2**	**K_2A_3**	**K_2A_4**	**K_2A_5**
K 3 	**K_3A_1**	**K_3A_2**	**K_3A_3**	**K_3A_4**	**K_3A_5**
K 4 	**K_4A_1**	**K_4A_2**	**K_4A_3**	**K_4A_4**	**K_4A_5**

3.5.3 Biological Evaluation

Numerous testing systems and protocols have been used to study 5-Lo inhibitors in different laboratories. Cell-free and cellular preparations have been employed as a primary screens, for *In-vitro* evaluation.[47] The most commonly used cell-free system is crude cytosolic fraction from broken RBL-1 (red blood leukocytes) cells,[48] wherein various broken neutrophil preparation are also used. The formation of 5-Lo product is generally

determined by radioimmunoassay or HPLC or bioassay. Inhibition of release of 5-Lo products from intact stimulated leukocytes is another widely used evaluation method. Most commonly the calcium ionophore A23187 is used to stimulate the production and release of 5-HETE and LTB_4 by neutrophils. Finally, the method, 5-Lo human whole blood assay (HWBL) developed by Carter *et al*[49] for the evaluation of 5-Lo inhibitors has been chosen as a method of choice mainly because of its versatility and easy accessibility. In this method the formation of 5-HETE and LTB_4 is stimulated by ionophore (A23187) and the amount of LTB_4 released is determined by Enzyme Immuno Assay (EIA).

3.6 RESULTS AND DISCUSSION

The library mixtures were screened using Human Whole blood Cell Assay (HWBL), the method described by Carter *et al*.[49] All nine mixtures synthesized in two sets were evaluated for their LTB_4 inhibitory activity at CytoMed, Inc. USA. The results of their evaluation are depicted in **Table 4**. The % inhibition figures at 30 µM were chosen for comparison in order to minimize the stastical experimental error. Two hit combinatorial mixtures, $\mathbf{K_4A_{1\text{-}5}}$ and $\mathbf{A_3K_{1\text{-}4}}$, each from one set, representing highest LTB_4 inhibitory activity were selected. At 30 µM concentration, the highest inhibition in the set one was shown by the mixture $\mathbf{K_4A_{1\text{-}5}}$ i.e. 31% inhibition, whereas the highest inhibition in the set two was shown by the mixture $\mathbf{A_3K_{1\text{-}4}}$ i.e. 37% inhibition. The other mixtures gave insignificant response as compared to the standard inhibitor. These mixtures showed either low or no inhibition with

Table 4: Results of LTB_4 Inhibitory Activity by HWBL Assay for Combinatorial Mixtures

Combinatorial mixture		% Inhibition of LTB_4 formation in human whole blood cell assay			
		Concentration			
		1 mM	**3 mM**	**10 mM**	**30 mM**
SET ONE	K_1A_{1-5}	-4	-1	2	30
	K_2A_{1-5}	25	12	15	20
	K_3A_{1-5}	13	8	13	26
	K_4A_{1-5}	-	10	24	**31**
SET TWO	A_1K_{1-4}	-6	16	26	19
	A_2K_{1-4}	-14	-5	2	8
	A_3K_{1-4}	20	27	25	**37**
	A_4K_{1-4}	2	12	4	-2
	A_5K_{1-4}	-1	-5	14	-

Fig 7

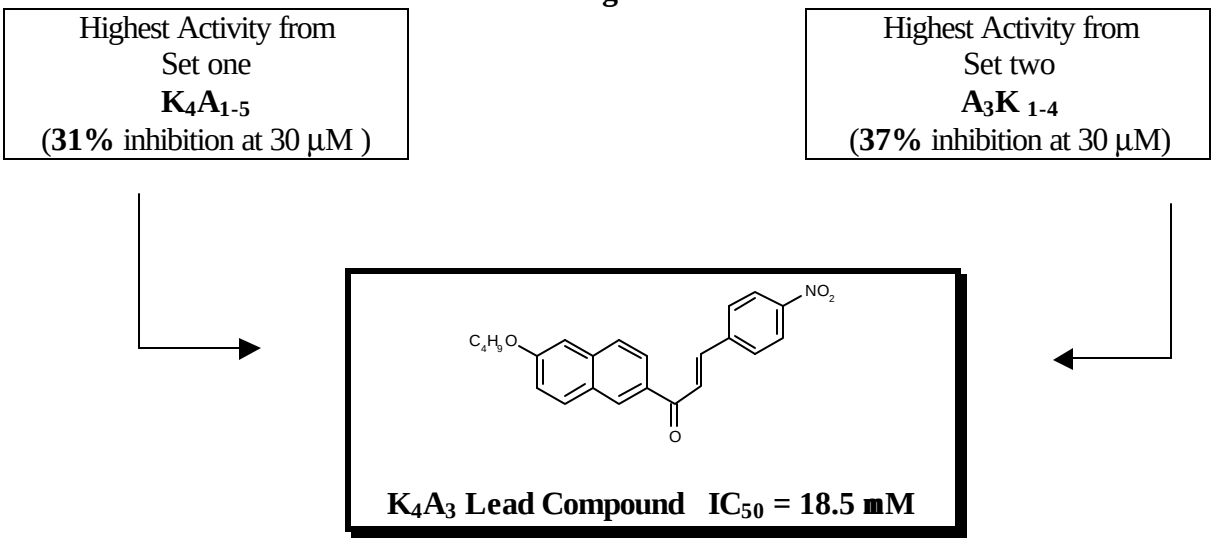


Table 5: Results of LTB_4 Inhibitory Activity by HWBL Assay for Individual Compounds

Individual Compounds	% Inhibition of LTB_4 Formation in Human Whole Blood Cell Assay				
	Concentration				
	1 mM	**3 mM**	**10 mM**	**30 mM**	**IC_{50} mM**
K_4A_3	23	25	36	62	18.5
K_1A_3	-	3	29	61	22.4
K_2A_3	Insignificant activity				
K_3A_3	-	11	24	29	68.4

rather normal biological variations associated with the assay. The two-dimensional deconvolution analysis of biological evaluation indicated the compound **K_4A_3** as a lead compound (as shown in **Fig. 7**). The compound **K_4A_3**, 1-(6-Butoxy-2-naphthyl)-3-(4-nitrophenyl)-prop-2-en-1-one, was resynthesised individually by condensing 6-butoxy-2-acetonaphthone and 4-nitrobenzaldehyde. The lead compound **K_4A_3** was evaluated for LTB_4 inhibitory activity by HWBL assay. The compound showed 62% inhibition at 30 μM concentration with an IC_{50} of 18.5 μM (**Table 5**). The results of inhibitory assay indicated that the nitro group in the aldehyde part enhances the activity. To verify this observation of the role played by the nitro group in the biological activity, all the compounds containing nitro group i.e. **K_1A_3**, **K_2A_3** and **K_3A_3** were synthesized individually and tested for their inhibitory potency. Compound **K_1A_3** and **K_3A_3** were active at 30 μM concentration with

IC_{50} values of 22.4 and 68.4 µM respectively (**Table 5**). Surprisingly the compound **K_2A_3** showed insignificant activity. This phenomenon indicates that the electron withdrawing functionality in the molecule is vital for the desired LTB_4 activity. In the nitro substituted compounds the inhibitory potency of ethers with varying chain lengths at the 6th position of naphthyl ring, the optimum activity was exhibited by the compound containing the *n*-butoxy substituent (**K_4A_3**) as compared to that of compounds containing methoxy (**K_1A_3**) and ethoxy (**K_2A_3**) groups. These results are quite parallel to the results obtained in the case of 6-alkoxy-2-naphthalenecarbohydroxamic acids reported by Sumeers *et al*[31] wherein the compound with butoxy substituent showed more activity as compared to any other alkoxy group.

The above results and discussion reveal that the electron withdrawing functionality in the aldehyde part and long chain ether moiety in the naphthyl part contributes significantly towards the LTB_4 inhibitory activity. Further manipulation of substituents, position of substituents and derivitisation of functional group with an aim to provide better LTB_4 inhibitor is in progress.

3.7 SUMMARY

We have successfully designed and synthesized a mini combinatorial library of naphthalene substituted chalcones by solution phase chemistry. Two dimensional deconvolution methodology was adopted for the biological testing of library. The biological evaluation of library mixtures for LTB_4 inhibitory activity (5-Lo inhibition) using human whole blood cell (HWBL) assay provided the lead compound 1-(6-Butoxy-2-naphthyl)-3-(4-nitrophenyl)-prop-2-en-1-one (**K_4A_3**) with an IC_{50} value of 18.5 µM as a

lead compound. Further manipulation of lead compound may provide more potent 5-Lo inhibitors.

Significance of the Work: Several reports have been published in search of a novel, potent and clinically active 5-Lo inhibitors with structural diversity and huge amount of efforts are currently going on in the pharma industry to develop more and more active molecules. In the present work[50] we have exploited the potential of the hitherto unknown naphtahalene chalcones and contributed to the ongoing research in this field. This information will be useful for the researchers in this field for the optimal designing of the said inhibitor. Simultaneously, the versatility of the combinatorial technique has also been successfully demonstrated for the synthesis of these chalcones and has thus saved time and human resources considerably. At this juncture of our project, it is noteworthy to mention that the combinatorial chemistry has really provided a lead candidate, which can be further explored, based on the present results to achieve the goal of efficient anti-inflammatory agents.

3.8 EXPERIMENTAL

Method of Library Preparation:

Stock solutions of 0.5 M of all individual reactants, ketones (**K_1** to **K_4**) and aldehydes (**A_1** to **A_5**), were prepared in methanol (20 mL). Stock solutions (10 mL) of all components from same reactant i.e. ketones (**K_1-K_4**) and aldehydes (**A_1-A_5**) were mixed separately to obtain **K_{1-4}** and **A_{1-5}**. In case of ketones the solutions of mixed components **K_{1-4}** was diluted to 50 mL with methanol to get 0.1 M of each reactant in solution. Solution (10 mL, 0.5 M) of individual reactant (**K** or **A**) and solution of mixed components of other reactant (**A_{1-5}** or **K_{1-4}**, 0.1 M, 10 mL) were mixed and aqueous NaOH was added (0.5 M, 1 mL). The reaction mixtures were stirred at room temperature for 48 h, concentrated to dryness in vacuo, neutralized with 1 N HCL and extracted with chloroform (2 X 40 mL). The combined organic layers were washed with water, brine and dried over anhydrous sodium sulphate. Concentration in vacuo furnished gummy or solid products, in quantitative yields.

HPLC Analysis:

The two representative combinatorial mixtures **K_2A_{1-5}** and **A_3K_{1-4}** were analyzed by HPLC. The HPLC of combinatorial library mixture was compared with the authentic mixture prepared by mixing, individually synthesized, equimolar amounts of compounds present in the library mixture. For the combinatorial mixture **K_2A_{1-5}** and **A_3K_{1-4}** the authentic mixtures (model mixtures) were prepared by mixing compounds **K_2A_1** to **K_2A_5** and **A_3K_1** to **A_3K_4** respectively. Both these combinatorial library mixtures showed identical HPLC profile when compared to their authentic mixtures prepared in the laboratory.

1-(6-Butoxy-2-naphthyl)-3-(4-nitrophenyl)-prop-2-ene-1-one (K_4A_3):

To a stirred solution of 6-butoxy-2-acetonaphthone (**K_4**, 2.42 gm, 10 mmol) and *p*-nitrobenzaldehyde (**A_1**, 1.51 gm, 10 mmol)in methanol (10 mL) was added aqueous NaOH (10 mmol, 2 mL). The reaction mixture was stirred at room temperature for 24 h. It was concentrated to dryness in vacuo, neutralized with 1 N HCL and extracted with chloroform (2 X 25 mL). The combined organic layer was washed with water, brine and dried over Na_2SO_4 and concentration of organic layer in vacuo followed by the silica gel column chromatographic purification of the residue furnished the pure compound **K_4A_3** in quantitative yield.

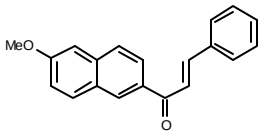
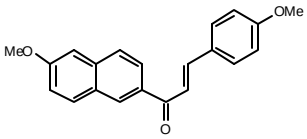
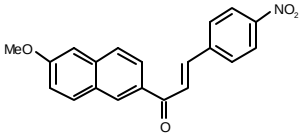
Compounds **K_2A_1** to **K_2A_5** and **A_3K_1** to **A_3K_4** were synthesized by analogues method.

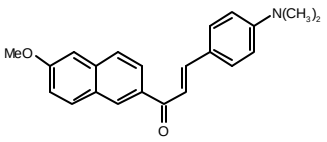
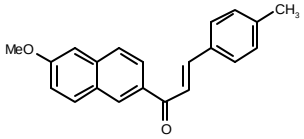
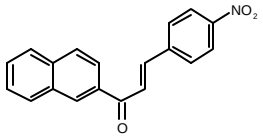
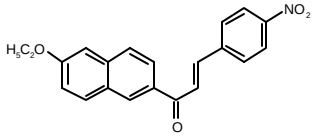
All the compounds were characterized using ^{1}H NMR, Mass and IR spectra and elemental analysis.

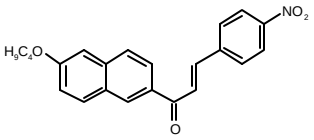
5-Lo Human Whole Blood Assay:

Human blood was collected in heparinised blood collection tubes and aliquoted in 1 mL portion into 1.5 mL microfuge tubes. 5 µl of test compound in DMSO was added to the blood sample and incubated for 15 minutes at 37 °C. Calcium ionophore A23187 (in DMSO, 50 µM final concentration) and the sample were incubated for 30 min. at 37 °C. The samples were centrifuged (1100 X g, 10 min. at 4 °C) and supernatant was assayed for LTB_4 using an EIA kit (Cayman Chemical). All results are mean of duplicate and in most cases triplicate determination.

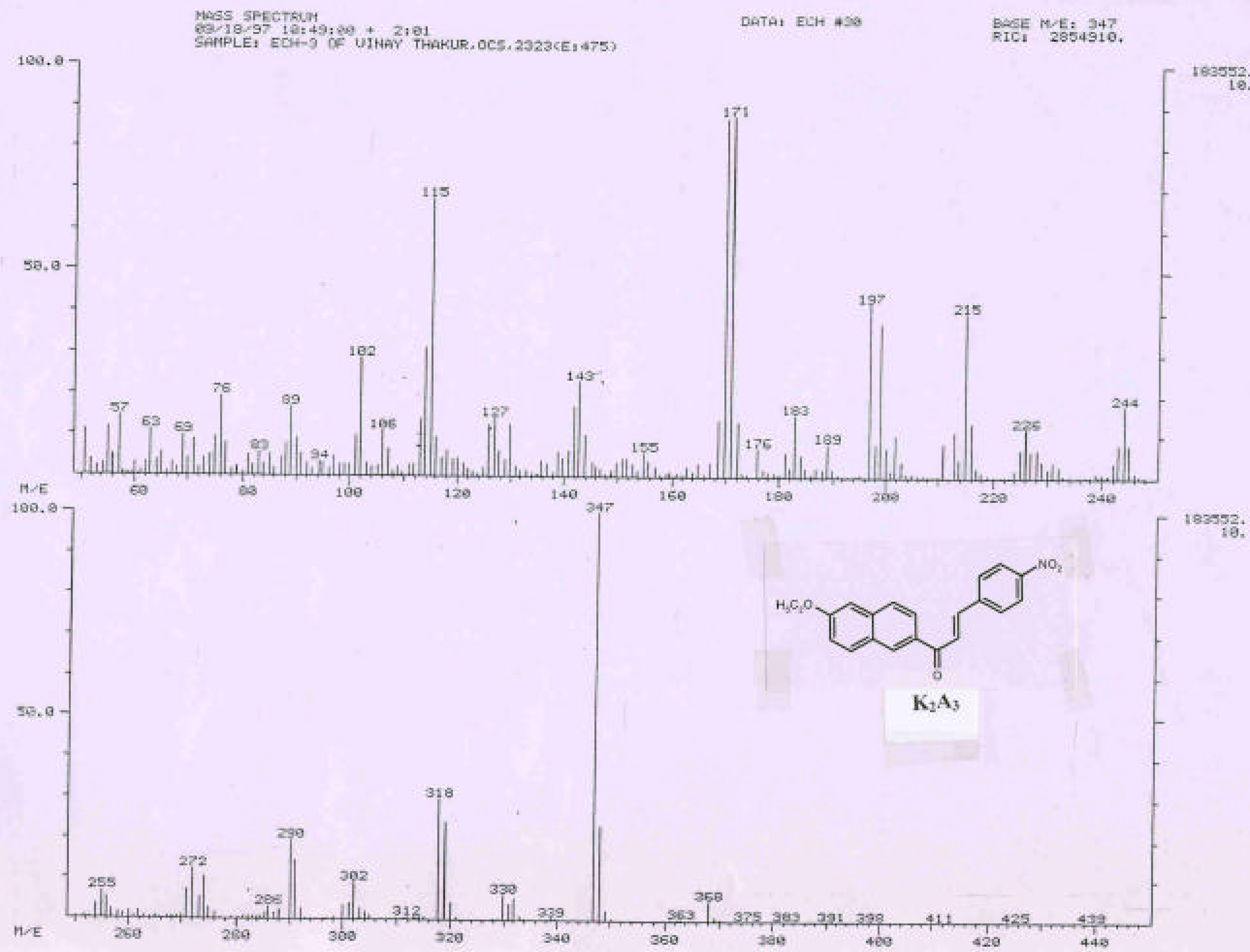
3.9 TABULATED SPECTRAL DATA

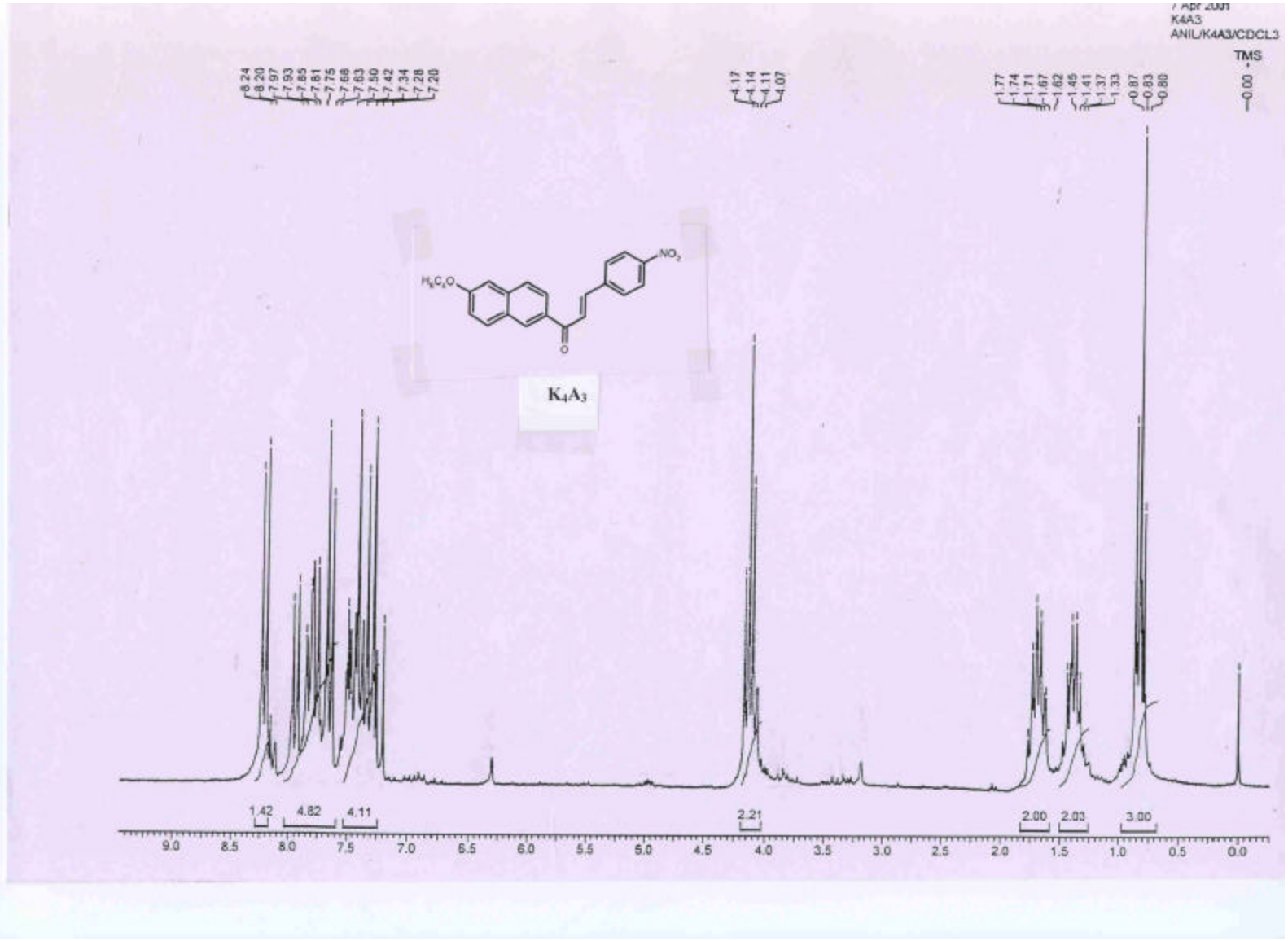
Sr. No.	Structure (Text Code)	PMR (δ); Mass spectral data
1	 **K_2A_1**	**^{1}H NMR** ($CDCl_3$, 200 MHz) δ 3.90 (s, 3H), 7.15 (d, *J* = 16 Hz, 1H), 7.25-7.60 (m, 9H), 7.70 (d, *J* = 8 Hz, 1H), 7.85 (d, *J* = 8 Hz, 1H), 7.95 (d, *J* = 8 Hz, 1H). **MS** (*m/e*): 271, 245, 229, 202, 185, 170, 142, 127, 114, 103, 77
2	 **K_2A_2**	**^{1}H NMR** ($CDCl_3$, 200 MHz) δ 3.85 (s, 3H0, 3.95 (s, 3H), 6.90 (d, *J* = 9 Hz, 1H), 7.05 (d, *J* = 16 Hz, 1H), 7.20-7.55 (m, 6H), 7.60-7.75 (m, 2H), 7.85 (d, *J* = 8 Hz, 1H), 7.95 (d, *J* = 8 Hz, 1H). **MS** (*m/e*): 318, 301, 290, 275, 259, 247, 211, 185, 161, 142, 133, 121, 101, 89, 77.
3	 **K_2A_3**	**^{1}H NMR** ($CDCl_3$, 200 MHz) δ 3.95 (s, 3H), 7.25 (d, *J* = 16 Hz, 2H), 7.35-7.60 (m, 4H), 7.70 (d, *J* = 8 Hz, 2H), 7.88 (d, *J* = 8 Hz, 1H), 8.00 (d, *J* = 8 Hz, 1H), 8.25 (d, *J* = 8 Hz, 2H). **MS** (*m/e*): 333, 316, 290, 274, 259, 228, 215, 197, 170, 157, 142, 127, 114, 102, 76, 63.

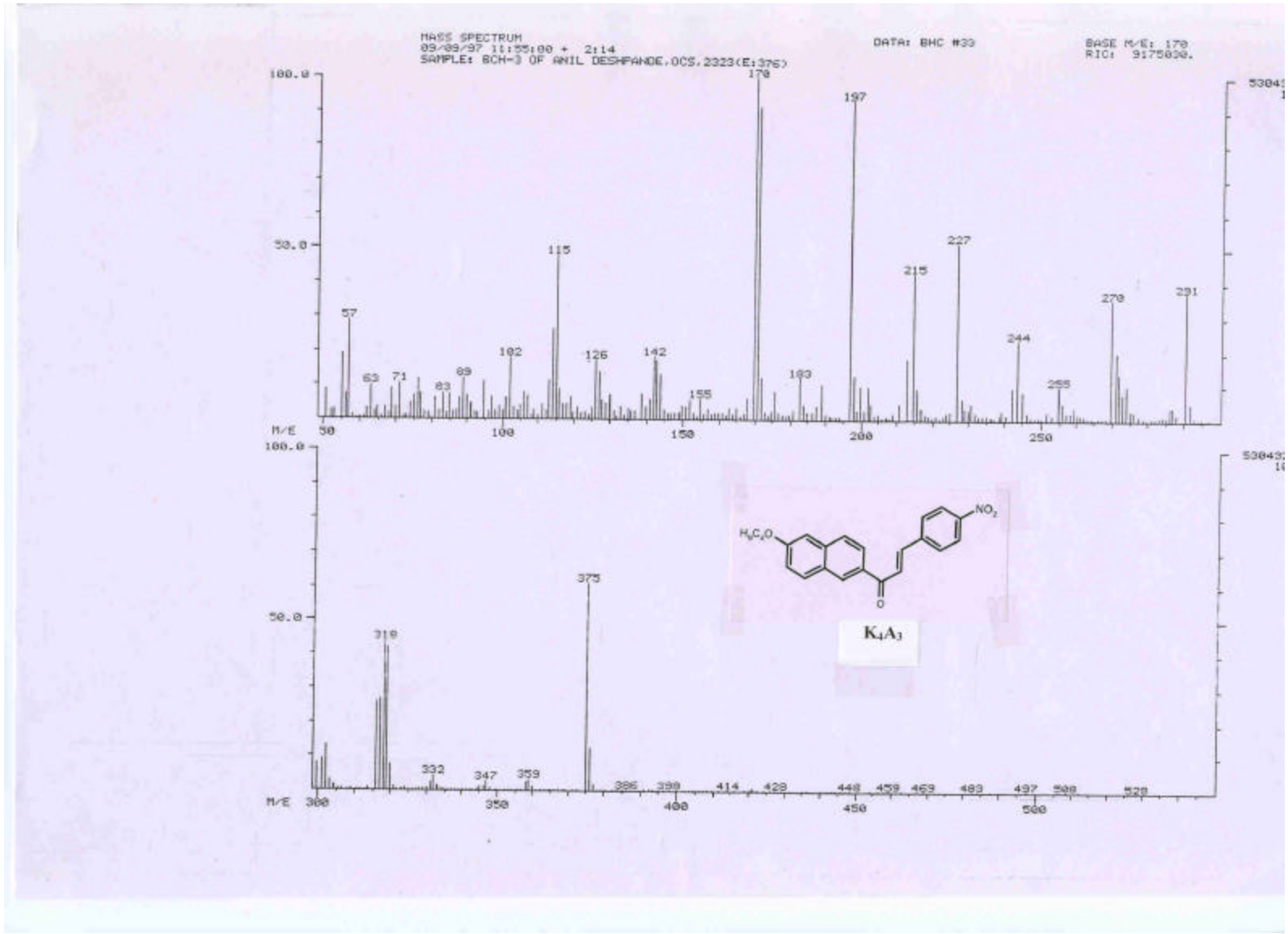
4	 **K_2A_4**	**^{1}H NMR** ($CDCl_3$, 200 MHz) δ 3.07 (s, 6H), 3.97 (s, 3H), 7.72 (d, *J* = 8 Hz, 2H), 7.43-7.65 (m, 5H), 7.75-7.95 (m, 3H), 8.10 (d, *J* = 8 Hz, 1H), 8.50 (s, 1H). **MS** (*m/e*):
5	 **K_2A_5**	**^{1}H NMR** ($CDCl_3$, 200 MHz) δ 2.43 (s, 3H), 3.98 (s, 3H), 7.15-7.30 (m, 4H), 7.50-7.75 (m, 3H), 7.75-7.95 (m, 3H), 8.10 (d, *J* = 8 Hz, 1H), 8.50 (s, 1H). **MS** (*m/e*): 302, 287, 274, 259, 243, 231, 211, 197, 185, 170, 158, 145, 127, 115, 91, 77.
6	 **K_1A_3**	**^{1}H NMR** ($CDCl_3$, 300 MHz) δ 6.70 (d, *J* = 7 Hz, 2H), 7.30-7.75 (m, 6H), 7.75-8.00 (m, 3H), 8.10 (d, *J* = 8 Hz, 1H), 8.20 (d, *J* = 8 Hz, 1H).
7	 **K_3A_3**	**^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.37 (t, *J* = 7 Hz, 3H), 4.25 (q, *J* = 7 Hz, 2H), 7.20-7.60 (m, 6H), 7.65-7.75 (d, *J* = 8 Hz, 2H), 7.75-7.90 (m, 2H), 7.95 (d, *J* = 8 Hz, 1H), 8.25 (d, *J* = 8 Hz, 1H). **MS** (*m/e*): 347, 330, 318, 290, 272, 255, 244, 226, 215, 183, 171, 155, 127, 115, 89, 63.
8		**^{1}H NMR** ($CDCl_3$, 200 MHz) δ 0.85 (t, *J* = 7 Hz,

	 **K_4A_3**	3H), 1.25-1.50 (m, 2H), 1.55-1.85 (m, 2H), 4.14 (t, *J* = 7 Hz, 2H), 7.15-7.55 (m, 5H), 7.60-7.90 (m, 4H), 7.95 (d, *J* = 8 Hz, 1H), 8.14 (d, *J* = 8 Hz, 1H), 8.22 (d, *J* = 8 Hz, 1H). **MS** (*m/e*): 375, 318, 291, 270, 255, 244, 227, 215, 197, 183, 170, 142, 126, 115, 102, 89, 71, 63.

SAMPLE NO. KCH-3 / CDCL3
10.00
5.38
12.47
11.04
5.76
9.89
9.74
8.45
4.31
13.49
50.80
16.43
12.14
PPM
K2A3
H3C
O
O
NO2


MASS SPECTRUM
09/18/97 10:49:00 + 2:01
SAMPLE: ECH-3 OF VINAY THAKUR.OCS.2323(E:475)
DATA: ECH #30
BASE M/E: 347
RIC: 2854910.
100.0
50.0
183552.
10.
57 63 69 76 83 89 94 102 106 115 127 143 155 171 176 183 189 197 215 226 244
M/E
60 80 100 120 140 160 180 200 220 240
255 272 286 290 302 312 318 330 339 347 363 368 375 383 391 398 411 425 439
260 280 300 320 340 360 380 400 420 440
NO2
H3C2O
O
K1A3


K4A3
ANIL/K4A3/CDCL3
TMS
8.24
8.20
7.97
7.93
7.85
7.81
7.75
7.68
7.63
7.50
7.42
7.34
7.28
7.20
4.17
4.14
4.11
4.07
1.77
1.74
1.71
1.67
1.62
1.45
1.41
1.37
1.33
0.87
0.83
0.80
0.00
H_5C_2O
NO_2
O
K_4A_3
1.42
4.82
4.11
2.21
2.00
2.03
3.00
9.0
8.5
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


MASS SPECTRUM
09/09/97 11:55:00 + 2:14
SAMPLE: BCH-3 OF ANIL DESHPANDE.OCS,2323(E:376)
DATA: BHC #33
BASE M/E: 170
RIC: 9175030.
100.0
50.0
M/E
57
63
71
83
89
102
115
126
142
155
170
183
197
215
227
244
255
270
291
319
332
347
359
375
386
399
414
428
448
459
469
483
497
508
528
H_3C_4O
NO_2
O
K_4A_3

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